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Адрес учредителя, издателя и редакции:

ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН».
Пр. Нахимова, 2, Севастополь, 299011, РФ.
Тел.: +7 8692 54-41-10. E-mail: *mbj@imbr-ras.ru*.
Сайт журнала: https://marine-biology.ru.

Адрес соиздателя:
Зоологический институт РАН.
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A. O. Kovalevsky Institute of Biology of the Southern Seas of Russian Academy of Sciences.
2 Nakhimov ave., Sevastopol, 299011, Russia.
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1 Universitetskaya emb., Saint Petersburg, 199034, Russia.

A. O. KOVALEVSKY INSTITUTE OF BIOLOGY OF THE SOUTHERN SEAS OF RAS
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COMPUTER METHODS FOR DETERMINING *MNEMIOPSIS LEIDYI* MOTILITY CHARACTERISTICS

Iu. S. Baiandina and O. N. Kuleshova

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
E-mail: *sepulturka@mail.ru*

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The paper considers capacities of common computer programs for analyzing the behavioral reactions to light of ctenophores, marine planktonic animals, under laboratory conditions. We showed that most image analysis programs do not automatically determine body contours of translucent objects, such as ctenophores. We developed a new method for determining basic motility characteristics of *Mnemiopsis leidyi* using ImageJ, Davinci Resolve video filters, and scripts written by the us. The developed method allows automatic calculating of such parameters as average and maximum speed, changes in direction of movement (locomotion vectors), and the percentage of time animals spend in certain regions of interest (ROI). The average speed of ctenophore movement was estimated in millimeter scale with high precision. The method is applicable for studying the behavior of various translucent marine animals. Computer scripts are available by request from the authors.

Keywords: *Mnemiopsis leidyi*, ctenophore, migration, motility characteristics, image analysis tools, ImageJ, wrMTrck_Batch, regions of interest (ROI), threshold

The species *Mnemiopsis leidyi* A. Agassiz, 1865 (Fig. 1) belongs to a distinct phylum Ctenophora. These planktonic predatory marine animals have eight meridional rows of locomotory ciliary comb plates (ctenes). Ctenophores are the largest animals swimming by means of cilia. Synchronous movement of cilia is coordinated by nervous system (Ringelberg, 2009 ; Schnitzler et al., 2012).

Fig. 1. An adult Black Sea ctenophore *Mnemiopsis leidyi*

Ctenophores can sense the direction of gravity and use it to modify their behavior and often assume a vertical position with their oral–aboral axis parallel to the gravity vector. A recent molecular-genomic study showed co-expression of photoprotein and opsin genes in developing photocytes of *Mnemiopsis* that suggests that light production and light reception are linked (Ruppert et al., 2004).

Changes in the level of illumination in a water column are considered to be the key factor regulating diel vertical migrations. There are some evidences of diel vertical migrations of *Mnemiopsis leidyi* (Kideys & Romanova, 2001 ; Mutlu, 1999 ; Wilson-Leedy & Ingermann, 2007 ; Zaika & Sergeeva, 1991) but the mechanisms and factors determining and triggering diel vertical migrations remain unclear (Haraldsson et al., 2014 ; Schnitzler et al., 2012).

The main reason for daily diel vertical migrations of most planktonic organisms from lighted surface layers to deeper waters is to avoid visually hunting predators (Hays, 2003 ; Ringelberg, 1995, 2009). Light, temperature, and food concentration can also affect migration patterns (Haney, 1988 ; Ringelberg, 1995).

The behavioral responses to light of marine animals could be studied by registration and analysis of their motility characteristics in a water column under laboratory conditions. Most of recent computer programs that provide general characteristics of the animals' movement [such as speed, distance, and time spent in regions of interest (hereinafter ROI)] use threshold filters to determine the contours of an object on video records (Franco-Restrepo et al., 2019). With the threshold method, moving contrasting objects over their entire surface are painted in black on a white background (the color scheme is converted into a binary) (Alsaif & Hamid, 2018). However, there are some issues for photo and video recording and further analysis of the movement of translucent animals because it is impossible to automatically select their fuzzy body contours. *Mnemiopsis leidyi* body is almost transparent since it consists of a relatively thick, jelly-like mesoglea (gel composed of water, cells, and collagen) sandwiched between two epithelial layers (Ringelberg, 1995). On the other hand, ctenes can possess light diffraction which can also complicate the automatic determination. This may be one of the reasons for the lack of experimental research in the behavioral reactions of this species.

The aim of the present work is to develop methods for movement registration and automated computer assessment of basic locomotion characteristics of the ctenophore *Mnemiopsis leidyi* in laboratory aquariums, such as average and maximum speed, changes in direction of movement (locomotion vectors), and the percentage of time animals spend in certain ROI, on the example of the investigation of the light effect on their behavior.

MATERIAL AND METHODS

Experimental design. Adult *Mnemiopsis leidyi* were sampled from the natural habitats in the Martynova Bay (Sevastopol, the Black Sea) and then transported to the laboratory, where those were held in 200-L (length 92 cm, width 55 cm, and height 40 cm) transparent tanks equipped with a flowing seawater system. For 12 h, ctenophores were acclimated to laboratory conditions. The water temperature in the aquariums corresponded to that in the sea. During the investigation, ctenophores were kept in still water because we experimentally found that even a minimal duct masks the animals' own movements. For each experiment ($n = 21$), three animals of different size were placed in the aquarium. Adult *Mnemiopsis* were divided into three size groups: large (with body length over 80 mm), medium-sized (with body length 60 to 80 mm), and small (body length less than 60 mm). Animals moved freely in a water column almost without contact with each other.

Experiments were conducted in a dark room. A light source was placed on a side wall of the aquarium, so that the upper third of the tank was illuminated. Light irradiance was measured with an irradiance sensor QSL-2101 (Biospherical Instruments Inc.). Four lighting regions (ROI) were distinguished in a water column: a region with relatively high illumination, 12 μmol quanta·m^{-2}·s^{-1} (1); with medium illumination, 5 µmol quanta·m^{-2}·s^{-1} (2); with low illumination, 2 µmol quanta·m^{-2}·s^{-1} (3); and a dark region, 0.5 µmol quanta·m^{-2}·s^{-1} (4) (Fig. 2). Control experiments were carried out in a completely dark room with no light source, *Mnemiopsis* speeds were evaluated visually, and the animals were almost motionless.

The movements of ctenophores were recorded in time-lapse mode (1 frame in 2 seconds; 300 frames in 10 minutes) using a camera mounted perpendicular to a front wall of the aquarium. We used a Nikon D5300 camera with a Nikon AF-S Micro-Nikkor 40mm lens and a Hoya 52mm PL polarizing filter. After turning on the light, the experiment was carried out during the time intervals as follows: 1–10, 31–40, and 51–60 minutes.

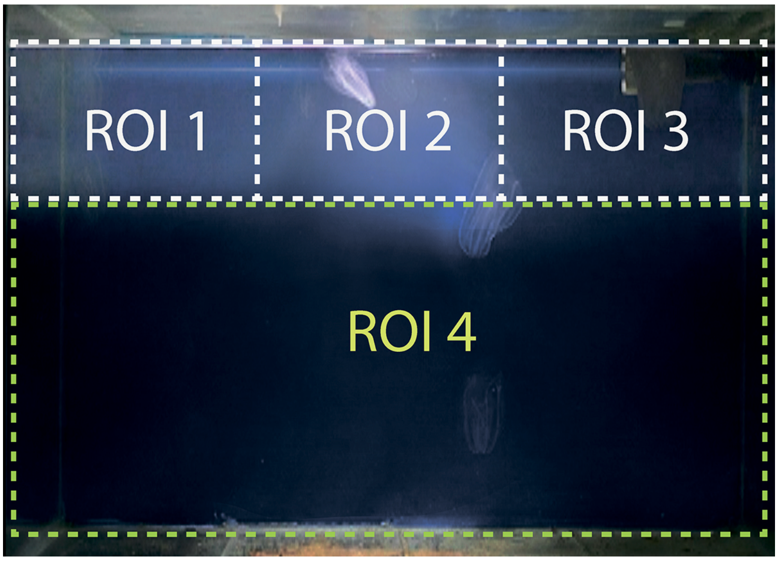

Fig. 2. Regions of interest (ROI): 1, a region with high level of illumination; 2, with medium level of illumination; 3, with low level of illumination; 4, a dark region

Hardware. We used computer equipment with the following parameters: MacBook Pro (13-inch, 2017, two Thunderbolt 3 ports). Processor: 2.3 GHz, Intel Core i5. RAM: 8 GB, 2133 MHz, LPDDR3. Graphics processor: Intel Iris Plus Graphics 640, 1536 MB. Operating system: macOS High Sierra 10.13.6.

Video analysis. The images were processed with the free access Davinci Resolve software (https://www.blackmagicdesign.com). Time-lapse frames for each experiment were combined in a video file with a frequency of 25 frames *per* second. The contour of an individual animal was manually selected on the first frame – using the "tracking" mask in the Davinci Resolve. For each animal in the aquarium, the contour determination process was repeated.

Sometimes the contours of the "tracking" masks can "jump" to the designated body contouring, incorrectly determining the trajectory of the animal movement. In such cases, the position of the mask has to be corrected manually.

The contrast of the image is adjusted using two color filters ("Brightness and Contrast" and "Gamma Correction") so that the animal is painted in white on a black background. Only one animal was visualized on the video record (Fig. 3).

To reduce the number of surveys and increase statistical significance, several animals were placed in experimental aquariums at once. Animal trajectories may overlap while moving, and animals overshadow each other; in this case, automatically detected object contours "jump" from animal to animal. In one video file, the movement of several animals was monitored using several “tracking” masks. Video files were saved as separate uncompressed AVI files for each animal (one mask – one animal – one video file) (Fig. 3) and analyzed in the ImageJ program (https://imagej.nih.gov/ij) using the installed wrMTrck_Batch plugin (http://www.phage.dk/plugins/wrmtrck.html) (Mutlu, 1999) which allows saving TXT files with X-Y coordinates of objects on each frame.

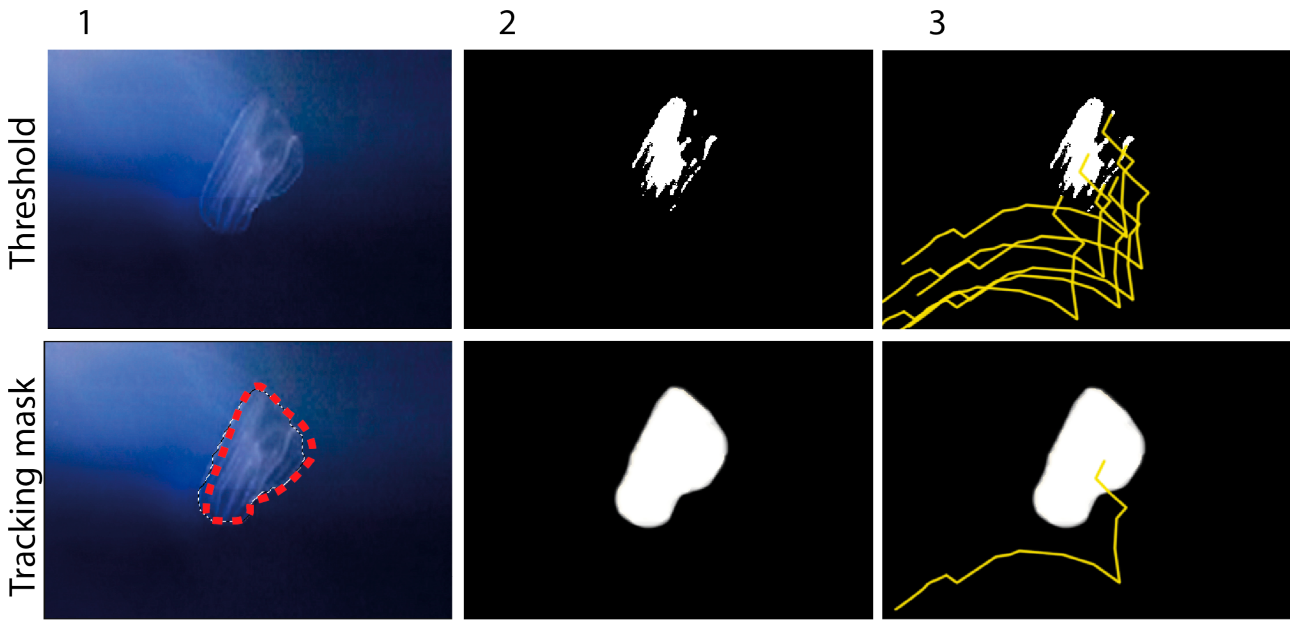

Fig. 3. Two methods for tracking *Mnemiopsis leidyi* movement during a video recording (enlarged video fragment is shown). With the threshold filter: 1, color video scheme converted to 8-bit; 2, application of the threshold filter; 3, plotting of the track of one of the objects. With the developed “tracking” mask method: 1, designation of the borders of a ctenophore body using a “tracking” mask in the Davinci Resolve program on the first frame of the video; 2, use of filters and conversion of the color scheme into a binary; 3, determination of a moving object and construction of its motion track for the entire time of movement in the AnimalTracker plugin

The scripts processing TXT data files. The scripts processing TXT data files were written to obtain the motion characteristics (such as speed, distance, and time spent in ROI) of the monitored objects (obtained in ImageJ). Bash (version 3.2.57(1)-release) (x86_64-apple-darwin17) and the programming language Python (version 3.6.7) were used. The software pipeline was designed and tested for Linux and MacOS operating systems.

Input:

- path to the directory containing files with motion coordinates;
- file path with coordinates describing ROI;
- path to the directory for generating results.

Output:

- files (TXT) for each track containing the original coordinates, ROI, difference of coordinate values between frames, instantaneous speeds, and motion vectors;
- a file that combines the results of all tracks (TXT);
- a file containing average speeds for each track (TXT).

The bash-script includes:

- receiving input data;
- definition of files with coordinates in the directory (*raw.txt);
- running a Python script for each file;
- combining results.

The Python script implements the following computational steps:

$$X = \{x_i\};\ i = \overline{1,l};\ Y = \{y_i\};\ i = \overline{1,l}\ , \tag{1}$$

where X and Y are set of coordinates for each frame;

l is a number of frames.

Calculation of difference of coordinate values between frames (Δx, Δy):

$$\forall i, \lfloor \Delta x_{i+1} = x_{i+1} - x_i,\ \Delta y_{i+1} = y_{i+1} - y_i \rfloor\ . \tag{2}$$

Calculation of motion vectors V (Fig. 4):

$$V = \begin{cases} \forall i, \lfloor |\Delta x_{i+1}| > |\Delta y_{i+1}| \to V[1] = "H", |\Delta x_{i+1}| > |\Delta y_{i+1}| \to V[1] = "V" \rfloor \\ \forall i, \lfloor |\Delta x_{i+1}| > 0 \to V[2] = "R", |\Delta x_{i+1}| < 0 \to V[2] = "L" \rfloor \\ \forall i, \lfloor |\Delta y_{i+1}| > 0 \to V[3] = "D", |\Delta y_{i+1}| < 0 \to V[3] = "U" \rfloor \end{cases}\ , \tag{3}$$

$$Z = \{z_j\};\ j = \overline{1,m};\ z_j = \{[zxs_j, zxf_j], [zys_j, zyf_j]\}\ , \tag{4}$$

where Z is the set of analyzed ROI;

zxs and zys are the zone beginning coordinates;

zxf and zyf are the zone end coordinates.

Calculation of ROI for each frame:

$$\forall i, j, \lfloor zxs_j < x_i \leq zyf_j, zys_j < y_i \leq zyf_j \to ROI = j \rfloor\ . \tag{5}$$

Instantaneous speed:

$$s_{i+1} = \sqrt{\Delta x_{i+1}{}^2 + \Delta y_{i+1}{}^2}\ . \tag{6}$$

Average speed:

$$avrs = \frac{\sum_{k=2}^{l} s_k}{l-1}\ . \tag{7}$$

The program is patented: Kuleshova O. N., Bayandina Iu. S. CtenophoraTrack. Certificate of state registration of the computer program 2020661100 Russian Federation; no. 2020617476; declared 13.07.2020; publ. 18.09.2020, Bull. no. 9.

Statistics. To compare the data, analysis of variance ANOVA was applied. Differences were considered statistically significant at $p \leq 0.05$.

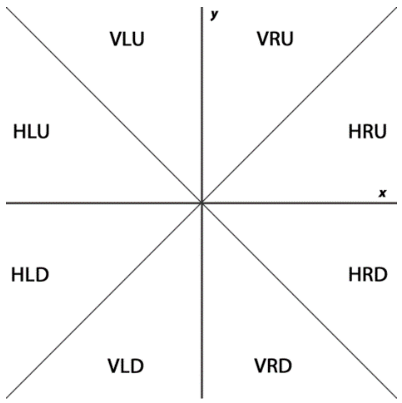

Fig. 4. Diagram of motion vectors in the X-Y coordinate system; letter combinations characterize motion vectors: H, horizontal movements; V, vertical; R, right; L, left; U, upward; D, downward movements

RESULTS

The developed method was successfully applied to determine the visible contours of ctenophores in a water column under various illumination. Using the macros and scripts, we analyzed the individual characteristics of the movement of the Black Sea ctenophores *Mnemiopsis leidyi*, such as average and maximum speed, changes in direction of movement (locomotion vectors), and the percentage of time animals spend in certain ROI. The average speed of ctenophore movement was calculated. The speed of large individuals is (0.54 ± 0.36) mm·s^{-1} (n = 21); of medium, (0.46 ± 0.38) mm·s^{-1} (n = 21); and of small, (0.84 ± 0.63) mm·s^{-1} (n = 21). During the first hour, the average speed was significantly higher for small-sized ctenophores compared to that of large and medium-sized animals. Most of the time, large individuals were in a highly lit area, avoiding an unlit one; medium and small animals migrated in a water column.

DISCUSSION

The type of software chosen for determining characteristics of animal movement depends on physical characteristics of the object and shooting conditions, as well as the quality of the resulting video material and its size.

Most programs used for determination of locomotion characteristics of biological objects were developed for investigating the behavior of mammals (Ringelberg, 1995) and fish (Larsen et al., 2015), for studying sperm motility (Tamm, 2014) and characteristics of other objects (Franco-Restrepo et al., 2019 ; Gulyás et al., 2016). In the most of software available, the threshold filter is used for distinguishing a moving object on the background. This filter replaces each pixel in the image with a black pixel (if the image intensity is lower than some fixed constant) or with a white one (if the image intensity is higher than this constant). For the precise determination, the object should be contrast to the background, have clear contours and uniform color, and be appropriately illuminated.

It was experimentally found that the methods of the threshold filter (such as the method based on the histogram shape, the method based on clustering, the method based on entropy, *etc.*) do not automatically determine body contours of translucent ctenophores in a water column. When converting the color scheme into a binary applying the threshold filter, individual parts of ctenophores remain distinguishable; programs count these parts as separate objects falsely identifying glare and irregular sections of lighting as moving objects; and the constructed track "jumps" from region to region (Fig. 3).

To estimate the effect of the illumination level on the change in a ctenophore behavior, it is necessary to determine contours of animal in heavily lit and darkened regions.

Applying the developed method with a "tracking" mask in Davinci Resolve, for better accuracy, it is possible to determine manually the body contour of an individual ctenophore, which differs it from special programs for tracking the movement of biological objects, and to track its movements during video recording in semi-automatic mode. The use of additional threshold algorithms is not required since the background is removed.

Video files processed in this way can be exported to almost any program for motion analysis. We tested programs for our purposes. Biological motion analysis programs have a number of benefits and limitations (Table 1).

Table 1. Comparison of programs for analyzing the movement of biological objects

Program	Types of analyses	Benefits	Limitations
EthoVision XT	Average speed, confidence intervals, area of objects, distances, and other motion characteristics	Real-time motion tracking. Ability to track multiple animals. Real-time track control	High cost. Need to purchase additional plugins. Specified types of analysis. Inability to save intermediate data
MouseMove	Distance, average speed, track curvature, and ROI	Free access. Plotting distance diagram, speed changes, *etc.*	Processes only one file at a time. Decreases the resolution of the source file
AnimalTracker	Total track length, average speed, trajectories, and percentage of time spent in ROI	Free access. Intuitive interface. Semi-automatic application of video filters and detection of moving objects. Flexible ROI setup. Saving coordinates	Processes only one file at a time
wrMTrck	Total distances, average speed, area, perimeter, and trajectories of objects. Coordinates, curvature of a track, *etc.*	Free access. The set of input parameters of the objects (their areas and changes over time, number of frames on which the object is monitored, maximum and minimum speeds, *etc.*). The movement of many objects in one video. Saving image files with motion tracks and background, video files with numbered objects	Processes only one file at a time. Does not determine the location of objects in ROI. Does not determine the motion vector
wrMTrck_Batch		All the benefits of wrMTrck. Batch processing of files. Automatic separation of the background and the use of the threshold filter. Automatic saving of the resulting tables with average values for all video files and for each file separately	Does not determine the location of objects in ROI. Does not determine the motion vector. Does not draw graphs of speeds and distances. Tracks are saved in raster format

EthoVision XT (information on acquisition options: https://www.noldus.com/animal-behavior-research/products/ethovision-xt) is an automatic, independent system designed to detect and analyze the movement of biological objects. EthoVision XT tracks the movement of one or more animals on video (in real time or pre-recorded). It has a variety of functions and types of motion analysis; it calculates average speed and confidence intervals, detects a change in direction of movement, and builds speed diagrams. For analysis, most types of video files (MPEG-1, MP4, AVI, *etc.*) are available. There are many additional plugins for specific research tasks (for example, connecting to a microscope and tracking several objects), but each plugin is supplied for a fee. The basic version of the program has limited functionality. In addition to the high cost of the program and its plugins, the disadvantages include the inability to save intermediate data, such as X-Y coordinates, instant speeds, *etc.* In the program, image tracks cannot be saved as separate files. There is no option to remove the background automatically.

MouseMove is a free access program developed in the LabVIEW 12.0 system [to download the program, see electronic supplementary material to (Samson et al., 2015)]. To run MouseMove.exe, installing an additional LabVIEW file is required. Creating a folder with video for analysis, *inter alia* a background video without animals, is pre-necessary. Then, it is required to perform the preparatory action algorithm developed by the authors in the ImageJ program. MouseMove accepts video in AVI with M-JPEG compression at 25 frames *per* second, a resolution of 640 × 480, but the program reduces the resolution down to 320 × 240 pixels. It provides such motion characteristics, as speed, distance, acceleration, curvature of the track, laterality (the number of turns to the left and right; the L / R ratio), and the time spent in ROI. MouseMove can be applied to analyze locomotor and social behavior of various animal species. However, the program has limitations on the input resolution of the analyzed video and cannot process certain types of behavior.

AnimalTracker (available for download at http://animaltracker.elte.hu/) is a plugin installed in the ImageJ program. It has three modules that work together: Tracker module processes video, facilitates the process of binarization of the image, and allows to get a TXT file with X-Y coordinates; TrackAnalyzer module visualizes the trajectories created by Tracker module; and ZoneDesigner can be configured to analyze different ROI. AnimalTracker calculates the following characteristics: total time of movement, time of absence of movement, distance and velocity vectors, their standard deviations, and maximum and minimum speed. Results can be saved as TXT files and spreadsheets. This software is convenient for determining locomotor and some characteristics of social and cognitive behavior. It is easy to use and involves the completion of several steps in a semi-automatic mode. However, the software recognizes only one animal at a time and does not allow processing several files at once.

WrMTrck (available for download at http://www.phage.dk/plugins/wrmtrck.html) is an ImageJ plugin written by Jesper Søndergaard Pedersen to determine movement parameters of *Caenorhabditis elegans* (Maupas, 1900) (Nussbaum-Krammer et al., 2015). Installing the plugin and using it do not require any additional actions from the researcher. Applying the plugin, it is possible to set the most suitable parameters for analyzing motion characteristics of various objects – minimum and maximum dimensions of the object in pixels, maximum speed of the object moving between frames, changing the visible area, and minimum number of frames during which the object moves. The parameter settings file can be saved and used for further analysis. The plugin monitors the movement of several objects at once on the frame and calculates the number of moving objects, determines characteristics of the movement of the objects separately and their average values. Moreover, processed videos can be saved in AVI; images can be saved in TIFF, JPEG, GIF, *etc.* The plugin does not track the location of objects in ROI and does not determine the motion vector.

WrMTrck_Batch (available for download with WrMTrck at the link above) is a modification of the wrMTrck plugin allowing to batch process files. The plugin automatically separates moving objects from the background and records additional parameters, such as X-Y coordinates, changes in shape and area of the object. After processing video files, WrMTrck_Batch automatically saves the resulting tables with the obtained characteristics in TXT format, motion tracks in PNG, and object movement frames for each video file separately in TIF. The plugin does not impose additional restrictions on the resolution of imported video files.

To determine the motion characteristics of *Mnemiopsis leidyi*, we chose the ImageJ program with the installed wrMTrck_Batch plugin: it allows to batch process video files and automatically save tables with coordinates for each moving object. This plugin was successfully tested by us to determine the motion characteristics of the Black Sea turbot sperm (Baiandina & Khanaychenko, 2019).

In its original version, wrMTrck_Batch plugin does not allow to obtain all the necessary movement characteristics. To process TXT data files, scripts were written. These scripts automate the calculation of animal motion characteristics (those are described in the "Material and methods" section). According to X-Y coordinates, the macros calculate and write into a new TXT file the values of instant and average speed for each track (one observation for one animal), average speed for all tracks, categorized values of direction vectors between all frames for each track, as well as finding an object in ROI for each frame and each track (ROI are set based on the experimental design by X-Y coordinates).

Thus, in our experiments, ctenophore movement was analyzed according to the scheme as follows:
1) video recording of the movement of ctenophores in aquariums in time-lapse mode;
2) processing of photographic materials in the Davinci Resolve – creating videos, determining individual animals with a "tracking" mask, and saving processed video clips as separate files;
3) running ImageJ with wrMTrck_Batch plugin – obtaining a separate folder with TXT files with the coordinates of movements of each animal on each frame;
4) running scripts for rendering new TXT files with summary tables of motility characteristics of each animal.

Conclusion. Automatic determination of motion parameters of a translucent ctenophore is almost impossible without preliminary "manual" processing of video files. The developed method allows automatic calculating of movement parameter set. The described algorithm helps to avoid many routine actions for processing each video file separately. It takes less than 1 min (for a computer with the configuration described in the paper) to create a summary table of the motion characteristics of 63 processed video records. The method was successfully applied for determining light-induced behavior motility characteristics of adult *Mnemiopsis leidyi* (Baiandina et al., 2022) in laboratory experiments and could be applied for behavioral study of various translucent marine animals.

This work was carried out within the framework of IBSS state research assignment "Regularities of formation and anthropogenic transformation of biodiversity and biological resources of the Sea of Azov–Black Sea basin and other areas of the World Ocean" (No. 121030100028-0).

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All applicable international, national, and institutional guidelines for the care and use of animals were followed.

REFERENCES

1. Alsaif K. I., Hamid R. H. Flora Zatoki Gdańskiej i wód przyleglych (Bałtyk Poludniowy). 6. Zielenice – Chlorophyta (red algae & brown algae). *International Journal of Computer Applications*, 2018, vol. 179, no. 16, pp. 10–13.
2. Baiandina Iu. S., Khanaychenko A. N. Optimization of the method for determining the motility characteristics of fish spermatozoa using ImageJ software and Excel macros. *Journal of Ichthyology*, 2019, vol. 59, iss. 1, pp. 127–130. https://doi.org/10.1134/S0032945219010016
3. Baiandina Iu. S., Kirin M. P., Krivenko O. V. Black Sea *Mnemiopsis leidyi* (Ctenophora) adult locomotion and light-induced behavior in laboratory experiments. *Journal of Sea Research*, 2022, vol. 180, art. no. 102152 (7 p.). https://doi.org/10.1016/j.seares.2021.102152
4. Franco-Restrepo J. E., Forero D. A., Vargas R. A. A review of freely available, open-source software for the automated analysis of the behavior of adult zebrafish. *Zebrafish*, 2019, vol. 16, no. 3, pp. 223–232. https://doi.org/10.1089/zeb.2018.1662
5. Gulyás M., Bencsik N., Pusztai S., Liliom H., Schlett K. AnimalTracker: An ImageJ-based tracking API to create a customized behaviour analyser program. *Neuroinformatics*, 2016, vol. 14, iss. 4, pp. 479–481. https://doi.org/10.1007/s12021-016-9303-z
6. Haney J. F. Diel patterns of zooplankton behavior. *Bulletin of Marine Science*, 1988, vol. 43, no. 3, pp. 583–603.
7. Haraldsson M., Båmstedt U., Tiselius P., Titelman J., Aksnes D. L. Evidence of diel vertical migration in *Mnemiopsis leidyi*. *PLoS ONE*, 2014, vol. 9, iss. 1, art. no. e86595 (10 p.). https://doi.org/10.1371/journal.pone.0086595
8. Hays G. C. A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migrations. *Hydrobiologia*, 2003, vol. 503, iss. 1–3, pp. 163–170. https://doi.org/10.1023/B:HYDR.0000008476.23617.b0
9. Kideys A., Romanova Z. Distribution of gelatinous macrozooplankton in the southern Black Sea during 1996–1999. *Marine Biology*, 2001, vol. 139, iss. 3, pp. 535–547. https://doi.org/10.1007/s002270100602
10. Larsen M. H., Johnsson J. I., Winberg S., Wilson A. D. M., Hammenstig D., Thörnqvist P.-O., Midwood J. D., Aarestrup K., Höglund E. Effects of emergence time and early social rearing environment on behaviour of Atlantic salmon: Consequences for juvenile fitness and smolt migration. *PLoS ONE*, 2015, vol. 10, iss. 3, art. no. e0119127 (17 p.). https://doi.org/10.1371/journal.pone.0119127
11. Mutlu E. Distribution and abundance of ctenophores and their zooplankton food in the Black Sea. II. *Mnemiopsis leidyi*. *Marine Biology*, 1999, vol. 135, iss. 4, pp. 603–613. https://doi.org/10.1007/s002270050661
12. Nussbaum-Krammer C. I., Neto M. F., Brielmann R. M., Pedersen J. S., Morimoto R. I. Investigating the spreading and toxicity of prion-like proteins using the metazoan model organism *C. elegans*. *Journal of Visualized Experiments*, 2015, vol. 95, art. no. e52321 (15 p.). https://doi.org/10.3791/52321
13. Ringelberg J. Changes in light intensity and diel vertical migration: A comparison of marine and freshwater environments. *Journal of the Marine Biological Association of the United Kingdom*, 1995, vol. 75, iss. 1, pp. 15–25. https://doi.org/10.1017/S0025315400015162
14. Ringelberg J. A. Decision-Making Mechanism. In: Ringelberg J. A. *Diel Vertical Migration of Zooplankton in Lakes and Oceans. Causal Explanations and Adaptive Significances.* Dordrecht ; Heidelberg ; London ; New York : Springer, 2009, pp. 49–70. https://doi.org/10.1007/978-90-481-3093-1_4
15. Ruppert E. E., Fox R. S., Barnes R. D. *Invertebrate Zoology: A Functional Evolutionary Approach.* 7th edition. Belmont, CA : Thomson-Brooks/Cole, 2004, 963 p.
16. Samson A. L., Ju L., Kim H. A., Zhang Sh. R., Lee J. A. A., Sturgeon Sh. A., Sobey Ch. G.,

Jackson Sh. P., Schoenwaelder S. M. Mouse-Move: An open source program for semi-automated analysis of movement and cognitive testing in rodents. *Scientific Reports*, 2015, vol. 5, art. no. 16171 (11 p.). https://doi.org/10.1038/srep16171

17. Schnitzler Ch. E., Pang K., Powers M. L., Reitzel A. M., Ryan J. F., Simmons D., Tada T., Park M., Gupta J., Brooks Sh. Y., Blakesley R. W., Yokoyama Sh., Haddock S. H. D., Martindale M. Q., Baxevanis A. D. Genomic organization, evolution, and expression of photoprotein and opsin genes in *Mnemiopsis leidyi*: A new view of ctenophore photocytes. *BMC Biology*, 2012, vol. 10, art. no. 107 (26 p.). https://doi.org/10.1186/1741-7007-10-107
18. Tamm S. L. Cilia and the life of ctenophores. *Invertebrate Biology*, 2014, vol. 133, iss. 1, pp. 1–46. https://doi.org/10.1111/ivb.12042
19. Wilson-Leedy J. G., Ingermann R. L. Development of a novel CASA system based on open source software for characterization of zebrafish sperm motility parameters. *Theriogenology*, 2007, vol. 67, iss. 3, pp. 661–672. https://doi.org/10.1016/j.theriogenology.2006.10.003
20. Zaika V. E., Sergeeva N. G. Diurnal changes of the population structure and vertical distribution of *Mnemiopsis mccradyi* Mayer (Ctenophora) in the Black Sea. *Gidrobiologicheskii zhurnal*, 1991, vol. 27, iss. 2, pp. 15–19.

КОМПЬЮТЕРНЫЕ МЕТОДЫ ОПРЕДЕЛЕНИЯ ХАРАКТЕРИСТИК ДВИЖЕНИЯ *MNEMIOPSIS LEIDYI*

Ю. С. Баяндина, О. Н. Кулешова

ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН»,
Севастополь, Российская Федерация
E-mail: *sepulturka@mail.ru*

Рассмотрены возможности основных современных компьютерных программ для анализа поведенческих реакций гребневиков, морских планктонных животных, в лабораторных условиях. Показано, что автоматическое определение контуров полупрозрачных объектов, таких как гребневики, недоступно в большинстве программ для анализа изображений. Разработан новый метод определения основных характеристик движения *Mnemiopsis leidyi* с помощью программы ImageJ, видеофильтров приложения Davinci Resolve и написанных авторами исполняемых программных кодов. Этот метод позволяет автоматизировать расчёт таких параметров, как средняя и максимальная скорость, изменение направления движения (векторы передвижения), процент времени, в течение которого животные находятся в определённых зонах интереса (regions of interest, ROI). Приведены значения средних скоростей движения гребневиков. Разработанный метод применим для изучения поведения различных полупрозрачных морских животных. Исполняемых программные коды доступны по запросу у авторов.

Ключевые слова: *Mnemiopsis leidyi*, гребневик, миграция, характеристики движения, анализ видеоизображений, ImageJ, wrMTrck_Batch, зоны интереса (ROI), threshold

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HEAVY METALS AND ARSENIC IN COMMERCIAL FISH OF THE SEA OF JAPAN, SEA OF OKHOTSK, AND BERING SEA: CURRENT STATUS (LITERATURE REVIEW)

M. K. Gamov[1], A. E. Ivanova[2], E. K. Mironova[1], and V. Yu. Tsygankov[1]

[1]Far Eastern Federal University, Vladivostok, Russian Federation
[2]Pacific branch of "VNIRO" ("TINRO"), Vladivostok, Russian Federation
E-mail: *gamovmota123456@gmail.com*

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The paper summarizes literature data on the concentrations of toxic elements – As, Pb, Cd, and Hg – in commercial fish of the Far Eastern seas – the Sea of Japan, Sea of Okhotsk, and Bering Sea. According to the analysis carried out, main commercial facilities and fishery basins meet the sanitary and hygienic standards. However, the existence of impact natural areas in fish ranges and on the routes of their migration contributes to an increase in concentrations of toxic elements in fishery objects. In some cases, the values exceed the maximum permissible levels. In this regard, it is necessary to continue monitoring of toxic microelements in commercial facilities and fishery basins of the Far Eastern seas.

Keywords: heavy metals, toxic elements, Far Eastern seas

The content of pollutants in marine ecosystems increases (Donets & Tsygankov, 2019), and this prioritizes food safety of seafood. Therefore, it is necessary to carry out chemical assessment of the quality of fish raw materials, in particular, from the standpoint of its pollution with heavy metals (Steblevskaya et al., 2016).

Heavy metals are characterized by high density and toxic effects even at very low concentrations (Duffus, 2002). Their intake into natural ecosystems occurs due to both natural processes and anthropogenic activities (Li et al., 2019). Heavy metals are considered hazardous because of their toxic and cumulative effects on living organisms and persistence in the environment.

Cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), and zinc (Zn) are essential trace elements; those are important for various biochemical and physiological functions (Dökmeci et al., 2014). Other metals – cadmium (Cd), mercury (Hg), and lead (Pb) – negatively affect living organisms even at relatively low concentrations (Bashkin & Kasimov, 2004 ; Hassan & Aarts, 2011).

Arsenic (As) is a carcinogenic chemical element with some properties of metals. In aquatic ecosystems, it can exist in both organic and inorganic (more toxic) forms (Soloduhina, 2014). Prolonged consumption of food containing As compounds can cause dangerous diseases (*e. g.*, coronary heart disease and neurological pathologies). Moreover, there may be physical and mental retardations in children and adolescents. Exposure to arsenic can cause diseases of the mucous membrane of the upper respiratory tract and emphysema, which increase the risk of lung cancer. Skin, liver, and bladder cancer may develop as well (Makarov, 2012).

Basically, cadmium, lead, mercury, and arsenic are accumulated in liver. This organ intensively accumulates metals, is a functional depot of these trace elements, and simultaneously participates in detoxification. A secondary position in terms of accumulation of toxic elements (Pb, As, and Cd) is occupied by muscle tissue. It can be attributed to accumulating organs as well, given that muscles make up a high percentage of body weight. Mostly, increased content of arsenic is recorded in gonads (Glazunova, 2007). Cadmium actively replaces calcium in cellular mechanisms regulating Ca concentration (through calcium channels). This toxicant enters the body mainly from the aquatic environment through gills which are actively involved in water–salt metabolism – regulate both absorption and excretion of water and salts (Chemagin et al., 2019). Lead is accumulated mainly in fish gills, liver, kidneys, and bones. In water bodies, Pb can be adsorbed by particles of bottom sediments; therefore, it will enter a body mostly through gills or with food. In the aquatic environment, under the effect of microorganisms, mercury is transformed into methylmercury which is taken up by a fish *via* the alimentary route and through gills during respiration, is intensively absorbed by tissues, and is accumulated in lipocytes. Moreover, methylation of inorganic Hg can occur in fish liver and intestines. Dissolved arsenic exists in trivalent and pentavalent forms. Fish absorb As from water through gills and intestines (Hrytsyniak et al., 2015). Analyzed organs and tissues accumulate various metals to varying degrees. The distribution of metals in fish is characterized by unevenness and depends on functional characteristics of organs, their cumulative activity, and chemical properties of a metal itself (Glazunova, 2007).

The issue of natural ecosystem pollution with heavy metals became especially acute in the mid-1960s – due to poisoning of people with mercury and cadmium compounds. They consumed food grown or caught in the environment polluted with Hg and Cd (Langston, 1990). Such cases confirm the need for monitoring water bodies aimed at detecting toxic elements and preventing dangerous situations.

In Russian Federation, the permissible level of heavy metals and arsenic is determined by the following regulatory documents controlling food safety: Sanitary Rules and Regulations 2.3.2.1078-01 (2002) and Technical Regulation of the Customs Union 021/2011 (2011).

Currently, in the Far Eastern fishery basin, about 99 % of all salmon are caught (out of the total catch in Russia), 100 % of crabs, over 90 % of flounders, over 40 % of herring, and about 60 % of molluscs. In terms of the volume of aquatic biological resources harvested by Russian fishermen, the Far Eastern basin ranks first (Pavlova et al., 2020). Therefore, annual studies of marine hydrobionts are required – in order to control commercial fishing areas.

To date, a large number of publications is focused on determining the trace element composition of hydrobionts in the Far Eastern seas (Chusovitina et al., 2020 ; Khristoforova & Kobzar, 2017, 2018 ; Khristoforova et al., 2015b ; Lebedev & Polyakova, 2019; *etc.*). Thereby, the aim of this work was to summarize the literature data on the content of toxic elements (cadmium, lead, arsenic, and mercury) in commercial fish of the Far Eastern seas of Russia.

Analytical techniques for determining heavy metals and arsenic

When determining the trace element concentration, the researchers used measuring instruments that differ in the way they work.

Predominantly, the atomic absorption was used which is based on the absorption of electromagnetic radiation of a certain wavelength by free atoms of the analyzed element. This technique involves using either electrothermal or flame atomizer. The electrothermal atomization is more sensitive to trace concentrations in the sample (from thousandths to tenths of $\mu g \cdot L^{-1}$) compared to the flame atomization (its sensitivity is much lower to concentrations from tenths to tens and hundreds of $\mu g \cdot L^{-1}$). At the same time, its detection limit is higher (from 10^{-1} to 10^{5} ng) than in the electrothermal variation (from 10^{-5} to 10 ng) (Burger et al., 2007 ; Khristoforova et al., 2015a, 2016 ; Kovekovdova & Simokon, 2004 ; Kovekovdova et al., 2016).

When determining mercury, the researchers carried out the stripping voltammetry and cold vapor atomic absorption. The core of the first technique is the isolation of the analyzed element from a dilute solution by electrolysis on a stationary indicator electrode, subsequent anodic dissolution of the obtained amalgam, and registration of the current–voltage curve. It allows determining the content at the level of tenths and hundredths of $\mu g \cdot L^{-1}$ (Khristoforova et al., 2015a, 2016). The cold vapor atomic absorption is based on the property of mercury to exist in the form of free atoms in the gas phase under normal conditions. The detection limit for this technique is 0.001–0.5 $\mu g \cdot mL^{-1}$ (Burger et al., 2007 ; Hwang et al., 2019 ; Kovekovdova & Simokon, 2004 ; Kovekovdova et al., 2016).

The core of the X-ray fluorescence is obtaining the spectrum of the studied material under X-rays. The detection limit is from 0.0001 % to 100 % (Chusovitina et al., 2020 ; Steblevskaya et al., 2013, 2016).

Toxic elements in commercial fish of the Sea of Okhotsk

The Sea of Okhotsk is one of the largest and deepest seas in Russia. Its area is 1,603 thousand km^2; volume, 1,318 thousand km^3; mean depth, 821 m; and maximum depth, 3,916 m (Dobrovolsky & Zalogin, 1982). It is characterized by impact geochemical conditions; those are formed by surface and underwater volcanism and postvolcanism of the Kuril Islands, as well as by upwellings delivering many chemical elements from the depths of the Kuril–Kamchatka Trench to the surface layer (Khristoforova et al., 2019b). The runoff of the Amur River carrying a large number of pollutants into the Sea of Okhotsk, active navigation, and oil production on the shelf are the factors which can negatively affect the trace element composition of marine ecosystems in this sea (Isakov & Kasperovich, 2007).

Pacific salmon (*Oncorhynchus* Suckley, 1861). It is the most valuable commercial fish: in terms of catch in the current century, Pacific salmon ranks the second–third (after pollock and herring). Its catches are 90 % provided by three species – the chum, pink, and sockeye salmon (Khristoforova et al., 2015b). These species are a suitable object for monitoring environmental pollution with trace elements due to their nutritional value, high abundance, and specificity of biological cycles and life strategies. While feeding in the ocean, fish make long seasonal migrations; as a result, trace elements can be accumulated in them in large amounts (Khristoforova et al., 2015b).

In 2013–2016, carcasses of the chum and pink salmon were investigated (Fig. 1). The content of toxic elements did not exceed the maximum permissible levels (hereinafter MPL) (TR TS 021/2011, 2011). Importantly, lead concentrations in carcass of the chum salmon sampled in 2013 (0.98 $mg \cdot kg^{-1}$ wet weight) approached the MPL (1.0 $mg \cdot kg^{-1}$ wet weight).

In the chum salmon tissues, trace element concentrations were higher than in the pink salmon tissues. This may be due to a longer life span and higher weight of the chum salmon (Zelenikhina et al., 2015).

In the chum salmon liver, high concentrations of lead were recorded: the values exceeded the MPL (TR TS 021/2011, 2011) (Fig. 2). Apparently, this is due to accumulating function of liver (Khristoforova et al., 2016). The content of mercury did not exceed the MPL.

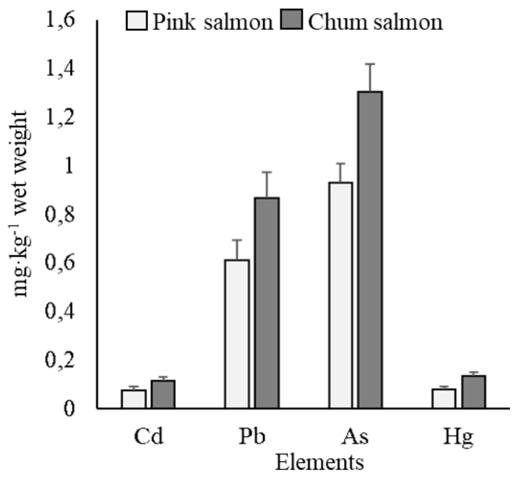

Fig. 1. Mean concentrations of heavy metals and arsenic in the pink and chum salmon carcasses, the Sea of Okhotsk (Khristoforova et al., 2015a)*

Such levels of accumulation are likely to be related to a long life span of the Pacific salmon and the fact that its feeding areas are located near spots characterized by abnormally high trace element concentrations (Khristoforova et al., 2016).

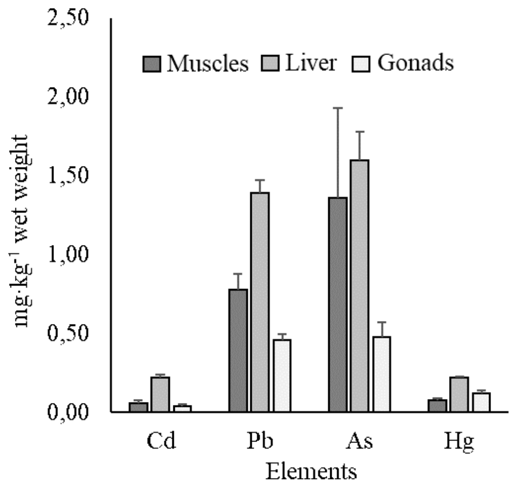

Fig. 2. Maximum mean concentrations of heavy metals and arsenic in the chum salmon organs, the Sea of Okhotsk (Khristoforova et al., 2016)

Sculpin (Cottidae Bonaparte, 1831). This family is one of the most typical and diverse in the northern Pacific Ocean. Cottidae lead benthic lifestyle and can make seasonal migrations (Matveev & Terentiev, 2016).

In 2013, representatives of the genus *Myoxocephalus* Tilesius, 1811 were sampled (Kovekovdova et al., 2013). When studying the trace element composition, a significant excess of the MPL was registered for cadmium in liver. Specifically, a value of 4.52 mg·kg^{-1} wet weight was recorded while the threshold is 0.7 mg·kg^{-1} wet weight. Moreover, arsenic, lead, and mercury were found in liver (Table 1). It can be concluded that liver is the main accumulating organ (Glazunova, 2007).

*__Note.__ Maximum permissible levels (TR TS 021/2011, 2011) of toxic elements in fresh, chilled, and frozen sea fish: Pb, 1.0; Cd, 0.20; As, 5.0; Hg, 0.50 mg·kg^{-1} wet weight. In fish caviar and milt: As, 1.0; Cd, 1.0; Hg, 0.2 mg·kg^{-1} wet weight. In fish liver: Cd, 0.7; Hg, 0.2 mg·kg^{-1} wet weight.

Arsenic is accumulated mainly in muscles and gonads. The values registered during the research (see Table 1) may indicate the effect of terrigenous and anthropogenic pollution since *Myoxocephalus* fish feed in coastal areas during their migration (Wheeler, 1983).

Table 1. Maximum concentrations of heavy metals and arsenic in organs of *Myoxocephalus* fish, the Sea of Okhotsk, 2013, mg·kg^{-1} wet weight (Kovekovdova et al., 2013)

Organ	Cd	Pb	As	Hg
Liver	4.52	0.05	0.84	0.09
Muscles	0.05	0.02	1.88	0.02
Gonads	0.60	0.02	1.42	0.01

Righteye flounder (Pleuronectidae G. Cuvier 1816). This family is characterized by a rather wide species diversity in the World Ocean. Mostly, Pleuronectidae representatives are found in coastal shallow water, intertidal zones, and shelf areas. Fish can make seasonal migrations. The righteye flounders can be predators (benthivorous) or fish with a mixed feeding type (Wheeler, 1983). When reaching high abundance, some Pleuronectidae representatives become economically valuable. However, those are currently considered as a minor commercial species encountered as by-catch (Datsky & Maznikova, 2017).

In 2013, Schrenk flounder *Pseudopleuronectes schrenki* (Schmidt, 1904) was sampled for the trace element analysis (Kovekovdova et al., 2013). In liver, an increased concentration of cadmium was registered (1.7 mg·kg^{-1} wet weight): the MPL (0.7 mg·kg^{-1} wet weight) was exceeded by 2.4 times. The content of arsenic in gonads (1.2 mg·kg^{-1} wet weight) also exceeded the MPL (1.0 mg·kg^{-1} wet weight) (Fig. 3). Such a high concentration of cadmium in Schrenk flounder liver is explained by its metabolic–accumulating function, as well as by possible fish feeding in the area of wastewater of mining enterprises.

In general, it is arsenic that is most actively accumulated in all the studied organs. Apparently, this is due to physiological peculiarities of the Schrenk flounder.

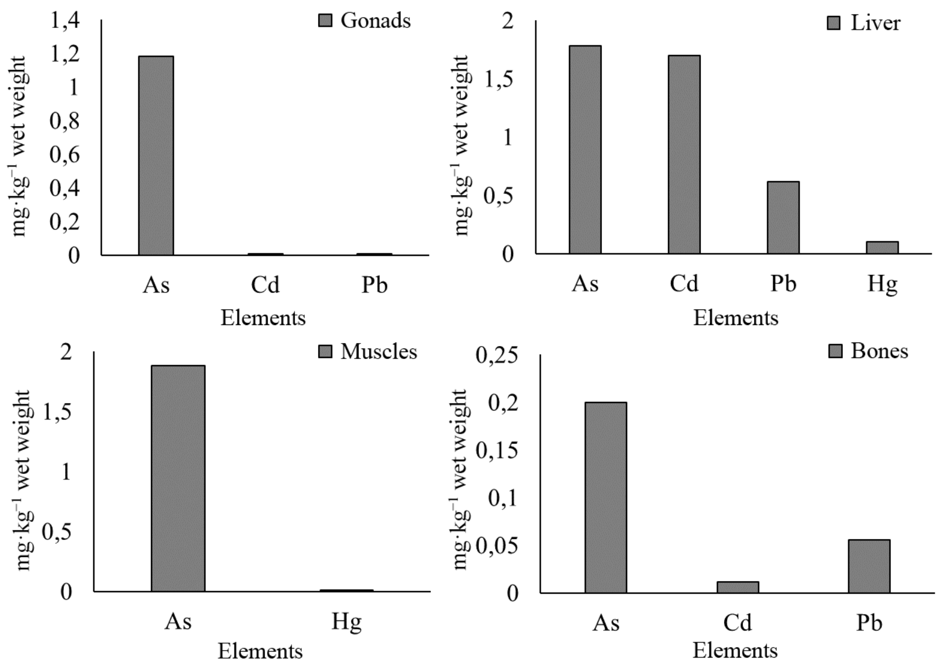

Fig. 3. Maximum concentrations of heavy metals and arsenic in organs of the Schrenk flounder *Pseudopleuronectes schrenki*, the Sea of Okhotsk (Kovekovdova et al., 2013)

Toxic elements in commercial fish of the Bering Sea

The Bering Sea is the largest of the Far Eastern seas washing the shores of Russia. Its area is 2,315 thousand km^2; volume, 3,796 thousand km^3; mean depth, 1,640 m; and maximum depth, 5,500 m. It is located in the northern Pacific Ocean and separated from it by the Aleutian and Commander islands; the Bering Strait connects it with the Chukchi Sea and Arctic Ocean (Shlyamin, 1958).

Anthropogenic load on the Bering Sea is exerted by rather active navigation causing local oil pollution. Coastal areas – the Anadyr Estuary, Ugolnaya Bay, and Kamchatka Peninsula shelf (Kamchatka Bay) – are subjected to the most intense load. The most polluted waters are those off the coast of Alaska (Balykin, 2006). Also, the trace element composition of waters can be affected by underwater volcanism – in particular, by activity of the Piip Volcano which is one of the largest (Astakhov et al., 2011). Importantly, near Anadyr, there is Ugolnye Kopi urban locality, with its mines and polluted industrial and domestic wastewaters. In some years, those brought to the Anadyr Estuary oil; petroleum products; sulfurous and hydrogen sulfide–containing gases; drill cuttings; mineralized formation water and wastewater from oil industry and well drilling; *etc.* (Poddubny, 2002).

Codfish (Gadidae Rafinesque, 1810). These pelagic fish are characterized by a wide species diversity and are widely spread in the oceans and seas of the Northern Hemisphere. Codfish are of high commercial importance. Gadidae representatives grow in size as long as they live, and fish life span averages 25 years. Codfish can be predators or plankton feeders (Orlov & Afanasiev, 2013).

The Pacific cod do not make long migrations; fish inhabit water areas of the Sea of Japan, Sea of Okhotsk, and Bering Sea keeping to the shore. The cod life span averages 10–12 years (Orlov & Afanasiev, 2013). For these reasons, codfish can act as bioindicators.

In order to study the trace element composition, the Pacific cod *Gadus macrocephalus* Tilesius, 1810 was sampled in the Aleutian Islands area in 2004 (Burger et al., 2007). The maximum values were recorded in liver (Fig. 4). The relatively high concentration of Cd in liver can be explained by high content of specific low molecular weight proteins – metallothioneins which have the capacity to bind cadmium (Alexeyeva & Tyunev, 2017).

In liver, the concentration of arsenic approaches the threshold of 5.0 mg·kg^{-1} wet weight. In muscles, lead and mercury prevail.

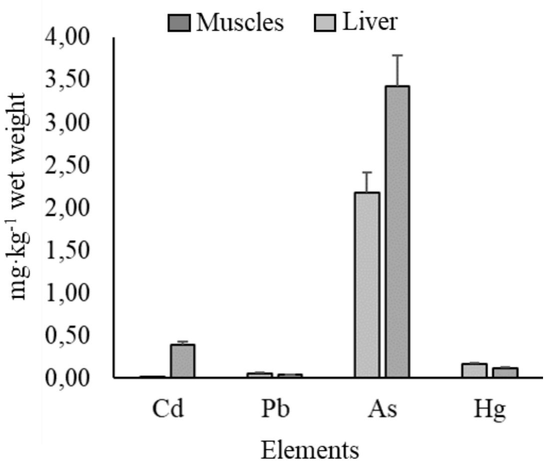

Fig. 4. Mean concentrations of heavy metals and arsenic in organs of the Pacific cod *Gadus macrocephalus*, the Bering Sea (Burger et al., 2007)

Righteye flounder (Pleuronectidae). Fish were sampled in the Aleutian Islands area in 2004 (Burger et al., 2007). In kidneys, the highest concentrations of lead and arsenic were recorded. Pb content (1.2 mg·kg^{-1} wet weight) exceeded the MPL (1.00 mg·kg^{-1} wet weight) (Fig. 5). In liver, cadmium, arsenic, and mercury prevailed. Cd concentration in liver (4.96 mg·kg^{-1} wet weight) was almost 7 times higher than the MPL (0.7 mg·kg^{-1} wet weight). In muscle tissue, Hg concentration was the highest since most of the mercury–binding proteins are there (Petukhov & Morozov, 1983).

In all analyzed organs, arsenic levels exceeded its threshold (MPL for sea fish is 5.00 mg·kg^{-1} wet weight, see note to Fig. 1). Specifically, in flounder kidneys, the value was 32.38 mg·kg^{-1} wet weight; in liver, 18.95; and in muscles, 19.45. The threshold was exceeded by almost 6.5, 4, and 6.5 times, respectively.

Fish were sampled on the Aleutian Islands – the islands of Adak, Kyska, and Amchitka. During World War II, military vehicles and weapons were tested there. As a result, lead, mercury, cadmium, and arsenic entered the water area, as well as petrochemicals, polyaromatic hydrocarbons, pesticides, and radioactive materials (Public Health Assessment, 2002).

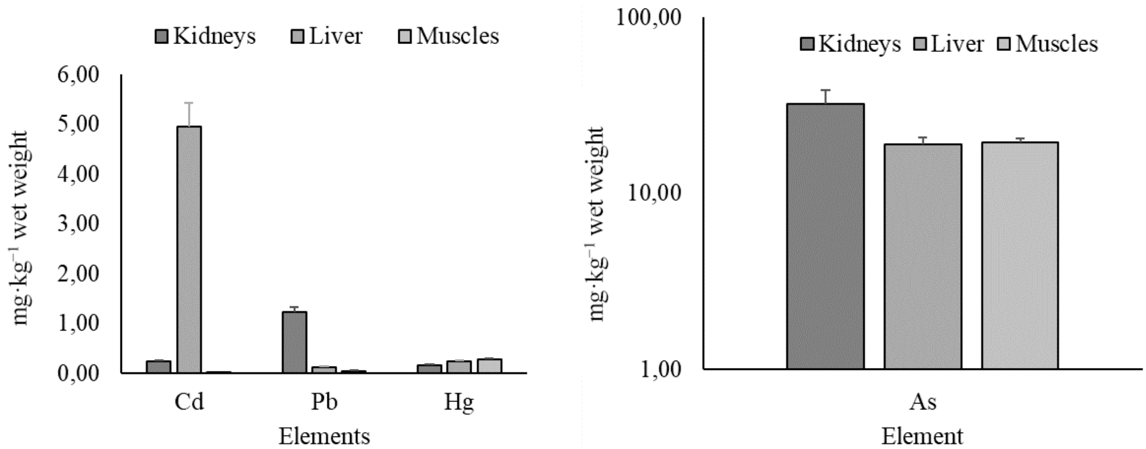

Fig. 5. Mean concentrations of trace elements in flounder, the Bering Sea (Burger et al., 2007)

Sculpin (Cottidae). In 2004, representatives of the genus *Myoxocephalus* were sampled in the Aleutian Islands area (Burger et al., 2007). The highest concentrations in fish liver were those of arsenic and cadmium. Importantly, the value for Cd (1.26 mg·kg^{-1} wet weight) exceeded the MPL (0.7 mg·kg^{-1} wet weight) (Fig. 6). Apparently, this is due to the fact that liver is involved in detoxification.

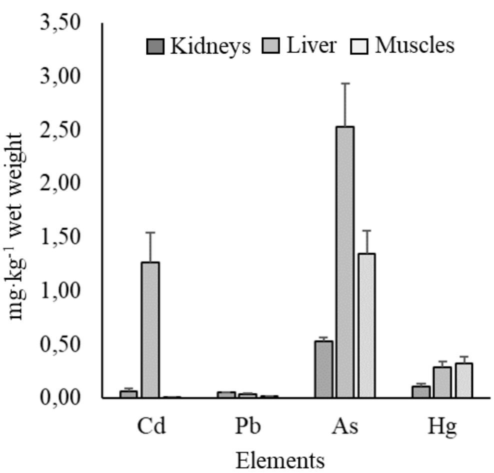

Fig. 6. Maximum mean concentrations of heavy metals and arsenic in organs of *Myoxocephalus* fish, the Bering Sea, 2004 (Burger et al., 2007)

Mercury is accumulated mostly in muscles; lead, in kidneys. In general, in *Myoxocephalus* fish, as in righteye flounders, increased concentrations may originate from the military activity on the Adak Island (Burger et al., 2007).

Toxic elements in commercial fish of the Sea of Japan

The Sea of Japan is a semi-enclosed sea of the Pacific Ocean. Its area is 1,062 thousand km^2; volume, 1,715 thousand km^3; mean depth, 1,750 m; and maximum depth, 3,720 m. From the neighboring Sea of Okhotsk, it is separated by the Sakhalin Island; from the Yellow Sea, by the Korean Peninsula. It washes the coasts of Russia, Japan, South Korea, and North Korea (Shuntov, 2001).

Due to a weak connection with the Pacific Ocean by several shallow straits, the Sea of Japan is under strong anthropogenic, technogenic, and terrigenous load. The affecting factors are domestic and industrial wastewaters, surface runoff from coastal areas, and consequences of burning fuel oil due to active navigation (Khristoforova, 1989).

Pacific salmon (*Oncorhynchus*). In 2013–2015, the pink salmon *Oncorhynchus gorbuscha* (Walbaum, 1792) was sampled (Kovekovdova et al., 2013, 2016). Its trace element composition complied with the thresholds; there was no noticeable excess of the MPL (Fig. 7). Apparently, this is due to a too short life span of the pink salmon – about 1.5 years (Khristoforova et al., 2019a).

Trace elements are mainly accumulated in liver, especially cadmium and arsenic. This is due to its metabolic–accumulating function.

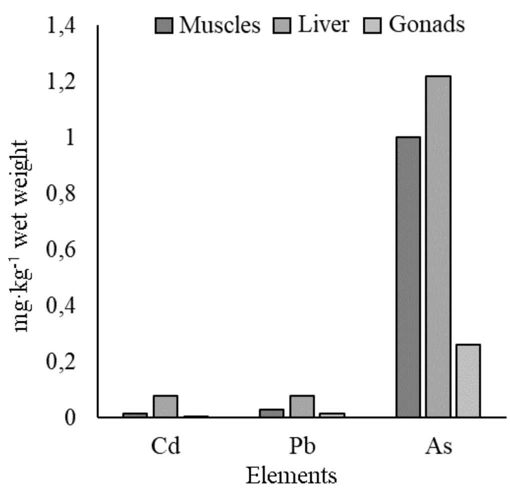

Fig. 7. Maximum concentrations of trace elements in organs and tissues of the pink salmon *Oncorhynchus gorbuscha*, the Sea of Japan, 2013–2015 (Kovekovdova et al., 2013, 2016)

Greenling (Hexagrammidae Gill, 1889). Representatives of this family lead predominantly benthic and coastal lifestyle. Fish are objects of both recreational and commercial fishing. Mostly, greenlings feed on benthos and plankton (Antonenko & Pushchina, 2002).

In 2004–2013, Hexagrammidae fish were sampled for the trace element analysis (Kovekovdova & Simokon, 2004 ; Kovekovdova et al., 2013). The excess of the MPL for cadmium (0.7 mg·kg^{-1} wet weight) was recorded in liver alone: the value was of 0.79 mg·kg^{-1} wet weight. In muscles, active accumulation of arsenic was registered (Fig. 8). Cadmium, lead, and mercury are accumulated predominantly in liver.

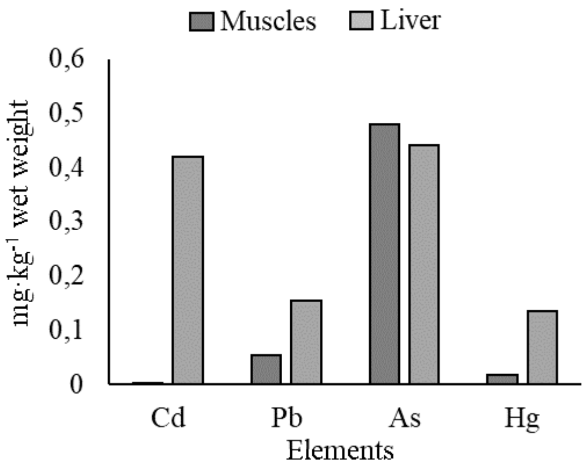

Fig. 8. Maximum concentrations of heavy metals and arsenic in organs and tissues of Hexagrammidae fish, the Sea of Japan, 2004–2013 (Kovekovdova & Simokon, 2004 ; Kovekovdova et al., 2013)

Herring (Clupeidae G. Cuvier, 1817). Fish are widely spread from the Subantarctic to Arctic. A typical representative of this family is the Pacific herring *Clupea pallasii* Valenciennes, 1847 having three ecological forms – marine, coastal, and lagoon–lake. Along the continent of Asia, herring is spread continuously from the Yellow Sea to the Bering Strait, *inter alia* in the Sea of Japan, Sea of Okhotsk, and Bering Sea. Fish feed mainly on zooplankton. The life span is up to 19 years (Naumenko, 2007).

The values for the trace element content in herring sampled in 2004–2013 generally complied with the MPL (Kovekovdova & Simokon, 2004 ; Kovekovdova et al., 2013). Importantly, cadmium concentrations in fish liver were rather high: the value was of 0.96 mg·kg^{-1} wet weight while the MPL is 0.7 mg·kg^{-1} wet weight (Fig. 9).

Interestingly, all the studied trace elements were localized in muscles (see Fig. 9). This can be explained by fish feeding in the areas of terrigenous and anthropogenic runoff.

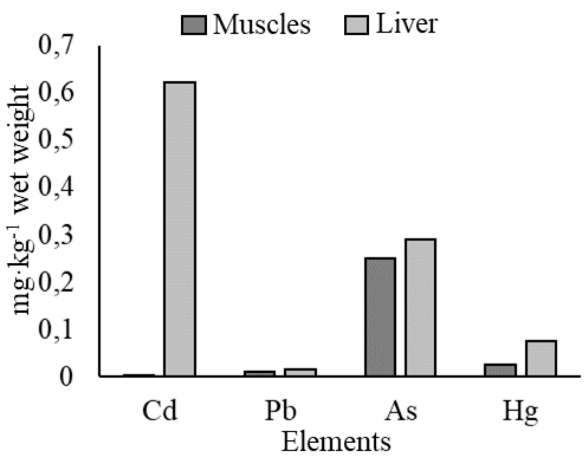

Fig. 9. Maximum concentrations of trace elements in organs and tissues of the Pacific herring *Clupea pallasii*, the Sea of Japan, 2004–2013 (Kovekovdova & Simokon, 2004 ; Kovekovdova et al., 2013)

Codfish (Gadidae). In 2013–2014, representatives of this family were studied – the Alaska pollock *Gadus chalcogrammus* Pallas, 1814 and saffron cod *Eleginus gracilis* (Tilesius, 1810) (Hwang et al., 2019 ; Kovekovdova et al., 2013).

Codfish are characterized by a wide species diversity. Those inhabit mainly the pelagic zone; some fish lead benthic lifestyle. By the feeding type, codfish are mainly predators (Napazakov et al., 2001).

As can be seen in Fig. 10, trace element concentrations in liver significantly exceed those in other organs. The MPL for arsenic was exceeded by almost 4 times. Mercury was accumulated predominantly in gonads. Muscles accumulated cadmium (the value was almost 45 times higher than the MPL) and lead (see Fig. 10). Such an active intake of trace elements and their subsequent accumulation in fish organs can originate from surface and river runoff, resuspension, and bioturbation of bottom sediments (Khristoforova, 1989).

Such values may result from rapidly developing industry of the Republic of Korea as well (some Gadidae representatives were sampled in its territorial waters) (Hwang et al., 2019). This country is quite rich in deposits located on its coast. Mainly, coal, iron, molybdenum, and lead–zinc ores are mined (Hwang et al., 2019). Apparently, these industries are the reason for the intake of polluted waters into the Sea of Japan.

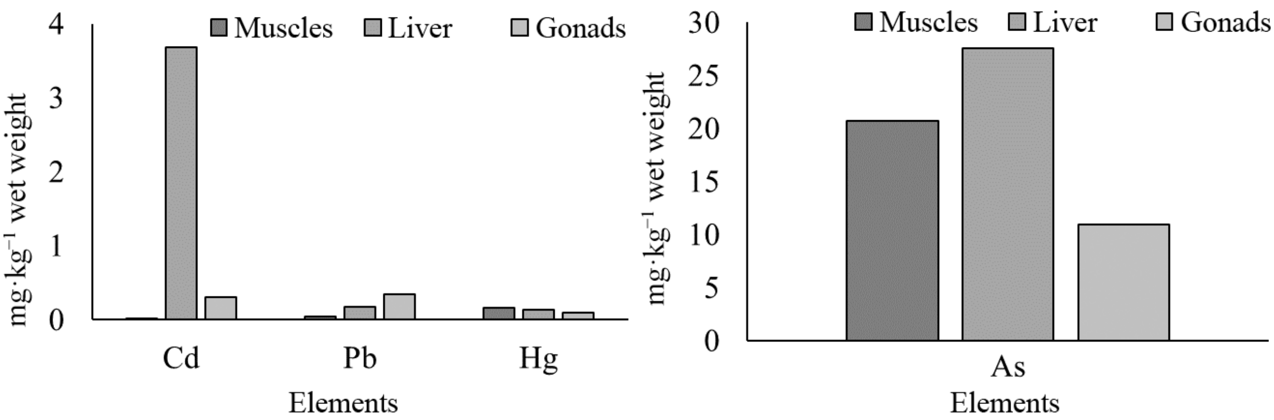

Fig. 10. Maximum concentrations of heavy metals and arsenic in organs and tissues of Gadidae fish, the Sea of Japan, 2013–2014 (Hwang et al., 2019)

Righteye flounder (Pleuronectidae). Representatives of this family were sampled in the Sea of Japan in 2004–2020 (Chusovitina et al., 2020 ; Kovekovdova & Simokon, 2004 ; Steblevskaya et al., 2013, 2016).

Based on the analyzed data, there was no excess of the MPL in righteye flounder organs. Cadmium and mercury were accumulated most actively in liver; arsenic, in muscles; and lead, in gills (Fig. 11).

Recorded concentrations of trace elements may indicate their accumulation in soils. Specifically, studies carried out by L. Kovekovdova *et al.* (2002, 2010) revealed an increase in arsenic content in organs and tissues of molluscs and fish in the Amur Bay due to its high concentration in soils.

The generalized data on the excess of the MPL for toxic trace elements in fish sampled in the Far Eastern seas are given in Fig. 12.

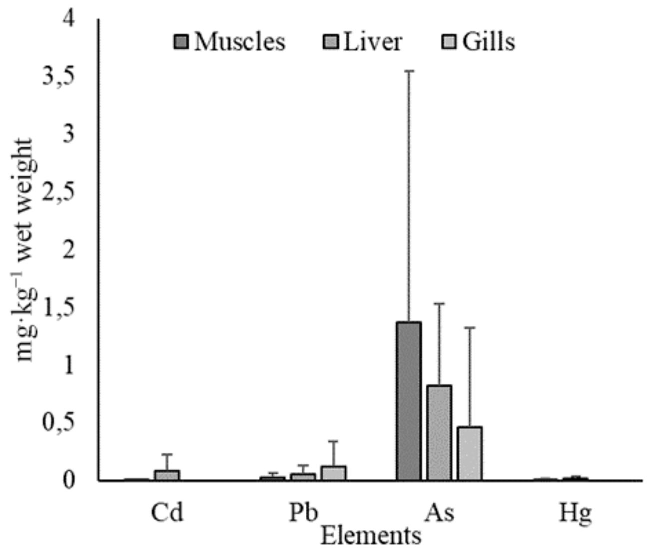

Fig. 11. Maximum mean concentrations of heavy metals and arsenic in organs of Pleuronectidae fish, the Sea of Japan, 2004–2020 (Chusovitina et al., 2020 ; Kovekovdova & Simokon, 2004 ; Steblevskaya et al., 2013, 2016)

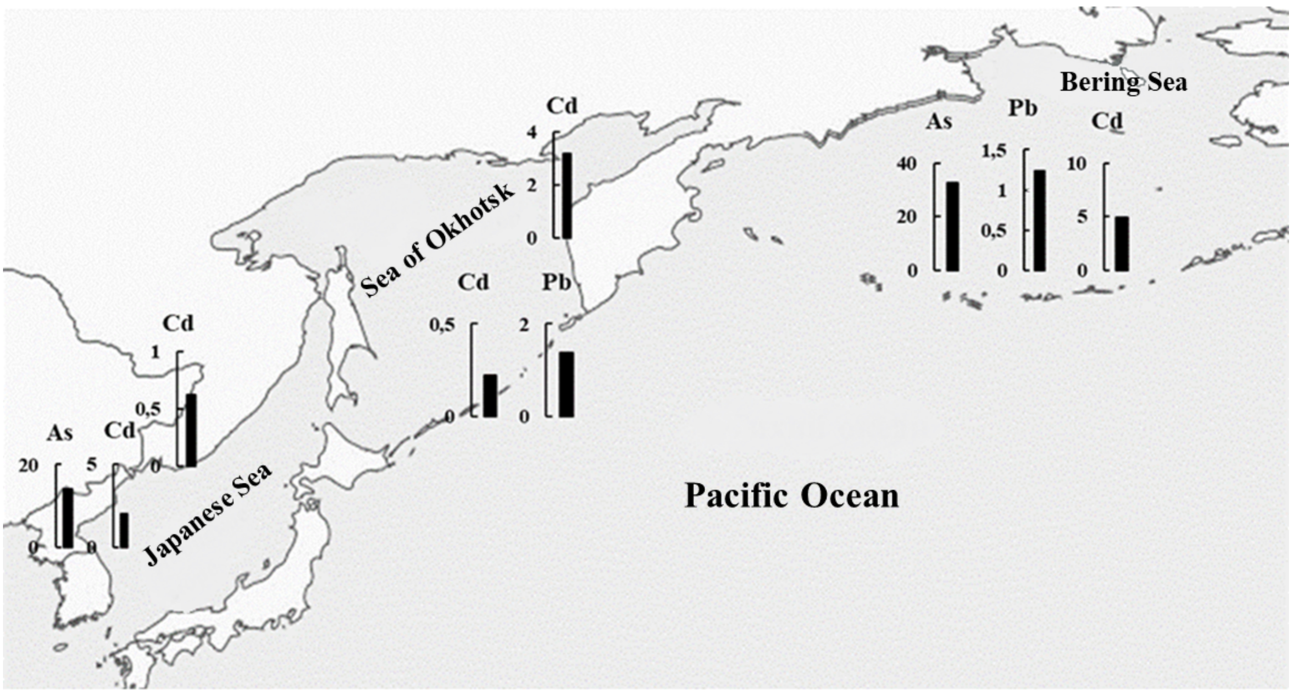

Fig. 12. Excess of the maximum permissible level (see note to Fig. 1) of toxic elements in fish of the Far Eastern seas: 1, maximum concentration; 2, maximum mean concentration; 3, mean concentration; 4, muscles; 5, gonads; 6, liver; 7, kidneys (Burger et al., 2007 ; Hwang et al., 2019 ; Khristoforova et al., 2016 ; Kovekovdova & Simokon, 2004 ; Kovekovdova et al., 2013)

Conclusion. In almost all studied fish, excess concentrations of such extremely toxic elements, as cadmium, lead, and arsenic, were revealed.

The highest cadmium content was recorded in organs of fish sampled in the Sea of Japan. Specifically, Cd concentration in muscles of codfish was 45 times higher than the maximum permissible level. Apparently, this is due to the fact that muscles make up a high percentage of body weight. Moreover, muscles are an accumulating organ; therefore, muscles are capable of accumulating significant concentrations of trace elements. Lead content in liver of the righteye flounder from the Sea of Okhotsk exceeded the MPL by 1.2 times. These fish are benthivorous; when feeding, they can accumulate adsorbed Pb from bottom sediments. High concentrations of arsenic were recorded in kidneys of the righteye flounder sampled in the Bering Sea (the MPL was exceeded by 6.5 times) and in liver of codfish from the Sea of Japan (the MPL was exceeded by 5.5 times). In the Far Eastern seas, there are areas with anomalous hydrochemical and geochemical conditions which can affect the trace element composition of hydrobionts. Food safety largely depends on the frequency of consumption of a particular product. This is especially relevant for trace elements which are capable of biomagnification and therefore can be accumulated over a lifetime.

Thus, consuming products from fish caught in the Far Eastern seas may be unsafe for human health. Regular monitoring is required to control the quality of seafood. In this regard, studies of the trace element composition of fish remain extremely relevant.

REFERENCES

1. Alexeyeva M. V., Tyunev A. V. Content and characteristics of toxic element dissemination in tissues and organs of the Baltic Sea fish. *Veterinary Science Today*, 2017, no. 3, pp. 62–64. (in Russ.)
2. Antonenko D. V., Pushchina O. I. The main biological features of greenlings (genus *Hexagrammos*) in Peter the Great Bay (Japan Sea). *Izvestiya TINRO*, 2002, vol. 131, pp. 164–178. (in Russ.)
3. Balykin P. A. *The State and the Resources of Fishing in the Western Bering Sea.* Moscow : VNIRO Publishing, 2006, 142 p. (in Russ.)
4. Bashkin V. N., Kasimov N. S. *Biogeokhimiya.* Moscow : Nauchnyi mir, 2004, 648 p. (in Russ.)
5. Wheeler A. *Key to the Fishes of Northern Europe : A Guide to the Identification of More Than 350 Species.* Moscow : Legkaya i pishchevaya promyshlennost', 1983, 432 p. (in Russ.)
6. Glazunova I. A. Soderzhanie i osobennosti raspredeleniya tyazhelykh metallov v organakh i tkanyakh ryb Verkhnei Obi. *Izvestiya Altaiskogo gosudarstvennogo universiteta*, 2007, no. 3, pp. 20–22. (in Russ.)
7. Hrytsyniak I. I., Yanovych D. O., Bekh V. V. Effect of non-essential elements (mercury, arsenic) on salmonids (Salmonidae) (review). *Rybohospodarska nauka Ukrainy*, 2015, no. 3, pp. 18–33. (in Ukr.)]. https://doi.org/10.15407/fsu2015.03.018
8. Dobrovolsky A. D., Zalogin B. S. *Morya SSSR.* Moscow : Izd-vo MGU, 1982, 192 p. (in Russ.)
9. Donets M. M., Tsygankov V. Yu. Current levels of pollutants in commercial hydrobionts of the Russian Far Eastern seas. *Vestnik of Far Eastern Branch of Russian Academy of Sciences*, 2019, no. 4, pp. 90–103. (in Russ.). https://doi.org/10.25808/08697698.2019.206.4.010

10. Zelenikhina G. S., Elnikov A. N., Tochilina T. G. The downstream migration of juvenile pink and chum salmon in Reydovaya River Iturup Island (South Kuril Islands) in spring–summer season of 2014. *Trudy VNIRO*, 2015, vol. 158, pp. 6–15. (in Russ.)
11. Isakov A. Ya., Kasperovich E. V. O zagryaznenii nefteproduktami Okhotskogo morya. *Polythematic Online Scientific Journal of Kuban State Agrarian University*, 2007, no. 26, pp. 1–6. (in Russ.)
12. Kovekovdova L. T., Kiku D. P., Kasyanenko I. S. Monitoring of water environment and food safety of commercial objects in the Far East fishery basin (toxic elements). In: *Morskie biologicheskie issledovaniya: dostizheniya i perspektivy* : v 3 tomakh : sb. materialov Vseros. nauch.-prakt. konf. s mezhdunar. uchastiem, priuroch. k 145-letiyu Sevastopol'skoi biologicheskoi stantsii, Sevastopol, 19–24 Sept., 2016. Sevastopol, 2016, vol. 3, pp. 111–114. (in Russ.)
13. Kovekovdova L. T., Simokon M. V., Kiku D. P. Microelemental composition of Far Eastern commercial fishes. *Regional Environmental Issues*, 2013, no. 2, pp. 72–75. (in Russ.)
14. Kovekovdova L. T., Ivanenko N. V., Simokon M. V. Osobennosti raspredeleniya As v komponentakh morskikh pribrezhnykh ekosistem Primor'ya. *Issledovano v Rossii*, 2002, vol. 5, pp. 1437–1445. (in Russ.)
15. Kovekovdova L. T., Simokon M. V. Assessment of metals and arsenic content in bottom sediments and fish tissues from the rivers and estuaries of Peter the Great Bay (Japan Sea). *Izvestiya TINRO*, 2010, vol. 160, pp. 223–235. (in Russ.)
16. Kovekovdova L. T., Simokon M. V. Tendencies in change of chemoecological situation in the coastal area of Primorye. Toxic elements in bottom sediments and aquatic organisms. *Izvestiya TINRO*, 2004, vol. 137, pp. 310–320. (in Russ.)
17. Lebedev L. E., Polyakova N. V. Heavy metals content of tissues and members of black plaice *Pseudopleuronectes obscurus* (Herzenstein, 1890) (Pleuronectidae) of Peter the Great Bay, Sea of Japan. *Scientific Journal of the Far East State Technical Fisheries University*, 2019, vol. 49, no. 3, pp. 70–77. (in Russ.)
18. Makarov V. N. Mysh'yak v biosfere Yakutii. *Nauka i tekhnika v Yakutii*, 2012, no. 1 (22), pp. 41–46. (in Russ.)
19. Matveev A. A., Terentiev D. A. Fishery, long-term dynamics of biomass, distribution and length composition of mass species of Cottidae on the west coast of Kamchatka. *Researches of Aquatic Biological Resources of Kamchatka and of the North-west Part of Pacific Ocean*, 2016, iss. 41, pp. 17–42. (in Russ.). https://doi.org/10.15853/2072-8212.2016.41.17-42
20. Napazakov V. V., Chuchukalo V. I., Kuznetsova N. A., Radchenko V. I., Slabinsky A. M., Nadtochy V. A. Feeding and some features of ecology of Gadidae fish in the western part of Bering Sea in the summer–autumn season. *Izvestiya TINRO*, 2001, vol. 128-3, pp. 907–928. (in Russ.)
21. Naumenko N. I. Far Eastern herring by eyes turned to XXI century (literature review, short excurse to the history of fishing and researches). *Researches of Aquatic Biological Resources of Kamchatka and of the North-west Part of Pacific Ocean*, 2007, iss. 9, pp. 185–190. (in Russ.)
22. Orlov A. M., Afanasiev P. K. Otolithometry as possible tool of the analysis of Pacific cod *Gadus macrocephalus* (Gadidae, Teleostei) population structure. *Amurian Zoological Journal*, 2013, vol. 5, no. 3, pp. 327–331. (in Russ.)
23. Pavlova S. A., Pavlov I. E., Shukshina T. G. East Basin – politically important fishing area.

Innovation Science, 2020, no. 6, pp. 179–182. (in Russ.)

24. Petukhov S. A., Morozov N. P. A contribution to the study of "species" differences in the trace element composition of fishes. *Voprosy ikhtiologii*, 1983, vol. 23, no. 5, pp. 870–872. (in Russ.)
25. Poddubny A. V. *Ekologicheskie problemy i ustoichivoe razvitie regionov* : uchebnoe posobie. Vladivostok : TIDOT DVGU, 2002, 143 p. (in Russ.)
26. SanPiN 2.3.2.1078-01. *Gigienicheskie trebovaniya bezopasnosti i pishchevoi tsennosti pishchevykh produktov.* Moscow : Goskomsanepidnadzor RF, 2002, 156 p. (in Russ.)
27. Soloduhina M. A. Arsenic in plants of natural and anthropogenic landscapes of Sherlovogorsky ore district of the Zabaikalsky Krai. *Mezhdunarodnyi zhurnal prikladnykh i fundamental'nykh issledovanii*, 2014, no. 11-3, pp. 377–382. (in Russ.)
28. Steblevskaya N. I., Polyakova N. V., Zhad'ko E. A., Chusovitina S. V. Microelement composition of tissues of some species of aquatic organisms of Peter the Great Bay (Northern Bay). *Vestnik of the Far East Branch of the Russian Academy of Sciences*, 2013, no. 5 (171), pp. 127–132. (in Russ.)
29. Steblevskaya N. I., Tchusovitina S. V., Polyakova N. V., Zhad'ko E. A. The study of microelement composition of tissues and organs of some commercial fish from the gulf of Peter the Great (Sea of Japan). *Problems of Fisheries*, 2016, vol. 17, no. 1, pp. 96–102. (in Russ.)
30. TR TS 021/2011. *Tekhnicheskii reglament Tamozhennogo soyuza o bezopasnosti pishchevoi produktsii.* Moscow : Gosstandart Rossii, 2011, 242 p. (in Russ.)
31. Khristoforova N. K. *Bioindikatsiya i monitoring zagryazneniya morskikh vod tyazhelymi metallami.* Leningrad : Nauka, 1989, 192 p. (in Russ.)
32. Khristoforova N. K., Kobzar A. D. Brown algae as accumulating indicators of sea water pollution by heavy metals. In: *Pollution of Marine Environment: Ecological Monitoring, Bioassay, Standardization* : collection of the papers of the Russian scientific conference with international participation devoted to 125th anniversary of prof. V. A. Vodyanitsky, Sevastopol, May 28 – June 1, 2018. Sevastopol : Kolorit, 2018, pp. 279–285. (in Russ.). https://doi.org/10.21072/978-5-60-400-20-8-7
33. Khristoforova N. K., Kobzar A. D. Assessment of ecological state of the Posyet Bay (the Sea of Japan) by heavy metals content in brown algae. *Samara Journal of Science*, 2017, vol. 6, no. 2 (19), pp. 91–95. (in Russ.)
34. Khristoforova N. K., Litvinenko A. V., Tsygankov V. Yu., Kovalchuk M. V. Some of the features of the microelement composition of pink salmon *Oncorhynchus gorbuscha* Walbaum, 1792 and masu *Oncorhynchus masou* Brevoort, 1856 from the Sakhalin–Kuril region. In: *Baltiiskii morskoi forum* : materialy VII Mezhdunar. Baltiiskogo mor. foruma. In 6 vols. Vol. 3. Vodnye bioresursy, akvakul'tura i ekologiya vodoemov : materialy VII Mezhdunar. nauch. konf. Kaliningrad : Izd-vo BGARF, 2019a, pp. 173–178. (in Russ.)
35. Chemagin A. A., Volosnikov G. I., Kyrov D. N., Liberman E. L. Heavy metals Hg, Cd, and Pb in the body of sterlet (*Acipenser ruthenus* L.), the Lower Irtysh River. *Vestnik Murmanskogo gosudarstvennogo tekhnicheskogo universiteta*, 2019, vol. 22, no. 2, pp. 225–233. (in Russ.). https://doi.org/10.21443/1560-9278-2019-22-2-225-233
36. Chusovitina S. V., Steblevskaya N. I., Polyakova N. V., Zhad'ko E. A. Distribution

of trace elements in organs and tissues of three species of flounder from the Peter the Great Gulf (the Sea of Japan). *Vestnik of the Far East Branch of the Russian Academy of Sciences*, 2020, no. 6 (214), pp. 70–77. (in Russ.). https://doi.org/10.37102/08697698.2020.214.6.008
37. Shlyamin B. A. *Beringovo more.* Moscow : Geografgiz, 1958, 96 p. (in Russ.)
38. Shuntov V. P. *Biologiya dal'nevostochnykh morei Rossii.* Vladivostok : TINRO-tsentr, 2001, vol. 1, 580 p. (in Russ.)
39. Astakhov A. S., Ivanov M. V., Li B. Ya. Hydrochemical and atmochemical mercury dispersion zones over hydrothermal vents of the submarine Piip Volcano in the Bering Sea. *Oceanology*, 2011, vol. 51, no. 5, pp. 826–835. https://doi.org/10.1134/S0001437011050031
40. Burger J., Gochfeld M., Shukla T., Jeitner Ch., Burke S., Donio M., Shukla Sh., Snigaroff R., Snigaroff D., Stamm T., Volz C. Heavy metals in Pacific cod (*Gadus macrocephalus*) from the Aleutians: Location, age, size, and risk. *Journal of Toxicology and Environmental Health, Part A*, 2007, vol. 70, iss. 22, pp. 1897–1911. https://doi.org/10.1080/15287390701551159
41. Datsky A. V., Maznikova O. A. Biological features of common fish species in Olyutorsky–Navarin region and the adjacent areas of the Bering Sea: 3. Righteye flounders (Pleuronectidae). *Journal of Ichthyology*, 2017, vol. 57, no. 2, pp. 228–256. https://doi.org/10.1134/S0032945217020060
42. Dökmeci A. H., Yildiz T., Ongen A., Sivri N. Heavy metal concentration in deepwater rose shrimp species (*Parapenaeus longirostris* Lucas, 1846) collected from the Marmara Sea Coast in Tekirdağ. *Environmental Monitoring and Assessment*, 2014, vol. 186, iss. 4, pp. 2449–2454. http://dx.doi.org/10.1007/s10661-013-3551-2
43. Duffus J. H. "Heavy metals" a meaningless term? (IUPAC Technical Report). *Pure and Applied Chemistry*, 2002, vol. 74, no. 5, pp. 793–807. https://doi.org/10.1351/pac200274050793
44. Hassan Z., Aarts M. G. Opportunities and feasibilities for biotechnological improvement of Zn, Cd or Ni tolerance and accumulation in plants. *Environmental and Experimental Botany*, 2011, vol. 72, iss. 1, pp. 53–63. https://doi.org/10.1016/j.envexpbot.2010.04.003
45. Hwang D. W., Shim K., Lee C. I. Concentrations and risk assessment of heavy metals in tissues of walleye pollock (*Gadus chalcogrammus*) captured from the northeastern coast of Korea. *Journal of Food Protection*, 2019, vol. 82, iss. 5, pp. 903–909. https://doi.org/10.4315/0362-028X.JFP-18-379
46. Khristoforova N. K., Tsygankov V. Yu., Boyarova M. D., Lukyanova O. N. Concentrations of trace elements in Pacific and Atlantic salmon. *Oceanology*, 2015a, vol. 55, iss. 5, pp. 679–685. https://doi.org/10.1134/S0001437015050057
47. Khristoforova N. K., Litvinenko A. V., Tsygankov V. Yu., Kovalchuk M. V., Erofeeva N. I. The trace-element content in the pink salmon *Oncorhynchus gorbuscha* (Walbaum, 1792) from the Sakhalin–Kuril region. *Russian Journal of Marine Biology*, 2019b, vol. 45, iss. 3, pp. 221–227. https://doi.org/10.1134/S1063074019030064
48. Khristoforova N. K., Tsygankov V. Yu., Boyarova M. D., Lukyanova O. N. Heavy metal contents in the pink salmon *Oncorhynchus gorbuscha* Walbaum, 1792 from Kuril oceanic waters during anadromous migration. *Russian Journal of Marine Biology*, 2015b, vol. 41, iss. 6, pp. 479–484. https://doi.org/10.1134/S1063074015060085
49. Khristoforova N. K., Tsygankov V. Yu.,

Lukyanova O. N., Boyarova M. D. The Kuril Islands as a potential region for aquaculture: Trace elements in chum salmon. *Environmental Pollution*, 2016, vol. 213, pp. 727–731. https://doi.org/10.1016/j.envpol.2016.03.027

50. Langston W. J. Toxic effects of metals and the incidence of metal pollution in marine ecosystems. In: *Heavy Metals in the Marine Environment* / R. W. Furness, Ph. S. Rainbow (Eds). Boca Raton : CRC Press, 1990, chap. 7, pp. 101–122. https://doi.org/10.1201/9781351073158
51. Li Ch., Zhou K., Qin W., Tian Ch., Qi M., Yan X., Han W. A review on heavy metals contamination in soil: Effects, sources, and remediation techniques. *Soil and Sediment Contamination: An International Journal*, 2019, vol. 28, iss. 4, pp. 380–394. https://doi.org/10.1080/15320383.2019.1592108
52. *Public Health Assessment* / Federal Facilities Assessment Branch ; Division of Health Assessment and Consultation ; Agency for Toxic Substances and Disease Registry (ATSDR). Adak, Alaska : Naval Air Facility, 2002. URL: https://www.atsdr.cdc.gov/hac/pha/adak/ada_toc.html [accessed: 01.04.2021].

ТЯЖЁЛЫЕ МЕТАЛЛЫ И МЫШЬЯК В ПРОМЫСЛОВЫХ РЫБАХ ЯПОНСКОГО, ОХОТСКОГО И БЕРИНГОВА МОРЕЙ: СОВРЕМЕННОЕ СОСТОЯНИЕ (ОБЗОР ЛИТЕРАТУРЫ)

М. К. Гамов[1], А. Е. Иванова[2], Е. К. Миронова[1], В. Ю. Цыганков[1]

[1]Дальневосточный федеральный университет, Владивосток, Российская Федерация

[2]Тихоокеанский филиал ФГБНУ «ВНИРО» («ТИНРО»), Владивосток, Российская Федерация

E-mail: *gamovmota123456@gmail.com*

В работе обобщены сведения о концентрациях токсичных элементов As, Pb, Cd и Hg в промысловых рыбах дальневосточных морей — Японского, Охотского и Берингова — на основе анализа литературных данных. Изучение показало, что в целом основные промысловые объекты и рыбохозяйственные бассейны соответствуют санитарно-гигиеническим нормативам, однако наличие импактных природных зон в ареалах и на пути миграции рыб способствует увеличению концентраций токсичных элементов в рыбных объектах промысла, а в некоторых случаях уровни превышают предельно допустимые концентрации. Необходимо продолжать мониторинг токсичных микроэлементов в промысловых объектах и рыбохозяйственных бассейнах дальневосточных морей.

Ключевые слова: тяжёлые металлы, токсичные элементы, дальневосточные моря

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GROWTH DYNAMICS OF THE BENTHIC DIATOM *ARDISSONEA CRYSTALLINA* (C. AGARDH) GRUNOW, 1880 (BACILLARIOPHYTA) UNDER COPPER IONS EFFECT

E. L. Nevrova and A. N. Petrov

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
E-mail: *el_nevrova@mail.ru*

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Increasing anthropogenic load on coastal ecosystems of the Black Sea determines the need for regular assessing the state of planktonic and benthic communities. Planktonic microalgae contributing up to 20–25 % of global primary production are traditionally used as test objects; however, the contribution of microphytobenthos is comparable to that of phytoplankton. Benthic diatoms are close-associated with bottom substrate, and most of them are highly sensitive to the effect of technogenic pollutants accumulating in sediments. The changes in physiological indicators of benthic Bacillariophyta may objectively reflect the negative effect of various toxicants; accordingly, benthic diatoms can be used as test objects in the indirect assessment of the marine environment quality. We aimed to study the growth dynamics of abundance of clonal strain cells for a new biotesting object – the diatom *Ardissonea crystallina* (C. Agardh) Grunow, 1880 (Bacillariophyta) – under the effect of various $CuSO_4{\cdot}5H_2O$ concentrations during 10-day laboratory experiments. This species is widespread in the Black Sea sublittoral and highly sensitive to the effect of different technogenic pollutants, *inter alia* heavy metals. As shown, at copper ions concentrations of 32–128 μg·L^{-1}, *A. crystallina* growth dynamics generally corresponds to the dose–response curve in a toxicological experiment. The correlation was found between a decrease in intensity of the culture growth and increase in toxicant concentration in the experimental medium. At copper ions concentration of 256–320 μg·L^{-1}, the ratio of alive cells in the clonal strain decreases gradually from 62–66 % (the 1st day) to 34–37 % (the 10th day); the indicators of an increase in cell abundance in the clonal strain are characterized by a negative trend – from −0.01 (on the 2nd day) to −0.34 (on the 10th day). At Cu^{2+} concentrations of 384 μg·L^{-1} and higher, drastic inhibition and subsequent death of *A. crystallina* cells were revealed. At 448–1,024 μg·L^{-1}, complete cell mortality was registered already on the 3rd day of the experiment. Statistical comparison of the ratio variability of *A. crystallina* alive cells and the specific growth in their abundance for the control and Cu^{2+} concentrations of 64–128 μg·L^{-1} showed as follows: at 32–128 μg·L^{-1}, the differences between the mean values of the test indicators were significant ($P = 0.002...0.020$). At 256 μg·L^{-1}, the changes in total abundance and alive cells ratio in the test culture significantly differ ($P = 0.002...0.014$) from those both at lower and higher copper concentrations. This fact allows to consider the toxicant level of 256 μg·L^{-1} as a critical one for *A. crystallina*: its exceeding will result in a sharp increase in cell mortality. Based on the results obtained, this benthic diatom can be recommended for use as a suitable test object in toxicological experiments, as well as for monitoring and indirect environmental assessment of coastal water areas subjected to technogenic pollution.

Keywords: toxicological experiment, copper ions, clonal strain, cell abundance, benthic diatom algae, Black Sea

Increasing anthropogenic load on the Black Sea ecosystem, especially manifested in coastal water area, including off the Crimean coast, determines the need for a regular assessment of current state of planktonic and benthic communities. As one of the objects for biotesting and bioindication, planktonic microalgae are traditionally used, due to relative simplicity of their cultivation and accounting during experiments (Ekologo-toksikologicheskie aspekty, 1985 ; Gelashvili et al., 2015 ; Nevrova, 2015 ; Spirkina et al., 2014). Importantly, the contribution of microphytobenthos (with its up to 99 % abundance and species richness being contributed by Bacillariophyta representatives) to the sea and ocean primary production is comparable to that of phytoplankton which determines up to 20–25 % of global production (Diatoms: Fundamentals and Applications, 2019 ; Kumar et al., 2015 ; The Diatom World, 2011). Along with a high rate of reproduction, benthic diatoms are characterized by adherence to certain microbiotopes and sensitivity to the effect of adverse environmental factors (Markina, 2009 ; Nevrova et al., 2015 ; Romanova et al., 2017). Therefore, a change in physiological parameters of benthic diatoms (cell growth, reproduction, and abundance) with a greater objectivity (compared to planktonic species) reflects the effect of various pollutants, and this allows to use benthic diatoms as convenient test objects in the indirect assessment of the marine environment quality (Anantharaj et al., 2011 ; Florence & Stauber, 1986 ; Gelashvili et al., 2015 ; Markina, 2009 ; Markina & Aizdaicher, 2006, 2007, 2011, 2019 ; Rijstenbil & Gerringa, 2002 ; Romanova et al., 2017 ; The Diatom World, 2011 ; Yan et al., 2014).

Approaches to using benthic diatoms for monitoring coastal water areas are developed insufficiently (Anantharaj et al., 2011 ; Leung et al., 2017 ; Nagajoti et al., 2010) since there are certain difficulties in their clone isolation, cultivation, and accounting during experiments (Nevrova et al., 2015 ; Petrov & Nevrova, 2020 ; Romanova et al., 2017). Researchers obtain new data on tolerance ranges of different marine diatom species when exposed to various toxicants (copper ions, surfactants, pesticides, *etc.*) (Aizdaicher & Reunova, 2002 ; Markina, 2009 ; Markina & Aizdaicher, 2007) and also develop methodological issues. Those include the investigation of clone cultivation peculiarities, determination of criteria for defining alive cells during visual assessment of morphological alterations and photographing, estimation of increase in cell abundance, ratio of alive and dead cells of a test object under different toxicant concentrations in a cultural medium, analysis of absorption of heavy metals by cells, *etc.* (Ahalya et al., 2003 ; Anantharaj et al., 2011 ; Leung et al., 2017 ; Spirkina et al., 2014). Of key importance is also expansion of knowledge on biology in terms of Bacillariophyta taxa development.

Copper sulfate is chosen as a model toxicant for experiments due to significance of copper compounds both in the biogeochemical cycle and hydrobiont metabolism. Copper is an essential trace element; it is actively involved in physiological processes – nitrogen metabolism, antioxidant protection (Cu/Zn superoxide dismutase), electron transfer in the mitochondrial respiratory chain of eucaryotes (cytochrome c oxidase), *etc.* (Gelashvili et al., 2015 ; Miazek et al., 2015 ; Smolyakov et al., 2010). Copper compounds are found in the Earth's crust in mass and form about 250 minerals; those are the most common technogenic pollutant both in aquatic environment and bottom sediments (Gelashvili et al., 2015 ; Smolyakov et al., 2010), including in the Black Sea coastal waters (Nevrova et al., 2015). Along with mercury ions, copper ions (Cu^{2+}) belong to the most environmentally hazardous substances; in increased concentrations, those become acutely toxic for most marine and freshwater hydrobionts (Ekologo-toksikologicheskie aspekty, 1985 ; Gelashvili et al., 2015).

An investigation of toxicological impact of Cu^{2+} in $CuSO_4$ on aquatic plants and planktonic Bacillariophyta revealed a growth inhibition at a concentration of copper compounds of about 0.1 $\mu g \cdot L^{-1}$ (Gelashvili et al., 2015). Moreover, the effect was studied of copper sulfate at different concentrations on cells of planktonic forms of *Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin, 1964, *Ditylum brightwellii* (West) Grunow ex Van Heurck emend. Dzhafarova, 1984, *Phaeodactylum tricornutum* Bohlin, 1897, and *Thalassiosira oceanica* Hasle, 1983 (Ahalya et al., 2003 ; Cid et al., 1995 ; Florence & Stauber, 1986 ; Kim & Price, 2017 ; Markina & Aizdaicher, 2006, 2011, 2019 ; Rijstenbil & Gerringa, 2002 ; Yan et al., 2014). Obtained results allowed suggesting a pronounced species-specific threshold resistance of diatom algae to copper ions.

Importantly, the value of copper maximum permissible concentration accepted for seawater is 5 $\mu g \cdot L^{-1}$ despite the fact that copper content in marine coastal areas can reach 50–100 $\mu g \cdot L^{-1}$ (Markina & Aizdaicher, 2019). In our opinion, for benthic diatoms, copper content in a water column is less ecologically significant than in bottom sediments: pollutants are accumulated in them while microalgae cells inhabit the surface of substrate particles in motile or attached form. As known, in silty sediments of the Black Sea coastal water areas, copper content can be of 0.4–11.2 $\mu g \cdot g^{-1}$ (Ovsyaniy et al., 2003); in technogenically polluted bays, concentrations can reach 20 and even 37 $\mu g \cdot g^{-1}$ of dry sediment (Burgess et al., 2009 ; Petrov & Nevrova, 2003 ; Petrov et al., 2005). As found, copper ions, along with ions of other heavy metals accumulated in soft bottom sediments, are the key factors for heavily polluted biotopes and affect both taxocene structure and spatial distribution of benthic diatoms (Petrov & Nevrova, 2003, 2004 ; Petrov et al., 2005).

Ardissonea crystallina (C. Agardh) Grunow, 1880 was chosen as a model object for the experiment for several reasons. The species cells are large, and this facilitates both their accounting during photoregistering and assessment of a vital state. Moreover, the species is easy to cultivate and is characterized by high vegetative reproduction rate and ability to form attached colonies. Earlier, *A. crystallina* was transferred from the class Fragilariophyceae to Coscinodiscophycea, and then to Mediophyceae; based on the results of molecular genetic analysis and sexual reproduction experiments, the taxonomic position of the species has been questioned. Apparently, *Ardissonea* (and other Toxariales representatives) can form a unique evolutionary group which is separated from other pennate diatoms and is characterized by an unusual way of sexual reproduction (Davidovich et al., 2017). Recently, some *Ardissonea* species were transferred into other genera; specifically, *Ardissonea crystallina* was transferred to *Synedrosphenia crystallina* (C. Agardh) Lobban & Ashworth comb. nov. (genus *Synedrosphenia* (H. Peragallo ex H. Peragallo et M. Peragallo, 1897–1908) Azpeitia, 1911, emend. Lobban & Ashworth) (Lobban et al., 2022). Nevertheless, taking into account the stick-shaped valve and the ability to form bunch-like colonies attached to substrate, as well as reckoning with the taxonomic system (Round et al., 1990), for our aims, we consider this species as belonging to Fragilariophyceae (until recently, it was referred to this class). Importantly, the present study continues a series of experiments focused on revealing species-specific tolerance of benthic diatoms representing three different Bacillariophyta classes – with their inherent morphological peculiarities and various lifestyle forms. Previously, we statistically confirmed the significance of selective assessment of cell distribution of diatom species belonging to three classes, including *A. crystallina*, in an experimental vessel (Petrov & Nevrova, 2020).

The aim of the work is to reveal the dynamics of growth and cell mortality of marine benthic diatom *Ardissonea crystallina* during 10-day experiments under a wide range of toxicant (copper ions) concentrations in the cultural medium and to evaluate the applicability of this species as a test object new for ecotoxicology.

MATERIAL AND METHODS

Study object. As a test object, *A. crystallina* clonal strain (Bacillariophyta) was used. The diatom cells were sampled from phytoperiphyton of an artificial substrate sampled in the Kazachya Bay (Sevastopol water area) in November 2018 at a 5-m depth. To obtain a clone line, a single cell was isolated with a micropipette under an MBS-10 binocular at ×40 magnification and rinsed 7 times with the medium (Gaisina et al., 2008 ; Petrov & Nevrova, 2020 ; Romanova et al., 2017). This marine benthic species occurs frequently in coastal areas; its cells are attached to substrate surface and form bunch-like colonies of 4–30 cells (Petrov & Nevrova, 2020). Valves are narrow-linear, 410 µm long, and 18 µm wide (see 1–6 in Fig. 1). Cell sizes are indicated as at the time of the beginning of cultivation.

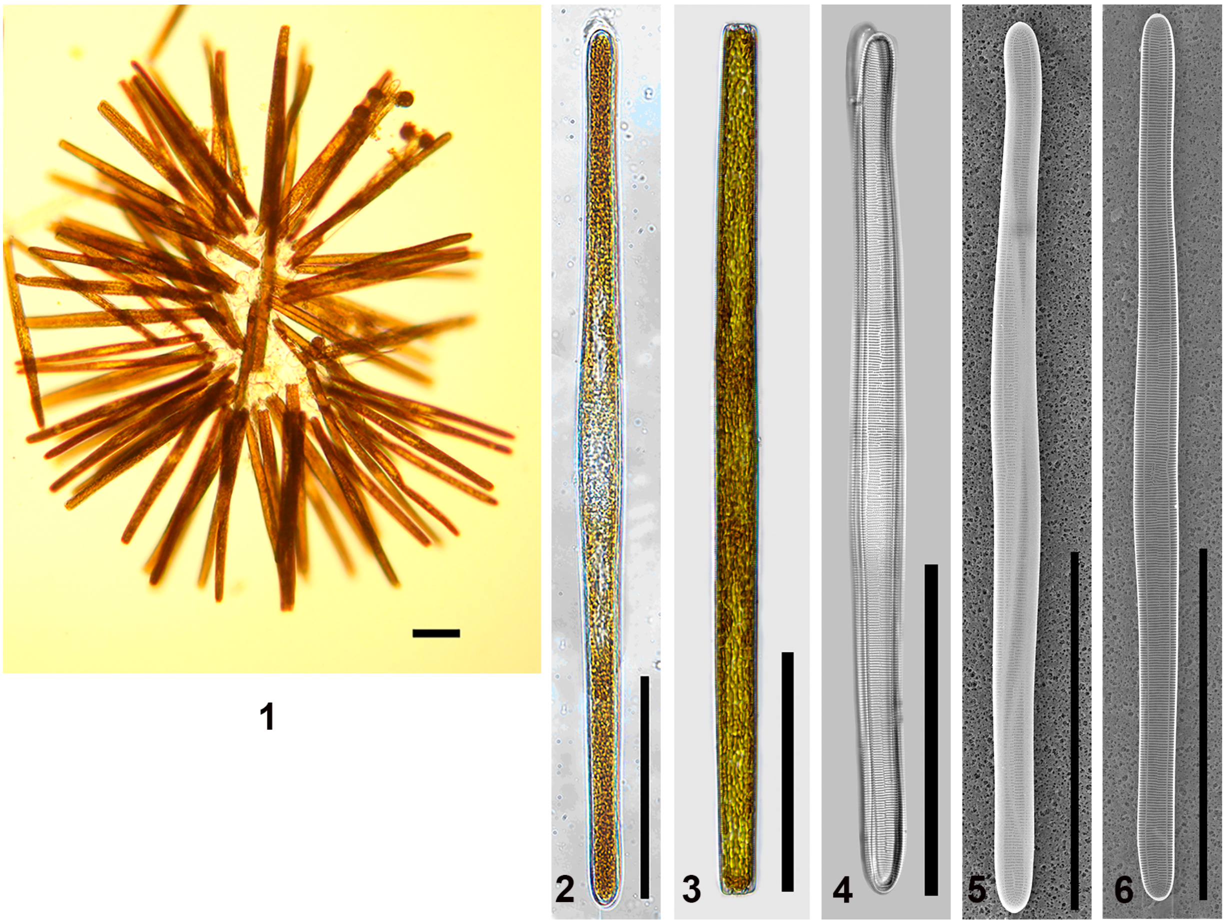

Fig. 1. Marine benthic diatom *Ardissonea crystallina* used in the experiment as a new test object: 1, colony of alive cells (LM, ×10, scale bar 10 µm); 2, alive cell, valve view (LM, ×60, scale bar 100 µm); 3, alive cell, band view (LM, ×60, scale bar 100 µm); 4, valve external view (LM, ×100, scale bar 100 µm); 5, valve external view (SEM, ×550, scale bar 100 µm); 6, valve internal view (SEM, ×550, scale bar 100 µm) (LM denotes light microscope; SEM, scanning electron microscope)

Cultivation. *A. crystallina* clone strain was cultured on a natural seawater medium by Goldberg (Andersen et al., 2005) modified for marine benthic diatoms (Petrov & Nevrova, 2020), at a constant temperature of (15 ± 2) °C, under scattered natural light. Seawater for the medium was sampled in a 12-mile zone off Crimean coast during research cruises of the RV "Professor Vodyanitsky", filtered through a 0.45-μm filter, and pasteurized three times at +75 °C; then, the medium was enriched with nutrients according to the protocol (Petrov & Nevrova, 2020).

Microphotography. During the experiment, alive cells were photoregistered with a Carl Zeiss Axiostar Plus light microscope equipped with an Achroplan ×10 objective and Canon PowerShot A640 camera (IBSS benthos ecology department). For taxonomic identification, micrographs of alive and cleaned diatom valves were taken under an inverted Nikon Eclipse Ts2R light microscope equipped with a Plan Fluor ×60 OFN25 DIC objective and an Infinity3-6UR camera, under a Carl Zeiss Primostar Plus light microscope with an N-Achroplan ×100 objective and an integrated camera (IBSS laboratory of biodiversity and functional genomics of the World Ocean), and under a Hitachi SU3500 scanning electron microscope. The species was identified according to the guide books (Guslyakov et al., 1992 ; Witkowski et al., 2000).

Experimental design. A stock solution was prepared with $CuSO_4{\cdot}5H_2O$ concentration of 40,000 μg·L^{-1} (10,240 μg·L^{-1} in terms of Cu^{2+} ions). For the experiments, a certain volume of the natural seawater medium, $CuSO_4{\cdot}5H_2O$ stock solution, and 1 mL of *A. crystallina* clonal strain inoculum were added to each 90-mm Petri dish with a micropipette dispenser so that the total volume of liquid in each dish was of 30 mL. To obtain test solutions with increasing copper ions concentrations (from 32 to 1,024 μg·L^{-1}), different aliquots of stock solution (from 0.09 to 3 mL) were added to Petri dishes (Table 1). The effect of each copper ions concentration was analyzed in triplicate. All the experiments lasted for 10 days. Petri dishes were sealed with Parafilm® to avoid contamination or evaporation of the test solution.

Table 1. Design of the experimental study of copper sulfate ($CuSO_4{\cdot}5H_2O$) effect on the growth of *A. crystallina* clonal strain

$CuSO_4{\cdot}5H_2O$ concentration in the test solution, μg·L^{-1}	Cu^{2+} concentration in the test solution, μg·L^{-1}	Volume of the added stock solution, mL	Volume of the medium, mL	Volume of the inoculum, mL
Control	0	0	29.00	1
125	32	0.09	28.91	1
250	64	0.19	28.81	1
500	128	0.38	28.62	1
1,000	256	0.75	28.25	1
1,250	320	0.94	28.06	1
1,500	384	1.13	27.87	1
1,750	448	1.31	27.69	1
2,000	512	1.50	27.50	1
4,000	1,024	3.00	26.00	1

We do not know any data on *A. crystallina* use in biotesting practice, peculiarities of a long-term cultivation, levels of resistance of cells of this species to copper impact, and maximum permissible concentrations of copper and other metals for marine bottom sediments. Therefore, to reveal the ranges of critical Cu^{2+} concentration, the experiment was carried out in two successive stages. At the first stage, in addition to the control (the natural seawater medium by Goldberg with no toxicant), *A. crystallina* was tested by doubly increasing toxicant concentrations – 32, 64, 128, 256, 512, and 1,024 $\mu g \cdot L^{-1}$ (in terms of Cu^{2+} ions).

According to results of the first stage, threshold concentrations of copper ions, at which a sharp cell elimination in the clonal strain begins, are in the range of 256–512 $\mu g \cdot L^{-1}$. In this regard, the second stage of the experiment was carried out: intermediate Cu^{2+} concentrations (320, 384, and 448 $\mu g \cdot L^{-1}$) were tested. Response to the toxicant effect on the clonal strain was assessed by the change in a ratio of alive cells (%). All the experiments were carried out in triplicate for each concentration and exposure time (in 1, 3, 5, 7, and 10 days). Cells were counted, and their vital state was assessed visually, on micrographs. A cell was defined as an alive one by valve shape and integrity, invariability of chloroplast structure and color, and divergence of cells after vegetative division. In case of cytoplasmic lysis, sharp chloroplast darkening, and valve destruction, a cell was defined as a dead one. Abundance of alive and dead cells at each exposure time was calculated from averaged data obtained in 12–16 random viewing fields which were photographed in experimental Petri dishes with a bottom area of about 5,700 mm^2. For all tested copper ions concentrations, both ratio of alive cells and growth (mortality) rate in cell abundance ($cells \cdot day^{-1}$) were determined; in toxicology, the second indicator is one of the basic since it allows assessing the state of microalgae populations (Filenko et al., 2006 ; Markina & Aizdaicher, 2007, 2019 ; Spirkina et al., 2014). The rate of cell growth for the culture was determined by a number of cell divisions (v) *per* day and calculated by the formula (Schlegel, 1987):

$$\nu = \frac{\ln N_{(t+\Delta t)} - \ln N_t}{\Delta t \times \ln 2}\,, \tag{1}$$

where N_t denotes mean cell abundance in the culture at time t (the 1st day of the experiment);

$N_{(t+\Delta t)}$ denotes mean cell abundance in the culture at time t + Δt (the 3rd, 5th, 7th, and 10th days);

Δt denotes exposure time (days).

Statistical data processing. The experimental results were statistically processed using standard routines of parametric and rank analysis of the statistical software package SigmaPlot 11.5 (2021).

For three independent replications for each toxicant concentration, variances were compared by the Fisher's test (ANOVA) for a significance level $P = 0.05$. Significance of differences in mean values of abundance and ratio of alive cells, as well as rate of cell growth at different exposure time were compared by Student's *t*-test – in case of normality of distribution mode and variances equality. To compare independent samples, with distribution mode differing from normal, the nonparametric Mann–Whitney *U* test, Holm–Šidák test (for equal sample sizes), and Dunn's test (for different sample sizes) were applied (SigmaPlot NG, 2021). The mean values of the indicators and standard errors of the sample (*SE*) are given in Fig. 2 and in Tables 2 and 3.

RESULTS AND DISCUSSION

At copper concentrations in experimental dishes from 32 to 128 $\mu g \cdot L^{-1}$ (in terms of Cu^{2+} ions), the change in the ratio of alive cells and an increase in *A. crystallina* abundance generally correspond to the dose–response curve in a toxicological experiment (Gelashvili et al., 2015). On the 1st day, for dishes with various Cu^{2+} ions concentrations, there were no statistically significant differences (P = 0.30…0.39) between the mean values of the indicator (the ratio of alive cells in 12–16 viewing fields amounted to 56–60 %). After the 1st day of the experiment, for copper ions concentrations of 32–128 μg·L^{-1}, a short period of cell adaptation was observed (for the control and at minimum Cu^{2+} concentration of 32 μg·L^{-1}), or the lack of a lag phase was registered. On the 3rd–7th days, an increase in the indicator values and reaching a plateau were revealed; on the 7th–10th days, a decrease in the values was recorded (Fig. 2, Table 2). From the 3rd to the 7th day (according to the mean values from viewing fields), no statistically significant effect (P = 0.18…0.93) of various toxicant concentrations was registered on an increase in abundance and ratio of alive cells. Significant decrease (P = 0.002…0.020) in the values of test indicators was noted on the 7th–10th days of the experiment; this can be directly caused by the negative effect of high toxicant concentrations in the experimental medium.

Table 2. Ratio (%) of *A. crystallina* alive cells (mean ± *SE*) at different toxicant concentrations (in terms of Cu^{2+} ions) and exposure time (results of the first and second stages are combined)

Cu^{2+} concentration, μg·L^{-1}	1st day	3rd day	5th day	7th day	10th day	*P* (7th day *vs.* 10th day)
Control	63 ± 3	67 ± 2	85 ± 2	84 ± 2	76 ± 3	0.071
32	58 ± 1	75 ± 4	86 ± 4	84 ± 1	81 ± 3	0.248
64	60 ± 6	79 ± 2	86 ± 1	80 ± 1	67 ± 4	**0.002**
128	60 ± 2	83 ± 2	83 ± 2	79 ± 4	65 ± 10	**0.003**
256	56 ± 2	77 ± 6	79 ± 1	77 ± 3	60 ± 1	**0.017**
320	58 ± 2	49 ± 1	47 ± 2	44 ± 1	46 ± 5	0.398
384	30 ± 5	22 ± 3	8 ± 1	0.8 ± 0.8	0.6 ± 0.6	0.412
448	21 ± 5	0.6 ± 0.4	0	1.0 ± 0.6	0.5 ± 0.5	–
512	13 ± 3	0.3 ± 0.1	0	0	0	–
1,024	3.6 ± 1	0.2 ± 0.1	0	0	0	–

Note: *P* is significance level of no differences between the mean values of the indicator when comparing the ratio of alive cells on the 7th and 10th days of the experiment. Statistically significant differences are highlighted in bold.

At copper ions concentrations of 256 μg·L^{-1} and higher, the ratio of alive cells in the clonal strain at all the stages of the experiment was significantly lower (P = 0.002…0.014) starting from the 3rd day than at 32–128 μg·L^{-1}. At 256–320 μg·L^{-1}, the ratio of alive cells decreased monotonically from 47–49 % (on the 3rd day) to 34–37 % (on the 10th day) – with no pronounced inflections in the model response curve. The critical toxicant concentration found during the experiments (256 μg·L^{-1}) can be considered as a threshold: upon reaching it, there is a statistically significant inhibition of growth activity and physiological state of the clonal strain.

At copper ions concentrations of 384 μg·L^{-1} and higher, a drastic inhibition of the clonal strain in dishes was observed already on the 1st day of the experiment. On the 5th day, the ratio of alive cells decreased to zero. For concentrations of 448–1,024 μg·L^{-1}, almost complete cell mortality was registered already on the 3rd day of the experiment (see Fig. 2, Table 2).

When determining cells abundance in viewing fields, the data variability differed much during the experiment. Specifically, coefficient of variation in *A. crystallina* samples during the 1st day of the experiment was up to 46 %; in 5 days, the value was 31 %. This fact could be due to uneven distribution in viewing fields when, along with single cells, there are aggregations of bunch-like colonies attached to dish bottom at one spot (see 1 in Fig. 1). The results of analysis showed that the variances of samples, when comparing three replicates, did not differ statistically: $P = 0.25\ldots0.28$ (in 1 day) and $P = 0.09\ldots0.23$ (in 5 days). All pairwise differences in mean abundance of *A. crystallina* cells between replicates both on the 1st and the 5th day of exposure are insignificant ($P_{exp} >> 0.05$). Apparently, variability of mean cell abundance in different replicates does not exceed the statistical error, and this allows to consider all the replicates (random cell samples) as belonging to one initially taken population with a similar variability of indicators (Petrov & Nevrova, 2020). This fact is of key importance for a valid comparison of differences in absolute cell abundance in dishes at different stages of the experiment and at different toxicant concentrations.

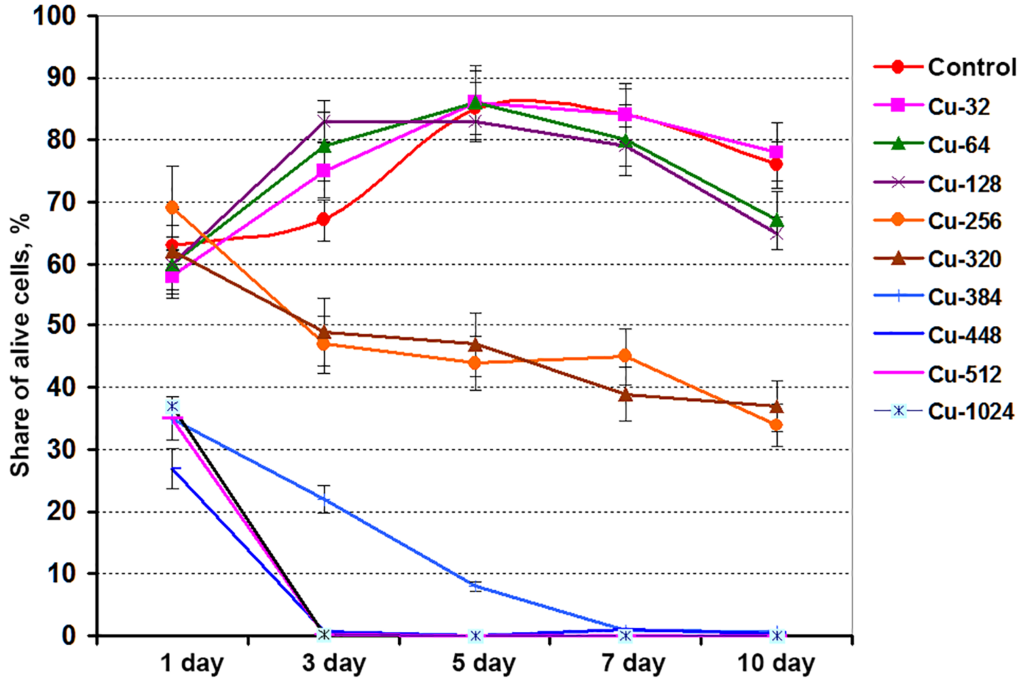

Fig. 2. Changes in the ratio (%) of alive cells (mean ± *SE*) in *A. crystallina* clonal strain during the experiment at different toxicant concentrations (in terms of Cu^{2+} ions)

One more indicator for assessing the toxic effect of copper on *A. crystallina* strain is the mean number of vegetative cell divisions *per* day (v). Comparison of mean values of specific cell growth in clonal strain at different toxicant concentrations is given in Table 3. At the first three days, the culture tends to have a positive increase at 32–128 $\mu g \cdot L^{-1}$. Importantly, along with a rise in toxicant concentrations, the daily growth rate rose; the highest specific increase was revealed at 128 $\mu g \cdot L^{-1}$. Apparently, at this concentration, primary stimulation by copper ions of cell growth and division occurs (Filenko et al., 2006). Specifically, compared with the control, the rate of *A. crystallina* cell division rose 18 times (from 0.17 to 3.0 divisions *per* day); already at 256 $\mu g \cdot L^{-1}$, it slowed down to almost zero. At toxicant concentrations of 320 $\mu g \cdot L^{-1}$ and higher, an increase in cell abundance became negative, and a rate of cell mortality rose (see Table 3). Importantly, in the control, the specific growth rate first increased (up to 0.42 divisions on the 7th day) and then decreased (down to 0.21 divisions on the 10th day); there was a noticeable cell mortality in the strain. In general, an increase in cell abundance was recorded up to the 5th day of the experiment at 32–128 $\mu g \cdot L^{-1}$. At higher copper ions concentrations and longer exposure time, negative specific growth rate was registered in the test culture.

Our experiments revealed higher resistance of benthic diatom *A. crystallina* to the impact of copper compared to species of planktonic microalgae. Specifically, as recorded in the study on copper chloride exposure on survival and reproduction of *Scenedesmus quadricauda* (Turpin) Brébisson, 1835 (Filenko et al., 2006), a noticeable decrease in total abundance and ratio of alive cells occurs already at copper concentration of 10–100 $\mu g \cdot L^{-1}$ at the exponential phase (10th–14th days). At the same time, after 14 days of the experiment, the ratio of actively dividing cells did not exceed 10 % already at 1–10 $\mu g \cdot L^{-1}$, and the remaining part of strain was at the resting stage and did not affect the growth rate in a test culture. When investigating the effect of copper ions concentrations of 50–100 $\mu g \cdot L^{-1}$ on another planktonic microalga, *Porphyridium purpureum* (Bory) K. M. Drew & R. Ross, 1965 (Markina & Aizdaicher, 2019), a pronounced inhibition of its growth and a decrease in photosynthetic pigments content as compared to the control were recorded in cells already on the 4th day. As revealed, heavy metals (copper and cadmium) inhibit cell growth and can damage the cell membrane; this results in a significant decrease in chlorophyll pigments in the benthic diatom *Amphora coffeaeformis* (C. Agardh) Kützing, 1844 at copper ions concentrations of 0.02–10 $\mu g \cdot L^{-1}$ (Anantharaj et al., 2011).

Table 3. Comparison of the specific growth in *A. crystallina* cell abundance (v, cells·day^{-1}) at different stages of experiment and different toxicant concentrations (in terms of Cu^{2+} ions)

Cu^{2+} concentration	Exposure time			
	1st–3rd days	3rd–5th days	5th–7th days	7th–10th days
Control	**0.17 ± 0.04**	**0.27 ± 0.07**	**0.42 ± 0.10**	**0.21 ± 0.06**
32	**1.15 ± 0.11**	**0.30 ± 0.03**	**0.03 ± 0.01**	–0.03
64	**1.60 ± 0.05**	**0.11 ± 0.01**	–0.12	–0.13
128	**3.00 ± 0.32**	**0.06 ± 0.01**	–0.13	–0.11
256	–0.01	–0.10	–0.19	–0.04
320	–0.02	–0.11	–0.25	–0.34
384	–0.04	–0.32	–0.47	
448	–0.48	–0.50		
512	–0.49	–0.50		
1,024	–0.47			

Note: values of positive increase in cell abundance are highlighted in bold.

An inhibition of growth and physiological state of *A. crystallina* and cells of other microalgae species can be associated with the negative effect of copper ions on the photosynthetic apparatus and with damage to chloroplast membranes which are involved in synthesis of amino acids and phytohormones affecting population growth (Kiseleva et al., 2012), as well as with the suppression of vegetative cell reproduction (Filenko et al., 2006). Apparently, higher resistance to the toxic effect of copper on benthic diatom *A. crystallina*, compared to planktonic species, is related to the presence of a thick silicified single-wall valve with pseudosepta; its complex areoles system provides contact with marine environment but does not allow penetration of microparticles into the cell (Lobban et al., 2022 ; The Diatom World, 2011). Such morphological peculiarities ensure sustainable development of benthic diatoms on soft bottom, with its levels of copper content being much higher than in a water column. Moreover, diatoms have a unique ability to bioaccumulate heavy metals up to values tens of thousands higher than those in the environment. Increased copper content in bottom sediments can also cause additional secretion of polysaccharide mucus in diatoms which is one of universal mechanisms for the detoxification of heavy metals, *inter alia* copper (Crespo et al., 2013 ; Miazek et al., 2015). It seems important to continue research on assessing the state of benthic diatoms to determine species-specific threshold concentrations of toxicants.

Conclusion. During 10-day toxicological experiments with a new test object, marine benthic diatom *Ardissonea crystallina*, various types of response to copper exposure were analyzed (changes in total cell abundance, ratio of alive cells, and specific population growth rate) at copper ions concentrations in the range of 32–1,024 μg·L^{-1}. At Cu^{2+} concentrations from 32 to 128 μg·L^{-1}, the dynamics of specific increase in abundance and survival of *A. crystallina* cells generally corresponds to the sigmoid dose–response curve in a toxicological experiment; specifically, there are a minimum period of a lag phase (the 1st day of the experiment), reaching a plateau (on the 3rd–7th days), and pronounced negative specific growth in cell abundance (from 7th to 10th day). As revealed, an increase in toxicant concentration results in a significant decrease in the ratio of alive cells and in specific growth in the test culture. For the first time, the threshold concentration was established (256 μg·L^{-1} in terms of Cu^{2+} ions): upon its reaching, a statistically significant inhibition of growth activity and physiological state of the test culture were recorded. This threshold concentration is significantly (3–10 times) higher than experimentally obtained threshold concentrations of copper which are critical for survival and growth of some planktonic microalgae. At 384 μg·L^{-1} and higher concentrations, a drastic inhibition and subsequent mortality of *A. crystallina* cells occurred already at the 1st day of the experiment; on the 5th–7th days, cell mortality was of 100 %. At 448–1,024 μg·L^{-1}, complete cell mortality was registered already on the 3rd day of the experiment.

Higher resistance of *A. crystallina* to the toxic effect of copper compared to planktonic species seems to result from morphological peculiarities of the valve which ensures sustainable development of benthic diatoms on soft bottom where copper content is much higher than in a water column. Obtained results allowed to recommend *A. crystallina* as a new test object for toxicological experiments with heavy metals, as well as for ecological monitoring in coastal marine areas under high technogenic pollution.

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REFERENCES

1. Aizdaicher N. A., Reunova Yu. A. The effect of detergents on growth of the diatom *Thalassiosira pseudonana* in culture. *Biologiya morya*, 2002, vol. 28, no. 5, pp. 362–365. (in Russ.)
2. Gaisina L. A., Fazlutdinova A. I., Kabirov R. R. *Sovremennye metody vydeleniya i kul'tivirovaniya vodoroslei* : uchebnoe posobie. Ufa : Izd-vo BGPU, 2008, 152 p. (in Russ.)
3. Gelashvili D. B., Bezel V. S., Romanova E. B., Bezrukov M. E., Silkin A. A., Nizhegorodtsev A. A. *Printsipy i metody ekologicheskoi toksikologii.* Nizhnii Novgorod : Nizhegorodskii gosuniversitet, 2015, 742 p. (in Russ.)
4. Guslyakov N. E., Zakordonets O. A., Gerasimyuk V. P. *Atlas diatomovykh vodoroslei bentosa severo-zapadnoi chasti Chernogo morya i prilegayushchikh vodoemov.* Kyiv : Naukova dumka, 1992, 115 p. (in Russ.)
5. Markina Zh. V. Influence of detergents and surface-active substances on unicellular algae growth, physiological and biochemical parameters (review). *Izvestiya TINRO*, 2009, vol. 156, pp. 125–134. (in Russ.)
6. Markina Zh. V., Aizdaicher N. A. The influence of detergents on the abundance dynamics and physiological state of the benthic microalgae *Attheya ussurensis* (Bacillariophyta) in laboratory culture. *Biologiya morya*, 2007, vol. 33, no. 6, pp. 432–439. (in Russ.)
7. Markina Zh. V., Aizdaicher N. A. *Phaeodactylum tricornutum* Bohlin bioassay of water quality of Amur Bay (the Sea of Japan). *Contemporary Problems of Ecology*, 2011, vol. 4, no. 1, pp. 74–79. (in Russ.). https://doi.org/10.1134/S1995425511010127
8. Markina Zh. V., Aizdaicher N. A. The effect of copper on the abundance, cell morphology and content of photosynthetic pigments in the microalga *Porphyridium purpureum*. *Morskoj biologicheskij zhurnal*, 2019, vol. 4, no. 4, pp. 34–40. (in Russ.). https://doi.org/10.21072/mbj.2019.04.4.03
9. Nevrova E. L. *Bentosnye diatomovye vodorosli (Bacillariophyta) Chernogo morya: raznoobrazie i struktura taksotsenov razlichnykh biotopov.* [dissertation]. Moscow, 2015, 445 p. (in Russ.). https://dlib.rsl.ru/01005555099
10. Nevrova E. L., Snigireva A. A., Petrov A. N., Kovaleva G. V. *Guidelines From Quality Control of the Black Sea. Microphytobenthos* / A. V. Gaevskaya (Ed.). Sevastopol ; Simferopol : N.Orianda, 2015, 176 p. (in Russ.). https://doi.org/10.21072/978-5-9907290-2-5
11. Ovsyaniy E. I., Romanov A. S., Ignatieva O. G. Distribution of heavy metals in superficial layer of bottom sediments of Sevastopol Bay (the Black Sea). *Morskoj ekologicheskij zhurnal*, 2003, vol. 2, no. 2, pp. 85–101. (in Russ.). https://repository.marine-research.org/handle/299011/710
12. Petrov A. N., Nevrova E. L. Anthropogenic press on the diatom algae taxocene structure (Sevastopol Bay as an example). In: *Modern Condition of Biological Diversity in Near-shore Zone of Crimea (the Black Sea Sector)* / V. N. Eremeev, A. V. Gaevskaya (Eds). Sevastopol : EKOSI-Gidrofizika, 2003, pp. 288–302. (in Russ.). https://doi.org/10.21072/966-02-3133-4
13. Petrov A. N., Nevrova E. L. Comparative analysis of taxocene structures of benthic diatoms (Bacillariophyta) in regions with different level of technogenic pollution (the Black Sea, Crimea). *Morskoj ekologicheskij zhurnal*, 2004, vol. 3, no. 2, pp. 72–83. (in Russ.). https://repository.marine-research.org/handle/299011/748

14. Petrov A. N., Nevrova E. L., Malakhova L. V. Multivariate analysis of benthic diatoms distribution across the multidimensional space of the environmental factors gradient in Sevastopol Bay (the Black Sea, Crimea). *Morskoj ekologicheskij zhurnal*, 2005, vol. 4, no. 3, pp. 65–77. (in Russ.). https://repository.marine-research.org/handle/299011/812
15. Petrov A. N., Nevrova E. L. Estimation of cell distribution heterogeneity at toxicological experiments with clonal cultures of benthic diatoms. *Morskoj biologicheskij zhurnal*, 2020, vol. 5, no. 2, pp. 76–87. (in Russ.). https://doi.org/10.21072/mbj.2020.05.2.07
16. Romanova D. Yu., Petrov A. N., Nevrova E. L. Copper sulphate impact on growth and cell morphology of clonal strains of four benthic diatom species (Bacillariophyta) from the Black Sea. *Morskoj biologicheskij zhurnal*, 2017, vol. 2, no. 3, pp. 53–67. (in Russ.). https://doi.org/10.21072/mbj.2017.02.3.05
17. Spirkina N. E., Ipatova V. I., Dmitrieva A. G., Filenko O. F. Comparative growth of microalgae cultures of species *Monoraphidium arcuatum* (Korsch.) Hind. and *Scenedesmus quadricauda* (Turp.) Bréb. *Bulletin of Moscow Society of Naturalists. Biological Series*, 2014, vol. 119, iss. 2, pp. 64–69. (in Russ.)
18. Filenko O. F., Marushkina E. V., Dmitrieva A. G. Assessment of impact of cooper on a model population of alga *Scenedesmus quadricauda* (Turp.) Bréb. by a microculture method. *Gidrobiologicheskii zhurnal*, 2006, vol. 42, no. 6, pp. 53–61. (in Russ.)
19. Schlegel H. G. *Allgemeine Mikrobiologie.* Moscow : Mir, 1987, 567 p. (in Russ.)
20. *Ekologo-toksikologicheskie aspekty zagryazneniya morskoi sredy.* Vol. 5 / S. A. Patin (Ed.). Leningrad : Gidrometeoizdat, 1985, 116 p. (in Russ.)
21. Ahalya N., Ramachandra T. V., Kanamadi N. Biosorption of heavy metals. *Research Journal of Chemical & Environmental Sciences*, 2003, vol. 7, iss. 4, pp. 71–79.
22. Anantharaj K., Govindasamy C., Natanamurugaraj G., Jeyachandran S. Effect of heavy metals on marine diatom *Amphora coffeaeformis* (Agardh Kutz.). *Global Journal of Environmental Research*, 2011, vol. 5, no. 3, pp. 112–117.
23. Andersen R. A., Berges J. A., Harrison P. J., Watanabe M. M. Recipes for freshwater and seawater media. In: *Algal Culturing Techniques* / R. A. Andersen (Ed.). Amsterdam ; Boston ; London : Elsevier Academic Press, 2005, pp. 429–538.
24. Burgess R. M., Terletskaya A. V., Milyukin M. V., Povolotskii M. I., Demchenko V. Y., Bogoslovskaya T. A., Topkin Yu. V., Vorobyova T. V., Petrov A. N., Lyashenko A. V., Ho K. T. Concentration and distribution of hydrophobic organic contaminants and metals in the estuaries of Ukraine. *Marine Pollution Bulletin*, 2009, vol. 58, no. 8, pp. 1103–1115. https://doi.org/10.1016/j.marpolbul.2009.04.013
25. Cid A., Herrero C., Torres E., Abalde J. Copper toxicity on the marine microalga *Phaeodactylum tricornutum*: Effects on photosynthesis and related parameters. *Aquatic Toxicology*, 1995, vol. 31, iss. 2, pp. 165–174. https://doi.org/10.1016/0166-445X(94)00071-W
26. Crespo E., Losano P., Blasco J., Moreno-Garrido I. Effect of copper, irgarol and atrazine on epiphytes attached to artificial devices for coastal ecotoxicology bioassays. *Bulletin of Environmental Contamination and Toxicology*, 2013, vol. 91, iss. 6, pp. 656–660. https://doi.org/10.1007/s00128-013-1122-4

27. Davidovich N. A., Davidovich O. I., Podunay Yu. A., Gastineau R., Kaczmarska I., Poulíčková A., Witkowski A. *Ardissonea crystallina* has a type of sexual reproduction that is unusual for centric diatoms. *Scientific Reports*, 2017, vol. 7, art. no. 14670 (16 p.). https://doi.org/10.1038/s41598-017-15301-z
28. *Diatoms: Fundamentals and Applications* / J. Seckbach, R. Gordon (Eds). Hoboken, New Jersey : Wiley ; Salem, Massachusetts : Scrivener, 2019, 679 p.
29. Florence T. M., Stauber J. L. Toxicity of copper complexes to the marine diatom *Nitzschia closterium*. *Aquatic Toxicology*, 1986, vol. 8, iss. 1, pp. 11–26. https://doi.org/10.1016/0166-445X(86)90069-X
30. Kim J. W., Price N. M. The influence of light on copper-limited growth of an oceanic diatom, *Thalassiosira oceanica* (Coscinodiscophyceae). *Journal of Phycology*, 2017, vol. 53, iss. 5, pp. 938–950. https://doi.org/10.1111/jpy.12563
31. Kiseleva A. A., Tarachovskaya E. R., Shishova M. F. Biosynthesis of phytohormones in algae. *Russian Journal of Plant Physiology*, 2012, vol. 59, iss. 5, pp. 595–610. http://doi.org/10.1134/S1021443712050081
32. Kumar S., Baweja P., Sahoo D. Diatoms: Yellow or golden brown algae. In: *The Algae World* / D. Sahoo, J. Seckbach (Eds). Dordrecht, Netherlands : Springer, 2015, pp. 235–258. (Book series: Cellular Origin, Life in Extreme Habitats and Astrobiology ; vol. 26). http://dx.doi.org/10.1007/978-94-017-7321-8_8
33. Leung P. T. Y., Yi A. X., Ip J. C. H., Mak S. S. T., Leung K. M. Y. Photosynthetic and transcriptional responses of the marine diatom *Thalassiosira pseudonana* to the combined effect of temperature stress and copper exposure. *Marine Pollution Bulletin*, 2017, vol. 124, iss. 2, pp. 938–945. http://doi.org/10.1016/j.marpolbul.2017.03.038
34. Lobban C. S., Ashworth M. P., Camacho T., Lam D. W., Theriot E. C. Revision of Ardissoneaceae (Bacillariophyta, Mediophyceae) from Micronesian populations, with descriptions of two new genera, *Ardissoneopsis* and *Grunowago*, and new species in *Ardissonea*, *Synedrosphenia* and *Climacosphenia*. *PhytoKeys*, 2022, vol. 208, pp. 103–184. https://doi.org/10.3897/phytokeys.208.89913
35. Markina Zh. V., Aizdaicher N. A. Content of photosynthetic pigments, growth, and cell size of microalga *Phaeodactylum tricornutum* in the copper-polluted environment. *Russian Journal of Plant Physiology*, 2006, vol. 53, no. 3, pp. 305–309. https://doi.org/10.1134/S1021443706030034
36. Miazek K., Iwanek W., Remacle C., Richel A., Goffin D. Effect of metals, metalloids and metallic nanoparticles on microalgae growth and industrial products biosynthesis: A review. *International Journal of Molecular Sciences*, 2015, vol. 16, iss. 10, pp. 23929–23969. https://doi.org/10.3390/ijms161023929
37. Nagajoti P. C., Lee K. D., Sreekanth T. V. M. Heavy metals, occurrence and toxicity for plants: A review. *Environmental Chemistry Letters*, 2010, vol. 8, iss. 3, pp. 199–216. http://doi.org/10.1007/s10311-010-0297-8
38. Rijstenbil J. W., Gerringa L. J. A. Interactions of algal ligands, metal complexation and availability, and cell responses of the diatom *Ditylum brightwellii* with a gradual increase in copper. *Aquatic Toxicology*, 2002, vol. 56, no. 2, pp. 115–131. https://doi.org/10.1016/s0166-445x(01)00188-6
39. Round F. E., Crawford R. M., Mann D. G. *The Diatoms. Biology and Morphology of the Genera.* Cambridge : Cambridge University Press, 1990, 747 p.

40. *SigmaPlot NG. USA* : [site]. 2021. https://sigmaplot.com [accessed: 15.01.2021].
41. Smolyakov B. S., Ryzhikh A. P., Romanov R. E. The fate of Cu, Zn, and Cd in the initial stage of water system contamination: The effect of phytoplankton activity. *Journal of Hazardous Materials*, 2010, vol. 184, iss. 1–3, pp. 819–825. https://doi.org/10.1016/j.jhazmat.2010.08.115
42. *The Diatom World* / J. Seckbach, J. P. Kociolek (Eds). Berlin ; Heidelberg ; New York : Springer Verlag, 2011, 533 p. (Book series : Cellular Origin, Life in Extreme Habitats and Astrobiology ; vol. 19). https://doi.org/10.1007/978-94-007-1327-7
43. Witkowski A., Lange-Bertalot H., Metzeltin D. *Diatom Flora of Marine Coasts. 1*. Rugell ; Königstein : Gantner Verlag : Koeltz Scientific Books, 2000, 925 p. (Iconographia Diatomologica : Annotated Diatom Micrographs ; vol. 7: Diversity-Taxonomy-Identification / H. Lange-Bertalot (Ed.)).
44. Yan J., Liu J., Li Y., Lang S. Effect of water current on the distribution of polycyclic aromatic hydrocarbons, heavy metals and benthic diatom community in sediments of Haihe estuary, China. *Environmental Science and Pollution Research*, 2014, vol. 21, no. 20, pp. 12050–12061. https://doi.org/10.1007/s11356-014-3145-8

ДИНАМИКА РОСТА БЕНТОСНОЙ ДИАТОМОВОЙ ВОДОРОСЛИ *ARDISSONEA CRYSTALLINA* (C. AGARDH) GRUNOW, 1880 (BACILLARIOPHYTA) ПРИ ВОЗДЕЙСТВИИ ИОНОВ МЕДИ

Е. Л. Неврова, А. Н. Петров

ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН»,
Севастополь, Российская Федерация
E-mail: *el_nevrova@mail.ru*

Увеличение антропогенной нагрузки на прибрежные экосистемы Чёрного моря определяет необходимость постоянной оценки состояния сообществ планктона и бентоса. В качестве тест-объектов традиционно используют планктонные диатомовые микроводоросли, вносящие до 20–25 % глобальной первичной продукции, между тем как вклад микрофитобентоса сопоставим по своей значимости. Диатомовые бентоса обладают высокой чувствительностью к влиянию техногенных поллютантов, накапливающихся в донных отложениях. Изменение физиологических параметров бентосных Bacillariophyta объективно отражает воздействие различных токсикантов, что позволяет применять их как тест-объекты при опосредованной оценке качества морской среды. Целью работы было изучить динамику численности клеток клоновой культуры нового для практики биотестирования вида морской микроводоросли *Ardissonea crystallina* (C. Agardh) Grunow, 1880 (Bacillariophyta) при воздействии разных концентраций $CuSO_4 \cdot 5H_2O$ в течение 10 суток. Данный вид микроводорослей характеризуется широкой встречаемостью в сублиторали Чёрного моря и высокой чувствительностью к различным техногенным поллютантам, включая тяжёлые металлы. Показано, что при концентрациях токсиканта от 32 до 128 мкг·л$^{-1}$ (в пересчёте на ионы Cu^{2+}) динамика роста *A. crystallina* в целом соответствует кривой отклика тест-объекта в токсикологическом эксперименте. Выявлено снижение интенсивности прироста культуры и возрастание концентраций токсиканта в экспериментальной среде. При концентрациях ионов меди в диапазоне от 256 до 320 мкг·л$^{-1}$ доля живых клеток в культуре монотонно уменьшается от 62–66 % (1-е сутки) до 34–37 % (10-е сутки); показатели прироста численности клеток в культуре демонстрируют отрицательную динамику в течение опыта — от –0,01 (на 2-е сутки) до –0,34 (на 10-е сутки). При концентрациях в культуральной среде ионов Cu^{2+} 384 мкг·л$^{-1}$ и выше происходило резкое угнетение и последующее отмирание клеток *A. crystallina*, а для 448–1024 мкг·л$^{-1}$ отмирание 100 % клеток отмечено уже на 3-и сутки эксперимента. Статистическое сравнение вариативности доли живых клеток *A. crystallina* и показателей удельного прироста их численности для контроля и концентраций ионов меди 64–128 мкг·л$^{-1}$ продемонстрировало, что только на 10-е сутки различия между средними значениями параметров достоверны (P = 0,002…0,020). Изменение общей численности и доли живых клеток в культуре при 256 мкг·л$^{-1}$ достоверно отличается (P = 0,002…0,014) от такового как при меньших, так и при более высоких концентрациях, что позволяет рассматривать этот уровень токсиканта как критический для обитания данного вида диатомовой водоросли: его превышение приводит к резкому усилению процесса отмирания клеток. С учётом полученных результатов вид *A. crystallina* может быть рекомендован для широкого использования в качестве тест-объекта в токсикологических экспериментах, а также при экологическом мониторинге и опосредованной оценке состояния прибрежных морских акваторий, подверженных техногенному загрязнению.

Ключевые слова: токсикологический эксперимент, ионы меди, клоновая культура, численность клеток, бентосные диатомовые водоросли, Чёрное море

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INVESTIGATION OF THE LONG-WAVELENGTH THRESHOLD OF SPECTRAL SENSITIVITY IN THE GRAY SEAL *HALICHOERUS GRYPUS* (FABRICIUS, 1791)

M. V. Pakhomov and A. A. Zaytsev

Murmansk Marine Biological Institute of the Russian Academy of Sciences, Murmansk, Russian Federation
E-mail: *edr750v@yandex.ru*

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In marine mammals, the absorption maxima of photopigments have a shift to the shorter-wavelength spectrum area compared to terrestrial mammals; this leads to a shift in the long-wavelength threshold of spectral sensitivity as well. In most publications focused on the investigation of long-wavelength sensitivity of marine mammals, only the absorption maxima of "red-sensitive" photopigments are given, but no data on maximum wavelengths of light emission that animals are able to perceive are provided. Therefore, this work was aimed at studying the long-wavelength thresholds of spectral sensitivity in a typical representative of earless seals – the gray seal *Halichoerus grypus* (Fabricius, 1791). During the experiment, a group of four gray seals was trained to press one of two buttons if a LED lamp located above it is switched on. In the lamp, there were groups of LEDs emitting monochromatic light in the wavelength range from 600 to 700 nm with a step of 10 nm and a luminous intensity of 0.5 cd. As shown, the lower perception threshold of light emission for the studied gray seals is light emission with a wavelength of 660 nm, and this confirms the data on the short-wavelength shift of the sensitivity peaks of photopigments in marine mammals. During prolonged exposure to extremely low-light conditions typical for the polar night, the long-wavelength perception threshold in the gray seals can increase from 660 to 670–680 nm.

Keywords: gray seal, vision, spectral sensitivity

The gray seals have a well-developed visual system which includes relatively large eyeballs and a developed visual area of the brain. Such anatomical peculiarities of the eye structure, as the tapetum and developed musculature of the iris, indicate the adaptation of the visual system to low-light conditions (Hanke et al., 2009). Most of the gray seals inhabit the Holarctic realm beyond the Arctic Circle, which is characterized by the midnight sun with its round-the-clock high illuminance and polar night with illuminance above 500 lux for only a few hours a day.

In marine mammals, the absorption maxima of photopigments have a shift to the shorter-wavelength spectrum area compared to terrestrial mammals, with a correlation found between feeding diving depth and spectral sensitivity of visual pigments (McFarland, 1971). Out of cetaceans, shallow-diving species have rhodopsin (Rh1) with an absorption maximum of about 500 nm; in deep-sea species, the absorption maximum shifts to 479 nm (Bischoff et al., 2012 ; Fasick & Robinson, 2000). Out of pinnipeds, deep-diving species, such as northern elephant seals *Mirounga angustirostris* (Gill, 1866) and Weddell seals *Leptonychotes weddellii* (Lesson, 1826), have an Rh1 absorption maximum of about 480 nm; shallow-diving species have an Rh1 absorption maximum close to that of terrestrial mammals. Many species of marine mammals lack the "red-sensitive" pigment (long-wavelength sensitive opsin, LWS), but if present, it has a short-wavelength shift to 530 nm; in terrestrial mammals, maximum absorption of the LWS pigment is about 560 nm (Levenson et al., 2006). All marine mammals lack the SWS1 photopigment (short-wavelength sensitive opsin 1) responsible for sensitivity to short-wavelength ("blue") photoactive emission (Peichl et al., 2001).

For most mammals, including humans, the long-wavelength threshold of visible light is emission with a wavelength of 760–780 nm (Sliney, 2016). Light in this range is perceived by long-wavelength (LWS) cone opsins, with their maximum sensitivity about 560–580 nm. When moving away from the maximum point, the sensitivity gradually decreases to zero; it is impossible to specify the exact spectral range threshold of visible emission. When perceiving reflective objects, the threshold strongly depends on the illuminance level, optical properties of a perceived object, the dark adaptation of the eye, and the state of the nervous system. When light sources are perceived at borderline wavelengths, the minimum brightness increases exponentially with wavelength; at extreme wavelengths, even under high brightness, those are perceived by humans as a very dim orange-red glow (Palczewska et al., 2014). The following effect was recorded as well: with continuous stimulation of retina with a laser at wavelengths of 1,060–1,064 nm, a feeling of red color arose; with pulsed exposure, it was a feeling of green color (Sliney et al., 1976). Nevertheless, researchers attribute it to the direct effect on retinal nerve cells rather than photopigments (Sliney, 2005).

Under natural conditions, animals do not actually encounter objects that emit light; in rare cases, it can be bioluminescence or open sources of fire. Mostly, eyes perceive objects reflecting light, the source of which is the Sun. Its maximum emission is about 500–550 nm; a short-wavelength spectrum area, from 350 nm, is actively absorbed by upper atmospheric layers, while the long-wavelength area, from 600 nm, is absorbed by its lower layer saturated with water vapor. Meanwhile, with an increase in emission wavelength, the degree of its absorption by the atmosphere rises as well (Kirk, 2015).

In the gray seal *Halichoerus grypus* (Fabricius, 1791), the LWS pigment–containing cones are present in the retina; those are distributed in the central fovea of the retina in a ratio of 1 : 200 in relation to the rods and have an absorption maximum about 530 nm (Braekevelt, 1986). Based on a calculation of a spectral sensitivity curve for long-wavelength photopigments (Lamb, 1995 ; Lewis, 1955), it is assumed that the long-wavelength threshold of spectral sensitivity for the gray seals varies within 650–700 nm.

The aim of the work was to reveal the long-wavelength threshold of spectral sensitivity of the visual system in the gray seal. In addition to theoretical significance, this information has practical importance since night video recording of seals using infrared light is of great interest.

MATERIAL AND METHODS

Four female gray seals were involved in the experiment: seals No. 1 and 2, at the age of 15; seals No. 3 and 4, at the age of 5. All of them represented one population and were caught on the Bolshoy Kiy Island (the eastern Varanger Fjord, the Barents Sea). At the beginning of the experiment, all the animals were healthy, and no deviations in behavior were observed. All these seals have already participated in several experimental studies of hearing (Litvinov & Pakhomov, 2019) and vision (Pakhomov, 2020) and were trained to work according to paradigms "stimulus–response", "choice of two", and "choice by pattern." In the present study, the animals had to designate the switched-on lamp by pressing the corresponding button; they worked according to a scheme of free choice from two stimuli – reinforced and stimulus-noise.

For the study, an experimental setup was developed (Fig. 1). On a platform (3), two lamps (4) and two buttons (5) were located at a distance of 1 m. In the identical lamps, there were groups of LEDs emitting light with different wavelengths. In each experiment, either the right or left lamp worked; the seal had to press the button located under the working lamp (this was considered as a correct choice). Correct choices were reinforced by food rewards with a preliminary bridge signal (one long whistle at a frequency of 3,200 Hz, with a duration of 1 s); mistakes were punished through a work termination for 15 "penalty" seconds with a preliminary bridge signal (two short whistles at a frequency of 3,200 Hz, with a duration of 0.25 s and a break of 0.5 s). Fish cut into 20-g pieces and placed in containers (2) served as a food reward.

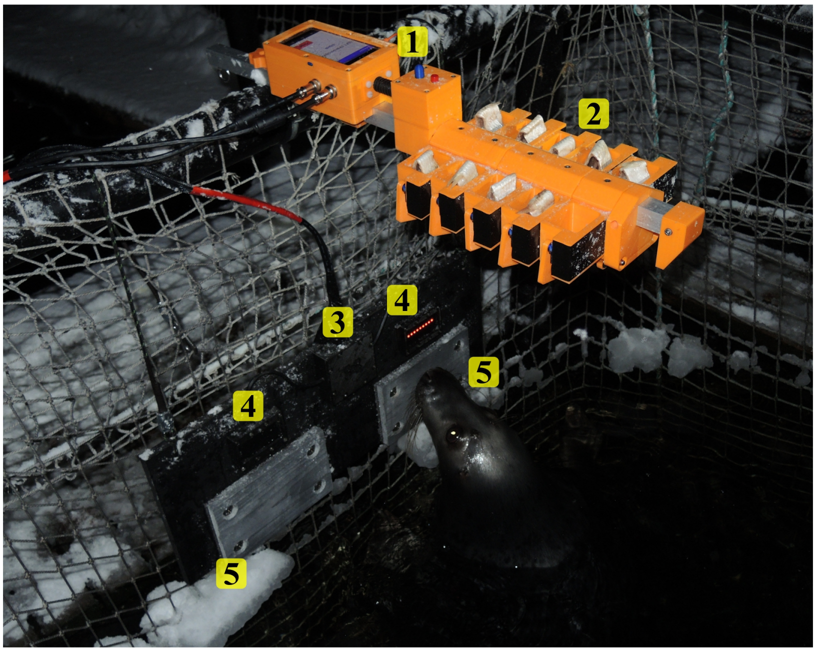

Fig. 1. Scheme of the experimental setup for determining the long-wavelength threshold of spectral sensitivity of the visual system of the gray seal: 1, control unit; 2, block of containers for fish feed; 3, distribution unit; 4, lamps; 5, buttons for interacting with the seal

The studies were carried out in September–February, when the gray seals first have a pupping season (November–December) and then a mating season (December–January). Accordingly, food motivation of the animals was significantly reduced. Therefore, firstly, the studied animals were on a special diet before, during, and after the experimental work; secondly, special attention was paid to the bridge signals. For the animals involved in the experiments, the bridge signals were associated during the initial adaptation to cage keeping and were maintained in a course of various researches. Immediately *prior* to the experiment, the work on consolidating the association was carried out with the gray seals: one long whistle was followed by a food reward; two short whistles were followed by a punishment through a work termination.

The source of the light stimulus were LEDs placed in a plastic case with a neutral filter cutting out 25 % of the light; this filter is designed for protecting LEDs from splashes, eliminating a halo around the working group of LEDs, and filtering out glare from the non-working groups. We used gallium-arsenide-phosphide LEDs with a narrow monochrome emission spectrum in the wavelength range of 600–700 nm with a step of 10 nm (for wavelengths of 600, 630, and 690 nm, produced by TZT; for all other wavelengths, produced by FSXSEMI). To check the LEDs for compliance with the declared characteristics, a Zolix Omni-λ300 spectroradiometer was applied. The luminous intensity of all LEDs did not change during the study and was calibrated for each "color" group by a value of 0.5 cd – with the light filter taken into account. Calibration and final measurements of the assembled lamps were carried out with a CEM DT-1308 luxmeter applied to determine the luminous intensity and with a two-tube spectroscope applied to determine the wavelength of the groups of LEDs.

To avoid glare and to increase the contrast of the emitted light, the platform (3) and the lamp cases (4) (see Fig. 1) were coated with anthracite-black matt waterproof paint.

For a human, the light of these LEDs is perceived in a range from amber (600 nm) to crimson (700 nm), and the threshold of perception is about 740–760 nm. Considering that and aiming to avoid the possibility of hints from the researcher, the system worked in a standalone mode. The researcher filled the containers with fish pieces, launched the system, and moved out of the seal visibility range. The general scheme of work was as follows. At the beginning of each cycle, the microcontroller-based control system paused for 5–30 s. If the gray seal pressed any button during this interval, there was a punishment through adding 5 "penalty" seconds to the waiting time. Then, the system pseudo-randomly, according to the Hellman sequence (Gerard et al., 2014) (no more than two identical combinations in a row and an equal number of right and left positions), switched on either the right or left lamp for 5 s. If the gray seal made a correct choice and pressed the button corresponding to the working lamp, he received a food reward. If the animal chose a non-working lamp, there was a punishment through a work termination for 15 s. Skipping a cycle was neither reinforced nor punished, but it was considered as an incorrect choice. After 10 cycles (emptying of the containers), the system terminated its work and signaled to the researcher. They came back to the cage, assessed whether the seal is ready to continue the experiment, filled the containers with fish pieces, and launched the continuation of the work. The system automatically ended the experiment in two cases: 1) if the gray seal missed five cycles in a row; 2) if the animal began to randomly press the buttons during a delay and gained more than two minutes of "penalty" time.

Each animal was usually subjected to 20–50 experiments *per* training – depending on its state and weather conditions. The ratio of correct choices to the total number of experiments was considered, as well as the reaction time of the gray seal. At the beginning of each training, the readings of illuminance, air temperature, humidity, and presence of precipitation or any other distractions were recorded. To reveal the randomness of correct choices, a binomial distribution for each sample of the corresponding wavelength was used. The resulting samples were subjected to multivariate analysis of variance – to determine correlations with weather conditions, date of the experiment, and stimulus position and order. Calculations and statistical data processing were carried out in Microsoft Excel 2019.

The work was carried out under conditions of the Murmansk Marine Biological Institute aquatic complex in the Polyarny town (the Kola Bay water area, the Tonya Cape vicinity). There, the studied gray seals are kept in individual cages under conditions as close to natural as possible. The experiment was conducted from September 2020 to February 2021 in two stages. At the preparatory stage (September–November), the animals were taught to choose the working lamp ignoring the non-working one. At the investigation stage (December–February), the seals were involved in the experimental work. Preparatory work was carried out twice a day – in the morning (before dawn) and in the evening (at dusk, with a total illuminance of not more than 15 lux). Experimental work was conducted twice a day as well – in the morning (at 7:00) and in the evening (in an hour after dark). For the experiment, the animals were chosen whose cages were the most darkened and where the level of illuminance at the platform location did not exceed 5 lux.

RESULTS

In total, 100 experiments were carried out with each gray seal for each group of LEDs during morning trainings, and 100 experiments were carried out during evening trainings. Fig. 2 shows the ratio of correct choices (touching the button under the switched-on lamp) by the gray seals depending on a wavelength of the given light stimulus.

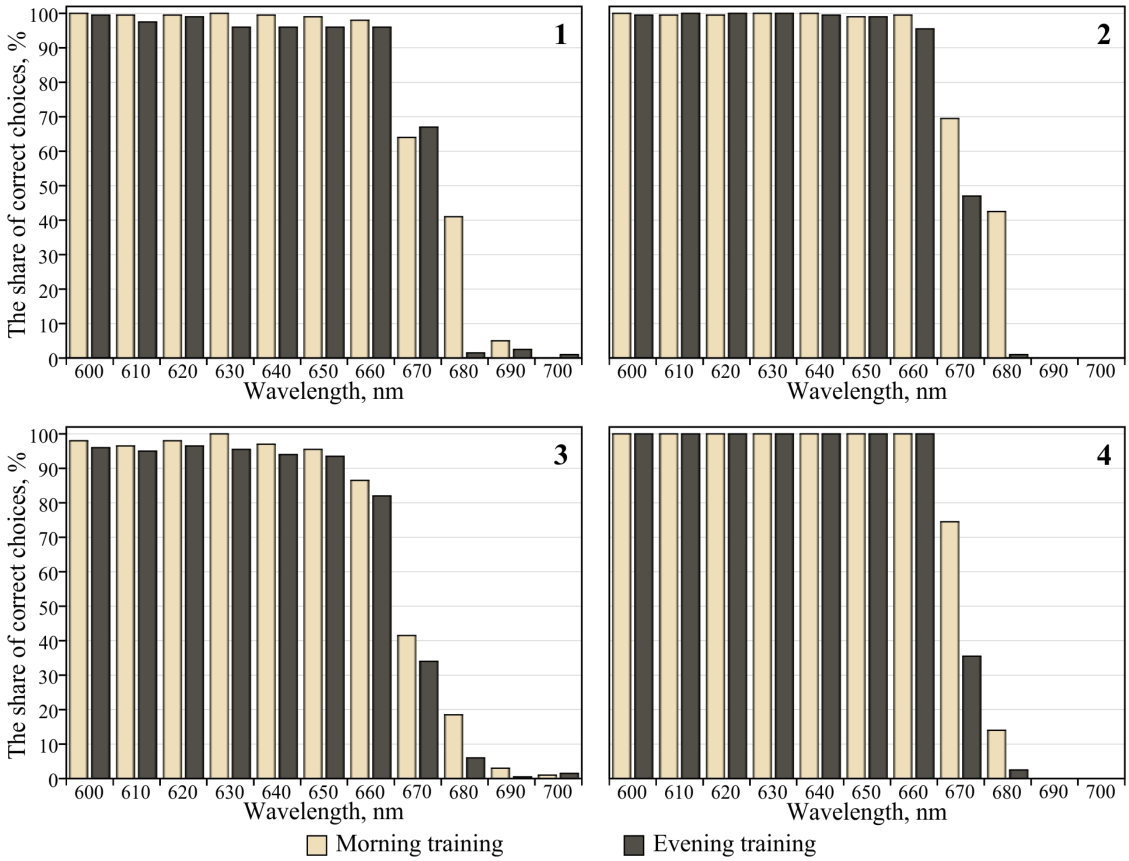

Fig. 2. Ratio of correct choices (touching the button under the switched-on lamp) of the gray seals No. 1–4 during morning and evening trainings

For all the gray seals, the ratio of correct choices (the choices of the switched-on lamp) at wavelengths of 600–660 nm was significantly higher than 75 %; for these samples ($n = 100$; $p < 0.05$), it indicates the non-randomness of the animal actions. Moreover, in this range, for all the seals, except for the seal No. 3, the ratio of correct choices was above 95 %; for the seal No. 4, the value was 100 %. The results of the binomial distribution were as close as possible to 1; for the seal No. 4, those were 1. This shows that the seals made their choices based on feelings rather than random guessing.

In this range, the ratios of correct choices during morning and evening trainings differed little. However, the gray seals made more mistakes during evening trainings. Mainly, those were mistakes when the animals pressed the buttons during the waiting time; to a lesser extent, those were mistakes when they ignored the given stimulus. The seal No. 1 often chose the non-working lamp instead of the nearby switched-on one. The ratio of this mistake made by the seal No. 1 was 2–3 %, while the ratio for other seals was less than 0.1 % of all the choices.

At 670 nm, the ratio of correct choices for all the gray seals decreased sharply and dropped significantly below 75 %, with the exception of the ratio for the seal No. 4: its ratio of correct choices was 74 % during morning trainings. During evening trainings, for all the seals, except for the seal No. 1, the ratio of correct choices decreased to the values below 50 %. For the seal No. 1, the ratio of correct choices during evening trainings was 67 % and was higher than during morning trainings (64 %).

At the same time, out of mistakes made by all the gray seals, the omission of the given stimulus prevailed. By the methods of multivariate analysis of variance, no significant correlations were revealed between the occurrence of the above mistake and weather conditions, date of the experiment, and stimulus position and order.

At 680 nm, the seals showed a reaction only during morning trainings. The ratios of correct choices of 15-year-old animals were 41 and 43 % for the seals No. 1 and 2, respectively, and were significantly higher than for younger ones (18 and 14 % for the seals No. 3 and 4, respectively).

When light stimuli with wavelengths of 690 and 700 nm were given, the gray seals showed no reaction and skipped these stimuli.

Data on the reaction time of the seals with a correct choice made are shown in Fig. 3 as a box plot. The mean, standard deviation of the mean, and extreme values for each wavelength are given.

At visible wavelengths, 600–650 nm, individual characteristics of the gray seals were revealed, such as mean reaction time and scatter of values. During morning trainings, the animals reacted faster than during evening ones. Starting from 660 nm, the mean reaction time and scatter of values began to increase.

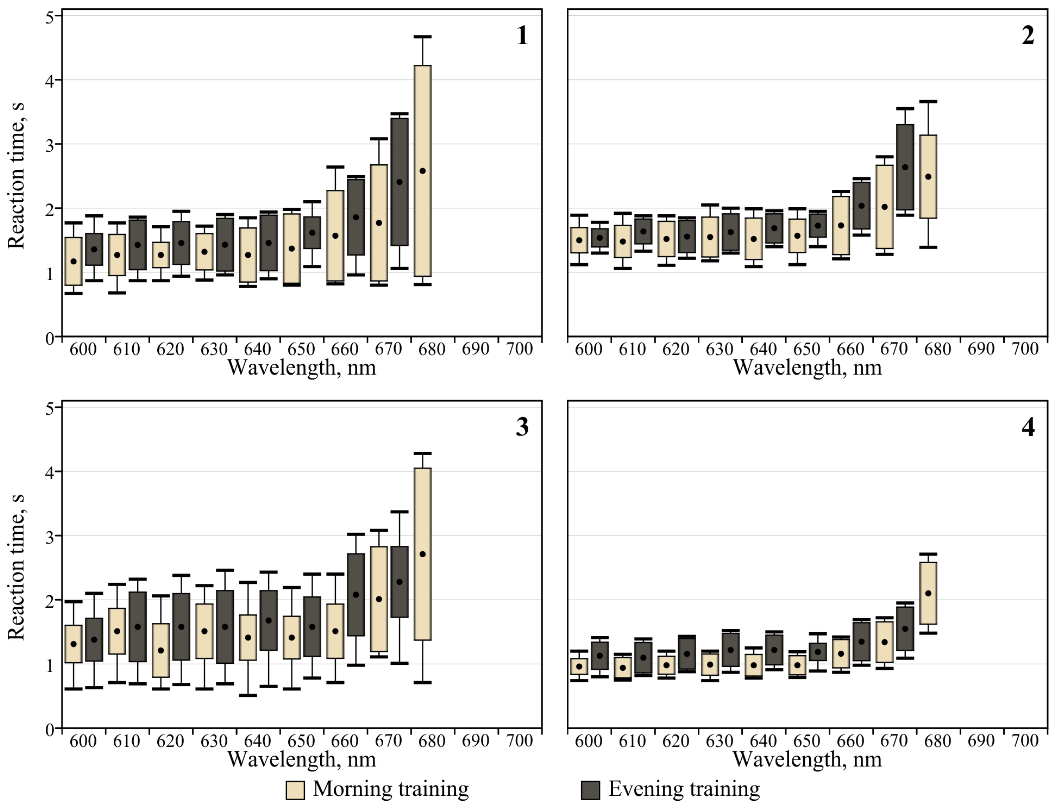

Fig. 3. Reaction time of the gray seals No. 1–4 to switching on the lamps during morning and evening trainings

Analyzing the relationship between the reaction time and external factors, we could establish the correlation with time of the day only; a weak correlation with weather conditions was revealed as well. With wind and sea waves of more 2 points on the Beaufort scale during the night before the experiment, the mean reaction time increased by 5–7.5 %, but the scatter of values did not change.

DISCUSSION

The results of the experiment show that the studied gray seals reliably perceive light with wavelengths up to 660 nm; the mean reaction time of the animals to a given stimulus and its scatter of values generally correspond to individual characteristics of these seals obtained in previous experiments with them (Litvinov & Pakhomov, 2019 ; Pakhomov, 2020). At a wavelength of 660 nm, for all the animals, the mean reaction time to the switched-on lamp and its scatter began to rise; this may result from the fact that the brain spends extra time processing the borderline signal.

At 670–680 nm, the ratio of correct choices significantly decreased but was not equal to zero, as was the case for wavelengths of 690 nm and higher. Considering that the choice is two-alternative, it can be concluded that such values indicate a high probability of random guessing of the switched-on lamp. Importantly, in case of guessing, the gray seals had to guess not only which of two lamps was switched on, but also when it would be switched on. Accordingly, with the lamp working time of 5 s and with taking into account the delay before switching on in a random range of 5–30 s, a probability of getting into a time interval when one of two lamps is working is about 17 %. Moreover, when pressing any button while the delay time had not yet expired, "penalty" 5 s were added to the delay time with the corresponding bridge signal. When gaining 120 s of "penalty" time, the system stopped the training. Thus, a probability of making a correct choice by chance was 8 %; according to the multiplication rule of probability, it was the product of a probability of guessing which of two lamps is switched on (50 %) and a probability of guessing when the lamp would be switched on (17 %). Therefore, the decrease in the ratio of correct choices when giving groups of LEDs with wavelengths of 670 and 680 nm can be explained by the fact that these emissions were the borderline for the gray seals: the animals pressed the correct button only when they were really able to distinguish a working lamp from a non-working one, preferring in case of doubts to wait for the next experiment. There, the effect could be explained by both short-term changes in weather conditions (drizzle fall, water evaporation, *etc.*) and physiological state of the seal, which primarily affects the degree of its focus on the task. This is also indicated by the difference between the results during morning and evening trainings: *prior* to morning trainings, the seals were under low-light conditions for a long time, and, due to dark adaptation, the sensitivity of photoreceptors was increased. The effect may result from own thermal noise of the retina as well: its intensity depends on many physiological peculiarities of the body and is taken into account by the brain when processing visual signals (Ala-Laurila et al., 2004).

Similar results were obtained in a number of behavioral response investigations with the South American sea lion [*Otaria byronia* (de Blainville, 1820)], the harbor seal [*Phoca vitulina* Linnaeus, 1758] (Griebel et al., 2006), and the harp seal [*Pagophilus groenlandicus* Erxleben, 1777] (Lavigne & Ronald, 1972). In all studied animals, the ratio of correct responses sharply decreased starting from 650–670 nm.

Conclusion:

1. For the gray seals studied, the long-wavelength threshold of the perception of light emission is light with a wavelength of 660 nm. This confirms the data on the short-wavelength shift of the sensitivity

peaks of photopigments in marine mammals. During prolonged exposure to extremely low-light conditions, the perception threshold in the gray seals increases from 660 to 670–680 nm.

2. According to the results obtained, the gray seals are unable to perceive light with a wavelength higher than 680 nm. Accordingly, the use of lamps operating in the near-infrared spectrum (750–1,000 nm) will be imperceptible to the seals, and this will allow using such lamps for night watching both domesticated and wild gray seals.

REFERENCES

1. Litvinov Yu. V., Pakhomov M. V. Investigation of the ability of gray seals to differentiate composite audio signals. *Vestnik Murmanskogo gosudarstvennogo tekhnicheskogo universiteta*, 2019, vol. 22, no. 2, pp. 249–257. (in Russ.). https://doi.org/10.21443/1560-9278-2019-22-2-249-257
2. Pakhomov M. V. Research on the ability of gray seals to differentiate light sources with different wavelengths. *Trudy Kol'skogo nauchnogo tsentra RAN*, 2020, vol. 11, no. 5, pp. 125–132. (Okeanologiya ; iss. 8). (in Russ.). https://doi.org/10.37614/2307-5252.2020.11.5.014
3. Ala-Laurila P., Donner K., Koskelainen A. Thermal activation and photoactivation of visual pigments. *Biophysical Journal*, 2004, vol. 86, iss. 6, pp. 3653–3662. https://doi.org/10.1529/biophysj.103.035626
4. Bischoff N., Nickle B., Cronin T. W., Velasquez S., Fasick J. I. Deep-sea and pelagic rod visual pigments identified in the mysticete whales. *Visual Neuroscience*, 2012, vol. 29, iss. 2, pp. 95–103. https://doi.org/10.1017/S0952523812000107
5. Braekevelt C. R. Retinal epithelial fine structure in the grey seal (*Halichoerus grypus*). *Acta Anatomica*, 1986, vol. 127, no. 4, pp. 4255–4261. https://doi.org/10.1159/000146295
6. Fasick J. I., Robinson P. R. Spectral-tuning mechanisms of marine mammal rhodopsins and correlations with foraging depth. *Visual Neuroscience*, 2000, vol. 17, iss. 5, pp. 781–788. https://doi.org/10.1017/S095252380017511X
7. Gerard C. J., Mackay H. A., Thompson B., McIlvane W. J. Rapid generation of balanced trial distributions for discrimination learning procedures: A technical note. *Journal of the Experimental Analysis of Behavior*, 2014, vol. 101, iss. 1, pp. 171–178. https://doi.org/10.1002/jeab.58
8. Griebel U., König G., Schmid F. Spectral sensitivity in two species of pinnipeds (*Phoca vitulina* and *Otaria flavescens*). *Marine Mammal Science*, 2006, vol. 22, iss. 1, pp. 156–166. https://doi.org/10.1111/j.1748-7692.2006.00010.x
9. Hanke F. D., Hanke W., Scholtyssek C., Dehnhardt G. Basic mechanisms in pinniped vision. *Experimental Brain Research*, 2009, vol. 199, pp. 299–311. https://doi.org/10.1007/s00221-009-1793-6
10. Kirk A. P. Chapter 2 – From nuclear fusion to sunlight. In: Kirk A. P. *Solar Photovoltaic Cells*. Amsterdam ; Boston ; London : Academic Press, 2015, pp. 9–24. https://doi.org/10.1016/B978-0-12-802329-7.00002-X
11. Lamb T. D. Photoreceptor spectral sensitivities: Common shape in the long-wavelength region. *Vision Research*, 1995, vol. 3, iss. 22, pp. 3083–3091. https://doi.org/10.1016/0042-6989(95)00114-F
12. Lavigne D. M., Ronald K. The harp seal, *Pagophilus groenlandicus* (Erxleben 1777). XXIII. Spectral sensitivity. *Canadian Journal of Zoology*, 1972, vol. 50, no. 9, pp. 1197–1206. https://doi.org/10.1139/z72-161
13. Levenson D. H., Ponganis P. J., Crognale M. A., Deegan J. F., Dizon A., Jacobs G. H. Visual pigments of marine carnivores: Pinnipeds, polar bear, and sea otter. *Journal of Comparative Physiology A*, 2006, vol. 192, pp. 833–843. https://doi.org/10.1007/s00359-006-0121-x

14. Lewis P. R. A theoretical interpretation of spectral sensitivity curves at long wavelengths. *Journal of Physiology*, 1955, vol. 130, iss. 1, pp. 45–52. https://doi.org/10.1113/jphysiol.1955.sp005391
15. McFarland W. N. Cetacean visual pigments. *Vision Research*, 1971, vol. 11, iss. 10, pp. 1065–1076. https://doi.org/10.1016/0042-6989(71)90113-1
16. Palczewska G., Vinberg F., Stremplewski P., Bircher M. P., Salom D., Komar K., Zhang J., Cascella M., Wojtkowski M., Kefalov V. J., Palczewski K. Human infrared vision is triggered by two-photon chromophore isomerization. *Proceedings of the National Academy of Sciences of the United States of America*, 2014, vol. 111, no. 50, pp. E5445–E5454. https://doi.org/10.1073/pnas.1410162111
17. Peichl L., Behrmann G., Kröger R. H. For whales and seals the ocean is not blue: A visual pigment loss in marine mammals. *European Journal of Neuroscience*, 2001, vol. 13, iss. 8, pp. 1520–1528. https://doi.org/10.1046/j.0953-816x.2001.01533.x
18. Sliney D. H., Wangemann R. T., Franks J. K., Wolbarsht M. L. Visual sensitivity of the eye to infrared laser radiation. *Journal of the Optical Society of America*, 1976, vol. 66, iss. 4, pp. 339–341. https://doi.org/10.1364/josa.66.000339
19. Sliney D. H. Exposure geometry and spectral environment determine photobiological effects on the human eye. *Photochemistry and Photobiology*, 2005, vol. 81, no. 3, pp. 483–489.
20. Sliney D. H. What is light? The visible spectrum and beyond. *Eye*, 2016, vol. 30, no. 2, pp. 222–231. https://doi.org/10.1038/eye.2015.252

ИССЛЕДОВАНИЕ ДЛИННОВОЛНОВОЙ ГРАНИЦЫ СПЕКТРАЛЬНОЙ ЧУВСТВИТЕЛЬНОСТИ У СЕРОГО ТЮЛЕНЯ *HALICHOERUS GRYPUS* (FABRICIUS, 1791)

М. В. Пахомов, А. А. Зайцев

Мурманский морской биологический институт Российской академии наук,
Мурманск, Российская Федерация
E-mail: *edr750v@yandex.ru*

У морских млекопитающих максимумы поглощения фотопигментов имеют сдвиг в более коротковолновую часть спектра относительно наземных млекопитающих; это обусловливает также и смещение длинноволновой границы спектральной чувствительности. В большинстве публикаций, посвящённых изучению длинноволновой чувствительности морских млекопитающих, приведены только максимумы поглощения «красночувствительных» фотопигментов, но нет данных о максимальных длинах волн светового излучения, которое животные способны воспринимать. Именно поэтому целью настоящей работы было исследовать длинноволновую границу спектральной чувствительности у типичного представителя настоящих тюленей — серого тюленя *Halichoerus grypus* (Fabricius, 1791). В эксперименте группу из четырёх серых тюленей обучили нажимать на одну из двух кнопок, если находящийся над ней светодиодный фонарь включён. В фонаре были расположены группы светодиодов, излучающие монохроматический свет в диапазоне длин волн 600–700 нм с шагом 10 нм и силой света 0,5 кд. Изучение показало, что длинноволновым пределом восприятия светового излучения для исследованных серых тюленей является свет с длиной волны 660 нм, что подтверждает данные о коротковолновом сдвиге пиков чувствительности фотопигментов у морских млекопитающих. При длительном пребывании серых тюленей в условиях крайне низкой освещённости, что характерно для полярной ночи, длинноволновая граница восприятия у них может увеличиваться с 660 до 670–680 нм.

Ключевые слова: серый тюлень, зрение, спектральная чувствительность

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SUSPENDED PARTICULATE MATTER AS A BIOCHEMICAL BARRIER TO HEAVY METALS IN MARINE FARM AREAS (SEVASTOPOL, THE BLACK SEA)

© 2022 **N. V. Pospelova, V. N. Egorov, V. Yu. Proskurnin, and A. S. Priymak**

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
E-mail: *nvpospelova@mail.ru*

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For recreational zones and marine farm areas, the investigation of chemical fluxes in coastal marine areas is of certain relevance. To study the role of suspended particulate matter in formation of biogeochemical barriers in marine farm areas, a method was proposed for estimating the fluxes of sedimentary self-purification of water from heavy metals (Co, Ni, Cu, Zn, Mo, Cd, and Pb) and arsenic (As). Based on the literature data on radioisotope dating of bottom sediments and the sedimentation rate, as well as considering our own estimates of the specific gravity of suspended particulate matter in water and concentrations of dissolved and suspended forms of heavy metals and As in the marine environment, the fluxes of biogeochemical self-purification of a marine farm water area from these trace elements were determined. As shown, the proposed methodological base is applicable for ecological regulation of coastal recreational zones in terms of pollution by trace elements.

Keywords: Black Sea, pollution, trace elements, self-purification, ecological regulation, suspended particulate matter, aquaculture

Industrial and recreational activities off the Crimean coast result in an increased entry of pollutants, *inter alia* heavy metals, into the marine environment. Suspended particulate matter (hereinafter SPM) is one of the indicators characterizing the quality of coastal marine water. Depending on its sources, SPM is divided into autochthonous and allochthonous. Allochthonous SPM enters the seas with coastal runoff due to coastal abrasion, resuspension of bottom sediments, river and industrial discharges, atmospheric precipitation, *etc.* As a result, various compounds, both nutrients and pollutants, including heavy metals, enter the marine environment. Biological processes, such as phyto- and zooplankton functioning (formation, destruction, and biochemical transformation of the organic component of suspension), play an important role in the formation of autochthonous SPM (Kukushkin & Parkhomenko, 2021). In the open sea areas, autochthonous SPM prevails, while near estuarine zones, allochthonous SPM predominates. In the coastal sea areas, which are not adjacent to estuarine zones of the rivers but are subject to coastal runoff of terrigenous SPM, waters are characterized by increased trophicity and production of biogenic (autochthonous) material (Gulin et al., 2013). In this publication, the researchers showed that autochthonous SPM prevails at the outer roadstead of Sevastopol, and it indicates a significant role of biotic processes in the transport of SPM there. The marine farm for cultivating bivalves certainly contributes to SPM formation in this water area. Since SPM is the primary trophic link in aquatic ecosystems, mariculture species interact with the sources of their constitutive, energy, and mineral nutrition contained in SPM, as well as with heavy metals, which can be both trace elements essential for metabolism and toxic pollutants of the marine environment.

According to modern views (Ergül et al., 2008 ; Stetsiuk, 2020 ; Turner & Millward, 2002), the living and abiogenic components of SPM concentrate chemicals of various biological significance, both in sorption and metabolic ways, with very high accumulation factors due to a large specific surface of fine-grained particles. Because of their negative buoyancy, SPM becomes the source of sedimentation processes and causes conservative chemical pollutants associated with SPM to deposit in bottom sediments. As a result, in marine farm areas, biogeochemical barriers may be formed affecting the chemical composition and self-purification of water from pollution.

Between SPM and metals in solution, adsorption/desorption and (co)precipitation occur; therefore, the ratios of trace elements in SPM and in dissolved fraction may vary depending on SPM composition, as well as on hydrological and hydrochemical parameters of seawater (Salomons & Förstner, 1984).

As known, heavy metals in water bodies do not degrade, unlike organic pollutants; instead, heavy metals are redistributed over the aquatic ecosystem components. At the same time, SPM has the greatest concentrating ability in relation to metals (due to its large specific surface area). In the process of sedimentation, suspended particles deposit pollutants in the bottom sediments. This allows considering SPM sedimentation as the main biogeochemical mechanism of self-purification of seawater (Bufetova, 2022 ; Egorov, 2019 ; Matishov et al., 2017).

The toxicological hazard of heavy metals and arsenic is normalized by sanitary and hygienic criteria. According to current regulations, water quality is estimated by maximum permissible concentrations (hereinafter MPC) for pollutants dissolved in water (Normativy kachestva vody, 2016). This diagnostic criterion shows threshold concentrations for population. Importantly, content of heavy metals and arsenic in water is formed by pools of dissolved and suspended forms of trace elements. Pools of suspended forms of metals are a biogeochemical barrier. This is of particular relevance in marine farm areas since such a barrier in ecotoxicological terms protects coastal waters and farm products from pollution. In this paper, a methodology for estimating the fluxes of biochemical self-purification of water from heavy metals and arsenic is proposed to study the role of SPM in the formation of biogeochemical barriers in marine farm areas. The method can further be used in the development of criteria for assessing the maximum permissible fluxes of water pollution in marine farm areas, taking into account sanitary and hygienic standards.

MATERIAL AND METHODS

Sampling area. The sampling was carried out at the outer roadstead of the Sevastopol Bay (44°37′13.4″N, 33°30′13.6″E) in 2020 (Fig. 1). The study area was chosen due to its proximity to the coastline (700 m) and location of a marine farm for cultivating mussels and oysters.

The water in this area is classified as mesotrophic; values of the water eutrophication index (E-TRIX) range from 1.63 to 4.33 (Kuftarkova et al., 2006). The mean depth in the marine farm area is 22 m. The long-term average temperature in February is +8 °C; salinity varies within 17.2–18.1 ‰, with temperature and salinity being uniform from the surface down to the bottom. The long-term average oxygen concentration in water is maximum in winter (7.3–7.7 mL·L^{-1}). The content of phosphates does not exceed 0.37 μM. The concentration of nitrates in surface waters in winter is on average higher (0.5–7.5 μM) than in summer due to the assimilation by fast-growing phytoplankton (Kapranov et al., 2020). As shown, the formation of the hydrochemical regime of the studied water area is affected by polluted water of the Sevastopol Bay (discharge of the Chernaya River) and Karantinnaya Bay (storm sewer wastewater), emergency discharge of domestic wastewater (up to 3 thousand m^3 of untreated wastewater *per* day), and storm runoff (Ivanov et al., 2002 ; Nemirovsky & Eremin, 2003).

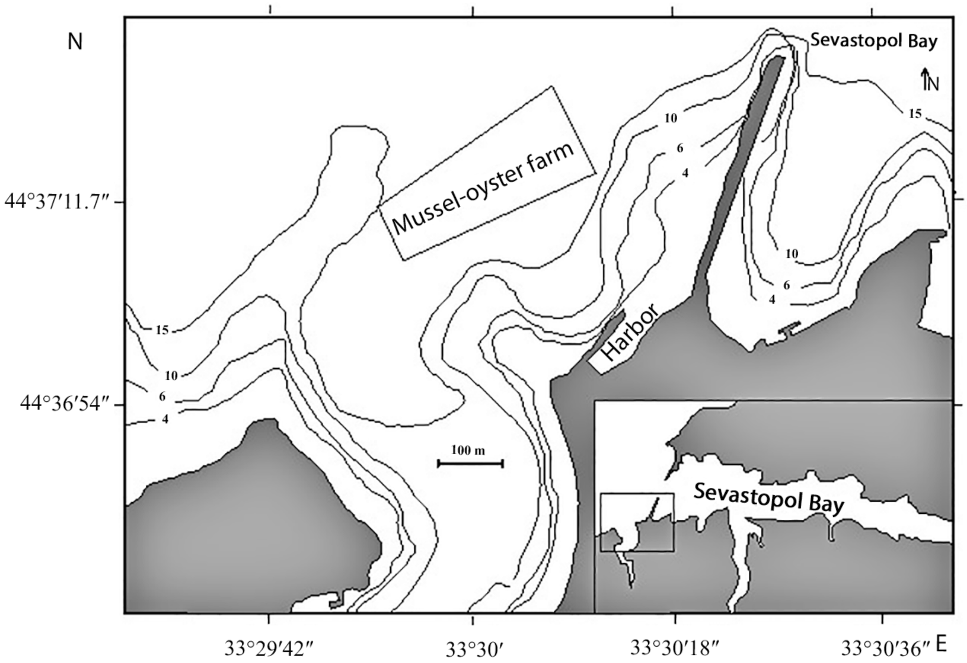

Fig. 1. Map of the study water area and sampling site

Sampling. In February 2020, water and SPM were sampled simultaneously to measure trace elements in them. In total, 50 L of seawater were sampled for analysis from the surface. The volume of samples was determined taking into account the expected concentration of SPM, so that the total mass of the collected SPM was 10–50 mg dry weight (hereinafter DW). SPM samples were concentrated using a 6-section vacuum filtration device (at a residual pressure of 0.4–0.6 atm) through the FMNTs-0.45 membrane filters. In total, 6 SPM samples and 6 water samples were treated.

Measurement of heavy metal content in seawater and SPM. Heavy metals and arsenic (hereinafter trace elements, TE) were isolated from seawater by the extraction concentration technique according to the guiding document RD 52.10.243-92 (Zolotov & Kuzmin, 1971). The technique consists in the extraction of complexes of the studied elements with carbon tetrachloride using sodium diethyldithiocarbamate (Na-DDC) as a chelating agent, which is followed by the destruction of the complexes with concentrated nitric acid and re-extraction of the elements in an aqueous solution.

SPM concentration was determined by the gravimetric method (Vityuk, 1983). Specifically, the FMNTs-0.45 membrane filters made of cellulose nitrate were kept for 30 min in a drying chamber at a temperature of +60 °C; then, those were kept for 2 h in a desiccator with a moisture absorber ($CaCl_2$). The weighing was carried out on an analytical scale with an accuracy of 0.1 mg.

After passing a certain volume of seawater through the filters (6 filters *per* sample) under vacuum (at a residual pressure of 0.4–0.6 atm), the filters were washed with deionized water, dried in the same way as described above, and weighed again. SPM concentration was calculated based on the difference between the weights of the filters before and after filtration, taking into account the volume of water passed, and expressed in terms of $mg \cdot L^{-1}$ or $mg \cdot m^{-3}$.

TE were isolated from the SPM samples by acid mineralization in accordance with the environmental regulatory document PND F 16.2.2:2.3.71-201. The mineralization was performed in a mixture of nitric acid and hydrogen peroxide heated on an electric stove; the filters were completely dissolved, and insoluble residues of SPM were separated by filtration through "Sinyaya lenta" filter papers. In parallel, a blank measurement was carried out with clean filters, using the same amounts of reagents as for the SPM samples.

TE in re-extracts from seawater and mineralized SPM were measured on the basis of IBSS core facility "Spectrometry and Chromatography" by inductively coupled plasma mass spectrometry using PlasmaQuant MS Elite mass spectrometer (Analytik Jena AG, Germany) in accordance with the international technical standard GOST R 56219-2014 and the operating manual for PlasmaQuant MS instruments. The spectrometer was calibrated for the measured elements using an IPC special multi-element standard solution IV (28 elements) (Inorganic Ventures, USA). The error in measurements of TE in seawater was no more than 15 %; in SPM, no more than 10 %.

Equations for assessing the fluxes of self-purification of water from trace elements *via* sedimentation. Accumulation factors (AF) of TE by SPM were calculated by the formula:

$$AF = 1,000 \times C_{\text{pm}}/C_{\text{d}} \,, \tag{1}$$

where C_{pm} is content of TE in SPM ($\mu g \cdot g^{-1}$ DW);

C_d is content of TE dissolved in seawater ($mg \cdot L^{-1}$).

A series of equations is proposed to evaluate the role of SPM in the formation of biogeochemical barriers against TE in coastal water areas. Theoretical analysis of the obtained results was carried out taking into account the recent views on the radioisotope and chemical homeostasis of marine ecosystems (Egorov, 2019).

The pool of TE in SPM (P_{pm}) under 1 m^2 of the farm in the 22-m water column ($\mu g \cdot m^{-2}$) was calculated as follows:

$$P_{\text{pm}} = C_{\text{pm}} \times 22 \times 1,000 \,. \tag{2}$$

The total pool of TE in seawater and SPM (P_{d+pm}) under 1 m^2 of the farm in the 22-m water column ($\mu g \cdot m^{-2}$) was calculated as follows:

$$P_{\text{d+pm}} = (C_{\text{d}} + C_{\text{pm}}) \times 22 \times 1,000 \,, \tag{3}$$

where C_{pm} is concentration of TE in SPM ($\mu g \cdot L^{-1}$).

According to the data in (Gulin et al., 2013), the sedimentation flux (F_{sed}) in the study area, calculated using the radioisotope dating method of bottom sediments, was equal to 664 $g\,DW \cdot m^{-2} \cdot year^{-1}$, or 1.82 $g\,DW \cdot m^{-2} \cdot day^{-1}$. Daily sedimentation flux of TE ($V_{sed/day}$) ($\mu g \cdot m^{-2} \cdot day^{-1}$), or the flux of self-purification of water, was calculated by the formula:

$$V_{\text{sed/day}} = F_{\text{sed}} \times C_{\text{pm}} , \tag{4}$$

where F_{sed} is sedimentation flux (g DW·m^{-2}·day^{-1}).

TE turnover (T_{pt}) (days) due to sedimentation processes in the 22-m layer was estimated using the following equation:

$$T_{\text{pt}} = P_{\text{d+pm}}/V_{\text{sed/day}} . \tag{5}$$

The time constant (p) (year^{-1}) was calculated as follows:

$$p = 365/T_{\text{pt}} . \tag{6}$$

Annual sedimentation flux (P_{ad}) of TE into bottom sediments (kg·ha^{-1}) was determined by the formula:

$$P_{\text{ad}} = V_{\text{sed/day}} \times 365 . \tag{7}$$

Also, TE content in SPM (N) (µg·g^{-1} DW) was calculated:

$$N = C_{\text{pm}}/m_{\text{pm}} , \tag{8}$$

where m_{pm} is SPM mass in seawater (mg·L^{-1}).

Statistical analysis. All results in the tables are presented as (mean ± standard deviation). The significance level was set as $p < 0.05$.

RESULTS

Content of trace elements in water and SPM. It was determined that in February 2020, SPM concentration in the study area amounted to 0.72 mg DW·L^{-1}. The results of measurements of TE concentrations in surface waters and in SPM within the analyzed area are given in Table 1.

Table 1. Trace element concentrations in the marine farm water (Sevastopol, February 2020)

TE	Trace element concentration (mean ± *SD*)					N (mean ± *SD*), µg·g^{-1} DW	MPC for fishery basins, µg·L^{-1}
	C_{d+pm}, µg·L^{-1} (% of MPC)	C_d (dissolved), µg·L^{-1}	% of total	C_{pm} (SPM), µg·L^{-1}	% of total		
1	2	3	4	5	6	7	8
Co	0.024 (0.4)	0.019 ± 0.001	79.2	0.005 ± 0.001	20.8	6.93 ± 0.07	5
Ni	0.940 (8.7)	0.866 ± 0.023	92.4	0.071 ± 0.001	7.6	99.76 ± 0.94	10
Cu	2.506 (45.0)	2.248 ± 0.063	89.7	0.258 ± 0.003	10.3	358.18 ± 4.03	5
Zn	22.477 (42.7)	21.374 ± 0.680	95.1	1.103 ± 0.018	4.9	1,532.40 ± 25.76	50
As	0.073 (0.3)	0.032 ± 0.005	44.1	0.041 ± 0.0004	55.9	56.37 ± 0.52	10
Mo	1.985 (198.5)*	1.982 ± 0.071	99.8	0.003 ± 0.0001	0.2	4.44 ± 0.11	1
Cd	0.029 (0.3)	0.026 ± 0.001	90.0	0.003 ± 0.0005	10.0	4.01 ± 0.07	10
Pb	0.555 (0.5)	0.481 ± 0.012	86.6	0.074 ± 0.001	13.4	103.27 ± 1.87	10

Note: *SD* denotes standard deviation; * denotes a value exceeding threshold limit value (MPC).

The data in Table 1 indicate that the ecotoxicological situation in February 2020, concerning water pollution in the marine farm area, can be regarded favorable by all TE (only a certain exceedance of MPC by molybdenum was registered). Importantly, the Russian regulations (Normativy kachestva vody, 2016) establish values mostly for freshwater economic objects; only for several TE, the MPC in marine water are additionally provided. For molybdenum, the MPC in marine water is not given.

In the column 2 of Table 1, the values in parentheses are TE concentrations compared to their MPC. Columns 4 and 6 show the ratios of dissolved and suspended forms in the total TE content in seawater. According to the data in Table 1, the total TE concentration in water ranged 0.024–22.477 $\mu g \cdot L^{-1}$. The values for dissolved forms were within 0.019–21.374 $\mu g \cdot L^{-1}$; for suspended forms, within 0.003–1.103 $\mu g \cdot L^{-1}$. Analyzed TE can be arranged in a sequence of their decreasing level in water as follows: Zn > Cu > Mo > Ni > Pb > As > Cd > Co. For SPM, the sequence was as follows: Zn > Cu > Pb > Ni > As > Co > Mo > Cd.

The data in Table 1 and the available literature material on the sedimentation rate of total (allochthonous and autochthonous) biogenic matter in the study area (Gulin et al., 2013) made it possible to propose a methodology for assessing the role of SPM in the formation of biogeochemical barriers in coastal waters (for example, in the marine farm area), based on the series of equations (2–7). The results of assessing the biogeochemical characteristics of the analyzed water area are summarized in Table 2.

Table 2. Biogeochemical characteristics of the turnover of trace elements in suspended particulate matter in the marine farm water area (Sevastopol, February 2020)

TE	AF, $\times 10^4$ DW	P_{d+pm}, $\mu g \cdot m^{-2}$	P_{pm}, $\mu g \cdot m^{-2}$	$V_{sed/day}$, $\mu g \cdot m^{-2} \cdot day^{-1}$	Parameters of the sedimentation TE turnover in the 22-m water column		P_{ad}, $kg \cdot ha^{-1}$
					T_{pt}, day	P_{ad}, $kg \cdot ha^{-1}$	
1	2	3	4	5	6	7	8
Co	30 ± 1	$5.3 \cdot 10^2$	$1.0 \cdot 10^2$	12.6	42.0	8.69	0.045
Ni	10 ± 0.3	$206.1 \cdot 10^2$	$15.6 \cdot 10^2$	181.6	113.5	3.22	0.663
Cu	16 ± 0.5	$547.8 \cdot 10^2$	$56.8 \cdot 10^2$	651.9	84.8	4.30	2.379
Zn	7 ± 0.2	$4{,}944.9 \cdot 10^2$	$242.7 \cdot 10^2$	2,788.9	177.4	2.06	10.180
As	180 ± 8	$16.1 \cdot 10^2$	$9.0 \cdot 10^2$	102.6	15.8	23.10	0.374
Mo	0.20 ± 0.02	$436.7 \cdot 10^2$	$0.7 \cdot 10^2$	8.1	5,404.7	0.07	0.029
Cd	15 ± 0.5	$547.8 \cdot 10^2$	$6.4 \cdot 10^2$	7.3	87.7	4.16	0.027
Pb	0.020 ± 0.001	$4{,}944.9 \cdot 10^2$	$122.1 \cdot 10^2$	187.9	65.0	5.62	0.684

Calculations showed that the AF values ranged $0.02 \cdot 10^4$ to $180.00 \cdot 10^4$ (column 2 in Table 2). The total pool of TE in water (P_{d+pm}) varied from $5.3 \cdot 10^2$ to $4{,}944.9 \cdot 10^2$ $\mu g \cdot m^{-2}$ (column 3); out of it, TE content in SPM was 0.2–55.9 % (column 6 in Table 1). The flux of self-purification of water *via* sedimentation ($V_{sed/day}$) ranged within 7.30–2,788.97 $\mu g \cdot m^{-2} \cdot day^{-1}$ (column 5 in Table 2). The TE turnover in the marine environment due to their concentration and elimination through the sorption and sedimentation processes mostly had a time scale from days to months or seasons. The only TE with the turnover on an annual time scale was Mo (column 6). Based on these data, we obtained the following sequence in ascending order for T_{pt} (days): As $<$ Co $<$ Pb $<$ Cd $<$ Cu $<$ Ni $<$ Zn $<$ Mo. Arsenic is the TE with the shortest SPM cycle (within 15 days); Co, Pb, Cd, and Cu are deposited with SPM within 1.5–2.5 months; a complete turnover of Ni and Zn in the cycle takes 3–6 months; and molybdenum has the slowest cycle (more than 14 years).

In column 7 of Table 2, we provide the estimates for the time constants of sedimentation exchange of TE content pools in the water layer 0–22 m. They showed: as a result of biogeochemical processes, TE content in this layer can be replaced 0.07–23.10 times a year. Taking into account this circumstance, a solution can be provided to the problem of ecological regulation of anthropogenic load on marine water areas, including regulation of the maximum permissible fluxes of TE entering waters of recreational zones. According to the results of calculations given in column 8 of Table 2, with the chemical composition of waters remaining at a stationary level in February 2020, the rate of sedimentation and deposition of various TE in deep layers of bottom sediments in each hectare of the studied farm water area ranged 0.027 to 10.180 kg·year^{-1}.

DISCUSSION

On SPM composition. Comparison of the results obtained in this work with literature material showed that our data on SPM are consistent with previously published information. Specifically, according to (Ryabushko et al., 2014), SPM content in the study area throughout the year varied 0.3 to 1.1 mg·L^{-1}; in our work, the value was 0.72 mg·L^{-1}.

Plankton is known to play an important role in the formation of autochthonous SPM in the surface layer of the Black Sea. Literature data on the phytoplankton ratio in SPM composition are extremely scarce. Researchers tend to show the ratio of mineral and organic components of SPM. For the Black Sea, the level of organic compounds in SPM ranges from 14 to 88 % (Vityuk, 1983). The ratio of detritus in SPM is significant as well. In the open Black Sea, at the outer roadstead of Sevastopol, SPM mainly consisted of autochthonous suspension, depending on the plankton composition (Gulin et al., 2013 ; Kukushkin & Parkhomenko, 2021); this indicates a notable role of biotic processes in the SPM transport into this area. In phytoplankton during our study, diatoms prevailed (*Pseudo-nitzschia* spp. and *Skeletonema costatum* (Greville) Cleve, 1873), as well as a coccolithophorid *Emiliania huxleyi* (Lohmann) W. W. Hay & H. Mohler, 1967. Wet biomass of phytoplankton in February 2020 in the marine farm area was about 0.1 mg·L^{-1} (Pospelova & Priimak, 2021). Assuming that phytoplankton DW averages 10 % of its wet biomass (Studenikina et al., 1999), we can conclude that dry biomass of microalgae in winter was 0.01 mg·L^{-1}, or 1.4 % of SPM mass. Apparently, the contribution of phytoplankton to SPM during this study was insignificant. However, it should be taken into account that small-celled species of microalgae prevailed. Those form a large surface area; therefore, they can play a key role in TE concentration from water.

In marine farm areas, cultivated molluscs can contribute to the formation of the chemical composition of seawater and SPM – due to peculiarities of the sorption and trophodynamic processes in them. Molluscs can release both dissolved and suspended organic and mineral substances into the farm water area (Lomakin et al., 2007 ; Pospelova et al., 2018).

An allochthonous contribution to SPM composition in the upper layer of the studied water area can be made by the water runoff of the Chernaya River (through the Sevastopol Bay), two emergency episodically operating wastewater outlets in the immediate vicinity of the farm, and wastewater from storm sewers entering directly the farm water area, *inter alia* from the Karantinnaya Bay (Ivanov et al., 2002 ; Nemirovsky & Eremin, 2003).

Content of dissolved and suspended forms of trace elements in water. According to different researchers, the values of concentrations of the studied TE for the Black Sea vary widely (Table 3). Comparison of our results with those available in literature showed as follows: the values of total concentration of Ni, Cu, Zn, Cd, and Pb in the marine farm area are within the ranges of variation of clarkes of these TE in the Black Sea, whereas content of Co and As in this work is lower than mean values for the Black Sea (Table 3). Concentrations of all TE, except for Zn, in SPM are comparable to the average data for the Black Sea.

Table 3. Variability ranges of trace element concentrations in the Black Sea, trace element content in suspended particulate matter of the Chernaya River, and clarkes in the Earth's crust

TE	TE concentration in the Black Sea			Clarkes in the Earth's crust, $\mu g \cdot g^{-1}$ (Kasimov & Vlasov, 2015)	TE concentration in SPM at the Chernaya River mouth, $\mu g \cdot g^{-1}$ (Malakhova et al., 2020)
	Total concentration, $\mu g \cdot L^{-1}$	Dissolved form, $\mu g \cdot L^{-1}$	Suspended form, $\mu g \cdot L^{-1}$ (% of total)		
Co	0.6–3.1[4)]	nd	$0.60 \cdot 10^{-3}$…0.10[1)]	15	14
Ni	0.4–23[4)]	0.47–0.70 (max 8.334)[2)]	0.005…0.26[1)] (0.01)[5)]	50	76
Cu	< 0.01–33[3)]	0.06–0.51 (max 7.75)[2)]	0.006…0.29[1)] (0.03)[5)]	27	877
Zn	0.42–108[3)]	max 54.53[2)]	0.001…0.91[1)] (0.25)[5)]	75	1,273
As	0.3–2.5[4)]	0.83–1.3[4)]	nd	5.6	14
Mo	0.67–3.74[6)]	nd	$0.3 \cdot 10^{-3}$…0.07[1)]	1.1	1
Cd	< 0.01–0.45[3)]	max 1.69[2)]	0.001…3.60[1)]	0.09	5
Pb	< 0.01–2.5[3)]	0.02–0.04 (max 8.08)[2)]	0.005…0.35[1)] (0.015)[5)]	17	196

Note: [1)]Yiğiterhan et al., 2011; [2)]Boran & Altinok, 2010; [3)]Sevost'yanova et al., 2016; [4)]Mitropolsky et al., 1982; [5)]Patin и Morozov, 1981; [6)]Nägler et al., 2011; nd denotes no data.

The ratio of suspended form of Ni, Cu, Zn, and Pb in the studied water area is higher than the average for the Black Sea. Importantly, the literature data used by us for comparison refer to the 1970–1980s, and the disagreement between the numbers is likely to result from both the difference in the techniques for determining SPM concentration and temporal changes in SPM composition.

Specifically, Cu, Zn, and As are geochemically mobile elements. Cu and Co are largely assimilated by the biota, while As is often associated with SPM. Ni is mostly in the dissolved phase in the marine environment. Pb is characterized by high activity of interaction with living matter (Mitropolsky et al., 2006). Mo is less biologically available in seawater than in freshwater (Howarth et al., 1988), and there is usually 10,000 times more dissolved Mo in seawater than in SPM (Dellwig et al., 2007 ; Howarth & Cole, 1985).

As known, there are no regulations for TE content in SPM of seawater; therefore, for a comparative assessment of the degree of environmental pollution, we used clarkes of chemical elements in the upper continental crust (Kasimov & Vlasov, 2015) (Table 3). The comparison of the obtained results showed that cadmium content in SPM of the farm was 44 times higher than mean clarkes of this element in the Earth's crust; zinc, 20 times; copper, 13 times; and lead, 6 times (Table 3). Cobalt content was 2 times lower. Apparently, this is due to the effect of both natural and anthropogenic factors. Natural factors include TE supply from bottom sediments, as well as with the waste products of cultivated molluscs and planktonic organisms. Anthropogenic factors will be considered in more detail.

An increase in the concentration of pollutants in the surface waters of the analyzed area is largely due to the effect of the adjacent polluted bays – the Karantinnaya Bay (Ryabushko et al., 2017) and Sevastopol Bay (Orekhova & Varenik, 2018 ; Sovga & Mezentseva, 2019). The Sevastopol Bay – a large semi-enclosed basin of the estuarine type – experiences certain anthropogenic pressure. In turn, its ecological state is affected by coastal sources of pollution, active navigation, and the Chernaya River runoff. At the same time, in the Sevastopol Bay, currents and winds develop which carry pollutants off the bay and contaminate the marine farm area (Kapranov et al., 2020). During our sampling, northeast winds prevailed in the Sevastopol region (https://weatherarchive.ru/Sevastopol/19-February-2020), which may evidence for the effect of the bay water on the marine farm area. In parallel with the data of our research, in February 2020, results were obtained on TE content at the Chernaya River mouth (Table 3) (Malakhova et al., 2020). Significant amounts of Cu, Zn, and Pb entered the Sevastopol Bay with SPM from the river. Concentrations of Co, Cu, and Pb in SPM of the river were higher than in SPM of the marine farm area; content of Ni, Zn, and Cd was comparable; and As and Mo were concentrated in SPM of the farm with a greater intensity than in SPM of the river. As known, in the mixing zone of river water and seawater, a geochemical barrier is formed. There, due to hydrological, physicochemical, and biological processes, more than 90 % of SPM of the river runoff is removed from water, and up to 90 % of suspended forms of Mn, Co, Ni, Cu, Zn, As, Cd, and Pb are eliminated from further migration, as well as 10–60 % of dissolved forms (Demina, 2015). So, the Chernaya River water could not make a significant contribution to TE content in the marine farm area.

Usually, in coastal zones, TE of aerosols (dissolved and suspended) are involved in biogeochemical cycles (Duce et al., 1991). The atmospheric component plays a notable role in the entry of pollutants, *inter alia* TE, into the Black Sea. Up to 83 % of Ni, Cu, Zn, Cd, and Pb fluxes into the Black Sea are provided by atmospheric fallout (Gubanov et al., 2004 ; Theodosi et al., 2013). The atmospheric contribution of Ni, Cu, and Pb to the marine environment of the Sevastopol region is comparable to the inflow of river runoff and sometimes even exceeds it (Ovsyanyi et al., 2001). Presumably, high content of suspended forms of Pb (predominantly of technogenic origin) is associated with the formation of aerosols with a high content of lead because of pollution from automobiles and from coal combustion; also, it is associated with industrial effluents. Arsenic is the substance of the first hazard class and is included in the group of chemical elements that must be detected during environmental monitoring, especially in marine farm areas. The results of our work showed that more than 50 % of As is present in water in suspended form. Similar data are published for the Chernaya River water (Malakhova et al., 2020). However, total concentrations of As and Pb in water did not exceed MPC (Table 1). The only heavy metal with content in water of 1.98 MPC was Mo. It is the most abundant transition metal in open sea water – due to the prevalence and low chemical reactivity of the molybdate ion (MoO_4^{2-}). Rivers supply oceans with most of molybdenum, mainly due to the weathering of continental material (Smedley & Kinniburgh, 2017).

Fluxes of trace elements through SPM. We assessed (Table 2) the dynamic parameters of the TE absorption by SPM by calculating the duration of biogeochemical cycles of these TE. Even with a lower ratio of metals in SPM, compared with that in the dissolved phase, SPM significantly (from several days to several years) accelerates the TE turnover.

The AF values obtained by us (Table 1) testify to high ability of SPM to concentrate TE. The ratio of metals in suspension (0.2–55.9 % of their total content in the aquatic environment) was lower than in the Sea of Azov (29–95.6 %) (Bufetova, 2022). It can be explained by SPM content in water: in the Black Sea, it is an order of magnitude lower than in the Sea of Azov. An increase in the ratio of metals in SPM with a rise in SPM content was also shown for the Sea of Japan coastal areas (Shulkin, 2007).

TE pool in SPM in the marine farm area ranged from 70 µg to 24 mg *per* m^2 of the water column; the values were minimum for Mo (70 µg) and Co (100 µg) and maximum for Zn (24 mg) and Pb (12 mg). The pool values made it possible to calculate the flux of self-purification of water from TE *via* sedimentation. Similar data on vertical fluxes of most of studied TE were obtained for the southeastern Black Sea (Ergül et al., 2008) and for the Caucasus coast (Denisov & Latun, 2018). The metal turnovers in the Sea of Azov are reported in (Matishov et al., 2017). There, the researchers showed the patterns of heavy metal concentration by bottom sediments; based on those, self-purification of water *via* sedimentation was characterized. In this work, the sedimentation turnover for a heavy metal in the aquatic environment (T_{pt}, years) was shown to reflect the time scale of self-purification of water *via* sedimentation. Different duration of biogeochemical cycles for various TE can be explained by the differences in their concentrations in solution and ratios of bioavailable forms (Demina, 2011). The calculation techniques proposed by us showed as follows: in the marine farm area, only due to sedimentation processes, water is purified from significant part of toxic elements during the day, and this undoubtedly makes a notable contribution to the purification of the coastal water area. Taking into account the fact that the marine farm for cultivating filter-feeding molluscs is located there, SPM can be considered as an additional factor for ensuring the safety of aquaculture products.

Our calculation of the sedimentation turnover for TE in the aquatic environment (T_{pt}, days) (Table 2) indicates the time scales of the processes of self-purification of the marine farm area *via* sedimentation, as well as the estimates for the time constants of sedimentation exchange of TE content pools in the entire water layer of the studied area. This will help in solving the problem of ecological regulation of anthropogenic pressure on marine water areas, *inter alia* regulation of the maximum permissible fluxes of TE which enter waters of recreational zones. Along with the known data on significant contribution of marine farms for cultivating bivalves to self-purification of coastal waters, another key factor should be taken into account – the participation of SPM in these processes.

Conclusion. The article presents assessment of the role of suspended particulate matter in marine farm areas as a biogeochemical barrier that contributes to the process of self-purification of the marine area from trace elements. Based on the obtained data on TE content in seawater and SPM in the marine farm area, the fluxes of their sedimentation turnover were determined; moreover, the method was proposed for assessing the process of self-purification of water from heavy metals (Co, Ni, Cu, Zn, Mo, Cd, and Pb) and arsenic (As) taking into account sanitary and hygienic standards. From a practical point of view, the results obtained can be used in the development of criteria for regulating the maximum permissible fluxes of water pollution into other water areas where marine farms are located.

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REFERENCES

1. Bufetova M. V. Assessment of the ability of suspended matter in the Sea of Azov to concentrate heavy metals. *Ecological Safety of Coastal and Shelf Zones of Sea*, 2022, no. 1, pp. 55–65. (in Russ.). https://doi.org/10.22449/2413-5577-2022-1-55-65
2. Vityuk D. M. *Vzveshennoe veshchestvo i ego biogennye komponenty.* Kyiv : Naukova dumka, 1983, 212 p. (in Russ.)
3. Gubanov V. I., Mal'chenko Yu. A., Kuftarkova E. A., Kovrigina N. P. Diagnosis of modern state of coastal water Sevastopol (the Black Sea) according to monitoring of chemical parameters. *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon i kompleksnoe ispol'zovanie resursov shel'fa*, 2004, iss. 10, pp. 141–148. (in Russ.)
4. Gulin S. B., Sidorov I. G., Gulina L. V. Biogenic sedimentation in the Black Sea: Radiotracer-derived study. *Morskoj ekologicheskij zhurnal*, 2013, vol. 12, no. 2, pp. 19–25. (in Russ.)
5. Demina L. L. Quantification of the role of organisms in the geochemical migration of trace metals in the ocean. *Geochemistry International*, 2015, vol. 53, no. 3, pp. 224–240. (in Russ.). https://doi.org/10.7868/S0016752515030048
6. Denisov V. I., Latun V. V. Flows of chemical elements in suspended matter fluxes in the shallow area of the Black Sea shelf (according to the sediment traps data). *Izvestiya vuzov. Severo-Kavkazskii region. Natural science*, 2018, no. 4 (200), pp. 77–85. (in Russ.). https://doi.org/10.23683/0321-3005-2018-4-77-85
7. Egorov V. N. *Theory of Radioisotope and Chemical Homeostasis of Marine Ecosystems.* Sevastopol : FITs InBYuM, 2019, 356 p. (in Russ.). https://doi.org/10.21072/978-5-6042938-5-0
8. Zolotov Yu. A., Kuzmin N. M. *Ekstraktsionnoe kontsentrirovanie.* Moscow : Khimiya, 1971, 272 p. (in Russ.)
9. Ivanov V. N., Subbotin A. A., Gubanov V. I., Kuftarkova E. A., Nemirovsky M. S., Parkhomenko A. V. Hydrochemical regime of Sevastopol coastal waters and its prospects for economical using. *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon i kompleksnoe ispol'zovanie resursov shel'fa*, 2002, no. 2 (7), pp. 134–143. (in Russ.)
10. Kasimov N. S., Vlasov D. V. Clarkes of chemical elements as comparison standards in ecogeochemistry. *Vestnik Moskovskogo universiteta. Seriya 5: Geografiya*, 2015, no. 2, pp. 7–17. (in Russ.)
11. Kuftarkova E. A., Gubanov V. I., Kovrigina N. P., Eremin I. Yu., Senicheva M. I. Ecological assessment of modern state of waters in the region of interaction of the Sevastopol Bay and part of the sea contiguous to it. *Morskoj ekologicheskij zhurnal*, 2006, vol. 5, no. 1, pp. 72–91. (in Russ.)
12. Lomakin P. D., Chepyzhenko A. I., Chepyzhenko A. A. Estimation to concentrations of total suspension and dissolved organic

matter of the artificial origin in the bays of the Crimea according to optical measurements. *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon i kompleksnoe ispol'zovanie resursov shel'fa*, 2007, no. 15, pp. 168–176. (in Russ.)

13. Malakhova L. V., Proskurnin V. Yu., Egorov V. N., Chuzhikova-Proskurnina O. D., Bobko N. I. Trace elements in the Chernaya River water and evaluation of their income with the riverine inflow into the Sevastopol Bay in winter 2020. *Ecological Safety of Coastal and Shelf Zones of Sea*, 2020, no. 3, pp. 77–94. (in Russ.). https://doi.org/10.22449/2413-5577-2020-3-77-94
14. Matishov G. G., Bufetova M. V., Egorov V. N. The regulation of flows of heavy metals into the Sea of Azov according to the intensity of sedimentation of water self-purification. *Nauka Yuga Rossii [Science in the South Russia]*, 2017, vol. 13, no. 1, pp. 44–58. (in Russ.). https://doi.org/10.23885/2500-0640-2017-13-1-44-58
15. Mitropolsky A. Yu., Bezborodov A. A., Ovsyanyi E. I. *Geokhimiya Chernogo morya.* Kyiv : Naukova dumka, 1982, 114 p. (in Russ.)
16. Mitropolsky O. Yu., Nasedkin E. I., Osokina N. P. *Ekoheokhimiia Chornoho moria.* Kyiv : Akademperiodika, 2006, 279 p. (in Ukr.)
17. Nemirovsky M. S., Eremin I. Yu. Water dynamics near Sevastopol city. *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon i kompleksnoe ispol'zovanie resursov shel'fa*, 2003, no. 9, pp. 59–66. (in Russ.)
18. *Normativy kachestva vody vodnykh ob"ektov rybokhozyaistvennogo znacheniya, v tom chisle normativy predel'no dopustimykh kontsentratsii vrednykh veshchestv v vodakh vodnykh ob"ektov rybokhozyaistvennogo znacheniya.* Prikaz Ministerstva sel'skogo khozyaistva RF ot 13.12.2016 no. 552. (in Russ.)
19. Ovsyanyi E. I., Romanov A. S., Minkovskaya R. Ya., Krasnovid I. I., Ozyumenko B. A., Tsymbal I. M. Basic polluting sources of sea near Sevastopol. *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon i kompleksnoe ispol'zovanie resursov shel'fa*, 2001, no. 2, pp. 138–152. (in Russ.)
20. Patin S. A., Morozov N. P. *Mikroelementy v morskikh organizmakh i ekosistemakh.* Moscow : Pishchevaya promyshlennost', 1981, 153 p. (in Russ.)
21. Pospelova N. V., Egorov V. N., Chelyadina N. S., Nekhoroshev M. V. The copper content in the organs and tissues of *Mytilus galloprovincialis* Lamarck, 1819 and the flow of its sedimentary deposition into bottom sediments in the farms of the Black Sea aquaculture. *Morskoj biologicheskij zhurnal*, 2018, vol. 3, no. 4, pp. 64–75. (in Russ.). https://doi.org/10.21072/mbj.2018.03.4.07
22. Pospelova N. V., Priimak A. S. The feeding of *Mytilus galloprovincialis* Lam. cultivating in coastal waters of Sevastopol. *Proceedings of the T. I. Vyazemsky Karadag Scientific Station – Nature Reserve of the Russian Academy of Sciences*, 2021, vol. 6, no. 1 (17), pp. 24–34. (in Russ.). https://doi.org/10.21072/eco.2021.17.03
23. Ryabushko L. I., Balycheva D. S., Popovichev V. N., Firsov Yu. K., Ryabushko V. I. The productive characteristics of experimental glass plates phytoperiphyton and phytoplankton in the Karantinnaya Bay (Crimean coastal waters of the Black Sea). *Algologiya*, 2014, vol. 24, no. 4, pp. 504–517. (in Russ.)
24. Sevost'yanova M. V., Pavlenko L. F., Korablina I. V. Current level of the Black Sea pollution by oil and heavy metals. In: *Morskie biologicheskie issledovaniya: dostizheniya i perspektivy* : v 3 tomakh : sb. materialov Vseros. nauch.-prakt. konf. s mezhdunar. uchastiem,

priuroch. k 145-letiyu Sevastopol'skoi biologicheskoi stantsii, Sevastopol, 19–24 Sept., 2016. Sevastopol, 2016, vol. 3, pp. 210–213. (in Russ.)

25. Sovga E. E., Mezentseva I. V. Ecological condition of the central part of Sevastopol Bay depending on the anthropogenic load level. *Ecological Safety of Coastal and Shelf Zones of Sea*, 2019, no. 3, pp. 52–60. (in Russ.). https://doi.org/10.22449/2413-5577-2019-3-52-60
26. Stetsiuk A. P. Mercury accumulation in suspended matter of foam and water of the Black Sea. *Morskoj biologicheskij zhurnal*, 2020, vol. 5, no. 3, pp. 74–84. (in Russ.). https://doi.org/10.21072/mbj.2020.05.3.07
27. Studenikina E. I., Aldakimova A. Ya., Gubin G. S. *Fitoplankton Azovskogo morya v usloviyakh antropogennykh vozdeistvii.* Rostov-on-Don : Everest, 1999, 175 p. (in Russ.)
28. Shulkin V. M. *Tyazhelye metally v rechnykh i pribrezhno-morskikh ekosistemakh* : avtoref. dis. … d-ra geogr. nauk : 25.00.36. Vladivostok, 2007, 37 p. (in Russ.)
29. Boran M., Altinok I. A review of heavy metals in water, sediment and living organisms in the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, 2010, vol. 10, no. 4, pp. 565–572. https://doi.org/10.4194/trjfas.2010.0418
30. Dellwig O., Beck M., Lemke A., Lunau M., Kolditz K., Schnetger B., Brumsack H.-J. Non-conservative behaviour of molybdenum in coastal waters: Coupling geochemical, biological, and sedimentological processes. *Geochimica et Cosmochimica Acta*, 2007, vol. 71, iss. 11, pp. 2745–2761. https://doi.org/10.1016/j.gca.2007.03.014
31. Demina L. L. Estimation of the role of global biological filters in the geochemical migration of trace elements in the ocean: The marginal filter of the ocean. *Doklady Earth Sciences*, 2011, vol. 439, pt. 1, pp. 981–985. https://doi.org/10.1134/S1028334X11070038
32. Duce R. A., Liss P. S., Merrill J. T., Atlas E. L., Buat-Menard P., Hicks B. B., Miller J. M., Prospero J. M., Arimoto R., Church T. M., Ellis W., Galloway J. N., Hansen L., Jickells T. D., Knap A. H., Reinhardt K. H., Schneider B., Soudine A., Tokos J. J., Tsunogai S., Wollast R., Zhou M. The atmospheric input of trace species to the World Ocean. *Global Biogeochemical Cycles*, 1991, vol. 5, iss. 3, pp. 193–259. https://doi.org/10.1029/91GB01778
33. Ergül H. A., Topcuoğlu S., Ölmez E., Kırbaşoğlu Ç. Heavy metals in sinking particles and bottom sediments from the eastern Turkish coast of the Black Sea. *Estuarine, Coastal and Shelf Science*, 2008, vol. 78, iss. 2, pp. 396–402. https://doi.org/10.1016/j.ecss.2008.01.006
34. Howarth R. W., Marino R., Lane J., Cole J. J. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. *Limnology and Oceanography*, 1988, vol. 33, iss. 4, pt. 2, pp. 688–701. https://doi.org/10.4319/lo.1988.33.4part2.0688
35. Howarth R. W., Cole J. J. Molybdenum availability, nitrogen limitation, and phytoplankton growth in natural waters. *Science*, 1985, vol. 229, iss. 4714, pp. 653–655. https://doi.org/10.1126/science.229.4714.653
36. Kapranov S. V., Kovrigina N. P., Troshchenko O. A., Rodionova N. Yu. Long-term variations of thermohaline and hydrochemical characteristics in the mussel farm area in the coastal waters off Sevastopol (Black Sea) in 2001–2018. *Continental Shelf Research*, 2020, vol. 206, art. no. 104185 (16 p.). https://doi.org/10.1016/j.csr.2020.104185

37. Kukushkin A. S., Parkhomenko A. V. Spatiotemporal variability of suspended particulate matter in the surface layer of the open part of the Black Sea. *Oceanology*, 2021, vol. 61, no. 2, pp. 272–282. https://doi.org/10.1134/S0001437021010124
38. Nägler T. F., Neubert N., Böttcher M. E., Dellwig O., Schnetger B. Molybdenum isotope fractionation in pelagic euxinia: Evidence from the modern Black and Baltic seas. *Chemical Geology*, 2011, vol. 289, iss. 1–2, pp. 1–11. https://doi.org/10.1016/j.chemgeo.2011.07.001
39. Orekhova N. A., Varenik A. V. Current hydrochemical regime of the Sevastopol Bay. *Physical Oceanography*, 2018, vol. 25, no. 2, pp. 124–135. https://doi.org/10.22449/1573-160X-2018-2-124-135
40. Smedley P. L., Kinniburgh D. G. Molybdenum in natural waters: A review of occurrence, distributions and controls. *Applied Geochemistry*, 2017, vol. 84, pp. 387–432. https://doi.org/10.1016/j.apgeochem.2017.05.008
41. Ryabushko V. I., Prazukin A. V., Gureeva E. V., Bobko N. I., Kovrigina N. P., Nekhoroshev M. V. Fucoxanthin and heavy metals in brown algae of genus *Cystoseira* C. Agardh from water areas with different anthropogenic influences (Black Sea). *Morskoj biologicheskij zhurnal*, 2017, vol. 2, no. 2, pp. 70–79. https://doi.org/10.21072/mbj.2017.02.2.07
42. Salomons W., Förstner U. *Metals in the Hydrocycle.* Berlin ; Heidelberg ; New York ; Tokyo : Springer-Verlag, 1984, 352 p. https://doi.org/10.1007/978-3-642-69325-0
43. Theodosi C., Stavrakakis S., Koulaki F., Stavrakaki I., Moncheva S., Papathanasiou E., Sanchez-Vidal A., Koçak M., Mihalopoulos N. The significance of atmospheric inputs of major and trace metals to the Black Sea. *Journal of Marine Systems*, 2013, vols 109–110, pp. 94–102. https://doi.org/10.1016/j.jmarsys.2012.02.016
44. Turner A., Millward G. E. Suspended particles: Their role in estuarine biogeochemical cycles. *Estuarine, Coastal and Shelf Science*, 2002, vol. 55, iss. 6, pp. 857–883. https://doi.org/10.1006/ecss.2002.1033
45. Yiğiterhan O., Murray J. W., Tuğrul S. Trace metal composition of suspended particulate matter in the water column of the Black Sea. *Marine Chemistry*, 2011, vol. 126, iss. 1–4, pp. 207–228. http://dx.doi.org/10.1016/j.marchem.2011.05.006

ВЗВЕШЕННОЕ ВЕЩЕСТВО КАК БИОГЕОХИМИЧЕСКИЙ БАРЬЕР ДЛЯ ТЯЖЁЛЫХ МЕТАЛЛОВ В РАЙОНАХ РАЗМЕЩЕНИЯ МОРСКИХ ФЕРМ (СЕВАСТОПОЛЬ, ЧЁРНОЕ МОРЕ)

Н. В. Поспелова, В. Н. Егоров, В. Ю. Проскурнин, А. С. Приймак

ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН»,
Севастополь, Российская Федерация
E-mail: *nvpospelova@mail.ru*

Исследования потоков химических веществ в прибрежных морских акваториях приобретают особенную актуальность в рекреационных районах и в местах размещения морских ферм. Для изучения роли взвешенного вещества в формировании биогеохимических барьеров в районах размещения морских ферм предложен метод оценки потоков седимен тационного самоочищения вод от тяжёлых металлов (Co, Ni, Cu, Zn, Mo, Cd и Pb) и мышьяка (As). На основе литературных данных по радиоизотопным датировкам донных отложений и скорости осадконакопления, а также на базе собственных оценок удельной массы взвешенного вещества в воде, концентраций растворённой и взвешенной форм тяжёлых металлов и мышьяка в морской среде определены потоки биогеохимического самоочищения акватории размещения морских ферм от тяжёлых металлов и мышьяка. Показано, что предложенная методическая база применима для экологического нормирования прибрежных рекреационных зон по фактору загрязнения тяжёлыми металлами и мышьяком.

Ключевые слова: Чёрное море, загрязнение, микроэлементы, самоочищение, экологическая регуляция, взвешенное вещество, аквакультура

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DETERMINATION OF CONTROL LEVELS OF RADIONUCLIDES ENSURING ACCEPTABLE ENVIRONMENTAL RISK IN THE BARENTS SEA WATER AND BOTTOM SEDIMENTS

© 2022 **N. A. Rosnovskaya, A. I. Kryshev, and I. I. Kryshev**

Research and Production Association "Typhoon", Obninsk, Russian Federation
E-mail: *rosnovskaya@rpatyphoon.ru*

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To assess the radioecological situation, criteria were developed ensuring acceptable environmental risk – control levels of radionuclides in the components of the natural ecosystem. The method was applied to evaluate the control levels of technogenic radionuclides in the Barents Sea water and bottom sediments. If these levels are not exceeded, marine biota is considered protected from radioactive contamination. Local concentration factors of ^{137}Cs, ^{90}Sr, and ^{239}Pu in the Barents Sea biota were estimated using the data of long-term observations. Moreover, the reference organisms were selected – a fish (cod), mollusc (mussel), aquatic plant (fucus), and marine mammal (harp seal). The values of ^{137}Cs, ^{239}Pu, and ^{90}Sr concentration factors were, respectively, as follows (L·kg^{-1}): in fish, 93, 262, and 12; in molluscs, 51, 1,180, and 21; in aquatic plants, 69, 732, and 19; and in marine mammals, 63, 222, and 14. The values of the water–sediment distribution coefficients of ^{137}Cs, ^{239}Pu, and ^{90}Sr were 426, 189,600, and 443 L·kg^{-1}, respectively. For most radionuclides and the reference organisms from the Barents Sea, the values of radionuclide concentration differ from the global average reference values. For the period of 1992–2020, there is no pronounced temporal trend for the concentration factors of all technogenic radionuclides in the Barents Sea fish; this indicates the establishment of equilibrium in the distribution of radioactivity between the components of the Arctic marine ecosystem. The control levels of radionuclides were as follows: in the Barents Sea water (Bq·L^{-1}), 115 for ^{137}Cs, 439 for ^{90}Sr, and 0.124 for ^{239,240}Pu; in the Barents Sea bottom sediments (kBq·kg^{-1} fresh weight), 48.9 for ^{137}Cs, 194 for ^{90}Sr, and 23.6 for ^{239,240}Pu. The contamination index for both water and bottom sediments of the Barents Sea was calculated using the monitoring data and reference levels. In 2006–2020, its values were several orders of magnitude lower than 1 and did not tend to increase or decrease. In terms of marine biota protection, the main contributor to the contamination index for the Barents Sea water is ^{239,240}Pu (up to 75 %) while the main contributor to the contamination index for the Barents Sea bottom sediments is ^{137}Cs (up to 90 %). To date, the ratio of the contributions of technogenic radionuclides to the contamination index for the Barents Sea water and bottom sediments is stable.

Keywords: Arctic, Barents Sea, water, bottom sediments, biota, control level, concentration factor, distribution coefficient, radionuclide

A correct interpretation of monitoring data requires their comparison with the criteria for assessing the radiation situation which ensure acceptable environmental risk. As such criteria, the control levels of radionuclides in the components of the natural ecosystem can be used since the measurement results can be directly compared with them. For assessing radiation and environmental impact on the ecosystem objects, recommendations were developed; those were based on the radiation situation monitoring (Otsenka radiatsionno-ekologicheskogo vozdeistviya, 2015) and calculation of control levels of radionuclides in seawater (Poryadok rascheta, 2016). The control levels defined in the recommendations (Poryadok rascheta, 2016) are based on the use of generalized values of concentration factors of radionuclides in biota, water–sediment distribution coefficients (Sediment Distribution Coefficients, 2004), and a standard selection of reference organisms (ICRP Publication 108, 2008); these control levels do not take into account regional specifics.

The values of the concentration factors of radionuclides in the components of the Arctic marine ecosystem may differ significantly from the global average values. The Arctic region is characterized by a harsh climate with low temperatures. In cold waters of the Arctic, the accumulation and excretion of radionuclides in hydrobionts occur slower than in water bodies of a temperate or warm climate. Moreover, a set of reference organisms during radioecological assessments for the Arctic seas differs from the standard one (Kryshev et al., 2022 ; Sazykina & Kryshev, 2011).

The presence of technogenic radionuclides in the Barents Sea is mainly due to the following factors: atmospheric fallout after nuclear weapon tests in the second half of the XX century; transport by currents of radioactive discharges from enterprises of Great Britain and France; presence in the Arctic seas of sunken or submerged nuclear and radiation hazardous facilities; and influx of radionuclides after the accident at the Chernobyl nuclear power plant in 1986 (Sarkisov et al., 2015 ; Sivintsev et al., 2005). Expeditionary surveys of the Barents Sea showed the presence in seawater of such long-lived technogenic radionuclides as ^{137}Cs, ^{90}Sr, and ^{239}Pu (Gwynn et al., 2016 ; Jensen et al., 2016).

The objective of the study is to determine the control levels of the technogenic radionuclide content in the Barents Sea water and bottom sediments, the non-exceeding of which ensures the radiation safety of marine biota. To do this, local concentration factors of technogenic radionuclides in biota and water–sediment distribution coefficients for the Barents Sea were estimated using long-term observational data. Moreover, reference species of the Barents Sea ecosystem were selected, as well as threshold values of the radiation dose rate below which no deterministic radiobiological effects for marine biota occur.

MATERIAL AND METHODS

The control level of the *i*-th radionuclide in seawater was calculated by the formula:

$$C_i = \frac{P}{\varepsilon_{a,i} \cdot \alpha_i \cdot CF_i + \varepsilon_{e,i} \cdot (\tau_w + 0.5 \cdot K_{d,i} \cdot \tau_s)}\,, \qquad (1)$$

where P is the threshold value of the dose rate above which deterministic radiobiological effects for a reference organism can occur, mGy·day^{-1};

$\varepsilon_{a,i}$ is the dose coefficient of internal exposure of the organism from the accumulated *i*-th radionuclide, (mGy·day^{-1})/(Bq·kg^{-1} fresh weight);

α_i is the quality factor associated with the relative biological effectiveness of radiation, dimensionless;

CF_i is the concentration factor of the *i*-th radionuclide in a reference organism, L·kg^{-1};

$\varepsilon_{e,i}$ is the dose coefficient of external exposure of the organism from seawater and bottom sediments, (mGy·day^{-1})/(Bq·kg^{-1} fresh weight);

$K_{d,i}$ is the water–sediment distribution coefficient for the *i*-th radionuclide, L·kg^{-1} fresh weight;

τ_w and τ_s are the fractions of time that a marine biota representative spends in a water column and near bottom, respectively, dimensionless.

The control level of the *i*-th radionuclide in marine sediments was calculated by the formula:

$$S_i = \frac{P}{\varepsilon_{a,i} \cdot \alpha_i \cdot CF_{s,i} + 0.5 \cdot \varepsilon_{e,i} \cdot \tau_s}\,, \tag{2}$$

where $CF_{s,i}$ is the ratio of the specific activity of the *i*-th radionuclide in a marine organism to its specific activity in bottom sediments, $kg \cdot kg^{-1}$ fresh weight.

The following criteria are used to select reference objects of the natural ecosystem: ecological significance of the object, accessibility for radioecological monitoring, exposure dose, radiosensitivity, and self-healing ability (Kryshev & Sazykina, 2012). When determining reference organisms of the Arctic seas, the availability of monitoring data is of particular importance since those allow estimating local values of the concentration factors of technogenic radionuclides in marine biota. Based on this, the following organisms were selected as reference ones for the Barents Sea: fish, cod *Gadus morhua* Linnaeus, 1758; bivalve, mussel *Mytilus edulis* Linnaeus, 1758; aquatic plant, *Fucus distichus* Linnaeus, 1767; and marine mammal, harp seal *Pagophilus groenlandicus* (Erxleben, 1777).

To determine the concentration factors of radionuclides in reference organisms of the Barents Sea and water–sediment distribution coefficients, a database was created on the content of radionuclides in its ecosystem components. The total error in determining the specific activity of radionuclides for the Barents Sea components is no more than 25 %. Most of the data were obtained as a result of expeditionary surveys under the Russian–Norwegian monitoring program for the Barents Sea in 2006–2020 (Gwynn et al., 2016 ; Jensen et al., 2016). Data for an earlier period (since 1992) were taken from the literature (Brown et al., 2004 ; Kryshev et al., 2002).

The database includes 107 values of specific activities for ^{137}Cs, 45 values for $^{239,240}Pu$, and 65 values for ^{90}Sr. The data on the radionuclide content in water, bottom sediments, and biota, obtained in one year and at one spot in the Barents Sea, were used to determine the concentration factors and distribution coefficients. Fig. 1 shows a map of the study area during the Russian–Norwegian monitoring and location of reference sampling sites.

To calculate the resulting local values of the concentration factors and distribution coefficients, statistical analysis was carried out – checking the normality of the distribution using the Shapiro–Wilk test (Kobzar, 2006). In the absence of a normal distribution, nonparametric statistics was applied (Kobzar, 2006).

The values of dose coefficients were determined using BiotaDC v.1.5.1 software tool (http://biotadc.icrp.org/) – a complement to (ICRP Publication 136, 2017). Marine organisms were approximated by ellipsoids with the following mass and ratios between the axes: cod, 2 kg, 1 / 0.2 / 0.2; harp seal, 130 kg, 1 / 0.24 / 0.24; mollusc, $1.64 \cdot 10^{-2}$ kg, 1 / 0.5 / 0.5; and aquatic plant, $6.5 \cdot 10^{-3}$ kg, 1 / 0.01 / 0.01. As the threshold values of the dose rate for biota, the following ones were set: 10 $mGy \cdot day^{-1}$ for molluscs; 1 $mGy \cdot day^{-1}$ for fish and aquatic plants (brown algae); and 0.1 $mGy \cdot day^{-1}$ for long-lived marine mammals (ICRP Publication 108, 2008 ; ICRP Publication 124, 2014). The values of the indicators τ_w and τ_s were conservatively taken equal to 0.5 for fish, aquatic plants, and marine mammals; for molluscs, the value $\tau_s = 1$ was accepted.

The value of the indicator α_i (the quality factor associated with the relative biological effectiveness of radiation) for ^{137}Cs and ^{90}Sr is equal to 1. As shown in (Sazykina & Kryshev, 2016), the quality factor of α-emitting radionuclides for biota averages 15. At the same time, it was found that for various α-emitting radionuclides, the values of the quality factor differ significantly: for radium isotopes, $\alpha_i = 5$; for plutonium and americium isotopes, $\alpha_i = 50$. Taking into account this result, when determining the control levels of ^{239}Pu in the Barents Sea water and bottom sediments, we used the value $\alpha_i = 50$.

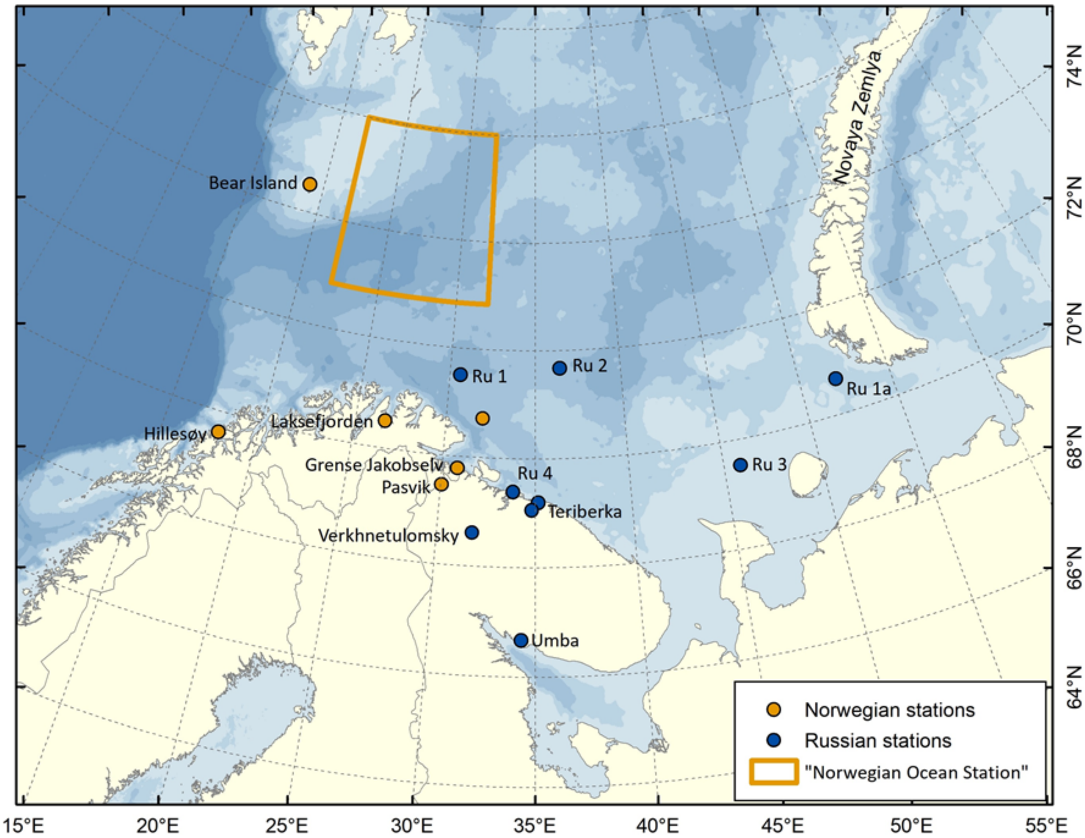

Fig. 1. Map of the study area and location of the reference sampling sites

RESULTS AND DISCUSSION

As a result of the radioecological database analysis, the concentration factors of ^{137}Cs, ^{90}Sr, and ^{239,240}Pu were determined in reference objects of the Barents Sea biota. The calculation results are given in Table 1. For comparison, it includes the values of the concentration factors of these radionuclides from the recommendations (Otsenka radiatsionno-ekologicheskogo vozdeistviya, 2015) based on the IAEA publication (Sediment Distribution Coefficients, 2004) and not taking into account regional specifics.

For fish, molluscs, and aquatic plants of the Barents Sea, the values of ^{137}Cs concentration factors are in good agreement (see Table 1) with the generalized global mean values from the recommendations (Otsenka radiatsionno-ekologicheskogo vozdeistviya, 2015), while for marine mammals, the local value of ^{137}Cs concentration factor is 3.3 times lower. For all reference organisms of the Barents Sea, the concentration factors of ^{90}Sr are 2–7 times higher than the generalized global average values. In the Barents Sea fish, the concentration factor of ^{239,240}Pu is higher than the value specified in the recommendations (Otsenka radiatsionno-ekologicheskogo vozdeistviya, 2015); in molluscs and aquatic plants, it is significantly lower.

Table 1. Long-term mean values of the concentration factors of radionuclides in the Barents Sea biota in 1992–2020

Radionuclide	Concentration factor according to monitoring data, L·kg^{-1}	Two-sided confidence interval (95 %), T1, T2	Concentration factor according to the recommendations (Otsenka radiatsionno-ekologicheskogo vozdeistviya, 2015), L·kg^{-1}
Fish			
^{137}Cs	93	76, 129	100
^{90}Sr	12	10, 27	3
^{239,240}Pu	262	197, 1,000	100
Molluscs			
^{137}Cs	51	26, 258	60
^{90}Sr	21	7, 56	10
^{239,240}Pu	1,180	912, 4,333	3,000
Aquatic plants			
^{137}Cs	69	58, 76	50
^{90}Sr	19	10, 31	10
^{239,240}Pu	732	449, 1,724	4,000
Marine mammals			
^{137}Cs	63	36, 86	210
^{90}Sr	14	1, 26	2
^{239,240}Pu	222	111, 333	280

The water–sediment distribution coefficients for the Barents Sea, determined as a result of the analysis of the radioecological database, are given in Table 2 in comparison with the values of this indicator from the recommendations (Otsenka radiatsionno-ekologicheskogo vozdeistviya, 2015) which are global mean values for marine ecosystems.

Table 2. Long-term mean values of the radionuclide water–sediment distribution coefficients in the Barents Sea in 2006–2020

Radionuclide	Distribution coefficient according to monitoring data, L·kg^{-1}	Two-sided confidence interval (95 %), T1, T2	Distribution coefficient according to the recommendations (Otsenka radiatsionno-ekologicheskogo vozdeistviya, 2015), L·kg^{-1}
^{137}Cs	426	362, 640	3,000
^{90}Sr	443	180, 720	1,000
^{239,240}Pu	189,600	56,360, 318,310	100,000

The average values of the water–sediment distribution coefficients of ^{137}Cs and ^{90}Sr for the Barents Sea according to the monitoring results (see Table 2) are lower than the values specified in the recommendations (Otsenka radiatsionno-ekologicheskogo vozdeistviya, 2015) by 7 and 2.3 times, respectively. The mean water–sediment distribution coefficient of ^{239,240}Pu for the Barents Sea is 1.9 times higher than the value recommended in (Otsenka radiatsionno-ekologicheskogo vozdeistviya, 2015); however, it has a wide confidence interval.

The concentration factors of technogenic radionuclides in the Barents Sea fish do not have a pronounced trend or a clear tendency to increase or decrease (Figs 2 and 3). This may indicate the establishment of equilibrium in the distribution of radioactivity between the components of the Arctic marine ecosystem.

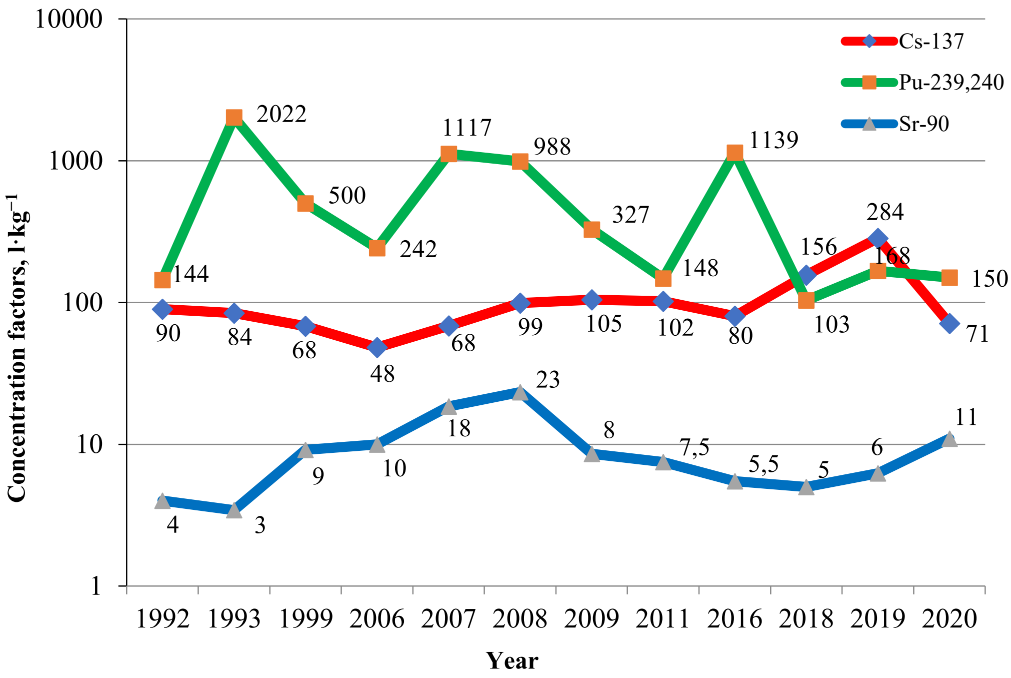

Fig. 2. Dynamics in the concentration factors of radionuclides in the Barents Sea fish in 1992–2020

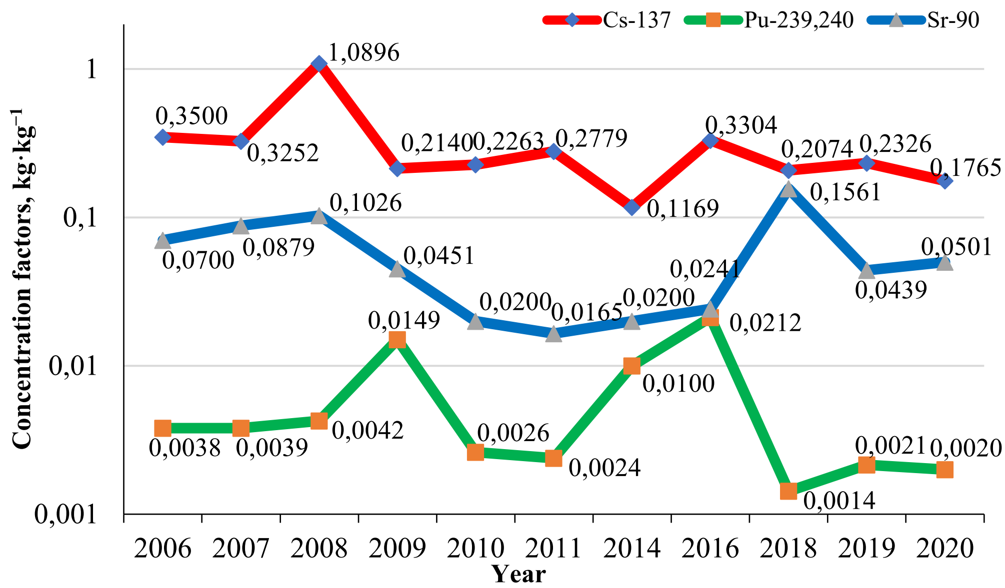

Fig. 3. Dynamics in the concentration factors of radionuclides in the Barents Sea fish from bottom sediments in 2006–2020

The calculated values of the concentration factors and distribution coefficients were used to determine the control levels of ^{137}Cs, ^{90}Sr, and $^{239,240}Pu$ in the Barents Sea water and bottom sediments. Control levels for each reference organism inhabiting the Barents Sea are given in Table 3.

Table 3. Control levels of radionuclides in the Barents Sea water and bottom sediments ensuring the radiation safety of the reference organisms

Organism	Control level in water, Bq·L^{-1}			Control level in bottom sediments, Bq·kg^{-1} fresh weight		
	^{137}Cs	^{90}Sr	^{239,240}Pu	^{137}Cs	^{90}Sr	^{239,240}Pu
Fish	8,78·10^{2}	4,37·10^{3}	1,05·10^{0}	3,75·10^{5}	1,94·10^{6}	2,00·10^{5}
Mollusc	5,53·10^{3}	1,45·10^{4}	2,34·10^{0}	2,35·10^{6}	6,41·10^{6}	4,43·10^{5}
Aquatic plant	9,20·10^{2}	1,36·10^{3}	3,77·10^{-1}	3,93·10^{5}	6,03·10^{5}	7,14·10^{4}
Marine mammal	1,15·10^{2}	4,39·10^{2}	1,24·10^{-1}	4,89·10^{4}	1,94·10^{5}	2,36·10^{4}

As the resulting control level of the radionuclide content in the Barents Sea water, its lowest value among all the values for reference organisms is taken: this ensures the safety of the most vulnerable link in the ecosystem. As seen from Table 3, the lowest values of control levels of all radionuclides are those for a marine mammal (harp seal). The control levels of technogenic radionuclides in the Barents Sea water ensuring the safety of marine biota (Bq·L^{-1}) are as follows: 115 for ^{137}Cs, 439 for ^{90}Sr, and 0.124 for ^{239,240}Pu. Reference levels of technogenic radionuclides in the Barents Sea bottom sediments (kBq·kg^{-1} fresh weight) are estimated as 48.9 for ^{137}Cs, 194 for ^{90}Sr, and 23.6 for ^{239,240}Pu.

For the Barents Sea, the contamination indices for water I_w and bottom sediments I_s were calculated by the formulas:

$$I_w = \sum_i \frac{C_{w,i}}{C_i}; \; I_s = \sum_i \frac{C_{s,i}}{S_i}, \tag{3}$$

where $C_{w,i}$ is the volumetric activity of the *i*-th radionuclide in seawater, Bq·L^{-1};

$C_{s,i}$ is the specific activity of the *i*-th radionuclide in bottom sediments, Bq·kg^{-1} fresh weight.

Fig. 4 shows the dynamics of the contamination indices for the Barents Sea water and bottom sediments by technogenic radionuclides in 2006–2020.

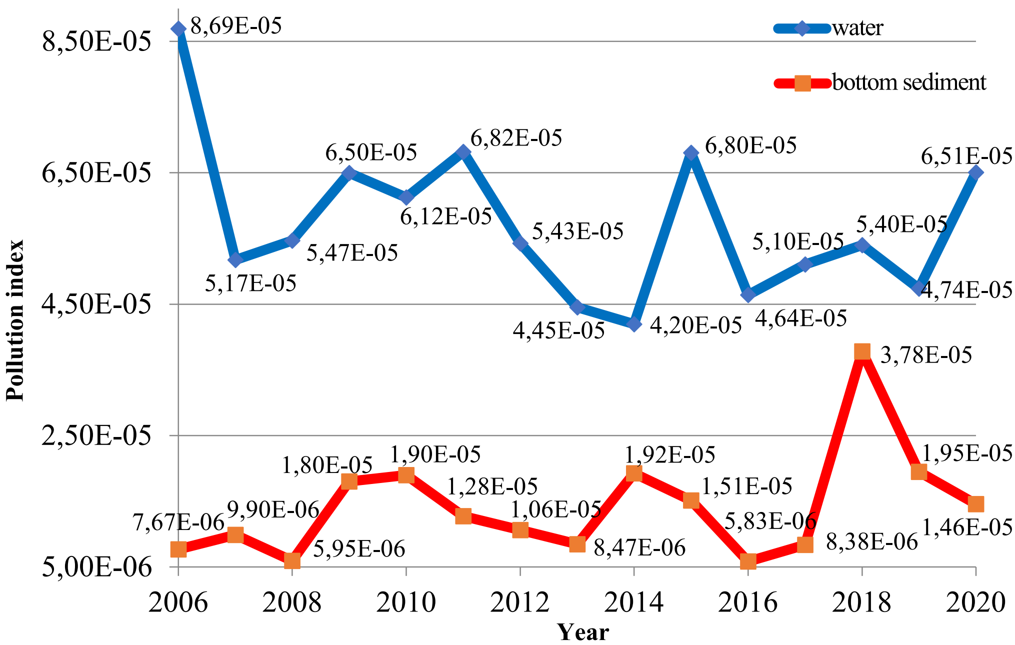

Fig. 4. Dynamics in contamination indices for the Barents Sea water and bottom sediments in 2006–2020

Fig. 4 shows that the contamination index for the Barents Sea water ranged from $4.2 \cdot 10^{-5}$ (2014) to $8.7 \cdot 10^{-5}$ (2006). The contamination index for the Barents Sea bottom sediments varied from $5.8 \cdot 10^{-6}$ (2016) to $3.8 \cdot 10^{-5}$ (2018). The contamination indices for both water and bottom sediments of the Barents Sea in 2006–2020 did not tend to increase or decrease.

The relative contribution of ^{137}Cs, ^{90}Sr, and ^{239,240}Pu to the contamination index for the Barents Sea water in 2006–2020 is shown in Fig. 5. The largest contribution to the contamination index for water was made by ^{239,240}Pu – 49–75 % in different years. Interestingly, the contribution of ^{137}Cs to the contamination index for the Barents Sea water varied within 16–41 %; the contribution of ^{90}Sr varied within 5–15 %. The contamination index for the Barents Sea bottom sediments is almost completely (up to 90 %) determined by the contribution of ^{137}Cs. In 2006–2020, the ratio of the contributions of technogenic radionuclides to the contamination index for the Barents Sea water and bottom sediments remained stable, without a noticeable tendency to change.

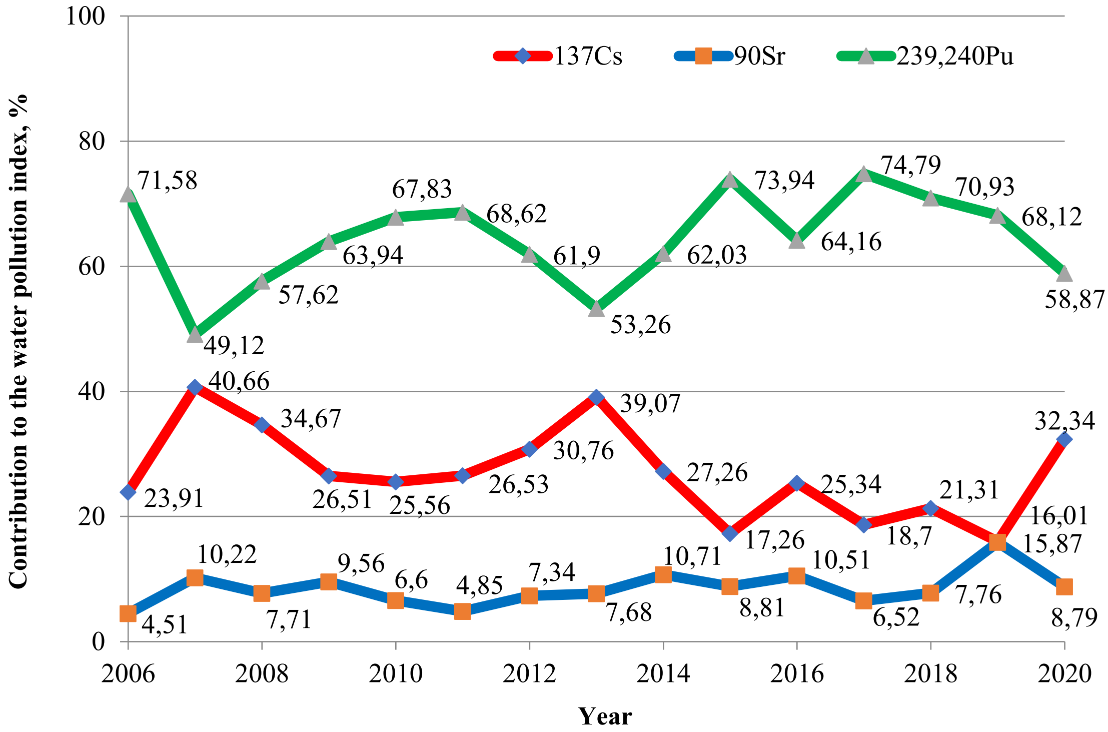

Fig. 5. Dynamics in the relative contribution of radionuclides to the contamination index of the Barents Sea water in 2006–2020

Conclusion. The concentration factors of technogenic radionuclides in the Barents Sea biota and the water–sediment distribution coefficients were determined using long-term observational data. According to the monitoring results, the water–sediment distribution coefficients of ^{137}Cs and ^{90}Sr for the Barents Sea are 7 and 2.3 times lower, respectively, than the global average values. For most of reference organisms of the Barents Sea, the values of the concentration factors of technogenic radionuclides differ from the reference values. The absence of a pronounced temporal trend for the concentration factors of all technogenic radionuclides in the Barents Sea fish in 1992–2020 indicates the establishment of equilibrium in the distribution of radioactivity between the components of the Arctic marine ecosystem.

Control levels of technogenic radionuclides in the Barents Sea water and bottom sediments are calculated, the non-exceeding of which ensures the safety of marine organisms. The control levels of radionuclides in the Barents Sea water ($Bq \cdot L^{-1}$) are 115 for ^{137}Cs, 439 for ^{90}Sr, and 0.124 for $^{239,240}Pu$. In the Barents Sea bottom sediments ($kBq \cdot kg^{-1}$ fresh weight), the values are 48.9 for ^{137}Cs, 194 for ^{90}Sr, and 23.6 for $^{239,240}Pu$.

In 2006–2020, the values of the contamination index for both water and bottom sediments of the Barents Sea, calculated using monitoring data and values of control levels, were several orders of magnitude lower than 1 and did not tend to increase or decrease. In terms of impact on natural biota, the main contribution to the contamination index for the Barents Sea water is made by $^{239,240}Pu$ (up to 75 %); to the contamination index for bottom sediments, by ^{137}Cs (up to 90 %). The ratio of the contributions of technogenic radionuclides to the contamination index of the Barents Sea water and bottom sediments is currently stable.

REFERENCES

1. Kobzar A. I. *Prikladnaya matematicheskaya statistika dlya inzhenerov i nauchnykh rabotnikov.* Moscow : Fizmatlit, 2006, 816 p. (in Russ.)
2. *Otsenka radiatsionno-ekologicheskogo vozdeistviya na ob"ekty prirodnoi sredy po dannym monitoringa radiatsionnoi obstanovki* : rekomendatsii R 52.18.820-2015 / Rosgidromet. Obninsk : FGBU "NPO "Taifun", 2015, 64 p. (in Russ.)
3. *Poryadok rascheta kontrol'nykh urovnei soderzhaniya radionuklidov v morskikh vodakh* : rekomendatsii R-52.18.852-2016 / Rosgidromet. Obninsk : FGBU "NPO "Taifun", 2016, 28 p. (in Russ.)
4. Sarkisov A. A., Sivintsev Yu. V., Vysotsky V. L., Nikitin V. S. *Atomnoe nasledie kholodnoi voiny na dne Arktiki. Radioekologicheskie i tekhniko-ekonomicheskie problemy radiatsionnoi reabilitatsii morei.* Moscow : IBRAE RAN, 2015, 699 p. (in Russ.)
5. Sivintsev Yu. V., Vakulovsky S. M., Vasilev A. P., Vysotsky V. L., Gubin A. T., Danilyan V. A., Kobzev V. I., Kryshev I. I., Lavkovsky S. A., Mazokin V. A., Nikitin A. I., Petrov O. I., Pologikh B. G., Skorik Yu. I. *Tekhnogennye radionuklidy v moryakh, omyvayushchikh Rossiyu. Radioekologicheskie posledstviya udaleniya radioaktivnykh otkhodov v arkticheskie i dal'nevostochnye morya ("Belaya kniga – 2000").* Moscow : IzdAT, 2005, 624 p. (in Russ.)
6. Brown J., Børretzen P., Dowdall M., Sazykina T., Kryshev I. The derivation of transfer parameters in the assessment of radiological impacts on Arctic marine biota. *Arctic*, 2004, vol. 57, no. 3, pp. 279–289. http://dx.doi.org/10.14430/arctic505
7. Gwynn J. P., Nikitin A. I., Shershakov V. M., Heldal H. E., Lind B., Teien H.-C., Lind O. C., Sidhu R. S., Bakke G., Kazennov A., Grishin D., Fedorova A., Blinova O., Sværen I., Liebig P. L., Salbu B., Wendell C. C., Strålberg E., Valetova N., Petrenko G., Katrich I., Logoyda I., Osvath I., Levy I., Bartocci J., Pham M. K., Sam A., Nies H., Rudjord A. L. Main results of the 2012 joint Norwegian–Russian expedition to the dumping sites of the nuclear submarine K-27 and solid radioactive waste in Stepovogo Fjord, Novaya Zemlya. *Journal of Environmental Radioactivity*, 2016, vol. 151, pt. 2, pp. 417–426. https://doi.org/10.1016/j.jenvrad.2015.02.003
8. ICRP Publication 108. Environmental protection: The concept and use of reference animals and plants. *Annals of the ICRP*, 2008, vol. 38, nos 4–6, 242 p.
9. ICRP Publication 124. Protection of the environment under different exposure situations. *Annals of the ICRP*, 2014, vol. 43, no. 1, 58 p.
10. ICRP Publication 136. Dose coefficients for non-human biota environmentally exposed to radiation. *Annals of the ICRP*, 2017, vol. 46, no. 2, 136 p.

11. Jensen L. K., Steenhuisen F., Standring W., Chen J., Leppanen A.-P., Nikitin A. I., Kryshev A. I., Gudnason K., Gwynn J., Stocki T., Joensen H. P. Monitoring of radioactivity in the Arctic. In: *AMAP Assessment 2015: Radioactivity in the Arctic*. Oslo, Norway : AMAP, 2016, pp. 35–57.
12. Kryshev A. I., Sazykina T. G. Comparative analysis of doses to aquatic biota in water bodies impacted by radioactive contamination. *Journal of Environmental Radioactivity*, 2012, vol. 108, pp. 9–14. https://doi.org/10.1016/j.jenvrad.2011.07.013
13. Kryshev A. I., Sazykina T. G., Katkova M. N., Buryakova A. A., Kryshev I. I. Modelling the radioactive contamination of commercial fish species in the Barents Sea following a hypothetical short-term release to the Stepovogo Bay of Novaya Zemlya. *Journal of Environmental Radioactivity*, 2022, vol. 244–245, art. no. 106825 (9 p.). https://doi.org/10.1016/j.jenvrad.2022.106825
14. Kryshev I. I., Sazykina T. G., Strand P., Brown J. E. Concentration factors of radionuclides in arctic marine biota. In: *Proceedings From the 5th International Conference on Environmental Radioactivity in the Arctic and Antarctic*, St. Petersburg, 16–20 June, 2002. Østerås : NRPA, 2002, pp. 322–325.
15. Sazykina T. G., Kryshev A. I. Manifestation of radiation effects in cold environment: Data review and modeling. *Radiation and Environmental Biophysics*, 2011, vol. 50, iss. 1, pp. 105–114. https://doi.org/10.1007/s00411-010-0336-7
16. Sazykina T. G., Kryshev A. I. Lower thresholds for lifetime health effects in mammals from high-LET radiation – Comparison with chronic low-LET radiation. *Journal of Environmental Radioactivity*, 2016, vol. 165, pp. 227–242. https://doi.org/10.1016/j.jenvrad.2016.10.013
17. *Sediment Distribution Coefficients and Concentration Factors for Biota in the Marine Environment.* Vienna : IAEA, 2004, 95 p. (IAEA Technical Reports Series ; no. 422).

ОПРЕДЕЛЕНИЕ В ВОДЕ И ДОННЫХ ОТЛОЖЕНИЯХ БАРЕНЦЕВА МОРЯ КОНТРОЛЬНЫХ УРОВНЕЙ СОДЕРЖАНИЯ РАДИОНУКЛИДОВ, ОБЕСПЕЧИВАЮЩИХ ПРИЕМЛЕМЫЙ ЭКОЛОГИЧЕСКИЙ РИСК

Н. А. Росновская, А. И. Крышев, И. И. Крышев

Научно-производственное объединение «Тайфун», Обнинск, Российская Федерация
E-mail: *rosnovskaya@rpatyphoon.ru*

Разработаны критерии оценки радиационной обстановки, обеспечивающие приемлемый экологический риск, — контрольные уровни содержания радионуклидов в компонентах природной среды. Метод применён для расчёта контрольных уровней содержания техногенных радионуклидов в воде и донных отложениях Баренцева моря, непревышение которых обеспечивает радиационную защиту морской биоты. Для этого выполнена оценка локальных коэффициентов накопления ^{137}Cs, ^{90}Sr и ^{239}Pu в биоте Баренцева моря с использованием многолетних данных наблюдений, а также выбраны представительные виды его экосистемы — рыба (треска), моллюск (мидия), водное растение (фукус) и морское млекопитающее (гренландский тюлень). Значения коэффициентов накопления ^{137}Cs, ^{239}Pu и ^{90}Sr составили соответственно (л·кг$^{-1}$): в рыбе — 93, 262 и 12; в моллюсках — 51, 1180 и 21; в водорослях — 69, 732 и 19; в морских млекопитающих — 63, 222 и 14. Значения коэффициентов распределения радионуклидов ^{137}Cs, ^{239}Pu и ^{90}Sr между водой и донными отложениями составили 426, 189 600 и 443 л·кг$^{-1}$ соответственно. Для большинства представительных организмов Баренцева моря значения коэффициентов накопления техногенных радионуклидов отличаются от справочных значений. Отсутствие выраженного временно́го тренда для коэффициентов накопления всех техногенных радионуклидов в рыбе Баренцева моря в 1992–2020 гг. указывает на установление равновесия в распределении радиоактивности между компонентами арктической морской экосистемы. Контрольные уровни содержания радионуклидов в воде Баренцева моря (Бк·л$^{-1}$) составляют 115 для ^{137}Cs, 439 для ^{90}Sr, 0,124 для ^{239,240}Pu; в донных отложениях (кБк·кг$^{-1}$ сырого веса) — 48,9 для ^{137}Cs, 194 для ^{90}Sr, 23,6 для ^{239,240}Pu. Значения индекса загрязнения как воды, так и донных отложений Баренцева моря, рассчитанные с использованием данных мониторинга и величин контрольных уровней, в 2006–2020 гг. были на несколько порядков ниже единицы и не имели существенной тенденции к повышению или снижению. Основной вклад в индекс загрязнения воды Баренцева моря с точки зрения воздействия на природную биоту вносит ^{239,240}Pu (до 75 %), в индекс загрязнения донных отложений — ^{137}Cs (до 90 %). Соотношение вкладов техногенных радионуклидов в индекс загрязнения воды и донных отложений Баренцева моря в настоящее время является постоянным.

Ключевые слова: Арктика, Баренцево море, вода, донные отложения, биота, контрольный уровень, коэффициент накопления, коэффициент распределения, радионуклид

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PHYTOPLANKTON PRIMARY PRODUCTION ON THE NORTHEASTERN SAKHALIN ISLAND SHELF IN SUMMER

P. P. Tishchenko

V. I. Il’ichev Pacific Oceanological Institute FEB RAS, Vladivostok, Russian Federation
E-mail: *eq15@poi.dvo.ru*

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The eastern Sakhalin Island shelf is the area of high biological production. Its key peculiarity is the presence of a feeding area for the Okhotsk–Korean population of gray whales. We aimed at determining the features of the formation of primary production in this area; thereby, on 7–9 July, 2016, hydrochemical studies on the northeastern Sakhalin Island shelf were carried out. At each station, water was sampled from surface and near-bottom layers; then, concentrations of chlorophyll *a*, nitrates, and phosphates were measured. Moreover, at each station, depth profiling was conducted by a Sea-Bird SBE 19plus and a Rinko-Profiler. Those profilers were equipped with sensors for pressure, temperature, electrical conductivity, chlorophyll fluorescence, dissolved oxygen, turbidity, and photosynthetically active radiation. Assimilation number for phytoplankton was measured *in situ* by ARO1-USB Rinko dissolved oxygen sensors (JFE Advantech Co., Ltd.). Phytoplankton primary production in the photic layer was determined by the light model based on the representation of the photosynthetic light-response curve in the modified model of the non-rectangular hyperbola. Most intensively, the primary production occurred in the area affected by the Amur River. In the photic layer, the values of integral primary production varied within 1.57–11.17 $g\,C\cdot m^{-2}\cdot day^{-1}$. The distribution area of the modified highly productive water of the Amur River reached the traverse of the southern boundary of the Piltun Bay; there, it was limited by cold salty water which had risen due to the eddy structure from deeper horizons. The ratio of the production spent on the food supply formation for the Okhotsk–Korean population of gray whales was 1.9 % of the total production of the studied water area.

Keywords: phytoplankton primary production, Amur River, Sakhalin Island, gray whale

The Sea of Okhotsk is a basin of high biological productivity. Here, the total annual production of organic matter varies within 17.85–23.9 billion tons wet weight, and out of it, 63–78 % is primary production (Shuntov et al., 2019). The Sakhalin Island shelf is one of the main productivity areas of the Sea of Okhotsk. In April–November, the southeastern slope is characterized by monthly mean values of phytoplankton primary production (hereinafter PP) of 0.4–0.6 $g\,C\cdot m^{-2}\cdot day^{-1}$ (Kasai & Hirakawa, 2015). During the phytoplankton bloom, PP can reach 4–6 $g\,C\cdot m^{-2}\cdot day^{-1}$ in the Piltun Bay area (Sorokin Yu. & Sorokin P., 1999) and 1.9 $g\,C\cdot m^{-2}\cdot day^{-1}$ abeam the southern boundary of the Chayvo Bay (Isada et al., 2009). Changes in water productivity on the eastern Sakhalin Island shelf are mostly related to the flow volume of the Amur River (Tskhay et al., 2015).

Moreover, the eastern Sakhalin Island shelf is of great interest since it is a feeding area for the Okhotsk–Korean population of gray whales. In the middle XX century, those were considered exterminated but later gray whales were found off the Sakhalin Island. To date, this population is included in the Red List of the International Union for Conservation of Nature.

Due to the importance of analyzing such a highly productive area of the World Ocean, on 7–9 July, 2016, hydrochemical studies of the northeastern Sakhalin Island shelf were carried out. The investigation was aimed at determining the features of the formation of PP there (Tishchenko et al., 2018).

MATERIAL AND METHODS

The work was carried out during the cruise of the RV "Professor Gagarinsky" in July 2016 (Tishchenko et al., 2018). There were 33 stations on the eastern slope of Sakhalin; their location is shown in Fig. 1. At each station, vertical profiling of the water column was carried out with Sea-Bird SBE 19plus V and Rinko-Profiler; those were equipped with sensors for pressure, temperature, electrical conductivity, chlorophyll fluorescence, dissolved oxygen, turbidity, and photosynthetically active radiation (hereinafter PAR). Moreover, at each station, water was sampled from surface and near-bottom layers with 5-L Niskin bottles, and concentrations of chlorophyll *a* (hereinafter Chl), nitrates, and phosphates were measured. A total of 66 water samples were taken to determine each parameter.

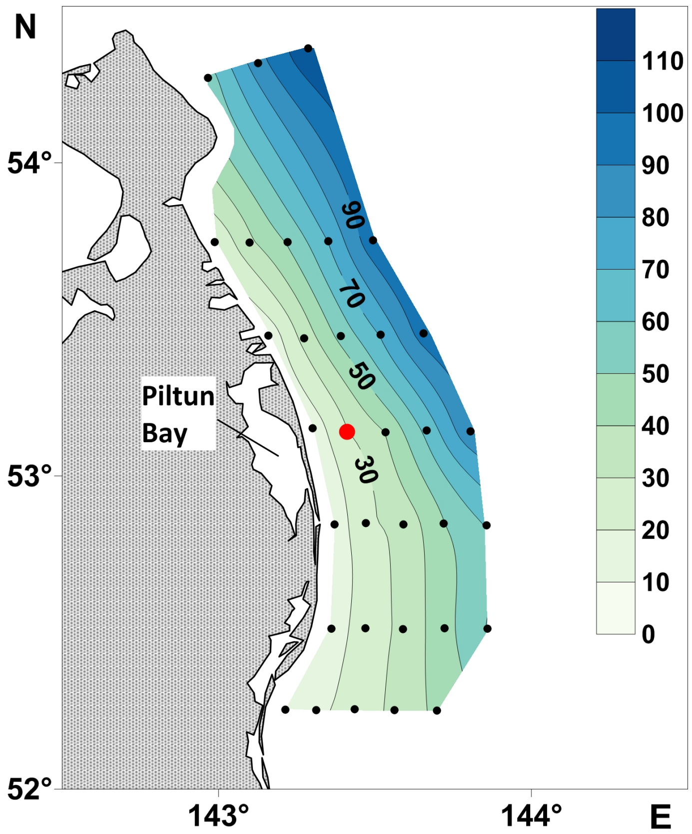

Fig. 1. Map of depth and location of stations during the study on the northeastern Sakhalin Island shelf (71st cruise of the RV "Professor Gagarinsky", 7–9 July, 2016). The red point denotes the station where the assimilation number for phytoplankton was measured

The nutrients were measured on the day of sampling in the laboratory onboard. Phosphates were determined by the Murphy–Riley method modified by Korolev (ascorbic acid was used as a reducing agent) (Metody gidrokhimicheskikh issledovanii, 1988). Nitrates were preliminarily reduced to nitrites in a cadmium reducer and then determined by the Griess method modified by Bendschneider–Robinson (Metody gidrokhimicheskikh issledovanii, 1988). Chl concentration in water samples, considering pheophytin content, was determined by the spectrophotometry. Water samples of about 1.5 L were preliminarily filtered through "Vladipor MFAS-OS-3" membrane filters, 35 mm in diameter, with a pore diameter of 0.8 μm. Then, the filters were dried, dissolved in 5 mL of 90 % acetone, and placed in a refrigerator. A day later, on a UV-3600 (Shimadzu) spectrophotometer, the absorbance of the extract was determined. *Prior* to pheophytin measuring, the extract was acidified with 2–3 drops of the prepared solution of hydrochloric acid in acetone. The concentrations were calculated using the formulas by Jeffrey and Humphrey (1975) and by Lorenzen (1967).

The thickness of the photic layer (hereinafter PhL) at each station was determined based on the data of a LI-COR QSP-2300L underwater PAR sensor. When probing the water column, vertical PAR profiles were obtained. The lower PhL boundary was taken as the depth of occurrence of 1 % PAR relative to the sensor readings in the surface water layer (1.5–2 m) (Ryther, 1956). For the dark time, the PhL thickness was determined from its dependence of the chlorophyll fluorescence maximum depth (Fig. 2). When having several extrema on the vertical profiles of chlorophyll, the depth of occurrence was used, which corresponded to the maximum of turbidity values. To calculate the PP, chlorophyll fluorescence data obtained during probing were corrected separately for each station (based on laboratory measurements of Chl by the spectrophotometry). The general trend of fluorescence *vs.* chlorophyll concentration is given in Fig. 3. The graph shows chlorophyll fluorescence measured by a Seapoint Chlorophyll Fluorometer at the depth of water sampling at the time of bathometer closure.

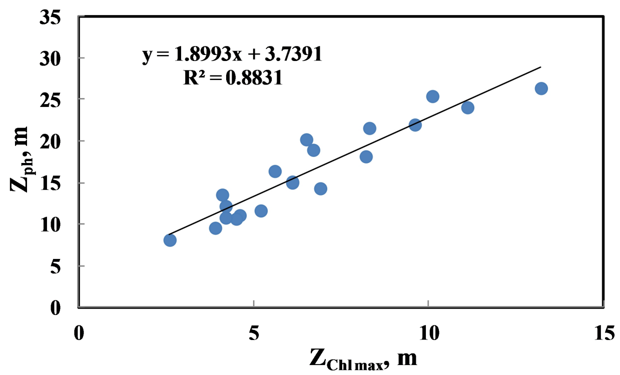

Fig. 2. Dependence of the photic layer depth, Z_{ph}, on chlorophyll maximum depth, $Z_{Chl\,max}$, during the study on the northeastern Sakhalin Island shelf

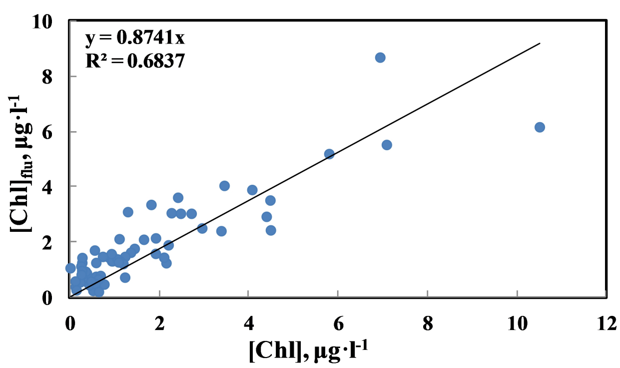

Fig. 3. Dependence of chlorophyll *a* concentration measured by a Seapoint Chlorophyll Fluorometer, $[Chl]_{flu}$, on the data of its laboratory measurements, [Chl]

To measure the assimilation number for phytoplankton (hereinafter An), water was sampled into 1.7-L bottles; to their necks, ARO1-USB Rinko optical oxygen sensors (JFE Advantech Co., Ltd.) were attached (their technical characteristics are given in Table 1). Using sensors hung overboard to a sampling depth (2 m), *in situ* for 2 h 40 min, oxygen was continuously recorded in the light and dark bottles with an interval of 1 min during a complete stoppage of the RV. This allowed to register temporal variability of oxygen content during incubation and eliminate the random error associated with the measurement of oxygen content in the bottles. At the same time, to determine An, linear approximation was applied to the entire data series; initial and final values of DO from the approximation equation were used for the initial and final moments of sample exposure. An was measured once (the spot is shown in Fig. 1).

Table 1. Technical characteristics of ARO1-USB Rinko dissolved oxygen sensors

Parameter	Dissolved oxygen	Temperature
Principle	Phosphorescence	Thermistor
Range	Air saturation 0 to 200 %	−3…+45 °C
Resolution	0.01–0.04 %	0.001 °C
Accuracy	Non-linearity ±2 % of full scale (at 1 atm, +25 °C)	±0.02 °C (0 to +35 °C)

Since the investigation was carried out within the framework of a complex research cruise, it was impossible to measure An at each station. The obtained An value was used to calculate the PP for the entire study area. Previously, a similar assumption was made by Yu. Sorokin and P. Sorokin (1999) when studying the PP of the Sea of Okhotsk. When carrying out the measurements, the slope of the time dependence of ΔO_2 did not change; thereby, the An value during the measurements was assumed to be constant in the daytime. The assimilation number was calculated according to the formula:

$$\mathrm{An} = \frac{dO_2}{[Chl] \cdot PQ \cdot t}\,, \qquad (1)$$

where $dO_2 = (O_{lf} - O_{df}) - (O_{l0} - O_{d0})$ is the difference between the final and initial differences in the readings of the sensors in the light and dark bottles, $mg \cdot L^{-1}$;

O_{l0} and O_{lf} are initial and final concentrations of oxygen in the light bottle, $mg \cdot L^{-1}$;

O_{d0} and O_{df} are initial and final concentrations of oxygen in the dark bottle, $mg \cdot L^{-1}$;

[Chl] is chlorophyll *a* concentration, $\mu g \cdot L^{-1}$;

PQ is photosynthetic coefficient;

t is exposure time, h.

PQ was taken equal to 1.42. This value corresponds to mesotrophic waters with the prevalence of diatoms (Laws, 1991 ; Smith et al., 2012), and it is characteristic of the study area (Orlova et al., 2004 ; Shevchenko & Ponomareva, 2013).

During the complex work on the northeastern Sakhalin Island shelf on the 71st cruise of the RV "Professor Gagarinsky", water was also sampled for determining the species composition of phytoplankton (Tishchenko et al., 2018). At the time of the study, diatoms accounted for more than 90 % of the total phytoplankton abundance (personal communication of Yu. Fedorets).

Based on the PhL thickness data and concentrations of Chl and An, phytoplankton primary production in the photic layer was determined. For the calculations, the representation of the photosynthetic light-response curve in the modified model of the non-rectangular hyperbola was used (Tishchenko et al., 2017, 2019 ; Zvalinsky, 2008 ; Zvalinskii et al., 2006) which is largely similar to the Vertically Generalized Production Model (VGPM) (Behrenfeld & Falkowski, 1997). Below, there is the derivation of the equation for calculating the integral PP in the PhL taken from (Tishchenko et al., 2019).

The formula for calculating primary production for the depth Z within the photic layer is as follows:

$$P = P^m \frac{1 + I_z/I_k}{2\gamma} \left\{ 1 - \sqrt{1 - \frac{4\gamma I_z/I_k}{(1 + I_z/I_k)^2}} \right\} , \tag{2}$$

where P^m is the rate of photosynthesis under light saturation, mg C·m^{-2}·day^{-2};

I_z is solar radiation at depth Z, µmol·m^{-2}·day^{-1};

I_k is the light constant corresponding to the light intensity at which the light-response curve goes to saturation (Talling, 1957), and it is equal to 10 % of PAR incident on water surface I_0, µmol·m^{-2}·day^{-1};

γ is the non-rectangular hyperbola parameter equal to 0.95 for real light-response curves for seaweed (Zvalinsky, 2008).

Within the photic layer (Z_{ph}), light intensity decreases exponentially with depth (Behrenfeld & Falkowski, 1997 ; Gordon & McCluney, 1975):

$$I_z = I_0 \cdot exp(-k_d \cdot Z) , \tag{3}$$

where k_d is the coefficient of diffuse attenuation of light;

Z is depth, m.

Taking into account that at the lower PhL boundary (*i. e.*, at a compensation depth Z_c) the light intensity $I_c \approx 1$ % PAR (Ryther, 1956) and the fact that $I_k = 0.1 I_0$, it was obtain:

$$\begin{aligned} k_d &= \frac{\ln(I_0/I_c)}{Z_c} = \frac{\ln(I_0/0.01I_0)}{Z_c} = 4.6/Z_c , \\ I_z/I_k &= \frac{I_0 \exp(-k_d Z)}{0.1 I_0} = 10 \exp(-4.6Z/Z_c) . \end{aligned} \tag{4}$$

When numerically integrating equation (2) from the surface horizon, corresponding to light saturation, to Z_c and considering equation (4), a coefficient of 0.66 is obtained which does not depend on the PhL thickness when the site depth is greater than the PhL depth. In this case, the equation for calculating the integral PP takes the following form:

$$P = 0.66 \cdot An \cdot C_{ch} \cdot T_d , \tag{5}$$

where An is the assimilation number for phytoplankton in the subsurface layer, mg C·(mg Chl·h)$^{-1}$;

C_{ch} is chlorophyll *a* content in the photosynthesis layer (Z_{ph}), mg·m^{-2};

T_d is the length of the day, h.

Chl content in the photosynthesis layer can be obtained by numerical integration of vertical chlorophyll profiles measured by probing equipment:

$$C_{ch} = \sum_i [Chl]_{Z_i} \,, \tag{6}$$

where

$$[Chl]_{Z_i} = (Z_{i+1} - Z_i)\frac{(Chl_{Z_i} + Chl_{Z_{i+1}})}{2} \,. \tag{7}$$

An integration step of 10 cm was used to obtain C_{ch}. The coefficient in equation (5) is close in value to that applied in VGPM (Behrenfeld & Falkowski, 1997) which is equal to 0.66125.

The main difference between the model used in the article and the VGPM is that photoinhibition is not directly taken into account here. However, it is taken into account indirectly when measuring An since the exposure time for the light and dark bottles is quite long. The second distinction is the description of the light-response curve by the non-rectangular hyperbola. The light-response curve comes to a state of saturation at a value equal to 10 % of PAR incident on surface. With this value, integration of equation (2) leads to the fact that the value of the coefficient in equation (5) is 0.66. The proposed model of photosynthesis showed good agreement with the modified model of the rectangular hyperbola which is capable of describing the photoinhibition of the process by the non-rectangular hyperbola, as well as with field data on CO_2 gas exchange in the leaves of land plants (Korsakova et al., 2018).

RESULTS AND DISCUSSION

Hydrological conditions, nutrients, and chlorophyll *a*. Hydrological conditions on the northeastern slope of Sakhalin in July are formed mainly by the Amur River runoff, which at that time goes around the northern tip of the island, passes along its northeastern coast southward, and forms an area of warm desalinated waters (Rutenko & Sosnin, 2014) (Figs 4, 5). Moreover, it causes a blast phytoplankton bloom (Prants et al., 2017 ; Tskhay et al., 2015). In some years in autumn, with the maximum Amur River runoff, desalinated waters can pass along the eastern coast southward and reach the Aniva Bay (Tskhay et al., 2015). In this case, the distribution area of these waters is limited by the traverse of the southern boundary of the Piltun Bay. There is a hypothesis that two relatively stable eddy formations may exist in this area of the shelf, and those limit further penetration of desalinated waters southward (Rutenko & Sosnin, 2014).

During our studies, desalinated warm waters of the Amur River (+13 °C, practical salinity (PS) 19) shifted southward along the Sakhalin Island shelf. Opposite the Piltun Bay, those collided with a core of cold salty waters (+1 °C, PS 32) and formed a hydrological front (Figs 4a, 5a). Near-bottom waters were characterized by a uniform decrease in temperature and an increase in salinity with depth (from +8 °C and PS 26 to −1.5 °C and PS 33). In the northern area under study, cold salty waters (+1 °C, PS 32.5) upwelled to the shelf which limited the distribution area of warm desalinated waters to depths of down to 20 m (Fig. 6b, d). In the surface layer, the area of warm desalinated waters was characterized by low content of nitrates and phosphates (Figs 7a, 8a) – no more than 1 and 0.2 μmol·L^{-1}, respectively. In the core of cold salty waters, concentration of nutrients rose significantly: nitrates, up to 11 μmol·L^{-1}; phosphates, up to 1.4 μmol·L^{-1}. With increasing depth, the content of nutrients in water rose: nitrates, up to 18 μmol·L^{-1}; phosphates, up to 1.6 μmol·L^{-1} (Figs 7b, 8b). Desalinated waters were characterized by an increased content of Chl – up to 12.9 μg·L^{-1} (Fig. 9a). In the near-bottom water layer, Chl content varied within 0.1–10.5 μg·L^{-1} (Fig. 9b). The highest concentrations in the near-bottom layer were observed at the nearest station, opposite the Piltun Bay. In the core of cold salty waters, concentrations were about 2 μg·L^{-1}; in the near-bottom layer, content decreased to 1 μg·L^{-1} in the northern area under study and remained at the level of 2 μg·L^{-1} in its southern area (Fig. 9b).

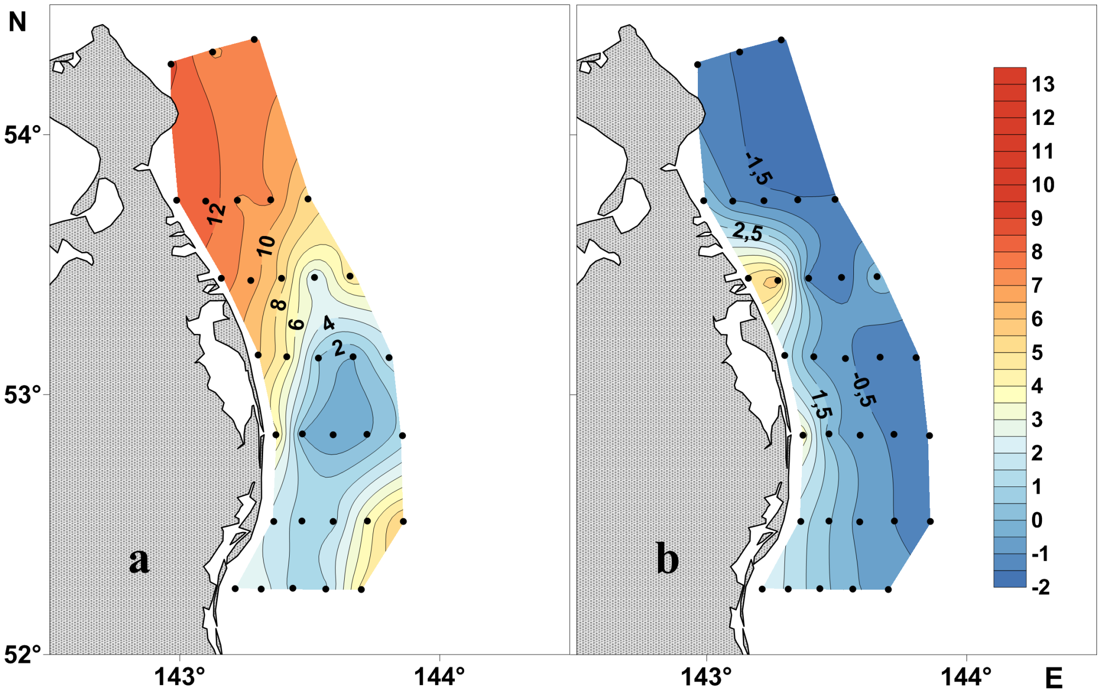

Fig. 4. Spatial distribution of water temperature, °C, on the northeastern Sakhalin Island shelf: a, surface horizon; b, near-bottom horizon (71st cruise of the RV "Professor Gagarinsky", 7–9 July, 2016)

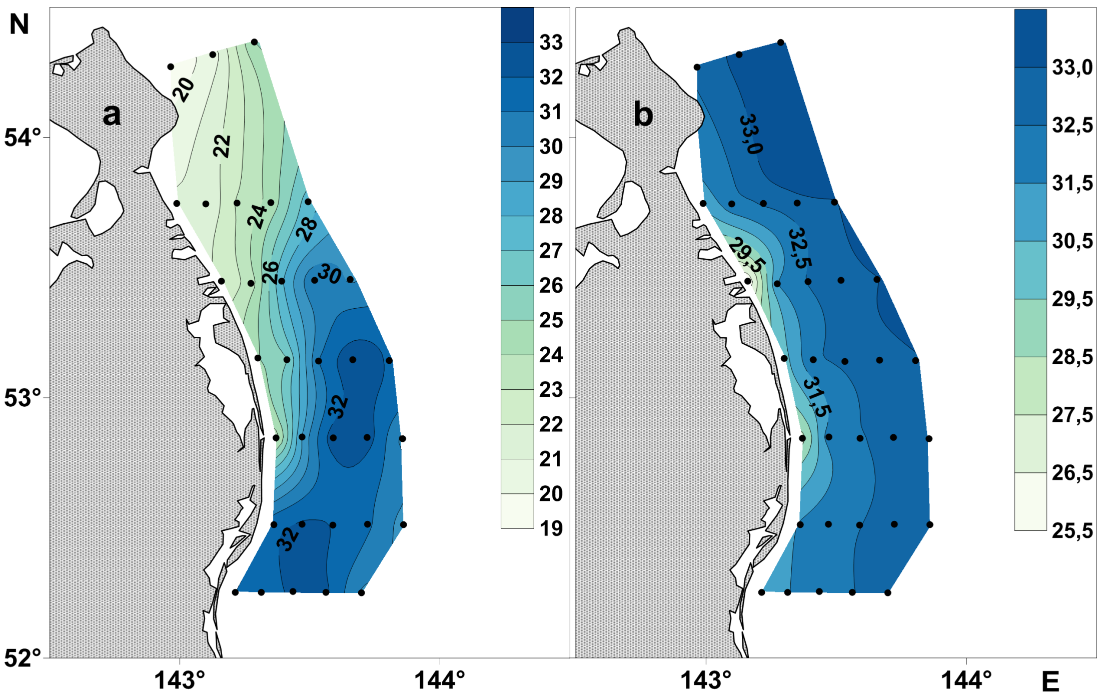

Fig. 5. Spatial distribution of practical salinity on the northeastern Sakhalin Island shelf: a, surface horizon; b, near-bottom horizon (71st cruise of the RV "Professor Gagarinsky", 7–9 July, 2016)

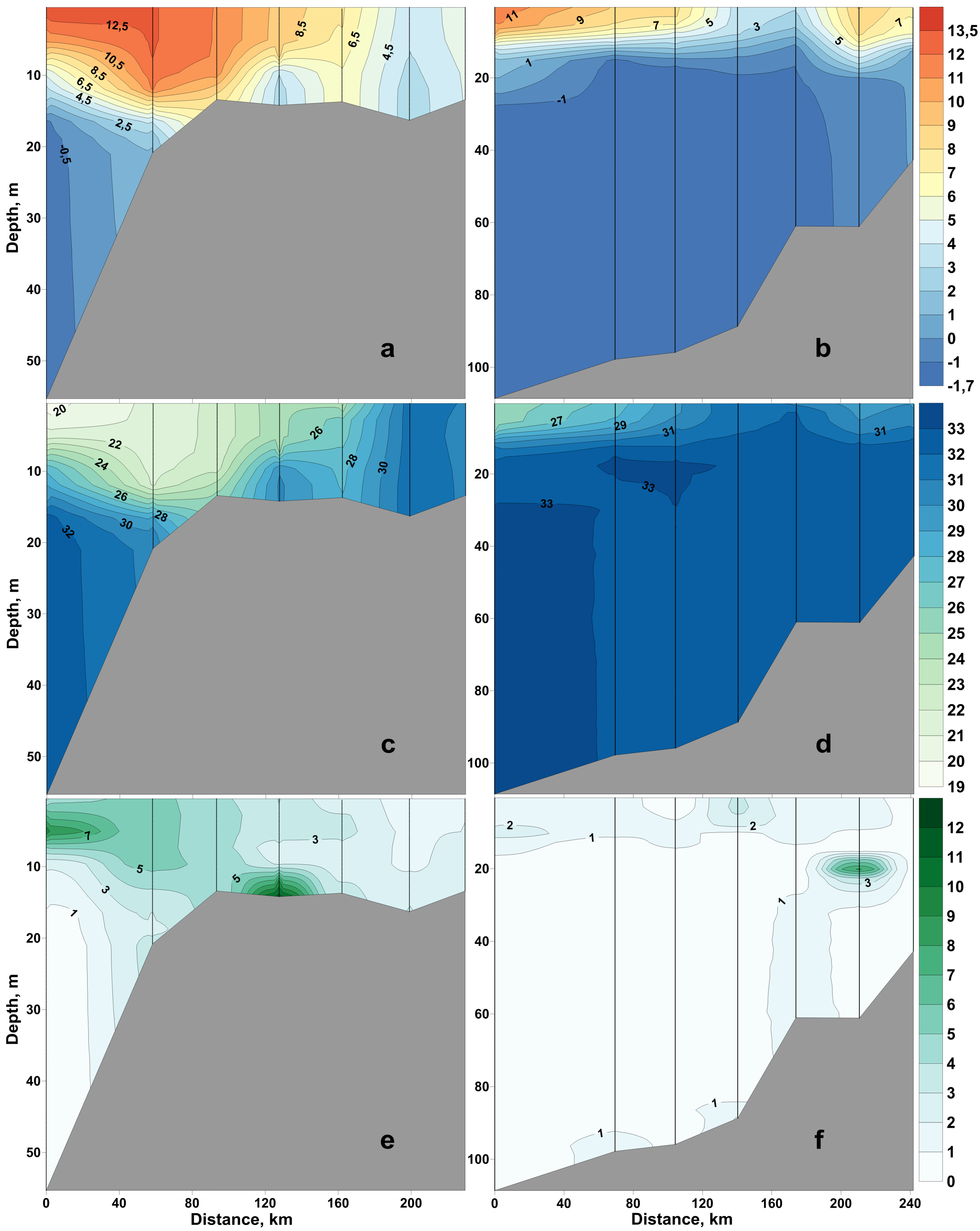

Fig. 6. Depth distribution of temperature, °C (a, b), practical salinity (c, d), and chlorophyll fluorescence, μg·L^{-1} (e, f), on meridional sections along the northeastern Sakhalin Island shelf through the coastal stations (a, c, e) and the deep-sea stations (b, d, f) (71st cruise of the RV "Professor Gagarinsky", 7–9 July, 2016). North is on the left

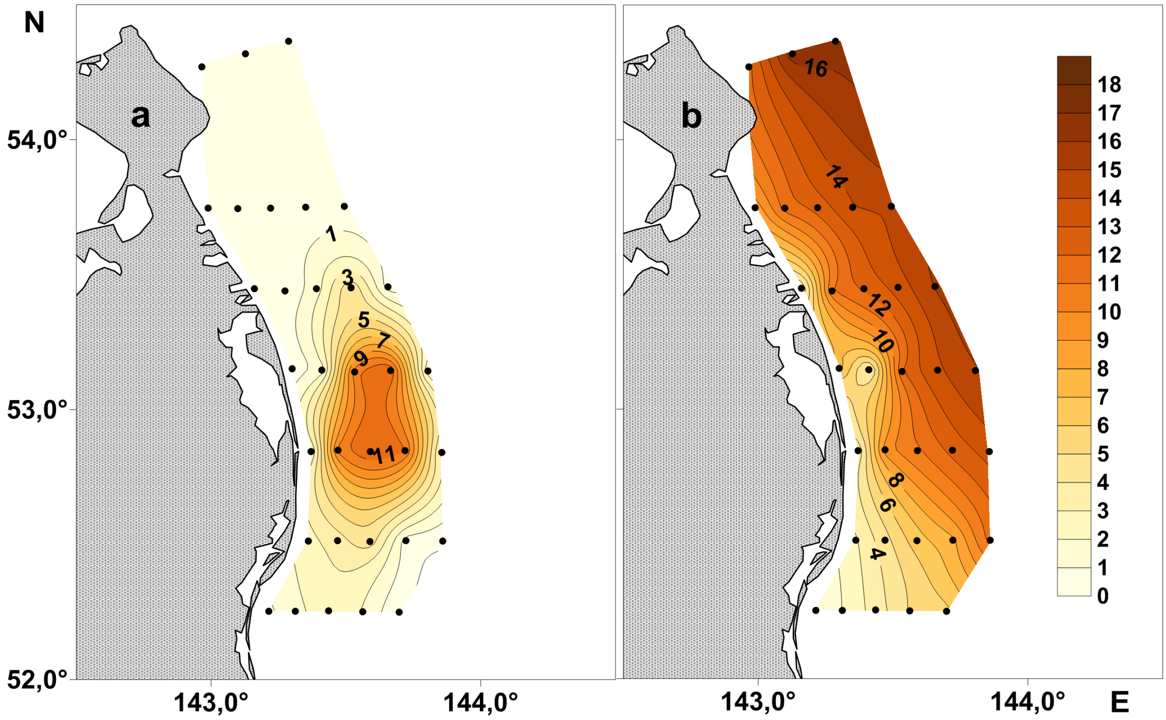

Fig. 7. Spatial distribution of nitrates, μmol·L^{-1}, on the northeastern Sakhalin Island shelf: a, surface horizon; b, near-bottom horizon (71st cruise of the RV "Professor Gagarinsky", 7–9 July, 2016)

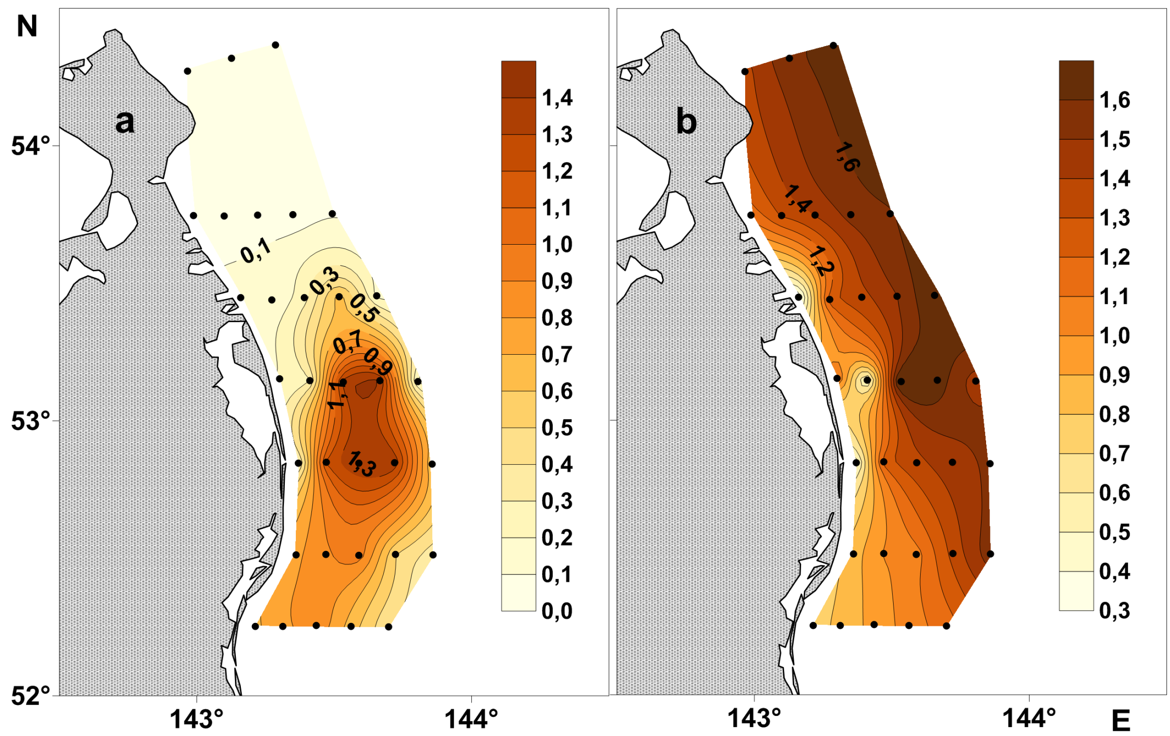

Fig. 8. Spatial distribution of phosphates, μmol·L^{-1}, on the northeastern Sakhalin Island shelf: a, surface horizon; b, near-bottom horizon (71st cruise of the RV "Professor Gagarinsky", 7–9 July, 2016)

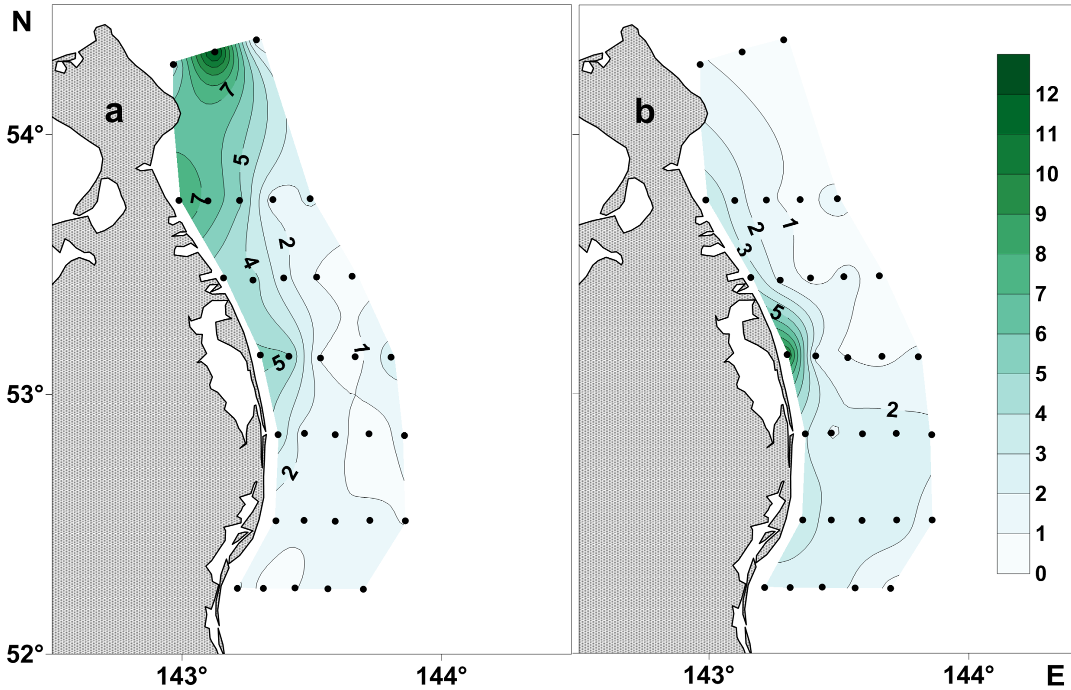

Fig. 9. Spatial distribution of chlorophyll *a*, μg·L^{-1}, on the northeastern Sakhalin Island shelf: a, surface horizon; b, near-bottom horizon (71st cruise of the RV "Professor Gagarinsky", 7–9 July, 2016)

The nature of spatial distributions of temperature and salinity in the surface layer indicates the penetration of cold salty waters there due to anticyclonic circulation which forms a hydrological front near the Piltun Bay (Figs 4a, 5a). The fact that the penetration of these waters was not a direct consequence of upwelling can be concluded from spatial distributions of temperature and salinity in the near-bottom water layer (Figs 4b, 5b). Moreover, it can be seen on hydrological sections (Fig. 6a–d): in spatial distribution of temperature and salinity in the southern area under study, no northward penetration of cold salty bottom waters was registered. Spatial changes in temperature and salinity were the most pronounced in the surface layer. Apparently, the mass of cold salty waters preventing the penetration of desalinated waters from the north initially rose to the surface from the lower horizons in the southeastern Sakhalin Island shelf and then shifted northward under the effect of an anticyclonic eddy.

The rise of waters from deeper horizons to the surface due to coastal upwelling (Prants et al., 2017) or the effect of an anticyclonic eddy (Rutenko & Sosnin, 2014) and their subsequent shift northward supplied large amounts of nitrogen and phosphorus into the surface layer. Specifically, their concentrations were comparable to the values corresponding to near-bottom water layers (Figs 7, 8). The desalinated water area was characterized by relatively low content of inorganic substances of nitrogen and phosphorus (Figs 7a, 8a). Presumably, this resulted from an increased photosynthetic activity of phytoplankton in the desalinated water area. Chl spatial distribution in the surface layer indirectly confirms this assumption (Fig. 9): high Chl concentrations were recorded in waters with low content of nutrients. In the northern area of the bay which is under maximum effect of the Amur River, Chl content in water reached 12.9 μg·L^{-1}. In the area of the hydrological front, it decreased to 2 μg·L^{-1}. Interestingly, in the area of cold salty waters, where the content of nutrients is maximum, Chl concentrations were about 2 μg·L^{-1} (Fig. 8a). Based on this, it can be assumed that the photosynthetic activity of phytoplankton is low in the core of cold salty waters. Apparently, intensive development of phytoplankton in these waters could result from their heating.

High values of chlorophyll fluorescence in the near-bottom water layer recorded at one of the coastal stations, opposite the Piltun Bay, have to be noted as well. Probably, sedimentation of organic matter to the bottom occurs here; this can also be assumed from the position of the isolines in the vertical fluorescence section (Fig. 6e).

Measurements of the assimilation number. During the entire exposure time, an increase in oxygen content in the light bottle was observed (Fig. 10a). The increase was non-linear. Specifically, during the first hour of exposure, the rate of O_2 increase was 0.22 mg·L^{-1}·h^{-1}; then, it slowed down to 0.05 mg·L^{-1}·h^{-1}. In the dark bottle, during the first hour of exposure, O_2 content increased as well (the rate was of 0.15 mg·L^{-1}·h^{-1}). Then, oxygen concentration began to decrease (the rate was of −0.11 mg·L^{-1}·h^{-1}).

Interestingly, the difference in readings between the light and dark bottles steadily increased with time. So, changes in the rate of decrease/increase in the level of oxygen in the samples obeyed the same laws and were not an experimental error. During the exposure, water temperature in the samples rose by 1.5 °C (Fig. 10b), and the increase was non-linear. The change in the difference in oxygen readings between the light and dark bottles occurred according to a linear law (Fig. 10c). Also, the linear increase in ΔO_2 values was not affected by a low temperature difference in the samples (Fig. 10b, c) which was noted earlier (Tishchenko et al., 2017). The maximum temperature difference between the light and dark bottles was of 0.508 °C, and this corresponded to the largest single deviations of ΔO_2 from the linear approximation reaching 0.038 mg·L^{-1} at ΔO_2 = 0.369 mg·L^{-1}. The total exposure time was 2 h 40 min. Only the initial and final values of the time dependence of ΔO_2 were used to calculate An, but the entire series of measurements is given in the article to prove the quality of the data obtained. Chlorophyll content in water before exposure was 3.85 μg·L^{-1}. According to the results of the experiment, the obtained value of the assimilation number was 9.66 mg C·(mg Chl·h)$^{-1}$. Such a high rate of carbon assimilation may be due to increased content of iron introduced by the Amur River waters (Nishioka et al., 2014 ; Shulkin & Zhang, 2014). The shadow growth of oxygen recorded during the experiment is a periodically observed phenomenon (Cherbadgy & Propp, 2008 ; Ettwig et al., 2012 ; Pamatmat, 1997 ; Pospíšil, 2007). There is no single and generally accepted explanation. Apparently, the shadow growth of O_2 results from the decomposition of hydrogen peroxide (Cherbadgy & Propp, 2008) and production of bacteria (Ettwig et al., 2012).

Phytoplankton primary production. Phytoplankton PP values varied from 1.57 to 11.17 g C·m^{-2}·day^{-1}. The nature of spatial variability of the PP coincided with that of Chl spatial distribution in the surface water layer: the highest PP values were confined to the northern area under study which is under maximum effect of the Amur River (Figs 9, 11). As shifting southward, the PP decreased and reached its minimum values in the southeastern area under study and in the area of cold salty waters. Obviously, this nature of the PP distribution corresponds to the period of maximum effect of the Amur River runoff since in August–September the value of phytoplankton production on the northeastern Sakhalin Island shelf is about 0.7–0.8 g C·m^{-2}·day^{-1}. Abeam the southern boundary of the Chayvo Bay, where the effect of the Amur River runoff is low, the obtained PP values correspond to the values typical for August–September (Isada et al., 2009). Thus, the phytoplankton bloom on the northeastern Sakhalin Island shelf is strongly dependent on the runoff volume of the Amur River, under the effect of which Chl content in the bloom area can vary fourfold (Tskhay et al., 2015).

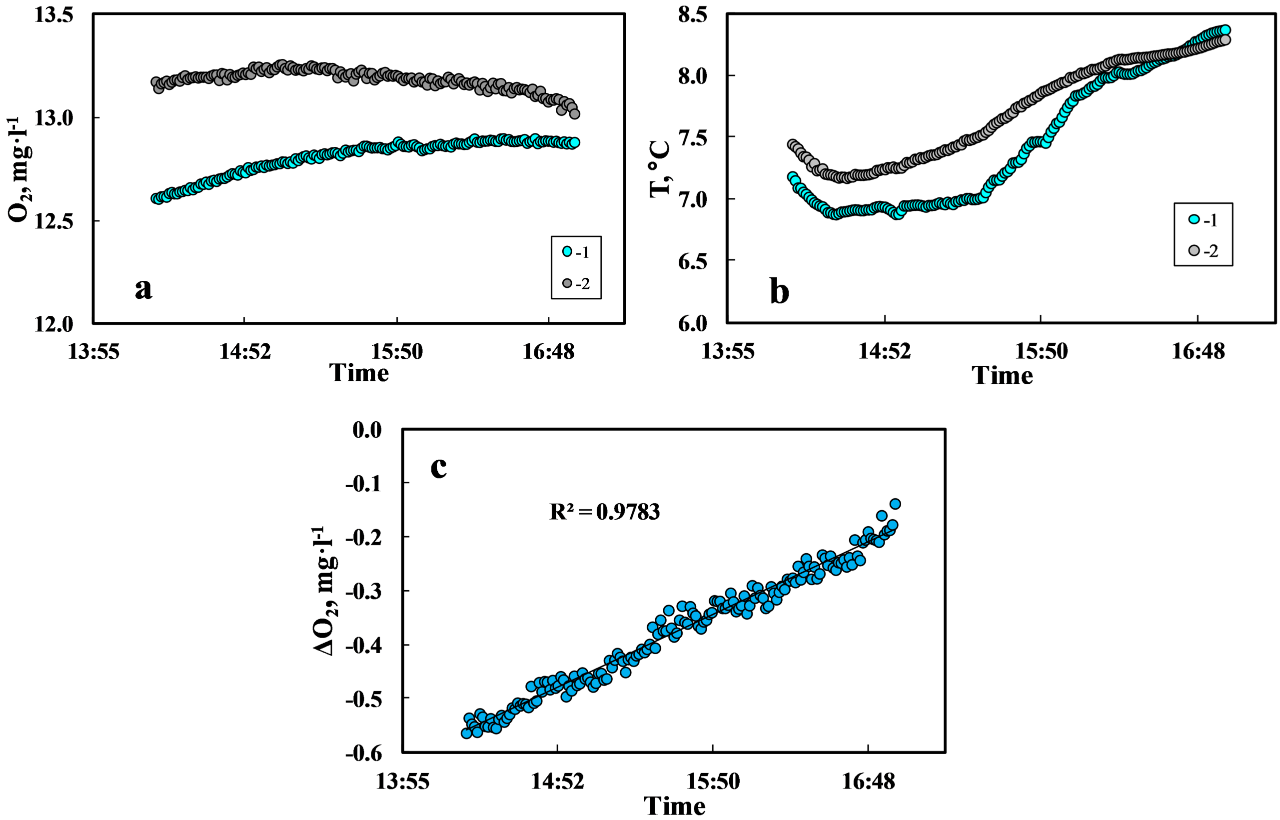

Fig. 10. Time variability of oxygen concentration (a), water temperature (b), and difference in readings for dark and light bottles (c) for ARO1-USB Rinko dissolved oxygen sensors on the northeastern Sakhalin Island shelf on 8 July, 2016: ● denotes a light bottle; ● denotes a dark bottle. The abscissa shows the local time

Apparently, the high production on the northeastern slope of the Sakhalin is due to the Amur River runoff since it is a source of iron (Nishioka et al., 2014 ; Shulkin & Zhang, 2014) actively involved in phytoplankton fertilization on the eastern island shelf (Kanna et al., 2018 ; Yoshimura et al., 2010). For the PP, iron is the key nutrient: its absence leads to formation of water areas with high nitrogen and phosphorus concentrations and low production in the photic layer in the open ocean (Martin & Fitzwater, 1988). The Amur River plays a significant role in the PP formation not only on the northeastern slope of Sakhalin Island studied in this work but in a large area of the Sea of Okhotsk and even in the Kuril Islands area (Nishioka et al., 2014). Our results emphasize the importance of the Amur River runoff in the PP formation.

Assessment of the feeding area for gray whales. The coastal zone from the Urkt Bay to the middle Chayvo Bay with depths down to 20 m, which is a feeding area for gray whales, covers about 600 km^2 (Bröker et al., 2020). With a mean PP value of 6.5 g C·m^{-2}·day^{-1}, the total phytoplankton production there will reach 3,900 t C·day^{-1}. Considering that carbon content is 10 % of the phytoplankton biomass, it corresponds to 39,000 t·day^{-1} wet weight of phytoplankton (Menden-Deuer & Lessard, 2000). Assuming that the biomass of the secondary link in the food chain averages 0.1 of the biomass of the primary link (Odum, 1971), the value of zooplankton/zoobenthos production in the feeding area of gray whales will be 3,900 t·day^{-1} wet weight. The biomass required for daily feeding of gray whale averages 409 kg·day^{-1} (Bröker et al., 2020). Based on general considerations, the coastal zone from the Urkt Bay to the Chayvo Bay can serve as a feeding area for 9,500 whales. This value is consistent with historical data: previously, the Okhotsk–Korean population of gray whales was estimated at 1,500–10,000 individuals (Berzin, 1974 ; Yablokov & Bogoslovskaya, 1984). In 2014–2015, the Okhotsk–Korean population was of 172–186 individuals (Bröker et al., 2020 ; Cooke et al., 2015). With a population of ~ 180 individuals, 736 t·day^{-1} wet weight of phytoplankton is required to form a food supply; it is 1.9 % of the total productivity of the studied water area.

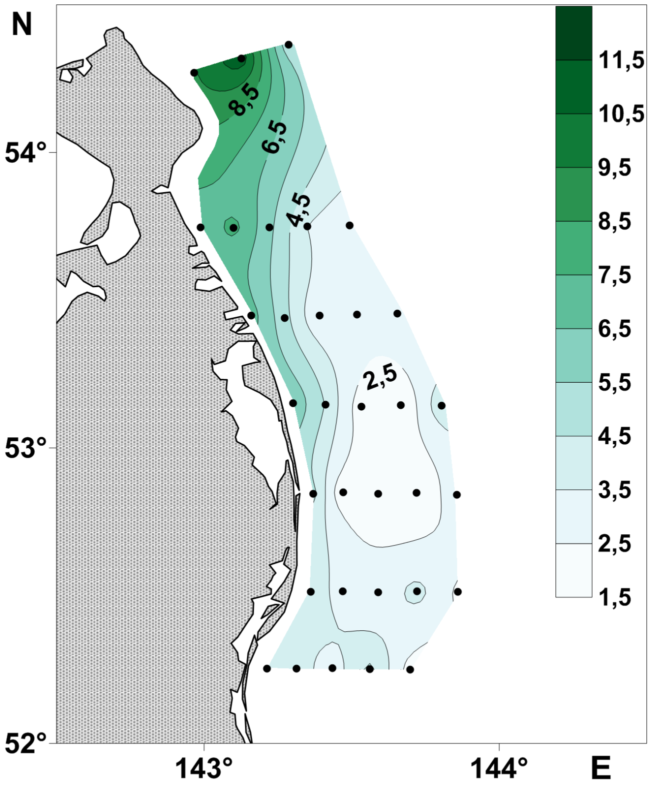

Fig. 11. Spatial distribution of phytoplankton primary production, g C·m^{-2}·day^{-1}, on the northeastern Sakhalin Island shelf (7–9 July, 2016)

Conclusion. As found during the study, the formation of phytoplankton primary production occurred most intensively in waters most affected by the Amur River runoff and coastal runoff. The effect of these waters extended up to the traverse of the southern boundary of the Piltun Bay; there, it was limited by cold salty water which had risen to the south of the study area due to the eddy structure from deeper horizons. The obtained high value of the assimilation number of phytoplankton characterizes the high rate of photosynthesis there. Primary production in the photic layer in the area of maximum effect of the Amur River reached 11.17 g C·m^{-2}·day^{-1}. The total phytoplankton primary production in the depth range down to 20 m, which is necessary for a food supply formation for gray whales, with a population of ~ 180 individuals, is 736 t·day^{-1} of phytoplankton wet weight, or 1.9 % of the total productivity of the studied water area.

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REFERENCES

1. Berzin A. A. Aktual'nye problemy izucheniya kitoobraznykh (na primere kitoobraznykh Tikhogo okeana). In: *Zoologiya bespozvonochnykh*. Moscow : VINITI, 1974, pp. 159–189. (Itogi nauki i tekhniki ; vol. 6). (in Russ.)
2. Zvalinsky V. I. Quantitative description of marine ecosystems. I. General approach. *Izvestiya TINRO*, 2008, vol. 152, pp. 132–153. (in Russ.)
3. Zvalinskii V. I., Lobanov V. B., Zakharkov S. P., Tishchenko P. Ya. Chlorophyll, delayed fluorescence, and primary production in the northwestern part of the Sea of Japan. *Oceanology*, 2006, vol. 46, iss. 1, pp. 27–37. (in Russ.). https://doi.org/10.1134/S0001437006010048
4. Korsakova S. P., Ilnitsky O. A., Plugatar Yu. V. Comparison of photosynthetic light-response curves models by the example of evergreen plant species. *Nauka Yuga Rossii [Science in the South Russia]*, 2018, vol. 14, no. 3, pp. 88–100. (in Russ.). https://doi.org/10.7868/S25000640180310
5. *Metody gidrokhimicheskikh issledovanii osnovnykh biogennykh elementov*. Moscow : VNIRO, 1988, 120 p. (in Russ.)
6. Orlova T. Yu., Selina M. S., Stonik I. V. Species composition of planktonic microalgae of coastal waters of Sakhalin Island, Sea of Okhotsk. *Biologiya morya*, 2004, vol. 30, no. 2, pp. 96–104. (in Russ.)
7. Rutenko A. N., Sosnin V. A. Hydrodynamic processes on the Sakhalin shelf in the coastal Piltun area of the grey whale feeding and their correlation with atmospheric circulation. *Meteorologiya i gidrologiya*, 2014, no. 5, pp. 74–93. (in Russ.). https://doi.org/10.3103/S1068373914050070
8. Tishchenko P. P., Tishchenko P. Ya., Zvalinsky V. I., Semkin P. Yu. The primary production of Amursky Bay (Sea of Japan) in the summer of 2008. *Biologiya morya*, 2017, vol. 43, no. 3, pp. 195–202. (in Russ.). https://doi.org/10.1134/S1063074017030117
9. Tishchenko P. Ya., Lobanov V. B., Shulkin V. M., Melnikov V. V., Tsoi I. B., Semkin P. Yu., Tishchenko P. P., Bannov V. A., Belous O. V., Vasileva L. E., Elovskaya O. A., Sagalaev S. G., Fedorets Yu. V. Comprehensive research of the coastal water area of the Sea of Japan and Sea of Okhotsk under the influence of river runoff (Cruise 71 of the RV "Professor Gagarinskii"). *Oceanology*, 2018, vol. 58, iss. 2, pp. 340–342. (in Russ.). https://doi.org/10.1134/S0001437018010150
10. Tishchenko P. P., Tishchenko P. Ya., Elovskaya O. A., Zvalinsky V. I., Fedorets Yu. V. Conditions for primary production of phytoplankton in the Vostok Bay (Japan Sea) in spring 2016. *Izvestiya TINRO*, 2019, vol. 198, pp. 164–185. (in Russ.). https://doi.org/10.26428/1606-9919-2019-198-164-185
11. Tskhay Zh. R., Shevchenko G. V., Chastikov V. N. Anomal'noe vliyanie stoka reki Amur na gidrologicheskie usloviya shel'fa o. Sakhalin v period pavodka 2013 goda. In: *Geodynamic Processes and Natural Hazards. Lessons of Neftegorsk* : vserossiiskaya nauchnaya konferentsiya s mezhdunarodnym uchastiem, 26–30 May, 2015, Yuzhno-Sakhalinsk, Russia : sbornik materialov : [in 2 vols]. Vladivostok : Dal'nauka, 2015, vol. 1, pp. 386–389. (in Russ.)
12. Cherbadgy I. I., Propp L. N. Photosynthesis and respiration of a deep-water periphyton community (Macclesfield Bank, South China Sea). *Biologiya morya*, 2008, vol. 34, no. 5, pp. 351–358. (in Russ.). https://doi.org/10.1134/S1063074008050064
13. Shevchenko O. G., Ponomareva A. A. Phytoplankton on the north-eastern coast of Sakhalin Island in August–September 2010. *Nauchnye trudy Dal'rybvtuza*, 2013, vol. 29, pp. 31–40. (in Russ.)
14. Behrenfeld M. J., Falkowski P. G. A consumer's guide to phytoplankton primary production models. *Limnology and Oceanography*, 1997, vol. 42, iss. 7, pp. 1479–1491. https://doi.org/10.4319/lo.1997.42.7.1479
15. Bröker K. C. A., Gailey G., Tyurneva O. Yu., Yakovlev Yu. M., Sychenko O., Dupont J. M.,

Vertyankin V. V., Shevtsov E., Drozdov K. A. Site-fidelity and spatial movements of western North Pacific gray whales on their summer range off Sakhalin, Russia. *PLoS ONE*, 2020, vol. 15, iss. 8, art. no. e0236649 (27 p.). https://doi.org/10.1371/journal.pone.0236649

16. Cooke J., Weller D., Bradford A., Sychenko O., Burdin A., Lang A. Updated population assessment of the Sakhalin gray whale aggregation based on the Russia–US photoidentification study at Piltun, Sakhalin, 1994–2014. *Western Gray Whale Advisory Panel Doc. WGWAP/16/17* : 16th meeting, Moscow, 22–24 Nov., 2015. [Moscow, 2015], 11 p.
17. Ettwig K. F., Speth D. R., Reimann J., Wu M. L., Jetten M. S., Keltjens J. T. Bacterial oxygen production in the dark. *Frontiers in Microbiology*, 2012, vol. 3, art. no. 273 (8 p.). https://doi.org/10.3389/fmicb.2012.00273
18. Gordon H. R., McCluney W. R. Estimation of the depth of sunlight penetration in the sea for remote sensing. *Applied Optics*, 1975, vol. 14, iss. 2, pp. 413–416. https://doi.org/10.1364/AO.14.000413
19. Isada T., Suzuki K., Liu H., Nishioka J., Nakatsuka T. Primary productivity and photosynthetic features of phytoplankton in the Sea of Okhotsk during late summer. In: *Proceedings of the Fourth Workshop on the Okhotsk Sea and Adjacent Areas* / M. Kashiwai, G. A. Kantakov (Eds). Sidney, B. C., Canada : PICES, 2009, pp. 72–75. (PICES Scientific Report ; no. 36).
20. Jeffrey S. W., Humphrey G. F. New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen*, 1975, vol. 167, iss. 2, pp. 191–194. https://doi.org/10.1016/S0015-3796(17)30778-3
21. Kanna N., Sibano Yu., Toyota T., Nishioka J. Winter iron supply processes fueling spring phytoplankton growth in a subpolar marginal sea, the Sea of Okhotsk: Importance of sea ice and the East Sakhalin Current. *Marine Chemistry*, 2018, vol. 206, pp. 109–120. https://doi.org/10.1016/j.marchem.2018.08.006
22. Kasai H., Hirakawa K. Seasonal changes of primary production in the southwestern Okhotsk Sea off Hokkaido, Japan during the ice-free period. *Plankton and Benthos Research*, 2015, vol. 10, iss. 4, pp. 178–186. https://doi.org/10.3800/pbr.10.178
23. Laws E. A. Photosynthetic quotients, new production and net community production in the open ocean. *Deep Sea Research Part A. Oceanographic Research Papers*, 1991, vol. 38, iss. 1, pp. 143–167. https://doi.org/10.1016/0198-0149(91)90059-O
24. Menden-Deuer S., Lessard E. J. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnology and Oceanography*, 2000, vol. 45, iss. 3, pp. 569–579. https://doi.org/10.4319/lo.2000.45.3.0569
25. Lorenzen C. J. Determination of chlorophyll and pheo-pigments: Spectrophotometric equations. *Limnology and Oceanography*, 1967, vol. 12, iss. 2, pp. 343–346. https://doi.org/10.4319/lo.1967.12.2.0343
26. Martin J. H., Fitzwater S. E. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. *Nature*, 1988, vol. 331, pp. 341–343. https://doi.org/10.1038/331341a0
27. Nishioka J., Nakatsuka T., Ono K., Volkov Yu. N., Scherbinin A., Shiraiwa T. Quantitative evaluation of iron transport processes in the Sea of Okhotsk. *Progress in Oceanography*, 2014, vol. 126, pp. 180–193. https://doi.org/10.1016/j.pocean.2014.04.011
28. Odum E. P. *Fundamentals of Ecology*. 3rd edition. Philadelphia, PA : W. B. Saunders Company, 1971, 574 p.
29. Pamatmat M. M. Non-photosynthetic oxygen production and non-respiratory oxygen uptake in the dark: A theory of oxygen dynamics in plankton communities. *Marine Biology*, 1997, vol. 129, pp. 735–746. https://doi.org/10.1007/s002270050216
30. Pospíšil P., Šnyrychová I., Nauš J. Dark production of reactive oxygen species in photosystem II membrane particles at elevated temperature: EPR spin-trapping study. *Biochimica et Biophysica Acta (BBA) – Bioenergetics*,

2007, vol. 1767, iss. 6, pp. 854–859. https://doi.org/10.1016/j.bbabio.2007.02.011
31. Prants S. V., Andreev A. G., Uleysky M. Yu., Budyansky M. V. Mesoscale circulation along the Sakhalin Island eastern coast. *Ocean Dynamics*, 2017, vol. 67, pp. 345–356. https://doi.org/10.1007/s10236-017-1031-x
32. Ryther J. H. The measurement of primary production. *Limnology and Oceanography*, 1956, vol. 1, iss. 2, pp. 72–84. https://doi.org/10.4319/lo.1956.1.2.0072
33. Smith L. M., Silver C. M., Oviatt C. A. Quantifying variation in water column photosynthetic quotient with changing field conditions in Narragansett Bay, RI, USA. *Journal of Plankton Research*, 2012, vol. 34, iss. 5, pp. 437–442. https://doi.org/10.1093/plankt/fbs011
34. Sorokin Yu. I., Sorokin P. Yu. Production in the Sea of Okhotsk. *Journal of Plankton Research*, 1999, vol. 21, iss. 2, pp. 201–230. https://doi.org/10.1093/plankt/21.2.201
35. Sorokin Yu. I., Sorokin P. Yu. Microplankton and primary production in the Sea of Okhotsk in summer 1994. *Journal of Plankton Research*, 2002, vol. 24, iss. 5, pp. 453–470. https://doi.org/10.1093/plankt/24.5.453
36. Shulkin V., Zhang J. Trace metals in estuaries in the Russian Far East and China: Case studies from the Amur River and the Changjiang. *Science of the Total Environment*, 2014, vol. 499, pp. 196–211. https://doi.org/10.1016/j.scitotenv.2014.08.015
37. Shuntov V. P., Ivanov O. A., Dulepova E. P. Biological resources in the Sea of Okhotsk Large Marine Ecosystem: Their status and commercial use. *Deep Sea Research Part II: Topical Studies in Oceanography*, 2019, vol. 163, pp. 33–45. https://doi.org/10.1016/j.dsr2.2019.01.006
38. Talling J. F. Photosynthetic characteristics of some freshwater plankton diatoms in relation to underwater radiation. *New Phytologist*, 1957, vol. 56, iss. 1, pp. 29–50. https://doi.org/10.1111/j.1469-8137.1957.tb07447.x
39. Yablokov A. V., Bogoslovskaya L. S. 20 – A review of Russian research on the biology and commercial whaling of the gray whale. In: *The Gray Whale: Eschrichtius robustus* / M. L. Jones, S. I. Swartz, S. Leatherwood (Eds). Orlando, FL : Academic Press, 1984, pp. 465–485. https://doi.org/10.1016/B978-0-08-092372-7.50026-1
40. Yoshimura T., Nishiona J., Nakatsuka T. Iron nutritional status of the phytoplankton assemblage in the Okhotsk Sea during summer. *Deep Sea Research Part I: Oceanographic Research Papers*, 2010, vol. 57, iss. 11, pp. 1454–1464. https://doi.org/10.1016/j.dsr.2010.08.003

ПЕРВИЧНАЯ ПРОДУКЦИЯ ФИТОПЛАНКТОНА НА СЕВЕРО-ВОСТОЧНОМ ШЕЛЬФЕ ОСТРОВА САХАЛИН В ЛЕТНИЙ ПЕРИОД

П. П. Тищенко

Тихоокеанский океанологический институт имени В. И. Ильичёва ДВО РАН, Владивосток, Российская Федерация
E-mail: *eq15@poi.dvo.ru*

Восточный шельф острова Сахалин относится к акваториям с высокой биологической продукцией. Его важная отличительная черта — наличие районов нагула для охотско-корейской популяции серых китов. Цель настоящей работы — определить особенности формирования первичной продукции в данном регионе. Для этого в период с 7 по 9 июля 2016 г. были проведены гидрохимические исследования северо-восточного шельфа острова Сахалин. На каждой станции с поверхностного и придонного горизонтов проводили отбор проб воды с последующими измерениями концентраций хлорофилла *a*, нитратов и фосфатов. Также на каждой станции проводили вертикальное зондирование водной толщи с помощью зондов Sea-Bird SBE 19plus V и Rinko-Profiler, оснащённых датчиками давления, температуры, электропроводности, флуоресценции хлорофилла, растворённого кислорода, мутности и фотосинтетически активной радиации. Датчиками кислорода ARO1-USB Rinko фирмы JFE Advantech Co., Ltd. в условиях *in situ* провели измерения, позволившие рассчитать ассимиляционное число фитопланктона. По результатам исследований определили первичную продукцию фитопланктона в фотическом слое. Для расчёта использовали представление световой кривой в модифицированной модели непрямоугольной гиперболы. Синтез первичной продукции происходил наиболее интенсивно в зоне влияния реки Амур, а значения интегральной первичной продукции в фотическом слое вод изменялись от 1,57 до 11,17 г С·м$^{-2}$·сут$^{-1}$. Область распространения модифицированных высокопродуктивных вод реки Амур достигала траверза южной границы залива Пильтун, где была ограничена холодными солёными водами, привнесёнными вихревой структурой из глубинных горизонтов. Доля продукции, затрачиваемой на формирование кормовой базы охотско-корейской популяции серых китов, составила 1,9 % от общей продукции рассматриваемой акватории.

Ключевые слова: первичная продукция фитопланктона, река Амур, остров Сахалин, серый кит

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RECORDS OF THE BROWN ALGA *HALOSIPHON TOMENTOSUS* (LYNGBYE) JAASUND (PHAEOPHYCEAE) IN THE SOUTH-EASTERN BALTIC SEA

© 2022 **A. A. Volodina**

Shirshov Institute of Oceanology of RAS, Moscow, Russian Federation
E-mail: *volodina.alexandra@gmail.com*

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For the first time, *Halosiphon tomentosus* (Lyngb.) Jaasund (*Chorda tomentosa* Lyngb.) was recorded in the South-Eastern Baltic Sea in May–June 2016, in several locations of the Sambia Peninsula northern coast. The species was found in the upper horizon of sublittoral on boulders in assemblages with green and brown algae (*Ulva intestinalis*, *Ulva prolifera*, *Cladophora glomerata*, *Ectocarpus siliculosus*, *Pylaiella littoralis*, and sometimes *Pseudolithoderma subextensum* and *Hildenbrandia rubra*). The species was recorded in 2017–2018 as well. The length of thread-like thalli ranged 3–30 cm, with the mean value of (9.2 ± 2.3) cm. The mean biomass was of (73.3 ± 41.9) g·m^{-2} in 2016 and (11 ± 8.8) g·m^{-2} in 2018. The reasons for *H. tomentosus* occurrence in the South-Eastern Baltic and its absence in the adjacent sea areas require further research.

Keywords: *Halosiphon tomentosus*, South-Eastern Baltic, new species records

The species composition of macroalgae of coastal fouling in the Russian area of the South-Eastern Baltic Sea is studied year-round since 2009. *Halosiphon tomentosus* (Lyngbye) Jaasund (*Chorda tomentosa* Lyngb.) (AlgaeBase, 2021) was recorded in May–June 2016 on the Sambia Peninsula northern coast – from Zelenogradsk town in the east to Lesnoy village in the west (Figs 1, 2). For the South-Eastern and Southern Baltic, *H. tomentosus* was not mentioned either at the early XX century (Lakowitz, 1907) or in the modern period until 2016 (Kostkevičiene & Sinkevičienė, 2008 ; Labanauskas, 2000 ; Pliński & Surosz, 2013 ; Volodina & Gerb, 2013, 2018).

Macroalgae quantitative sampling was carried out, and communities were described in the locations of *H. tomentosus* record from depths of 0–0.50 m on 0.01-m^2 sites. The macroalgae samples were weighed. The length of thread-like thalli ranged from 3 to 30 cm, with the mean value of (9.2 ± 2.3) cm. The color varied from golden to brown. In all the habitats, the species was recorded in the upper horizon of sublittoral on boulders or concrete structures at depths of 0–0.20 m. The ratio of the species in samples averaged 18 %. Air-dried phytomass ranged from less than 0.01 to 407 g·m^{-2}; its values averaged (73.3 ± 41.9) g·m^{-2} in 2016 and (11 ± 8.8) g·m^{-2} in 2018. The projective cover of the species was low – 0.5–5 % (Table 1).

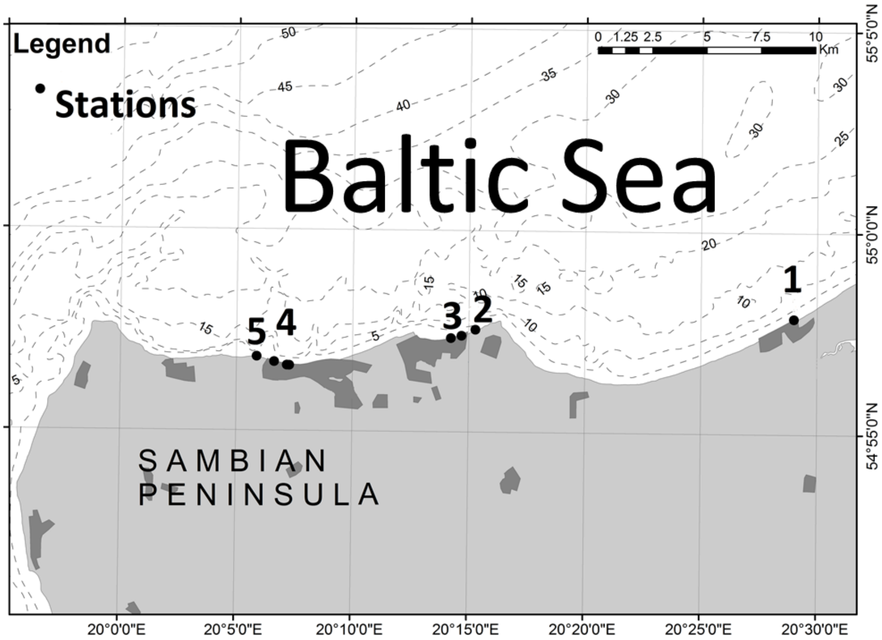

Fig. 1. Location of *Halosiphon tomentosus* sampling stations in the South-Eastern Baltic in 2016: 1, Zelenogradsk town; 2, Zaostrovie village; 3, Pionersky town; 4, Otradnoe village; 5, Lesnoy village

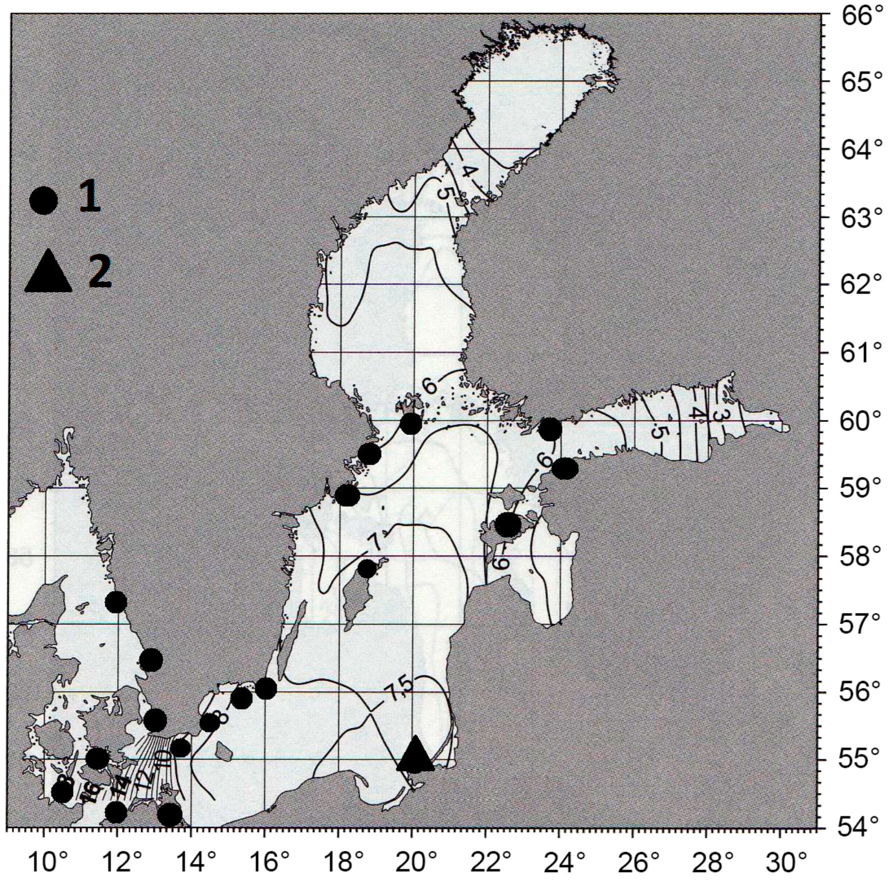

Fig. 2. *Halosiphon tomentosus* distribution in the Baltic Sea and salinity on the sea surface (practical salinity units, PSU) in December (1954–2000) (Dubravin, 2014); 1, *H. tomentosus* distribution in the Baltic Sea according to (Kontula & Fürhapter, 2012); 2, species record in the South-Eastern Baltic (2016–2018)

The highest value of *H. tomentosus* phytomass was registered on 5 June, 2016, in the area between Otradnoe and Lesnoy villages. On the locations studied, the species occurred in assemblages of annual green (*Ulva intestinalis* Linnaeus, 1753, *Cladophora glomerata* (Linnaeus) Kützing, 1843, and *Ulva prolifera* O. F. Müller, 1778) and brown macroalgae (*Pylaiella littoralis* (Linnaeus) Kjellman, 1872 and *Ectocarpus siliculosus* (Dillwyn) Lyngbye, 1819), as well as in assemblages involving corticated perennial red and brown macroalgae (*Hildenbrandia rubra* (Sommerfelt) Meneghini, 1841 and *Pseudolithoderma subextensum* (Waern) S. Lund, 1959).

Table 1. Algae occurrence, species projective cover in assemblages, and species ratio in biomass (%) in *Halosiphon tomentosus* habitats

No.	Species	Occurrence, %	Projective cover, %	Species ratio in total biomass, %
1	*Cladophora glomerata*	93.3	20–95	46
2	*Ectocarpus siliculosus*	40	0–0.5	2
3	*Pylaiella littoralis*	46.6	0.5	6
4	*Ulva intestinalis*	69	50–100	16
5	*Ulva prolifera*	13.3	1–5	12
6	*Pseudolithoderma subextensum*	29	0–0.5	–
7	*Hildenbrandia rubra*	20	0–1	–
8	*Halosiphon tomentosus*	68.4	0.5–5	18

H. tomentosus diagnostic feature is a thread-like thallus densely covered along its entire length with multicellular golden-brown hairs. In the Baltic Sea, thalli up to 1 m long and 4 mm wide occur in sublittoral on boulders and shell rocks at depths of 1–15 m (Pankow, 1990).

H. tomentosus is a typical representative of the Arctic cold-boreal North Atlantic flora. In 2015, the species was recorded in the north-western Black Sea (the Odesa Bay). This habitat is the southernmost spot of *H. tomentosus* range (Minicheva, 2015).

The species occurs in seas varying in salinity – from 35 PSU (the North Sea) to 6 PSU (the Baltic Sea) (Hällfors & Heikkonen, 1992). Winter (+7 °C) and spring temperatures (+5…+13 °C) in the Baltic Sea (Dubravin, 2014) are suitable for its widespread distribution up to the Gulf of Bothnia. L. Zenkevich (1963) classified *H. tomentosus* as a species characteristic of the Baltic Sea. According to current floristic lists, *H. tomentosus* distribution in the Baltic Sea is concentrated in its western and south-western areas. The species is also recorded in the Bornholm Basin, the Western Gotland Basin, and the North-Eastern Baltic (the Gulf of Finland and Gulf of Riga) (Kontula & Fürhapter, 2012 ; Möller et al., 2010) (see Fig. 2). The reasons for *H. tomentosus* occurrence in the South-Eastern Baltic and its absence in the adjacent sea areas require further research. Apparently, the species is not recorded there due to the fact that monitoring is carried out mainly in the second half of summer – in the period of maximum macroalgae development when there are no *H. tomentosus* sporophytes.

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REFERENCES

1. Volodina A. A., Gerb M. A. Macrophytes of the seacoast of the South-Eastern part of the Baltic Sea (Kaliningrad Region). *Izvestiya Kaliningradskogo gosudarstvennogo universiteta*, 2013, no. 28, pp. 129–135. (in Russ.)
2. Volodina A. A., Gerb M. A. Macroalgae suggested for inclusion in the new edition of the Red Book of the Kaliningrad region. *Vestnik Baltiiskogo Federal'nogo universiteta im. I. Kanta. Seriya: Estestvennye i meditsinskie nauki*, 2018, no. 3, pp. 74–85. (in Russ.)
3. Zenkevich L. A. *Biologiya morei SSSR*. Moscow : AN SSSR, 1963, 739 p. (in Russ.)
4. Dubravin V. F. *Evolutions of Hydro-meteorological Fields in the Baltic Sea.* Kaliningrad : Kapros, 2014, 438 p. (in Russ.)
5. Minicheva G. G. New invader in the Black Sea: Brown algae *Chorda tomentosa* Lyngb. *Al'gologiya*, 2015, vol. 25, iss. 3, pp. 323–329. (in Russ.). https://doi.org/10.15407/alg25.03.323
6. *AlgaeBase.* World-wide electronic publication, National University of Ireland, Galway / M. D. Guiry, G. M. Guiry (Eds) : [site], 2021. URL: http://www.algaebase.org [accessed: 26.10.2021].
7. Hällfors G., Heikkonen K. *Chorda tomentosa* Lyngbye in Finnish coastal waters. In: *Phycological Studies of Nordic Coastal Waters : A Festschrift Dedicated to Prof. Mats Wærn on His 80th Birthday* / I. Wallentinus, P. Snoeijs (Eds). Uppsala : Opulus Press AB, 1992, pp. 79–84. (Acta Phytogeographica Suecica ; [no.] 78).
8. Kontula T., Fürhapter K. Checklist documentation and distribution data for Baltic Sea macrophyte species [including] Table 7.1: Checklist of Baltic Sea Macrophyte Species. In: *Checklist of Baltic Sea Macro-species* / T. Kontula, J. Haldin (Eds). Helsinki : Helsinki Commission, 2012, pp. 9–12 ; 24–98. (Baltic Sea Environment Proceedings ; no. 130).
9. Kostkevičiene J., Sinkevičienė Z. A preliminary checklist of Lithuanian macroalgae. *Botanica Lithuanica*, 2008, vol. 14, no. 1, pp. 11–27.
10. Labanauskas V. Communities of marine macrophytobenthos along the Lithuanian coastline of the Baltic Sea. *Botanica Lithuanica*, 2000, vol. 6, no. 4, pp. 401–413. (in Lithuanian).
11. Lakowitz K. *Die Algenflora der Danziger Bucht. Ein Beitrag zur Kenntnis der Ostseeflora.* Leipzig : W. Engelmann, 1907, 141 p.
12. Möller T., Kotta J., Martin G. Mapping of structural and functional characteristics of reef habitats. In: *The First International Conference on Biodiversity. BioSciencesWorld 2010* : proceedings, March 7–13, 2010, Cancun, Mexico / P. Dini, M. Popescu (Eds). Los Alamitos ; Washington ; Tokyo : IEEE Computer Science : CPS, 2010, pp. 150–154. https://doi.org/10.1109/BioSciencesWorld.2010.27
13. Pankow H. *Ostsee–Algenflora.* Jena, Germany : G. Fischer Verlag, 1990, 648 p.
14. Pliński M., Surosz W. *Flora Zatoki Gdańskiej i wód przyleglych (Bałtyk Poludniowy).* 6. *Zielenice – Chlorophyta (Red Algae & Brown Algae).* With the English key for the identification to the genus. Gdansk : Wydawnictwo Uniwersytetu Gdańskiego, 2013, 146 p. (in Polish and English).

НАХОДКИ *HALOSIPHON TOMENTOSUS* (LYNGBYE) JAASUND (PHAEOPHYCEAE) В ЮГО-ВОСТОЧНОЙ БАЛТИКЕ

А. А. Володина

Институт океанологии имени П. П. Ширшова РАН, Москва, Российская Федерация
E-mail: *volodina.alexandra@gmail.com*

Первая находка *Halosiphon tomentosus* (Lyngb.) Jaasund (*Chorda tomentosa* Lyngb.) была сделана в юго-восточной части Балтийского моря в мае — июне 2016 г. на северном побережье Самбийского полуострова на нескольких участках. Вид был встречен в верхнем горизонте сублиторали на валунах в ассоциациях преимущественно с зелёными и бурыми водорослями (*Ulva intestinalis*, *Ulva prolifera*, *Cladophora glomerata*, *Ectocarpus siliculosus*, *Pylaiella littoralis*, изредка *Pseudolithoderma subextensum*, *Hildenbrandia rubra*). Также вид был обнаружен в 2017–2018 гг. Длина нитевидного слоевища варьировала от 3 до 30 см, в среднем равняясь (9,2 ± 2,3) см. Средняя биомасса составила (73,3 ± 41,9) г·м$^{-2}$ в 2016 г. и (11 ± 8,8) г·м$^{-2}$ в 2018 г. Причины появления *H. tomentosus* в Юго-Восточной Балтике и его отсутствия в прилегающих районах Балтийского моря требуют дальнейшего изучения.

Ключевые слова: *Halosiphon tomentosus*, Юго-Восточная Балтика, новые местонахождения вида

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CHRONICLE AND INFORMATION

ON THE ANNIVERSARY OF D. SC. VITALY RYABUSHKO

On 15 February, 2022, D. Sc. Vitaly Ryabushko celebrated his 75th birthday – the chief researcher of IBSS department of aquaculture and marine pharmacology.

V. Ryabushko began his scientific career in 1971 in Dalniye Zelentsy (the Murmansk Region) in the underwater research group headed by M. Propp. Since 1974, he worked as the researcher at the Institute of Marine Biology (Vladivostok). He was lucky to participate in numerous sea and land expeditions, to scuba dive into the abyss of the Arctic, Atlantic, Indian, and Pacific oceans, and to explore the depths of the Barents Sea, Sea of Japan, Sea of Okhotsk, South China Sea, and Black Sea. During the research cruise to the Kuril Islands, he dived into a sea volcano crater on the Iturup Island. During the expedition to the Indian Ocean, he became interested in the search for biologically active substances in hydrobionts.

In 1984, Vitaly Ryabushko moved from Vladivostok to Sevastopol at the invitation of the director of IBSS and began to work as the scientific secretary. Currently, he heads the aquaculture and marine pharmacology department. Under his leadership, studies of biologically active compounds in hydrobionts have been developed.

The unique experimental material obtained during underwater research formed the basis of his dissertations and the monograph "The Energy Exchange in Echinoderms (Type Echinodermata)" (2000). This work generalizes little-known original data on the experimental metabolism study in representatives of all classes of echinoderms under natural and laboratory conditions. It is an important contribution to world science.

The name of Vitaly Ryabushko is well known both in scientific circles and business community. The staff of the department he heads applies the latest developments in marine aquaculture – in the technology of bivalve cultivation – and professionally advises businessmen on a wide range of issues.

His success in scientific, methodological, pedagogical, and social activities is determined by high professionalism and constant creative search. V. Ryabushko is the author of more than 300 scientific publications, *inter alia* one authored and two collective monographs, and more than 80 patents in the USSR, Ukraine, and Russia.

Since 2014, he is the chairman of the specialized dissertation council in hydrobiology at IBSS; so far, about 30 PhD theses and D. Sc. dissertations have been defended. Moreover, he is a member of the dissertation council in oceanology at the Murmansk Marine Biological Institute.

Vitaly Ryabushko pays considerable attention to the training of qualified scientific personnel: under his scientific supervision, five researchers defended their PhD theses. Currently, he supervises the preparation for the defense of one D. Sc. dissertation and three PhD theses and professionally advises younger colleagues.

V. Ryabushko serves on editorial boards and councils of four Russian scientific journals. He heads the Sevastopol branch of the Ovchinnikov Russian Biotechnology Society and is the member of the Russian Hydrobiological Society and Russian Professorial Assembly. He is the academician of the Russian Ecological Academy, Petrovsky Academy of Sciences and Arts, and Crimean Academy of Sciences. Vitaly Ryabushko has been awarded many times for fundamental and applied results of his scientific activity – with a diploma from the Chairman of the Leninsky District Administration for his achievements and for being listed on the Board of Honor of the Leninsky District of Sevastopol; a medal of the Russian Ecological Academy "150th Anniversary of the Birth of V. Vernadsky"; two certificates of honor of the President of the Russian Academy of Sciences; four gold medals of the International Salon of Inventions and New Technologies "New Times"; a diploma of the Federal Agency for Fishery; *etc.*

V. Ryabushko is a specialist with high demands on himself, scientific adherence to principles, originality of approaches to research, and ability to generate new ideas, as well as goodwill, modesty, and decency.

Grateful students and colleagues congratulate their leader and mentor, who is an example of professionalism, creativity, and devotion to his work! We wish great success, new victories, and outstanding achievements!

К ЮБИЛЕЮ ДОКТОРА БИОЛОГИЧЕСКИХ НАУК ВИТАЛИЯ ИВАНОВИЧА РЯБУШКО

15 февраля 2022 г. исполнилось 75 лет Виталию Ивановичу Рябушко — д. б. н., гл. н. с. отдела аквакультуры и морской фармакологии ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН». Виталий Иванович является автором более чем 300 научных работ, председателем диссертационного совета по специальности «гидробиология» при ФИЦ ИнБЮМ, академиком Российской экологической академии, Петровской академии наук и искусств и Крымской академии наук.

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ON THE ANNIVERSARY OF D. SC. LARISA RYABUSHKO

On 14 July, 2022, D. Sc. Larisa Ryabushko celebrated her 75th birthday – the chief researcher of IBSS aquaculture and marine pharmacology department.

L. Ryabushko was born on 14 July, 1947, in Gelendzhik (Krasnodar Krai). In 1975, she graduated from the university in Rostov-on-Don. In 1974, she began her scientific and organizational activities at the Institute of Marine Biology of the Far Eastern Scientific Center of the Academy of Sciences of the Soviet Union (Vladivostok). Being a professional museum worker at that time, she was involved in organization of the first marine reserve in the USSR (now, the Far Eastern Marine Biosphere Reserve) and in creation of the Museum of Sea Nature and Its Conservation on the Popov Island (Peter the Great Bay, Sea of Japan). Larisa Ryabushko began her full-fledged scientific activity in 1977 with studying benthic diatoms in the seas of the Far East.

Since 1985, she has been working at IBSS, starting as a senior engineer and now being a chief researcher. Based on the material of her study of benthic diatoms, she successfully defended her PhD thesis “Diatoms of the Upper Sublittoral of the Northwestern Sea of Japan” in 1986. L. Ryabushko has a wide range of scientific interests: it covers investigating systematics, floristics, and ecology of marine microalgae of the Sea of Japan; Black, Aegean, and Mediterranean seas; seas of the Far East; and seas of Antarctica. Samples she has taken (more than 1,000 items) were included in IBSS museum collection. Based on the material of three research cruises on the RV “Akademik A. Kovalevsky” (1989, 1990, and 1993) aimed at studying microphytobenthos of the Zernov *Phyllophora* Field and banks of the Aegean Sea and Mediterranean coast of Egypt, she obtained new scientific results, which were included in her D. Sc. dissertation. She collected and processed extensive material on species diversity of microphytobenthos of the Black Sea coastal zones and dynamics of its structural and functional characteristics. In 2009, Larisa Ryabushko successfully defended her D. Sc. dissertation “Microphytobenthos of the Black Sea” in hydrobiology. In 2013, her monograph of the same name was published. She did a great work on the inventory of microalgae of the Black Sea benthos (including potentially dangerous ones), phytoplankton and microphytobenthos of the Sea of Azov, and the Black Sea benthos.

In 2015–2016, L. Ryabushko, in collaboration with her student A. Begun (Institute of Marine Biology, Vladivostok), published a 2-volume synopsis and atlas “Diatoms of the Microphytobenthos of the Sea of Japan” – with detailed description of size characteristics, ecology, and geography of taxa and with micrographs.

Larisa Ryabushko is the author of more than 220 scientific publications, *inter alia* 10 monographs, 3 of which are collective, and the co-author of 1 patent and 3 database registration certificates. She presented her scientific reports at national and international congresses, symposiums, and conferences in Russia, Ukraine, Belarus, Greece, the Netherlands, Germany, Montenegro, and Turkey.

Since 2011, she has been successfully leading a non-structural group of 9 employees of IBSS aquaculture and marine pharmacology department. They investigate floristics and ecology of planktonic and benthic microalgae, cyanobacteria, and microscopic marine fungi. Under her leadership, the team effectively solves advanced problems in algology.

L. Ryabushko has good scientific and organizational skills. Over the years, she led the grant of the International Foundation For Science, the Ukrainian–Turkish project, and the RFBR grant aimed at studying diatoms and cyanobacteria of periphyton of experimental synthetic polymeric materials in the Black Sea. She makes a significant contribution to scientific activities of the department and IBSS.

Larisa Ryabushko is a member of two dissertation councils – in hydrobiology at IBSS and in oceanology at the Murmansk Marine Biological Institute. She pays considerable attention to the training of researchers. In 2008–2011, she was PhD thesis consultant for a colleague from Institute of Marine Biology (Vladivostok). Under her scientific supervision, four researchers successfully defended their PhD theses. To date, one more work is being prepared.

L. Ryabushko is the member of the Ovchinnikov Russian Biotechnology Society and Russian Professorial Assembly, as well as academician of the Russian Ecological Academy and Petrovsky Academy of Sciences and Arts. She serves on the editorial board of the journal “Issues of Modern Algology” (Moscow).

For her merits in scientific activity, training of researchers, and achievements in hydrobiology, Larisa Ryabushko was awarded a diploma of the President of the Russian Academy of Sciences, as well as several diplomas and certificates of appreciation from the Sevastopol City Council, the Legislative Assembly of Sevastopol, and the Ministry of Science and Higher Education of the Russian Federation.

Students, colleagues, and friends sincerely admire her purposefulness, initiative, high professionalism, and devotion to science; they really appreciate her help and support in work! The staff of IBSS wishes Larisa Ryabushko good health, inexhaustible energy, new scientific achievements, family well-being, and fulfillment of desires!

К ЮБИЛЕЮ ДОКТОРА БИОЛОГИЧЕСКИХ НАУК ЛАРИСЫ ИВАНОВНЫ РЯБУШКО

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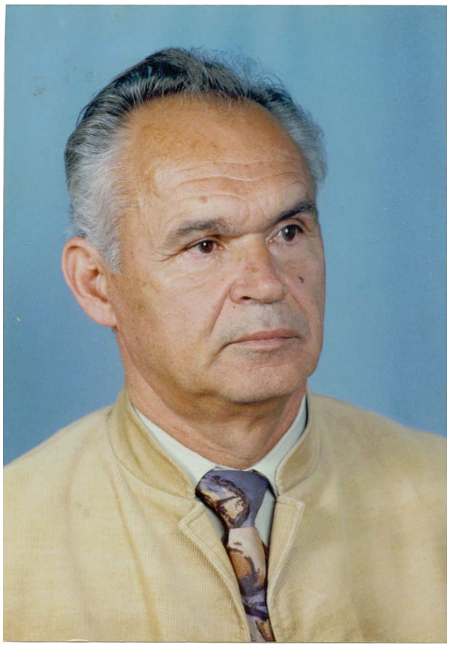
SCIENTIFIC ACTIVITY OF D. SC., PROF. ERNEST SAMYSHEV (TO HIS 85th BIRTHDAY)



On 28 October, 2022, Ernest Samyshev – D. Sc., Prof., the head of IBSS marine ecosystems functioning department – celebrates his anniversary.

E. Samyshev began his scientific activity after graduating from the faculty of ichthyology of the Kaliningrad Technical Institute for the Fishery Industry in 1963. He worked as an assistant and at the same time studied hydrobiology at the PhD graduate school of this institute under the guidance of professor N. Gaevskaya and her deputy, associate professor N. Berezina. In 1965–1969, he participated in the International Program for Investigating the Tropical Atlantic (ISITA) and carried out a wide range of trophoecological and biochemical studies of zooplankton and content of suspended organic matter in different seasons of the year. The obtained results formed the basis of his PhD thesis "Trophological and Biochemical Aspects of the Study of Seston Components in the Tropical Zone of the Eastern Atlantic" defended in 1970 at IBSS of the Academy of Sciences of the Ukrainian SSR. In 1968, he began working at the Atlantic branch of the All-Union Research Institute of Fisheries and Oceanography (AtlantNIRO) and resumed the work of the hydrobiology sector there, which had ceased to function by that time for a number of reasons.

Since 1974, he worked at the Sea of Azov–Black Sea Research Institute of Fisheries and Oceanography (AzCherNIRO) as a head of the fish food base laboratory. Later, it was renamed on his initiative into the hydrobiology laboratory.

In 1979–1988, benthologists of this laboratory under the guidance of Ernest Samyshev carried out fundamental studies which made it possible to assess the negative contribution of three components (eutrophication, soil dumping during dredging, and bottom fishing causing destruction of bottom biocenoses and resedimentation) to the transformation of various biocenoses. Despite attempts to counteract, E. Samyshev showed civil courage, publicizing these results at scientific forums, and finally achieved a ban on the barbaric method of sprat fishing in the Black Sea and commercial mussel fishing in the Black Sea with dredges.

He paid special attention to research in the Antarctic. It was he who organized the world's first comprehensive monitoring in the Cooperation Sea area (1976–1987). This allowed to study the main structural and functional characteristics of the Antarctic krill and other key components of the community in its range, as well as to assess their functional role and to analyze scale of reproduction and assimilation of matter and energy in the pelagic zone. Ernest Samyshev's conclusion on the significant trophic imbalance of the Antarctic pelagic community is very important for science.

The results of AzCherNIRO research in the Antarctic, carried out under his guidance, served as the basis for his D. Sc. dissertation "Antarctic Krill and the Structure of the Plankton Community in Its Range" defended in 1987 at the Institute of Oceanology of the Academy of Sciences of the Soviet Union. By decision of the specialized council, the dissertation was published in 1991 by the "Nauka" publishing house.

In 1988–1990, under the guidance of E. Samyshev (deputy director for research at AzCherNIRO and head of the mariculture department), the technology for breeding so-iuy mullet in the Black Sea was developed and implemented.

His scientific and organizational activities during years of work at AzCherNIRO are impressive. He was expert (1974–1983) and member of the bureau (1984–1989) of the Ichthyological Commission of the USSR Ministry of Fisheries; scientific consultant of the Mariculture Council under the State Committee for Science and Technology (1988–1990); member of the working group on Antarctic krill of the Scientific Committee on Antarctic Research (SCAR) (1990–1991); and permanent head of the Kerch branch of the All-Union Hydrobiological Society (1975–1989).

In 1990, Ernest Samyshev began to work as a chief researcher of the marine ecosystems functioning department at IBSS. Since 1991, he heads this department. In the same 1990, under his guidance and with his participation, a comprehensive monitoring of Donuzlav Lake was carried out. Its results were used in the biological justification for the development of this lake for fish breeding and commercial cultivation of so-iuy mullet and European flounder.

He took part in the development and implementation of the program of a unique experiment to study the annual cycle of the main components of the Black Sea pelagic ecosystem. This experiment was carried out in 1992–1993 by the staff of the Ukrainian Scientific Center of Ecology of Sea (Odesa) of the Ministry of Environmental Protection, with the involvement of IBSS specialists.

While working at IBSS, he continued his Antarctic research. Professor Samyshev became the author of projects on bioresources in the National Antarctic Scientific Program of Ukraine (1996–2000, 2001–2010), scientific supervisor of biological research in the Ukrainian Antarctic expeditions (1997, 1998, 2002), and author and scientific supervisor of monitoring projects of the current state of coastal ecosystems in the Ukrainian Antarctic station "Akademik Vernadsky" area. Based on this environmental monitoring, a holistic view of the structural organization of the aquatic ecosystem was obtained for the poorly studied coast of the Argentine Islands archipelago adjacent to the station.

Ernest Samyshev calculated the nutritional requirements of the main heterotrophs – bacterioplankton, ciliates, mesozooplankton, krill, and Thaliacea – in the Antarctic plankton under different scenarios for the Atlantic sector of the Antarctic. The results of long-term studies of the Antarctic ecosystem under his guidance

E. Samyshev at the highest point of Galindez Island (Argentine Islands archipelago, Atlantic sector of the Antarctic) during Soviet and Ukrainian expeditions are presented in the monograph “Structural and Functional Organization of the Antarctic Plankton” prepared for publication. In general, the data on his Antarctic research formed a theoretical basis for further study and understanding of the Antarctic ecosystem functioning.

Professor Samyshev is a productive supervisor for many young researchers. Under his scientific guidance, 10 PhD theses were defended. It is worth quoting here the fragment of the review in support of the nomination of Ernest Samyshev as a corresponding member of the National Academy of Sciences of Ukraine in 2012. The review was written by his student, now a professor at the University of British Columbia (Vancouver, Canada) and director of the Institute for the Oceans and Fisheries – Evgeny Pakhomov. “To sum up professor Samyshev’s accomplishments over the past three decades, I would like to point out that most of his works either broke the foundations and habitual ideas, or shook them. He is undoubtedly an innovator in many ways; at the same time, his conclusions are based on the most solid, reproducible data. The novelty and merits of his research have been tested and are undeniable, and this is well known among researchers within the countries of the former Soviet Union and abroad. In general, and this is by no means an exaggeration, I consider professor Samyshev one of the best (perhaps, in the top ten) biological oceanographers and marine ecologists in the world.”

Dear Ernest Samyshev, we wish you good health, cheerfulness, happiness, new achievements, and creative successes!

Leading researcher of IBSS marine ecosystems functioning department,
PhD N. Minkina

О НАУЧНОЙ ДЕЯТЕЛЬНОСТИ Д. Б. Н., ПРОФ. ЭРНЕСТА ЗАЙНУЛЛИНОВИЧА САМЫШЕВА (К 85-ЛЕТИЮ СО ДНЯ РОЖДЕНИЯ)

28 октября 2022 г. свой юбилей отметил Эрнест Зайнуллинович Самышев — руководитель отдела функционирования морских экосистем ФИЦ ИнБЮМ, доктор биологических наук, профессор, автор более чем 270 научных работ, лауреат Государственной премии Украины в области науки и техники 2007 г. Сфера его научных интересов — гидробиология, экология, биоценология, антропогенная трансформация морских экосистем, марикультура рыб и беспозвоночных.

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CENTENARY OF THE RESEARCH VESSEL "PERSEY"

The research vessel "Persey" was built a hundred years ago. It is a vessel with an interesting fate and a tragic end. First of all, it is the first Soviet vessel designed for marine scientific research. But it has another important historical hypostasis: it is the first vessel of the Knipovich Polar Research Institute of Marine Fisheries and Oceanography (PINRO).

There were two PINRO predecessors, two institutes – Plavmornin and GOIN. Plavmornin (the Floating Marine Research Institute) was formed by the Government Decree signed on 10 March, 1921, by V. I. Lenin, the head of state. The main task of the institute was to carry out constant, systematic, and planned fisheries research in the northern seas. After a series of civil upheavals (World War I, the October Revolution, and Russian Civil War), in February 1920, when Soviet power was established in the North, the industry of the Kola Peninsula was destroyed, with fisheries being dramatically affected. After the revolution, Russia was politically isolated, and the country needed to show its presence in the Arctic and to begin economic development of the Barents Sea biological resources.

The scientific base of Plavmornin was located in Moscow, while the expeditionary one was located in Arkhangelsk, and it was there that "Persey" was built. The vessel construction is a separate layer of history. The RV was built in the hull of an unfinished wooden hunting schooner (an Arkhangelsk fisherman E. V. Moguchy began to construct it back in 1917). A unique research vessel was built in a short time. It had seven scientific laboratories, winches for various purposes, trawl equipment, and navigation instruments. The national flag was raised on 7 November, 1922. Prince V. M. Golitsyn, one of the first employees of the institute, created the expeditionary flag of the ship based on the constellation Perseus. Later, the blue seven-star flag became the official emblem of PINRO.

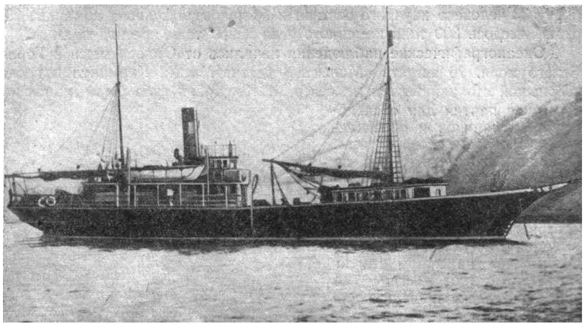
The expedition activity of the RV "Persey" began in 1923. For a long time, it was the only vessel of PINRO, and its deployment on the freezing White Sea created certain difficulties for the institute to work efficiently. Specifically, only after the White Sea was free from ice, it was possible to reach the Barents Sea.

In 1929, Plavmornin and the Murmansk Biological Station were merged – a new institute was created in the city of Aleksandrovsk. GOIN (the State Oceanographic Institute) was located on the coast of the non-freezing Ekaterininskaya Harbor of the Kola Bay (the Barents Sea). In 1934, after several reorganizations in the industry, a new institute began its work in Murmansk, with an abbreviation widely known in fisheries science – PINRO.

The RV "Persey" served the needs of science from 1923 to 1941. The ship was sunk on 10 July, 1941, during a German aerial attack, in the Eina Bay (the Motovsky Bay), when she was carrying military cargo for the defenders of the Rybachy Peninsula. Over 18 years of its scientific activity, "Persey" completed 90 research cruises to the White, Barents, Kara, and Greenland seas and determined the destiny of many people. The participants of the cruises later became academicians and corresponding members of the USSR Academy of Sciences, D. Sc., heads of large institutions, and founders of scientific directions. Such well-known researchers as I. I. Mesyatsev, N. A. Maslov, Yu. Yu. Marty, M. M. Adrov, I. G. Yudanov, N. N. Zubov, L. A. Zenkevich, S. A. Zernov, M. V. Klenova, A. I. Rossolimo, A. A. Shorygin, V. K. Soldatov, V. V. Shuleikin and many others worked on board the vessel.

For the history of fisheries science, the RV "Persey" is a legendary ship. Articles and books were written about her; poems and songs were composed; and the anniversaries were celebrated. The cruises aboard the RV "Persey" laid a solid foundation for continuity for future generations of researchers.

To discover more about the history of fisheries research cruises and about the RV "Persey", you can turn on the video (see "Suplemetary" tab: https://marine-biology.ru/mbj/article/view/378). The narrator is Tatiana Pashkova – leading specialist of PINRO scientific and organizational department and Honorary Worker of Fisheries of Russia.



K. M. Sokolov, T. E. Pashkova, and A. N. Benzik, Polar branch of "VNIRO"
("PINRO" named after N. M. Knipovich)

СТОЛЕТНИЙ ЮБИЛЕЙ НАУЧНО-ИССЛЕДОВАТЕЛЬСКОГО СУДНА «ПЕРСЕЙ»

В 1922 г. было построено НИС «Персей» — первое советское судно, созданное специально для морских научных исследований, и первый корабль Полярного научно-исследовательского института морского рыбного хозяйства и океанографии имени Н. М. Книповича.

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IN MEMORIAM: YURI MORDVINOV (17.06.1937 – 18.09.2022)

On 18 September, 2022, D. Sc. Yuri Mordvinov passed away at the age of 86. He worked at the A. O. Kovalevsky Institute of Biology of the Southern Seas for many years.

Yu. Mordvinov was born in Baku in 1937. All his life and scientific activity were connected with the sea. After finishing school in 1952, he entered the Azerbaijan Fishery College. Having received a diploma in ichthyology and fish farming, he was assigned to the Baltgosrybtrest Expeditionary Fishing Department (Kaliningrad). In 1959–1964, he studied at the Kaliningrad Technical Institute for the Fishery Industry. In 1965, Yuri Mordvinov entered the PhD graduate school at IBSS (Sevastopol) specializing in ichthyology (fish functional morphology). After graduating, he began his scientific career in the nekton department. In 1969, he defended his PhD thesis “Functional Foundations of the External Structure of Pinnipeds.” Since 1977, he worked as a senior researcher in the nekton department. On the initiative of its head, D. Sc. Yuri Aleyev, the nekton department was transformed in 1980 into the theory of life forms department. Later, it was headed by Yu. Mordvinov. Since 1993, he headed the ecomorphology structural laboratory. In 1996, together with his colleagues, he moved to the shelf ecosystems department and worked there for about seven years. In 1994, he defended his D. Sc. dissertation “Functional Morphology of Aquatic Birds and Semi-aquatic Mammals.”

In his scientific activity, three main directions can be distinguished – functional morphology, biohydrodynamics, and ecomorphology. Those are related to the analysis of the system of morphological adaptations of animals representing various taxonomic ranks. Yuri Mordvinov studied the main patterns of formation and development of nekton adaptations in secondary aquatic vertebrates – reptiles, birds, and mammals. Diverse experiments were aimed at determining parameters of the total hydrodynamic resistance and were carried out in a large biohydrodynamic channel at IBSS both with living objects and model ones. The scope of his scientific interests also covered research of ecological and morphological features of birds of varying degrees of specialization for inhabiting aquatic environment considering their swimming, flight, and terrestrial locomotion. Obtained results contributed much to the solution of several fundamental problems of ecology and evolution.

Over the years of his scientific activity, Yu. Mordvinov both carried out experimental work and conducted field studies. He took part in expeditions to the Far East, Dalniye Zelentsy, Commander Islands, Bermuda, and Antarctic, as well as to the Voronezh and Gasan-Kuli (now Hazar) reserves.

The results of his research are reflected in 70 scientific publications and summarized in the monograph “Functional Morphology of Swimming Birds and Semi-aquatic Mammals” (1984). Yury Mordvinov was the speaker at numerous conferences and symposiums, participated in the integrated interdepartmental program of research of the USSR Ministry of the Fisheries on marine mammals, and for many years was a member of IBSS specialized scientific council. Moreover, he supervised diploma projects in biology sharing his knowledge and experience with students from various universities, was the chairman of the State Examination Board at the faculty of natural sciences at the Simferopol State University, and advised younger colleagues at IBSS.

Both in science and life, Yuri Mordvinov was an active, principled, straightforward, and at the same time benevolent person. The bright memory of a talented scientist and a reliable friend will forever remain in our hearts.

Colleagues

ПАМЯТИ ЮРИЯ ЕГОРОВИЧА МОРДВИНОВА (17.06.1937 – 18.09.2022)

18 сентября 2022 г. ушёл из жизни д. б. н. Юрий Егорович Мордвинов. Он изучал основные закономерности становления и развития нектонных адаптаций у вторичноводных позвоночных из классов рептилий, птиц и млекопитающих и опубликовал 70 научных работ.

Учредитель и издатель журнала:
Федеральное государственное бюджетное учреждение науки
Федеральный исследовательский центр
«Институт биологии южных морей
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