1871
ИнБЮМ – IBSS

ISSN 2499-9768 print

МОРСКОЙ БИОЛОГИЧЕСКИЙ ЖУРНАЛ

MARINE BIOLOGICAL JOURNAL

Vol. 6 No. 1

2021

МОРСКОЙ БИОЛОГИЧЕСКИЙ ЖУРНАЛ
MARINE BIOLOGICAL JOURNAL
включён в перечень рецензируемых научных изданий, рекомендованных ВАК Российской Федерации.
Журнал реферируется международной библиографической и реферативной базой данных Scopus (Elsevier),
международной информационной системой по водным наукам и рыболовству ASFA (ProQuest),
Всероссийским институтом научно-технической информации (ВИНИТИ),
а также Российским индексом научного цитирования (РИНЦ) на базе Научной электронной библиотеки elibrary.ru.
Все материалы проходят независимое двойное слепое рецензирование.

Редакционная коллегия

Главный редактор
Егоров В. Н., акад. РАН, д. б. н., проф., ФИЦ ИнБЮМ
Заместитель главного редактора
Солдатов А. А., д. б. н., проф., ФИЦ ИнБЮМ
Ответственный секретарь
Корнийчук Ю. М., к. б. н., ФИЦ ИнБЮМ

Адрианов А. В., акад. РАН, д. б. н., проф., ИБМ ДВО РАН
Азовский А. И., д. б. н., проф., МГУ
Генкал С. И., д. б. н., проф., ИБВВ РАН
Денисенко С. Г., д. б. н., ЗИН РАН
Довгаль И. В., д. б. н., проф., ФИЦ ИнБЮМ
Зуев Г. В., д. б. н., проф., ФИЦ ИнБЮМ
Коновалов С. К., чл.-корр. РАН, д. г. н., ФИЦ МГИ
Мильчакова Н. А., к. б. н., ФИЦ ИнБЮМ
Миронов О. Г., д. б. н., проф., ФИЦ ИнБЮМ
Неврова Е. Л., д. б. н., ФИЦ ИнБЮМ
Празукин А. В., д. б. н., ФИЦ ИнБЮМ
Руднева И. И., д. б. н., проф., ФИЦ ИнБЮМ
Рябушко В. И., д. б. н., ФИЦ ИнБЮМ
Самышев Э. З., д. б. н., проф., ФИЦ ИнБЮМ
Совга Е. Е., д. г. н., проф., ФИЦ МГИ
Трапезников А. В., д. б. н., ИЭРиЖ УрО РАН
Финенко З. З., д. б. н., проф., ФИЦ ИнБЮМ
Arvanitidis Chr., D. Sc., HCMR, Greece
Bat L., D. Sc., Prof., Sinop University, Turkey
Ben Souissi J., D. Sc., Prof., INAT, Tunis
Kociolek J. P., D. Sc., Prof., CU, USA
Magni P., PhD, CNR-IAS, Italy
Moncheva S., D. Sc., Prof., IO BAS, Bulgaria
Pešić V., D. Sc., Prof., University of Montenegro, Montenegro
Zaharia T., D. Sc., NIMRD, Romania

Editorial Board

Editor-in-Chief
Egorov V. N., Acad. of RAS, D. Sc., Prof., IBSS
Assistant Editor
Soldatov A. A., D. Sc., Prof., IBSS
Managing Editor
Kornyychuk Yu. M., PhD, IBSS

Adrianov A. V., Acad. of RAS, D. Sc., Prof., IMB FEB RAS, Russia
Arvanitidis Chr., D. Sc., HCMR, Greece
Azovsky A. I., D. Sc., Prof., MSU, Russia
Bat L., D. Sc., Prof., Sinop University, Turkey
Ben Souissi J., D. Sc., Prof., INAT, Tunis
Denisenko S. G., D. Sc., ZIN, Russia
Dovgal I. V., D. Sc., Prof., IBSS
Finenko Z. Z., D. Sc., Prof., IBSS
Genkal S. I., D. Sc., Prof., IBIW RAS, Russia
Kociolek J. P., D. Sc., Prof., CU, USA
Konovalov S. K., Corr. Member of RAS, D. Sc., Prof., MHI RAS, Russia
Magni P., PhD, CNR-IAS, Italy
Milchakova N. A., PhD, IBSS
Mironov O. G., D. Sc., Prof., IBSS
Moncheva S., D. Sc., Prof., IO BAS, Bulgaria
Nevrova E. L., D. Sc., IBSS
Pešić V., D. Sc., Prof., University of Montenegro, Montenegro
Prazukin A. V., D. Sc., IBSS
Rudneva I. I., D. Sc., Prof., IBSS
Ryabushko V. I., D. Sc., IBSS
Samyshev E. Z., D. Sc., Prof., IBSS
Sovga E. E., D. Sc., Prof., MHI RAS, Russia
Trapeznikov A. V., D. Sc., IPAE UB RAS, Russia
Zaharia T., D. Sc., NIMRD, Romania
Zuyev G. V., D. Sc., Prof., IBSS

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://mbj.marine-research.org.

Адрес соиздателя:

Зоологический институт РАН.
Университетская наб., 1, Санкт-Петербург, 199034, РФ.

Founder, Publisher, and Editorial Office address:

A. O. Kovalevsky Institute of Biology of the Southern Seas
of Russian Academy of Sciences.
2 Nakhimov ave., Sevastopol, 299011, Russia.
Tel.: +7 8692 54-41-10.
E-mail: *mbj@imbr-ras.ru*.
Journal website: https://mbj.marine-research.org.

Co-publisher address:

Zoological Institute Russian Academy of Sciences.
1 Universitetskaya emb., Saint Petersburg, 199034, Russia.

© ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН», 2021
© Зоологический институт РАН, 2021

A. O. KOVALEVSKY INSTITUTE OF BIOLOGY OF THE SOUTHERN SEAS OF RAS
ZOOLOGICAL INSTITUTE OF RAS

МОРСКОЙ БИОЛОГИЧЕСКИЙ ЖУРНАЛ

MARINE BIOLOGICAL JOURNAL

2021 Vol. 6 no. 1

Established in February 2016 SCIENTIFIC JOURNAL *4 issues per year*

CONTENTS

Scientific communications

Chronicle and information

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 1, pp. 3–16
https://doi.org/10.21072/mbj.2021.06.1.01

SCIENTIFIC COMMUNICATIONS

UDC [551.464.621:581.13](265.54.04)

PECULIARITIES OF TEMPORAL VARIABILITY OF DISSOLVED OXYGEN CONTENT IN EELGRASS *ZOSTERA MARINA* LINNAEUS, 1753 MEADOWS IN THE VOEVODA BAY (THE AMUR BAY, THE SEA OF JAPAN)

© 2021 **Yu. A. Barabanshchikov[1], P. Ya. Tishchenko[1], P. Yu. Semkin[1], V. I. Zvalinsky[1], T. A. Mikhailik[2], and P. P. Tishchenko[1]**

[1]V. I. Il'ichev Pacific Oceanological Institute FEB RAS, Vladivostok, Russian Federation
[2]Saint Petersburg branch of VNIRO (GosNIORKh named after L. S. Berg),
Saint Petersburg, Russian Federation
E-mail: *biw90@mail.ru*

Received by the Editor 14.08.2020; after reviewing 10.11.2020;
accepted for publication 11.03.2021; published online 23.03.2021.

Currently, the shallow basins with *Zostera marina* L. meadows are considered as absorbers of atmospheric carbon dioxide, capable of restraining an increase in its concentration. Due to its high primary productivity, eelgrass releases a large amount of oxygen into the environment. To establish the peculiarities of production activity in shallow-water basins, covered with *Z. marina* meadows, we conducted monitoring of hydrological and production indicators with different measurement intervals on the example of the Voevoda Bay (the Amur Bay, the Sea of Japan). Observations were carried out for eight and a half months (22.09.2012–07.06.2013). Measurements of temperature, salinity, chlorophyll fluorescence, and turbidity were carried out in *Z. marina* meadows at a depth of 4 m every three hours by a Water Quality Monitor hydrological station. Dissolved oxygen content was determined every hour by an optical oxygen sensor ARO-USB. Two types of oxygen concentration variability were established: 1) seasonal variability, mostly resulting from seasonal variations in the environment; 2) daily variability during the freeze-up period, mostly determined by the intensity of photosynthetically active radiation penetration into sub-ice water. In the autumn season, low oxygen concentrations, up to hypoxic level, were recorded. In the winter and spring seasons, the oxygen content was, as a rule, at 100–130 % of saturation. High daily variability was observed during the freeze-up period, with no snow coverage. In February, the range of daily fluctuations of oxygen concentration reached 730 $\mu mol \cdot kg^{-1}$, with 3-fold supersaturation regarding atmospheric O_2. As established, the maximum rate of oxygen production, relative to 1 g of *Z. marina* wet weight, is 6.5 mg $O_2 \cdot h^{-1} \cdot g^{-1}$. High daily dynamics of oxygen in seawater is analyzed in relation to eelgrass physiological peculiarities (air lacunae play an important role in oxygen dynamics in the environment), as well as to short-period tides.

Keywords: *Zostera marina* L., macrophytes, dissolved oxygen, Voevoda Bay, Amur Bay, Sea of Japan

To date, there is ample evidence that global changes, such as deoxygenation and acidification, are taking place in the World Ocean (Dore et al., 2009 ; Schmodtko et al., 2017). These changes affect both coastal waters and the open areas of the oceans, and they are explained from different positions.

Most researchers relate global changes in the open ocean to warming, resulting in reducing the oxygen solubility and leading to water stagnation, *i. e.* a decrease in the rate of oxygen transfer from the surface horizons to the deeper ocean layers. Changes in coastal waters are mainly explained by eutrophication (their nutrient enrichment), as well as by an increase in the intensity of production and destruction of organic matter (De Jonge et al., 2002). The acidification of the World Ocean is usually related to an increase in carbon dioxide concentration in the atmosphere (Dore et al., 2009).

Shallow-water basins with *Zostera marina* Linnaeus, 1753 meadows are considered as absorbers of atmospheric carbon dioxide, capable of restraining an increase in its concentration due to eelgrass high productivity (Blue Carbon…, 2009). Obviously, as a result of *Z. marina* photosynthetic activity, not only carbon dioxide is absorbed and organic matter is formed (vegetative and generative shoots and rhizomes), but also oxygen is released into the environment; it is confirmed by direct observations of oxygen production, the gross values of which can reach 2 mg $O_2 \cdot h^{-1}$ per 1 gram of wet weight (Berger, 2011). It was previously established that biogeochemical processes in the Voevoda Bay (the Amur Bay, the Sea of Japan), where eelgrass meadows grow, are determined by the seasonal variability of the production/destruction of organic matter (Barabanshchikov et al., 2015). It was assumed that in the winter season, the intensity of production processes at the bottom is higher than at the surface due to *Z. marina*. The predominance of production activity in the bottom horizons in the winter period causes an increase in oxygen concentration and a decrease in the partial pressure of carbon dioxide at the bottom compared to that of the surface layer waters (Barabanshchikov et al., 2015). It was also found that eelgrass production activity has both seasonal variability and daily one (Kharlamenko et al., 1994).

The aim of this work is to establish the peculiarities of the production activity manifestation of shallow-water basins, covered with *Z. marina* meadows, on the example of the Voevoda Bay, by monitoring hydrological and production indicators with different measurement intervals.

MATERIAL AND METHODS

The Voevoda Bay is located in the eastern seaward area of the Amur Bay (Peter the Great Bay, the Sea of Japan), deeply going into the western coast of Russky Island (Fig. 1).

The Voevoda Bay in its northern area includes the Kruglaya and Melkovodnaya bays. Water area of the Voevoda Bay is about 4 km^2, of the Melkovodnaya Bay – about 2.4 km^2 (Lotsiya severo-zapadnogo…, 1996). The shores, adjacent to the entrance capes of the bays, are elevated and deep; in the inner area, they are low. Off the low-lying shores, the coastal area is shallow; depths of 10 m are usually about 1 km from the coast. The bottom is rocky; off the low-lying shores, it is sandy and sandy-silty. From the east, the Russkaya River flows into the bay; this river is about 6 km long and is fed mainly by rain; the flow rate varies 0.2 to 2.0 $m^3 \cdot s^{-1}$ throughout the year. In the Voevoda Bay, 6 sites were revealed, covered with *Z. marina* meadows, which are located in the upper sublittoral at depths of no more than 10 m and have silty and sandy-silty soils. *Z. marina* meadows are located in calm, wave-protected areas: in the southern shallow waters of the Voevoda, Kruglaya, and Melkovodnaya bays (Fig. 1). The largest areas of eelgrass meadows were registered in the tail-end sites of the Kruglaya and Melkovodnaya bays, with an area of 900×400 m and 1000×500 m, respectively. In this water area, the density of *Z. marina* biomass reaches 3 kg of wet weight per 1 m^2 with a projective cover of 100 %. On average, for the entire Voevoda Bay, eelgrass biomass was of 0.5–1.5 kg of wet weight per 1 m^2 (Barabanshchikov et al., 2018).

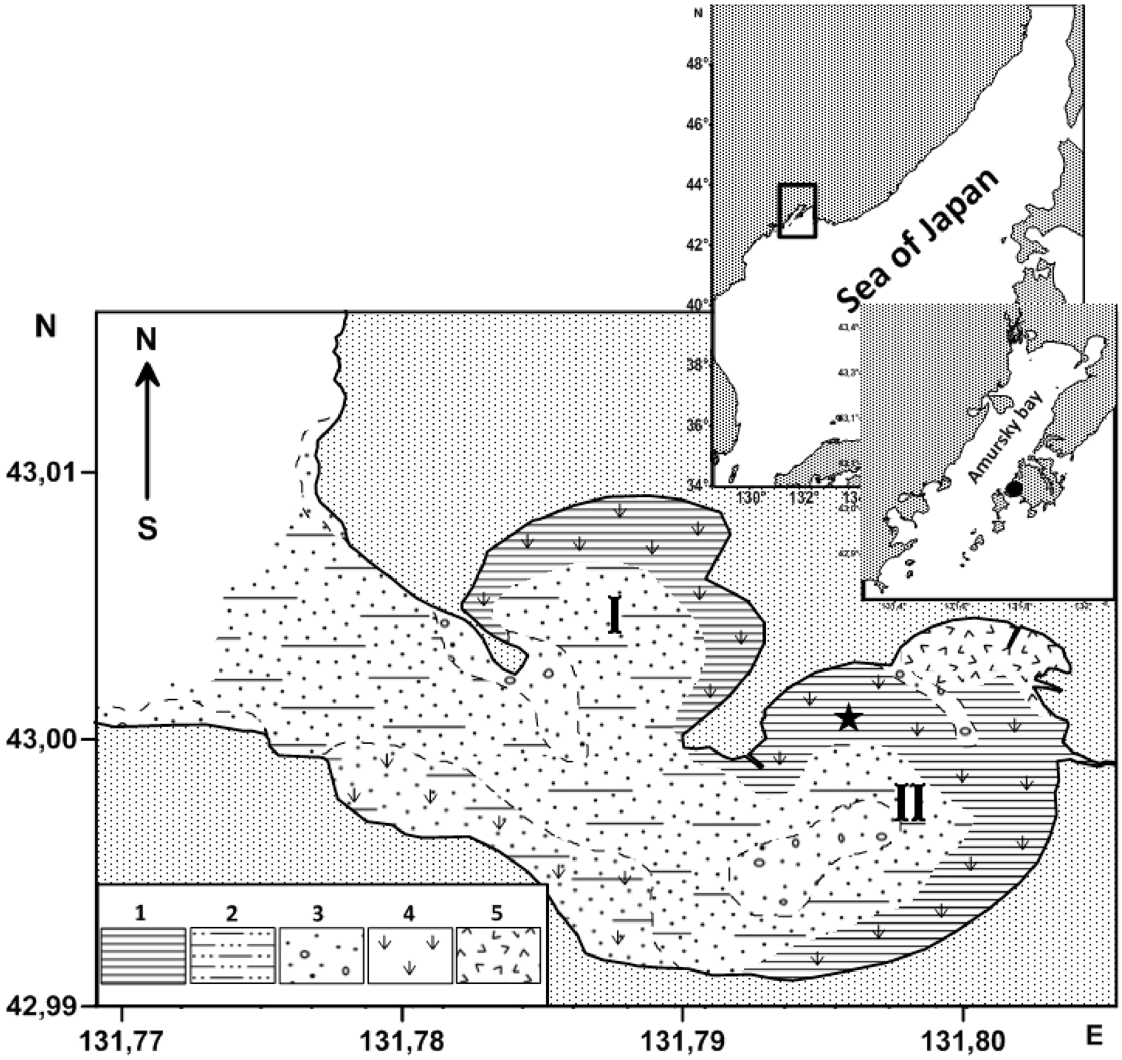


Fig. 1. Map of bottom landscapes and bottom biocenoses in the Voevoda Bay: I – the Kruglaya Bay, II – the Melkovodnaya Bay; 1 – clayey silt; 2 – clayey silt, sandstone; 3 – sand, stone; 4 – *Zostera marina* L. meadows; 5 – oyster *Crassostrea gigas* colony; ★ – Water Quality Monitor hydrological station setting point

A Water Quality Monitor hydrological station (WQM, Wet Labs) was set in the Melkovodnaya Bay (see Fig. 1) at a depth of 4 m for the period 22.09.2012–07.06.2013. The coordinates were 42°59.978′N, 131°47.659′E. The station was equipped with sensors of pressure, temperature, electrical conductivity, chlorophyll fluorescence, and turbidity. The WQM station was equipped with an automatic system for flushing the sensors with a fluid for biofouling destruction and for cleaning the optical windows. At the WQM station, a fast-response optical oxygen sensor ARO-USB (Japan) was installed. Its readings were compared with those obtained by an automated version of the Winkler method (Talley et al., 2004) within the temperature range of +5…+29 °C. The introduced correction depended on temperature; the readings of the oxygen sensor were corrected in accordance with the empirical relation:

$$[O_2]_{cor}(ml \cdot l^{-1}) = [O_2]_{meas} - 1.075 + 0.04 \cdot t\,, \tag{1}$$

where $[O_2]_{cor}$ and $[O_2]_{meas}$ are corrected and measured oxygen concentrations, $mL \cdot L^{-1}$;

t is temperature, °C.

When obtaining the relation (1), nine measurements were used; $R^2 = 0.97$.

For eight and a half months, every 3 hours, the readings of the sensors of temperature, salinity, chlorophyll *a* fluorescence, and turbidity were recorded for 5 minutes with an interval of 1 second. The ARO-USB sensor recorded oxygen concentration at 1-hour interval. For each day, the average values of the sensor readings were obtained.

To analyze the results, the intensity (I) of photosynthetically active radiation, falling on the ice surface, was estimated for February 2013. Its value, depending on the season and astronomical characteristics, was calculated for a cloudless sky by the formula:

$$[I] = [I_0] \cdot \sin(h) \,, \tag{2}$$

where h is the Sun height above the horizon;

$[I_0]$ is solar constant when the Sun is at its zenith, $[I_0] \approx 1670$ μmol·m^{-2}·s^{-1} (Kirk, 1992).

The Sun height h for the Northern Hemisphere was determined by the basic astronomical formula (Barteneva et al., 1971):

$$sin(h) = cos(\phi) \cdot cos(\delta) \cdot cos(\tau) + sin(\phi) \cdot sin(\delta) \,, \tag{3}$$

where φ is geographic latitude;

δ is declination, δ = 23°×cos(2π×(N+9)/365) (N is the ordinal number of the day of the year, starting from 1 January);

τ is hour angle, τ = 2π×t/24 (t is time of the day, hour).

RESULTS

Seasonal variability of bottom water hydrological indicators of the Voevoda Bay at the WQM station for the period 22.09.2012–07.06.2013, based on the daily average results (Fig. 2), is typical for the latitudes studied.

When setting the station, the bottom water temperature was about +20 °C, and the salinity was about 31.5 ‰. The characteristic features of the autumn season are a decrease in water temperature and significant fluctuations in salinity, caused by coastal freshwater runoff and strong wind mixing of water. From 9 December to 2 April, the bay is covered with ice (this interval is marked with red vertical lines in Fig. 2). During the freeze-up period, there are no small-scale temperature and salinity fluctuations, since there is no wind mixing. Temperature of the bottom water during this period is close to the freezing point. Salinity of the bottom water increases December to early March, reaching 34.20 ‰, which is associated with ice formation. From the beginning of March until the ice cover break-up, a decrease in salinity is observed, resulting from ice melting in the daytime [its salinity is about 6 ‰ (Zvalinsky et al., 2010)] and meltwater inflow through the ice pores into ice water. After the ice cover break-up, a seasonal increase in temperature occurs, as well as a decrease in salinity, accompanied by fluctuations in these indicators due to wind mixing.

The results of measurements of dissolved oxygen content and the saturation degree in the bottom water with the ARO-USB sensor are shown in Fig. 3a and b, respectively. The saturation degree was calculated by the equation, proposed in (Weiss, 1970). The results obtained demonstrate two types of oxygen concentration variability and its saturation degree: 1) long-term variability due to seasonal variations in the environment; 2) short-term (daily) variability. It should be noted that long-term variability in the water saturation degree with oxygen slightly deviate from the state of 100 % of saturation, with the exception of the autumn season and January 2013. Short-term variations are, as a rule, positive deviations regarding the "baseline" of long-term ones. In our case, the "baseline" is of 100–130 % of the oxygen saturation degree (Fig. 3b). The range of these variations can be extremely wide, reaching in some cases 3-fold water supersaturation regarding atmospheric oxygen.

Temporal variability of chlorophyll fluorescence and turbidity (Fig. 3c, d) demonstrates two seasonal peaks: in November and in early April, at the time of ice cover break-up in the bay. The results obtained confirm the fact of spring and autumn phytoplankton bloom, which is typical for the Sea of Japan open water area (Yamada & Ishizaka, 2005) and for its coastal area (Tishchenko et al., 2020a). It should be noted that the autumn phytoplankton bloom did not affect the oxygen content (see Fig. 3). On the contrary, the spring phytoplankton bloom caused positive fluctuations in the O_2 concentration. This phenomenon was accompanied by a high turbidity (see Fig. 3d), which was obviously caused by the inflow of aerosol, accumulated on the ice surface in the winter season, during the period of ice cover break-up and its intensive melting.

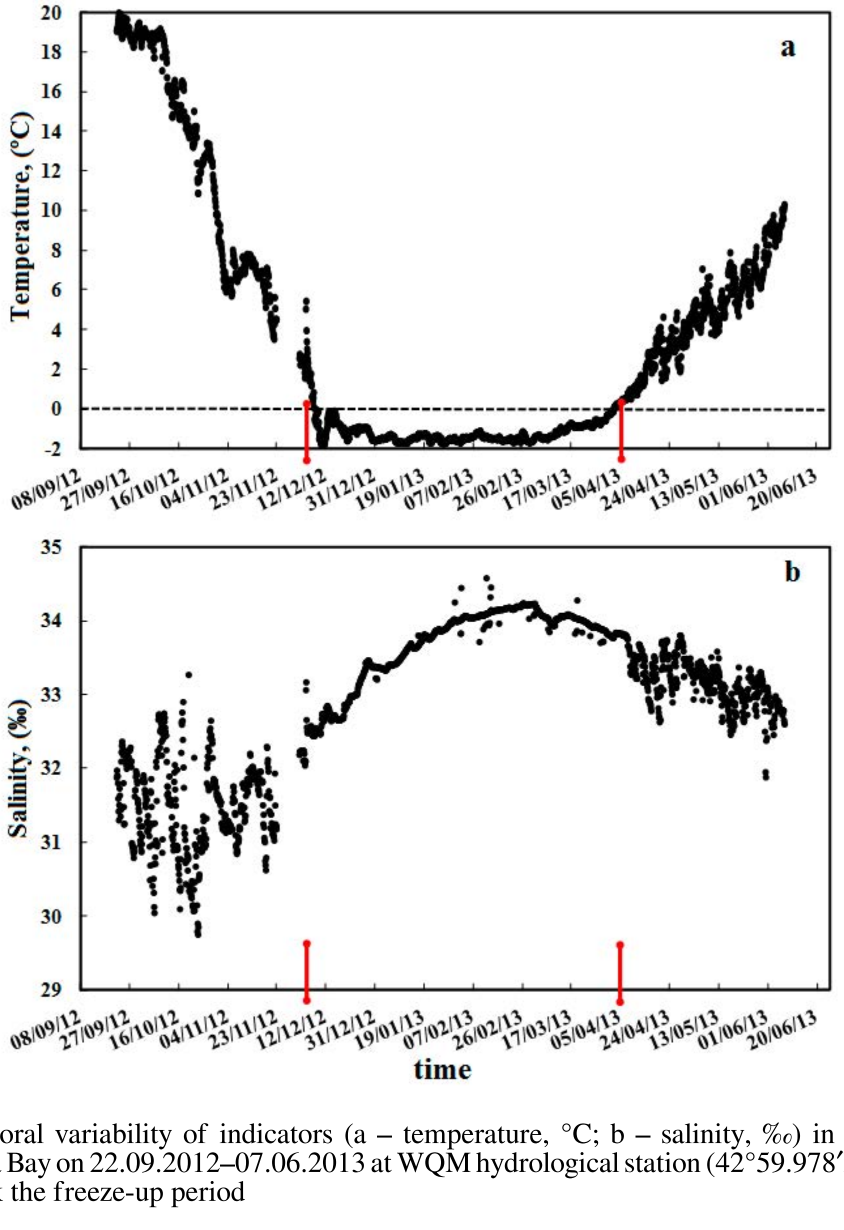


Fig. 2. Temporal variability of indicators (a – temperature, °C; b – salinity, ‰) in the bottom water of the Voevoda Bay on 22.09.2012–07.06.2013 at WQM hydrological station (42°59.978′N, 131°47.659′E). Red lines mark the freeze-up period

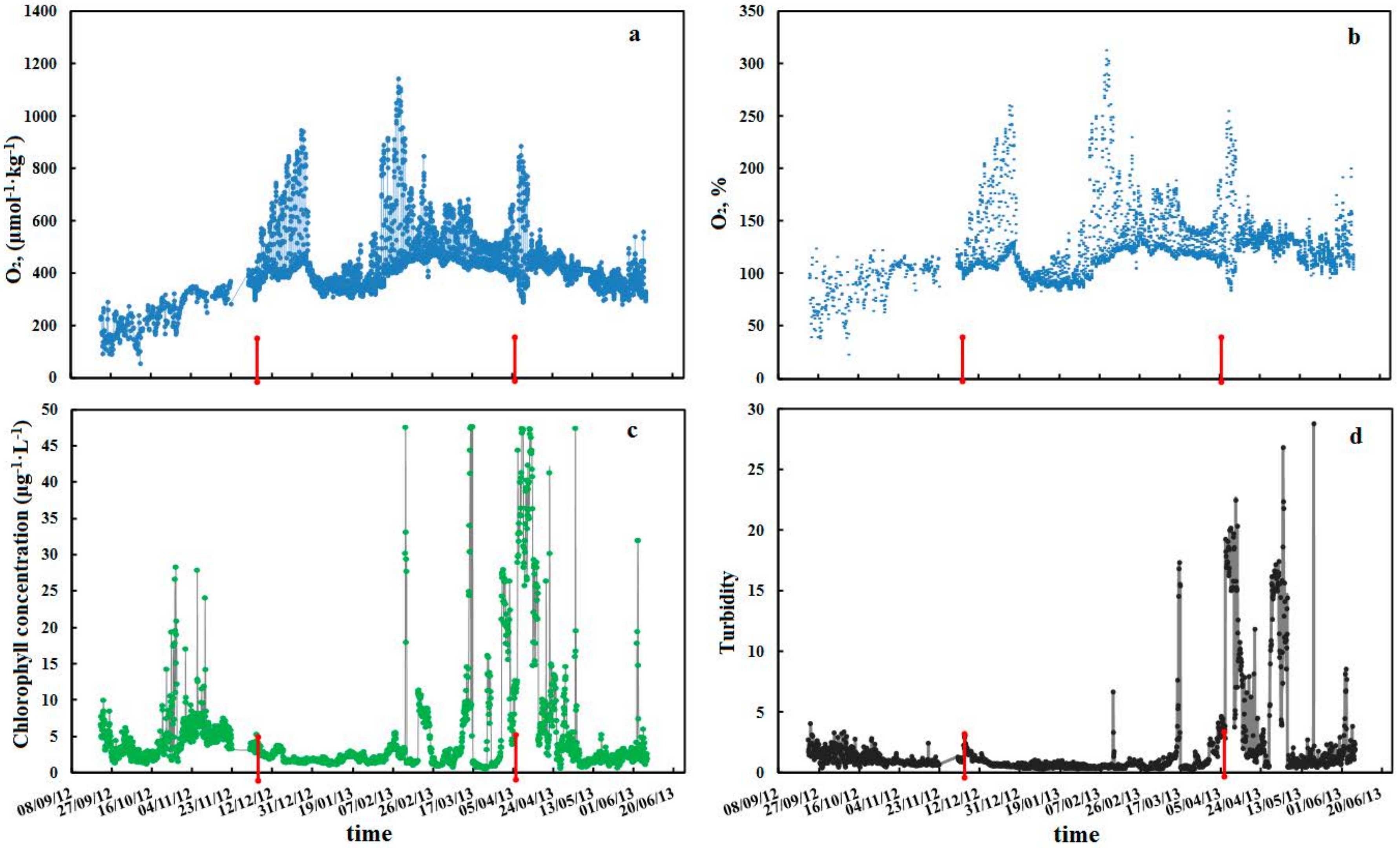


Fig. 3. Temporal variability of indicators (a – dissolved oxygen content, μmol·kg^{-1}; b – oxygen saturation degree, %; c – chlorophyll concentration, μg·L^{-1}; d – turbidity, Formazin Turbidity Unit, FTU) in the bottom water of the Voevoda Bay on 22.09.2012–07.06.2013 at WQM hydrological station (42°59.978′N, 131°47.659′E). Red lines mark the freeze-up period

It was found that diurnal anomalies in the oxygen content were related to changes in daylight (Fig. 4a). In our opinion, diurnal variations in the dissolved oxygen concentration (Fig. 4b) were associated with the photosynthetic activity of *Z. marina* and its physiological characteristics. The ranges of diurnal variations in the seawater saturation degree with oxygen were approximately 2–2.7 times higher than the values of the "baseline" (see Fig. 3b). Similar amplitudes of diurnal variations in the water saturation degree with oxygen in *Z. marina* meadows have been registered earlier (Long et al., 2019). The difference between our data and the results of the work, cited above, is in the value of the O_2 concentration of the "baselines". According to our measurements, this value is in the range of 100–130 % of saturation; in (Long et al., 2019), it is in the range of 50–75 % of saturation.

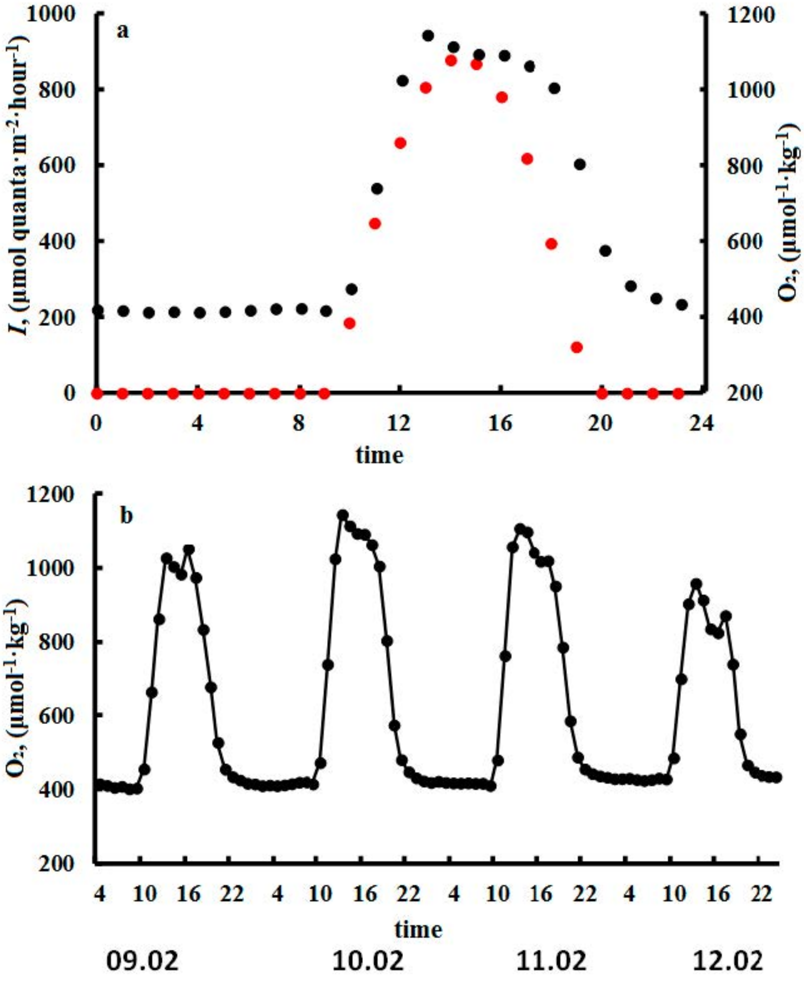


Fig. 4. Daily variability in the Voevoda Bay: a – light intensity, I, μmol quanta·m^{-2}·h^{-1} (red dots) and oxygen concentration, μmol·kg^{-1} (black dots) (10.02.2013); b – oxygen concentration, μmol·kg^{-1} (09.02.2013–12.02.2013)

DISCUSSION

An important peculiarity of *Z. marina* is its high production capacity: the average "net production" is 4.8 g C·m^{-2}·day^{-1} (McRoy, 1974). Eelgrass high productivity forms conditions for water supersaturation regarding atmospheric oxygen, up to the formation of air bubbles with an increased O_2 content on the surface of *Z. marina* leaves (Long et al., 2019). On the other hand, *Z. marina* high productivity results in the formation of silty sediments with significant concentrations of organic matter (Van Katwijk et al., 2010). Organic matter diagenesis in sediments in the areas of eelgrass growth leads to sulfate reduction, which can cause the meadows' die-off (Pedersen et al., 2004). The study of the Voevoda Bay sediment confirmed that sulfate reduction is an important manifestation of early organic matter diagenesis in this basin (Tishchenko et al., 2020a, b). In other words, the reduced sediments of the bay remove oxygen from the aquatic environment. Therefore, the long-term variability of the oxygen content in water is determined by the ratios of fluxes due to the oxygen production in the environment (photosynthesis), oxygen invasion/evasion at the water – atmosphere interface, and oxygen absorption by sediments.

At the beginning of monitoring (September 2012), the O_2 content was low: about 160 $\mu mol \cdot kg^{-1}$, or 60 % of saturation. High temperature and intensive decomposition of generative *Z. marina* leaves, intensively shedding in late July – early August (Lysenko, 1985 ; Paimeeva, 1984), were the main reasons for the low oxygen concentration and water saturation degree. Fluctuations in salinity and the O_2 content (see Figs 2, 3) were caused by wind mixing, which is a common phenomenon in late September – early October and leads to increased oxygen absorption by sediments. The minimum O_2 concentration (56 $\mu mol \cdot kg^{-1}$, or 24 %), corresponding to hypoxia, was recorded on 10 October. Then, an intensive increase in its content began; by early November, concentration increased up to 350 $\mu mol \cdot kg^{-1}$, or 110 % of saturation. A sharp decrease in temperature during this period (+18 to +6 °C) determined an increase in oxygen solubility and, accordingly, an increase in the atmospheric oxygen inflow into the aquatic environment. After generative leaves shedding, the production of *Z. marina* vegetative leaves increased (Lysenko, 1985), which also led to an increase in the O_2 concentration and even to water supersaturation with oxygen. The autumn peak in chlorophyll content (see Fig. 3c) did not affect the O_2 concentration. This fact indicates as follows: the oxygen flux, caused by *Z. marina* production/destruction at the water – atmosphere and water – bottom interface, was significantly higher than the oxygen production, caused by phytoplankton bloom. In December, immediately after the freeze-up period, high-amplitude positive fluctuations of oxygen were registered. The oxygen concentration (the "baseline") was slightly above 100 % of saturation and tended to increase up to 132 %, or 478 $\mu mol \cdot kg^{-1}$. During the freeze-up period, ice eliminated wind mixing and limited the effect of the atmosphere on the O_2 content in water; therefore, its production, resulting from sub-ice photosynthesis, and its removal by reduced sediments maintained the oxygen balance in water. Previously, we found that for ice with no snow coverage (60 cm thick), the flux of photosynthetically active radiation was sufficient to ensure sub-ice photosynthesis for all the Voevoda Bay depths (Barabanshchikov et al., 2015). It is obvious that in December 2012, the oxygen photosynthetic production dominated the respiratory processes and the O_2 removal by reduced sediments. According to the weather website (WindGURU, 2020), there was snow on 30 December (up to 3.3 mm in 3 hours), which led to a sharp decrease in the flux of photosynthetically active radiation into the water column. The oxygen concentration level began to decrease and reached values of 312–360 $\mu mol \cdot kg^{-1}$, or about 90 % of saturation (see Fig. 3a, b). At the end of January, the snow on the ice surface began to disappear, the "baseline" again reached 100 % and even slightly exceeded it. The oxygen content in sub-ice water in the period February to March (before ice cover break-up) was at the level of 103–133 %; at the same time, positive anomalies of O_2 concentration were observed in the daytime (Fig. 4). According to the weather website (WindGURU, 2020), there was rain on 6 April (up to 0.9 mm in 3 hours), and strong north wind (13 $m \cdot s^{-1}$) was blowing. The wind and positive air temperatures led to both ice cover break-up and fast ice break-up.

The ice cover break-up and melting resulted in a sharp change in the illumination of water, causing a peak of phytoplankton bloom and an anomalous O_2 concentration. During this period, negative oxygen anomalies were also observed, which seemed to be associated with the beginning of the wind movement of water in the Voevoda Bay and an increase in the rate of the O_2 removal by reduced sediments. The subsequent spring decrease in oxygen concentration was mainly due to a seasonal increase in temperature; however, water remained supersaturated (about 120 %) regarding atmospheric oxygen until the end of monitoring.

To estimate the maximum rate of O_2 production, the maximum diurnal oxygen anomaly on 10.02.2013 was chosen (see Fig. 4b). For the interval 9:00–13:00, an empirical fourth-order relationship of oxygen concentration on time was obtained. A time derivative of this relationship gave the rate of an increase in the O_2 content in the aquatic environment – 307 $\text{mmol}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$. By calculation method for average depth of the Melkovodnaya Bay (2 m), the production rate of 614 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ was obtained. Obviously, this rate value depends on the density of *Z. marina* meadows (in our case, 3 kg of wet weight per 1 m^2) (Barabanshchikov et al., 2018). As a result, the maximum oxygen production rate per 1 g of *Z. marina* wet weight was obtained – 6.5 mg $O_2\cdot\text{h}^{-1}\cdot\text{g}^{-1}$; it was approximately three times higher compared to that from (Berger, 2011). The resulting rate value corresponded to 11:00 local time.

A similar calculation for the descending branch of the oxygen anomaly gave the maximum rate of oxygen “removal” from the aquatic environment, relative to 1 g of *Z. marina* wet weight: −5.0 mg $O_2\cdot\text{h}^{-1}\cdot\text{g}^{-1}$; it corresponded to 19:00 local time. This high rate of the O_2 “removal” from the environment is surprising, since *Z. marina* and other seagrass use oxygen for respiration in the range of 10–30 % of the maximum production value (Borum et al., 2007). In our case, the “removal” was 76 % of the production. We use quotation marks to indicate the rate, at which the oxygen concentration decreases, because we believe this value to be apparent and be the result of complex processes, caused by *Z. marina* anatomical and physiological peculiarities, as well as short-term changes in the external environment (tides).

Zostera marina is a higher vascular perennial plant with a rhizome, on which vegetative and generative shoots develop (Paimeeva, 1984). One of eelgrass anatomical features is the air lacunae in its leaves, rhizome, and roots. *Z. marina* physiological peculiarities make it possible to accumulate oxygen in the air lacunae in the leaves due to photosynthesis, to transport oxygen to the roots, and to provide an aerobic microenvironment in the immediate vicinity of the roots in anaerobic sediments (Borum et al., 2007). The oxygen release into the aquatic environment by vascular plants can be provided by two mechanisms: diffusion and bubble formation (Colmer, 2003). We assume that at the initial stage, with the onset of daytime, photosynthesis in *Z. marina* leaf cells increases the partial pressure of oxygen in the air lacunae. This process causes oxygen diffusion into the seawater. By 11:00, the partial pressure of oxygen in seawater reaches 2-fold water supersaturation regarding the basic oxygen supersaturation, with its value being higher in the air lacunae. Direct measurements of the partial pressure of oxygen in the air lacunae indicate a value of more than 200 % (Sand-Jensen et al., 1985). We assume that in this state, *Z. marina* leaves release oxygen-enriched air bubbles, that float to the inner ice surface and go into the ice pores and further into the atmosphere. A similar possibility of gas exchange between the atmosphere and sea ice has been demonstrated for carbon dioxide (Semiletov et al., 2004). By 13:00, the process of gas transfer becomes dominant; therefore, further photosynthesis does not lead to an increase in oxygen concentration in seawater. It should be noted that irregular semidiurnal fluctuations of the water level with an amplitude of 30–50 cm are characteristic of Peter the Great Bay, including the Voevoda Bay (Supranovich & Yakunin, 1976). The values of hydrostatic pressure, registered by the WQM station from 9 February to 12 February 2013, indicate that the maximum amplitude of the anomalies for the studied bay is 46 mbar (Fig. 5), which is consistent with the previously presented data (Supranovich & Yakunin, 1976).

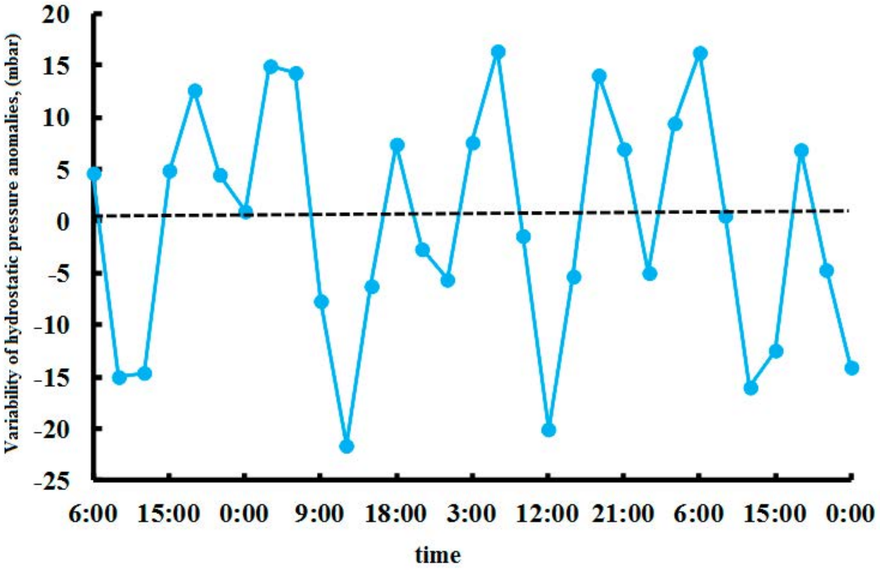


Fig. 5. Variability of hydrostatic pressure anomalies, mbar, in the Voevoda Bay for 09.02.2013–12.02.2013

Tides can be described as short-term variability in the environment, capable of influencing the O_2 concentration in seawater, since hydrostatic pressure anomalies affect the partial pressure of oxygen in *Z. marina* air lacunae. From 06:00 to 12:00, the Voevoda Bay experiences a low tide, during which negative hydrostatic pressure anomalies arise, causing the oxygen transfer from *Z. marina* into the seawater *via* the bubble mechanism (Long et al., 2019). From 06:00 to 9:00 on 10.02.2013, the positive anomaly (14.3 mbar) changed to a negative anomaly (−7.7 mbar) due to the low tide (see Fig. 5). During this time interval, the O_2 concentration in the aquatic environment practically did not change. With the onset of daylight (about 09:00), a sharp increase in the oxygen content began. The maximum oxygen supersaturation (314 %) was recorded on 10.02.2013 at 13:00 (see Fig. 3b), and the maximum rate of an increase in the O_2 concentration with 2-fold supersaturation of water regarding air oxygen was registered at 11:00. We assume that after 11:00, due to water supersaturation with oxygen and a negative hydrostatic pressure anomaly (−21.6 mbar at 12:00), oxygen was released from *Z. marina* into the marine environment *via* the bubble mechanism. From 12:00 to 18:00, an increase in hydrostatic pressure was recorded, which pushed air bubbles with an increased oxygen content from seawater into the ice pores and further into the atmosphere. After noon, photosynthesis weakens, the intensity of photosynthetically active radiation decreased (see Fig. 4a); these factors, in combination with increasing hydrostatic pressure, contribute to the oxygen flow from seawater to *Z. marina* aerenchyma and then along the air lacunae to the roots and sediments (Borum et al., 2007). The internal oxygen flow through the air lacunae in the eelgrass can be both diffusional and supramolecular. For example, the internal air flow rate for a reed (also a vascular plant) reaches 50 $mL \cdot min^{-1}$ (Colmer, 2003). As established, oxygen, supplied by *Z. marina* to the reduced sediments of the bay, is spent on the oxidation of hydrogen sulfide and sulfides (Borum et al., 2007); accordingly, the observed rate of oxygen removal cannot be used to assess the rate of its consumption for eelgrass respiration. It should also be noted that an almost unchanged level of oxygen concentration is observed at night (see Fig. 4b). The rate of oxygen consumption for *Z. marina* respiration is 20 % of the maximum production (Borum et al., 2007). For the existing population density of the eelgrass meadows in the Voevoda Bay, the rate of oxygen consumption was of -61 mmol·m^{-3}·h^{-1}. Such a rate had to be recorded by the oxygen sensor, and then we could observe a decrease in its content in water at night, but it was not the case. Therefore, it was assumed that the amount of oxygen in the air lacunae is quite high, and they play the role of a buffer system in maintaining a stable oxygen concentration at night.

Conclusion. Two types of oxygen concentration variability in the Voevoda Bay environment have been established: 1) long-term variability, caused by seasonal variations in the environment; 2) daily variability during the freeze-up period, determined by the intensity of photosynthetically active radiation penetration into sub-ice water. In the autumn period, low O_2 concentrations, up to hypoxic level, were observed; in the winter and spring seasons, the oxygen content was recorded at 100–130 % of saturation. High daily variability was registered during the freeze-up period, with no snow coverage. In February, the amplitude of daily fluctuations in the O_2 concentration reached 730 μmol·kg^{-1}, with 3-fold supersaturation regarding atmospheric oxygen. It was established that the maximum rate of oxygen production, relative to 1 g of *Z. marina* wet weight, is 6.5 mg O_2·h^{-1}·g^{-1}. The daily dynamics of oxygen in seawater is affected by eelgrass physiological peculiarities: air lacunae in its shoots can play the role of a buffer system in maintaining a stable oxygen concentration at night. Semidiurnal tides in the water area of the bay, affecting the air lacunae, increase or decrease the oxygen flow from *Z. marina* into the seawater in the corresponding period of low tide or high tide.

This work has been carried out with the financial support of the RFBR grant (No. 20-05-00381-a) and fundamental scientific research programs (topics AAAA-A17-117030110042-2 and AAAA-A17-117030110038-5).

REFERENCES

1. Barabanshchikov Yu. A., Tishchenko P. Ya., Semkin P. Yu., Volkova T. I., Zvalinsky V. I., Mikhailik T. A., Sagalaev S. G., Sergeev A. F., Tishchenko P. P., Shvetsova M. G., Shkirnikova E. M. Seasonal hydrological and hydrochemical surveys in the Voevoda Bay (Amur Bay, Japan Sea). *Izvestiya TINRO*, 2015, vol. 180, pp. 161–178. (in Russ.)
2. Barabanshchikov Yu. A., Tishchenko P. Ya., Semkin P. Yu., Mikhailik T. A., Kosyanenko A. A. Conditions of forming for therapeutic mud in the Voevoda Bay (Amur Bay, Japan Sea). *Izvestiya TINRO*, 2018, vol. 192, pp. 167–176. (in Russ.). https://doi.org/10.26428/1606-9919-2018-192-167-176
3. Barteneva O. D., Polyakova E. A., Rusin N. P. *Rezhim estestvennoi osveshchennosti na territorii SSSR*. Leningrad : Gidrometeoizdat, 1971, 239 p. (in Russ.)
4. Berger V. Ya. Production of eel grass *Zostera marina* Linnaeus, 1753 in the White Sea. *Biologiya morya*, 2011, vol. 37, no. 5, pp. 362–366. (in Russ.). https://doi.org/10.1134/S1063074011050038
5. Zvalinsky V. I., Mar'yash A. A., Stonik I. V., Shvetsova M. G., Sagalaev S. G., Begun A. A., Tishchenko P. Ya. Production and hydrochemical characteristics of ice, under-ice water and sediments in the Razdolnaya River estuary (Amursky Bay, Sea of Japan)

during the ice cover period. *Biologiya morya*, 2010, vol. 36, no. 3, pp. 186–195. (in Russ.). https://doi.org/10.1134/S106307401004005X
6. *Lotsiya severo-zapadnogo berega Yaponskogo morya.* Saint Petersburg : GUNIO MO, 1996, 360 p. (in Russ.)
7. Lysenko V. N. *Produktsiya makrobentosa soobshchestva Zostera marina v severo-zapadnoy chasti Yaponskogo morya.* [dissertation]. Vladivostok, 1985, 187 p. (in Russ.)
8. Paimeeva L. G. *Biologiya Zostera marina L. i Zostera asiatica Miki Primor'ya.* [dissertation]. Vladivostok, 1984, 183 p. (in Russ.)
9. Supranovich T. I., Yakunin L. P. *Gidrologiya zaliva Petra Velikogo.* Leningrad : Gidrometeoizdat, 1976, 198 p. (in Russ.
10. Tishchenko P. Ya., Medvedev Ye. V., Barabanshchikov Yu. A., Pavlova G. Yu., Sagalayev S. G., Tishchenko P. P., Shvetsova M. G., Shkirnikova Ye. M., Ulanova O. A., Tibenko Ye. Yu., Orekhova N. A. Organic carbon and carbonate system in the bottom sediments of shallow bights of the Peter the Great Bay (Sea of Japan). *Geokhimiya*, 2020a, vol. 65, no. 6, pp. 583–598. (in Russ.). https://doi.org/10.31857/S001675252005012X
11. Tishchenko P. Ya., Khodorenko N. D., Barabanshchikov Yu. A., Volkova T. I., Mar'yash A. A., Mikhaylik T. A., Pavlova G. Yu., Sagalayev S. G., Semkin P. Yu., Tishchenko P. P., Shvetsova M. G., Shkirnikova Ye. M. Diagenesis of organic matter in eelgrass (*Zostera marina* L.) vegetated sediments. *Okeanologiya*, 2020b, vol. 60, no. 3, pp. 393–406. (in Russ.). https://doi.org/10.31857/S0030157420020112
12. Kharlamenko V. I., Lysenko V. N. Produktsionnyye protsessy i rol' mikrogeterotrofov v soobshchestve zostery. In: *Ekosistemy issledovanii pribrezhnogo soobshchestva zaliva Petra Velikogo.* Vladivostok : DVO AN SSSR, 1994, pp. 6–16. (in Russ.)
13. *Blue Carbon. The Role of Healthy Oceans in Binding Carbon : A Rapid Response Assessment* / C. Nellemann, E. Corcoran, C. M. Duarte, L. Valdés, C. De Young, L. Fonseca, G. Grimsditch (Eds). Birkeland : Trykkeri AS, 2009, 80 p. (GRID-Arendal, United Nations Environment Programme).
14. Borum J., Sand-Jensen K., Binzer T., Pedersen O., Greve T. M. Oxygen movement in seagrasses. In: *Seagrasses: Biology, Ecology and Conservation* / A. W. D. Larkum, R. J. Orth, C. M. Duarte (Eds). Dordrecht : Springer, 2007, chap. 10, pp. 255–270. https://doi.org/10.1007/978-1-4020-2983-7_10
15. Colmer T. D. Long-distance transport of gases in plants: A perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell & Environment*, 2003, vol. 26, iss. 1, pp. 17–36. https://doi.org/10.1046/j.1365-3040.2003.00846.x
16. De Jonge V. N., Elliot M., Orive E. Causes, historical development, effects and future challenges of a common environmental problem: Eutrophication. *Hydrobiologia*, 2002, vol. 475/476, pp. 1–19. https://doi.org/10.1023/A:1020366418295
17. Dore J. E., Lukas R., Sadler D. W., Church M. J., Karl D. M. Physical and biochemical modulation of ocean acidification in the central North Pacific. *Proceedings of National Academy of Sciences of the United States of America*, 2009, vol. 106, no. 30, pp. 12235–12240. https://doi.org/10.1073/pnas.0906044106
18. Kirk J. T. The nature and measurement of the light environment in the ocean. In: *Primary Productivity and Biogeochemical Cycles in the Sea* / P. G. Falkowski, A. D. Woodhead, K. Vivirito (Eds). New York : Plenum Press, 1992, pp. 9–29. https://doi.org/10.1007/978-1-4899-0762-2_2
19. Long M. H., Sutherland K., Wankel S. D., Burdige D. J., Zimmerman R. C. Ebullition of oxygen from seagrasses under supersaturated conditions. *Limnology and Oceanography*, 2019, vol. 65, iss. 2, pp. 314–324. https://doi.org/10.1002/lno.11299
20. McRoy C. P. Seagrass productivity: Carbon uptake experiments in eelgrass, *Zostera marina*. *Aquaculture*, 1974, vol. 4, pp. 131–137. https://doi.org/10.1016/0044-8486(74)90028-3
21. Pedersen O., Binzer T., Borum J. Sulphide intrusion in eelgrass (*Zostera marina* L.). *Plant, Cell & Environment*, 2004, vol. 27, iss. 5, pp. 595–602. https://doi.org/10.1111/j.1365-3040.2004.01173.x

22. Sand-Jensen K., Revsbech N. P., Jørgensen B. B. Microprofiles of oxygen in epiphyte communities on submerged macrophytes. *Marine Biology*, 1985, vol. 89, iss. 1, pp. 55–62. https://doi.org/10.1007/BF00392877
23. Schmodtko S., Stramma L., Visbeck M. Decline in global oceanic oxygen content during the past five decades. *Nature*, 2017, vol. 542, pp. 335–339. https://doi.org/10.1038/nature21399
24. Semiletov I., Makshtas A., Akasofu S.-I., Andreas E. L. Atmospheric CO_2 balance: The role of Arctic sea ice. *Geophysical Research Letters*, 2004, vol. 31, iss. 5, art. L05121 (4 p.). https://doi.org/10.1029/2003GL017996
25. Talley L. D., Tishchenko P., Luchin V., Nedashkovskiy A., Sagalaev S., Kang D.-J., Warner W., Min D.-H. Atlas of Japan (East) Sea hydrographic properties in summer, 1999. *Progress in Oceanography*, 2004, vol. 61, iss. 2–4, pp. 277–348. https://doi.org/10.1016/j.pocean.2004.06.011
26. Tishchenko P. Ya., Tishchenko P. P., Lobanov V. B., Mikhaylik T. A., Sergeev A. F., Semkin P. Yu., Shvetsova M. G. Impact of the transboundary Razdolnaya and Tumannaya rivers on deoxygenation of the Peter the Great Bay (Sea of Japan). *Estuarine, Coastal and Shelf Science*, 2020c, vol. 239, art. no. 106731 (12 p.). https://doi.org/10.1016/j.ecss.2020.106731
27. Van Katwijk M. M., Bos A. R., Hermus D. C. R., Suykerbuyk W. Sediment modification by seagrass beds: Muddification and sandification induced by plant cover and environmental conditions. *Estuarine, Coastal and Shelf Science*, 2010, vol. 89, iss. 2, pp. 175–181. https://doi.org/10.1016/j.ecss.2010.06.008
28. Weiss R. F. The solubility of nitrogen, oxygen and argon in water and seawater. *Deep Sea Research and Oceanographic Abstracts*, 1970, vol. 17, iss. 4, pp. 721–735. https://doi.org/10.1016/0011-7471(70)90037-9
29. Yamada K., Ishizaka J., Nagata H. Spatial and temporal variability of satellite estimated primary production in the Japan Sea from 1998 to 2002. *Journal of Oceanography*, 2005, vol. 61, pp. 857–869. https://doi.org/10.1007/s10872-006-0005-2
30. *WindGURU* [weather archive] : site. United States, 2020. URL: http://old.windguru.cz [accessed: 20.07.2020].

ОСОБЕННОСТИ ВРЕМЕННОЙ ИЗМЕНЧИВОСТИ СОДЕРЖАНИЯ КИСЛОРОДА В ЗАРОСЛЯХ *ZOSTERA MARINA* LINNAEUS, 1753 В БУХТЕ ВОЕВОДА (АМУРСКИЙ ЗАЛИВ, ЯПОНСКОЕ МОРЕ)

Ю. А. Барабанщиков[1], П. Я. Тищенко[1], П. Ю. Семкин[1], В. И. Звалинский[1], Т. А. Михайлик[2], П. П. Тищенко[1]

[1]Тихоокеанский океанологический институт имени В. И. Ильичёва ДВО РАН,
Владивосток, Российская Федерация
[2]Санкт-Петербургский филиал ВНИРО (ГосНИОРХ имени Л. С. Берга),
Санкт-Петербург, Российская Федерация
E-mail: *biw90@mail.ru*

В настоящее время мелководные бассейны с лугами *Zostera marina* L. рассматривают в качестве поглотителей атмосферного углекислого газа, способных сдерживать увеличение его концентрации. Благодаря своей высокой продуктивности взморник выделяет в среду большое количество кислорода. Для установления особенностей проявления продукционной активности мелководных бассейнов, покрытых лугами *Z. marina*, на примере бухты Воевода (Амурский залив, Японское море) проведён мониторинг гидрологических и продукционных параметров с различными интервалами измерений. Наблюдения продолжались 8,5 мес. (22.09.2012–07.06.2013). В зарослях *Z. marina* на глубине 4 м измеряли температуру, солёность, флуоресценцию хлорофилла и мутность с помощью гидрологической станции Water Quality Monitor с интервалом 3 часа. Малоинерционным оптическим кислородным датчиком ARO-USB определяли концентрацию растворённого кислорода с интервалом 1 час. Установлено два типа изменчивости содержания кислорода в среде: 1) долгопериодная изменчивость, обусловленная сезонными изменениями среды; 2) суточная в период ледостава, определяемая интенсивностью проникновения фотосинтетически активной радиации в подлёдную воду. В осенний сезон отмечены низкие концентрации кислорода, достигающие уровня гипоксии. Для зимнего и весеннего периодов содержание кислорода находилось, как правило, на уровне 100–130 % от насыщения. Высокую суточную изменчивость наблюдали в период ледостава, в отсутствие снега. В феврале амплитуда суточных колебаний концентрации кислорода достигала 730 мкмоль·кг$^{-1}$, с трёхкратным пересыщением по отношению к атмосферному O_2. Установлено, что максимальная скорость продукции кислорода, отнесённая к 1 г сырой массы *Z. marina*, составляет 6,5 мг O_2·час$^{-1}$·г$^{-1}$. Суточная динамика кислорода в морской воде проанализирована в связи с физиологическими особенностями *Z. marina* (воздухоносные полости в побегах играют важную роль в динамике кислорода в среде), а также в связи с короткопериодными приливами.

Ключевые слова: *Zostera marina* L., макрофиты, растворённый кислород, бухта Воевода, Амурский залив, Японское море

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 1, pp. 17–33
https://doi.org/10.21072/mbj.2021.06.1.02

UDC 574.65

STUDY OF FOLLING COMMUNITIES SUCCESSION UNDER CONDITIONS OF THE DEVICE OF CONTROLLED WATER FLOW

© 2021 **A. Yu. Zvyagintsev[1], S. I. Maslennikov[1], A. K. Tsvetnikov[2], A. A. Begun[1], and N. I. Grigoryeva[1]**

[1]A. V. Zhirmunsky National Scientific Center of Marine Biology, FEB RAS, Vladivostok, Russian Federation
[2]Institute of Chemistry, FEB RAS, Vladivostok, Russian Federation
E-mail: *ayzvyagin@gmail.com*

Received by the Editor 16.03.2020; after reviewing 29.07.2020;
accepted for publication 11.03.2021; published online 23.03.2021.

For testing the anticorrosive and antifouling protective coatings, a ground stand is developed: the device of controlled water flow. The relevance of the study is undeniable, given the practical significance of the problem. The stand is connected to the main of sea running water. The device makes it possible to imitate the motion of aqueous flow around the vessel, thus simulating the conditions of moving amphibious facility. The aim of this work is to present for the first time the new device, created by us, which received a positive decision of Rospatent (Federal Service for Intellectual Property). For two months, full-scale field tests were carried out. They have showed essential qualitative and quantitative differences in the composition of fouling communities on the experimental plates, placed into the device of controlled water flow and suspended in the water column on the pier of the Zapad marine biological station of the National Scientific Center of Marine Biology, FEB RAS. Benthic diatoms predominate in the periphyton community under the conditions of the device of controlled water flow; there is practically no zoofouling. Phytocenosis of green algae, which is common for a vessel variable loadline or a hydraulic structure drainage zone, is presented on the plates from the open bay. The efficiency of using the device of controlled water flow, created by us, is shown for studying the patterns of formation of the fouling communities in different hydrodynamic flows. The main practical conclusion is that the device can be used to verify the properties of protective coatings on the substrates tested, *inter alia* antifouling and anticorrosive coatings.

Keywords: device, controlled flow, fouling community, green algae, diatoms

The study of formation of fouling communities is one of the necessary aspects of identifying the patterns of their functioning. In world practice, a detailed description of methods for investigating the periphyton of experimental plates is possible only in a large analytical review; its compilation is not the aim of this work. The importance of analyzing the dynamic aspect of the fouling communities of operating vessels is obvious, since the problem is of high practical significance. To the middle of the XX century, the annual damage from vessels fouling was estimated at millions of dollars (Marine Fouling and Its Prevention, 1952); to date, the values are only increasing. The Institute of Marine Biology of the Far Eastern Branch of the Russian Academy of Sciences (FEB RAS) has developed a methodology for studying the fouling communities succession of operating vessels afloat using surface-supplied diving equipment. To study the dynamic aspects of the fouling, the surface of the vessel's hull was used. Significant results have already been obtained by this methodology (Zvyagintsev, 2005). In 1986, specialists of both the Institute of Marine Biology and Central Research Institute “Prometey” manufactured a ship device for biological and corrosion tests (Ostrikov et al., 1998), intended for exposing removable plates on the vessel’s hull during its operation. The device was successfully tested during the Vietnamese cruise of the RV “Akademik Aleksandr Nesmeyanov” in 1986.

In world practice, the devices of controlled water flow have been used for many times to solve mariculture problems, as well as for other purposes (Fitzsimmons, 2000 ; Patent no. US8245440B2, 2012 ; Pearce et al., 2002 ; etc.). The aim of this work is to present for the first time a new device, developed by us, which received a positive decision of Rospatent (Federal Service for Intellectual Property) (Patent no. 2728490, 2019). The objectives set were as follows: to give an illustrated description of the device of controlled water flow (hereinafter DCWF); to describe the hydrological conditions of the testing both in the device and in the open bay area; to give the readings of average water flow velocities for each gradation of the device electric motor; to carry out a comparative analysis of the structure of the fouling communities in the DCWF and in the open bay; to substantiate the effectiveness of using our device to identify the patterns of formation of the fouling communities in different hydrodynamic flows; and to provide practical conclusions and recommendations for using the DCWF.

MATERIAL AND METHODS

Design of the device of controlled water flow. A method for testing the properties of protective coatings in seawater flow and the device for its simulating have been developed by us (Fig. 1).

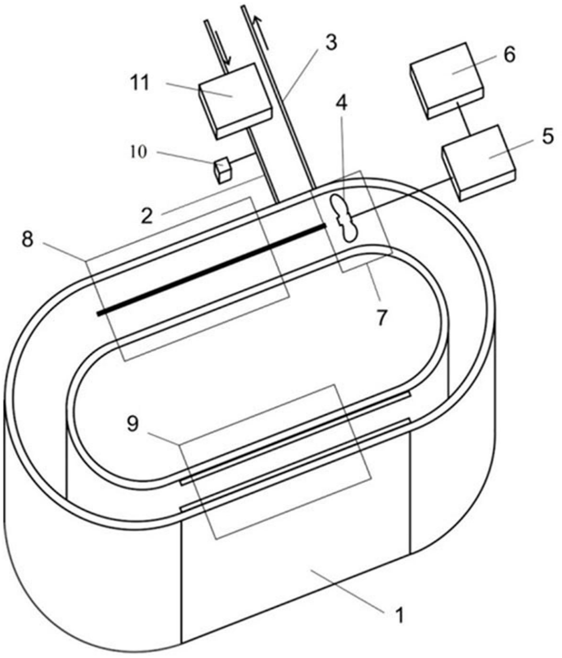


Fig. 1. Device of controlled water flow:
1 – o-shaped container of variable cross-section;
2 – water supply pipe;
3 – spillway drain pipe;
4 – main screw propeller;
5 – electric motor power plant;
6 – electric motor on-off control unit with electric motor speed control unit;
7 – screw-steering group zone;
8 – imitation of screw-steering group surfaces;
9 – midship and different foreship areas, *inter alia* bulbous bow;
10 – continuous salinometer and thermometer;
11 – seawater conduit with specified parameters of purification and treatment or without purification

Together with specialists from the Institute of Chemistry, FEB RAS, design proposals and a draft study of a year-round marine stand were made for testing the peculiarities of the fouling formation on the plates with different composition of coatings, the adhesion of marine organisms on the surface at different water flow velocities, and the effect of coatings on the occurrence of turbulent flows. The device of controlled water flow (the stand) is a complex of technical devices for simulating seawater flow with as complete set of factors as possible, including biotic and abiotic components. The DCWF can be used for testing and verifying the properties of protective coatings on various substrates, *inter alia* antifouling and anticorrosion coatings. Moreover, it allows to carry out experiments on the effect of the water flow velocity and its duration both on attachment and development of separate hydrobionts and peculiarities of the fouling community formation and functioning. The device can be used for load testing of installations and mechanisms in seawater flow of constant and variable force, as well as for modeling storm load and currents (tidal, wind, and constant ones). Successful testing of the DCWF operation on experimental plates has been carried out; preliminary results have been obtained.

The device, developed by us (Patent no. 2728490, 2019), consists of: underwater intake from a pumping station (centrifugal pump, 12.5 $m^3 \cdot h^{-1}$, 900 W); seawater conduit with specified parameters of purification and treatment or without purification; spillway drain pipe; electric motor on-off control unit; control unit of electric motor speed and seawater supply and discharge valves; o-shaped container of variable cross-section for modeling areas with different water flow velocities; main screw propeller; imitators of steering column and vessel side from a given material with any protective coating or protector (see Fig. 1). The DCWF allows to simulate all conditions of a moving vessel simultaneously, with imitation of the surfaces of screw-steering group, midship, and bulbous bow.

Hydrological conditions of the test. The research was carried out in the Tikhaya Zavod Bay, Vostok Bay (Peter the Great Bay, the Sea of Japan). Seawater characteristics were measured by SeaGuard RCM system (Aanderaa). The device was installed on the pier of the eastern cape of the Tikhaya Zavod Bay at the depths of 1.0–1.5 m. The average long-term values of water temperature and salinity are given for the middle area of Vostok Bay; seasonal and diurnal – for the Tikhaya Zavod Bay. Variations in tide rise are given for Vladivostok.

This area of the bay is characterized by significant thermohaline fluctuations in the surface and bottom water layers, especially in rainy years. In Vostok Bay, water temperature in the 0–6-m layer varies +7.4 to +25.9 °C June to September (Grigoryeva & Kashenko, 2010). By the surface, salinity values vary in a wide range of 0.5–34.0 ‰; at the bottom, they are slightly less: 19.2–35.2 ‰. Long-term periods of desalination were recorded during two or three summer months, as in other Peter the Great Bay shallow areas.

The pattern of Vostok Bay currents is determined by wind, drift, and tidal currents. The Primorsky Current affects this pattern as well, which is manifested in the stability of quasi-stationary flows in the water area. In Peter the Great Bay, irregular semidiurnal tides are registered, with maximum fluctuations of 40–50 cm (Sostoyanie i ustoichivost' ekosistem…, 2001). Data about constant currents in Vostok Bay are rather contradictory (Ivashchenko, 1993 ; Khristoforova et al., 2004). According to one data, the main water transfer is carried out west to east and is of anticyclonic character; according to others, it is of cyclonic one. According to our archival material, water movement in the Tikhaya Zavod Bay has a predominantly cyclonic direction. The velocity of currents here varies 2–18 $cm \cdot s^{-1}$, increasing up to 6–26 $cm \cdot s^{-1}$ towards the bay exit.

During the period of experiments, we have carried out three series of measurements of flow velocities, generated in the device, on 11 and 22 October. All the measurements were carried out both near the outlet and at a distance of 1.0–1.5 m. In separate series, flow velocities were determined in the main (VIII) mode. In addition, we have carried out measurements when immersing the upper part of the device only, where the sensor is located.

Four steel rectangular plates, 20×20 cm each, were simultaneously installed vertically on the buoy in the Tikhaya Zavod Bay at a depth of 1 m and in the DCWF. The plates were exposed for 2 months (early September to late October 2019). On each plate, macrofouling samples were collected on an area of 10×10 cm in triplicate. A total of 24 quantitative samples were collected and processed. Macrofouling samples were treated by the generally accepted procedure for processing benthic samples in laboratory conditions (Zhadin, 1969). During laboratory processing of fresh living material, all components of zoo- and phytoperiphyton were extracted and counted; then, they were divided into separate groups. After samples treating, macrophytes were placed in the herbarium or fixed with 4 % formalin, and zoofouling – with 70 % alcohol; later, they were transferred to specialists for determination. The taxonomic affiliation of the foulers was established by the staff of the National Scientific Center of Marine Biology, FEB RAS. Animals, identified to species lever or to higher taxonomic rank, were counted and weighed to the nearest 0.01 g after drying on filter paper. Sedentary polychaetes in tubes and amphipods in shells were weighed with these formations, which are the products of their vital activity. After the identification of systematic groups was completed, lists of species were compiled, with the quantitative indicators of each species and its location.

Laboratory and microscopic processing of microperiphyton on the experimental plates was carried out according to generally accepted methods (Ryabushko, 2013 ; Ryabushko & Begun, 2015) at the marine microbiota laboratory of the National Scientific Center of Marine Biology. The quantitative abundance of microalgae was assessed by the Visloukh scale. Identification of the species composition was carried out on temporary aqueous preparations under an Olympus BX41 light microscope, using a UPLanFl 100×/1.30 objective (Japan) and oil immersion (Guiry & Guiry, 2020).

Statistical processing of the material. The samples were grouped using Ward's method of cluster analysis (ward.D2) based on the Bray – Curtis metric and the subsequent application of the bootstraping to assess the probability of a node appearing on the dendrogram (Shitikov & Rozenberg, 2013). Unbiased bootstrap probabilities were estimated by fitting parametric models using the maximum likelihood estimation. To check the reliability of the appearance of the tree structure fragments, the ANOSIM procedure was applied with the calculation of the general statistics (global R, *GR*). For the selected groups, species contribution to intragroup similarity and intergroup dissimilarity was determined (the SIMPER procedure). Pairwise comparison of various quantitative characteristics of the fouling (average species number in a sample; biomass; the Margalef species diversity index; the Pielou evenness index; and the Shannon – Wiener diversity index) was carried out by the Mann – Whitney test [a nonparametric analogue of the Student's *t*-test (null hypothesis H_0: there is no relative shift in distributions)].

RESULTS AND DISCUSSION

Hydrological monitoring in the area of the device testing. The data of a diurnal survey in October 2019 indicate as follows: in the area of the eastern cape of the Tikhaya Zavod Bay, velocities of currents by the water surface varied 0.02 to 9.7 $cm \cdot s^{-1}$, averaging 1.1 $cm \cdot s^{-1}$; at low tide, the currents were of northern and northeastern directions, while at high tide – of southeastern, southern, and southwestern (Fig. 2). Water temperature and salinity values changed as well: temperature decreased by 2.2 °C at night, and salinity increased by 1.5 ‰ with the tide.

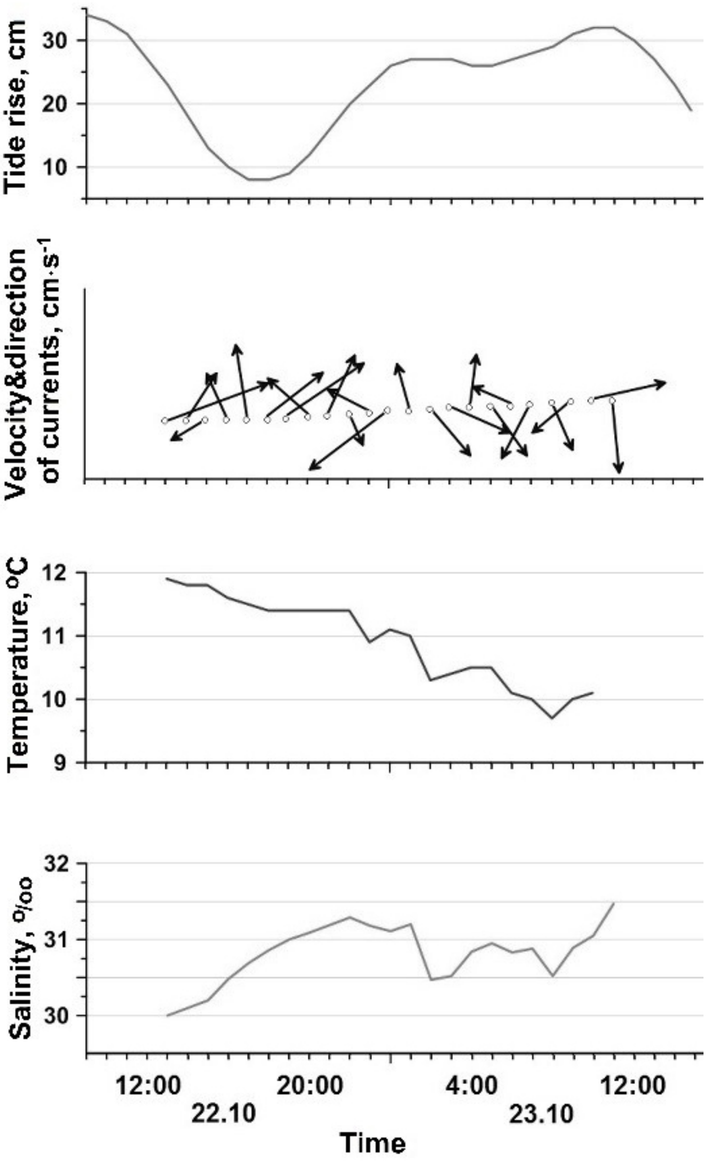


Fig. 2. Daily variations of indicators in tide rise (cm), velocity (cm·s^{-1}) and direction of currents (degrees), temperature (°C), and water salinity (‰) near the eastern cape of the Tikhaya Zavod Bay on 22–23 October, 2019

The readings of the average flow velocities for each power gradation (mode) are shown in Fig. 3. On 11 October, the series of measurements showed good convergence of the results; on 22 October, some instability of the flow was noted in the VI, VIII, and X modes. The minimum average velocities were 2.6–2.7 cm·s^{-1}; the maximum ones – 55.1–65.0 cm·s^{-1}. When carrying out a series of measurements with variable mode switching, the velocity in the VIII mode varied 21.0–32.7 cm·s^{-1}, averaging 24.5, 22.0, and 29.0 cm·s^{-1} (the values correspond to the numbers of the series of experiments). The average velocity of the main mode (VIII) with a separate long exposition was 51.7 cm·s^{-1}. It should be noted that the velocity is underestimated by the case of the device itself. To eliminate this interference, measurements were carried out when immersing the upper part of the device only. The average flow velocity at a distance of 1.0–1.5 m was 78.8 cm·s^{-1}, and near the outlet – 84.3 cm·s^{-1}. The maximum recorded value was 91.2 cm·s^{-1}.

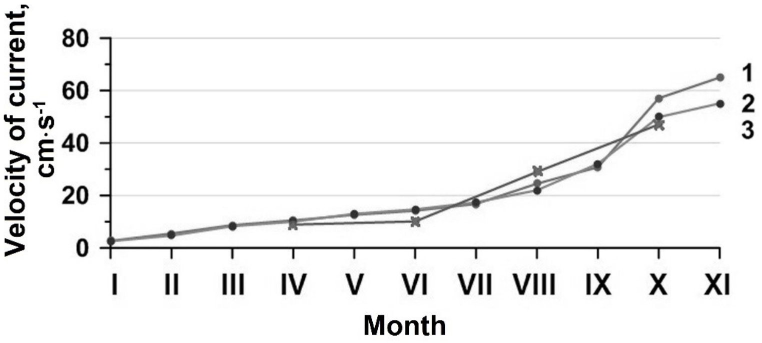


Fig. 3. Changes in flow velocities depending on the set power. 1, 2 – series of the measurements on 11.10.2019; 3 – series of the measurements on 22.10.2019

During the 3-month period of studying the fouling formation (September to November), temperature and salinity of water, entering the device, were measured hourly (Fig. 4). Temperature naturally decreased +22.0 to +1.9 °C (water was gradually cooling down). In the first half of September, salinity underwent significant fluctuations (26.2–33.7 ‰) due to rains; then, the general background levelled off, and indicator varied in a range of 31.2–34.1 ‰.

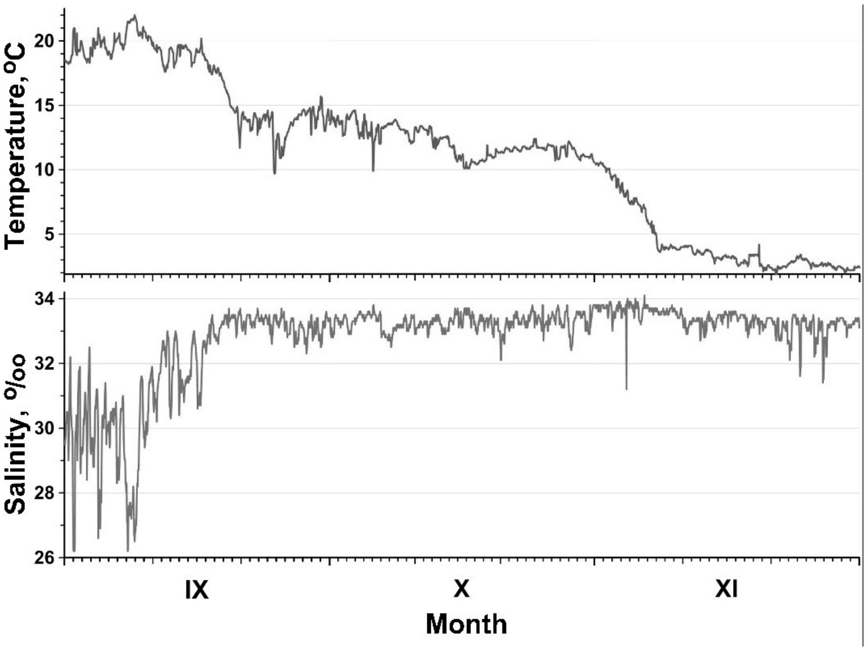


Fig. 4. Fluctuations of temperature (°C) and salinity (‰) of water, entering the device on September – November

Comparative analysis of the fouling communities on the experimental plates under conditions of the device of controlled water flow and the open Tikhaya Zavod Bay. During the period of experiments, 12 hydrobiont species were recorded on the plates from different areas of exposition, 2 of which were green algae (Table 1). Of the attached forms of zoofouling, bay barnacle *Amphibalanus improvisus* (Darwin, 1854) was recorded, as well as polychaetes *Hydroides ezoensis* Okuda, 1934 and *Neodexiospira alveolata* (Zachs, 1933). In addition to macroalgae development, mass diatom development was registered in the fouling on the plates from both biotopes studied; a total of 16 Bacillariophyta species were identified (see Tables 5, 6).

Table 1. Taxonomic composition of macrofouling on the plates from the device of controlled water flow and under the conditions of open Tikhaya Zavod Bay

	Device of controlled water flow	Open Tikhaya Zavod Bay
Algae		
Ulva linza Linnaeus, 1753	+	+
Ulva fenestrata Postels & Ruprecht, 1840	+	+
Animalia		
Hydroides ezoensis Okuda, 1934	–	+
Neodexiospira alveolata (Zachs, 1933)	–	+
Amphibalanus improvisus (Darwin, 1854)	–	+
Caprella cristibrachium Mayer, 1903	–	+
Caprella acanthogaster Mayer, 1890	–	+
Caprella algaceus Vassilenko, 1967	–	+
Ampithoe lacertosa Spence Bate, 1858	–	+
Pontogeneia intermedia (?) Gurjanova, 1938	–	+
Monocorophium acherusicum (Costa, 1853)	–	+
Lacuna turrita A. Adams, 1861	–	+

We attributed green algae *Ulva linza* Linnaeus, 1753 and *Ulva fenestrata* Postels & Ruprecht, 1840, as well as bay barnacles *Amphibalanus improvisus*, to background-forming macrofouling species. Taxonomic composition and quantitative distribution of the fouling communities from the DCWF and from the open Tikhaya Zavod Bay differ significantly (see Figs 5, 6). Thus, on the plates from the device, diatoms predominated, forming the basis of biomass; on the plates from the open bay, green algae *Ulva linza* prevailed. The mobile forms, represented by gastropods and amphipods, were of minimal biomass or were found in qualitative samples only.

Results of the statistical analysis. The analyzed sample is clearly divided into two groups with a very high intragroup similarity of 91.2 and 94.7 %, with an intergroup dissimilarity of 70.8 % (Fig. 7, Table 2): group I – samples from the plates exposed in the Tikhaya Zavod Bay (Z1–Z12); group II – samples from the DCWF (U1–U12). In the group I, the main contributor to the similarity of the samples is *Ulva linza* (52.8 %); it is followed by *Amphibalanus improvisus*, the contribution of which is 2.7 times less (19.4 %). In the group II, the main contributor is the complex of diatoms (66.9 %); the contribution of *U. linza* is two times less (32.9 %). The same organisms determine group dissimilarity: the contribution of diatoms is 31.1 %; *U. linza* – 26.8 %; *A. improvisus* – 16.2 %. The isolation of these groups is significant from the standpoint of statistics, as evidenced by the results of the ANOSIM procedure (GR (1.000 ± 0.120); quantile of permutations is ± 95 %; $p = 0.001$; number of permutations is 999).

The fouling associations on the plates, exposed in the Tikhaya Zavod Bay and on the DCWF, are statistically significantly different in all quantitative indicators (Table 3). The first association is characterized by larger values of the average biomass, species number in the sample, and value of the Margalef index, while the second one – by higher values of the Pielou and Shannon – Wiener indices. The latter indicates that the second group has greater species distribution evenness by ranks and species diversity from the standpoint of information theory; this is an "artifact", which is explained by a much smaller number of settled organisms and a lower biomass of the dominant species (the complex of diatoms, in this case). It should be emphasized as follows: the absolute values of all ecological indices, *inter alia* the average species number, are extremely low in comparison with those of both most natural communities and the fouling associations with a longer duration of development.

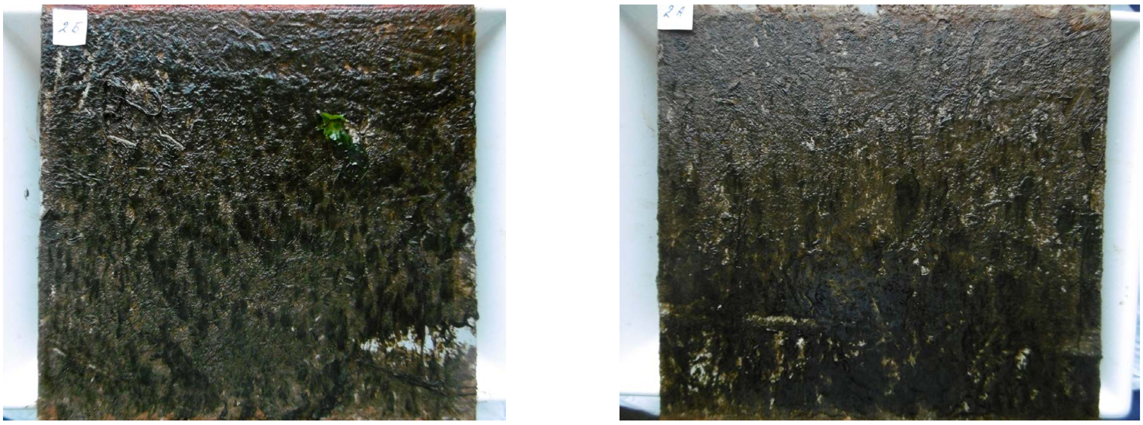


Fig. 5. View of fouling on the plates from the device of controlled water flow. Diatom taxocen in the form of macrocolonies

Fig. 6. View of fouling on the plates from the open Tikhaya Zavod Bay. *Ulva linza* + *Amphibalanus improvisus* community

Table 2. Distribution of average similarity (dissimilarity) within (between) groups based on contribution of each fouling species on the plates from the device of controlled water flow and from the open Tikhaya Zavod Bay

Taxon	I/II	$\bar{\delta}_i$	$\bar{\delta}_i / SD(\delta_i)$	CN, %	CCN, %
Group I. Average similarity: 91.2					
Ulva linza	5148	48.14	21.38	52.82	52.82
Amphibalanus improvisus	99.7	17.70	22.25	19.42	72.23
Hydroides ezoensis	16.3	10.48	8.90	11.49	83.72
Neodexiospira alveolata	7.8	9.20	13.62	10.09	93.82
Group II. Average similarity: 94.7					
Bacillariophyta	1983	63.30	19.85	66.86	66.86
Ulva linza	115	31.13	16.88	32.88	99.74
Groups I–II. Average dissimilarity: 70.8					
Bacillariophyta	0.15/1983	21.99	20.56	31.08	31.08
Ulva linza	5148/115	18.93	12.67	26.75	57.84
Amphibalanus improvisus	99.7/0.0	11.47	16.39	16.21	74.04
Hydroides ezoensis	16.3/0.0	7.14	7.35	10.10	84.14
Neodexiospira alveolata	7.83/0.0	6.05	10.32	8.55	92.69

Note: species are arranged in decreasing order of percent contribution; δ_i — measure of similarity (dissimilarity); *SD* – standard deviation; CN – percent contribution; CCN – accumulated percent.

Table 3. Quantitative characteristics of fouling communities on the plates from the device of controlled water flow and from the open Tikhaya Zavod Bay and results of pairwise comparison of indicators (Mann – Whitney test; probability of H_0 validity is shown)

Group	*S* ± *SE*	B ± *SE*, g·m^{-2}	*R* ± *SE*	*e* ± *SE*	*H’* ± *SE*, bit·g^{-1}
I. *Ulva linza*	6.4 ± 0.3	5277 ± 209	0.63 ± 0.03	0.073 ± 0.004	0.19 ± 0.01
II. Diatomea	2.2 ± 0.1	2101 ± 108	0.15 ± 0.01	0.299 ± 0.019	0.32 ± 0.02
Mann – Whitney test	0.000	0.000	0.000	0.000	0.000

Note: *S* – average species number in the sample; B – biomass; *R* – Margalef index; *e* – Pielou evenness index; *H’* – Shannon – Wiener diversity index; *SE* – standard error.

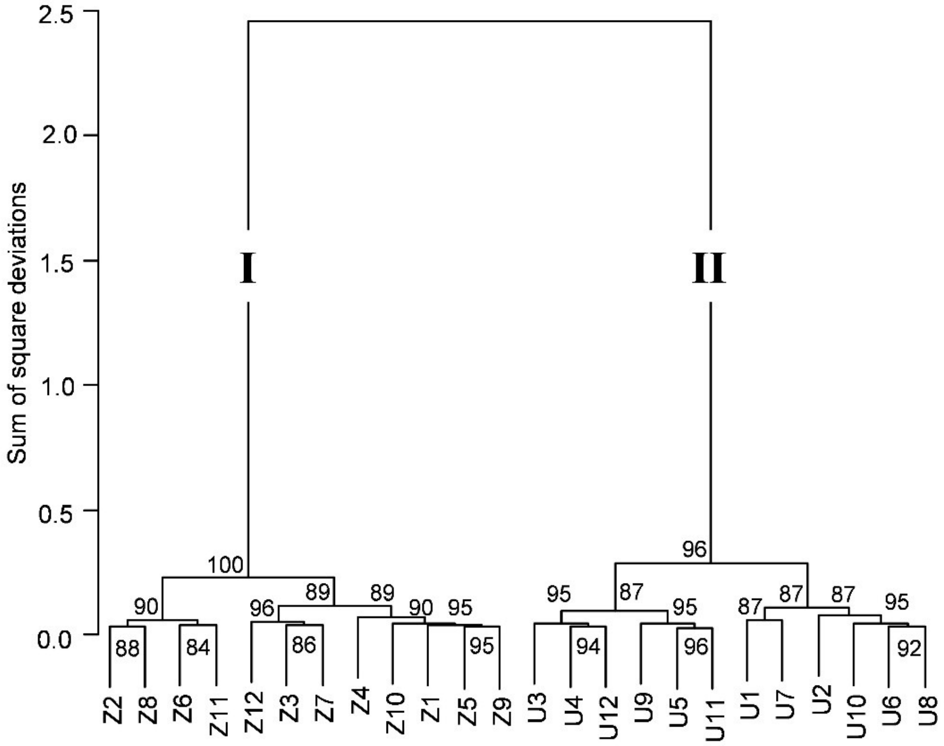


Fig. 7. Dendrogram of the plates similarity (ward.D2 method) with Bray – Curtis similarity index (the fourth-root transformation) based on phyto- and zoofouling biomasses. The best estimations of unbiased bootstrap probabilities are shown in dendrogram nodes (number of permutations is 1000). Roman numerals denote allocated groups

The identified associations have only three common components: the complex of diatoms, *U. linza*, and *U. fenestrata*. The first two were found in all samples; the third one was recorded with a frequency of 50 and 17 % in the first and second groups, respectively. The average biomass of microalgae on the plates, exposed on the DCWF, was thousands of times higher than in natural conditions, while that of *U. linza* macrophytes, on the contrary, was almost 45 times lower. Naturally, such differences are significant from the standpoint of statistics (the results of the Mann – Whitney test: the probability of H_0 validity $p = 0.000$) (Fig. 8, Table 4). Biomass of *U. fenestrata* in both cases was approximately the same ($p = 0.260$). Another difference, qualitative one, is the complete absence of macrozoobenthos on the plates from the DCWF, whereas in natural conditions, 10 macrozoobenthos species were found on the plates, and *A. improvisus* can be considered as a subdominant species (taking into account 100 % occurrence and high biomass).

Thus, the statistical analysis proves cardinal differences (qualitative and quantitative ones) in the fouling associations on the plates, exposed in the Tikhaya Zavod Bay and on the DCWF. These differences, in fact, are of a succession nature. It is known that on objects, that have gotten to the sea, bacteria and microalgae settle in the first phases of succession; they form a mucous film, which facilitates the subsequent colonization of the substrate by various hydrobionts (Zvyagintsev, 2005). Our experiment clearly shows the "delay" of the succession under conditions of the DCWF.

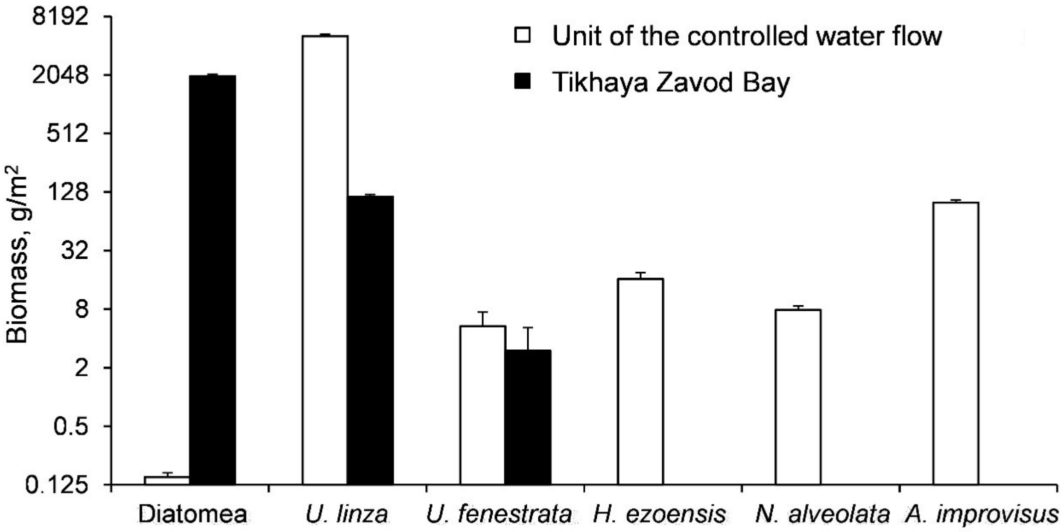


Fig. 8. Biomass of some foulers on the plates from the device of controlled water flow and from the open Tikhaya Zavod Bay (logarithmic scale). Error bars denote standard error

Table 4. Taxonomic composition and biomass (± standard error) of fouling communities on the plates from the device of controlled water flow and from the open Tikhaya Zavod Bay

Taxon	Device of controlled water flow	Open Tikhaya Zavod Bay
	Microalgae	
Bacillariophyta	1983 ± 107	0.15 ± 0.02
	Algae	
Ulva linza Linnaeus, 1753	115 ± 6	5148 ± 210
Ulva fenestrata Postels & Ruprecht, 1840	3.0 ± 2.1	5.3 ± 2.1
	Animalia	
Hydroides ezoensis Okuda, 1934	–	16.3 ± 2.7
Neodexiospira alveolata (Zachs, 1933)	–	7.8 ± 0.8
Amphibalanus improvisus (Darwin,1854)	–	100 ± 6
Caprella cristibrachium Mayer, 1903	–	0.03 ± 0.01
Caprella acanthogaster Mayer, 1890	–	0.02 ± 0.01
Caprella algaceus Vassilenko, 1967	–	0.01 ± 0.01
Ampithoe lacertosa Spence Bate, 1858	–	0.02 ± 0.01
Pontogeneia intermedia (?) Gurjanova, 1938	–	0.01 ± 0.01
Monocorophium acherusicum (Costa, 1853)	–	0.01 ± 0.01
Lacuna turrita A. Adams, 1861	–	0.01 ± 0.01

Note: a dash (–) means that no organisms were found.

Diatoms of microperiphyton on the experimental plates. On the surface of the experimental plates under conditions of the DCWF, the presence of dark brown microperiphyton was noted, with the predominance of macrocolonies of diatoms, forming the basis of the biomass of the fouling. In the microperiphyton community, 14 species were identified, represented by different life forms: mobile and immobile; attached and free-living; and solitary and colonial (Table 5). In relation to water salinity, marine forms predominated (7 species), as well as brackish-water and marine ones; in relation to active reaction of the environment, alkaliphiles prevailed (Fitzsimmons, 2000). In the phytogeographic aspect, widespread cosmopolitan species predominated (Ryabushko & Begun, 2015).

Table 5. Composition, ecological and geographical characteristics, and relative quantitative abundance of diatoms of microperiphyton from the device of controlled water flow (November 2019)

Taxon	Ecological characteristic			Phytogeographical characteristic	Quantitative abundance
	Life form	Salinity	Saprobity		
Berkeleya rutilans (Trentepohl ex Roth) Grunow, 1880	B	BM	–	C	ms
Cylindrotheca closterium (Ehrenberg) Reimann & J. C. Lewin, 1964	BP	BM	β	C	r
Gomphonemopsis pseudexigua (Simonsen) Medlin in Medlin & Round, 1986	B	M	–	ABT	of
Licmophora abbreviata C. Agardh, 1831	B	M	β	AB	ms
Licmophora flabellata (Greville) C. Agardh, 1831	B	M	β	BT	of
Melosira lineata (Dillwyn) C. Agardh, 1824	BP	BM	α	ABT	r
Melosira moniliformis var. *subglobosa* (Grunow) Hustedt, 1927	BP	BM	α	AB	of
Navicula directa (W. Smith) Ralfs, 1861	B	BM	–	C	r
Nitzschia hybrida f. *hyalina* Proschkina-Lavrenko, 1963	BP	BW	–	Bo	of
Nitzschia longissima (Brébisson) Ralfs, 1861	B	M	–	C	r
Odontella aurita (Lyngbye) C. Agardh, 1832	BP	M	–	C	r
Parlibellus delognei (Van Heurck) E. J. Cox, 1988	B	M	–	C	r
Rhabdonema arcuatum (Lyngbye) Kützing, 1844	BP	M	–	C	r
Tabularia tabulata (C. Agardh) Snoeijs, 1992	B	BM	α	C	of

Note. Confinement to habitat: B – benthic; BP – benthic and planktonic. Confinement to salinity: M – marine; BM – brackish-water and marine; BW – brackish-water. Confinement to saprobiology: α – alpha-mesosaprobiont; β – beta-mesosaprobiont. Phytogeographic characteristics: C – cosmopolitan; AB – arctic-boreal; ABT – arctic-boreal-tropical; BT – boreal-tropical; Bo – boreal. Relative quantitative abundance is given according to the Visloukh scale (Diatomovye vodorosli SSSR…, 1974): ms – mass; l – a lot of; of – often; r – rarely.

Two colonial diatom species were characterized by the maximum quantitative development: *Berkeleya rutilans*, which forms tubular mucous microcolonies, and *Licmophora abbreviata*, which develops on a substrate with stems of a polysaccharide matrix (Fig. 9). The quantitative abundance of these species in the fouling on the plates from the DCWF was assessed as "mass". Both of these species are typical epiphytes, which form in natural conditions a mutual connection with the phorophyte – the same as in terrestrial plants with their epiphytes (Ryabushko, 2013). The above-mentioned species are the main component of diatom periphyton of anthropogenic substrates in the water of Peter the Great Bay (the Sea of Japan), as well as in the coastal areas of Japan (Kasim & Mukai, 2006), China (Checklist of Marine Biota of Chinese Seas, 2008), and Korea (Lee et al., 2015).

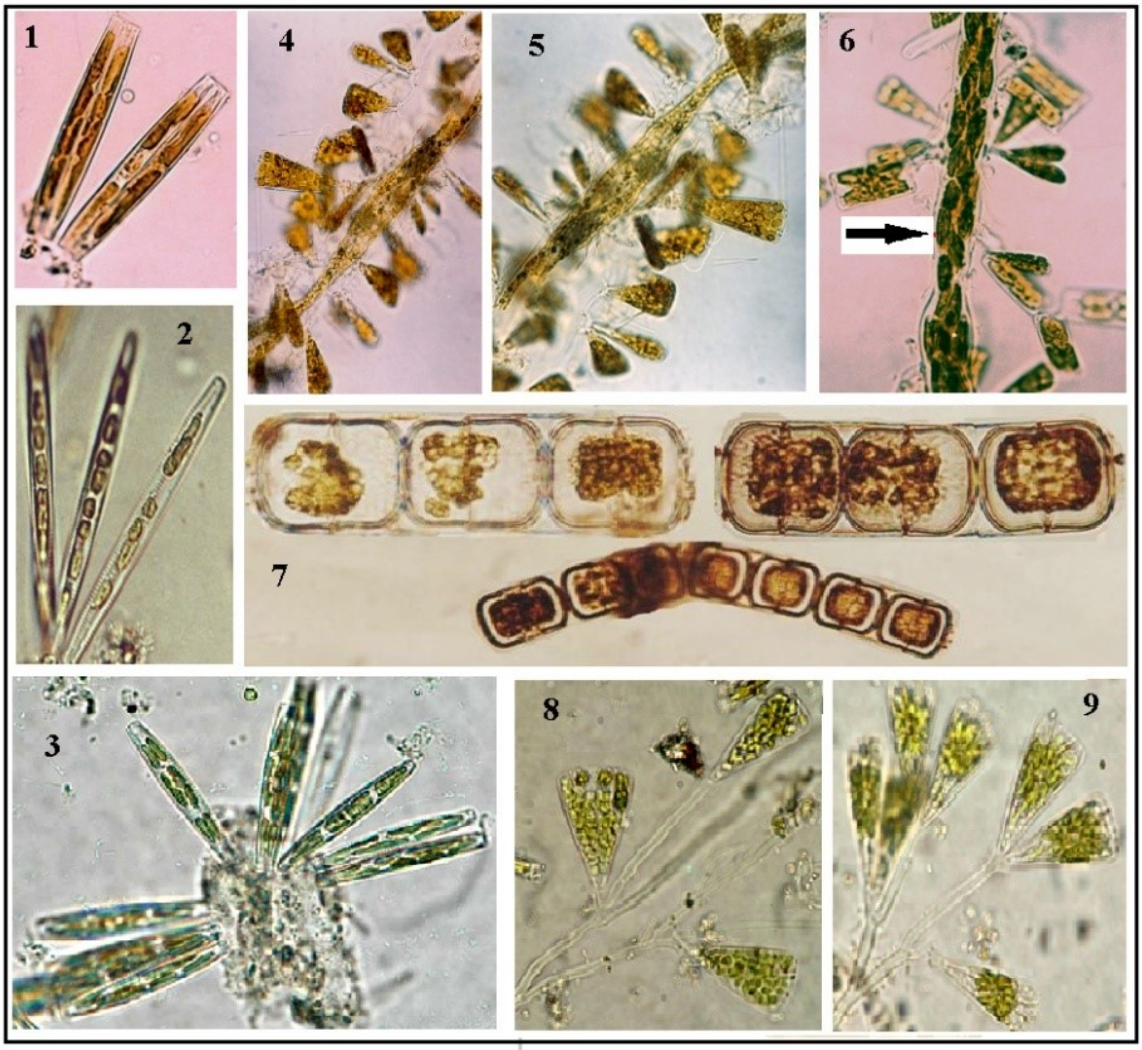


Fig. 9. View of the mass colonial forms of diatoms from microperiphyton on the experimental plates from the device of controlled water flow and the Tikhaya Zavod Bay in November 2019: 1–3 – *Tabularia tabulata*; 4, 5, 8, 9 – *Licmophora abbreviata*; 6 – *Berkeleya rutilans* (the arrow indicates the tubular mucous macrocolony of this species); 7 – *Melosira lineata*

High quantitative indicators of diatoms *B. rutilans* and *L. abbreviata* on the experimental plates from the DCWF are determined, first of all, by the presence of favorable hydrological and hydrochemical conditions. Thus, in the late autumn period, in most of the shallow bays of Peter the Great Bay, the maximum concentrations of nitrites, nitrates, phosphates, and silicon are recorded, which are the most important biogenic elements, limiting the development of diatoms.

Diatom species, found in the microperiphyton on the plates from the DCWF, are eurythermal – occurring year-round regardless of water temperature, since water masses are subject to significant convective mixing in the coastal area of Peter the Great Bay in late autumn (Ryabushko & Begun, 2015).

Of all the mass species with a known saprobiological characteristic, in the microperiphyton on the plates from the DCWF, diatom *L. abbreviata* is recorded: a beta-mesosaprobiont and an indicator of moderate organic pollution of water. Mass development of this species and the absence of indicators of high organic pollution of water is primarily associated with no desalination and no anthropogenic load during the research period, since water intake for the DCWF was carried out at a greater depth at a distance of about 100 m from the pier.

On the surface of macrofouling on the experimental plates, exposed on the pier of the Zapad marine biological station of the National Scientific Center of Marine Biology in the coastal area of the Tikhaya Zavod Bay, a light brown plaque about 1 mm thick was observed. Microscopy showed that the plaque consists entirely of macrocolonies of diatoms. In total, 12 diatom species and intraspecific taxa were registered, represented by various life forms: mobile and immobile; attached and free-living; and solitary and colonial (Table 6). A quantitative analysis of the microperiphyton on the experimental plates showed that two colonial diatom species, *Melosira lineata* and *Odontella aurita*, reached their maximum development; their relative abundance was estimated as "mass" (Fig. 9). These species are benthic and planktonic, or tychopelagic, capable of dwelling both in phytoplankton and microphytobenthos, in fouling of stony soils and macrophytes, reaching mass development in late autumn and winter (Ryabushko, 2013). According to the data of previous studies in the water of Peter the Great Bay (the Sea of Japan), these microalgae are an integral component of the periphyton of the experimental plates, made of different materials (Ryabushko & Begun, 2015). The remaining 10 diatom species, recorded in the microperiphyton on the plates from the Tikhaya Zavod Bay (Table 6), are typical epiphytes, developing on macrophytes or solid substrates.

Table 6. Species list, ecological-geographical characteristics, and relative abundance of diatoms from microperiphyton on the plates from the Tikhaya Zavod Bay (November 2019)

Taxon	Ecological characteristic			Phytogeographical characteristic	Quantitative abundance
	Life form	Salinity	Saprobity		
Arachnodiscus ehrenbergii J. W. Bailey ex Ehrenberg, 1849	BP	M	–	C	r
Berkeleya rutilans (Trentepohl ex Roth) Grunow, 1880	B	BM	–	C	of
Cocconeis stauroneiformis H. Okuno, 1957	B	M	–	AB	of
Gomphonemopsis pseudexigua (Simonsen) Medlin in Medlin & Round, 1986	B	M	–	ABT	r
Licmophora abbreviata C. Agardh, 1831	B	M	β	AB	of
Licmophora flabellata (Greville) C. Agardh, 1831	B	M	β	BT	r
Melosira lineata (Dillwyn) C. Agardh, 1824	BP	BM	α	ABT	ms
Navicula directa (W. Smith) Ralfs, 1861	B	BM	–	C	of
Nitzschia longissima (Brébisson) Ralfs, 1861	B	M	–	C	r
Odontella aurita (Lyngbye) C. Agardh, 1832	BP	M	–	C	ms
Rhabdonema arcuatum (Lyngbye) Kützing, 1844	BP	M	–	C	r
Tabularia tabulata (C. Agardh) Snoeijs, 1992	B	BM	α	C	l

Note: as for Table 5.

Of all the species with a known saprobiological characteristic, in the microperiphyton on the plates from the Tikhaya Zavod Bay, two alpha-mesosaprobiont species were registered – indicators of high organic pollution of water. These are *M. lineata* and *T. tabulata*; the quantitative abundance of the latter was assessed at the level of "a lot of". These are brackish-water and marine ubiquist species with high saprobity indices, tolerant to adverse environmental conditions (oil, chemical, and thermal pollution) and capable of switching from autotrophic nutrition to heterotrophic or mixed one (Mitra et al., 2014).

In this paper, for the first time, the new device of controlled water flow, developed by us, is presented, for which a positive decision of Rospatent has already been received. Our experiment is an illustration of the capabilities of the DCWF. The results of its testing in biotopes of different abiotic conditions were obtained in just two months. Such a huge difference in the composition of the fouling on the plates under conditions of the DCWF and the open Tikhaya Zavod Bay can be explained as follows. It is known that the fouling of operating vessels forms most rapidly during anchorages in ports, where fouling larvae successfully settle on the vessel's hull (Marine Fouling and Its Prevention, 1952). Zoofouling is practically absent on the plates under conditions of a controlled water flow; only 2 green algae species and 12 diatom species are registered in phytoperiphyton. It should be concluded that for successful settling of macrofouling larvae, it is necessary to periodically stop the water flow, simulating a vessel anchorage. But this is already the task of our subsequent work.

The possibility of an "artifact", introduced by the device itself, has to be taken into account, in particular the negative effect of the pump on the fouling larvae. An indirect confirmation of their survival after passing through the water intake pumps of thermal power stations, as well as after passing ballast water, is the subsequent formation of a full-fledged fouling. At the same time, numerous literature data indicate that a significant part of zooplankton is injured when water passes through industrial cooling systems. However, the results of studies, carried out at the cooling ponds of the Kursk, Kalinin, and Smolensk Nuclear Power Plants (Suzdaleva et al., 2007), indicate that a noticeable number of injured individuals is registered not in every zooplankton sample, collected in a discharge area. The total ratio of injured zooplankton individuals in a water supply system, which is very high at the beginning of a cooling reservoir operation, is gradually decreasing. For example, at present, zooplankton die-off in the cooling system of the Kursk NPP, as a rule, does not exceed 45 % of the abundance of organisms at the water intake. In most cases, the ratio is significantly lower. In some periods, zooplankton die-off is only 3–5 %, which is less than when eaten by fish in natural conditions. Thus, at the beginning of operation, the ratio of dead zooplankton in the cooling system of the Zmievskaya Power Plant (Ukraine) was 75–80 %; in the subsequent period, it decreased to 20 %. None of the researchers registered 100 % die-off of fouling larvae. This fact can serve as an explanation for the mass development of macrofouling of the cooling systems of enterprises after passing through the pumps: for its formation, a limited number of larvae is enough. In our experiment, we do not yet have an idea of how the device affects fouling larvae; therefore, we cannot ignore the probability of mass die-off of meroplankton after passing through the pumps. In this situation, more research is required on the adequacy of our device. The study of plankton survival after passing through the pumps of the DCWF, developed by us, is planned to be carried out in the near future.

Conclusion. A new device of controlled water flow, developed by us, is presented, which received a positive decision of Rospatent; its capabilities are described. During 2-month experiment on testing the DCWF in biotopes with different abiotic conditions, results were obtained, that demonstrate significant differences in the composition of the fouling on the plates under conditions of the device and the open Tikhaya Zavod Bay.

Diatoms predominate in the fouling community under conditions of the DCWF; zoofouling is practically absent. On the plates from the open bay, there is a community of green algae *Ulva linza* and bay barnacles *Amphibalanus improvisus*, which is common for a vessel variable loadline or a hydraulic structure drainage zone. The latter is an introduced species; it has been successfully acclimatized in Peter the Great Bay. The statistical analysis proves cardinal differences (qualitative and quantitative ones) in the fouling associations on the plates, exposed in the Tikhaya Zavod Bay and on the DCWF; in fact, these differences are of a succession nature.

A developed microperiphyton community was registered on the experimental plates from both areas studied, and there were significant differences in the composition of dominant taxa, their quantitative indicators, and saprobiological characteristics. This is mainly due to different hydrodynamics of the controlled water flow and open water, as well as level of eutrophication, which determines the development of taxa, tolerant to high nutrient load. Based on the foregoing, it can be argued about the effectiveness of using our DCWF for studying the patterns of formation of the fouling communities in different hydrodynamic flows. The main practical conclusion is as follows: the device is recommended to verify the properties of protective coatings on the substrates tested, *inter alia* antifouling and anticorrosive coatings.

This work was carried out with partial financial support of the grant "Comprehensive program of fundamental scientific research of the FEB RAS "Far East" for 2018–2020" (No. 18-3-014, part 2).

Acknowledgement. The authors are grateful to Yu. Kulchin, E. Subbotin, and A. Nikitin (the Institute of Automation and Control Processes), as well as to A. Nikolenko (the National Scientific Center of Marine Biology), who took part in organizing the work and collecting material. Also, the authors are grateful to A. Moschenko (Far Eastern Regional Hydrometeorological Research Institute) for help in statistical processing of the material.

REFERENCES

1. Grigoryeva N. I., Kashenko S. D. Study on interannual and seasonal variations of thermohaline conditions in the Vostok Bay (Peter the Great Bay, Japan Sea). *Izvestiya TINRO*, 2010, vol. 162, pp. 242–255. (in Russ.)
2. *Diatomovye vodorosli SSSR. Iskopaemye i sovremennye*. Vol. 1 / A. I. Proshkina-Lavrenko (Ed.). Leningrad : Nauka, 1974, 402 p. (in Russ.)
3. Zhadin V. I. *Metody gidrobiologicheskogo issledovaniya.* Moscow : Vysshaya shkola, 1969, 191 p. (in Russ.)
4. Zvyagintsev A. Yu. *Marine Fouling in the North-Western Part of the Pacific Ocean.* Vladivostok : Dal'nauka, 2005, 432 p. (in Russ.)
5. Ivashchenko E. A. Tsirkulyatsiya vod zaliva Petra Velikogo. In: *Geograficheskie issledovaniya shel'fa dal'nevostochnykh morei.* Vladivostok : Izd-vo DVGU, 1993, pp. 31–61. (in Russ.)
6. Ostrikov V. V., Zvyagintsev A. Yu., Mikhailov S. R., Metel'skaya R. N., Pukas G. P., Kurdin A. V. *Sudovoe ustroistvo dlya biologicheskikh i korrozionnykh ispytanii* : Avt. svid-vo no. 1415626 ot 08.04.1988. Moscow : VNIIPI Gos. kom. SSSR po delam izobret. i otkr., [1988], 3 p. (in Russ.)
7. Patent no. 2728490 Rossiiskaya Federatsiya, MPK G01M 10/00, G01N 17/00 (2006.01). *Sposob issledovaniya svoistv zashchitnykh pokrytii v potoke morskoi vody i ustanovka dlya ego osushchestvleniya* : no. 2019142558 : appl. 16.12.2019 : publ. 29.07.2020, Byul. no. 22 / Maslennikov S. I., Tsvetnikov A. K., Nikolenko A. Yu., Nikitin A. I., Subbotin E. P. ; assignees: OOO "Morskoi biotekhnopark", OOO "Vladforum". 2 p. (in Russ.)
8. Ryabushko L. I. *Microphytobenthos of the Black Sea.* Sevastopol : EKOSI-Gidrofizika, 2013, 416 p. (in Russ.)
9. Ryabushko L. I., Begun A. A. *Diatoms of Microphytobenthos of the Sea of Japan.* Sevastopol ; Simferopol : N. Orianda, 2015, vol. 1, 288 p. (in Russ.)
10. *Sostoyanie i ustoichivost' ekosistem dal'nevostochnykh morei Rossii* : otchet o NIR. Tikhookeanskii okeanologicheskii institut ; ruk. Lobanov V. B. Vladivostok, 2001, 290 p. (in Russ.)

11. Suzdaleva A. L., Popov A. V., Kuchkina M. A., Fomin D. V., Minin D. V. Izmenenie khimicheskogo sostava vody i planktona pri prokhozhdenii cherez sistemu tekhnicheskogo vodosnabzheniya AES. *Bezopasnost' energeticheskikh sooruzhenii*, 2007, vol. 16, pp. 201–215. (in Russ.)
12. Khristoforova N. K., Naumov Yu. A., Arzamastsev I. S. Heavy metals in bottom sediments of Vostok Bay (Japan Sea). *Izvestiya TINRO*, 2004, vol. 136, pp. 278–289. (in Russ.)
13. Shitikov V. K., Rozenberg G. S. *Randomizatsiya i butstrep: statisticheskii analiz v biologii i ekologii s ispol'zovaniem R.* Tolyatti : Kassandra, 2014, 314 p. (in Russ.)
14. *Checklist of Marine Biota of Chinese Seas* / J. Y. Liu (Ed.). Beijing : Science Press, Academia Sinica, 2008, 1267 p.
15. Fitzsimmons K. Tilapia aquaculture in Mexico. In: *Tilapia Aquaculture in the Americas* / B. A. Costa-Pierce, J. E. Rakocy (Eds). Baton Rouge : The World Aquaculture Society, 2000, vol. 2, pp. 171–183.
16. Guiry M. D., Guiry G. M. *AlgaeBase.* World-wide electronic publication. Galway : National University of Ireland, 2020. URL: https://www.algaebase.org [accessed: 03.12.2020].
17. Kasim M., Mukai H. Contribution of benthic and epiphytic diatoms to clam and oyster production in the Akkeshi-ko estuary. *Journal of Oceanography*, 2006, vol. 62, pp. 267–281. https://doi.org/10.1007/s10872-006-0051-9
18. Lee S. D., Yun S. M., Park J. S., Lee J. H. Floristic survey of diatom in the three Islands (Baeknyeong, Daecheong, Socheong) from Yellow Sea of Korea. *Journal of Ecology and Environment*, 2015, vol. 38, iss. 4, p. 563–598. https://doi.org/10.5141/ecoenv.2015.059
19. *Marine Fouling and Its Prevention* : prepared for Bureau of Ships, Naval Department / Woods Hole Oceanographic Institution. Menasha : Georg Banta Publ. Co., 1952, 388 p. (Contribution / Woods Hole Oceanographic Institution ; no. 580). https://doi.org/10.1575/1912/191
20. Mitra A., Flynn K. J., Burkholder M., Berge T., Calbet A., Raven J. A., Granéli E., Glibert P. M., Hansen P. J., Stoecker D. K., Thingstad F., Tillmann U., Våge S., Wilken S., Zubkov M. V. The role of mixotrophic protists in the biological carbon pump. *Biogeosciences*, 2014, vol. 11, iss. 4, pp. 995–1005. https://doi.org/10.5194/bg-11-995-2014
21. Patent no. US8245440B2 United States, IPC A01G31/00; A01G7/00; A01H13/00; *Aquaculture Raceway Integrated Design* : US82410610A·: appl. 25.06.2010 : publ. 21.08.2012 / M. Kacira, P. Li, R. D. Ryan, P. M. Waller ; appl. Arizona Board of Regents of University of Arizona. 2012. 1 p. https://patents.google.com/patent/US8245440B2/en
22. Pearce C. M., Daggett T. L., Robinson S. M. C. Effect of binder type and concentration on prepared feed stability and gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture*, 2002, vol. 205, iss. 3–4, pp. 301–323. https://doi.org/10.1016/S0044-8486(01)00685-8

ИССЛЕДОВАНИЕ ФОРМИРОВАНИЯ СООБЩЕСТВ ОБРАСТАНИЯ В УСЛОВИЯХ УСТАНОВКИ РЕГУЛИРУЕМОГО ПОТОКА ВОДЫ

А. Ю. Звягинцев[1], С. И. Масленников[1], А. К. Цветников[2], А. А. Бегун[1], Н. И. Григорьева[1]

[1]Национальный научный центр морской биологии имени А. В. Жирмунского ДВО РАН, Владивосток, Российская Федерация

[2]Институт химии ДВО РАН, Владивосток, Российская Федерация

E-mail: *ayzvyagin@gmail.com*

Для испытания противокоррозионных и антиобрастающих защитных покрытий разработан наземный стенд — установка регулируемого потока воды. С учётом практической значимости проблемы актуальность проведённого исследования не вызывает сомнений. Стенд подключён к магистрали морской проточной воды. Устройство позволяет имитировать движение водного потока вокруг судна, тем самым моделирует условия движущегося плавающего средства. Цель настоящей работы — впервые представить созданную нами новую установку, на которую получено положительное решение Роспатента. Проведены натурные полевые испытания, продолжавшиеся два месяца. Они показали существенные качественные и количественные различия в составе сообществ обрастания на экспериментальных пластинах, помещённых в установку и подвешенных в толще воды в бухте Тихая Заводь (залив Восток) на пирсе МБС «Запад» Национального научного центра морской биологии ДВО РАН. В условиях установки регулируемого потока воды в сообществе перифитона доминируют бентосные диатомовые водоросли; зоообрастание практически отсутствует. На пластинах из открытой бухты представлен обычный для переменной ватерлинии судов либо осушной зоны гидротехнических сооружений фитоценоз зелёных водорослей. Показана эффективность использования созданной нами установки для исследования закономерностей формирования сообществ обрастания в разных гидродинамических потоках. Основной практический вывод — то, что установку можно использовать для испытания и проверки свойств защитных покрытий на тестируемых субстратах, включая противообрастающие и антикоррозийные покрытия.

Ключевые слова: установка, регулируемый поток, сообщество обрастания, зелёные водоросли, диатомовые водоросли

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 1, pp. 34–40
https://doi.org/10.21072/mbj.2021.06.1.03

UDC 594.121/.124-155.7

THE FIRST RECORD OF ROCK-BORING MOLLUSC *PETRICOLA LITHOPHAGA* (RETZIUS, 1788) INSIDE THE VALVES OF OYSTERS *CRASSOSTREA GIGAS* (THUNBERG, 1793), CULTIVATED IN CRIMEA (THE DONUZLAV BAY, THE BLACK SEA)

© 2021 **M. A. Kovalyova and O. Yu. Vyalova**

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

Received by the Editor 06.02.2020; after reviewing 06.02.2020;
accepted for publication 11.03.2021; published online 23.03.2021.

The number of mollusc farms off the coast of Crimea and the Caucasus has increased significantly in recent years. The cultivation of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) requires monitoring of mollusc health and parasitological control of mariculture farms. The aim of this work was to study species composition of epibionts and endobionts, associated with shells of cultivated oyster *C. gigas*, as well as to identify species, damaging shells. Commercial oysters with visual shell damage were collected on a mariculture farm in the Donuzlav Bay (Crimea, the Black Sea) and brought to the laboratory alive chilled. As a result of 22 oysters' examination, 14 macrozoobenthos species and live specimens of rock-boring mollusc *Petricola lithophaga* (Retzius, 1788) were found. The size of rock-borers varied 9 to 16 mm; their age was about two years. Prolonged presence of *P. lithophaga* inside oyster valves can cause degradation of shell calcareous layer and even death of the mollusc host; this fact is of great importance for the Black Sea mariculture. Considering *P. lithophaga* annual development cycle, during the period of mass larval settlement (July to October), it is recommended to inspect the shells of cultivated oysters. Further detailed studies will allow to develop measures for prevention and protection of bivalve molluscs from infestation with *P. lithophaga*.

Keywords: mariculture, commensals, infauna, shell boring, oysters, Black Sea

Crassostrea gigas (Thunberg, 1793) is a euryhaline species, resistant to salinity changes in a wide range of 12–34 ‰; large specimens withstand even short-term desalination. Due to its tolerance to salinity, this oyster species is one of the most popular mariculture objects in the world. Since the middle of the XX century, *C. gigas* is actively cultivated in the Black Sea: off the coast of Crimea and the Caucasus (Kholodov et al., 2010). The main problem for mariculture farms is the threat of destructive epizootics. Parasitic infestation can lead to deterioration of shell appearance and its thinning, inhibition of cultivated mollusc growth, and even its death. As known for the Black Sea, on shells of 1-year old oysters larger than 3–4 cm, several rock-boring species can be found, such as sponges of the family Clionaidae [*Pione vastifica* (Hancock, 1849)], polychaetes of the family Spionidae [*Polydora websteri* Hartman in Loosanoff & Engle, 1943 and *Polydora ciliata* (Johnston, 1838)], and others (Milashevich, 1916 ; Pirkova & Demenko, 2008).

The need for parasitological control of mariculture farms, detailed study of biology and species composition of potential parasites and commensals of cultivated molluscs, and their distribution in mariculture areas becomes obvious and urgent.

The aim of this work is to study species composition of epibionts and endobionts, associated with shells of commercial oyster *C. gigas*, cultivated in the Donuzlav Bay (the Black Sea), as well as to identify rock-boring species and to assess the overall condition of shells and the degree of their damage.

MATERIAL AND METHODS

The object of the study was the bivalve mollusc *C. gigas*, grown on an oyster farm in the Donuzlav Bay. This water area is located in western Crimea and cuts into the land for 30 km. The oyster farm is located in the central bay area (45°23.7′N, 33°07.1′E) (Fig. 1). The depth under the farm is 5–15 m. Oysters were grown in suspended culture, in cages of 100–150 individuals at a depth of 2–3 m.

In October 2019, 22 specimens of commercial oyster were sampled, with visual shell damage, noted during a preliminary examination. In a laboratory, shells linear parameters were measured, and live weight of each oyster was registered. Oyster height was estimated as the maximum distance between a shell lock and a growing edge. Shell length was measured at its widest part, perpendicular to the height (Nair & Nair, 1986). At first, all biofoulers were collected from shell surface for further species identification; only then rock-boring molluscs were removed from oyster shells. Some macrozoobenthos specimens were identified to species level (Kiseleva, 2004).

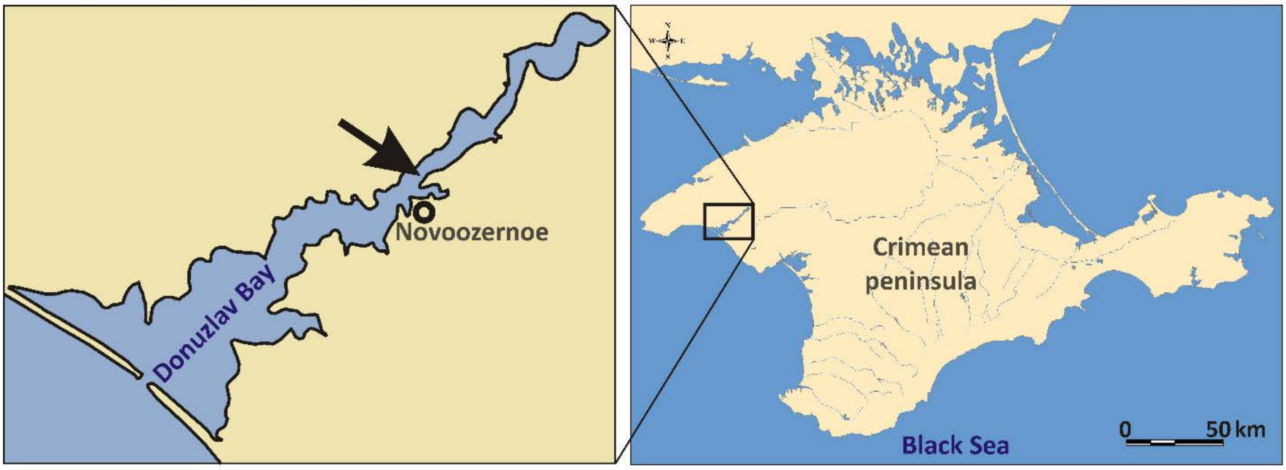


Fig. 1. Area of *Petricola lithophaga* finding in *Crassostrea gigas* valves (the Donuzlav Bay, the Black Sea). The arrow indicates mariculture farm location

RESULTS

In total, 22 *C. gigas* specimens were examined, with a shell height of 65–150 mm and weight of 38–151 g. On oyster valves, 14 macrozoobenthos species were found and identified: polychaetes *Nereis zonata* Malmgren, 1867, *Platynereis dumerilii* (Audouin & Milne Edwards, 1834), *Hydroides dianthus* (Verrill, 1873), *Dorvillea rubrovittata* (Grube, 1855), *Prionospio cirrifera* Wirén, 1883, *Polydora websteri* Hartman in Loosanoff & Engle, 1943, and Phyllodocidae g. sp.; molluscs

Bittium reticulatum (da Costa, 1778), *Rissoa parva* (da Costa, 1778), *Mytilus galloprovincialis* Lamarck, 1819, *Mytilaster lineatus* (Gmelin, 1791), *Modiolula phaseolina* (Philippi, 1844), and *Petricola lithophaga* (Retzius, 1788); and crustacean *Amphibalanus improvisus* (Darwin, 1854). Moreover, representatives of sponges, bryozoans, turbellaria, and nemerteans were registered, which could not be identified.

In the cavities on the outer side of oyster valves (95–145 mm in size), 7 specimens of the rock-borer *P. lithophaga* were found, with a shell length of 9–16 mm. In five oysters, 1 specimen of the rock-borer was recorded in each; in one oyster – 2 specimens (Table 1). Thus, the number of oysters, infested with *Petricola*, amounted to 27 % of the total number of examined individuals.

Table 1. Morphometric characteristics of oysters *C. gigas* and rock-boring molluscs *P. lithophaga*, found inside their shells

No.	Oyster shell height, mm	Oyster shell length, mm	Total mollusc weight, g	Number of *P. lithophaga*, ind. per 1 oyster	*P. lithophaga* shell length, mm
1	95	45	65	1	9
2	110	40	80	1	12
3	110	40	81	1	16
4	120	65	100	1	12
5	130	60	110	1	14
6	145	55	98	2	13.11

In *C. gigas* valves, infested with *P. lithophaga*, an increased calcification on inner surface, at rock-borer penetration sites, was noted, as well as formation of characteristic rounded bulges (blisters). Live rock-boring molluscs were found inside the blisters. Large rock-borers greatly thinned the valve and were clearly visible, when we examined inner shell surface (Fig. 2). All found *P. lithophaga* were located in the central part of oyster shell or near its shell lock.

DISCUSSION

Previously, representatives of various taxonomic groups were registered among *C. gigas* parasites and pests: viruses, bacteria, parasitic fungi, protozoa, helminths, molluscs, and crustaceans (Gaevskaya & Lebedovskaya, 2010). This list also includes species of a special ecological group: rock-borers. Among the species dangerous to *C. gigas*, the most known are those of the families Clionaidae (sponges) and Spionidae (polychaetes), as well as several Mollusca representatives. The activity of rock-borers not only affects the overall condition of damaged molluscs, but also deteriorates oyster shell appearance, which reduces its commercial value.

Our finding of the rock-boring mollusc *P. lithophaga* in oyster shells in the Crimean Peninsula water area is of particular interest. Dense *C. gigas* shells served as a substrate for this infauna species, which usually inhabits rock ground, such as limestone, marl, *etc.* (Milashevich, 1916).

Four rock-boring mollusc species are known for the Black Sea: *Pholas dactylus* Linnaeus, 1758, *Barnea candida* (Linnaeus, 1758), *Rocellaria dubia* (Pennant, 1777), and *P. lithophaga* (Golikov & Starobogatov, 1972). Single cases of *P. lithophaga* and *R. dubia* finding in the valves of the Black Sea mussels and oysters were recorded (Gaevskaya, 2006 ; Kholodkovskaja, 2003). In the valves of oysters *C. gigas*, cultivated in Crimea, *P. lithophaga* was found for the first time.

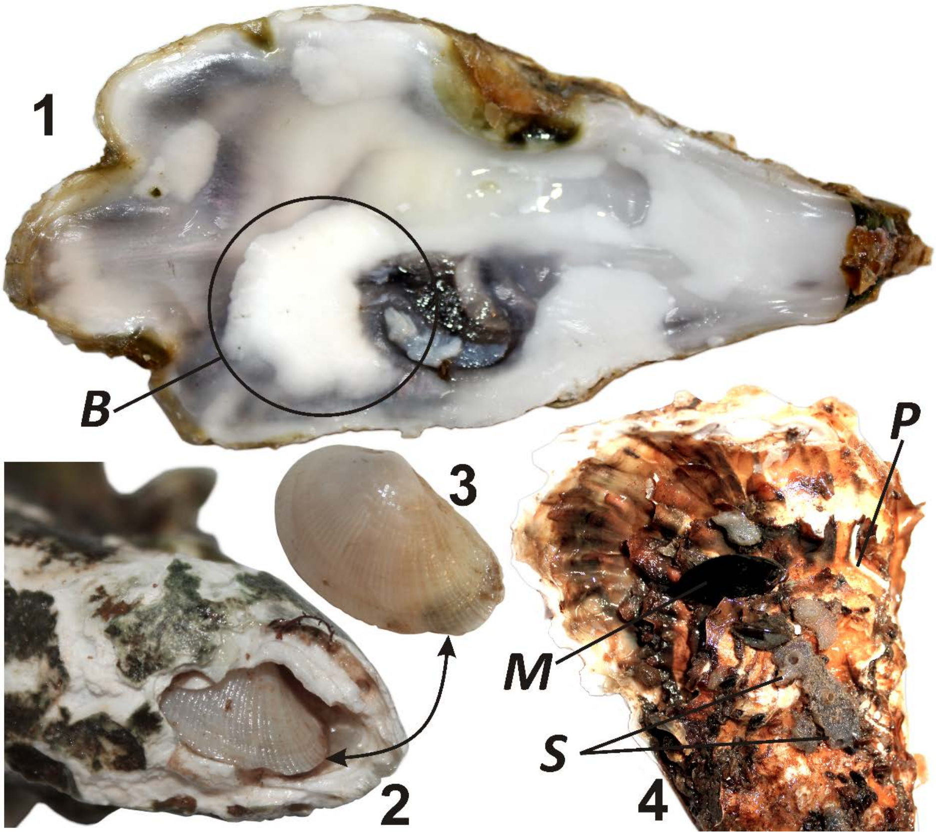


Fig. 2. *Petricola lithophaga* inside *Crassostrea gigas* valves:
1 – inner side of oyster valve;
2 – oyster shell, damaged by a rock-borer;
3 – *P. lithophaga* appearance (shell length of 13 mm);
4 – biofoulers on oyster shell.
Abbreviations:
B – blister (abnormal formation on oyster valve at rock-borer penetration site);
S – sponges;
P – polychaetes;
M – mussels

P. lithophaga range is European Atlantic Coast and northern Africa (it is distributed up to England in the north and up to Morocco in the south), the Sea of Marmara, and Mediterranean, Aegean, and Black seas (Golikov & Starobogatov, 1972). In the Mediterranean Basin, the species is poorly studied and, as a rule, is included only in general lists of benthic animals (Crocetta et al., 2013 ; Ricci et al., 2015 ; Sen et al., 2010 ; Zenetos et al., 2005). Off the coast of Crimea, this species was found in the upper sublittoral zone in the biotope of limestone boulders in the waters near Tarkhankut Peninsula, Sevastopol, Karadag Nature Reserve, and Dvuyakornaya Bay (Kovalyova, 2012, 2015). The degree of damage, caused by *Petricola*, is largely determined by the density of its population, which can be quite high in favorable biotopes. For the Black Sea, the maximum abundance of this mollusc was recorded in the Sevastopol Bay: the density and biomass averaged (185 ± 15) ind.$\cdot$m^{-2} and (144.7 ± 12) g$\cdot$m^{-2}, respectively (Kovalyova, 2012). In the limestones of the Donuzlav water area, *P. lithophaga* has not been previously found; there are no quantitative data on this mollusc in this area.

The maximum recorded *P. lithophaga* shell length is 30 mm (Golikov & Starobogatov, 1972). As established previously, when the representative of this species reaches one year, the size of its shell is 7–10 mm (Kovalyova, 2017). The individuals found by us were noticeably larger (9–16 mm);

their age was about two years. Probably, *P. lithophaga* larvae settled on oyster shells just after the latter appeared on the farm in 2017. Oyster shell height (Lisitskaya et al., 2010 ; Pirkova & Demenko, 2008) at that time was no more than 20–35 mm.

Prolonged presence of *P. lithophaga* inside oyster valves can cause first degradation of shell calcareous layer and then even death of a mollusc host. A case is described of finding a bivalve rock-borer *R. dubia* in *Venus verrucosa* Linnaeus, 1758 valves (Trigui El-Menif et al., 2005). The authors point out as follows: the presence of bored cavities increases the risk of infestation of a mollusc host with pathogens and makes it vulnerable to predators. As it is assumed, even some forms of competition for food may occur between a parasitic mollusc and a mollusc host, especially in cases when their siphons are in close proximity to each other. The negative effect of parasitic molluscs may also be as follows: as a rock-borer grows, a mollusc host has to make more and more effort in moving the valves to sustain itself. Moreover, some rock-borers [*P. lithophaga* is among them (Morton & Scott, 1988)] secrete an acid when boring a hole, and its effect on a mollusc host remains unstudied.

Conclusion. The fact of *P. lithophaga* finding on an oyster farm, located in the Donuzlav Bay, is not only of scientific interest: it has important applied significance for the Black Sea mariculture. Coastal water areas of Crimea are characterized by the presence of favorable conditions for increasing the number of mariculture farms and the volume of growing bivalve molluscs. Various shell damage, *e. g.* its boring, can lead not only to deterioration of oyster commercial appearance, but also to its death. In such conditions, monitoring studies of cultivated molluscs and parasitological control become relevant. The case of rock-borer finding in the shells of live oysters on a mariculture farm indicates the need for further research on this phenomenon, as well as for development of comprehensive diagnostic methods, prevention of infestation with rock-borers, and protection of cultivated molluscs. A detailed study of the development cycle of rock-boring molluscs will allow predicting the timing of their appearance on coastal mariculture farms and the timing of preventive measures to preserve cultivated oysters.

This work has been carried out within the framework of IBSS government 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. AAAA-A18-118020890074-2).

Acknowledgement. The authors are grateful to N. Boltachova, senior researcher, PhD, for the identification of polychaetes and to A. Nadolny, researcher, PhD, for his help in the design of the figures.

REFERENCES

1. Gaevskaya A. V. *Parasite, Diseases, and Pests of Mussels (Mytilus, Mytilidae). II. Mollusca.* Sevastopol : EKOSI-Gidrofizika, 2006, 100 p. (in Russ.)
2. Gaevskaya A. V., Lebedovskaya M. V. *Parasites and Diseases of the Giant Oyster (Crassostrea gigas) in Aquaculture.* Sevastopol : EKOSI-Gidrofizika, 2010, 218 p. (in Russ.)
3. Golikov A. N., Starobogatov Ya. I. *Opredelitel' fauny Chernogo i Azovskogo morei.* Kyiv : Naukova dumka, 1972, vol. 3, 166 p. (in Russ.)
4. Kiseleva M. I. *Polychaetes (Polychaeta) of the Black and Azov seas.* Apatity : Izd-vo Kol'skogo nauchnogo tsentra RAN, 2004, 409 p. (in Russ.)
5. Kovalyova M. A. Preliminary data on the distribution of the rock-boring mollusk *Petricola lithophaga* (Philippson 1788) along the western coast of Crimea. *Naukovi zapysky Ternopilskoho natsionalnoho pedahohichnoho universytetu imeni Volodymyra Hnatiuka*, 2012, vol. 51, no. 2, pp. 132–136. (in Russ.)

6. Lisitskaya E. V., Boltachova N. A., Lebedovskaya M. V. New Ukrainian fauna species *Polydora websteri* (Hartman, 1943) (Polychaeta: Spionidae) from the coastal waters of Crimea (Black Sea). *Morskoj ekologicheskij zhurnal*, 2010, vol. 9, no. 2, pp. 74–80. (in Russ.)
7. Milashevich K. O. *Fauna Rossii i sopredel'nykh stran* : in 6 vol. Petrograd : Tipografiya Imperatorskoi akademii nauk, 1916. Vol. 1. Molluski russkikh morei : Molluski Chernogo i Azovskogo morei, 312 p. (in Russ.)
8. Pirkova A. V., Demenko D. P. Cases of shell disease in the giant oyster *Crassostrea gigas* (Bivalvia) cultivated in the Black Sea. *Biologiya morya*, 2008, vol. 34, no. 5, pp. 359–364. (in Russ.) https://doi.org/10.1134/S1063074008050076
9. Kholodkovskaja E. V. Study of symbionts faunae of commercial Black Sea bivalve mollusks. *Ekologiya morya*, 2003, no. 63, pp. 66–72. (in Russ.)
10. Kholodov V. I., Pirkova A. V., Ladygina L. V. *Cultivation of Mussels and Oysters in Black Sea* / V. N. Eremeev (Ed.) ; A. O. Kovalevsky Institute of Biology of the Southern Seas of the National Academy of Sciences of Ukraine. Sevastopol : [DigitPrint], 2010, 424 p. (in Russ.)
11. Crocetta F., Bitar G., Zibrowius H., Oliverio M. Biogeographical homogeneity in the eastern Mediterranean Sea. II. Temporal variation in Lebanese bivalve biota. *Aquatic Biology*, 2013, vol. 19, no. 1, pp. 75–84. https://doi.org/10.3354/ab00521
12. Kovalyova M. A. Morphometric features of the shell and distribution of *Petricola lithophaga* (Retzius, 1788) (Mollusca: Veneridae) along the Crimean coast (Black Sea). *Acta Zoologica Bulgarica*, 2015, vol. 67, no. 4, pp. 587–590.
13. Kovalyova M. Reproductive biology of the rock-borer *Petricola lithophaga* (Retzius, 1788) (Bivalvia: Veneridae) in the Black Sea. *Molluscan Research*, 2017, vol. 37, iss. 3, pp. 159–166. https://doi.org/10.1080/13235818.2017.1279475
14. Morton B., Scott P. J. B. Evidence for chemical boring in *Petricola lapicida* (Gmelin, 1791) (Bivalvia: Petricolidae). *Journal of Molluscan Studies*, 1988, vol. 54, iss. 2, pp. 231–237. https://doi.org/10.1093/mollus/54.2.231
15. Nair N. U., Nair N. B. Height-Length relation of shells in the Indian blackwater oyster *Crassostrea madrasensis* (Preston) of the Cochin Harbour. *Fishery Technology*, 1986, vol. 1, no. 231, pp. 27–31. http://aquaticcommons.org/18508
16. Trigui El-Menif N., Guezzi Y., Le Pennec M., Boumaiza M., Le Pennec G. Infestation of the clam *Venus verrucosa* by Sipunculoidea and the lithophagus bivalve, *Gastrochaena dubia*. *Acta Adriatica*, 2005, vol. 46, no. 1, pp. 83–90. https://hrcak.srce.hr/81
17. Ricci S., Antonelli F., Sacco Perasso C. Aspects of biodeterioration of lapideous submerged artefacts: 3D methodologies application. *ISPRS – International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences*, 2015, vol. 40/5W5, pp. 191–194. https://doi.org/10.5194/isprsarchives-XL-5-W5-191-2015
18. Sen S., Sivrikaya H., Yalcin M., Bakir A. K., Ozturk B. Fouling and boring organisms that deteriorate various European and tropical woods at Turkish seas. *African Journal of Biotechnology*, 2010, vol. 9, no. 17, pp. 2566–2573.
19. Zenetos A., Vardala-Theodorou E., Alexandrakis C. Update of the marine Bivalvia Mollusca checklist in Greek waters. *Journal of the Marine Biological Association of the United Kingdom*, 2005, vol. 585, iss. 4, pp. 993–998. https://doi.org/10.1017/S0025315405012014

ПЕРВОЕ ОБНАРУЖЕНИЕ МОЛЛЮСКА-КАМНЕТОЧЦА *PETRICOLA LITHOPHAGA* (RETZIUS, 1788) В СТВОРКАХ КУЛЬТИВИРУЕМЫХ В КРЫМУ УСТРИЦ *CRASSOSTREA GIGAS* (THUNBERG, 1793) (ЗАЛИВ ДОНУЗЛАВ, ЧЁРНОЕ МОРЕ)

М. А. Ковалёва, О. Ю. Вялова

Федеральный исследовательский центр «Институт биологии южных морей
имени А. О. Ковалевского РАН», Севастополь, Российская Федерация
E-mail: *kovalmargarita@mail.ru*

За последние годы значительно увеличилось количество ферм по выращиванию моллюсков у берегов Крыма и Кавказа. Культивирование тихоокеанской устрицы *Crassostrea gigas* (Thunberg, 1793) требует проведения мониторинга здоровья моллюсков и паразитологического контроля морских ферм. Целью данной работы было изучить видовой состав эпибионтов и эндобионтов, ассоциированных с раковинами культивируемых устриц *C. gigas*, а также выявить виды, повреждающие раковины. Товарные устрицы с визуальными повреждениями раковины собраны на морской ферме в заливе Донузлав (Крым, Чёрное море) и доставлены для дальнейшего изучения в лабораторию в живом виде охлаждёнными. В результате обследования 22 устриц обнаружены 14 видов макрозообентоса и экземпляры живых моллюсков-камнеточцев *Petricola lithophaga* (Retzius, 1788). Размеры моллюсков-камнеточцев варьировали от 9 до 16 мм, возраст составлял около двух лет. Длительное нахождение *P. lithophaga* внутри створок устриц может вызывать деградацию известкового слоя раковины и даже гибель моллюска-хозяина; данный факт имеет большое значение для марикультуры Чёрного моря. Принимая в расчёт годовой цикл развития камнеточца-петриколы, в период массового оседания его личинок (с июля по октябрь) можно рекомендовать проводить осмотр раковин культивируемых устриц. Выполнение дальнейших детальных исследований позволит разработать мероприятия по профилактике и защите двустворчатых моллюсков от заражения камнеточцем *P. lithophaga*.

Ключевые слова: марикультура, комменсалы, инфауна, перфорирование раковины, устрицы, Чёрное море

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 1, pp. 41–57
https://doi.org/10.21072/mbj.2021.06.1.04

UDC [504.5:620.267](261.243)

ASSESSMENT OF RADIATION STATE OF MARINE ENVIRONMENT IN THE LENINGRAD NPP AREA ACCORDING TO LONG-TERM MONITORING DATA (1973–2019)

© 2021 **I. I. Kryshev, T. G. Sazykina, N. N. Pavlova, I. V. Kosykh, A. A. Buryakova, and A. I. Kryshev**

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

Received by the Editor 05.11.2020; after reviewing 17.12.2020;
accepted for publication 11.03.2021; published online 23.03.2021.

The aim of the study was to conduct a radioecological assessment of the Leningrad NPP marine cooling reservoir – Koporye Bay of the Gulf of Finland. According to the international basic safety standards, accepted at the IAEA General Conference, this issue is of particular relevance due to the need to justify protection from technogenic radiation exposure both to humans and the environment. The assessment was based on the long-term radioecological monitoring data (1973–2019) within the Leningrad NPP observation area: radionuclides concentration in seawater, bottom sediments, and hydrobionts. The reference levels of radionuclides content in seawater and bottom sediments were used as indicators of the radiation state of the marine environment; their calculation procedure is defined in the Recommendations R 52.18.852-2016 and R 52.18.873-2018, issued by the Federal Service for Hydrometeorology and Environmental Monitoring (the Ministry of Natural Resources and Environment of the Russian Federation). These recommendations, developed by RPA "Typhoon" specialists, contain a methodology for assessing the radioecological state of the marine environment by the level of radionuclides activity, based on the principles, ensuring the maintenance of favorable environment, safety of marine hydrobionts, and radiation protection of humans. In the presence of various radionuclides in the marine environment, the sum of technogenic radionuclide activity ratios in seawater (bottom sediments) to the corresponding reference levels shall be below 1. According to monitoring data in the early period of NPP operation (1973–1985), a wide spectrum of technogenic radionuclides was observed in the marine ecosystem components. Along with ^{137}Cs, significant contributors to the contamination of seawater and bottom sediments were ^{54}Mn and ^{60}Co. In contrast to reference levels for ^{137}Cs, reference levels for ^{54}Mn and ^{60}Co in seawater are determined by an environmental criterion, not a radiation-hygienic one. The presence of technogenic radionuclides in algae was registered at distances, exceeding 10 km from the NPP. Biogenic transfer of corrosion radionuclides (^{54}Mn, ^{60}Co, and ^{65}Zn) by fish into rivers, flowing into the Koporye Bay, was noted. The Chernobyl disaster led to a noticeable increase in the pollution of the Koporye Bay with technogenic radionuclides. In May – December 1986, the sum of technogenic radionuclide activity ratios in seawater to the reference levels exceeded the pre-accidental level by 100 times, and in bottom sediments – by 30 times. In 1986, ^{137}Cs and ^{134}Cs were the main contributors to the marine ecosystem radioactive contamination. Currently, the technogenic radioactivity of seawater and bottom sediments of the Koporye Bay is mainly determined by ^{137}Cs; its level is relatively constant, which indicates the stability of the radioecological situation in the Leningrad NPP marine cooling reservoir.

Keywords: Koporye Bay of the Gulf of Finland, Leningrad NPP, radioecological monitoring, seawater, bottom sediments, radionuclide reference levels, environmental and radiation-hygienic criteria, integral indicator of pollution, long-term dynamics

In accordance with the modern paradigm of radiation safety, not only humans, but also other organisms must be protected from radiation risks; it is necessary to confirm (not to assume) that the environment is protected from industrial radiation exposure (Kryshev & Sazykina, 2013, 2014, 2018 ; Radiation Protection…, 2014).

The Leningrad NPP (hereinafter LNPP) is located 80 km west of Saint Petersburg, on the Koporye Bay coast (the Gulf of Finland, the Baltic Sea). Koporye Bay is used as a cooling reservoir for the LNPP since 1973. Currently, the nuclear power plant operates two power units with RBMK-1000 reactors and one power unit with a WWER-1200 reactor. In 2021, it is planned to launch another power unit with a WWER-1200 reactor. Two power units with RBMK-1000 reactors were shut down for decommissioning. LNPP operation is accompanied by releases of radioactive material into the atmosphere and discharges into the marine environment. In this area, other enterprises of the nuclear-industrial complex are also located: A. P. Aleksandrov Scientific Research Technological Institute with a complex of experimental nuclear installations and Leningrad Office of "North-Western Territorial District" branch of "Federal Ecological Operator", which processes and stores radioactive waste.

To date, a unique experience of environmental radioecological monitoring has been accumulated in the LNPP area, including marine cooling reservoir, which is exposed to the effect of radioactive material discharge and a set of non-radiational factors (thermal discharge, chemical pollution, eutrophication, and mechanical injury of organisms in the NPP water intakes) (Kryshev, 2017 ; Kryshev & Ryazantsev, 2010 ; Ekologo-geofizicheskie aspekty…, 1992). Territorially, the monitoring object was the marine cooling reservoir and the adjacent zone of the catchment area with rivers, flowing into the Koporye Bay.

The Koporye Bay of the Gulf of Finland with an area of 255 km^2 is of shallow semi-enclosed water bodies type, with an extended watershed boundary with the main water area. Salinity varies 2 to 4 ‰. The bottom is mainly sandy, and sometimes silt and stones are found. Mean depth is about 12 m. Maximum depths (up to 27 m) are registered on the border with the main water area. The LNPP discharges heated water to the eastern Koporye Bay with the area of about 50 km^2 and mean depth of 5 m. Three rivers (Sista, Kovashi, and Voronka) flow into the eastern Koporye Bay; their total mean annual flow rate is about 10 m^3·s^{-1}. Taking into account their pollution by industrial and domestic wastewater, it can be considered that it is the eastern Koporye Bay (which is used as a cooling reservoir), that is the most affected by anthropogenic factors in the LNPP area.

The aim of our work was to assess the radioecological state of the Leningrad NPP marine cooling reservoir – the Koporye Bay of the Gulf of Finland – based on long-term radioecological monitoring data (1973–2019) and criteria, providing the preservation of a favorable environment and hydrobionts, as well as human radiation protection.

MATERIAL AND METHODS

Radioecological monitoring. For more than 47 years, environmental departments of Roshydromet (Russian Federal Service for Hydrometeorology and Environmental Monitoring), Scientific Research Technological Institute, V. G. Khlopin Radium Institute, and the LNPP are carrying out monitoring of the technogenic radionuclide content in seawater, bottom sediments, and marine biota of the Koporye Bay (Blinova, 1998 ; Bondarenko et al., 2013 ; Vakulovskii & Nikitin, 1984 ; Kryshev, 2017 ; Kryshev & Blinova, 1991 ; Kryshev & Ryazantsev, 2010 ; Radiatsionnaya obstanovka…, 1992–2020 ; Radioaktivnoe zagryaznenie raionov AES, 1990). To analyze radionuclide content, standard methods of sampling, radiochemical analysis, and radiometric and spectrometric measurements are used (Metodicheskie rekomendatsii…, 1980, 1986 ; Nastavlenie gidrometeorologicheskim stantsiyam…, 2015 ; Rukovodstvo po organizatsii…, 1990).

The most detailed radioecological monitoring was carried out in the period, when LNPP with RBMK-1000 reactors reached maximum power (1973–1985), as well as in the first years after the Chernobyl disaster (Kryshev & Blinova, 1991 ; Kryshev & Ryazantsev, 2010). Monthly, radionuclide content was determined in the samples of the NPP intake and discharge channels. Monthly during the growing season of water plants (April to October) at 5 points of the coastal water area and year-round in the discharge channels of heated water, specific activity was determined in the samples of seawater, bottom sediments, and water plants. Annually, specific activity was measured in 20–30 fish samples from the nets of the NPP water intakes and from the catches of fishermen (Blinova, 1998). The results of the NPP cooling reservoir monitoring in the pre-Chernobyl period are summarized for further analysis (Table 1).

In addition to radionuclides (see Table 1), ^{51}Cr, ^{58}Co, ^{95}Zr, ^{95}Nb, and ^{134}Cs were determined in bottom sediment samples in 1–5 % of cases (Kryshev & Blinova, 1991). In the samples of marine plants in the Koporye Bay, along with radionuclides, a wider range of technogenic radionuclides was recorded, than in seawater and bottom sediment samples, in 4–28 % of cases: ^{51}Cr, ^{58}Co, ^{59}Fe, ^{95}Zr, ^{95}Nb, ^{131}I, ^{134}Cs, ^{141}Ce, and ^{144}Ce (Radioaktivnoe zagryaznenie raionov AES, 1990).

Table 1. Technogenic radionuclides content in components of the marine cooling reservoir at different distances from the Leningrad NPP (1973–1985)

Sampling location	Radionuclides				
	^{90}Sr	^{137}Cs	^{54}Mn	^{60}Co	^{65}Zn
Seawater, mBq·L^{-1}					
Discharge channels	30 ± 3 (100 %)	32 ± 6 (100 %)	38 ± 14 (5 %)	33 ± 11 (7 %)	< MDA
1–3 km from the LNPP	27 ± 2 (100 %)	18 ± 2 (100 %)	31 ± 13 (4 %)	30 ± 9 (4 %)	< MDA
3–10 km	23 ± 2 (100 %)	11 ± 2 (100 %)	< MDA	< MDA	< MDA
Over 10 km	23 ± 2 (100 %)	10 ± 2 (100 %)	< MDA	< MDA	< MDA
Reference level in seawater (Poryadok rascheta…, 2016)	16 800	1220	270	610	116

Continue on the next page…

Sampling location	Radionuclides				
	^{90}Sr	^{137}Cs	^{54}Mn	^{60}Co	^{65}Zn
Bottom sediments, Bq·kg^{-1} of wet weight					
Discharge channels	3.5 ± 1.6 (100 %)	4.0 ± 1.7 (100 %)	8 ± 3 (25 %)	8 ± 3 (12 %)	4.4 ± 2.1 (6 %)
1–3 km from the LNPP	2.0 ± 0.9 (100 %)	2.1 ± 1.0 (100 %)	14 ± 6 (10 %)	10 ± 4 (12 %)	11 ± 5 (5 %)
3–10 km	2.1 ± 1.1 (100 %)	2.0 ± 1.0 (100 %)	6 ± 3 (12 %)	7 ± 3 (7 %)	< MDA
Over 10 km	1.5 ± 0.8 (100 %)	1.6 ± 0.7 (100 %)	0.2 ± 0.1 (7 %)	< MDA	< MDA
Reference level in bottom sediments (Poryadok rascheta…, 2019)	2500	1500	810	1800	2400
Water plants, Bq·kg^{-1} of wet weight					
Discharge channels	1.8 ± 0.6 (100 %)	5.2 ± 1.4 (100 %)	18 ± 6 (43 %)	17 ± 5 (41 %)	13 ± 4 (12 %)
1–3 km from the LNPP	1.7 ± 0.7 (100 %)	4.8 ± 1.5 (100 %)	15 ± 5 (31 %)	15 ± 5 (33 %)	10 ± 4 (11 %)
3–10 km	1.7 ± 0.7 (100 %)	3.3 ± 1.5 (100 %)	5.2 ± 1.9 (30 %)	7 ± 3 (19 %)	10 ± 4 (11 %)
Over 10 km	1.2 ± 0.5 (100 %)	1.6 ± 0.7 (100 %)	5.2 ± 2.0 (10 %)	4.4 ± 1.8 (19 %)	7 ± 3 (7 %)
Fish, Bq·kg^{-1} of wet weight					
Discharge channels					
Common roach *Rutilus rutilus* (Linnaeus, 1758)	2.3 ± 0.7 (100 %)	1.7 ± 0.5 (100 %)	18 ± 6 (30 %)	11 ± 3 (52 %)	70 ± 27 (43 %)
Koporye Bay					
Common roach *R. rutilus*	1.8 ± 0.7 (100 %)	1.5 ± 0.5 (100 %)	1.5 ± 0.6 (7 %)	10 ± 3 (14 %)	60 ± 21 (28 %)
Baltic herring *Clupea harengus membras* Linnaeus, 1760	1.3 ± 0.5 (100 %)	2.0 ± 0.7 (100 %)	0.3 ± 0.1 (4 %)	5.4 ± 1.9 (12 %)	12 ± 5 (8 %)
Common perch *Perca fluviatilis* Linnaeus, 1758	1.5 ± 0.5 (100 %)	2.7 ± 0.8 (100 %)	6.3 ± 2.5 (20 %)	10 ± 3 (20 %)	14 ± 5 (10 %)
Rivers					
Common roach *R. rutilus*	1.7 ± 0.7 (100 %)	1.3 ± 0.5 (100 %)	3.7 ± 1.3 (17 %)	1.9 ± 0.7 (17 %)	34 ± 11 (17 %)

Note: in parentheses, the frequency of radionuclide detection above MDA according to monitoring data is indicated; MDA is the minimum detectable activity (5 mBq·L^{-1} for seawater; 0.2 Bq·kg^{-1} for bottom sediments; 0.6 Bq·kg^{-1} for water plants and fish).

The Chernobyl disaster had a significant effect on the radiation state in the LNPP area, including the Koporye Bay (Blinova, 1998 ; Kryshev & Blinova, 1991 ; Kryshev & Ryazantsev, 2010 ; Radioaktivnoe zagryaznenie raionov AES, 1990). The radioactive cloud of the accidental release reached the LNPP area on 28.04.1986. An atmospheric fallout resulted in the pollution of the Koporye Bay catchment area, seawater, bottom sediments, and biota. After a decay of ^{131}I (half-life of 8.04 days) and other short-lived radionuclides, the main radioecological significance in this part of the distant Chernobyl trace was acquired by ^{134}Cs (half-life of 2.06 years) and in particular the long-lived ^{137}Cs (half-life of 30 years); thus, ^{137}Cs content in the marine ecosystem components increased by 6–250 times, compared with the pre-accidental level (Table 2).

Table 2. Dynamics of ^{137}Cs average annual content in the coastal marine ecosystem components in the Leningrad NPP area (1985–1990)

Ecosystem component		Average data for monthly observations					
		1985	1986*	1987	1988	1989	1990
Seawater, mBq·L^{-1}		10 ± 3	1300 ± 570	290 ± 110	130 ± 40	56 ± 18	52 ± 15
Bottom sediments, Bq·kg^{-1} of wet weight		1.5 ± 0.6	49 ± 19	19 ± 8	10 ± 4	10 ± 3	8 ± 3
Water plants, Bq·kg^{-1} of wet weight		0.8 ± 0.3	200 ± 97	17 ± 7	25 ± 8	14 ± 6	12 ± 5
Fish, Bq·kg^{-1} of wet weight	Baltic herring *C. harengus membras*	1.7 ± 0.5	27 ± 11	36 ± 15	19 ± 7	14 ± 5	25 ± 11
	Common perch *P. fluviatilis*	3.5 ± 1.1	22 ± 10	123 ± 41	126 ± 39	113 ± 31	116 ± 39

Note: * denotes averaged data for May – December 1986.

The highest levels of ^{137}Cs contamination for almost all marine ecosystem components (except for predatory fish species) were recorded in 1986. For predatory fish, the effect of trophic levels was observed since 1987, which was manifested in an increased accumulation of radiocesium a year after the accidental pollution, compared with the accumulation by non-predatory species. For most marine ecosystem components, there was a gradual decrease in ^{137}Cs content; however, it remained 5–30 times higher, than the pre-accidental level, even in 1990.

The current values of radionuclides content in the coastal marine ecosystem components of the Koporye Bay of the Gulf of Finland were determined (Table 3) (Bondarenko et al., 2013 ; Radiatsionnaya obstanovka…, 1992–2020).

According to the long-term monitoring data, more than 30 years after the Chernobyl disaster, ^{137}Cs content in bottom sediments, seawater, and fish tissues (common perch) in the Koporye Bay still exceeds the pre-accidental level. At the same time, the content of corrosion radionuclides of the LNPP origin in seawater and bottom sediments has noticeably decreased. Tritium and carbon-14, both of natural and technogenic origin, have acquired particular significance.

Table 3. Radionuclide content in the coastal marine ecosystem components in the Leningrad NPP area (2000–2019)

Ecosystem component		Radionuclide					
		^{90}Sr	^{137}Cs	^{60}Co	^{54}Mn	^{14}C	^{3}H
Seawater, mBq·L^{-1}		12 ± 2	20 ± 4	26 ± 7*	< MDA	–	21 000 ± 10 000
Reference level for seawater		16 800	1220	610	270	137	86 800 000
Bottom sediments, Bq·kg^{-1} of wet weight		0.70 ± 0.16	19 ± 3	1.5 ± 0.7	0.9 ± 0.4	–	–
Reference level for bottom sediments		2500	1500	1800	810	41	–
Fish, Bq·kg^{-1} of wet weight	Common roach *R. rutilus*	0.20 ± 0.08	1.2 ± 0.3	–	–	150 ± 50**	56 ± 17**
	Common perch *P. fluviatilis*	0.30 ± 0.10	6.2 ± 1.3	–	–	63 ± 31**	38 ± 11**

Note: a dash (–) denotes no data; * denotes data for 2002–2004 (Radiatsionnaya obstanovka…, 1992–2020); ** denotes data for 2012 (Bondarenko et al., 2013).

Methods for assessing the radiation state of the marine environment. The reference levels of technogenic radionuclides in seawater and bottom sediments were used as criteria for assessing the radiation state of the LNPP marine cooling reservoir; their calculation procedure is defined in the Roshydromet Recommendations (Poryadok rascheta…, 2019, 2016). There, for the first time in domestic and world practice, a methodology is given for assessing the quality of the marine environment on the basis of environmental and radiation-hygienic principles, that provide the preservation of a favorable environment and biological diversity, protection of aquatic ecosystems, and human radiation protection.

The environmental criterion for assessing the reference levels of radionuclide content in seawater is considered to be the maximum permissible dose (P_{max}) on marine biota objects. Its values are as follows: 1.0 mGy·day^{-1} for marine vertebrates and 10 mGy·day^{-1} for marine invertebrates and water plants (Otsenka radiatsionno-ekologicheskogo vozdeistviya…, 2015 ; Environmental protection…, 2009).

Due to huge species diversity of the biosphere and the practical impossibility of assessing the radiation effect on each flora and fauna component, the methodology of environmental radiation safety has been developed for a relatively small number of representative biota species (Otsenka radiatsionno-ekologicheskogo vozdeistviya…, 2015 ; Environmental protection…, 2009). The reference level of the *i*-th radionuclide in seawater for the *k*-th representative marine biota object $A_{i,k,ec}$, Bq·L^{-1}, is calculated by the formula (Kryshev et al., 2017 ; Poryadok rascheta…, 2016):

$$A_{i,k,\mathrm{ec}} = \frac{P_{max,k}}{(DCF_{i,k,1} \cdot CF_{i,k,2} + DCF_{i,k,2} \cdot \alpha'_{k,2} + 0.5 \cdot DCF_{i,k,2} \cdot K_{d,i,3} \cdot \alpha'_{k,3}) \cdot \tau}\,, \qquad (1)$$

where $P_{max,k}$ is criterion of the maximum permissible dose on the *k*-th representative marine biota object, $mGy \cdot day^{-1}$;

$DCF_{i,k,1}$ and $DCF_{i,k,2}$ are dose conversion factors for internal and external exposure, respectively, of the *k*-th representative marine biota object to the *i*-th radionuclide, $(\mu Gy \cdot hour^{-1})/(Bq \cdot kg^{-1}$ of wet weight);

$CF_{i,k,2}$ is accumulation coefficient of the *i*-th radionuclide in the *k*-th representative marine biota object, $L \cdot kg^{-1}$;

$K_{d,i,3}$ is distribution coefficient of the *i*-th radionuclide between seawater and bottom sediments, $L \cdot kg^{-1}$;

$\alpha'_{k,2}$ and $\alpha'_{k,3}$ are the ratios of time, that the *k*-th representative marine biota object spends in the water and at the bottom, respectively, dimensionless coefficients;

τ is conversion factor, equal to $2.4 \cdot 10^{-2}$ $(mGy \cdot day^{-1})/(\mu Gy \cdot hour^{-1})$.

In accordance with regulatory documents (Otsenka radiatsionno-ekologicheskogo vozdeistviya…, 2015 ; Environmental protection…, 2009), the following marine biota objects were selected as reference ones: marine fish, molluscs, crustaceans, algae, and mammals. Non-exceeding the minimum $A_{i,k,ec}$ value for all representative organisms in the marine ecosystem guarantees sustainable functioning and preservation of the ecosystem species diversity as a whole. For most of technogenic radionuclides, detected by the radioecological monitoring system in the LNPP area, fish are critical organisms; they are characterized by the lowest values of the reference levels of radionuclides content in seawater (Poryadok rascheta…, 2016). As representative fish species of the marine cooling reservoir, common roach *Rutilus rutilus* (Linnaeus, 1758) and common perch *Perca fluviatilis* Linnaeus, 1758 were chosen (Radioaktivnoe zagryaznenie raionov AES, 1990), since they are available for catching throughout the year both in areas of discharge water distribution and in other areas, *inter alia* basins of rivers, flowing into the sea bay. These species spawn in the cooling reservoir, including the discharge channels; juveniles live in the channels constantly in significant quantities; large specimens accumulate in the area of heated water distribution, where they are fished in commercial quantities. It is essential that these species breed in thickets of aquatic plants, that accumulate radionuclides. Roe and juveniles of common roach and common perch experience increased dose loads and high temperature (above +30 °C in summer).

When calculating the reference levels of radionuclides in seawater, it is taken into account that it is not consumed for drinking purposes. As a radiation-hygienic criterion, radiation dose limit from seafood consumption is used for a critical population group, which is characterized by significant seafood consumption (Kryshev et al., 2017 ; Poryadok rascheta…, 2016 ; Sazykina & Kryshev, 1999).

The reference level of the *i*-th radionuclide in seawater according to the radiation-hygienic criterion $A_{i,human}$, $Bq \cdot L^{-1}$, is calculated by the formula:

$$A_{i,human} = \frac{DL_{10\%}}{E_i \sum CF_{i,k,2} \cdot R_k}\,, \tag{2}$$

where $DL_{10\%}$ is a part of the dose limit (DL) for the critical population group from consumption of seafood, containing the *i*-th radionuclide, equal to 10 %, or 0.1 $mSv \cdot year^{-1}$ (according to Sanitary rules and regulations SanPiN 2.6.1.2523, DL is of 1 $mSv \cdot year^{-1}$);

E_i denotes dose coefficients for the *i*-th radionuclide, Sv·Bq^{-1}, when assessing the dose per person from seafood consumption according to SanPiN 2.6.1.2523;

$CF_{i,k,2}$ is accumulation coefficient of the *i*-th radionuclide in the *k*-th seafood, L·kg^{-1};

R_k denotes values of the annual consumption of the *k*-th seafood by the critical population group.

In order to provide environmental safety, it is recommended to take the minimum value $A_{i,min}$, calculated according to the radiation-hygienic and environmental criteria, as a reference level of radionuclide content in seawater:

$$A_{i,min} = min\{A_{i,human}, A_{i,k,ec}\} \ . \quad (3)$$

In the presence of a mixture of radionuclides in seawater, it is necessary to fulfill the condition of non-exceeding the environmentally safe level to provide the radiation protection of humans and marine biota (Poryadok rascheta…, 2016):

$$\sum_i \frac{A_{v,i}}{A_{i,min}} \leq 1 \ , \quad (4)$$

where $A_{v,i}$ is activity concentration of the *i*-th radionuclide in seawater, Bq·L^{-1}.

The fulfillment of the condition (4) provides both environmental and radiation-hygienic safety, since the total contamination of seawater by technogenic radionuclides in this case will not result in exceeding the safe radiation level for biota and human radiation safety standards.

The reference technogenic radionuclide activity ratios for marine bottom sediments are determined in a similar way (Poryadok rascheta…, 2019).

The sum of technogenic radionuclide activity ratios, observed in seawater and bottom sediments, to the values of the reference levels will be further interpreted as an integral indicator of pollution of a reservoir with technogenic radionuclides. The use of ratios, similar to (4), and the corresponding indicators (indices) of pollution is widespread in the practice of radioecological monitoring (Radiatsionnaya obstanovka…, 1992–2020).

RESULTS

The distribution of the integral indicators of seawater and bottom sediments pollution with technogenic radionuclides in the Koporye Bay at different distances from the LNPP, calculated based on the long-term monitoring data (see Table 1) in the pre-Chernobyl period, indicates that the contamination values both of seawater and bottom sediments were the highest in the NPP discharge channels and the lowest at a distance over 10 km (Fig. 1). With the distance from the NPP, there is a gradual decrease in the level of contamination by technogenic radionuclides of the coastal zone of the eastern Koporye Bay. The main contributors to seawater contamination in the Koporye Bay coastal zone, close to the NPP, are ^{137}Cs (52–55 %), ^{54}Mn (14–17 %), and ^{60}Co (7–14 %). The contribution of ^{90}Sr to seawater contamination in this area is 4–8 %. At a distance over 10 km, the contribution of ^{137}Cs to technogenic radioactive contamination increases up to 85 %, and of ^{90}Sr – up to almost 15 %. Approximately the same radionuclide composition and spatial distribution of technogenic radionuclide contamination are registered for bottom sediments.

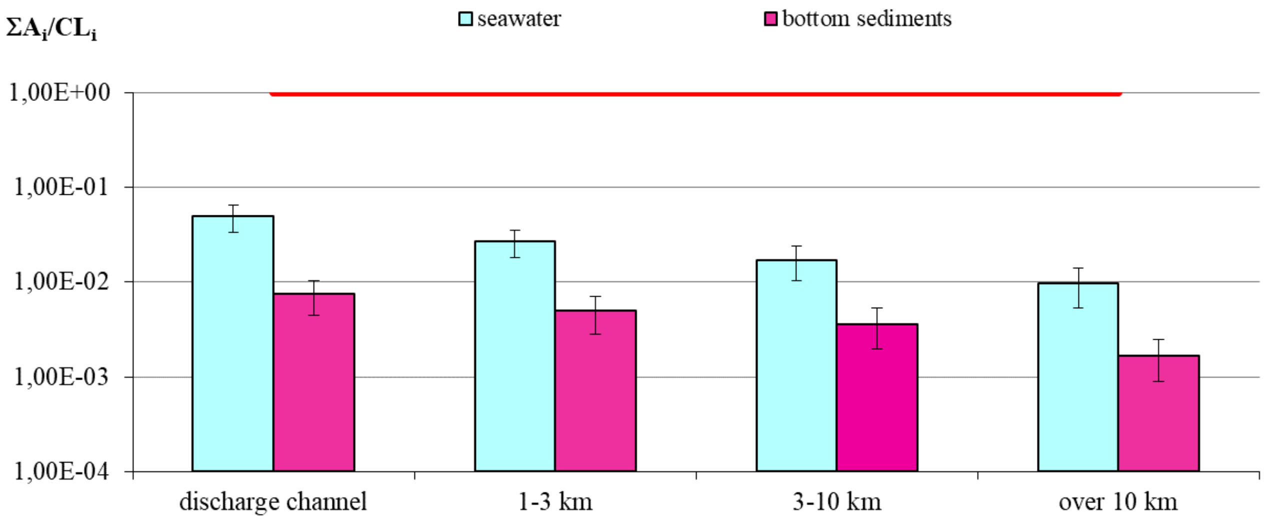


Fig. 1. Sum of technogenic radionuclide activity ratios, observed in coastal seawater and bottom sediments of the Koporye Bay in the Leningrad NPP area, to the reference levels (1973–1985). The upper horizontal line corresponds to the fulfillment of the condition (4), providing the environmental and radiation-hygienic safety of the marine environment

Taking into account the non-equilibrium of radioecological processes after the Chernobyl disaster and following the recommendations (Poryadok rascheta…, 2019, 2016), the sum of observed technogenic radionuclide activity ratios to the reference levels in 1986–1990 was calculated for the studied marine ecosystem on the basis of the monitoring data (Blinova, 1998 ; Kryshev & Ryazantsev, 2010 ; Radioaktivnoe zagryaznenie raionov AES, 1990). The dynamics of variations of this indicator is characterized by reaching the maximum values in May – December 1986 (Fig. 2), close to the value for seawater,

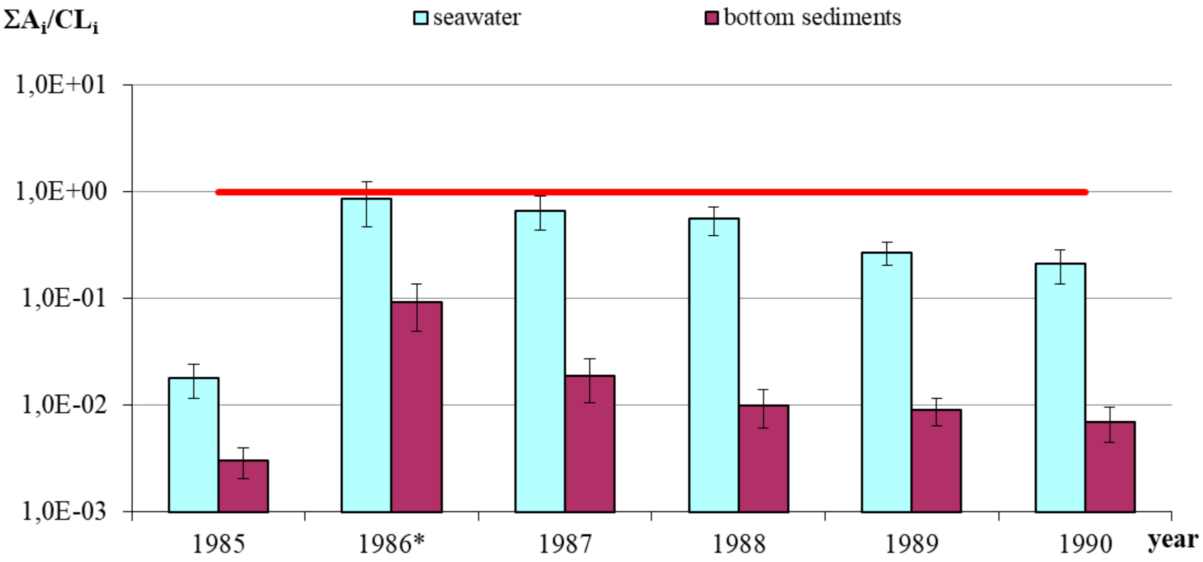


Fig. 2. Dynamics of changes of the sum of technogenic radionuclide activity ratios, observed in seawater and bottom sediments of the Koporye Bay in the Leningrad NPP area, to the reference levels before and after the Chernobyl disaster (1985–1990); * – according to data for May – December 1986. The upper horizontal line corresponds to the fulfillment of the condition (4), providing the environmental and radiation-hygienic safety of the marine environment

which corresponds to the fulfillment of the condition (4), providing environmental and radiation-hygienic safety. The main contributors to the radioactive contamination of the reservoir in 1986 were ^{137}Cs and ^{134}Cs, the reference levels for which are limited by the radiation-hygienic criterion. The rest of the technogenic radionuclides (^{95}Zr, ^{95}Nb, ^{131}I, ^{90}Sr, *etc.*) made a total contribution of less than 2 %. In subsequent years, there was a decrease in the level of contamination, but even in 1990 the value was an order of magnitude higher than the pre-accidental one.

The current dynamics of radioactive contamination of seawater and bottom sediments of the Koporye Bay coastal zone in the Leningrad NPP area is generally characterized by a relatively stable level of technogenic radionuclide activity ratios (Fig. 3).

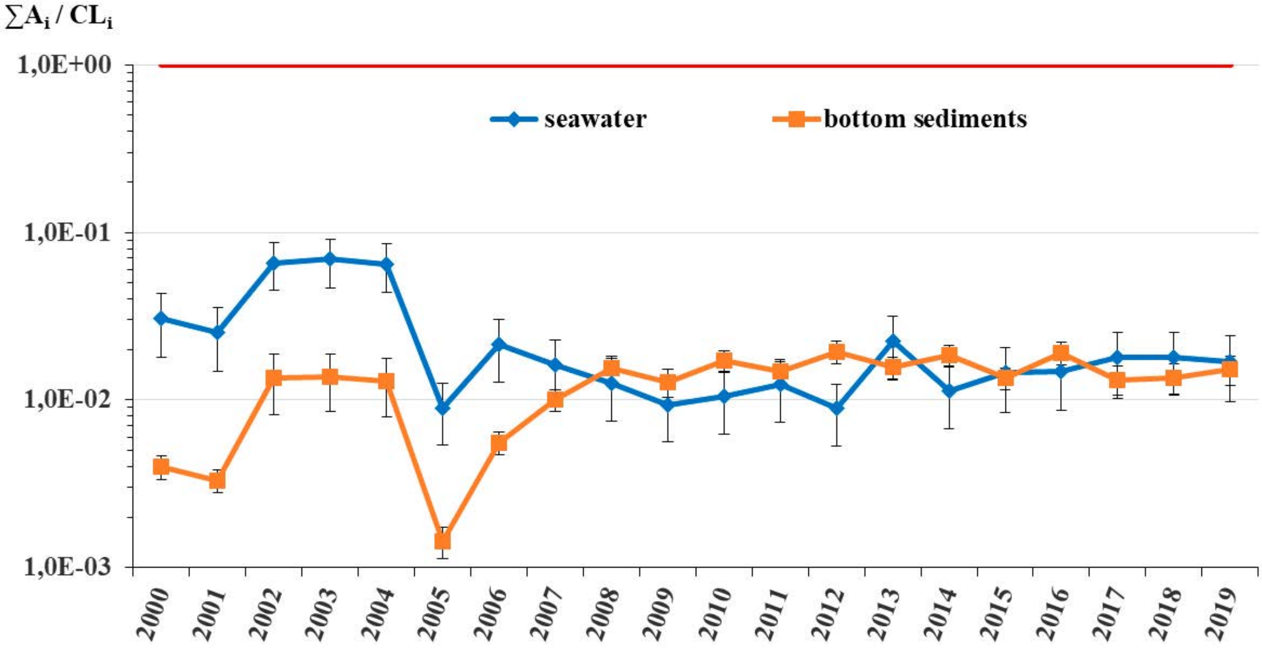


Fig. 3. Dynamics of changes of the sum of technogenic radionuclide activity ratios, observed in seawater and bottom sediments of the Koporye Bay in the Leningrad NPP area, to the reference levels (2000–2019). The upper horizontal line corresponds to the fulfillment of the condition (4), providing the environmental and radiation-hygienic safety of the marine environment

In 2002–2004, an increase was noted in the sum of technogenic radionuclide activity ratios, observed in seawater and bottom sediments of the Koporye Bay coastal zone, to the reference levels; that was associated with ^{60}Co presence in these marine ecosystem components in amounts, comparable to those of ^{137}Cs. In subsequent years, seawater contamination was mainly due to ^{137}Cs and was approximately at the same level. Similar dynamics was observed for bottom sediments.

DISCUSSION

Before the recommendations R 52.18.852-2016 2016 (Poryadok rascheta…, 2016) and R 52.18.873-2018 (Poryadok rascheta…, 2019) were published, there were no regulatory and methodological documents in the country and in the world regarding the criteria for assessing the radiation state of the marine environment, non-exceeding of which provides radiation safety and preservation of a favorable environment, as well as hydrobionts and humans. This created difficulties in solving environmental problems and using atomic energy at sea.

The main source of additional exposure of population due to contacts with the marine environment is seafood consumption. Seawater is practically not consumed in Russia for drinking purposes. For this reason, intervention levels of separate radionuclides content in drinking water cannot be applied to assess the radiation state of seawater (these levels are published in the radiation safety standards NRB-99/2009 (Appendix P-2a); if they are exceeded, certain protective measures should be taken).

Currently, the priority is to provide human safety, but the scientific community also shows considerable interest in regulation and scientific substantiation of the radiation state of the environment (Kryshev & Sazykina, 2013, 2018 ; Environmental protection…, 2009 ; Radiation Protection…, 2014). With radioactive contamination of the sea, hydrobionts can receive higher radiation doses, than humans, in a number of cases, for example with external exposure from bottom sediments. In addition, separate groups of organisms accumulate radionuclides from the environment with high accumulation rates, which are not typical for humans.

The methodology for assessing the indicators of the radiation state of the marine environment based on environmental and radiation-hygienic criteria is developed in the number of publications (Gusev, 1975 ; Kryshev et al., 2017 ; Sazykina & Kryshev, 1999, 2001 ; Kryshev & Sazykina, 2002 ; Sazykina & Kryshev, 2002a, b). Values of the reference levels in seawater for radionuclides, detected by radioecological monitoring systems in different periods of the LNPP operation, are given in Table 4 (Poryadok rascheta…, 2016).

For ^{54}Mn, ^{60}Co, ^{95}Zr, ^{95}Nb, ^{141}Ce, and ^{144}Ce, the limiting levels are the reference ones, calculated according to the environmental criterion. For ^{137}Cs, ^{90}Sr, ^{3}H, and ^{14}C (the most common in the environment), the values of the reference levels in seawater are determined by radiation-hygienic limits. Radionuclides, that are characterized by high accumulation rates in marine biota (^{14}C and ^{65}Zn) and in bottom sediments (^{54}Mn, ^{60}Co, ^{95}Zr, and ^{95}Nb), have the lowest values of the reference levels.

The reference levels of technogenic radionuclide activity ratios in the marine environment components can be directly compared with the data of direct measurements, which makes it possible to use them in the practice of radioecological monitoring. An integral indicator of the radiation state is the sum of technogenic radionuclide activity ratios, observed in seawater and bottom sediments, to the values of the reference levels. Based on the long-term radioecological monitoring data (1973–2019) for the Koporye Bay of the Gulf of Finland in the LNPP area, this indicator was calculated for the first time. Analysis of monitoring data allows us to draw the following conclusions.

During the period, when LNPP with RBMK-1000 reactors reached maximum power (1973–1985), its influence on radionuclides activity in seawater was traced in the discharge channels and the adjacent water area at a distance up to 3 km (Table 1). Only in rare cases (up to 4 %), trace amounts of corrosion radionuclides were determined in the observation area, adjacent to the NPP. Constant ^{137}Cs and ^{90}Sr presence in seawater is explained by the global processes of technogenic radiation background formation. An increase in these radionuclides specific activity was noted in the discharge channels of the 1st and 2nd stages of the LNPP and the adjacent water area.

Table 4. Reference levels of radionuclide content in seawater according to environmental and radiation-hygienic criteria

Radionuclide	Reference levels of activity concentration in water, Bq·L^{-1}		
	according to environmental criterion	according to radiation-hygienic criterion	minimum value, that meets both environmental and radiation-hygienic criteria (Poryadok rascheta…, 2016)
^{137}Cs	180	1.22	1.22
^{134}Cs	70	0.838	0.8382
^{90}Sr	490*	16.8	16.82
^{54}Mn	0.27	1.91	0.272
^{60}Co	0.61	0.628	0.612
^{65}Zn	11	0.116	0.1162
^{95}Zr	0.31	62.7	0.312
^{95}Nb	0.74	83.7	0.742
^{131}I	420	8.04	8.042
^{141}Ce	2.0	14.2	2.02
^{144}Ce	0.72	1.94	0.722
^{3}H	1 000 000*	86 800	86 800
^{14}C	72	0.137	0.1372

Note: * – according to the criterion of classification as liquid radioactive waste.

The LNPP effect on radionuclides activity in sea bottom sediments was traced at a distance up to 10 km; a decrease in the technogenic radionuclides activity in bottom sediments and a decrease in the frequency of detection of radionuclides of the power plant origin with distance from the NPP were recorded.

Due to the accumulation processes, the probability of detecting technogenic radionuclides in water plant samples is significantly higher than in water samples. The LNPP effect on radionuclides activity in marine plants was traced at a distance over 10 km. A decrease in radioactive contamination of marine plants with a distance from the LNPP was recorded. In general, water plants are an informative bioindicator; that makes it possible to trace the spread of technogenic radionuclides in the NPP area.

The highest levels of technogenic radionuclide activity ratios in fish tissues were registered in the LNPP discharge channels. Corrosion radionuclides (^{54}Mn, ^{60}Co, and ^{65}Zn) were recorded in several cases in fish tissue samples from the Koporye Bay, along with the constantly present ^{137}Cs and ^{90}Sr. The biological transfer of trace amounts of these radionuclides into the rivers, flowing into the Koporye Bay, is of certain interest.

The Chernobyl disaster led to a noticeable increase in the Koporye Bay contamination with technogenic radionuclides. The value of the sum of observed technogenic radionuclide activity ratios of Chernobyl origin in seawater in May – December 1986 to the values of the reference levels increased in comparison with that of the pre-accidental period by about 100 times. During this period, the indicator was close to the value, at which the condition (4), providing the preservation of a favorable environment, is violated. The increase in the value was mainly associated with cesium radioisotopes, the reference levels for which in seawater are limited by the radiation-hygienic criterion.

The formation of current dynamics of contamination of the marine ecosystem components with technogenic radionuclides is still affected by the consequences of the Koporye Bay contamination by the “Chernobyl” ^{137}Cs. At the same time, a decrease in the LNPP discharges to the bay is registered. According to the long-term monitoring data, more than 30 years after the Chernobyl disaster, ^{137}Cs concentration in bottom sediments, seawater, and fish tissues of the Koporye Bay still exceeds the pre-accidental level, while the content of corrosion radionuclides of the LNPP origin in bottom sediments and seawater has significantly decreased. Carbon-14 and tritium, both of natural and technogenic origin, are of particular importance in the practice of radioecological monitoring in this area.

Conclusion. Analysis of the long-term radioecological monitoring data (1973–2019) in the Leningrad NPP area allows to conclude that technogenic radionuclide activity ratios in the marine ecosystem components of the Koporye Bay of the Gulf of Finland under normal NPP operating conditions do not exceed the reference levels, which are presented in the Roshydromet Recommendations and provide the preservation of a favorable environment. Several characteristic periods can be distinguished in the dynamics of the radiation state in this area. In the early period of NPP operation (1973–1985), a wide range of technogenic radionuclides was registered in the marine ecosystem components. Along with ^{137}Cs, significant contributors to the contamination of seawater and bottom sediments were ^{54}Mn and ^{60}Co, the reference levels for which in seawater, in contrast to those for ^{137}Cs, are determined by an environmental criterion, not a radiation-hygienic one. The widest range of technogenic radionuclides is recorded in algae – an informative bioindicator, that allows tracing the spread of technogenic radionuclides in the NPP area at a distance over 10 km. Biogenic transfer of corrosion radionuclides (^{54}Mn, ^{60}Co, and ^{65}Zn) by fish into rivers, flowing into the Koporye Bay, was traced.

After the Chernobyl disaster, there was a significant increase in cesium radioisotopes content in the marine ecosystem components. After a decay of relatively short-lived “Chernobyl” radionuclides, ^{137}Cs acquired the main radioecological significance in this area; its content increased in May – December 1986, compared with the pre-accidental level: in seawater – by 130 times, in algae – by 250 times, and in bottom sediments – by 30 times. For predatory fish, the effect of radiocesium accumulation at trophic levels was observed. The maximum specific activity of ^{137}Cs in fish muscles was recorded a year after the accidental pollution – in 1987; the values remained high, and even in 1990 they were 3–8 times higher than the activities of non-predatory species.

Currently (2010–2019), the radioactive contamination of the marine ecosystem components of the Koporye Bay is determined mainly by ^{137}Cs presence; according to the monitoring data, its level is relatively constant, which indicates the stability of the radiation state in the LNPP marine cooling reservoir. Taking into account the NPP potential radiation hazard, as well as the state of joint effect on the marine biota of technogenic ionizing radiation and a set of non-radiational factors (thermal discharge, chemical pollution, and injury of hydrobionts in the NPP water intakes), it can be concluded that it is necessary to continue and develop radioecological monitoring of the LNPP marine cooling reservoir.

The work was carried out within the framework of Roshydromet research and technological work, theme 4.3.1 "Modernization and development of state monitoring of the radiation state in the Russian Federation territory" (2020–2024).

REFERENCES

1. Blinova L. D. *Radioekologicheskii monitoring atmosfery i gidrosfery v raione raspolozheniya ob"ektov yadernogo kompleksa (na primere goroda Sosnovyi Bor)*. [dissertation]. Obninsk, 1998, 166 p. (in Russ.)
2. Bondarenko L. G., Bystrova A. A., Gavrilov V. M., Dushin V. N., Lebedeva A. V., Osipov V. V., Savelieva I. M., Sadykin A. D., Stepanov A. V., Tishkov V. P. Influence of the Leningrad Nuclear Power Plant and other enterprises of the region on a radioecological condition of the Koporsky Bay of the Gulf of Finland. In: *Yadernaya i radiatsionnaya bezopasnost' Rossii* : tematicheskii sbornik / Gosudarstvennaya korporatsiya po atomnoi energii "Rosatom". Moscow : GK "Rosatom", 2013, iss. 15, pp. 115–125. (in Russ.)
3. Vakulovskii S. M., Nikitin A. I. Radioactive contamination of the marine environment near the Leningrad Nuclear Power Station in 1982. *Atomnaya energiya*, 1984, vol. 56, iss. 3, pp. 153–155. (in Russ.)
4. Gusev D. I. Health criteria for estimating acceptable contamination of coastal waters by radionuclides. In: *Impacts of Nuclear Releases Into the Aquatic Environment* : proceedings of symposium, Otaniemi, 30 June – 4 July, 1975. Vienna : IAEA, 1975, pp. 363–373. (in Russ.)
5. Kryshev A. I., Sazykina T. G., Kryshev I. I., Kosykh I. V. Reference levels of radioactive contamination of water bodies based on the environmental criteria. *Meteorologiya i gidrologiya*, 2017, vol. 42, no. 5, pp. 91–97. (in Russ.). https://doi.org/10.3103/S1068373917050090
6. Kryshev I. I. Stanovlenie otechestvennogo radioekologicheskogo monitoringa. In: *XLVI Mezhdunarodnye radioekologicheskie chteniya, posvyashchennye deistvitel'nomu chlenu VASKhNIL V. M. Klechkovskomu* : sbornik materialov, Obninsk, 30 Nov., 2017. Obninsk : FGBNU VNIIRAE, 2017, pp. 113–135. (in Russ.)
7. Kryshev I. I., Blinova L. D. Radioekologicheskii monitoring morskoi sredy v raione raspolozheniya Leningradskoi AES. In: *Atomnaya energetika na more. Ekologiya i bezopasnost'.* Moscow : Yadernoe obshchestvo SSSR, 1991, pp. 266–271. (in Russ.)
8. Kryshev I. I., Ryazantsev E. P. *Ekologicheskaya bezopasnost' yaderno-energeticheskogo kompleksa Rossii*. 2nd ed., revised and added. Moscow : IzdAt, 2010, 496 p. (in Russ.)
9. Kryshev I. I., Sazykina T. G. Radiation safety of the environment: Request for harmonization of Russian and international regulation documents with consideration of federal laws and new international basic safety standards. *Radiatsiya i risk*, 2013, vol. 22, no. 1, pp. 47–61. (in Russ.)
10. Kryshev I. I., Sazykina T. G. Radiatsionnaya bezopasnost' biosfery: nauchnye i normativno-metodicheskie aspekty. In: *XLII Radioekologicheskie chteniya, posvyashchennye deistvitel'nomu chlenu VASKhNIL V. M. Klechkovskomu* : sbornik materialov, Obninsk, 3 Dec., 2013. Obninsk : GNU VNIISKhRAE, 2014, pp. 31–76. (in Russ.)

11. Kryshev I. I., Sazykina T. G. Radiation protection of the natural environment. Review. *Radiatsiya i risk*, 2018, vol. 27, no. 3, pp. 113–131. (in Russ.). https://doi.org/10.21870/0131-3878-2018-27-3-113-131
12. *Metodicheskie rekomendatsii po sanitarnomu kontrolyu za soderzhaniem radioaktivnykh veshchestv v ob"ektakh vneshnei sredy* / A. N. Marei, A. S. Zykova (Eds). Moscow : MZ SSSR, 1980, 337 p. (in Russ.)
13. *Metodicheskie rekomendatsii po opredeleniyu radioaktivnogo zagryazneniya vodnykh ob"ektov* / S. M. Vakulovskii (Ed.). Moscow : Gidrometeoizdat, 1986, 78 p. (in Russ.)
14. *Nastavlenie gidrometeorologicheskim stantsiyam i postam.* Iss. 12. *Nablyudeniya za radioaktivnym zagryazneniem komponentov prirodnoi sredy* : rukovodyashchii dokument RD 52.18.826-2015 / Rosgidromet. Obninsk : FGBU "NPO "Taifun", 2015, 96 p. (in Russ.)
15. *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.)
16. *Poryadok rascheta kontrol'nykh urovnei soderzhaniya radionuklidov v donnykh otlozheniyakh morskikh vodnykh ob"ektov* : rekomendatsii R 52.18.873-2018 / Rosgidromet. Obninsk : FGBU "NPO "Taifun", 2019, 29 p. (in Russ.)
17. *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.)
18. *Radiatsionnaya obstanovka na territorii Rossii i sopredel'nykh gosudarstv v 1991–2019 gg.* : ezhegodnik / Rosgidromet. Obninsk : FGBU "NPO "Taifun", 1992–2020. (in Russ.)
19. *Radioaktivnoe zagryaznenie raionov AES* / I. I. Kryshev (Ed.). Moscow : Yadernoe obshchestvo SSSR, 1990, 150 p. (in Russ.)
20. *Rukovodstvo po organizatsii kontrolya sostoyaniya prirodnoi sredy v raione raspolozheniya AES* / Gos. kom. SSSR po gidrometeorologii ; K. P. Makhon'ko (Ed.). Leningrad : Gidrometeoizdat, 1990, 264 p. (in Russ.)
21. Sazykina T. G., Kryshev I. I. On the estimation of control concentrations of radionuclides in sea water with concentration for hygienic and radioecological criteria. *Atomnaya energiya*, 1999, vol. 87, iss. 4, pp. 302–307. (in Russ.)
22. Sazykina T. G., Kryshev I. I. Radioekologicheskie kriterii normirovaniya tekhnologicheskikh sbrosov v morskuyu sredu. In: *Problemy radioekologii i pogranichnykh distsiplin* : [sbornik dokladov] / A. V. Trapeznikov, S. M. Vovk (Eds). Zarechnyi : [S. n.], 2001, iss. 4, pp. 92–106. (in Russ.)
23. *Ekologo-geofizicheskie aspekty monitoringa raionov AES* : [sb. st.] / V. A. Borzilov, I. I. Kryshev (Eds). Moscow : Mosk. otd-nie Gidrometeoizdata, 1992, 228 p. (Trudy Instituta eksperimental'noi meteorologii ; iss. 19 (152)). (in Russ.)
24. Environmental protection: The concept and use of reference animals and plants. *Annals of the ICRP*, 2009, vol. 38, no. 4–6, 242 p. (ICRP Publication ; 108).
25. Kryshev I. I., Sazykina T. G. Assessment of permissible low-level releases of radionuclides into the marine environment. In: *Issues and Trends in Radioactive Waste Management* : Proceedings of an International Conference on Vienna, Austria, 9–13 Dec., 2002. Vienna : IAEA-CN-90, 2003, pp. 17–20.
26. *Radiation Protection and Safety of Radiation Sources : International Basic Safety Standards.* Vienna : International Atomic Energy Agency,

2014, 471 p. (IAEA safety standards series ; no. GSR part 3).

27. Sazykina T. G., Kryshev I. I. Methodology for radioecological assessment of radionuclides permissible levels in the seas – protection of human and marine biota. *Radioprotection*, 2002a, vol. 37, C1, pp. C1-899–C1-902. https://doi.org/10.1051/radiopro/2002221

28. Sazykina T. G., Kryshev I. I. Assessment of radiological impact on marine biota in the OSPAR region (Marina Update Project). In: *Proceedings From the International Conference on Radioactivity in the Environment*, 1–5 Sept., Monaco : extended abstracts. Østerås : NRPA, 2002b, pp. 565–568.

ОЦЕНКА РАДИАЦИОННОЙ БЕЗОПАСНОСТИ МОРСКОЙ СРЕДЫ В РАЙОНЕ РАСПОЛОЖЕНИЯ ЛЕНИНГРАДСКОЙ АЭС ПО ДАННЫМ МНОГОЛЕТНЕГО МОНИТОРИНГА (1973–2019)

И. И. Крышев, Т. Г. Сазыкина, Н. Н. Павлова, И. В. Косых,
А. А. Бурякова, А. И. Крышев

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

Целью работы было оценить радиоэкологическое состояние морского водоёма — охладителя Ленинградской АЭС — Копорской губы Финского залива. Согласно международным основным нормам безопасности, принятым на генеральной конференции МАГАТЭ, этот вопрос имеет особую актуальность в связи с необходимостью обоснования защиты от промышленного радиационного воздействия не только человека, но и окружающей среды. Как исходные материалы для оценки использованы данные многолетнего мониторинга (1973–2019) содержания радионуклидов в морской воде, донных отложениях и гидробионтах в зоне наблюдения Ленинградской АЭС. В качестве показателей радиационного состояния морской среды применены контрольные уровни содержания радионуклидов в морской воде и донных отложениях, порядок расчёта которых определён в Рекомендациях Росгидромета Минприроды России Р 52.18.852-2016 и Р 52.18.873-2018. В этих рекомендациях, разработанных специалистами ФГБУ НПО «Тайфун», представлена методика оценки радиоэкологического состояния морской среды по уровню активности радионуклидов на основе принципов, обеспечивающих сохранение благоприятной окружающей среды и морских гидробионтов, а также радиационную защиту человека. При наличии в морской среде смеси радионуклидов должно быть выполнено условие непревышения единицы для суммы отношений наблюдаемых концентраций техногенных радионуклидов в морской воде (донных отложениях) к соответствующим значениям контрольных уровней. По данным мониторинга в ранний период эксплуатации АЭС (1973–1985), в компонентах морской экосистемы присутствовал широкий спектр техногенных радионуклидов. Наряду с ^{137}Cs заметный вклад в загрязнение морской воды и донных отложений вносили ^{54}Mn и ^{60}Co, контрольные уровни для которых в морской воде, в отличие от таковых ^{137}Cs, определяются по экологическому, а не радиационно-гигиеническому критерию. Зарегистрировано присутствие техногенных радионуклидов в водорослях на расстоянии свыше 10 км от АЭС. Отмечен биогенный перенос коррозионных радионуклидов (^{54}Mn, ^{60}Co, ^{65}Zn) рыбой в ре́ки, впадающие в Копорскую губу. Чернобыльская авария привела к заметному увеличению загрязнения Копорской губы техногенными радионуклидами. По сравнению с доаварийным периодом значение суммы отношений наблюдаемых концентраций техногенных радионуклидов в морской воде к соответствующим значениям контрольных уровней в мае — декабре 1986 г. возросло в 100 раз, а в донных отложениях — в 30 раз. Основной вклад в загрязнение компонент морской экосистемы в 1986 г. вносили ^{137}Cs и ^{134}Cs. В настоящее время загрязнение морской воды и донных отложений Копорской губы определяется в основном ^{137}Cs и находится, по данным наблюдений, на относительно постоянном уровне, свидетельствующем о стабильности радиоэкологической обстановки в морском водоёме — охладителе АЭС.

Ключевые слова: Копорская губа Финского залива, Ленинградская АЭС, радиоэкологический мониторинг, морская вода, донные отложения, контрольные уровни радионуклидов, экологический и радиационно-гигиенический критерии, интегральный показатель загрязнения, многолетняя динамика

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 1, pp. 58–66
https://doi.org/10.21072/mbj.2021.06.1.05

UDC 595.371

ADDITIONAL DATA ON MORPHOLOGY AND DISTRIBUTION OF *MELITOIDES VALIDA* (SHOEMAKER, 1955) (AMPHIPODA, MELITIDAE)

© 2021 **V. S. Labay**

Sakhalin Branch of the Russian Research Institute of Fisheries and Oceanography,
Yuzhno-Sakhalinsk, Russian Federation
E-mail: *v.labaj@yandex.ru*

Received by the Editor 08.05.2020; after reviewing 18.06.2020;
accepted for publication 11.03.2021; published online 23.03.2021.

The genus *Melitoides* Gurjanova, 1934 (Amphipoda, Melitidae) includes three species from the Arctic and northwestern Pacific: *Melitoides makarovi* Gurjanova, 1934, *M. valida* (Shoemaker, 1955), and *M. kawaii* Labay, 2014. *M. makarovi* and *M. kawaii* only were recorded until recently in the seas of the Russian Far East. Only two specimens of *M. valida* were found once near the Arctic coast of Alaska; therefore, the morphological description of the species was incomplete, which led to difficulties with its generic identification. For the first time, *M. valida* was found in the seas of the Russian Far East in September 2018 on the shelf of the Sea of Okhotsk, near the North-Eastern Sakhalin Island at the depth of 29 m on the sand bottom. Detailed re-description of the species was carried out using optical and electronic scanning microscopes by the Coleman protocol. The material collected is stored at the Crustacea collection of the Zoological Museum of Far Eastern Federal University (Vladivostok). The specimen from the Sea of Okhotsk is identical to the specimens of the type series from the Arctic coast of Alaska in the form of dorsal carination (with several teeth on posterior margin of pleon segments 2, 3 and urosomites 1, 2), in the structure of pereopods 1–7, especially in the form of propodus of pereopods 2 (palm with distinct posterior-distal tooth, as well as with three large and one small obtuse palmar teeth). *M. valida* description has been substantially supplemented, and information on its range has been expanded.

Keywords: Amphipoda, Melitidae, *Melitoides valida*, Sea of Okhotsk, North-Eastern Sakhalin

The genus *Melitoides* Gurjanova, 1934 was described by E. F. Gurjanova (1934) for the new species *Melitoides makarovi* Gurjanova, 1934 from the Kara Sea. This species was also subsequently noted in the Bering Sea (Gurjanova, 1951). Only two Amphipoda species of the genus *Melitoides*, *M. makarovi* and *M. kawaii* Labay, 2014, were recorded until recently in the seas of the Russian Far East (Labay, 2014). Another species of the genus, *Melitoides valida* (Shoemaker, 1955), was found only once in Arctic waters (Alaska coast on the Chukchi and Beaufort seas border) (Shoemaker, 1955): as *Melita valida* Shoemaker, 1955 (Jarrett & Bousfield, 1996). The latter species was described incompletely and only by two specimens (Shoemaker, 1955). This species was not noted in other Amphipoda collections from the Arctic and Far Eastern seas. Because of the incompleteness of description, Jarret & Bousfield (1996) have not established the exact generic affiliation of the species, and it was referred to the genus *Melitoides* "tentatively": as *Melitoides* (?) *valida* (Shoemaker).

Labay (2014) finally approved this species in the genus *Melitoides*, based on morphological analysis, despite the presence of several features that combine it with the species of the genus *Quasimelita* Jarrett & Bousfield, 1996.

Another *M. valida* specimen was found in benthos samples, collected on the shelf of North-Eastern Sakhalin in September 2018. This finding allows not only to significantly expand the range of the species, but also to make its full description in accordance with the requirements of modern systematics.

MATERIAL AND METHODS

The material was collected from the bottom sediments of the Sakhalin Island shelf (53°18′42.495″N, 143°17′31.469″E) in the bottom survey on the RV "Dmitry Peskov". The specimens were dissected under a stereoscopic microscope, and their appendages and mouth parts were mounted in glycerol gel slides. Illustrations were made under an optical microscope with a photo digital camera DCM-500 and digitally prepared by the Coleman protocol (2003). All the material collected is kept in 70 % ethanol and stored at the Crustacea collection of the Zoological Museum of Far Eastern Federal University (Vladivostok).

Setae definitions are based on Watling (1989). Scanning electron microscope images of surface sculpture were made by a NeoScope JCM-5000 SEM (JEOL) on pre-dissected alcohol-dried material, coated with gold.

The following abbreviations are used on the plates: Pr denotes pereon; Pl – pleon; Ur – urosomite.

Systematics. *Melitoides valida* (Shoemaker, 1955) (Figs 1, 2, 3, 4, 5).

Syn.: *Melita valida* Shoemaker, 1955: 50 (Shoemaker, 1955). – Barnard, 1958: 62. – Barnard & Barnard, 1983: 666;
Abludomelita valida (Shoemaker) Karaman, 1981: 40;
Melitoides valida (Shoemaker, 1955) Jarrett & Bousfield, 1996: 34. – Labay, 2014: 269.

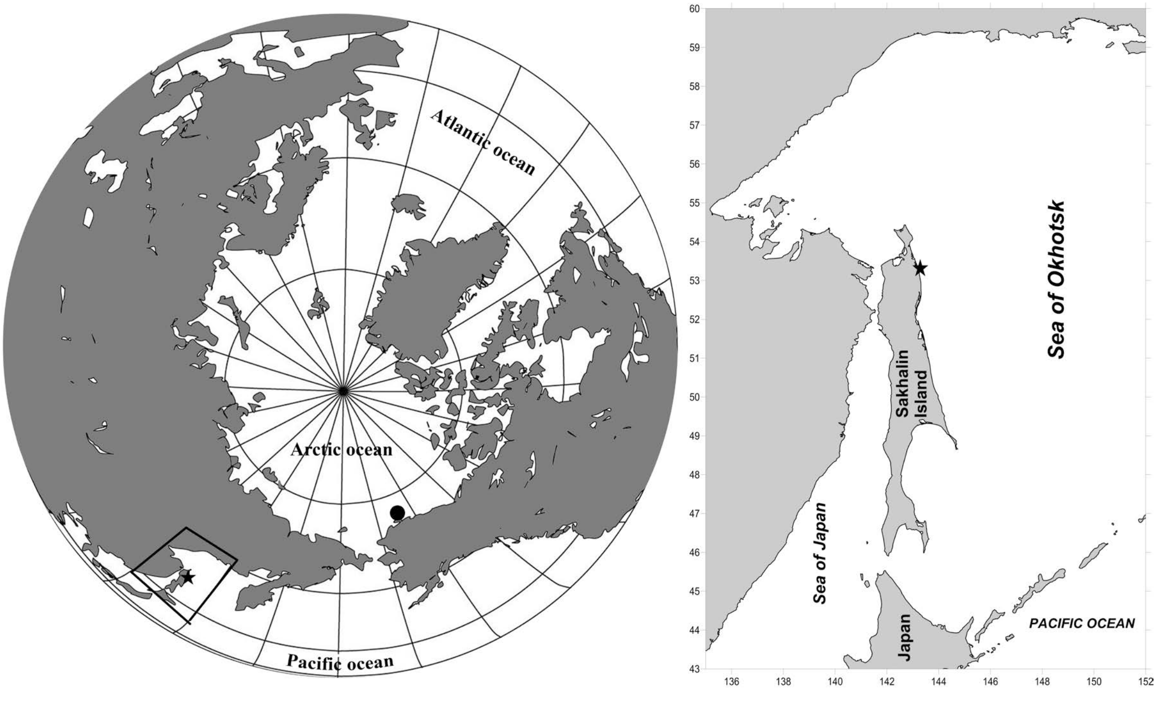


Fig. 1. Distribution of *Melitoides valida* (Shoemaker, 1955): ● is a type locality; ★ is a new location on the north-eastern shelf of the Sakhalin Island

Material. Male, 14 mm, X 53091/Cr-2310, north-eastern shelf of the Sakhalin Island, Sea of Okhotsk, Russian Far East (53°18′42.495″N, 143°17′31.469″E; 29 m), sand, M. G. Rogotnev, 11.09.2018.

Type locality. Beach at Point Barrow, Alaska (Shoemaker, 1955).

Description. *Male* (14 mm). Medium size species. Vital body color unknown. Pereon segments without teeth (Figs 2A, 5). Pleon segment 1 without teeth; pleon segment 2 with 4–5 small teeth, pleon segment 3 with 7 small teeth. Urosomite 1 with a medial group of 3 post-dorsal teeth; urosomite 2 with two pairs of small teeth and with single cuspidate seta among each lateral pair.

Head (Fig. 2A): subequal to pereonites 1 and 2 together with an indistinct rostrum; eyes absent; inferior antennal sinus shallow; cusp indistinct.

Antenna 1 (Fig. 2B): 0.45 times of body length, slender; flagellum exceeding 1.5 times length of the peduncle, 23–25 articles, few very thin and short setae scattered along flagellum; peduncle article 1 double width and slightly longer than article 2, article 3 0.38 times length of article 2; accessory flagellum 4-articulate.

Antenna 2 (Fig. 2C): flagellum 0.47 times as long as peduncle, 9 articles, bundle of thin setae on each article; peduncle article 5 1.2 times shorter than article 4, articles 1 + 2 + 3 subequal to article 5, articles 2 with protruding lobe almost reaching the distal end of next article.

Mouthparts.

Labrum (Fig. 2D): short and rounded, frontal margin convex, densely covered by minute setae.

Mandible (Fig. 2E, F): incisor crenulate and stretching in a rounded tip, left and right lacinia are present; raker setae stout and plumose, numerous (9–10), as long as incisor; molar rectangular triturative, chewing plate flattened; palp 3-articulate, palp segments 2 and 3 setose, article 1 with protruding lobe, the percentage of total length of the articles of the palp is 16 % (article 1), 44 % (article 2), and 40 % (article 3).

Labium (Fig. 2G): inner lobes large, well defined, rounded; outer lobes rounded at distal margin.

Maxilla 1 (Fig. 2H): inner plate subtriangular, lined with a row of 6 plumose setae; outer plate elongated, 1.3 times as long as inner plate, apically with 2 rows of 9 toothed strong setae (anterior row of 5 bifurcate setae and posterior row of 4 comb setae); palp 2-articulate, article 2 2 times as long as article 1, article 2 apically with two rows of 9 cuspidate setae each.

Maxilla 2 (Fig. 2I): inner plate slightly shorter than outer plate, facial row of setae of inner plate reduced, tip covered with numerous plain setae; outer plate with same numerous plain setae.

Maxilliped (Fig. 3A): compact; inner plates medium, fully cleft, distal margin with a row of 4 teeth, inner and distal margins with long setae; outer plates reaching 0.9 of palp article 2, inner margin lined with a row of short stout paddle-like setae longer toward the apex, apical row with long thin specific setae, which plumose in the middle part; palp 4-articulate, the percentage of total length of the articles of the palp is 15 % (article 1), 44 % (article 2), 21 % (article 3), and 20 % (article 4); article 2 with numerous setae along inner margin; article 3 bilobed, tip of article 3 felted, with a row of plain setae along apical indentation; article 4 straight, with nail, inner surface with a dense row of tiny setae.

Pereopod 1 (Fig. 3B): coxa medium long, extended and rounded anterior-distally; basis straight with a rare row of long simple setae along distal part of anterior margin and with long setae along posterior margin; ischium subrectangular with two bunches of long simple setae at posterior-distal corner; merus subrectangular, with dense group of short setae along distal part of posterior margin, distal margin with a row of long setae; carpus long and broad, 1.1 times as long as propodus, 6 oblique rows of long plumose

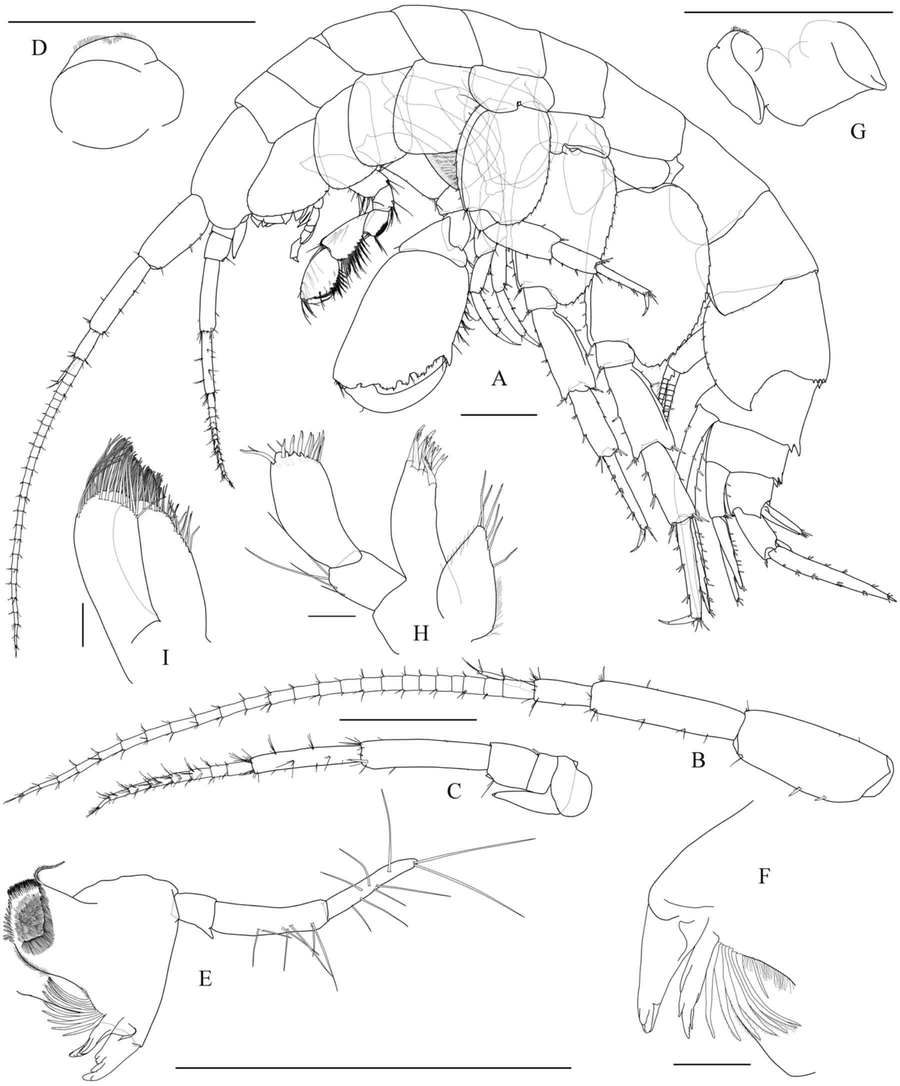


Fig. 2. *Melitoides valida* (Shoemaker, 1955), male: A – lateral view; B – antenna 1; C – antenna 2; D – upper lip; E – left mandible; F – incisor of right mandible; G – lower lips; H – maxilla 1; I – maxilla 2. Scale bars: A, B, C, D, E, and G – 1 mm; F, H, and I – 0.1 mm

setae along outer posterior margin, 3 transverse rows of long plumose setae along anterior margin, with a row of long plumose setae along anterior and posterior parts of distal margin; propodus slightly extended distally, with 7 oblique rows of long plumose setae along posterior margin, with 5 tufts of long setae along anterior margin, and with a dense bunch of long simple setae at anterior-distal corner, palmar margin slightly oblique, with a row of short thin simple setae along palmar; dactylus as long as palm, curved.

Pereopod 2 (Fig. 3C, D): coxa relatively short, subrectangular, anterior angle rounded; basis substraight, wide, subequal to coxa in length, with rare long plain setae along posterior margin and with few rare short setae along anterior margin; merus subrectangular with short triangular protrusion at posterior-distal corner, carpus cup-shaped, with developed posterior lobe, almost 3.8 times shorter than propodus; propodus with subparallel anterior and posterior margins, palm oblique, with distinct posterior-distal tooth and with 3 large and 1 small obtuse palmar teeth, posterior margin with 8 tufts of short setae, palm with rare small cuspidate setae; dactylus inflated, as long as palm, curved to the inside of propodus, outer margin rarely setose.

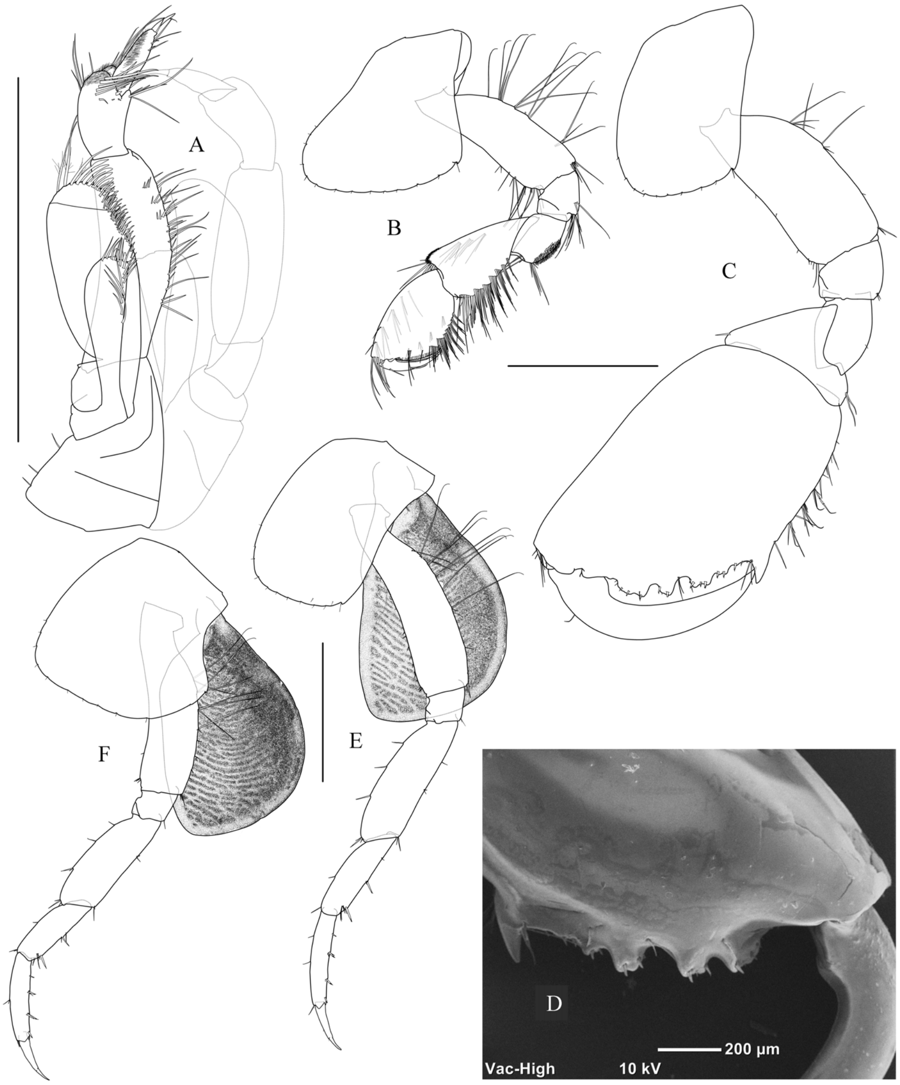


Fig. 3. *Melitoides valida* (Shoemaker, 1955), male: A – maxilliped; B – pereopod 1; C – pereopod 2; D – palmar margin of propodus of pereopod 2; E – pereopod 3; F – pereopod 4. Scale bars: A, B, C, E, and F – 1 mm; D – 0.2 mm

Pereopod 3 (Fig. 3E): coxa subequal to coxa 2, subrectangular, with rounded anterior and posterior corners; basis long, anterior margin concave, posterior margin convex, with long plain setae along posterior margin; merus long; carpus 0.63 times as long as merus, with 3 cuspidate setae along posterior margin; propodus linear, more narrow and slightly longer than carpus, with 3 small stout setae along posterior margin; dactylus medium (0.5 of propodus length), with nail.

Pereopod 4 (Fig. 3F): coxa as long as coxa 3, wide, excavated posteriorly, leg is similar to that of pereopod 3.

Pereopods 5–7 elongated from pereopod 5 to pereopod 6; pereopod 6 subequal to pereopod 7.

Pereopod 5 (Fig. 4A): coxa rounded anteriorly, hind margin with angled posterior lobe; basis short and broad, with rounded posterior wing, rounded posterior-distally, width 0.75 times length, hind margin crenulated, anterior margin with short cuspidate setae; merus slightly broad medially, with 2 cuspidate setae along posterior margin and with 3 small stout setae along anterior margin, and with a cluster of 2–3 cuspidate setae at posterior-distal and anterior-distal corners each; carpus 0.69 times as long as merus, with 2 groups of 2 cuspidate setae at anterior margin and with single clusters of cuspidate setae at anterior and posterior-distal corners each; propodus linear, more narrow than carpus and 1.12 times as long as carpus; dactylus medium (0.44 of propodus length), with nail.

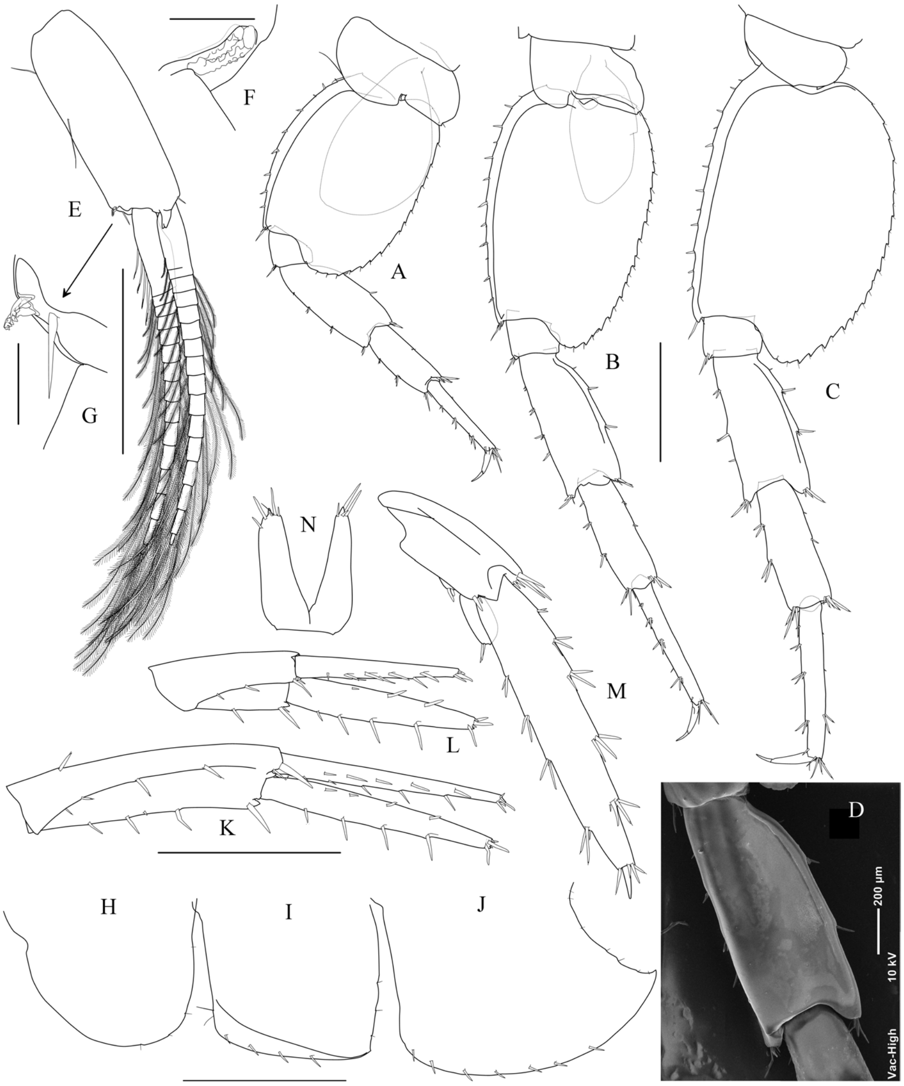


Fig. 4. *Melitoides valida* (Shoemaker, 1955), male: A – pereopod 5; B – pereopod 6; C – pereopod 7; D – merus of pereopod 7; E – pleopod 3; F – coupling spines of pleopod 2; G – coupling spines of pleopod 3; H – epimer 1; I – epimer 2; J – epimer 3; K – uropod 1; L – uropod 2; M – uropod 3; N – telson. Scale bars: A, B, C, E, H, I, J, K, L, M, and N – 1 mm; D – 0.2 mm; F and G – 0.1 mm

Pereopod 6 (Fig. 4B): coxa with angled hind margin and with short and narrow anterior lobe lowered down; basis broad, with rounded posterior wing, slightly narrowed distally, width 0.64 times as length, posterior-distal corner rounded, hind margin crenulated, anterior margin with small cuspidate setae; merus long, with a rigid rib along posterior margin on the outer surface (Fig. 3D), with 3–4 groups of short cuspidate setae along anterior and posterior margins each, and with single clusters of 3–4 cuspidate setae at anterior and posterior-distal corners each; carpus 0.8 times as long as merus, with 2–3 rare cuspidate setae at anterior and posterior margins each and with a single cluster of cuspidate setae at anterior and posterior-distal corners each; propodus linear, more narrow and slightly longer than carpus; dactylus small (0.32 times length of propodus), with nail.

Pereopod 7 (Fig. 4C, D): coxa small, without anterior lobe, with rounded posterior margin; basis short and broad, with rounded posterior wing, rounded posterior-distally, width 0.67 times length, hind margin crenulated, with small cuspidate setae along anterior margin; merus long, slightly broadened medially, with a rigid rib along posterior margin on the outer surface, with 3 clusters of small cuspidate setae along anterior and posterior margins each, and with single clusters of 3–5 cuspidate setae at anterior and posterior-distal corners each; carpus short, 0.88 times as long as merus, with two groups of cuspidate setae along anterior and posterior margins each and with single clusters of 4–5 cuspidate setae at anterior and posterior-distal corners each; propodus linear, more narrow than carpus and 1.12 times as long as carpus, with 3–4 groups of short cuspidate setae along anterior margin; dactylus small (0.39 times length of propodus), with nail.

Coxal gills 2–6. Coxal gills 6 distinctly smaller than gills 2–5.

Epimeral plates (Fig. 4H, I, J): plate 1, hind corner not acuminate, rounded posterior-distally; plate 2 with oblique crest, with quadrate posterior-distal corner, lower margin not serrated, with 4 cuspidate setae; plate 3 hind corner produced, acute, posterior margin concave, ventral margin convex, with a row of 7 strong cuspidate setae.

Pleopods (Fig. 4E, F, G): normal; peduncle of pleopods 1 and 2 with 2 specific toothed coupling strong setae on the inner corner, which hidden in a specific recess (Fig. 4F), peduncle of pleopod 3 with 2 specific toothed coupling strong setae and with 1 simple stout seta on the inner corner, distal margin of peduncle posteriorly with specific stopper process; coupling basis of inner ramus with 4–5 specific stout two-pointed plumose setae along inner margin: pleopod 1 (5), pleopod 2 (5), and pleopod 3 (4).

Uropod 1 (Fig. 4K): peduncle without interramal spur, armed with 5 stout cuspidate setae along inner and outer margins; rami subequal in length, shorter than peduncle length; rami with tiny spine-form setae along inner and outer edges each, both rami with few tiny simple stout setae at the tip.

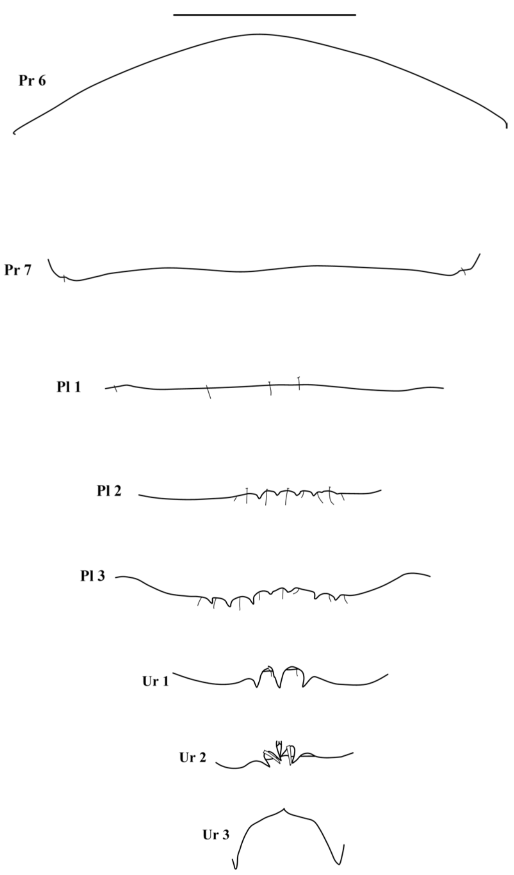


Fig. 5. *Melitoides valida* (Shoemaker, 1955), male: dorsal carination. Scale bar is 1 mm

Uropod 2 (Fig. 4L): peduncle shorter than outer ramus (0.77 ramus length); inner ramus shorter than outer, both with numerous simple stout setae along inner and outer edges each; uropod 2 1.47 times shorter than uropod 1.

Uropod 3 (Fig. 4M): peduncle subequal to peduncle of uropod 2, with a group of 2 small stout cuspidate setae at the inner margin, with a row of 5 stout cuspidate setae at posterior-distal margin; elongated 2-articulate outer ramus (6 times as long as inner ramus), with 5 tufts of small cuspidate setae along lateral margin, with 4 tufts of cuspidate setae along medial margin of article 1, article 2 minutely, 1.5–2 times longer than crowning setae, 10.5 times shorter than article 1; inner ramus short and sub-oval with 2 cuspidate setae subapically.

Telson (Fig. 4N): fully cleft; lobes diverging distally, with acute tips; lateral and medial notches in almost identical position; each lobe with 2 stout setae in the lateral and medial notches each.

Female unknown.

Ecology. *Melitoides valida* was found on the shelf of North-Eastern Sakhalin at a depth of 29 m on the sand bottom. Specimens of the type series were found on pebble beach at Point Barrow (Alaska) (Shoemaker, 1955).

Distribution. Recorded from eastern Arctic (Alaska coast on the Chukchi and Beaufort seas border; the Arctic Ocean) and from northern Pacific (the Sea of Okhotsk, near the North-Eastern Sakhalin Island) (Fig. 1).

Remarks. The specimen from the Sea of Okhotsk is identical to the specimens of the type series from Point Barrow (Alaska) in the form of dorsal carination, in the structure of pereopods 1–7, especially in the form of propodus of pereopod 2, and in the structure of telson and maxilliped.

The results were obtained within the framework of the state assignment of the Ministry of Education and Science of the Russian Federation (publication number 6.9551.2017/8.9).

REFERENCES

1. Barnard J. L. Index to the families, genera and species of the gammaridean Amphipoda (Crustacea). *Allan Hancock Foundation Publications. Occasional Papers*, 1958, no. 19, 145 p.
2. Barnard J. L., Barnard C. M. *Freshwater Amphipoda of the World. I. Evolutionary Patterns. II. Handbook and Bibliography.* Mount Vernon, Virginia : Hayfield Associates, 1983, 830 p.
3. Coleman C. O. “Digital inking”: How to make perfect line drawings on computers. *Organisms, Diversity & Evolution*, 2003, vol. 3, iss. 4, pp. 303–304. http://dx.doi.org/10.1078/1439-6092-00081
4. Gurjanova E. Neue Formen von Amphipoden des Karischen Meeres. *Zoologischer Anzeiger*, 1934, vol. 108, pp. 122–130.
5. Gurjanova E. F. *Bokoplavy morei SSSR i sopredel'nykh vod (Amphipoda, Gammaridea).* Moscow ; Leningrad : AN SSSR, 1951, 1032 p. (Opredeliteli po faune morei ; vol. 41). (in Russ.)
6. Jarrett N. E., Bousfield E. L. The Amphipod superfamily Hadzioidea on the Pacific coast of North America: Family Melitidae. Part I. The *Melita* group: Systematic and distributional ecology. *Amphipacifica. Journal of Systematic Biology*, 1996, vol. 2, no. 2, pp. 3–74.
7. Karaman G. S. Redescription of *Melita planaterga* Kunkel, 1910 from Bermuda with revision of the genera *Melita* Leach and *Abludomelita* n. gen. *Poljoprivreda i šumarstvo, Titograd*, 1981, vol. 27, no. 1, pp. 29–50.
8. Labay V. S. Review of amphipods of the *Melita* group (Amphipoda: Melitidae) from the coastal waters of Sakhalin Island (Far East of Russia). II. Genera *Quasimelita* Jarrett and Bousfield, 1996 and *Melitoides* Gurjanova, 1934. *Zootaxa*, 2014, vol. 3689, no. 3, pp. 237–280. http://dx.doi.org/10.11646/zootaxa.3869.3.3
9. Shoemaker C. R. Amphipoda collected at the Arctic Laboratory, Office of Naval Research, Point Barrow, Alaska, by G. E. McGinite. *Smithsonian Miscellaneous Collections*, 1955, vol. 128, no. 1, pp. 1–78.
10. Watling L. A classification system for crustacean setae based on the homology concept. In: *Functional Morphology of Feeding and Grooming in Crustacea* / B. E. Felgenhauer, L. Watling, A. B. Thistle (Eds). Rotterdam, Netherlands : A. A. Balkema, 1989, pp. 15–26. (Crustaceans issues ; vol. 6).

ДОПОЛНИТЕЛЬНЫЕ СВЕДЕНИЯ О МОРФОЛОГИИ И РАСПРОСТРАНЕНИИ *MELITOIDES VALIDA* (SHOEMAKER, 1955) (AMPHIPODA, MELITIDAE)

В. С. Лабай

Сахалинский филиал Всероссийского научно-исследовательского института
рыбного хозяйства и океанографии, Южно-Сахалинск, Российская Федерация
E-mail: *v.labaj@yandex.ru*

Род *Melitoides* Gurjanova, 1934 (Amphipoda, Melitidae) объединяет три вида амфипод из Арктики и северо-западной части Тихого океана: *Melitoides makarovi* Gurjanova, 1934, *M. valida* (Shoemaker, 1955) и *M. kawaii* Labay, 2014. Только виды *M. makarovi* и *M. kawaii* были отмечены ранее в дальневосточных морях России. *M. valida* обнаружен единожды у арктического побережья Аляски в количестве двух экземпляров; в связи с этим его морфологическое описание было неполным, что привело к трудностям с родовой идентификацией. По данным сборов в сентябре 2018 г. на шельфе Охотского моря у Северо-Восточного Сахалина на глубине 29 м на песках, впервые в дальневосточных морях России обнаружен редкий вид бокоплава *M. valida*. С использованием оптического и электронного сканирующего микроскопов по протоколу Коулмана проведено подробное переописание вида. Собранный материал передан на хранение в коллекцию ракообразных Зоологического музея Дальневосточного федерального университета (г. Владивосток). Экземпляр из Охотского моря идентичен образцам типовой серии с арктического побережья Аляски по форме дорсальной каринации (несёт несколько зубчиков на заднем крае плеосомальных сегментов 2, 3 и уросомальных сегментов 1, 2), по структуре переоподов 1–7, особенно по форме проподуса переоподов 2 (пальмарный край с отчётливым заднедистальным зубцом и с тремя крупными и одним мелким тупыми пальмарными зубцами). Существенно дополнено видовое описание *M. valida* и расширены сведения о его ареале.

Ключевые слова: амфипода, Melitidae, *Melitoides valida*, Охотское море, Северо-Восточный Сахалин

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 1, pp. 67–81
https://doi.org/10.21072/mbj.2021.06.1.06

UDC [579.61:615]:582.26/.27-119.2

MARINE AND FRESHWATER MICROALGAE AS A SUSTAINABLE SOURCE OF COSMECEUTICALS[*]

© 2021 **T. V. Puchkova[1], S. A. Khapchaeva[1], V. S. Zotov[1],
A. A. Lukyanov[2], and A. E. Solovchenko[2,3]**

[1]Federal research centre "Fundamentals of Biotechnology" of RAS, Moscow, Russian Federation
[2]Lomonosov Moscow State University, Moscow, Russian Federation
[3]Derzhavin Tambov State University, Tambov, Russian Federation
E-mail: *solovchenko@mail.bio.msu.ru*

Received by the Editor 01.12.2020; after reviewing 31.01.2021;
accepted for publication 11.03.2021; published online 23.03.2021.

A prominent feature of stress-tolerant microalgae is their versatile metabolism, allowing them to synthesize a broad spectrum of molecules. In microalgae, they increase stress resilience of these organisms. In human body, they exhibit anti-aging, anti-inflammatory, and sunscreen activities. This is not surprising, given that many of the stress-induced deleterious processes in human body and in photosynthetic cell are mediated by the same mechanisms: free-radical attacks and lipid peroxidation. It is also worth noting, that the photosynthetic machinery of microalgae is always at risk of oxidative damage since high redox potentials and reactive molecules are constantly generated during its functioning. These risks are kept at bay by efficient reactive oxygen species elimination systems including, *inter alia*, potent low-molecular antioxidants. Therefore, photosynthetic organisms are a rich source of bioactive substances with a great potential for curbing the negative effects of stresses, acting on human skin cells on a day-to-day basis. In many cases these compounds appear to be less toxic, less allergenic, and, in general, more "biocompatible" than most of their synthetic counterparts. The same algal metabolites are recognized as promising ingredients for innovative cosmetics and cosmeceutical formulations. Ever increasing efforts are being put into the search for new natural biologically active substances from microalgae. This trend is also fueled by the growing demand for natural raw materials for foods, nutraceuticals, pharmaceuticals, and cosmetology, associated with the global transition to a "greener" lifestyle. Although a dramatic diversity of cosmeceuticals was discovered in macrophyte algae, single-celled algae are on the same level or even surpass them in this regard. At the same time, a large-scale biotechnological production of microalgal biomass, enriched with the cosmeceutical compounds, is more technically feasible and economically viable than that of macrophyte biomass. The autotrophic cultivation of microalgae is generally simpler and often cheaper than that of heterotrophic microorganisms. Cultivation in bioreactors makes it possible to obtain more standardized raw biomass, quality of which is less dependent on seasonal factors. Microalgae biotechnology opens many possibilities to the "green" cosmeceutical production. However, a significant part of microalgae chemo- and biodiversity remains so far untapped. Consequently, bioprospecting and biochemical characterization of new algal species and strains, especially those isolated from habitats with harsh environmental conditions, is a major avenue for further research and development. Equally important is the development of approaches to cost-effective microalgae cultivation, as well as induction, extraction, and purification of cosmeceutical metabolites. World scientific community is rapidly accumulating extensive information on the chemistry and diverse effects of microalgae substances and metabolites; many substances of microalgal origin are extensively used in the cosmetic industry. However, the list of extracts and individual chemicals, isolated from them

---

[*]Preprint was published at https://doi.org/10.20944/preprints202012.0696.v1 on 28 December, 2020.

and thoroughly tested for safety and effectiveness, is not yet very large. Although excellent reviews of individual microalgal cosmeceutical groups exist, here we covered all the most important classes of such compounds of cosmeceutical relevance, linking the patterns of their composition and accumulation with the relevant aspects of microalgae biology.

Keywords: carotenoids, chlorophylls, lipids, mycosporine-like amino acids, antioxidants, UV screens

Microalgae are a large and diverse group of unicellular, prokaryotic, and eukaryotic microorganisms. They can grow in freshwater or seawater and play a key role in aquatic ecosystems as the primary producers (Masojídek et al., 2013). Microalgae are characterized by the presence of versatile metabolic pathways, capable of producing a broad spectrum of molecules. Many of these metabolites exert a plethora of beneficial effects on human health, particularly on skin condition and functioning (Algal Green Chemistry…, 2017 ; Marine Cosmeceuticals…, 2011 ; Thomas & Kim, 2013). Since the second half of the XX century, ever increasing efforts are being put into the search for new natural biologically active substances from microalgae. This trend is also fueled by the growing demand for natural raw materials for foods, pharmaceuticals, and cosmetology, associated with the global transition to a "greener" lifestyle (Algal Green Chemistry…, 2017 ; García et al., 2017 ; Marine Cosmeceuticals…, 2011).

As commercial demand increases, microalgae are cultivated at a large scale under different conditions (Borowitzka & Vonshak, 2017). This gives rise to differences in chemical composition of raw materials from microalgal biomass and, hence, to problems in the process of mass production of cosmetic extracts from the point of view of standardization. Therefore, growing conditions and climatic fluctuations / seasonality, in case of outdoor biomass production, must be taken into account in the process of biomass development for cosmetic lines (Carlsson et al., 2007 ; Marine Cosmeceuticals…, 2011).

Microalgae are a rich source of various compounds of commercial interest (Kijjoa & Sawangwong, 2004), especially those needed for cosmetics (Table 1): pigments, polysaccharides, and fatty acids (Borowitzka, 2013 ; Thomas & Kim, 2013). Most of the commercially promising active substances of microalgae are secondary metabolites, that accumulate in cells under unfavorable culture conditions for growth (Mulders et al., 2014 ; Solovchenko, 2013). Some of the metabolites have a chemical structure, which is not found in terrestrial organisms and has a function that is not yet understood. The high potential of microalgae as raw materials for the pharmaceutical and cosmetic industries is associated with the presence of substances, serving for environmental stress acclimation, which have formed during evolution (Solovchenko, 2010).

Table 1. Microalgal ingredients for the cosmetic industry and its main suppliers (Couteau & Coiffard, 2018)

Microalgae	Ingredients	Suppliers
Phaeodactylum tricornutum	Megassane	Soliance (merged with Givaudan Active Beauty)
	Depollutine	
Skeletonema costatum	Costalane	
Pyrocystis noctiluca		Microphyt
Chlorella	Dermochlorella D	CODIF Technologie Naturelle
	Dermochlorella DP	
	Agility chlorella	Roquette
		SetAlg
Odontella		Innov'Alg

The autotrophic cultivation of microalgae is generally simpler and often cheaper than that of heterotrophic microorganisms. It can be even economically efficient since microalgae can grow autotrophically (Algal Green Chemistry…, 2017 ; Masojídek et al., 2013). Cultivation in bioreactors makes it possible to obtain more standardized raw biomass, quality of which is less dependent on seasonal factors (Borowitzka, 1999 ; Zittelli et al., 2013). World scientific community has rapidly accumulated extensive information on the chemistry and diverse effects of substances and metabolites of microalgae (Coates et al., 2013 ; García et al., 2017 ; Levine, 2018). Many substances of microalgal origin have found extensive use in the cosmetic industry. However, the list of extracts and individual chemicals, isolated from them and thoroughly tested for safety and effectiveness, is not yet very large (Scott, 2015). Although excellent reviews of individual microalgal cosmeceutical groups exist (Fox & Zimba, 2018 ; Gong & Bassi, 2016 ; Julius, 2018 ; Mimouni et al., 2018 ; Morançais et al., 2018 ; Novoveská et al., 2019), here we covered all the most important classes of such compounds of cosmeceutical relevance (Eom & Kim, 2013), linking the patterns of their composition and accumulation with the relevant aspects of microalgae biology.

Structural and reverse polysaccharides

The bulk of the carbohydrates that make up algae are polysaccharides: up to 55 % of the dry matter (Algal Green Chemistry…, 2017 ; Morançais et al., 2018). A widespread structural polysaccharide, cellulose is a major component of cell wall of many algal species (3–18 % of the cell dry weight). It is a linear homopolymer of β-glucose molecules, linked by β-1,4 glycosidic bonds. Other frequently encountered polysaccharides of microalgae are divided into two groups according to the type of sugar bonds in their polymer chains. These are α-1,4-glucans (starch and floridean starch) and β-1,3-glucans (chrysolaminarin and paramylon) (Julius, 2018). α-glucans, such as α-1,4-glucans, are found in green, charophyte, glaucophyte, dinophyte, cryptomonad, and red microalgae, as well as in cyanobacteria. The latter are characterized by a high degree of branching, resembling in this regard glycogen: the evolutionary oldest reserve glucan (Julius, 2018). Cryptomonad starch, as in red algae, contains more amylopectin (branched molecules with α-1,4 and α-1,6 bonds) than amylose (linear chains with α-1,4 bonds). Starch of chlorophytes contains both amylose and amylopectin (Algal Green Chemistry…, 2017). It differs from the starch of higher plants by a lower molecular weight of amylose and amylopectin and a smaller size of granules. In cosmetics, mostly α-1,4- and α-1,6-glucans are used (Kijjoa & Sawangwong, 2004). β-1,3-glucans, *e. g.* paramylon, are synthesized by euglenophytes and Pavlovaceae from haptophytes. Representatives of the genera *Astasia* and *Euglena* accumulate paramylon to more than 50 % of the cell dry weight. Chrysolaminarin is a water-soluble glucan, a reserve product of golden, yellow-green, and diatom microalgae. This is a colorless substance similar to laminarin of kelps (Julius, 2018). Microalgae also contain more exotic chemically modified, *e. g.* sulfated, polysaccharide species with unique physical and chemical properties, valued in the cosmetic industry (Arad & van Moppes, 2013 ; Silva et al., 2012).

Lipids

Microalgae represent an important "green" source of lipids, enriched with biologically active long-chain polyunsaturated fatty acids (hereinafter PUFA), such as γ-linolenic, arachidonic, and eicosapentaenoic (hereinafter EPA), docosahexaenoic acid (hereinafter DHA), and stearidonic acid – fatty acids, exerting vitamin F activity (Cohen & Khozin-Goldberg, 2010 ; Lee et al., 2013 ; Marine Macro- and Microalgae…, 2018 ; Mimouni et al., 2018 ; Ward & Singh, 2005). The lipids are divided into neutral and polar. Neutral lipids are mainly triacylglycerides, which are primarily accumulated in chloroplast or cytosolic lipid bodies, normally accumulated by microalgae in response to stresses (Solovchenko, 2012). Under those conditions, lipid content in oleaginous microalgae cells, such as *Schizochytrium* sp., *Pavlova lutheri*, *Isochrysis*, and *Nannochloropsis*, can reach 50–70 % of the cell dry weight. *Lobosphaera incisa* is capable of accumulating arachidonic acid up to 60 % of the total fatty acids (Solovchenko et al., 2008). Certain microalgae species are known to accumulate EPA and DHA up to 3–5 % of the cell dry weight (Khozin-Goldberg et al., 2011). The genus *Schizochytrium* is a rich source of DHA (up to 37.7 % of the total fatty acids) (Cohen & Khozin-Goldberg, 2010 ; Mimouni et al., 2018). Microalgae, such as *Rhodomonas salina*, *Tetraselmis suecica*, *Thalassiosira pseudonana*, *Phaeodactylum tricornutum*, *Porphyridium cruentum*, *Nannochloropsis oculata*, and *Nannochloropsis gaditana*, are also intensively studied as potential sources of PUFA (Borowitzka, 2013 ; Solovchenko et al., 2008). A high EPA content was found in red microalgae, where it can reach 50 % of the total fatty acids (Cohen, 1999).

Accumulation of PUFA can be enhanced through the exposure of microalgae to various abiotic stresses, such as extreme salinities, temperatures, and shortage of N and P in the medium. Low-temperature stress for algae is one of the effective strategies for increasing PUFA. As a part of the adaptation to low temperature, microalgae increase PUFA production to maintain membrane fluidity. Cultivation of microalgae in bioreactors under controlled conditions allows to better standardize PUFA profiles of the algal lipid extracts.

Microalgae *Pavlova lutheri* and *Phaeodactylum tricornutum* show an increase in EPA content by about 20–30 % with a decrease in the cultivation temperature to +15 and +10 °C, respectively. On the other hand, high PUFA levels are observed within cell lipids when microalgae are grown under favorable conditions (Solovchenko et al., 2014). The production of “algal oil” by biotechnological methods for the purposes of the food and cosmetic industry has been proved for certain species: *Porphyridium cruentum* and *Crypthecodinium cohnii* (USA), *Schizochytrium* sp. (USA), and *Ulkenia* sp. (Germany) (Dufossé et al., 2005 ; Pulz & Gross, 2004 ; Spolaore et al., 2006). DHA is essential for humans as a major PUFA of brain cell membrane lipids, retina, heart muscle, and sperm; it is also important for the development of young children (Borowitzka, 2013 ; Cohen & Khozin-Goldberg, 2010 ; García et al., 2017 ; Kijjoa & Sawangwong, 2004).

In the cosmetic industry, “algal oil”, a concentrate of the essential ω-3 and ω-6 PUFA, is becoming more widespread. For infant formulations, “algal oil” from the dinoflagellate *Crypthecodinium cohnii* is used (30 % PUFA of the cell dry weight with DHA comprising approximately 50 % of the total PUFA). The technology for DHA obtaining from *Crypthecodinium* by Martek company (USA) is based on aseptic heterotrophic cultivation of the proprietary algal strain. OmegaTech (USA) produces a cheaper “algal oil” from *Schizochytrium* sp. (branded “DHA Gold”), which is approved for the production of nutraceuticals and food products and is used in skin care products, especially natural cosmetics. German company Nutrinova produces DHA from *Ulkenia* sp. (branded “DHA Active”) (Pulz & Gross, 2004). Food supplements, containing microalgal DHA, are used for the prevention and treatment of diseases, associated with impaired brain activity, heart attack, and age-related visual impairment (Ward & Singh, 2005). EPA from *Porphyridium cruentum*, *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Nannochloropsis* sp., and *Nitzschia laevis* is in demand for the prevention and treatment of lipid metabolism disorders. In cosmetics, this product is an important ingredient for restoring the water-lipid mantle of the skin (Dufossé et al., 2005 ; Spolaore et al., 2006).

Sterols perform a variety of functions in marine organisms, *inter alia* chemical defenses against attack by other organisms. Bioactive molecules, as steroid hormones, bile acids, and various biotoxins including steroid and triterpene saponins, can be considered as products of biotransformation of sterols. The structural closeness of algal sterols to the sterols, commonly used in cosmetic chemistry, allows to use them as emulsion bases and raw materials for obtaining, for example, vitamin D and creating new medical preparations and cosmetics on their basis. Microalgal sterols can be components of the cell wall, *e. g.* in *Isochrysis galbana* and *Pavlova lutheri*. The main sterols of these microalgae include clionasterol, 4α-methyl poriferast-22-enol, poriferasterol, methylpavlovol, and epicampesterol. Thus, *Pavlova lutheri* can produce significant amounts of sterols (*ca.* 100 $mg \cdot g^{-1}$ total cell lipids), which can be further increased by ultraviolet (hereinafter UV) exposure of the microalga (Mimouni et al., 2018).

Algal sterols are promising precursors for vitamin D synthesis or as a part of emulsion bases in the production of soft dosage forms; they are potential agents for the treatment of atherosclerosis and have antitumor and anti-inflammatory effects. Thus, desmosterol of microalgae is a versatile precursor giving rise to many biologically active steroids (Marine Macro- and Microalgae…, 2018).

Pigments

Chlorophylls are pigments that absorb light in the blue and red regions of the visible spectrum. They are central to photochemical conversion of light energy in photosynthesis. Within photosynthetic cells, chlorophylls are always bound to proteins. Chlorophylls *a*, *b*, *d*, and *f* have a long apolar phytol chain, lacking in chlorophylls *c*. All microalgal taxa contain chlorophyll *a*, whereas the composition of chlorophylls depends on the algal group (Barbosa & Roque, 2019).

The antimicrobial, anti-inflammatory effect of chlorophyll-based drugs, their ability to stimulate not only hematopoiesis, but also the healing of wounds and ulcers, has long been known. As an antiseptic additive, chlorophyll is popular in cosmetics for oily skin and skin with acne, as well as for care products for oily scalp (Freitas et al., 2019 ; Mu et al., 2019). Chlorophyll derivatives 13-hydroxy-phaeophytin and 13-hydroxy-phaeopharnesin, isolated from the cyanobacterium *Spirulina* and green microalga *Chlorella*, demonstrated a significant lipid-reducing activity in the model of differentiated adipocytes 3T3-L1. The experimental data suggests that these compounds are promising for development of nutraceuticals with a lipid-control activity or a cosmetic ingredient with lipolytic activity (Freitas et al., 2019). The pronounced deodorizing properties of chlorophyll derivatives were the basis for their widespread use as an active component of hygiene products, used for oral care, and deodorants in natural cosmetics. In the cosmetics of the natural direction of skin care, chlorophyll is also used as a pigment. Production of chlorophyll-based cosmeceutical additives is a very promising direction for substituting chlorophyll preparation from higher plants.

Carotenoids are natural pigments that convey yellow, orange, or red hue to organisms, containing them. Chemically, they are a class of tetraterpenoids with a C_{40} backbone ubiquitously present in the photosynthetic apparatus of plants, microalgae, and cyanobacteria (Gong & Bassi, 2016 ; Sun et al., 2018). Carotenoids are divided into carotenes, the hydrocarbons devoid of oxygen, and xanthophylls, which contain oxygen (Gong & Bassi, 2016 ; Morançais et al., 2018 ; Novoveská et al., 2019 ; Sun et al., 2018). Around 750 natural carotenoids were isolated from various biological sources, of which about 200 were found in algae; nearly 30 of them were involved in photosynthesis of microalgae (Gong & Bassi, 2016). These are among the most diverse and widespread pigments in nature.

Carotenoids also found extensive use in the foods, nutraceuticals, pharmaceuticals, medicines, and cosmetic industry due to their antioxidant, antibacterial, antiviral, antifungal, anti-inflammatory, and antitumor properties (Black et al., 2020 ; Mulders et al., 2014 ; Novoveská et al., 2019). The antioxidant activity of carotenoids determines their application as functional food and cosmetics ingredients, as well as safe colorants (Boer, 2014). Currently, carotenoids, derived from microalgae, dominate certain segments of the natural pigment market (Novoveská et al., 2019). Overall, microalgal carotenoid production is considered as an important business opportunity for the healthcare and cosmetic industry of the future. The main carotenoids, currently commercially used in the world, are β-carotene, astaxanthin, lutein, canthaxanthin, zeaxanthin, and fucoxanthin (Gong & Bassi, 2016 ; Morançais et al., 2018). Lycopene and canthaxanthin are also biotechnologically important carotenoids. Natural carotenoids occur in microalgae as a mixture of *cis-trans* and optical isomers, whereas synthetic carotenoids are mostly in the free form. Natural carotenoids are preferred in cosmetic applications over their synthetic counterpart due to safety and higher bioavailability.

β-carotene is a yellow-orange strongly polar carotenoid. It is synthesized by photosynthetic organisms; it participates in light harvesting and photoprotection of chlorophyll and in prevention of damage to DNA by active oxygen forms (Davidi et al., 2015 ; Telfer, 2002). In nature, β-carotene is the most common precursor of vitamin A and a powerful antioxidant (Black et al., 2020). β-carotene is used as a food coloring agent, as well as in medicines, nutraceuticals, cosmetics, and feed (Novoveská et al., 2019). Commercial production of microalgal β-carotene employs diverse technologies from shallow ponds to advanced photobioreactors. β-carotene from *Dunaliella salina* (Fig. 1) was the first product, commercially obtained from microalgae (Lamers et al., 2010 ; Ye et al., 2009). The content of β-carotene in *Dunaliella salina* biomass reaches 10–14 % under stress conditions. Commercial companies, producing β-carotene from microalgae, include Aqua Carotene (USA), Nature Beta Technologies (Israel), Cognis Nutrition & Health (Australia), Cyanotech (USA), and Parry Nutraceuticals (India).

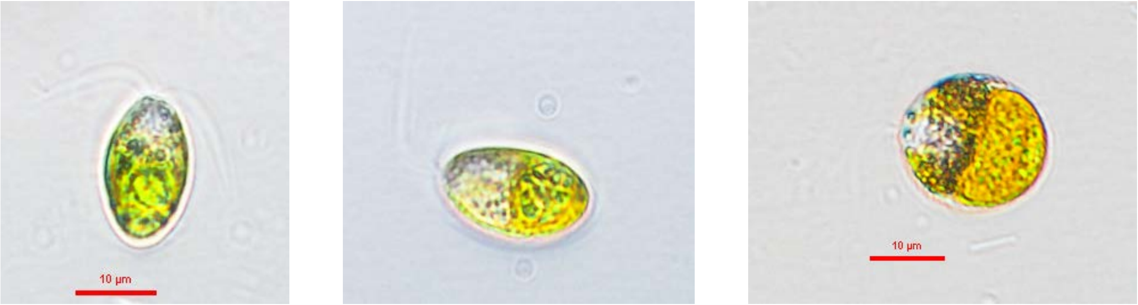


Fig. 1. Changes in *Dunaliella salina* cell morphology (left to right) in the course of high light and salinity stress-induced accumulation of β-carotene. Courtesy of Dr. Elena Seliwanova

Astaxanthin (3,3-dihydroxy-β-carotene-4,4-dione) is an oxygenated derivative of β-carotene. It is biosynthesized by some species of microalgae, fungi, and plants; this carotenoid gives salmon, shrimp, and lobsters, as well as their consumers, *e. g.* birds, their distinctive coloration (Novoveská et al., 2019). The high stress resilience of the astaxanthin-producing microalgae is a good marketing legend for the cosmetic industry, where extracts from these microalgae are offered as skin care products (Solovchenko, 2012). The natural pigment, represented mainly by the *3S,3′S* isomer, in antioxidant activity also exceeds its synthetic counterpart, which is a racemate comprised by all possible optical isomers (Han et al., 2013).

Antioxidant activity of astaxanthin exceeds considerably that of other carotenoids such as β-carotene, protecting the lipid structures of the cell, especially cell membrane phospholipids. The unique astaxanthin structure facilitates its accumulation in cell membranes. Unlike other antioxidants, which are located either inside or outside the lipid bilayer of the membrane, astaxanthin molecules have a unique ability to be located across the lipid bilayer of the membrane, protecting it from the attacks of charged and uncharged reactive oxygen species (Hussein et al., 2006 ; Naguib, 2000). Astaxanthin protects microalgal cells from exposure to high light intensity and from harmful UV radiation, decreasing the formation of reactive oxygen species. This is also the basis for the use of *Haematococcus* extracts in protective skin care products (Cornish & Garbary, 2010 ; Tanaka et al., 2012).

Unlike β-carotene, astaxanthin is not a precursor of vitamin A, so it can be taken up safely without the risk of side effects, associated with vitamin A overdose. Astaxanthin has pronounced anti-inflammatory and antitumor effects and a rare ability to penetrate the blood-brain barrier; the latter characteristic determines its efficiency in prevention and treatment of central nervous system diseases (Goiris et al., 2012 ; Tanaka et al., 2012). For commercial cosmeceutical needs, astaxanthin is used in various forms: pills, capsules, syrups, oils, soft gels, creams, biomass dry powder, and granular powder (Cornish & Garbary, 2010 ; Thomas & Kim, 2013).

Haematococcus pluvialis (Fig. 2) is the most widely used as a producer of natural astaxanthin, although other microalgae, including *Chlorococcum* sp., *Chlorella zofingiensis*, *Botryococcus braunii*, *Chlamydomonas nivalis*, *Scotiellopsis oocystiformis*, and *Chloromonas nivalis*, are capable of synthesizing astaxanthin (Chubchikova et al., 2011). In *Haematococcus*, astaxanthin is predominantly esterified by fatty acids [$C_{16:0}$, $C_{18:2}$, and $C_{18:1}$ (Zhekisheva et al., 2005)]. Under stress conditions (nitrogen depletion, as well as high light intensity or salinity), *Haematococcus pluvialis* can accumulate astaxanthin up to 5–6 % of the cell dry weight during stress-induced transition of green vegetative cells into astaxanthin-rich resting haematocysts (Boussiba, 2000 ; Chekanov et al., 2016).

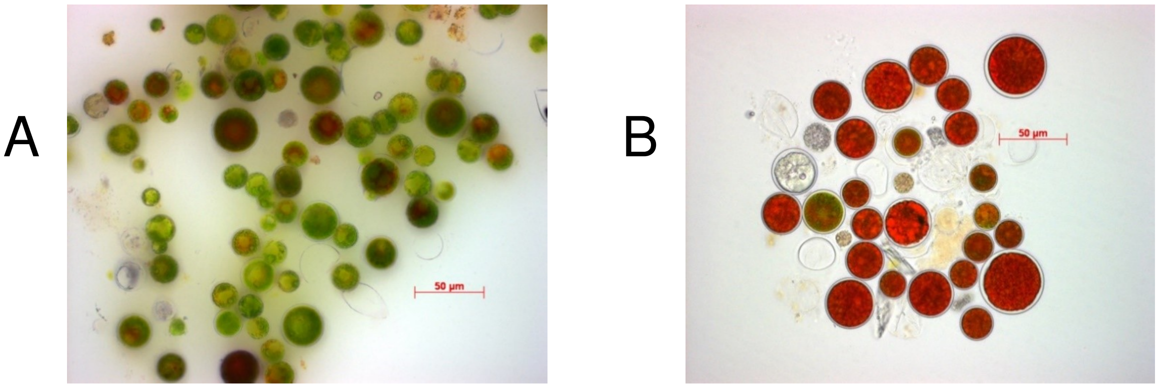


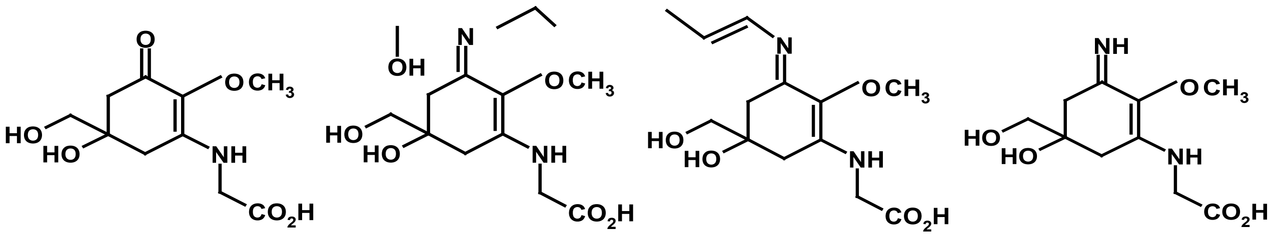
Fig. 2. Accumulation of astaxanthin in *Haematococcus pluvialis*: A – green vegetative cells, where accumulation has just begun; B – astaxanthin-rich haematocysts

Astaxanthin patents are related to food, feed, and nutraceutical, which are currently the main market driver for the pigment. Algatech (Israel), Nutrex Hawaii (USA), Cyanotech (USA), Jingzhou Natural Astaxanthin Inc. (China), Algaetech International (Malaysia), and Parry Nutraceuticals (India) are the main suppliers of microalgal astaxanthin on the market (Cornish & Garbary, 2010 ; Kijjoa & Sawangwong, 2004). Currently, astaxanthin from *Haematococcus* accounts for several percent of the global carotenoid market (Li et al., 2011) as a food coloring agent and a cosmetics ingredient.

Fucoxanthin is an accessory pigment in chloroplast of brown algae, phytoplankton, brown seaweed, and diatoms, giving them a brownish or olive-green color. Microalgae *Phaeodactylum tricornutum* and *Isochrysis galbana* are the main commercially significant producers of fucoxanthin. Structural peculiarity of this pigment includes the presence of an unusual double allyl carbon and two hydroxyl groups, which are thought to increase its high energy transfer efficiency (80 %) and strong antioxidant activity. Fucoxanthin beneficial effects include antioxidant, antitumor, antidiabetic, and other activities (Kijjoa & Sawangwong, 2004 ; Novoveská et al., 2019). In cosmetics, it is used to whiten and improve skin condition, as well as a natural antioxidant and lipolytic agent.

Mycosporine-like amino acids

Mycosporine-like amino acids (hereinafter MAA) are secondary metabolites, found in marine organisms of any climate zone, including microalgae, especially affected by high fluxes of solar radiation or hypersaline conditions (Gröniger et al., 2000 ; Shick & Dunlap, 2002). Over the past 30 years, cyanobacteria from the orders Synechococcales, Chroococcales, Oscillatoriales, and Nostocales have been studied for the presence of new MAA, while the orders Gloeobacterales, Spirulinales, Pleurocapsales, and Chroococcidiopsidales remain scarcely investigated in this regard. MAA are low-molecular mass, colorless, uncharged, and water-soluble molecules. MAA possess a similar backbone, but differ in functional groups; they include cyclohexenone or cyclohexenimine ring, conjugated with an amino alcohol group or a nitrogen subgroup of an amino acid (Shick & Dunlap, 2002) [Fig. 3 and (Wada et al., 2015)].



Mycosporyne-glycin (λ_{max} = 330 nm)

Shinorine (λ_{max} = 334 nm)

Palythene (λ_{max} = 360 nm)

Palythine (λ_{max} = 320 nm)

Fig. 3. Typical mycosporine-like amino acids and their absorption maxima

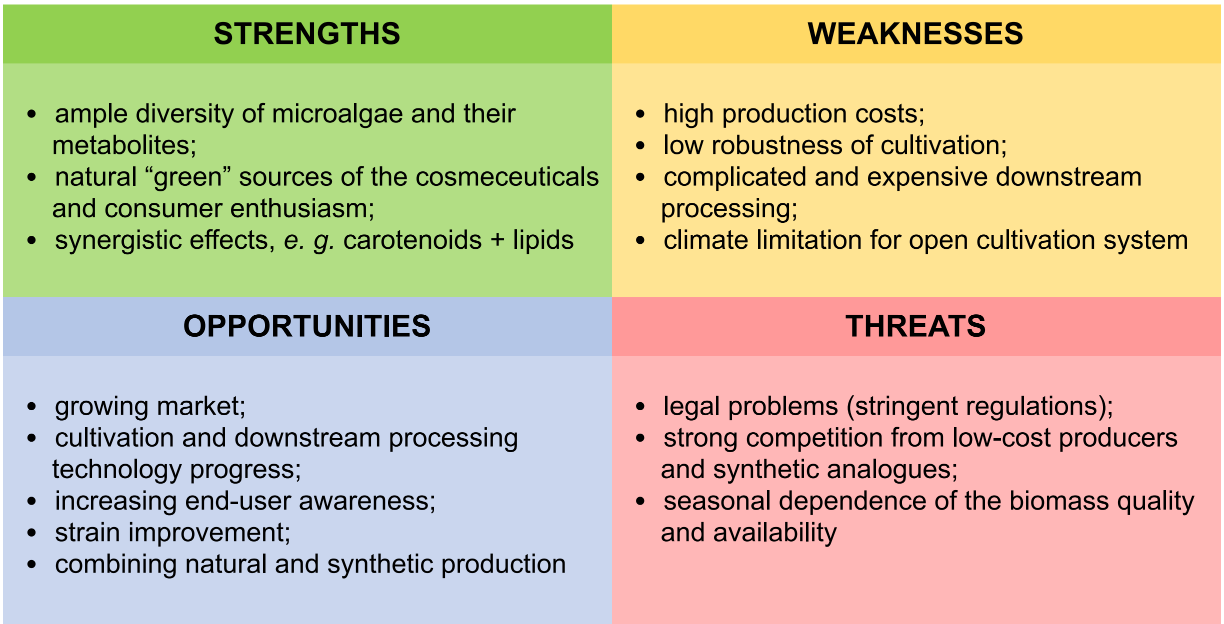
Prolonged exposure to UV radiation causes skin photoaging and several other disorders, *inter alia* fine and coarse wrinkling, and increases the risk of skin cancer. The most noticeable disorders include erythema, edema, blisters, sunburn cell formation, phototoxic reactions, photo-allergy, photo-sensitivity, and acute photo-immunosuppression (Brenner & Hearing, 2008). Sunscreens are commonly applied to reduce the harmful effects of UV on the skin. MAA are promising alternative UV-absorbing compounds of natural origin, which are highly soluble in water and do not generate reactive oxygen species upon absorption of UV radiation. More than 30 MAA from various organisms have been characterized (Gröniger et al., 2000 ; Torres et al., 2006). In addition to photoprotective properties, there is substantial evidence that MAA protect skin from aging and can exert antioxidant and anti-inflammatory activity; MAA can also inhibit protein glycation and collagenase activity. MMA anti-photoaging activity is thought to be related with reduction lipid peroxidation, a determinant of the aging process (de la Coba et al., 2009). Application of 0.005 % MAA in lecithin liposomes on the inner side of the forearm inhibited UVA-stimulated lipid peroxidation by 37 %; four-week treatments improved the skin elasticity and smoothness by 10 % and 12 %, respectively (Schmid et al., 2006). The tested MAA formulation was as effective as the standard cream, containing 1 % synthetic UV filters, Parsol® 1789 and 4 % UVB filters, Neo Heliopan® AV (Schmid et al., 2006). MAA also inhibited the UV-enhanced activity of elastase, which leads to the decomposition of elastin and the formation of wrinkles by 82.5 % as compared to unprotected UVA irradiated cells (Ryu et al., 2014). In addition, MAA can protect the skin from photoaging by regulating the expression level of genes, associated with inflammation, such as COX-2. Treatment of the model cells with Myc-Gly caused a two-fold decline in COX-2 mRNA levels (Suh et al., 2014).

A promising alternative to existing chemical and physical sunscreen filters is the use of multifunctional MAA, which are also suitable for cosmetics formulations (Godlewska et al., 2017 ; Suh et al., 2014). Experiments with cell culture models demonstrated UV-protective effects in HaCaT human keratinocyte cell line (Ishihara et al., 2017). Application of MAA prevented the UV-induced reduction of trans-urocanic acid and UV-stimulated histidine build-up. A crude methanol extract of cyanobacterium *Aphanizomenon flos-aquae*, enriched in MAA, showed a level of UVA protection as compared to a commercial skin care product with a sun protection factor SPF = 4 and a UVA/UVB protection ratio of 0.95 (Torres et al., 2006).

Conclusion and outlook

Microalgae are naturally equipped in terms of metabolic plasticity to cope with diverse stresses. They synthesize a broad spectrum of molecules, exerting potent beneficial effects on many aspects of human body functioning. This is scarcely surprising given that many of the stress-induced deleterious processes in the human body and in a photosynthetic cell are mediated by the same mechanisms, such as free-radical attacks and lipid peroxidation. It is also worth noting that the photosynthetic machinery of microalgae is always at risk of oxidative damage since high redox potentials and reactive molecules are constantly generated during its functioning. These risks are kept at bay by efficient reactive oxygen species elimination systems including potent low-molecular antioxidants.

Therefore, photosynthetic organisms are a rich source of bioactive substances with a great potential for curbing the negative effects of stresses on human skin cells from day to day. In many cases these compounds appear to be less toxic, less allergenic, and, in general, more “biocompatible” than most of their synthetic counterparts. Although a dramatic diversity of cosmeceuticals was discovered in macrophyte algae, single-celled algae are on the same level or even surpass them in this regard. At the same time, the large-scale biotechnological production of microalgal biomass, enriched with the cosmeceutical compounds, is more technically feasible and economically viable than that of macrophyte biomass (Fig. 4).



STRENGTHS	WEAKNESSES
• ample diversity of microalgae and their metabolites; • natural “green” sources of the cosmeceuticals and consumer enthusiasm; • synergistic effects, *e. g.* carotenoids + lipids	• high production costs; • low robustness of cultivation; • complicated and expensive downstream processing; • climate limitation for open cultivation system
OPPORTUNITIES	**THREATS**
• growing market; • cultivation and downstream processing technology progress; • increasing end-user awareness; • strain improvement; • combining natural and synthetic production	• legal problems (stringent regulations); • strong competition from low-cost producers and synthetic analogues; • seasonal dependence of the biomass quality and availability

Fig. 4. SWOT analysis of production of cosmeceuticals from microalgal sources [modified from (Novoveská et al., 2019)]

Even such a brief review makes obvious the advantages and the potential of microalgal biotechnology for the "green" cosmeceutical production. However, a significant part of the chemo- and biodiversity of microalgae remains so far untapped. Consequently, bioprospecting and biochemical characterization of new algal species and strains, especially those isolated from habitats with harsh environmental conditions, is a major avenue for further research and development. As important is the development of efficient approaches to cost-effective cultivation of microalgae, as well as induction, extraction, and purification of cosmeceutical metabolites.

This research was funded by the Ministry of Science and Higher Education of the Russian Federation (grant No. RFMEFI60419X0213).

Acknowledgement. Critical reading of the manuscript by Dr. Ranga Rao Ambati is greatly appreciated.

REFERENCES

1. *Algal Green Chemistry: Recent Progress in Biotechnology* / R. P. Rastogi, D. Madamwar, A. Pandey (Eds). Amsterdam : Elsevier, 2017, 336 p.
2. Arad S., van Moppes D. Novel sulfated polysaccharides of red microalgae: Basics and applications. In: *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*. 2nd ed. / A. Richmond, Q. Hu (Eds). Chichester : Wiley-Blackwell, 2013, chap. 21, pp. 406–416. https://doi.org/10.1002/9781118567166.ch21
3. Barbosa A. J., Roque A. C. Free marine natural products databases for biotechnology and bioengineering. *Biotechnology Journal*, 2019, vol. 14, iss. 11, art. no. 1800607 (8 p.). https://doi.org/10.1002/biot.201800607
4. Black H. S., Boehm F., Edge R., Truscott T. G. The benefits and risks of certain dietary carotenoids that exhibit both anti- and pro-oxidative mechanisms – A comprehensive review. *Antioxidants*, 2020, vol. 9, iss. 3, art. no. 264 (31 p.). https://doi.org/10.3390/antiox9030264
5. Boer L. Biotechnological production of colorants. In: *Biotechnology of Food and Feed Additives* / H. Zorn, P. Czermak (Eds). Berlin ; Heidelberg : Springer, 2013, pp. 51–89. (Advances in Biochemical Engineering/Biotechnology, 2014, vol. 143). https://doi.org/10.1007/10_2013_241
6. Borowitzka M. A. Commercial production of microalgae: Ponds, tanks, and fermenters. *Journal of Biotechnology*, 1999, vol. 70, iss. 1–3, pp. 313–321. https://doi.org/10.1016/S0168-1656(99)00083-8
7. Borowitzka M. A. High-value products from microalgae – Their development and commercialisation. *Journal of Applied Phycology*, 2013, vol. 25, pp. 743–756. https://doi.org/10.1007/s10811-013-9983-9
8. Borowitzka M. A., Vonshak A. Scaling up microalgal cultures to commercial scale. *European Journal of Phycology*, 2017, vol. 52, iss. 4, pp. 407–418. https://doi.org/10.1080/09670262.2017.1365177
9. Boussiba S. Carotenogenesis in the green alga *Haematococcus pluvialis*: Cellular physiology and stress response. *Physiologia Plantarum*, 2000, vol. 108, iss. 2, pp. 111–117. https://doi.org/10.1034/j.1399-3054.2000.108002111.x
10. Brenner M., Hearing V. J. The protective role of melanin against UV damage in human skin. *Photochemistry and Photobiology*, 2008, vol. 84, iss. 3, pp. 539–549. https://doi.org/10.1111/j.1751-1097.2007.00226.x
11. Carlsson A., Van Beilen J., Möller R., Clayton D. *Micro- and macro-algae: Utility for industrial applications : outputs from the EPOBIO project* / D. Bowles (Ed.) ; CNAP, University of York. Newbury, UK : CPL Press, 2007, 82 p. https://www.etipbioenergy.eu/images/epobio_aquatic_report.pdf
12. Chekanov K., Lukyanov A., Boussiba S., Aflalo C., Solovchenko A. Modulation of photosynthetic activity and photoprotection in *Haematococcus pluvialis* cells during their conversion into haematocysts and back. *Photosynthesis Research*, 2016, vol. 128, pp. 313–323. https://doi.org/10.1007/s11120-016-0246-x
13. Chubchikova I., Drobetskaya I., Minyuk G., Dantsyuk N., Chelebieva E. Screening of green

microalgae as potential source of nature keto-carotenoids. 2. Features of growth and secondary carotenogenesis in the representatives of the genus *Bracteacoccus* (Chlorophyceae). *Morskoj ekologicheskij zhurnal*, 2011, vol. 10, no. 1, pp. 91–97.

14. Coates R. C., Trentacoste E., Gerwick W. H. Bioactive and novel chemicals from microalgae. In: *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*, 2nd ed. / A. Richmond, Q. Hu (Eds). Chichester : Wiley-Blackwell, 2013, chap. 26, pp. 504–531. https://doi.org/10.1002/9781118567166.ch26
15. Cohen Z. [Production of polyunsaturated fatty acids by the microalga] *Porphyridium cruentum*. In: *Production of Chemicals by Microalgae* / Z. Cohen (Ed.). Boca Raton ; London ; New York : CRC Press, 1999, pp. 1–24. https://doi.org/10.1201/9781482295306
16. Cohen Z., Khozin-Goldberg I. Searching for polyunsaturated fatty acid-rich photosynthetic microalgae. In: *Single Cell Oils. Microbial and Algal Oils*. 2nd ed. / Z. Cohen, C. Ratledge (Eds). Urbana, IL : AOCS Press, 2010, pt. 3, chap. 10, pp. 201–224. https://doi.org/10.1016/B978-1-893997-73-8.50014-1
17. Cornish M. L., Garbary D. J. Antioxidants from macroalgae: Potential applications in human health and nutrition. *Algae*, 2010, vol. 25, iss. 4, pp. 155–171. https://doi.org/10.4490/algae.2010.25.4.155
18. Couteau C., Coiffard L. Microalgal application in cosmetics. In: *Microalgae in Health and Disease Prevention* / I. A. Levine, J. Fleurence (Eds). London : Academic Press, 2018, chap. 15, pp. 317–323. https://doi.org/10.1016/B978-0-12-811405-6.00015-3
19. Davidi L., Levin Y., Ben-Dor S., Pick U. Proteome analysis of cytoplasmatic and of plastidic β-carotene lipid droplets in *Dunaliella bardawil*. *Plant Physiology*, 2015, vol. 167, iss. 1, pp. 60–79. https://doi.org/10.1104/pp.114.248450
20. de la Coba F., Aguilera J., De Galvez M., Alvarez M., Gallego E., Figueroa F., Herrera E. Prevention of the ultraviolet effects on clinical and histopathological changes, as well as the heat shock protein-70 expression in mouse skin by topical application of algal UV-absorbing compounds. *Journal of Dermatological Science*, 2009, vol. 55, iss. 3, pp. 161–169. https://doi.org/10.1016/j.jdermsci.2009.06.004
21. Dufossé L., Galaup P., Yaron A., Arad S. M., Blanc P., Murthy K. N. C., Ravishankar G. A. Microorganisms and microalgae as sources of pigments for food use: A scientific oddity or an industrial reality? *Trends in Food Science & Technology*, 2005, vol. 16, iss. 9, pp. 389–406. https://doi.org/10.1016/j.tifs.2005.02.006
22. Eom S.-H., Kim S.-K. Cosmeceutical applications from marine organisms. In: *Cosmeceuticals and Cosmetic Practice* / P. K. Farris (Ed.). Chichester : John Wiley & Sons, Ltd., 2013, pp. 200–208.
23. Fox J. M., Zimba P. V. Minerals and trace elements in microalgae. In: *Microalgae in Health and Disease Prevention* / I. A. Levine, J. Fleurence (Eds). London : Academic Press, 2018, pp. 177–193. https://doi.org/10.1016/B978-0-12-811405-6.00008-6
24. Freitas S., Silva N. G., Sousa M. L., Ribeiro T., Rosa F., Leão P. N., Vasconcelos V., Reis M. A., Urbatzka R. Chlorophyll derivatives from marine cyanobacteria with lipid-reducing activities. *Marine Drugs*, 2019, vol. 17, iss. 4, art. no. 229 (18 p.). https://doi.org/10.3390/md17040229
25. García J. L., de Vicente M., Galán B. Microalgae, old sustainable food and fashion nutraceuticals. *Microbial Biotechnology*, 2017, vol. 10, iss. 5, pp. 1017–1024. https://doi.org/10.1111/1751-7915.12800
26. Godlewska K., Dmytryk A., Tuhy Ł., Chojnacka K. Algae as source of food and nutraceuticals. In: *Prospects and Challenges in Algal Biotechnology* / B. Tripathi, D. Kumar (Eds). Singapore : Springer, 2017, pp. 277–294. https://doi.org/10.1007/978-981-10-1950-0_10
27. Goiris K., Muylaert K., Fraeye I., Foubert I., De Brabanter J., De Cooman L. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *Journal of Applied Phycology*, 2012, vol. 24, pp. 1477–1486. https://doi.org/10.1007/s10811-012-9804-6
28. Gong M., Bassi A. Carotenoids from microalgae: A review of recent developments. *Biotechnology Advances*, 2016, vol. 34, iss. 8, pp. 1396–1412.

https://doi.org/10.1016/j.biotechadv.2016.10.005

29. Gröniger A., Sinha R., Klisch M., Häder D. Photoprotective compounds in cyanobacteria, phytoplankton and macroalgae – A database. *Journal of Photochemistry & Photobiology B: Biology*, 2000, vol. 58, iss. 2–3, pp. 115–122. https://doi.org/10.1016/S1011-1344(00)00112-3
30. Han D., Li Y., Hu Q. Astaxanthin in microalgae: Pathways, functions and biotechnological implications. *Algae*, 2013, vol. 28, iss. 2, pp. 131–147. https://doi.org/10.4490/algae.2013.28.2.131
31. Hussein G., Sankawa U., Goto H., Matsumoto K., Watanabe H. Astaxanthin, a carotenoid with potential in human health and nutrition. *Journal of Natural Products*, 2006, vol. 69, iss. 3, pp. 443–449. https://doi.org/10.1021/np050354+
32. Ishihara K., Watanabe R., Uchida H., Suzuki T., Yamashita M., Takenaka H., Nazifi E., Matsugo S., Yamaba M., Sakamoto T. Novel glycosylated mycosporine-like amino acid, 13-O-(β-galactosyl)-porphyra-334, from the edible cyanobacterium *Nostoc sphaericum* – protective activity on human keratinocytes from UV light. *Journal of Photochemistry and Photobiology B: Biology*, 2017, vol. 172, pp. 102–108. https://doi.org/10.1016/j.jphotobiol.2017.05.019
33. Julius M. L. Carbohydrate diversity in microalgae: A phylogenetically arranged presentation. In: *Microalgae in Health and Disease Prevention* / I. A. Levine, J. Fleurence (Eds). London : Academic Press, 2018, pp. 133–144. https://doi.org/10.1016/B978-0-12-811405-6.00006-2
34. Khozin-Goldberg I., Iskandarov U., Cohen Z. LC-PUFA from photosynthetic microalgae: Occurrence, biosynthesis, and prospects in biotechnology. *Applied Microbiology and Biotechnology*, 2011, vol. 91, iss. 4, pp. 905–915. https://doi.org/10.1007/s00253-011-3441-x
35. Kijjoa A., Sawangwong P. Drugs and cosmetics from the sea. *Marine Drugs*, 2004, vol. 2, iss. 2, pp. 73–82. https://doi.org/10.3390/md202073
36. Lamers P. P., van de Laak C. C. W., Kaasenbrood P. S., Lorier J., Janssen M., De Vos R. C. H., Bino R. J., Wijffels R. H. Carotenoid and fatty acid metabolism in light-stressed *Dunaliella salina*. *Biotechnology and Bioengineering*, 2010, vol. 106, iss. 4, pp. 638–648. https://doi.org/10.1002/Bit.22725
37. Lee J.-C., Hou M.-F., Huang H.-W., Chang F.-R., Yeh C.-C., Tang J.-Y., Chang H.-W. Marine algal natural products with anti-oxidative, anti-inflammatory, and anti-cancer properties. *Cancer Cell International*, 2013, vol. 13, art. no. 55 (7 p.). https://doi.org/10.1186/1475-2867-13-55
38. Levine I. A. Algae: A way of life and health. In: *Microalgae in Health and Disease Prevention* / I. A. Levine, J. Fleurence (Eds). London : Academic Press, 2018, chap. 1, pp. 1–10. https://doi.org/10.1016/B978-0-12-811405-6.00001-3
39. Li J., Zhu D., Niu J., Shen S., Wang G. An economic assessment of astaxanthin production by large scale cultivation of *Haematococcus pluvialis*. *Biotechnology Advances*, 2011, vol. 29, iss. 6, pp. 568–574. https://doi.org/10.1016/j.biotechadv.2011.04.001
40. *Marine Cosmeceuticals: Trends and Prospects* / S.-K. Kim (Ed). Boca Raton : CRC Press, 2011, 432 p. https://doi.org/10.1201/b10120
41. *Marine Macro- and Microalgae : An Overview* / F. X. Malcata, I. S. Pinto, A. C. Guedes (Eds). Boca Raton : CRC Press, 2018, 342 p. https://doi.org/10.1201/9781315119441
42. Masojídek J., Torzillo G., Koblížek M. Photosynthesis in microalgae. In: *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*, 2nd ed. / A. Richmond, Q. Hu (Eds). Chichester : Wiley-Blackwell, 2013, chap. 2, pp. 21–35. https://doi.org/10.1002/9781118567166.ch2
43. Mimouni V., Couzinet-Mossion A., Ulmann L., Wielgosz-Collin G. Lipids from microalgae. In: *Microalgae in Health and Disease Prevention* / I. A. Levine, J. Fleurence (Eds). London : Academic Press, 2018, pp. 109–131. https://doi.org/10.1016/B978-0-12-811405-6.00005-0
44. Morançais M., Mouget J.-L., Dumay J. Proteins and pigments. In: *Microalgae in Health and Disease Prevention* / I. A. Levine, J. Fleurence (Eds). London : Academic Press, 2018, pp. 145–175. https://doi.org/10.1016/B978-0-12-811405-6.00007-4
45. Mu N., Mehar J. G., Mudliar S. N., Shekh A. Y. Recent advances in microalgal bioactives for food,

feed, and healthcare products: Commercial potential, market space, and sustainability. *Comprehensive Reviews in Food Science and Food Safety*, 2019, vol. 18, iss. 6, pp. 1882–1897. https://doi.org/10.1111/1541-4337.12500

46. Mulders K. J. M., Lamers P. P., Martens D. E., Wijffels R. H. Phototrophic pigment production with microalgae: Biological constraints and opportunities. *Journal of Phycology*, 2014, vol. 50, iss. 2, pp. 229–242. https://doi.org/10.1111/jpy.12173
47. Naguib Y. Antioxidant activities of astaxanthin and related carotenoids. *Journal of Agriculture and Food Chemistry*, 2000, vol. 48, iss. 4, pp. 1150–1154. https://doi.org/10.1021/jf991106k
48. Novoveská L., Ross M. E., Stanley M. S., Pradelles R., Wasiolek V., Sassi J. F. Microalgal carotenoids: A review of production, current markets, regulations, and future direction. *Marine Drugs*, 2019, vol. 17, iss. 11, art. no. 640 (21 p.). https://doi.org/10.3390/md17110640
49. Pulz O., Gross W. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*, 2004, vol. 65, pp. 635–648. https://doi.org/10.1007/s00253-004-1647-x
50. Ryu J., Park S.-J., Kim I.-H., Choi Y. H., Nam T.-J. Protective effect of porphyra-334 on UVA-induced photoaging in human skin fibroblasts. *International Journal of Molecular Medicine*, 2014, vol. 34, iss. 3, pp. 796–803. https://doi.org/10.3892/ijmm.2014.1815
51. Schmid D., Schürch C., Zülli F. Mycosporine-like amino acids from red algae protect against premature skin-aging. *Euro Cosmetics*, 2006, vol. 9, pp. 1–4.
52. Scott R. Marine ingredients: Latest actives from the deep. *Personal Care*, 2015, vol. 4, pp. 43–44.
53. Shick J., Dunlap W. Mycosporine-like amino acids and related gadusols: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annual Review of Physiology*, 2002, vol. 64, pp. 223–262. https://doi.org/10.1146/annurev.physiol.64.081501.155802
54. Silva T. H., Alves A., Popa E. G., Reys L. L., Gomes M. E., Sousa R. A., Silva S. S., Mano J. F., Reis R. L. Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. *Biomatter*, 2012, vol. 2, iss. 4, pp. 278–289. https://doi.org/10.4161/biom.22947
55. Solovchenko A. *Photoprotection in Plants: Optical Screening-based Mechanisms.* Berlin ; Heidelberg : Springer, 2010, 167 p. https://doi.org/10.1007/978-3-642-13887-4
56. Solovchenko A. Physiological role of neutral lipid accumulation in eukaryotic microalgae under stresses. *Russian Journal of Plant Physiology*, 2012, vol. 59, pp. 167–176. https://doi.org/10.1134/S1021443712020161
57. Solovchenko A., Khozin-Goldberg I., Didi-Cohen S., Cohen Z., Merzlyak M. Effects of light intensity and nitrogen starvation on growth, total fatty acids and arachidonic acid in the green microalga *Parietochloris incisa*. *Journal of Applied Phycology*, 2008, vol. 20, pp. 245–251. https://doi.org/10.1007/s10811-007-9233-0
58. Solovchenko A., Lukyanov A., Solovchenko O., Didi-Cohen S., Boussiba S., Khozin-Goldberg I. Interactive effects of salinity, high light, and nitrogen starvation on fatty acid and carotenoid profiles in *Nannochloropsis oceanica* CCALA 804. *European Journal of Lipid Science and Technology*, 2014, vol. 116, iss. 5, pp. 635–644. https://doi.org/10.1002/ejlt.201300456
59. Solovchenko A. E. Physiology and adaptive significance of secondary carotenogenesis in green microalgae. *Russian Journal of Plant Physiology*, 2013, vol. 60, pp. 1–13. https://doi.org/10.1134/s1021443713010081
60. Spolaore P., Joannis-Cassan C., Duran E., Isambert A. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, 2006, vol. 101, iss. 2, pp. 87–96. https://doi.org/10.1263/jbb.101.87
61. Suh S.-S., Hwang J., Park M., Seo H. H., Kim H.-S., Lee J. H., Moh S. H., Lee T.-K. Anti-inflammation activities of mycosporine-like amino acids (MAAs) in response to UV radiation suggest potential anti-skin aging activity. *Marine Drugs*, 2014, vol. 12, iss. 10, pp. 5174–5187. https://doi.org/10.3390/md12105174
62. Sun T., Yuan H., Cao H., Yazdani M., Tadmor Y., Li L. Carotenoid metabolism in plants:

The role of plastids. *Molecular Plant*, 2018, vol. 11, iss. 1, pp. 58–74. https://doi.org/10.1016/j.molp.2017.09.010

63. Tanaka T., Shnimizu M., Moriwaki H. Cancer chemoprevention by carotenoids. *Molecules*, 2012, vol. 17, iss. 3, pp. 3202–3242. https://doi.org/10.3390/molecules17033202
64. Telfer A. What is β-carotene doing in the photosystem II reaction centre? *Philosophical Transactions of the Royal Society B. Biological Sciences*, 2002, vol. 357, iss. 1426, pp. 1431–1440. https://doi.org/10.1098/rstb.2002.1139
65. Thomas N. V., Kim S.-K. Beneficial effects of marine algal compounds in cosmeceuticals. *Marine Drugs*, 2013, vol. 11, iss. 1, pp. 146–164. https://dx.doi.org/10.3390%2Fmd11010146
66. Torres A., Enk C. D., Hochberg M., Srebnik M. Porphyra-334, a potential natural source for UVA protective sunscreens. *Photochemical & Photobiological Sciences*, 2006, vol. 5, iss. 4, pp. 432–435. https://doi.org/10.1039/B517330M
67. Wada N., Sakamoto T., Matsugo S. Mycosporine-like amino acids and their derivatives as natural antioxidants. *Antioxidants*, 2015, vol. 4, iss. 3, pp. 603–646. https://doi.org/10.3390/antiox4030603
68. Ward O. P., Singh A. Omega-3/6 fatty acids: Alternative sources of production. *Process Biochemistry*, 2005, vol. 40, iss. 12, pp. 3627–3652. https://doi.org/10.1016/j.procbio.2005.02.020
69. Ye Z.-W., Jiang J.-G., Wu G.-H. Biosynthesis and regulation of carotenoids in *Dunaliella*: Progresses and prospects. *Biotechnology Advances*, 2009, vol. 26, iss. 4, pp. 352–360. https://doi.org/10.1016/j.biotechadv.2008.03.004
70. Zhekisheva M., Zarka A., Khozin-Goldberg I., Cohen Z., Boussiba S. Inhibition of astaxanthin synthesis under high irradiance does not abolish triacylglycerol accumulation in the green alga *Haematococcus pluvialis* (Chlorophyceae). *Journal of Phycology*, 2005, vol. 41, iss. 4, pp. 819–826. https://doi.org/10.1111/j.0022-3646.2005.05015.x
71. Zittelli G., Biondi N., Rodolfi L., Tredici M. Photobioreactors for mass production of microalgae. In: *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*. 2nd ed. / A. Richmond, Q. Hu (Eds). Chichester : Wiley-Blackwell, 2013, chap. 13, pp. 225–266. https://doi.org/10.1002/9781118567166.ch13

МОРСКИЕ И ПРЕСНОВОДНЫЕ МИКРОВОДОРОСЛИ КАК ВОЗОБНОВЛЯЕМЫЙ ИСТОЧНИК СОЕДИНЕНИЙ С КОСМЕЦЕВТИЧЕСКОЙ АКТИВНОСТЬЮ[*]

Т. В. Пучкова[1], С. А. Хапчаева[1], В. С. Зотов[1],
А. А. Лукьянов[2], А. Е. Соловченко[2,3]

[1]Федеральный исследовательский центр «Фундаментальные основы биотехнологии» РАН,
Москва, Российская Федерация
[2]Московский государственный университет имени М. В. Ломоносова, Москва, Российская Федерация
[3]Тамбовский государственный университет имени Г. Р. Державина, Тамбов, Российская Федерация
E-mail: *solovchenko@mail.bio.msu.ru*

Важная особенность экстремофильных и стресс-толерантных микроводорослей — их универсальный метаболизм, позволяющий им синтезировать широкий спектр биомолекул. Данные соединения повышают устойчивость клеток микроводорослей к неблагоприятным факторам. В организме человека биологически активные вещества способны замедлять старение и оказывать противовоспалительное и фотопротекторное действие. Это неудивительно, если учесть, что многие повреждения, вызываемые стрессами в организме человека и в фотоавтотрофных клетках, опосредуются одними и теми же механизмами, такими как атаки свободных радикалов и перекисное окисление липидов. Фотосинтетический аппарат клеток микроводорослей

---

[*] Препринт опубликован 28 декабря 2020 г.: https://doi.org/10.20944/preprints202012.0696.v1.

всегда подвержен риску окислительного повреждения, поскольку в процессе его функционирования постоянно генерируются высокие окислительно-восстановительные потенциалы и реакционноспособные молекулы. Этим факторам риска противостоят эффективные системы элиминации активных форм кислорода, включающие, среди прочих компонентов, мощные низкомолекулярные антиоксиданты. Как следствие, фототрофные организмы являются богатым источником биологически активных веществ с большим потенциалом для сдерживания негативных последствий стрессов, действующих на клетки кожи человека изо дня в день. Во многих случаях эти соединения оказываются менее токсичными, менее аллергенными и в целом более «биосовместимыми», чем большинство их синтетических аналогов. Те же самые метаболиты водорослей признаны перспективными ингредиентами для инновационных косметических средств и космецевтических рецептур. Исследователи прилагают всё больше усилий для поиска новых природных биологически активных веществ из микроводорослей. Поддерживает эту тенденцию и растущий спрос на натуральное сырьё для пищевых продуктов, а также нутрицевтики, фармацевтики и косметологии, связанный с глобальным переходом на «зелёные» (возобновляемые) источники сырья. В водорослях-макрофитах было обнаружено поразительное разнообразие соединений с космецевтическими эффектами, но одноклеточные водоросли не уступают им и даже превосходят их в этом отношении. В то же время крупномасштабное биотехнологическое производство биомассы микроводорослей, обогащённой космецевтическими соединениями, проще технически и выгоднее, чем производство или сбор биомассы макрофитов. Культивирование автотрофных микроводорослей, как правило, проще и дешевле, чем культивирование гетеротрофных микроорганизмов. Выращивание в биореакторах позволяет получать более стандартизированную сырую биомассу, качество которой в меньшей степени зависит от сезонных факторов. Биотехнология открывает множество возможностей для производства возобновляемого космецевтического сырья, однако значительная часть биоразнообразия микроводорослей и добываемых из них компонентов остаётся неизученной. Следовательно, поиск и получение биохимической характеристики новых видов и штаммов водорослей, особенно выделенных из местообитаний с суровыми условиями окружающей среды, — это одно из наиболее актуальных направлений дальнейших исследований. Не менее важна разработка подходов к рентабельному культивированию микроводорослей, а также к индукции, экстракции и очистке космецевтически активных метаболитов. Мировое научное сообщество стремительно накапливает информацию о химии и разнообразном действии соединений и метаболитов из микроводорослей; многие экстрагируемые из них вещества уже нашли широкое применение в косметической промышленности. Между тем перечень экстрактов и отдельных химических веществ, выделенных из них и тщательно проверенных на безопасность и эффективность, пока не очень велик. В литературе имеются содержательные обзоры по отдельным классам космецевтических субстанций из микроводорослей, но работы, охватывающие все основные группы таких соединений, встречаются редко. В данной статье рассмотрены наиболее важные классы химических веществ из клеток микроводорослей, обладающих космецевтическим потенциалом. Освещены закономерности состава и накопления этих веществ в связи с аспектами биологии микроводорослей.

Ключевые слова: каротиноиды, хлорофиллы, липиды, микоспорин-подобные аминокислоты, антиоксиданты, УФ-защитные соединения

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 1, pp. 82–101
https://doi.org/10.21072/mbj.2021.06.1.07

UDC [574.583:579](262.5.04:292.471-14)

SEASONAL DYNAMICS AND SPATIAL DISTRIBUTION OF STRUCTURAL INDICATORS OF THE BACTERIOPLANKTON COMMUNITY OF THE SEVASTOPOL BAY (THE BLACK SEA)

© 2021 **O. A. Rylkova[1] and I. G. Polikarpov[2]**

[1]A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
[2]Ecosystem-based Management of Marine Resources Program, Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, Kuwait City, State of Kuwait
E-mail: *ol.rylkova@yandex.ru*

Received by the Editor 31.12.2019; after reviewing 17.06.2020;
accepted for publication 11.03.2021; published online 23.03.2021.

Bacterioplankton community determines formation of a significant part of the secondary production and mineralization of organic matter in aquatic ecosystems, as well as responds quickly to any changes in the environment. Data on the state of the microbial community are required for understanding the processes of substance and energy flow transfer in aquatic ecosystems; this is especially important for coastal waters, where significant negative transformations have occurred in recent decades. The aim of this study was to investigate and analyze changes in structural indicators of the bacterioplankton community in different areas of the Sevastopol Bay (the Black Sea) during 1992–2005. Bacterial abundance was determined by direct microscopy, using adsorption (erythrosine) or fluorescent (acridine orange) stains; biomass was calculated using a conversion factor ($2{\cdot}10^{-14}$ g $C{\cdot}cell^{-1}$) or by direct cell measurements. Cell morphotypes were determined by scanning electron microscopy. The total abundance of microorganisms varied $0.2{\cdot}10^{6}$ to $10{\cdot}10^{6}$ $cells{\cdot}mL^{-1}$; biomass – 2 to 201 $mg\ C{\cdot}m^{-3}$. In the morphological structure of bacterioplankton community, cocci (0.36–0.86 μm in diameter) with a volume of 0.02–0.27 μm^3 and rod-shaped cells (0.6–1.2 μm length; 0.2–0.4 μm width) with a volume of 0.50–0.65 μm^3 prevailed. Maximum values of the bacterioplankton abundance, biomass, and cell size in the Sevastopol Bay were registered in summer and autumn (June to October), while minimum values were recorded in winter and spring. The observed values of bacterioplankton quantitative indicators were comparable with the values for various coastal water areas of the World Ocean, *inter alia* the Black Sea. The dynamics of bacterioplankton structural indicators of the Sevastopol Bay during the annual cycle was determined by abiotic and biotic environmental factors. High correlation (86 %, $p < 0.01$) between the hydrological, hydrochemical, and biological variables confirms the non-random nature of the relationship between them. The discriminant analysis revealed significant differences in the structure of bacterioplankton communities for the bay areas with different intensity of water exchange, degree of general pollution, and distance from the open sea. Significantly smaller bacterial cell volume in 2004 [(0.16 ± 0.05) μm^3] compared with that of 2005 [(0.20 ± 0.03) μm^3] (paired t-test, $p < 0.05$) was probably related to intense microorganisms' grazing by phagotrophic protozoa. The obtained data on the structure of the bacterioplankton community can be used for forecasting the state of the Sevastopol Bay ecosystem, as well as for developing and verifying mathematical models of coastal ecosystems functioning.

Keywords: bacterioplankton, abundance, biomass, morphology, abiotic and biotic factors, Sevastopol Bay, Black Sea

In modern research, bacterioplankton is considered as a source of organic carbon for consumers and biogenic elements for primary producers. Assessment of the state of the microbial community is an integral part of comprehensive studies of any aquatic ecosystem (Bul'on, 2002 ; Kopylov & Kosolapov, 2011).

Coastal water, experiencing maximum anthropogenic load, have always been the object of increased interest for researchers. In the middle of the XX century, the Sevastopol Bay water area (the Crimean Peninsula southwestern tip) has been chosen as a research site for studying the dynamics of meteorological, hydrological, hydrochemical, and biological parameters (Gorbenko, 1977 ; Ivanov et al., 2006 ; Morochkovskii & Koval'chuk, 1993). The regime of the bay is determined by water circulation and intensity of water exchange with the Black Sea, as well as by river and storm runoff and industrial wastewater. Moreover, shipping activity is intense in the bay. As a result of the combination of these factors and due to significant length of the bay (more than 7 km), non-uniform fields of distribution of various hydrological, hydrochemical, and other characteristics are formed in its water. The level of pollution increases from the mouth of the bay to its tail-end areas (Gubanov et al., 2015 ; Ivanov et al., 2006 ; Orekhova & Varenik, 2018 ; Khorolich, 1986).

Microbiological research in the Sevastopol Bay started in 1966. The average annual density of bacterioplankton in the bay water was of $0.5 \cdot 10^6$ cells·mL^{-1}, varying $0.4 \cdot 10^6$ to $0.7 \cdot 10^6$ cells·mL^{-1}, which corresponded to the level of oligotrophic water (Gorbenko, 1977). A decade later (1976), the bay was already characterized as a mesotrophic water area, with the average annual bacterial abundance up to $1.0 \cdot 10^6$ cells·mL^{-1} (with variations $0.6 \cdot 10^6$ to $1.4 \cdot 10^6$ cells·mL^{-1}) and the biomass 86–507 mg of wet weight per m^3 (Shumakova, 1980). A negative effect on the ecological situation in the bay resulted from lowering the water exchange due to the construction of a barrier pier in 1978 (Khorolich, 1986). In 1982–1983, the average annual bacterial abundance increased up to $3 \cdot 10^6$ cells·mL^{-1} ($1.31 \cdot 10^6$–$4.4 \cdot 10^6$ cells·mL^{-1}) in the mouth of the bay and up to $3.7 \cdot 10^6$ cells·mL^{-1} ($2.2 \cdot 10^6$–$7.6 \cdot 10^6$ cells·mL^{-1}) in its center. Similar bacterioplankton abundance was recorded in 1988–1989 (Chepurnova et al., 1993). The obtained data on the density of microorganisms were considered previously as characteristic of eutrophic sea water areas (Sorokin, 1973). Thus, the results of long-term microbiological studies in the Sevastopol Bay indicated an increase in its trophicity.

Our aim was to study and analyze the long-term dynamics (1992–2005) of structural indicators and further changes in the bacterioplankton community in the Sevastopol Bay areas, differing in distance from the open sea.

MATERIAL AND METHODS

The Sevastopol Bay is a semi-enclosed estuary with constrained water exchange. The bay is about 7.5 km long and up to 0.85 km wide. The maximum depth is about 20 m; it decreases up to 4–5 m from the mouth of the bay to its tail-end areas. The bay eastern end is the estuary of the Chernaya River, and up to 80 % of its runoff occurs during floods in autumn and winter (Morochkovskii & Koval'chuk, 1993). Based on the data on the distribution of hydrological (temperature, salinity, pH, alkalinity, and transparency) and hydrochemical (concentration of oxygen, phosphates, and silicon, as well as concentration of nitrate, nitrite, and ammonium nitrogen) indicators, the Sevastopol Bay water area is conventionally divided into four zones with a graduation from “weak” to “very strong” pollution (Fig. 1A). This gradient is explained by an increase in anthropogenic load with distance from the open sea to the bay tail-end areas (Ivanov et al., 2006 ; Ovsyanyi et al., 2000). The choice of sampling stations in our study corresponded to that zoning: st. 2, 5 were located in the zone of "weak" pollution, st. 3 – in the zone of "strong" one, and st. 4 – in the zone of "very strong" one (Fig. 1B).

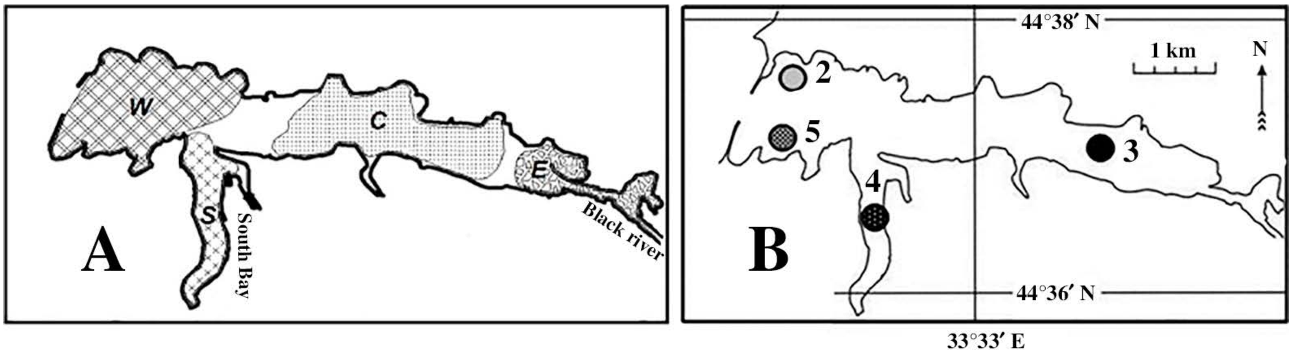


Fig. 1. Map of the Sevastopol Bay: A – zoning according to the distribution of hydrological and hydrochemical indicators [W – western area, zone of "weak" pollution; E – eastern area, "moderate" pollution; C – central area, "strong" pollution; S – southern area, "very strong" pollution (Ivanov et al., 2006)]; B – location of sampling stations

Water was sampled from the surface layer by a 10-L Niskin bottle. In total, 372 water samples were processed during the research period (January 1992 to December 2005). We used the data on bacterioplankton (kindly provided by V. Ponomarenko, 1992) and phytoplankton (L. Manzhos and Yu. Bryantseva, 1998–1999). The data on hydrological and hydrochemical indicators for 1998 and 1999 are given according to (Ovsyanyi et al., 2000).

The total bacterial abundance in water (N, $\cdot 10^6$ cells·mL^{-1}) was determined by direct counting after filtration. The cells were stained with erythrosine (Rodina, 1965) on Sartorius nitrocellulose filters (pore diameter of 0.2 μm; Germany) and with acridine orange (Hobbie et al., 1977) on track membranes (thickness of 12 μm, pore diameter of 0.2 μm; production of Joint Institute for Nuclear Research (JINR), Dubna, Russian Federation), which were stained with Sudan Black.

To remove non-living particles and microorganisms, that got into solutions during preparation and storage, all reagents for staining and fixing microorganisms were prefiltered through the Sartorius filters (Germany) (pore diameter of 0.2 μm) (Brock, 1987). When staining with erythrosine, bacteria were counted under a Biolam light microscope (LOMO, Russian Federation) at a magnification 1350× using phase contrast (Rodina, 1965). When using acridine orange, the preparations were viewed under the epifluorescence mode of a JenaLumar fluorescence microscope (Carl Zeiss Jena, Germany) with an excitation range of 470–490 nm and a transmission range of 500–520 nm, at a magnification 1000× (Hobbie et al., 1977). On each filter, depending on the density of bacteria, 10–20 fields of view were calculated to obtain data with an error of no more than 20 % at a 95 % significance level (Lebedeva & Shumakova, 1969).

Bacterioplankton biomass (B, mg·m^{-3}) was calculated taking into account the abundance of cocci and rods in the sample (Potapova & Korolevskaya, 1991 ; Romanenko & Dobrynin, 1973). Cell size was determined using an ocular micrometer, measuring at least 50 cells, stained with acridine orange. Cocci volume was calculated by the formula for the volume of a sphere ($V = \frac{1}{6}\pi d^3$); rods volume – by the formula for the volume of a cylinder ($V = \frac{1}{4}\pi d^3 h$). The carbon content in bacterial cells was taken equal to 11 % of the raw biomass, according to (Troitskii & Sorokin, 1967). No conversion factor for shrinkage of the cells, stained with acridine orange, was introduced. In the lack of data on the cell sizes, the biomass was determined by taking the carbon content in one bacterial cell equal to 20 fg ($2{\cdot}10^{-14}$ g $\mathrm{C{\cdot}cell^{-1}}$) (Lee & Furman, 1987).

To detail the morphological structure of the Sevastopol Bay bacterioplankton in 2019, samples were taken for examination under a scanning electron microscope. When preparing the samples, 30–50 mL of water was fixed for 1 hour with a 6 % glutaric dialdehyde solution (Merck, Germany), prepared in phosphate buffer or sterile seawater (final concentration of 2.5 %). The sample was concentrated on a track membrane with a pore diameter of 0.2 μm (JINR, Dubna, Russian Federation). Then, dehydration was carried out in a series of ethanol dilutions of 20, 30, 50, 75, 96, and 100 % (Bratbak, 1993). A Leica EM CPD300 dryer (Germany) was used to dry the samples at the critical point (1.5–2.5 h). For deposition (Au/Pd; 0.5–1.0 min.), a Leica EM ACE200 vacuum coater (Germany) was used. The samples were examined under a Hitachi SU3500 scanning electron microscope (Japan) at a magnification 35000×.

RESULTS

In recent decades, methods for determining bacterioplankton quantitative indicators have undergone significant changes because of the use of various stains (from adsorptive erythrosine to fluorochromes – acridine orange, proflavine, fluorescein, DAPI, *etc.*), as well as various microscopy (light and fluorescence). In this regard, we have previously conducted relevant intercalibration studies. It was shown that the bacterial abundance in the Sevastopol Bay, with microscopic counts of cells, was significantly higher – by (1.92 ± 0.23) times (paired t-test, $p < 0.05$) – after staining with erythrosine, compared with the bacterial abundance in the samples, stained with acridine orange (Ryl'kova et al., 2003). When comparing long-term data, we divided the values of the bacterial abundance, obtained *prior* 1998, by the coefficient.

Variations in the bacterioplankton abundance in the Sevastopol Bay areas. During our research period (1992–2005), the overall density of bacteria in the bay changed by two orders of magnitude: $0.1{\cdot}10^6$ to $10{\cdot}10^6$ $\mathrm{cells{\cdot}mL^{-1}}$. At the same time, 1998 and 2002 were characterized by the maximum variations of the bacterioplankton abundance in all the sites of the water area studied (Table 1). In the open areas of the bay (st. 2, 5), the average annual abundance ($N_{avg./year}$) varied from $1.0{\cdot}10^6$ $\mathrm{cells{\cdot}mL^{-1}}$ (1999, st. 2, 5; 2005, st. 5) to $2.2{\cdot}10^6$ $\mathrm{cells{\cdot}mL^{-1}}$ (1992, st. 2, 5). In the tail-end areas (st. 3, 4), the values of $N_{avg./year}$ were higher: from ($1.1{\cdot}10^6 \pm 0.3{\cdot}10^6$) $\mathrm{cells{\cdot}mL^{-1}}$ (2005, st. 4) to ($3.6{\cdot}10^6 \pm 2.0{\cdot}10^6$) $\mathrm{cells{\cdot}mL^{-1}}$ (2002, st. 3) (Table 1).

A significant difference was recorded in the density of bacterioplankton (with an increase in the value from the mouth of the bay to the tail-end): in 1998 and 1999 – between st. 2, 5 and st. 3, 4; in 2003–2005 – between st. 2, 5 and st. 3 (in all cases, paired t-test, $p < 0.05$). In 1992 and 2002, the differences in the average annual abundance of microorganisms were insignificant between all the stations studied; in 2005, the differences were insignificant between st. 2, 5 and tail-end st. 4 (Table 1, Fig. 1B).

Since the bacterial biomass was calculated using the conversion factor ($2{\cdot}10^{-14}$ g $\mathrm{C{\cdot}cell^{-1}}$) (Lee & Furman, 1987), variations in this indicator coincided with those in the total bacterioplankton abundance. The range of biomass fluctuations over the entire research period was 2.2–200.9 mg $\mathrm{C{\cdot}m^{-3}}$, and the average annual values varied from (20.7 ± 3.8) mg $\mathrm{C{\cdot}m^{-3}}$ (1999, st. 2) to (72.6 ± 40.6) mg $\mathrm{C{\cdot}m^{-3}}$ (2002, st. 3) (Table 1).

Table 1. Abundance ($\cdot 10^6$ cells$\cdot$mL^{-1}), biomass (mg C$\cdot$m^{-3}), and average cell volume (μm^3) of bacterioplankton in the Sevastopol Bay (numerator denotes mean value ± confidence interval; denominator denotes minimum and maximum values)

Indicator	Year of research	**Average annual value** Range of value changes			
		Station 2	Station 5	Station 3	Station 4
Bacterioplankton abundance, $\cdot 10^6$ cells$\cdot$mL^{-1}	1992	**2.2 ± 0.5** 0.6–3.6	**2.2 ± 0.4** 1.1–3.8	n/d	**1.9 ± 0.3** 1.0–2.9
	1998	**2.1 ± 0.8** 0.7–3.8	**1.1 ± 0.2** 0.4–2.1	**3.2 ± 1.0** 1.6–5.3	**2.6 ± 1.3** 0.9–7.5
	1999	**1.0 ± 0.2** 0.5–1.3	**1.0 ± 0.3** 0.4–2.2	**2.2 ± 0.8** 0.2–4.5	**1.9 ± 0.7** 0.6–4.9
	2002	**2.1 ± 0.7** 0.7–8.1	n/d	**3.6 ± 2.0** 0.9–10.0	n/d
	2003	**2.1 ± 0.3** 1.2–3.7	n/d	**2.9 ± 0.5** 1.2–4.7	n/d
	2004	**1.3 ± 0.2** 0.6–2.0	**1.2 ± 0.2** 0.7–1.6	**1.6 ± 0.1** 0.6–2.3	**1.2 ± 0.3** 0.2–2.2
	2005	**1.2 ± 0.2** 0.6–2.5	**1.0 ± 0.3** 0.3–2.3	**1.6 ± 0.3** 0.1–3.8	**1.1 ± 0.3** 0.4–2.2
Bacterial biomass, mg C$\cdot$m^{-3}	1992	**43.0 ± 10.8** 12.4–72.29	**43.5 ± 8.3** 22.8–76.8	n/d	**37.0 ± 6.6** 19.5–57.1
	1998	**41.9 ± 15.6** 14.2–75.1	**20.7 ± 3.0** 7.8–30.1	**63.7 ± 19.2** 31.9–105.5	**51.9 ± 26.1** 18.1–150.7
	1999	**20.7 ± 3.8** 9.4–26.5	**21.3 ± 6.2** 8.0–44.7	**43.3 ± 15.9** 4.3–90.7	**37.1 ± 14.2** 12.8–98.4
	2002	**42.5 ± 17.0** 13.2–162.4	n/d	**72.6 ± 40.6** 17.4–200.9	n/d
	2003	**42.9 ± 5.9** 23.0–74.8	n/d	**56.9 ± 10.3** 24.8–94.5	n/d
	2004	**26.6 ± 3.2** 11.4–39.0	**24.1 ± 3.4** 13.6–31.2	**31.3 ± 2.9** 11.6–45.5	**21.3 ± 6.5** 4.8–43.9
	2004*	**27.3 ± 11.4** 2.4–91.3	**18.9 ± 6.8** 6.6–44.7	**27.4 ± 8.6** 2.6–104.6	**28.8 ± 16.2** 6.0–92.2
	2005	**24.2 ± 4.2** 11.2–50.8	**20.4 ± 6.4** 6.4–46.7	**31.5 ± 6.8** 2.2–76.2	**22.2 ± 6.0** 8.4–43.8
	2005*	**25.4 ± 5.3** 7.0–50.2	**22.8 ± 7.4** 5.1–45.2	**37.1 ± 13.8** 3.2–174.5	**27.5 ± 8.8** 6.6–63.9
Average cell volume, μm^3	2004	**0.16 ± 0.05** 0.02–0.51	**0.14 ± 0.05** 0.02–0.38	**0.16 ± 0.05** 0.02–0.52	**0.20 ± 0.08** 0.05–0.56
	2005	**0.18 ± 0.02** 0.11–0.27	**0.20 ± 0.03** 0.14–0.33	**0.20 ± 0.03** 0.11–0.42	**0.23 ± 0.05** 0.17–0.45

Note: * – biomass, calculated by cell measurements; without an asterisk – biomass, calculated according to (Lee & Furman, 1987); n/d – no data.

Data, obtained in 1998–1999 with simultaneous surveys of hydrological and hydrochemical indicators [water temperature, salinity, density, pH, alkalinity, and transparency; dissolved oxygen content; and nutrients concentration (Ivanov et al., 2006 ; Ovsyanyi et al., 2000)] and biological indicators (bacterio- and phytoplankton abundance and biomass), allowed for multivariate statistical analysis. The correlation between two sets of variables was 86 % ($p < 0.001$), which indicates a significant relationship between biological indicators and environmental factors. Discriminant analysis (with the same datasets used) showed significant differences between three stations in the Sevastopol Bay (Fig. 2), located in the zones of "weak", "strong", and "very strong" pollution (see Fig. 1A), according to the zoning, proposed in (Ivanov et al., 2006 ; Ovsyanyi et al., 2000).

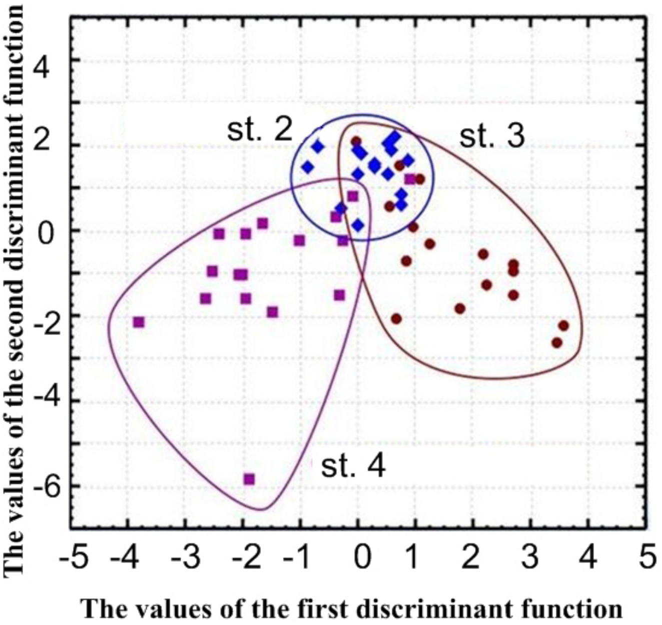


Fig. 2. Values of the first and second discriminant functions in the analysis of physical, hydrochemical, and biological variables (for more information, see the text) for stations 2, 3, and 4 in the Sevastopol Bay in 1998–1999. Sets of indicators for all the stations were significantly different ($p < 0.001$)

Seasonal variations in the bacterioplankton abundance in the Sevastopol Bay. Variations, observed in planktonic communities throughout the annual cycle, usually do not correspond to calendar seasons; therefore, the term "biological seasons" is often used (Usachev, 1947). To analyze the annual dynamics of the bacterial abundance in the Sevastopol Bay, we identified three periods: winter-autumn – November to February at water temperature (T) of (9.0 ± 0.95) °C; spring – March to May at T of (12.7 ± 1.43) °C; and summer-autumn – June to October at T of (24.4 ± 0.33) °C.

Analysis of long-term data has shown as follows: in all research periods, the highest, but variable values of the bacterioplankton abundance in the bay were recorded June to October (in 78 % of cases). The minimum quantitative indicators of microorganisms were confined to the autumn-winter (67 % of samples) and spring (30 % of samples) periods. Seasonal dynamics of variations in the bacterial abundance for 1992–2005, on average for the bay, corresponded to the temperature curve: November to January, the bacterial abundance was $0.9 \cdot 10^6$–$1.4 \cdot 10^6$ cells·mL^{-1}; then, when the water warmed up, there was a gradual increase up to $1.4 \cdot 10^6$–$1.5 \cdot 10^6$ cells·mL^{-1}; at maximum temperatures, June to October, a sharp increase occurred – up to $2.1 \cdot 10^6$–$2.7 \cdot 10^6$ cells·mL^{-1} (Fig. 3A). In summer months in some years (1998, 2002), water temperature in the bay reached maximum values (higher than +28 °C), and the density of microorganisms increased up to $8.1 \cdot 10^6$–$10 \cdot 10^6$ cells·mL^{-1}. The maximum variability in abundance was observed at st. 3 (Table 1).

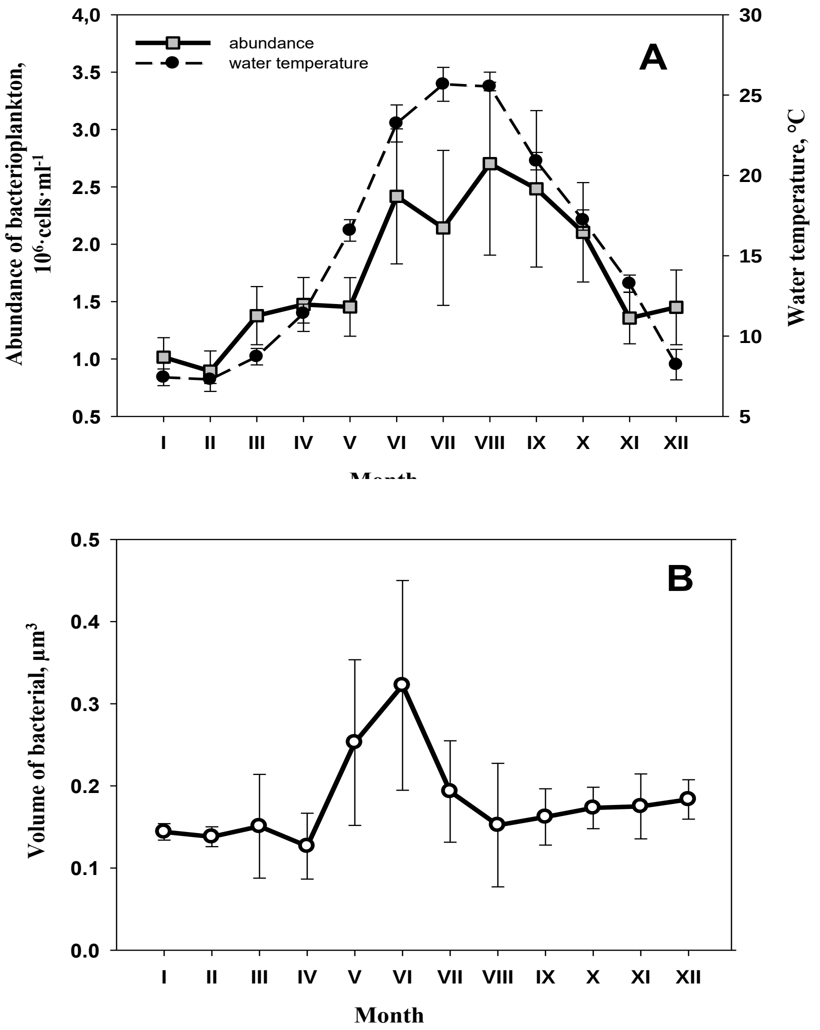


Fig. 3. Seasonal changes in bacterioplankton structural indicators, averaged for the entire water area of the Sevastopol Bay: A – bacterioplankton abundance ($\cdot 10^6$ cells·mL^{-1}) and water temperature (°C) (1992–2005); B – average bacterial cell volume (μm^3) (2004–2005). Data are presented with 95 % confidence interval

Bacterioplankton morphological structure. According to the data of fluorescence microscopy, in bacterioplankton morphological structure, cocci predominated (69–96 %) with a diameter of 0.36–0.86 μm and a volume of 0.02–0.27 μm^3, as well as rod-shaped cells with a length of 0.61–1.24 μm, a width of 0.25–0.45 μm, and a volume of 0.50–0.65 μm^3. In spring, large cocci with a diameter of more than 1 μm and a volume of 0.52–0.55 μm^3 were found at all the stations. In summer, in the entire water area of the bay, large rods with a length of more than 2.0 μm, a width of more than 1.0 μm, and a volume of up to 1.65 μm^3 were recorded as well. Seasonal changes in the cell abundance and biomass, taking into account the contribution of various morphological groups, are shown in Fig. 4A, B. In spring and summer, abundance of rods was of $0.07 \cdot 10^6$ to $1.1 \cdot 10^6$ cells·mL^{-1} (5–25 % of the total bacterial abundance), and biomass reached 5.2–90.1 mg C·m^{-3} (their contribution to the total biomass ranged 7 to 82 %).

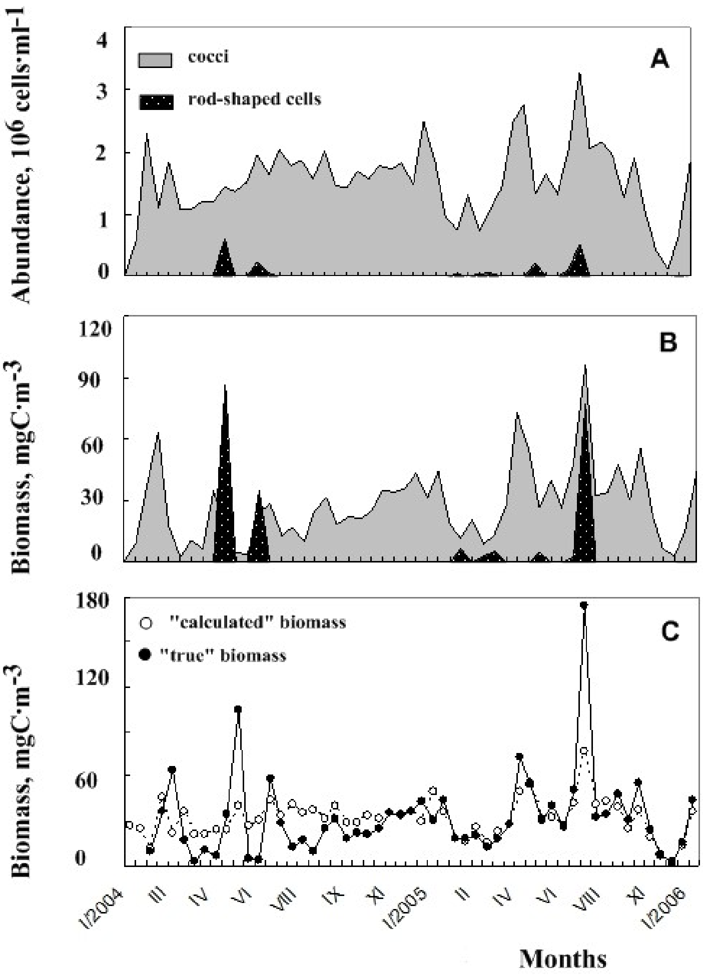


Fig. 4. Bacterioplankton quantitative indicators in the Sevastopol Bay (station 3, 2004–2005):
A – bacterioplankton abundance, considering two groups of cells (cocci and rod-shaped cells);
B – bacterioplankton biomass, considering two groups of cells;
C – ratio of the values of "true" bacterioplankton biomass [calculated by cell measurements] and "calculated" one [using the conversion factor ($2 \cdot 10^{-14}$ g C·cell^{-1}) (Lee & Furman, 1987)]

To detail the morphotypes of microorganisms, bacterioplankton samples, collected in 2019, were examined by electron microscopy. The data obtained made it possible to enlarge the list of bacterioplankton groups, previously recorded in the water area. Along with cocci and rod-shaped cells (Fig. 5B, D, E), the sizes of which fit into the ranges of values, obtained by fluorescence microscopy, thinner rods were registered, with a length of 0.92–1.21 μm and a width of 0.15–0.18 μm (Fig. 5C), as well as convoluted forms (attributed by us to spirilla), with a length of 1.55–2.13 μm and a width of 0.22–0.37 μm (Fig. 5A). In the research of 2004–2005, the last two morphotypes (thin rods and convoluted forms) were probably attributed by us to rod-shaped cells. Undoubtedly, analysis of natural bacterioplankton by electron microscopy will make it possible to study the morphology of cells in more detail.

Variations in the average bacterial cell volume. For all the stations in the Sevastopol Bay water area in 2004, the average bacterial cell volume ($V_{avg.}$) was characterized by sharp variations in values (at different stations, $V_{avg.max}/V_{avg.min}$ had changed by 11–26 times). In 2005, the variability was lower by an order of magnitude ($V_{avg.max}/V_{avg.min}$ had changed by 2–4 times) (Table 1). For the entire water area, microbial cell volume was significantly lower (paired t-test, $p < 0.05$) in 2004 compared to 2005: (0.16 ± 0.05) and (0.20 ± 0.03) μm^3, respectively.

Variations in the average bacterial cell volume for the water area throughout the research period had a clearly pronounced seasonal trend. In May and June, due to the appearance of large cells in the bacterioplankton community, an increase in $V_{avg.}$ was registered: up to (0.25 ± 0.10) and (0.32 ± 0.13) μm^3, respectively. For the rest of the year, the value was almost twice as low (Fig. 3B).

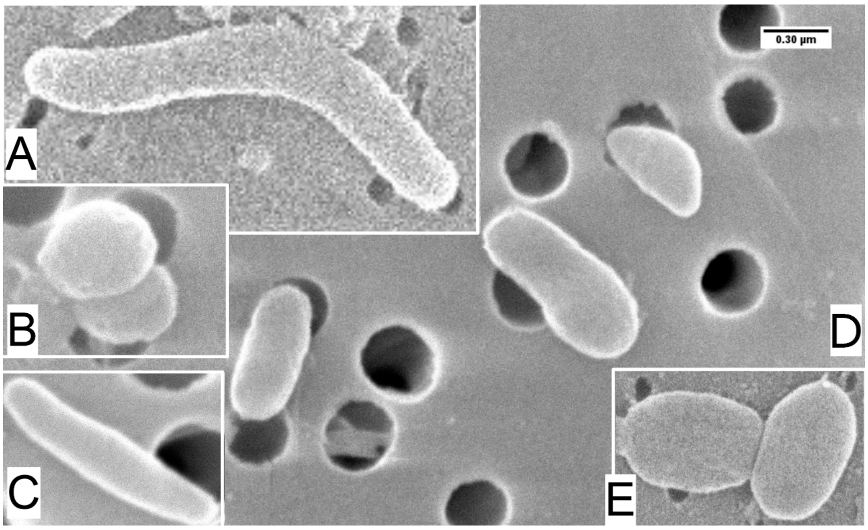


Fig. 5. Morphotypes of bacterioplankton cells of the Sevastopol Bay by electron microscopy:
A – convoluted forms (spirilla);
B – rounded forms (cocci);
C – rod-shaped forms (thin rods);
D and E – rod-shaped forms (thick rods)

Approaches to calculating bacterioplankton biomass and dynamics of this indicator in the Sevastopol Bay water area. Bacterial biomass is calculated using various conversion factors (Bratbak, 1985 ; Lee & Furman, 1987), making it possible to go directly from the bacterial abundance to the biomass in carbon units. As noted above, we assumed in this study that the carbon content in one bacterial cell is 20 fg ($2 \cdot 10^{-14}$ g C·cell^{-1}) (Lee & Furman, 1987).

In 2004–2005 along with the calculation of biomass using the conversion factor (Lee & Furman, 1987), we performed direct cell measurements, calculated the “true” biomass, and compared the values obtained (see Table 1). The graphs of seasonal changes in the “calculated” biomass turned out to be smoother. In spring and summer, with a significant number of larger bacterial cells in the samples, the values of the “calculated” biomass were 2–2.5 times lower compared to those of the biomass, determined by cell measurements. Conversely, in April – May and August – October 2004, with smaller cells predominating in the samples, the values of the “calculated” biomass were lower than those of the “true” one (Fig. 4A, B, C). However, statistical analysis of the entire dataset did not reveal significant differences (paired t-test, $p > 0.05$) between bacterioplankton biomass values, obtained by these methods: (27.3 ± 4.3) and (30.5 ± 7.5) mg C·m^{-3} for the “calculated” and “true” biomass, respectively.

When carrying out a detailed study of the correlations within the microbial community, it is more relevant to measure bacterial cells. In the absence of such measurements, it is permissible to use conversion factors, accepted in microbiology (Bratbak, 1985 ; Lee & Furman, 1987).

DISCUSSION

In the coastal sea areas, experiencing maximum anthropogenic load (bays, lagoons, and estuaries), indicators of the total bacterioplankton abundance are characterized by high values with their pronounced variability, regardless of geographic location and climatic conditions (Kopylov & Kosolapov, 2011 ; Prakticheskaya ekologiya pribrezhnykh regionov…, 1990 ; Heidelberg et al., 2002). As a rule, at the end of unicellular algae bloom and their subsequent die-off, the bacterioplankton abundance increases significantly (Myrzov et al., 1999). Its maximum values are recorded in summer and autumn months, when water temperature is high. With increasing distance from the coast, bacterial content decreases (Kopylov & Kosolapov, 2011 ; Prakticheskaya ekologiya pribrezhnykh regionov…, 1990 ; Heidelberg et al., 2002). For example, in the Chesapeake Bay (North America) (the estuary of the Susquehanna River, flowing into the Atlantic Ocean), abundance of microorganisms varied within the range of $1 \cdot 10^6$–$20 \cdot 10^6$ cells·mL^{-1} (Heidelberg et al., 2002). For the Gulf of Burgas (the western Black Sea), the density of the bacterial community was of $1 \cdot 10^6$–$12 \cdot 10^6$ cells·mL^{-1} (Prakticheskaya ekologiya pribrezhnykh regionov…, 1990). Close N values were recorded in the Gelendzhik and Anapa bays (the northeastern Black Sea): microorganisms' content in summer period reached $12.7 \cdot 10^6$ and $14.2 \cdot 10^6$ cells·mL^{-1}, respectively. These values correspond to the level of hypereutrophic water (Selifonova, 2015 ; Sorokin, 1973). For the estuaries of the Cananéia River (Brazil coast, the Atlantic Ocean) and the Elbe River (North Sea coast), the density of the planktonic microbial population was lower; the values corresponded to the levels of mesotrophic and eutrophic water: $0.2 \cdot 10^6$–$3.8 \cdot 10^6$ and $1.8 \cdot 10^6$–$4.8 \cdot 10^6$ cells·mL^{-1}, respectively (Barrera-Alba et al., 2009 ; Karrasch et al., 2003).

In general, the order of magnitude of the total bacterial abundance, that we obtained in the Sevastopol Bay ($1 \cdot 10^6$–$10 \cdot 10^6$ cells·mL^{-1}), is comparable with the data, known for similar polluted water areas of the World Ocean, including the Black Sea (Table 2).

Table 2. Bacterial abundance in sea surface layer from different coastal areas of the World Ocean (bays, gulfs, and estuaries)

Research area	Bacterial abundance, $\cdot 10^6$ cells·mL^{-1}	Reference
Chesapeake Bay (Atlantic Ocean)	1–20	(Heidelberg et al., 2002)
Gulf of Burgas (Black Sea)	1–12	(Prakticheskaya ekologiya pribrezhnykh regionov…, 1990)
Anapa Bay (Black Sea)	2.8–8.6* (max 14.2)	(Selifonova, 2015)
Gelendzhik Bay (Black Sea)	2.8–7.1* (max 12.7)	(Selifonova, 2015)
Sevastopol Bay (Black Sea)	0.1–10	Own data
Elbe River estuary (North Sea)	1.8–4.8	(Kopylov & Kosolapov, 2011 ; Karrasch et al., 2003)
Cananéia River estuary (Atlantic Ocean)	0.2–3.8	(Kopylov & Kosolapov, 2011 ; Barrera-Alba et al., 2009)

Note: * – average values for the research period are given.

The main natural inhabitants of aquatic bacteriocenoses in marine areas are normally coccoid forms, which carry out the final stages of decomposition of more persistent organic compounds (Bogdanova, 2015 ; Ponomareva, 1978). Thus, in relatively low-contaminated areas of the Kola Bay, in bacterial communities gram-positive bacteria predominated, more often coccoid forms; in more eutrophic areas, rod-shaped bacteria prevailed (Bogdanova, 2015 ; Ponomareva, 1978). In the northeastern Black Sea (Golubaya Bay), small coccoid forms predominated; regardless of the season, their ratio was maximum at coastal stations (up to 83 %) (Mosharova & Sazhin, 2007). Our data on the ratio of morphotypes (cocci prevalence, 69–96 %) and bacterial cell volume (0.02–0.27 μm^3 for cocci; 0.50–0.65 μm^3 for rods) in the Sevastopol Bay are consistent with the results of other authors. Predominance of coccoid forms in all the sites of the bay water area indirectly indicates its relatively stable state.

Along with typical autochthonous marine microflora (cocci and rod-shaped cells), convoluted forms are often recorded in coastal waters. These are, first of all, conditionally pathogenic microorganisms of the genus *Vibrio*. Similar data were obtained by other methods (not by direct cell counting on filters). Thus, in the Kola Bay intertidal zone, occurrence of vibrios was registered (by the method of successive dilutions and cultivation) only in water areas, close to the household wastewater collector (Bogdanova, 2015)]. By the fluorescence *in situ* hybridization, vibrios were found off the North Sea coast; in summer, they accounted for only 2.2 % of the total bacterial abundance; in winter, their abundance decreased significantly (Oberbeckmann et al., 2012). The same seasonality of the genus *Vibrio* representatives was noted when cultivating samples from coastal waters off the Caucasian coast (the eastern Black Sea) (Janelidze et al., 2011). Along with the standard indicators of the marine environment quality, the indication of opportunistic microflora abundance is required; however, such works are related to sanitary and microbiological studies, not to environmental ones. The absence of convoluted forms in our samples (2004–2005) was possibly due to the methodological limitations of direct microscopic counting of bacterioplankton (filtration of a small water volume and difficulty of identification at a microscope magnification 1000×), as well as to the location of the sampling sites at a considerable distance from the household wastewater collector. The identification of the bacterial convoluted forms, that we have registered in 2019 (preliminarily attributed to spirilla), requires additional researches by electron microscopic and molecular methods.

Analysis of long-term dynamics of the bacterioplankton abundance at st. 2 in the Sevastopol Bay (1966–2007) showed cyclical variability of this indicator (Ryl'kova, 2013). We have found a similar trend in other sites of the bay water area (see Table 1, Fig. 6). The periods of an increase in the bacterial abundance were followed by the periods of a relatively stable state of the community.

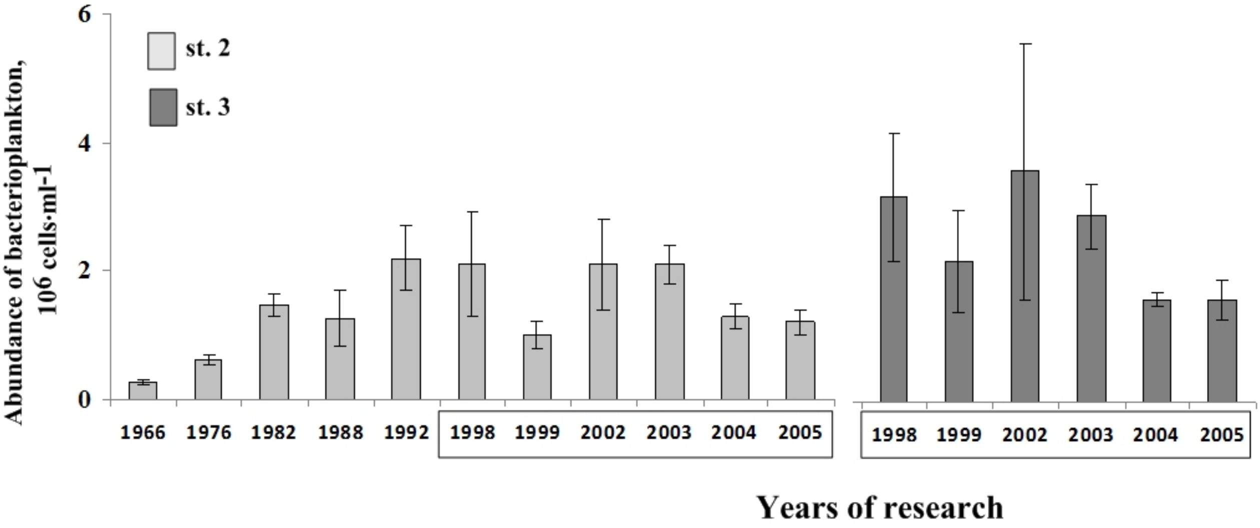


Fig. 6. Interannual dynamics of average annual bacterioplankton abundance in the open (st. 2) and tail-end areas (st. 3) of the Sevastopol Bay (data for 1966–1988 – according to (Gorbenko, 1977 ; Chepurnova et al., 1993 ; Shumakova, 1980); data for 1992 provided by V. A. Ponomarenko)

The established similarity of the interannual dynamics of the bacterioplankton abundance at various sites of the water area could be due to similar hydrological and meteorological conditions, recorded for the entire Sevastopol Bay (Table 3) (Ivanov et al., 2006).

Table 3. Main hydrological and meteorological peculiarities in the Sevastopol Bay in 1998–2004 [according to (Ivanov et al., 2006)]

Year	Hydrological and meteorological conditions
1998	A large volume of the river water runoff was observed, as well as an emergency wastewater discharge from the Chernorechensky Reservoir
1999	The period was characterized by intensive seawater advection, which positively affected chemical composition and quality of the water in the bay. However, against the background of high temperatures in summer months due to the weakening of hydrodynamic processes at the tail-end stations, hypoxia was observed
2002	In summer, high air and water temperatures were recorded; in autumn, due to heavy rainfall, unusually high water level was registered
2003	An anomalous decrease in sea level was noted; water runoff from the bay prevailed, which slowed down the water exchange between the bay and the sea. Frequent upwellings and low water temperatures were recorded
2004	The sea level rise was observed; clean seawater with increased salinity flowed into the bay, resulting from advection

Conditions, observed in the bay in 1998, 2002, and 2003 (heavy rainfall, emergency discharge, high summer temperatures, and slowing down of water exchange), stimulated the intensive development of heterotrophic microflora, especially in summer months. Throughout this research period, high values of $N_{\text{avg./year}}$ were revealed, and more significant variations in the abundance during the annual cycle were identified (1.9–4.4 times).

On the contrary, in 1999 and 2004, against the background of the sea level rise and intense advection, the water quality in the bay improved, which contributed to a decrease in $N_{\text{avg./year}}$ and lower variability in the abundance of microorganisms (1.3–1.9 times) (Table 1, Fig. 6).

It is interesting that in 1998, 2002, 2003, and in 1999, 2004, 2005, we obtained close minimum values of the bacterioplankton abundance ($0.4 \cdot 10^6$–$1.6 \cdot 10^6$ and $0.1 \cdot 10^6$–$0.7 \cdot 10^6$ cells·mL^{-1}, respectively), as well as a decrease in the maximum values from $2.1 \cdot 10^6$–$10.0 \cdot 10^6$ to $1.3 \cdot 10^6$–$3.8 \cdot 10^6$ cells·mL^{-1} (Table 1). As known, a small variation in the lower values of the abundance of microorganisms indicates a sufficient supply of assimilable organic matter, while the higher values are determined by the temporary supply of allochthonous or autochthonous organic matter, *e. g.* during phytoplankton blooms (Romanenko, 1985).

Cyclicity in the long-term dynamics of the bacterioplankton abundance, found at st. 2 in the Sevastopol Bay (Ryl'kova, 2013) and confirmed by us for other sites of the water area, was also observed off the coast of Bulgaria, in the northwestern and northeastern Black Sea (Mosharova & Sazhin, 2007 ; Prakticheskaya ekologiya pribrezhnykh regionov…, 1990). This may indicate a large ecological capacity of coastal waters and a capacity of ecosystems to stabilize and restore under reduced effect of negative environmental factors and anthropogenic load (Ryl'kova, 2013).

Hydrometeorological conditions are known to determine significant variability of the hydrochemical situation in the Sevastopol Bay, which affects the level of development of hydrobionts (Ivanov et al., 2006). Multivariate statistical analysis, carried out in 1998–1999 (see Fig. 2), confirmed a high correlation between biological and abiotic variables, as well as significant differences for three stations (st. 2, 3, 4): in the sites of the bay with different hydrological and hydrochemical regimes, non-uniform conditions were formed for the development of the biotic component.

In modern research, estimates of the eutrophication index E-TRIX are often used to determine the trophic status of the water area (taking into account the concentrations of dissolved oxygen, total phosphorus, and sum of mineral forms of nitrogen and chlorophyll *a*) (Vollenweider et al., 1998). At st. 2, 5 (near the open sea), the value is 5.10; at st. 3 (in the tail-end area), E-TRIX rises up to 5.7, which is characteristic of water areas with a high trophic level and satisfactory water quality (E-TRIX values 5 to 6) (Gubanov et al., 2015 ; Slepchuk et al., 2017). The intensity of the water area chronic pollution with petroleum products (for st. 2 and 3, 80 and 180 mg per 100 g, respectively) and heavy metals increases from open water deep into the bay (Osadchaya et al., 2004). Bacterioplankton quantitative indicators also increase similarly, which indicates an increase in the trophicity of water (see Table 1).

In certain seasons, special conditions for hydrobionts were formed in the bayhead areas. Thus, in the area of st. 3, the effect of the Chernaya River runoff, transporting an additional amount of allochthonous organic matter, was strong. For st. 4, limited water exchange and frequent blockage of polluted water are typical, as well as presence of industrial and household wastewater and storm runoff (Gubanov et al., 2015 ; Ivanov et al., 2006). Such conditions are favorable for the development of microorganisms and can result in a rapid increase in their abundance. It was assumed that at bayhead st. 3 and 4, seasonal ecotone zones form in some years. The life strategy of biotic complexes of ecotones should provide the system with a stable existence in an unstable environment, usually characterized by an increase in diversity and density of organisms, an increased frequency, and a wide range of variations in its indicators (in some cases, by an increase in biological productivity as well). Under certain conditions, after a state of temporary imbalance, new relatively stable structures can form (Ekotony v biosfere, 1997). Actually, at these sites of the water area, there was a strong variability in the values of the bacterial biomass and abundance (Table 1).

However, the dynamics of bacterioplankton development depends not only on abiotic environmental factors, but also on the biotic component of the plankton community. As known, after winter-spring phytoplankton bloom and its subsequent die-off, a large amount of dissolved organic matter is released. In a month, as a rule, an increase in the bacterioplankton abundance occurs, as well as in abundance of all other groups of heterotrophs of the microplankton community (Myrzov et al., 1999). It was established for the Sevastopol Bay that seasonal changes in the biomass of phyto- and bacterioplankton occurred in antiphase: after a period of active microalgae vegetation, there was an increase in bacterioplankton biomass (Lopukhina et al., 2006 ; Ryl'kova, 2010). The observed increase in the bacterial abundance in spring (Fig. 3A) was likely to result from supply of microorganisms with nutrients due to the previous development of the phytoplankton complex (Bul'on, 2002 ; Kopylov & Kosolapov, 2011 ; Church, 2008).

One of the main reasons for the decrease in the bacterioplankton content in marine and freshwater ecosystems is its grazing by ic flagellates and ciliates (Sherr et al., 1992). During the warm period of the year, high values of the phagotrophs abundance were noted in various coastal ecosystems; the ranges of their annual fluctuations can be 6–10 times or more, while seasonal values of the bacterial abundance are more static (Tsai et al., 2013). The effect of consumers on the development of the microbial community can be confirmed by a change in the size structure of bacteria (Golubkov, 2013). Experiments have shown that ciliates and colorless flagellates prefer large bacteria, which are usually actively growing cells or dividing ones. Thus, the bacterial community, with active grazing by phagotrophs, is often represented by small, slowly growing cells. In the absence of consumers, large bacteria become more abundant (Gonzalez et al., 1990 ; Simek & Chrzanowski, 1992).

We have previously found that in March – April 2004 and 2005, the abundance of bacteria consumers increased significantly in the Sevastopol Bay. In the spring of 2004, the abundance of colorless flagellates was the highest and amounted to $10{\cdot}10^3$–$43{\cdot}10^3$ cells·mL^{-1} (biomass was of 42.7–1525.5 mg·m^{-3}); the abundance of ciliates reached $0.9{\cdot}10^3$–$8.0{\cdot}10^3$ cells·mL^{-1} (biomass was of 16.0–99.0 mg·m^{-3}). During the annual cycle, a significant negative correlation was revealed between the abundance of phagotrophs and bacteria at all the stations studied. In the spring of 2005, the biomass of bacteria consumers decreased (for flagellates – on average 8 times; for ciliates – 3 times). However, in 2005, the correlation between the abundance of bacteria and organisms, feeding on them, was statistically insignificant (Lopukhina et al., 2006 ; Ryl'kova, 2010).

An increase in the average bacterial cell volume up to 0.27 and 0.32 μm^3 (due to the appearance of large cocci and rods) in May – June 2004 and 2005 (Fig. 3B) was probably related to a decrease in the phagotrophs abundance during this period of the year (Lopukhina et al., 2006 ; Ryl'kova, 2010) and could be considered as a response of bacteria to the grazing process, contributing to the restoration of the microbial community structure. Moreover, with an active development of communities of colorless flagellates and ciliates, in general in 2004, the average bacterioplankton cell volume was (0.16 ± 0.05) μm^3; in a year, with no "outbursts" of phagotrophs development registered, the bacteria were significantly larger: on average (0.20 ± 0.03) μm^3.

Thus, a significant negative correlation between the abundance of microorganisms and their consumers, as well as a significant decrease in the average bacterioplankton cell volume during mass development of ciliates and heterotrophic flagellates, indicates the presence of direct trophic relationships of the predator – prey type, which confirms the importance of the grazing process in regulation of the bacterioplankton abundance. Similar results are available in literature, concerning the role of the microbial "loop" in the functioning of plankton communities in marine and freshwater ecosystems (Bul'on, 2002 ; Golubkov, 2013 ; Kopylov & Kosolapov, 2011 ; Myrzov et al., 1999 ; Gonzalez et al., 1990 ; Sherr et al., 1992 ; Tsai et al., 2013).

Another reason for the decrease in the bacterial abundance during the annual cycle can be viral infection of the microbial community (Golubkov, 2013 ; Kopylov & Kosolapov, 2011 ; Proctor & Fuhrman, 1990). Seasonal changes in the virus concentration are closely related to changes in the abundance of their hosts (Lymer et al., 2008 ; Sanda & Larsen, 2006). In warm spring-summer period, the maximum abundance of virus particles in aquatic ecosystems is recorded; in winter, the virus concentration is typically low (Jacquet et al., 2010). In spring, an increase in virus content is preceded by an intensive development of the phytoplankton community (Maurin et al., 1997). In summer, an increase in the abundance of viruses is caused by the high bacterial abundance (Filippini et al., 2008). Viral infection, causing lysis of host cells, was likely one of the reasons of a decrease in the abundance of microorganisms, recorded by us in the middle of summer (Fig. 3A).

The data obtained allow us to point out the indicator role of bacterioplankton in monitoring studies of aquatic ecosystems and can be used in the ecological zoning of the water area against an abiotic background (Barrera-Alba et al., 2009 ; Janelidze et al., 2011 ; Lopukhin et al., 2008).

Conclusion. In the Sevastopol Bay during the entire research period (1992–2005), the bacterial abundance varied $0.2 \cdot 10^6$ to $10 \cdot 10^6$ cells·mL^{-1}, the biomass – 2 to 201 mg C·m^{-3}. In the morphological structure of bacterioplankton in the bay, cocci predominated (69–96 %), with a diameter of 0.36–0.86 μm and a volume of 0.02–0.27 μm^3, as well as rod-shaped cells with a length of 0.61–1.24 μm, a width of 0.2–0.4 μm, and a volume of 0.50–0.65 μm^3. The minimum values of the bacterioplankton abundance, biomass, and cell volume were recorded in the open areas of the bay (st. 2, 5) in winter; the maximum values were noted in the tail-end (st. 3, 4) in summer months.

The relevance of direct measurements of bacterioplankton cells is highlighted to determine the "true" biomass and to conduct a more detailed study of the microplankton community. In the absence of such measurements, it is permissible to use conversion factors for calculations.

A significant relationship between biological indicators and abiotic environmental factors is shown (correlation 86 %, $p < 0.001$). Discriminant analysis revealed significant differences in bacterioplankton quantitative indicators for three stations, located at the sites of the water area with different water exchange rates, level of total pollution, and the distance from the open sea.

A significant negative correlation between the abundance of microorganisms and their consumers, as well as a significant decrease in the average bacterioplankton cell volume during mass development of ciliates and heterotrophic flagellates, indicates the presence of direct trophic relationships of the predator – prey type, which confirms the importance of the grazing process in regulation of the bacterioplankton abundance.

This work has been carried out within the framework of IBSS government research assignment "Investigation of mechanisms of controlling production processes in biotechnological complexes with the aim of developing scientific foundations for production of biologically active substances and technical products of marine genesis" (No. AAAA-A18-118021350003-6).

Acknowledgement. The authors express their sincere gratitude to V. Ponomarenko, L. Manzhos, Yu. Bryantseva, N. Gavrilova, and V. Gubanov for providing data on various groups of planktonic organisms; V. Parchevsky – for assistance in statistical data processing; A. Borovkov and R. Gevorgiz – for constructive comments, that allowed to improve the initial version of the text and figures; V. Lishaev – for help in working with an electron microscope.

REFERENCES

1. Bogdanova O. Yu. Taksonomicheskii sostav mikrobnogo naseleniya litorali Kol'skogo zaliva. *Evraziiskii Soyuz Uchenykh*, 2015, no. 4–10 (13), pp. 8–11. (in Russ.)
2. Brock T. *Membrane Filtration* : transl. from Engl. Moscow : Mir, 1987, 464 p. (in Russ.)
3. Bul'on V. V. Struktura i funktsiya mikrobial'noi "petli" v planktone ozernykh system. *Biologiya vnutrennikh vod*, 2002, no. 2, pp. 5–14. (in Russ.)
4. Golubkov S. M. The role of consumers in food web dynamics and functioning of aquatic ecosystems. *Zhurnal Sibirskogo federal'nogo universiteta. Biologiya*, 2013, no. 6 (4), pp. 335–353. (in Russ.)
5. Gorbenko Yu. A. *Ekologiya morskikh perifitonnykh organizmov.* Kyiv : Naukova dumka, 1977, 252 p. (in Russ.)
6. Gubanov V. I., Gubanova A. D., Rodionova N. Yu. Diagnoz trofnosti vod Sevastopol'skoi bukhty i vzmor'ya g. Sevastopolya. In: *Aktual'nye problemy akvakul'tury*

v sovremennyi period : materialy mezhdunar. nauch. konf., Rostov-on-Don, 28.09.2015 – 02.10.2015. Rostov-on-Don : AzNIIRKH, 2015, pp. 64–67. (in Russ.)

7. Ivanov V. A., Ovsyanyi E. I., Repetin L. N., Romanov A. S., Ignat'eva O. G. *Gidrologo-gidrokhimicheskii rezhim Sevastopol'skoi bukhty i ego izmeneniya pod vozdeistviem klimaticheskikh i antropogennykh faktorov.* Sevastopol, 2006, 91 p. (Preprint / MHI of the NAS of Ukraine). (in Russ.)
8. Kopylov A. I., Kosolapov D. B. *Mikrobnaya "petlya" v planktonnykh soobshchestvakh morskikh i presnovodnykh ekosistem.* Izhevsk : Knigograd, 2011, 330 p. (in Russ.)
9. Lebedeva M. N., Shumakova G. V. K voprosu o dostovernosti dannykh, poluchennykh metodom pryamogo ucheta bakterii na fil'trakh. *Mikrobiologiya*, 1969, vol. 38, iss. 2, pp. 351–357. (in Russ.)
10. Lopukhina O. A., Ryl'kova O. A., Gavrilova N. A., Gubanov V. V. Vklad razlichnykh grupp mikrogeterotrofov v summarnuyu biomassu mikroplanktona Sevastopol'skoi bukhty. In: *Sostoyanie i problemy produktsionnoi gidrobiologii* : sb. nauch. rabot po materialam dokl. na mezhdunar. konf. "Vodnaya ekologiya na zare XXI veka", posvyashch. 100-letiyu so dnya rozhd. G. G. Vinberga. Moscow, 2006, pp. 189–198. (in Russ.)
11. Morochkovskii V. A., Koval'chuk Yu. L. Gidrologo-gidrokhimicheskii rezhim Sevastopol'skoi bukhty. In: *Ikhtiofauna chernomorskikh bukht v usloviyakh antropogennogo vozdeistviya.* Kyiv : Naukova dumka, 1993, pp. 17–24. (in Russ.)
12. Mosharova I. V., Sazhin A. F. Bacterioplankton in the northeastern part of the Black Sea during the summer and autumn of 2005. *Okeanologiya*, 2007, vol. 47, no. 5, pp. 720–728. (in Russ.). https://doi.org/10.1134/S0001437007050098
13. Murzov S. A., Gavrilova N. A., Samyshev E. Z. Geterotrofnyi nanoplankton i infuzorii v Sevastopol'skoi bukhte: sostav, obilie, raspredelenie i ikh izmenchivost'. In: *Akvatoriya i berega Sevastopolya: ekosistemnye protsessy i uslugi obshchestvu.* Sevastopol : Akvavita, 1999, pp. 121–130. (in Russ.)
14. Ovsyanyi E. I., Kemp R. B., Repetin L. N., Romanov A. S. Gidrologo-gidrokhimicheskii rezhim Sevastopol'skoi bukhty v usloviyakh antropogennogo vozdeistviya (po nablyudeniyam 1998–1999 gg.). In: *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon i kompleksnoe ispol'zovanie resursov shel'fa.* Sevastopol : EKOSI-Gidrofizika, 2000, pp. 79–103. (in Russ.)
15. Orekhova N. A., Varenik A. V. Current hydrochemical regime of the Sevastopol Bay. *Morskoi gidrofizicheskii zhurnal*, 2018, vol. 34, no. 2, pp. 134–146. (in Russ.). https://doi.org/10.22449/0233-7584-2018-2-134-146
16. Osadchaya T. S., Alemov S. V., Shadrina T. V. Ecological quality of Sevastopol Bay bottom sediments: Retrospective and present-day state. *Ekologiya morya*, 2004, iss. 66, pp. 82–87. (in Russ.)
17. Ponomareva L. V. *Sanitarno-mikrobiologicheskaya kharakteristika Kol'skogo zaliva* : avtoref. dis. … kand. biol. nauk. Saratov, 1978, 20 p. (in Russ.)
18. Potapova N. A., Korolevskaya T. V. On procedure to determine biomass of bacterial cells. *Gidrobiologicheskii zhurnal*, 1991, vol. 27, no. 1, pp. 83–88. (in Russ.)
19. *Prakticheskaya ekologiya pribrezhnykh regionov. Chernoe more* / V. P. Keondzhyan, A. M. Kudin, Yu. V. Terekhin (Eds). Kyiv : Naukova dumka, 1990, 250 p. (in Russ.)
20. Rodina A. G. *Metody vodnoi mikrobiologii (prakticheskoe rukovodstvo).* Moscow : Nauka, 1965, 364 p. (in Russ.)

21. Romanenko V. I., Dobrynin E. G. Opredelenie udel’nogo vesa sukhikh bakterial’nykh kletok *Ps. denitrificans*. *Biologiya vnutrennikh vod: informatsionnyi byulleten’*, 1973, no. 16, pp. 2–10. (in Russ.)
22. Romanenko V. I. *Mikrobiologicheskie protsessy produktsii i destruktsii organicheskogo veshchestva vo vnutrennikh vodoemakh.* Leningrad : Nauka, 1985, 294 p. (in Russ.)
23. Ryl’kova O. A., Polikarpov I. G., Saburova M. A. Comparison between fluorescence and absorption stain methods for quantitative direct count of the marine heterotrophic bacteria. *Ekologiya morya*, 2003, iss. 64, pp. 109–115. (in Russ.)
24. Ryl’kova O. A. *Strukturnye i funktsional’nye pokazateli bakterioplanktona v pribrezhnykh vodakh Kryma* : avtoref. dis. … kand. biol. nauk : 03.00.17. Sevastopol, 2010, 23 p. (in Russ.)
25. Ryl’kova O. A. Long-term changes of the bacterioplankton abundance in the coastal zone of Sevastopol (Black Sea). *Morskoj ekologicheskij zhurnal*, 2013, vol. 12, no. 1, pp. 74–79. (in Russ.)
26. Selifonova Zh. P. *Strukturno-funktsional’naya organizatsiya ekosistem zalivov i bukht Chernogo i Azovskogo morei (Rossiiskii sektor)* : avtoref. dis. … d-ra biol. nauk : 03.00.10. Murmansk, 2015, 52 p. (in Russ.)
27. Slepchuk K. A., Khmara T. V., Man’kovskaya E. V. Comparative assessment of the trophic level of the Sevastopol and South bays using E-TRIX index. *Morskoi gidrofizicheskii zhurnal*, 2017, no. 5 (197), pp. 67–77. (in Russ.). https://doi.org/10.22449/0233-7584-2017-5-67-78
28. Sorokin Yu. I. Bakterial’naya produktsiya v vodoemakh. In: *Obshchaya ekologiya. Biotsenologiya. Gidrobiologiya*, 1973, vol. 1, pp. 47–101. (in Russ.)
29. Troitskii A. S., Sorokin Yu. I. K metodike rascheta biomassy bakterii v vodoemakh. *Trudy Instituta biologii vnutrennikh vod*, 1967, iss. 15, pp. 85–90. (in Russ.)
30. Usachev P. I. Obshchaya kharakteristika fitoplanktona morei SSSR. *Uspekhi sovremennoi biologii*, 1947, vol. 23, iss. 2, pp. 265–288. (in Russ.)
31. Khorolich N. G. Raschet vodoobmena melkovodnogo zaliva (bukhty) s morem. *Trudy Gosudarstvennogo okeanograficheskogo instituta*, 1986, iss. 168, pp. 113–118. (in Russ.)
32. Chepurnova E. A., Shumakova G. V., Gutveib L. G. Bacterioplankton. In: *Plankton Chernogo morya* / A. I. Kovalev, Z. Z. Finenko (Eds). Kyiv : Naukova dumka, 1993, pp. 110–142. (in Russ.)
33. Shumakova G. V. Seasonal dynamics of the bacterioplankton number, biomass and production in the Sevastopol Bay. *Ekologiya morya*, 1980, iss. 1, pp. 28–33. (in Russ.)
34. *Ekotony v biosfere* / V. S. Zaletaev (Ed.). Moscow : Izd-vo RASKhN, 1997, 329 p. (in Russ.)
35. Barrera Alba J. J., Gianesella S. M. F., Moser G. A. O., Saldanha-Correa F. M. P. Influence of allochthonous organic matter on bacterioplankton biomass and activity in a eutrophic, sub-tropical estuary. *Estuarine, Coastal and Shelf Science*, 2009, vol. 82, iss. 1, pp. 84–94. https://doi.org/10.1016/j.ecss.2008.12.020
36. Bratbak G. Bacterial biovolume and biomass estimation. *Applied and Environmental Microbiology*, 1985, vol. 49, no. 6, pp. 1488–1493. https://doi.org/10.1128/AEM.49.6.1488-1493.1985
37. Bratbak G. Microscope methods for measuring bacterial biovolume: Epifluorescence microscopy, scanning electron microscopy, and transmission electron microscopy. In: *Handbook of Methods in Aquatic Microbial Ecology* / P. F. Kemp, B. F. Sherr,

E. B. Sherr, J. J. Cole (Eds). Boca Raton : CRC Press, 1993, chap. 36, pp. 309–316. https://doi.org/10.1201/9780203752746

38. Church M. J. Resource control of bacterial dynamics in the sea. In: *Microbial Ecology of the Oceans* / D. L. Kirchman (Ed.) ; 2nd ed. Hoboken, New Jersey : John Wiley & Sons, 2008, chap. 10, pp. 335–382. https://doi.org/10.1002/9780470281840.ch10
39. Filippini M., Buesing N., Gessner M. Temporal dynamics of freshwater bacterio- and virioplankton along a littoral–pelagic gradient. *Freshwater Biology*, 2008, vol. 53, iss. 6, pp. 1114–1125. https://doi.org/10.1111/j.1365-2427.2007.01886.x
40. Gonzalez J. M., Sherr E. B., Sherr B. F. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *Applied and Environmental Microbiology*, 1990, vol. 56, no. 3, pp. 583–589. https://doi.org/10.1128/AEM.56.3.583-589.1990
41. Heidelberg J. F., Heidelberg K. B., Colwell R. R. Seasonality of Chesapeake Bay bacterioplankton species. *Applied and Environmental Microbiology*, 2002, vol. 68, no. 11, pp. 5488–5497. https://doi.org/10.1128/AEM.68.11.5488-5497.2002
42. Hobbie J. E., Daley R. J., Jasper S. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Applied and Environmental Microbiology*, 1977, vol. 33, no. 5, pp. 1296–1307. https://doi.org/10.1128/AEM.33.5.1225-1228.1977
43. Jacquet S., Miki T., Noble R., Peduzzi P., Wilhelm S. Viruses in aquatic ecosystems: Important advancements of the last 20 years and prospects for the future in the field of microbial oceanography and limnology. *Advances in Oceanography and Limnology*, 2010, vol. 1, iss. 1, pp. 97–141. https://doi.org/10.1080/19475721003743843
44. Janelidze N., Jaiani E., Lashkhi N., Tskhvediani A., Kokashvili T., Gvarishvili T., Jgenti D., Mikashavidze E., Diasamidze R., Narodny S., Obiso R., Tediashvili A. Microbial water quality of the Georgian coastal zone of the Black Sea. *Marine Pollution Bulletin*, 2011, vol. 62, iss. 3, pp. 573–580. https://doi.org/10.1016/j.marpolbul.2010.11.027
45. Karrasch B., Ullrich S., Mehrens M., Zimmermann-Timm H. Free and particle-associated extracellular enzyme activity and bacterial production in the Lower Elbe Estuary, Germany. *Acta hydrochimica et hydrobiologica*, 2003, vol. 31, iss. 4–5, pp. 297–306. https://doi.org/10.1002/aheh.200300505
46. Lee S. S., Furman J. A. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Applied and Environmental Microbiology*, 1987, vol. 53, no. 6, pp. 1298–1303. https://doi.org/10.1128/aem.53.6.1298-1303.1987
47. Lopukhin A. S., Wilson J. G., Sysoeva I. V., Bryantseva Ju. V., Ryl'kova O. A., Kemp R. B. Verification of the heterotrophic-photoautotrophic index in Sevastopol Bay, Black Sea. *Doklady Earth Sciences*, 2008, vol. 423, no. 9, pp. 1520–1524. https://doi.org/10.1134/S1028334X08090456
48. Lymer D., Lindström E. S., Vrede K. A. Variable importance of viral-induced bacterial mortality along gradients of trophic status and humic content in lakes. *Freshwater Biology*, 2008, vol. 53, iss. 6, pp. 1101–1113. https://doi.org/10.1111/j.1365-2427.2008.02015.x
49. Maurin N., Amblard C., Bourdier G. Phytoplanktonic excretion and bacterial reassimilation in an oligotrophic lake: Molecular weight fractionation. *Journal Plankton Research*, 1997, vol. 19, iss. 8, pp. 1045–1068. https://doi.org/10.1093/plankt/19.8.1045
50. Oberbeckmann S., Fuchsv B. M., Meiners M., Wichels A., Karen H. Wiltshire K. H., Gerdts G. Seasonal dynamics and modeling of a *Vibrio* community in coastal

waters of the North Sea. *Microbial Ecology*, 2012, vol. 63, no. 3, pp. 543–551. https://doi.org/10.1007/s00248-011-9990-9

51. Proctor L. M., Fuhrman J. A. Viral mortality of marine bacteria and cyanobacteria. *Nature*, 1990, vol. 62, no. 343, pp. 60–62. https://doi.org/10.1038/343060a0
52. Sanda R. A., Larsen A. Seasonal variations in virus-host populations in Norwegian coastal waters: Focusing on the cyanophage community infecting marine *Synechococcus* spp. *Applied and Environmental Microbiology*, 2006, vol. 72, pp. 4610–4618. https://doi.org/10.1128/AEM.00168-06
53. Sherr B. F., Sherr E. B., McDaniel J. Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. *Applied and Environmental Microbiology*, 1992, vol. 58, no. 8, pp. 2381–2385. https://doi.org/10.1128/AEM.58.8.2381-2385.1992
54. Simek K., Chrzanowski T. H. Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. *Applied and Environmental Microbiology*, 1992, vol. 58, no. 11, pp. 3715–3720. https://doi.org/10.1128/AEM.58.11.3715-3720.1992
55. Tsai A. Y., Gong G. C., Hung J. Seasonal variations of virus- and nanoflagellate-mediated mortality of heterotrophic bacteria in the coastal ecosystem of subtropical western Pacific. *Biogeosciences*, 2013, vol. 10, iss. 5, pp. 3055–3065. https://doi.org/10.5194/bg-10-3055-2013
56. Vollenweider R., Giovanardi F., Montanari G., Rinaldi A. Characterization of the trophic conditions of marine coastal waters, with special reference to the NW Adriatic Sea: Proposal for a trophic scale, turbidity and generalized water quality index. *Environmetrics*, 1998, vol. 9, iss. 3, pp. 329–357. https://doi.org/10.1002/(SICI)1099-095X(199805/06)9:3%3C329::AID-ENV308%3E3.0.CO;2-9

СЕЗОННАЯ ДИНАМИКА И ПРОСТРАНСТВЕННОЕ РАСПРЕДЕЛЕНИЕ СТРУКТУРНЫХ ПОКАЗАТЕЛЕЙ БАКТЕРИОПЛАНКТОННОГО СООБЩЕСТВА БУХТЫ СЕВАСТОПОЛЬСКАЯ (КРЫМ, ЧЁРНОЕ МОРЕ)

О. А. Рылькова[1], И. Г. Поликарпов[2]

[1]Федеральный исследовательский центр «Институт биологии южных морей
имени А. О. Ковалевского РАН», Севастополь, Российская Федерация
[2]Кувейтский институт научных исследований, Эль-Кувейт, Государство Кувейт
E-mail: *ol.rylkova@yandex.ru*

Бактериопланктон определяет формирование значительной части вторичной продукции и минерализации новообразованного органического вещества в водных экосистемах и быстро реагирует на любые изменения в окружающей среде. Данные о состоянии микробиального сообщества исключительно важны для понимания процессов переноса вещества и потока энергии в водных экосистемах, что особенно актуально для прибрежных акваторий, где в последние десятилетия произошли существенные негативные трансформации. Целью нашей работы было изучить долговременные изменения структурных показателей бактериопланктона в различных участках бухты Севастопольская (Чёрное море) в период 1992–2005 гг. Численность бактерий определяли прямым микроскопическим методом, используя адсорбционный (эритрозин) или флуоресцентный (акридиновый оранжевый) красители; биомассу рассчитывали с применением коэффициента ($2\cdot10^{-14}$ г С·кл.$^{-1}$) или по непосредственным промерам клеток. Для определения морфотипов клеток использовали сканирующую электронную микроскопию. Показано, что диапазон общей численности микроорганизмов составил $0{,}2\cdot10^{6}$–$10\cdot10^{6}$ кл.·мл$^{-1}$;

биомассы — 2–201 мг С·м$^{-3}$. В морфологической структуре бактериопланктона преобладали кокки (диаметр — 0,36–0,86 мкм) объёмом 0,02–0,27 мкм^3 и палочковидные клетки (длина — 0,6–1,2 мкм, ширина — 0,2–0,4 мкм) объёмом 0,50–0,65 мкм^3. Максимальные значения всех переменных зарегистрированы в летний и осенний периоды года (с июня по октябрь), минимальные приурочены к зимнему и весеннему сезонам. Полученные величи́ны количественных показателей бактериопланктона сопоставимы со значениями для различных акваторий Мирового океана, включая Чёрное море. Динамику структурных показателей бактериопланктона бухты Севастопольская в течение годового цикла определяли абиотические и биотические факторы. Значимо высокая корреляция (86 %, $p < 0{,}01$) между гидролого-гидрохимическими и биологическими переменными подтверждает неслучайный характер взаимосвязи между ними. Дискриминантный анализ выявил достоверные различия в структуре бактериопланктонных сообществ между участками бухты с разной интенсивностью водообмена, степенью общей загрязнённости и удалённостью от открытого моря. Достоверно меньший объём клеток бактерий в 2004 г. [(0,16 ± 0,05) мкм^3] по сравнению с таковым в 2005 г. [(0,20 ± 0,03) мкм^3] (парный t-тест, $p < 0{,}05$) был связан, вероятно, с интенсивным выеданием микроорганизмов фаготрофными простейшими. Полученные данные о структуре сообщества бактериопланктона могут быть использованы при прогнозировании состояния экосистемы бухты Севастопольская, а также при разработке и верификации математических моделей функционирования прибрежных экосистем.

Ключевые слова: бактериопланктон, численность, биомасса, морфология, абиотические и биотические факторы, бухта Севастопольская, Чёрное море

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 1, pp. 102–114
https://doi.org/10.21072/mbj.2021.06.1.08

UDC 581.526.325(262.5.04/.05:292.471)

DEVELOPMENT OF PHYTOPLANKTON IN THE WINTER-SPRING PERIOD IN THE COASTAL WATERS OF CRIMEA

© 2021 **Z. Z. Finenko, I. M. Mansurova, I. V. Kovalyova, and E. Yu. Georgieva**

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

Received by the Editor 10.10.2019; after reviewing 20.03.2020;
accepted for publication 11.03.2021;

The analysis of phytoplankton in the winter-spring period is important for investigating peculiarities of its annual dynamics and the Black Sea ecosystem overall functioning. Phytoplankton state in the winter-spring period in the Black Sea shelf zone is less studied than that of the summer-autumn season; conducting such a research is especially important for solving several problems, related to the productivity of the last links of the food chain, the formation of water hydrochemical regime, and the carbon cycle in the sea. The aim of the work is to assess the effect of seasonal conditions on the development of phytoplankton and its production estimates in the winter-spring period in the coastal waters of Crimea. The article presents the results of studies of hydrophysical (water temperature, density, and relative transparency) and biological indicators (chlorophyll *a* concentration, its fluorescence, taxonomic composition, and phytoplankton production estimates) in the Black Sea shelf zone in January – April 2016–2019. The studies were carried out at 50 stations, located in the coastal waters of Crimea from the Karkinitsky Bay to the Kerch Strait. Chlorophyll *a* concentration was measured by the standard fluorometric method, species composition was determined by microscopy, and phytoplankton specific growth rate was calculated according to the previously developed model. In winter (January – February), the values of chlorophyll *a* content and upper mixed layer depth were the highest (0.42–0.52 $\text{mg}\cdot\text{m}^{-3}$ and 44–58 m, respectively); in spring (March – April) they were 2–3 times lower. In January – February, the coccolithophore species *Emiliania huxleyi* (Lohmann) W. W. Hay & H. P. Mohler, 1967 predominated; in March – April, in different years, either dinoflagellates and diatoms or coccolithophores, dinoflagellates, and diatoms prevailed. In winter, chlorophyll *a* vertical distribution at most stations was uniform; in spring, unimodal profiles with a depth maximum prevailed, the location of which was not related to temperature and density gradients. Relative changes in chlorophyll *a* concentration and fluorescence with depth were usually the same. Phytoplankton production and daily production/biomass ratio (P/B) increased from winter to spring. There was no correlation between the values of integral production, biomass, and maximum specific growth rate of algae. Maximum specific growth rate was the least variable indicator. During the winter-spring period, algae in the photosynthetic zone divided on average once every 2–5 days.

Keywords: taxonomic composition, phytoplankton abundance and biomass, chlorophyll *a*, fluorescence, algae maximum specific growth rate, temperature, water density, Black Sea

Winter-spring period is the season of great importance for the Black Sea ecosystem: convective mixing of the upper sea layer is observed, and active transport of nutrients from deeper sea layers to the photosynthetic zone occurs, where nutrients, being consumed by phytoplankton, change their state from dissolved to suspended (Krivenko & Parkhomenko, 2014). This process results in formation of new production, supporting the growth of heterotrophs (Krivenko et al., 1998). The intensity of this formation depends on climatic conditions. As it is considered, the transport of nutrients is more intensive in cold and severe winters, and the conditions for organic matter biosynthesis during photosynthesis are more favorable than in mild ones (Finenko et al., 2009 ; Mikaelyan et al., 2017). Primary production, formed in the winter-spring period, determines the regeneration processes throughout the year, *inter alia* in the warm season.

A wide range of research in phytoplankton community is required for solving several problems, related to the productivity of the final links of the food chain, the formation of water hydrochemical regime, and the carbon cycle in the sea. Number of seasonal phytoplankton investigations, carried out off the Crimean Peninsula, is rather high, but planktonic algae state in the winter-spring period has been poorly studied (Arashkevich et al., 2015 ; Stelmakh, 2010 ; Finenko at al., 2019 ; Mikaelyan et al., 2017). Since there is no long-term monitoring, it is difficult to understand the effect of climatic conditions on the level of development of planktonic algae. At the same time, the results of direct and satellite measurements of chlorophyll *a* concentration in the surface layer indicate the presence of interannual changes in the development of phytoplankton (Finenko at al., 2014 ; Yunev et al., 2002). Physical and geographical conditions affect the development of plankton community, with its peculiarities in deep and shallow sea areas, but there is clearly not enough data for carrying out the biogeochemical zoning.

The aim of the work is to assess the effect of seasonal conditions on the level of the development of phytoplankton and its production estimates in the winter-spring period in the coastal waters of Crimea.

MATERIAL AND METHODS

Phytoplankton studies were carried out during the 83rd, 84th, 93rd, and 106th cruises of the RV "Professor Vodyanitsky" in the coastal waters of Crimea from the Karkinitsky Bay to the Kerch Strait at stations with a total depth of 16–93 m, as well as on three transects, which began at Cape Tarkhankut, the southern coast of Crimea, and the Kerch Strait and ended in the open Black Sea (January – May 2016–2019) (Fig. 1). The measurements of chlorophyll *a* (hereinafter Chl) concentration, its fluorescence, water temperature, density, and relative transparency were performed at 52, 11, 38, 23, and 34 stations, respectively (Table 1). Vertical profiles of these indicators were established for 75–100 % of the total number of stations.

Samples were taken with a plastic bathometer or with a cassette of CTD bathometers of a Neil Brown Mark III probe. When choosing the depths, a vertical profile of fluorescence or temperature [the data, obtained from a Neil Brown Mark III or Ocean Seven 320 Plus (Idronaut) probe] and relative water transparency were taken into account. Continuous fluorescence monitoring in the shelf zone was carried out throughout the water column, while in deep-water areas – up to 100–150 m. The data on temperature and salinity, measured by high-precision submersible digital probes, were vertically interpolated with a step of 1 m and used for calculating potential density by the UNESCO formula. The lower boundary of the upper mixed layer (hereinafter UML) is considered as a depth, at which potential water density exceeds surface water density by 0.07 $kg \cdot m^{-3}$ (Kubryakov et al., 2019). The depth of the mixed layer for each station was estimated by the depth of the maximum water density gradient. Relative water transparency was determined in the daytime by a Secchi disk. When irradiance was not measured at various depths, the depth of the euphotic zone (Z, m) was calculated as follows:

$$Z = 3 \times S \,, \tag{1}$$

where S is the depth of Secchi disk visibility, m.

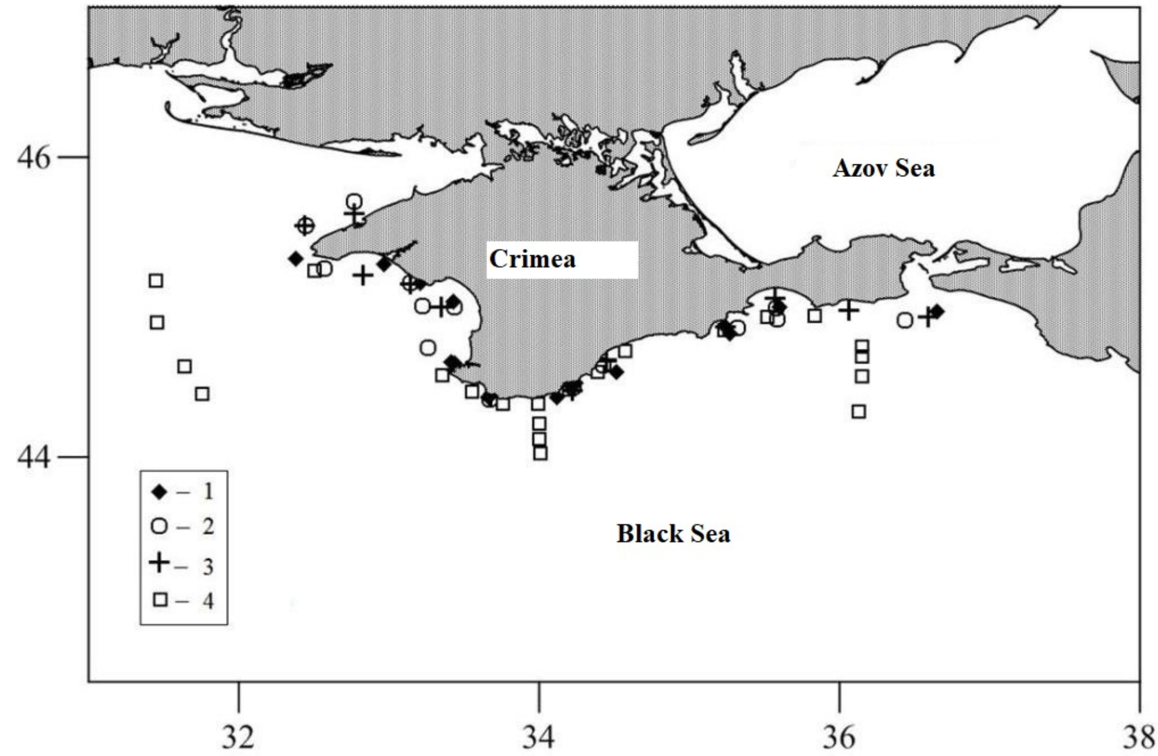


Fig. 1. Map of the stations, where the work was carried out: 1 – 27 January to 03 February, 2016; 2 – 19 to 25 April, 2016; 3 – 28 March to 04 April, 2017; 4 – 18 April to 13 May, 2019

Seawater samples of 0.25–1.50 L were filtered immediately after sampling at a vacuum (< 0.2 atm) through Sartorius membrane filters with a pore diameter of 0.65 μm and a working surface diameter of 47 mm or through glass microfiber filters GF/F (Whatman) with a 22-mm working surface. Comparison of the results, obtained with two types of filters used, showed their similarity. The filters were dried on filter paper in the dark for 15 minutes, folded in four with a sediment inward, and foil-wrapped; then, they were stored in a freezer at −18 °C for no longer than three weeks. Measurements of pigment concentration were performed under laboratory conditions onshore. Chl was extracted with 3–5 ml of a 90 % aqueous solution of acetone. To improve pigment extraction, the filters were mechanically rubbed with a glass rod and stored in a refrigerator in the dark at +8 °C for 18 hours (Phytoplankton Pigments in Oceanography…, 1997). Then, the filters were rubbed again and centrifuged by a laboratory clinical centrifuge OPn-3 UKhL 4.2 for 5 minutes at 3000 rpm. Acetone extracts were put into a quartz cuvette, in which fluorescence measurements were performed before and after acidification with two drops of 1.2 M HCl. Fluorescence measurements were carried out using a laboratory fluorometer. A KGM 12-100 halogen lamp was used as a source of excitation of pigment fluorescence. Fluorescence was excited by a CC8 blue-light filter with a maximum transmission of 440–450 nm. Fluorescence was recorded by an FEU-27 photomultiplier, fed from a stabilized voltage source of a VS-22 type. A red-light filter KS17 with a wavelength of 670 nm was used to record fluorescence signal. The signal from FEU load resistance was fed through a preamplifier to the input of a UT60A digital multimeter, used as a recording device. The fluorometer was pre-calibrated, as in (Yunev & Berseneva, 1986 ; Lorenzen, 1967), based on chromatographically pure chlorophyll *a* by Sigma (USA); its initial concentration was determined by a Specord UV-Vis spectrophotometer with the specific light absorption coefficient of 87.67 $L \cdot g^{-1} \cdot cm^{-1}$ (Phytoplankton Pigments in Oceanography…, 1997).

Table 1. Data on the work, carried out in the coastal waters of Crimea in the winter-spring period

Cruise No.	Dates	Total number of stations / Number of stations with vertical profiles with determination of:					
		depth of Secchi disk visibility	temperature	density	chlorophyll *a*	fluorescence	phytoplankton
83	27 January – 03 February, 2016	10/10	15/15	8/8	16/14	7/7	14/0
84	19–25 April, 2016	12/11	13/13	5/5	14/13	4/4	13/0
93	28 March – 04 April, 2017	–	–	–	–	–	13/0
106	19 April – 1 May, 2019	12/7	10/10	10/10	22/11	–	–
In total		34/28	38/38	23/23	52/39	11/11	40/0

To calculate phytoplankton production estimates (biomass, specific growth rate, and maximum and integral photosynthesis rates), we used the models, described earlier (Finenko et al., 2018 ; Finenko at al., 2019).

To determine phytoplankton taxonomic composition and quantitative characteristics, 2-L water samples were concentrated by reverse filtration through track-etched membrane filters with a pore diameter of 1 µm. The resulting concentrate (40–50 mL) was fixed with 0.1 mL of Lugol's iodine. The samples were stored in a refrigerator at +8 °C. Determinations of species composition and phytoplankton cells size were carried out under a light trinocular microscope XY-B2 using a Nauman's chamber. Biomass was calculated by cell volume, using the standard method (Radchenko et al., 2010).

RESULTS

Chlorophyll a content in the upper mixed layer and predominant phytoplankton species. In January – February, in the shelf zone off the western coast of Crimea from Cape Tarkhankut to Cape Fiolent, the UML extended from the surface to the bottom (16–90 m, on average (44 ± 34) m); only at one station in the Kalamitsky Bay (depth of 26 m), the UML thickness was 14 m. In winter, in all the studied areas, the coccolithophore species *Emiliania huxleyi* (Lohmann) W. W. Hay & H. P. Mohler, 1967 predominated (52–94 % of the total phytoplankton abundance). Its ratio in the total biomass was 24–57 %. At several stations in the eastern area, *Skeletonema costatum* (Greville) Cleve, 1873 prevailed in abundance and biomass (60–70 and 26–30 %, respectively).

Chl content in the UML varied 0.40 to 0.60 mg·m^{-3}, averaging (0.47 ± 0.07). Off the southern coast of Crimea, with a depth range of 70–80 m, the UML thickness varied 50 to 80 m; in the deep-water area, it was 37 m, averaging (58 ± 18) m. Mean Chl concentration for this area in the UML was (0.42 ± 0.11) mg·m^{-3}. Off the eastern coast, with station depth of 25–60 m, the lower boundary of the UML was on average at (31 ± 9) m, and Chl content was (0.52 ± 0.18) mg·m^{-3}. In general, mean Chl values in the UML in all the studied areas did not differ significantly. No correlation was established between the UML depth and mean Chl concentration in it.

Work, carried out at the end of April 2016 on the shelf off the western coast of Crimea, showed that the UML thickness and Chl concentration in it decreased significantly, compared to the estimates of the winter period: up to (15 ± 12) m and (0.15 ± 0.08) mg·m^{-3}, respectively. Off the southern and eastern coast of Crimea, mean values of the UML and Chl content in it were the same: (16 ± 2) and (14 ± 4) m and (0.22 ± 0.04) and (0.22 ± 0.09) mg·m^{-3}, respectively. Mean values of Chl concentration in these areas are slightly higher than those off the western coast.

In late April – early May 2019, the UML thickness in the coastal and deep-water areas decreased, compared to that of 2016, and averaged (9 ± 4) m. Chl content in the eastern sea area, near the Kerch Strait, did not change during this period; however, in the western area, it was almost 1.5 times higher, and in the central area – 2 times higher than in the spring of 2016.

Thus, in the coastal waters of Crimea, Chl values were usually higher in winter than in spring.

In the early spring of 2017, *E. huxleyi* predominated in abundance off the southern coast of Crimea (47–57 %), while in other areas different Flagellata species prevailed (36–69 %). In the early spring period, at most stations in the western area and at several stations in the central and eastern areas, dinoflagellate *Heterocapsa triquetra* (Ehrenberg) F. Stein, 1883 predominated in biomass (18–59 %), as well as diatoms *Coscinodiscus janischii* A. W. F. Schmidt, 1878 (29–64 %), *Chaetoceros curvisetus* Cleve, 1889 (23 %), and *Pseudosolenia calcar-avis* (Schultze) B. G. Sundström, 1986 (29 %).

At the end of April 2016, *E. huxleyi* again predominated in abundance (41–96 %) in most of the water area; only at two stations, the diatom *Pseudo-nitzschia delicatissima* (Cleve) Heiden, 1928 prevailed (35–36 %). In the western and central areas, algae of different taxonomic groups predominated in biomass: coccolithophore *E. huxleyi* (22–50 %), dinoflagellates *Ceratium furca* (Ehrenberg) Claparède & Lachmann, 1859 (46–51 %) and *Ceratium tripos* (O. F. Müller) Nitzsch, 1817 (20 %), and diatoms *P. delicatissima* (13 %) and *P. calcar-avis* (27–43 %). Throughout the eastern area, *P. calcar-avis* prevailed (23–32 %).

Vertical distribution of chlorophyll a concentration. In winter and spring, Chl vertical distribution was analyzed by its content at different depths and by continuously recorded fluorescence. Comparison showed as follows: in 64 % of cases, change in relative values of Chl concentration and fluorescence with depth was of the same character, whereas in 36 % it was multidirectional (Fig. 2). The reason for this discrepancy may be that with an increase in Chl content with depth, relative fluorescence (normalized to a chlorophyll unit) decreases. Generally, vertical profiles of Chl fluorescence were less variable than the profiles of its concentration. It should be noted that for the entire dataset, no reliable correlation was revealed between Chl content and its fluorescence.

In winter, the lower boundary of the mixed layer almost reached the bottom or was 10–20 m higher. Temperature and density gradients in the water column were low: as a rule, they did not exceed 0.1 degree·m^{-1} and ranged 0.01–0.10 kg·m^{-3}·m^{-1}, respectively. Under such conditions, Chl vertical distribution at 60 % of the total number of stations was uniform; in other cases, Chl concentration either increased with depth or decreased. At several stations, maximum content was registered in the 0–10-m layer (deeper its values decreased).

In spring, the stability of the water column increased, and this resulted in a decrease of the UML thickness on average 3-fold, compared to that of the winter of 2016; temperature and density gradients remained low. Under these conditions, a distribution of Chl along the depths was uniform only in 30 % of cases; at the other stations, Chl content increased mainly from the surface to the lower boundary of the photosynthetic zone. In the winter of 2016, the photosynthesis layer averaged (25 ± 5) m;

in the spring, the value ranged 21 to 51 m, averaging (35 ± 10) m. In winter, this layer contains 12 $mg \cdot m^{-2}$ of Chl, which is 63 % of the integral value in the UML. In other words, 37 % of Chl from its total content is outside the photosynthetic zone. In spring, the total Chl content in the photosynthetic zone averages 7 $mg \cdot m^{-2}$, and all of it is in the euphotic layer. In the spring of 2019, the stability of the water column was higher than in the spring of 2016; the UML thickness averaged (9 ± 4) m; temperature and density gradients remained at the same level. Meanwhile, a decrease in the UML thickness did not result in an increase in Chl concentration in the surface layer. At most stations, unimodal vertical distribution of Chl was observed. In the 0–20-m layer, its content varied slightly; deeper, an increase was registered, with a maximum at 30–48 m, being on average (38 ± 6) m (Fig. 3). As a rule, Chl maximums were recorded at a depth, where 0.2–1.0 % of light from the surface penetrates. Chl concentration at its maximum reached 1.23 $mg \cdot m^{-3}$ and was on average 2.8 times higher than Chl content in the UML. The maximums are not related to temperature and density gradients; they may result from algae adaptation to low light intensities, which is possible only with weak turbulent mixing.

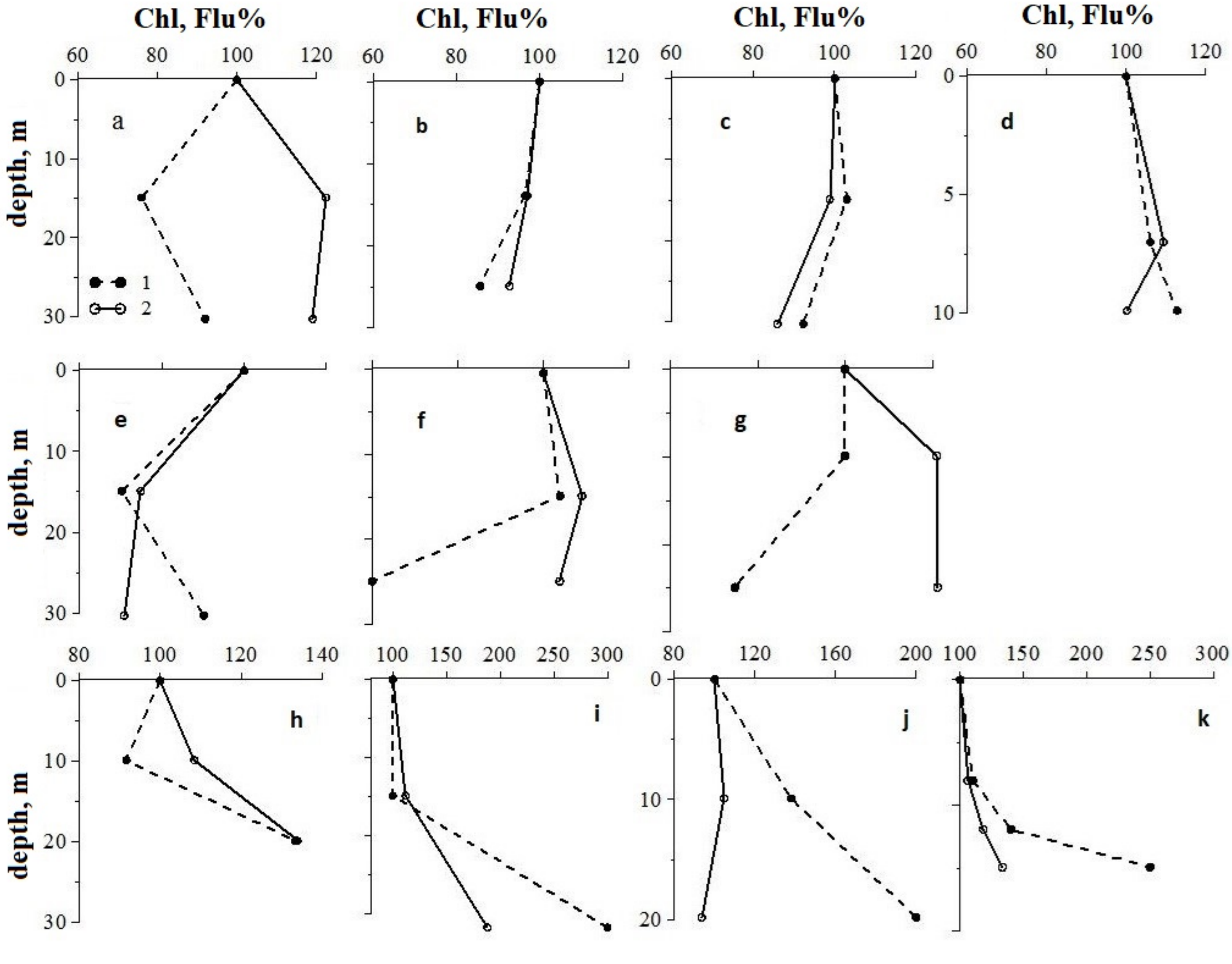


Fig. 2. Vertical distribution of chlorophyll *a* concentration (1) and fluorescence (2) in relative units (% of surface values) in January – February 2016 (a – at Cape Tarkhankut; b – in the Kalamitsky Bay; c – at Cape Fiolent; d – off the coast of Yevpatoriya; e, f – in Yalta area; g – in Alushta area) and at the end of April 2016 (h, i – in the Karkinitsky Bay; j – in the Kalamitsky Bay; k – off the coast of Yevpatoriya)

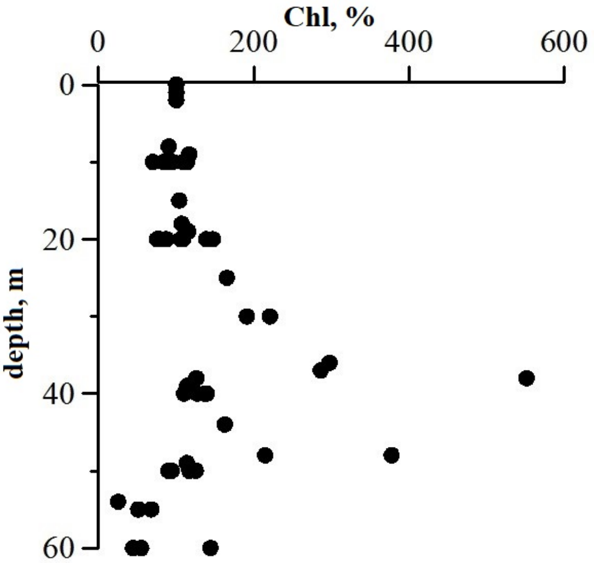


Fig. 3. Vertical distribution of chlorophyll *a* concentration in relative units (% of surface values) in spring 2019

It is apparent from the above data, that in spring the interannual differences in Chl content in the UML amounted 1.5–2.0 times for the western and southern sea areas. During that period, a vertical Chl structure with a depth maximum begins to form. In some cases, algae are able to adapt to extremely low irradiance (0.06 mol·m^{-2}·day^{-1}). Off the southern coast of Crimea, Chl concentration in the photosynthetic zone and outside it was the same; in the eastern area, only 30 % was in the irradiance zone.

In winter, at stations within the depth range of 16–50 m, the UML in 70 % of cases extended to the bottom; at 2 stations, it was at a depth of 14 and 31 m. At the same time, vertical distribution of Chl content was uniform only in 60 % of cases, and in the rest it either increased with depth or decreased. At stations within the depth range of 50–100 m, the lower boundary of the UML either reached the bottom or was at 42–53 m. When the UML reached the bottom, vertical distribution of Chl was uniform, or concentrations were the highest in the surface layer and decreased with depth.

Phytoplankton production estimates. The main production estimates of the phytoplankton community varied considerably in winter and spring (Table 2). In winter, phytoplankton physiological activity was low: primary production averaged 94 mg C·m^{-2}·day^{-1}, and daily production/biomass ratio (P/B) for the photosynthesis layer was 0.2. At the same time, the values of phytoplankton maximum specific growth rate and Chl concentration were relatively high. Low intensity of solar radiation, minimum temperature, and relatively shallow depth of the photosynthetic zone were the main limiting factors: because of them, algae in the photosynthetic zone divided once every 5 days.

In spring, mean intensity of solar radiation increased almost 4-fold, and the thickness of the photosynthetic zone increased 1.4 times on average. As a result, in 2016, the values of primary production and daily P/B ratio increased on average 2 times compared to those in winter. In 2019, primary production and biomass in the water column almost doubled compared to that of the spring of 2016; it resulted in P/B ratio and phytoplankton maximum specific growth rate remaining at the same level.

It allows us to conclude that the change in production, biomass, and maximum phytoplankton growth rate in the winter-spring period are not always synchronized. In spring, in the photosynthetic zone, algae divide approximately once every 2 days. Values of P/B and maximum specific growth rate differ insignificantly, which indicates that the onset of light saturation by the growth rate is observed at extremely low irradiance.

Table 2. Phytoplankton production estimates in the coastal waters of Crimea in the winter-spring period (numerator denotes minimum and maximum values; denominator denotes mean value ± standard deviation)

Season	PP, mg C·m^{-2}·day^{-1}	B, mg C·m^{-3}	B_i, mg C·m^{-2}	P/B, day^{-1}	μ, day^{-1}
Winter (2016)	49.8–143.3 93.9 ± 29.2	10.4–26.9 15.8 ± 4.3	218.0–659.3 412.1 ± 127.3	0.21–0.24 0.23 ± 0.01	0.59–0.73 0.66 ± 0.04
Spring (2016)	50.6–315.1 174.1 ± 71.9	4.7–18.0 11.8 ± 4.5	118.2–735.1 411.1 ± 200.1	0.35–0.52 0.44 ± 0.07	0.41–0.68 0.50 ± 0.08
Spring (2019)	208.2–522.4 309.1 ± 123.9	14.2–36.9 22.4 ± 9.0	450.5–1283.4 752.1 ± 353.8	0.39–0.46 0.44 ± 0.03	0.49–0.58 0.55 ± 0.03

Note: PP is phytoplankton integral primary production; B is phytoplankton mean biomass in the photosynthetic zone; B_i is integral biomass in the photosynthetic zone; P/B is daily production/biomass ratio for the photosynthetic zone; μ is phytoplankton maximum specific growth rate.

DISCUSSION

In the coastal waters of Crimea, Chl concentration in the UML in winter varied within narrow limits, averaging (0.47 ± 0.12) mg·m^{-3}. These values are 2.0–2.5 times lower than in deep-water areas (Finenko et al., 2017). Discrepancies in Chl values can result from different mechanisms of nutrient input into the photosynthetic zone. In the deep-water area, they come mainly with the rise of deep water, whereas in the coastal areas they inflow due to coastal runoff and mineralization of organic matter by heterotrophs. In winter, at low water temperatures, their metabolism is minimal, which results in a low mineralization rate. The weak flow of nutrients determines low Chl concentrations and phytoplankton biomass values in the coastal area. On the contrary, in deep-water areas, the water column stability decreases in winter, and dynamic activity of water increases; as a result, favorable conditions are created for the nutrient input into the photosynthetic zone.

In winter, the thickness of the mixed layer in Crimean coastal waters reached 75 m. Under these conditions, maximum Chl content was registered, and three types of its vertical distribution were observed. At most stations (60 %), it was uniform. At the other stations, maximum Chl content either increased with depth or decreased. During this period, deep-water areas are characterized by a uniform distribution of Chl in the UML (30–40 m), which is limited by the main pycnocline (Finenko et al., 2005). Below the mixed layer, Chl concentration decreases sharply.

In spring, a relatively high interannual variability of Chl content was observed in deep-water areas, with mean values in April varying, according to satellite data, 0.28 to 1.48 mg·m^{-3} (Finenko at al., 2014). In the coastal areas, variability of Chl concentration during this period was lower, with mean values within the range of 0.19–0.33 mg·m^{-3}. At this time, the UML decreases on average 3.5-fold compared to winter value, and the stability of the water column increases. These conditions are believed to be favorable for spring development of phytoplankton (Chiswell, 2011); however, in the coastal waters of Crimea in 2016, it was not registered. The results of hydrochemical measurements showed that the amount of inorganic nitrogen and phosphorus compound in the UML is low: mean nitrates concentration was (0.21 ± 0.11) μM, and phosphates one – (0.04 ± 0.02) μM. In the Black Sea, the Michaelis – Menten half-saturation constants (Ks) for nitrates in spring are on average (0.15 ± 0.05) μM (Krivenko, 2008), and for phosphates – (0.035 ± 0.010) μM (Parkhomenko, 2009). As can be seen, concentrations of these substances in water and Ks values are approximately the same. Therefore, they could have a limiting effect on the development of phytoplankton.

In spring, a depth Chl maximum begins to form in the coastal and deep-water areas (Finenko et al., 2005). During this period, the number of stations with a uniform distribution of Chl was 2 times less than in winter; at the other stations, the distribution was mainly unimodal. The depth maximum was recorded at mean temperature of (9.0 ± 0.6) °C and conditional density of (14.3 ± 0.2); it was on average 2.8 times higher than Chl concentration in the UML. According to the results of fluorescence measurements in the southern sea area, the maximum fluorescence intensity was observed at depths with temperature of (6.9 ± 0.3) °C and conditional density of (14.4 ± 0.1); the maximum fluorescence intensity was on average 2.7 times higher than that near the surface (Krivenko, 2008). The location of the depth maximum was closely related to the boundary of the nitratocline layer. In general, in the southern sea area and off the coast of Crimea, the depth maximum is at the same conditional density and has the same intensity of development; it is not related to density gradients.

Phytoplankton maximum specific growth rate depends on abiotic factors, as well as on size and taxonomic composition of phytoplankton (Chen & Liu, 2010). In the coastal areas, the values of the specific growth rate, calculated for the layer with optimal light conditions, varied within narrow limits during the study period. In winter, coccolithophore *E. huxleyi*, with a small cell size, predominated, and the maximum growth rate was on average (0.66 ± 0.04) day^{-1}. Generally, in spring, after mild winters, relatively large algae prevailed: at half of the stations, it was diatom *P. calcar-avis*, and at quarter – dinoflagellates of the genus *Ceratium*. With the given taxonomic composition of the phytoplankton community, the maximum growth rate decreased and averaged (0.50 ± 0.08) day^{-1}; daily P/B ratio for the photosynthesis layer increased on average 2 times. In winter, the rate of algae division is 3 times lower in the photosynthesis layer than that near the surface. In spring, these values differed insignificantly. One of the reasons for the multidirectional change is as follows: in winter, intensity of solar radiation is low, and algae growth rate rapidly decreases with depth. In spring, intensity of solar radiation increases several times, and the growth rate in the photosynthetic zone changes very little; as a result, P/B ratio turns out to be higher than in winter and approaches the values of the maximum growth rate. The maximum values of the growth rate, recorded in January – April in the Sevastopol Bay (Finenko et al., 2017), and those calculated by us in the coastal waters of Crimea were the same, while the taxonomic composition of the phytoplankton community was different. In the bays near Sevastopol in February – April, with the predominance of diatoms, the maximum growth rate varied 0.40 to 0.75 day^{-1}, averaging 0.50 day^{-1}; in the open coastal area opposite the Kruglaya Bay, it was slightly higher: on average 0.85 day^{-1} (Stelmakh et al., 2009). In the Sevastopol Bay, with dinoflagellates predominating in the community, the growth rate was on average 1.5 times lower than with the predominance of diatoms (Stelmakh, 2016). Thus, the values of the specific growth rate, obtained by the dilution method and calculated by us, proved to be quite close. The previously developed model for assessing phytoplankton specific growth rate can be used for rapid determination of the functional activity of the phytoplankton community.

Conclusions:

1. In winter, in the coastal waters of Crimea, chlorophyll *a* concentration and the depth of the upper mixed layer are the highest, and Chl content in the UML does not significantly differ at the western, southern, and eastern coast. During that period, in the studied water area, the coccolithophore *E. huxleyi* mostly predominated. In spring, Chl concentration and the depth of the UML were 2–3 times lower than in winter. In March – April, in different years, either dinoflagellates and diatoms or coccolithophores, dinoflagellates, and diatoms prevailed.
2. In winter, vertical distribution of Chl concentration at most stations was uniform. In spring, unimodal profiles with a depth maximum prevailed, location of which was not related to temperature and density gradients; Chl content was on average 3 times higher than in the UML. Generally, relative changes in Chl concentration and fluorescence with depth have the same character. Vertical profiles of Chl fluorescence were less variable than the profiles of its content; for the entire dataset, no reliable correlation was revealed between them.
3. The values of phytoplankton production and daily P/B ratio increase from winter to spring. The values of integral production, biomass, and specific growth rate vary disproportionately to each other. Functional estimates of the phytoplankton community in the coastal and deep-water areas are approximately the same. In spring, in the photosynthetic zone, algae divide almost once every 5 days. P/B ratio and maximum specific growth rate values differ insignificantly, which indicates as follows: the onset of light saturation by the growth rate is observed at extremely low irradiance.

This work has been carried out within the framework of IBSS government research assignment "Functional, metabolic, and toxicological aspects of hydrobionts and their populations existence in biotopes with different physical and chemical regimes" (No. AAAA-A18-118021490093-4), as well as within the framework of the RAS project "Influence of physical and chemical processes on the change in species composition and productivity of marine phytoplankton" (No. AAAA-A18-118020790209-9).

Acknowledgment. The authors are grateful to N. Minina, leading engineer, for collecting samples and determining chlorophyll *a* concentration during the 106th cruise of the RV "Professor Vodyanitsky" and to N. Rodionova, leading engineer, for determining the hydrochemical indicators during the 84th cruise of the RV "Professor Vodyanitsky".

REFERENCES

1. Arashkevich E. G., Louppova N. E., Nikishina A. B., Pautova L. A., Chasovnikov V. K., Drits A. V., Podymov O. I., Romanova N. D., Stanichnaya R. R., Zatsepin A. G., Kuklev S. B., Flint M. V. Marine environmental monitoring in the shelf zone of the Black Sea: Assessment of the current state of the pelagic ecosystem. *Oceanologiya*, 2015, vol. 55, no. 6, pp. 964–970. (in Russ.). https://doi.org/10.7868/S0030157415060015
2. Krivenko O. V. Contents and uptake of inorganic nitrogen in the Black Sea. *Morskoj ekologicheskij zhurnal*, 2008, vol. 7, no. 4, pp. 13–26. (in Russ.)
3. Krivenko O. V., Gevorgiz N. S., Finenko Z. Z. Spatial distribution of the chlorophyll fluorescence in the Southern Black Sea during late spring. *Morskoj ekologicheskij zhurnal*, 2008, vol. 7, no. 1, pp. 31–45. (in Russ.)
4. Krivenko O. V., Parkhomenko A. V. Upward and regeneration fluxes of inorganic nitrogen and phosphorus in the deep-water areas of the Black Sea. *Zhurnal obshchei biologii*, 2014, vol. 75, no. 5, pp. 394–408. (in Russ.). https://doi.org/10.1134/S2079086415030044

5. Kubryakov A. A., Belokopytov V. N., Zatsepin A. G., Stanichnyi S. V., Piotukh V. B. The Black Sea mixed layer depth variability and its relation to the basin dynamics and atmospheric forcing. *Morskoj gidrofizicheskij zhurnal*, 2019, vol. 35, no. 5, pp. 449–468. (in Russ.). https://doi.org/10.22449/0233-7584-2019-5-449-468
6. Parkhomenko A. V. Seasonal variability of mineral phosphorus uptake by microplankton in deep-water area of the Black Sea. *Morskoj ekologicheskij zhurnal*, 2009, vol. 8, no. 2, pp. 5–23. (in Russ.)
7. Radchenko I. G., Kapkov V. I., Fedorov V. D. *Prakticheskoe rukovodstvo po sboru i analizu prob morskogo fitoplanktona* : uchebno-metodicheskoe posobie dlya studentov biologicheskikh spetsial'nostei universitetov. Moscow : Mordvintsev, 2010, 60 p. (in Russ.)
8. Stelmakh L. V. Cell size effect on seasonal variability of phytoplankton growth rate in surface coastal waters of the Black Sea. In: *Morskie biologicheskie issledovaniya: dostizheniya i perspektivy* : v 3 t. : 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. 2, pp. 451–454. (in Russ.)
9. Stelmakh L. V. Specific phytoplankton growth rate in deep-water of the Black Sea in different seasons. *Morskoj ekologicheskij zhurnal*, 2010, vol. 9, no. 3, pp. 83–87. (in Russ.)
10. Stelmakh L. V., Kuftarkova E. A., Babich I. I. Seasonal variations of phytoplankton growth rate in coastal waters of the Black Sea (near Sevastopol). *Morskoj ekologicheskij zhurnal*, 2009, vol. 8, no. 1, pp. 67–80. (in Russ.)
19. Finenko Z. Z., Mansurova I. M., Suslin V. V. Dynamics of chlorophyll *a* concentration in the Black Sea on satellite data. *Morskoj biologicheskij zhurnal*, 2019, vol. 4, no. 2, pp. 87–95. (in Russ.). https://doi.org/10.21072/mbj.2019.04.2.09
12. Finenko Z. Z., Stelmakh L. V., Mansurova I. M., Georgieva E. Ju., Tsilinsky V. S. Seasonal dynamics of structural and functional characteristics of the phytoplankton community in the Sevastopol Bay. *Sistemy kontrolya okruzhayushchei sredy*, 2017, no. 9, pp. 73–82. (in Russ.). https://doi.org/10.33075/2220-5861-2017-3-73-82
13. Finenko Z. Z., Suslin V. V., Churilova T. Ya. The regional model to calculate the Black Sea primary production using satellite color scanner SeaWIFS. *Morskoj ekologicheskij zhurnal*, 2009, vol. 8, no. 1, pp. 81–106. (in Russ.)
14. Finenko Z. Z., Churilova T. Ya., Lee R. I. Vertical distribution of chlorophyll and fluorescence in the Black Sea. *Morskoj ekologicheskij zhurnal*, 2005, vol. 4, no. 1, pp. 15–46. (in Russ.)
15. Finenko Z. Z., Kovalyova I. V., Suslin V. V. A new approach to estimate phytoplankton biomass and its variability in the Black Sea surface water layer based on satellite data. *Uspekhi sovremennoi biologii*, 2018, vol. 138, no. 3, pp. 294–307. (in Russ.). https://doi.org/10.7868/S0042132418030079
16. Yunev O. A., Berseneva G. P. Fluorimetric method of determination of chlorophill *a* and pheophytin *a* concentration in phytoplankton. *Gidrobiologicheskii zhurnal*, 1986, vol. 22, no. 2, pp. 89–95. (in Russ.)
17. Chen B., Liu H. Relationships between phytoplankton growth and cell size in surface oceans: Interactive effects of temperature, nutrients and grazing. *Limnology and Oceanography*, 2010, vol. 55, pp. 965–972. https://doi.org/10.4319/lo.2010.55.3.0965
18. Chiswell S. M. Annual cycles and spring blooms in phytoplankton: Don't abandon Sverdrup completely. *Marine Ecology Progress Series*, 2011, vol. 443, pp. 39–50. https://doi.org/10.3354/meps09453
19. Finenko Z. Z., Kovalyova I. V., Suslin V. V. Use of satellite data for the estimation of the specific growth rate of phytoplankton in the surface layer of the Black Sea. *Russian Journal of Marine Biology*, 2019, vol. 45, iss. 4, pp. 313–319. https://doi.org/10.1134/S1063074019040059
20. Finenko Z. Z., Suslin V. V., Kovaleva I. V. Seasonal and long-term dynamics of the chlorophyll concentration in the Black Sea according to satellite observations. *Oceanology*, 2014, vol. 54, no. 5, pp. 596–605. https://doi.org/10.1134/S0001437014050063

21. Krivenko O. V., Burlakova Z. P., Eremeeva L. V. Basic characteristics of biotic nitrogen cycle in the open western part of the Black Sea. In: *Ecosystem Modeling as a Management Tool for the Black Sea* / L. I. Ivanov, T. Oguz (Eds). Dordrecht, Netherlands : Springer, 1998, pp. 121–136.
22. Lorenzen C. V. Determination of chlorophyll and phaeopigments: Spectrophotometric equations. *Limnologica*, 1967, no. 12, pp. 343–346.
23. Mikaelyan A. S., Chasovnikov V. K., Kubryakov A. A., Stanichny S. V. Phenology and drivers of the winter–spring phytoplankton bloom in the open Black Sea: The application of Sverdrup's hypothesis and its refinements. *Progress in Oceanography*, 2017, vol. 151, pp. 163–176. https://doi.org/10.1016/j.pocean.2016.12.006
24. *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods* / S. W. Jeffrey, R. F. C. Mantoura, S. W. Wright (Eds). Paris : UNESCO Publishing, 1997, 661 p. (Monographs on oceanographic methodology ; 10).
25. Yunev O. A., Vedernikov V. I., Basturk O., Yilmaz A., Kideys A. E., Moncheva S., Konovalov S. K. Long-term variations of surface chlorophyll *a* and primary production in the open Black Sea. *Marine Ecology Progress Series*, 2002, vol. 230, pp. 11–28. https://doi.org/10.3354/meps230011

РАЗВИТИЕ ФИТОПЛАНКТОНА В ЗИМНЕ-ВЕСЕННИЙ ПЕРИОД В ПРИБРЕЖНЫХ ВОДАХ КРЫМА

З. З. Финенко, И. М. Мансурова, И. В. Ковалёва, Е. Ю. Георгиева

Федеральный исследовательский центр «Институт биологии южных морей
имени А. О. Ковалевского РАН», Севастополь, Российская Федерация
E-mail: *zosim_finenko@mail.ru*

Исследование фитопланктона в зимне-весенний период имеет важное значение для изучения особенностей его годовой динамики и функционирования экосистемы Чёрного моря в целом. Состояние фитопланктона в шельфовой зоне в зимне-весенний период по сравнению с таковым в летне-осенний сезон изучено слабо, поэтому проведение подобного исследования особенно важно для решения ряда проблем, связанных с продуктивностью конечных звеньев пищевой цепи, формированием гидрохимического режима вод и циклом углерода в море. Цель работы — оценить влияние сезонных условий на развитие фитопланктона и его продукционные показатели в зимне-весенний период в прибрежных водах Крыма. В статье представлены результаты исследований гидрофизических (температура, плотность, относительная прозрачность воды) и биологических параметров (концентрация хлорофилла *a*, его флуоресценция, таксономический состав и продукционные характеристики фитопланктона) в шельфовой зоне Чёрного моря в январе — апреле 2016–2019 гг. Исследования проведены на 50 станциях, расположенных в прибрежных водах Крыма от Каркинитского залива до Керченского пролива. Концентрация хлорофилла *a* определена стандартным флуориметрическим методом, видовой состав — с помощью микроскопирования; удельная скорость роста фитопланктона рассчитана по разработанной ранее модели. Зимой (январь — февраль) концентрация хлорофилла *a* и глубина верхнего квазиоднородного слоя были максимальными (0,42–0,52 мг·м$^{-3}$ и 44–58 м соответственно), весной (март — апрель) — в 2–3 раза ниже. В январе — феврале доминировала примнезиевая водоросль *Emiliania huxleyi* (Lohmann) W. W. Hay & H. P. Mohler, 1967; в марте — апреле в разные годы преобладали динофитовые и диатомовые водоросли либо примнезиевые, динофитовые и диатомовые. Зимой вертикальное распределение хлорофилла *a* на большинстве станций было равномерным; весной преобладали одномодальные профили с глубинным максимумом, расположение которого не было связано с градиентами температуры и плотности. Относительное изменение концентрации хлорофилла *a* и флуоресценции с глубиной имело, как правило, одинаковый характер. Продукция фитопланктона и суточный коэффициент P/B (production/biomass ratio) повышались от зимы к весне. Корреляция между величинами интегральной продукции, биомассой и максимальной удельной скоростью роста водорослей отсутствовала. Максимальная удельная скорость роста была наименее изменчивым показателем. В течение зимне-весеннего периода водоросли в зоне фотосинтеза делились в среднем 1 раз в 2–5 суток.

Ключевые слова: таксономический состав, численность и биомасса фитопланктона, хлорофилл *a*, флуоресценция, максимальная удельная скорость роста водорослей, температура, плотность воды, Чёрное море

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 1, pp. 115–116
https://mbj.marine-research.org

CHRONICLE AND INFORMATION

TO THE JUBILEE OF LIDIYA SALEKHOVA

On 29 January, 2021, our dear Lidiya Salekhova, one of the oldest employees of the A. O. Kovalevsky Institute of Biology of the Southern Seas, turned 90 years old. Friends and colleagues heartily congratulate L. Salekhova and share the highlights of her biography.

Her whole life is connected with Sevastopol. Here she was born in 1931 and went to school in 1938. She did not leave her native city during the Great Patriotic War: she survived the hungry years of siege and occupation by fascists and together with other Sevastopol schoolchildren helped the defenders. Sharing her memories, Lidiya Salekhova talks about the empty soup in the besieged Sevastopol, incessant shelling and bombing, long road along the Laboratornoe highway in a vain attempt to evacuate, and mass shootings of citizens in the already captured city. On the day of its liberation, she together with other Sevastopol citizens cheered loudly and sincerely the Soviet soldiers.

After the end of the war, L. Salekhova returned to her studies; in 1949, she graduated from school No. 4. In 1950, she entered the faculty of ichthyology of the A. I. Mikoyan Moscow Technical Institute for the Fishery Industry; in 1955, she received a diploma of ichthyologist and fish breeder and came back to native Sevastopol.

Lidiya Salekhova began her scientific career in August 1955 in the ichthyological laboratory of the Sevastopol Biological Station of the Academy of Sciences of the Soviet Union as an intern. After completing practice in December 1955, she was hired as a laboratory assistant. As a young specialist, she studied sex inversion, reproduction, and development of the annular sea bream *Diplodus annularis*. In November 1956, she received a position of junior researcher at the already transformed Institute of Biology of the Southern Seas of the Academy of Sciences of the Soviet Union. In 1958, her first research cruise took place on the RV “Akademik A. Kovalevsky” – to the Adriatic Sea.

On 24 June, 1966, L. Salekhova successfully defended PhD thesis at the dissertation council of the Kaliningrad Technical Institute for the Fishery Industry. On 17 December, 1966, the scientific degree of candidate of biological sciences was awarded to her.

From 1969 to 1977, Lidiya Salekhova took part in seven IBSS cruises on the Mediterranean, Balearic, Adriatic, Aegean, and Black seas, collecting material on various types of Mediterranean migrants: fish of the family Sparidae. She studied morphological, morphophysiological, and biological characteristics of three fish species of this family, carried out a comparative analysis of their variability in the Black Sea, and characterized different geographical groupings of *Spicara flexuosa*. The results obtained were of great importance for identifying the role of the environment in the process of microevolution of these fish and formed the basis of her monograph "Sparidae fishes of the seas of the Mediterranean Basin" (1979).

Since 1976, L. Salekhova worked in IBSS ichthyology department in the position of a senior researcher. She wrote more than 70 scientific papers, became the author of one individual and two collective monographs, and was an editor and compiler of scientific publications. She prepared a biological part for several species guides: of Black Sea fish, Black Sea fish larvae, fish families of the Mediterranean Sea. Lidiya Salekhova took part in research cruises on the RV "Akademik A. Kovalevsky" and "Professor Vodyanitsky" to the seas of the Mediterranean Basin and to the Atlantic Ocean. For a number of years, she supervised the practice, as well as coursework and diploma work of students of biological faculties of several universities in the country and worked with schoolchildren of the Junior Academy of Sciences of the city.

Dear Lidiya Pavlovna, we remember you as a highly qualified specialist, who made a significant contribution to the study of fish biology in the seas of the Mediterranean Basin. You have always been distinguished by your high capacity for work, dedication and scrupulousness in scientific research, as well as exceptional intelligence, sensitivity, and kindness to people. We express our admiration for your professional and human qualities, and we wish you good health and active longevity!

Colleagues from IBSS ichthyology department

К ЮБИЛЕЮ ЛИДИИ ПАВЛОВНЫ САЛЕХОВОЙ

В январе 2021 г. отметила юбилей Лидия Павловна Салехова — одна из старейших сотрудниц ИнБЮМ. Л. П. Салехова является автором более чем 70 научных публикаций, в том числе монографии «Смаридовые рыбы морей Средиземноморского бассейна».

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 1, pp. 117–118
https://mbj.marine-research.org

TO THE MEMORY OF LIDIYA OVEN
(06.05.1930 – 09.01.2021)

On 9 January, 2021, after a long illness, D. Sc. Lydia Oven passed away. She had worked for many years in the ichthyology department of the A. O. Kovalevsky Institute of Biology of the Southern Seas.

L. Oven was born on 6 May, 1930, in the village of Pervomaisk, Pervomaisk district, Gorky region. Her family was large, and parents, wishing their children a better life, instilled in them a love of learning from childhood. After graduating from school in 1948, Lydia Oven entered the faculty of biology of Lomonosov Moscow State University.

In 1953, she received a diploma of zoologist and ichthyologist. After a year of work at the department at the university, she moved to Crimea and started PhD graduate studies at the Karadag Biological Station. L. Oven studied the problems of fish reproduction in the Black Sea, mainly the peculiarities of fertility and multiple spawning. She described the main features of reproduction of Black Sea fish of 18 families. This material later formed the basis of her monograph “Peculiarities of oogenesis and nature of spawning of marine fish” (1976).

On 11 June, 1963, Lydia Oven successfully defended PhD thesis at the Odessa I. I. Mechnikov National University. On 15 February, 1964, the scientific degree of candidate of biological sciences was awarded to her.

L. Oven began her scientific career at the A. O. Kovalevsky Institute of Biology of the Southern Seas in 1964 as a senior researcher. Since 1966, she took an active part in research cruises in the Red, Mediterranean, and Adriatic seas. In 1985, she was the head of an Atlantic expedition in the Guinea region.

Lidiya Oven paid much attention to the study of the development and maturation of germ cells in fish of warm latitudes during the spawning. She described in detail various oogenesis types and the corresponding spawning types.

In 1979, L. Oven defended D. Sc. dissertation. In 1980–1987, she was the head of IBSS ichthyology department. During the period of work at the institute (1964–2007), she became the author of more than 100 scientific publications, *inter alia* three collective and two individual monographs. Under her supervision, several PhD theses on the peculiarities of reproduction of various fish species were defended.

We will remember Lydia Oven as a highly qualified specialist, a wonderful leader, a creative soul, and a very good person.

Colleagues from IBSS ichthyology department

ПАМЯТИ ЛИДИИ СЕРГЕЕВНЫ ОВЕН (06.05.1930 – 09.01.2021)

Ушла из жизни д. б. н. Лидия Сергеевна Овен, долгие годы проработавшая в отделе ихтиологии ИнБЮМ. Л. С. Овен — автор более чем 100 научных публикаций, в том числе трёх коллективных и двух личных монографий.

ISSN 2499-9768

Вниманию читателей!

Институт биологии южных морей
имени А. О. Ковалевского РАН,
Зоологический институт РАН

издают
научный журнал

Морской биологический журнал
Marine Biological Journal

- МБЖ — периодическое издание открытого доступа. Подаваемые материалы проходят независимое двойное слепое рецензирование. Журнал публикует обзорные и оригинальные научные статьи, краткие сообщения и заметки, содержащие новые данные теоретических и экспериментальных исследований в области морской биологии, материалы по разнообразию морских организмов, их популяций и сообществ, закономерностям распределения живых организмов в Мировом океане, результаты комплексного изучения морских и океанических экосистем, антропогенного воздействия на морские организмы и экосистемы.
- Целевая аудитория: биологи, экологи, биофизики, гидро- и радиобиологи, океанологи, географы, учёные других смежных специальностей, аспиранты и студенты соответствующих научных и отраслевых профилей.
- Статьи публикуются на русском и английском языках.
- Периодичность — четыре раза в год.
- Подписной индекс в каталоге «Пресса России» — Е38872. Цена свободная.

Заказать журнал

можно в научно-информационном отделе ИнБЮМ.
Адрес: ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН», пр. Нахимова, 2, г. Севастополь, 299011, Российская Федерация.
Тел.: +7 8692 54-06-49.
E-mail: *mbj@imbr-ras.ru*.

A. O. Kovalevsky Institute of Biology
of the Southern Seas of RAS,
Zoological Institute of RAS

publish
scientific journal

Морской биологический журнал
Marine Biological Journal

- MBJ is an open access, peer reviewed (double-blind) journal. The journal publishes original articles as well as reviews and brief reports and notes focused on new data of theoretical and experimental research in the fields of marine biology, diversity of marine organisms and their populations and communities, patterns of distribution of animals and plants in the World Ocean, the results of a comprehensive studies of marine and oceanic ecosystems, anthropogenic impact on marine organisms and on the ecosystems.
- Intended audience: biologists, ecologists, biophysicists, hydrobiologists, radiobiologists, oceanologists, geographers, scientists of other related specialties, graduate students, and students of relevant scientific profiles.
- The articles are published in Russian and English.
- The journal is published four times a year.
- The subscription index in the “Russian Press” catalogue is E38872. The price is free.

You may order the journal

in the Scientific Information Department of IBSS.
Address: A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, 2 Nakhimov avenue, Sevastopol, 299011, Russian Federation.
Tel.: +7 8692 54-06-49.
E-mail: *mbj@imbr-ras.ru*.