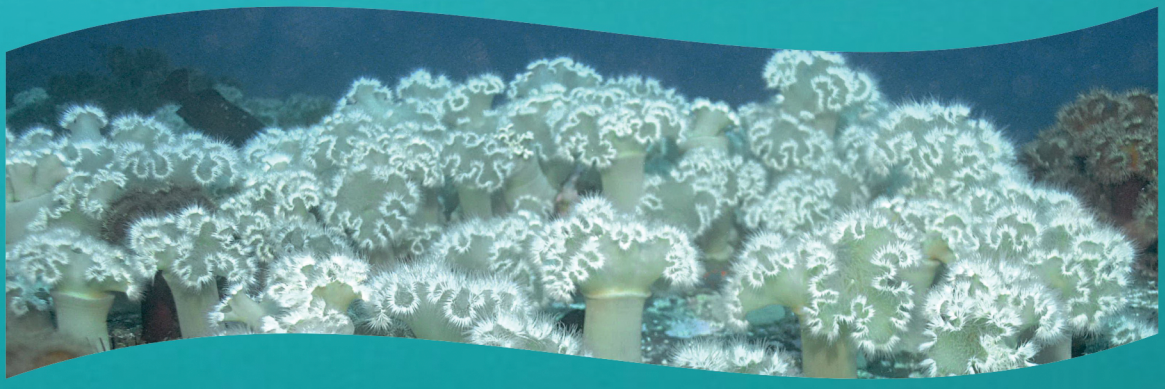
Abstracts
of the International Conference
Marine Biology in the 21st Century:
Achievements and Development Outlook
(in Commemoration of the 100th Anniversary
of the Birth of Academician Alexey V. Zhirmunsky)
October 6-8, 2021
Vladivostok, Russia



Тезисы докладов
Международной конференции
Морская биология в 21 веке:
достижения и перспективы развития
(к 100-летию со дня рождения
академика Алексея Викторовича Жирмунского)
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2021

Far Eastern Branch of the Russian Academy of Sciences
A.V. ZHIRMUNSKY NATIONAL SCIENTIFIC CENTER
OF MARINE BIOLOGY
SCIENTIFIC AND EDUCATIONAL COMPLEX
PRIMORSKY AQUARIUM

Дальневосточное отделение Российской академии наук
НАЦИОНАЛЬНЫЙ НАУЧНЫЙ ЦЕНТР МОРСКОЙ БИОЛОГИИ
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ABSTRACTS
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Greeting from the Chair of the Conference Organizing Committee

Dear Friends and Colleagues,

The third decade of the 21st century was designated by the United Nations as the Decade of Ocean Science for Sustainable Development (2021–2030). This decision clearly demonstrates the importance of marine scientific research to ensuring the sustainability of social, economic and environmental development of mankind. The global challenges that we are facing now, such as climate change or the search for new renewable resources, require international coordination and collaboration to build up capacity for scientific investigations and new technology growth.

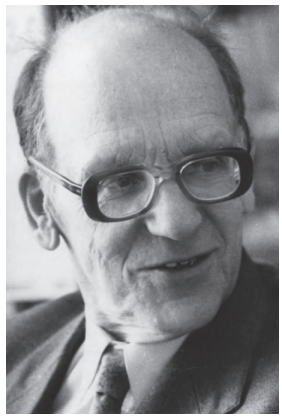
The ocean covers two-thirds of our planet, and today we know much more about it than we have ever done in human history. Yet we still know very little about this underwater universe, which is full of secrets to be discovered. Ocean exploration requires considerable financial support, expensive ships and equipment, submersible robots and satellite technologies. Marine science tasks can be efficiently performed only by joint action. The year 2020 marked the 50th anniversary of the National Scientific Center of Marine Biology, but currently, we are focusing on our next 50 years. We see our future as conducting interdisciplinary investigations in partnership with marine research institutions worldwide.

This conference aims to strengthen scientific and professional contacts, coordinate the development of marine scientific research and increase the spread of knowledge on marine biology. The entire team of the National Scientific Center of Marine Biology and the Primorsky Aquarium is keenly interested in holding the conference and is doing its best to make the event a successful and fruitful experience. We are looking forward to meeting members of the global community of marine biologists, researchers and public aquaria staff. Our mission is to study marine life and educate the public for the sake of sustainable human development in harmony with the ocean.

We are planning to make the conference "Marine Biology in the 21st century" a regular event and a permanent forum for experts to share their opinions. Since the present pandemic restrictions do not allow us to physically get together in Vladivostok, I urge you to join us this year online, through video communication platforms. COVID-19 has shown how illusionary the borders that divide us are. We can shut down cities or even countries but we will continue to live on one common planet, with one common ocean. Science and education have no boundaries. Let's break borders between us and combine our efforts to explore and preserve the ocean!

Inessa Dyuizen
Corresponding Member of the Russian Academy of Sciences,
Director of the A.V. Zhirmunsky National Scientific Center of Marine Biology

Academician Alexey V. Zhirmunsky



The 100th anniversary of the birth of Alexey Viktorovich (October 15, 1921 – October 20, 2000) is celebrated in 2021. The founder and the first Director of the Institute of Marine Biology, since the very moment of its creation until 1989, was Alexey Viktorovich Zhirmunsky, he was an outstanding biologist, Academician of the Russian academy of sciences, one of the leading scientists in marine biology and physiology of marine invertebrates. He headed the Institute for 18 years (1970–1988), and is the Honorary director of the IMB FEB RAS since 1988. Despite the fact that Alexey Viktorovich was a director, he continued his research on physiological ecology and supervised the Laboratory.

The Institute of Marine Biology and, subsequently, the National Scientific Center of Marine Biology were named after Academician Alexey V. Zhirmunsky.

The Far Eastern Branch of Russian Academy of Sciences established the award "for the work in the field of ecology" named by A.V. Zhirmunsky in order to encourage scientists for their works, discoveries of importance for basic and applied research, the results of which were published in monographs and the leading national and international journals.

Academician Alexey Viktorovich Zhirmunsky was an outstanding organizer of marine biological science in the Russian Far East. His greatest services to the country and science were the foundation of the Institute of Marine Biology (IMB) FEB RAS, a first-class academic institution, as well as the academic journal "Russian Journal of Marine Biology", published in Vladivostok, of which the editor-in-chief he was from 1975 to 1989. The results of his activities were the creation of a scientific space, which did not exist in Vladivostok before, including the first marine reserve in the country, and a museum, and several biological stations, and the Young Academy of Marine Biology, and two university departments, and regular all-Union and international conferences and schools, and even the Akademkniga store. (Nadezhda K. Khristoforova, Far Eastern Federal University, Vladivostok, Russia).

Pigmentation and enzymes expressed in pigment cells throughout development and in cell cultures of embryos of the sand dollar *Scaphechinus mirabilis*

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Pigmentation, a natural mechanism, plays an important role in photoprotecting sea urchin embryos and larvae from the harmful effects of solar UV radiation, hypoxia, pathogens, metals and toxicants and might be useful as a marker of environmental stresses. The use of sea urchin embryos and gametes in testing developmental and production effects has been successfully developed by a number of laboratories worldwide.

A new *in vitro* technology without transfecting foreign genes into the sea urchin embryos was developed with the use of the specific components of the coelomic fluids from sea urchins. The expression of two genes (the polyketide synthase – *pks* and sulfotransferase – *sult*) was evaluated associated, possibly, with biosynthesis of naphthoquinone pigments in the sand dollar *Scaphechinus mirabilis* by quantitative real-time-PCR (qRT-PCR). *In vivo*, the highest level of the *pks* and *sult* expression in sand dollar embryos (the Sea of Japan, Russia) was detected at the blastula and gastrula stages. *In vitro*, for the first time, pigment cell number in sand dollar cells cultivated under different conditions (in different culture media) was estimated: the number of pigment cells was higher when the cells were cultured in sea urchin coelomic fluids rather than in seawater. We chose the sand dollar *S. mirabilis* from available species of regular and irregular

Echinoids because the sand dollar embryos and larvae contain many pigment cells in their body. The gene expression profiles for the two genes tested, the *pks* and *sult*, were differed in gametes and throughout the development. In unfertilized eggs and spermatozoids, only trace amounts of both transcripts were detected. The highest *pks* level of expression was observed at the gastrula stage, while the maximum *sult* level was found at the earlier stage of development – at the blastula stage. After the gastrula stage, the level of *pks* expression fell by more than three times at the prism stage and increased at the pluteus stage, but did not reach the previous peak level at the gastrula stage. The level of *sult* expression fell after gastrulation and increased in abundance at the prism and pluteus stages equally but also did not reach the level of *sult* expression at the blastula stage.

To study the effect of different culture conditions on the development of pigment differentiation in the sand dollar cell cultures, we tested three types of media: sea water (*SW)*, the coelomic fluid preparations of normal (*CFn*) and wounding (*CFw*) sea urchins. Pigment cells of a blastula-derived culture were detected in all media tested at all times of cultivation. Pigment cell number was dependent on the coelomic fluid used: more than a two-fold increase in the number of pigment cells was detected after the 10 day-cultivation in the *CFw* relative to that of cells cultivated in the *CFn*. After 10 day-cultivation, a marked decrease in pigment cell number was observed in all tested media.

The gene expression profiling associated with the induction of pigment differentiation has also revealed significant differences between the expression levels of genes studied in sand dollar cells cultivated in different culture media during 4 days. The *pks* expression in sand dollar cells cultivated in the coelomic fluids was increased reliably in comparison to that of cells cultivated in *SW*; at that, the expression level in cells cultivated in *CFw* was significantly (more 2-fold – in 2.1 times) higher than that in cells cultivated in *SW*. These results coincide with our data about the number of pigment cells cultivated in different culture media. The *sult* had a similar trend to that of the *pks* expression profile.

In contrast with our *in vivo* data obtained previously for the sea urchin *Strongylocentrotus intermedius*, the timing of the specific *pks* and *sult* expression differs at the blastula and gastrula stages in the sand dollar *S. mirabilus*, which indicates the specificity of pigment cell appearance in these two echinoid species. Nevertheless, we have confirmed the previously observed fact that the culture medium composition affects the rate of appearance of echinoid pigment cell precursors in culture.

As shown in this study, pigment differentiation in cultured sand dollar cells grown in coelomic fluids was more intense than that in cells grown in *SW*. A significant difference in the proportion of pigment cells grown in the *CFw* in comparison to that in the cells cultivated in the *CFn* could be connected to the specific components of coelomic fluids: a change in the protein composition in the sea urchin coelomic fluid after injury in comparison to the norm and the considerable shift of the absorption maxima for some proteins was previously reported.

However, there is an alternative explanation for these effects, which is connected with carbonic anhydrases (CAs). CA activity has been reported to be involved in the calcification process in numerous invertebrates. In the common littoral crab *Carcinus maenas*, CA activity is highest during the growth of calcite in the pigmented layers, although it is impossible to exclude a limited role for CA in the calcification process. Recently, very low concentrations of a specific inhibitor of biomineralization and a potent inhibitor of CAs, acetazolamide, has been found to inhibit pigment cell precursor differentiation, as well as the production of echinochrome in echinoid larvae. The authors suggest that some isoforms of CAs might be implicated in the formation of echinochrome, providing a possible explanation for the effect of acetazolamide

on the number of pigment cells in sea urchin larvae. Currently, a potential role of CAs in echinoid larval pigment cells remains unknown and requires further study and analysis. The activity of such CAs in larval pigment cells appears to be restricted to sea urchins only.

Numerous attempts to develop cell lines from commercially important marine invertebrate species have been reported but all of these have been unsuccessful yet. Thus, we could not use any echinoid cell line. In this study, q-RT-PCR and cell culture methods have been developed for the quantitative assessment of pigment cell precursor differentiation in sand dollar primary cell cultures.

This assay is a useful tool for assessing the production of naphthoquinone pigments throughout development and in cell cultures of these sand dollars. The findings contribute to the understanding of pigment biology of Echinoid cells and create opportunities for commercial production of natural antioxidants of marine origin.

Smoltification of chum salmon (*Oncorhynchus keta*) in Primorsky Aquarium

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The chum salmon (*Oncorhynchus keta*) is one of three species of anadromous salmon in Primorsky Krai. It comes into the rivers flowing to Peter the Great Bay of the Sea of Japan to spawn from August to October. Two fish hatcheries, Ryazanovsky and Barabashevsky, were built in southern Primorye to maintain chum salmon population.

In May, the juvenile salmon from these hatcheries are released into rivers. If a river is small, the juveniles reach its estuary rather quickly, in several days. There, the stage of smoltification, or parr-smolt transformation, begins, and the fish bodies undergo a series of changes to adapt to life in marine habitat.

In order to show different life stages of chum salmon in the exhibits of the Primorsky Aquarium, some of the fish are kept under freshwater conditions, while others are transferred to sea water. Annually, in February or March, the Aquarium buys chum salmon alevins with an average body length of 35 mm and a weight of 0.39 g, from fish hatcheries. By June, the alevins develop into parrs about 5.7 mm long, with a mean weight of 3.5 g. At that stage, some fish are left in freshwater aquariums, while some are being transformed into smolts to be kept in marine aquariums, by using a specially developed technique of smoltification.

This technique is carried out as follows: seawater (33‰) is added daily to the freshwater aquarium with fish by dripping, increasing the salinity by 2–3‰ a day. When the salinity reaches 16–18‰, the adding of saltwater is stopped for one day and then renewed at the same pace. Thus, the transfer of chum salmon from fresh water to sea water takes 15–16 days. Years of this technique application have proven 100% survival of salmon annually.

As the fish grow in the marine aquarium, the total biomass increases, and some individuals have to be removed. They are relocated directly to the freshwater Upper Amur tank to serve as prey for predatory fish (Siberian taimen, burbot, etc.). Observations show that the saltwater chum salmon can live in this freshwater tank for up to 30 days, successfully feeding, but eventually being eaten up by carnivores because of their small size.

Within two years, the chum salmon individuals that stay in the marine aquarium can grow up to 49 cm long, with a body weight of 1383 g, while their counterparts in the freshwater aquarium reach only 23 cm and 162 g. The growth rate in marine environment is much higher than in fresh water.

Thus, the results obtained indicate that the technology of keeping different life stages of chum salmon in aquaria has been thoroughly developed and can be successfully applied.

Retinal ganglion cell topography and spatial resolution in the masked greenling *Hexagrammos octogrammus* (Pallas, 1914)

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Of all animal sensory systems, the visual system is the most important. It plays a crucial role in adaptation to environmental conditions. Studying peculiarities of the visual system structure and function and its relationship to a species' ecology is of great theoretical and applied interest. The above fully applies to the topography of retinal neurons and spatial resolution (SR) in vertebrates. In the retina of many vertebrates, there are so-called specialized visual areas. These areas are characterized by increased of retinal neurons (in particular, ganglion cells (GC)) and spatial resolution (SR). The presence and localization of specialized visual areas are species-specific and are related to a species' ecology and visual behavior. SR largely determines a more integral parameter, visual acuity. The latter, in turn, plays an important role in visual behavior, in particular, defense reactions, food search, inter- and intraspecific communication. Despite a large number of works on GC topography and visual acuity in bony fishes, many of their groups remain poorly studied in this respect.

We studied GC topography and estimated SR in a hexagrammid fish, the masked greenling *Hexagrammos octogrammus*.

To examine GC topography, a series of retinal wholemounts were obtained and DAPI stained. The SD of cells was studied using stereological analysis based on a series of confocal-microscopic images of retinal fragments.

To estimate the proportion of non-ganglion cells in the inner retinal and ganglion cell layers, a series of retinal wholemounts was obtained and analyzed. The cells were observed and studied by light microscopy using differential interference contrast.

To estimate eye optics parameters and calculate SR, a series of optical eye sections were obtained in the sagittal plane using computer-assisted tomography. SR was calculated based on GC SD and lens radius.

The proportion of non-ganglion cells in the inner retinal and ganglion cell layers was 17.63%. The total number of GCs ranged from 455073 to 688613. The maximum GC SD was found in the temporal quadrant of the retina (so-called area retinae temporalis) and ranged from 9637 to 12685 cells/mm^2. The minimum GC SD was observed in the ventro-nasal periphery and ranged from 824 to 1977 cells/mm^2. The maximum SR ranged from 3.18 to 5.02 cycles per degree.

The masked greenling is an ambush predator attacking its prey at a relatively close distance. The specialized visual area in the temporal retina provides an increased visual acuity in the frontal binocular visual field. This presumably facilitates the detection of prey near the bottom. The present estimates of the maximum SR correspond to a 1-mm prey detection horizon of 36–57 centimeters. The real prey detection horizon can be considerably lower as it depends on the environmental conditions (water turbidity, light level, visual complexity of the environment, prey mobility, etc.) and does not necessarily involve all GC types in a particular visual reaction associated with feeding behavior. For all this, the average prey size of adult masked greenlings (shrimp, amphipods, winged clams, small fish) varies from 0.5 to 9 centimeters. Thus, the spatial resolution in this species is functionally sufficient for efficient detection and catch of ambush prey.

Interestingly, we found no pronounced visual streak, a horizontal or (lessoften) oblique area of increased retinal cell density. Such a specialized visual zone is found in many coastal species. According to Hughes' theory, vertebrates inhabiting open spaces with a pronounced horizon (such as coastal fish species) tend to possess a visual streak, while species preferring enclosed, visually complex environments have concentric visual zones. Apparently, despite the presence of pronounced "water-bottom" and "water-air" visual horizons in masked greenling habitats, local features of the visual environments are of crucial importance. As mentioned above, the masked greenling is an ambush predator preferring cluttered spaces such as rocky gorges, bottom sites covered with algae or seagrass etc. Thus, its local visual environment, although structurally complex, is quite isotropic, at the same time, a pronounced visual horizon may be absent. This may account for the lack of a pronounced visual streak in this species.

Immune parameters of peripheral blood of beluga whales (*Delphinapterus leucas*) from the Primorsky Aquarium

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Belugas (*Delphinapterus leucas*) are one of the first marine mammals successfully introduced by humans into aquariums. At present, however, there are often cases of various immune pathologies of belugas, which can lead to their death. *D. leucas* are kept in the Primorsky Aquarium in pools with flowing sterilized seawater and in open enclosures in the bay of Paris, so they are of considerable interest for practical research. Due to the weak knowledge of the immunity of these animals as a whole and the of the individual variability of their immune parameters in various conditions, the first stage in assessing their health is monitoring the basic immunological parameters of peripheral blood to determine their natural values and the boundaries of the conditional norm.

The purpose of this work was to analyze some parameters of the peripheral blood of belugas and identify on their basis possible features in different groups of animals. We analyzed the leukocyte formula, *in vitro* phagocytosis and reactive oxygen species (ROS) by spontaneous nitroblue tetrazolium reduction test (NBT-test).

Blood of seven individuals of *D. leucas* was provided by M.V. Chistyayeva, a veterinarian of the A.V. Zhirmunsky branch of the Scientific and Educational Complex "Primorsky Aquarium". Three individuals lived in pools with flowing sterilized seawater in the Exposition Building of the aquarium (the conditional group "oceanarium"), and four lived in open enclosures in Paris Bay (the conditional group "enclosures"). Among the studied belugas, five individuals were female and two were male.

To assess the leukocyte formula, smears were prepared in a generally accepted way, and fixed with methanol. After that, the preparations were stained according to the May-Grünwald method and placed in a "Biomount" medium. The smears were analyzed on a Zeiss Axio Imager A1 fluorescence microscope at a magnification of ×100. On each preparation, different types of white blood cells were counted among 100 cells, and then their percentage was determined. The analysis of leukocyte formulas did not show significant differences ($p>0.05$, Mann–Whitney test) between the groups "aquarium" and "enclosures", as well as individuals of different sexes. The distribution of the white blood cell ratio in the blood of belugas was like the distribution in terrestrial

mammals: all animals had a neutrophil profile (53±8.2%), a higher content of eosinophils compared to human blood (8.5±1.9%), which, however, is within the normal range for many domesticated animals. The studied belugas were characterized by an increased proportion of basophils (3.7±1%) compared to the literature data (<1%). An increase in the number of basophils usually indicates possible inflammatory or allergic processes, but such conditions have not been reported under regular veterinary control. Probably, the increase in basophils detected in all the studied animals is not associated with immune pathologies and only expresses the individual variability characteristic of the studied group of belugas.

Leukocyte preparations were prepared in a generally accepted way and used for the analysis of ROS and phagocytic activity. Then they were left in a wet incubation chamber at 23°C for 40 minutes to adhere the cells to the glass. For the NBT-test, a 0.004% NBT solution prepared in phosphate buffered saline (PBS) was added to the adhered cells. After 30 minutes, the preparations were washed with PBS, fixed for 1 hour with a 4% solution of paraformaldehyde (PFA) and after washing, they were enclosed in Mowiol. The absorbed NBT was detected using a microscope in the cytoplasm of cells in the reduced form in the form of dark blue diformazane is within the normal range granules. The proportion of stained cells from the total number of white blood cells was calculated to determine the concentration of cells with ROS. The analysis of this indicator did not give significant differences ($p>0.05$, the Mann–Whitney test), which probably indicates the stability of this indicator to differences in the environmental conditions of the studied groups, as well as its resistance to sexual characteristics.

Staphylococcus sp. strain labeled with fluorescin-5-isothiocyanate (FITC) was added to glass-adhered leukocytes for *in vitro* phagocytosis. After 20, 40, 60 and 80 minutes, the reaction was stopped by fixing with a 4% PFA solution. After washing from PFA, the preparations were enclosed in Mowiol medium and analyzed on a Zeiss Axio Imager A1 fluorescence microscope. The number of phagocytic bacteria per cell (phagocytic index, PI) and the proportion of active phagocytes (phagocytic activity, PA) among the total number of leukocytes (at least 100 cells) were counted in each animal. The PI index had no significant differences from 20 to 80 minutes of incubation with the antigen ($p>0.05$, Friedman's ANOVA), but PA had maximum values at the point of 60 minutes ($p<0.05$, Friedman's ANOVA). At the same time, the PA of white blood cells of belugas from the aquarium was significantly higher than that of animals from the aviary ($p<0.05$, Mann–Whitney test). As known, the intensity of the immune response and homeostasis of the body depends on the PA of cells, so it can be assumed that the immune response of belugas from the aquarium is more intense than that of belugas from the aviary, with an equal PI index. This difference between the groups may be caused by a higher diversity of pathogens in aquariums than in. For example, the number of microorganisms in the exhaled air of bottlenose dolphins from aquariums is thousands times higher than that of bottlenose dolphins from enclosures.

In conclusion, the evaluation of the parameters of the peripheral blood of belugas did not show differences between individuals of different sexes in any of the studied parameters, and showed resistance to differences in the conditions of detention for the leukocyte formula, ROS and PI. A higher PA value was found for the "aquarium" group compared to the "aviary" group, which may be one of the mechanisms of adaptation of innate immunity to conditions that, according to the literature data, are characterized by a large microbial load.

The studies were carried out on the site of the Shared Equipment Facility "Primorsky Aquarium", A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS (Vladivostok, Russia).

Review of animal diversity in hypersaline waters of the world in connection with the problem of desalination of seawater

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Hypersaline habitats, being poly-extreme, are among the most extreme. At the same time, they are quite widespread, especially in arid and subarid zones. There are several types of hypersaline habitats in the World Ocean (ice pore water, lagoons, marine lakes, splash water pools). At present, desalination of seawater leads to the creation of such habitats, and the need for desalination is growing due to the increasing shortage of fresh water in the world. Currently, there are more than 16 thousand seawater desalination plants on the planet, as a result of which 142 million m^3 of brine are produced per day. In most cases, this brine is discharged into the sea; areas of hypersaline waters can be formed in coastal sea areas as a result of this discharge. This often leads to negative consequences, reducing biodiversity and disrupting the normal functioning of ecosystems. Studying the possibility of the existence of animals at salinity higher than oceanic is necessary to predict the possible consequences of this discharge for marine ecosystems. There is also an alternative to dumping brine into the sea, this is its use for the development of hypersaline aquaculture, and this can not only protect marine ecosystems from negative impacts but make desalination more economically viable by obtaining aquaculture products. Authors' long-term research and analysis of world literature have shown that there are a large number of animal species that can not only exist in hypersaline waters but also reach very high numbers in them. In hypersaline habitats, representatives of 12 types, 25 classes, 83 orders, 455 genera, and 809 species of free-living animals have been recorded. The upper limit of the salinity range of occurrence of representatives of animal types differs Cnidaria, Ctenophora, Xenacoelomorpha, Bryozoa – 100 g/L, Annelida – 160 g/L, Platyhelminthes – 190 g/L, Chordata (Pisces) – 220 g/L, Nematoda – 280 g/L, Kinorhyncha – 50 g/L, Rotifera, Mollusca – 310 g/L, Arthropoda – more than 310 g/L. The number of animal types does not depend on salinity in the range from 35 to 100 g/L, with a further increase in salinity, the number of types decreases, and at the highest salt concentration, only Arthropoda was noted. With an increase in salinity above 35 g/L, the number of classes, orders, genera, and species gradually decreases. The diversity of both free-living and parasitic taxa of all levels decreases with increasing salinity at all spatial scales. Quantitatively, this regularity for taxa of different levels manifests itself in somewhat different ways. With an increase in salinity, the total global diversity of species decreases somewhat more strongly than in a separate water body, which is probably due to the presence of a large number of widespread species among

superhalotolerant animals. The decrease in the number of parasitic species with increasing salinity occurs faster than that of free-living ones. The salinity range from 35 to 100 g/L, often not it, but other factors determine the number of living animal species. Conclusions: 1. The impact of brine discharge on marine ecosystems may not be primarily due to the direct increase in salinity; 2. There are many species of animals suitable for cultivation in the resulting brines.

The study was supported by the Russian Science Foundation (grant no.18-16-00001).

The behavior responses of Black Sea *Mnemiopsis leidyi* to a sharp change in light intensity

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Ctenophora is one of the most ancient metazoan lineages. Ctenophores are planktonic predatory gelatinous marine animals. The species *Mnemiopsis leidyi* belongs to the order Lobata. The animals of this order are the largest animals using cilia to move. Synchronous movement of the cilia is coordinated by the nervous system. The complex of the aboral organ is the main sensory structure of the animal, it is regarded as a primitive brain of the ctenophore. It controls the position of the animal in space and its movement and, possibly, is a light sensor.

The comparative morphology and genomics data indicate the ability of ctenophores to sense light. The putative photosensitive organ was found in the various ctenophore species (*M. leidyi*, *Pleurobrachia pileus*, *Bolinopsis mikado*). However, the animal reaction to illumination changes has not been studied yet.

We developed the design of a laboratory experiment to reveal the light-induced behavior of adult *M. leidyi* from the Black Sea.We collected adult *M. leidyi* from the Black Sea (Martinova Bay, Sevastopol) in February–April and May–June 2019. In the laboratory, we placed animals in 200 L (length – 92 cm, width – 55 cm, height – 40 cm) transparent tanks. Animals moved freely in the water column practically without contacting each other. The water temperature in the aquariums corresponded to that in the sea. We divided animals into three size groups: large size with a total body length of more than 80 mm, medium – more than 60 mm, but less than 80 mm, small – less than 60 mm. A total of 104 experiments were performed. We conducted experiments in a dark room. A light source was placed on the sidewall of the aquarium, so that the upper third of the aquarium was illuminated. The movements of the ctenophores we recorded in time-lapse mode (1 frame in 2 seconds, 300 frames in 10 minutes) using a camera mounted perpendicular to the front wall of the aquarium. After turning on the light, the experiment was carried out during the following time intervals: 1–10, 31–40, and 51–60 minutes. Video analysis was carried out using the following programs: Davinci Resolve video editor, ImageJ image analysis program with wrMTrck_Batch plug-in, CtenophoraTrack data analysis program.

We observed that ctenophores of all sizes moved with the greatest speed immediately after turning on the light. Average speed up to ~ 6–7 mm s^{-1} can be considered specific for the *M. leidyi* adults under rest or active feeding. Ctenophore reacts to a sharp

change in light intensity as a stress stimulus, briefly increasing the rate several times (up to 40 mm sec^{-1}). Analysis of motion vectors revealed no directional movement from the light source or towards it. Maximum and average rates decrease significantly for two minutes after the light is turned on, and movement speeds return to the original values within an hour. We also found the features of *M. leidyi* locomotion of different size groups. Small animals are more active than large ones. The mean movement rates of small comb jellies (<60 mm) were 0.7±0.84 mm s^{-1} and 0.18±0.19 mm s^{-1} – for large animals (>80 mm) when averaging the data for the entire observation period (from February to June).

Thus, we can conclude that adult lobate *M. leidyi* instantly responds to a sharp increase in illumination, perceiving light as an irritant. Adult ctenophores fast increase the movement speed and then adapt to a new illumination level, slowing down the speed to normal.

Conservation genetics of marine biodiversity

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Biodiversity is a dynamic outcome of ongoing evolutionary-genetic processes in permanently changing environment. A growing body of evidence shows that human activities may cause multiple harmful genetic consequences, thus, increasing the risk of extinction, especially for heavily exploited commercially valuable species. Therefore, scientific investigations aimed to prevent and correct the detrimental human made transformations of natural populations, which are responsible for large-scale biodiversity losses, should be based on fundamental genetic principles and methodology. The present report, using the example of marine algae, invertebrates, and vertebrates, focuses on some high-priority evolutionary-genetic issues including intraspecific variability, population structure, hybridization and introgression, inbreeding and outbreeding depression, and microbiome structure that are directly relevant for the objectives of conservation and monitoring of biodiversity in the world's oceans. Due to the fact, however, that humans represent the most powerful evolutionary force on Earth, the current technical and scientific advances may not be sufficient enough to protect biodiversity without the complementary development of the ethical paradigm in the human–nature interaction.

The importance of dolphin therapy for children's health

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Currently, aquariums became very important for the society. In addition to their contribution to the ecological education, preservation and reproduction of rare and endangered species, they also bring enormous benefits to human health.

Results of fundamental research indicate a critical state of health in representatives of all age groups, especially in children. Statistics show that the percentage of child disability has doubled since the end of the 20-th century to the present.

Nowadays, important role in the improvement of the human health belongs to dolphins. Dolphin therapy is a promising direction in the development of aquariums. The importance of this prospect signified by the positive influence of dolphins on the human body. Interaction with dolphins can have a significant psychotherapeutic and correctional influence for people.

Dolphin's ultrasound effects not only an individual organs or tissues, but also the cells of the human body, improving intercellular metabolism and fluid circulation. Dolphin's ultrasound signals activate the work of the brain and the whole organism through sensory sound stimulation and contribute to the speech and sensorimotor development of children with a disability.

In terms of psychological and emotional correction of children with disabilities, following take places: the ability to get out of self-isolation (in children with autism), fulfilling the needs for emotional contact, acquiring adaptation skills in society, getting out of depression, stimulation of the endorphins production and replenishing the deficit of positive emotions (in those experiencing maladjustment).

Dolphin therapy has a positive effect on the psychological and physical condition and can be used as a tool for recovery and rehabilitation process of children and adults.

Molecular species composition and thermotropic behavior of the main polar lipids of *Ulva lactuca* during rapid and slow cold acclimation

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The study of adaptive abilities of marine organisms, in particular algae, to temperature change is an important scientific task. The results of these studies could be also useful for mariculture. *Ulva lactuca* is a widespread and valuable commercial species of green algae, which grow in the intertidal zone and vegetates throughout the year.

According to the concept of homeoviscous adaptation, ectothermic organisms, including plants, adjust to alteration in ambient temperature due to changes in fatty acid composition of membrane lipids. This process is aimed to maintain the functionally active liquid-crystalline state of the biomembrane lipid matrix. The most abundant polar lipids of *U. lactuca* are glycolipids which mainly compose thylakoid membranes of chloroplasts: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG). However, extraplastidial membranes of *U. lactuca* are composed of phospholipids and betaine lipid 1,2-diacylglycero-O-4´-(*N,N,N*-tri-methyl)-homoserine (DGTS).

The purpose of this research was to study the changes in molecular species composition and thermotropic behavior of glycolipids and DGTS of *U. lactuca* at different rates of cold acclimation of algae.

Algae were collected in summer at seawater temperature of 19°C and cooled to 4°C (seawater temperature in winter) at rates of 15°C/day (rapid acclimation) and 2°C/day (slow acclimation). The molecular species composition of the individual studied lipids was investigated by high performance liquid chromatography – mass spectrometry (HPLC-MS). The crystal-to-liquid crystal phase transition of the individual studied lipids was investigated by differential scanning calorimetry (DSC).

It was found that at both rates of cold acclimation in the thermal transition of MGDG, which is the major and the most unsaturated lipid in photosynthetic membranes, there were an increase in intensity of the high-temperature peak and a decrease in intensity of the low-temperature peak, which was the main peak on the thermogram of MGDG in summer. This is consistent with seasonal changes and contradicts the classical concept of homeoviscous adaptation. This effect is possibly targeted to inhibit photosynthetic activity when cooling seawater. Changes in thermotropic behavior of other glycolipids

DGDG, SQDG and betaine lipid DGTS at cold acclimation, in contrast to MGDG, were generally aimed at lowering the phase transition temperature. The thermogram profiles of glycolipids were complex, and at cold acclimation they resembled a fit for those winter profiles. In contrast to photosynthetic glycolipids, the peak maximum temperature of thermal transition (T_{max}) of the extraplastidial lipid DGTS exactly reached the winter value at both rates of cold acclimation.

The composition of molecular forms of MGDG was relatively stable when temperature changes. The only predominant molecular form of MGDG was polyunsaturated fatty acid (PUFA)/PUFA, percentage of which was more than 75% of the total. However, with cold acclimation, as well as with a seasonal decrease in temperature, a slight decrease in the percentage of this molecular form was noticeable. And only with a longer cold acclimation, as with the change of season, an increase in the content of the monounsaturated fatty acid (MUFA)/PUFA form was observed. In the molecular forms composition of the most saturated glycolipid SQDG with cold acclimation, as well as with a seasonal decrease in temperature, there was an increase in the percentage of one of the dominant forms of saturated fatty acid (SFA)/MUFA with a decrease in percentage of the predominant form of SFA/PUFA. But only with a longer cold acclimation, as well as with a seasonal decrease in temperature, a significant decrease in the percentage of form of SFA/SFA was observed. Changes in the molecular forms composition of DGDG and DGTS were more complicated than those of MGDG and SQDG, possibly due to a greater variety of molecular forms. The data obtained in the middle of the slow acclimation period showed that changes in the molecular forms composition of the studied lipids were not unidirectional.

In conclusion, both rapid and slow cold acclimation caused approximately the same effect on thermotropic behavior of the major polar lipids of *U. lactuca*, which was close to the seasonal changes. At different rates of seawater cooling the redistribution in composition of molecular forms of polar lipids was complicated. However, it was noticeable that with a longer cold acclimation the trends of changes in the molecular forms composition of MGDG and, in part, SQDG were more similar to seasonal ones, than during 1-day acclimation. The acclimation times chosen in this experiment were not sufficient to achieve seasonal effects.

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Microsatellite DNA assessment of multiple paternity in the white-edged rockfish, *Sebastes taczanowskii*

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Multiple paternity is widespread in wildlife. It is one of the crucial characteristics of the genetic interbreeding systems and models of sexual selection. Data on the number of sexual partners may shed light not only on the evolution of these mechanisms, but also on the rate of genetic drift, speciation, and maintenance of genetic diversity in a population. Multiple mating can be detected by direct observation. However, this approach does not provide understanding of the presence and level of polyandry. The use of microsatellite markers with a high level of polymorphism and relevant statistical methods allow identification of multiple paternity in many fish species.

Some species of the genus *Sebastes* are characterized by polyandry, internal fertilization, and viviparous, which makes them interesting models for testing mating systems and reproductive tactics. Paternity has also been described from 15 grouper species studied to date. In 12 species of the genus *Sebastes*, multiple mating with several males was recorded during underwater observations, but no genetic analysis of paternity was carried out. The biology of white-edged rockfish, *Sebastes taczanowskii*, is relatively well studied, but the genetic aspects of its reproductive system still remain poorly understood. The presence or lack of multiple paternity in wild *S. taczanowskii* was verified through genotypic assignment of females to their broods. The microsatellite primers, developed for closely related *Sebastes* species, were assessed using a sample of adults, and the protocol was optimized to provide reliable and reproducible results (Batishcheva, Brykov, 2021). Five microsatellite DNA loci were used to preliminary estimate the level of polyandry in 10 female *S. taczanowskii* and their offspring. Then, using the method of molecular genetic identification based on fragmentary analysis, we determined the genotypes of 10 pregnant females and juveniles collected by dissecting the females' gonads. The examination showed that the embryonic development of eggs was at different stages of ontogenesis. A comparison between the genotypes of the offspring and their mothers made it possible to identify origin and estimate the number of alleged fathers. Based on the offspring and mothers' genotypes, we could simulate the genotypes of males and appraise the level of closely related crosses. The methods of exclusion and kinship reconstruction were used for the parentage analysis (Jones, Ardren, 2003; Jones et al., 2004, 2010; Flanagan, Jones, 2019). As a result, it has become evident that most females mated with more than one male. Polyandry is probably a very common strategy in white-edged rockfish inhabiting waters of the Far East. The level of polyandry

and genetic diversity in *S. taczanowskii* in this study is similar to those reported for other rockfish species: *S. schlegeli* (Gao et al., 2018), *S. atrovirens*, *S. brevispinis*, *S. diploproa*, *S. elongates*, *S. goodie*, *S. jordani*, *S. proriger*, *S. ruberrimus*, *S. rufus* (Hyde et al., 2008), *S. inermis* (Gonzalez et al., 2009), *S. alutus* (Van Doornik et al., 2008), *S. melanops* (Karageorge, Wilson Jr, 2017), *S. maliger* (Johansson et al., 2012; Gray et al., 2015), and *S. caurinus* (Johansson et al., 2012).

Such a strategy can: (1) improve the genetic diversity of offspring from a single female; (2) potentially decrease the likelihood of incomplete fertilization of eggs in a brood; (3) reduce inbreeding; (4) increase the effective population size; (5) increase the rate of genetic drift; and (6) contribute to maintaining the genetic diversity in the population (Hyde et al., 2008; Sogard et al., 2008; Van Doornik et al., 2008). The obtained data on the number of sexual partners are important for understanding the evolution of models of sexual selection and reproductive behavior in Far Eastern rockfish and allow further comparison of the levels of polyandry in different species within the same genus.

Biogeographic patterns of marine planktonic cyanobacteria in coastal habitats – what can we inferfrom 'omics' based approach?

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The structure of marine planktonic cyanobacterial communities in coastal habitats can strongly influence rates and fluxes of coastal carbon cycling. Biogeographic patterns of marine planktonic cyanobacterial assemblages were elucidated from four coastal habitats namely, Baltic Sea (BL), Monterey Bay (MB), South China Sea (SCS) and Sundarbans (SB) based on deep phylogeny of 16S rRNA sequences generated using next generation sequencing and Sanger sequencing approaches. Based on 16S rRNA phylogeny, four major taxonomic orders of marine planktonic cyanobacteria were recovered in varying proportions with several novel 16S rRNA sequences in each of the four targeted habitats. Members of the order Synechococcales were dominant in all the habitats (94% sequences) while the orders Chroococcales and Oscillatoriales were only detected in SB and SCS, respectively. In the phylogenetic tree, *Synechococcus*-like sequences showed overwhelming dominance in SB and they were found in three other habitats. *Prochlorococcus*-like sequences were found in sizeable number in MB and SCS but were absent in SB and coastal BL. *Synechococcus*-like sequences were represented by three major marine clusters (5.1, 5.2, and 5.3). Three novel clades as part of *Synechococcus* cluster were detected only in SB and one novel clade in BL. These clades could have potential functional significance in coastal carbon cycling. Interestingly, cultured based approaches also revealed a new species of *Synechococcus*, *Synechococcus moorigangaii* strain CMS01 from SB which has the ability to grow across a range of salinity and metabolize different forms of nitrogen as well as fix di-nitrogen. The genome of *Synechococcus moorigangaii* strain CMS01 revealed presence of genes coding for essential metabolic proteins, transport proteins and other cellular apparatus, genome also codes for proteins involved in flagella and pilus formation which was never reported previously in any coastal species belonging to this genus. This reflects the potential unexplored functional diversity of marine planktonic cyanobacteria in coastal habitats which have consequences for ecosystem functioning. Overall the study has revealed that majority of the marine planktonic cyanobacterial OTUs were found to be exclusive to each habitat, whereas some were shared by two or more habitats based on beta-diversity analysis.

Marine wilderness concept for arctic and pacific geoographical research

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Biodiversity, adaptation strategies, factors and conditions for the life formation, composition of the environment and other characteristics of functional ecology in modern world usually depend on the anthropogenic impacts. Is there an alternative to this? Which title could belong to the nature not affected by human influence? 'Wilderness' – this concept has an overseas origin. This term appeared after centuries of practice to denote the most mysterious and inaccessible places for man. It was offered into science by american environmentalists (O. Leopold, R. Muir, R. Marshall).

Wilderness was approved officially at the national level in the United States through the 1964 Wilderness Act. The idea of wild nature is transmitted to a person intuitively, figuratively, the logic of a person interprets it with the meaning that natural ecosystems are always more than a resource, it is a life space that is not intended for human needs (R. Nash). From our modern perspective, the image of "wild nature" conveys the holistic meaning of the areas across the planet which are free from the significant influence of human economic activity.

In contrast to the West, the idea of wildlife conservation was substantiated by Russian scientists from a cognitive and research position, considered historically, consolidated as a special nature conservation area, developed in a practical sense by the organization of 'zapovednik (reserve) network' (Kozhevnikov G.A., Dokuchaev V.V., Borodin I.P.). In the official list of national specially protected natural areas, 1*b* category 'wilderness' has been replaced by 1*a* category 'zapovedniks'. The idea of 'reserved nature' was successfully implemented during the Soviet period, but it was significantly corrected after the disappearance of the USSR. What can be noted in the context of understanding the wildlife of the seas and oceans? The concept of marine wildlife is primarily aimed at identifying such ecosystems in which undisturbed areas of nature and populations of organisms are indeed preserved that do not experience a critical anthropogenic impact.

The concept of marine wildlife represents a fundamentally new basis for marine spatial planning and regulation of economic activities. It can organically include various strategies for the conservation of ocean wildlife, conservation of biosphere functions and ecosystem services, provide practical formats for the conservation of biodiversity, implement the principles of the ecosystem approach, identify the specifics of interaction in the land-ocean contact zone, implement integrated management of the coastal zone etc.

Many studies have established approximate scales for identifying wildlife: global marine ecosystems (or ecoregions of the world) include coastal regions from river basins to continental shelves and extend far beyond ocean currents. FAO developed a Concept and compiled a list of vulnerable marine ecosystems. Indicator groups and habitat areas have been identified to assess marine areas and their status (FAO, 2009).

According to modern estimates, more than 70% of the Earth's surface is occupied by marine ecosystems, two-thirds of which require special measures to preserve biodiversity (Jones et all., 2020), and only about 13% of the marine space can be considered "wild". It's worth noticing here that the definition of specially protected marine areas is still poorly substantiated. As a rule, it is based on the impact of certain nature uses on biodiversity (Horta e Costa et al., 2016). In Russian research activities, the concept of marine wildlife is practically not used. Despite this, it should be noted that Russian research institutes have carried out large-scale complex biological and oceanological studies of all seas surrounding our country, as well as far beyond them, including identification of vulnerable marine ecosystems (Spiridonov et al., 2018).

The landscape-bionomic zoning was carried out (from global to local ecosystems) by the example of the Barents Sea. The creation of a system for the conservation of biological resources is proposed at the hierarchical level (Matishov et al., 2009). Such scientifically grounded recommendations are focused on the rational use of deep-water zones for fishing and the protection of the marine environment outside the territorial waters. Integrated marine area management plans have been developed for many areas such as the Barents Sea (2010). They are based on national interests in the resources of the World Ocean in the system of balanced marine nature management.

Extensive studies on the geography of the World Ocean (Bogdanov, 1978) confirm in an empirical representation many facts of the use of ocean resources, and convey the general trend of the rapid disappearance of populations and species, simplification of the structure of communities and the functionality of ecosystems. Despite this, methodological techniques for assessing 'wild' and 'vulnerable' nature are associated with the justification or regulation of sustainable fishing in the deep-water regions of the World Ocean (Ardron et al., 2014). We believe that based on the idea of wilderness, many international initiatives to establish marine protected areas beyond the limits of national jurisdiction acquire completely different meaning.

The main task of our work can be conveyed by the thesis of the need to develop methods for the delimitation and mapping of marine wildlife of the Arctic and Pacific marine ecosystems based on the use of satellite images, the organization of interactive mapping and processing of positioning data of sea vessels. The possibility of integrating scientific and informational support for monitoring the state of the marine environment, substantiating marine planning and organizing information on commercial populations of aquatic organisms is approved.

It is proposed to identify the tools for registering the taxonomic composition and the degree of knowledge of marine flora and fauna, as well as the methods of processing

quantitative indicators for assessing economic activity in coastal areas (as a potential threat to the development and pollution of the sea) and density of maritime transport in the water areas.

There is also considered availability of facilities for the extraction of mineral resources and operational information on emergencies during transportation, including other factors of the possible threat of pollution of the marine environment, the study of Arctic environmental problems based on the idea of conservation of wildlife.

The contribution of apoptosis and necrosis in freezing injury of marine invertebrate cells

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Marine invertebrate embryos and larvae are strongly influenced by their environment and often used as bioindicators in ecotoxicological studies. Bivalve mollusk and Echinoderm cells are a promising tool for researches of oxidative stress, UV light perturbations and senescence. The development of cryopreservation methods for marine invertebrate cells is important for these studies, but freezing-thawing leads to different cell injuries, abnormalities in mitosis and cytoskeleton disassembly. To improve the cryopreservation outcomes of the cells, we focused on the morphological and functional alterations in cells of mussel *Mytilus trossulus* and sea urchin *Strongylocentrotus intermedius* and identifying the predominant pathway of their cell death after freezing-thawing.

Apoptosis and necrosis can occur independently, sequentially, or simultaneously. The induction of these two processes is regulated by many of biochemical factors, and the degree of stimulation often determines whether the cells die via apoptosis or necrosis. Necrosis, which is characterized by cell swelling and a loss of cell membrane integrity, is triggered by multiple stresses, such as osmotic shock, hypothermia, hypoxia, and mechanical or chemical injuries. In contrast, apoptosis is a highly organized and evolutionarily conserved cellular process that occurs under normal physiological conditions and is accompanied by gene expression of caspases and other enzymes responsible for controlled cellular disruption. Many stress factors are well-known initiators of necrosis and apoptotic cell death. Necrosis is frequently observed during the cryopreservation of mammalian cells. Apoptosis may also be initiated by a variety of extracellular and intracellular stresses, including exposure to extreme temperatures. Very few studies have analyzed the mechanisms underlying cell death pathways in marine invertebrate cell cultures after a cold shock. Freezing-thawing and the use of cryoprotectants result in different cell disturbances in cells that could lead to cell death.

To estimate the effect of cryopreservation on the sea urchin and the mussel cells and identify the predominant cell death pathways, we analyzed cell viability (via MTT, H_2DCFDA and DAPI staining), caspase activity (via flow cytometry and spectrophotometry), the level of apoptosis (via annexin V and TO-PRO™-3 staining), and cell ultrastructure alterations (via transmission electron microscopy). Using general caspase detection, we found that the level of caspase activity was low in unfrozen cells, whereas the number of dead cells and cells in late apoptosis increased significantly after

freezing-thawing. The data using annexin V-binding assay revealed a high apoptosis level in all tested samples, even in unfrozen cells (about 66%). Thus, annexin V assay appears to be unsuitable for sea urchin embryonic cells.

Apoptosis was not a main death pathway in mussel cells, but it was induced in a significant part of these cells (up to 24%) immediately after thawing and depended mostly on the cryoprotectant used. These results assume that physical cell disruption but not freezing-induced apoptosis or necrosis is the predominant reason of cell death in cultures after freezing-thawing with any cryoprotectant combination.

We also examined whether apoptotic inducers, such as staurosporine (a natural antibiotic), camptothecin (a topoisomerase I inhibitor), and mitomycin C (a drug of the camptothecin family), that are routinely used for chemical induction of apoptosis in mammalian cells, can induce apoptosis in cultivated molluscan larval cells. Only staurosporine resulted in an evident apoptotic increase in molluscan cell cultures: 9.06% early apoptotic cells in comparison with 5.63% in control frozen-thawed cells and 20.6% late apoptotic cells in comparison with 10.68% in controls. Camptothecin did not significantly induce molluscan cell apoptosis but did cause a slight increase in the number of active cells after thawing. Mitomycin C produced similar results, but its effect was less pronounced. In addition, we hypothesize that the use of the apoptotic inhibitors could reduce apoptosis. We tested three apoptotic inhibitors used for mammalian cells, such as Y-27632, cyclic pifithrin-α, and CHIR99021 in order to reduce apoptosis after cryopreservation; however, our attempts were failed. Development of this direction is important for understanding the mechanisms of marine organisms' cold susceptibility and improving the cryopreservation outcomes of their cells.

NW Pacific biodiversity and biogeography studies help understanding faunal changes in the Arctic Ocean

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In the past six years, the biology of the bathyal, abyssal and hadal faunas of all size classes (meio-, macro-, and megabenthos) of the NW Pacific have been intensively investigated based on a total of four Russian-German and German-Russian expeditions with both RV *Akademik M.A. Lavrentyev* as well as RV *Sonne.* These expeditions have provided a wealth of faunistic data on the systematics, evolution and biogeography of benthic organisms from the Sea of Japan, Sea of Okhotsk, the NW Pacific abyssal plain and the Kuril-Kamchatka Trench (KKT) as well. In the north, the KKT is connected to the Aleutian Trench (AT) and to the Bering Sea by the Kamchatka Strait (191 km wide and 4420 m depth). The aim of our study was to deliver a sound biogeographic baseline study of the composition and distribution of benthic organisms in the northeastern (NE) part of the Kuril-Kamchatka Trench and the Aleutian Trench where only six stations were sampled in the past with RVs *Vityaz* and *Akademik Mstislav Keldysh*. We plan to sample three of the previously sampled stations in the NE Kuril-Kamchatka Trench, the Kamchatka Strait and in the AT from board of RV *Sonne* in summer of 2019 in order to complement our previous benchmark biodiversity studies which will serve as a background for future identifications of biogeographic changes in the NW Pacific. From our four expeditions to the NW Pacific deep sea (SojaBio, SokhoBio, and KuramBio I and II), we have mobilised 7,042 unique deep-sea taxa distribution records to open-access databases, of which 1,723 records were at the species level. Using this dataset and data collected from open-access databases, we analyzed species richness, endemism rates, and faunal community composition in the shallow and deep NW Pacific and adjacent Arctic Ocean. Our results supported the hypothesis that, although biodiversity is increasing in the tropics and coastal depths (especially in the Philippines) is highest, but decreases at the equator and at depths below ca. 2000 m. Despite the high species richness around the eastern Philippines, the Yellow Sea and Gulf of Tonkin had the highest benthic species endemism rates (ca. 70%), while the Aleutian Islands had the highest pelagic endemism rates (ca. 45%) among all the different ecoregions. Our generalized linear models (GAMs) showed that the combined effects of all environmental predictors provided the best model for species richness in both the shallow and deep seas of the NW Pacific. These results provides baselines for marine conservation plans and inform the policy, as species richness and knowledge of endemic hotspots are necessary prerequisites for preventing biodiversity loss.

Wetlands and formation of water quality: a review of coastal salt marshes

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Coastal wetlands contribute to formation of seawater quality. There are several types of coastal wetlands, for example, salt marshes. The most common type of the marshlands (i.e., salt marshes and tidal marshes), is a coastal ecosystem in the upper coastal intertidal zone between land and open saltwater or brackish water, over which it floods tidally. The theory of polyfunctional role of the biota (sum of organisms in the marshlands) on water self-purification that was developed by S. Ostroumov in a series of publications (starting year 2000 and later) helps to better understand and analyze functions of organism dwellers on the salt marshes toward formation and improvement of seawater quality. In this paper, the role of the organisms in coastal wetlands toward seawater self-purification is reviewed, discussed and concluded.

Marine Research Laboratory of the Zoological Institute of the Russian Academy of Sciences in the study of marine ecosystems of the Arctic and Far Eastern Seas in the 1960s–1980s under the direction of A.N. Golikov

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The year 2021 marks the 90th anniversary of the birth of Alexander Nikolaevich Golikov (1931–2010), an outstanding Russian marine biologist, one of the leading specialists in the field of biocoenology, general and local hydrobiology, malacology and biogeography. Alexander Nikolaevich was the head of the Laboratory of Marine Research of the Zoological Institute (ZIN) from 1965 to 1992. During these years, the seas of the Arctic, Far East and Antarctic were the main research areas of the Laboratory. Wide use of light diving equipment and the development of a special quantitative diving method of collecting material under the direction of A.N. Golikov in the early 1960s ('*a new era in the exploration of the far eastern seas*' – see Kussakin, Chavtur, 2000) allowed scientists to study quantitatively places that could not be accessed by research vessel gear such as rocky and stony grounds of inshore sea areas from the shoreline to 30–40 meter depth. Since 1962, the laboratory staff conducted a series of expeditions using light diving equipment, first in the Far East in the Sea of Japan, and then in other regions of Far Eastern and Arctic seas.

Far Eastern seas. In seas of the Far East, the most detailed study was conducted in the Possjet Bay in the Sea of Japan, Kurile Islands, southern and eastern Sakhalin, Shantarskie Islands and all large bays of the eastern part of the Sea of Okhotsk, Eastern Kamchatka, Commander Islands, Karaginskii Island, Olutorskii Bay, Providence Bay and Bering Strait. The study of biodiversity and phylogeny of separate taxa of marine far eastern invertebrates was conducted from 1962 to 1989 along with the study of distribution patterns of marine organisms, composition and structure of biocoenoses and long-time chages in benthic communities. Along with the Laboratory of Marine Research staff members (Golikov A.N., Scarlato O.A., Gruzov E.N., Sirenko B.I., Buzhinskaya G.N., Smirnov A.V., Platonova T.A., Savitskaya K.V., Potin V.V., Kuntsevych Z.V., Novikov O.K., Sheremetevskii A.M. et al.) the expeditions were joined by scientists from other ZIN laboratories (Mamkaev Yu.V., Kuznetsova G.A. (artist)) and Saint Petersburg State University (Minichev Yu.S.).

Possjet expedition in 1962 was the first big one for the Laboratory of Marine Research after its establishment in 1960. The work in this region continued in 1965 and 1966. Shallow-water bays were explored for the first time, their species composition was researched along with population dynamics of dominant species, the peculiarities of their ontogenesis, ecology and production. Members of said expedition offered their scientific recommendations regarding the creation of managed underwater facilities focused on the farming of several varieties of commercial shellfish. The results were published (Baranova, 1967, 1971).

The second major expedition was the Sakhalin Expedition of the Zoological Institute in 1963. Shallow waters of the Sakhalin coast by the Sea of Japan, Aniva Bay, Busse Lagoon and the Sea of Okhotsk coast of Sakhalin Island, including Terpeniya Bay, were examined in detail. After studying the distribution of more than 570 species of animals and plants, biogeographic zonation of the shallow-water zones of the South Sakhalin shelf was conducted and vertical zonation in the distribution of life forms in connection with hydrological conditions was specified (Golikov, 1985).

In 1969 Zoological Institute conducted a major expedition to the southern Kuril Islands to study the composition and distribution of flora and fauna in shallow coastal areas near the islands of Kunashir, Iturup and Paramushir. The information on the structure of bottom communities of the upper parts of the Kuril Islands shelf was obtained for the very first time. The expedition managed to gather a vast array of zoological material which helped scientists describe many new species previously unknown to zoologists and review different invertebrate groups. Young employees of the Marine Biology Institute, Far East Branch of the USSR Academy of Science, joined the expedition with V.I. Lukin as their leader. They learned the techniques of the quantitative diving method and later applied them in Far East sea expeditions in the 1970s and 1980s.

In 1975 the members of the Laboratory, led by A.N. Golikov, conducted further research on the ship *Raduga* on the eastern shelf of Kamchatka and Commander Islands. They used light diving equipment and vessel gear to research bottom communities in several regions of the Kamchatka shelf from the littoral zone to the 150-meter depth (Golikov, Scarlato, 1982).

Arctic. From the end of 1960 and up to 1985, several expeditions took place in the White Sea, which was the most accessible for this kind of work. The seasonal dynamics of bottom fauna, as well as abundance, biomass and productivity of aquatic ecosystems were studied in different areas of the White Sea using the quantitative diving method. Benthic communities of the upper shelf near Franz Josef Land were firstly studied in details in the 1970s and the early 1980s during three expeditions of the Laboratory. Light diving quantitative method and traditional vessel gear techniques were used to study benthos of the east and the south-east parts of the Laptev Sea, New Siberian Islands and the west part of the East-Siberian Sea in 1973. Using light diving equipment, the members of the Laboratory under the direction of A.N. Golikov studied benthos near

Wrangel Island and in the Chukchi Sea in 1976. Also, in 1986 and 1989, hydrobiological researches were conducted in the east part of the East-Siberian Sea, the west part of the Bering Sea and the east and central parts of the Chukchi Sea.

From the 1990s onward until the present time the Laboratory is focusing on researching sea fauna of the Arctic Region.

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Polar lipidomic profile of endophytic microalgae *Laminariocolax aecidioides*, *Streblonema corymbiferum* and *Streblonema* sp. (Ectocarpales: Phaeophyceae)

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The molecular species composition of polar lipids of brown endophytic microalgae *Laminariocolax aecidioides*, *Streblonema corymbiferum* and *Streblonema* sp. was investigated for the first time. It was shown that the composition of extraplastid polar lipids reflects the systematic position of the alga.

Laminariocolax aecidioides, *Streblonema corymbiferum* and *Streblonema* sp. are closely related species of filamentous brown endophytic microalgae belonging to the Chordariaceae family. These species of microalgae are distributed all over the world and have a wide range of macrophyte hosts. Identification of filamentous endophytes is difficult due to their microscopic size and the similarity of thalli. Information about their biochemical and, in particular, lipid composition is almost completely absent. The aim of this work is to establish the molecular composition of the polar classes of lipids of three species of endophytes and to establish the chemotaxonomic differences between them.

Spores of *S. corymbiferum* and *Streblonema* sp. were isolated from samples of brown macroalgae *Eualaria fistulosa* (Laminareales), spores of *L. aecidioides* were isolated from samples of brown macroalgae *Undaria pinnatifida* (Laminareales), and were grown as free-living cultures. The total lipid extract was obtained by the Bligh and Dyer method. The lipidomic profile of microalgae samples were investigated by HPLC-MS/MS. Lipid molecular species were identified based on precision masses and the detailed interpretation of MS/MS spectra allowed to confirm the structural details both the polar head and fatty acid composition of lipid species.

Analysis of the lipid composition of endophytic microalgae *L. aecidioides*, *S. corymbiferum* and *Streblonema* sp. showed the presence of 9 classes of polar lipids, including 5 classes of phospholipids (PL), 3 classes of glycolipids (GL) and 1 class of betaine lipids (BL).

The identified PL classes included phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), and phosphatidylhydroxyethylglycine (PHEG). PC and PE were abundant of molecular species, containing polyunsaturated fatty acids (PUFAs) with 20 carbon atoms in one or two positions, meanwhile, PE had a higher degree of unsaturation. The main molecular species of

PI contained 16:0 FA in one position and 18:1, 18:2, 18:3 in the other. PHEG contained two main molecular species 20:5/20:4 and 20:4/20:4. The main molecular species of PG were C18/C18 and C18/C16 with different degree of unsaturation.

GL is represented by monogalactosyldiacyglycerol (MGDG), digalactosyldiacyglycerol (DGDG), and sulfoquinovosyldiacyglycerol (SQDG). SQDG was characterized by the highest degree of saturation among GL. The main molecular species of SQDG contained predominantly 16:0 in sn-1 and C18 monounsaturated fatty acids (MUFAs) and PUFAs in the sn-2 position. MGDG was abundant of molecular species with C18 PUFAs in both positions, as well as C20:C18. The main species of DGDG included C20/C18, C18/C18, and C18/C16 with a predominance of PUFAs.

The only identified betaine lipid diacylglyceryltrimethylhomoserine (DGTS) was characterized by a predominance of saturated fatty acids (SFAs) and MUFAs with 14, 16 and 18 carbon atoms.

The composition of the molecular species of lipids contained in photosynthetic mebrans (MGDG, SQDG, DGDG, and PG) differs in all species of endophytes, while the molecular composition of extraplastid lipids (PC, PE, PI, PHEG, DGTS) is similar in *S. corymbiferum* and *Streblonema* sp., but differs in *L. aecidioides*. Thus, the composition of the molecular species of extraplastid membrane lipids depends on the systematic position of the alga, while the differences in the composition of polar plastid lipids are more dependent on other factors.

Educational program for younger students at the Primorsky Aquarium

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The Primorsky Aquarium sees education in marine biology and ecology, study and protection of aquatic ecosystems as one of its important roles. In view of this role, an environmental biology club 'Belyok' was organized in 2016.

A program called 'How the Fringed Blenny Studied the Glass House' has been developed for 6–7-year-old children. It is now run within the educational project "Growing up at the Aquarium". The main goal of the program is to generate respect for nature in children, to make them perceive nature as a value worthy of appreciation, understanding, and preservation by humans. Being such a value for children, nature is regarded by them more as a subject or a partner. The age period of 6–7 years is chosen as the main audience, since this age is considered to be the most significant for developing children's higher feelings, which henceforth motivate their behavior. Analysis of environmental education practices for younger students and related literature shows that their personal attitude towards nature is being formed and developed through physical and mental activity. For five years already the studio has been creating conditions that develop caring attitude towards nature in children, instilling in them the desire to learn more about nature as a whole, as well as about living organisms and to avoid destructive actions against those organisms. The Primorsky Aquarium has all the necessary facilities to do this kind of pedagogical activity.

When the program was at the stage of development, best practices in career guidance and projects in environmental education for families with pre-school children were analyzed and the following conclusions were made.

The first conclusion is that there are many ways to learn about professions, but there are few programs related to professions in natural sciences (which are, in addition, not so widely popular) that could arouse child's interest by uniting the efforts of a specialist in the profession and a teacher. During club meetings on professions, a teacher helps children to understand the qualities that are required for people who provide the welfare of animals.

The second conclusion is that there is a demand from parents to participate in some kind of leisure educational programs that would stimulate children's interest in nature.

The educational program 'How the Fringed Blenny Studied the Glass House' for senior pre-school and primary school students was developed in 2016, taking into account all these conclusions. Its key ideas were aimed at solving two problems: 1) to foster intimate relationship, dialogue, and equal cooperation between a child and

nature; and 2) to offer environmental tourist activities to help families to know more about nature, and to create learning opportunities for children so that they could master useful skills and communicate with natural objects, particularly aquatic organisms.

The first problem can be dealt with by telling younger students about nature-related careers that require caring attitude toward living organisms. A syllabus in animal welfare jobs in the Primorsky Aquarium as well as a web-site on Google have been created; and for five years appropriate methodology has been developed to immerse students into the program. The methodology incorporates teacher's handbooks, techniques for stimulating students' educational and creative activities, and diagnostic test packs.

The first unit of the syllabus is aimed at adaptation. It includes a set of games to get children used to the new surrounding and people (static exhibits and those with live organisms; a teacher and peers). Another component of the unit consists of a series of diagnostic tests for students and their parents that identify the interests of students, their preferred activities, and attitude towards nature. This unit has constantly evolved with time.

The second unit is devoted to educational and creative activities, as well as to development of environmental values. During club meetings students learn about aquatic biodiversity and nature-related careers, which drums up their interest to the sea. Children study the outward appearance of various organisms, their behavior and ecology. They also visit several scientific and cultural institutions, where they can meet specialists and find new facts about nature and its conservation. Creative activities help children to express their emotions towards live aquatic world.

The third unit combines assessment activities: a range of tests, which evaluate individual changes in children after the program, and feedback from their parents.

It became obvious with time that the best way to master the skills and knowledge obtained through 'How the fringed blenny studied the glass house' program, as well as to sustain the families' interest in local marine species was to include a week-long field unit into the syllabus. This unit, called 'Mysteries of Seacoast' was developed in collaboration with the Environmental Education Center of the Far Eastern Marine Reserve – Branch of the NSCMB FEB RAS.

The three units and the additional field unit of the program are well-structured to form a successive process fostering children's respect for nature. This process is believed to have good prospects for even further development.

Warm-water species of invasive mollusks off eastern Kamchatka (the Avacha Gulf)

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In 2017, staff members from the Kamchatka branch of the Pacific Geographical Institute conducted the first survey of Bechevinskaya Bay, situated on the eastern coast of the Kamchatka Peninsula, in the north of the Avacha Gulf. The survey aimed to discover the Japanese weathervane scallop, whose dead shells had been previously found on the bay bottom by divers.

Cutting 11 km into the Shipunsky Peninsula, Bechevinskaya Bay has a maximum depth of 54 m and a maximum width of no more than 2 km. Though quite close to the capital of Kamchatka, the bay was inaccessible to researchers for a long time because of the submarine base located there from the 1960s until 1996. In 2019, exploration of the bay resumed that resulted in the finding of a small-scale aggregation of the Japanese weathervane scallop, *Mizuhopecten yessoensis* (Jay, 1857).

According to the literature, *M. yessoensis* is a western Pacific low-boreal species that lives in the Sea of Japan off the northern coast of the Korean Peninsula, in Primorye region (the Sea of Japan coast of Russia) north to Rudnaya Bay, off western Sakhalin, northern Honshu and Hokkaido, and in the south of the Sea of Okhotsk (Scarlato, 1981; Kafanov, 1991; Arzamascev et al., 2001). The northern boundary of the species' range is the Gulf of Patience off the eastern coast of Sakhalin and the southern part of Iturup Island (Evseev, Yakovlev, 2006). Being aquacultured, *M. yessoensis* is now introduced to the coastal areas of northern China (the Yellow Sea, Liaoning and Shandong Provinces) (Lutaenko et al., 2013). It is found at depths of 0.5 to 83 m; off the Japanese Islands the species is recorded at depths of up to 311 m. Young Japanese weathervane scallops under one year of age have a shell length of 18–22 mm and are attached to algae, sea grasses and rocks with the byssus (Egorova, Sirenko, 2010). According to various sources, the life span of this mollusk ranges from 15 to 22 years (Yavnov et al., 2000).

All found specimens were larger than 100 mm in length due to which we were not certain if they belonged to a self-reproducing population or if they had once been transferred there as juveniles through hull fouling on naval vessels from Primorye. To date, it has been over 24 years since the last warship left the bay. In 2020 it was studied once more, and another aggregation of *M. yessoensis* was encountered containing young mollusks under five years of age.

The scallops occurred in a bathymetric range of 8–25 m on mud and pebble bottoms. The water temperature recorded at a depth of 8 m at one of the collection sites was 14°C,

which provided an optimal environment for the species. Numerous large old shells of *M. yessoensis* were observed at the collection sites. A total of 14 and 28 live specimens of the mollusk were caught during the first and the second survey, respectively, with a shell length ranging from 55 to 174 mm. The total weight of the largest specimen was 752 g. The density in the scallop aggregations was quite low: 1 individual per 8–10 m of the bottom.

The main threats to the encountered population, in our opinion, are predation from sea otters and poaching by divers. The aquarium observations have revealed that the scallop quickly digs itself into the sediment, leaving no traces of its presence on the surface. If the mollusk fails to hide, it becomes an easy prey to hermit crabs. At present, the species is facing another threat: bottom dredging planned for the construction of a gas terminal.

During the analysis of benthic samples obtained from a depth of 25 m in 2020, fresh shells of *Macoma incongrua* (Martens, 1865) were found. It is a subtropical low-boreal bivalve species, whose northern distribution limit is eastern Sakhalin and the South Kuril shallow waters (Scarlato, 1981). This is probably not a complete list of invasive species brought in by naval vessels from Primorye. Undoubtedly, the unique habitats of Bechevinskaya Bay require protection and more detailed study.

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Emperor Chain ecosystems – biodiversity, resources and perspectives

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Seamounts complexes, such as the Emperor Chain, are areas with high biological productivity of benthic and pelagic communities, including industrially important accumulations of marine organisms that support commercial fishery. Uncontrolled commercial fishery in the neutral waters of the North Pacific seriously threatens the natural balance, as at least 75% of the fish stocks on the seamounts are decreasing. Seamounts and guyots are of interest from a biogeographic point of view, since various communities of bottom and pelagic fauna are associated with them. According to the modern concepts, oceanic risings can be "stepping stones" for resettlement and enrichment of deep-sea fauna, as well as refuges and faunal centers; they can become biogeographic barriers and have a significant impact on the formation of the oceanic fauna. In the North Pacific, marine species dispersed by currents, meet seamounts and guyots of the Emperor Chain on their way; however, the biogeographic role of this process is unclear. The newest data obtained from the the expedition of the A.V. Zhirmunsky National Scientific Center of Marine Biology (FEB RAS) with participation of the Shirshov Institute of Oceanology (RAS) aboard the RV *Akademik M. Lavrentyev* (cruise no. 86) in 2019–2021 show the significant role of the Sponges and Octocorals as key groups in the deep-water bottm ecosystems of the Emperor Chain. The landscape-ecological situation and the bottom fauna were studied in the depth range from 2182 to 338 m depth using the ROV "Comanche". The data obtained in the present research regarding the intricate distribution of the biotic complexes and key taxa of the Octocorallia and Porifera in the studied areas of the Emperor Chain seamounts indicate the importance of such local conditions as substrate diversity and a complicated system of currents, which, in combination with depth, determine the state of the community. In the near future, complex biological studies of VMEs in the area of the Emperor Chain are required, together with hydrological, geological and geophysical surveys, to obtain pioneering data on the state of biological and other resources in the North Pacific.

Parasite fauna of ray and stingrays (Pisces: Elasmobranchii) from the coastal water of Vietnam

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About 105 species of the elasmobranches, namely 54 species of ray and stingrays from 25 genera and 51 species of sharks from 28 genera, permanently or temporarily occur in the East Sea. Data on the fauna of cestodes infecting 45 species of the elasmobranches caught off the coast of Malaysia (Borneo) are presently known, i.e. these helminths are studied only for 40% of the Elasmobranchii fauna in this region. There are 80 species of cestodes from 41 genera and 6 orders. Moreover, larvae of 4 cestode species from 2 genera and 2 orders (Trypanorhyncha: Pseudophyllidea) were also recorded in the teleost fishes in the Gulf of Tonkin. The fauna of monogeneans infecting elasmobranches in the East Sea are almost not studied. Four monogenean species from Monocotylidae are only described from rays in the Gulf of Tonkin off China. Until our research started in 2019, the fauna of parasites of the elasmobranches off the coast of Vietnam had not been studied.

In total 109 specimens of 11 batoids (ray and stingrays) species from the families Rajidae (1 species / 33 specimens), Dasyatidae (7/16), Gymnuridae (2/2) and Glaucostegidae (1/6) caught off the coast of central Vietnam (Nha Trang, Kui Nyon, Da Nang, Hue and Quang Binh) were studied in 2019 and 2021. The batoids were identified using molecular genetic data. About 33 species of parasites from 9 orders and 4 higher taxa (Monogenea, Cestoda, Nematoda and Crustacea) were found in 7 species of ray and stingrays.

The most species was recorded among cestodes. In total, 16 cestode species from 5 orders were found for the first time in the East Sea in *Maculabatis gerrardi* (Gray, 1851), *Okamejei hollandi* (Jordan et Richardson, 1909), *Hemitrygon bennettii* (Müller et Henle, 1841), *Glaucostegus typus* (Anonymous [Bennett], 1830), *Neotrygon* spp. and *Pateobatis* spp. These species are from the following orders: Lecanicephalidea – 4 species/3 genera, Trypanorhyncha – 6/4, Rhinebothriidea – 4/2, Diphyllidea – 1/1, Onchoproteocephalidea – 1/1. Eight of these cestode species was identified as new species for science. The representatives of the order Lecanicephalidea and the genus *Rhinebothrium* Linton, 1890 (Rhinebothriidea) were found in the East Sea for the first time.

The monogeneans of the genus *Calicotyle* Diesing, 1850 in *Okamejei hollandi* as well as *Monocotyle tritestis* Young, 1967 and *Heterocotyle chinensis* Timofeeva, 1983 (Monocotylidae) and new species of the genus *Hypanocotyle* in *Hemitrygon bennettii* were recorded for the first time in these fish not only in the East Sea, but in all area of their distribution. Moreover, the representative of the new genus of monogeneans of the family Hexabothriidae was identified in *Maculabatis gerrardi*.

Larvae of two species of Nematoda were found in the studied rays. The larva of *Raphidascaroides nipponen*sis Yamaguti, 1941 (Raphidascarididae) was recorded in *Okamejei hollandi* for the first time, adult specimens of this nematode was previously found in the East Sea in the teleost fish *Halieutaea stellata* (Vahl, 1797). The other nematode larva found in *Glaucostegus typus*, *Gymnura japonica* (Temminck et Schlegel, 1850) and *Neotrygon* sp. was not identified.

The fauna of parasitic crustaceans was very diverse and represented by 5 species of Copepoda, 1 species of the genus *Caligus* Müller O.F., 1785 and larvae of Gnathiidae (Isopoda).

Note that stingrays of the genus *Hemitrygon* Müller et Henle, 1838 have been poorly studied not only in the East Sea, but also in their nature range in the World Ocean; some data on parasites are known only for 4 of 10 valid species. Thus, we studied parasites from *H. bennettii* for the first time.

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Effects of the proteins involved in regeneration on the antioxidant enzyme activity of phagocytes in the holothurian *Eupentacta fraudatrix*

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Cells of the immune system play an important role in the recovery processes in tissues, and they are considered as regulators of regeneration (Yushkov, 2017). In vertebrates, macrophages have ability to undergo activation to pro-inflammatory M1 (classically activated) or anti-inflammatory M2 (alternatively activated) phenotypes (Italiani, Boraschi, 2014). M1 macrophages can contribute to inflammation and immunostimulation; M2 macrophages contribute to the resolution of inflammation and immunosuppression (Dolmatova, Dolmatov, 2021). Two types of macrophages use different metabolic pathways for energy generation: M1 type uses glycolysis, and M2 cells use oxidative metabolism (Dolmatova, Dolmatov, 2020). Reactive oxygen species (ROS) are modulators of degenerative-inflammatory phase during regeneration (Kozakowska et al., 2015), in turn, antioxidant enzymes, including catalase and glutathione reductase (GR) can catalyze different types of ROS (Tan et al., 2016), and glutathione-S-transferase (GT) detoxifies xenobiotics (Habig et al., 1974).

Of note, two types of macrophages have different ratios of reduced-to-oxidized glutathione (GSH/GSSG) (M1>M2), and GSH depletion can switch macrophages from the M1 towards the M2 phenotype (Peterson et al., 1998). A switching from M1 to M2 type is considered as a target for newly developed pharmacological agents (Saqib et al., 2018).

Holothurians have a high capacity to regenerate. Several groups of proteins were also shown to play a role in the regeneration (Dolmatov, 2021). In addition, the holothurian *Eupentacta fraudatrix* has two types of phagocytes (P1 and P2) which can be obtained by gradient centrifugation (Dolmatova et al., 2004) and which are known to have different functional activities and markers similar to the M1 and M2 macrophages respectively.

The object of the study was research on the influence of some proteins whose synthesis changes during regeneration on the activity of antioxidant system enzymes of *E. fraudatrix* phagocytes. Specimens of the holothurian *E. fraudatrix* with a body length of 4 to 6 cm were collected in winter in Vostok Bay (Peter the Great Bay, Sea of Japan) using a light diving equipment.

On the first stage of the experiment, the animals were given a superficial wound and injected with phosphate buffered saline with the addition of 36 g/L NaCl (PBSN) or the extract of the holothurians known as a stimulator of the regeneration (Dolmatova et al., 2014). After 1 day, the coelomic fluid of the holothurians was sampled and analyzed for the protein molecular mass distributions by HPLC. Separate fractions were collected and frozen. The content of proteins was determined by Bradford method.

On the second stage, the animals were injected with PBSN (control) or given a superficial wound and injected with PBSN or proteins selected. After 1 day, the coelomic fluid of the holothurians was sampled and processed for isolation of P1 and P2 phagocytes (Dolmatova et al., 2007). Their concentrations were determined in Goryaev chamber, and activities of antioxidant enzymes were assessed by spectrophotometric methods.

At stage 1, chromatogram analysis revealed that the most significant changes when the extract was added to the wounded animal occurred with the concentrations of two unidentified proteins, the level of the first (Protein 1) increasing compared to control, and the second (Protein 2), detected when one extract was injected, by contrast, disappeared. These proteins also had opposite concentration-dependent effects on the concentration of coelomic cells in the coelomic fluid. These two proteins were used in the second stage of the experiments. Injury of holothurian led to an almost 10-fold increase in the activity of catalase in P1 and, conversely, to a decrease in its activity in P2 phagocytes. Injection of Protein 1 at the lowest concentration studied led to the return of catalase activity to the control level, the increase in concentration caused a significantly smaller increase in the activity of the enzyme in P1 and a decrease in its activity in P2 cells. At the same time, the activity of the enzyme in P1 remained higher than in P2 all the time. Protein 2 at a medium concentration stimulated activity in P2 and decreased activity in P1 phagocytes.

GR activity of P1 and P2 cells significantly decreased and increased respectively in wounded animals compared to controls. Protein 1 suppressed the enzyme activity compared with control and wounded animals in both types of phagocytes at the lowest of concentrations and significantly stimulated the activity in P1, but not in P2, at the average concentration. A further increase in concentration was accompanied by a decrease in enzyme activity in P1 below its level at wounding, but with no change in P2 phagocytes. Protein 2 at the highest concentration significantly suppressed the enzyme activity compared to that at wounding in both cell types, and at the average concentration also suppressed it, but the enzyme activity in P2 cells was higher than in P1.

GT activity significantly increased at wounding compared with control in P1 but not in P2 phagocytes. The low concentration of Protein 1 significantly decreased enzyme activity in both types of phagocytes, and with increasing its concentration there was a return of enzyme activity to the level at wounding (medium concentration) and even an increase in enzyme activity in P2. However, a significantly higher activity in P1 compared with that in P2 was maintained. Protein 2 significantly reduced enzyme activity

compared with wounding at both concentrations studied in an inverse concentration relationship, with activity in P2 being higher than in P1 (while an inverse ratio of enzyme activity in the two phagocyte types was observed in the control).

Thus, the changes in enzyme activity in phagocytes during wounding are consistent with the ideas about the participation of the antioxidant enzyme system in wound healing, and the opposite changes in the glutathione-dependent enzymes GR and GT confirm the previously obtained data on the different level of reduced glutathione in these two types of phagocytes (Dolmatova et al., 2021).

Changes in the activity of antioxidant enzymes under the influence of proteins isolated from animals after wounding testify to changes in the functional activity of phagocytes during wounding and recovery. It is noteworthy that the ratio of enzyme activity in the two types of phagocytes changed under the influence of Protein 2.

The data obtained indicate multidirectional changes in the activity of antioxidant enzymes in the two types of phagocytes during tissue damage and the possibility of preferential stimulation of the functional activity of P2 phagocytes under the action of the protein mediator of the wound-healing preparation.

Academician Alexey Viktorovich Zhirmunsky – founder of experimental marine ecology

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The founder of the Institute of Marine Biology (currently A.V. Zhirmunsky National Scientific Center of Marine Biology, FEB RAS) Alexei Viktorovich Zhirmunsky (15.10.1921 – 20.10.2000) was born into a humanitarian family. His father, Viktor Maksimovich Zhirmunsky (1891–1971)) was an authoritative philologist, and his mother Tatyana Nikolaevna Zhirmunskaya (nee Yakovleva (1903–1999) was a member of the Union of Artists of the USSR, a candidate of art history. It would seem that fate predetermined Alexei Viktorovich a penchant for humanitarian pursuits.

However, under the influence of maternal relatives, he chose the path of a naturalist. Undoubtedly, the influence of grandfather Nikolai Nikolayevich Yakovlev – a geologist, one of the founders and the first chairman of the Russian Paleontological Society. He took his fifteen-year-old grandson on an expedition to collect fossil invertebrate fauna. No less interesting is the personality of his great-grandfather Alexander Yakovlevich Gerd (1841–1888) – a Russian teacher, the founder of the teaching method of natural science as a scientific discipline and a method of practical and laboratory work. Since 1877, he headed the educational institution of the prince Obolenskaya, and in 1879, when this institution was transformed into a gymnasium of the Ministry of Public Education, Gerd was appointed chairman of the pedagogical council of this gymnasium. Many famous women of Russia graduated from this gymnasium. Including N.K. Krupskaya, whose youthful portrait Tatyana Nikolaevna became famous for. They were introduced by the grandmother of Alexei Viktorovich. The history of the maternal family is extremely interesting. No less interesting is the history of the paternal family.

So, the admission of Alexei Viktorovich in 1939 to the Faculty of Biology of Leningrad University looks quite logical. It was experimental science that attracted him and he graduated from university with a degree in animal physiology. He devoted his scientific life to her.

Interest in marine biology began to form after marine expeditions. There were many of them: to the Crimea, to the Karadag and Sevastopol biological stations, to study cytological adaptations of marine invertebrates, to the Sea of Japan for research on the ecology of invertebrates, to the Murmansk marine biological station "Dalnie Zelentsy" in the Barents Sea, on Soviet-Chinese expeditions to the South Chinese and Yellow Seas.

Alexey Viktorovich began to realize his conviction of the need to develop marine biology in the Far East in 1965 by writing a note to the Presidium of the Far Eastern Branch of the Siberian Branch of the USSR Academy of Sciences.

Under the leadership of Aleksey Viktorovich in 1967, the Department of Marine Biology of the Far Eastern Branch of the Siberian Branch of the USSR Academy of Sciences was created, which consisted of two laboratories – the Laboratory of Experimental Ecology (headed by A.V. Zhirmunsky) and the Laboratory of Systematics and Marine Hydrobiology (headed by OG Kusakin). In January 1970, on the basis of the Department, the Institute of Marine Biology (IMB) of the Far Eastern Branch of the Siberian Branch of the USSR Academy of Sciences was organized, the director of which was Alexei Viktorovich.

Three periods can be distinguished in the work of Alexei Viktorovich. In the early years (1939–1958), under the leadership of Professor D.N. Nasonova, comparative physiological studies of the muscular and nervous systems of animals. In 1957, his key work on the adaptation of the Black Sea anemones to increased salinity was published in the "Reports of the USSR Academy of Sciences". This work initiated his long-term interest in two key environmental factors for marine organisms: salinity and temperature. In 1971 he defended his doctoral dissertation on the importance of cell heat resistance in the distribution of benthic animals in the upper zones of the sea in the specialty "cytology". The laboratory of ecological physiology, headed by him at the Institute of Marine Biology, dealt with these problems until the end of the 2000s of the XXI century.

The third period in the work of Alexei Viktorovich is associated with general theoretical problems of natural science. He was interested in them from an early age. In particular, he read popular lectures on the origin and development of life on Earth through the Knowledge Society. They formed the basis of his popular brochure published by the Society for the Dissemination of Political and Scientific Knowledge of the RSFSR. Since the late 1970s, there have been works on the regulation of the functions of mammals, including humans – on reflexology. In 1980, an article on critical periods in human ontogenesis was published in the "Reports of the USSR Academy of Sciences". It was followed by a long-term series of articles and monographs on critical levels in the development of living and natural systems in general. During these years, he published a series of articles on thorium and the practice of conservation. They were timed to coincide with the establishment of the Far Eastern Marine Reserve.

Based on his own experience, Alexey Viktorovich was well aware of the importance of the formation of interests precisely in adolescence. He passed on the pedagogical talent of his great-grandfather A.Ya. Gerda. Overloaded with scientific and administrative duties, he devoted a lot of time to working with schoolchildren, which he treated with dedication and passion: he read popular lectures through the Knowledge Society, met with groups of students and teachers in schools and, finally, created Malaya Academy of Marine Biology for high school students. Her students had to undergo summer biological practice in nature.

Alexey Viktorovich knew, respected and loved many biologists. He is the author of many articles and obituaries about his colleagues and fellow associates. The first was an article dedicated to the 60th birthday of his teacher, D.N. Nasonov. Later, his obituary was published in the "Bulletin of the USSR Academy of Medical Sciences". He published articles about personalities until his last days.

Alexey Viktorovich's activity as director of IBM was multifaceted. He is an outstanding organizer of marine biological science in the Russian Far East. The results of his activities are the creation of a scientific space, which was not previously in Vladivostok, which included the country's first marine reserve, a museum, several biological stations from Posyet to Kamchatka, the Small Academy of Marine Biology and two university departments, as well as regularly held all-Union and international conferences and schools.

Regulation of gene activity in the development of moon jellyfish *Aurelia aurita*

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Study of diversity and plasticity of marine invertebrate development is an important source of knowledge and progress in various aspects of fundamental and applied biosciences. Though the processes of differentiation and aging of the multicellular organisms are known to be irreversible, the unique processes of reprogramming/reverse development were described in a certain species of Cnidarians. In this project we aimed to describe the model of gene regulation of reverse development of jellyfish *Aurelia aurita*. At the beginning, we established the laboratory model of *A. aurita* originally collected on the Black sea and characterized in details the morphological processes of its direct and reverse development. We also re-sequenced *A. aurita* genome with a low coverage and confirmed the phylogenetic identity of our model specie. To determine the major factors of the life cycle reprogramming we collected samples on various developmental stages of *A. aurita* and performed deep sequencing of mRNA transcriptomes. Using modern bioinformatic approaches, a spectrum of genes that alter activity in the processes of forward and reverse development of the jellyfish *A. aurita* was identified. Functional study revealed multiple signaling pathways and transcription factors, orchestrated in the course of development, which were compared to the existing data on other Cnidarians. Specific activation of TGF-beta signaling pathway genes (e.g. *BMPR2, SMAD6*) during strobilation and wnt genes in reverse development is discussed.

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From Cytarabin to Blenrep® – 50 years story of "Marine Drugs"

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In 2019, the scientific and medical community celebrated the 50th anniversary of the introduction of the very first marine-derived drug, Cytarabine, into clinics. Cytarabine (aka Ara-C, Cytosar-U®) was first isolated from a marine sponge and is known to kill cancer cells by blocking DNA polymerase function. In 1969, the U.S. Food and Drug Administration (FDA) have approved the drug for the treatment of leukemia. This marine drug, which still belongs to the mainstay of leukemia therapy, and by now has probably saved many thousands of lives, made its way into clinics in less than 20 years after the original prototype molecules, namely spongothymidine and spongouridine, were reported by Bergmann and Feeney in 1951. Some years later, in 1976, another marine drug, Vidarabine (Ara-A, Vira-A®), was approved for the treatment of Herpes simplex virus. However, after that, the clinical development of marine-derived drugs was less successful, and for another almost 40 years, no other compounds were approved by drug authorities. Moreover, the rapid development of methods of high-throughput screening, and especially computational approaches to rational drug design, made some scientists believe that the search for new bioactive molecules from natural sources was an activity of the past. Thus, by the end of the last century, a part of the scientific community was rather skeptic on whether new natural products and, in particular, marine natural products, still harbored the potential to make new drugs. However, having passed this transition period, the situation has dramatically changed, and at the beginning of the 21st century, marine drugs entered a time of *renaissance*. Nowadays, while many terrestrial animals and plants are already well investigated, marine inhabitants have become the main source of new chemical compounds. Moreover, due to the specific (and often extreme) environmental conditions, marine inhabitants often possess particular biochemistry which results in unique secondary metabolites. These organisms are mainly produced by marine invertebrates like sponges and tunicates (Ascidia), and marine fungi and bacteria, the latter often being the true producers of small bioactive molecules of interest.

Currently, the potential for marine natural products as drug candidates has been recognized all over the world, and the field is constantly growing and developing. Additionally, the development of new chemical and physicochemical approaches and

tools has led to the isolation and structure elucidation of novel minor marine secondary metabolites, which could not be isolated/detected in the past. The number of structures isolated each year has almost doubled over the past 20 years. This is illustrated by the fact that according to the report of John Faulkner, 869 new structures were isolated from marine organisms during the year 2000. A decade later, in 2010, the number had risen to 1003 substances per year, as reported by John Blunt et al., and a very recent report by Anthony Carroll et al. described 1554 new molecules isolated in the year 2018. Moreover, the development of new and improved organic synthesis methods made possible the synthesis of promising active compounds in the amounts required for further preclinical and clinical studies.

Tremendous progress in the clinical development of marine-derived drugs has been achieved over the past 20 years. For example, during this period, 13 out of 15 currently used drugs derived from marine origin have been approved by their corresponding authorities. Remarkably, five of them have been approved within the last three years. Many more molecules are currently undergoing different stages of clinical and pre-clinical trials.

Recovery of a symbiotic soft coral *Sinularia heterospiculata* after a heat stress

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Most corals are symbiotic animals whose tissues contain intracellular dinoflagellates or zooxanthellae that carry out photosynthesis and, thus, provide the host organism with nutrients it needs. Stressful environmental conditions result in photo-oxidative stress, which triggers the initial stages of coral bleaching. A stress causes photoinhibition and damage to the photosynthetic apparatus of zooxanthellae cells. These damages are manifested as changes in the lipid profile of the thylakoid membrane and pigments of zooxanthellae. Moreover, when the host coral is exposed to adverse conditions (for example, the rise of sea surface temperature), disturbances occur also in its tissues, which destroys the symbiotic relationships between the partners. The zooxanthellae leave the polyp, thus, depriving it of the main source of vital nutrients. In nature, water temperature increases can have a seasonal pattern. Among corals, some species that are more resistant to high temperatures and can recover. Coral reef restoration programs are implemented in different regions across the world's oceans. However, the lack of knowledge about the coral physiology limits the development of this field of research. The coral restoration research is of high priority for the conservation of coral reef ecosystems. The study of lipid changes in the coral organism during bleaching and subsequent restoration after stress will extend our knowledge about the functioning mechanisms of cell membranes in corals and their endosymbionts.

To study the process of coral restoration after damage caused by increased water temperature, we set up an experiment with recovery of a partly bleached soft coral cultivated under experimental conditions. As part of the study, the patterns of changes in lipid contents, number of symbiotic dinoflagellates, and contents of photostatic pigments (chlorophylls *a*, *b*, and carotenoids) of the soft coral *Sinularia heterospiculata* were assessed. The number of symbiont cells was determined by flow fluorocytometry.

The experimental *S. heterospiculata* lost a significant part of its symbionts during the short-term heat exposure (two days). After the colonies were heated to 30°C, the proportion of carotenoids and chlorophyll *b* significantly increased from 110.3±12.3 and 97.5±23.9 μg to 187.9±33.22 and 136.9±35.5 μg, respectively (HSD test, $p<0.5$). Further heating (bleaching period, 32°C) induced a significant reduction (HSD test, $p<0.05$) in the levels of chlorophylls *a* and *b* and carotenoid after a two-day exposure (day 8 of experiment). The levels of carotenoid and chlorophyll *b* in the experimental

colonies reduced to 117.9±18.7 and 46.3±9.3 μg. The chlorophyll *a* level reduced from 158.7±18.0 to 77.8±19.1 μg. After the bleaching period, the chlorophyll levels sharply decreased and remained unchanged during the recovery period. The number of carotenoids, on the contrary, returned to the initial value, despite the decrease in the zooxanthellae density. Carotenoids play an important role in all photosynthetic systems. In algae, carotenoids are found in light harvesting antenna complexes and are involved in photosynthesis. They transfer the harvested light energy to chlorophylls. Moreover, carotenoids also act as antioxidants, as they can protect the cell from oxidation by reactive singlet oxygen and radicals. In symbionts of *S. heterospiculata* exposed to heat stress, carotenoids presumably perform the antioxidant function and, therefore, their amount increases relative to symbiont proportion.

A decrease of chlorophyll-positive cells (zooxanthellae cells) was observed immediately after the two-day heat exposure (bleaching period, 32°C). The proportion of dinoflagellate cells decreased from 33.0±3.7% (day 0 of experiment) to 20.9±3.0% (day 8). After 7 days of recovery (27°C, day 22), the proportion of hosted dinoflagellates increased to 49.8±2.9%. At this moment, the amount of photosynthetic pigments did not significantly change. Thus, the increased zooxanthellae population had a reduced photosynthetic capacity and could not provide the host's organism with the necessary level of nutrients, as a result of which, after 21 days of recovery, the zooxanthellae was expelled. After the following 14 days of recovery (27°C, day 36), the proportion of dinoflagellates decreased to 16.8±2.8% and remained at this level until the end of the experiment. In the experimental tank, a flow-through water system was used. Which supplied natural plankton from Peter the Great Bay. Therefore, heterotrophic nutrition (predation on plankton, intake of dissolved and particulate organic matter) became an alternative source of nutrients for the coral *S. heterospiculata*. This mode of nutrition is suggested as a compensative mechanism that helps *S. heterospiculata* to survive bleaching events, as evidenced by the stable total lipid content recorded during the experiment. The total lipid content did not decrease and remained stable throughout the experiment period (HSD test, $p>0.5$). Thus, the duration of the heat exposure was sufficient for the loss of a large number of symbionts, while the viability of the coral was preserved.

As a result, the amount of chlorophylls *a* and *b* decreased. The number of carotenoids, on the contrary, increased relative to their proportion in hosted symbionts, which is probably associated with the complementary function of carotenoids as antioxidants. Due to the reduced photosynthetic activity of dinoflagellate symbionts, their restored population could not fully meet the demands of the coral for nutrients. Subsequently, the contribution of heterotrophic nutrition increased in the soft coral, which allowed it to maintain its vital functions, as evidenced by the stable level of lipids.

Development of test systems for rapid immunodetection of phycotoxin microcystin-LR

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Intensification of international contacts in the modern world market for fish products and seafood has necessitated the monitoring of contamination of seafood by toxic compounds at different stages of its production and consumption. One highly toxic marine contaminant is the phycotoxin microcystin-leucine-arginine (MC-LR) produced by cyanobacteria, mainly *Microcystis aeruginosa*. As part of plankton and benthos, cyanobacteria serve as a food source for mollusks and fish and may accumulate in them. Consumption of products contaminated with MC-LR causes acute and chronic toxic effects to human health. A transfer of phycotoxins along food chains leads to serious danger to humans. Therefore, the development of rapid, sensitive, and reliable techniques of MC-LR control in food products is extremely urgent. In this study, an immunochromatographic analysis (ICA) and a fluorescence polarization immunoassay (FPIA) were proposed as promising immunotechniques for rapid determination of MC-LR in seafood.

Specific reagents for ICA and FPIA were obtained. For indirect ICA of MC-LR, colloidal gold nanodispersed carriers were synthesized and characterized. Two types of nanomarkers were obtained, differing in shape and dimensional characteristics, namely spherical gold nanoparticles (AuNPs) and gold nanoflowers (AuNFs). Characterization of AuNPs and AuNFs by transmission electron microscopy showed that the preparations contained non-aggregated objects with different surface geometries with an average diameter of about 30 and 90 nm for AuNPs and AuNFs, respectively. The obtained nanoparticles were conjugated with secondary antibodies by physical adsorption. For the FPIA, ethylenediamine fluorescein thiocarbamate (EDF) was used as a fluorescent label. The EDF-labeled MC-LR (the so-called tracer) was synthesized by carbodiimide activation and purified by thin layer chromatography. Three clones of monoclonal antibodies to MC-LR were characterized by enzyme-linked immunosorbent assay (ELISA). The clone with the highest binding affinity was selected for further experiments. As a result of indirect competitive ELISA, it was demonstrated that MC-LR limit of detection (LoD) was 0.01 ng/mL, and the working range of the detected concentrations was 0.03–0.13 ng/mL. Such a high sensitivity allowed for the development of rapid immunoanalytical methods for detecting MC-LR.

The production of the immunochromatographic test systems consisted of applying test components onto membrane carriers, assembling a multimembrane composite, and cutting the multimembrane composite into test strips. A test strip consisted of a plastic support and several membrane carriers, including a working membrane. Immunoreagents were applied to the working membrane. Namely, test zone was formed by the application of MC-LR–BSA conjugate, and anti-species antibodies were immobilized to form a control zone. The analysis was based on the movement of specific reagents with a liquid flow along the membranes followed by the formation of specific complexes and, accordingly, colored zones on the test strip.

The indirect ICA consisted of two stages: preliminary incubation of the sample containing the analyte with specific antibodies in solution and then immersion and incubation of the test strip with the reaction mixture. The assay conditions have been optimized to achieve a low MC-LR LoD and high analytical signals. For this purpose, the concentrations of specific reagents, the assay duration, the composition of the reaction medium, the types of membrane carriers, and other parameters were varied. As a result of the optimization, the instrumental LoDs of MC-LR were 0.1 and 0.2 ng/mL in the case of AuNFs and AuNPs, respectively. The visual LoD was 1 ng/mL in both cases, and the time of analysis was 18 min.

The obtained data allowed proceeding to the approbation of the developed test systems for the determination of MC-LR content in real samples. Among the tested samples were samples of fish (cod) and seafood (shrimp, squid, mussels, and octopus) purchased in Moscow supermarkets. Because seafood is a complex matrix, it was necessary to implement sample preparation prior to analysis aimed at the extraction of MC-LR from the samples. For this, a procedure was proposed consisting of sample homogenization, extracting, centrifugation, and evaporation of the residue. The precipitates redissolved in the buffer were used for the ICA. It was demonstrated that the test systems allowed for the determination of MC-LR in contaminated samples. The disappearance of the colored line in the analytical zone was observed, correlating with an increase of MC-LR concentration in the samples. Therefore, the testing confirmed the applicability of the developed ICA for the determination of MC-LR in seafood.

At the next stage of the study, the FPIA as a rapid homogeneous method of MC-LR detection was implemented. The FPIA is based on the competitive interaction of the analyte and its labeled derivative with specific antibodies and the measurement of the fluorescence polarization (mP) value of the tracer. A small tracer molecule rotates rapidly and, accordingly, has a low mP. When the tracer binds to the antibody, a high molecular weight complex is formed, which is characterized by slow rotation and high mP. Therefore, the mP reflects the ratio of the bound and free fractions of the tracer and is inversely proportional to the concentration of the analyte in the sample. Before implementing the FPIA, it was necessary to characterize the functional properties of the tracer. Its working dilution was selected so the fluorescence intensity of the tracer solution several times exceeded the intensity of the background signal. Then the working

concentration of specific antibodies was selected based on the assessment of the efficiency of their binding to the tracer. The antibody concentration providing 80% MC-LR binding was selected as a working dilution.

The optimization of the FPIA included the selection of the duration and sequence of the immunochemical interactions, the ionic strength and pH of the reaction medium, and the presence of a detergent. According to the calibration curve obtained under optimized conditions, the detection limit for of MC-LR was 7.5 ng/mL, and the analysis time was only 10 min. The developed immunoanalytical systems can be recommended for sensitive and rapid point-of-care screening for MC-LR.

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Biotechnological potential of psychrotolerant bacteria associated with Antarctic ribbon worms (Nemertea)

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Cultivable microorganisms from Antarctic environments are of great interest among scientific community for potential biotechnology applications. The geographic isolation and harsh environmental conditions, including low temperatures, high incidence of UV radiation, nutrient deficit, and freezing and thawing cycles, resulted in a microbiome that cannot be found anywhere else on the planet. Physiological adaptations allowing microorganisms to thrive in the Antarctic make them a source of unique cold-active enzymes with high catalytic efficiency, antifreeze-nucleating proteins, and exopolysaccharides. Special interest causes secondary metabolites of Antarctic bacteria exhibiting antimicrobial and antiproliferative functions as a source for new antibiotic and anticancer drugs elaborating. Numerous pigments of Antarctic bacteria with different metabolic activities, such as antimicrobial activity, photoprotective function, biodegradability, and extracellular sunscreen, have wide biotechnological potential.

This study aimed to identify bacteria isolated from the Antarctic ribbon worms (Nemertea) and evaluate their potential for producing metabolites with biotechnological interest. The material was collected during the 79th cruise of the research vessel "Akademik Mstislav Keldysh" to the Southern Ocean, December 2019 – May 2020. Bacterial isolates were identified with PCR amplification, sequencing, and phylogenetic analyses of partial 16S rRNA gene fragments. From 32 bacterial isolates recovered from the nemertean samples, 28 were identified. They were distributed in three phyla: Proteobacteria (62% of isolates), Actinobacteria (31% of isolates), and Firmicutes (7% of isolates). Identified bacteria belonged to 8 genera: *Pseudoalteromonas*, *Pseudomonas*, *Alteromonas*, *Cobetia*, *Psychrobacter*, *Salinicoccus*, *Staphylococcus*, and *Kocuria*.

Two strains closely related to *Psychrobacter piscatorii* are of special interest in this study. The strains of this species exhibit an extraordinarily high catalase activity. Moreover, one of the *P. piscatorii* strains found in a current study produces a dark brown pigment actively releasing into the environment. Another isolated *Psychrobacter* strain closely relates to *Psychrobacter marincola*, which is a source of polysaccharides with anticancer activity. Further studies of the isolated strains may result in the extraction of new bioactive compounds for biotechnological and pharmaceutical industry.

Influence of the extracts from marine algae on the antioxidant system status at acute stress

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In the genesis and development of various disorders, including diseases of the digestive, circulatory, neuroendocrine, and immune systems, the impact of stress factors (physical, chemical, psychoemotional, etc.) plays a significant role. At early stage the antioxidant system is activated due to excess of reactive oxygen species (ROS) generated at stress impact. Prolonged exposure to stress factors leads to a misalignment of the antioxidant-prooxidant balance towards to increase generation of free radicals. Failure to manage the excessive production of ROS by the antioxidant defense system is the basis for the impairment of multiple physiological reactions in the body. The employment of natural complexes with antioxidant properties can impact both the generation of ROS and the functional state of organism helping to prevent stress-induced disorders.

Marine algae are of great interest as a source of biologically active compounds. It is known that algae-derived preparations exhibit a wide range of pharmacological activities (antibacterial, antiviral, antitumor, antimicrobial, hepatoprotective, etc.). The algae composition includes easily digestible proteins, amino acids, lipids, polysaccharides, carotenoids, polyphenols, minerals, etc. Polyphenols as compounds with high antioxidant activity possess a great importance. Phlorotannins is a prevailing group of polyphenolic compounds in seaweed. It contains a large number of conjugated double bonds with high mobility and free hydroxyl groups, because of that, they have the ability to inactivate free radicals, neutralizing their damaging effects to the body.

In previous studies, we found that extracts of a number of marine macrophytes belonging to different taxonomic groups exhibited a pronounced protective effect under various experimental models (toxic hepatitis, hyperlipidemia, stress exposure).

The goal of this study was a comparative research of the preventive effect of water-ethanol extracts isolated from the thallus of brown alga – *Sargassum pallidum* (Turner) C. Agardh, green alga – *Ulva lactuca* Linnaeus, 1753, red alga – *Ahnfeltia tobuchiensis* (Kanno et Matsubara) Makienko on the indices of the antioxidant defense system of the liver and blood in mice under experimental stress exposure. The algae selected for the study are the most widespread in the seas of the Far East and are typical representatives of their divisions and the main mass species.

The algae were collected in August–September in the coastal waters of the Peter the Great Bay of the Sea of Japan at the marine experimental station of the POI FEB RAS (Popov Island). The air dry algae thallus was extracted with 70% ethyl alcohol

by the method of re-percolation. The yield of the extract made 1 liter from 1 kg of raw material. The obtained extract was evaporated under vacuum to dryness, and the total polyphenols (PP) contents was estimated by the Folin–Chocalteu method.

The experiment was carried out on white outbred male mice with a body weight 20–30 g that were kept under the standard conditions of a vivarium. Acute stress was simulated by suspending animals in the upright position by their neck dorsal skin fold for 24 h. The control animals were also kept under the standard vivarium conditions. The preparations were introduced directly into the stomach through a feeding tube two times: immediately before the vertical suspension and 6 hours after the first administration. Prior to administration, the alcohol was removed from the extracts on a vacuum evaporator; the extract was then diluted to the required volume with distilled water. Preparations were administered as water suspension. The therapeutic dose made 100 mg of total PP / kg of body weight. The animals from the "pure stress" group were treated with distilled water in a volume equal to that of the administered preparations.

Stress impact has led to the formation of all characteristic attributes, such as adrenal hypertrophy, involution of the thymus and spleen, ulceration of the gastric and intestinal mucosa. While evaluation of the state of the antioxidant defense system of animals subjected to stress impact it was revealed a decrease in the level of antiradical activity (ARA) in blood plasma by 46%, the activity of superoxide dismutase (SOD) was by 40% below the control level. There was also noted a decrease of reduced glutathione (G-SH) level in the liver by almost 2 times while the activity of glutathione reductase (GRx) declined by 26%. The activity of another key enzyme of the glutathione cycle, glutathione peroxidase (GPx), was also reduced by 35%. Such changes in the parameters of the antioxidant defense system might be defined as its depletion. Impairment of the antioxidant defense system under stress impact were also manifested by an increase of malondialdehyde (MDA) level by 68%, which confirms the activation of lipids peroxidation and causes an increase of cell membranes permeability.

The administration of algae extracts under exposure to the acute stress was accompanied by a decrease in the severity of destructive changes in the internal organs in comparison with the "pure stress" group. Ulcerative manifestations of the gastric mucous were absent. The explored antioxidant parameters in all groups of animals taking algae extracts stabilized and approached the control values. When compared with the "pure stress" group significantly increased the level of ARA (by 73–80%) and SOD activity (by 46–60%), while the content of MDA decreased (by 32–42%). Parameters of the glutathione cycle in mice treated with extracts from *U. lactuca* and *A. tobuchiensis* remained significantly lower than the control values and were inferior to similar parameters in the group of animals treated with *S. pallidum* extract. The activity of GPx and GRx in blood was lower, by 17–21% compared to ones in the group of mice receiving *S. pallidum* extract. Because the content of polyphenols in the extract of the brown alga *S. pallidum* is significantly higher than one in the extracts of *U. lactuca* and *A. tobuchiensis*, respectively, it has a higher level of antiradical activity.

The administration of the seaweed extracts demonstrated promising effect of diminishing the consequences of acute stress impact. This manifested in preservation of the parameters of the antioxidant defense system that is important for most of the vital processes in the body. Data above propose seaweed *S. pallidum*, *U. lactuca*, and *A. tobuchiensis* are promising objects for creating drugs that can preserve the body antioxidant defense under acute stress preventing stress-induced disorders.

Alexei Zhirmunsky's idea to create a reserve in the Vostok Bay came to life

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With great appreciation, the author pays tribute to the first director of the Institute of Marine Biology, Academician Alexey Viktorovich Zhirmunsky, for entrusting her with the matters of great importance such as establishment of Vostok Bay Reserve in the water area of the Vostok Bay (the Peter the Great Bay, Sea of Japan), and Caldera Crater Reserve on Ushishir Islands, Kuril Islands (Pacific Ocean). He also supported the author's proposal to conduct a hydrometeorological study of the Vostok Bay and provided a comprehensive support during the study.

Director of the Institute of Marine Biology, Academician Alexey Zhirmunsky came up with the idea of creating a protective area in the Vostok Bay almost simultaneously with the creation of Vostok Biological Station by the Institute of Marine Biology, then the Far East Science Center, of the USSR Academy of Sciences on the shore of the bay in 1970. The location for the Biostation was chosen very well. The Biostation is located on a flat cape and fenced with a hill from the south, and from the north, east and west it is surrounded by the waters of Vostok Bay. The purity of the bay waters and its biota diversity had induced Vostok Biostation's development as a ground for biological and ecosystem studies on the coast of the Sea of Japan. The Biostation became, according to Academician Vladimir L. Kasyanov, "a marine biologists' window to Europe, America and Asia at once". Intensive studies in the field of genetics, embryology, physiology, ecology, ethology and other aspects of marine biology research had been carried out here both by FEB RAS scientists and specialists from other Russian and foreign institutes. Those studies provided the background for growth of the Institute's scientific research in the field of mariculture biological foundations, and since 1982, studies for improvement of the existing biotechnologies and creation of new ones for industrial farming of marine aquatic organisms, and experimental projects for cultivation of such valuable invertebrates as the Pacific mussel (*Crenomytilus grayanus*) and the Yesso scallop (*Patinopecten yessoensis*) had been carried out at Vostok Biostation. So, the Vostok Bay was a ground for scientific research by the FEB RAS Institute of Marine Biology, on the one hand, and a promising area for industrial shellfish farming, on the other hand. In addition, according to N.K. Khristoforova, the Vostok Bay was one of the cleanest water areas in the Sea of Japan. To ensure suitable conditions for farming of marine invertebrates, it was decided to introduce a protective status in the Vostok Bay.

The author of the presented paper, under the guidance and with the direct support of Academician A.V. Zhirmunsky, started to establish a reserve in the Vostok Bay in August 1987. Necessary documents had been drawn up: a scientific justification for the reserve creation, a reserve regulation; letters to concerned organizations, draft decisions of the executive committees of the Councils of People's Deputies, "approval sheets" for directors of enterprises and organizations located on the Vostok Bay shore. The author personally attended various sessions and meetings of executive committees of Nakhodka, Partisansk, Primorsky Regional Councils of People's Deputies. Based on the approval sheets, Decisions of Nakhodka and Partisansk Executive Committees of the Soviets of People's Deputies were adopted. As a result of almost two years of efforts, in April 1989 the long-awaited Decision of the Executive Committee of the Primorsky Regional Council of People's Deputies "On Establishment of Vostok Bay Comprehensive Marine Reserve in Peter the Great Bay, Sea of Japan from 20.04.1989" was adopted under No. 131, whereunder the reserve included 1.82 thousand hectares of the Bay aquatic area, in the south limited with the line connecting Cape Puschin and Elizarov. The establishment of the reserve was reported the same year at National Scientific and Engineering Problems of Sea Farming conference which was held at TINRO, Vladivostok (Alexey Zhirmunsky, Larissa Gayko. Establishment of the mariculture reserve in Vostok Bay // National Scientific and Engineering Problems of Sea Farming: abstract. Vladivostok: TINRO, 1989. P. 13–14).

It should also be noted that during the water area preparation for future reserve (1987–1989), Alexey supported the author's idea to conduct an expedition to the Vostok Bay in order to study the bay hydrometeorological condition before introducing the reserve status. The expedition findings serve as a reference for further monitoring of the bay hydrological condition variability.

In conclusion, I would like to add that the cherished dream of Alexei Zhirmunsky had been realized. Vostok Bay Reserve has existed for more than three decades and successfully fulfills its main task – to support the existing natural state of the bay waters and preserve its natural biodiversity. The etablishment of Vostok Bay Reserve is a significant contribution to the protection of marine nature, as well as a precedent for creation of similar reserves.

Effect of temperature conditions on dynamics of catches (abundance) of the pink salmon *Oncorhynchus gorbuscha* based on retrospective data (Sea of Japan, Strait of Tartary)

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The long-term (1980–2018) data on the water temperature regime and associated synoptic processes in favorable and unfavorable years of the abundance formation and the pink salmon *Oncorhynchus gorbuscha* return to the rivers of the Sea of Japan coast in Primorsky Krai from Nevelsky Strait in the north to the Tumannaya River in the south have been analyzed. Catches of Primorye pink salmon in 1980–1999 were lower than in 2000–2019. The sea water temperature in the coastal areas of these regions in May–July 1980–2018 was analyzed according to the data of coastal weather stations Sovetskaya Gavan, Sosunovo, and Rudnaya Pristan. It is shown that low catches of Primorye pink salmon at the end of the past century were accompanied by negative temperature anomalies in the coastal waters and increasing catches during the first two decades of the new century were accompanied by positive temperatures anomalies. Pink salmon returns to rivers at the sea-water temperature 8.6–14.9°C, and values below 3.0–4.0°C are one of the reasons of redistribution of anadromous migratory flows of pink salmon in the Primorye subzone. The synoptic and hydrological situation at different intensity of pink salmon return is described using concrete examples. With a high frequency of cold air troughs typical for the first cold stage of the Far Eastern monsoon caused by the Okhotsk anticyclone, the pink salmon return was delayed and extremely high catches were recorded when the second (warm) stage of the summer monsoon strengthened due to spreading of the Hawaiian anticyclone in the Sea of Japan. The results may be used for short-term prediction of the dynamics of the Primorye pink salmon return. Catches of Primorye pink salmon in 1980–1999 were lower than in 2000–2019. An increase in the stock and catches of pink salmon coincided with a positive trend of water temperature anomalies in the coastal area in May, June, and July 1980–2018: negative temperature anomalies were mainly recorded during the period of low catches (until 2000), later positive temperature anomalies began to prevail. During the same months, in the seaward part of the regions positive water temperature anomalies prevail in the Strait of Tartary and their manifestations are insignificant in their northern part of the Sea of Japan.

The survival of pink salmon juveniles was maximal in 2009 and 2015 that is confirmed by multiplicity of the return. During the downstream migration of juveniles in May 2009, the surface water layer temperature was higher than the average one for the period of survey and the maximum increase (Δtw = 1.5°C) was recorded in the north both in the coastal zone of (Sovetskaya Gavan coastal station) and seaward that is probably due to a stronger effect of warm waters of the Tsushima current on the region of reproduction. The synoptic situation corresponded to favorable conditions for the survival of juveniles when the SFET center was located at 55° N and warm air masses propagated in the front zone of the surface cyclones.

In 2012, the survival of juveniles was low. In this year, the center of cold air masses near the surface was located somewhat to the east from the center of extremely low H500 values and the surface water temperature was low. Thus, a significant decrease (Δtw = −1.6…−2.1°C) at Sosunovo in June–July indicates a stronger effect of the cold Primorye current. In June 2018, when the survival of juveniles was medium, the first (cold) stage of the Far Eastern monsoon was active and determined the features of the thermal regime in the Sea of Japan and adjacent regions. A decrease in the water temperature in the Strait of Tartary (Δtw within −0.5…−1.0°C) did not have a crucial effect on the pink salmon survival. The maximum pink salmon return was observed in 2010 and 2016 at high positive water temperature anomalies. Thus, in June 2010, the maximum increase in the temperature was recorded at Sovetskaya Gavan coastal weather stations (Δtw = 3.4°C), at other stations towards the south Δtw was 0.3 and 1.5°C, respectively and in July, it was 2.1, 2.4 and 2.3°C, i.e., in July, the water temperature along the coast simultaneously increased over the entire area. In the seaward direction, positive temperature anomalies from the north to the south were 1.7, 0.8 and 0.1°C in June and 1.6, 0.7 and 1.1°C in July. The maximum pink salmon return was observed in 2010 and 2016 at high positive water temperature anomalies. Thus, in June 2010, the maximum increase in the temperature was recorded at Sovetskaya Gavan coastal weather stations (Δtw = 3.4°C), at other stations towards the south Δtw was 0.3 and 1.5°C, respectively and in July, it was 2.1, 2.4 and 2.3°C, i.e., in July, the water temperature along the coast simultaneously increased over the entire area. In the seaward direction, positive temperature anomalies from the north to the south were 1.7, 0.8 and 0.1°C in June and 1.6, 0.7 and 1.1°C in July. During the period of low pink salmon return in 2009, negative water temperature anomalies prevailed almost in the entire surveyed area: in June, 0.5 (Sovetskaya Gavan), −1.1 and −0.9°C (Rudnaya Pristan and seaward), and in July the water temperature decreased even more in the south, Δtw = −1.7 and −1.3°C. An increase in the water temperature was recorded in the north: in June in the Strait of Tartary (Δtw = 0.9°C) and in June at Sovetskaya Gavan; a slight decrease in the temperature was recorded at Sosunovo in June and July (0.1 and 0.2°C). The maximum return of Primorye pink salmon in 2010 and 2016 was probably associated with redistribution of anadromous migratary flows due to the favorable temperature regime of sea waters in the surveyed region as a result of which fish from other Japanese stocks entered the rivers of Primorsky Krai.

Based on the analysis of synoptic processes the schemes of favorable and unfavorable climatic conditions for anadromous migration of Primorye pink salmon were made. An early beginning of the second stage of the summer Far Eastern monsoon during the anadromous migration leads to the formation of warm regimes and occurrence of thermal extrema on the surface of the Sea of Japan that creates favorable conditions for pink salmon return to the Primorye coast. When the effect of the Hawaiian anticyclone increases, regions with extremely high air temperature are formed over the Sea of Japan that is a prerequisite for extremely high catches of pink salmon. The delay of return is observed when a high-altitude trough is formed over the Sea of Okhotsk and a high-pressure region appears in the surface layer.

The thermal regime of sea waters formed under the effect of cold and warm stages of the Far Eastern monsoon has a considerable effect on the anadromous migration of pink salmon but further detail studies of this issue are required. The results obtained in the study may be used for a short-term prediction of the dynamics of the Primorye pink salmon return.

Primorsky Aquarium facilities for 'AQUAlaboratory' high school programs

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Environmental education has been developing at a remarkable rate in recent years and is considered the most important means of overcoming the global environmental crisis. One of the major tasks of school education is to raise students' environmental awareness by broadening their knowledge of natural science and improving their skills in applying this knowledge to real-world problems and situations. Museums, zoos, and aquaria have become centers of educational innovations that test new methods of environmental education and foster awareness and conservation actions.

The Primorsky Aquarium – Branch of the NSCMB FEB RAS (A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences) offers a range of educational programs, from lessons and hands-on activities to large-scale projects. The vast facilities of the Primorsky Aquarium, which can be used to implement all these programs, include modern learning laboratories with research microscopes and equipment for sample preparation, lecture and video rooms with multimedia devices. Exhibits of the Aquarium show a paleontological collection and unique collections of live aquatic animals and plants representing all the climatic zones, from the Arctic and the Antarctic to the tropical seas. The 'Evolution of Life in the Ocean' exhibit has a variety of touch screen displays and lifelike models of prehistoric marine creatures. All these features combine to form a unique educational environment, ready to realize any ideas and projects in environmental education.

The 'Growing up at the Aquarium' project for children aged between 7 and 17 years started in 2018. It incorporates four clubs meeting weekly during the school year. The project is outstanding in its continuity and succession: the children graduating from one club can proceed their education in the next one.

The 'AQUAlaboratory' is the fourth and the last grade of the project. It provides a two-year course for high school students 14–17 years old. The first- and second-year curricula include lab lessons, theoretical lectures and exhibit tours, hands-on outdoor activities, and watching science documentaries. The teenagers carry out research projects on ecology, marine biology, paleontology, botany, for instance, research on the effect of detergents on the growth and development of cultured microalgae, ciliates, planarians and daphnia.

While studying in the 'AQUAlaboratory', the teenagers receive the knowledge of natural sciences and fundamental laws of nature and, as a result, develop their

environmental awareness, enhance their ability to analyze human impact on nature, set goals and make meaningful decisions.

The 'AQUAlaboratory' course is implemented in collaboration with specialists from the School of Natural Sciences of the Far Eastern Federal University; the NSCMB FEB RAS; and the Geological Institute FEB RAS.

Rhizocephalan barnacles (Crustacea: Cirripedia) from Peter the Great Bay of the Sea of Japan

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Rhizocephalan barnacles (Crustacea: Cirripedia: Rhizocephala) are extremely simplified parasites that infest mostly decapods and some other crustaceans. The structure of parasites is so reduced that only the morphology of the larvae reveals them as Crustacea. The rhizocephalan female consists of two functional parts: an external reproductive body (the externa) connected through a stalk to an internal system of trophic rootlets (the interna). Since the number of morphological characters of the externa is very limited, molecular analysis is required to correctly identify the rhizocephalan species. Larval morphology also provides the important information for taxonomy and phylogeny of this group. To study Rhizocephala, an integrative approach was used, including molecular and morphological methods that maximize the number of taxonomic characters.

At present, at least ten rhizocephalan species belonging to four families occur in Russian waters of the Sea of Japan. Recently, two new species, *Lernaeodiscus rybakovi* and *L. kasyanovi*, parasitizing the porcellanid crab *Pachycheles stevensii* were found. Two *Lernaeodiscus* species differ well by molecular markers, by the size, color, the morphology of receptacles, by the retinacula type on the internal cuticle, and the presence of distinct marginal lobes in *L. kasyanovi*. Molecular analysis confirmed the monophyly of the genus *Lernaeodiscus* and revealed that *L. rybakovi* is the sister taxon to *L. ingolfi*, while *L. kasyanovi* – the sister taxon to *L. porcellanae*. *Lernaeodiscus kasyanovi* is rather rare compared to *L. rybakovi* (their prevalence on the crab *P. stevensii* is 2% *vs* 30%, respectively). The presence of developing eggs in a number of *P. stevensii* infested females (an extremely rare phenomenon) and the absence of embryos in the *L. kasyanovi* infested females indicates that the degree of control of the parasite over the host can vary even within the same genus.

Molecular and morphological methods were used to describe rhizocephalan *Peltogaster reticulata* parasitizing the hermit crab *Pagurus minutus* in Peter the Great Bay. It was shown that *P. reticulata* is the sister taxon to *P. postica* infesting the same host in southwestern Japan.

Peltogaster lineata inhabiting hermit crab *Pagurus brachiomastus* is the second *Peltogaster* species from Peter the Great Bay. It differs well from *P. reticulata* by size and color of the externa. In *P. reticulata*, mature externa is green, olive or brown, but in *P. lineata*, it is white, yellow or pale. Moreover, externa of *P. reticulata* is

considerably longer (length:width ratio is 3.5–4:1 in *P. reticulata vs* 2.5:1 in *P. lineata*). The morphology of the externa of *P. lineata* from Japanese, Korean and Russian waters is very similar. The host preference of *P. lineata* is changing to the north from *P. filholi+P. nigrivittatus+P. maculosus* in Japan to *P. filholi+P. brachiomastus* in Korea, and to *P. brachiomastus* in Russia. It is likely that Peter the Great Bay is the northern border of *P. lineata* area.

It was found that the spider crab *Pugettia* aff. *ferox* from Peter the Great Bay is simultaneously infested by two rhizocephalans, *Parasacculina pilosella* and *Sacculina pugettiae*. The two rhizocephalans differ well by the morphology of the external cuticle, the shape and position of receptacles, and the structure of the colleteric glands. Molecular analysis revealed that these rhizocephalans are unrelated and should be placed in different genera and families. *Sacculina pugettiae* is clustered within the monophyletic clade of Sacculinidae, whereas the second parasite is nested in the genus *Parasacculina*, belonging to the family Polyascidae and thus should be named *Parasacculina pilosella*. The intensity of two-species multiple infestations reached four externae per host. It is the first finding of multi-species infestation of a single crab specimen.

The complete larval development of all studied rhizocephalans with kentrogonid type of development includes five naupliar and one cypris stage. All nauplii can be divided into peltogastrid-type larvae (families Peltogastridae and Peltogasterellidae) or sacculinid-type larvae (families Sacculinidae and Polyascidae) differing by the presence or the absence of the flotation collar, the dorsal setae on the head shield, the structure of the frontolateral horns, appendages, and furca. The sexual dimorphism of cyprids is undoubtedly associated with different functions of male and female larvae.

Peter the Great Bay (the northwestern Sea of Japan) is characterized by significant fluctuations in water temperature throughout the year, reaching as low as −1.9ºC in winter, and the presence of ice-cover in December–May. The reproductive season of rhizocephalan parasites in this area coincides with the summer – the most favorable time for their embryonic development, larval release and settlement. Mature externae with developing embryos in the mantle cavity occur during summer – early autumn producing multiple larval generations per reproductive season.

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Importance of the discovery of a full fossil imprint of a euconodont animal in Russia

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Academician A.V. Zhirmunsky encouraged our paleontological studies, and, thus, we dedicate the present report to his honor and in his memory. Fossil imprints of soft tissues of such taxonomically challenging organisms as euconodont animals are found extremely rarely in geological records. Their scarce, microscopic remains – tooth-shaped elements referred by C. Pander to as conodonts and rounded attachment plates termed as H elements – are much more common. The appearance of these animals remained unclear for a long time, although their conodont (euconodont) elements are very widely spread in all types of rocks of marine origin and constitute the most important orthostratigraphic group of fossils, used as a basis for detailed zonal delineation of Paleozoic and Triassic sediments. It is, therefore, not surprising that every discovery of fossil imprint of a euconodont animal is actually considered a sensation similar to a discovery of a mammoth. The first imprints attributed to *Clydagnathus windsorensis* (Globensky) on the basis of conodonts were found in 1983–1993 in the Lower Carboniferous Shrimp bed in the Granton District, near Edinburgh, Scotland. In 1995, an imprint with dental elements from *Promissum pulchrum* Kovacs-Endrody was also found in the Upper Ordovician Soom shales in South Africa. In the fossil euconodont animals from Scotland and South Africa, only the internal parts of the head, trunk, and tail segments are available for examination. The first euconodont animal fossil imprint in Russia was discovered in 1998 by A.V. Zhuravlev in the Lower Carboniferous deposits at the Kozhim River (Northern Urals). The imprint was left by an individual with a long (ca. 4.8 mm), narrow (0.3–0.4 mm), and worm-like body. The head, the trunk, and the tail can be discerned on it thanks to the unique case of the excellently preserved external surface. Complete data on the external morphology of euconodont animals has been obtained and published in the literature for the first time. The data confirms the hypothesis that these animals belong to a separate phylum Euconodontophylea Kassatkina et Buryi, 1997.

Our work was awarded as significant by the resolution of the Russian Academy of Sciences of November 4, 1999, Earth Sciences (Geology, Geophysics, Geochemistry). In the section Stratigraphy, Paleontology, Geochronology. The structure and evolution of the Earth (page 85).

Identification of population structure of Japanese sea cucumbers, *Apostichopus japonicus*, from Peter the Great Bay, Sea of Japan, using microsatellite loci

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The Japanese sea cucumber, *Apostichopus japonicus*, is a marine invertebrate of the phylum Echinodermata, whose range occupies coastal waters of the northern parts of the Yellow and East China Seas, most of the Sea of Japan coast, the eastern Pacific coast of Japan, and the southernmost Sea of Okhotsk (Levin, 1982). Microsatellite markers (Kanno et al., 2005) and single nucleotide polymorphisms (Du et al., 2012) are used to describe the genetic variability and population structure of this species, and many are under development. A study of the Japanese sea cucumber samples from the coast of South Korea and the obtained statistical values have demonstrated the isolation of the populations relative to each other (Kim et al., 2008). Chinese researchers also report similar values of genetic distances (Chang et al., 2009).

The aim of our research was to identify the population structure of *A. japonicus* on the basis of five samples collected in Peter the Great Bay, Sea of Japan, using five microsatellite loci.

Values of genetic distances between groups of *A. japonicus* individuals were obtained. The maximum F_{ST} value was 0.3100 (P<0.001) for populations from Vostok Bay and Cape Krasny. The minimum F_{ST} was 0.0066 (P<0.05) for individuals from Possjet Bay in 2015 and 2018.

An analysis of the structure of genotype distribution showed the most optimal division of the studied populations into four clusters, if based on the likelihood function. On the basis of the F_{ST} and R_{ST} values and also on the genotype distribution data, the samples can be divided into four groups:

1) individuals of *A. japonicus* from Vostok Bay;
2) individuals from Fedorov Bay;
3) a sample from Cape Krasny;
4) samples from Possjet Bay for different years.

A high level of heterogeneity was found between the samples, which is possible in several cases. First, it can be caused by stochastic processes, where larvae from a few breeding individuals settle in a certain locality.

Selection may be another factor responsible for heterogeneity over a relatively large geographic distance. One more factor determining the high level of heterogeneity may be the selection pressure varying for the different loci of quantitative traits and microsatellites linked to them. This selection can determine the differential survival rate of larvae in certain localities and, as a consequence, the formation of heterogeneity.

To remove the effect of stochastic processes, the number of individuals in samples and the number of Japanese sea cucumber samples should be increased. To detect the effect of multidirectional selection by loci of quantitative traits, it is necessary to test the differential survival rates of larvae with various combinations of microsatellite loci under experimental conditions.

Assessment of antimicrobial activity of sand dollar *Scaphechinus mirabilis*

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The marine environment is aggressive; organisms are forced to produce substances with a vast range of biological activity. In this regard, hydrobionts are a source of biologically active compounds, the chemical structure of which differs significantly from the structure of medicinal substances obtained from terrestrial plants and animals. At the same time, substances of marine origin often have a considerably more significant effect than known biologically active compounds from plants and animals on land. Marine hydrobionts, as sources of biologically active compounds, are represented by various types, classes, species, and subspecies of marine organisms. These can be jellyfish, fish (sea bass, catfish, flounder), sea mushrooms, actinomycetes, and sea urchins. The study of the mechanisms and features of their immunity, providing antibacterial, antiviral, antiparasitic protection, allows us to enter the field of entirely new ideas and obtain innovative chemical compounds (molecules). Cyclic and linear peptides isolated from marine hydrobionts can exhibit powerful antioxidant, antimicrobial, cytotoxic, anti-inflammatory properties; act as specific ion channel blockers. Sea urchins have an innate immune system and are considered a potential source for discovering new antimicrobial substances. Spinochrome naphthoquinoid pigments are a characteristic biochemical synapomorphy of sea urchins, caused by genes encoding polyketide synthase enzymes, which are necessary for the biosynthesis of these compounds. They are believed to have antioxidant activity.

At the moment, many scientific studies have been carried out on the study of embryology, ecology, and the influence of isolated substances from sea urchins. We have also evaluated the antimicrobial activity of an extract from the sand dollar *Scaphechinus mirabilis*. The extract from the sea urchin was obtained by alcohol extraction. The fragments of sea urchin were incubated for one hour on a magnetic stirrer in 70% alcohol. After filtration, the extract solution was dried, the precipitate was dissolved in 20% DMSO. The antimicrobial effect of the obtained extract from *S. mirabilis* has been tested for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *MRSA*, *Escherichia coli K12* and *Pectobacterium carotovorum*. Antimicrobial activity was recorded by the presence of transparent zones of no growth around drops with the extract.

In the course of our work, it was determined that the extract obtained from the sea urchin *S. mirabilis* has inhibition of the growth of *S. aureus*, *MRSA* and *P. carotovorum*. The data obtained indicate that the sea urchin *S. mirabilis* produces antimicrobial substances of an unspecified nature and requires further and more detailed study.

Investigation of the transduction of the recombinant adeno-associated virus of the mammalian hippocampus in the mesencephalic tegmentum of juvenile chum salmon *Oncorhynchus keta*

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Adeno-associated viruses, capable of invading vertebrate cells and found to be relatively non-pathogenic in lower vertebrates. Thus, these agents can be very suitable for transferring genes into fish cells for vaccination or for other experimental studies where the expression of specific genes is present.

To assess the potential of adenoviral transduction into the brain cells of juvenile chum salmon *Oncorhynchus keta*, we used recombinant adeno-associated viral (AAV) vectors of the mammalian hippocampus. The aim of this work was to study AAV transduction into the cells of the mesencephalic tegmentum of juvenile chum salmon *O. keta* after one week.

We used ready-made recombinant adeno-associated viruses of the mouse hippocampus.

Delivery of the gene *in vivo* was carried out by intracranial injection of a GFP-containing vector directly into the mesencephalic tegmentum of one-year-old juvenile chum salmon *O. keta*. Evaluation of the level of infectious efficacy was performed 1 week after injection of the vector.

Using confocal scanning microscopy, the expression of the adeno-associated vector was assessed in different zones of the tegmentum of juvenile *O. keta*, followed by IHC analysis of immunofluorescence of the neuron-specific protein HuCD in combination with staining of DAPI nuclei.

Studies on juvenile chum salmon *O. keta* have shown for the first time efficient transduction of genes of recombinant mammalian hippocampal AAV in mesencephalon and diencephalon cells 1 week after a single injection.

The expression of GFP+ cells, nuclei and granules, expression of calcium binding protein of neuronal differentiation HuCD, as well as co-expression of GFP+/HuCD+ in the diencephalon and in the mesencephalon were shown.

The data obtained indicate the highest number of GFP+/ HuCD+ cells in the anterior hypothalamic bay, which is apparently due to intensive anterograde intracellular transport.

The presence of a large number of GFP+ and GFP+/HuCD+ cells in the dorsomedial and dorsolateral tegmentum is associated with the spread of the adenoviral vector as a result of post-traumatic neurogenic processes.

The presence of a minimum number of GFP+ and GFP+/HuCD+ cells in the mesencephalic reticular formation is due to the minimum density of cell distribution in this area and limited retrograde intracellular transport in the caudal direction.

Usage of molecular markers: from biodiversity assessment and modern general biology paradigm validation to seafood mislabeling detection

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Application of molecular genetic markers is very fruitful in many directions. One is validation of modern paradigms, the Synthetic Theory of Evolution (STE) plus Biological Species Concept (BSC) and another is Biodiversity estimation for the variety of social needs including false labelling of marine species as food sources.

Methods: Vast sequence data on nDNA and mtDNA gathered and subjected to meta-analysis and a variety of software applications.

The main issues of the report are focused on 5 items. (1) A combination of nDNA and mtDNA markers best suits the hybrid identification and estimates of genetic introgression. (2) The available facts on nDNA and mtDNA diversity seemingly make obvious the introgression among many taxa, although, it is evident that introgression may be quite restricted or asymmetric, thus holding at least the "source" taxon (taxa) intact. (3) If we accept that sexually reproducing species in marine and terrestrial realms are introgressed, as it is still evident for many cases, then we should recognize that the BSC, in terms of complete lack of gene flow among species, is inadequate due to the fact, that many zoological species are not biological species yet. However, vast modern molecular data proved that sooner or later they will definitely become biological species (Kartavtsev, 2013, 2018; Hedges et al., 2015). (4) The recent investigation of fish taxa divergence (Kartavtsev, 2017, 2018) using BOLD database shows that most gene trees are basically monophyletic and interspecies reticulations are rare. (5) A variety of evidence are available globally that proved a fraud of seafood; thus, scientists must to develop a molecular control for this and similar industry spheres.

Abnormal plankton organisms as bioindicators in marine waters exposed to environmental pollution

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Bioindication using chaetognaths (Chaetognatha) is based on the unique structure of these animals and the specifics of their tissue responses. Chaetognaths are distinguished by a combination of a primitive general organization and highly differentiated and peculiar tissues.

Due to the unique features of these animals, their tissue organization is of high scientific interest. The relevance of the study of cells, tissues, and the general morphology of chaetognaths is undoubted, because their different tissues respond differently to anthropogenic impacts and natural fluctuations. The study of the morphology of healthy and abnormal animals is crucial to identify the characteristic changes caused by certain effects and also to develop an approach to monitoring the ecological condition of a marine body of water.

For this purpose, the structure of a widely distributed epiplanktonic species, *Aidanosagitta macilenta* (family Sagittidae), was subjected to a histological examination. Also the ultrastructure of the skin and intestinal epithelium in *A. macilenta* was studied. The tissues of abnormal animals exposed to radiation were examined on a histological level (the animals were obtained from the focus of radioactive contamination in Chazhma Bay).

Changes in the skin and intestinal epithelium were found in the chaetognaths exposed to anthropogenic radioactive impact. These changes are revealed by external examination and are manifested as damaged intestinal and skin epithelium. Histological sections show detachment of skin epithelial cells from the surface of the epithelium. Apparently, intercellular junctions (cell–cell bridges) become destroyed as a result of radiation exposure. The intestine undergoes significant damage; intestinal epithelial cells decrease in volume, and manifest shrinkage and compaction. The muscle layer is detached from the basement membrane of skin epithelium and, as a result, the massive ventral nerve ganglion sometimes becomes separated together with the epithelium. Characteristic changes are observed in the fins: the fin margin looks indented (acquiring the shape of a "cock's crest") due to a partial destruction of the fin plate, but sometimes the fin plate is completely destroyed. A conclusion can be drawn that cytoplasm of the epithelial cells covering the fin plat as well as, possibly, other components of the fin

plate are easily destroyed, with the rays remaining undamaged as the most durable structures: as a result, the cock's crest pattern is formed, which can be considered an indicator of radioactive contamination of the marine environment. The chemical composition of the rays (the presence of elastoidin) provides their strength. This quality prevents them from being destroyed by radiation impact.

The revealed changes are manifested only under this effect and are not observed during exposure to other damaging effects. For this reason, they can be used for the bioindication of radioactive contamination of marine waters.

Many planktonic organisms do not show external damages during radiation exposure: these are found neither in phytoplankton nor in planktonic animals protected by their chitinous carapace (Kassatkina et al., 1993). Other planktonic animals affected by radiation (e.g., tunicates) cannot stay in the plankton anymore: they die and eventually become destroyed. In contrast, chaetognaths are distinguished by high viability and do not die when exposed to a damaging effect, but continue their existence, feeding and reproduction, thus, representing a convenient object for bioindication. It is probable that such a high level of viability is achieved thanks to the simplicity of their body structure and the ability to absorb dissolved organic matter (Sorokin, Wyskwarzev, 1973).

Thus, chaetognaths are a unique object that, due to their characteristic tissue responses under certain effects and high viability, can be successfully used for ecological monitoring of marine bodies of water. This is confirmed by the respective patent for invention (Stolyarova, Kassatkina, 2012).

The histological examination showed more clearly the damages described through visual observations under a binocular microscope (Kassatkina, Stolyarova, 2010). New details of the recorded changes and their specifics at the tissue level were elucidated. These observations allow a conclusion that penetrating radiation affects the tissue systems of marine arrowworms, causing characteristic damage to them. The histological analysis of these abnormal animals revealed some features that are characteristic only of individuals that have been exposed to radioactive impact. The major trends of changes are the weakening of cell–cell connections (detachment of skin epithelial cells from epithelial surface) and connections between neighboring tissues (detachment of the muscle layer from the skin epithelium). Nevertheless, despite the profound changes and structural disorders, marine arrowworms remain viable, continue their life cycle, and retain all life functions, including feeding and reproduction. In cases where the intestinal epithelium is destroyed under the effect of radiation, arrowworms also maintain their life activity. This is the evidence of the high adaptability of chaetognaths, which allows them to survive various adverse conditions. Marine arrowworms are characterized by a combination of primitive organization of the body structure, and high specialization of tissues. Such a unique feature of the tissue structure and general organization makes marine arrowworms a convenient object to be potentially used as a bioindicator of various impacts of both anthropogenic origin and natural fluctuations in the marine

environment. Histological and cytological studies of chaetognaths can be considered as an independent or supplementary method of bioindication, which also allows explanation data obtained by visual examination.

The anthropogenic impact on marine ecosystems has increased significantly in recent years and is now observed on a global scale, as reported by the international programs of UNESCO, IUBS, and DIVERSITAS. Man-made disasters with subsequent contamination of the marine environment with radionuclides have a major impact primarily on planktonic biota. In this regard, chaetognaths are most promising organisms to be used as bioindicators of pollution in the seas and oceans.

Variability of zooplankton communities over the Emperor Seamounts Chain, Northwest Pacific

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Seamounts are present in all oceans of the world and are considered one of the least-known habitats on earth. The growing interest in studying the biota of seamounts has several explanations. One of them is the high concentrations of commercially valuable fish and invertebrate species that are found here. Such richness is suggested to be associated in part with the baric disturbances occurring above and around submarine rises (Uchida et al., 1986; Rogers, 1994). Another explanation for the high interest in seamounts, especially in biogeography, is the great diversity of species on their summits, the geomorphological features, and the degree of geographical isolation. The study of seamount biota, its sources, and also routes of transport and dispersal may shed light on some controversial issues in marine biogeography (Parin et al., 1997; Vinogradov, 1997).

The Emperor Seamount Chain is apparently the world's most promising region for wildlife research. It has a form of a very long ridge extending from south to north in the northwestern Pacific Ocean and consists of seamounts and guyots that vary greatly in shape, summit depth, degree of geomorphological isolation, and oceanography. The Emperor Seamounts are the site of the largest documented catches of fish and invertebrate compared to other seamount waters in the world (Clark et al., 2007). These seamounts are located at the border between the boreal and tropical biogeographic zones of distribution of marine biota, with different faunal complexes of species mixing here. Therefore, the role of the Emperor Seamount Chain in the distribution of species in the northwestern Pacific Ocean can be very significant. This study provides the first description of the faunal composition of copepods and other zooplankton in the Emperor Seamounts area for which there has been little information to date.

The study was based on zooplankton samples collected from July 13 to August 11, 2019 at 29 stations in the area of the Koko (171° E, 35° N), Jingu (171° E, 38° N), Nintoku (170° E, 40° N), and Suiko (170° E, 44° N) guyots. Sampling was performed during daylight or night hours by vertical net (opening diameter 0.6 m; mesh size 200 μm). For each station, the epipelagic (0–200 m) and mesopelagic (200–800 to 1000 m) zones were considered separately. A total of 47 plankton samples were obtained. The samples were fixed with a 4% formaldehyde solution. Simultaneously with sampling, hydrological data (current velocity, depth, water temperature, salinity, oxygen concentration, chlorophyll *a* level, turbidity, etc.) were measured at each

station using a SBE 19 plus V2 SeaCAT Profiler CTD from the surface to the depth of immersion. Copepods, as the dominant group of zooplankton, were subjected to more detailed taxonomic analysis using the Marine Planktonic Copepods database (Razouls et al., 2005–2021).

We found a total of 89 different zooplankton taxa. Among them, we identified species from four genera of the order Amphipoda, seven genera of the order Pteropoda, 39 genera of the subclass Copepoda, and also larvae of benthic invertebrates and fish. Of the 22 large taxonomic groups of zooplankton, copepods were the most important group in terms of species diversity (58 species), although some proportion of the animals found were not identified to a lower taxonomic level.

The structure of the zooplankton communities over the Emperor Seamounts changed from the dominance of small-sized tropical copepods with a small proportion of large-sized species to the dominance of large-sized oceanic copepods with a small proportion of small-sized species along our route from south (Koko) to north (Suiko). The structure of the community of smaller in size copepods was associated with the high-temperature region; the community of larger in size copepods, with the low-temperature region. The highest number of zooplankton taxa was recorded from the "transitional zone" (Jingu and Nintoku), while the highest concentration of zooplankton with largest values of abundance and biomass was observed in the Suiko area.

Plankton species from various zoogeographic regions of the ocean, brought by changing systems of currents, can be found in the waters over seamounts. Furthermore, both seamounts and the vortex zone around them can be a source of local plankton populations (Fedosova, 1980; Schulenberger, 1982). Local populations of some tropical/subtropical copepods (*Calocalanus pavo*, *Paraeuchaeta aequatorialis*, *Pleuromamma gracilis*, and *P. quadrungulata*) were observed in the plankton at the summit of Jingu Guyot. Local populations of wide-boreal copepods (*Euchaeta acuta*, *Paraeuchaeta barbata*, and *Pleuromamma robusta*) were recorded from plankton over the northern slope of Suiko Guyot. Populations of cephalopod larvae and colonial salps were found in plankton over the northern slope of Nintoku Guyot.

In the distribution of planktonic organisms, passive transport by currents is a crucial factor. The biogeographic structure of the copepod fauna varied in the south-to-north direction from Koko over Jingu and Nintoku to Suiko. The number of tropical species characteristic decreased in the direction from Koko to Suiko, and the number of boreal species increased. The indicator species of waters of the warm North Pacific Current, which flows at the boundary between tropical and subarctic waters, forming a wide transitional area between 35° and 42° N (Ivanov, Sukhanov, 2015), were *C. pacificus*, *Euchirella rostrata*, and *Lucicutia flavicornis* identified in the plankton over Jingu and Nintoku. We observed a shift of tropical and subarctic waters in the transitional zone from Jingu (38° N) to Nintoku (40° N). The transitional area between the boreal and tropical epipelagic zones in the northwestern Pacific Ocean has narrowed and ran between 38 and 40° N, as inferred from the data obtained.

Based on the analysis of the physical properties and distribution of water masses, we determined the position of the northern boundary of the Subarctic Front (the zone of mixing of Subarctic Water and Subtropical Water) in summer for the present time. The analysis of data on the distribution of copepod species structure (Brodsky, 1957; Fedosova, 1980; Vinogradov, 1997; present study) and the information on oceanological processes observed during numerous oceanographic studies in the northwestern Pacific Ocean (Ivanov, Sukhanov, 2015) made it possible to determine the temperature limits of species distribution: boreal copepods were recorded from in Subarctic Water at a water temperature of 10–12ºC and below (Suiko); tropical/subtropical copepods were found in Subtropical Water at a temperature of 14ºC and above (Koko); the area of detection of boreal and tropical/subtropical copepods together was located in the Subarctic Front within a temperature range from 10–12 to 14ºC (Jingu and Nintoku).

In conclusion, effects of the factors responsible for the disturbance of the latitudinal zoning in the marine environment are directly determined by the pattern of interaction of land masses, winds, and currents. Accordingly, with a shift of the latitudinal zones, the boundaries of distribution of some marine organisms, belonging to a certain latitudinal/zonal complex of species, will also move. Extensive studies are required to clarify the biogeographic boundaries on the basis of distribution of plankton communities and to prevent possible damage to the vulnerable marine ecosystems of seamounts.

The "Sea Reflection" contest as a form of environmental education at the Primorsky Aquarium

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Being a branch of the A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, the Primorsky Aquarium has a high cultural and educational potential and resources. One of the institution's main focuses is engaging the general public with environmental education through various events: themed tours and environmental holiday celebrations, lectures and exhibitions.

In 2018, the Aquarium launched an educational initiative for preschool and school children from Primorsky Krai – a creativity contest called "Sea Reflection". The event commemorates World Ocean Day, which is annually celebrated on June 8. The "Sea Reflection" contest seeks to raise awareness in children and youths about the importance of biodiversity conservation and nature protection through individual creative activity. The contest categories (Essay, Video Art and Drawing) and their themes help contestants show the main purpose of World Ocean Day – to remind the global community of the key role the world oceans play in its everyday life, put a spotlight on their problems and the necessity to sustainably use living marine resources.

In the Drawing category of the 5th "Sea Reflection" contest young artists were offered the following themes: "The Primorsky Aquarium, My Family and I", "Sea Patterns", "My Encounters with Marine Inhabitants", "Sea as a Home for Us". In their art works, participants shared their views on the life inhabiting the world's oceans, showed the beauty and biodiversity of the underwater world and described their experiences of the Primorsky Aquarium exhibits.

The themes in the Essay category – "You are Live, You Can Breathe", "Ocean Voyagers and Ocean Voyages", "I Live by the Sea" – gave the children an opportunity to plunge into the world of sea voyages and discoveries, learn about people committed to the sea and get an insight into the history of their homeland. The contestants could choose any of the suggested genres (essay, poem, discursive essay and short story) to express their love for the sea and their homeland.

The Video Art category is the most technically demanding of the three since it requires specific computer and program skills. This year's themes – "A Day at the Primorsky Aquarium", "When the Sea Got Sick", "Everything Depends on Us", "Preserving the Sea of Japan", "Scenes from the Life of Sea Creatures" were represented in 3-minute cartoons and videos such as "The Journey of Spotted Seal Dulcinea", "Marine Life: Interview with Illarion Fishson", "Protecting Lights" and so on.

All entries feature an integration of science and creativity: the depictions (or descriptions) of animals are in line with reality, recreating their natural appearance, behaviors, coloration, their functions in ecosystems and explaining human impact on their populations, in particular, and on nature, in general. All eligible works are judged by a professional jury. Depending on the contest category, it may comprise qualified philologists, teachers, cultural experts and artists.

The contest finale is an award ceremony that traditionally takes place in the Aquarium's conference hall. Its participants include winners, their parents and teachers as well as a jury panel. The event sees an opening of the exhibition of drawings by contestants in the Aquarium's art gallery. The gallery also houses the interactive touch screen showing the best essays and videos submitted to the "Sea Reflection" contest. The exhibition runs for a month, drawing special attention from Aquarium visitors.

Over the five years of the initiative's existence, the number of entrants has increased from 110 in 2018 to more than 700 in 2021, which indicates its significance and relevance to the environmental education provided by the Primorsky Aquarium.

Tolerance to hypersalinity stress in ark clams *Anadara kagoshimensis*

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Salinity is one of the most important environmental factors influencing survival ofaquatic organisms. In this key, sea salinification is an emerging concern for World coastal aquaculture. Bivalve mollusks are osmoconformers, which are unable to maintain a constant level of internal osmolarity. Therefore, the level of salinity tolerance of bivalves is ensured by hemocyte cellular adaptation to changes of environment osmolarity. Increased environmental salinity was shown to affect hemolymph cellular composition and physiological state of hemocytes. Ark clam, *Anadara kagoshimensis* (the Arcidae family), is an invasive species in the Black Sea region which is naturally distributed throughout Indian and the Pacific Ocean. Functional state of the ark clam hemocytes have never been investigated in the Black Sea mollusks, which are acclimated to lower salinity conditions. The aim of the present work was to examine the effect of hypersalinity on hemolymph cellular composition and functional parameters of the ark clam (*A. kagoshimensis*) hemocytes, which inhabit hyposalinic Black Sea region.

Ark clams (shell length 36.5±1.5 mm, body mass 19.6±1.7 g) were captured in Laspi bay (Sevastopol) at a depth of 17 m in August 2020. Nineteen ark clams were randomly divided into three groups. In the control group (5 specimens) water salinity was equal to the Black Sea level (15 ppm); in two experimental groups, the salinity level increased up to 35 ppm (7 specimens) and 45 ppm (7 specimens). Water salinity in the experimental tank was increased with the speed 0.8±0.2 ppm per hour by the addition of the Red sea salt (France); the salinity 35 ppm was reached after 6 days incubation and maintained at that level for 2 days. Then, the salinity was similarly increased up to 45 ppm and maintained at that level for 2 days. At the end of the exposure period, hemolymph was withdrawn from the extrapalliar cavity of the individual mollusk with 2-mL syringe and a 25-gauge needle. The osmolarity of hemolymph samples (50µl) was determined using the Astori OsmoSpecial 1 osmometer. Hemocytes were analyzed using flow cytometric and light microscopic techniques.

Smears of hemolymph samples were dried on air at least 24 h and then fixed and dyed with May Grünwald and Giemsa stain solutions. Hemocytes were viewed on a light microscope (Biomed PR-2 Lum) equipped with a camera (Levenhuk C NG Series). Approximately 1000 cells per smear were examined for morphological analysis. For flow cytometric characterization, washed hemolymph cells were diluted in

sterile seawater to adjust hemocyte concentration in suspension to $1–2·10^6$ cells mL^{-1}. All flow cytometric measurements were performed with Cytomics FC500 flow cytometer (Beckman Coulter) equipped with the blue argon laser (excitation at 488 nm). Cell suspensions were dyed with DNA-binding fluorochrome SYBR Green I (final concentration in the probe 10 μM) according to previously used standard protocol, and hemocytes were readily differentiated from other particles in the suspension on the basis of its fluorescence. The number of dead cells in suspension was investigated using the analysis of the fluorescence of hemocytes stained with Propidium iodide (PI). 10 μl of 200 μg mL^{-1} PI stock solution (Sigma Aldrich) was added to 1 ml hemocyte suspension, and the cells were incubated in the dark for 30 min at 4ºC. The percentage of dead hemocytes was evaluated on the histograms of PI fluorescence in the channel FL4 of cytometer. Differences between groups were analyzed in Statistica software version 8.0 (Statsoft Inc., Tulsa, OK, USA). The analysis of variance (ANOVA) was used to determine the significance of differences and the Tukey test was applied to compare the means whenever the data were significant, and $p<0.05$ was considered statistically significant. The results are expressed as mean±SEM.

The osmolarity of ark clam hemolymph was equal to seawater and increased gradually when the environment became hyperosmotic. Hemolymph osmolality of mollusks acclimated to 35 ppm was 1052.7±9.2 mOsm L^{-1}. In mollusks acclimated to salinity 45 ppm hemolymph osmolality increased more than 2.5 times compared to control ($p<0.05$).

At normal osmotic conditions ark clam hemolymph contained two hemocyte types: granular (erythrocytes) and agranular cells (amebocytes). Exposure to a hypersaline environment did not significantly influence hemolymph cellular composition, whereas the morphology of cells substantially changed. In both experimental groups (35 ppm and 45 ppm) we observed invaginations of the erythrocyte membrane, as well as an increase of euchromatin level in nuclei and their optical density. At 35 ppm diameter of erythrocytes decreased on nearly 2%, but at 45 ppm it was 10% larger compared to control values ($p<0.05$). For amebocytes, hypersaline environment led to cell enlargement in both experimental groups on about 24 and 34% respectively ($p<0.05$). Similarly to cellular diameter, the size of erythrocyte nuclei decreased significantly at salinity 35 ppm (4.5±0.03 μm) and increased at 45 ppm (4.9±0.03 μm) ($p<0.05$). The nuclear diameter of amebocytes gradually increased with salinisation of the environment ($p<0.05$).

The increase of environmental salinity was not accompanied with hemocyte mortality in the first experimental group (35 ppm). No significant changes in the level of dead cells were observed. At salinity 45 ppm the number of dead cells was significantly higher compared to control probes ($p<0.05$).

Based on presented results we may speculate that *A. kagoshimensis* is tolerant to salinity 35 ppm. This hypothesis is confirmed by the wide distribution of the species (from 12 ppm in the Azov Sea to 38 ppm in the Adriatic Sea). Bivalve mollusks are unable to maintain a constant osmolarity of the internal environment and fluctuations

of external salinity affect of normal cells functioning. In general, mollusks response to a negative impact of salinization by closing their valves in conditions of short-time exposure, but long-time survival in hyperosmotic conditions frequently leads to raised mortality of mollusks. In the present work, increasing salinity was not associated with mortality ofark clams. whereas a high mortality rate was observed in *Venerupis corrugate* at similar salinity conditions (35–42 ppt) . Therefore, ark clams are supposed to have a pronounced tolerance salinity by both 35 and 45 ppm.

The work on the influence of hypersalinity on the hemocyte morphology and functional parameters supported by State Assignment (state registration no. 121041400077-1) and identification of cells type has been investigated with the support of Grants Council of the President of the Russian Federation (project no. MK-609.2020.4).

Ichthyoplankton of the Black Sea at the beginning of the summer spawning season

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The study examines species diversity and spatial distribution of ichthyoplankton on the shelf and deep-water areas of the Black Sea off the Crimean Peninsula and along the shore of the Caucasus at the beginning of the summer spawning season in June 2020. In the coastal water area ichthyoplankton research were carried out off the south-western part of the Crimean Peninsula (Sevastopol). Samples were collected in the mode of vertical hauls using a reverse conical net BR-80/113 and a conical net IKS-80 (mouth opening 0.5 m^2, mesh 400 μm). The analysis of hydrological regime and surface circulation employed satellite infrared (IR) images of the sea surface, and altimetry data from (Marine Portal, http://dvs.net.ru/mp/index.shtml) and expeditionary data from vertical soundings using an CTD "SBE 911plus", Indronaut Os320Plus and Acoustic Doppler Current Profiler. Supplementary materials included concurrent data from hydrologic observations in the coastal waters of Sevastopol.

The aim of the research is to study the species composition structure of ichthyoplankton in different regions of the northern part of the Black sea in connection with the peculiarities of the hydrological regime against the climatic changes.

The greatest impact of the climatic changes in recent decades has been observed in the ecosystem of the seas of the temperate zone, where many fish species exhibit strong seasonal patterns of spawning. Environmental changes contributed to the prolongation of the spawning season for both temperate and warm-water fish species and led to the simultaneous presence of their larvae in ichthyoplankton. Latitudinal shifts in the distribution of natural fish populations, as well as changes in the spatial distribution as a response to climate and hydrological regime changes are documented for both pelagic and benthic fish species (Asch, 2015; Walsh et al., 2015; Auth et al., 2017; Rogers, Dougherty, 2019). Due to climate warming, changes in the regime characteristics of the Black Sea waters (primarily coastal) have been observed in the Black Sea (Belokopytov, 2017). Long-term and interannual variability in the spawning phenology of warm-water and temperate-water fish species have resulted from changes in the hydrological regime of the Black Sea waters observed against the background of climatically induced changes in recent decades, such as a rise in surface temperature, an increase in the heat content of the upper active water layer, degradation of the "classic" cold intermediate layer, a change in "haline phases", a strengthen in the intensity of the general circulation of the sea and regional differences in the seasonal vorticity and time of existence of coastal

circulation structures. While the species composition, spawning periods and spawning intensity are determined by the parameters of the current and previous hydrological seasons and their regional inertia, the nature of the spatial distribution of ichthyoplankton is depended on the activity of the Black Sea Main Current (Rim Current), different-scale quasi-stationary anticyclonic and cyclonic eddies, local coastal thermodynamic structures of synoptic nature (coastal upwellings, mushroom-shaped currents).

In ichthyoplankton samples eggs and larvae of 34 fish species from 21 families were identified, including 4 species of temperate water and 30 species of warm water fish. The number of species of fish eggs and larvae in open and coastal waters was of the same order. At open waters stations 26 species of fish eggs and larvae from 18 families were recorded, and at coastal waters stations (water area of Sevastopol) – 25 fish species from 17 families. In open waters, the average number of eggs was 28.8, and the number of larvae was 2.64 ind./m^2. The eggs of anchovy *Engraulis encrasicolus* (73%) were dominant; its larvae accounted for 22% of the total number of larvae of all encountered species. The percentage of anchovy larvae from their eggs in the samples was 2.8, which is quite comparable with the data (Dekhnik, 1973). The highest abundance and species diversity of ichthyoplankton were recorded on the western shelf of the Crimean Peninsula. In the open waters of the western shelf, the mean abundance of eggs was 101.1 ind./m^2, larvae – 9.1 ind./m^2, and the maximum abundances were 274 and 20 ind./m^2, respectively. In coastal waters the mean number of eggs was 161.5 ind./m^2, larvae – 25.1 ind./m^2, and the maximum number of fish eggs and larvae reached 368 and 85 ind./m^2, respectively.

An increase in the dynamic activity of the Rim Current and, first of all, the vorticity of quasi-stationary anticyclonic gyres, have led to an increase in water exchange between coastal and open waters. As a result, in the ichthyoplankton of the open waters of the Crimean Peninsula shelf the number of species of fish eggs and larvae of typical inhabitants of coastal biocenoses ("other" species) has increased. The share of fish eggs of "other" species in open waters of the shelf compared to 1950–1970 has changed from 4.3 to 10%, and the proportion of their larvae – from 7.9 to 74% (Dekhnik, 1979).

The indices of species diversity (Odum, 1986) of ichthyoplankton collected in open shelf waters, where anchovy eggs and larvae dominated, were quite comparable with those of coastal waters and were twice as high as those for open shelf waters at the period of active spawning of thermophilic fish species in July 1988 (Klimova, 2006).

The most favorable conditions for the survival of ichthyoplankton in June 2020 were formed in the coastal water area of Sevastopol, where the maximum evenness (0.53) and the minimum dominance of certain species (0.27) were observed, and the species diversity index was 2.44. The high abundance of eggs and larvae, including commercial species, both in the open and coastal regions of the western shelf of the Crimean Peninsula, is likely to evidence the restoration of the fishery significance of this region as a nursery area of the Black sea natural fish populations.

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Correspondence in the activity of energy metabolism oxydoreductases in brain and heart compartments of the Black Sea scorpionfish *Scorpaena porcus* Linnaeus, 1758 under acute hypoxia

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The fish brain and heart are main organs provided the survival in aquatic environment with constantly varying O_2 level (PwO_2) and periodically arising episodes of hypoxia/anoxia. Moreover, the primary cause of fish death during O_2 lack is a violation of ATP brain homeostasis, regardless of the general resistance of certain species to O_2 starvation. Experiencing a raised need in O_2 to ensure their metabolism the oxyphilic structures of brain and heart differ significantly in origin, cytoarchitectonics and specificity of blood (arterial or venous) supply, and this may tell upon their metabolic activity.

Oxydoreductases – malate dehydrogenase (MDH, L-malate: NAD oxidoreductase, EC 1.1.1.37) and lactate dehydrogenase (LDH, L-lactate: NAD oxidoreductase, EC 1.1.1.27) are involved in the energy production, regulate the cell redox potential and serve as markers of the oxidative and glycolytic capacity of energy metabolism. Each change in the hydrochemical characteristics of the aquatic habitat, primarily in PwO_2, cause shifts between aerobic and anaerobic pathways of energy metabolic processes in which oxidoreductases are involved.

The Black Sea scorpionfish is a benthic ("sit-and-wait") ambush-predator characterized by an especial tolerance to O_2 deficiency. It is well known that one of the most striking differences between hypoxia-tolerant and hypoxia-intolerant animals is the ability of the latter to maintain brain and heart energy status in the face of a diminished energy supply. Such hypoxia-tolerant vertebrates as scorpiofish become unique study object which could give the information about the mechanisms that are invoked during O_2 starvation for survival. Of particular interest is to elucidate the features of the biochemical strategy for maintaining energy metabolism. So on, the aim of this study was to exam the activity of energy metabolism oxydoreductases in the brain and heart compartments of scorpionfish under acute hypoxia.

The objects of study were mature Black Sea scorpionfish *Scorpaena porcus* Linnaeus, 1758 (16 exemplars, 13–17 cm long, stage of gonad maturity VI-II) in summer period. In the course of the experiments, each scorpionfish was kept in a separate aquarium-stand with sea water which allows to stabilize the temperature as in

the sea (21–23°C) and oxygen concentration (21–23°C, 5.6–6.7 mg $O_2 \cdot l^{-1}$). After period of adaptation (24 h) O_2 concentration was decreased during 15–20 minutes till 0.9–1.2 mg $O_2 \cdot l^{-1}$ (acute hypoxia) by nitrogen pumping. Experimental fish group were exposed to hypoxic condition during 90 minutes. O_2 concentration in sea water was monitored potentiometrically using the oxymeter DO Meter ST300D RU ("Ohaus", USA). Tissues were sampled immediately after fish decapitation and stored at −80°C in a freezing chamber (Farma 900 Series, TermoScientific, USA). The brain samples were divided into medulla oblongata (B1) and forebrain, diencephalon, midbrain (B2). The heart was separated into two chambers – atrium (HA) and ventricle (HV). Activity of cytoplasmic MDH and LDH was measured spectrophotometrically.

Fish brain and heart had the near level of energy metabolism. At the same time, B1, as compared to B2, was characterized by increased MDH activity (40%, $p<0.05$) and relatively low LDH activity (on 21%), which, apparently, could be associated with more intensive energy exchange in B1 to provide continuously basic cardiorespiratory reflexes. Despite the principal HV functional role in providing of heart output and blood pressure, the MDH activity in it only tended to decrease compared to the atrium in the absence of differences in LDH activity. Obviously, the ventricle could be predisposed to anaerobic glycolysis, which increases the reliability of its energy supply under hypoxic conditions.

In this way, the highest values of MDH activity in B1 and HA should be noted compared to B2 and HV. LDH activity was comparable both in all structures of the brain and in the chambers of the heart.

Exposure to acute hypoxia elicited a close similarity in multidirectional changes of oxidoreductases activity in the brain and heart compartments. There were two strategies for energy metabolism reorganization in the form of a decrease in MDH activity in B1 and HA (39 and 68%, $p<0.05$, respectively) and simultaneous rise in LDH activity in B2 and HV (94 and 87%, $p<0.05$, respectively). Dropped MDH activity in B1 could be determined by functional rearrangements of it nuclei function associated with the development of bradycardia providing an optimal perfusion-ventilation ratio in gill apparatus. In turn, increased LDH activity in B2 and HV indicated a transition to anaerobic glycolysis, which ensured the maintenance of its energy supply and functions. In particular, during O_2 starvation fish heart has the ability to enhance the stroke volume noticeably and to maintain cardiac output preferentially due to HV function. Under hypoxia the MDH/LDH ratio in the brain and heart compartments diminished proportionally, indicating whole decrease in ATP production.

Thus, in scorpionfish brain and heart are characterized by similar level of energy metabolism according to manifested oxidoreductases activity. At the same time, with PwO_2 fall brain structures and heart chambers significantly differ in MDH and LDH action. Under acute hypoxia a correspondent rearragement of oxidoreductases activity occures between brain and heart compartments, which have a diverse functional load. It is known that in fish O_2 starvation inhibits the electrical activity of the brain and causes

a decrease in the heart rate (bradycardia). Apparently, two strategies of oxidoreductases hypoxic "behavior" – drop in MDH activity and rise in LDH activity – are closely interrelated with structures that pass into the physiological suppression mode (B1, HA), and that retain its functional role/need in macroergs (HV, B2). Fish as well as mammals are characterized by a close temporal relationship between respiratory activity and cardiac contractions in the form cardiorespiratory coupling and cardiorespiratory synchronization (CRS). The anatomical substrate of CRS manifestation is very close proximity of the cardio- and respiratory centers in the brainstem, including elements of the motor nuclei of the trigeminal, facial, glossopharyngeal, and vagal cranial nerves. Apparently, partially physiological coupling, synchronization and correspondence of brain compartment (B1) and heart chambers manifest itself at the level of energy metabolism. During hypoxia in scorpionfish enhanced anaerobic glycolysis with increased LDH activity support such compartments on which the survival/preservation of organism integrity could depend foremost. Noteworthy is the fact that whole correspondence of the energy metabolism of the brain and heart during normoxia and hypoxia is carried out against the background of a multiple difference in O_2 level in arterial and venous blood.

Some addition to feeding ecology of nudibranch mollusk *Coryphella verrucosa* (Nudibranchia: Coryphellidae)

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Coryphella verrucosa is an amphiboreal species widespread in the North Atlantic and can be found on the coast of North America, where it is usually a common species. In the eastern seas of Russia, it is distributed up to the Sea of Japan, but rather rare. Inhabits rocky ground, in the littoral zone, occurs at depths of up to 15 meters, as well as at depths of up to 70 meters in the Far Eastern seas. *C. verrucosa* forms massive clusters among the hydroid overgrowth of mussel cultures.

According to previously published data, it is known that *C. verrucosa* does not specialize in a particular type of food. The situation may differ in different waters area and biotopes. In the White Sea, this species usually feeds on *Obelia longissima* hydroid polyps, but can also feed on *Clava multicornis* and *Coryne loveni*. In the waters of the British Isles, *C. verrucosa* feeds on the hydroids *Tubularia indivisa*, in New England, it can switch to feeding on the ascidians *Botryllus schlosseri* and the hydroids *Hydractinia echinata*. Cases of attacks on individuals of their own species have been recorded. *C. verrucosa* may also feed on the soft corals *Alcyonium digitaturn*, such as those found on the east coast of North America. On the western coast of Sweden, cases of *C. verrucosa* feeding on the sphingopolips *Aurelia aurita* were recorded, the species was found on the surface of old polyps, from which jellyfish had already emerged, but such cases are episodic and are explained by the fact that the life cycle of these species occurs at different times of the year, but at the same time slightly overlaps.

The study of the composition of fatty acids can be added to experimental methods and the analysis of the contents of the stomach of mollusks. That method has proven itself well in various studies of the food webs of benthic communities. The method of biochemical tracers, such as lipid and fatty acids analysis, provides essential information on marine food webs. Fatty acids have been used as markers to trace predator-prey relationships in the world's ecosystems for more than 40 years.

Fatty acids are an important component of organisms. They are an integral part of triglycerides and phospholipids. Lipid content from bacteria, microalgae, higher plants and marine invertebrates is widely used in food web research. A comparison of the prey and predator fatty acids markers seems to be useful for understanding of mechanisms of metabolic processes. For nudibranchs identified the transfer of fatty acids markers between marine trophic levels in lipidomic studies as well as symbiotic relationships between organisms.

In the present study, the composition and percentage of fatty acids of the nudibranch *C. verrucosa* collected during the 56th scientific expedition of the RV *Academic Oparin*, from Broughton Bay of the Simushir island. From the data it is possible to cancel some of the peculiarities of the content of fatty acids of the species Analysis showed a high content of 16:1n-7, as well as eicosenoic, docosenoic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. The feature of this case is the high content of eicosenoic and docosenoic acids. Studies of the composition and content of fatty acids of sea anemones indicate a high content of these particular fatty acids and, mainly, in storage (neutral) lipids. On this basis, it can be assumed that the diet of *C. verrucosa* in the study area is possibly related to sea anemones. During the collection of material, it was noted that sea anemones are part of the littoral community, which may also indicate their relationship with *C. verrucosa.*

Thus, it can be said that observational data, experimental data and, in addition, analysis of fatty acid content in aggregate can more accurately describe interactions within communities of living organisms. The analysis of fatty acids of *C. verrucos*a indicates possible food interactions of the mollusk with sea anemones, which broadens the range of food of the species and indicates a high adaptive ability in food and the peculiarity of its habitat in the study area.

Techniques and practical experience of cartilaginous fishes training in aquaria

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Specialists who work with sharks and rays in aquaria, should know the biology and ethology of these fishes and must also clearly understand the character traits of each individual. Some elements of their natural behaviour can be stimulated by training animals through a variety of techniques.

Sharks and rays are among the most popular animals in aquaria. Since they are relatively new species for Russia to keep, breed and display in aquaria, any Russian facility having these cartilaginous fishes needs to use new techniques for their maintenance and training. Despite a widespread belief that sharks and rays cannot be taught, many public aquaria have repeatedly demonstrated that it's not true. Moreover, training is necessary for large fishes, including sharks and rays, to ensure their own safety and the safety of personnel. Besides, participation of these animals in shows may be a wonderful marketing strategy attracting lots of visitors to an aquarium.

In the *Moskvarium*, training is conducted for four species: the grey bamboo shark (*Chiloscyllium griseum*), the honeycomb stingray (*Himantura uarnak*), the cowtail stingray (*Pastinachus sephen*), and the bluespotted ribbontail ray (*Taeniura lymma*). These fishes are readily available on the market and are quite often displayed in aquaria.

The major goals of this work were as follows:

1. Teaching cartilaginous fishes a range of behaviours.
2. Creating a new show for visitors based on these behaviours.

To achieve these goals, we focused on:

1. Teaching cartilaginous fishes to react to an acoustic signal (a clicker).
2. Using the effect of tonic immobility.
3. Using targets.

Training techniques for sharks and rays has proven to be successful and applicable for any public aquaria, where these animals are kept. Training of dangerous cartilaginous fishes guarantees more safety for the staff and the animals. Shark and ray shows and feedings may promote education and research, which are the key values for each aquarium.

Changes of the intertidal macrobenthic belt-forming communities in Izmeny Bay (Kunashir Island, South Kurile Islands): a survey of 1963 repeated in 1991 and 2011

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Izmeny Bay is located on the southern coast of Kunashir Island. A sandy beach in Izmeny Bay is protected from a strong wave action. The tides reach 1.8 m in the bay. Species composition of the intertidal macrobenthos is poorer as compared to that in the many other areas of Kunashir Island. In the area of Golovino Village, the intertidal zone was exposed to domestic effluents and effluents from sea food processing, especially in 1963 and 1991. In 1991 and 2011, survey of the intertidal zone was conducted at one of locations studied by Oleg G. Kussakin in 1963 (1.5 km eastward of Golovnino Village, 43.732680° N, 145.542164° E). Macrobenthos is not found in the upper intertidal subzone.

In 1963, most of the middle intertidal subzone was occupied by the belt-forming community dominated by a bivalve mollusk *Ruditapes philippinarum* with biomass of the dominant species 114.0 g wet wt m^{-2}, and a gastropod species *Batillaria cumingii* was the subdominant species (about 70 g wet wt m^{-2}). In 1991, *B. cumingii* became the dominant species of the community (445.6 g wet wt m^{-2}), and biomass of *R. philippinarum* reduced as compared to 1963 (15.6 g wet wt m^{-2}). In 2011, biomass of *B. cumingii* reduced eight times as compared to 1991 (54.6 g wet wt m^{-2}), and *R. philippinarum* became the subdominant species (31.4 g wet wt m^{-2}). Besides, a reduction of species richness took place in this community from 1963 to 1991 (1963 – 11 species, 1991 – 5 species). However, more than 10 macrobenthic species (preliminary data) were found in the *B. cumingii* community in 2011. In 1991, biomass of macrobenthos increased to a third of that observed in the 1963 (329.0 g wet wt m^{-2} and 503.6 g wet wt m^{-2} in 1963 and 1991, respectively), but in 2011, biomass of macrobenthos of the *B. cumingii* community again reduced (121.1 g wet wt m^{-2}).

The lowest part of the middle intertidal subzone and the upper part of the lower intertidal subzones are occupied by the belt-forming community dominated by the eelgrass *Zostera japonica*. Biomass of macrobenthos increased in this community in 1991 as compared to 1963 (1280.5 g wet wt m^{-2} and 1942.1 g wet wt m^{-2} in 1963 and 1991, respectively). In 2011, the biomass of macrobenthos was almost comparable to that reported in 1991 (1957.4 g wet wt m^{-2}). In 1963, 26 macrobenthic species were found

in the *Z. japonica* community, whereas in 1991 there were only 20 species. In 2011, the number of species increased again (at least, not less than 25 species were found in this community).

The *Zostera marina* belt is widespread in the lower part of the lower intertidal subzone. Biomass of macrobenthos increased a little bit in this community (2384.0 g wet wt m^{-2}, 2422.8 g wet wt m^{-2}, and 2439.6 g wet wt m^{-2} in 1963, 1991, and 2011, respectively). In both 1963 and 1991, 15 plant and animal species were found in the *Z. marina* community. However, more than 25 macrobenthic species (preliminary data) were found in the community in 2011. Besides, change of subdominant species took place (*Macoma incongrua* – in 1963 and *R. philippinarum* – in 1991 and 2011) in the *Z. japonica* and *Z. marina* communities.

Over almost 50 years, the quantitative and qualitative changes of the intertidal biota took place in Izmeny Bay. Reduction of species richness and increase of biomass of macrobenthos have been recorded from 1963 to 1991 due to the fact that this area has been exposed to anthropogenic impact. It conforms to the observed earlier tendency on the other South Kurile Islands, when eutrophication of biotopes takes place and increase of the macrobenthic biomass is observed, and at the same time, species richness steadily declines under the effect organic pollutions. However, in 2011, the biomass of macrobenthos did not increase and even reduced in the *B. cumingii* community. In addition, the species richness reached the level of 1963 in the *B. cumingii* community and exceeded probably the 50 years level in the eelgrass communities. The 1994 earthquake did not appear to affect the distribution of the intertidal communities. At present, recovery of the intertidal macrobenthic belt-forming communities apparently occurs in Izmeny Bay.

About the multi-species fishing during the fishery of arabesque greenling in the Primorye subzone from Cape Povorotny till Cape Zolotoy

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The work is based on the materials of research bottom trawl surveys and control trawlings carried out in TINRO scientific expeditions on vessels such as BMRT, BATM, SRTM, RTMS, STM, RS, MRTC and MRS from 1978 till 2020. 115 expeditions were conducted. The total number of trawlings was 3713.

Fishing was carried out by bottom trawl. In order to compare the results of trawling of different vessels with each other, they had to be brought to a unique CPUE (catch per unit of effort). For this purpose, the catches of all trawls were recalculated, first, for an hour of trawling, and secondly, for such a catch as would be obtained under these conditions by a 27.1-meter trawl at a ship speed of 2.7 knots.

Rational organization of specialized fishing of the species, with the implementation of control over associated by-catch species, is possible through the allocation of areas of its fishing accumulations. Such areas in the distribution of a particular species have at least three characteristics. First, there would be concentrated commercial accumulations of this species. Secondly, these are the areas where this species dominates in catches. Because if it does not dominate (the highest catches are not provided), then this is a specialized fishery not of it, but of another species that dominates here; and this species is only a species of by-catch. Third, in the entire aquatory of the areas of commercial accumulations of this species, this species forms a stable share in the catches, not lower than a certain and significant value.

The abundance of each numerous species is characterized by an interannual variability. This is important for the objects of specialized fishing, since their share in catches changes in the years of different abundance, including aquatory of the areas of fishing accumulations. In addition, the proportion of by-catch to the catch of the harvested species depends mathematically on the abundance of the harvested species.

The period of high catches of greenling was recorded by us from 1990 till 1994. Apparently, the previous period of high abundance of greenling ended in 1978, the duration of which was not determined by us, since the surveys had just begun that year. The years of the average abundance of greenling can be considered the period

from 1995 to 2007. The remaining two periods, from 1979 till 1989 and from 2008 till 2020, are generally years of relatively low abundance of greenling.

The average catch of greenling during the periods of high abundance was 375 kg, average abundance – 106 kg and low abundance – 41 kg. The maximum catches of greenling reached: in periods of low abundance – 925 kg, average abundance – 2509 kg, and high abundance – 5135 kg.

Since the catches of greenling vary significantly not only during periods of its different abundance, but also within such periods, it is more correct to analyze the dynamics of catches by years, rather than by periods. Based on the analyzed materials, the years of low abundance of greenling were attributed to those years when its average catch was less than 50 kg. In the years of average abundance, the average catch of greenling varied from 50 to 150 kg, and in the years of high abundance it was more than 150 kg.

Comparison of the average catches of greenling using a two-sample *Z*-test showed that in the years of high and medium abundance of greenling, as well as in the years of its medium and low abundance, these average catches significantly differ, since the calculated Z_p values are significantly higher than the critical one. Based on the reliability of the differences between the variation series of catches in the years of high, medium and low abundance, it is possible to determine the parameters of the areas of commercial accumulations of greenling in each of these categories of years.

In the areas of commercial accumulations of greenling, its stable share in catches in the years of high abundance was 50% of the biomass of fish catches, in the years of medium abundance – 30% and in the years of low abundance – 10%.

The square of areas of commercial accumulations of greenling in the years of its high, medium and low abundance also differed. According to the Rosenbaum criterion, the square of commercial accumulations of greenling was significantly higher in the years of high abundance than in the years of average abundance; and in the years of average abundance than in the years of low abundance. The same thing applied to the average values of the square of greenling's commercial accumulations, according to the Student's criterion. But the variability of deviations from the average value of the square of greenling's commercial accumulations, according to the Fisher's criterion, in the years of different abundance, as a rule, did not significantly differ. That is, in each category of years, the sizes of the areas of greenling's commercial accumulations were equally stable.

In general, during the years of high abundance, the aquatory occupied by commercial accumulations of greenling averaged 21.33% of the total square of the research area. In the years of average abundance, it was on average 9.08%, and in the years of low abundance – 3.84%.

The correlation analysis showed that there was a close direct relationship between the increase in the greenling's abundance and the increase in the size of the area of its fishing accumulations. In this case, the Spearman's correlation coefficient was 0.86, and the Bravet-Pearson's correlation coefficient was 0.71.

After determining the parameters and sizes of the areas of greenling's commercial accumulations, for these units of area, you can determine the share of greenling in catches and the share of by-catch of other fish species (or groups of species), for each category of years that differs in the value of catches.

Share of greenling and by-catch species (groups of species) (% of ichthyomass) on the specialized greenling's fishery (within areas with fishing accumulations), respectively, in the years of low, medium and high abundance of greenling

Greenling's abundance	Ratio of biomass of greenling and by-catch species (groups of species)								
	Greenling	Flounders	Sculpins	Pollock	Skates	Pacific cod	Saffron cod	Herring	Others
Low	30	20	24	11	5	2	2	1	5
Medium	76	8	3	8	1	1	1	1	1
High	84	6	3	2	1	1	1	1	1

Now, based on the Table according to the formula constructed by us, it is possible to determine the quota of each group of by-catch in the proposed fishing of greenling.

$$x = (a \times 100 \div b) \times Q \div 100,$$

where x – the quota of this group of by-catch (tons or kg), a – the share of this group of by-catch, respectively, in year of high, medium or low abundance of greenling (%) according to the Table, b – the share of greenling in the catches also, respectively, in year of high, medium or low its abundance (%) according to the Table, Q – the quota for catch of greenling issued an official permission.

So, in 2022, to the fishery in the northern Primorye (from Cape Povorotny till Cape Zolotoy) it recommended 5.4 thousand tons of greenling. According to the forecast, 2022 is expected to be a year of low abundance of greenling. Using the formula, we can determine the quota of each group of by-catch to 5.4 thousand tons of greenling. We have obtained the following results: flounders are expected to catch 3.6 thousand tons, sculpins – 4.32 thousand tons, pollock – 1.98 thousand tons, skates – 900 tons, pacific cod – 360 tons, saffron cod – 360 tons, herring – 180 tons and others – 900 tons.

Lipid complexes from marine algae as hypolipidemic agents in a high-fat diet

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Seaweed is now widely recognized as a new source of natural biologically active substances and pharmaceutic remedies for the prevention and/or treatment of many diseases. Seaweed is self-sustaining, with a large biomass and highly available for harvesting raw material. The process of separation of biologically active substances is not complicated with using sophisticated equipment. Separated substances possess a pronounced pharmacological activity and usually have low toxicity. Marine hydrobionts synthesize many secondary metabolites with unique structure that are absent in terrestrial plants. This applies to polyphenolic metabolites namely phloroglucinol polymers – phlorotannins, as well as to the lipid structures containing essential polyunsaturated fatty acids (PUFAs) of the n-3 type.

We studied the effect of lipid complexes isolated from the thallus of seaweeds: brown algae – *Saccharina japonica* and *Sargassum pallidum* (Turner) C. Agardh, green alga – *Ulva lactuca* Linnaeus, 1753, red alga – *Ahnfeltia tobuchiensis* (Kannoet Matsubara) on biochemical parameters of blood and liver of rats at an experimental high-fat diet model (hypercholesterol diet with a fat load).

Algae samples were collected in the autumn in coastal waters of Alekseeva Bay, Popov Island, Peter the Great Bay (Sea of Japan). The algae were thoroughly cleaned of epiphytes and sand particles, washed first with sea water, then with tap water, and then squeezed and immersed in boiling water for 2 min to inactivate the enzymes. Further, thallus was dried at temperature below 50°C, after which it was ground using a laboratory mill to a particle size of 0.5–1 mm. Isolation of the lipid complex was carried out by the standard Bligh and Dyer method for the isolation of lipids from plant and animal raw materials. Lipid complexes appeared as a green-brown oily mass. The main fractions were glycolipids, neutral lipids and phospholipids. Triacylglycerols predominated among neutral lipids. Neutral lipids also content the total fraction of diacylglyce rols+monoacylglycerols, free fatty acids, free sterols, and sterol esters. The phospholipid fraction of the lipid complexes of *S. japonica* and *A. tobuchiensis* was characterized by the presence of the following phospholipids: phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidic acid. In the phospholipid fraction of *S. pallidum* and *U. lactuca*, from mentioned above, there was no phosphatidylcholine. In addition, phospholipid fraction from *U. lactuca* contained phosphatidylserine.

The main component of saturated fatty acids was palmitic acid, while monounsaturated fatty acids were represented by palmitoleic and oleic acids. It should be noted that cis-vaccenic (18:1 n-7) acid is present in green algae of the genus *Ulva*, which is its taxonomic feature. Among the polyunsaturated fatty acids, prevailed the representatives of the n-6 type (linoleic and arachidonic fatty acids) and n-3 (α-linolenic, eicosapentaenoic, docosahexaenoic fatty acids), which are part of glycolipids and phospholipids.

In the local and foreign pharmaceutical industry the fat of valuable marine fish species usually serve as a source for manufacturing of non-medicine lipid-lowering remedies,at the same time, seaweed could also be a raw material source for obtaining lipid complexes with hypolipidemic properties.

The experiment was carried out on outbred white male rats kept under standard vivarium conditions and on a standard diet. The development of alimentary dyslipidemia was carried out by feeding the animals with a high-fat diet for 30 days (Formula: common feed 78%, beef fat 29%, cholesterol 2%). The experimental group of animals administered a lipid complex of seaweed at a dose of 1 g/kg of body weight in addition to a high-fat diet. Ten intact animals randomly selected as a blank group and fed common feed. Instead of lipid complex of seaweed blank animals received Vaseline oil in equal amount. The effect of a high-fat diet feeding was accompanied by an increase in plasma levels of total lipids, total cholesterol, low-density lipoproteins, cholesterol/phospholipids ratios and atherogenicity, as well as a decrease in total phospholipids, high-density lipoprotein cholesterol levels, as well as a mismatch in the ratio of liver neutral lipid fractions. These changes confirmed the formation of dyslipidemia and disturbance of the liver lipid metabolism.

The administration of seaweed lipid complexes into a high-fat diet of animals was accompanied by a pronounced tendency to restore the studied biochemical parameters. There was a decrease in total lipids, total cholesterol and an increase in total phospholipids in blood plasma, which led to a decrease in the cholesterol/phospholipid ratio. The level of triacylglycerols and the readings of LDL and HDL did not differ from the control values. The values of LDL cholesterol and HDL cholesterol in blood plasma, as well as the atherogenic index were at the control level. The amount of free fatty acids and cholesterol in the liver decreased, while their esters increased. A decrease of elevated plasma cholesterol levels under the action of n-3 PUFAs is related to an increase of the activity of the enzyme lecithin: cholesterol acyltransferase (LCAT). It is known that n-3 PUFAs inhibit hepatic synthesis of triacylglycerols and increase their excretion, as well as cholesterol in bile and feces. In addition, PUFAs are deposited in the form of cholesterol esters, which is confirmed by an increase of this biochemical indicator in the liver. Thus, metabolic alterations under the influence of lipid complexes of seaweeds contribute to the restoration of the ratio of lipoproteins in direction to an increase in HDL level in blood plasma. Due to the ability of exogenous "sea" lipids to be included in metabolism, one can assume their active influence on most vital processes.

The biological effect of such natural complex set as lipid complexes of seaweed should be considered as the result of the sum of all components of their composition. However, at present, the value of medicines from marine aquatic organisms in most cases mainly is associated with the properties of PUFA n-3. The presence of phospholipids of marine origin with reparative properties in lipid complexes of seaweed, as well as polyunsaturated fatty acids of the n-3 and n-6 families, apparently, determines their high biological activity.

Seaweed can be exploited as a source for the production of drugs with lipid-lowering properties.The use of lipid complexes containing “sea” lipids isolated from seaweed will make it possible to effectively prevent metabolic disorders at high calorie nutrition impact.

Study on the long-term changes of macrozoobenthos in the Yellow Sea and East China Sea

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This study focuses on the long-term changes of macrozoobenthos in the South Yellow Sea and East China Sea, based on the analysis of historic references and field works of the current situation. The results showed, (1) in the Yellow Sea, the differences are significant on the biomasses in different seasons and the community structures among different periods and different areas, in the southern area; in the eastern area the brittle star *Ophiura sarsii vadicola* Djakonov, 1954 is the most obvious species with its abundance increasing, while in the western area five polychaete worm species had obvious abundance increasing; the environmental factors such as depth, temperature and salinity can only partially explain the variation of macrobenthic communities, and the PDO and SOI are two significant climate indices relative with the long-term changes of the macrobenthos, the long-term changes of macrozoobenthos were mainly affected by human activities; the nearshore species gradually became small and the community structure became simple, while the area off coast with species remained stable; (2) in the East China Sea, there were no significant regional differences in the macrobenthic communities; the nearshore communities were positively in spring and negative in summer correlated with the contents of total nitrogen and total organic carbon; the area off the coast with the communities were positively in both spring and summer correlated with the contents of total nitrogen and total organic carbon; a bottom branch of the Kuroshio Current invaded the East China Sea from the northeastern Taiwan Island significantly affected the macrobenthic community structure, although the invasion started only from about 2000, the diversity and distribution of the bottom fishes and the influence of the bottom branch of the Kuroshio indicate that the distribution of macrobenthic communities in the sea is closely related to the invasion of the Kuroshio, it is suggested that the Kuroshio sub-community had been formed in the coatal area.

Molecular analyses of the gill symbiosis of the bathymodiolin mussel *Gigantidas platifrons*

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Animal-microbe symbioses have played fundamental roles in the animals' adaptation, ecology, and evolution. To maintain the stability and efficiency of the symbiosis, the host must be able to regulate and interact with the symbiont at the tissue, cell, and molecular levels. Although the deep-sea bathymodiolin mussels have been intensively studied as a model of animal-bacteria symbiosis, it remains challenging to assess the host-symbiont interactions due to the complexity of the symbiotic tissue – the gill. Using cold-seep mussel *Gigantidas platifrons* as a model, we isolated the symbiont harboring bacteriocytes and profiled the transcriptomes of the three major parts of the symbiosis – the gill, the bacteriocyte, and the symbiont. This breakdown of the complex symbiotic tissue allowed us to characterize the host-symbiont interactions further. Our data showed that the gill's non-symbiotic parts play crucial roles in maintaining and protecting the symbiosis; the bacteriocytes supply the symbiont with metabolites, control symbiont population, and shelter the symbiont from phage infection; the symbiont dedicates to the methane oxidation and energy production. This study demonstrates that the bathymodiolin symbiosis interacts at the tissue, cellular, and molecular level, maintaining high efficiency and harmonic chemosynthetic micro niche.

Macrobenthos structure in the Kara Sea and Laptev Sea (based on materials from expeditions in 2012–2014)

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Since the 1990s, interest to the Arctic seas ecosystems has increased due to the growth of anthropogenic impact – the intensification of oil and gas field development in the shelf zone. Such activities are impossible without environmental monitoring. Its traditional object is marine benthos, which rather slowly reacts to changes in the environment. In 2012–2014 one of the most detailed for the entire observation period hydrobiological studies were undertaken in the southwestern Kara Sea and Laptev Sea. A significant part of these seas was covered with a grid of stations. As a result, it became possible to characterize the benthos structure in this area in the most complete way and to compare the results obtained with the results of previous studies.

Sampling was carried out from the R/V "Dalnie Zelentsy" at 119 (depths 4–415 m) and 84 stations (depths 9.8–345 m) in the SW Kara Sea and Laptev Sea respectively in 2012–2014. Samples were taken with a 0.1 m^2 van Veen grab (3 replicates). All animals were identified to the species, counted and weighed (up to 0.001 g). The environmental data including the sediment granulometry, near-bottom temperature, salinity, oxygen concentration and pH were determined at each station (3 replicates) during the same surveys. The granulometric analysis was performed using the standard procedure.

We used respiration rate R_i for every taxon to analyze the distribution of macrobenthos:

$$R_i = A_i B^{0.75} N^{0.25},$$

where B_i and N_i are biomass (g wet wt m^{-2}) and abundance (ind. m^{-2}) of the *i*-th species and A_i is the taxon-specific coefficient of respiration intensity.

The similarity of the stations in respect of biotic characteristics was assessed using a cluster analysis and multi-dimensional scaling. The results of cluster analysis were subjected to the SIMPROF procedure. The significance of differences between cluster characteristics was verified by ANOSIM analysis. The species diversity in each cluster was estimated using the Shannon index and the Hurlbert rarefaction index for 100 individuals (*ES* (100)). Evenness of species diversity inside clusters was calculated using the Pielou index.

Mantel test was applied to estimate the correlation between the distribution of environmental parameters and macrobenthos distribution. To identify abiotic characteristics that best explain the distribution of macrobenthos in the analyzed region, the BEST procedure was employed.

To assess macrobenthos ecological quality in distinguished clusters AZTI Marine Biotic Index (AMBI) and its multivariate extension, M-AMBI were applied.

The data obtained allows to speak of the absence of serious changes in the structure of macrobenthos in the studied areas of the Kara and Laptev Seas at least over the past several decades. Taxonomic diversity, ranges of fluctuations of quantitative indicators, taxonomic groups of macrobenthos most significant in terms of quantitative characteristics turned out to be very similar to the data of previous studies. In total, we registered 425 and 473 macrobenthic taxa in the studied areas of the Kara Sea and Laptev Sea respectively. Most of the macrobenthos species in the Kara and Laptev Seas belonged to polychaetes (27 and 28%, respectively), crustaceans (22 and 27.5%, respectively) and mollusks (19 and 18% respectively). The total biomass of benthos in the studied areas of the Kara and Laptev Seas in 2012–2014 varied from 3 to 512 g/m^2 and from 2.8 to 353 g/m^2, respectively. The dominant taxonomic groups of macrobenthos were bivalves, polychaetes, and echinodermates in the Kara Sea and bivalves, echinodermates and crustaceans in the Laptev Sea (by biomass), polychaetes and bivalves in both areas (by abundance).

11 benthic associations were identified within the considered areas in the Kara and Laptev Seas. These associations, apparently, did not always correspond to benthic communities (in a traditional sense). In several cases, they probably combined several benthic communities with a similar structure, but different species dominating by biomass. The characteristics of most clusters (spatial position, species dominant by biomass, typical species, total density and biomass) had obvious similarities with biosystems described earlier. In this regard, the deep-sea benthic association (depths mostly over 150 m) in the Kara Sea should be noted, in which the echinodermates *Ophiopleura borealis*, *Molpadia borealis*, *Elpidia glacialis* and small bivalves *Yoldiella* sp. occupied an essential position. The communities of these taxa are represented on the maps of almost all previous works. A similar situation was observed with regard to associations in which the main role was played by bivalve mollusks (mainly at depths less than 100 m) – *Portlandia aestuariorum*, *Portlandia arctica*, *Serripes groenlandicus*, *Astarte* sp.

The benthic associations described in the work differed substantially in species richness. At the same time, with a few exceptions, variations in the indices of species diversity were less pronounced and quite logically reflected changes in the structure of bottom communities on different sediments and along the salinity gradient, particularly in the Kara Sea, where material was collected also in the estuarine areas. Relatively low values of Shannon and Pielou indices (0.83 and 0.24, respectively) were obtained for the *Portlandia aestuariorum+Cyrtodaria kurriana* community located in

the low salinity area, while in the rest of the communities the Shannon index varied from 1.94 to 4.11 (excluding the oligomix community of cumaceans). The highest values of the Shannon index (4 and above) were obtained for associations located on mixed sandy-silty soils in the Kara Sea and silty-sandy soils in the Laptev Sea at depths less than 100 m and 60 m, respectively.

Spatial arrangement of most of the identified benthic associations showed certain zoning associated with salinity conditions, characteristics of bottom sediments and depth. We identified these abiotic factors as the most significant (using BEST procedure) for heterogeneity of macrobenthos distribution in the study area, and so they were recognized in previous studies also.

Although the AMBI index assessed the ecological quality of most identified benthic associations as "undisturbed", and the M-AMBI index assigned the ecological status "Good" and "High", some calibration of the AMBI index when employed in Arctic waters may not be superfluous. First of all, this concerns the clarification of ecological status of some widespread species and correction of the ecological group assigned to it. In general, the experience of using AMBI indices for detecting environmental impacts in Arctic marine ecosystems in this work is assessed as quite successful. The obtained results can be very promising for tracking future changes in the ecosystem of the Kara and Laptev Seas.

Thus, the conducted studies demonstrated no serious changes in the macrobenthos structure in the Kara and Laptev Seas at the beginning of the 21st century. Despite the differences in sampling methods and data processing, the main trends, noted in the macrobenthic distribution in 2012–2014, were similar to those noted before. The identified abiotic factors most significant for distribution of benthic organisms – salinity, depth and sediment characteristics, were the same as reported in previous researches. Apparently, the state of macrobenthos in the study areas of the Seas at the beginning of the 21st century is close to the long-term norm.

Alexey V. Zhirmunsky as a malacologist

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"Academician Zhirmunsky devoted his life to marine biology research, building international cooperation … founding the Institute of Marine Biology in the Far East, and was truly an ***Academician of The Sea***.".

Li-chun (Chu) Wu, a retiree
Los Altos, California, USA
(*co-author of A.V. Zhirmunsky in the 1960s*)

This year, 2021, marks the 100th anniversary of the birth of Prof. Alexey Viktorovich Zhirmunsky (October 15, 1921 – October 20, 2000), member of the USSR/Russian Academy of Sciences (Academician), founder and first Director of the Institute of Marine Biology in Vladivostok, an outstanding biologist, physiologist, ecologist and organizer of the Far Eastern Marine Biosphere Reserve, *Soviet Journal of Marine Biology* and the Marine Biological Station *Vostok*. The Institute, re-organized in 2016 as the National Scientific Center of Marine Biology, was established in 1970 (and its predecessor, Department of Marine Biology within the USSR Academy of Sciences, in 1967) and celebrated the 50th anniversary in 2020. The IMB/NSCMB became home for various activities including conferences, science societies, and informal seminars in the 1970s–1990s as well as international meetings, especially since the 1990s, and despite Prof. A.V. Zhirmunsky stepped down as Director of the institute since 1988, he was very active in these events and gatherings. I was especially lucky to travel with him and two other colleagues to my very first international meeting in Beijing, China in 1994, to participate in a global change project, and to get his support in the organization of the Institute Museum in the 1990s but this time, I would like to emphasize his role as malacologist and one of the founder of the Russian Far East Malacological Society in 1994.

A.V. Zhirmunsky was specialist in physiological ecology, and a number of his early papers were based on studies of marine invertebrates and mollusks. In a series of papers published in the 1950s–1970s, he presented results on interrelations between the vertical distribution of some common species of the bottom animals living in the upper subtidal zone of the Chinese seas, White Sea, Black Sea and the Sea of Japan in dependence on temperature conditions, substrate and animal mobility; these data were compared with

cell heat resistance (the thermostability), which may be used as a species characteristic (e.g., *Zhirmunsky A.V.* 1960. Studies of the temperature adaptations in invertebrates of the South China Sea. *Cytology*, v. 2, p. 675–691; *Zhirmunsky A.V.* 1969. A comparative study of cellular thermostability in mollusks of the White Sea in relation to the vertical distribution of species and history of fauna formation. *Journal of General Biology*, v. 30, p. 685–702; *Zhirmunsky A.V.* 1973. Vertical distribution and cellular heat resistance of bottom animals from the Possyet Bay (Japan Sea). *Helgoländer Wissenschaftliche Meeresuntersuchungen*, Bd. 24, S. 247–255). In the latter paper, he considered 15 species of bottom animals including gastropods (2 species) and bivalve mollusks (9 species).

A.V. Zhirminsky spent four months in 1959–1960 in expeditions to Hainan and Qingdao (China) during the period of intensive Russian-Chinese marine biological researches. He published results of these expeditions in several papers in the Russian and Chinese languages on the physiological ecology of both gastropod and bivalve mollusks jointly with Chinese specialists (e.g., *Zhirmunsky A.V., Chu Li-Chun*. 1960. Thermal stability of ciliated epithelium in the sympatric tropical mollusks of the genus *Nerita* in relation to temperature conditions of their habitat. *Cytology*, v. 2, p. 478–482; *Zhirmunsky A.V., Chu Li-Chun*. 1963. The cell thermostability of sympatric species of *Donax* in relation to the temperature conditions of their habitat. *Acta Zoologica Sinica*, v. 15, p. 21–27).

Mollusks have always been the most important object of various research in the IMB including such aspects as ecology, physiology, genetics, biochemistry, reproduction, etc. A.V. Zhirmunsky supported all these studies, and initiated several projects, publication of comprehensive monographs and several conferences as well as invited to the IMB many leading figures in these fields. In the 1970s, Vladimir L. Kasyanov organized the Laboratory of Embriology and specialized in reproductive biology of bivalves and echinoderms; Alexander I. Kafanov and Vladimir V. Gulbin were taxonomists contrubuted to biogeography, paleontology and faunistic studies of bivalves and gastropods; Laboratory of Invertebrate Cultivation, Laboratory of Benthic Ecology, Laboratory of Genetics and Laboratory of Shelf Ecology contributed much to ecological, genetic and aquaculture aspects and molluscan biofouling. Zhirmunsky paid special attention to biology and cultivation of the Pacific mussel *Mytilus trossulus* and co-authored several papers, and prepared a special volume of the *Soviet Journal of Marine Biology/Biologiya Morya* (introductory paper: *Zhirmunsky A.V.* 1986. Problems of biology and cultivation of the mussel *Mytilus edulis*. *Biologiya Morya*, no. 4, p. 3–6). In fact, his first paper on ecological physiology of mussels was published in 1960 and dealt with reaction of the Black Sea mussels to heat (*Zhirmunsky A.V.* 1960. Sensitivness of the Black Sea mussels and their ciliated epithelium to effect of elevated temperature. *Doklady (Reports) of the USSR Academy of Sciences*, v. 133, p. 683–685). In 1979, he presented a talk about studies of bivalve mollusks in the IMB at the all-Soviet meeting on mollusks in Leningrad (St. Petersburg) (abstract was published in English later in

the US journal: *Zhirmunsky A.V.* 1984. Studies of bivalve mollusks at the Institute of Marine Biology. *Malacological Review*, v. 17, p. 126), and he took part in these research during the 1970s–1980s and co-authored a number of papers on ecology of biofouling communities dominated by mollusks, mollusk' aquaculture, mollusk' growth, cell thermostability (e.g., *Zhirmunsky A.V., Krasnov E.V., Zolotarev V.N.* 1977. Studies of growth temperatures of scallops estimated by isotope method. S. Motoda (ed.). *Proceedings of the Second Soviet-Japan Joint Symposium on Aquaculture, November 1973, Moscow, USSR*. Tokyo: Tokai University. P. 151–163). In 1980, he co-authored a paper on first finding of the abalone *Haliotis discus* in Russian Far Eastern unique Moneron Island in *Priroda* journal (Nature).

A significant chapter on mollusks was included in the identification book *Animals and Plants of Peter the Great Bay* (1976) published under editorship and by initiative of A.V. Zhirmunsky; it was first book of this kind published by the IMB and it is still in use by amateurs and zoologists. His special attention to conservation of nature and natural environments led to organization of the first marine reserve in Russia, Far Eastern Marine Biosphere Reserve which is the richest area in terms of biodiversity of mollusks. Finally, A.V. Zhirmunsky fully supported founding of the Russian Far East Malacological Society in Vladivostok (1994), and made many efforts to establish the journal of the society, the *Bulletin of the RFEMS* (first published in 1996), in that difficult financial situation of transformation to a market economy of the 1990s. So, he is a co-founder of the society, along with Prof. A.I. Kafanov. I would like to merely mention that he could care even about this small society while his many projects were much more grand and massive. In addition, the Institute Museum (IMB) founded in 1994 received much support from A.V. Zhirmunsky, and the molluscan collection is a major part of this museum. In my archive, there is a written note from Zhirmunsky suggesting to make a separate malacological library of the RFEMS/IMB Library to promote research and assist with bibliographic search.

The real value of great intellectuals is not just the value of their scientific contribution but the impact. Alexey Viktorovich was inspiring for many, and his role in science and development of research organizations and other enterprises/facilities/initiatives was huge and amazing. We are lucky to have malacology among numerous fields he impacted and promoted.

Ecology of the starfish *Asterias amurensis* in Peter the Great Bay (Sea of Japan)

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The Northern Pacific starfish *Asterias amurensis* Lütken, 1871 is indigenous to the coastal waters of Russia, Japan, Korea and Northern China. In the 20th century, it was brought via ballast water to the southern coast of Australia, as well as to Alaska and the Aleutian Islands, Europe and the American state of Maine (Shah, Surati, 2013). As a result, study of the ecology of the starfish *A. amurensis* in Peter the Great Bay, in the center of its natural range, is of particular interest in order to predict the further spread of this invader to new habitats.

This study was carried out in two areas of Peter the Great Bay (Sea of Japan): a southwestern region extending from the southern border of the Southern Part of the Far Eastern Marine Reserve to the northern border of Baklan Bay, including Possjet Bay, and an eastern region extending from Putyatin Island to Cape Likhachev, including Vostok Bay. ROVs Sub Fighter 3000 and Mirage were used. Video recording was carried out on 222 transects with a total length of 38,596 m at depths from 2.0 to 80.7 m in the southwestern region and on 68 transects with a length of 11,994 m at depths from 2.1 to 67 m in the eastern region. 2310 video samples were analyzed totally, including 1722 video samples from a total area of 25,107 m^2 in the southwestern region and 588 video samples from a total area of 10,135 m^2 in the eastern region. The surveyed water area was 1,167.7 km^2 in the southwestern region and 441.3 km^2 in the eastern one.

In the samples, we counted the number of starfish *A. amurensis* and measured the maximum distance between the ends of the rays. The types of bottom substrates such as silt, silty sand, sand, shell rock, and hard substrates that include all fractions larger than sand were visually determined for each video sample.

The starfish *A. amurensis* was encountered in the depth range from 3.5 m to 70.8 m in the southwestern region, and in the depth range of 4.1–50.0 m in the eastern region. The average occurrence of the starfish in the southwestern region is highest on shell rock and silts (67.4 and 64.3% respectively), comparable on silty sand and hard substrates (47.6 and 45.3%) and lowest on sand, 23.4%. At depths below 70 m, its occurrence decreases to 10.0%; below 80 m, *A. amurensis* was not found.

The recorded average density of *A. amurensis* aggregations in the southwestern region is minimal at depths of up to 10 m (0.030±0.006 ind/m^2), increases with depth to maximum in the depth range of 40–50 m (0.468±0.109 ind/m^2), then decreases to

0 at depths of more than 80 m. When considering the relationship of starfish aggregation density and substrate type, the average density was minimal on sand, and the maximum was recorded on shell rock, 1.652±1.026 ind/m^2. Its values were 0.388±0.039 ind/m^2 on silt, 0.709±0.285 ind/m^2 on silty sand, 0.365±0.179 ind/m^2 on hard substrates. The maximum average densities of aggregations on hard substrates, shell rock and silty sand are observed in the depth range of 40–50 m.

The average population density of the starfish *A. amurensis* in the whole southwestern region is estimated at 0.220 ind/m^2. At depths of up to 30 m, where research is usually carried out using SCUBA, a little more than a quarter of the population lives, and the average population density is estimated at 0.150 ind/m^2. The main part of the population (49.1%) occurs in the depth range of 30–50 m with an average density of 0.336 ind/m^2. In the eastern region, the average density of aggregations does not exceed 0.004–0.006 ind/m^2 and decreases to 0 at depths greater than 50 m. When considering the relationship to substrate type, the maximum density of aggregations was recorded on silts (0.030±0.012 ind/m^2); and minimal, on sand (0 ind/m^2). The average population density of *A. amurensis* in the entire eastern region is estimated at 0.00044 ind/m^2, i.e. lower by more than two orders of magnitude than in the southwestern region.

The difference in the starfish *A. amurensis* aggregation density between the two regions rapidly increases with depth. At depths of less than 20 m, the average density of aggregations is 70 times higher in the southwestern region; at depths of 20–30 m it is higher by more than three orders of magnitude; at depths of 30–50 m the difference is 2 times higher than at depths of 20–30 m, and at depths of more than 50 m in the eastern region, *A. amurensis* was not found.

The size of the starfish in the population ranges from 1.6 to 62.5 cm. The average size of *A. amurensis* is minimal at depths less than 10 m (9.6±1.6 cm) due to the large proportion of juveniles; it increases with depth, reaching maximum at depths of 40–50 m (24.5±0.5 cm), then begins to decrease with increasing depth and at depths of 70–80 m is 14.4±1.1 cm. The distribution of the biomass of *A. amurensis* in the southwestern region depends on two factors: an increase in the average aggregation density at depths of more than 40 m and an increase in the average size of individuals at these depths. As a result, in the southwestern region, the maximum average biomass of the starfish at depths of more than 40 m reaches 200 g/m^2 in the eastern part of Possjet Bay and around Cape Gamov, and it exceeds 500 g/m^2 off Astafiev and Srednaya bays.

In the current study density of starfish aggregations sharply decrease at great depths in the northeastern direction, from Possjet Bay to Vostok Bay in Peter the Great Bay. This is consistent with the findings of other authors, who noted that in 1934 this starfish was not recorded in Kievka Bay, which is located 80 km northeast of Vostok Bay (Proceedings of the Hydrobiological Expedition of the Zoological Institute of the USSR Academy of Sciences, 1934), while in 2001–2004 it was recorded there only at depths of up to 7 m (Galysheva and Pustovalova, 2009). In Kit Bay, which is located 50 km northeast of Kievka Bay, *A. amurensis* was not encountered during 4 years of studies (Gabaev, 2018).

Detritus plays a significant role in the diet of *A. amurensis* (Yamamoto, 1967); it occurs in 70–80% of individuals and occupies up to 30–40% of the food bolus volume (Tsikhon-Lukanina and Nikolaeva, 2007). Fatty acid markers suggest that the food sources of starfish in the upper trophic levels of the family *Asteriidae* are more diverse (Latyshev et al., 2001), and depending on the availability of food, they can include in the diet both a variety of animal food, including detritus feeders, and detritus and epibiont film with associated microorganisms. It can be assumed that detritus is the main source of food for *A. amurensis* outside of accumulations of mollusks in natural ecosystems. This explains the high densities of this species in Possjet Bay at isobaths with a predominance of silt and silty sand, where the starfish do not experience a shortage of food resource even at their high biomass comparable to the total biomass of benthos in these areas (Klimova, 1980; Ozolinsh, 2004).

Cortically localized maternal messenger RNAs in sea urchin unfertilized eggs, a transcriptomic approach

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Maternally accumulated mRNAs and proteins play different roles in early development of animals, cell cycle progression, cell architecture maintenance, metabolism and patterning. In some animals, mRNAs required for early development, especially for embryo patterning, are may be localized in oocyte particular regions. Later on, these localized mRNAs participate in specification of particular regions in embryos. In sea urchin eggs, vegetal region is determinated by maternal factors, from which Dishevelled protein has only been known to be localized in vegetal pole of unfertilized eggs. We suggest that determination of vegetal region of sea urchin eggs and specification of other embryonic regions should require some or many localized maternal factors both mRNAs and proteins. The goal of this study is analysis of localized maternal mRNAs that are potentially necessary for early sea urchin development. To detect localized transcripts, we performed transcriptomic analysis of unfertilized eggs and their isolated cortical layers. We detected a pool of cortically-enriched transcripts. Using gene ontology analysis, we found 62 gene probes corresponded to 27 unique genes. Further analysis showed that among 17 found terms the most prevalent 'Cellular components' category is nucleic acid binding (20 genes). The most prevalent 'Biological processes' categories are DNA metabolic process (7 genes) and nucleic acid metabolic process (9 genes). We suppose that found cortically-associated transcripts coding nucleic acid binding proteins may be necessary for cell specification in early sea urchin development.

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Intrabody TTX distribution and possible way of its migration in ribbon worms *Cephalothrix* cf. *simula* (Palaeonemertea: Nemertea)

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The data of tetrodotoxin and its analogs (TTXs) distribution in highly toxic animals is of great interest due to its contribution to the understanding of entering ways of the toxin in animal's body, toxin migration and accumulation in tissues and cells, and its functions. In 2004 Tanu with colleagues (Tanu et al., 2004) investigated TTX's intrabody distribution in nemerteans on undescribed species of the *Cephalothrix* genus for the first time. They studied the toxin's intrabody distribution only in the foregut region and described only TTX-positive cells, not defining all cell types of TTX-positive tissues and organs. In the current work, four regions of toxic *Cephalothrix* cf. *simula* were studied: (1) the precerebral region, (2) the foregut region, (3) the middle and (4) the posterior intestine regions. At the light-optical level, all cell types of TTX-positive tissues (namely cephalic gland, integument, intestinal epithelium, glandular epithelium of proboscis, blood and nephridial systems) were described. Using confocal laser scanning microscopy with anti-TTX antibodies and cryosections, cell types accumulating the toxins were identified. In the current study, we obtained new data on toxin localization. An intense label was found in the glandular epithelium of the proboscis, protonephridia, epidermis, and intestinal epithelium. The medium intensity label was detected in the cephalic glands, lateral nerve, and oocytes. Weak TTX-like immunoreactivity was observed in the musculature of the body wall and proboscis and the endothelium of blood vessels. According to the data obtained, TTX in *C*. cf. *simula* enters the intestine with food.

Absorption of the main amount of TTX occurs in the foregut, where free toxin enters the intestinal cells. The structural features of the circulatory system allow the toxin to migrate mainly to the organs of the anterior part of the worm, in particular to the glandular systems producing epidermal mucus, effectively protecting nemerteans from predators. At the same time, cells accumulating TTX contribute to toxicity in different ways: some types of cells can maintain a constant concentration of toxin in the epidermal mucus, releasing granular secretions in one granule, while others can rapidly secrete large amounts of TTX-containing serous mucous secretions in response to stress. In the current study, we revealed TTX-like compounds in granules of bacillary cells contained in the glandular epithelium of proboscis.

Bacillary cells associated in pairs with pseudocnid-containing cells form a glandular system taking part in prey retention. That is, the "sticky" component, which is part of the secretion of bacillary cells, can enhance the adhesion of pseudocnidae to the surface of the victim's body, while the toxic component has an immobilizing effect on the victim.

Toxicity and immunogenicity investigation of Tick-borne encephalitis virus chimeric antigen incorporated in immunostimulating complexes based on triterpene glycosides from *Cucumaria japonica*

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Tick-borne encephalitis is a dangerous feral nidal zoonotic infection that is widespread on the territory of the Russian Federation. More than 80 years have passed since the discovery of the tick-borne encephalitis virus, but this dangerous disease has not yet eliminated and currently poses a serious challenge to public health. Vaccination – is the most effective way to cure tick-borne encephalitis at preset time.

All vaccines used for vaccination against tick-borne encephalitis in Russia and Europe is a whole-virion type, and contain aluminum hydroxide as adjuvant. Fabrication whole-virion vaccines is associated with the production and processing of a large amount of dangerous viral material. It makes the vaccine production process are complex and expensive. At the same time, the use of commercial available whole-virion vaccines allows achieving a high protection degree only in 53% of vaccinated people, even if triple immunization performed. In addition, approximately 30% of cases have adverse reactions to the vaccine. Thus, the development of a new safety vaccine against tick-borne encephalitis with high effectiveness and a safe production process is an important goal.

To the develop anti-encephalitis vaccine of new generation, we applied an subunit antigens-based approach. Utilization of highly purified subunit protective antigens, instead of the whole virus, allow to increase the safety and effectiveness of vaccines. Most of such antigens are proteins and can be obtained using a recombinant strategy. Unlike analogues isolated from a native virus, recombinant proteins have a significant advantage in terms of safety and cost of production. On the other hand, the use of purposefully designed recombinant chimeric antigens with specified properties for vaccination is a new promising approach to improving the effectiveness of subunit vaccines (Sanina et al., 2017). At the moment, there are no subunit vaccines for tick-borne encephalitis.

In order to develop a subunit vaccine against tick-borne encephalitis, we obtained a recombinant chimeric protein (Golotin, 2018) consisting of the III domain of the tick-borne encephalitis virus protein E and the HSP70 protein from *Yersinia pseudotuberculosis* (EIII-HSP70). To construct the chimeric protein, a fragment of the c-DNA of

the tick-borne encephalitis virus, the strain "Dalnegorsk" with a length of 450 bp and the gene of the protein HSP70 from *Y. pseudotuberculosis*, strain 488, with a length of 2100 bp were used.

At the first stage, we perform studies of the toxicity of the obtained antigen. It was found that the protein EIII-HSP70 has an extremely low acute toxicity – its lethal dose significantly exceeds 1 mg per mouse. It was shown that the subacute toxicity of the EIII-HSP70 protein against laboratory mice is manifested at doses of 150 mcg per mouse and higher, and is expressed in the cessation of weight gain by growing animals. The EIII-HSP70 antigen did not have pyrogenic and hemolytic activity at doses up to 1 mg per mouse and 2.4 mg/ml, respectively. Thus, the obtained chimeric antigen demonstrated high safety for laboratory mice.

At the next stage of research, the chimeric antigen EIII-HSP70 was introduced into immunostimulating complexes and its immunogenicity and protective activity were investigated. The need for the introduction of the chimeric antigen obtained by us was dictated by the fact that subunit antigens, have weak immunogenicity at common. Therefore, their practical application in a vaccines requires the introduction of an adjuvant. One of the most effective adjuvants currently used are immunostimulating complexes (ISCOM). Immunization with an antigen in the composition of ISCOM increases the formation of specific antibodies tenfold and allows inducing reactions of cellular and humoral immunity. The main drawback of ISCOM is toxicity due to the presence of Quil A saponins that destroy cell membranes (Kersten, Crommelin, 1995). Earlier, we carried out work on optimizing the composition of classical ISCOMs in order to reduce their toxicity. In the course of these works, we obtained a new type of immunostimulating complex – a tubular immunostimulating complex (TI-complex). The composition of classical ISCOM was modified by replacing QuilA saponins with high hemolytic activity with the triterepene glycoside cucumarioside A2-2 from the holothuria *Cucumaria japonica*, the toxicity of which completely disappears with the addition of cholesterol. The immunologically inactive phospholipids in the ISCOM were replaced by monohalactosyldiacylglycerol isolated from various marine macrophytes of the Sea of Japan, which has a wide range of biological activity. The obtained TI-complex have structure of tubular nanoparticles, which fundamentally differed from classical immunostimulating complexes, whose particles have a vesicular structure (Kostetsky et al., 2011).

Determination of specific antibodies level in the serum of mice immunized with the chimeric antigen EIII-HSP70 showed that even a dose of 1 mcg per mouse it leads to the effective formation of specific antibodies directed against the whole antigen. However, for the effective formation of specific antibodies directed against domain III of the tick-borne encephalitis virus E-protein, immunization with a chimeric antigen at a dose of at least 20 micrograms per mouse was necessary. Immunization with a chimeric antigen as part of the TI-complex allowed to increase the number of antibodies against the whole antigen by 1.4-5.5 times. The greatest adjuvant effect on the formation of antibodies against the whole antigen was observed when using a TI-complex based on

MGDH from *Sargassum pallidum*. An adjuvant effect on the formation of antibodies against the E-protein domain III of the tick-borne encephalitis virus was observed only when using a TI-complex based on MGDH from *Ulva lactuca*.

At the final stage, we determined the protective activity of the chimeric antigen in the model infection of immunized mice with tick-borne encephalitis at a dose of 10 LD50. It was shown that immunization with the EIII-HSP70 protein at a dose of 20 micrograms per mouse can achieve a 20% protective effect. However, the introduction of the chimeric protein EIII-HSP70 into the composition of the TI-complex did not lead to an increase in its protective activity.

Training of *Phoca largha* and *Delphinapterus leucas* in the Primorsky Aquarium

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The investigation of marine mammals is important area of activity of the Shared Equipment Facility "Primorsky Aquarium". These animals are of scientific interest and applied value for evaluation prospects for use in various area of human activity. Due to the habits of marine mammals it is rather difficult to study them. However, in the conditions in captivity a wide range of studies can be performed. For example, it is possible to carry out round-the-clock ethological observations and long-term veterinary research, including the study of behavior, physiology, biochemistry and histology. Additionally, it makes possible to observe seals (in aquatic and terrestrial environments) and beluga whales in aquatic environments taking a video and audio recording of behavioral elements.

Phoca largha, a resident species the Sea of Japan, and a beluga whale (*Delphinapterus leucas*) were selected as model species for the further research. The open-air complex for keeping seals and beluga whales was organized on the basis of the Shared Equipment Facility "Primorsky Aquarium" in Paris Bay, Primorsky region, Russky Island. Animals are kept in their natural environment within the fenced area.

Training of 5 seals (2 females and 3 males) for the research began in April 2015, at the age of ~1.5 months. A habituation to a diet, training reflexes to the name, conditioned signals, command gestures and voice were performed during the current study. Moreover, one of the female seals gave birth and reared the pup independently. The pup had natural stages of molting and hunger.

Training of 4 beluga whales (females) for the research purposes began in January 2020. Currently, these animals have developed skills that are needed for an experimental work. Animals perform exercises in open-air complex, delimited by a conditional partition on the surface of a water pool. Studies the lateral behavior in terms of side preference (i.e., left, right) were carried out with beluga whales.

The training of animals is carried out in three parallel processes, which are 1) adaptation to housing conditions, 2) nutritional adaptation, and 3) educational training. The feeding adaptation for seals and beluga whales was developed to be as close to natural diet as possible. Diet adjustments were created considering the seasonal and age characteristics of the animals and also took into account the analysis of physical condition, activity, food motivation. At the same time, the weight and composition of the feed, the amount and availability of calories and the diet were variable and could be modified. For this, the behavior of seals and beluga whales was monitored daily and their condition was assessed by biological sample collection, weighing (for seals).

Further training of animals will be performed. This will include strengthening already acquired skills, socialization, improving of stress resistance, developing special forms of behavior for conducting experiments in various biotechnical systems, studying of cognitive abilities, as well as skills necessary for the applied use in various area of human activity of seals and beluga whales.

Biodiversity in coastal marine and deep-sea habitats associations of free-living nematodes on the intertidal zone of Vietnam

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The paper summarizes the results of meiobenthic studies on the intertidal zone of Vietnam along the coastline from the North to the South. The main locations of our studies were the Red river estuary, Mekong delta, Nha Trang Bay, Islands of Con Dao and Phu Quok.

The studies include quantitative estimation of meiobenthic groups in different localities, seasonal changes and long-term succession, small-scale spatial structure with special reference to free-living nematodes. In terms of taxonomy and diversity, nematodes of Vietnam are one of the best investigated groups of meiofauna. Great investigation effort of many taxonomists was reviewed by V.G. Gagarin and V.A. Gusakov in 2017 and 2018. Two lists of 127 and 208 species names make the diversity of this group on the coasts and estuaries of Vietnam pretty well described to be used as a model for further ecological studies. The number of meiobenthic studies was undertaken along the coast of Vietnam by several groups of researches. Quantitative data on meiobenthos of different sites were published; taxonomic composition of nematodes and harpacticoid copepods were described in a number of habitats.

Our own studies in Vietnam are conducted since 2007 and are focused on the structure and dynamics of the communities of free-living nematodes. In these studies nematodes' assemblages were described in mangrove communities of different species composition and on non-mangrove intertidal flats of fine and coarse sands. The number of hypothesis were formulated and tested on this material: does local diversity of nematodes in tropical zone exceeds the same of higher latitudes; how different species of mangrove trees structure the nematodes' assemblages; are there prominent patterns in taxonomic composition and diversity of nematodes along the geographical gradient from North to South Vietnam in the same habitats; in which extent seasonality is present in tropical meiobenthic communities and several others.

Local diversity patterns and taxonomic composition of nematodes were compared along the gradient from North to South Vietnam. Similarities and differences in taxonomic composition were highlighted for the same types of mangrove biotopes. The local diversity is comparable or higher that known form temperate intertidal.

The role of mangrove trees in structuring nematodes' communities was described in details for two locations and the hypothesis that the type of root system is critical was

confirmed. Mangrove trees determine the spatial structure of the communities of nematodes along both vertical and horizontal axes. Comparative analysis of the data from sites of different mangrove species and sediment composition evaluates the relative influence of biotic and abiotic factors on the structure of nematode communities.

The long-term observations on the succession on newly established mangroves clearly show the dynamics of nematodes assemblages along the time. The stable set of genera that inhabited mangroves of different kind was separated from overall diversity as well as the spectrum of families typical for mangroves were described.

Prominent seasonal changes occur in meiobenthic abundance, composition of high taxa and species structure of nematodes assemblages.

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Meiobenthos at methane seeps on the Koryak slope of the Bering Sea

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Recently discovered methane seeps on the Koryak slope of the Bering Sea at depths 400–700 m are the northernmost chemosynthetic habitats (~61° N) known to date in the Pacific. In general, the identified communities can be divided into three types: (1) Communities of the first type are encountered at depths of 695–647 m surrounded by a background community of Ophiuroidea+Macrura Natantia. They are characterized by the development of numerous settlements of the symbiotrophic bivalve pliocardiin *Calyptogena pacifica*. (2) Communities of the second type are encountered at depths of 429–417 m within the background community *Protoptilum*/*Asteronyx+Brisaster latifrons*. Among the specialized forms, *C. pacifica* is represented in a small amount. (3) Communities of the third type are noted at depths of 400–402 m within the community dominated by the anemone *Sagartiogeton* cf. *californicus* (Sagartiidae). Numerous and extensive seeps, often more than 1 m in size, are marked by widespread bacterial mats. Single calyptogens are present on the periphery of seeps. No apparent reaction of macrofauna to such seeps is observed. The aims of this study are: (1) to describe the meiofauna community composition at methane seeps and adjacent area; (2) to determine the composition of assemblages of the dominant meiofaunal taxon, the nematodes, and (3) to study the contribution of products of chemosynthetic origin to the nutrition of the dominant groups of meiofauna.

A total of 17 major meiofaunal taxa, excluding copepod nauplii, were recorded in the methane seeps: Nematoda, Copepoda, Polychaeta, Hydrozoa, Ostracoda, Priapulida, Kinorhycha, Bivalvia, Acarina, Ophiuroidea, Gastrotricha, Cumacea, Kamptozoa, Nemertea, Caudofoveata, Isopoda with a strong dorninance of Nematoda (more than 88% of total abundance). Meiofaunal abundances ranged between from 347 ind./10 cm^2 (seep) and 5561 ind./10 cm^2 (periphery sediment). There were significant differences in the abundance of meiobenthos at different locations.

The density of nematodes ranged from 336 ind./10 cm^2 (seep) to 5506 ind./10 cm^2 (periphery sediments). A total of 78 nematode genera were identified. Nematode dominant genusnera was *Daptonema*, subdominant genus was *Sabatieria*. Similarity and diversity analyses are done to compare the different locations.

The depleted isotopic signatures suggest that chemosynthetically derived organic matter is an important carbon source for copepods and nematodes at seeps and is also included in the diet of organisms from the periphery of seeps.

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Prospects for regular replenishment of the "Tropical Seas" exposition in the Primorsky Aquarium using aquaculture methods

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The high demand for coral fish, the tightening of fishing rules and the problems of coral reefs preserving are stimulate the work of artificial breeding of ornamental species around the world. There are about one and a half hundred species of coral ornamental fish in the "Tropical Seas" exposition in the Primorsky Aquarium. Some of them are kept in the laboratories of the Scientific Adaptation Building. The works on artificial reproduction is also being carried out there.

The tomato clownfish *Amphiprion frenatus* was chosen as object for test of the breeding technology. A pre-formed pair of clowns was planted in a 390 L tank with the necessary equipment. Environment parameters: temperature 25–26°C, pH 8.1–8.2, salinity 35–36‰. Spawning was observed every 14–15 days. The larvae were collected in 10 L cages in the same aquarium. For the first 5 days the feeding of the larvae is carried out by the saltwater rotifer *Brachionus plicatilis* 6–8 pcs/ml together with microalgae of the genus *Nannochloropsis*. On the 5th day, fresh nauplii *Artemia salina* 4–6 pcs/ml were added to the mixture of algae and rotifer. The larval period lasts 10 days. After that the most vulnerable but short period of metamorphosis begins. On the 15th day the survival rate was 80%. At this time you can switch entirely to brine shrimp nauplii and add crushed dry food with a high protein content. On the 25th day, the fry can take grated shellfish meat and shrimp caviar.

This technology has been successfully applied to other amphiprion species *A. percula*, *Premnas biaculeatus.* And also adapted for fish larvae from other families: Bristle-tail file-fish *Acreichthys tomentosus* and Longspined porcupinefish *Diodon holocanthus*. This made it possible to grow replacement individuals, which can be included in the exhibition as needed. Cases of spawning of Banggai cardinal fish *Pterapogon kauderni* have been also successful. For the first time among the aquariums in Russia, we have obtained the offspring of a Zebra shark *Stegostoma fasciatum*. Our specialists managed to find the right husbandry parameters and diet, due to which the cub is healthy and grows quickly. At the moment, monitoring of the development of the next clutch of a pair of Zebra sharks and eggs of the Zebra bullhead shark *Heterodontus zebra* continues.

Promising objects for further breeding works are Goldtail demoiselle *Chrysiptera parasema*, Sapphire devil *C. cyanea* and Whitetail dascyllus *Dascyllus aruanus* from

the family *Pomacentridae*. Currently, the larvae period with selection of feed and environmental parameters are being studied.

The generation of fish reared in aquaculture is more viable, not subject to stress and adapted to captivity from the first days. Long lifespan and wellbeing of farmed fish can be expected under optimal conditions.

A current knowledge of composition and ecology of the free-living nematodes of the family Microlaimidae from the Far Eastern seas

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The Microlaimidae family is an exclusively marine and widespread family of free-living nematodes from coastal to deep-water zones of the World Ocean. It includes 14 genera and more than 120 species., The fauna of microlaimids of the European seas shelf is the most studied during the entire period of research of the free-living nematodes of the World Ocean.

The genus *Microlaimus* de Man, 1880 is one of the most species-rich. As a result of several revisions carried out in recent years (Bussau, Vopel, 1999; Kovalyev, Tchesunov, 2005; Leduc, Wharton, 2008; Kovalyev, Miljutina, 2009; Leduc, 2016), the new list of the genus *Microlaimus* is represented by 83 species, which takes into account the latest nomenclature changes. Over the past decades, an active discussion in the scientific literature has touched upon a number of issues: the description of large-scale patterns (latitudinal, depth gradient, primary production, etc.), the diversity of nematodes in deep-water areas (Rex, 1981, 1997; Lambshead, 1993; Rex et al., 1993, 2001; Rex, Etter, 1998; Danovaro et al., 1999; Lambshead et al., 2000, 2001, 2003; Lambshead, Boucher, 2003).

The most studied region is the North European coasts or the North Sea where found are 44 microlaimids species. The majority of the species (30) on the North Sea coast (the coasts of Sweden, Belgium, and Britain) at depths from 0.5 to 175 m in sandy sediment. Although many microlaimids species prefer specific environmental conditions, some have extensive ranges, inhabiting sediments with different densities.

Most notably, *Microlaimus honestus* De Man 1922, is the most widespread among all species, which were found in almost all oceans. The highest frequency of occurrence were recorded in the Atlantic Ocean (North and Baltic seas) and the Arctic Ocean (White and Norwegian seas). In addition, *M. honestus* was recorded in the southern part of the Indian Ocean shelf, which indicates that the species may be confined to a wide temperature range.

The basis of the material of this work was a database compiled mainly from publications that provide quantitative data on nematodes of the Far Eastern seas from different depths, as well as some unpublished data from employees of the Department of Ecology of the FEFU and collections of microlaimid obtained by the authors in 2016–2018. The soft sediment selected in the waters of the Sea of Japan, Okhotsk, Bering, and East Siberian seas were studied.

The species composition of nematodes of the genus *Microlaimus* has been identified, which according to modern data includes 13 species, of which 8 species were recorded for the first time for the fauna of the Far Eastern seas. The fauna of the microlaimid of the studied area is poorer than that adjacent to the European coast of the Northern, White, and Norwegian seas, which may be due to the insufficient study of the Pacific nematofauna.

The results of a comprehensive analysis of morphological, biogeographic and ecological data for Far Eastern nematodes carry out a taxonomic revision of the genus *Microlaimus* de Man, 1880 and significantly improve knowledge of the evolution within a large group of nematodes.

Methods of Brine Shrimp (*Artemia salina*) raising in Primorsky Aquarium

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A group of specialists from the Animal Food Supply Department of the Primorsky Aquarium – Branch of the NSCMB FEB RAS was charged with cultivating live food for marine organisms, including starting food for the propagation of rare and expensive fish species. New methods of live food raising, previously not described, have been applied in the Primorsky Aquarium.

Special attention has been paid to the cultivation of *Artemia salina*, the most essential live feed for marine and freshwater organisms of the Primorsky Aquarium. The volume of brine shrimp cultivation is the highest; it constitutes 90% of all live food in the Aquarium.

For Primorsky Aquarium, the most effective way to raise *A. salina* has proven to be a three-step technique.

The three-step technique of brine shrimp cultivation runs as follows. The first step is incubation of dry resting eggs (cysts) for two days up to a stage of nauplius. The second is rearing of the nauplii up to two-week-old young shrimps and the third, raising of the two-week-olds up to fully mature shrimps.

This technology involving cyclical movement of batches of eggs, larvae or young shrimps from one container to another decreases the mortality of shrimps caused by high concentration of food remains and metabolites in overstocked containers. At every step, brine shrimp batches are weighed and counted, and the quantitative dynamics in the raised culture is controlled.

At step 2, nauplii are reared to two-week-old young shrimps in the main farm of sixteen cylindrical 250-l containers, plus additional two cylindrical 2000-l tanks, all with conical bottoms. The main farm of sixteen containers is divided into four zones. The batches of nauplii are entered into each zone one after another in one week interval, each batch for two weeks. The full rearing cycle of the farm requires 4 m^3 of purified seawater.

The application of the continuous *A. salina* production technique has resulted in 1.6–1.8 kg yield per one rearing cycle at the full capacity of all the containers and tanks.

As of now, the keepers of the Primorsky Aquarium have used nauplii and adult *A. salina*, as well as other live food, to successfully propagate the tropical fishes *Acreichthys tomentosus*, *Amphiprion ocellaris*, *Amphiprion frenatus*, and *Premnas biaculeatus*. The two-day-old nauplii and adult shrimps have been used as a starting food to breed the seawater tropical fishes *Diodon holocanthus* and *Pterapogon kauderni* and the freshwater tropical fishes: *Corydoras aeneus*, *Symphysodon aequifasciatus*, *Herichthys carpintis*, *Cyphotilapia frontosa*, and *Labeotropheus fuelleborni*.

Benthic diatoms off Crimean coast (Black Sea): taxonomic diversity and structure of taxocenes at the locations under varying levels of technogenic pollution

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The taxonomic diversity and structure of benthic diatom taxocenes were investigated in Crimean nearshore habitats with varying levels of technogenic pollution. The material for this study was sampled along the coast of Crimea between 1984 and 2016 at 16 sites at depths ranging from 0.5 to 50 m. In total 793 benthic diatom species and intraspecific taxa, belonging to 736 species, 130 genera, 53 families, 27 orders and 3 classes of Bacillariophyta were registered. Taxonomic Distinctness indices (TaxDI, AvTD and VarTD) were used to assess the structure of hierarchical diatom trees for these locations. TaxDI values were evaluated and compared for environmentally pristine and areas. Structure of diatom taxocenes from heavily polluted sites can be described as having low species richness and a large proportion of mono- and oligospecies branches, closed at the family and order level on hierarchical tree. In locations under a moderate level of anthropogenic impact, the structure was characterised by relatively high species richness and an equal ratio of oligo- and poly-species branches closed at the genus level, with the presence of monospecies branches converging at the family or order level on hierarchical tree. Structure of taxocenes in pristine sites were distinguished by a predominance of poly-species taxa, the highest species richness and taxonomic branches closed at different hierarchical levels. Based on the calculation of AvTD and VarTD indices, it was evident that the appearance or elimination of a large number of closely related species has a less significant effect on taxonomical trees than that the finding or disappearing of a few species with a distant phylogenetic relationship. In summary, when the species richness of a taxocene based on poly-species branches formed by phylogenetically allied species is higher, while the average value of Δ^+ is lower. Conversely, if the species richness of the taxocene based on oligo- or monospecies branches with a distant phylogenetic relationship is relatively low, the value of Δ^+ will be higher.

Based on the calculation of AvTD and VarTD indices, it was shown that the appearance or disappearance of a large number of closely related benthic diatom species has a less significant effect on the taxonomic tree structure than that the finding or elimination of single species with a distant phylogenetic relationship. It is also shown that the high

species richness of diatom taxocene from pristine water areas, formed by phylogenetically allied species, was described by a low AvTD value, that was located on the graph below the average expected value for the diatom flora of the Black Sea. Conversely, the low species richness of diatom taxocene from moderately or heavily polluted locations, based on oligo- or monospecific branches with a distant phylogenetic relationship, was characterized by a higher AvTD value located on the graph near or significantly higher than the average expected value for the Black Sea' flora of Bacillariophyta.

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Formation of favorable hydrological structures within feeding migrations for Japanese flying squid in the northwestern part of the Sea of Japan according to satellite observations in 2018–2020

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In recent years the decreasing trend for stock of Japanese flying squid (JFS) in the Pacific region as the result of shift in the climate and hydrological regimes is noted. The shifts in hydrological conditions also make the geography changes of JFS fishing areas. The comparison of the jigging fishing areas for the JFS (from official statistical data of daily catches for fishing vessels) and the elements of the hydrological conditions deciphered from infrared satellite images for the period 2018–2020 in the northwestern part of Sea of Japan was studied. The data from the AQUA, TERRA satellites (MODIS spectroradiometer), obtained and processed at the Center for Collective Use of the Regional Satellite Environmental Monitoring of the Far Eastern Branch of the Russian Academy of Sciences (CCP) was studied too. As result was found that the scools of JFS mainly was confined to the areas of upwelling eddies, where the rising of biogens in the upper layers of the sea is occurs, and the food base for fishes and JFS is formed. The maximum JFS catches were observed on the periphery of warm mesoscale anticyclonic eddies bordering with subarctic waters. The JFS scools were concentrated in the central part of eddies, if its had a spiral view structure. If the subtropical waters stream had the form of a mushroom-shaped flow, the largest catches of JFS were observed inside stream and in the zone of anticyclonic and cyclonic turbulence of the dipole. In the frontal zone between subtropical and subarctic waters the JFS fishing areas were on the warm side of the front zone. The sea surface temperature (SST) in the fishing areas ranged from 12 to 22°C. The absolute values of SST did not significantly affect for JFS scool forming, but only limited the spatial distribution of JFS. Was noted that the timing of the typhoons coming in the region indirectly affects the beginning of the migration of JFS, what probably associated with an increase in the advection of warm waters. In 2018–2019, the early time of typhoons coming caused the early dates of the start of JFS migrations and formation of scools in Primorye waters. In 2020, the late time of typhoons coming delayed the start of migration and JFS fishing. This information can be useful for forecasting of the timing start migrations and formation JFS fishing grounds in Primorye waters.

The telencephalon of Actinoptherygian fish – as a special variant in the evolution of the central nervous system of vertebrates

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Telencephalon is the most complex part of the brain of vertebrates. It is here that the highest centers of representation of all sensory systems are located and associative zones that control behavior and thinking are formed. In the hemispheres of the telencephalon there are dorsal (pallial, cortical) and ventral (subpallial, striatal) divisions. The formation of the telencephalon in the evolution of various groups of vertebrates went in two fundamentally different directions – inverted and everted. A characteristic feature of the structure of the inverted terminal brain is the presence of lateral (lateral) ventricles in the paired hemispheres. This type of brain has round-mouthed, cartilaginous and lungfish fish, amphibians, reptiles, birds and mammals. The hemispheres of the everted type are devoid of lateral ventricles and they are separated by an unpaired medial ventricle. The only group of vertebrates with this type of the brain – are ray-finned fish (Actinopterygii). In this regard, there are certain difficulties regarding the homology of the brain structures of ray-finned fish and other vertebrates. This is important because recently fish have been widely used as model objects in neurophysiological and neuroembryological studies.

The poster presents information on the morpho-functional organization of the telencephalon of ray-finned fish. There is a clear evolutionary trend in the development of the cytoarchitectonics of the brain: from simply arranged hemispheres in multi-finned and sturgeon fish, to more differentiated ones in teleost fish. Thus, in the *Polypterus ornatipinnis* area, the dorsal hemisphere is not differentiated into zones, and the main mass of neurons is concentrated under the surface of the hemisphere. The ventral region is divided into three zones: dorsal (Vd), ventral (Vv) and lateral (VI). In chondrostei, the most studied representatives of which are sturgeons, three or four zones are already differentiated in the dorsal region: medial (Dm), dorsolateral (Dd+l), often divided into dorsal (Dd) and lateral (D1), and central (Dc). In bony fish, there are various variants of the structure of the pallium and subpallium: from poorly differentiated in the lower ones, to the most complex complex in representatives of higher bony fish, where up to a dozen and a half separate zones are distinguished in the telencephalon hemispheres. The analysis of the neural organization of the showed that there are no significant changes in the process of their evolution at this level of the brain organization. The majority of

neurons belong to the varieties of non-specialized iso- and allodendritic neurons. Highly differentiated stellate short-axon (idiodendritic) neurons, characteristic of the central nervous system of higher vertebrates and sharks, were not found in ray-finned fish. This indicates a certain trend in the development of the brain of ray-finned fish, which can be defined as evolutionarily-conservative.

Due to the peculiarities of the embryogenesis of the everted telencephalon of ray-finned fish, the most difficult question is the homology of their hemispheres with the structures of the inverted brain. Some researchers propose to carry out a direct homology of brain zones of this two types brain , others consider it impossible to make any comparisons, considering the brain of ray-finned fish as a special variant telencephalon formed in the evolution of vertebrates.

Most researchers, based on data on morphology, histochemistry, connections, development and genetics of the vertebrate brain, believe that a solution to this problem can be found. Currently, it is shown that in the structures of the fish telencephalon there are zones of representation of all the main analyzer systems. The dorsal (Dd) and lateral (Dl) zones of the pallium receive afferents from the subpallium, the thalamus and the brain stem. Through these connections, the pallium receives polysensory information (visual, somatosensory, gustatory, from the system of lateral line organs). The medial (Dm) zone has reciprocal connections with the preglomerular complex and thalamus nuclei. The central zone of the pallium (Dc) receives connections from other zones of the pallium, the preoptic region of the hypothalamus, the preglomerular complex and the cerebellum.The representation of olfactory projections is limited to the ventral region of the hemisphere and the posterior-dorsal (Dp) zone of the pallium. The lateral (Dl) pallium of fish and the hippocampus (MP) of mammals contain centers associated with the processes of memory and learning. The medial pallium (Dm) is also known as the area of the pallial amygdala terrestrial vertebrates are functionally connected with the emotional sphere of the work of the brain. Damage to different parts of the hemispheres caused serious changes in the behavioral activity of fish.

Thus, the telencephalon is the integrating center of the fish brain. Recently, a significant argument in solving the question of the homology of brain structures of different types has been the study of the expression of regulatory genes that control the development of certain structures of the hemispheres. It is shown that the Dlx1, Dlx2, Dlx5, as well as Nkx2,1 and Gsh 1/2 genes are expressed in the subpallium of bony fish, controlling the development of the striatum, part of the amygdala, and septum. The genes Pax6, Emx1/2, Tbr1-2, Neug2, are expressed in the dorsal region. Comparison with similar studies on terrestrial vertebrates showed that the basic character of the division of the hemispheres of all vertebrates into ventral, subpallial, and dorsal, palluial regions is preserved throughout the evolution of vertebrates. Thus, the Dp zone in the hemispheres of ray-finned fish is considered as a possible homologue of the lateral pallium. The greatest disagreements concern the dorsal region (Dd) of the pallium: from the recognition of the homology of this region with the dorsal pallium of tetrapods,

to the statement of the fact that this region of the hemispheres is unique for ray-finned fish and, as a result, the lack of homology with the structures of the hemispheres of terrestrial vertebrates. The caudal parts of the Dm zone and part of the Dc zone are compared with the striatum, noting that other parts of these zones include components of the dorsal pallium. The rostral sections of the medial zone are homologated with a part of the amygdala. There are no special difficulties regarding the subpallium. Its structures, which are not subjected to the everted process, preserve the basic plan of the structure in the entire range of vertebrates. The ventral zones Vd and Vv are compared with the area of the septal nuclei, the zone Vl – with the olfactory tubercle, and the caudal sections Vs, Vp, Vi – with part of the amygdala.

In general, the telencephalon of Actinopterigian fish has of significant differences from that of other vertebrate groups. This should be taken into account when interpreting the data obtained during morpho-functional studies of the central nervous system on different representatives of vertebrates.

30 years of HAB monitoring on Russia's east coast: results and lessons

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NSCMB has been actively involved in research on harmful algal blooms (HABs) for over 30 years. Main tasks for HABs are distinguished as: a fundamental understanding of causative species; revealing the dynamics, mechanisms and vectors; formation of a collection of phycotoxin producers on the basis of the Marine Biobank NSCMB FEB RAS http://marbank.dvo.ru in order to provide scientific research with available legitimate biological samples in accordance with international protocols for the collection, cataloging, storage and sharing of biomaterials; formation of a database (data on past and current HABs events) to identify trends that can be correlated with environmental conditions. The east coast of Russian is unique not only for its size and biodiversity but also for the fact that it has some of the highest levels of marine phycotoxins not only in the Russian Federation, but also in the world and the site of HAB's incidents that led to death marine fauna and humans. HAB monitoring program on the east coast of Russia during the period 1999–2021 revealed 47 species which is known as potentially toxic and bloom-forming microalgae. The changes and trends in the composition, abundances and distribution of toxic microalgae are shown as follows: a decreasing of diatom component of phytoplankton; new bloom forming flagellate species were detected and new toxin producing species were revealed. For the last two decades some species of harmful algae (e.g., toxic dinoflagellates, prymnesiophytes and pelagophytes benefiting water column stratification/increased temperatures, warm water benthic dinoflagellates and cyanobacteria) became common. HABs monitoring, which was launched in 2007 revealed the real threat of shellfish poisoning: phycotoxins as PST, AST (domoic acid), DTX-1, YTX, AZA-2, PTX-2 were detected in shellfish. But still there are no practical methods for analyzing phycotoxins and as a consequence it is impossible to register poisons by any of these toxins in medical or sanitary organization. All of these are the reasons of not having data about real economic impacts and harm for people's health for Far Eastern region of Russia. Low level of scientific knowledge and general ignorance of HABs problem can be explained by two of the most important reasons: a huge length of unpopulated coast and the absence of a national program for HABs monitoring and management. The question raised in the report is if Russia will fit into the world HAB's (PICES, WESTPAC) strategy and what are the prospects?

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Project work in the individualized setting

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In 2018, the Primorsky Aquarium, which is a branch of the A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, launched an environmental education project called "Growing up at the Aquarium". The project is focused on the study and conservation of aquatic ecosystems in Russia and across the world. Over 50 children attend the project's sessions every week. The project participants are divided into age groups, sessions are conducted both in group and one-on-one settings.

At first, the "Growing up at the Aquarium" comprised four stages: the environmental biology club "Belyok", the ecology club "Sea Guardians", the "Ichthyoclub"and the marine biology club "AQUAlaboratory". In 2020, Aquarium educators analyzed the results of the students' group work and the methodological experience acquired in the course of the project and decided to expand the "Growing up at the Aquarium" with one more club, "Researcher", offering classes to children ages 10 to 12. The club curriculum was implemented between October 2020 and May 2021. Based on the positive experience from the existing curricular of the "Ichthyoclub" and "Guardians of the Sea", a tutorial method of teaching, namely individual project-based learning, was chosen to serve as the methodological model.

The project-based learning method aims to enhance students' learning and critical thinking skills, as well as to develop their abilities to build knowledge and use information sources through project work. By the tutorial method we mean organized activities intended to train a child to develop and complete his/her individual education program.

At the initial meetings of the club, the participants were introduced to the project-related terminology including such terms as "learning project", "research project", "project importance", "goal", "objectives", "object of research", "subject of research", "hypothesis", "end product". Group work involved play and hands-on activities and debates and was aimed to result in pilot projects. The individual support for projects was provided using the following tools: an individual education program (IEP), personal resource map, project progress map, consultations with professionals supervising the project and one-on-one tutorials. During the group sessions, an educational situation was assessed to identify if the students had enough potential to fulfill their projects and what weak points might affect their success. After the pilot projects were successfully defended, the educators began providing individualized support for the students.

The one-on-one tutorial included the following steps:

1) Developing an IEP (choosing a project theme and an idea of the end product, defining the project goal and objectives, creating a timeline for the project components, etc.).

2) Planning the sequence and types of activities and adding this information to the IEP to make it efficient, tailored to the child's skills, interests and preferences and adaptable to changing needs.

3) Developing a personal resource map. The map enabled students to analyze the available educational resources of the institution, discover resource deficiencies and choose a project implementation path.

At the final session of the "Researcher" curriculum, the participants presented end products of their projects. In the reflective phase of the course, outcomes of the project work were collaboratively analyzed by the students and tutors to find out if the results conformed to the initial idea and how new skills and knowledge could be used in the student's personal and social life. In the assessment phase, a group reflection was held by the club educators to evaluate the efficiency of the delivered curriculum and its contribution to their personal and professional development.

Based on the above-mentioned reflections and assessment results, we have drawn the following conclusions:

1) The interim monitoring of each participant's progress, which was performed through IEPs, personal resource maps and project progress maps, made it possible to keep both the students and educators motivated in completing projects.

2) The individualized approach let the tutors understand the children's research interests, judging from their personal demands and learning experience.

Study of chemico-biotic interactions in the biosphere and hydrosphere and contribution to scientific basis of protection of biodiversity of organisms including marine hydrobionts

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Ecological and environmental research conducted by the co-authors led to obtaining new experimental data on chemico-biotic interactions including biological effects of man-made chemicals and emergent chemical pollutants on biological organisms. Some of the new data contributed to development of an innovative approach to understanding function of aquatic ecosystems, and multi-functional role of aquatic biodiversity in maintaining and improving water quality (water self-purification). These considerations were published in a series of publications of the first of the co-authors of this abstract (namely, S.A.Ostroumov) in the journals 'Doklady Biological Sciences', 'Russian Journal of General Chemistry', 'Hydrobiologia', 'Water Resources', and 'Russian Journal of Ecology', as well as in some other academic journals. As a result, these publications formed an interdisciplinary qualitative theory of ecosystem-driven and polyfunctional (multifunctional) biota-driven water self-purification.

The results and conclusions of this theory might be applied to further development of the scientific basis for protection of biodiversity including biodiversity of aquatic organisms.

The traditional approach to protection of biodiversity is based on the principle of conservation of the diversity of species of biological organisms. When the existence and fate of the populations of given species is endangered, the situation is considered bad and the conclusion follows that the protection measures are needed. When the existence of the given species is not endangered, the situation is considered as acceptable and it is thought that no worries about this species are needed.

The new data obtained and the innovative theoretical considerations mentioned above lead us to a conclusion that the traditional approach needs to be corrected and supplemented. In the presentation, we plan to prove that the additional input to the scientific basis of protection of biodiversity is needed, and additional attention and protection should be given to quantitative parameters of ecosystem function of the populations of the species under consideration. An example is the water-filtering activity of filter-feeders (however it is only one of many examples). We plan to give new examples in support of this consideration, in addition to previously published examples.

A relevant result of the abovementioned theory of ecosystem-driven and biota-driven water self-purification is a better understanding of the indispensable role of biota in maintaining water quality of water resources. The practically important conclusion is that it is absolutely necessary to protect aquatic biodiversity in order to protect water quality. It is important for both freshwater and marine water systems.

New data on reproduction and early ontogenesis of the antlered sculpin, *Enophrys diceraus* (Cottidae) in Peter the Great Bay

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Reports on spawning of the antlered sculpin (*Enophrys diceraus),* its larvae development and timing of their appearance in plankton in Peter the Great Bay were already done earlier (Sokolovsky, Sokolovskaya, 1999; Panchenko, 2005). However, such important aspects of the biology of this species as the timing and conditions of spawning, eggs development and the transition of the pelagic larval stage have not been sufficiently investigated. Our year-round complex ichthyoplanktonic and ichthyological study conducted in 2017–2021 and supplemented investigations with using SCUBA techniques starting from 2019 made it possible to highlight these issues in more detail. The research was carried out on a weekly basis in the Zhitkov Bay located on Russky Island and in the adjacent waters of the Paris Bay.

During the SCUBA diving performed in November–December 2019 and March–May 2020 we found several clutches of antlered sculpin eggs. The period from January to February was not covered by diving operations due to ice conditions. The first two clutches in this area were found on November 29, 2019, while the procreators were spotted in this location during October–November. The determining factor for the onset of spawning was probably the bottom water temperature because we found a clutch of this species at an earlier period in previous years in another area of the Peter the Great Bay. It was discovered during investigations in the Vostok Bay at a depth of 7 m with water temperature of about 3°C on November 22, 1999 (Panchenko, 2005). The clutch was spread over a stone in an irregular oval shape measuring 30x20 cm in size with 3 cm thickness in the middle. Such a large size of the clutch indicated that the eggs were laid by several females. Mature eggs in the gonads of different females of antlered sculpin vary in colour (Sokolovsky, Sokolovskaya, 1999; our data) so in different areas of the clutch the color of eggs laid by different females changed from orange to purple while the eggs diameter varied from 1.7–2.0 to 1.8–2.1 mm. An adult antlered sculpin was spotted near the clutch. Protection of eggs, usually done by males, is a characteristic feature of many species of fish of the Cottidae family (Nikolsky, 1950; Andriyashev, 1954; Gorbunova, 1964; Munehara at al., 1994; Hayakawa, Munehara, 1996; Panchenko, 2001; and others). We found that protection of antlered sculpin eggs was also carried out by males.

In our diving research in the autumn-winter period in Zhitkov Bay and in the adjacent water area, we observed males and females of antlered sculpin species. Some of the males were seen near clutches found at a depth of 6–11 m guarding them. The seabed in the area of the location of spawning grounds of antlered sculpin that we found was muddy. However, the eggs were usually attached to solid surfaces like upper parts of rocks, between stones or on mussel druses, oysters, etc. Only one clutch was found loose on the muddy bottom. It was located near a flooded metal structure on which it was probably deposited but slid off as a result of an unsuccessful placement onto the substrate. A male sculpin was initially found above the clutch but it probably stopped guarding it because of the failed placement of the clutch so we removed the eggs for the subsequent incubation in the aquarium. In springtime, only antlered sculpin males continuing to guard the eggs were found in the area of the spawning grounds.

The first clutches of antlered sculpin were found at the end November 2019 at water temperature of 2.3°C. The latest eggs finding was recorded on April 10, 2020 at a depth of 11 m and the bottom layer water temperature of 2.0°C. Visually, the size of this clutch appeared decreased which was possibly due to the onset of hatching of larvae. We did not find any egg clutches and/or individuals of antlered sculpin in the area of the spawning grounds later than this period, apparently, due to the end of hatching and the protected fish species departure.

It is known from literary sources that larvae of the antlered sculpin in the Peter the Great Bay appear in ichthyoplankton at the end of April (Sokolovsky, Sokolovskaya, 1999). Our research showed that the onset of hatching may occur much earlier in some years. In 2020 the first antlered sculpin larvae were found at the fish-luring light stations in the Zhitkov Bay already on March 23rd with the bottom water temperature of −0.6°C (+2.4°C at the surface). Taking into account the cases of finding clutches up to the 10th of April in the current year, the hatching period could have lasted for several weeks. Hatching of larvae of this species in the Peter the Great Bay begins already at a negative water temperature. Apparently, the development of eggs initiates, albeit slowly, in winter (lasting for about 4 months) and it does not stop with the bottom water temperature staying below 0°C (a drop to −1.7°C in January–February was recorded). Such temperature conditions are common in the area from mid-December to the end of March.

In other years, the timing of the onset of larvae appearance at a fish-luring light stations varied from late March to the second decade of May, which, we believe, depends not only on hydrological conditions, but also on the proximity of the developing clutches to plankton collection sites. It is possible that in 2018, when the first larvae having the size approaching the size of transition to bentic life mode were observed only in May, the spawning of the antlered sculpin near the place of collection in the Zhitkov Bay did not happen for some reason.

The earliest prelarvae caught by us had large pinkish-red yolk sacs (the colour of developing eggs) and a body length of 7.8–8.0 mm (*TL*). According to literature data it is known that hatching occurs at smaller sizes of 6.3–6.5 mm (*SL*) in April–May

(Sokolovsky, Sokolovskaya, 1999). The significant disparity in size with the literature data is probably due to the fact that, according to our observations made during the incubation of eggs, the prelarvae are inactive for some time (1–2 days) and do not rise from the bottom before becoming part of the plankton. Also, we measured living species while earlier descriptions were made on fixed material. Hatching from the developing clutch collected by us in winter 2020 under aquarium conditions (SAC of the Primorsky Aquarium) began on March 2, 2021 at water temperature of about 5°C. Development of eggs under such conditions was obviously significantly accelerated so the live larvae had a body length of about 5.5 mm (*TL*), which would undoubtedly be less in natural conditions.

As shown by the analysis of our catchings, larvae of antlered sculpin usually remain in plankton until the second half of May with surface water temperatures rising to 7–8°C. By this time they reach a body length of 13.0–14.0 mm (*TL*). Larvae of this size already have well-formed fins and show first signs of pigmentation characteristic of bottom settled juvenile fish. In the course of observations of larvae development in aquarium conditions, carried out in the spring of 2017 in the environment of the NSCMB aquarium, it was also noted that already at a length of about 14 mm (*TL*) postlarvae preferred to spend most of their time lying on the bottom of the aquarium only occasionally rising up for picking crustaceans offered as food. Meanwhile, in the paper by A.S. Sokolovsky and T.G. Sokolovskaya (1999) it was reported that the transfer of antlered sculpin to the bentic mode of life happened at a much greater body length (over 20 mm). Considering the fact that the collection of the larvae described in their study was carried out not only at the surface, but also with the help of oblique towing of ichthyoplankton sampler from bottom, one can assume that some already bentic juveniles also got into their catches.

Investigation a lateral behavior of spotted seals (*Phoca largha)*

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Lateral behavior is manifested in the asymmetric actions of animals as side preferences or in the use of the left or right limbs performing certain actions. Lateralization is a common feature of vertebrates. The lateralization may depend on gender and age, performed activities and the conditions of keeping, however, for Pinnipedia this poorly studied. Thus, the purpose of this study was to investigate the asymmetric behavior of spotted seals (*Phoca largha* Pallas, 1811) in natural conditions with restricted area, free preference in choosing the left or right side using objects stimuli in the training process. The long-term studies of the lateral behavior of *Phoca largha* were carried out in the Shared Equipment Facility "Primorsky Aquarium" in the NSCMB FEB RAS. *Phoca largha* is a resident inhabitant of Peter the Great Bay and a widespread member of the true seals. The data on the lateralized behavior of 6 seals were analyzed, the effect of various objects stimuli on the preference of the side (right, left) was established.

The studies were carried out using the specially designed experimental facility, which was modified for the physiological characteristics of seals for to mark the objects stimuli. The animal was given a choice of 2 identical objects on the left and right sides, one of which a seal had to mark by touching the nose. Three series of the experiment were carried out in the period from 2015 to 2021. Volumetric plastic figures of the same color were used as objects stimulus.

According to the obtained results, it was shown that spotted seals exhibit lateral behavior in relation to the choice of the objects stimuli. It was found that the preference for the choice is influenced by uncontrollable environmental factors, as well as the age and gender of the seals. Females demonstrated greater lability compared to males, the degree of laterality of which did not change. It was found that in one studied group of seals there are right-lateralized and left-lateralized. Also, our data indicate that spotted seals demonstrate an individual lateralization and dynamic of the lateral behavior.

Taxonomic composition and seasonal changes of meiofauna communities inhabiting rhizomes of intertidal seagrass meadows in Tien Yen Estuary (South China Sea, Vietnam)

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This study investigated the seasonal changes of the meiofauna community inhabiting rhizomes of tropical seagrass meadows within Tien Yen District of Quang Ninh Province, Vietnam. Sampling was conducted in 2015, in April (dry season) and October (rainy season) on intertidal flats at the Ha Dong (HD) and Cua Song (CS) in seagrass meadows comprising *Zostera japonica*, Con Mat (CM) in *Halophila ovalis* seagrass meadows. Seagrasses were manually collected, the rhizomes were cut in by 10x10x10 cm frame and were washed gently with estuary water and were sieved through sieves with 1000 μm and 32 μm mesh sizes to remove the sediment. At each site three stations and triplicate samples were randomly taken for meiofauna communities. Meiofauna found in the seagrass rhizomes within Tien Yen estuary consists of 9 groups. Taxa identified from the samples of studied area, were: Nematoda, Harpacticoida, Kinorhyncha, Ostracoda, Gastropoda, Polychaeta, Oligochaeta, Amphipoda, Isopoda. It is considered that the abundance and diversity of meiofauna due to seagrass cover density and rhizome pattern. The more powerful the rhizomes at fine sediments also contain more organic material - it is due to the ability of substrate to bind more organic material that become one of the factors that support the increase of meiofauna in seagrass beds.

Overall, 9 meiofaunal taxa were found in April, the averaged meiofauna density was 787±121 inds. per 10 cm^2, nematodes was the most abundant phyla in each sampling station. The highest diversity and abundance of meiofauna was found at station HD, taxonomic composition included 8 groups, nematodes made 66% of the total meiobenthos density. Harpacticoids and polychaetes accounted for 27 and 4%, respectively. The smallest variety (6 groups) and density (249±93 inds. per 10 cm^2) were registered at the station CM. Nematodes dominated (73%), the second dominant taxa were harpacticoids (15%).

In October, after heavy rains, there were significant changes in the communities of meiobenthos from the rhizomes of seagrasses. The mean meiofauna density for each site over the two seasons showed an decrease from spring to autumn at two

times (362±111 inds. per 10 cm^2), the most noticeable change through the seasons was the reducing of the taxonomical composition. At HD total of 5 different meiofaunal taxa were recorded, Nematodes were dominant group and comprised 50% of the total meiobenthos density, followed by Harpacticoida 26% and Polychaeta. At CS low diversity in the community in *H. ovalis* was found (two groups were only identified (nematodes 62% and harpacticoids 36%) compared to that of in April, moreover, compared to April, the proportion of nematodes decreased, and the harpacticoid increased. At CM the meiofauna community constituted also only two taxa among which nematodes were the most dominant (93%), followed by Polychaeta (7.0%). When comparing meiobenthos communities in seagrass rhizomes in April with communities in grass-free areas, it turned out that the density in rhizomes on HD and CS is higher than on CM (249 inds. per 10 cm^2 *vs* 1003 inds. per 10 cm^2). At the HD and CS stations, the grass cover was denser than at the CM station.

Total diversity and density of meiofaunal taxa were higher in spring within each studied station. In general, in October, the density and taxonomic diversity of meiobenthos in rhizomes were lower than in free areas.

Thus, in the Tien Yen estuary a high variability of the meiofauna communities in the horizontal scale and in time was found. It could be explained by the high spatial heterogeneity of environmental conditions and their pronounced seasonal variations. It is well known that seagrass cover density and rhizome features of each seagrass significantly affect the structure and activity of communities by changing the properties of bottom sediments and transporting the organic matter into them. This organic matter provides nearly unlimited resources making the microbe-consisting food supply available resulting in an increase in the number of meiobenthic animals. Our study highlights that the precipitation (heavy rainfalls) in the intertidal zone of the Tien Yen estuary is the main factor responsible for seasonal changes of density, diversity and composition of meiofauna assemblages. During heavy rainfalls, fresh water penetrates and intermix the upper layers of sea bottom sediments. In this case, small sediment particles are washed out at some places and accumulate at others, causing the sediment structure to change and, moreover, the species is mechanically washed out, resulting in a loss of abundance and taxonomic composition in the meiobenthos community. Our results confirm that in the rainy season (October), the meiofauna density noticeably decreased, and the taxonomic composition and dominant taxa, as well as such parameters of assemblages as diversity and structure, changed as compared to April.

Ascertainment of specific threshold resistance for the Black Sea benthic diatoms (Bacillariophyta) under the copper ions impact in an experiment

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The increased anthropogenic impact on coastal marine ecosystems stipulates the relevance of monitoring and the application of ecotoxicological methods to determine the response of various groups of hydrobionts. Benthic diatoms are promising test objects due to their abundant in microphytobenthos and pronounced sensitivity to pollutants accumulated in soft-bottom substrates. The selection of different benthic diatom species, analysis of their morpho-physiological responses and determination of the tolerance ranges to the impact of heavy metals and other pollutants, is one of the important tasks in biotesting and indirect assessment of the marine environment quality.

The resistance of clonal strains of three benthic diatom species belonging to three different classes of Bacillariophyta [*Thalassiosira excentrica* Cleve 1903, *Ardissonea crystallina* (C. Agardh) Grunow 1880 and *Pleurosigma aestuarii* (Bréb. in Kütz.) W. Smith 1853] under the influence of $CuSO_4 \cdot 5H_2O$ have been studied for 10-day laboratory experiments. The work aimed to assess changes in the share (%) of alive cells, the absolute number and specific growth rate of cells in the clonal strain at different exposures and copper ions concentrations range up 32 to 1024 μg L^{-1}.

Regarding on *T. excentrica*, the threshold concentration of copper ions was rated at 128 μg L^{-1}, since the share of alive cells in the test-culture decreased from 95–97% to 70% already by the 5th day of exposure considerably and significantly ($P<0.01$) and fell down up to 42% by 10 day. For the concentrations of Cu^{2+} 256 μg L^{-1} and higher, a sharp inhibition of the clonal culture was detected already starting from 1st day of the experiment, the share of alive cells decreased to almost 0% by days 3–5.

As for *A. crystallina*, the threshold concentration of copper ions was 256 μg L^{-1}. For concentration of 384 μg L^{-1}, there occur a sharp decrease in the share of alive cells and subsequent dying off the culture; for concentrations of 448 μg L^{-1} and higher complete cell death was registered already by the 3rd day of the experiment.

For *P. aestuarii* the higher tolerance to copper among the tested species was revealed. For threshold concentration of 320 μg L^{-1} Cu^{2+} the share of alive cells in test-culture decreased sharply already by the 3rd day, and it was 10% by the 5th day. While the toxicant concentration was up to 384 μg L^{-1} and higher, inhibition of diatom cell was noted within 1st day and the percentage of alive cells dropped to 0 by the 3–5th day.

Dynamics of increase in the absolute cells number for the tested diatom clonal strains at low concentrations of copper ions (up to 64 μg L^{-1} for *T. excentrica*, up to 128 μg L^{-1} for *A. crystallina*, and up to 256 μg L^{-1} for *P. aestuarii*) can be described by sigmoid response curve. The augmentation in the cells number in different clonal strains increases 3–8 times within the period of 1–5 days compared with the initial values, attain to maximum and reaching plateau by 5–7 days. Decreasing by 10–25% due to the toxicant impact and apparently due to aging of test-cultures was observed in the subsequent period. Regarding every tested species, a significant ($P<0.05$) close conformity between the decreasing of absolute values of growth indices and the increasing in the toxicant concentration was revealed. These dependences are species-specific taking into account the threshold concentrations of the toxicant. The most drastic sigmoid curve of cell growth was appeared for *T. excentrica*, and the smoothest shape of sigmoid was observed for *A. crystallina*.

Assessment of the specific growth rate in the cells number (as one of the principal biotest parameters) was carried out based on the quantity of cell divisions per day (*V*) (Spirkina et al., 2014). Parameters of the direct relationship between an increase in toxicant concentration and a decrease in the intensity of specific growth rate in tested diatom species were determined.

Considering *T. excentrica*, the greatest positive specific growth rate was revealed only for the period of 1–3 days in the range of 32–128 μg L^{-1} Cu^{2+} concentrations. At the threshold of toxicant concentration (128 μg L^{-1}), the growth rate of *T. excentrica* cells was still positive, but it was reduced by 12 times in comparison with the control. Sharply negative specific cell growth (up to −0.43) was recorded for concentration 256 μg L^{-1} of copper ions and higher after 1st day of the experiment.

As for *A. crystallina*, the highest specific growth rate was also noted in the period of 1–3 days for Cu^{2+} concentrations of 32–128 μg L^{-1}, but the specific growth rate becomes negative already starting from the 5th day. For threshold concentration 256 μg L^{-1} and higher in the further exposure periods, the clonal strain *A. crystallina* was characterized by negative growth rates (up to −0.34).

Species *P. aestuarii* was characterized by a positive specific growth rate for Cu^{2+} concentrations of 32–256 μg L^{-1} within the period of 1–7 days, while the maximum value of specific growth rate (0.89) was revealed from 3 to 5 days. The specific growth rate in the strain becomes negative starting from the threshold concentration of 320 μg L^{-1}.

Thus, a certain specific threshold resistance was revealed. This fact appeared in the increasing of tested species' resistance parameter to the threshold concentration of toxicant (copper ions), depending on the taxonomic class of benthic diatoms. This phenomenon probably caused by more sophisticated development of morphological structure of valves, areolae and raphe systems over the evolution. The revealed regularity would be proved more reasonable only after conducting the extended experiments with various representatives of Bacillariophyta.

Taking into account the obtained results, the tested benthic diatom species can be recommended as new test-objects for toxicological experiments, as well as for environmental monitoring and indirect assessment the state of coastal marine areas under technogenic pollution of bottom biotopes.

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Popularization of science and engineering through case study activities at the Primorsky Aquarium

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Popularization of scientific ideas, engineering solutions and social initiatives in the area of the World Ocean exploration, sustainable use of nature resources and natural heritage protection become an important priority of modern cultural and creative industries, which include the activities of aquariums.

In what way do we introduce modern students to the wonderlands of science? In the field of popularizing science and engineering for a wide audience, we think it possible to offer two main approaches. One of them simplifies scientific ideas and inventions to vulgarization, and acquaintance with them turns into a show of scientific miracles or a sphere of thoughtless entertainment. Another approach involves demonstrating science and engineering as a strenuous intellectual activity that not only generates new knowledge and helps people solve their problems, but also causes bright positive emotions, motivates people to seek and discover the truth, brings pleasure from the meaningful knowledge application, brings joy from using resources of their own intelligence, and inspires collective creative work. We need to search for and implement the practices of high-tech education, enlightenment and popularization, inspiring students to engage in science and technology, preparing them for the future. Such practices include educational research and projects. With proper organization, research and project activities can not only provide new knowledge and form the creative skills of students, but also promote their personal and professional self-determination in science-intensive, high-tech and socially important areas of activity.

The partnership project called 'Research and Project Ideas Lab' developed by the Environment Education Service of the Primorsky Aquarium which is under way since 2020, focuses on involving grades 8–11 students in working on current tasks of Russian science and high-tech technologies, orienting students to promising labor markets in their native region through support of educational research and projects.

The main objectives of the project:

- familiarization of students with modern research, technological solutions of the best scientific, industrial, environmental, cultural and educational organizations of the Primorsky Krai (Territory);
- inclusion of human, intellectual and technological resources of scientific, innovative organizations and institutions of culture and education in the structure of research and project activities of students;

- organization of assistance to students in choosing topics of educational research and projects that are interesting, significant, promising and relevant for implementation;
- providing methodological support to school teams in acquiring knowledge and skills in research and project work;
- opening an opportunity to receive professional advice from specialized experts;
- offering methodological support to teachers – curators of school teams;
- creating conditions for demonstrating research and project skills as part of participating in the project competition program.

The project tested the practice of organizing research and project activities of students using case technology. The following partner organizations of the Primorsky Krai took part in the development of cases, accompanied by the implementation of case tasks by students: Botanical Garden-Institute of the Far Eastern Branch, Russian Academy of Sciences (FEB RAS); Far Eastern State Marine Reserve; National Park "Land of the Leopard"; G.I. Nevelskoy Maritime State University; V.K. Arsenyev Museum of the History of the Far East; A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS; LLC "Composite Architecture"; LLC "Fakir"; Primorsky Aquarium – Branch of the NSCMB FEB RAS; V.I. Ilyichev Pacific Oceanological Institute FEB RAS.

The cases developed according to a single template contain a description of specific situations: facts and scientific data are given; real problems are formulated that the organization is working to solve; approaches and solutions to these problems are specified. Completing case assignments requires students to show initiative, creative attitude, independence, and the use of research and project skills. School teams need to analyze the recommended information and offer the best solution to the problem. The project uses three types of case tasks: research, business plan, and social project.

The main results of the project:

- 2020–2021 school year has seen more than 200 students from 18 municipalities of the Primorsky Krai involved in the project;
- 14 cases were developed, 16 webinars were held;
- a collection of scientific and methodological project materials "Research and Project Ideas Lab": case practices of ecological and environmental activities" has been prepared;
- methodological tools for popularizing scientific, cultural and innovative activities based on the use of case studies have been developed, tested and implemented;
- social networking services have been tested for remote support of research and project work of students;
- a successful practice of interaction of the Primorsky Aquarium with schools and partner organizations has been developed.

Participation in the project expands the educational space of the school, transforms scientific and innovative content into an educational resource, offers solutions for organizing project and research activities of students based on relevant regional topics.

The project developers see a significant potential for using case studies to be applied to popularizing science and science-intensive technologies.

Methods of enhancing the biodiversity of Russian Far East marine fishes in the Primorsky Aquarium

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Despite high level of public aquarium industry development and application of modern methods for fish catching, transporting, and keeping, many boreal fishes have not been displayed yet, sometimes because of their secretive life and sometimes due to high sensitivity of their adult individuals to external environment. Additionally, most commercial aquaria prioritize entertainment of visitors and thus feature eye-catching exhibits with colourful tropical species. The Primorsky Aquarium, located on the coast of the Sea of Japan, believes that one of its main goals is to distribute information about fish living in the northern Pacific. Of 118 tanks and aquaria, more than third (46) are placed in the exhibits on the Russian Far East seas.

The most frequently applied method of replenishing live exhibits for aquaria is taking younger fish from the wild. The younger the fish are, the better they adapt to changes in environment and the less stressed they become while being transported or transferred to different aquaria. Other means to increase the biodiversity in the exhibits of the Primorsky Aquarium are fish propagation and raising fish from eggs, larvae and juveniles caught in the coastal waters. Slightly more than a third (37) of the 90 Russian Far East marine species of fish have been caught only mature. Most of the fishes have also been collected at early stages of their ontogenesis, particularly as larvae. It has been found that the closer to settlement the larvae are taken, the higher is their survival rate. However, some species, for example Osmeridae (smelt) and Engraulidae (anchovy), have larvae that are very sensitive to catching and transporting at any age. Microalgae, the rotifers *Brachious plicatilis*, or nauplii of the brine shrimp *Artemia salina* have been given as food to fish larvae and juveniles in accordance with their life stage.

Rearing of marine fishes in aquaria is always rather difficult, and one of the complications is that there is almost no information on what are the exact conditions for rearing a particular species. Hence, every detail on how to reproduce and raise marine fishes is of high practical, as well as of theoretical interest.

Fish eggs are regularly collected from the coastal zone of Russky Island and Muraviev-Amursky Peninsula, and up to now the larvae of 24 fish species from 10 families have hatched from these eggs: sandfishes (Trichodontidae) – 1 species, sand

eels (Hypoptychidae) – 1 species, halfbeaks (Hemirhamphidae) – 1 species, gunnels (Pholidae) – 1 species, toadfishes (Psychrolutidae) – 1 species, poachers (Agonidae) – 1 species, sea snails (Liparidae) – 2 species, cottids (Cottidae) – 7 species, pricklebacks (Stichaeidae) – 9 species. Many of these fishes spawn from late autumn to winter, and the larvae hatch in winter or spring. Others spawn in spring, and hatching takes place in late spring. In winter water temperature in Peter the Great Bay goes below zero. The temperature of incubation in the Primorsky Aquarium is several degrees higher, hence, the eggs develop faster, and the larvae hatch one to two months earlier than in the wild. If everything goes right, these larvae also grow faster, to a size suitable for being exhibited.

Seven species of Russian Far East marine fish have spawned in the Primorsky Aquarium: the Agassiz's snailfish *Liparis agassizii* (fam. Liparidae), two sculpins – the tentacled sculpin *Porocottus allisi* and the Derjavin's sculpin *Radulinopsis derjavini* (fam. Cottidae), the green cockscomb *Alectrias benjamini* (fam. Stichaeidae), and two greenling species, the masked greenling *Hexagrammos octogrammus* and the arabesque greenling *Pleurogrammus azonus* (fam. Hexagrammidae).

The larvae of the Agassiz's snailfish (SL 10–15 mm) were caught in April and May in Zhitkov Bay. They became mature in autumn at a length (SL) of 226–385 mm, and their fecundity was about 50 000 eggs. They spawned in the Aquarium from late December to early April at a temperature from 1.2 to 2.8°C while, according to published data, in Peter the Great Bay they spawned when the water temperature was below zero, from December till January. It has been reported that the ratio between the sexes should be 1:1 for normal reproduction. This ratio is perfect to eliminate competition between males happening if they are too many and to ensure that females will spawn all their eggs (if there are not enough males, females cannot spawn well and may die). The larvae of the Agassiz's snailfish are pelagic, and in three weeks after hatching they begin to settle.

The larvae of over 100 fish species from 29 families have been taken in Zhitkov Bay of Russky Island. They have been scooped with dip nets out from eelgrass beds near the shore or collected with light traps from the middle of the bay by specialists of the Laboratory of Ichthyology of the NSCMB FEB RAS. The larvae of 50 species have been successfully reared and their ontogenesis monitored in aquaria of the Science and Acclimation Building. Development of early stages in some local fish species has been scarcely studied up to now, and the observations of the ontogenesis allow identifying the taxonomic positions of the larvae and juveniles as they grow. If a species identified is absent from the exhibits, its larvae and juveniles are raised up to reach suitable sizes and then transferred to one of the public display aquaria of the Main Building.

On the whole, the three-year collaboration between the Primorsky Aquarium and the Laboratory of Ichthyology of the NSCMB FEB RAS has proven to be useful for both sides. On the one hand, it enables considerable expansion of ontogenetic studies for fishes inhabiting Peter the Great Bay; on the other hand, it greatly enhances the biodiversity in the exhibits of the Primorsky Aquarium.

The diversity of fish retinal ganglion cells and ways to study it

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Retinal ganglion cells (GC) are output retinal neurons. They participate in visual signal processing and send information to the brain. In all vertebrates including fishes, they come in several types differing in structure and function. Different GC types form parallel pathways conducting different types of visual information to the respective brain centers. Classical approaches to fish GC classification were based on a limited number of traits and focused on either GC structure or physiology. More recent classifications consider a larger number of parameters and use automated algorithms to identify potential GC types. The available data suggest that there may be at least 15–20 GC types in the fish retina. The major differences between them include dendritic field size, level of dendrite stratification in the retina, dendrite course and arborization, overall structural complexity, receptive field size, type of response, preferable stimuli, and central projections. At least large (and probably all) fish GC types are non-randomly distributed in the retina forming so-called mosaics. Neighboring cells of the same type "respect" each other's space, while those of different types do not do so. Some GC types are present in all fish species studied, while others are only found in a limited number of species. In the lecture, both classical and up-to-date approaches to GC classification will be considered, and available fish GC classifications will be reviewed.

Molecular markers of adult neurogenesis in the brain of trout, *Oncorhynchus mikiss*

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The distribution of several molecular markers in the telencephalon, tectum, cerebellum, and brainstem of trout *Oncorhynchus mikiss*, identifying neural stem/progenitor cells, neuroblasts, and radial glia was investigated. The presence of all these types of cells determines specific conditions for the development of processes of adult neurogenesis in the brain of a 2 year old trout *O. mikiss*. The distribution of glutamine synthetase (GS), a molecular marker of neural stem cells (NSCs) in the telencephalon of trout, revealed a large population of radial glia (RG), corresponding to the adult-type NSCs. Radial glia predominated in the pallial region of the telencephalon, while in the subpallial region, RG was found in the lateral and ventral zones. In the optic tectum RG fibers were widespread and localized both in the marginal layer and in the periventriclure gray layer. In the cerebellum, a heterogeneous population of GS-producing cells was identified: in the lateral and dorsolateral regions of the cerebellum GS-producing glutamatergic cells were identified, which are obviously involved in the metabolism of glutamate. Another population is represented by Purkinje cells in the ganglionic layer of the basolateral part of the cerebellar body, corresponding to projection glutamatergic neurons. GS-producing cells of the neuroepithelial type involved in constitutive neurogenesis were identified in the dorsal matrix zone (DMZ) and the superficial layers of granular eminence and in the periventricular layer. Doublecortin immunolabeling revealed a large population of neuroblasts formed in the postembryonic period, which is indicative of intense adult neurogenesis in the trout brain. The pallial and subpallial regions of the telencephalon contained numerous DC+ cells and their clusters. In the tectum, DC+ cells were found not only in the periventricular gray layer (SGP) and longitudinal torus (TL) containing proliferating cells, but also in the layers containing differentiated neurons: the central gray layer (SGC), periventricular gray and white layers (SGAP) and superficial white layer (SSA) DC+ cells were identified. In the cerebellum, a heterogeneous population of DC + cells was revealed, both in the dorsal (DMZ) and basal (BMZ) matrix zones, and in the granular layer of the cerebellar body and granular eminences. The features of DC+ cells in the cerebellum make it possible to distinguish differentiated and undifferentiated cell phenotypes. In the brainstem of trout, a population of DC+ cells was identified in the viscero-sensor nuclei of the cranial nerves and motor parts of the reticular formation, which indicates the emergence of cells of this type in the postembryonic period. The study of the localization patterns of vimentin and nestin

in the telencephalon and tectum of trout showed the presence of neuroepithelial neural stem cells (eNSCs) in the periventricular matrix zones of the brain. In the cerebellum and brainstem of trout, however, in the region of the *crista cerebellaris*, vim+ RG were identified, which indicates the presence of adult neural stem cells (aNSCs) in this region of the brain. Nestin+ RG cells were detected in the dorsal reticular formation and near the IV ventricular zone. Another feature of the expression of vimentin and nestin in the projection neurons of the cerebellum and brainstem was detected. Expression of vimentin was identified in the main nucleus of the trigeminal nerve, reticulo-spinal cells and neurons of the ventro-lateral part of the reticular formation, and nestin was also found in the nucleus of VII nerve. In the cerebellum of an adult trout, constitutive neurogenic niches were identified containing vim+ eNSCs in the periventricular region and granular eminences, as well as populations of large vims+ Purkinje projection neurons and evrydendroid cells (EDCs) in the dorsal and lateral zones of the cerebellar body. Nestin+ cells were found in granular eminences and cerebellar DMZ. The presence of vimentin and nestin in functionally heterogeneous cell types of adult trout opens up new functional properties of these proteins and their heterogeneous participation in intracellular motility and adult neurogenesis.

Species composition and distribution of pelagic larvae of bivalves in Far Eastern Marine Reserve

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Taxonomic composition and distribution in plankton of bivalve mollusks larvae, the most abundant group of meroplankton organisms, have been already studied in Peter the Great Bay (Sea of Japan) in sufficient detail (Korn, Kulikova, 1997; Kulikova et al., 2013, 2014; and others). At the same time, the main attention is devoted to the larval plankton of Amursky, Ussuriysky and Vostok bays. In the southern part of Peter the Great Bay (Possjet Bay), studies were carried out mainly with regard to the larvae of commercial bivalves (Kolotukhina, Semenikhina, 1998; Radovets, 2007; Radovets, Khristoforova, 2008; and others). Much less attention has been paid to the study of slightly polluted reserved waters. Thus, there is some information on meroplankton status in some areas of southern and eastern regions of the Far Eastern Marine Reserve (Kulikova et al., 2004; Kolotukhina et al., 2015).

The aim of the work was to study taxonomic composition and distribution of larvae of bivalve mollusks in plankton of eastern region of the Far Eastern Marine Reserve.

The work was carried out in mid-July 2019 at 8 stations located at Lev Cape, in Srednyaya, Astafieva and Spaseniya bays, at Hildebrandt Island and at Bolshoi Pelis Island: in Zapadnaya and Molchansky bays and at the southern tip of the island. Plankton samples were taken by plankton net in the upper 10 m water layer, except for the station located in Zapadnaya Bay, where the sampling depth was 7 m only. A total of 24 plankton samples were collected and processed. The material was processed by standard method (Kulikova et al., 2013, 2014). The earliest bivalve larvae at the veliger stage were not identified due to their small size. In identifying of the larvae at a later stage (the "velikonch stage"), identification guides and literature data were used (Kasyanov et al., 1983; and others).

At the same time measurements of temperature of surface water layers with a Checktem thermometer were conducted. Also, water samples were taken for hydrochemical analysis, which was carried out according to the accepted methodology (Summer training field practice for the quality of natural waters assessing, 2010). The relationship between the quantitative distribution of larvae and hydrochemical parameters was determined using Microsoft Excel using the Pearson correlation coefficient.

In total, 36 taxa of bivalve larvae were identified in plankton, among them 25 were identified to the species, 4 to the genus, and 7 to the family. Larvae of early stages

and larvae, the identification of which caused difficulties, were attributed to the group Bivalvia sp. The total number of larvae varied from 717 ind./m^3 at station 6, located at Hildebrandt Island, up to 2580 ind./m^3 at station 8 in Astafieva Bay. Most of all were *Swiftopecten swiftii* (Bernardi, 1858), *Crassostrea gigas* (Thunberg, 1793) and family Hiatellidae – up to 1175–1415, 448–460, and 143 ind./m^3, respectively. At the same time, peaks in the density of *S. swiftii* larvae were noted at the southern tip of Bolshoi Pelis Island and in Molchansky Bay. Maximum numbers of the oyster larvae were recorded in Astafieva and Srednyaya bays. Peak density of the Hiatellidae larvae was noted in Srednyaya Bay. The taxonomical composition was dominated by representatives of family Mytillidae: *Mytilus trossulus* (A.A. Gould, 1850), *Mytilus galloprovincialis* (Lamarck, 1819), *Mytilus coruscus* (Gould, 1861), *Crenomytilus grayanus* (Dunker, 1853), *Modiolus kurilensis* (F.R. Bernard, 1983), *Modiolus modiolus* (Linnaeus, 1758), *Adula falcatoides* (Habe, 1955), *Musculista senhousia* (Benson, 1842), *Septifer keenae* (Nomura, 1936) and not identified to the species larvae of the family.

Their number was: 7–64 ind./m^3 – for *M. trossulus*, 12–50 ind./m^3 – for *M. coruscus*, 3–36 ind./m^3 – for *C. grayanus*, 2–66 ind./m^3 – for *M. kurilensis*, 2–43 ind./m^3 – for *M. modiolus*, 2–45 ind./m^3 – for *A. falcatoides*, 5–22 ind./m^3 – for *S. keenae*. At the same time, most of the larvae of *M. trossulus* and *S. keenae* were recorded in Srednyaya Bay, *M. coruscus* and both species of modiolus – in Zapadnaya and Molchansky bays of Bolshoi Pelis Island, *C. grayanus* in Astafieva Bay, *A. falcatoides* – in Spaseniya Bay. Larvae of *M. galloprovincialis* and *M. senhousia* were rare, early of mytilids larvae were found in the amount of 2–32 ind./m^3.

The next in terms of species diversity was family Mactridae: *Mactra chinensis* Philippi, 1846, *Mactra veneriformis* (Reeve, 1854), *Spisula sachalinensis* (Schrenck, 1862), *Mactromeris polynyma* (Stimpson, 1860) and not identified larvae of the family. The number of their larvae was 2–13 ind./m^3 in Srednyaya and Spaseniya bays.

Family Veneridae included three representatives in plankton: *Ruditapes philippinarum* (Adams et Reeve, 1850), *Mercenaria stimpsoni* (Gould, 1861) and *Protothaca* sp. Their number was: 7–20 ind./m^3 for *R. philippinarum*, 7–60 ind./m^3 for *M. stimpsoni*, 3–88 ind./m^3 for *Protothaca* sp. The abundance of *R. philippinarum* was maximal in Srednyaya Bay, *M. stimpsoni* – near Hildebrandt Island, *Protothaca* sp. – in Astafieva Bay.

Family Pectinidae incorporated larvae of three species: *S. swiftii*, *Chlamys farreri* (Jones et Preston, 1904), *Mizuhopecten yessoensis* (Jay, 1857) and not identified larvae of the family. The larvae of *C. farreri* and *M. yessoensis* were found in the amount of 2–16 ind./m^3. Scallops larvae were found in Srednyaya, Spaseniya, and Astafieva bays.

The families: Glycymerididae, Lasaeidae, Kelliellidae, Teredinidae, Solenidae, Pholadidae, Myidae, Cardiidae and Tellinidae each included one or two representatives in plankton. Among them, the larvae of *Glycymeris yessoensis* (G.B. Sowerby III, 1889) were the most abundant – up to 56 ind./m^3 in Molchansky Bay. Larvae of *Kellia*

japonica (Pilsbry, 1895), *Alveinus ojianus* (Yokoyama, 1927), *Teredo navalis* (Linnaeus, 1758), *Solen krusensterni* (Schrenck, 1867), *Zirfaea pilsbryi* (H.N. Lowe, 1931), *Mysella* sp., *Mya* sp., *Pholas* sp., and larvae of the families Cardiidae and Tellinidae were found in amount of 2–23 ind./m^3 in bays of Bolshoi Pelis Island, in Srednyaya, Spaseniya, Astafieva bays, near Lev Cape and Hildebrandt Island.

The significant prevalence of *S. swiftii* larvae in plankton is obviously associated with the presence of adult settlements of the species in eastern part of the reserve (Lebedev, 2015). Most of the species that we have noted are also typical inhabitants of the protected water areas (Skarlato, 1967; Lebedev et al., 2004; and others). However, some of the studied larvae belong to the species that are not included in the list of adult mollusks inhabiting Peter the Great Bay due to their rare occurrence, habitat in the fouling biotope, or drift by currents from adjacent waters. These are *T. navalis*, *M. veneriformis*, and *M. modiolus*. Comparison of the obtained results with the data for July 2012 (Ivin et al., 2014; Kolotukhina et al., 2015) showed that the species composition of bivalve larvae in meroplankton of protected areas has changed a little. However, in 2012 larvae of Mactridae family, *R. philippinarum*, *S. krusensterni*, and *K. japonica*, prevailed. The maximum density of larvae (over 2000 ind./m^3) was provided by *A. ojanus*, *M. senhousia*, *R. philippinarum* and Polychaeta. Such differences can be caused both by changes in hydrological and hydrochemical regimes of the area and by natural interannual fluctuations in the abundance of larvae of bivalves (Kulikova et al., 2013, 2014; and others). The Pearson correlation coefficient showed the presence of a weak positive relationship between the number of larvae in plankton and the temperature of surface water layers (R=0.30). The average BOD_5 value for the area was 0.93 mg O_2/L, which indicates the relative purity of the studied region.

Study of benthic communities of the Piip Volcano by fatty acid trophic markers

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Fatty acids (FAs), which are major constituents of most lipids, are particularly useful trophic markers. They are generally synthesized at low trophic levels, with certain FAs being characteristic of specific groups of microorganisms: bacteria, phytoplankton, macroalgae, vascular plants or zooplankton. These marker FAs accumulate with minimal modification in consumers tissue and can be traced through the food web providing insight into trophic relationships. Therefore, FAs have been applied successfully for revealing trophic relationships between pelagic and benthic organisms and in particular deep-sea benthos (Dalsgaard et al., 2003; Howell et al., 2003; Drazen et al., 2008a, b; Kelly, Scheibling, 2012; Kharlamenko et al., 2013).

On cruise 82 voyage of the R/V *Akademik M.A. Lavrentyev*, comprehensive studies of ecosystems associated with hydrothermal vents and cold seeps in the Bering Sea were carried out. The researchers believe that fluid secretions lead to a significant increase in the productivity of bottom waters and sediment due to the production of chemosynthesis and methanotrophy (Galkin, Ivin, 2019; Rybakova et al., 2020). However, the role of bacterial organic matter in animal nutrition and its transmission routes to high trophic levels have not yet been studied. To estimate the trophic relationships between benthic animals and understand the ecological processes that determine the structural and functional biodiversity in the communities of the Piip Volcano method based on FA markers was applied.

With this aim, the FA composition of the most abundant megabenthos species was determined, including new species which have not been the subject of similar studies. Special attention was paid to polyunsaturated FAs (PUFAs) – phytoplankton markers and the monounsaturated FAs (MUFA) – bacterial markers. Since methane is the main gas of the hydrothermal fluid in this ecosystem, confirmation of the contribution of methanotrophy in representatives of megafauna was primarily looked for. However, the specific FA markers (16:1n-6; 16:1n-8) characterized for methanotrophs (Bowman et al., 1993) were not found in the tissues of all the studied animals. Thus, the hypothesis about the possible influence of methanotrophic bacteria that could serve as food for benthic fauna and support the existence of this ecosystem has not been confirmed. On the other hand, animals of several taxonomic groups: Bivalva, Gastropoda, Isopoda, Leptostraca and Amphipoda have high level MUFA (16:1n-7 and 18:1n-7) – markers of sulfide-oxidizing chemoautotrophic bacteria (Nichols et al., 1987; Bowman

et al., 1993). The proportion of these two acids in FA composition of bacterial mats in the hydrothermal vents and cold seeps in the Bering Sea reaches 41.7% and indicates the dominance of sulfur bacteria. Thus, it can be confidently stated that these animals feed at the expense of organic matter created by bacteria in the process of chemosynthesis on the basis of reduced components of hydrothermal fluids. Other animals from the background substrate, have the high content of PUFA – phytoplankton markers (Dalsgaard et al., 2003; Kelly, Scheibling, 2012), which clearly indicates the photosynthetic origin of their food. The analysis of FA composition allows dividing the animals of the background landscape into several trophic groups: detritophages, sestonophages, predators. The obtained results show that the proportion of organic matter of photosynthetic origin was different for different groups of animals. The animals in each of these groups have their own characteristic features of FA composition. Epizoanthus, Actiniaria, Corallimorpharia have a high content of zooplankton markers (C20:1-22:1) and PUFA n-3, feeding from suspension with fresher food (crustaceans). Amphipods, isopods depend on bacteria, but some of the food is obtained by eating live or dead hydrothermal macro and meiobenthos animals.

In general, the results of this study emphasize the complexity of food chains in the ecosystem of the Piip Volcano and reinforce the need for a more detailed description of the trophic niches occupied by each species and their interactions.

Environmental drivers of benthic community composition in the NW Pacific and the Arctic Ocean

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In this study we examine patterns of benthic community composition and species richness along the NW Pacific and Arctic Seas, and show a decreasing trends towards the higher latitudes and deeper waters, peaking in coastal waters of the eastern Philippines. The most dominant taxa were Arthropoda, Mollusca, Cnidaria, Echinodermata, and Annelida. We found that depth, not temperature, is the main driver of community composition along the NW Pacific and neighboring Arctic Seas. Depth has been previously suggested as a factor driving speciation in benthic fauna. Following depth, the most influential environmental drivers of community composition along the NW Pacific and the Arctic Ocean (AO) were Silicate, light, and currents. For example, Silicate in Hexactinellida, Holothuroidea and Ophiuroidea; and light in Cephalopoda and Gymnolaemata had the highest correlation with benthic distribution and community composition. In this study we show that different benthic communities might respond differently to future climatic changes based on the taxon-specific biology, physiology, and ecology characteristics. The management of species for conservation and habitat preservation should take an adaptive approach and apply measures that take the differences of different benthic communities in responding to the future climate changes into account. This multiple-scale prioritization approach facilitates better understanding of the impact of future climate-driven environmental changes on community composition that is crucial for implementing appropriate management strategies for the conservation and sustainable utilization of the NW Pacific and Arctic marine ecosystems.

Ecological forms of the Primorie`s cherry salmon (*Oncorhynchus masou* (Brevoort))

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The coastal cherry salmon (one of the masou groups from the Sea of Japan mainland coastal rivers) lives in all the rivers flowing down from the eastern slopes of the Sikhote-Alin mountain system and the East Manchurian Mountain region. Almost all masou groups develop according to the anadromous type: breeding occurs on a stony-pebble substrate in large tributaries, or in the upper part of mountain rivers, feeding of juveniles continues in the river environment for one or two years, smoltification in the river and seaward migration occurs in the spring. For spawning, masou enters the mountain rivers from the sea at different times: in the north – from second half of May to early June, and in the south of the Primorie territory, the spawning grounds are filled from the end of August to the end of September. These differences in reproduction timing are due to the presence of two seasonal ecological forms (races) in the anadromous masou: there are cherry salmon race with early spawning in the north and late spawning in the south of Primorie territory. Previous studies have shown significant differences between them in many morphological indicators. According to the exterior indicator, a high-bodied and low-bodied masou are distinguished.

It was found that the young masou from an early age has a different rate of linear growth. The main part of the juveniles develops according to the anadromous type, but some individuals are ahead of their peers in terms of the linear growth intensity and the speed of puberty. From such individuals, precocious males are later formed, maturing in rivers, never going out to sea. These males actively participate in spawning with anadromous females. There are 2–6 precocious males within the spawning nest area and each female. As a rule, they all die after spawning.

Very rarely, males are observed on spawning grounds, which are noticeably larger than the masou parr young, and precocious (neotenic) males. These are salmon migrating to rivers (male jackies). When studying the scales of such fish, their life history was deciphered. On the sclerites of fish scales, characteristic spawning marks are formed, which indicate their spawning at the parr stage. Other males do not have similar marks. Such fish, after living in the river, enter the marine environment, and then return again and take part in spawning with anadromous females.

In the 1980s, in the reservoirs of the Artemovsky hydroelectric complex, residential form of masou was discovered. There are 14 females with a body weight of 92 to 1750 g

were captured. These fish lived for more than four years long without going to sea, near Vladivostok, the riverine sima was preserved in freshwater systems isolated from the sea in the basins of the Pionerskaya and Bogataya rivers and locked drinking reservoirs.

There are 4 ecological forms among the masou groups in the southern part of the range. They are divided at the epigenetic level: 1. the **anadromous form**, spends one or two years in the river and after the sea period matures in the river, and dies after spawning. 2. **precocious form of males**. Males mature at the parr stage in the river without going out to sea. They they die after the first or second spawning, as well as anadromous males. 3. intermediate, **rapidly maturing male form** (male jackies). In size, they occupy an average position between anadromous and precocious males. After the river period, they feed in the sea for several months before maturing, and then all die after spawning. 4. the **riverine form** of both sexes individuals. Formation of mature individuals is possible in this grouping, similar to the those masou, which always develops according to the anadromous type. Females are divided into two groups: anadromous and very rarely – riverine.

Livestock experiments on the masou in a closed water supply cycle conditions were conducted in the Primorsky Aquarium in Russian Island, Vladivostok. Cherry salmon individuals that were caught in the rivers of Southern Primorie were raised in tanks with certain parameters of water composition and lighting mode. The temperature of 8–12°C remained shortly after capture, then a few days later it was raised to 12°C. The fish evolved and grew very quickly. A year later, at the age of 2+, rapid maturation began in individuals of both sexes. Maturation was accompanied by changes in the body color, the mating color began to appear. Some individuals were transferred to the content in salt water, but the mating color and maturation process in these fish was preserved. When the period of full maturation and life cycle ended, all individuals died, as well as all wild individuals in nature. Our task was not to provide conditions for the spawning of mature masou individuals. The riverine masou group was formed in the locked Pioneer drinking reservoir (Sedanka River basin) after its isolation from the sea since 1936. To date, the riverbed has preserved its small population and natural spawning grounds. In winter, it lives within reservoir area. Masou, caught in the Sedanka River, and then grown up in the Primorsky Aquarium conditions, began to develop rapidly according to its anadromous type. The strategy of existence in the "river–lake–river" system was replaced by the previous strategy of anadromous salmon "river–sea–river". Since fish with different life strategies are not reproductively isolated, we should not expect them to form stable genetic differences.

So, Cherry salmon in its species range, from the northern towards the southern border, has significant changes in life strategies. From the northern coastal rivers (Tumnin. Koppi, Samarga basins), to the south – (rivers of Peter the Great Bay), the percentage of precocious males is rapidly increasing, from 4 to 46%. Further south, new elements in the biological structure of populations and resident forms appear. In China, in the lower right tributary of the Tumangan River (Yijiang River), the northernmost grouping of

residential riverine Cherry salmon population occurs. If we move further to the southern border of the genus *Oncorhynchus* range, we see the emergence of species derived from the wild masou. There are young species *O. rhodurus* appears in Biwa Lake (Japan), and *O. masou formosanus* riverine population on Taiwan Island. This fact necessitates a deeper study of the new Pacific salmon forms formation mechanisms with its full life cycle, including methods of molecular genetics. Masou salmon in the Amur River basin on the northern border of its range along the mainland coast has not been studied at all.

Herpetological collection of the Primorsky Aquarium

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The collection of amphibians and reptiles of the Tropical Rain Forest Department was started in 2014, two years before the Aquarium was opened, and included two amphibian and two reptile species at that time. Now this collection contains 109 species.

In 2016 the Tropical Rain Forest exhibit had five large equally-sized terraria. However, it is constantly developing, and has significantly grown for the past seven years, being regularly renovated and thus always stirring up visitors' interest. Amphibians, reptiles, and arthropods of tropical rain forest are now displayed in 23 terraria of different sizes.

The main goals of the Primorsky Aquarium's herpetological collection are: to show the diversity of tropical forest fauna to visitors, to study the biology of the animals kept and to breed rare and endangered species.

These goals determine the following tasks to perform:

- to add new species to the collection regularly;
- to develop the best techniques of animal care and to provide the conditions enabling animals to live as naturally as possible;
- to develop breeding techniques;
- to diagnose diseases and traumas, provide veterinary care.

The Tropical Rain Forest Department staff members raise live food for the animals displayed in the exhibit, which guarantees high quality of the diet, good health and successful reproduction.

The offspring from more than a half of the captive animals has been received. The breeding of amphibians is much more complicated than that of reptiles. The first step is to prepare them through hibernation, which stimulates maturation of the gonads. The hibernation period has two stages, the first is a gradual decrease in the temperature, humidity and amount of food, and the second is a stepwise increase in these values. When males are sexually ready, they start to produce advertisement calls (vocalization). Both males and females prepared for reproduction are moved into a rain chamber-special enclosure filled with water and provided with an artificial rain system. The ratio between males and females should be determined according to species. After amplexus, egg-releasing and fertilization of eggs, the adults are relocated to their terraria and the tadpoles are raised in the rain chamber. Sometimes their enclosures are dried up to stimulate metamorphosis.

The herpetological collection of the Primorsky Aquarium is completely ready to provide animals for research carried out at its Shared Equipment Facility and for exchange with other zoos and aquaria.

Ecosystems of aquatic extreme habitats: diversity and common features

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In the biosphere, there is a wide variety of aquatic extreme habitats (hypersaline, thermal, alkaline, acidic, underground, subglacial, ultraoligotrophic, etc.), which occupy a significant part of the hydrosphere. For a long time, they were perceived as something exotic, not paying due attention to their study. In recent years, the situation has changed due to the trends governing the development of environmental science and the growing interest in the use of their biological resources. Existence in an inhospitable environment requires various adaptations, including biochemical ones. Therefore, the interest of aquaculture and biotechnology specialists in the aquatic organisms inhabiting them for the creation of new technologies is understandable. Summarizing the author's long-term experience in studying extreme ecosystems, primarily hypersaline waters, as well as the data available in the world literature, the authors give a brief overview of extreme aquatic habitats. Based on this, general patterns in the formation of the structure and functioning of ecosystems of such habitats are formulated. It is shown that extreme habitats, as a rule, are extreme not by one factor, but by several at once, i.e. poly-extreme. This is considered in detail in this report for cases of hypersaline waters (surface, deep-sea, and sub-ice) and black smokers. An important feature of extreme ecosystems is a large diversification of energy input to them, in comparison with 'normal' aquatic ecosystems (marine, freshwater). It involves three mechanisms of phototrophy (bacterial proton pump, anoxygenic and oxygenic photosynthesis), as well as a wide variety of chemolithotrophic mechanisms. The extremities for existence in such habitats are often added by their high temporal variability. The ability of populations, communities, and ecosystems to exist in a wide range of environmental factors is ensured by the fact that they can exist in alternative states. Most aquatic organisms that exist under such conditions have dormant stages, which ensure conservation in the most severe periods, incompatible with the existence of active stages.

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Some adaptations of pricklebacks (family Stichaeidae) to reproduction in Peter the Great Bay (the Sea of Japan)

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Fishes of the family Stichaeidae are widely distributed in waters of the North Pacific. The Sea of Japan is home to up to 38 species of this family, of which 23 species have been recorded from Peter the Great Bay. Members of the family Stichaeidae inhabit mostly shallow waters, the intertidal and subtidal zones, but some species are found also on the continental slope. Larvae of pricklebacks are pelagic, show pronounced phototaxis and, thus, can concentrate near a light source, which allows catching them with the use of illumination equipment in the dark. These species can disperse quite widely through pelagic larvae despite the sedentary life of their adults. The duration of the pelagic stage can range from 2–3 weeks to several months, depending on the species and habitat conditions. As our study shows, this characteristic is quite variable in some species.

Our ichthyoplankton survey in Zhitkov Bay, Russky Island, in the period from March to December 2017–2020 with the use of fish-luring systems, light traps, surface hauls of a IKS-80 plankton net, and also diving operations in the period 2019–2020, made it possible to study the dynamics of the planktonic stage of development in 17 identified stichaeid fish species. The timing of spawning and hatching were determined for some of them.

It has been found that some prickebacks in the waters of Peter the Great Bay, which is characterized by a wide variety of habitat conditions, show a spawning strategy and timing of early development that are not typical for them in other parts of the range, and can be conditionally divided into three groups: (1) species spawning in autumn, hatching in autumn–winter, and having a prolonged planktonic stage of development; (2) species spawning in autumn or winter, hatching in late winter and early spring, and having a planktonic stage from 1 to 3 months; (3) species spawning in spring, hatching from April to June, and having a planktonic stage lasting from 2 weeks to 1 month. The same species exposed to milder hydrological conditions, e.g., off southern Hokkaido Island or northern Honshu Island, where water temperatures in winter do not fall below 1–2°C, spawn typically at a later time (winter–spring) but at a similar temperature. Their larvae hatch earlier in winter and spring and more rapidly complete the planktonic stage of development at higher temperatures.

Typical representatives of group 1 in Peter the Great Bay are two species of the genus *Chirolophis*. Species of the genera *Opistocentrus* and *Lumpenus*, and also the species *Alectrias cirratus* and *Stichaeus ohriamkini*, can be attributed to group 2. Group 3 includes representatives of the genus *Stichaeus* (except *S. ohriamkini*), *Stihaeopsis*, *Ernogrammus* and also the *Alectrias benjamini.*

Thus, due to the ecological plasticity (a shift in the spawning period, a change in the duration of incubation and the timing of the pelagic stage), species of this family not only can spawn under optimal temperature conditions, but also either survive the adverse winter period at the stage of eggs, or use for early stages of development favorable feeding conditions in the second half of the autumn. For some species that have small eggs and spawn in rapidly warming shallow water, it became more advantageous to use the period of sharp spring warming with the subsequent peak in abundance of forage plankton for breeding. Due to these factors, the time of incubation and, accordingly, the protection of offspring, as well as the time of the planktonic larvae stage, are significantly reduced.

The Primorsky Aquarium: modern trends in its educational and awareness-raising activities

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The Primorsky Aquarium is a branch of the A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences. The Aquarium was open to general public on September 3rd, 2016. The integral part of the Aquarium is an educational block consisting of lecture rooms, learning laboratories, a movie theatre and a library equipped with modern equipment. A conference hall, classrooms and exhibit collections facilitate the implementation of unique educational programs on marine biology and ecology. The Aquarium serves as a teaching ground for preschool and school children and university students providing them with an opportunity to participate in scientific research process.

The first educational project, *Lesson at the Aquarium*, was launched right after the opening of the Aquarium in October, 2016. The concept of the *Lesson at the Aquarium* project implies a single class or a series of classes held in the framework of a particular school subject on the basis of the Aquarium exhibits for the purpose of in-depth study of the subject. The lesson necessarily corresponds to particular themes from the school curriculum and includes additional research and creative tasks beyond the curriculum.

On the basis of the Federal State Educational Standards a total of 13 lessons have been developed for children of primary, secondary and upper secondary schools on such subjects as Science, Biology and Geography.

Distinctive features of the project classes are as follows:

- Classes are held at one or, rarely, two of the exhibits;
- Each lesson has its scenario reflecting the specifics of the exhibit and a developed worksheet;
- During the classes a special emphasis is made on self-directed work of pupils;
- Children have an opportunity to work with educational films (the 'Evolution of Life in the Ocean' and 'Rivers and Lakes' exhibits), microscopes (the 'Microworld' exhibit), a touch pool and interactive displays ('The Sea of Japan' exhibit);
- Pupils are enabled to perform research in the learning laboratories.

Being conducted at the Aquarium exhibits, the classes give children a chance to see live marine and freshwater animals in conditions most similar to the ones of their natural habitats. The content of the Aquarium exhibits corresponds to the themes of

the lessons and includes topics on nature protection and commercial fishery. To investigate microorganisms and cellular texture of different organisms, the Aquarium has learning laboratories with modern microscopy equipment.

The Aquarium classes are to be attended by an organized group of school children accompanied by their teacher and one parent. On average, over 400 schoolchildren take *Lessons at the Aquarium* exhibits every Wednesday. During the implementation of the project almost 30,000 children have taken part in it. To date, the *Lesson at the Aquarium* has developed into the *Educational Environment* project and gained larger audience. On Wednesdays the Aquarium is visited by groups of preschoolers for whom our specialists hold entertaining excursions.

The Aquarium is developing cooperation with secondary and higher educational institutions of Vladivostok and Primorsky Krai. University students specializing in such fields as Biology, Ecology, Ichthyology, Architecture and Engineering are given lectures by their teachers at the Aquarium exhibits. The Russian language classes conducted at the Aquarium are extremely popular among foreign students studying Biology in various universities of Vladivostok. The significant feature of this language program is its applicability: students study the specialty language being surrounded by aquatic inhabitants. Such interactive lectures contribute to a better assimilation of both vocabulary and biological knowledge.

The second large scale project of the Primorsky Aquarium, *Growing up at the Aquarium*, began its work in October 2018. Its main idea is to raise humanity in children aged 5–15 through the creation of an educational space which is focused on the manifestation of such qualities such as kindness, empathy, compassion, responsibility, and helps to develop in children personal responsibility and cognitive interest in the science of marine biology.

The *Growing up at the Aquarium* project objectives are as follows:

- To acquaint children with science as a way of knowing the world;
- To generate conditions for cultivating environmental literacy among children aged 5–15;
- To create conditions for raising children's awareness about the unicity of aquatic inhabitants and forming careful attitude to them;
- To provide necessary conditions for nurturing in children aged 5–15 the best human qualities, team working skills and personal responsibility in interaction with Nature on the basis of their cognitive interest in marine biology;
- To produce an emotive environment for children with disabilities;
- To implement programs, the content of which is based on the child's cognitive interest in marine biology in the context of its continuous study.

The project implies the continuous study of marine biology and consists of 4 learning stages, each of them is designed for children of particular age and will be held from October to May. At each stage children get information from various fields of marine biology.

The first of them is "Belyok", an environmental biology studio for children aged 5 to 7, which started its work 3 years ago. Here children get acquainted with such professions as biologist-divers, marine mammal trainers, marine biologists, paleontologists, ichthyologists that are related to providing the aquatic dwellers of the Aquarium with comfortable living conditions.

One of the main goals of the kids' studio is to instill a positive attitude to the environment in children and lead them to understanding of intrinsic value of any living being. Since most studio alumni want to know more about the aquatic world and its inhabitants, they pass to the new learning stages of the *Growing up at the Aquarium* project.

The following stage, a "Sea Guardians" art studio, is designed for children at the age of 8–9. Its purpose is to cultivate empathy in a child. Pupils will learn about the harmony of the surrounding world and see the beauty of marine inhabitants. With the help of collaborative and individual art projects children will understand how to tell people about the problems of the World Ocean, and what each individual can do to protect it. The participants will express their feelings through creative art works and share their experiences with peers within scheduled meetings.

The third learning stage, "Ichthyology Club", is aimed at children aged 10–11. During the classes children will "dive" into the world of aquarium fish to become familiar with their behaviors. The pupils will learn how to produce conditions favorable to certain fish species and collectively choose plants and fishes to add to their common fish tank. This stage is designed to plunge a pupil into research activity and involve him into the process of creating a habitat comfortable for his/her pet fish. A child who knows special aspects of keeping of aquatic inhabitants will never do any harm to them and learn what 'taking care of someone' means.

"AQUAlaboratory", the fourth stage of the project, invites school children aged 12 and older and is intended to develop personal responsibility and team working skills in children. The classes will be held in the Aquarium's learning laboratories equipped with research microscopes. Children will study microscopic organisms constituting plankton and investigate a complex life cycle of marine invertebrates and specifics of their anatomy.

One of the "AQUAlaboratory" sections is dedicated to the evolution of life in the ocean. Using the paleontological collection and exhibits of the Aquarium, pupils will trace how anthropods, mollusks and chordates have been changing in the course of millions of years. The stage also includes paleontological excursions to the Zhitkov Peninsula of Russky Island where Triassic layers crop out. A separate section of the program deals with dinosaurs and ichthyosaurs, and the final part of the stage is dedicated to man and his place on Earth.

The project is designed to bring up small biologists, starting with children of preschool age. And even if in future the project alumni choose some other professions, not connected with natural sciences, they will turn into environmentally educated people taking good care of our planet.

Pattern of effects of environmental factors on shell growth rates in the scallop *Mizuhopecten yessoensis*

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Understanding of tolerance limits and optimal ranges of values of environmental factors for a species is important for addressing many theoretical and practical issues, including, in particular, cultivation. The major environmental factors that affect growth of marine mollusks are water temperature and salinity, as well as food composition and abundance.

Shell growth rates in relationship to seasonal changes of environmental factors were studied in a wild population of the commercial Yesso scallop *Mizuhopecten yessoensis* (Jay, 1857) inhabiting Amur Bay (Peter the Great Bay, Sea of Japan, Russia). The growth rates of the scallop shell have been found to change significantly during the year, in accordance with the seasonal variations in the environmental parameters. However, not all factors are of equal ecological importance for scallop.

It appeared that the main exogenous factors causing the seasonal variations in the scallop growth rates are the water temperature, which was too high in July and August (>18°C) and too low in December–March (<4°C), and the water salinity, which was too low (<30 psu) for this stenohaline species in summer.

The relationships between the daily shell increments of Yesso scallop and the studied environment factors are obviously nonlinear. The relationship of the daily shell increments in Yesso scallop with the water temperature can be described by a dome-shaped curve. The largest increments were observed at 8–16°C.

The dependence of daily shell increments on the water salinity was also best described by a dome-shaped curve, showing the optimal range of 32.5–33.5 psu. A significant reduction in shell growth rates was observed when the salinity decreased below 32.0 psu. Low values of daily shell increments at high water salinity, 34.0 psu, were most likely due to the low winter water temperature for the species, since the stable water salinity of approximately 34.0 psu in the study area is usually observed during the cold season. This is a result of the combined simultaneous effects of various factors on scallops in the natural environment.

It was found that food availability is not a limiting factor for the scallop growth in the study area: a phytoplankton biomass of 3.5–6.0 g m^{-3} provided high scallop growth rates. The largest daily shell increments were observed with a phytoplankton biomass of about 6 g m^{-3}. With a decrease in the phytoplankton biomass to <2 g m^{-3}, as well as

with an increase to >11 g m^{-3}, the daily shell increments reduced. The decrease in scallop growth rates with a phytoplankton biomass <2.0 g m^{-3} can be explained by insufficient food availability for the species. It is suggested that the large aggregates of phytoplankton (>11 g m^{-3}) led to clogging of the scallop gills. The revealed relationship, approximated by dome-shaped curves, evidently indicates that both insufficient and excessive effect of the factor negatively affects scallop growth.

In wild populations, even under optimal values of one or more factors, scallop daily shell increments can be extremely low. These are the examples of a combined simultaneous effect of various factors on an organism in nature. According to the Leibig's law of the minimum, the Blekhman's law of limiting factors and the Shelford's law of tolerance (the law of optimum), the success of an organism depends on a complex of conditions in the habitat, while the inability to thrive is determined by an insufficiency or, conversely, an excess of any factor. Therefore, based on the results obtained and on these well-known laws, I propose to describe mathematically the result of the multiplicative (combined) effect of several environmental factors on the daily shell increment as a product of the functions of its dependence on each of the factors:

$$I_t = a \times F(x_1) \times F(x_2) \times F(x_3) \times ...,$$

where I_t is the daily shell increment for a t-th day. Constant a is the maximum possible daily shell increment for the species in the study area. $F(x_i)$ is the function of the dependence of the daily shell increment on the i-th factor. x_i is the value of the i-th factor in t-th day.

To successfully use the proposed model, the following conditions should be satisfied for the selection of functions: (1) $0 \leq F(x_i) \leq 1$; (2) with the optimum value of the i-th factor (x_{iopt}), this function should reach the maximum, i.e. $F(x_{iopt}) = 1$, or close to 1; (3) when approaching the lower or upper limit of the i-th factor in the tolerance range for the species, $F(x_i)$ should tend to 0; (4) the types of functions are determined from empirical observations. When these conditions for selecting the type of functions are met, the growth rates will be maximum (equal or close to the constant a) only under the full set of favorable factors; if there is a lack or excess of at least one of the factors, growth will decelerate to some extent.

Hemocytes of the Manila clam *Ruditapes philippinarum* (Adams et Reeve, 1850) as a potential biomarker of heavy metal pollution in marine environment monitoring

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To estimate the potential of Manila clam hemocytes as a biomarker for heavy metal pollution, we studied the cellular composition of the hemolymph, hemocyte DNA content, and heavy metal content in Manila clams from Novik Bay.

Thirty-two clams were collected in mid-July 2018 at four different sites in Novik Bay (Uzkiy Cape, Cape of Elagin, Cape of Staritsky, and a site near the settlement of Podnozhie). Hemolymph was taken from the adductor muscle using a 1 ml syringe with a 21-gauge needle containing 0.3 M EDTA solution to prevent cell aggregation. Hemolymph samples were fixed in an equal volume of 8% paraformaldehyde solution (PFA) so that hemolymph cells were suspended in 4% PFA. For morphological analysis and classification of hemocytes, PFA-fixed cell suspensions were partially dropped on slides, air dried, stained with azure-eosin after Romanowsky–Giemsa, and examined with a light AxioImager A1 microscope (Carl Zeiss). The number and shape of cell nuclei were also visually examined. Another part of the fixed hemolymph was used for flow cytometry. The cell suspensions were standardized to ~10^6 cells per 1 ml of 0.02 M phosphate buffered saline (PBS), washed twice in PBS and analyzed with an Accuri C6 flow cytometer (Becton Dickinson). The forward and side light scattering values (FSC and SSC) were estimated to determine the cell size and complexity. Cell aggregates were discriminated on the dot-plot FSC-A vs FSC-H. For DNA content analysis, PFA-fixed cell suspension of *Ruditapes philippinarum*'s spermatozoa obtained from the spawning gonads was added (~10^5 cells) to each hemolymph sample as a haploid standard. The mixed suspensions were stained with 0.0001% DAPI solution and analyzed with a CytoFLEX flow cytometer (Beckman Coulter). The heavy metal (Fe, Mn, Cu, Zn, Cd, and Ni) content was determined in tissues of the Manila clams used in the haemolymph analysis, as well as of other bivalves sampled in the same sites. To do so, the molluscs were dried at a temperature of 85°C for 2–3 days until no noticeable changes in weight could be detected. The tissue specimens were mechanically homogenized and analyzed with a Shimadzu AA-6800 atomic absorption spectrophotometer.

Proceeding from the microscopic analysis, five structural hemocyte types were identified, 1) blast-like cells, 2) hyalinocytes, 3) basophilic granulocytes, 4) neutrophilic

granulocytes, and 5) eosinophilic granulocytes. By means of flow cytometry, hemocytes were divided into three "subpopulations" according to their forward and side light scattering values, 1) region R1 (small cells of low complexity), 2) region R2 (large cells of intermediate complexity), and 3) region R3 (large cells of high complexity). The number of hemocytes assigned to different structural types was compared to the flow cytometry results using Spearman's correlation analysis. The comparison suggests that R1 is composed by blast-like cells and small hyalinocytes; R2, by a mix of large hyalinocytes, slightly granular basophilic granulocytes, and degranulated eosinophilic granulocytes; and R3, by the largest granulocytes of different affinity containing multiple and/or highly refractory inclusions.

The proportions of blast-like cells, hyalinocytes, and granulocytes in the hemolymph of Manila clams from all sites varied greatly and were not correlated to the heavy metal levels in mollusc tissues. For example, the greatest Fe levels were detected in the molluscs from the Cape of Elagin and Podnozhie sites, but their hemograms differed dramatically showing the predominance of hyalinocytes and eosinophilic granulocytes in the first and second samples, respectively. On the other hand, the hemograms of the specimens from Uzkiy Cape and Cape of Elagin were generally similar although the former had significantly lower Cd levels than the latter. At the same time, the clams from Uzkiy Cape displayed the similarly high Mn levels comparable to those from Cape of Elagin and Podnozhie. Of all the samples, the molluscs from Cape of Staritsky displayed the lowest metal levels while their hemograms had intermediate parameters. We propose that high haemogram variability in the Manila clam population in Novik Bay is related to the differences in the immune status or/and asynchronous spawning of its constituent subpopulations rather than differences in heavy metal impact.

The analysis of DNA content showed that the distribution of cells in R1, unlike those in other regions, corresponds to that of the mitotic cycle. Blast-like cells displaying high proliferative activity (as determined by flow DNA cytometry) were apparently divided into two size groups. The proportion of R1-cells in the active phases of the cell cycle (S, G_2, and M) was significantly positively correlated with the number of blast-like cells ($r=0.518$, $p<0.05$); however, none of these parameters were correlated with heavy metal content. Among all structural types, binucleated hemocytes were found, as well as cells with a pleomorphic nucleus. Some nuclei contained heterogeneous chromatin or displayed signs of karyorexis and karyolysis. Deviant DNA profiles were regularly observed in samples from all sites. In some specimens, a portion of cycling R1-cells showed a decrease in the DNA content. Their frequency of occurrence histogram contained an additional peak closed to 3 *c* position in the middle of the S-phase plateau and demonstrated a selective loss of the G_1/M-cells. This pattern may indicate either aneuploidy or apoptosis; both phenomena are known to be associated with genotoxic heavy metal effects and are widely used as a tool for detecting continuous water pollution. These two scenarios are complementary rather than mutually exclusive. Hemocytes can receive an altered number of chromosomes as a result of mitotic

errors; afterwards, these cells are eliminated by apoptosis or differentiate ignoring the cell cycle checkpoints. When aligning DNA profiles of blast-like cells (region R1) and differentiated cells (regions R2 and R3), the peak of differentiated cells was occasionally shifted "to the right" (relative to the diploid G_1-value of blast-like cells) or even reached tri- and tetraploid values. In such specimens, the proportion of hemocytes with doubled and pleomorphic nuclei increased; they comprised both young hyalinocytes and mature eosinophilic granulocytes. All these genetic abnormalities may be due to toxic effects of heavy metals. In Novik Bay, *R. philippinarum*, as well as *Callista brevisiphonata*, showed low potential for bioaccumulation compared with *Modiolus kurilensis* and *Panopea abrupta.* At all locations presently examined, maximum permissible concentrations of heavy metals were not exceeded. However, the level of bioaccumulated metals in mollusc tissues does not always correlate directly with their content in the environment. In *R. philippinarum*, aneuploidy displayed stronger correlation with sediment pollution than with bioaccumulation level. Thereby, the examination of aneuploidy and other DNA cell cycle alterations in Manila clam hemocytes may be used for marine environmental monitoring in impact waters of the Far East.

Maintenance and cultivation of some exhibiting algae species (*Saccharina japonica, Saccharina cichorioides, Undaria pinnatifida, Sargassum pallidum, Sargassum myiabei, Cystoseira crassipes, Codium yezoense, Ulva fenestrata*)

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Seaweeds, among other macrophytes, are widely used in expositions of the Primorsky Aquarium both for their natural beauty and as elements necessary for normal life activity of hydrobionts. Due to seasonality of our climate, it is very difficult or even impossible to replenish expositions with wild algae in late autumn, winter and early spring. To solve this problem in the Primorsky Aquarium we have worked out methods of keeping or growing some basic algae species used in exposition aquariums.

Japanese kelp and cichorioides (*Saccharina japonica*, *Saccharina cichorioides*) are species for which the full cycle cultivation technique has been worked out. Kelp zoospores are obtained from large kelp thalli (from 2 m and larger). Rope lines are used as a substrate for the zoospores settling and gametophytes and sporophytes further cultivation. When young sporophytes reach 10–15 cm in size, they are thinned out and grown further on vertically placed rope lines under active illumination and water exchange until they reach the right exposing size.

Undaria pinnatifida also belongs to the species for which the full cycle cultivation technique has been perfected. In general, the process is almost exactly the same as in kelp cultivation. The difference is in the substrate used both for settling of zoospores and further gamete and sporophyte cultivation. Experiments showed that the best substrate for seed settling is the giant oyster (*Crassostrea gigas*) flaps.

Sargassum pallidum, *Sargassum myiabei*, and *Cystoseira crassipes* are species for which only the rearing of juvenile thalli and the preservation of adults are carried out. Young algae collected in wild nature are reared on natural substrate under controlled conditions. These species large thallus are mostly collected without substrate and kept either free-floating or planted on fixed horizontal rope lines, which gives better results. This technique helps to keep the algae in an active vegetative state for a long time due to steady light and water exchange and avoid tangling between separate alga strings, resulting in less trauma during transplanting.

Codium yezoense is a species that propagates vegetative. Adult algae are divided into small pieces (size 5–7 cm), which are planted on the substrate in quite warm low-flow water (t=14–18°C). As to substrate, the best results are achieved with Mytilidae, oyster shells and small pebbles. After a new thallus has fully attached to the substrate in about a month, active growth begins.

Ulva fenestrata also belongs to the species reproducing by thallus division. In general, the technique of cultivation and propagation of this species practically does not differ from that of codium, except for binding to a certain type of substrate, which allows Ulva planting on vertical rope lines (also together with Laminaria).

At present, we are working on optimal conditions for *Costaria costata* and *Agarum clathratum* cultivation.

Assessment of the hepatoprotective effect of lipid complex from marine red algae *Ahnfeltia tobuchiensis* against CCl_4 induced hepatotoxicity in mice

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Liver is an important organ that metabolizes toxins, synthesizes nutrients, and promotes hematopoiesis. Due to these functions, liver ailments continue to be among the main threats to public health. Nowadays, people have increased liver load because of the work pressure, imbalanced diet, excessive drinking, and viral infections, which causes a series of liver diseases. The metabolism by the liver of various chemical agents such as carbon tetrachloride (CCl_4) cause severe damage to the liver cells over time.

Although liver disease is caused by a variety of different etiologies, the pathogenesis of chronic liver disease is relatively uniform. The healthy liver is prompted by various triggers (extensive alcohol abuse, hepatitis infection, metabolic disorders, genetic diseases, environmental toxins and drug consumption) to undergo hepatic fibrogenesis resulting in an injured, fibrotic liver. The prevalence of liver diseases necessitates effective and cost-efficient treatments.

One of the best candidates for preventing of such liver pathologies could be lipid complexes containing essential phospholipids with fatty acids substitutes represented by the n-3 fatty acids with one of the mechanism of action to be through cellular membrane stabilization.

The marine environment may be explored as a rich source for novel antifibrotic drugs. A number of marine derived compounds are shown to prevent formation of the reactive oxygen species (ROS) and possess anti-inflammatory activity. Marine derived Omega-3 long-chain polyunsaturated fatty acids (Omega-3 PUFAs) especially; eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have long been reported for their valuable hepatoprotective effects and their ability to decrease hepatic injury. They have a variety of proposed mechanisms of action; the most significant of which would be; modulating cell proliferation, regulating fatty acid metabolism, inhibiting lipogenesis as well as suppressing inflammation and oxidative stress.

In the course of searching for hepatoprotective (HP) agents of lipid nature from marine sources, we evaluated the lipid complex of marine red alga, *Ahnfeltia tobuchiensis* (AT) for HP activity. Algae samples were collected in the autumn in coastal waters of Alekseeva bay, Popov Island, Peter the Great bay (Sea of Japan). Isolation of the lipid complex was carried out by the standard Bligh and Dyer method. Obtained lipid extract from AT (ATL) contained 56% of membrane active fraction (sum of glyco- (GL)- and

phospholipids (PL)). PL and GL made 25.7 and 30.5% of total lipids accordingly. Fraction of PL consisted of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatydilinosit (PI). PC and PE are among the most important structural bricks of membranes that are providing the spatial organization of the membrane matrix and the functioning of membrane structures. The fatty acids pattern of GL and PL was presented mainly by unsaturated fatty acids where prevailed PUFA of omega-3 (α-linolenic and eicosapentaenoic fatty acids) and omega-6 (linoleic and arachidonic fatty acids) groups. Monounsaturated fatty acids were represented by oleic acid and palmitic was the main among saturated ones.

HP potential of the obtained preparation was assessed using the model of CCl_4 induced liver damage in outbred male mice (25–30g). All animals were housed in a well-ventilated environment, received standard mice food pellets and water was provided ad libitum throughout the experimental period. Mice were injected in the dorsal neck fold with 2 ml/kg of a 50% solution of CCl_4 in olive oil for 4 days. ATL was administered intragastrically in a dose of 80 mg of total lipids/kg of body mass. Mice were divided into four groups of ten animals. Group 1 was normal control, group 2 – CCl_4 group; group 3 – CCl_4+withdrawal for 7 days; group 4 – CCl_4+ ATL for seven days.

After 4 days, the administration of CCl_4 produced severe liver damage characterized by significant elevation ($P<0.001$) in serum ALT by 400%, liver Mass Index (LMI) by 35% and total lipids amount in the liver by 3 times, compared with the control group. The neutral lipids pattern in the liver was changed to the direction of an increase by 20–24% ($p<0.01$) in the amount of triacylglycerols (TAG), cholesterol (Chol), and free fatty acids (FFA) due to enhanced flow of lipids due to the peripheral lipolysis in fat tissue – stress response to toxin.

The mechanism behind CCl_4-hepatotoxicity appears to be oxidative stress initiated by CCl_4-derived reactive free radical metabolites, including trichloromethyl (CCl_3) and trichloromethyl peroxyl ($OOCCl_3$). The former is responsible for the covalent binding to cell components like GSH, resulting in its depletion, whereas the latter initiates the lipid peroxidation process with the consequent formation of by-products such as, TBARS, resulting in loss of membrane integrity and fibrosis development.

We observed double increase in the TBARS level ($p<0.001$). Hepatic reduced glutathione (GSH) content and the level of antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GRx), Superoxide dismutase (SOD) were strikingly depleted (by 25–48%, $p<0.01$) in CCl_4-intoxicated mice, compared with the normal rats. Also, we observed reduction of the levels of the main structural phospholipids PC and PE by 7–12% ($p<0.01$) with concurrently increasing by 58–70% ($p<0.001$) of lyso-PC and lyso-PE. This indicated a high level of free radical generation due to reducing dehalogenation of CCl_4 by CYP_2E_1, which are actively included in the phospholipid fatty acid chains, disorganizing the structure of cellular membranes.

After 7 days of CCl_4 withdrawal, most of the controled biochemical parameters in the mice liver (group 3) did not recover to the control value, indicating a continuing toxic impact and insufficiency of the body resources to counteract the toxic pathology.

Furthermore, the level of the antioxidant enzymes GPx, GRx, SOD as well as GSH content were expressed by further reduction. The neutral lipids pattern remains misbalanced, which indicates further progress of catabolic processes in the liver. Serum ALT remains increased by 2 times ($p<0.001$), while the level of PC and PE remains reduced and even underwent to the further declining while lyso-forms of these phospholipids continue to grow up, indicating the high level the structure disorder of cellular membranes.

The administration of the ATL during CCl_4 withdrawal (group 4) exhibited significant HP activity by reducing the CCl_4 caused changes and led to the recovery of almost all studied biochemical parameters to the control value. We observed a restoration of the LMI and total lipids amount and neutral lipids pattern balance, which means improvement of fatty liver. TBARS level returned to normal and as well as the level of antioxidant enzymes GPx, GRx, SOD and GSH pool together with the level of PC and PE which indicates the reduction of the free radical activity and restoration of the spatial organization of the membrane matrix.

The results obtained by this study suggest that a lipid complex enriched with marine phospholipids and omega-3 fatty acids from AT is a promising source for effective hepatoprotectors of lipid nature.

Synaptamide provides anti-inflammatory and analgesic effects after the peripheral nervous system injury in rats

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Polyunsaturated fatty acids (PUFA) are neuroprotective compounds that can directly or indirectly modulate neurological activity in peripheral and central nervous system due to their "classic" anti-inflammatory properties. Docosahexaenoic acid (DHA) is a PUFA that is metabolized in the central nervous system into highly active compounds, such as resolvins and neuroprotectins, associated with both regulation of the inflammatory process and structural and metabolic integrity recovery in injured neural tissue. At present, there is a growing interest in the study of the neurotropic activity of polyunsaturated fatty acid ethanolamides (N-acylethanolamines). N-docosahexaenoylethanolamine (DHEA), or synaptamide, an endogenous metabolite of docosahexaenoic acid, is a promising compound with anti-inflammatory activity. Synaptamide is a structural analogue of N-arachidonylethanolamine (anandamide), the potent endocannabinoid produced from arachidonic acid. Anandamide has antinociceptive effects mediated predominately through the cannabinoid receptor 1 (CB1). However, it is believed that synaptamide does not primarily function as an endocannabinoid, mainly due to its weak binding to cannabinoid receptors. Synaptamide is known to have neurogenic, neuritogenic and synaptogenic effects in neuronal cell cultures, as well as inhibit the LPS-induced release of proinflammatory cytokines in microglial cells. Synaptamide activates the G-protein coupled receptor GPR110 (ADGRF1), leading to an increase in cAMP production in neural cells. Considering the anti-inflammatory potential of synaptamide and other N-acylethanolamines, it is suggested that these compounds could be used for the treatment of multiple conditions in the nervous system, including neuroinflammation and neuropathic pain.

Chronic constriction injury (CCI) of rat right hind limb sciatic nerve was used as a model of neuropathic pain syndrome, which is accompanied by inflammation, sensitization, and activation of nociceptive neurons in the spinal cord. In the present study, we hypothesize that administration of synaptamide reduces neuroinflammation and oxidative stress, which contributes to the attenuation of neuropathic pain by inhibiting the NO and substance P (SP) release.

The experimental rats were randomly divided into groups: "Sham+vehicle" – vehicle-treated sham-operated animals (n=14); "Sham+Syn" – synaptamide-treated sham-operated rats (n=14); "CCI+vehicle" – vehicle-treated animals with chronic constriction injury of the sciatic nerve (n=14); "CCI+Syn" – synaptamide-treated rats with chronic

constriction injury of the sciatic nerve (n=14). Sham-operated rats underwent sciatic nerve exposure surgery without ligating the nerve trunk. The effect of synaptamide (4 mg/kg/day, 35 days) on neuropathic pain development was examined by the cold allodynia and mechanical hyperalgesia testing. Spinal cord samples were extracted for immunohistochemical studies on ionized calcium-binding adaptor molecule 1 (iba-1, microglia marker), glial fibrillary acidic protein (GFAP, astroglia marker), neuronal nitric oxide synthase (nNOS) and Substance P (SP) on 35th day after the surgery. Sciatic nerve samples were examined for myelin basic protein (MBP) staining. The concentration of interleukin 1 beta (IL-1β) and interleukin 6 (IL-6) in the lumbar spinal cord was determined by ELISA. Astrocyte culture was used to evaluate the activity of antioxidant enzyme superoxide dismutase (SOD) following synaptamide treatment.

Behavioral testing revealed a sharp increase in cold allodynia and mechanical hyperalgesia as early as the first week after surgery in "CCI+vehicle" group, whereas in "CCI+Syn" group these indicators were significantly less pronounced in several time-points. Synaptamide administration enhanced remyelination process in the site of sciatic nerve injury (33.4±1.1% in "CCI+Syn", compared to 28.4±0.9% in "CCI+vehicle" group). Further, synaptamide suppressed the CCI-induced increase in the activity of iba-1+ microglia (13.1±0.5% in "CCI+Syn", compared to 15.3±0.7% in "CCI+vehicle" group) and the number of nNOS+ neurons (58307±5206 cells per mm^3 in "CCI+Syn", compared to 80288±4287 cells per mm^3 in "CCI+vehicle" group) in the dorsal horns of the spinal cord, and also reduced the concentration of IL-1β in the spinal cord (169.3±4 pg/mg of protein in "CCI+Syn", compared to 236.9±9.3 pg/mg of protein in "CCI+vehicle" group) 35 days after surgery. Moreover, synaptamide treatment resulted in decrease of reactive astrogliosis in the spinal cord dorsal horns to 20.8±1.3%, which occurred simultaneously with a decrease in the SP level (9.8±0.5%) compared to vehicle-treated animals (30.2±2.2% and 13.4±0.9% for GFAP and SP staining area, respectively). In addition, synaptamide increased SOD activity up to 68.6±0.8%, compared to control (50.6±0.9%) in astrocyte culture. Thus, synaptamide provides anti-inflammatory and neuroprotective effects in both peripheral and central nervous system after the sciatic nerve injury.

Currently, various PUFAs and some of their highly active metabolites are used in multiple studies focused on the treatment of the peripheral and central nervous system injuries. However, there are very few studies that use synaptamide, and they are mostly limited by studying its effects on axonal growth, synaptogenesis, and neural stem cells development. Considering previously described anti-inflammatory properties of synaptamide and its effect on pain hypersensitivity and the properties of glial cells, observed in our study, synaptamide is a promising compound for the treatment of the peripheral nervous system injuries.

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Constitutive and reparative neurogenesis in the cerebellum of juvenile masu salmon, *Oncorhynchus masou*

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Teleosts are a useful model for studying the mechanisms that provide processes of constitutive neurogenesis and neuronal regeneration, because the formation of new cells in their central nervous system (CNS) continues throughout life. The aim of our work was to study the ratio of radial glia cells and neuroepithelial cells in the cerebellum of juvenile *Oncorhynchus masou* and their participation in the regulation of reparative neurogenesis. We also investigated participation in the processes of neuronal regeneration glutamine synthetase and hydrogen sulfide. To achieve this goal, we analyzed the comparative distribution of the expression of glutamine synthetase, vimentin, nestin and cystathionine β-synthase in the cerebellum of juvenile chum salmon *O. masou* two days after applying mechanical damage. To study, immunoperoxidase labeling was performed on frozen, free floating brain sections. The study was carried out on 45 one-year-old juveniles of the masu salmon *O. keta* with a body length of 11–13 cm and a weight of 25–35 g.

At two days after a traumatic brain damage to the cerebellum of juvenile masu salmon, the expression of nestin and vimentin was revealed in many neurogenic zones, containing neuroepithelial and glia cerebellar precursors. They differed in their topographic features and cellular composition. It was found that reorganization of neuroepithelial constitutive neurogenic niches and the formation of reactive neurogenic niches heterogeneous in their cellular composition occurred in all areas of the cerebellum. In our opinion, reflects different neurogenic abilities, as well as plastic properties of various neuroanatomical zones of the brain. Precursors of both types are the main sources of neurons, playing an important role in the plasticity during ontogenesis and CNS homeostasis in the cerebellum of juvenile *O. keta*. The detection of neuroepithelial cells and radial glia cells is an important finding, as it shows a certain proportion of embryonic and adult precursors. These facts show different contributions of these cells in the acute post-traumatic period. After a mechanical damage lost neurons are actively replaced due to the proliferative activity of radial glia cells and neuroepithelial cells in the neurogenic zone of cerebellum. The heterogeneous pattern of progenitor cells and the different mechanisms of activation of such cells are critically important for the formation of adequate post-traumatic programs associated with neuronal regeneration.

In the acute post-traumatic period the expression of glutamine synthetase, a neuronal stem cells molecular marker, was detected in heterogeneous pool of cells located

within the neurogenic niches. They have mostly a neuroepithelial phenotype, along with which precursors of the glial phenotype. The maximum number of glutamine synthetase-positive radial glia cells was found as part of heterogeneous neurogenic niches. In order to utilize exclude the increasing excitotoxicity accompanied by an intense neuroimmune reaction of secondary inflammation and the toxic effects of glutamate, cells were activated in the cerebellum of juvenile *O. masou*, which regulated the cerebellar post-traumatic homeostasis of glutamate.

Hydrogen sulfide (H_2S) is a proneurogenic factor, producing by cystathionine β-synthase, which reduces destructive excitotoxicity effects and oxidative stress. In the brain cells of juvenile chum salmon H_2S increased glutamine synthetase production. Also, H_2S may be involved in maintaining the microenvironment in neurogenic zones. Thus provides optimal circumstances for the functioning of neurogenic niches during constitutive and reparative neurogenesis. In the cerebellum of juvenile *O. masou* we studied the H_2S distribution, being the enzyme that catalyzes the reaction producing H_2S from L-Cysteinein, as well as in fish after a traumatic brain damage. At two days after a traumatic brain damage to the cerebellum presence of cystathionine β-synthase showed the expression in neural progenitors in the cerebellum of juvenile *O. masou*. The number of cystathionine β-synthase-positive cells increased multifold in the acute post-traumatic period. Induction of cystathionine -synthase in large cells indicates the involvement of H_2S in the decrease in the production of reactive oxygen species, weakening of the toxic effects of extracellular glutamate and also detention of the oxidative stress development and a reduction in excitotoxicity. We consider H_2S as an active factor, the numerous known effects of which can be supplemented by involvement in the regulation of constitutive and reparative neurogenesis. This suggests a synergistic neutralizing effect of glutamine synthetase and H_2S, aimed at reducing the toxic effects of glutamate. We consider such effects as neuroprotective, significantly affecting the cellular microenvironment and their participation in the processes of neuronal regeneration.

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Biological oceanography and the development of IOCAS

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The Institute of Oceanology, Chinese Academy of Sciences (IOCAS) is a comprehensive oceanographic research institution, it developed from a Laboratory of marine biology. Biological oceanography plays an important role in the development of the institute. 2020 was the occasion of the 70th anniversary of the IOCAS, to review its development history from the perspective of biological oceanography will deepen our understanding of the connotation, rule of development and the importance of interdisciplinary research in marine science. It will be helpful for us to keep clear mind, have a right development strategy and take the leadership in marine science, especially in the situation of demand for supporting to blue economy and challenges from the increase of marine institutions.

Long term changes of plankton in China coast

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Based on the comprehensive analysis of the response of the China coastal ecosystems to global climate change in recent 20–30 years, the direct and indirect impacts of climate change on plankton in China's coast were comprehensively assessed. Under the background of global warming, the phytoplankton community structure was changed obviously. The species number and abundance of dinoflagellates were increasing gradually, and the dominant position of diatom in the community was declining. In the northern part of China coast, some warm-water phytoplankton species expanded northward, and the proportion of warm-water species in total phytoplankton community increased. The proportion of micro-phytoplankton decreased in some areas, while the proportion of nano-phytoplankton increased. For the zooplankton, the small jellyfish group was the group with the most significant changes among the zooplankton functional groups. In the past two decades, the pelagic ecosystem showed the phenomenon and trend of "gelatinization", and the outbreaks of small jellyfish, tunicates and other gelatinous organisms occurred frequently. Some warm-water species of zooplankton appeared northward. It was found that the abundance, distribution and seasonal change of the abundance peak of zooplankton in China coast were closely correlated with the large-scale climate index, and the correlation was positive or negative depending on the species, functional group and sea area.

Benthic foraminifera of the genus *Ammonia* from the intertidal sandy/silty bottom of Sishili Bay, Yellow Sea

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Modern benthic foraminifera from the intertidal zone of Sishili Bay, Yellow Sea coast (northern China), were studied in three areas. There the intertidal zone is mainly represented by sandy beaches and sandy/silty bottom. Samples of bottom sediments for studying foraminifera from these areas were collected at 9 stations of 3 transects. Species of the genus *Ammonia* constitute the major part of the foraminifera fauna on the intertidal silty/sandy bottom of Sishili Bay (from 70 to more than 90% of the total foraminifera assemblage). Their abundance varies significantly depending on the part of the intertidal zone. The smallest number of specimens, usually single live individuals, was recorded from the upper, drier, layer of the zone. The largest number of tests of this genus was recorded from the middle layer. The foraminifera fauna in all samples from the middle and lower layers of the silty/sandy intertidal zone is abundant and consists mainly of live individuals. When processing this material, we faced a major challenge in identification of species belonging to the genus *Ammonia*. One of the most abundant and widely distributed species among modern foraminifera is *Ammonia beccarii* (Cushman, 1931). Its range covers shallow waters of the southern and northern seas, the Atlantic and Pacific coasts, and coastal areas of ocean islands. Due to such cosmopolitanism and an extremely high degree of variability of forms, many experts worldwide express doubts that these forms belong to a single species, *A. beccarii*. Identification of its various morphotypes has been the subject of serious debates over the past 50 years. Recent studies by B.W. Hayward, M. Holzmann, J. Pawlowski and other leading researchers have contributed to establishing the significant morphological criteria that will help us identify the *Ammonia* morphotypes from Sishili Bay.

Effect of N-docosahexanoylethanolamine obtained from squid *Berryteuthis magister* on hippocampal plasticity in a murine model of neuroinflammation

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Chronic neuroinflammation is an integral pathogenetic component in the development of various neurological and neurodegenerative diseases. A detailed study of the neuroinflammation mechanisms and the development of drugs that reverse the neuroinflammation negative effect on memory and cognitive processes are priority areas in modern neurobiology and neuropharmacology. One of the promising pharmacologically active compounds is N-docosahexanoylethanolamine (DHEA, synaptamide), a derivative of docosahexaenoic acid, the source of which is a variety of marine organisms. Synaptamide is an endogenous metabolite and structural analogue of anandamide, an essential endocannabinoid derived from arachidonic acid. The very small number of studies devoted to the biological activity of synaptamide dictates the need for further detailed studies of such a promising compound. Our study is aimed to elucidate the synaptamide pharmacological activity in neuroinflammation caused by bacterial lipopolysaccharide (LPS) stimulation. LPS-induced neuroinflammtion is a common model, which simulates neuroinflammation in the development of many neurological diseases. We used it to study the effect of synaptamide on the memory state and the main functional, morphological and biochemical parameters of experimental animals.

N-docosahexanoylethanolamine was obtained from by-products of squid *Berryteuthis magister* caught in the Bering Sea. Male C57BL/6 mice (3 months old) were used for the experiments. Neuroinflammation was induced by intraperitoneal (i.p.) injections of bacterial lipopolysaccharides (LPS, *Escherichia coli* O111:B4, Sigma-Aldrich, St. Louis, MO, USA). The mice (n=80) were divided into the following treatment groups: "Veh" (n=20) – i.p. saline and water by subcutaneous (s.q.) injection; "LPS" (n=20) – i.p. LPS and water by s.q. injection; "LPS+Syn" (n=20) – i.p. LPS and synaptamide by s.q. injection; "Syn" (n=20) – i.p. saline and synaptamide by s.q. injection. The i.p. saline or LPS (750 mg/kg) injections were administered for seven consecutive days. Synaptamide was injected as an aqueous emulsion at a dose of 10 mg/kg daily for seven consecutive days. Working memory in mice was assessed using the spontaneous alternation test in the Y-maze. We used the passive avoidance test to assess the effects of

neuroinflammation and synaptamide treatment on the long-term memory of experimental animals. In addition, long-term memory was assessed with the novel object recognition test. Electrophysiological recording was performed to evaluate the effect of synaptamide on hippocampal synaptic plasticity in neuroinflammation. To assess LPS- and synaptamide induced changes in neuronal morphology Golgi–Cox staining followed by Sholl analysis was used.

We found that synaptamide treatment prevented the LPS-mediated decrease in spatial working memory during Y-maze testing. However, synaptamide treatment did not cause a significant recovery of locomotor activity, which was reduced in neuroinflammation. In addition, synaptamide improved the parameters of long-term memory in animals with neuroinflammation, as shown in the passive avoidance test and the novel object recognition test. For example, in synaptamide-treated animals with neuroinflammation, the step-through latency in the passive avoidance test was significantly higher than in vehicle-treated animals (81.44±3.07 vs. 52.33±9.63, $p=0.005$). In a novel object recognition test, the time spent with a novel object in the "LPS" group was significantly lower than the time in the "LPS+Syn" group (4.14±1.48 vs. 12.87±3.83, $p=0.047$). Synaptamide administration prevented a decrease in the recognition index in mice with neuroinflammation. Thus, synaptamide administration significantly improved memory indices in animals with neuroinflammation.

Sholl analysis revealed a decrease in CA1 pyramidal neuron complexity when exposed to LPS. Thus, the degree of dendrite branching at a distance of 125–260 μm from the soma was significantly reduced ($p<0.05$) compared to the "Veh" group. At the same time, in the "LPS+syn" group, the degree of arborization was significantly higher than in the "LPS" group, at a distance of 130–255 μm from the soma ($p<0.05$). Synaptamide administration in vehicle-treated mice did not significantly affect the degree of arborization. A Kruskal–Wallis test revealed a significant difference among the groups in the mean number of intersections in the CA1 pyramidal neurons' dendritic tree ($p=0.015$). The subsequent Dunn's test showed that synaptamide administration prevented an LPS-mediated decrease in mean intersection number: 35.66±5.52 in "LPS" vs. 67.83±9.22 in "LPS+Syn", $p<0.05$. Synaptamide administration prevented the LPS-induced decrease in dendrite length: 1474.32±110.63 in "LPS" vs. 2345.43±215.91 in "LPS+Syn", $p<0.05$. A Kruskal–Wallis test showed a significant overall group difference in dendrite spine density ($p=0.039$). There was a decrease in the density of mushroom dendritic spines upon induction of neuroinflammation, which was blocked by synaptamide treatment: 2.90±0.22 in "LPS" vs. 8.799±0.97 in "LPS+Syn", $p<0.001$. Synaptamide treatment not only prevented the LPS-mediated decrease in the number of mushroom spines but also increased the density of both thin and mushroom spines compared to control (Veh: 4.55±0.65 vs. Syn: 7.38±1.04, $p=0.038$ – thin spines; Veh: 5.30±0.48 vs. Syn: 12.93±2.17, $p=0.005$ – mushroom spines). Thus, synaptamide treatment prevented LPS-induced changes in neuronal morphology and reversed neurodegeneration.

To study the effects of LPS and synaptamide treatment on synaptic plasticity, long-term potentiation was examined in the CA1 area of mouse hippocampal slices. Tetanic stimulation of the Schaffer collateral-commissural fibers produced long-term potentiation of field excitatory postsynaptic potentials (EPSPs) in the CA1 area. The normalized field EPSP slopes in the "LPS" and "LPS+Syn" groups amounted to 95.40±7.94% vs. 157.00±19.03% ($p=0.01$) of baseline values immediately after tetanus. At 40 min after tetanization, EPSP slopes for "LPS" and "LPS+Syn" were 103.40±17.62% vs. 174.33±0.02% ($p=0.009$). Thus, we showed that synaptamide treatment restored the LPS-impaired hippocampal long-term potentiation.

N-docosahexanoylethanolamine (synaptamide) administration to animals significantly improved hippocampus-dependent memory, prevented synaptic plasticity impairments, neuronal degeneration, and neurogenesis deterioration. The likely basis of the phenomena described above is the powerful anti-inflammatory activity of synaptamide, as shown in our study and several previous works.

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The radiolarian abundances and diversity in the Pleistocene deposits of the submarine Vityaz Ridge, Northwest Pacific

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Radiolarians are marine siliceous microfossils widely used in biostratigraphy. The radiolarian biostratigraphic data can help the age assignment of the sedimentary rocks, identification of the geological and tectonic processes of the development of the marine floor (e.g., Motoyama, 1996). In addition, radiolarians are sensitive to changes in the water salinity and temperature (e.g., Tochilina, 1985; Afanasieva, Amon, 2006). Therefore, the stratigraphic and paleoecological study of radiolaria in the sediments is also essential for the reconstruction of paleoceanographic and paleoclimatic events.

This study presents data on the radiolarian fauna from the Pleistocene sediments of the submarine Vityaz Ridge (SVR) and the southern part of the paraxial zone of the Kuril-Kamchatka Trench (KKT) opposite the Bussol Strait. The SVR is a continuation of the Lesser Kuril Ridge and divided into the southern and northern plateaus by the Bussol graben (Vasiliev et al., 2001; Kulinich et al., 2007).

In this study, 25 dredge samples were investigated from the SVR (24 samples) and the paraxial zone of the KKT (1 sample). These samples were collected during the 37th, 41st, and 52nd cruises of the R/V "Akademik M. A. Lavrentyev" in 2005, 2006, and 2010, respectively, and the 4th cruise of the R/V "Akademik Alexander Nesmeyanov" in 1984. The radiolarian slides prepared according to the method described by Lipman (1979) and Tochilina (1985).

In the studied sediments, we found radiolarians: Polycystine (60 taxa from 32 genera of Spumellaria and 50 taxa from 34 genera of Nassellaria) and colonial radiolarians (6 taxa from 2 genera of Collodaria).

Gelasian radiolarians (Early Pleistocene) were found in the deposits of the northern plateau of the SVR. The total radiolarian abundance (TRA) (up to 426 skeletons/$g_{dry\ sediment}$) and radiolarian diversity are low. Radiolarian list includes Polycystine few species: *Stylacontarium acquilonium* (Hays), *St. pachydermum* Chen, Zhang, Zhang, Liu, *Thecosphaera microsphaera* Nakaseko, *Lithelius minor* Jørgensen, *Spongurus pylomaticus* Riedel, *Spongopyle osculosa* Dreyer, *Sethocorys* sp., *Cycladophora* cf. *sphaeris* Popova, *Ceratospyris borealis* Bailey. These species are widely distributed in the subarctic and transitional areas of the North Pacific (Kamikuri et al., 2007; Liu et al., 2017).

Calabrian radiolarians (Early Pleistocene) are common throughout the SVR and in the deposits of the paraxial zone of the KKT. The assemblage includes Polycystine and Collodaria. Spumellaria dominate (to 64.3%) in the deposits of the southern and northern plateau of the SVR and the paraxial zone of the KKT. Nassellaria dominates (to 55.5%) in the deposits of the Bussol graben region. Collodaria has a high total abundance (19.2%) in the deposits of the southern plateau of the SVR. TRA reaches 2966 skeletons/$g_{dry\ sediment}$, and the radiolarian diversity increases up to 95 taxa. The predominant genera are Cycladophora (up to 37.8%), Lychnocanoma (up to 19.6%), Acrosphaera (up to 19.2%), Spongopyle (up to 17.5%), Tholospyra (up to 10.2%), Streblacantha (up to 10.2%), Ceratospyris (up to 10.2%).

Cold-water species *Cycladophora davisiana* Ehrenberg (up to 35.1%), *Acrosphaera arktios* (Nigrini) F.1 (up to 15.4%), *C. borealis* Bailey (up to 10.0%) dominate. Other cold-water species *Botryosrtobus aquilonaris* (Bailey) (up to 5.7%), *Stylochlamidium bensoni* Kamikuri (up to 5.8%), *Spongotrochus glacialis* Popofsky (up to 4.7%), *Stylatractus universus* Hays (up to 3.8%), *A. arktios* (Nigrini) F.2 (up to 3.8%), *Eucyrtidium matuyamai* Hays (up to 1.9%), *Rhizoplegma boreale* Cleve (up to 0.9%), and *Spongodiscus biconcavus* Haeckel (up to 0.9%), widely distributed in the subarctic North Pacific (Bailey, 1856; Kamikuri et al., 2007; Itaki et al., 2008), are presented. Furthermore, the assemblage includes *Streblacantha circumtexta* Jørgensen (up to 10.2%), *Stichopilium bicorne* Haeckel (up to 6.1%), *Dictyophimus macropterus* (Ehrenberg) (up to 1.5%), *Dictyophimus* cf. *hertwigii* Haeckel (up to 3.0%), *Artostrobium botryocyrtium* (Haeckel) (up to 1.1%), and *Cromyomma villosum* Haeckel (up to 0.9%), distributed both in the boreal and subtropic areas (Takahashi, 1991; Popova-Goll, Goll, 2006; Boltovskoy, Correa, 2016). The cosmopolitan species *Stylotrochus bipedius* Vasilenko (up to 0.9%), *Stylotrochus tripedius* Vasilenko (up to 0.9%), *Pterocorys hirundo* Haeckel (up to 4.9%) and *Spongurus pylomaticus* Riedel (up to 3.5%) (Dinkelman, 1974; Nakaseko, Nishimura, 1982; Vasilenko, 2019; Rogers, 2020) are also presented.

Calabrian–Middle Pleistocene radiolarians were found in the deposits of the southern and northern plateaus of the SVR. The assemblage includes Polycystine and Collodaria. In the deposits of the southern plateau of the SVR, the assemblage has a low abundance and is represented by a few Spumellaria taxa of Stylatractus, Haliomma and Spongodiscus genera. In the deposits of the northern plateau of the SVR, the total radiolarian abundance is 2745 skeletons/$g_{dry\ sediment}$. The generic and species composition is not rich. The preservation of the skeletons is moderate to good. The richness of the Polycystine genera is as follows: Lychnocanoma (up to 20.0%), Cycladophora (up to 18.0%), Thecosphaera (up to 6.0%), Streblacantha (up to 6.0%), Pterocorys (up to 6.0%), Ceratospyris (up to 6.0%). The colonial radiolarians of genus Acrosphaera are sporadic specimens represented (up to 2.0%). *Lychnocanoma sakaii* Morley et Nigrini (up to 20.0%) and *C. davisiana* Ehrenberg (up to 18.0%) dominate; these are typical for the subarctic and transitional areas (the Sea of Japan, the Sea of Okhotsk and

the Bering Sea) (Tochilina, 1985; Morley et al., 1995; Matul et al., 2002; Kamikuri et al., 2007; Matul, 2009; Matsuzaki et al., 2014). *St. circumtexta* Jørgensen (up to 6.0%), *Pterocorys hirundo* Haeckel (up to 6.0%), *C. borealis* Bailey (up to 6.0%), *Hexacontium pachydermum* Jørgensen (up to 4.0%), *Sp. glacialis* Popofsky (up to 4.0%), *Echinomma* ex gr. *leptodermum* Jørgensen (up to 2.0%), *St. bensoni* Kamikuri (up to 2.0%), and *A. arktios* (Nigrini) F.2 (up to 2.0%) are presented in the assemblage, also widely distributed in the subarctic and transitional areas of the Pacific.

Late Pleistocene radiolarians were found in the deposits of the southern plateau of the SVR. The assemblage is represented by Polycystine radiolarians. The abundance of radiolarians is 1732–2950 skeletons/$g_{dry\ sediment}$. Nassellaria dominate, of which the genera Lychnocanoma (up to 29.0%), Cycladophora (up to 27.0%), and Ceratospyris (up to 9.5%) prevail. *L. sakaii* Morley et Nigrini (29.0–37.1%) and *C. davisiana* Ehrenberg (19.6–27.0%) continue to dominate, the species richness of these species increased. Furthermore, the assemblage includes *Thecosphaera dedoensis* Nakaseko (1.0–2.5%), *Ech. leptodermum* Jørgensen (1.5–3.1%), *Stylodictya validispina* Jørgensen (1.5–2.1%), *St. venustum* Bailey (1.0–4.5%), *Sp. glacialis* Popofsky (3.1%), *Pt. hirundo* Haeckel (0.5–4.1%), *Eucoronis nephrospyris* Haeckel (0.5–2.1%), and *C. borealis* Bailey (4.5–6.2%). All these species are widely distributed in the deposits of the Bering Sea, the Sea of Okhotsk, the Detroit and Meiji Guyots (Kamikuri et al., 2007; Itaki et al., 2008; Matul et al., 2009; Liu et al., 2017).

In the Early Pleistocene, there was a relatively high total radiolarian abundance and high species diversity. At the same time, there was no pronounced dominance of any species. This species diversity and the absence of dominants suggest the presence of stressful conditions. In the Late Pleistocene, while maintaining a relatively high total radiolarian abundance, the species diversity was significantly reduced, and dominants appear. Therefore, the formation of the modern northern Pacific arctic-boreal radiolarian community began in the early Pleistocene and finally took shape by the late Pleistocene.

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Tetrodotoxins secretion and voltage-gated sodium channel adaptation in ribbon worm *Kulikovia alborostrata* (Takakura, 1898) (Nemertea)

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Nemertea is a phylum of marine worms, counting more than 1300 species, most of which are active predators. Nemerteans are subdivided into three phylogenetic groups – pilidiophora, palaeo-, and hoplonemerteans. Many species of these worms bear various toxins, including tetrodotoxin (TTX) – a potent low molecular weight neurotoxin of bacterial origin. With various physicochemical and immunohistochemical methods and mouse bioassay TTX was found in some nemertean species from all three phylogenetic groups.

Despite the more than 30 years of studying TTX in nemerteans, many questions regarding its functions, mechanisms ensuring its accumulation and usage remain unclear. For many TTX-secreting animals, the function of the toxin as the predators' deterrent was suggested. The realization of this function in ribbon worms supposes the recovery of TTX in secreting cells through migration from the tissues of the internal environment. In the current research, using 17 specimens of the ribbon worm *Kulikovia alborostrata*, we studied the dynamics of TTXs concentration in the secretion produced at different time intervals and toxins localization at different stages of the excretion process. To accumulate TTXs and specifically use them as antipredator defense or for prey immobilizing during hunting, animals should have molecular mechanisms ensuring resistance to the toxin. The resistance mechanisms known for some TTX-bearing animals represent mutations in TTX targets – voltage-gated sodium (NaV) channels in the region of the selective filter. In the current research, for the first time, a search for the amino acid substitutions, leading to a decrease of the affinity of the NaV1 channel to TTX in nemerteans, was performed.

For TTXs detection, the immunohistological study with anti-TTX antibody and HPLC-MS/MS were conducted. For NaV1 structure investigation, PCR amplification with specific primers followed by Sanger sequencing was used. The study revealed that in response to an external stimulus, subepidermal TTX-positive cells released secretions actively to the body surface. The post-release toxins recovery in these cells was low for TTX and high for 5,6,11-trideoxyTTX during captivity. According to the data

obtained low probability of targeted usage of TTX as a repellent and a possible targeted 5,6,11-trideoxyTTX secretion by TTX-bearing nemerteans was suggested. The Sanger sequencing revealed the identical sequences of the P-loop regions of NaV1 domains I–IV in all 17 studied individuals. The amino acid substitutions probably contributing to nemertean channel resistance to TTX was showed.

Expression of glutathione S-transferase classes in Bivalvia

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Glutathione S-transferase (GST) is one of the critical proteins in xenobiotic metabolism and is a cellular protector under various intoxications, hypoxic conditions, parasite infestation, etc. Three subfamilies of glutathione S-transferases are currently known: cytosolic, mitochondrial (kappa class) and microsomal (MAPEG class). The cytosolic family has about 36 classes, representing which varies in different organisms, some being specific to plants, animals, fungi and bacteria. It has been shown that each of GST classes may lead to a different expression profile depending on the negative factor or the type of tissue. Therefore, the study of the representation of glutathione S-transferases in the genome is necessary for comprehensive and correct analysis in toxicological and ecological studies. However, there are very few studies on GST encoding genes in marine invertebrates.

The mussel *Mytilus galloprovincialis* L. 1819, an essential commercial object, is exposed to some negative stresses in cultivation. At the same time, this claim has not been thoroughly studied using genomics and transcriptomics methods, and only three classes of cytosolic glutathione S-transferases (GST) have been described for this species.

This study sequenced seven complete *M. galloprovincialis* transcriptomes from gill tissues (GenBank accession numbers: SRR13013753, SRR13013754, SRR13013755, SRR13013756, SRR13013757, SRR13013758, SRR13013759). As a result, 83 transcripts were detected that revealed homology to genes encoding GST. Amino acid sequences of the protein were predicted using TransDecoder based on the nucleotide sequences. We classified all obtained nucleotide sequences by phylogenetic analysis. For *M. galloprovincialis*, all three known subfamilies of this GST gene were found for the first time: mitochondrial (kappa class), microsomal (MAPEG class) and cytosolic (alpha, pi, sigma – were known for this mussel earlier; mu, omega, rho, tau, theta classes were found for the first time). Two isoforms were detected for the sigma class. The exon-intron structure of each gene based on the genome for *M. galloprovincialis* (GCA_900618805.1) was studied. Some classes were duplicated in the genome. The interspecies amino acid variability for each class was detected, which connected with the species divergence and the evolution of GSTs genes.

The obtained results suggest a greater representation of different GST classes in Bivalvia than previously thought. This question requires further investigation.

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Bivalve mollusk's collection from China in the Zoological Museum, Science and Educational Museum, Far Eastern Federal University (Vladivostok)

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Chinese bivalve molluscan fauna is one of the richest in the world. Marine bivalve mollusk's diversity in all Chinese seas is estimated at the level of 1104 species, with 87 and 175 species known for Bohai and Yellow seas, respectively, and 337 species are identified for subtropical East China Sea (Xu, Zhang, 2011); in Taiwan, 759 species of bivalves are known. For comparison, about 288 species of bivalves were found in all Russian Far Eastern seas (Lutaenko, 2013), over 1470 – in Japan, and over 450 – in Korea (Higo et al., 1999; Lee, Min, 2002). So, China and Japan are global hotspots of the world marine molluscan biodiversity but due to overexploitation of biological resources and high coastal population density, biota of these countries experiences strong decline and modifications. In this respect, historical collections stored in museums play an increasing role in understanding of the past and present state of biodiversity. Korea, Japan and China are three countries lie next to the eastern Russia and molluscan samples from these regions are very important for Russian malacologists in terms of taxonomic and biogeographic intercomparisons. We report here about the bivalve collection of the Zoological Museum, Science and Educational Museum of the Far Eastern Federal University.

In total, 378 lots of Chinese bivalve mollusks are available in the museum, and more than 140 species are represented (some lots are not identified up to species level). A majority of lots are from Yellow Sea and South China Sea, and only few are available from East China Sea:

Yellow Sea	211 lots	64 species
South China Sea	154 lots	81 species
Including Hong Kong	141 lots	74 species
East China Sea	3 lots	3 species

A few lots contain fresh-water mollusks (Unionidae – 5 lots, Cyrenidae (Corbiculidae) – 4 lots). Most of the collection consists of dry shells. All samples are labelled

and listed in a systematic card catalog containing detailed information on each lot. All information is also in an electronic database created in the Access software. This allows to quickly sort, group and select objects in accordance with the user's request. An illustrated paper was published based on the collection from Hong Kong (*Lutaenko K.A., Volvenko I.E.* On the fauna of bivalve mollusks of Hong Kong (South China Sea) // Bulletin of the Russian Far East Malacological Society, 2013, v. 17, pp. 79–141) including several new records for this local fauna.

Most species-rich families in the collection are Veneridae (35 species, 94 lots) and Arcidae (26 species, 94 lots), other families are represented by less than ten species (Ostreidae, Mytilidae, Mactridae, Pectinidae, Cardiidae, Tellinidae, Solenidae, Corbulidae, Donacidae, Psammobiidae, Semelidae, Lucinidae, Pharidae, Ungulinidae, Myidae), and the remaining families contain one species only (Carditidae, Mesodesmatidae, Chamidae, Myidae, Isognomonidae, Pteriidae, Solecurtidae, Anomiidae, Glauconomidae, Glycymerididae, Gryphaeidae, Kelliidae Laternulidae, Pinnidae, Plicatulidae, Spondylidae).

In Yellow Sea, many samples were collected around Qingdao and Yantai and neighboring areas of Shandong Province. Few samples are available from Hainan Island (South China Sea). Important collection from South China Sea mostly consists of samples from Hong Kong. Some mollusks were bought in markets of Qingdao, Yantai, Hong Kong, and Dalian. A maority of samples were collected by K.A. Lutaenko (NSCMB FEB RAS), some jointly with his Chinese (Zhang Junlong, Chen Linlin, Li Baoquan and others) or Russian (E.M. Sayenko, A.V. Raschepkina) colleagues, fresh-water mollusks were contributed by E.I. Schornikov (NSCMB FEB RAS) and O.A. Burkovsky (formerly, a staff member of the Zoological Museum) and Shu Gao (Nanjing University).

Somatic cells supporting gametogenesis in deep-sea clam *Calyptogena pacifica*

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In bivalve molluscs, as well as in other Metazoa, the developing germ cells are closely associated with specialized somatic cells (Guraya, 1995, 1998). These are intraacinar accessory (in males) or follicular (in females) cells, which create a specialized microenvironment around developing germ cells and participate in regulation of their development (Pipe, 1987; Eckelbarger, Davis, 1996a, b; Yurchenko, Vashchenko, 2010). In addition to accessory and follicular cells, there are extraacinar cells, mainly, adipogranular (ADG) cells and cells of vesicular-connective tissue (VCT-cells), that are crucial for nutrient accumulation and storage (Lowe et al., 1982; Peek, Gabbott, 1990; Mathieu, Lubet, 1993). Some bivalve molluscs accumulate nutrients in the muscles and use these nutrients to supply germ cells development (Mathieu, Lubet, 1993; Galap et al., 1997; Barber, Blake, 2006; Vite-García, Saucedo, 2008).

Species of the genus *Calyptogena* are often dominant in deep-sea communities, reaching a density of up to several hundreds of individuals per square meter (Barry et al., 1996, 2007; Fujikura et al., 2002). These molluscs have reduced digestive system and maintain chemosynthetic bacterial endosyrnbionts in their gills. It significantly differs the *Calyptogena* genus from other bivalves. At the same time, their fecundity is rather high (Berg, 1985 in Fiala-Medioni, Le Pennec, 1989) that allow suggesting well-developed "infrastructure" to support gametogenic processes.

The gonad consists of numerous acini united by an excurrent duct. On a section, we found acini at various stages of reproductive cycle (active gametogenesis or post-spawning) that was not described in shallow-water species with a clear reproductive cycle. The acini submerged into haemocoel filled with haemocytes. There are several types of haemocytes, namely, erythrocytes and granulocytes, in the interacinar space in both males and females. There were no any other cell types as ADG-cells or VCT-cells. Bundles of muscle cells running in various directions were observed in the interacinar space. The myofibers within a bundle may differ by their electron density. In both males and females, the acinar lumen was lined with developing germ cells and somatic cells. The morphology of accessory cells in males is very variable. Sometime these cells have enlarged basal part spanning up to several tens of micrometers. On the other hand, bodies of accessory cells often extended towards the acinus lumen. The developing germ cells located in small pockets along the accessory cells. Accessory cells were described in molluscs, but they were significantly smaller (Eckelbarger, Davis, 1996; Yurchenko,

Vashchenko, 2010). The somatic cells are located along the acinar wall and do not make follicles around developing oocytes. It should notice that, as a rule, in molluscs the follicular cells form a thin layer of somatic cells around the developing oocyte – follicle that is defines the name of the cells – "follicular". In *C. pacifica* the intraacinar somatic cells are not follicular. Their shape varies from cuboidal to slightly elongate. In general, somatic cells arranged into two layers in the proximity of privetellogenic oocytes and form a single layer beneath vitellogenic oocytes. Endoplasmic reticulum is well-developed and could occupy till half of cytoplasm volume. The mature gametes are evacuated from an acinus through the excurrent duct. The duct is lined with epithelium continuous with the one, lining the acini. It should be noted that at the region of transition of the acinus into the excurrent duct, the cells lining the acinus are enriched in lipids.

Thus, *C. pacifica* demonstrates a well-developed system of somatic cells involved in gametogenesis. All cell types presented in *C. pacifica* were been described in shallow-water molluscs (Franco et al., 2010; Agnese et al., 2013; Rosati, 2018; and others) however, the studied species showed the absence of interacinar stationary (ADG-, VCT) cells and typical intraacinar female follicular cells. As it has been shown in pectinides, haemocytes during gametogenesis, deliver nutrients from an adductor muscle or intestinal loop (the main storage places in the scallops) to the acini with germ cells (Le Pennec et al., 1991). Because no interacinar stationary cells and other places of nutrient deposition were observed in *C. pacifica*, the transport of nutrients is probably carried out from the gills and muscle fibres by haemocytes circulating in a highly developed haemocoel.

The presence of specific somatic cells in the female acini of *C. pacifica*, different from the typical follicular cells described for other molluscs, does not exclude their involvement in vitellogenesis. This is evidenced by intercellular contacts between oocytes and somatic cells and a highly developed endoplasmic reticulum.

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Ice microalgal flora of the Peter the Great Bay water areas

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For the first time for the Russian coast of the Sea of Japan a study of the species composition and quantitative characteristics of the algal flora of sea ice, as its most important autotrophic microcomponent has been carried out. The study is focused on algal flora of sea ice and under-ice water in Voevoda and Novik bays (Russky Island, Peter the Great Bay, Sea of Japan) in the winter of 2020 and 2021.

As a result of the research, 88 species of microalgae from 50 genera and 7 phylums were identified. The main dominant species in the biotopes of ice and under-ice water have been established. The vast majority of them are diatoms: *Chaetoceros socialis*, *Cylindrotheca closterium*, *Entomoneis gigantea* var. *decussata*, *Nitzschia frigida*, *Nitzschia* sp., *Thalassiosira gravida*, *T. nordenskioeldii*; as well as a species of cryptophyte microalgae *Plagioselmis* sp.

Subdominants, having a significant proportion in the taxonomic and quantitative structure of the community: diatoms *Coscinodiscus* sp., *Navicula distans*, *N. granii*, *N. septentrionalis*, *Pseudo-nitzschia fraudulenta*, *P. pungens*; dinoflagellates *Amphidinium sphenoides*, *Protoperidinium depressum*; and unidentified prasinophytes.

The taxonomic structure of ice algal flora is similar to the qualitative composition of under-ice water in winter, which is confirmed not only by our results, but also by other botanical works on the floristic composition of sea water in winter (Konovalova et al., 1989; Mel'nikov, 1989; Pautova, 2000; Ponomareva, 2017). The community is based on diatoms (up to 99%), both in species and quantitative composition. Dinoflagellates are in second place in terms of numbers in 2020 (up to 14%), in 2021 – representatives of green algae (up to 4%). Chrysophytic and euglena algae are the next most abundant. For the first time, the fact of the dominance of cryptophyte algae in under-ice water (up to 67%) in the Peter the Great Bay, has been noted.

A tendency has been established in the distribution of the abundance and biomass of the algal flora over the layers of ice cores. So, in 2020, in Voevoda Bay, the maximum number of algae was observed in the upper layer of the ice core, which gradually decreased closer to the border with the under-ice water. At the same time, the average number of algae in ice exceeded the number of cells in under-ice water by 7.3 times. In Novik Bay, the abundance was evenly distributed throughout the core. At the same time, the number of cells in under-ice water exceeded the average number in ice by 13 times. The distribution of biomass in Voevoda Bay is similar to the abundance, and in Novik Bay the volume of biomass in under-ice water exceeded the average

value in ice by 9.4 times. In 2021, in Voevoda Bay, there was a reverse situation in the distribution of the population compared to the previous year. In the upper layers of the core, it was minimal, gradually increasing as it moved down to the boundary with the under-ice water. In under-ice water, the number of cells was 7.4 times higher than in ice. In Novik Bay, the maximum number of algae was observed in the upper layer of the core, but at the same time, in general, it was evenly distributed over all layers. Under ice, the number of cells was 3 times higher than in ice. The biomass in the under-ice water of Voevoda Bay exceeded the average for the ice core by 3 times, and in Novik Bay – by 12 times.

It was also established that some species of algae "prefer" ice as a habitat and reproduction. In 2020, these were the species of the ice-neritic complex – diatoms *T. nordenskioeldii*, *N. frigida*, as well as species of genera *Navicula*, *Pseudo-nitzschia* and *Protoperidinium*. In 2021, ice-neritic species were distinguished in this respect *N. frigida*, *E. gigantea* var. *decussata*, *T. gravida*, *D. confervacea*, as well as other species encountered from the phylums Chrysophyta, Chlorophyta and Haptophyta.

Thus, a sufficiently high level of species and quantitative abundance of the algal flora of the ice cover in Voevoda and Novik bays was established, comparable to that for a plankton biotope. Further research of sea ice in the Peter the Great Bay is required, since at the given geographical latitude only in this water area a stable ice cover can form for a long time.

Electron microscopy of the XXI century: methods of cryofixation in the study of aquatic invertebrates

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Conventional chemical fixation and embedding, as the standard methods for morphological studies of tissues by TEM, are suitable for most studies of aquatic invertebrates despite several limitations including artifacts caused by hyperosmotic fixatives and loss of immunoreactivity. The rapid combined physical and chemical fixation of specimens achieved using cryomethods is, however, indispensable for high-resolution studies involving immunocytochemistry, electron tomography and 3D reconstructions, quantitative assessments of cytoplasmic elements, and correlative light and electron microscopy (CLEM). Cryofixation immediately arrests all biochemical, physiological and dynamic processes underway in the sample in their present state, resulting in both excellent preservation of the specimen's ultrastructure as well as its antigenicity. Cryofixation involves extremely rapid cooling of specimens, creating an amorphous, or "non-crystalline" state of water containing no detectable ice crystals, a process dependent on pressure, medium composition and temperature. High pressure freezing with subsequent freeze substitution (HPF/FS) is widely used in cell and developmental biology.

Cryofixation and cryopreparation of individuals *in toto* preserve structures and the arrangement of cellular components with fewer artifacts in cell and organelle morphological studies. The cryopreparation methods are also the best way to preserve tissue epitopes for immuno-electron microscopy (IEM). However the expensive HPF machines are not widely distributed in laboratories and institutions. The problems are reduced by development of alternative cryofixation methods. "Self-pressurized rapid freezing" (SPRF) employs plunge freezing of specimens in a sealed copper tube into a cryogen such as nitrogen slush (−210°C) and liquid nitrogen (−196°C). SPRF with cooled acetone on dry ice (−78°C) as the cryogen is one more newly developed method named "DryIce SPRF". Although with this relatively high temperature amorphous water cannot be formed, it was demonstrated that the ultrastructural and antigenicity results after DryIce SPRF on nematode *Caenorhabditis elegans* are perfectly comparable with those achieved using HPF and SPRF. A huge advantage of DryIce SPRF over other techniques is its use of affordable, easily available and safe products. Review is based on the study of modern methodological literature and the personal experience of the author.

Possible relationships between seabird colony population changes and anthropogenic pressures in and around Magadan and the Staritskogo Peninsula

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This study looked the census of seabird colonies around Magadan conducted in 2009 and 2019 to document changes and possible relationships with anthropogenic pressures. The Staritskogo Peninsula and its surrounding four bays are the most affected by anthropogenic pressures on the coastline and islands in the Magadan region. There are no islands in the Svetlaya and Nagaeva bays. Kekurny Islet is located at the edge of Gertnera Bay. Vdovushka Island and the small Three Brothers Islets are located in the Veselaya Bay. The regional center, the city of Magadan, is located on the isthmus of the Staritskogo Peninsula between Nagaeva Bay and Gertnera Bay. Urban development occupies the entire isthmus. The shores of Nagaeva Bay near the city are the territory of commercial and fishing ports; in the shallow part of the bay there is a city beach. The shallow Gertnera and Veselaya bays are traditional places of recreation for regional residents. The entire coastline from Chorny Cape (Gertnera Bay) to Ostrovnoy Cape (Nagaeva Bay) is constantly used by people for recreation and fishing from rowboats and motor boats. In contrast, there are no permanent settlements on the territory of Staritskogo Peninsula and the surrounding bays' coastlines, except for the lighthouse on the Chirikova Cape and the dacha district along the coast in Gertnera Bay. Lack of roads, mountainous terrain, difficult hiking, and steep banks leave the landscape of the peninsula almost unaffected by anthropogenic impact.

The Magadanka River, which flows through the city, and the nearby Dukcha River both flow into Gertnera Bay. Magadanka River has always served as a collector for the disposal of industrial and municipal wastewater. In the 1990's, a number of industrial enterprises were closed in the city, which had introduced aggressive chemical pollution into the river. Since 1992, wastewater from the main part of the city has been mechanically treated and discharged into Gertnera Bay through an underwater pipeline between Gertnera Bay and Veselaya Bay. The treatment facilities did not provide standard wastewater treatment. The wastewater discharge continued until the end of August 2018. A municipal solid waste (MSW) landfill is located at a distance of about 4 km from the mouth of the Dukcha River. The facility's capacity is 54.5 thousand tons of waste per year. Humpback Salmon (*Oncorhynchus gorbuscha*) enter for spawning in the Dukcha River. A licensed fishing zone of salmon is open near the mouth of the Dukcha River.

The following seabird species breed in 27 colonies on the seashores and islands: Pelagic Cormorant (*Phalacrocorax pelagicus*), Slaty-backed Gull (*Larus schistisagus*), Black-legged Kittiwake (*Rissa tridactyla*), Common Murre (*Uria aalge*), Spectacled Guillemot (*Cepphus carbo*), Tufted Puffin (*Lunda cirrhata*), Horned Puffin (*Fratercula corniculata*), and Parakeet Auklet (*Cyclorrhynchus psittacula*). There are no seabird colonies in Svetlaya Bay or Nagaeva Bay, nor on Vdovushka Island.

An urban population of Slaty-backed Gulls formed in the city of Magadan, and successfully breeds on the roofs of city buildings, despite the constant attempts of building owners to destroy nests. The productivity of urban gulls is significantly higher than that in the surrounding natural colonies; the growth rate of urban colonies is about 13% per year. The number of nesting gulls has tripled over the past decade (see Table). The growth of the urbanized population is facilitated by the MSW landfill and the food-rich waters of Nagaeva and Gertnera bays. The growth of the urban population could also be due to simultaneous emigration of gulls from the surrounding natural colonies.

Numbers of seabirds in colonies around the Staritskogo Peninsula (individuals)

Species and census year	City of Magadan	Coast		Island colonies		
		Gertnera Bay	Staritskogo Peninsula	Ostrovnoy Cape	Three Brothers Islets	Kekurny Islet
Phalacrocorax pelagicus 2009	–	800	694	160	376	2
Phalacrocorax pelagicus 2019	–	5512	592	320	430	114
Larus schistisagus 2009	1024	1244	704	3202	733	88
Larus schistisagus 2019	3454	5140	1084	3548	738	146
Rissa tridactyla 2009	–	–	212	1352	9702	204
Rissa tridactyla 2019	–	1384	90	3248	13442	600
Uria aalge 2009	–	–	–	362	13667	–
Uria aalge 2019	–	–	–	1579	17745	1
Cepphus carbo 2009	–	–	102	1650	10	–
Cepphus carbo 2019	–	24	12	174	114	6
Lunda cirrhata 2009	–	–	102	175	245	–
Lunda cirrhata 2019	–	–	60	180	1060	30
Fratercula corniculata 2009	–	–	–	14	–	–
Fratercula corniculata 2019	–	26	30	88	52	2
Cyclorrhynchus psittacula 2009	–	–	–	–	–	–
Cyclorrhynchus psittacula 2019	–	–	–	–	25	–

Currents carry urban wastewater to the Three Brothers Islets, and the number of seabirds rapidly continues to grow in this colony. This colony is now almost four times the size of the colony on the Ostrovnoy Cape, which lies outside the influence of the city's wastewater. Over the past decade, the species composition of this colony has expanded with the reappearance of Horned Puffins and Parakeet Auklets, the last sightings of which were recorded here over 60 years ago.

Anthropogenic pressure on seabird colonies from 2009 to 2019 remained constant; there were no periods of decrease or increase. It consisted of the regular presence of people in the areas of colonies and the flow of nutrients from wastewater and from the MSW landfill into the sea. Wastewater likely makes the benthos of Gertnera Bay more productive, which is reflected in the explosive growth in bottom-feeding breeding Pelagic Cormorants. The total number of breeding seabirds (taking into account the urban population of the Slaty-backed Gull in the city of Magadan) increased from 36 thousand individuals in 2009 to 57.6 thousand individuals in 2019. The distribution of colonies along the coast of the Staritskogo Peninsula has changed little, except for an increase in the number of small colonies in the Chirikova Cape area and in the most inaccessible spots for landing people on the coast.

The influence of environmental factors on spawning of sea urchins with planktotrophic larva

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Many marine invertebrates with external fertilization (broadcast spawners) have feeding (planktotrophic) larvae. The success of the reproduction of such species is determined by two main factors:

1) The spawning period should be timed with the seasons, when sets of most important abiotic (e.g. temperature, salinity) and biotic (phytoplankton abundance) conditions are favourable for offspring development and survival.

2) For successful fertilization, an external stimulus is required, which would ensure the simultaneous release of gametes into the water by the sufficient numbers of individuals of both sexes.

Most species of sea urchins are typical representatives of broadcast spawners with planktotrophic larva, therefore, for decades, they have been widely used as model organisms to identify the environmental variables that trigger and synchronize spawning in the populations of such animals. Prior to the beginning of our studies, a number of exogenous cues were discussed in the literature that claim to be synchronizers of spawning in sea urchin populations (an increase in phytoplankton concentration, changes in water temperature, photoperiod, phases of the lunar cycle, the presence of gametes in the environment). However, most inferences were drawn from the studies of sea urchins' reproductive cycles and therefore provided only indirect evidence of the nature of such stimuli due to the low frequency of animal sampling for gonadal analysis (usually once for month) and insufficient environmental data. Diving observations of sea urchin spawning events in the field were rare, and no one spawning event could be directly linked to a certain environmental cue.

Our studies were intended to test the hypothesis that phytoplankton induces spawning in natural populations of sea urchins with planktotrophic larvae. The prerequisite for these studies was our previous research, which showed that the timing of gonadal maturation and spawning, as well as the completeness of spawning were different in the populations of the sea urchin *Strongylocentrotus intermedius* inhabiting under conditions of relatively high and low phytoplankton abundance.

The field studies were conducted in two bays in the northwestern Sea of Japan, Kievka Bay (low phytoplankton abundance) and Alekseev Bay, Popov Island (high

phytoplankton abundance). The objects of study were two cohabiting sea urchin species, *S. intermedius* and *Mesocentrotus nudus*. The timing of the studies was chosen to coincide with the spawning seasons of these echinoids. For *S. intermedius*, it was May–September in Alekseev Bay and July–September in Kievka Bay. The spawning season of *M. nudus* in both bays was July–August. Environmental variables (phytoplankton measured as chlorophyll a concentration, temperature, salinity, oxygen concentration, photosynthetically active radiation and tidal level) were measured with a multi-parameter sonde in sea urchins' habitats.

The research was carried out in 2 stages. At the first stage, the state of the gonads of *S. intermedius* sea urchins was analyzed from 4 sites in Kievka Bay with sea urchin sampling at intervals of every 4 days on average and daily measurement of environmental variables. At the second stage, round-the-clock continuous (over the spawning season) video recording of the spawning behavior of *S. intermedius* and *M. nudus* was performed in parallel with gonadal analysis and automatic datalogging of environmental variables.

Studies of the role of endogenous (the degree of gonadal maturity) and exogenous (environmental variables) factors in triggering and synchronization of spawning in natural populations of sea urchins have shown that:

1) Phytoplankton is the most important factor because under conditions of low phytoplankton abundance, spawning does not occur.

2) Despite the high level of gonad maturity, under conditions of low phytoplankton abundance, the spawning period of sea urchins lasted 2 months or more, while under conditions of high phytoplankton abundance, mass spawning was recorded which lasted less than a month.

3) Males and females showed significant increases in the locomotion rate 35 min before the start of spawning and continued to actively move during spawning.

4) Males are more sensitive to external stimulus than females; therefore, during mass spawning, they began to actively move first, in the absence of other spawners. Males began to spawn first, and could spawn alone and in groups without females.

5) On a vertical surface, both echinoids moved strictly upward, whereas on a flat food substrate, their movement was multidirectional. Spatial distribution analysis showed that although neither echinoid formed spawning aggregations on flat surfaces, the males and, to a much lesser extent, nonspawners approached females *during mass* spawning.

6) Despite the males started spawning earlier and finished spawning later than females, the dynamics of male and female numbers in both species was well synchronized, so that the maximum numbers of simultaneously spawning sexes coincided.

7) Most of the spawning episodes coincided with the phases of the new or full moon, however, since under the conditions of the same lunar cycle, the completeness of spawning in different populations of sea urchins was different, this factor can be considered only additional.

8) Most of the spawning episodes occurred at dusk and at night, which indicates the participation of the circadian rhythm in the regulation of spawning.

We showed that two sea urchin species with planktotrophic larvae display similar reproductive adaptations aimed at enhancing reproductive success. The high sensitivity of sea urchins, primarily males, to some environmental cue(s), most likely phytoplankton, may be considered a large-scale adaptation characteristic for many broadcast spawners with planktotrophic larvae and is apparently one of the prerequisites for the development of mass spawning events. The nighttime and new and full moon phases apparently to be modulating factors increasing the probability of mass spawning. The longer spawning duration in males compared with females, longer duration of sperm release during mass spawning events compared with that during solitary male spawning, longer durations of sperm release and total time of spawning in males inhabiting the bay with higher levels of phytoplankton and approach of males and females during mass spawning may be considered small-scale adaptations that promote the likelihood of fertilization.

Proliferation and migration of neuronal progenitor cells in telencephalon during constitutive neurogenesis and after traumatic injury of juvenile masu salmon, *Oncorhynchus masou*

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The investigation of neurogenesis in adult animals is a significant area of neurobiology and developmental biology. For the clarification of its mechanism, it is important to identify various types of cells with stem properties in the brain of adult animals, because of the heterogeneity of this process, which manifests a range of features, both at the level of individual constitutive neurogenic niches and in various species. In the central nervous system of amniotes, postembryonic populations of tissue-specific stem cells usually have a glial, radial glial, or astrocytic phenotype. Neuroepithelial cells play a significant role in mammalian embryonic neurogenesis, while glial stem cells are the main source of neurons in the postembryonic stages of development. In contrast to mammals, neuroepithelial-like stem cells/progenitor cells are present in the brain of teleosts throughout life.

The aim of the study is a comparative analysis of the distribution of glial-type neural stem cells (aNSCs) markers, such as vimentin and glial fibrillar acid protein (GFAP), as well as the proliferation marker bromodeoxyuridine (BrdU) and migratory neuronal precursor doublecortin, in the pallial and subpallial zones of the intact telencephalon in juvenile *Oncorhynchus masou* and after mechanical injury.

The immunohistochemical labeling with antibodies to vimentin, GFAP and doublecortin in the pallium and subpallium of intact fish revealed single, small, round and oval immunopositive cells, that correspond to a persistent pool of neuronal and/or glial progenitors. The distribution of BrdU+ cells inintact juvenile *O. masou* indicates a high intensity of the processes of constitutive neurogenesis in the pallium. The maximum number of BrdU+ cells and nuclei was detected in dorsal pallial zone; the minimum, in medial pallium ($p<0.05$). After the traumatic injury of the telencephalon, an increase in the number of BrdU+ cells and nuclei and the intensity of their labeling was observed in all areas of the pallium of juvenile *O. masou*; significant differences from the control group ($p<0.05$) were detected in medial pallium.

The studied molecular markers: vimentin, GFAP, and doublecortin in the pallium and subpallium of intact juvenile *O. masou* allowed only single immunopositive cells to be identified. A subsequent analysis of these cells detected in the territory of the pallial

and subpallial matrix zones showed that they are mainly of the same type, small rounded and oval cells, detected in all variants of immunolabeling, which allows us to consider them as a persistent pool of neuronal and/or glial precursors present in the palial and subpallial matrix zones of juvenile *O. masou*.

The GFAP immunolabeling pattern in the telencephalon of juvenile *O. masou* after injury significantly differed from the GFAP labeling in intact animals. Heterogeneous cell clusters, additional radial glia fibers, and single small intensely GFAP-labeled cells in the parenchyma appear in dorsal and lateral pallial and dorsal subpallial areas. All of these GFAP+ elements appear *de novo* as a result of activation of the resident glial type aNSCs and their subsequent slow proliferation in response to damage. This is most pronounced in lataral pallial and dorsal subpallial zones, where heterogeneous clusters were detected, including both GFAP-positive and GFAP-negative cells, radial glia fibers, as well as GFAP-positive granules. We believe that these GFAP-immunopositive structures are reactive neurogenic niches containing glial aNSCs that occur in response to damage. A detailed study of the cellular composition and patterns of extracellular immunolocalization of vimentin in the pallium and subpallium of juvenile *O. masou* after damage indicates a multiple increase in the processes of constructive metabolism. Another important consequence is the multifold increase in the pool of Vim+ neural progenitor cells (NPCs) generated after trauma. The presence of local clusters of intensely BrdU-labeled cells of similar sizes and shapes indicates the proliferation of progenitor cells, increasing the total number of cells involved in the reparative process. Thus, post-traumatic vimentin and GFAP expression patterns indicate a similar increase in the expression of intermediate filament proteins in the damaged pallium and subpallium of juvenile *O. masou*. After injury, Vim+ cells with neuroepithelial morphology were identified in dorsal and medial pallial, as well as in dorsal and ventral subpallial areas; the glial glia-like morphology, in lateral pallium.

In the pallium of *O. masou* after injury, intense DC-labeling of cells was observed in the periventricular layers of the brain. For an intact subpallialium, patterns of cluster distribution of DC+ cells are more typical. A characteristic feature of DC labeling after injury is the appearance of the pattern of cell migration from the neurogenic zone of the pallium deep into the brain parenchyma. In the subpallium, a significant increase in the number of DC+ cells in the post-traumatic period was found in the dorsal area. This confirms the simultaneous process of radial migration of numerous new generated neuroblasts as a result of the traumatic process. The appearance of intensely DC-labeled cells in the brain parenchyma indicates the activation of resident NSCs after the traumatic process. In the parenchymal zone after trauma, the occurrence of closely spaced pairs of intensely DC-labeled cells located outside the proliferative zones is characteristic. Thus, as a result of the traumatic process, an additional pool of highly active aNSCs in the pallium, as well as patterns of mass migration of newly formed DC+ neuroblasts migrating from neurogenic zones to the deep layers of the parenchyma, are formed in *O. masou*.

The obtained data actualize various questions for further studies of adult neurogenesis in the brain of Pacific salmon; in particular, how does the ratio of neuroepithelial and glial precursors change during the adulthood of *O. masou*? How does the rate of proliferation of pallial and subpallial cells change in the later stages of postembryonic development of the *O. masou* brain, because the brain size of an adult *O. masou* is many times larger than that of a juvenile? From what types of neural and/or glial precursors during the *O. masou* life circle is the structure of various subregions of the telencephalon formed? Answers to these questions can significantly advance current knowledge about the neurogenesis of salmon fish, as convenient models for brain development research.

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Feeding preferences of shallow-water spionids (Annelida: Polychaeta: Spionidae) inferred from fatty acid biomarkers

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Polychaetes are a substantial group of animals occurring in nearly every marine habitat from intertidal to deep-sea. They often dominate in benthic communities in numbers of specimens and are among the most 'species-rich' groups in communities of soft bottom sediments, being an important trophic level in food webs in marine ecosystems. It is considered that species richness is associated with trophic diversity. High trophic diversity is found only in food webs with high species richness, which, in turn, affects the functioning of the ecosystems.

The fatty acid composition of organisms is known to be influenced by diets and is therefore an indicator of food ingested. Significant success has been achieved in exploring food sources and trophic interactions between species in aquatic communities using fatty acid biomarker approach. We analyzed fatty acid composition of seven species of spionid polychaetes co-occurring in a small shallow-water area in the Peter the Great Bay of the Sea of Japan, but living in different microhabitats and using different feeding strategies: free-crawling *Laonice* aff. *cirrata*, tube-dwelling *Dipolydora cardalia*, *D. carunculata*, shell-boring opportunists *D. bidentata* boring into shells of the scallop *Mizuhopecten yessoensis* and various gastropods occupied by hermil crabs, and shell-boring commensals including *Polydora brevipalpa* from the scallop *M. yessoensis*, *P. glycymerica* from bivalve *Glycymeris yessoensis* and hermit crab *Pagurus capillatus*, and *D. commensalis* burrowing exclusively into gastropod shells inhabited by hermit crabs *P. capillatus* and *P. brachiomastus*. Likewise, we conducted fatty acid analysis of the bivalves and hermit crabs hosting commensal spionids to determine the trophic relationships between polychaetes and their hosts. Using the method of fatty acid biomarkers, we elucidate the spectrum and diversity of food sources of spionids to ascertain how spionids from soft bottom sediments can live together in the same environment, especially if they belong to the same trophic level.

Comparative fatty acid analysis of the spionids revealed significant interspecific and intraspecific differences, indicating different food preferences of the polychaetes. A principal component analysis divided the examined species into five groups: suspension feeders, deposit feeders, omnivorous, surface deposit feeders, and sub-surface deposit feeders. High amounts of 20:5n-3, C16polyunsaturated fatty acids, and 14:0, as well as a ratio of 16:1n-7 to 16:0 close to 1, indicated a predominance of planktonic

and benthic diatoms in the diet of suspension feeders, *P. brevipalpa*, *D. bidentata*, and *D. carunculata*. High concentration of 18:1n-9 together with a high level of 22:6n-3 was found in hermit crab commensal *P. commensalis*. These trophic markers suggest a high contribution of decaying organic matter in its diet. The tube-dwelling *D. cardalia*, which collects food particles from the surrounding water and from bottom surface by tentacles, occupied an intermediate position between herbivorous and omnivore species. The fatty acid composition of *P. glycymerica*, which is a commensal of bivalve *G. yessoensis* living on sand sediments, distinguished this species from other polychaetes. The free-crawling *Laonice* aff. *cirrata* is known to swallow soft bottom sediments. It differed by high concentrations of 20:1n-11, 20:4n-6 and 22:6n-3, which suggests the consumption of meiobenthos inhabiting soft bottom sediments, including nematodes, foraminifera, and other protozoa. This species can also feed on mixed food sources, including bacteria and benthic microalgae, because it was particularly rich in 18:1n-7, specific to bacteria, and significant amount of 20:5n-3, typical for microalgae. Analysis of fatty acid revealed that some species are capable to adapt their diet depending on the habitat conditions. Thus, *D. bidentata*, living in the scallop shells, consumed mainly diatoms, and individuals from the shells occupied by hermit crabs shared their food with the host crab. These finding are consistent with the morphological and behavioral peculiarities of the spionids species.

Lipids play a key role in animal physiology. The predominance of triacylglycerols (TAG), followed by phospholipids and then sterols recorded herein, is common for shallow-water polychaetes, while deep-water species different principally in lipid class distribution. The increased accumulation of TAG in females observed in the present study is probably related to the accumulation of energy and organic matter for gametogenesis and the oocyte maturation. The storage lipid content can also give an insight into the intensity of the feeding. Particularly high proportion of TAG, energy storage lipids, detected in the commensal *P. commensalis* suggests a sporadic food supply. These lipids could be accumulated in *P. commensalis* in response to a period of repletion followed by periods of forced starvation, which is a common situation for individuals of this species awaiting a change of hermit crab in the shell.

In conclusion, evidence from fatty acid markers showed that polychaete species consume a variety of foods available in their habitats. It appears that benthic spionid polychaetes occupy distinct dietary niches, which are probably the result of special feeding strategies and can mitigate inter- and intraspecific competition between sympatric species. The revealed features of the fatty acid composition are in consistent with the specificity of their lifestyle and organization, as well as with the structure of the palps of the species. Lipids play a key role in physiology and can provide an indication of the intensity of feeding of animals.

The use of Co1 and 16S mitochondrial markers in the study of molecular genetic relationships among squid of the family Gonatidae (Cephalopoda: Teuthida: Oegopsida)

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Squids of the family Gonatidae are abundant cephalopods in subpolar and polar waters, are key components in pelagic and near-bottom marine and oceanic communities, and in certain areas, e.g., in the North Pacific Ocean, are found in high-density commercial concentrations. In spite of high ecological and economic importance of the gonatid squid, the species diversity among them remains poorly understood. To resolve some controversial issues associated with the CO1 tree topologies earlier obtained for the Gonatidae, we added another mitochondrial gene marker, the 16S, and included more individuals into the analysis of molecular genetic diversity among species within the family. In this research, Neighbor-Joining and Bayesian-trees were used to construct phylogenetic relationships among the gonatid species, and species hypothesis-free automatic barcoding gap discovery (ABGD) methods were used to analyze differences among the species groups. Squid samples from our collection were analyzed together with sequence data for the gonatid squid and two outgroup species available in the GenBank.

Squid samples from our collection formed 14 specific groups using species hypothesis-free ABGD for the CO1 marker with Barcode gap distance = 0.025: 1) *Gonatus kamtschaticus*; 2) *Gonatus madokai*; 3) *Gonatus* cf. *berryi*; 4) *Gonatus* sp.; 5) *Gonatus pyros*; 6) *Gonatus onyx*; 7) *Gonatus tinro*; 8) *Gonatopsis octopedatus*; 9) *Gonatopsis japonicus*; 10) *Boreoteuthis borealis* Large form; 11) *Boreoteuthis borealis* Small form; 12) *Boreoteuthis makko*; 13) *Berryteuthis magister*; 14) *Okutania anonycha*.

Squid samples from our collection formed 10 specific groups using species hypothesis-free ABGD for the 16S marker with Barcode gap distance = 0.008: 1) *Gonatus kamtschaticus*, *Gonatus madokai* (p-distances between them were Co1=4.4%, 16S=0.4%); *Gonatus* cf. *berryi*, *Gonatus* sp., (p-distances between them were Co1=6.1%, 16S=0.8%); 2) *Gonatus pyros*; 3) *Gonatus onyx*; 4) *Gonatus tinro*; 5) *Gonatopsis octopedatus* and *Gonatopsis japonicus* (p-distances between them were Co1=3%, 16S=0.3%); 6) *Boreoteuthis borealis* Large form; 7) *Boreoteuthis borealis* Small form; 8) *Boreoteuthis makko*; 9) *Berryteuthis magister*; 10) *Okutania anonycha*.

Tree topologies obtained using Co1 and 16S for the gonatid squid from our collection were generally concordant, and reflected the existing views on the species subdivision with the family in the North Pacific Ocean. However, the ABGD approach suggested that Co1 better reflected subdivision of species into the species groups, and 16S had less discriminative power. Therefore, the use of squid from our collection alone yielded clear genetic differentiation among the species of the Gonatidae.

However, further inclusion of available sequence data for the gonatid species from the GenBank not only added new valuable information on the genetic subdivision among the family members, but also revealed several inconsistencies, which should be considered in molecular phylogenetic studies of that group of squid. Sequence analysis for the gonatid squid from both our collection and the GenBank suggested that, based on Co1 and 16S tree topologies, such inconsistencies were revealed for the following taxa: (1) *Gonatus berryi* (and *Gonatus californiensis*), (2) *Gonatus pyros*, and (3) *Gonatus fabricii* (and *Gonatus steenstrupi*).

The gonatid species identified as *G. berryi* or *Gonatus* cf. *berryi* appeared in three different branches on Co1 and in two clades on 16S trees. Those branches yielded bootstrap support of about 50% and showed high p-distance values when compared with other species clades. On the Co1 tree, one *G. berryi* individual from the GenBank formed a separate lineage close to *G. antarcticus* group; one clade included *G. berryi* and *G. californiensis* from the GenBank and *Gonatus* cf. *berryi* from our collection, and one clade consisted of *Gonatus* cf. *berryi* from our collection alone. On the 16S tree, *G. berryi* formed two groups: one included specimens from our collection, and another consisted of specimens from the GenBank and from our collection. The split of *G. berryi* into three lineages on the Co1 and two lineages on the 16S trees may indicate the existence of hidden taxonomic differences among the examined squid at a species level. The fact that several individuals of *G. berryi* from the northwestern Pacific Ocean clustered together with the North American *G. californiensis* may suggest erroneous species identification and/or a much wider geographic range for the latter species.

The gonatid species identified as *G. pyros* formed two sister clades on the Co1 and 16S trees. Those clusters had high bootstrap support of >90; however, p-distances between them were small. One group of *G. pyros* consisted of individuals from our collection, which were captured in the northwestern Pacific Ocean, and of individuals from the GenBank. Another group comprised animals from the GenBank alone. Further investigation of such a subdivision is needed.

The third case of inconsistency, which may indicate an existing taxonomic ambiguity in the Gonatidae, was associated with the gonatid squid from the North Atlantic, all of them come from the GenBank. On the Co1 tree, all individuals of *G. fabricii* and *G. steenstrupi* appeared in a single clade with high bootstrap support of about 99. On the 16S tree, one *G. fabricii* individual formed an individual branch, and one squid erroneously appeared in one clade with small *B. borealis*. No 16S sequences were available in the GenBank for *G. steenstrupi*.

Occurrence and distribution of cephalopods in the upper epipelagic in Gulf of Alaska and adjacent waters in winter of 2019

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The cephalopod fauna of the Gulf of Alaska (GoA) and adjacent waters is comprised from about 35 species from 14 families. Cephalopods are an important component of the pelagic food web – they are consumers of zooplankton and a significant food source for fishes, squids, marine mammals and seabirds. Little is known about Cephalopods distribution and distribution in winter. To address fish and squid winter ecology knowledge gap, an expedition on research vessel "Professor Kaganovsky" was carried out in February–March 2019. Our study is based on 64 epi pelagic (0–40 m) trawl catches and 60 Juday plankton nets (0–250 m). We encountered nine squid species (Teuthida) from five families and one octopus (Octopoda). Majority (~90%) of squid was caught at night. The dominant species by abundance and biomass were from Gonatidae and Onychoteuthidae families. Species from families Enoploteuthidae, Chiroteuthidae, Cranchiidae, and Bolitaenidae were caght sporadically. All encouteres species were characteristic for the Pacific subarctic zone.

Boreoteuthis borealis (Sasaki, 1923) syn. *Gonatopsis borealis* – boreopacific gonate squid. All *B. borealis* was caught at night (17:40 to 04:55, here and herein local time), it the most abundant squid and it was encountered in 40.6% of total trawls. In total, 1665 specimens were caught – from 2 to 277 specimens per trawl. This species is active diel vertical migrator and comes up to pelagic layer at night, Maximum catches (n>100) were observed from 18:40 по 23:40. Size ranged from 21 to 130 mm which is juvenile and sub-adult stages. In plankton nets two larval were caught – 4.0 and 4.6 mm.

Gonatus onyx (Young, 1971) – one-hooked gonatus. It is a mesopelagic squid that comes to epipelagic layer at night. It was encountered in 23.4% trawls with catches from 1 to 35 speciemnt per trawl. In total 81 specimens were caught, size range from 33 to 95 mm. The largest specimen was female, II stage of maturity. Two larvae (each 4.0 mm) were caught in plankton net at one station.

Gonatus madokai (Kubodera et Okutani, 1977) – long-armed gonatus. This is an abundant, mesopelagic species in North Pacific. It was encountered in 7.8% trawls, in total 7 specimens were caught ranging from 45 to 55 mm and one specimen 155 mm. This species was caught only in to the north of 53° N.

Okutania anonycha (Pearcy et Voss, 1963) syn. *Berryteuthis anonychus* – smallfin gonate squid. This is common, mesopelagic species in North Pacific. None were caught in trawls, however it was present in stomachs of sockeye salmon (n=4), coho (n=2), and chinook (n=3). All specimens in stomachs were encountered on the east of 144° longitude. Specimen's size ranged from 42 to 78 mm.

Onychoteuthis borealijaponica (Okada, 1927) – boreal clubhook squid. This is a common species in North Pacific. It was the second most abundant species (after *B. borealis*). and it was encountered in 26.6% of total trawls. In total, 254 specimens were caught – from 1 to 101 specimens per trawl. Size ranged from 64 to 235 mm. Size range was bimodal and included specimens born in fall (64–110 mm) and spring/summer (>110 mm). Majority (n=105) of specimens <110 mm were males and juveniles. Larger specimens were at stage II and III stages of maturity. Most of squids (n=231) were caught to the south of 50° N.

Onykia robusta (Verrill, 1876) syn. *Moroteuthis robusta* – robust clubhook squid. This is one of the largest squids. One specimen was caught in southern area, it was a male (III stages of maturity) with mantle size of 346 mm.

Abraliopsis felis (McGowan et Okutani, 1968). This is a common squid of North Pacific. It was present in 7.8% of total trawls, 2–5 specimen per trawl. It total, 17 specimens were caught, size range was from 34 to 43 mm. It was encountered in southern areas of GoA.

Chiroteuthis calyx (Young, 1972). This is a common species of boreal Pacific. It was present in 7.8% of total trawls, 1–15 specimen per trawl. Size ranged from 38 to 60 mm.

Taonius borealis (Nesis, 1972), syn. *Belonella borealis*. This is a cranch squids (Cranchiidae) inhabiting deep waters (up to 3400 m) of north Pacific. One specimen was caught – female VII stages of maturity, size 600 mm. Remains of this species were found in two shark (*Squalis acanthias*) stomachs. One larvae, size 7.0 mm, was caught in plankton net. These results confirm, for the first time, that this species can reproduce in GoA in winter.

Japetella diaphana (Hoyle, 1885). This is a pelagic octopus usually brought to GoA with subarctic current. Only one specimen was encountered (34 mm) in the southern GoA area. One larvae was caught in the net collecting samples for micro plastic study.

Gonatid squids is typical for boreal zone. Species of this family were caught in all night and some evening trawls. Mass vertical migration to epipelagic zone is characteristic only boreopacific gonate squid (*B. borealis*). Another common squid – smallfin gonate prefer to stay in mesopelagic zone in winter. Boreal clubhook squid (*O. borelaijaponica*) is very abundant in the GoA and occasionally can be found in coastal waters of Alaska and British Columbia. This species using GoA as nursing and feeding ground in winter, *C. calyx* has the same strategy. Squids that continuously (from larvae to spawning adults) live in GoA are cranch squids. In winter, female *T. borealis* continue egg incubation with larvae present in mesopelagic zone.

Squids species diversity is at its peak in late fall in the North Pacific. Species inhabiting GoA in winter-spring period attempt to maximize resources utilization – boreal squids is growing; boreopacific gonate squid, one-hooked gonatus and *T. borealis* are spawning. In the southern GoA, *A. felis* is mating. With movement of oceanic water masses, early ontogenetic stages of subtropical *J. diaphana* can also drift to GoA. In general, GoA in winter period, is inhabited by species of squids that are able to grow and spawn in cold temperatures.