1871
ИнБЮМ – IBSS

ISSN 2499-9768 print

# МОРСКОЙ БИОЛОГИЧЕСКИЙ ЖУРНАЛ

# MARINE BIOLOGICAL JOURNAL

**Vol. 8 No. 2**
**2023**

300 years
Russian Academy of Sciences

# МОРСКОЙ БИОЛОГИЧЕСКИЙ ЖУРНАЛ
# MARINE BIOLOGICAL JOURNAL

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Journal website: https://marine-biology.ru.

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2023 Vol. 8 no. 2

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

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ИнБЮМ – IBSS

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*SCIENTIFIC COMMUNICATIONS*

UDC 591.524.12-045.52(262.5)

# SPATIAL-TEMPORAL DYNAMICS OF THE ZOOPLANKTON ASSEMBLAGE STRUCTURE IN COASTAL WATERS NEAR SEVASTOPOL IN THE SPRING–AUTUMN PERIOD

© 2023 **E. A. Galagovets and I. Yu. Prusova**

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Received by the Editor 16.12.2020; after reviewing 01.04.2021;
accepted for publication 16.02.2023; published online 31.05.2023.

Zooplankton of the coastal water area near Sevastopol are quite well studied. However, there are few comprehensive investigations of local zooplankton assemblages involving the characterization of all the taxa forming them. Moreover, previous research was mainly based on material sampled in the Sevastopol Bay at only one or two stations – at the bay mouth and/or in its apex, and there was no analysis of zooplankton spatial variability within the bay. The aim of this work is to characterize the spatial-temporal dynamics of zooplankton communities in the Sevastopol Bay and the adjacent open coastal waters in the spring–autumn 2013. We analyzed zooplankton sampled in April–November 2013 in the western, central, and eastern Sevastopol Bay, as well as at three stations in the adjacent open coastal area: two miles from the Sevastopol Bay mouth, near the Uchkuevka village, and at the Kruglaya Bay mouth. To assess spatial-temporal differences in the taxonomic structure of zooplankton assemblages, we applied analysis of similarities (ANOSIM), used nonparametric multidimensional scaling (MDS), and determined the contribution of individual taxa to the Bray–Curtis dissimilarity between sample groups (SIMPER). When analyzing beta diversity, the Shannon index was applied. As revealed, during the study period, there were spatial-temporal differences in the abundance and taxonomic structure of zooplankton communities between various areas of the Sevastopol Bay and the adjacent open coastal waters. The highest degree of dissimilarity in the taxonomic structure of zooplankton was recorded between the central–eastern bay and the open coastal area. When comparing assemblages of these water areas, $R$ values (ANOSIM) were 0.926, 0.572, and 0.761 ($p < 0.03$) in spring, summer, and autumn, respectively. The mean total abundance of zooplankton in the bay in all seasons was higher than in the open coastal water area: (5.3 ± 1.9), (16.3 ± 2.7), and (8.3 ± 1.4) thousand ind.·m$^{-3}$ *vs.* (0.8 ± 0.3), (4.6 ± 1.2), and (3.4 ± 1.3) thousand ind.·m$^{-3}$ in spring, summer, and autumn, respectively (mean ± $SE$; $p < 0.006$). There was a tendency towards higher density values in the central Sevastopol Bay. A change in the level of diversity and, accordingly, in the degree of complexity of zooplankton assemblage was revealed in the spatial-temporal aspect. In spring, the lowest level of diversity was registered, with a mean (± $SE$) value of the Shannon index $H'$ of 1.09 ± 0.16. In summer and autumn, the values increased to 1.94 ± 0.11 and 1.48 ± 0.09, respectively. In summer–autumn period, the values of $H'$ were higher in the open coastal area (2.07 ± 0.09) and lower in the inner water area (1.53 ± 0.09). As determined, the differences in the taxonomic structure between the communities of the compared water areas were driven by three dominant taxa in spring, nine in summer, and five in autumn.

**Keywords:** zooplankton, copepods, taxonomic structure, diversity, Sevastopol Bay

Zooplankton of the coastal water area near Sevastopol are quite well studied. Specifically, the dynamics of abundance and biomass of fodder zooplankton before and after the invasion of ctenophores *Mnemiopsis leidyi* A. Agassiz, 1865 and *Beroe ovata* Bruguière, 1789 is investigated [Datsyk et al., 2012; Hubareva et al., 2004], and long-term alterations in the Copepoda taxocene structure in 1976–1996 are described [Gubanova et al., 2002]. Much attention is given to the analysis of ecology of individual taxa, in particular, invasive species, and their effect on the structure of the zooplankton assemblage [Altukhov, Gubanova, 2006; Gubanova, 2000, 2003; Gubanova et al., 2016, 2019, 2020; Seregin, Popova, 2016]. Comprehensive studies of zooplankton communities in coastal waters near Sevastopol, including the characteristics of all the taxa that form them, are not so numerous; those were carried out mainly using material from the Sevastopol Bay and an external water area in the 1980s [Belyaeva, Zagorodnyaya, 1988; Kovalev, 1980] and early 2000s [Datsyk et al., 2012; Gubanova, 2003; Temnykh et al., 2008; Zagorodnyaya et al., 2007]. Previous investigations were based mostly on material sampled at one or two stations – at the bay mouth and/or in its apex (eastern area), while the analysis of the spatial variability of zooplankton within the bay was not carried out. However, ecological conditions in the bay are heterogeneous: the western area is characterized by a more intensive water exchange with the open sea; the eastern area is replenished with freshwater from the Chernaya River; and the central area is affected by a large amount of wastewater, both storm water runoff and industrial and household sewage [Gubanov et al., 2015; Pavlova et al., 1999]. This is bound to affect the state of assemblages in these water areas.

The aim of this study is to characterize the spatial-temporal dynamics of zooplankton communities in the Sevastopol Bay and adjacent open water areas in the spring–autumn 2013.

## MATERIAL AND METHODS

The studies covered the coastal areas of Sevastopol (Fig. 1) – the Sevastopol Bay (sta. 1–7) and three stations in the open coastal waters: the areas two miles from the bay mouth (sta. M), near the Uchkuevka village (sta. U), and at the Kruglaya Bay mouth (sta. K). The works were carried out from April to November 2013 (Table 1). Zooplankton were sampled using a 0.1-$m^2$ Juday net fitted with 132-µm mesh. Vertical net hauls were taken at all stations in the first half of the day in the 10–0-m layer. The sea surface temperature was measured at the time of sampling. The samples were preserved in 4% solution of buffered formalin. The organisms were identified and measured under MBS-9 microscope at 10–140-fold magnification. Mass species were counted using a Bogorov chamber in 1/20 or 1/10 of the initial sample taken with 1-mL and 5-mL Stempel pipettes in several replicates, depending on the abundance of planktonic organisms in a sample. To count rare taxa, the entire sample volume was examined. Adult and juvenile copepods (including naupliar stages) were identified down to the species level; other animals were identified down to the genus, family, or order level (whenever possible). A total of 45 samples were analyzed.

Graphical and statistical analysis was performed using PRIMER v5.2.4 and SigmaPlot 12.5 software packages. Spatial-temporal variations in the taxonomic structure of zooplankton assemblages were assessed based on the algorithm for comparing the degree of variability of rank similarities (the statistic $R$) in the ANOSIM program applying the Bray–Curtis similarity index $S$ ($S = 100\%$, if compared samples are completely similar; $S = 0$, if compared samples are completely dissimilar [Clarke, Warwick, 2001]). The test statistic $R$ characterizes observed dissimilarities in the structure of communities *between* sampling areas compared with dissimilarities between samples *within* each area. $R$ value varies from −1 to 1. $R = 1$, if *all* replicates at the sampling site are more similar to each other than to *any* sample from another area. $R$ value is close to zero, if the similarity between samples within and between water areas on average is the same [Clarke, Warwick, 2001]. To analyze beta diversity, the Shannon index $H'$ ($\log e$) was applied. When ordinating by the method of nonparametric multidimensional scaling (MDS), the Bray–Curtis indices were used with 10 permutations for determining the lowest value of the stress index. When performing the procedures of cluster analysis, MDS, and SIMPER (determining the contribution of individual taxa to similarity/dissimilarity between groups of samples), the Bray–Curtis similarity matrix was constructed based on the initial data transformed to the power of 0.5 [Clarke, Warwick, 2001]. When constructing a matrix for cluster and MDS analysis, the data for each season for each station (*inter alia* for conditional sta. B2 and B3) were preliminarily averaged. Differences between the mean values of the Shannon index were assessed using Student's $t$-test (ANOVA) for a significance level of $p = 0.05$, and between the mean abundance values, according to the Mann–Whitney rank test for a significance level of $p < 0.01$. To designate certain sites of the water area studied, the following abbreviations were used: B1, the western Sevastopol Bay (sta. 1); B2, the central bay (sta. 2–4); B3, the eastern bay (sta. 5–7); and MKU, the open coastal water area (sta. M, K, U).

**Table 1.** Sampling dates and areas, number of the samples analyzed

| Season 2013 | Sea surface temperature range, °C | Site, sampling depth | | | | | |
|---|---|---|---|---|---|---|---|
| | | The Sevastopol Bay, 10–15 m | | | Two miles from the coast, 50 m | The Kruglaya Bay, 20 m | Uchkuevka, 50 m |
| | | sta. 1 | sta. 2–4 | sta. 5–7 | M | K | U |
| | | B1 | B2 | B3 | MKU | MKU | MKU |
| | | Number of samples | | | | | |
| Spring (11.04–25.04) | +10.3…+11.9 | 2 | 2 | 1 | 1 | 1 | 2 |
| Summer (11.07–19.09)* | +23.0…+25.4 | 5 | 2 | 3 | 3 | 3 | 3 |
| Autumn (11.10–14.11) | +13.7…+15.5 | 4 | 6 | 3 | 1 | – | 3 |

**Note:** B1, the western Sevastopol Bay (sta. 1); B2, the central bay (sta. 2–4); B3, the eastern bay (sta. 5–7); MKU, the open coastal water area (sta. M, K, U). *, according to the classification of hydrological seasons in the neritic zone of the Black Sea proposed by V. Greze *et al.* [1971], the September data were referred to summer season data.

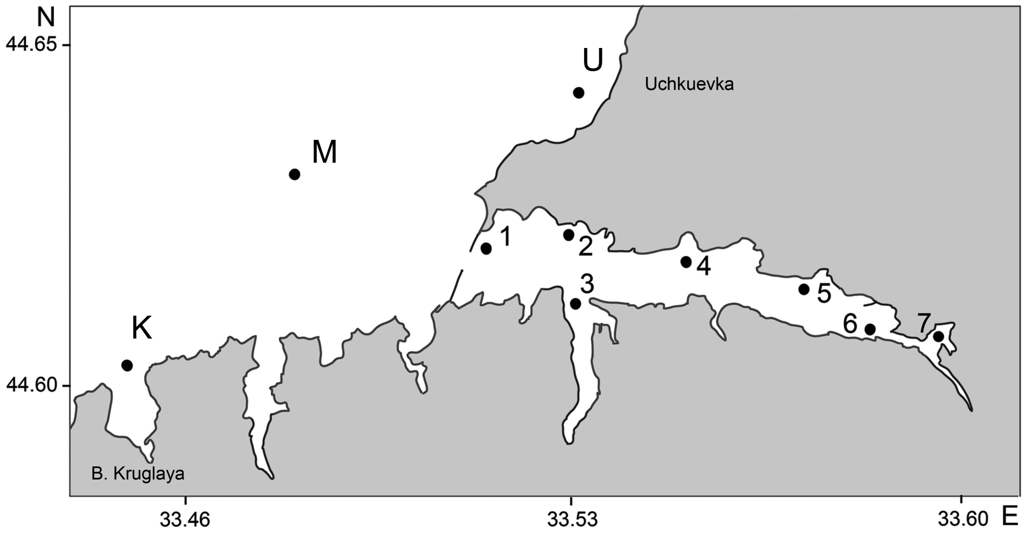

**Fig. 1.** The map of sampling stations: sta. K, the Kruglaya Bay mouth; sta. M, two miles from the Sevastopol Bay mouth; sta. U, near the Uchkuevka village; sta. 1–7, in the Sevastopol Bay

## RESULTS AND DISCUSSION

**Taxonomic composition and mean abundance of zooplankton.** In analyzed material, 26 zooplankton taxa were recorded (Table 2); out of them, 16 were identified down to the species level. The cells of the flagellate *Noctiluca scintillans* were taken into account as well. Holoplankton was represented mainly by copepods, and their contribution to the total population was 25.5, 74.4, and 87.0% in spring, summer, and autumn, respectively (Table 3). A relatively small proportion of Copepoda in spring was due to high density of Rotifera in this season (see Table 3). Out of meroplankton organisms, the most abundant were larvae of Cirripedia, Polychaeta, and Mollusca (Bivalvia and Gastropoda), with the contribution to the total abundance of zooplankton within 2.5–10.1, 0.8–4.2, and 1.4–5.6%, respectively. The total mean abundance (excluding *N. scintillans*) was the highest in summer and the lowest in spring (Table 3).

**Table 2.** Taxonomic composition, mean abundance, and occurrence of zooplankton taxa in the studied water area in the spring–autumn 2013

| Taxon | Mean abundance, ind.·m$^{-3}$ | Standard error (*SE*), ind.·m$^{-3}$ | Occurrence in samples, % | Season | | |
|---|---|---|---|---|---|---|
| | | | | Spring | Summer | Autumn |
| Copepoda | | | | | | |
| *Oithona davisae* Ferrari & Orsi, 1984 | 3,488.2 | 831.7 | 91 | + | + | + |
| *Acartia clausi* Giesbrecht, 1889 | 795.1 | 120.4 | 100 | + | + | + |
| *Acartia tonsa* Dana, 1849 | 449.4 | 144.2 | 38 | – | + | + |
| *Paracalanus parvus* (Claus, 1863) | 379.9 | 68.7 | 91 | + | + | + |
| *Centropages ponticus* Karavaev, 1895 | 205.2 | 44.6 | 82 | + | + | + |
| *Pseudocalanus elongatus* (Boeck, 1865) | 71.3 | 17.5 | 56 | + | + | + |
| *Oithona similis* Claus, 1866 | 44.9 | 18.4 | 36 | + | + | + |
| *Calanus euxinus* Hulsemann, 1991 | 36.7 | 9.8 | 60 | + | + | + |
| Harpacticoida | 13.2 | 5.0 | 49 | + | + | + |
| *Cyclopina gracilis* Claus, 1863 | 0.5 | 0.4 | 4 | + | + | – |
| *Pontella mediterranea* (Claus, 1863) | 0.3 | 0.2 | 13 | + | – | – |
| Other groups of zooplankton | | | | | | |
| Rotifera | 422.8 | 336.9 | 20 | + | + | + |
| *Penilia avirostris* Dana, 1852 | 288.0 | 130.2 | 58 | – | + | + |
| Cirripedia (nauplii) | 283.0 | 61.8 | 91 | + | + | + |
| *Oikopleura dioica* Fol, 1872 | 247.4 | 59.2 | 73 | + | + | + |
| Bivalvia (larvae) | 189.0 | 63.6 | 98 | + | + | + |
| Polychaeta (larvae) | 177.4 | 67.0 | 84 | + | + | + |
| Gastropoda (larvae) | 106.3 | 19.9 | 84 | + | + | + |
| *Evadne spinifera* P. E. Müller, 1867 | 84.2 | 33.0 | 31 | – | + | – |
| Hydrozoa (larvae) | 55.5 | 35.1 | 29 | – | + | + |

Continue on the next page…

| Taxon | Mean abundance, ind.·m$^{-3}$ | Standard error (*SE*), ind.·m$^{-3}$ | Occurrence in samples, % | Season | | |
|---|---|---|---|---|---|---|
| | | | | Spring | Summer | Autumn |
| *Parasagitta setosa* (J. Müller, 1847) | 47.6 | 17.4 | 67 | + | + | + |
| *Pleopis polyphemoides* (Leuckart, 1859) | 31.1 | 14.9 | 38 | − | + | + |
| *Pseudevadne tergestina* (Claus, 1877) | 22.6 | 12.8 | 16 | − | + | − |
| Decapoda (larvae) | 14.5 | 4.9 | 53 | − | + | + |
| Isopoda | 10.8 | 3.8 | 44 | − | + | + |
| Nematoda | 4.1 | 3.3 | 16 | + | + | + |
| *Noctiluca scintillans* (Macartney) Kofoid & Swezy, 1921 | 242.5 | 87.1 | 29 | + | + | − |

**Table 3.** Seasonal mean values of the absolute and relative abundance of different taxonomic groups of zooplankton in the spring–autumn 2013

| Taxon | Mean abundance ± *SE*, ind.·m$^{-3}$ | | | Proportion in the total abundance, % | | |
|---|---|---|---|---|---|---|
| | Spring | Summer | Autumn | Spring | Summer | Autumn |
| Appendicularia | 2 ± 1 | 241 ± 96 | 384 ± 104 | 0.1 | 2.3 | 5.6 |
| Chaetognatha | 0 | 91 ± 39 | 24 ± 9 | 0 | 0.9 | 0.4 |
| Cirripedia | 352 ± 159 | 262 ± 106 | 270 ± 82 | 10.1 | 2.5 | 3.9 |
| Cladocera | 0 | 985 ± 347 | 27 ± 7 | 0 | 9.3 | 0.4 |
| Copepoda | 896 ± 150 | 7,898 ± 1,976 | 6,044 ± 998 | 25.7 | 74.6 | 87.3 |
| Hydrozoa | 0 | 123 ± 81 | 10 ± 6 | 0 | 1.2 | 0.1 |
| Decapoda | 0 | 31 ± 11 | 4 ± 2 | 0 | 0.3 | 0.1 |
| Isopoda | 0 | 21 ± 8 | 5 ± 2 | 0 | 0.2 | 0.1 |
| Mollusca | 49 ± 18 | 590 ± 153 | 96 ± 21 | 1.4 | 5.6 | 1.4 |
| Nematoda | 1 ± 0.7 | 9 ± 8 | 1 ± 0.5 | 0.0 | 0.1 | 0.0 |
| Polychaeta | 146 ± 71 | 304 ± 151 | 52 ± 18 | 4.2 | 2.9 | 0.8 |
| Rotifera | 2,044 ± 1,646 | 30 ± 22 | 4 ± 3 | 58.6 | 0.3 | 0.1 |
| In total (without *Noctiluca scintillans*) | 3,491 ± 1,797 | 10,583 ± 2,467 | 6,922 ± 1,143 | – | – | – |
| *N. scintillans* | 974 ± 328 | 113 ± 58 | 0 | – | – | – |

**Sample grouping.** The degree of similarity of the sampling areas (and, accordingly, the composition of zooplankton assemblages in these water areas) was analyzed by the MDS ordination based on the data on the abundance of organisms in the samples (Fig. 2).

In all seasons, the stations within the bay (groups B2 and B3) and in the open coastal area (sta. M, K, U) were grouped separately within the ordination plane, which reflects a certain degree of dissimilarities in the taxonomic structure between zooplankton communities in the bay and in the open coastal waters. The assemblage at the station at the bay mouth (sta. B1) was similar in species composition to the communities both in the inner bay area and in the open coastal area, which resulted from the intermediate location of the sta. B1. In terms of the degree of similarity of the species composition, this station was grouped in spring and summer with the stations of the open coastal water area, and in autumn, with the stations of the bay (see Fig. 2).

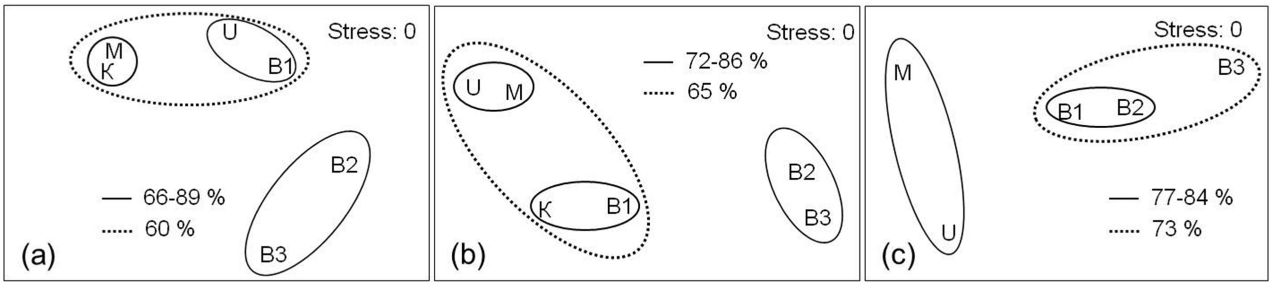

**Fig. 2.** Results of MDS ordination analysis; grouping of stations based on the taxonomic structure of zooplankton assemblages: a, spring; b, summer; c, autumn. Data on the abundance of zooplankton taxa averaged for each area were used. Solid and dashed lines correspond to the level (%) of grouping of areas (stations) by the results of cluster analysis

Taking into account the results of ordination, the degree of similarity between the identified groups of samples was analyzed (Table 4). The communities of the areas B2 and B3 were the most similar: the Bray–Curtis index *S* in the group B2 compared with the group B3 varied within 53–67%; in other compared groups, the value was lower in all seasons. The dissimilarities between the zooplankton assemblages in the bay waters and the open coastal area are confirmed by high values of the test statistic *R*, which assesses the degree of variability of average values of rank similarities for combinations of all pairs of stations from different groups compared with the variability of similarities between any pair of stations from the same group.

**Table 4.** Results of the test for spatial-temporal differences in the taxonomic structure of zooplankton assemblages when comparing groups of samples based on the abundance of taxa in the spring–autumn 2013

| Areas under comparison | Spring | | | Summer | | | Autumn | | |
|---|---|---|---|---|---|---|---|---|---|
| | *S*, % | *R* | *p* | *S*, % | *R* | *p* | *S*, % | *R* | *p* |
| B2 *vs.* B3 | 63.4 | 1.000 | 0.333 | 53.2 | −0.083 | 0.400 | 67.5 | 0.243 | 0.143 |
| B2 + B3 *vs.* MKU | 35.9 | 0.926 | **0.029** | 42.6 | 0.572 | **0.003** | 47.2 | 0.761 | **0.001** |
| B1 *vs.* B2 + B3 | 53.2 | 0.333 | 0.200 | 51.8 | 0.336 | **0.008** | 65.3 | 0.181 | 0.134 |
| B1 *vs.* MKU | 59.7 | 0.107 | 0.400 | 50.7 | 0.358 | **0.011** | 55.1 | 0.145 | 0.257 |
| | Global *R* | | *p* | Global *R* | | *p* | Global *R* | | *p* |
| The entire water area studied | 0.608 | | **0.022** | 0.425 | | **0.001** | 0.427 | | **0.005** |

**Note:** *S*, the Bray–Curtis similarity index; *R*, the test statistic (see “Material and Methods” section); *p*, the probability of acceptance of the hypothesis that there are no differences between the compared mean values ($p_0 = 0.05$). Statistically significantly different results are highlighted in bold.

For these areas, *R* values in spring, summer, and autumn were 0.926, 0.572, and 0.761, respectively; the dissimilarities between them were significant (Table 4). In the compared groups (B1 *vs.* B2 + B3; B1 *vs.* MKU), *R* values were low, from 0.107 to 0.358, indicating a low degree of dissimilarities within these groups. The generalizing value of *R* (global *R*), which characterizes the level of spatial differences between groups for each season as a whole, was significantly higher in spring than in summer and autumn (see Table 4). Global *R* values calculated for each of the areas – MKU, B1, B2, and B3 – were 0.790, 0.814, 0.992, and 0.556, respectively (at $p < 0.002$), and those were generally higher than the values of spatial variations.

The results obtained indicate that: 1) the taxonomic structure of zooplankton in the studied water areas is heterogeneous, and the degree of similarity between the communities of the central and eastern bay is higher than that for the western bay and the open coastal waters; 2) the highest degree of dissimilarity is recorded between the central–eastern bay and the open coastal area in all seasons; 3) spatial variability of zooplankton structure is higher in spring than in summer and autumn; and 4) seasonal variability of the structure is more pronounced than the spatial one.

**Diversity index.** Distinct seasonal differences in the values of the Shannon diversity index were registered for zooplankton assemblages of the open coastal water area (MKU): the mean value of *H’* in summer was significantly higher than in spring and autumn ($p < 0.001$) (Fig. 3). At the sta. B1 in summer, the value of *H’* was higher than in spring ($p = 0.016$); at the stations within the bay (sta. B2 + B3), seasonal differences in the mean values of *H’* were not revealed. For the entire water area studied, the mean value of *H’* in summer (1.94 ± 0.11) was significantly higher than in spring and autumn (1.09 ± 0.16 and 1.48 ± 0.09, respectively) ($p < 0.0041$).

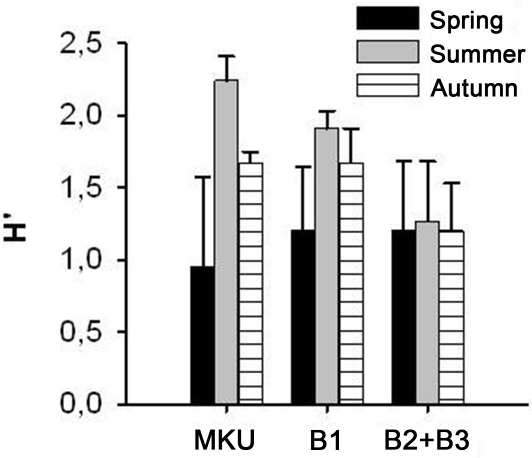

**Fig. 3.** Variability of mean values (mean ± *SE*) of the Shannon diversity index in the Sevastopol Bay (B1, B2 + B3) and the open coastal water area (MKU) in the spring–autumn 2013

The spatial dynamics of the Shannon diversity index values was as follows: in the summer–autumn period, the values of *H’* were higher in the open coastal area (2.07 ± 0.09) and lower in the water area within the bay (1.53 ± 0.09). In summer, the value of *H’* for each of three examined water areas (MKU, B1, and B2 + B3) differed significantly ($p < 0.001$), being the highest for the open coastal waters (2.26 ± 0.06) and the lowest for the area within the bay (1.66 ± 0.15). In autumn, at the stations within the bay (sta. B2 + B3), the value of *H’* (1.28 ± 0.11) was significantly lower than at the bay mouth (1.78 ± 0.15) and in the open coastal area (1.64 ± 0.04) ($p = 0.041$ and $p = 0.048$, respectively). In spring, the differences were insignificant.

Thus, lower values of the Shannon diversity index and, accordingly, lower taxonomic diversity of the community were revealed in spring throughout the entire water area. The degree of the assemblage complexity tended to increase towards the open coastal water area in summer and autumn. The stability of this trend is confirmed by the fact that a similar pattern of diversity variability in the bay water area was observed earlier on the material of 1981–1983, based on the analysis of spatial-temporal dynamics in the number of zooplankton species [Belyaeva, Zagorodnyaya, 1988].

**Taxa determining dissimilarities between assemblages.** Using the SIMPER procedure, discriminatory taxa were identified, the contribution of which to dissimilarities between the zooplankton communities of the open coastal area (sta. MKU), within the bay (sta. B2 + B3), and at the bay mouth (sta. B1) was the most significant and accounted for about 50% dissimilarities between the compared groups of samples (Table 5). In spring, the main contribution to the dissimilarity between groups of samples was made by 2–3 taxa prevailing in the assemblage; in autumn, there were more discriminatory taxa; and in summer, their number was maximum. The obtained results confirm the conclusions about the nature of prevalence and the degree of complexity of communities, which were made on the basis of the analysis of the spatial-temporal variability in the diversity index.

**Table 5.** Taxa with the largest contribution to the dissimilarity between zooplankton assemblages of different sites of the Sevastopol Bay and the open coastal water area in the spring–autumn 2013

| Areas under comparison | Spring | | Summer | | Autumn | |
|---|---|---|---|---|---|---|
| | Taxon | Contribution to dissimilarity, % | Taxon | Contribution to dissimilarity, % | Taxon | Contribution to dissimilarity, % |
| B2 + B3 *vs.* MKU | Rotifera | 34.0 | *O. davisae* | 25.5 | *O. davisae* | 28.2 |
| | *N. scintillans* | 21.4 | *P. avirostris* | 8.2 | *A. clausi* | 9.9 |
| | | | *A. tonsa* | 8.0 | Cirripedia | 9.5 |
| | | | *P. parvus* | 5.5 | *P. parvus* | 8.5 |
| | | | *O. dioica* | 5.4 | | |
| B1 *vs.* MKU | Cirripedia | 20.6 | *O. davisae* | 18.2 | *O. davisae* | 16.0 |
| | *N. scintillans* | 19.0 | *A. tonsa* | 9.7 | *A. clausi* | 15.7 |
| | Rotifera | 10.3 | Cirripedia | 8.4 | *O. dioica* | 13.0 |
| | | | *C. ponticus* | 6.3 | *P. parvus* | 9.5 |
| | | | *O. dioica* | 5.1 | | |
| | | | Bivalvia | 4.4 | | |
| B1 *vs.* B2 + B3 | Rotifera | 35.8 | *O. davisae* | 17.5 | *O. davisae* | 22.2 |
| | *N. scintillans* | 18.3 | *A. tonsa* | 8.3 | *A. clausi* | 12.2 |
| | | | *P. avirostris* | 7.5 | *O. dioica* | 9.7 |
| | | | Cirripedia | 6.7 | Cirripedia | 7.1 |
| | | | *P. parvus* | 6.4 | | |
| | | | *C. ponticus* | 5.7 | | |

Throughout the entire study period, the taxa determining dissimilarities between assemblages were five Copepoda species (*Acartia clausi*, *Acartia tonsa*, *Centropages ponticus*, *Oithona davisae*, and *Paracalanus parvus*), cladoceran *Penilia avirostris*, appendicularian *Oikopleura dioica*, Bivalvia and Cirripedia larvae, as well as rotifers and the flagellate *N. scintillans* (Table 5). These taxa had the highest values of the mean abundance (see Table 3). The composition of groups of discriminatory taxa in various seasons was different (Table 5), and this indicates seasonal differences in the structure of zooplankton communities (Fig. 4).

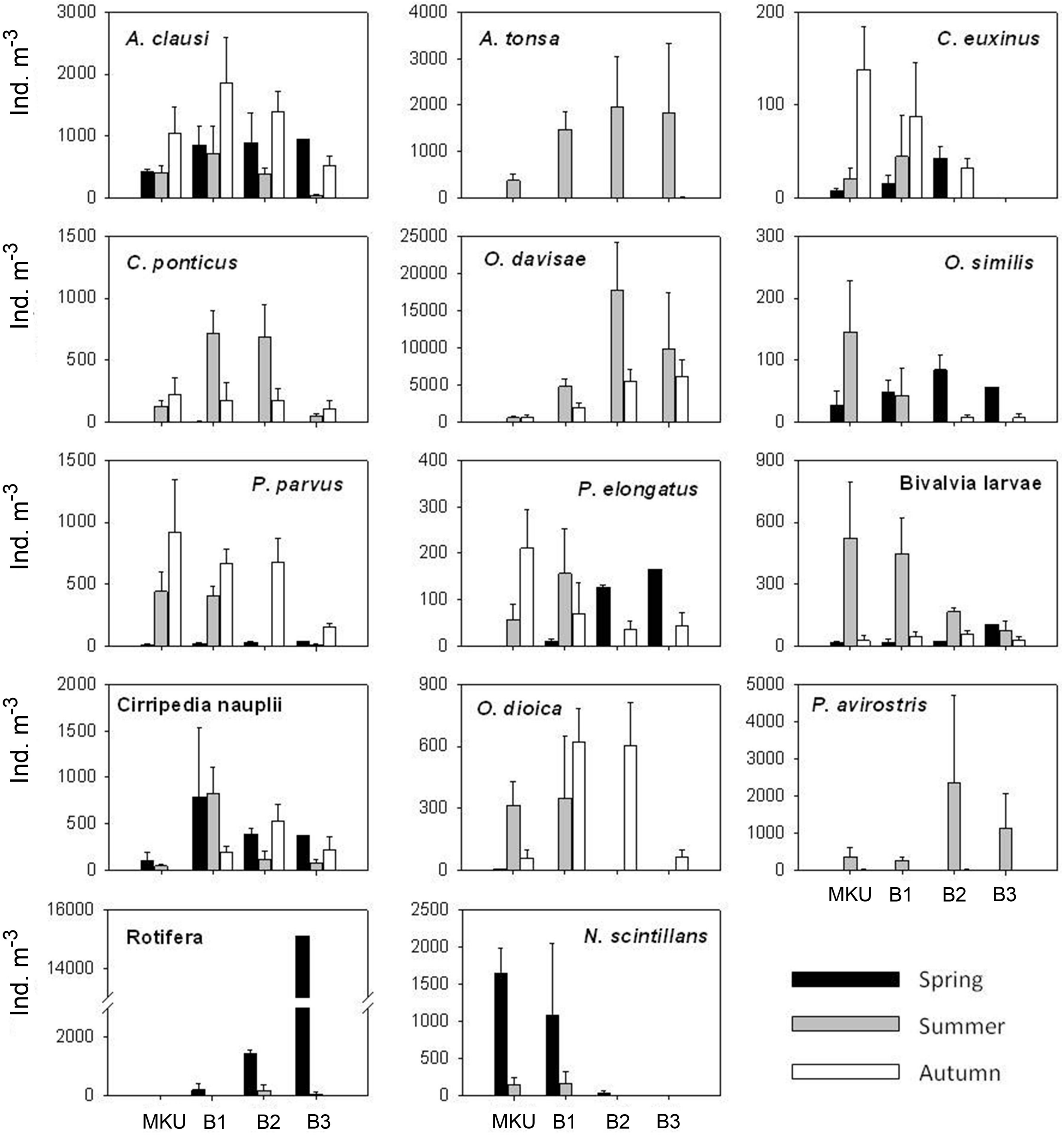

**Fig. 4.** Spatial-temporal dynamics of the mean abundance (mean ± *SE*) of the main Copepoda species and other most abundant taxa in the Sevastopol Bay (B1, B2, B3) and the open coastal water area (MKU) in the spring–autumn 2013

**Dynamics of the zooplankton assemblage structure.** In spring, Copepoda communities were distinctly dominated by *A. clausi* (74.8–89.3% of the total abundance of copepods in spring *vs.* 0.3–38.9% in summer and autumn), with almost complete absence of *C. ponticus* (0–0.4% in spring *vs.* 1.6–6.9% in summer) and a small contribution of other species (Fig. 5b). In summer–autumn, *O. davisae* prevailed (21.6–88.1% in summer and autumn *vs.* 0.3–4.8% in spring); it was the most common species in the studied material (Table 2), with the highest density values in summer (up to 24,950 ind.·m$^{-3}$ in August at sta. 6). An important element in the assemblages of the summer period was *A. tonsa* (9.4–17.6% of the total Copepoda abundance), which was absent from plankton in spring and recorded as single individuals at two out of seven stations in autumn. *Calanus euxinus*, *Oithona similis*, and *Pseudocalanus elongatus* were present in plankton throughout the entire study period; their density was low (Fig. 4), and their contribution varied within 0.4–4.3, 0.1–7.1, and 0.1–13.1%, respectively. Therefore, the role of these species in the spatial-temporal dynamics of the zooplankton structure was insignificant.

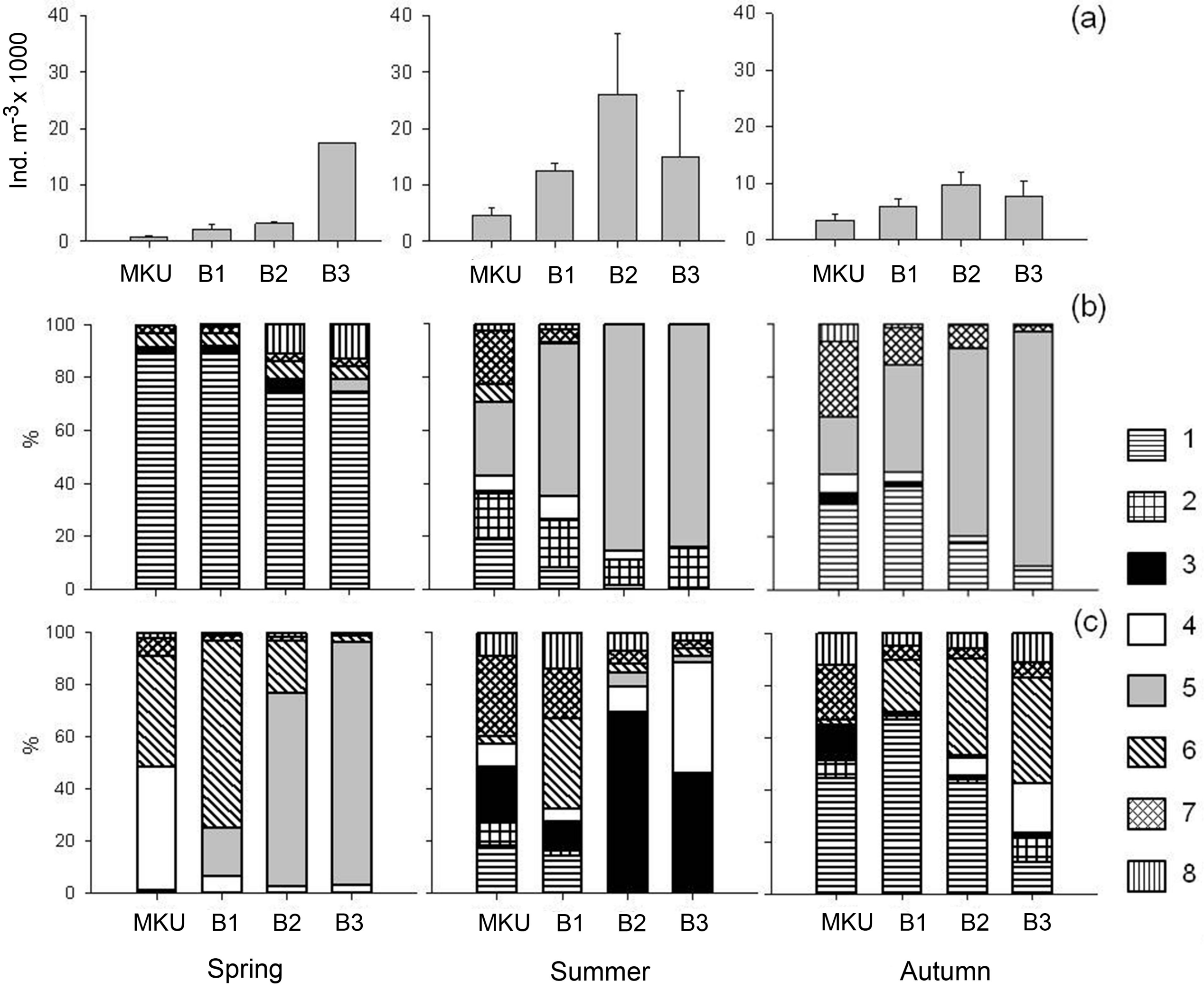

**Fig. 5.** Spatial-temporal dynamics of the total abundance (mean ± *SE*) of zooplankton (a) and taxonomic structure of Copepoda (b) and other taxa of forage zooplankton (c) in the Sevastopol Bay (B1, B2, B3) and the open coastal water area (MKU) in the spring–autumn 2013. For (b): 1, *Acartia clausi*; 2, *Acartia tonsa*; 3, *Calanus euxinus*; 4, *Centropages ponticus*; 5, *Oithona davisae*; 6, *Oithona similis*; 7, *Paracalanus parvus*; 8, *Pseudocalanus elongatus*. For (c): 1, *Oikopleura dioica*; 2, *Parasagitta setosa*; 3, *Penilia avirostris*; 4, Polychaeta larvae; 5, Rotatoria; 6, Cirripedia nauplii; 7, Bivalvia larvae; 8, Gastropoda larvae

Among the groups of other organisms, the largest range of seasonal density variations was found in Rotifera: from mean values of thousands of ind.·m$^{-3}$ in spring to minimum values in summer and autumn (see Table 3). Rotifers were noted only in the bay water area (B1, B2, and B3) and not registered in the open coastal area. The spring peak of their abundance, 15,110 ind.·m$^{-3}$, was recorded in the area B3 (Fig. 4); it was 86.2% of the total abundance of zooplankton there. In spring, the communities differed significantly from those of summer ($p = 0.0034$) in higher population density of *N. scintillans*: (974 ± 328) ind.·m$^{-3}$ *vs.* (113 ± 58) ind.·m$^{-3}$ in summer. In autumn, this flagellate was absent in the plankton.

Spatial heterogeneity of the taxonomic structure of the Copepoda assemblage was determined mainly by the variability in the proportions of *A. clausi*, *A. tonsa*, *C. ponticus*, *O. davisae*, and *P. parvus*. In summer and autumn, the degree of spatial dissimilarity in the structure was more pronounced than in spring (Fig. 5b). In the group of other organisms, spatial variations in spring were mainly driven by the variability of the contribution of rotifers and Cirripedia larvae; in summer, *P. avirostris* and Polychaeta and Bivalvia larvae; and in autumn, *O. dioica* and Bivalvia larvae (Fig. 5c).

The mean abundance of total zooplankton in all seasons in the bay (B1 + B2 + B3) was significantly higher than in the open coastal area: (5.3 ± 1.9), (16.3 ± 2.7), and (8.3 ± 1.4) thousand ind.·m$^{-3}$ *vs.* (0.8 ± 0.3), (4.6 ± 1.2), and (3.4 ± 1.3) thousand ind.·m$^{-3}$ in spring, summer, and autumn, respectively (mean ± *SE*; $p < 0.006$). A similar ratio was registered separately for communities of copepods and other organisms, as well as for *A. tonsa* ($p = 0.0080$), *O. davisae* ($p = 0.0004$), and Cirripedia ($p = 0.0003$). The mean abundance of *A. clausi*, *C. ponticus*, *O. dioica*, and *P. avirostris* was also higher in the bay (Fig. 4), but the differences were not significant. The average population density of *C. euxinus*, *O. similis*, *P. parvus*, *P. elongatus*, *N. scintillans*, and Bivalvia larvae was slightly higher in the open coastal waters (Fig. 4), but the differences were not significant. Assessing the spatial variability of abundance values within the bay water area, it should be noted as follows. In summer and autumn, the total mean abundance of zooplankton in the central area (B2) was higher than in the eastern (B3) and western (B1) areas (Fig. 5a), but taking into account the variability values, these differences were not significant.

The revealed trend of increasing values of the assemblage abundance in the central Sevastopol Bay, compared to the values in its eastern and western areas, is consistent with previously reported results. Specifically, based on the material of 1981–1983, maximum zooplankton abundance and biomass were registered in the center of the bay [Belyaeva, Zagorodnyaya, 1988]. Spatial dissimilarities in zooplankton abundance values might be related to different environmental conditions in various bay areas, namely, to the level of eutrophication due to anthropogenic pollution. Based on the study of the distribution of phosphates, silicates, nitrates, nitrites, ammonium ions, and amount of suspended matter in the surface layer in 1998–2000, the western bay was assigned earlier to the areas with the level of weak pollution; the eastern bay, moderate pollution; and the central bay, heavy pollution [Lopukhin et al., 2007]. The central bay area may be characterized by a higher level of water trophicity, resulting in higher plankton abundance. In relatively clean waters of the open coastal area, lower zooplankton densities were recorded. Apparently, lower zooplankton density in the open coastal waters results from the fact that sampling was carried out only in the 10–0-m layer, but not in the entire inhabited water column.

Further studies in the waters of the Sevastopol Bay and open coastal areas, along with the analysis of hydrochemistry data, will supplement the obtained results and reveal the spatial patterns of the formation of zooplankton communities within this area.

**Conclusion.** Between various sites of the Sevastopol Bay area and the adjacent open coastal waters, spatial-temporal variability in the quantitative values and taxonomic structure of zooplankton assemblages was revealed in the spring–autumn 2013. In all seasons, the total mean zooplankton abundance in the bay was higher than in the open coastal water area. A tendency towards higher abundance values in the central bay was recorded. The highest level of variability in the zooplankton taxonomic structure was registered between the central–eastern bay and the open coastal waters. In the summer–autumn period, an increase in the diversity and, accordingly, in the level of community complexity from the eastern bay towards the open coastal area was noted. The lowest level of the assemblage diversity was observed in spring. As determined, the dissimilarities in the taxonomic structure between the communities of the compared water areas were due to three dominant taxa in spring, nine in summer, and five in autumn, including five Copepoda species (*Acartia clausi*, *Acartia tonsa*, *Centropages ponticus*, *Oithona davisae*, and *Paracalanus parvus*), cladoceran *Penilia avirostris*, appendicularian *Oikopleura dioica*, Bivalvia and Cirripedia larvae, rotifers, and the flagellate *Noctiluca scintillans*.

*This work was carried out within the framework of IBSS state research assignment "Structural and functional organization, productivity, and sustainability of marine pelagic ecosystems" (No. 121040600178-6).*

**Acknowledgement.** The authors are grateful to S. Ignatyev and D. Altukhov for their help in zooplankton sampling and to anonymous reviewers for their valuable comments.

## REFERENCES

1. Altukhov D. A., Gubanova A. D. *Oithona brevicornis* Giesbrecht in the Sevastopol Bay in October, 2005 – March, 2006. *Morskoj ekologicheskij zhurnal*, 2006, vol. 5, no. 2, pp. 32. (in Russ.). https://repository.marine-research.ru/handle/299011/865
2. Belyaeva N. V., Zagorodnyaya Yu. A. Zooplankton of the Sevastopol Bay in 1981–1983. *Ekologiya morya*, 1988, iss. 29, pp. 77–84. (in Russ.). https://repository.marine-research.ru/handle/299011/3844
3. Greze V. N., Baldina E. P., Bileva O. K. Dinamika chislennosti i produktsii osnovnykh komponentov zooplanktona v neriticheskoi zone Chernogo morya. *Biologiya morya*, 1971, iss. 24, pp. 12–49. (in Russ.). https://repository.marine-research.ru/handle/299011/1592
4. Gubanov V. V., Gubanova A. D., Rodionova N. Yu. Diagnosis of water trophicity in the Sevastopol Bay and its offshore. In: *Current Issues in Aquaculture* : proceedings of the International Scientific Conference, Rostov-on-Don, 28 September – 02 October, 2015. Rostov-on-Don : FGBNU "AzNIIRKh", 2015, pp. 64–66. (in Russ.)
5. Gubanova A. D. *Acartia tonsa* Dana in the Sevastopol Bay: Appearance, seasonal dynamic, size structure. *Ekologiya morya*, 2000, iss. 51, pp. 55–58. (in Russ.). https://repository.marine-research.ru/handle/299011/4299
6. Gubanova A. D. Long-term changes in the specific composition and abundance of genera *Acartia* Dana copepod in the Sevastopol Bay. In: *Modern Condition of Biological Diversity in Near-shore Zone of Crimea (the Black Sea Sector)* / V. N. Eremeev, A. V. Gaevskaya (Eds) ; NAS Ukraine, Institute of Biology of the Southern Seas. Sevastopol :

EKOSI-Gidrofizika, 2003, pp. 94–104. (in Russ.). https://repository.marine-research.ru/handle/299011/1467

7. Gubanova A. D., Altukhov D. A., Popova E. V., Garbazey O. A. *Oithona davisae*, the new species of copepods in the Black Sea: Long-term dynamics, spatial distribution and impact on zooplankton community. 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. 1, pp. 154–157. (in Russ.)
8. Hubareva E. S., Svetlichny L. S., Romanova Z. A., Abolmasova G. I., Anninsky B. E., Finenko G. A., Bat L., Kideys A. Zooplankton community state in the Sevastopol Bay after the invasion of ctenophore *Beroe ovata* in the Black Sea (1999–2003). *Morskoj ekologicheskij zhurnal*, 2004, vol. 3, no. 1, pp. 39–46. (in Russ.). https://repository.marine-research.ru/handle/299011/732
9. Datsyk N. A., Romanova Z. A., Finenko G. A., Abolmasova G. I., Anninsky B. E. Zooplankton community structure in the inshore waters of the Crimean coasts (Sevastopol area) and trophic relations in the food chain zooplankton–*Mnemiopsis* in 2004–2008. *Morskoj ekologicheskij zhurnal*, 2012, vol. 11, no. 2, pp. 28–38. (in Russ.). https://repository.marine-research.ru/handle/299011/1210
10. Zagorodnyaya Yu. A., Temnykh A. V., Moryakova V. K. Holoplankton seasonal dynamics in inshore waters of the Black Sea in 2002. *Morskoj ekologicheskij zhurnal*, 2007, vol. 6, no. 1, pp. 31–43. (in Russ.). https://repository.marine-research.ru/handle/299011/894
11. Kovalev A. V. Sezonnye izmeneniya zooplanktona v Sevastopol'skoi bukhte. *Gidrobiologicheskii zhurnal*, 1980, vol. 16, no. 6, pp. 9–14. (in Russ.). https://repository.marine-research.ru/handle/299011/2866
12. Lopukhin A. S., Ovsyany E. I., Romanov A. S., Kovardakov S. A., Sysoeva I. V., Bryantseva Yu. V., Ryl'kova O. A., Gavrilova N. A., Gubanov V. V., Lopukhin S. A., Kamenir Yu. G., Gomis C., Wilson J. G., Kemp R. B. Seasonal peculiarities of hydrologic-hydrochemical structure of Sevastopol Bay water, microplankton and distribution of its biochemical components (the Black Sea, observations of 2004–2005). *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zoni i kompleksnoe ispol'zovanie resursov shel'fa*, 2007, no. 15, pp. 74–109. (in Russ.)
13. Pavlova E. V., Ovsyany E. I., Gordina A. D., Romanov A. S., Kemp R. B. Modern state and tendencies of change in Sevastopol Bay ecosystem. In: *Akvatoriya i berega Sevastopolya: ekosistemnye protsessy i uslugi obshchestvu* / E. V. Pavlova, N. V. Shadrin (Eds). Sevastopol : Akvavita, 1999, chap. 2, pp. 70–95. (in Russ.). https://repository.marine-research.ru/handle/299011/5248
14. Seregin S. A., Popova E. V. Long-term dynamics of abundance of the copepod-invader, *Oithona davisae*, in the coastal waters of the Black Sea. *Rossiiskii zhurnal biologicheskikh invazii*, 2016, vol. 9, no. 3, pp. 90–100. (in Russ.)
15. Temnykh A. V., Zagorodnyaya Yu. A., Moryakova V. K. Spatial variability of seasonal dynamics of zooplankton in coastal waters of southwestern Crimea in 2002 and 2003. *Ekologiya morya*, 2008, iss. 75, pp. 23–30. (in Russ.). https://repository.marine-research.ru/handle/299011/4795
16. Clarke K. R., Warwick R. M. *Change*

*in Marine Communities: An Approach to Statistical Analysis and Interpretation.* 2nd ed. Plymouth : PRIMER-E Ltd., 2001, 171 p.

17. Gubanova A. D., Polikarpov I. G., Saburova M. A., Prusova I. Yu. Long-term dynamics of mesozooplankton by the example of the Copepoda community in Sevastopol Bay (1976–1996). *Oceanology*, 2002, vol. 42, no. 4, pp. 512–520.
18. Gubanova A. D., Garbazey O. A., Popova E. V., Altukhov D. A., Mukhanov V. S. *Oithona davisae*: Naturalization in the Black Sea, interannual and seasonal dynamics, and effect on the structure of the planktonic copepod community. *Oceanology*, 2019, vol. 59, no. 6, pp. 912–919. https://doi.org/10.1134/S0001437019060079
19. Gubanova A., Drapun I., Garbazey O., Krivenko O., Vodiasova E. *Pseudodiaptomus marinus* Sato, 1913 in the Black Sea: Morphology, genetic analysis, and variability in seasonal and interannual abundance. *PeerJ*, 2020, vol. 8, art. no. e10153 (26 p.). https://doi.org/10.7717/peerj.10153

# ПРОСТРАНСТВЕННО-ВРЕМЕННАЯ ДИНАМИКА СТРУКТУРЫ СООБЩЕСТВА ЗООПЛАНКТОНА В ПРИБРЕЖНЫХ ВОДАХ У СЕВАСТОПОЛЯ В ВЕСЕННЕ-ОСЕННИЙ ПЕРИОД

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Зоопланктон прибрежной зоны у Севастополя изучен довольно хорошо, однако комплексных работ с характеристикой всех таксонов, формирующих зоопланктонные сообщества данного региона, немного. Кроме того, проведённые ранее исследования в основном базировались на материалах, собранных в Севастопольской бухте на одной или двух станциях (у входа и/или в кутовой части), при этом анализ пространственной изменчивости зоопланктона внутри акватории бухты выполнен не был. Цель настоящей работы — охарактеризовать пространственно-временну́ю динамику сообществ зоопланктона Севастопольской бухты и прилегающих открытых вод в весенне-осенний период 2013 г. Материалом послужили пробы зоопланктона, собранные с апреля по ноябрь 2013 г. в западной, центральной и восточной частях Севастопольской бухты, а также на трёх станциях в открытом прибрежье — в двух милях от входа в бухту, возле посёлка Учкуевка и у входа в бухту Круглая. Оценку пространственно-временны́х различий таксономической структуры сообществ зоопланктона проводили с использованием процедур анализа сходства (ANOSIM), непараметрического многомерного шкалирования (MDS) и определения вклада отдельных таксонов в сходство/различие Брея — Кёртиса между группами проб (SIMPER). При анализе бета-разнообразия применяли индекс Шеннона. Исследование показало, что в рассматриваемый период между разными частями акватории Севастопольской бухты и прилегающего открытого прибрежья имелись пространственно-временны́е различия в количественных показателях и таксономической структуре зоопланктонных сообществ. Наибольший уровень различий в таксономической структуре зоопланктона отмечен между центрально-восточной частью бухты и открытым прибрежьем. При сравнении сообществ этих акваторий значения тестовой статистики $R$ (ANOSIM) весной, летом и осенью составили 0,926; 0,572 и 0,761 ($p < 0,03$) соответственно. Средняя численность суммарного зоопланктона во все сезоны в бухте была выше, чем в открытом прибрежье, — (5,3 ± 1,9), (16,3 ± 2,7) и (8,3 ± 1,4) тыс. экз.·м$^{-3}$ против (0,8 ± 0,3), (4,6 ± 1,2) и (3,4 ± 1,3) тыс. экз.·м$^{-3}$ весной, летом и осенью соответственно (среднее ± $SE$; $p < 0,006$). Отмечена тенденция к более высоким величинам плотности в срединной части бухты. Выявлено изменение уровня разнообразия и, соответственно, степени сложности сообщества зоопланктона в пространственно-временно́м аспекте. Наиболее низкий уровень разнообразия зарегистрирован весной при средней (± *SE*) величине индекса Шеннона *H’* 1,09 ± 0,16; летом и осенью значения возросли до 1,94 ± 0,11 и 1,48 ± 0,09 соответственно. В летне-осенний период величи́ны *H’* были выше в зоне открытого прибрежья (2,07 ± 0,09) и ниже в акватории внутри бухты (1,53 ± 0,09). Определено, что различия в таксономической структуре между сообществами сравниваемых акваторий весной обусловлены тремя, летом — девятью, осенью — пятью доминирующими таксонами.

**Ключевые слова:** зоопланктон, копеподы, таксономическая структура, разнообразие, Севастопольская бухта

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 2, pp. 18–41
https://marine-biology.ru

UDC [502.175:504.5](265.54.04)

# ORGANIC MATTER IN THE ECOSYSTEM OF THE VLADIMIR BAY (THE SEA OF JAPAN): FOOD RESOURCE AND ENVIRONMENTAL RISK FACTOR

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Received by the Editor 09.12.2021; after reviewing 06.04.2022;
accepted for publication 16.02.2023;

To understand the accumulation process resulting from the input of toxic substances and elements into water areas, the study of the organic matter content in the aquatic environment and bottom sediments is of great importance. Moreover, such investigation is significant for identifying negative alterations in the ecosystem and potential environmental risks driven by the nature management. We aimed at analyzing the importance of organic matter as a factor of the environmental contamination in the Vladimir Bay (the Sea of Japan) and determining the toxicity of hydrobionts and the environmental risk to both the ecosystem and human health. This complex work was carried out applying chemical, analytical, microbiological, and hydrobiological techniques; it included mathematical and cartographic data processing, as well as calculation of accumulation factor and sediment quality guideline quotient. Seawater, bottom sediments, and macrobenthos sampled in the Vladimir Bay in July 2014 were analyzed. Chemical, ecological, and microbiological parameters of the bay waters were determined; those allowed to estimate the organic matter accumulation in the aquatic environment and bottom sediments and to establish the trophic status of the ecosystem in summer. Mass macrobenthic species and groups were identified; indices of benthic population abundance were determined indicating high levels of biomass. In surface waters, the abundance of heavy metal resistant groups of bacteria was estimated. In bottom sediments, heavy metal content and its spatial distribution were determined. Heavy metal concentrations in the sea urchin gonads were quantified. The results of the microbiological assessment were compared with maximum permissible concentrations (MPC), and a significant pollution of the bay waters (more than 3 MPC) in terms of Cd was revealed. For Ni, Cu, Zn, and Cd, background concentrations were exceeded in the bottom sediments; for Cd and Zn, clarke content was exceeded. The correlation between concentrations of organic matter in the bottom sediments and heavy metal content there was checked, as well as the correlation between heavy metal concentrations in the bottom sediments and in the sea urchin gonads (a statistically significant correlation was revealed for Zn). For the bay bottom sediments, the contamination factor $C_f$ was determined; its values characterize the contamination with zinc as high, and with cadmium and copper – as very high. Also, the degree of contamination $C_d$ was estimated; its values evidence for an average degree of contamination for the bottom sediments in total. The environmental risk quotients were calculated. According to SQG-Q value, the bay bottom sediments are classified as moderately polluted. As established, Cd and Pb content in the sea urchin gonads from the Vladimir Bay does not exceed the permissible values set in Technical Regulation of the Customs Union 021/2011. However, based on ILCR value, it can be concluded that there is a carcinogenic risk arising from consuming the sea urchin gonads.

**Keywords:** Vladimir Bay, Sea of Japan, organic matter, trophicity, bottom sediments, macrobenthos, heavy metals, accumulation factors, environmental risk, human health risk

High concentrations of organic matter (hereinafter OM) in marine ecosystems often result from natural processes occurring there. Specifically, OM is accumulated in “trap bays”: water areas deeply incised into the coast, with a relatively narrow mouth and limited or specific water exchange. Such water areas are accumulators of OM, which is a food resource generally determining the trophic status of the environment in the ecosystem. Marine ecosystems, which have a high trophic status and are characterized by great variety of ecotopes, are key areas for the development of mariculture and the rational use of natural populations. Moreover, those are the areas of natural formation and conservation of biodiversity.

OM accumulation in a marine ecosystem has a pronounced positive effect up to a certain level of values for OM dissolved in water and accumulated in bottom sediments. When exceeding this level, negative consequences arise. In addition to more pronounced and short-term manifested eutrophication, there is a less noticeable and more extended in time accumulation of toxic substances and elements entering a bay or a bight. For bottom sediments, this effect is especially significant. The processes of OM transformation and destruction at the boundary of bottom sediments and a water column and in sediments are the main cause of both the mobilization of heavy metals (hereinafter HM) into pore and bottom waters and the binding of elements [Shulkin, 2004; Zhang et al., 2014]. Entering a water column, HM cause its secondary pollution; then, HM are accumulated in organisms *via* food chains. The analysis of element migration in various ecosystem components is an important direction of toxicological studies; it allows to assess both toxic effects for organisms of different trophic levels [Saroop, Tamchos, 2021; Vashchenko et al., 2010] and risks to human health [Birch, 2011; Donets et al., 2020].

The Vladimir Bay is located on the eastern coast of the Primorsky Krai. This water area is deeply incised into the coast; its mouth is relatively narrow. In central areas of bights, the depth exceeds 20 m; at the bay mouth, the value is 40 m. The currents within the bay are mainly due to wind-wave processes. The effect of the Primorsky Current is insignificant since the bay water area is quite closed. There are no currents formed by a river flow because of the lack of full-flowing rivers entering the bay. This water area is under anthropogenic load since 1907, when the first settlement occurred, Veselyi Yar. Later, because of the deployment of military facilities on the bay coast, new settlements arose: Timofeevka (1932), Nord-Ost, and Rakushka (1934). In 1934–1999, the Vladimir-Olginsk naval base of the Pacific Fleet was located in the bay water area (now, it is disbanded).

A few marine biological studies of the Vladimir Bay cover particular commercial macrobenthic species [Gavrilova et al., 2006; Kulepanov, Ivanova, 2006] and some invasive species [Lutaenko, Kolpakov, 2016]. Macrobenthic composition, abundance, and distribution are described by the authors [Galysheva et al., 2018]. Several scientific works are devoted to the analysis of Neogene deposits [Vashchenkova, Tsoy, 2014] and the prospects for mariculture development [Gavrilova et al., 2019]. There are no published data on hydrochemical and microbiological studies by other researchers. Complex works have not been carried out.

The aim of this work is to analyze the level of organic matter as a possible factor in the formation of environmental toxicity in the Vladimir Bay and to assess the contamination of hydrobionts and the environmental risk to both the ecosystem and human health.

Research objectives are: 1) to analyze OM content in the aquatic environment in terms of chemical and microbiological indicators and to assess the trophic status of the water area; 2) to determine the granulometric composition of the bottom sediments and OM content in them; 3) to assess the composition and abundance of macrobenthic organisms; 4) to assess HM content in environmental components and macrobenthic organisms; and 5) to analyze the relationship between OM and HM accumulation in the bay bottom sediments, as well as to assess the environmental risk for the organisms inhabiting it and the toxicological risk driven by their consumption by humans.

## MATERIAL AND METHODS

**Sampling.** The data of the analysis of seawater, bottom sediments, and macrobenthos sampled in the Vladimir Bay (the Sea of Japan) (Fig. 1) in July 2014 served as the material for the work, as well as the authors' information on other water areas of the Primorsky Krai [Galysheva, 2010]. In total, 17 complex stations were surveyed, covering different bay areas down to a depth of 25 m (the depth limit is associated with diving descents). Seawater, bottom sediments, and macrobenthos were sampled at the entire net of stations. Water for microbiological analysis (due to limited possibility of ensuring the seeding sterility in the field) was sampled at particular stations (sta. 2, 4, 5, 8, and 11–14), which cover all bights of the bay, and at the control station (C) outside the bay. The sea urchin *Strongylocentrotus intermedius* (A. Agassiz, 1864) was chosen to serve as a bioindicator based on the following criteria: its high biomass and frequency of occurrence in the bay; sensitivity to various toxicants, *inter alia* HM, at all stages of the life cycle; and the fact that humans consume gonads [Vaschenko et al., 2005]. Sea urchins were sampled at sta. 2, 7, 10–12, and 14 out of the total quantitative macrobenthic samples. In total, we analyzed 26 samples of surface water (17 for hydrochemical parameters and 9 for microbiological indicators), 17 samples of bottom sediments, 102 quantitative samples of macrobenthos, and 18 samples of the sea urchin gonads. When carrying out chemical, ecological, and microbiological analysis, a triplicate of the measurement of each indicator was observed for each sample (Table 1). There were six macrobenthic samples at each station. Primary analysis was carried out in a field laboratory; final one, in the laboratories of the UNESCO international chair in marine ecology and the chair in soil science of the Institute of the World Ocean (School) of the Far Eastern Federal University (FEFU).

**Hydrochemical analysis.** Seawater was sampled from the surface 50-cm layer according to [Rukovodstvo po khimicheskomy analizu, 2003]. Oxygen dissolved in water and $BOD_5$ values were determined in accordance with the Winkler method. Permanganate oxidizability was established by the Skopintsev technique. To determine two forms of phosphorus (organic and mineral), the Morphy–Riley method was applied [Rukovodstvo po khimicheskomy analizu, 2003]. The analysis was carried out on the day of sampling, in triplicate for each indicator.

**Microbiological analysis.** Water was sampled from the surface layer into sterile plastic containers. The samples were transported to the field laboratory and analyzed on the day of sampling, in compliance with terms of storage and transportation according to international technical standards GOST 31861-2012 and GOST 31942-2012. The abundance of colony-forming heterotrophic microorganisms (hereinafter CHM) in 1 mL of water was determined applying the Koch plate count technique in a medium for marine microorganisms (MMM) with the addition of 1.5% agar [Yoshimizu, Kimura, 1976]. The abundance of metal-resistant forms in a CHM community was established by the plate count technique in the MMM with addition of metal salts at concentrations inhibiting the growth of sensitive forms of bacteria. As additives, metal chlorides (Zn, Cu, Cd, Ni, and Co) and lead nitrate were used [Bezverbnaya et al., 2003]. Resistance testing was carried out for each toxic additive (element by element) for all samples taken (in triplicate).

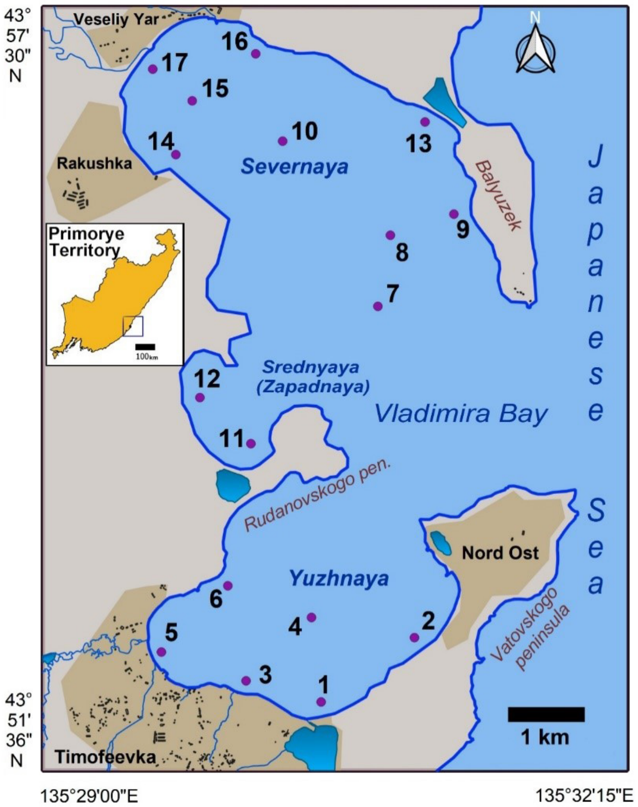

**Fig. 1.** The map of sampling in the Vladimir Bay

**Table 1.** Amount of factual material for chemical, ecological, and microbiological assessment

| Sample type | Surface water for hydrochemical analysis | Surface water for microbiological analysis | Bottom sediments | Sea urchins |
|---|---|---|---|---|
| Number of samples | 17 | 9 | 17 | 18 |
| Assessment parameters | DO, $BOD_5$, PO, $P_{total}$, $P_{min}$, $P_{org}$ (6 parameters) | CHM, groups of bacteria resistant to heavy metals – Cu, Cd, Ni, Co, Zn, Pb (7 parameters) | Granulometric composition, OM, Cu, Cd, Ni, Zn, Pb, Fe, Cr (9 parameters) | Cu, Cd, Ni, Zn, Pb, Cr (6 parameters) |
| Number of parameter determinations | 306 | 189 | 459 | 324 |

**Note:** abbreviations for the parameter names are given below in the text – in the sections describing different types of analysis.

**Granulometric analysis of bottom sediments.** The particle size of surface bottom sediments was determined mechanically (GOST 12536-79), while the amount of the finest fractions in samples with silt prevalence was established by the pipette method [Kachinsky, 1958]. The sediment type was determined by the dominant fraction.

**Analysis of organic matter content in bottom sediments.** The total content of organic carbon ($C_{org}$) was determined by the Tyurin method modified by the Central Research Institute of Agrochemical Services for Agriculture (GOST 26213-91).

**Assessment of composition and quantitative indicators of macrobenthos.** Sampling was carried out by scuba divers from a motor boat in the horizon of the upper sublittoral (3–27 m). At each station, epibenthos was sampled (using a hydrobiological frame with an area of 1 $m^2$), as well as infauna (using a diving gear grab with a capture area of 0.025 $m^2$). Sampling with each tool was carried out in triplicate. In total, there were 102 samples. Primary processing was carried out in the field laboratory. Unidentified animal species were fixed with a 4% formaldehyde solution, and plants were placed in herbarium; to each species, a code was assigned. Further identification was carried out in the laboratories of the FEFU Institute of the World Ocean, and the data of primary processing were supplemented. All indicators are recalculated to 1 $m^2$.

**Analysis of heavy metal content in bottom sediments and hydrobionts.** In bottom sediments, the total content of Fe, Cd, Ni, Cr, Cu, Zn, and Pb was determined. Sampled sediments were treated with a mixture of hydrochloric, hydrofluoric, perchloric, and nitric acids. The procedure was followed by determination of the total content of elements by flame atomic absorption spectrometry (AAS) (the regulatory document PND F 16.1:2.2:2.3.36-02) on a Shimadzu AA-6800 atomic absorption spectrophotometer (Japan).

At least three specimens of the sea urchin *S. intermedius* were selected for the analysis at each station from each macrobenthic sample in which this species occurred. A total of 18 specimens were sampled. Gonads were preliminarily dried in an oven at a temperature of +85 °C and grinded. Then, samples of 0.40–0.50 g were dried to a constant weight and subjected to acid decomposition with concentrated high-purity nitric acid. After that, these samples were transferred to a 2% nitric acid solution. HM content (Zn, Cu, Pb, Cr, Cd, and Ni) was determined by AAS on a Shimadzu AA-6800. The accuracy was controlled by analyzing standard samples (NIST 2976). The determination error did not exceed 15%.

**Calculation of accumulation factor and sediment quality guideline quotient.** Indices of HM contamination of bottom sediments (the contamination factor $C_f$ and the degree of contamination $C_d$) were determined in accordance with the algorithm proposed by Hakanson [1980] and successfully tested in various water areas [Chakraborty et al., 2014; Liang et al., 2018; Saroop, Tamchos, 2021; Vashchenko et al., 2010; *etc.*]:

$$C_f = C/C_\mathrm{b}\,, \tag{1}$$

$$C_d = \sum C_f\,, \tag{2}$$

where C is the mean concentration of a substance in bottom sediments;

$C_b$ is the background concentration of this substance in bottom sediments [Shulkin, 2004].

An integral assessment of potential toxicity of bottom sediments in the Vladimir Bay was carried out applying the sediment quality guideline quotient, SQG-Q [Birch, 2011; MacDonald et al., 1996; Vashchenko et al., 2010; *etc.*]. This index allows characterizing the toxicity of accumulated pollutants for marine hydrobionts in abiotic components of a studied biotope and is determined by the formula:

$$SQG-Q = \sum PEL-Q/n\,, \tag{3}$$

where ∑PEL-Q is the ratio of the mean concentration of a toxicant in bottom sediments to the PEL (probable effect level) for the same toxicant [MacDonald et al., 1996];

6n is the number of toxicants.

To determine individual carcinogenic risk when exposed to non-threshold toxicants, a calculator establishing incremental lifetime cancer risk, ILCR, was used (http://www.popstoolkit.com/tools/HHRA/Carcinogen.aspx). This coefficient is widely applied in Canada and the USA. ILCR index estimates incremental lifetime carcinogenic risk when a product is consumed over a certain period of time.

**Cartographic and statistical data processing.** Maps of the distribution of OM and HM content in bottom sediments were built by the kriging interpolation in Surfer software package (Golden Software). The final design of the cartographic material was carried out in QGIS program based on the digitization of raster topographic maps of the studied area, the access to which is free.

To determine the strength of the relationship between the analyzed indicators, we used the Pearson correlation coefficient, the assessment of linear approximation trends, and the value of the approximation reliability $R^2$.

## RESULTS

**Ecological assessment of the aquatic environment.** The well-being of natural waters and OM amount there can be estimated by values of the content of oxygen dissolved in water (hereinafter DO), BOD$_5$, permanganate oxidizability (hereinafter PO), and $P_{org}$ concentration. DO content depends on two groups of processes: some increase it (release during photosynthesis and adsorption from the atmosphere), while others decrease (consumption for the oxidation of organic substances). According to DO value, conclusions are drawn about the intensity of the self-purification in water bodies and the oxidation of dissolved OM [Khristoforova et al., 2015]. Chemical and environmental control of the Vladimir Bay state showed sufficient DO content in its surface waters and the lack of areas with the values below the summer standard of 6 mg $O_2 \cdot L^{-1}$ [Prikaz Minsel'khoza Rossii no. 552, 2016]. The mean value was 8.79 mg $O_2 \cdot L^{-1}$, and the minimum one was 7.22 mg $O_2 \cdot L^{-1}$ (Table 2).

**Table 2.** Hydrochemical parameters and trophic status of the Vladimir Bay surface waters (mean ± *SD*)

| Sta. | Temperature, °C | DO, mg $O_2 \cdot L^{-1}$ | BOD$_5$, mg $O_2 \cdot L^{-1}$ | PO, mg $O \cdot L^{-1}$ | $P_{min}$, µg$\cdot L^{-1}$ | $P_{org}$, µg$\cdot L^{-1}$ | $P_{total}$, mg$\cdot L^{-1}$ | Trophic status |
|---|---|---|---|---|---|---|---|---|
| 1 | +21.4 | 9.06 ± 0.04 | 1.04 ± 0.05 | 1.12 ± 0.02 | 27.78 ± 0.36 | 256.69 ± 6.72 | 284.47 ± 1.89 | O |
| 2 | +20.5 | 8.42 ± 0.07 | 1.13 ± 0.05 | 1.12 ± 0.07 | **109.12** ± 8.64 | 44.34 ± 1.16 | 153.46 ± 10.03 | M |
| 3 | +20.7 | 8.69 ± 0.22 | 1.46 ± 0.05 | 1.44 ± 0.28 | 13.89 ± 1.44 | 809.57 ± 67.76 | 823.46 ± 67.76 | O |
| 4 | +22.2 | 9.10 ± 0.18 | 1.80 ± 0.11 | 1.60 ± 0.07 | **75.39** ± 0.19 | 516.00 ± 32.89 | 591.39 ± 12.05 | M |
| 5 | +22.3 | 8.90 ± 0.07 | 1.43 ± 0.06 | 2.08 ± 0.04 | **61.50** ± 1.49 | 230.45 ± 12.81 | 291.95 ± 16.30 | M |
| 6 | +22.1 | 8.54 ± 0.28 | 0.81 ± 0.29 | 1.36 ± 0.18 | **93.25** ± 3.80 | 22.13 ± 9.52 | 115.38 ± 2.50 | M |
| 7 | +21.2 | 8.93 ± 0.11 | 1.37 ± 0.16 | 1.76 ± 0.09 | 23.81 ± 6.46 | 5.09 ± 0.06 | 28.09 ± 1.09 | O |
| 8 | +21.0 | 8.98 ± 0.08 | 1.51 ± 0.23 | 3.52 ± 0.22 | 43.65 ± 6.08 | 87.36 ± 14.81 | 131.01 ± 5.95 | O |

Continue on the next page…

| Sta. | Temperature, °C | DO, mg $O_2 \cdot L^{-1}$ | $BOD_5$, mg $O_2 \cdot L^{-1}$ | PO, mg $O \cdot L^{-1}$ | $P_{min}$, $\mu g \cdot L^{-1}$ | $P_{org}$, $\mu g \cdot L^{-1}$ | $P_{total}$, $mg \cdot L^{-1}$ | Trophic status |
|---|---|---|---|---|---|---|---|---|
| 9 | +21.4 | 9.23 ± 0.12 | 1.27 ± 0.12 | 1.92 ± 0.10 | **53.57** ± 9.94 | 489.17 ± 21.27 | 542.74 ± 22.76 | M |
| 10 | +20.8 | 8.84 ± 0.14 | 1.46 ± 0.10 | 0.16 ± 0.10 | **51.58** ± 8.56 | 71.94 ± 10.24 | 123.52 ± 10.19 | M |
| 11 | +19.0 | 9.19 ± 0.16 | 2.24 ± 0.11 | 3.36 ± 0.15 | **69.44** ± 13.25 | 42.85 ± 7.63 | 112.29 ± 5.62 | M |
| 12 | +19.0 | 9.26 ± 0.35 | 1.38 ± 0.2 | 1.36 ± 0.14 | **71.42** ± 5.53 | 190.59 ± 12.6 | 262.01 ± 12.24 | M |
| 13 | +21.0 | 8.77 ± 0.40 | 1.32 ± 0.11 | 3.60 ± 0.51 | 39.68 ± 5.01 | 8.98 ± 0.11 | 48.66 ± 5.12 | O |
| 14 | +19.9 | 7.22 ± 0.26 | 0.34 ± 0.02 | 2.24 ± 0.09 | 41.66 ± 4.01 | 194.15 ± 13.44 | 235.81 ± 17.65 | O |
| 15 | +19.8 | 8.82 ± 0.15 | 1.02 ± 0.19 | 1.28 ± 0.26 | **63.49** ± 11.13 | 45.06 ± 16.96 | 108.55 ± 23.11 | M |
| 16 | +20.2 | 8.48 ± 0.44 | 2.71 ± 0.20 | 2.88 ± 0.15 | 21.82 ± 2.01 | 15.61 ± 3.18 | 36.98 ± 4.80 | O |
| 17 | +19.1 | 8.50 ± 0.34 | 3.90 ± 0.43 | 2.56 ± 0.08 | 31.74 ± 5.37 | 192.84 ± 17.95 | 224.58 ± 14.82 | O |

**Note:** M, mesotrophic; O, oligotrophic. The values of the concentration of mineral phosphorus compounds for the stations with mesotrophic status are highlighted in bold.

$BOD_5$ indicates the presence of easily oxidizable OM in water, most often being waste products of hydrobionts or entering with municipal wastewater. At most stations, $BOD_5$ levels corresponded to the standard of 2.1 mg $O_2 \cdot L^{-1}$ [Prikaz Minsel'khoza Rossii no. 552, 2016], varying within 0.34–1.80 mg $O_2 \cdot L^{-1}$. Only at three stations (sta. 11, 16, and 17) the values of biochemical oxygen consumption exceeded the standard and amounted to 2.24, 2.71, and 3.90 mg $O_2 \cdot L^{-1}$, respectively.

PO reflects the content of hardly oxidizable substances in water (decomposition products of dead organisms, as well as oil, heavy fuel oil, and other hydrocarbons entering water with surface flushing and runoff). For the purest waters, PO values usually do not exceed 3–5 mg $O \cdot L^{-1}$ and may vary by season [Khristoforova et al., 2015]. The values of PO ranged from 0.16 to 3.60 mg $O \cdot L^{-1}$, reaching a maximum at sta. 13. To date, PO value is not standardized for fishery basins.

Phosphorus is one of the elements determining the productivity of natural waters. The concentration of its organic ($P_{org}$) and mineral ($P_{min}$) compounds is an important component of the OM balance in the aquatic environment. During the period of our study, summer values of total concentrations of phosphorus compounds ($P_{total}$) were high. At 50% of the analyzed stations, those exceeded 200 $\mu g \cdot L^{-1}$. The contribution of organic and mineral forms of phosphorus to its total content was different: at 8 stations, where $P_{total}$ values were higher than 200 $\mu g \cdot L^{-1}$, its organic compounds with concentrations above 100 $\mu g \cdot L^{-1}$ prevailed. A maximum of 809.57 $\mu g \cdot L^{-1}$ was detected in the Yuzhnaya Bay, at sta. 6. In accordance with the water quality standards for fishery basins [Prikaz Minsel'khoza Rossii no. 552, 2016], the trophic status can be judged by the concentration of phosphate ions ($P_{min}$): up to 50 $\mu g \cdot L^{-1}$ phosphates are characteristic of oligotrophic waters; 50–150 $\mu g \cdot L^{-1}$, of mesotrophic (those are highlighted in bold in Table 2); and more than 200 $\mu g \cdot L^{-1}$, of eutrophic. Eutrophic values were not revealed. The ratio of the stations with oligotrophic and mesotrophic status was approximately 1 : 1 (see Table 2).

CHM can serve as a microbiological indicator of the total OM level in the aquatic environment, describing water quality of the marine ecosystem and allowing both to rank OM level in the environment and to classify pollution. According to a saprobity scale, waters with CHM content of up to $10^3$ cells·mL$^{-1}$ are oligosaprobic; up to $10^5$, mesosaprobic (subdivided into categories of a lower rank); with abundance of $10^6$ cells·mL$^{-1}$ and more, polysaprobic [Obshchaya i sanitarnaya mikrobiologiya, 2004]. The ecological and bacteriological assessment of surface waters revealed a uniform distribution of CHM abundance at a level of $10^3$ CFU·mL$^{-1}$ in the surface layer (Table 3). This allows to consider waters as α-mesosaprobic, *i. e.*, enriched with OM. The control station outside the bay was characterized by the same level of heterotrophic bacteria.

**Table 3.** Abundance of colony-forming heterotrophic microorganisms (CHM) in the Vladimir Bay surface waters

| Station | 2 | 4 | 5 | 8 | 11 | 12 | 13 | 14 | Control (C) |
|---|---|---|---|---|---|---|---|---|---|
| CHM (× $10^3$ CFU·mL$^{-1}$) | 1.5 | 5.3 | 7.2 | 1.2 | 1.5 | 5.2 | 3.8 | 7.3 | 4.5 |

**Organic matter in bottom sediments.** The content of organic carbon ($C_{org}$) in soft sediments varied from 0.11 to 3.64% *per* 100 g of dry sample weight. The minimum values were recorded in pebbles (fine psephite) and sands (fine-grained psammite) of the shallowest stations (depth of 3–5 m), while the maximum values were registered in silts (siltstones) at depths of 10, 15, 20, and 25 m (Tables 4, 5, Fig. 2).

The spatial distribution of $C_{org}$ values in the surface layer of the bay bottom sediments has a characteristic manifestation and is determined by a combination of factors: remoteness from the coast, bottom topography, intensity of hydrodynamics and sedimentogenesis, and granulometric composition of bottom sediments. The maximums are confined to central areas of the Severnaya and Yuzhnaya bays and to the apex of the Srednyaya Bay, *i. e.*, to zones of accumulation with a prevalence of siltstone fractions and the lowest water dynamics. There, active sedimentation occurs of both small remains of organisms and OM – suspended and dissolved in water.

**Table 4.** Granulometric composition of the Vladimir Bay bottom sediments (excluding silt fractions)

| Sta. | Depth, m | Content of sediment fractions (mm), % | | | | | | | | Sediment type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | > 10 | 10–5 | 5–2 | 2–1 | 1–0.5 | 0.5–0.25 | 0.25–0.1 | < 0.1 | |
| 1 | 3 | 0 | 0.5 | 0.5 | 0.3 | 0.6 | 21 | 67.3 | 8.9 | Fine-grained psammite |
| 2 | 3 | 0 | 0 | 0.7 | 0.7 | 0.5 | 38.3 | 59.7 | 0.1 | Fine-grained psammite |
| 3 | 3 | 0 | 0 | 2.1 | 2.8 | 3.3 | 24.1 | 50.8 | 16.1 | Fine-grained psammite |
| 5 | 4 | 0 | 0 | 3.7 | 5.7 | 5.7 | 26.5 | 55.5 | 2.9 | Fine-grained psammite |
| 6 | 6 | 0 | 0 | 2.5 | 12.3 | 17.3 | 39.5 | 26.6 | 1.8 | Coarse-grained psammite |
| 8 | 27 | 0 | 0 | 0.9 | 0.6 | 1.3 | 17.8 | 75 | 3.7 | Fine-grained psammite |
| 9 | 5 | 0 | 0 | 0.8 | 1.2 | 3 | 24.7 | 66.5 | 3 | Fine-grained psammite |
| 10 | 20 | 39.8 | 2.1 | 4.4 | 5.9 | 5.1 | 14.3 | 26.1 | 2.3 | Fine psephite |
| 13 | 5 | 0 | 0 | 1.6 | 1.8 | 1.6 | 4 | 83.3 | 6.8 | Fine-grained psammite |
| 14 | 5 | 68.5 | 0 | 4.6 | 8 | 6.5 | 8.1 | 2.8 | 1.5 | Fine psephite |
| 17 | 5 | 0 | 0 | 0.6 | 4.3 | 8.8 | 23.8 | 56.9 | 4.8 | Fine-grained psammite |

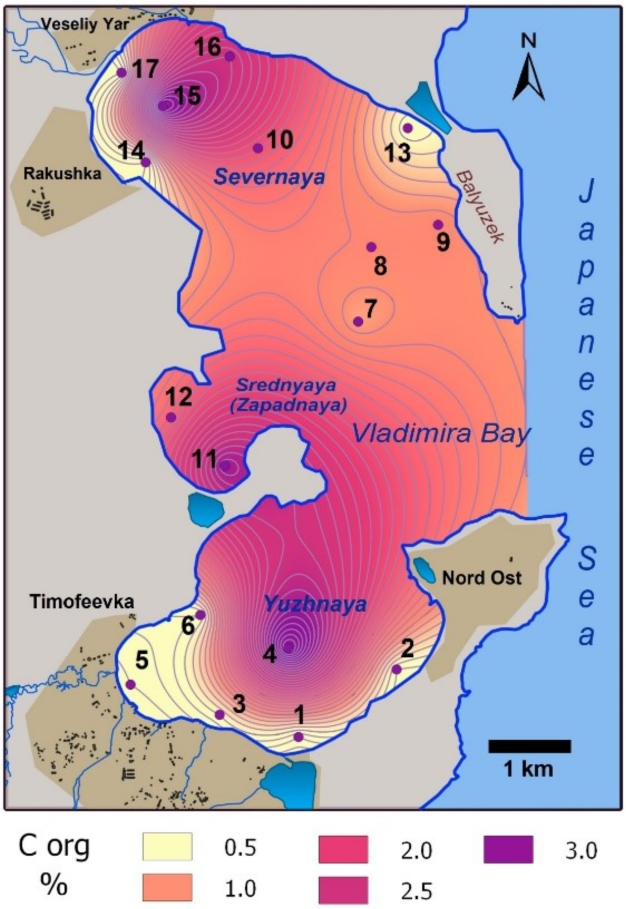

**Fig. 2.** Map of organic carbon ($C_{org}$) distribution in the Vladimir Bay bottom sediments

**Table 5.** Granulometric composition of silt fractions of the Vladimir Bay bottom sediments

| Sta. | Depth, m | Content of sediment fractions (mm), % | | | | | | Sum of particles | | Sediment type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.00–0.25 | 0.25–0.05 | 0.05–0.01 | 0.01–0.005 | 0.005–0.001 | < 0.001 | < 0.01 | > 0.01 | |
| 4 | 21 | 1 | 36 | 30 | 18 | 4 | 11 | 33 | 67 | Pelitic siltstone |
| 7 | 24 | 30 | 43 | 11 | 4 | 7 | 5 | 16 | 84 | Fine-grained psammite |
| 11 | 5 | 1 | 12 | 50 | 18 | 10 | 9 | 37 | 63 | Psammitic siltstone |
| 12 | 10 | 1 | 66 | 20 | 7 | 2 | 4 | 13 | 87 | Psammitic siltstone |
| 15 | 18 | 1 | 15 | 59 | 13 | 7 | 5 | 25 | 75 | Pelitic siltstone |
| 16 | 7 | 1 | 28 | 61 | 3 | 2 | 5 | 10 | 90 | Psammitic siltstone |

**Characteristics of macrobenthos.** In total, 15 macrobenthic taxa of the highest rank were identified: 11 phyla of marine animals (Porifera, Cnidaria, Nemertea, Annelida, Sipuncula, Arthropoda, Mollusca, Brachiopoda, Bryozoa, Echinodermata, and Chordata) and 4 divisions of plants (Rhodophyta, Ochrophyta, Chlorophyta, and Tracheophyta). The total species richness of the macrobenthos of the Vladimir Bay sublittoral includes at least 63 species: 45 species of invertebrates and 18 species of plants. The highest species richness was recorded in the classes Florideophyceae, Gastropoda, Bivalvia, Malacostraca, Polychaeta, Phaeophyceae, and Asteroidea.

The mean biomass of macrobenthos at stations varied from 40.7 to 2,442.3 g·m$^{-2}$. The minimum value was registered at sta. 4, in the central Yuzhnaya Bay, where one Bivalvia species and one Polychaeta species were noted. At 30% of stations, the mean biomass reached 500 g·m$^{-2}$, and the rest were characterized by high values (from 500 g·m$^{-2}$ to maximum ones). Thus, the general background of biomass values can be described as evenly high. The values of mean biomass for the Vladimir Bay are similar to those for the Vostok Bay (Table 6).

**Table 6.** The values of mean biomass and abundance of macrobenthos in different water areas off the Primorsky Krai marine coast

| Area | Mean biomass ± *SD*, g·m$^{-2}$ | Mean settlement density ± *SD*, ind.·m$^{-2}$ |
|---|---|---|
| Udobnaya Bight | 4,523.9 ± 9,324.2 | 108.5 ± 90.3 |
| Kievka Bight | 1,634.9 ± 954.5 | 159.1 ± 124.6 |
| Rudnaya Bight | 1,173.5 ± 1,268.6 | 77.0 ± 54.4 |
| Nakhodka Bay (except for Nakhodka Bight) | 1014.9 ± 825.2 | 250.6 ± 365.4 |
| Vostok Bay | 864.5 ± 662.0 | 170.2 ± 762.0 |
| Vladimir Bay | 841.2 ± 583.0 | 57.4 ± 12.3 |
| Trinity Bay | 473.7 ± 627.1 | 93.7 ± 80.4 |
| Zolotoy Rog Bay | 204.2 ± 50.1 | 115.0 ± 4.9 |

**Note:** *SD* is standard deviation. The data on all the water areas, except for the Vladimir Bay, are given according to [Galysheva et al., 2018].

The dominant macrozoobenthic species with mean biomass at station of more than 100 g·m$^{-2}$ included species typical for the Sea of Japan: sea urchin *S. intermedius* (frequency of occurrence was 44%); starfish *Asterina pectinifera* (Muller & Troschel, 1842) (36%); bivalves *Mizuhopecten yessoensis* (Jay, 1857) (12%), *Crenomytilus grayanus* (Dunker, 1853) (8%), and *Pododesmus macrochisma* (Deshayes, 1839) (4%); and holothurian *Cucumaria japonica* Semper, 1868 (4%). In macrophytobenthos, the following species prevailed: brown algae *Desmarestia viridis* (O. F. Müller) J. V. Lamouroux, 1813 (frequency of occurrence was 24%), *Costaria costata* (C. Agardh) De A. Saunders, 1895 (16%), and *Saccharina japonica* (Areschoug) C. E. Lane, C. Mayes, L. D. Druehl & G. W. Saunders, 2006 (12%), as well as seagrasses *Zostera marina* Linnaeus, 1753 (16%) and *Phyllospadix iwatensis* Makino, 1931 (12%).

**Heavy metals in the environment.** The level of HM content in surface waters was determined by the abundance of metal-resistant microorganisms, which, due to their exceptional specificity, allow to assess properly environmental pollution with these elements [Bezverbnaya et al., 2003; Kalitina et al., 2015]. In general, lead-resistant bacteria turned out to be the least common group (those were detected at two stations only). The most common ecological and trophic group occurring at all stations in high concentrations (from $10^2$ to $10^3$ CFU·mL$^{-1}$) were cadmium-resistant bacteria (Table 7).

Interestingly, Cu- and Zn-resistant microorganisms were also found at almost all stations and formed a general background with lower cell concentration. However, at the control station outside the bay, the abundance of Zn-resistant bacteria was two orders of magnitude higher. In general, bacteria resistant to several metals were simultaneously registered in microbial communities of the Vladimir Bay surface waters at most stations. The least common were Pb-, Ni-, and Co-resistant bacteria.

**Table 7.** Distribution of the abundance of heavy metal resistant microorganisms (CFU·mL$^{-1}$) in the Vladimir Bay surface waters (mean ± *SD*)

| Sta. | Ecological and trophic groups of microorganisms | | | | | |
|---|---|---|---|---|---|---|
| | Cu | Cd | Ni | Co | Zn | Pb |
| 2 | $(7.0 \pm 0.09) \times 10^2$ | $(1.8 \pm 0.1) \times 10^2$ | $(1.2 \pm 0.28) \times 10^2$ | 0 | $(2.0 \pm 0.12) \times 10$ | $(5.0 \pm 0.1) \times 10^2$ |
| 4 | $(2.4 \pm 0.09) \times 10^3$ | $(1.8 \pm 0.17) \times 10^3$ | $(2.0 \pm 0.1) \times 10^2$ | 0 | $(5.0 \pm 0.2) \times 10$ | $(2.0 \pm 0.02) \times 10$ |
| 5 | $(1.02 \pm 0.12) \times 10^3$ | $(2.08 \pm 0.1) \times 10^3$ | 0 | $(8.2 \pm 0.06) \times 10^2$ | $(2.0 \pm 0.02) \times 10$ | 0 |
| 8 | $(1.08 \pm 0.08) \times 10$ | $(1.0 \pm 0.03) \times 10^2$ | 0 | 0 | 0 | 0 |
| 11 | $(1.0 \pm 0.28) \times 10^2$ | $(1.7 \pm 0.02) \times 10^2$ | $(2.3 \pm 0.1) \times 10^2$ | $(4.0 \pm 0.2) \times 10^2$ | $(2.0 \pm 0.04) \times 10$ | 0 |
| 12 | $(1.1 \pm 0.1) \times 10^2$ | $(9.0 \pm 0.1) \times 10^2$ | $(2.0 \pm 0.1) \times 10^2$ | $(1.0 \pm 0.31) \times 10^3$ | $(1.2 \pm 0.05) \times 10$ | 0 |
| 13 | 0 | $(1.0 \pm 0.2) \times 10^2$ | $(6.0 \pm 0.3) \times 10$ | 0 | $(2.0 \pm 0.02) \times 10$ | 0 |
| 14 | $(3.5 \pm 0.3) \times 10^2$ | $(1.8 \pm 0.12) \times 10^3$ | 0 | $(1.8 \pm 0.2) \times 10^2$ | 0 | 0 |
| C | $(1.6 \pm 0.3) \times 10^2$ | $(9.0 \pm 0.2) \times 10^2$ | 0 | $(2.0 \pm 0.08) \times 10$ | $(1.1 \pm 0.3) \times 10^2$ | 0 |

When assessing the ecological state of bottom sediments, it is recommended to analyze the total content and the concentration of acid-soluble (conditionally total) and mobile forms of HM. Depending on HM form under determination, pollution is assessed *via* comparison with the clarke content of the element in the Earth's crust, or with maximum permissible concentration (MPC) and approximate permissible concentration established for soils, or with a background. We determined the total concentrations compared with the clarke content of the element in the Earth's crust [Vinogradov, 1962] and background concentrations for Peter the Great Bay [Shulkin, 2004].

The concentrations of the analyzed compounds (Cd, Ni, Pb, Cu, Zn, Fe, and Cr) varied in different ranges. Specifically, the multiplicity of the difference between the maximum and minimum values ranged from 1.9 to 70.3, indicating the most even background of bottom concentrations for Cr and the most varying background for Cu (Table 8). For Pb, Fe, and Zn, the max/min ratio was 3.6, 4.6, and 6.7 times. There was more than 13-fold variation in the range of Ni content in bottom sediments. The highest concentrations of HM compounds in bottom sediments were as follows: Cd, 0.75 μg·g$^{-1}$; Ni, 33.0; Pb, 10.9; Cu, 21.1; Zn, 115.3; Fe, 17,437; and Cr, 16.26 μg·g$^{-1}$.

For almost all elements, the total content of HM in bottom sediments was below the clarke value. An exception was Cd: for this element, an excess of clarke values was noted at many stations. Maximum concentrations of Cd occurred at stations with the highest values of $C_{org}$. For several stations, an excess of the clarke value for Zn was registered. Interestingly, the maximums are associated with increased OM concentrations in bottom sediments and the prevalence of the fine silt fraction as well. Apparently, this contributes to accumulation of these elements from the aquatic environment and concentration in accumulative zones of the bay. For all studied stations, iron and zinc prevail in the range of concentrations, while the content of cadmium is always minimum (compared to that of other metals). The general sequence of decreasing HM concentrations in bottom sediments is as follows: Fe > Zn > Cu > Cr > Pb > Ni > Cd. At several stations, Cr and Pb "switched places" in this sequence, while the rest of the pattern was repeated. When comparing our data with the descending clarke sequence of these elements, Fe > Zn > Cr > Ni > Cu > Pb > Cd [Vinogradov, 1962], it can be seen that positions of iron, zinc, and cadmium coincide in two sequences.

**Table 8.** Concentrations of heavy metals (μg·g$^{-1}$) in the Vladimir Bay bottom sediments (mean ± *SD*)

| Station | Cd | Ni | Pb | Cu | Zn | Fe | Cr |
|---|---|---|---|---|---|---|---|
| 1 | **0.75** ± 0.08 | 2.5 ± 0.1 | 3.0 ± 0.2 | 10.9 ± 0.1 | 26.7 ± 0.5 | 4,681 ± 154 | 7.45 ± 0.11 |
| 2 | 0.00 | 2.5 ± 0.2 | 3.4 ± 0.2 | 10.2 ± 4.0 | 34.9 ± 1.0 | 6,893 ± 2,579 | 7.80 ± 0.04 |
| 3 | 0.45 ± 0.03 | 3.8 ± 0.2 | 7.5 ± 0.2 | 10.3 ± 0.1 | 74.5 ± 0.4 | 13,168 ± 151 | 4.43 ± 0.38 |
| 4 | 0.69 ± 0.03 | 3.7 ± 0.4 | 8.3 ± 0.1 | 9.4 ± 0.1 | **115.3** ± 8.7 | **17,437** ± 169 | **16.26** ± 0.11 |
| 5 | 0.35 ± 0.01 | 3.8 ± 0.2 | 7.4 ± 0.1 | 10.1 ± 0.2 | 61.6 ± 0.4 | 14,952 ± 138 | 2.74 ± 0.67 |
| 6 | 0.19 ± 0.01 | 3.2 ± 0.1 | 4.5 ± 0.1 | 12.7 ± 1.4 | 45.6 ± 0.8 | 6,721 ± 19 | 2.04 ± 0.13 |
| 7 | 0.00 | 2.9 ± 0.1 | 4.1 ± 0.1 | 2.0 ± 0.1 | 30.3 ± 0.1 | 10,571 ± 165 | 12.00 ± 0.27 |
| 8 | 0.42 ± 0.03 | **33.0** ± 0.7 | 9.2 ± 0.1 | 16.2 ± 0.1 | 97.4 ± 0.2 | 14,708 ± 77 | 9.00 ± 0.44 |
| 9 | 0.26 ± 0.01 | 2.8 ± 0.1 | 6.3 ± 0.1 | 5.8 ± 0.1 | 35.8 ± 0.2 | 7,085 ± 34 | 3.32 ± 0.20 |
| 10 | 0.11 ± 0.01 | 4.6 ± 0.1 | 8.5 ± 0.1 | 1.6 ± 0.1 | 17.3 ± 0.1 | 4,289 ± 4 | 11.19 ± 0.07 |
| 11 | 0.73 ± 0.01 | 2.6 ± 0.1 | 3.2 ± 0.1 | 10.8 ± 0.1 | 35.7 ± 0.1 | 5,533 ± 19 | 5.59 ± 0.11 |
| 12 | 0.72 ± 0.01 | 2.7 ± 0.2 | 3.3 ± 0.1 | 11.2 ± 0.1 | 36.3 ± 0.1 | 5,609 ± 3 | 5.91 ± 0.09 |
| 13 | 0.35 ± 0.01 | 3.4 ± 0.1 | 7.1 ± 0.2 | **21.1** ± 0.4 | 28.5 ± 0.1 | 6,262 ± 8 | 2.04 ± 0.04 |
| 14 | 0.64 ± 0.01 | 2.8 ± 0.1 | 3.4 ± 0.1 | 10.7 ± 0.1 | 65.3 ± 0.1 | 11,622 ± 216 | 6.63 ± 0.07 |
| 15 | 0.44 ± 0.01 | 5.0 ± 0.1 | **10.9** ± 0.1 | 18.5 ± 0.1 | 113.6 ± 0.5 | 12,378 ± 12 | 13.46 ± 0.07 |
| 16 | 0.06 ± 0.01 | 3.2 ± 0.1 | 3.1 ± 0.2 | 1.4 ± 0.2 | 22.5 ± 0.1 | 3,781 ± 32 | 0.66 ± 0.07 |
| 17 | 0.19 ± 0.01 | 3.4 ± 0.1 | 5.8 ± 0.2 | 0.3 ± 0.1 | 39.2 ± 0.1 | 11,052 ± 43 | 2.68 ± 0.07 |
| Max/min | 13.6 | 13.2 | 3.6 | 70.3 | 6.7 | 4.6 | 24.64 |
| Clarke | 0.13 | 58 | 16 | 47 | 83 | 46,500 | 83 |
| $\mathrm{BG}_{\mathrm{sands}}$ | 0.1 ± 0.05 | 15 ± 3 | 20 ± 5 | 8 ± 2 | 30 ± 7 | – | – |
| $\mathrm{BG}_{\mathrm{aleuropelite}}$ | 0.2 ± 0.1 | 45 ± 5 | 35 ± 5 | 2 ± 3 | 100 ± 10 | – | – |

**Note:** clarke content is given according to [Vinogradov, 1962]; background concentrations of metals (BG) are given for the coastal bottom sediments of the northwestern Sea of Japan [Shulkin, 2004]. Maximum concentrations of heavy metal compounds are highlighted in bold.

HM spatial distribution over the surface of the Vladimir Bay bottom is characterized by four types. "Unipolar" is revealed for Ni; it has one area of maximum values: at the Severnaya Bay mouth, at a depth of 25 m (Fig. 3). "Bipolar" distribution has two areas of maximum concentrations; it is registered for Pb, Cr, and Cu. "Tripolar" is typical for the most common metals: Zn and Fe; the maximums of their concentrations are recorded in the zones of the most pronounced sedimentogenesis. "Vector" distribution is noted for Cd; it is characterized by a clear gradient of its concentration decrease in bottom sediments inward from the coast in almost all areas of the bay.

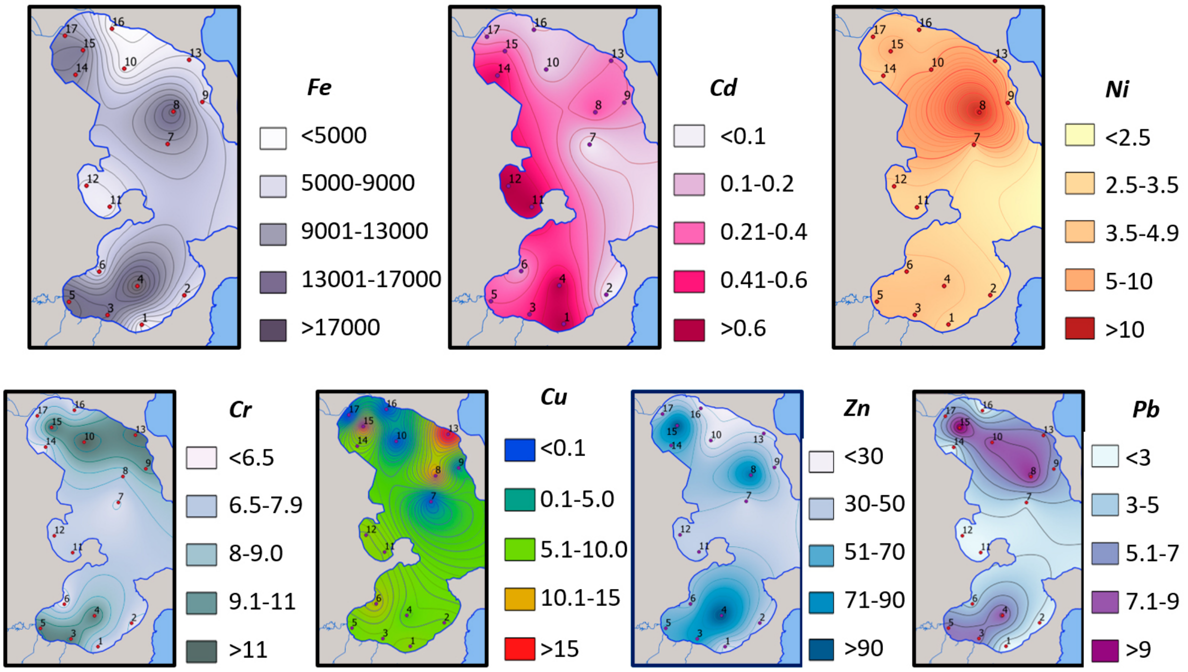

**Fig. 3.** Maps of the distribution of heavy metal concentrations ($\mu g \cdot g^{-1}$) in the Vladimir Bay bottom sediments (the stations are indicated on the maps with numbers)

**Heavy metals in the sea urchin gonads.** In *S. intermedius* gonads, the concentrations of the same six HM were determined, as in bottom sediments (excluding Fe). The range of concentrations was as follows: Pb, 2.36–13.46 $\mu g \cdot g^{-1}$ dry weight; Cd, 0.22–0.62; Cu, 3.05–5.12; Cr, 1.34–2.32; Ni, 0.89–4.65; and Zn, 32.51–118.94 $\mu g \cdot g^{-1}$ dry weight. This range was narrower than that for bottom sediments. The maximum variability (5.70 times) was recorded for Pb content (Table 9). Out of all HM, the highest concentrations are typical for zinc; the lowest ones, for cadmium. Nickel, copper, lead, and chromium occupy an intermediate position, replacing each other in a sequence of descending concentrations at different stations. On average, decreasing concentrations form the following sequence: Zn > Pb > Cu > Ni > Cr > Cd.

**Table 9.** Heavy metal concentrations in *Strongylocentrotus intermedius* gonads ($\mu g \cdot g^{-1}$ dry weight) from the Vladimir Bay

| Station | Metals | | | | | |
|---|---|---|---|---|---|---|
| | Pb | Cd | Cu | Cr | Ni | Zn |
| 2 | 2.36 ± 0.44 | 0.36 ± 0.02 | 3.30 ± 0.77 | 1.58 ± 0.21 | 2.65 ± 1.20 | 70.11 ± 16.58 |
| 7 | 3.89 ± 0.85 | **0.62 ± 0.02** | 3.21 ± 0.48 | **2.32 ± 0.31** | 2.58 ± 0.67 | 101.83 ± 21.37 |
| 10 | 5.89 ± 2.23 | 0.51 ± 0.08 | 3.99 ± 0.49 | 2.16 ± 0.14 | 2.05 ± 0.58 | **118.94 ± 28.67** |
| 11 | 2.82 ± 0.21 | 0.28 ± 0.03 | 3.05 ± 0.37 | 1.38 ± 0.16 | **4.65 ± 2.21** | 84.85 ± 15.68 |
| 12 | 3.01 ± 0.21 | 0.59 ± 0.08 | 3.58 ± 0.25 | 1.68 ± 0.11 | 1.54 ± 0.58 | 92.41 ± 15.81 |
| 14 | **13.46 ± 1.19** | 0.22 ± 0.03 | **5.12 ± 0.06** | 1.34 ± 0.47 | 0.89 ± 0.71 | 32.51 ± 21.47 |
| Max/min | 5.70 | 2.81 | 1.68 | 1.73 | 5.22 | 3.66 |

**Note:** the mean values based on the results of three replicates and *SD* are given. Maximum values are highlighted in bold.

## DISCUSSION

In general, the oxygen regime of the Vladimir Bay (DO and $BOD_5$) is normal; there are no hypoxia zones. Nevertheless, in the apex of the Srednyaya Bay and in the apex of the Severnaya Bay, areas with a high level of $BOD_5$ were recorded, which determines intensive DO consumption for the oxidation of easy-to-decompose OM. When analyzing the trace of hard-to-decompose OM (*inter alia* household wastewater from villages, containing synthetic surfactants and other compounds), it can be concluded that this trace is not significant in terms of PO values.

The values of the content of phosphorus in organic forms are quite high and reach a maximum, 809.57 $\mu g \cdot L^{-1}$, in the Yuzhnaya Bay, near the village of Timofeevka. However, the concentrations of mineral phosphorus compounds ($P_{min}$), according to which the trophic status is classified, generally characterize the Vladimir Bay waters in summer as mesotrophic. An even CHM distribution in surface waters at the level of $10^3$ $CFU \cdot mL^{-1}$ (including the control station outside the bay) indicates α-mesosaprobic level of OM [Obshchaya i sanitarnaya mikrobiologiya, 2004]. Comparison of microbiological data with a trophicity scale shows oligomesotrophic level, and this coincides with our direct chemical assessment. Thus, the water mass of the bay in summer has mesotrophic level of OM, which is sufficient to maintain the abundance of organisms in biocenoses formed there. Moreover, the water mass successfully "utilizes" incoming OM of both natural and household origin. In general, villages and the coastal strip insignificantly affect the formation of eutrophication conditions.

The content of organic carbon in soft sediments reached 3.64% of dry sample weight. In the series of maximum $C_{org}$ values obtained for different water areas of the Primorsky Krai marine coast [Galysheva, 2010], the Vladimir Bay is between the Nakhodka Bay (except for Nakhodka Bight) and the Amur Bay (Fig. 4).

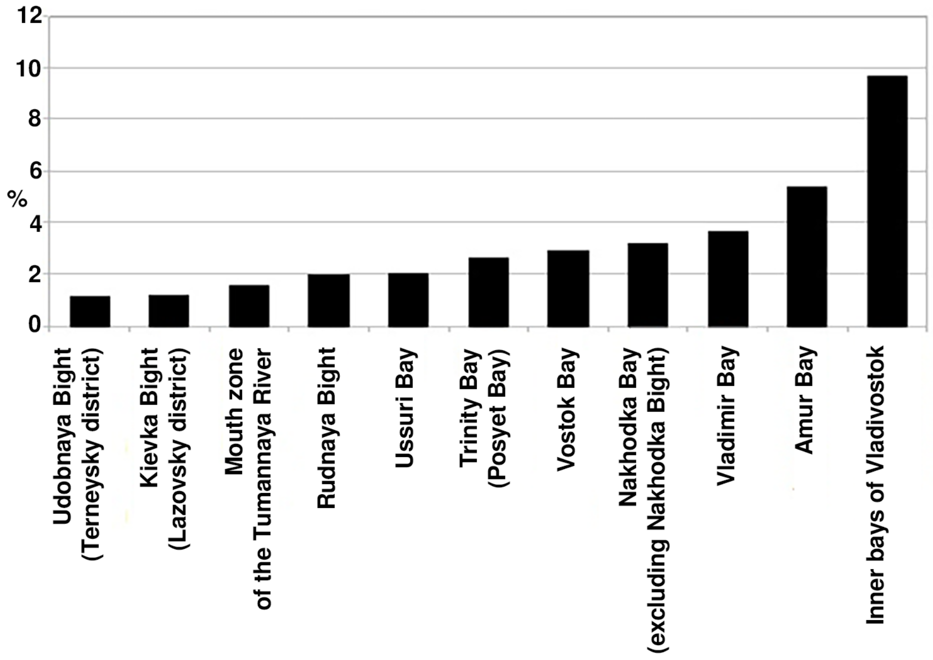

**Fig. 4.** Range of the maximum values of organic carbon ($C_{org}$) content (% *per* 100 g of dry sample weight) recorded in the bottom sediments off the Primorsky Krai marine coast

Accordingly, the Vladimir Bay can be attributed to marine areas with a relatively high OM content in soft sediments. This indicates the severity of the processes of OM accumulation in the bay ecosystem even under conditions of oligomesotrophic level of seawater. The reasons for the formation of conditions for OM accumulation in the environment of the bay are natural, since the anthropogenic load there is weak (compared to the load on the Nakhodka and Amur bays).

Metal-resistant microorganisms, a part of heterotrophs, can help in assessing the level and nature of the technogenic pressure on the bay surface waters. The abundance of Cd-, Pb-, Co-, and Ni-resistant forms of bacteria reflects mainly the technogenic load on the environment from which those are sown, while the abundance of Cu- and Zn-resistant forms serves as an indicator of biological natural or anthropogenic load [Khristoforova et al., 2018]. Copper and zinc are part of the molecules of living organisms; Cu and Zn enter the environment during the decomposition of waste products or the death of the organisms themselves. Municipal wastewater with faecal effluent also contains these microelements at high concentrations. In general, the microbial association of the Vladimir Bay was mostly characterized as Cd-resistant. When checking ecological and physiological properties of colonies grown on a Cd-enriched substrate, more than half of cultivated heterotrophic bacteria showed a high degree of resistance to this HM.

To transit from microbiological data to an ecological assessment of the degree of pollution for the marine area, a scale is used that shows correlations between the microbial index (the proportion of a particular metal-resistant group as a percentage of CHM) and MPC of heavy metals for fishery basins [Bezverbnaya et al., 2003]. When recalculating the absolute abundance of metal-resistant groups in the format of the microbial index and comparing it with a scale for assessing the degree of contamination, we can conclude as follows. A relatively even background of Cd-resistant bacteria indicates a significant degree (> 3 MPC) of cadmium contamination in the Vladimir Bay waters (Table 10). “Vector” distribution of Cd concentrations in bottom sediments shows the gradient of its content decrease from the coast towards the central area of the bay, and this gives grounds to assume the presence of a coastal source of cadmium.

**Table 10.** Assessment of the ecological state of the Vladimir Bay surface waters on a scale of correlation of the microbial index with the maximum permissible concentration (MPC) for heavy metals in fishery basins

| Station | Cd | Cu | Zn | Pb | Ni | Co |
|---|---|---|---|---|---|---|
| 2 | ▲ | Δ | * | * | * | ○ |
| 4 | ▲ | * | * | ○ | * | ○ |
| 5 | ▲ | * | * | ○ | ○ | * |
| 8 | Δ | ○ | ○ | ○ | ○ | ○ |
| 11 | ▲ | ○ | * | ○ | Δ | Δ |
| 12 | Δ | ○ | * | ○ | * | * |
| 13 | Δ | ○ | * | ○ | * | ○ |
| 14 | ▲ | ○ | ○ | ○ | ○ | * |
| C | Δ | ○ | * | ○ | ○ | ○ |

**Note:** C denotes the control station outside the bay. The symbols indicate the degree of pollution: ○, background (< MPC); *, insignificant (≈ 1 MPC); Δ, tangible (1–3 MPC); ▲, significant (> 3 MPC).

The correlation analysis of the relationship between OM in the bay bottom sediments and HM concentrations, grain-size characteristics, and sediment depth confirmed known dependences of an increase in OM content with a rise in proportion of the finest fractions and stabilization of hydrodynamic conditions associated with depth (Fig. 5) [Romankevich et al., 2009]. A positive correlation between an increase in HM content due to a rise in $C_{org}$ concentration in bottom sediments (at low values of the Pearson coefficient) was revealed for four elements: Zn, Cd, Cr, and Pb. The data on Cu, Fe, and Ni content do not show a correlation with the values of OM content. Thus, only in relation to four out of the seven microelements, one can assume the effect of the accumulated OM concentration as a possible factor of the binding and deposition in the composition of bottom sediment.

The values of the contamination factor $C_f$ (Table 11) are ranked as follows (in terms of pollution levels): $C_f < 1$, low; $1 \le C_f < 3$, moderate; $3 \le C_f < 6$, high; and $C_f \ge 6$, very high [Hakanson, 1980; Vashchenko et al., 2010]. The Vladimir Bay bottom sediments are characterized by a low level of Pb and Ni contamination (only one value at sta. 8 for nickel is > 1). Interestingly, this location has a high level of Zn pollution as well, while other studied spots are characterized by moderate (10 stations) and low (6 stations) pollution with this compound. Bottom sediments are the most polluted with Cd and Cu: high and very high Cd and Cu pollution was recorded for sta. 9 and 4, respectively. It is worth noting as follows. Sta. 4, 12, and 15, which are located in the central areas of the Severnaya and Yuzhnaya bays and in the apex of the Srednyaya Bay (there, OM is actively accumulated), are also characterized by high levels of Cd and Cu pollution. For Zn and Ni, such a relationship was not revealed.

**Table 11.** Assessment of the ecological state of the Vladimir Bay bottom sediments by the values of the contamination factor, the degree of contamination, and the sediment quality guideline quotient

| Station | $C_f$ | | | | | $C_d$ | SQG-Q |
|---|---|---|---|---|---|---|---|
| | Cd | Ni | Pb | Cu | Zn | | |
| 1 | 7.47 | 0.17 | 0.15 | 1.36 | 0.89 | 10.04 | 0.29 |
| 2 | 0.00 | 0.17 | 0.17 | 1.28 | 1.16 | 2.78 | 0.24 |
| 3 | 4.52 | 0.25 | 0.38 | 1.29 | 2.48 | 8.92 | 0.31 |
| 4 | 3.47 | 0.08 | 0.24 | 4.70 | 1.15 | 9.64 | 0.33 |
| 5 | 3.52 | 0.25 | 0.37 | 1.26 | 2.05 | 7.46 | 0.29 |
| 6 | 1.86 | 0.21 | 0.23 | 1.59 | 1.52 | 5.41 | 0.31 |
| 7 | 0.00 | 0.19 | 0.21 | 0.25 | 1.01 | 1.66 | 0.08 |
| 8 | 4.16 | 2.20 | 0.46 | 2.03 | 3.25 | 12.09 | 0.53 |
| 9 | 2.56 | 0.19 | 0.32 | 0.73 | 1.19 | 4.98 | 0.17 |
| 10 | 1.13 | 0.31 | 0.43 | 0.20 | 0.58 | 2.64 | 0.08 |
| 11 | 3.67 | 0.06 | 0.09 | 5.40 | 0.36 | 9.58 | 0.29 |
| 12 | 3.61 | 0.06 | 0.09 | 5.60 | 0.36 | 9.73 | 0.30 |
| 13 | 3.46 | 0.23 | 0.36 | 2.64 | 0.95 | 7.63 | 0.48 |
| 14 | 6.43 | 0.19 | 0.17 | 1.34 | 2.18 | 10.30 | 0.31 |
| 15 | 2.22 | 0.11 | 0.31 | 9.25 | 1.14 | 13.02 | 0.51 |
| 16 | 0.28 | 0.07 | 0.09 | 0.70 | 0.23 | 1.36 | 0.06 |
| 17 | 1.91 | 0.23 | 0.29 | 0.04 | 1.31 | 3.77 | 0.06 |

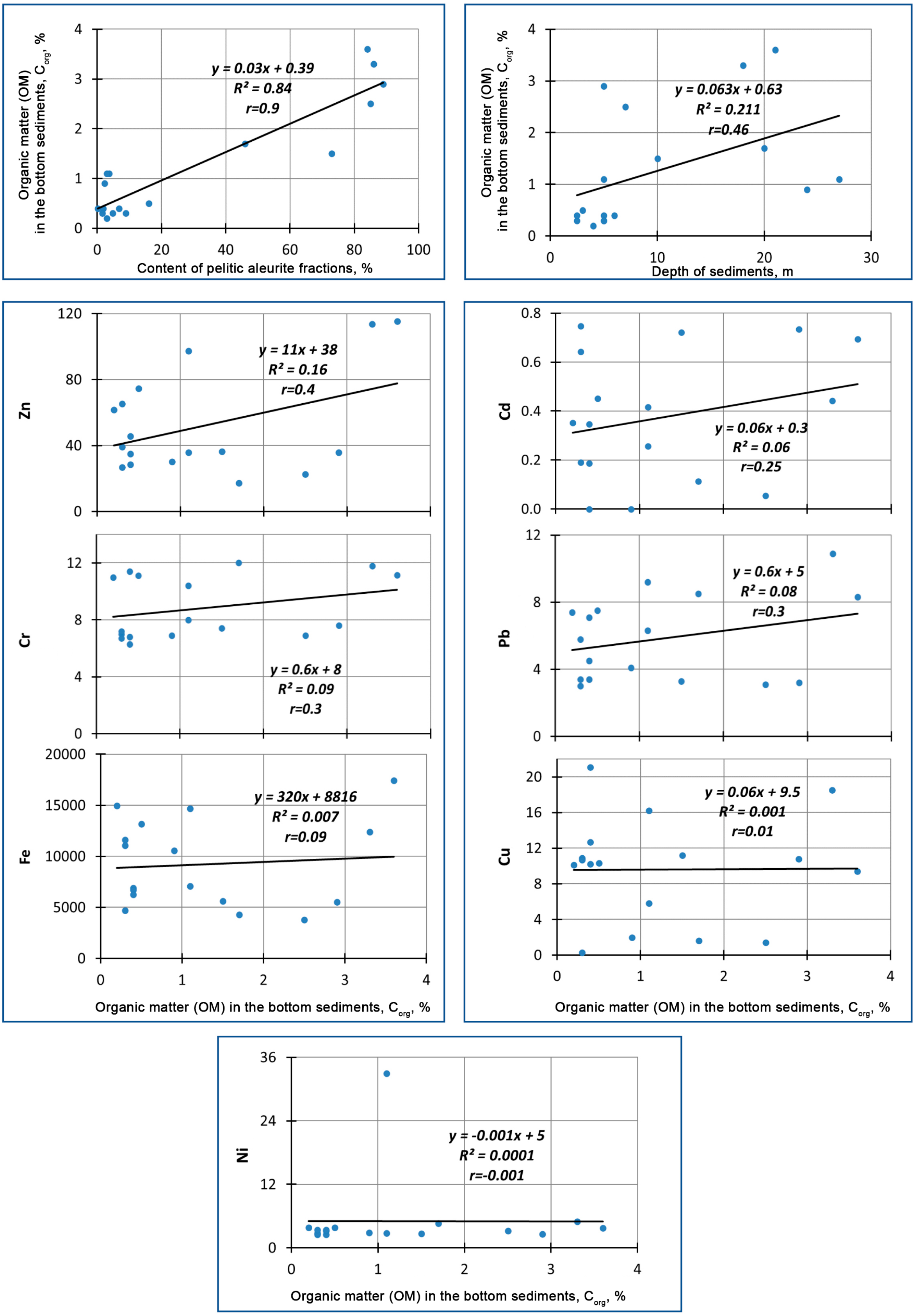

**Fig. 5.** Correlation analysis of the relationship of organic matter (OM) in the Vladimir Bay bottom sediments with heavy metal concentrations, grain-size characteristics, and depth of sediments ($R^2$, the coefficient of determination; $r$, the Pearson correlation coefficient)

Increased OM concentration in sediments, which is characteristic of finely dispersed fractions, determines a decrease in the content of inorganic carbon and in pH value and, accordingly, a change in the redox potential of bottom sediments. A drop in pH values enhances the migration ability of elements included in the composition of sediments; moreover, it is a risk factor for secondary pollution during accumulation of toxic elements and their compounds in sediments. Thus, based on the values of the microbial index and $C_f$, it can also be concluded that Cd enters the bay water from coastal sources and is deposited in bottom sediments. However, $C_{org}$ accumulation in sediments can contribute to HM binding and then release HM into a water column later (with a change in the redox potential of sediments themselves and a decrease in pH values) [Shulkin, 2004].

The values of the degree of contamination $C_d$ for sediments (see Table 11) are ranked in accordance with the total value of $C_f$ for the background area, which is equal to 6 [Vashchenko et al., 2010], as follows: $C_d < 6$, low degree of HM contamination; $6 \le C_d < 12$, moderate; $12 \le C_d < 24$, high; and $C_d > 24$, very high. Most of the stations (8 out of 17) are characterized by a moderate degree of contamination of bottom sediments. High contamination is recorded for sta. 8 and 15.

SQG-Q values (Table 11) are ranked as follows [MacDonald et al., 1996; Vashchenko et al., 2010]: SQG-Q ≤ 0.1, non-toxic sediments, the lowest probability of observing negative biological effects; 0.1 < SQG-Q < 1, moderately toxic sediments, average probability of observing negative biological effects; and SQG-Q > 1, highly toxic sediments, high probability of observing negative biological effects. Despite the fact that the Vladimir Bay bottom sediments are HM-contaminated, in general those can be characterized as moderately toxic. The highest potential toxicity was registered at sta. 8.

In the sea urchin gonads, Zn was accumulated at maximum concentrations. In the bottom sediments, Zn content was high as well (and even exceeded the clarke values at three stations). However, when analyzing the correlation of pairs of values (Zn in sediments – Zn in *S. intermedius* gonads) obtained for each station, a clear inverse relationship was revealed, with the Pearson correlation coefficient $r = -0.96$ (Fig. 6). In other words, the higher Zn concentration in gonads, the lower observed Zn content in bottom sediments.

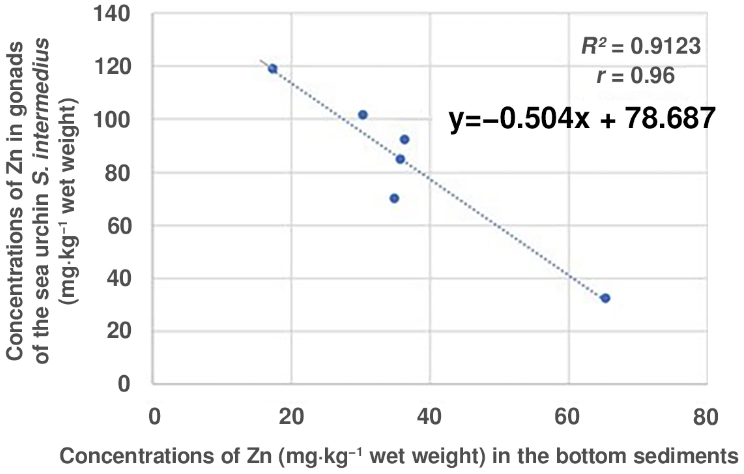

**Fig. 6.** Correlation analysis of the relationship of Zn concentration values in the bottom sediments and in gonads of the sea urchin *Strongylocentrotus intermedius*

Out of all forms of metals in the environment, living organisms are capable of absorbing and assimilating only biologically available ones, wherein marine invertebrates can regulate the intake of zinc into their bodies [Shulkin, 2004]. Apparently, despite high Zn concentrations in bottom sediments, the content of its bioavailable form for hydrobionts in water and marine sediments is low. Accordingly, subsequent alimentary absorption by sea urchins also determines its low concentrations in gonads. A similar pattern was noted earlier for mytilids from the Muravyov-Amursky Peninsula coast [Shulkin, 2004]. The absorption of Zn in a bioavailable form by sea urchins is likely to occur from bottom waters during continuous passage of water through body cavities (the same as for all hydrobionts).

Sea urchin roe is not only a valuable food product, but also a highly effective therapeutic and prophylactic supplement. Given this fact, one of the tasks for ecologists is to determine whether roe quality complies with existing standards and to assess the health hazard when consuming it as food. Technical Regulation of the Customs Union *On Food Safety* (TR TS 021/2011) determines permissible levels of certain toxic HM; the values are given in mg·kg$^{-1}$ wet weight. The recalculation of the values obtained by us in terms of wet weight and comparison of these results (Table 12) with the established permissible levels showed as follows. Cd and Pb content in the sea urchin gonads at any sampling station in the Vladimir Bay does not exceed the determined permissible values.

**Table 12.** Pb and Cd concentrations (mg·kg$^{-1}$ wet weight) in gonads of the sea urchin *Strongylocentrotus intermedius* from the Vladimir Bay (mean ± *SD*)

| Station | Pb | Cd |
|---|---|---|
| 2 | 0.52 ± 0.09 | 0.08 ± 0.00 |
| 7 | 0.86 ± 0.19 | 0.14 ± 0.00 |
| 10 | 1.31 ± 0.49 | 0.11 ± 0.02 |
| 11 | 0.63 ± 0.05 | 0.06 ± 0.01 |
| 12 | 0.67 ± 0.05 | 0.13 ± 0.02 |
| 14 | 2.99 ± 0.26 | 0.05 ± 0.01 |
| PL | 10.00 | 2.00 |

**Note:** PL denotes a permissible level (Technical Regulation of the Customs Union 021/2011).

However, we assessed the overall health risk from continuous consumption of the sea urchin roe from the Vladimir Bay during the summer season applying the ILCR calculator. It indicated the existence of carcinogenic risk. ILCR value, if taking into account actual Cd and Pb concentrations in gonads, was $5.27 \cdot 10^{-5}$. The permissible value, which is a criterion for the safety of a marine product, is $10^{-5}$.

**Conclusions:**

1. The Vladimir Bay ecosystem with a relatively high level of organic matter in summer successfully “utilizes” organic matter of both natural and household origin. The effect of villages and the coastal strip on the formation of eutrophication conditions is insignificant. Chemical and environmental indexes indicate mesotrophic status of surface waters. Ecological and microbiological assessment in terms of the abundance of colony-forming heterotrophic microorganisms showed α-mesosaprobic level of the water quality. The conversion of microbiological data into chemical data in accordance with a comparison scale indicates the oligomesotrophic status of the studied water horizon.

2. According to the maximum $C_{org}$ value in the Vladimir Bay, it belongs to marine areas with high content of organic matter in bottom sediments. Maximum concentrations were recorded in central and apex areas of its bights.
3. Mass macrozoobenthic species are *Strongylocentrotus intermedius*, *Asterina pectinifera*, *Mizuhopecten yessoensis*, *Crenomytilus grayanus*, *Pododesmus macrochisma*, and *Cucumaria japonica*. Mass species of macrophytobenthos are *Desmarestia viridis*, *Costaria costata*, *Saccharina japonica*, *Zostera marina*, and *Phyllospadix iwatensis*. Mean biomass of benthos is (841 ± 583) $g \cdot m^{-2}$, and this indicates high levels of bioresources in the bay. In macrozoobenthos, echinoderms prevail, primarily sea urchins.
4. In general, microbial communities of the Vladimir Bay surface waters are characterized by simultaneous presence of ecological and trophic groups resistant to various metals. Comparison of the results of the microbiological assessment with the MPC scale indicates a significant contamination of the bay surface waters with Cd (> 3 MPC). The general background with a lower cell abundance is formed by Cu- and Zn-resistant microorganisms.
5. For most heavy metals (HM), the total content in bottom sediments was below the clarke values. For several HM, background concentrations were exceeded. The sequence of decreasing concentrations of the studied HM in sediments is as follows: Fe > Zn > Cu > Cr > Pb > Ni > Cd. Horizontal spatial distribution of HM concentrations in bottom sediments is characterized by four types: “unipolar,” “bipolar,” “tripolar,” and “vector.” In the sea urchin gonads, HM content varied less than in the bottom sediments. On average, decreasing concentrations form the following sequence: Zn > Pb > Cu > Ni > Cr > Cd.
6. The correlation analysis confirmed the dependence of an increase in the concentration of organic matter in bottom sediments on an increase in the proportion of the finest fractions and depth. A positive correlation was registered for $C_{org}$ and four elements (Zn, Cd, Cr, and Pb), in relation to which the effect of the accumulated organic matter concentration is assumed as a possible factor of the binding and deposition in the composition of bottom sediment. An analysis of the relationship between HM concentrations in sediments and the sea urchin gonads showed a high negative correlation for Zn. Apparently, the inverse relationship is due to biologically unavaliable form of zinc compounds in bottom sediments.
7. $C_f$ characterizes the contamination of the bay bottom sediments with zinc as high, and with cadmium and copper, as very high. $C_d$ value indicates a moderate degree of HM contamination for bottom sediment in general. According to SQG-Q, the bay sediments are classified as moderately toxic.
8. The risk to human health, determined by total presence of Cd and Pb in the sea urchin gonads, showed compliance with the permissible values specified in Technical Regulation of the Customs Union *On Food Safety*. Meanwhile, according to ILCR value, it is possible to predict the existence of carcinogenic risk driven by long-term consumption of gonads of the sea urchin *S. intermedius* from the Vladimir Bay.

*The work was supported by the Ministry of Science and Higher Education of the Russian Federation (project FZNS-2023-0011).*

## REFERENCES

1. Bezverbnaya I. P., Dimitrieva G. Yu., Tazaki K., Vatanabe H. Evaluation of sea water quality in the coastal zone of Primor'e using the method of microbial indication. *Vodnye resursy*, 2003, vol. 30, no. 2, pp. 222–231. (in Russ.)
2. Vaschenko M. A., Almyashova T. N., Zhadan P. M. Long-term and seasonal dynamics of the gonad state in the sea urchin *Strongylocentrotus intermedius* under anthropogenic pollution (Amursky Bay, Sea of Japan). *Vestnik Dal'nevostochnogo otdeleniya Rossiiskoi akademii nauk*, 2005, no. 1 (119), pp. 32–42. (in Russ.)
3. Vashchenko M. A., Zhadan P. M., Almyashova T. N., Kovalyova A. L., Slinko E. N. Assessment of the contamination level of bottom sediments of Amursky Bay (Sea of Japan) and their potential toxicity. *Biologiya morya*, 2010, vol. 36, no. 5, pp. 354–361. (in Russ.)
4. Vashchenkova N. G., Tsoy I. B. Composition and conditions for formation of Neogene deposits at the Primorye continental slope in the area of Vladimir Bay in the Sea of Japan. *Okeanologiya*, 2014, vol. 54, no. 4, pp. 518–529. (in Russ.). https://doi.org/10.7868/S0030157414030149
5. Vinogradov A. P. Srednie soderzhaniya khimicheskikh elementov v glavnykh tipakh izverzhennykh gornykh porod zemnoi kory. *Geokhimiya*, 1962, no. 7, pp. 555–571. (in Russ.)
6. Gavrilova G. S., Kucheryavenko A. V., Odintsov A. M. Results and prospects of the scallop *Mizuhopecten yessoensis* cultivation in the Vladimir Bay (Japan Sea). *Izvestiya TINRO*, 2006, vol. 147, pp. 385–396. (in Russ.)
7. Gavrilova G. S., Sukhin I. Yu., Turabzhanova I. S. First experience of cage cultivation of hatchery-produced juvenile scallop *Mizuhopecten yessoensis* at eastern coast of Primorye. *Izvestiya TINRO*, 2019, vol. 197, pp. 208–218. (in Russ.). https://doi.org/10.26428/1606-9919-2019-197-208-218
8. Galysheva Yu. A. Features of macrobenthos distribution in coastal marine ecosystems at Primorye (Japan Sea). *Izvestiya TINRO*, 2010, vol. 163, pp. 286–296. (in Russ.)
9. Galysheva Yu. A., Serdyuk U. I., Khristoforova N. K. Structure and distribution of macrobenthos in the sublittoral zone of the Vladimir Bay, Japan Sea. *Izvestiya TINRO*, 2018, vol. 192, pp. 145–156. (in Russ.). https://doi.org/10.26428/1606-9919-2018-192-145-156
10. Donets M. M., Tsygankov V. Yu., Kulshova V. I., Elkhoury J., Boyarova M. D., Gumovskiy A. N., Gumovskaya Yu. P., Bogatov V. V., Prozorova L. A., Chernova E. N., Lysenko E. V., Ngo X. Q. Food safety of bivalves from the South Vietnam: Organochlorine compounds and heavy metals as risk factors for human health. *Meditsinskii akademicheskii zhurnal*, 2020, vol. 20, no. 2, pp. 45–58. (in Russ.). https://doi.org/10.17816/MAJ34285
11. Kalitina E. G., Mikhailik T. A., Semkin P. Yu., Barabanshchikov Yu. A., Zorin S. A. Features of microbiological composition for water from the Razdolnaya River (Southern Primorye). *Izvestiya TINRO*, 2015, vol. 180, pp. 187–197. (in Russ.)
12. Kachinsky N. A. *Mekhanicheskii i mikroagregatnyi sostav pochvy, metody ego izucheniya* : monograph. Moscow : AN SSSR, 1958, 193 p. (in Russ.)

13. Kulepanov V. N., Ivanova N. V. Specific structure and quantitative distribution of macrophytes in the sublittoral of Vladimir Bay (North Primorye). *Izvestiya TINRO*, 2006, vol. 146, pp. 136–149. (in Russ.)
14. Lutaenko K. A., Kolpakov E. V. The extension of the distributional range of an invasive mussel, *Mytilus galloprovincialis* (Bivalvia: Mytilidae) in the Sea of Japan. *Byulleten' Dal'nevostochnogo malakologicheskogo obshchestva*, 2016, vol. 20, no. 1, pp. 57–76. (in Russ.)
15. *Obshchaya i sanitarnaya mikrobiologiya s tekhnikoi mikrobiologicheskikh issledovanii* / A. S. Labinskaya, L. P. Blinkova, A. S. Eshchina (Eds). Moscow : Meditsina, 2004, 576 p. (in Russ.)
16. *Ob utverzhdenii normativov kachestva vody vodnykh ob"ektov rybokhozyaistvennogo znacheniya, v tom chisle normativov predel'no dopustimykh kontsentratsii vrednykh veshchestv v vodakh vodnykh ob"ektov rybokhozyaistvennogo znacheniya* : prikaz Minsel'khoza Rossii ot 13.12.2016 no. 552 [v red. ot 10.03.2020]. (in Russ.). URL: https://docs.cntd.ru/document/420389120 [accessed: 30.11.2021].
17. Romankevich E. A., Vetrov A. A., Peresypkin V. I. Organic matter of the World Ocean. *Geologiya i geofizika*, 2009, vol. 50, no. 4, pp. 299–307. (in Russ.)
18. *Rukovodstvo po khimicheskomy analizu morskikh i presnykh vod pri ekologicheskom monitoringe rybokhozyaistvennykh vodoemov i perspektivnykh dlya promysla raionov Mirovogo okeana.* Moscow : VNIRO, 2003, 202 p. (in Russ.)
19. Khristoforova N. K., Emelyanov A. A., Berdasova K. S., Degteva Yu. E. Ecological characteristic of water in the Eastern Bosporus Strait by oxygen parameters. *Izvestiya TINRO*, 2015, vol. 181, pp. 161–168. (in Russ.)
20. Khristoforova N. K., Emelyanov A. A., Efimov A. V. Bioindication of pollution in the coastal marine waters at Russky Island (Peter the Great Bay, Japan Sea) by heavy metals. *Izvestiya TINRO*, 2018, vol. 192, pp. 157–166. (in Russ.). https://doi.org/10.26428/1606-9919-2018-192-157-166
21. Shulkin V. M. *Metally v ekosistemakh morskikh melkovodii.* Vladivostok : Dal'nauka, 2004, 279 p. (in Russ.)
22. Birch G. F. Indicators of anthropogenic change and biological risk in coastal aquatic environments. In: *Geochemistry of Estuaries and Coasts* / G. Shimmield (Ed.). London ; Waltham ; San Diego : Academic Press, 2011, pp. 235–270. (Series: Treatise on Estuarine and Coastal Science ; vol. 4). http://dx.doi.org/10.1016/B978-0-12-374711-2.00411-3
23. Chakraborty P., Ramteke D., Chakraborty S., Nagender Nath B. Changes in metal contamination levels in estuarine sediments around India – An assessment. *Marine Pollution Bulletin*, 2014, vol. 78, iss. 1–2, pp. 15–25. https://doi.org/10.1016/j.marpolbul.2013.09.044
24. Hakanson L. An ecological risk index for aquatic pollution control. A sedimentological approach. *Water Research*, 1980, vol. 14, iss. 8, pp. 975–1001. https://doi.org/10.1016/0043-1354(80)90143-8
25. Liang B., Qian X., Peng S., Liu X., Bai L., Cui B., Bai J. Speciation variation and comprehensive risk assessment of metal(loid)s in surface sediments of intertidal zones. *International Journal of Environmental Research and Public Health*, 2018, vol. 15, iss. 10, art. no. 2125 (16 p.). https://doi.org/10.3390/ijerph15102125

26. MacDonald D. D., Scott Carr R., Calder F. D., Long E. R., Ingersoll Ch. G. Development and evaluation of sediment quality guidelines for Florida coastal waters. *Ecotoxicology*, 1996, vol. 5, iss. 4, pp. 253–278. https://doi.org/10.1007/BF00118995
27. Saroop Sh., Tamchos S. Monitoring and impact assessment approaches for heavy metals. In: *Heavy Metals in the Environment: Impact, Assessment, and Remediation* / V. Kumar, A. Sharma, A. Cerdà (Eds). Amsterdam ; Oxford ; Cambridge : Elsevier, 2021, pp. 57–86. http://dx.doi.org/10.1016/B978-0-12-821656-9.00004-3
28. Yoshimizu M., Kimura T. Study of intestinal microflora of salmonids. *Fish Pathology*, 1976, vol. 10, iss. 2, pp. 243–259. https://doi.org/10.3147/jsfp.10.243
29. Zhang Ch., Yu Zh., Zeng G., Jiang M., Yang Zh., Cui F., Zhu M., Shen L., Hu L. Effects of sediment geochemical properties on heavy metal bioavailability. *Environment International*, 2014, vol. 73, pp. 270–281. https://doi.org/10.1016/j.envint.2014.08.010

# ОРГАНИЧЕСКОЕ ВЕЩЕСТВО В ЭКОСИСТЕМЕ ЗАЛИВА ВЛАДИМИРА (ЯПОНСКОЕ МОРЕ): РЕСУРС ПИТАНИЯ И ВОЗМОЖНЫЙ ФАКТОР ЭКОЛОГИЧЕСКОГО РИСКА

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Исследование содержания органического вещества в водной среде и донных отложениях крайне важно для понимания аккумуляционного процесса в отношении поступающих в среду акватории токсических веществ и элементов, а также выявления негативных изменений в экосистеме и потенциальных экологических рисков при осуществлении природопользования. Цель работы — проанализировать значение органического вещества как фактора контаминации среды залива Владимира (Японское море) и оценить токсичность гидробионтов и экологический риск для экосистемы и здоровья человека. Комплексная работа выполнена с применением химико-аналитических, микробиологических и гидробиологических методов; она включает математическую и картографическую обработку данных и расчёт коэффициентов загрязнения и экологического риска. Проведён анализ проб морской воды, донных отложений и макробентоса, отобранных в заливе Владимира в июле 2014 г. Определены химико-экологические и микробиологические параметры вод залива, позволяющие оценить процессы накопления органического вещества в водной среде и донных отложениях и установить трофический статус экосистемы в летний период. Выявлены массовые виды и группы макробентоса, определены показатели обилия донного населения, свидетельствующие о высоких уровнях биомассы. Установлена численность металлрезистентных групп бактерий в поверхностных водах. Оценено содержание и пространственное распределение тяжёлых металлов (ТМ) в донных отложениях. Установлены концентрации ТМ в гонадах морских ежей. Результаты микробиологической оценки соотнесены с ПДК; они выявляют значительное загрязнение вод залива (более 3 ПДК) в отношении Cd. Для Ni, Cu, Zn и Cd в донных отложениях обнаружено превышение фоновых концентраций, для Cd и Zn — превышение кларковых значений. Проверена корреляционная связь концентраций органического вещества в донных отложениях с содержанием в них ТМ, а также связь концентраций ТМ в грунтах и гонадах морских ежей (значимая корреляция выявлена в отношении Zn). Для донных отложений определены фактор загрязнения $C_f$, характеризующий их загрязнение в заливе цинком как высокое, а кадмием и медью — как очень высокое, а также степень загрязнения $C_d$, свидетельствующая о среднем уровне загрязнения донных осадков в целом. Проведён расчёт индексов экологического риска. По величине SQG-Q грунты залива отнесены к категории умеренно токсичных. Установлено: согласно нормативам Технического регламента Таможенного союза «О безопасности пищевой продукции» 021/2011, содержание Cd и Pb в гонадах морских ежей из залива Владимира не превышает допустимых уровней, однако по значению ILCR (индекс нарастающего пожизненного риска) можно сделать прогноз об опасности возникновения онкологических заболеваний при употреблении гонад в пищу.

**Ключевые слова:** залив Владимира, Японское море, органическое вещество, трофность, донные отложения, макробентос, тяжёлые металлы, коэффициенты накопления, экологический риск, риск здоровью человека

1871
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Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 2, pp. 42–54
https://marine-biology.ru

UDC [582.261.1-11:551.311.8](292.471)

# REPRODUCTIVE BIOLOGY AND THE LIFE CYCLE OF THE DIATOM *NITZSCHIA* CF. *THERMALOIDES* INHABITING MUD VOLCANOES OF CRIMEA

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Received by the Editor 15.01.2021; after reviewing 17.02.2021;
accepted for publication 16.02.2023; published online 31.05.2023.

In biology, it is important to study the ability of organisms to exist under extreme conditions, to which the phase of the life cycle, related to sexual reproduction and the possibility to leave the next generation, is especially sensitive. *Nitzschia* cf. *thermaloides* Hustedt was found in high abundance in samples from puddles and lakes formed in the areas of action of mud volcanoes of the Bulganak mud volcano field (Kerch Peninsula, Crimea). Individual clones were isolated from the samples by the micropipette technique and introduced into a culture; their crossing made it possible to initiate heterothallic sexual reproduction. The aim of this work was to study reproductive biology and the life cycle of the alga from a biotope with extremely high levels of irradiance, temperature, and salinity. For the first time for this species, a description of the sexual process corresponding to IB2a type according to Geitler classification is given. The cardinal points have been defined, which represent critical cell sizes that determine the transition from one phase of the life cycle to another. The full range of cell sizes is given, from the initial ones that appeared as a result of sexual reproduction to the smallest ones observed when kept in the culture. The main postulates of the life-cycle theory are considered allowing to analyze the natural population of the alga. The population of *N.* cf. *thermaloides* was found to exist in the sampling site for a long time and to be represented by cells in all phases of the life cycle.

**Keywords:** diatoms, *Nitzschia* cf. *thermaloides*, mud volcanoes, sexual reproduction, life cycle

Diatoms are known not only for their great species diversity, but also for their significant evolutionary plasticity, which allowed them to adapt to existence on the bottom and in water column of marine and freshwater basins, in soils, and on wet surfaces. Some diatom species are tolerant to conditions of extreme temperature [Nikulina, 2010], salinity [Nevrova, Shadrin, 2005; Senicheva et al., 2008], and high irradiance, including ultraviolet light [Peletier et al., 1996]; in the absence of light, they can grow mixotrophically [Abdullin, Bagmet, 2016]. In samples of water flowing out of mud volcanoes of the Bulganak mud volcano field (Kerch Peninsula, Crimea), 15 microalgal species were registered; out of them, 6 represented the phylum Bacillariophyta, including *Nitzschia* cf. *thermaloides* Hustedt, 1955 [Ryabushko, Bondarenko, 2020]. Due to this finding, a number of questions arose concerning the fundamental aspects of the biology of these species living under unique conditions of temperature, salinity, and irradiance. First of all, we were interested in reproductive biology of diatoms and their response to extreme conditions. What is the lifespan of populations found in the mud volcanoes? What are the rates of division of diatoms in these biotopes? Are the species capable of reproducing in generations under these conditions, passing through all phases of the life cycle, *inter alia* the process of sexual reproduction? These questions determined the aim of our work.

## MATERIAL AND METHODS

The objects of the study were isolated from samples taken on 15.10.2020 in the Bulganak mud volcano field at the points with the coordinates given in Table 1.

**Table 1.** Sampling points in the Bulganak mud volcano field (Kerch Peninsula, Crimea), 15.10.2020; water salinity in the samples

| Mud volcano | GPS coordinates | | Water salinity, ‰ |
|---|---|---|---|
| | N | E | |
| Andrusov | 45°25′35.9″ | 36°28′38.9″ | 30, 35, 68 |
| Pavlov | 45°25′33.0″ | 36°28′44.1″ | 18, 35, 56 |
| Tishchenko | 45°25′33.3″ | 36°28′25.4″ | 20 |
| Central Lake | 45°25′23.7″ | 36°28′38.4″ | 19, 26 |

Four mud volcanoes were explored: Andrusov (Fig. 1A), Pavlov (Fig. 1B), Tishchenko (Fig. 1C), and Central Lake (Fig. 1D). The Bulganak mud volcano field belongs to mud volcanic formations with dispersed centers of eruptions (Fig. 2); hills and gryphons (local centers of mud volcanic activity) are located in a relatively small area. There are hills slightly rising above the surrounding landscape, from which gas and liquid silt are released, spreading in a thin layer and forming shallow puddles, and mud lakes of a smaller or larger area (for example, Central Lake). The second morphotype of volcanoes is cone-shaped hills, excreting rather viscous mud flowing down the slopes (Tishchenko mud volcano).

Brines of the mud volcanoes are of the hydrocarbonate-sodium type (hydrocarbonate-chloride sodium or chloride-hydrocarbonate sodium); those are characterized by an increased content of bromine (85–210 mg·L$^{-1}$) and iodine (23–45 mg·L$^{-1}$) [Matyunina, 2019]. The chemical composition of the brines of the mud volcanoes is variable: it can change significantly even within a few hours. The mineralization of erupted brines does not exceed 20–23 g·L$^{-1}$ [Kayukova, 2018; Kurishko et al., 1968]. However, under conditions of high irradiance and intense winds, due to water evaporation in effluent brines, a noticeable increase in the concentration and precipitation of salts is recorded locally. Mineralization can reach 110 g·L$^{-1}$ [Kurishko et al., 1968].

In puddles and mud lakes, diatoms form a biofilm of a characteristic brown-green color (Fig. 1A). The biofilm is located both on the surface of the liquid phase and on the bottom in the spots where the water column is shallow and the suspension is mostly settled. Depending on the location, the area of continuous biofilm can be of several square decimeters or more.

Single cells of *N.* cf. *thermaloides* were isolated from samples delivered to the laboratory by micropipette technique using inverted microscopes Nib-100 (China) and Altami INVERT 3 (Russia) (Fig. 3); those gave rise to 15 clonal cultures. The cultures were kept in 100-mL glass Erlenmeyer flasks in a modified ESAW medium [Polyakova et al., 2018] and inoculated into fresh medium every 10–14 days. Conditions of maintaining and the method of clones naming are described in more detail in [Davidovich et al., 2017]. The clones were kept and crossed in glass Petri dishes (40–50 mm in diameter) in a medium with the same salinity as they were in the samples. Initial salinity of the modified ESAW medium (36‰) was adjusted to the required level either by diluting it with distilled water or by adding sodium chloride. Salinity was measured with an RHS-10ATC refractometer (China). In the samples, salinity differed significantly depending on a sampling point (see Table 1). Specifically, in three samples of mud flowing out of the Andrusov mud volcano, which were taken on an area of < 1 $m^2$, salinity was 30, 35, and 68‰. Apparently, the reason for such noticeable differences are salts occurring in the samples which were deposited on the underlying surface because of water evaporation. In a sample taken directly from the vent (Tishchenko mud volcano), salinity was 20‰.

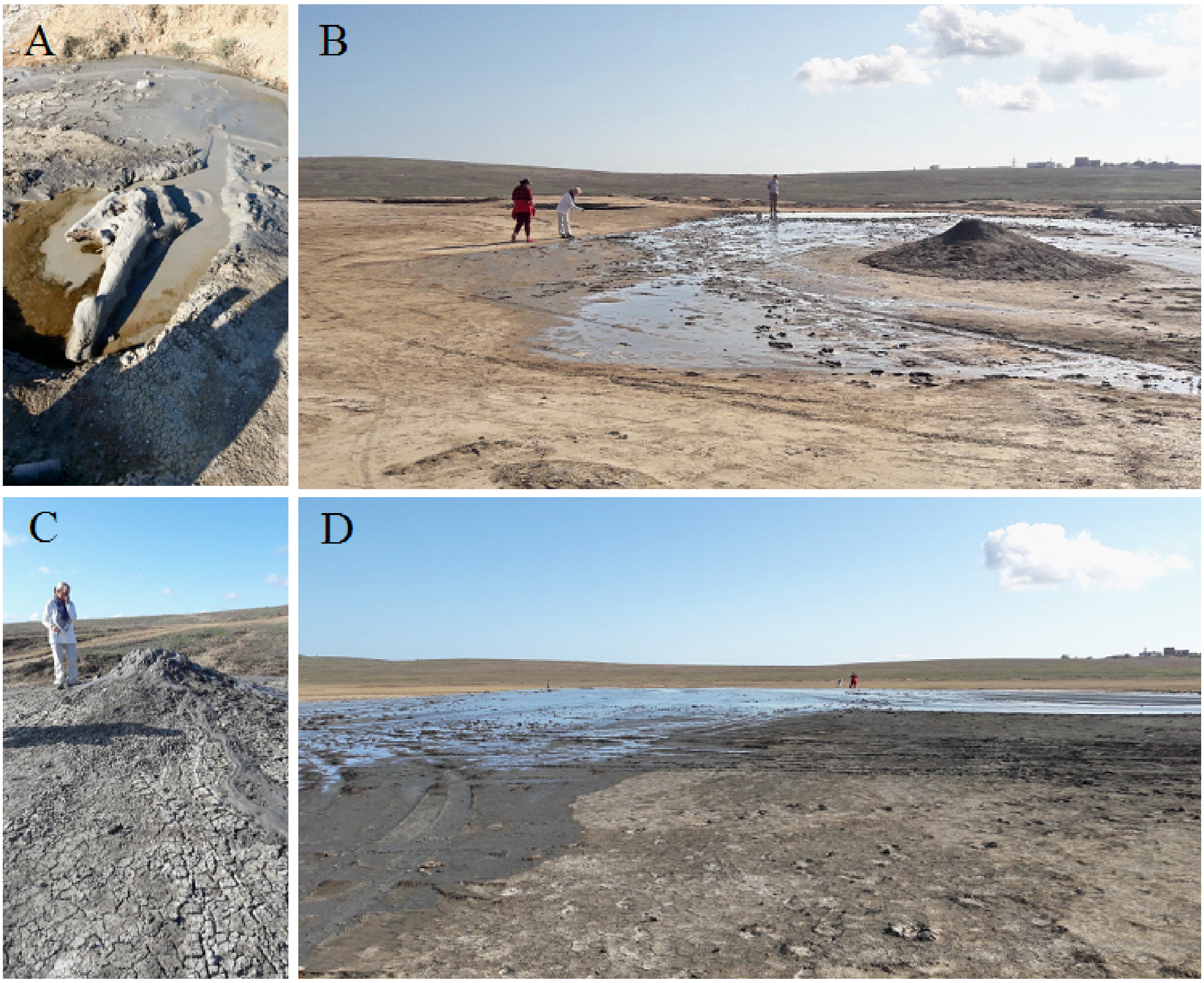

**Fig. 1.** The mud volcanoes – Andrusov (A), Pavlov (B), Tishchenko (C), and Central Lake (D) – of the Bulganak mud volcano field during the sampling period (Kerch Peninsula, Crimea)

For electron microscopy, frustules were boiled in hydrogen peroxide (35%) and washed (7–8 times) with distilled water; then, cleansed valves were placed on an object stub and coated with gold. Microphotography was carried out under a Hitachi SU3500 scanning electron microscope (Japan). Cell length and width were measured using a calibrated eyepiece ruler by direct observation under a microscope or a Moticam 1080 digital camera (China) with the supplied software. The obtained data were statistically processed in Microsoft Excel. Mean values (*M*) are given with a standard error (*SE*) and sample size (*N*): *M* ± *SE*.

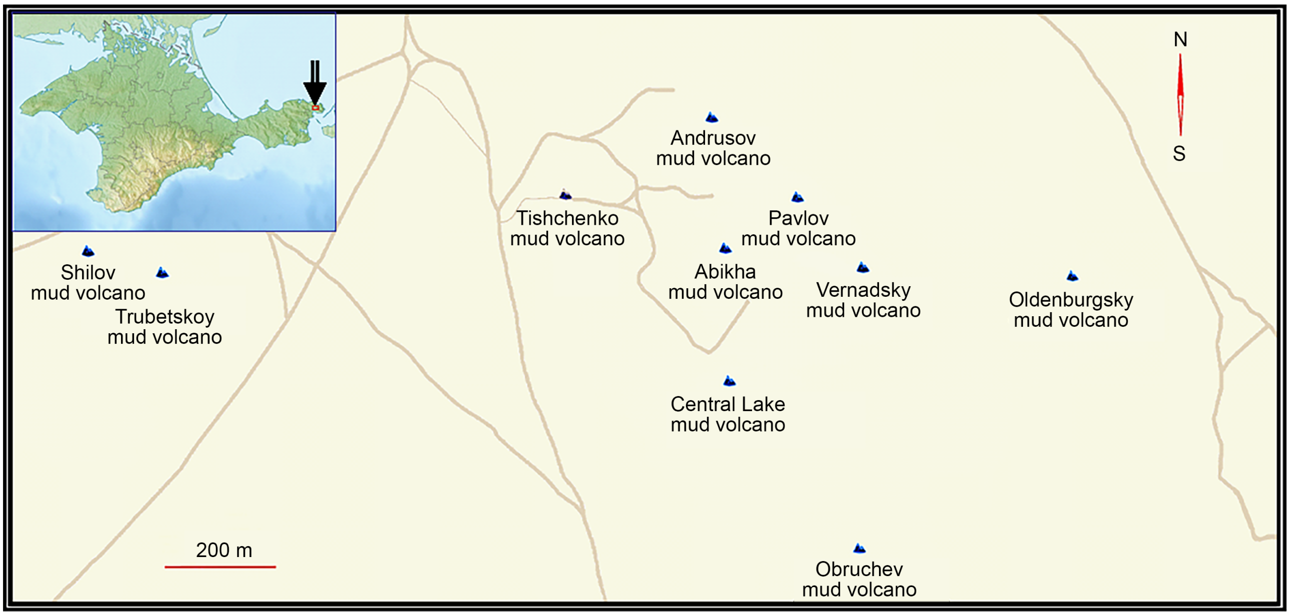

**Fig. 2.** Location map of the mud volcanoes of the Malo-Tarkhan (left cluster) and the Bulganak (right cluster) mud volcano fields (Kerch Peninsula, Crimea)

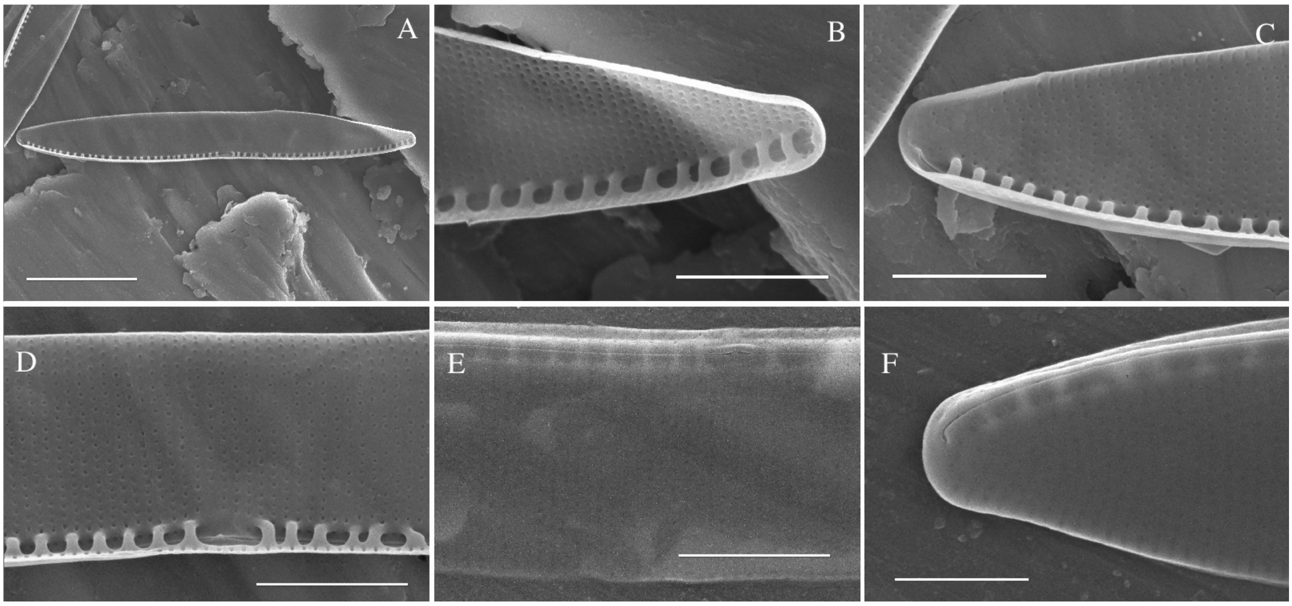

**Fig. 3.** *Nitzschia* cf. *thermaloides*, scanning electron microscopy. Internal (A–D) and external (E–F) valve view. Scale bar: A, 10 μm; B–E, 3 μm; F, 2 μm

## RESULTS

For the first time, we observed *N.* cf. *thermaloides* sexual reproduction in samples with the nutrient medium added even before the isolation of individual clones. In total, 17 initial cells were found and measured, their mean apical size turned out to be (68.7 ± 0.5) μm (maximum was 71.0 μm). The length of the valves of the empty open frustules of parental cells, lying next to them, averaged (37.4 ± 0.7) μm ($N = 14$; maximum was 40 μm). Even these preliminary data allowed concluding as follows: in the process of sexual reproduction, there was a twofold increase in size, and the cell length of slightly more than 70 μm was obviously close to the maximum species-specific length. Subsequent measurements of cells (not only from the natural population, but also obtained by crossing the isolated clones) made it possible to identify the sizes that determine the phases of *N.* cf. *thermaloides* life cycle (Table 2).

**Table 2.** The apical size of *Nitzschia* cf. *thermaloides* vegetative, gametangial, and initial cells

| Cells | Sample size (*N*) | Cell length, μm | |
|---|---|---|---|
| | | min | max |
| Vegetative | 1,190 | 15 | 84 |
| Gametangial | 103 | 33 | 43 |
| Initial | 202 | 56 | 84 |

Measurement of cells from the natural population (*N* = 502) showed that their length varied from 15 to 74 μm. The histogram of the distribution of cell sizes in terms of the frequency of occurrence in the natural population is shown in Fig. 4. By crossing the isolated clones, initial cells were obtained; their maximum size was 84 μm. The length of the largest gametangial cells was 43 μm (52% of the maximum size); this is the transition boundary from the pre-reproductive to the reproductive phase of the life cycle.

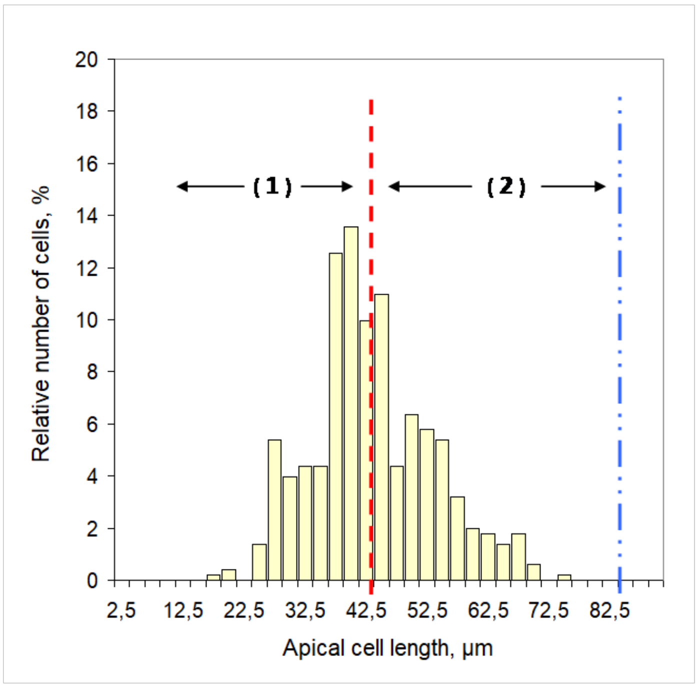

**Fig. 4.** Distribution of *Nitzschia* cf. *thermaloides* cell length (*N* = 502) by frequency of occurrence in the natural population of the mud volcanoes of the Bulganak mud volcano field. The dash-dotted line defines the maximum species-specific size. Cells smaller than the critical limit (a dashed line) are in the reproductive phase (1) and represent the effective fraction of the population. Larger cells, the size of which is between a dashed and a dash-dotted line, are in the pre-reproductive phase of the life cycle (2)

There was a direct correlation between length of parental and daughter cells (Fig. 5). After isolation, the clones were tested for crossability/non-crossability in pairwise combinations, and 15 clones randomly selected from the samples turned out to be sexually compatible strictly in accordance with their mating types. For a sample of 10 clones, a table of cross-mating is given (Table 3). The clones were unevenly distributed by mating types, in a ratio of 4 : 11.

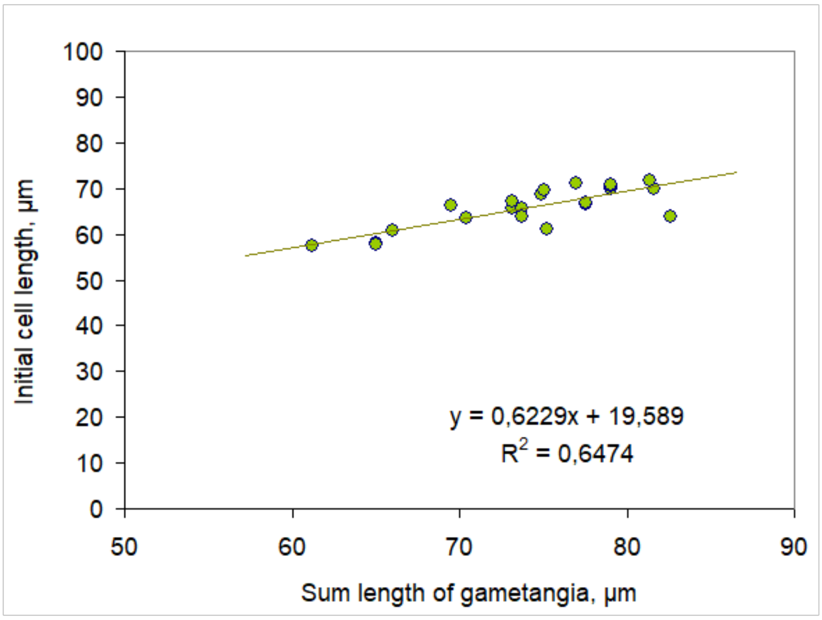

**Fig. 5.** Dependence of the initial cell length on the gametangial cell length in *Nitzschia* cf. *thermaloides* ($N$ = 18)

**Table 3.** Cross-mating table (shows reproductive compatibility of *Nitzschia* cf. *thermaloides* clones isolated from the mud volcanoes)

| Mud volcano | Clone | | 0.1026-YB | 0.1026-YD | 0.1020-OA | 0.1020-OC | 0.1029-YE | 0.1029-YF | 0.1029-YG | 0.1025-YA | 0.1020-OE | 0.1020-OF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mt | mt1 | mt2 | mt1 | mt2 | mt2 | mt2 | mt2 | mt1 | mt2 | mt2 |
| Andrusov | 0.1026-YB | mt1 | | | | | | | | | | |
| Andrusov | 0.1026-YD | mt2 | **3** | | | | | | | | | |
| Pavlov | 0.1020-OA | mt1 | **0** | **2** | | | | | | | | |
| Pavlov | 0.1020-OC | mt2 | **2** | **0** | **3** | | | | | | | |
| Pavlov | 0.1029-YE | mt2 | **3** | **0** | **3** | **0** | | | | | | |
| Pavlov | 0.1029-YF | mt2 | **2** | **0** | **–** | **0** | **0** | | | | | |
| Pavlov | 0.1029-YG | mt2 | **3** | **0** | **3** | **0** | **0** | **0** | | | | |
| Central Lake | 0.1025-YA | mt1 | **0** | **2** | **0** | **2** | **3** | **3** | **3** | | | |
| Central Lake | 0.1020-OE | mt2 | **3** | **3** | **3** | **0** | **0** | **0** | **0** | **3** | | |
| Central Lake | 0.1020-OF | mt2 | **2** | **3** | **3** | **0** | **0** | **0** | **0** | **2** | **0** | |

**Note:** mt, mating type; mating types (mt1 and mt2) are distinguished conventionally and marked in blue and red, respectively, for ease of perception. The table shows the maximum scores for the intensity of auxospore formation, obtained as a result of 3–5 times repeated experiments: 0, no auxospores were observed; 1, single; 2, non-abundant; 3, abundant. Dash indicates lack of data.

In *N.* cf. *thermaloides*, the sexual process is preceded by pairing of parental cells (Fig. 6A); the formation of pairs could be observed on the second day after mixed inoculations were made. Cells in pairs were arranged more or less parallel to each other and showed a stable relationship. At the same time, cells did not secrete any mucus and did not form mucous capsules. Gametogenesis in parental cells (gametangial cells) usually began on the third day (after the light–dark period completion) in the morning and proceeded in pairs almost simultaneously, but not synchronously. Initially, the cell protoplast contracted along the apical axis (Fig. 6B). Resulting from transapical division, two identical gametes were formed in each gametangial cell (Fig. 6C). The gametes slightly increased in size, which is more typical of the gametes that were formed first (Fig. 6D). At the end of the formation, the gametes fused in pairs without any order (Fig. 6E, F); this resulted in the formation of two zygotes. The zygotes began bipolar growth, extending parallel to the apical axis of the gametangial valves (Fig. 6G). From this point on, cells should be called auxospores (Fig. 6H). Growing auxospores were usually arranged side by side, more or less parallel to each other and to the valves of parental cells; occasionally, auxospores were lined up along one axis (Fig. 6I). There were gametangial pairs characterized by disordered orientation of auxospores and valves of parental cells, for example, due to deformation of the perizonium, which determines the bipolar direction of auxospore growth (Fig. 6J).

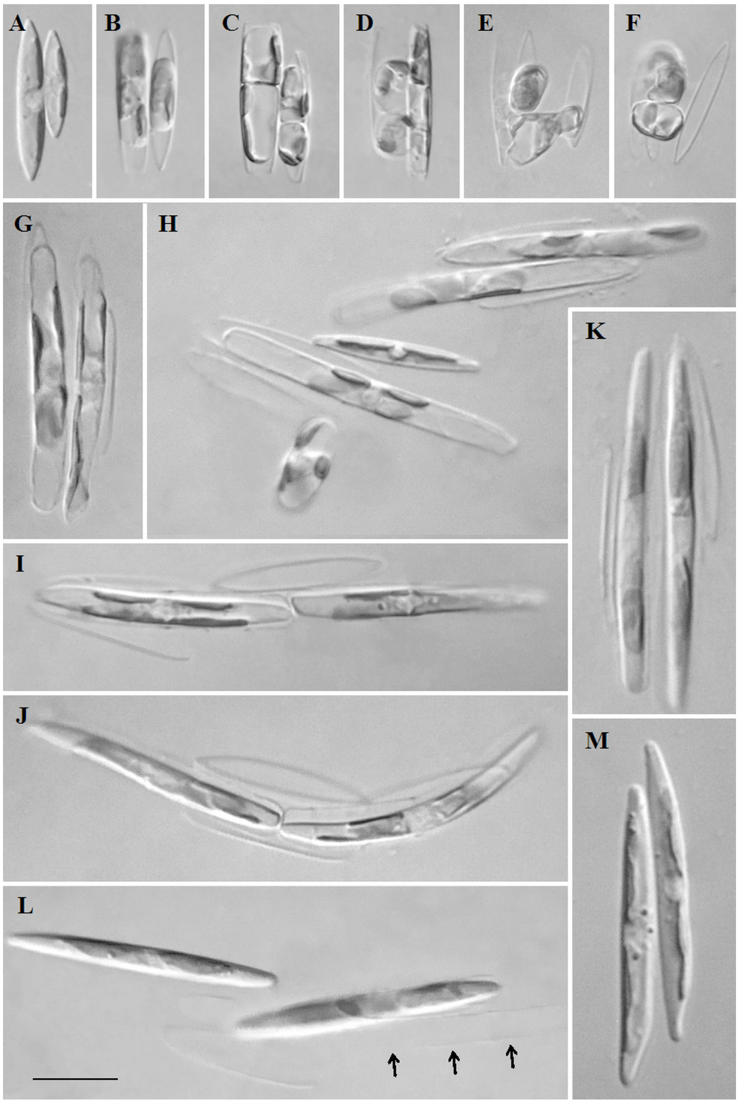

**Fig. 6.** The process of *Nitzschia* cf. *thermaloides* sexual reproduction: A, formation of pairs of gametangial cells; B, the beginning of gamete formation, the protoplast contracts along the apical axis; C, as a result of transapical division, two identical gametes are formed; D, gametes slightly increase in diameter; E, F, gametes fuse in pairs without any order; G, bipolar-growing auxospores; H, usual arrangement of auxospores – side by side and parallel to each other and to the valves of gametangia; I, occasionally, auxospores are located along one axis; J, developmental disorders result in disordered orientation of auxospores and valves of parental cells; K, inside fully formed auxospores, which have reached a size close to the maximum species-specific, the valves of initial cells begin to deposit (first epitheca; then hypotheca); L, initial cells, that recently left the perizonium (indicated by arrows); M, initial cells return to normal mitotic divisions, giving rise to a new generation of clones. Scale bar: 20 μm

With a sufficient duration of the light period (October–November), on the same day, auxospores that had grown to the maximum size could be detected; inside them, the valves of initial cells began to be deposited (Fig. 6K) – first epitheca, and then, after acytokinetic meiosis, hypotheca. The process of auxospore formation continued on the fourth day. Since initial cells proceeded to mitotic division, in the mixed cultures one could detect both initial cells that had recently left the perizonium (Fig. 6L) and the first post-initial cells morphologically slightly different from them (Fig. 6M).

Interestingly, syngamy was not always successful. Sometimes, only one pair of gametes fused; therefore, in some gametangial pairs, only one zygote and, accordingly, one auxospore was formed. Aborting the development of one of auxospores led to the same result. Sometimes, not a single gametangial pair could reach syngamy, even in those rare cases when gametangial cells had formed not pairs, but triplets. However, in general, the process of auxospore formation was intensive, and large cells of the new generation could be observed in mixed cultures in high abundance.

## DISCUSSION

The theory of the life cycle of diatoms is quite well developed [Chepurnov et al., 2004; Drebes, 1977; Gastineau et al., 2014; Poulíčková, Mann, 2019; Roshchin, 1994; Round et al., 1990; *etc.*]. During the main part of their life cycle, these microalgae are in a diploid state, carrying out mitotic divisions; only gametes are haploid. Sexual reproduction, accompanied by meiosis, is an obligatory attribute of the life cycle in almost all diatoms, with rare exceptions. At the same time, one of the most remarkable features of these microalgae is that their life cycle is closely related to cell size. The following main key points regarding the vast majority of diatom species must be taken into consideration for performing an analysis of the natural population studied by us:

- Passing through all phases of the life cycle depends on the change in cell sizes.
- Due to the peculiarities of the structure and the way of frustule formation, the apical size in pennates and cell diameter in centric diatoms decrease with each vegetative division. In clones, a decrease in the mean size and an increase in the range of variation in cell sizes occur.
- Restoration of initial (maximum) cell sizes in a population is carried out in the process of sexual reproduction due to rapidly growing zygotes called auxospores.
- In the life cycle, pre-reproductive, reproductive, and post-reproductive phases can be distinguished (the latter one is not observed in all species).
- Duration of the pre-reproductive phase of the life cycle ranges from several months to several years, depending on the species and the cell division rate.
- Transition from the pre-reproductive to the reproductive phase is related to overcoming the critical size (one of the cardinal points of the life cycle), which in most diatoms is close to half the maximum species-specific size.
- Transition to the reproductive phase does not mean an unconditional entry into the sexual process. Its implementation requires a favorable combination of environmental factors and presence of a sexual partner (in the case of heterothallic reproduction, which prevails in most pennate diatoms). Most cells that have reached the reproductive phase continue to divide vegetatively and decrease in size.

- Regardless of the size of parental cells, auxospores usually grow to the size close to the maximum species-specific one. However, in some species, the size of daughter (initial) cells depends on the size of parental cells. In such cases, the size of the arisen initial cells may be less than the critical limit, which allows them to enter the sexual process immediately. All this results in two-stage auxospore formation.

Thus, two important events occur in the process of sexual reproduction in a diatom population: restoration of initial cell sizes and appearance of a new generation of clones with new genetic "patiences." Worth noting that a small number of Bacillariophyta species (extremely insignificant compared to the total number of those studied) do not change cell sizes during vegetative division. Apparently, for this reason, the sexual process has never been observed in them. Those include species which are model in many aspects but sexually exceptional: *Phaeodactylum tricornutum* Bohlin, 1897 and *Thalassiosira pseudonana* Hasle & Heimdal, 1970. These are the first two diatoms with a complete genome sequenced [Armbrust et al., 2004; Montsant et al., 2005].

Considering the above principles, it is possible to analyze cell distribution in the natural population of *N.* cf. *thermaloides* (see Fig. 3) by the life cycle phases. At the time of sampling, slightly more than a half of cells in the population were in the pre-reproductive phase. The effective fraction of the population accounted for 46%. The process of sexual reproduction occurs in the population, as evidenced by the finding of cells whose size was close to the maximum species-specific one. Obviously, appearance of such cells could result only from sexual reproduction. The size spectrum covers all phases of the life cycle: cells in the pre-reproductive and reproductive phases are equally represented. This state of the population can be called balanced. Moreover, given that it usually takes several years for diatoms to go through the entire life cycle [Mann, 1988], we can conclude that *N.* cf. *thermaloides* population is not ephemeral and inhabits this spot for quite a long time.

The trend characteristic of most diatoms was confirmed once again: the size boundary (the cardinal point), passing which cells reach the reproductive phase, is close to half the maximum species-specific size (in most of the species studied, it ranges from 45 to 55%) [Davidovich, 2000]. A consequence of the discovered positive correlation between the length of parental and daughter cells (see Fig. 4) is unequal duration of the life cycle of individual clones.

Isogamous sexual reproduction, accompanied by the formation of two gametes in each gametangial cell, was recorded in at least 15 genera of diatoms, phylogenetically both close to the genus *Nitzschia* Hassall, 1845 (for example, *Achnanthes* Bory, 1822) and distant from it (for instance, *Dickieia* Berkeley ex Kützing, 1844 or *Berkeleya* Greville, 1827), considering the largest phylogenetic tree constructed from data for 11 genes in 1,151 diatom taxa [Nakov et al., 2018]. However, in addition to number of gametes and their behavior, an important role in the classification of types of the sexual process is played by the following details: presence or absence of copulatory mucus secreted by gametangial cells; division of the cell protoplast in the apical or transapical plane during the formation of gametes and presence or absence of rearrangement of the latter; direction of movement of gametes; and mutual orientation of axes of auxospores and valves of gametangial cells. Given all the details, the sexual process observed in *N.* cf. *thermaloides* can be categorized as IB2a according to Geitler classification [Geitler, 1973].

In the mating system of the species, heterothallism prevails. Apparently, it is the only way to reproduce, given that no cases of homothallism have been revealed in clonal cultures so far.

In the future, the response of *N.* cf. *thermaloides* to quite variable (extreme for many other diatoms) conditions of temperature and high irradiance is to be studied. Moreover, the limits of the halotolerance of this species living under conditions of relatively high salinity, typical for puddles and lakes of mud volcanoes, are to be established.

**Conclusion.** In water bodies formed by emissions from the mud volcanoes of the Kerch Peninsula, *Nitzschia* cf. *thermaloides* is represented as a stable population containing cells in all phases of the life cycle (pre-reproductive, reproductive, and post-reproductive). The analysis of the size distribution of cells in the population, which became possible due to the established cardinal points of the life cycle, shows that the population is not ephemeral and is capable of reproducing in generations, despite living under extreme conditions.

*This work was carried out within the framework of KSS – Nature Reserve of RAS – Branch of IBSS state research assignment "Study of fundamental physical, physiological, biochemical, reproductive, population, and behavioral characteristics of marine hydrobionts" (No. 121032300019-0). Isolation of clones, measurements, and observations were carried out using microscopes, digital cameras, laboratory glassware, and software purchased at the expense of grants from the Russian Foundation for Basic Research "Data finding on reproductive biology of the genus Ardissonea, which are crucial for understanding of the diatom phylogeny" (No. 15-04-00237_a) and "Study of the species specificity of the evolutionary transition from oogamy to neogamous sexual reproduction in polar centric diatoms (Mediophyceae)" (No. 19-04-00070_a).*

**Acknowledgement.** We express our gratitude to colleagues from IBSS, L. Ryabushko and her co-authors, for their pioneering work that inspired us to carry out this study, and to V. Lishaev, for his help with SEM microphotography. We are grateful to the driver of the Karadag Scientific Station V. Sirota, a regular member of our expeditions.

## REFERENCES

1. Abdullin Sh. R., Bagmet V. B. Myxotrophy of cyanobacteriae and algae inhabiting caves. *Zhurnal obshchei biologii*, 2016, vol. 77, no. 1, pp. 54–62. (in Russ.)
2. Davidovich N. A., Davidovich O. I., Podunay Yu. A. Diatom culture collection of the Karadag scientific station (Crimea). *Morskoj biologicheskij zhurnal*, 2017, vol. 2, no. 1, pp. 18–28. (in Russ.). https://doi.org/10.21072/mbj.2017.02.1.03
3. Kayukova E. P. Khimicheskii sostav zhidkoi fazy gryazevykh sopok severo-vostochnoi chasti Kerchenskogo poluostrova (Vostochnyi Krym). In: *Geologiya, geoekologiya, evolyutsionnaya geografiya* : collective monograph. Vol. 17 / E. M. Nesterov, V. A. Snytko (Eds). Saint Petersburg : Izd-vo RGPU im. A. I. Gertsena, 2018, pp. 186–190. (in Russ.)
4. Kurishko V. A., Mesyats I. A., Terdovidov A. S. Gidrogeologiya gryazevogo vulkanizma Kerchenskogo poluostrova. *Geologicheskii zhurnal*, 1968, vol. 8, iss. 1, pp. 49–59. (in Russ.)
5. Matyunina V. A. Khimicheskii sostav rassolov Bulganakskogo i Malo-Tarkhanskogo sopochnykh polei (Vostochnyi Krym) // *Materialy Mezhdunarodnogo molodezhnogo nauchnogo foruma "Lomonosov-2019"* / I. A. Aleshkovsky, A. V. Andriyanov, E. A. Antipov (Eds). Moscow : MAKS Press, 2019, pp. 1–2.

(in Russ.). URL: https://lomonosov-msu.ru/archive/Lomonosov_2019/data/15896/92330_uid343668_report.pdf [accessed: 11.11.2020].

6. Nevrova E. L., Shadrin N. V. Benthic diatoms in Crimean saline lakes. *Morskoj ekologicheskij zhurnal*, 2005, vol. 4, no. 4, pp. 61–71. (in Russ.). https://repository.marine-research.ru/handle/299011/821
7. Nikulina T. V. Biodiversity of algae of hot springs from Kuril Islands (Russia). *Al'gologia*, 2010, vol. 20, no. 3, pp. 334–356. (in Russ.). https://doi.org/10.1615/InterJAlgae.v12.i4.10
8. Polyakova S. L., Davidovich O. I., Podunay Yu. A., Davidovich N. A. Modification of the ESAW culture medium used for cultivation of marine diatoms. *Morskoj biologicheskij zhurnal*, 2018, vol. 3, no. 2, pp. 73–80. (in Russ.). https://doi.org/10.21072/mbj.2018.03.2.06
9. Roshchin A. M. *Zhiznennye tsikly diatomovykh vodoroslei.* Kyiv : Naukova dumka, 1994, 171 p. (in Russ.)
10. Ryabushko L. I., Bondarenko A. V. Microalgae of mud volcano of the Bulganak sopochnoe field on the Crimean Peninsula. *Morskoj biologicheskij zhurnal*, 2020, vol. 5, no. 1, pp. 64–77. (in Russ.). https://doi.org/10.21072/mbj.2020.05.1.07
11. Senicheva M. I., Gubelit Yu. I., Prazukin A. V., Shadrin N. V. Fitoplankton gipersolenykh ozer Kryma // *The Black Sea Microalgae: Problems of Biodiversity Preservation and Biotechnological Usage* / Yu. N. Tokarev, Z. Z. Finenko, N. V. Shadrin (Eds) ; NAS of Ukraine, Institute of Biology of the Southern Seas. Sevastopol : EKOSI-Gidrofizika, 2008, pp. 93–99. (in Russ.). https://repository.marine-research.ru/handle/299011/5521
12. Armbrust E. V., Berges J. A., Bowler Ch., Green B. R., Martinez D., Putnam N. H., Zhou Sh., Allen A. E., Apt K. E., Bechner M., Brzezinski M. A., Chaal B. K., Chiovitti A., Davis A. K., Demarest M. S., Detter J. Ch., Glavina T., Goodstein D., Hadi M. Z., Hellsten U., Hildebrand M., Jenkins B. D., Jurka J., Kapitonov V. V., Kröger N., Lau W. W. Y., Lane T. W., Larimer F. W., Lippmeier J. C., Lucas S., Medina M., Montsant A., Obornik M., Parker M. Sch., Palenik B., Pazour G. J., Richardson P. M., Rynearson T. A., Saito M. A., Schwartz D. C., Thamatrakoln K., Valentin K., Vardi A., Wilkerson F. P., Rokhsar D. S. The genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution, and metabolism. *Science*, 2004, vol. 306, iss. 5693, pp. 79–86. https://doi.org/10.1126/science.1101156
13. Drebes G. Sexuality. In: *The Biology of Diatoms* / D. Werner (Ed.). Oxford : Blackwell Scientific Publications, 1977, pp. 250–283. (Botanical Monographs ; vol. 13).
14. Chepurnov V. A., Mann D. G., Sabbe K., Vyverman W. Experimental studies on sexual reproduction in diatoms. *International Review of Cytology*, 2004, vol. 237, pp. 91–154. https://doi.org/10.1016/S0074-7696(04)37003-8
15. Davidovich N. A. Species-specific sizes and size range of sexual reproduction in diatoms. In: *Proceedings of the 16th International Diatom Symposium*, Greece, 25 August – 1 September, 2000 / A. Economou-Amilli (Ed.). Greece : University of Athens, 2000, pp. 191–196.
16. Gastineau R., Davidovich N. A.,

Hallegraeff G. M. Reproduction in microalgae. In: *Reproductive Biology of Plants* / K. G. Ramawat, J.-M. Mérillon, K. R. Shivanna (Eds). Boca Raton : CRC Press, 2014, pp. 1–28. https://doi.org/10.1201/b16535

17. Geitler L. Auxosporenbildung und Systematik bei pennaten Diatomeen und die Cytologie von *Cocconeis*-Sippen. *Österreichische Botanische Zeitschrift*, 1973, vol. 122, no. 5, pp. 299–321.
18. Mann D. G. Why didn't Lund see sex in *Asterionella*? A discussion of the diatom life cycle in nature. In: *Algae and the Aquatic Environment: Contributions in Honour of J. W. G. Lund* / F. E. Round (Ed.). Bristol, UK : Biopress Ltd., 1988, pp. 384–412.
19. Montsant A., Jabbari K., Maheswari U., Bowler C. Comparative genomics of the pennate diatom *Phaeodactylum tricornutum*. *Plant Physiology*, 2005, vol. 137, iss. 2, pp. 500–513. https://doi.org/10.1104/pp.104.052829
20. Nakov T., Beaulieu J. M., Alverson A. J. Accelerated diversification is related to life history and locomotion in a hyperdiverse lineage of microbial eukaryotes (diatoms, Bacillariophyta). *New Phytologist*, 2018, vol. 219, iss. 1, pp. 462–473. https://doi.org/10.1111/nph.15137
21. Peletier H., Gieskes W. W. C., Buma A. G. J. Ultraviolet-B radiation resistance of benthic diatoms isolated from tidal flats in the Dutch Wadden Sea. *Marine Ecology Progress Series*, 1996, vol. 135, pp. 163–168. https://doi.org/10.3354/meps135163
22. Poulíčková A., Mann D. G. Diatom sexual reproduction and life cycles. In: *Diatoms: Fundamentals and Applications* / J. Seckbach, R. Gordon (Eds). Hoboken, New Jersey : Wiley ; Salem, Massachusetts : Scrivener Publishing LLC, 2019, pp. 245–272. https://doi.org/10.1002/9781119370741.ch11
23. Round F. E., Crawford R. M., Mann D. G. *The Diatoms. Biology and Morphology of the Genera.* Cambridge, UK : Cambridge University Press, 1990, 747 p.

# БИОЛОГИЯ ВОСПРОИЗВЕДЕНИЯ И ЖИЗНЕННЫЙ ЦИКЛ ДИАТОМОВОЙ ВОДОРОСЛИ *NITZSCHIA* CF. *THERMALOIDES*, НАСЕЛЯЮЩЕЙ ГРЯЗЕВЫЕ ВУЛКАНЫ КРЫМА

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В биологии актуально изучение способности организмов к существованию в экстремальных условиях, к которым особенно чувствительна фаза жизненного цикла, связанная с половым размножением и возможностью оставить следующее поколение. В пробах, которые были взяты из луж и озёр, образующихся в местах действия грязевых вулканов Булганакского сопочного поля (Керченский полуостров, Крым), обнаружена в большом количестве *Nitzschia* cf. *thermaloides* Hustedt. Из проб микропипеточным способом выделены и введены в культуру отдельные клоны, скрещивание которых позволило инициировать гетероталлическое половое воспроизведение. Целью работы было изучить биологию воспроизведения и жизненный цикл водоросли из биотопа с экстремально высокими уровнями освещённости, температуры и солёности. Впервые для данного вида приведено описание полового процесса, соответствующего типу IB2a по классификации Гайтлера. Определены кардинальные пункты — критические размеры клеток, обусловливающие переход из одной фазы жизненного цикла в другую. Приведён полный диапазон размеров клеток — от инициальных, появившихся в результате полового воспроизведения, до самых маленьких, наблюдавшихся при содержании в культуре. Рассмотрены основные положения теории жизненного цикла диатомовых водорослей, позволяющие выполнить анализ их природной популяции. Установлено, что популяция *N.* cf. *thermaloides* существует в месте отбора проб длительное время и представлена клетками, находящимися во всех фазах жизненного цикла.

**Ключевые слова:** диатомовые, *Nitzschia* cf. *thermaloides*, грязевые вулканы, половое воспроизведение, жизненный цикл

Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 2, pp. 55–73
https://marine-biology.ru

UDC 574.5(262.5)

# STATE OF THE ICHTHYO-, MESO-, AND MACROPLANKTON COMPLEXES OFF THE CRIMEAN PENINSULA (THE BLACK SEA) IN CONNECTION WITH THE HYDROBIOLOGICAL REGIME FEATURES IN OCTOBER 2016

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Received by the Editor 14.07.2020; after reviewing 20.11.2020;
accepted for publication 16.02.2023; published online 31.05.2023.

The changes in the Black Sea hydrological regime recorded since 1990s have altered the state of epipelagic complexes of marine organisms, primarily the seasonal variability of their biological cycles. This largely affected the spawning phenology of natural fish populations, as well as ichthyoplankton species diversity and spatial distribution, and established trophic relationships within the plankton community. The interactions between links of the food chain in epipelagic complexes, as well as their seasonal and interannual variations, ultimately affect fish spawning efficiency, especially that of mass commercial species, and determine the replenishment of their new generations. To establish ichthyoplankton species composition, abundance, and spatial distribution, the study was carried out in shelf and open areas of the Black Sea (the Crimean coast) during the 89th cruise of the RV "Professor Vodyanitsky" (30 September – 19 October, 2016). Eggs and larvae of fish and the biomass of meso- and macroplankton were analyzed. Ichthyoplankton and macroplankton were sampled with Bogorov–Rass net (inlet area of 0.5 $m^2$; mesh size of 300 μm) by vertical sampling technique. In the shelf areas, sampling was carried out from the bottom up to the surface, while in the deep-sea areas, from the lower boundary of the oxygen zone up to the surface. Ichthyoplankton was fixed with 4% neutralized formaldehyde and investigated under microscope to determine taxonomic composition and, if possible, to analyze contents of fish larvae intestines. Species composition and spatial distribution of ichthyo-, meso-, and macroplankton in October 2016 were studied, as well as the feeding of fish larvae of the Black Sea off the Crimean coast. The research covered the initial phase of the autumn hydrological season. In samples, eggs and larvae of 9 warm-water fish species and 6 temperate-water fish species were found. The mean abundance of eggs was 2.92 $\text{ind.}\cdot\text{m}^{-2}$, and the mean abundance of larvae was 3.56 $\text{ind.}\cdot\text{m}^{-2}$. The low percentage (30%) of dead eggs of the warm-water European anchovy *Engraulis encrasicolus* and the presence of its different-sized larvae evidenced the ongoing productive spawning. The zooplankton biomass increased from the shelf towards the deep-sea areas. Small plankton organisms prevailed in the shelf areas providing enough food for fish larvae to survive. Despite the significant biomass of gelatinous plankton feeders in October 2016, their effect on ichthyoplankton complexes of the Black Sea was apparently minor.

**Keywords:** ichthyoplankton, mesoplankton, gelatinous macroplankton, feeding of fish larvae, species diversity, spatial distribution, Black Sea

Climate change is one of the main factors affecting spawning phenology, spatial distribution, species structure, and trophic relationships in marine ecosystems [Richardson et al., 2009; Robinson et al., 2014; Turan et al., 2016; Vinogradov et al., 1999]. An increase in temperature in seas with a well-pronounced seasonality, *inter alia* in the Black Sea, led to an inversion of life cycles, a shift in the reproduction timing, and an expansion in the species diversity of hydrobionts at all trophic levels, from primary producers to predators [Auth et al., 2017; Fincham et al., 2013; McQueen, Marshall, 2017; Parsons, Lalli, 2002; Purcell et al., 2007]. The autumn hydrological season (October–November) is usually characterized by a restructuring of the surface current system from the summer type of circulation to the winter one; also, it is distinguished by maximum temporal gradients of the sea surface temperature [Artamonov et al., 2018; Troshchenko, Subbotin, 2018]. Interestingly, autumn ichthyoplankton can be formed by both warm-water and temperate-water fish species. In October, spawning of temperate-water fish begins (their eggs prevail in ichthyoplankton), and spawning of warm-water fish continues. However, small abundance of alive eggs and the absence of larvae of younger age groups indicate that the spawning is unproductive. In October, on a shelf and in deep-sea areas, eggs of temperate-water species *Sprattus sprattus* (Linnaeus, 1758) and *Merlangius merlangus* (Linnaeus, 1758) are common, as well as larvae of *Engraulis encrasicolus* (Linnaeus, 1758) of older age groups and juveniles of *Syngnathus schmidti* Popov, 1928. Eggs, larvae, and juveniles of coastal species brought there by a current are found singly [Dekhnik, 1973; Dekhnik, Pavlovskaya, 1979; Gordina et al., 1991; Klimova et al., 2010].

For gelatinous macroplankton, the autumn hydrological season is important for several reasons. For the scyphomedusa *Aurelia aurita* (Linnaeus, 1758), it is a significant stage characterizing the success of spring generation; for *Mnemiopsis leidyi* A. Agassiz, 1865, it is the time of maximum elimination under the pressure of *Beroe ovata* Bruguière, 1789 [Finenko et al., 2003]. For many species of mesozooplankton, October is a period of active growth and reproduction against the backdrop of weakened predation by ctenophores. Although, with a decrease in *M. leidyi* abundance in a plankton community, a trend towards a rise in the trophic role of jellyfish was recorded; its predatory pressure on zooplankton in recent years usually does not reach a threshold critical for maintaining prey populations [Anninsky et al., 2016].

Unfortunately, the Black Sea ichthyoplankton in the shelf and open waters at the beginning of the autumn hydrological season was practically not analyzed due to its low abundance and species diversity [Dekhnik, Pavlovskaya, 1979; Klimova, Podrezova, 2018; Klimova et al., 2010, 2019]. The latest published data of ichthyo-, meso-, and macroplankton studies in the western sector of the Black Sea, including the area off the Crimean Peninsula, date back to October 2005 [Klimova et al., 2010]. Therefore, the results of complex plankton research carried out in October 2016 both in the shelf and deep-sea areas of the Black Sea off the Crimean Peninsula, from Cape Tarkhankut to the Kerch Strait, during the transition from the summer hydrological season to the autumn one are of particular interest.

The aim of the work is to analyze species composition, abundance, and spatial distribution of ichthyo-, meso-, and gelatinous macroplankton in October 2016 against the backdrop of climatic variability of the Black Sea hydrological regime.

## MATERIAL AND METHODS

Plankton studies were carried out during the 89th cruise of the RV "Professor Vodyanitsky" (September 30 – October 19, 2016) in the shelf and deep-sea areas of the Black Sea off the Crimean Peninsula in the western (Cape Tarkhankut – the city of Alupka) and eastern sectors (Cape Meganom – the Kerch Strait) in the water area with coordinates from N43.22° to N45.15° and from E31.24° to E36.26° (Fig. 1).

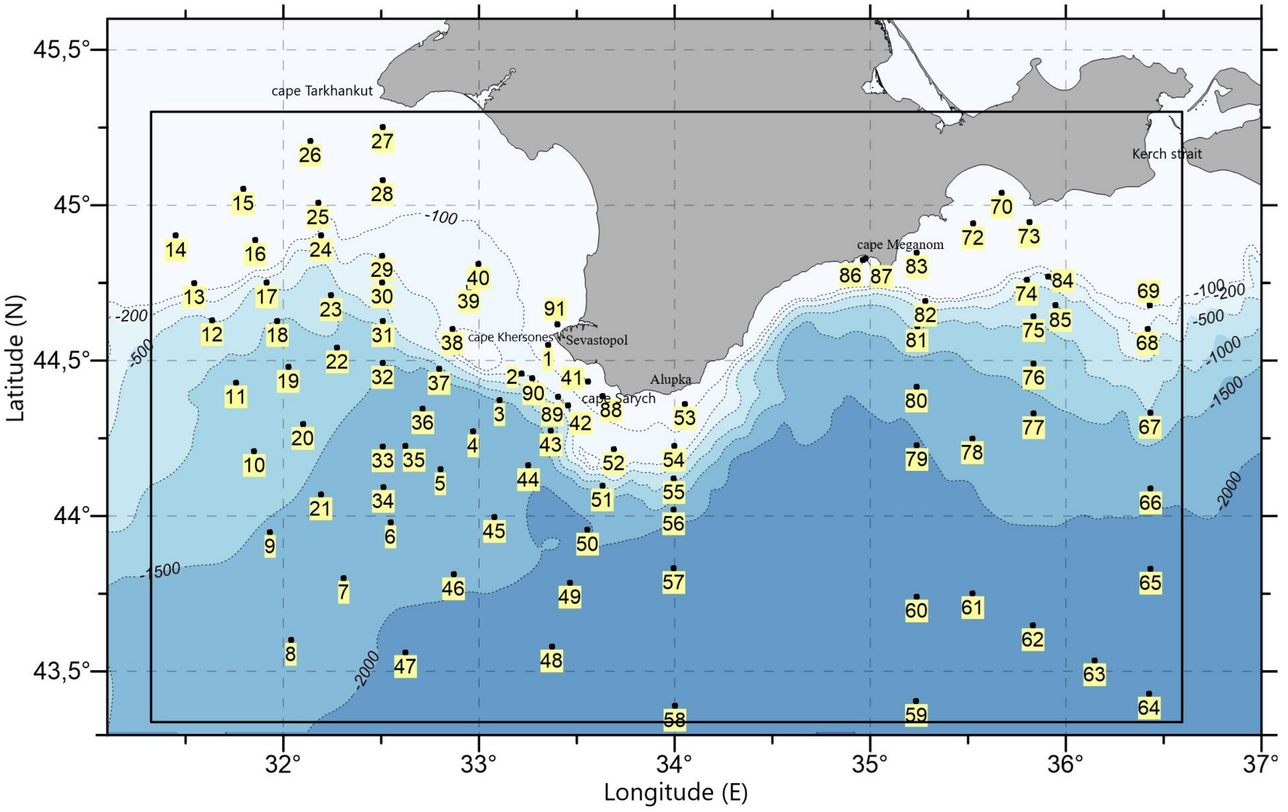

**Fig. 1.** Schematic map of sampling stations in the 89th cruise of the RV "Professor Vodyanitsky"

Ichthyo- and macroplankton, as well as large crustacean plankton (*Calanus euxinus* Hulsemann, 1991) was sampled with an inverse conical Bogorov–Rass net (BR-80/113; inlet area of 0.5 $m^2$; mesh size of 300 μm). Total mesozooplankton was sampled with a Juday net (inlet area of 0.1 $m^2$; mesh size of 112 μm). Plankton was sampled by vertical hauls from the bottom to the sea surface in the shelf area and from the lower boundary of the oxygen zone ($\sigma_t = 16.2$, according to Sea-Bird 911plus CTD) to the sea surface in open waters.

With BR-80/113, sampling was carried out in one replication at 62 stations, including 39 stations in the deep-sea (> 200 m) epipelagic zone, 19 stations on the outer shelf (50–200 m), and 4 stations on the inner shelf (< 50 m). Large gelatinous macroplankton was filtered through a sieve with a 4-mm mesh from freshly taken samples and analyzed *in vivo* onboard the RV for taxonomic composition, abundance, oral-aboral body length of ctenophores, and umbrella diameter of jellyfish. The remaining plankton fraction, *inter alia* small (< 5 mm) gelatinous, meso-, and ichthyoplankton, was fixed and processed under stationary conditions. Species composition and abundance of early size–age stages of gelatinous, large copepods, and ichthyoplankton were determined under MBS-10 at a magnification of 8 × 2 and 8 × 4. The body weight of the organisms was calculated using known linear weight ratios [Aninsky et al., 2013]. Fish eggs and larvae were identified according to [Dekhnik, 1973; D'Ancona, 1933; Russell, 1976].

Mesoplankton was sampled with a Juday net in duplicate on the outer shelf (sta. 1, N44.56°, E33.34°) and in the deep-sea areas (sta. 3, N44.37°, E33.07°), fixed, and identified under stationary conditions under a microscope (Fig. 1).

All plankton samples were fixed in 4% (v/v) formaldehyde buffered with borates.

The study of fish larvae feeding was carried out on a fixed material according to the technique of L. Duka and V. Sinyukova [1976]. Larvae were divided into size groups: *E. encrasicolus*, in accordance with [Dekhnik, 1960]; *S. schmidti*, in accordance with [Gordina et al., 1991].

Using the formulas proposed by Yu. Odum [1986], diversity indices were calculated: species diversity [Shannon, Weaver, 1949], species richness [Simpson, 1949], dominance [Margalef, 1958], and evenness [Pielou, 1966].

To analyze the hydrological regime and the structure of surface currents, we used expeditionary data of vertical profiling with Sea-Bird 911plus CTD and data of acoustic Doppler current profiler ADCP 300 kHz WorkHouse [Artamonov et al., 2018]. Additionally, we used material of satellite observations of the sea surface temperature (hereinafter SST); maps of geostrophic and surface currents [Copernicus Marine Service, 2019; Marine Portal of the Marine Hydrophysical Institute, 2020; National Center for Environmental Information, 2018] for August–October 2016; and data of regular observations on water temperature and salinity in the coastal areas of Sevastopol and Karadag [Troshchenko, Subbotin, 2018; Troshchenko et al., 2019].

The relationship between the distribution of the studied plankton components and certain abiotic environmental factors (seawater temperature and salinity) was measured by ANOSIM in PAST 4.0 statistical program.

## RESULTS

**Features of the hydrological regime.** The period of the survey (September 30 – October 19, 2016) corresponded to the initial phase of the autumn hydrological season. Intensive cooling of the sea upper layer with temperature gradients of more than 3–3.5 °C *per* month began in the third 10-day period of September and lasted until mid-December [Artamonov et al., 2018; Copernicus Marine Service, 2019; Marine Portal of the Marine Hydrophysical Institute, 2020; National Center for Environmental Information, 2018; Troshchenko, Subbotin, 2018; Troshchenko et al., 2019]. The autumn hydrological season is characterized by a restructuring of the surface current system from the summer type of circulation to the winter one. However, satellite observation data for the previous period and the time of the survey, geostrophic circulation calculations [Copernicus Marine Service, 2019; Marine Portal of the Marine Hydrophysical Institute, 2020; National Center for Environmental Information, 2018], and instrumental observations [Artamonov et al., 2018] indicate that the surface water circulation system in the study area in the first 10-day period of October 2016 corresponded to the summer one, with the preservation of its main features [Klimova et al., 2019]. Active meandering of the Rim Current jet along the continental slope contributed to the preservation of two quasi-stationary synoptic formations: the Sevastopol anticyclone (hereinafter SevAC) over the great topographic trough west of the Heracles Peninsula and the Crimean anticyclone (hereinafter CrAC) on the shelf south of the Feodosiya Gulf [Ivanov, Belokopytov, 2011]. To the south of the Rim Current area, in the western and eastern sectors of the survey, peripheral areas of western and eastern cores of the main cyclonic gyre (hereinafter MCG) with weak and unstable currents were traced (Fig. 2).

The general picture of the seasonal spatial distribution of the SST with a tendency to increase its values from the northwest (areas of the earliest and most intense SST decrease) to the east was disturbed by a strong storm on 9–10 October (Fig. 2). As a result, in the second 10-day period of October, background SST values throughout the study area decreased by 2–3 °C [Artamonov et al., 2018].

Thus, in the western sector, before the storm, the site of increased SST values (+21.0…+21.5 °C) corresponded to the SevAC zone; the site of minimum SST values (+19.0…+19.5 °C) was limited to a small shelf zone in the northwestern sea area [Artamonov et al., 2018] (Fig. 3).

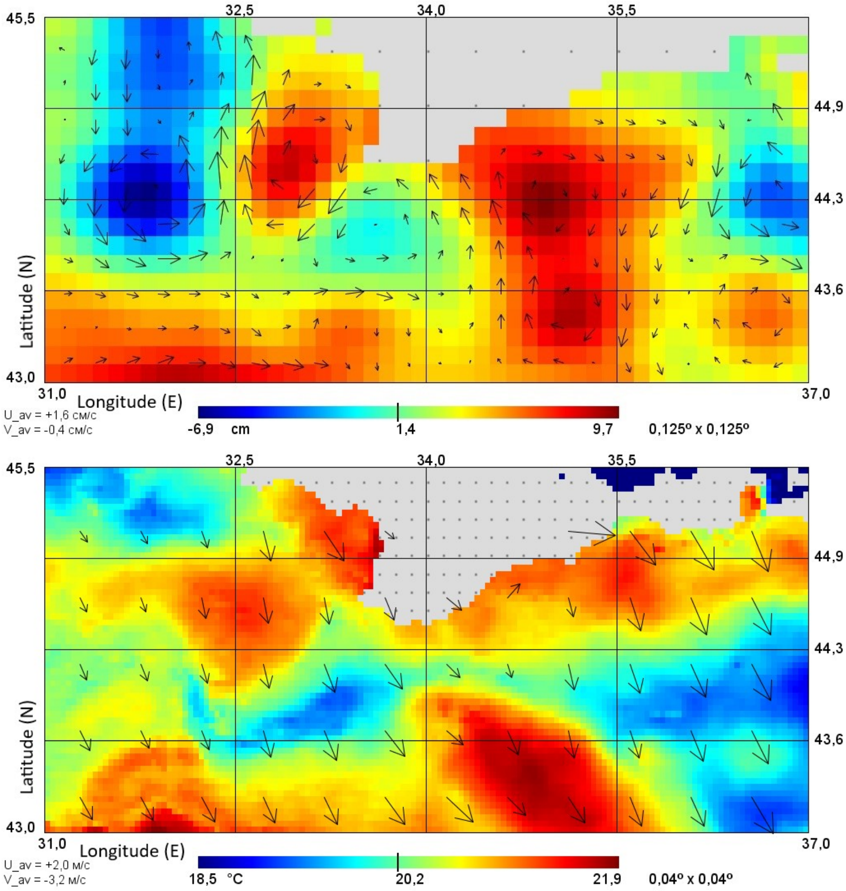

**Fig. 2.** Compositional maps of geostrophic currents (top) and sea surface temperature (bottom), 20–30 September, 2016 [Copernicus Marine Service, 2019; National Center for Environmental Information, 2018]

In the eastern sector of the survey, ichthyoplankton was sampled in the second 10-day period of October 2016. Background SST values dropped after the storm to +16…+16.5 °C. In the shelf area from Cape Sarych to Sevastopol, the values decreased to +17 °C (Fig. 4).

Against the backdrop of seasonal cooling of the sea surface layer and intensification of wind-wave mixing, the thickness of the upper quasi-homogeneous layer increased everywhere compared to that of the summer period. The maximum values of the thicknesses of the upper quasi-homogeneous layer (up to 28–30 m) were registered in the SevAC and CrAC zones, while the minimum values (up to 12–16 m) were recorded in areas of water rise on the northern peripheries of the western and eastern cores of the MCG.

Over the entire study area, cold intermediate layer differed significantly from "classical" one [Ivanov, Belokopytov, 2011] in terms of the core temperatures. The range of its spatial temperature variability was 8.30–8.52 °C: up to 8.30–8.36 °C (minimum values), in the areas of water rise on the MCG periphery; up to 8.42–8.44 °C, in the SevAC; and up to 8.5–8.52 °C, in CrAC. The variability of the depth of the cold intermediate layer core also corresponded to the position of main circulation structures. The maximum depth (up to 85–90 m) was noted above the great topographic trough in the SevAC zone; the values up to 80 m were recorded in the CrAC zone; and the minimum depth was registered on the periphery of the western (up to 35–40 m) and eastern (up to 40–45 m) cores of the MCG.

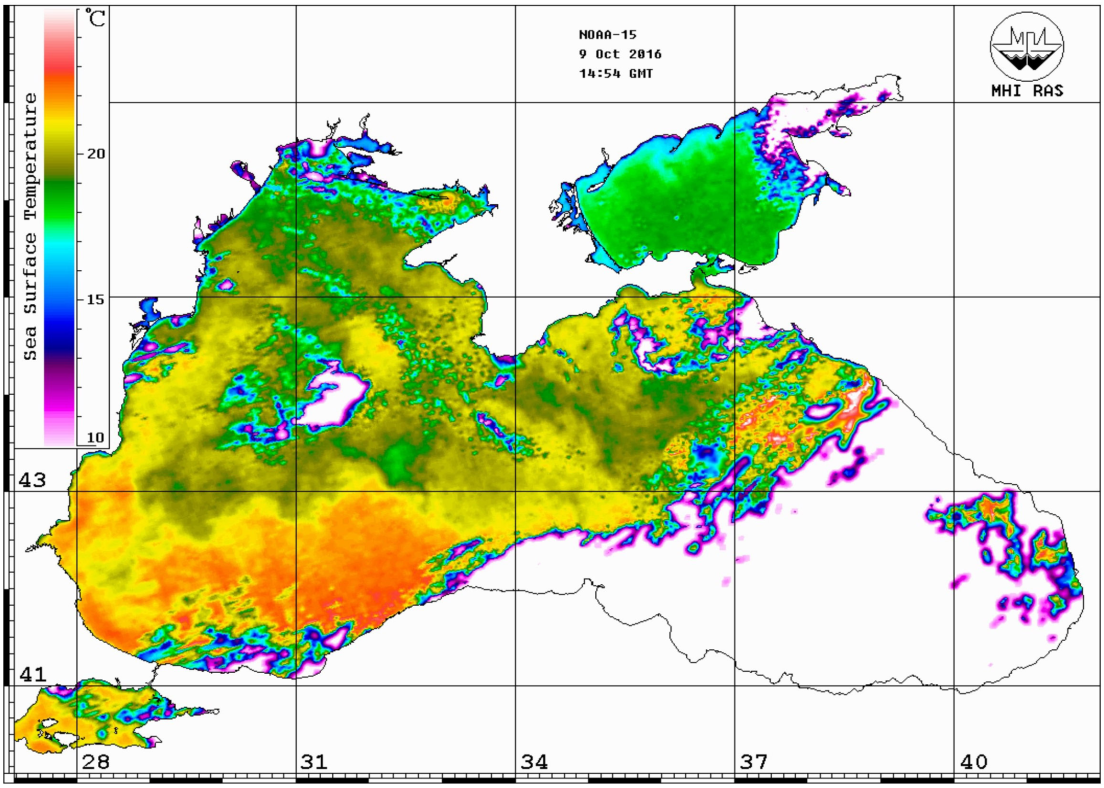

**Fig. 3.** Sea surface temperature according to NOAA-15 data (09.10.2016)

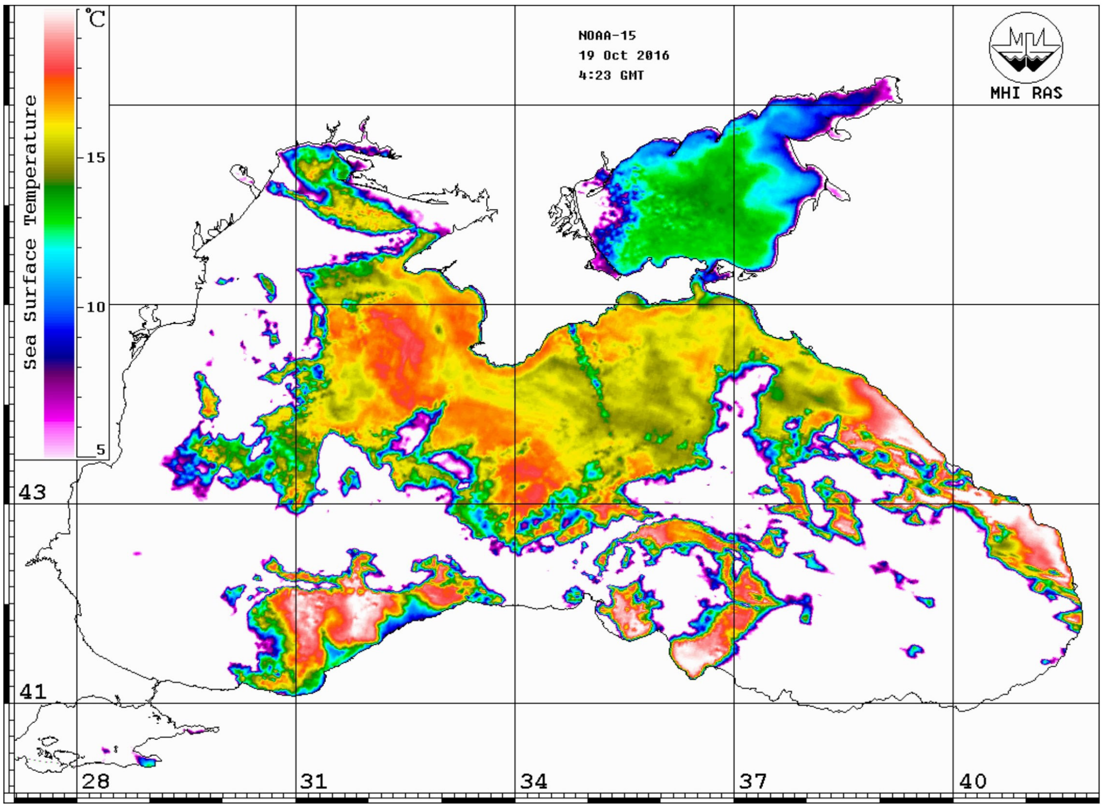

**Fig. 4.** Sea surface temperature according to NOAA-15 data (19.10.2016)

The spatial distribution of salinity of surface waters was determined by the proximity to the main sources of desalination, the direction of advection of desalinated waters, and the position of quasi-stationary dynamic formations. Low water salinity (< 18‰) was recorded in the SevAC and CrAC zones, where transformed desalinated waters from the northwestern sea area and from the Kerch Strait occurred. The most saline waters (18.45–18.50‰) corresponded to the peripheral areas of the MCG.

**Ichthyoplankton.** In ichthyoplankton, eggs and larvae of 15 fish species representing 9 families were identified (9 species of warm-water and 6 species of temperate-water fish). The mean abundance of eggs was 2.92, and larvae, 3.56 ind.·m$^{-2}$ (Table 1).

**Table 1.** Species composition and relative abundance (%) of fish eggs and larvae off the Crimean Peninsula in October 2016

| Species | Eggs | Larvae |
|---|---|---|
| **Family: Engraulidae**<br>*Engraulis encrasicolus* (Linnaeus, 1758) | 27.0 | 39.4 |
| **Family: Clupeidae**<br>*Sprattus sprattus* (Linnaeus, 1758) | 42.5 | 0.8 |
| **Family: Gadidae**<br>*Merlangius merlangus* Linnaeus, 1758 | 20.5 | 5.3 |
| *Trisopterus luscus* (Linnaeus, 1758) | 4.5 | 10.7 |
| Gadidae sp. | – | 3.6 |
| **Family: Lotidae**<br>*Gaidropsarus mediterraneus* (Linnaeus, 1758) | 4.5 | 0.8 |
| *Molva macrophthalma* (Rafinesque, 1810) | – | 0.8 |
| **Family: Syngnathidae**<br>*Syngnathus schmidti* Popov, 1928 | – | 28.1 |
| **Family: Scorpaenidae**<br>*Scorpaena porcus* Linnaeus, 1758 | – | 0.8 |
| **Family: Gobiidae**<br>*Pomatoschistus marmoratus* (Risso, 1810) | – | 0.8 |
| *Pomatoschistus minutus* (Pallas, 1770) | – | 3.7 |
| *Pomatoschistus pictus* (Malm, 1865) | – | 1.8 |
| *Gobius* sp. | – | 0.8 |
| **Family: Mugilidae**<br>*Mugil cephalus* Linnaeus, 1758 | 1.0 | 1.8 |
| **Family: Bothidae**<br>*Arnoglossus kessleri* Schmidt, 1915 | – | 0.8 |
| Mean abundance in a catch, ind.·m$^{-2}$ | 2.92 ± 1.64 | 3.56 ± 1.63 |

In the western sector of the survey (Cape Tarkhankut – the city of Alupka), ichthyoplankton was sampled in the first 10-day period of October, when the surface water circulation system, as mentioned earlier, corresponded to the summer one, with the preservation of its main features. At seawater temperature reaching +19.0…+21.5 °C, eggs and larvae of 10 fish species were recorded; their mean abundance was 3.4 and 4.7 ind.·m$^{-2}$, respectively (Fig. 5).

Above the depths of more than 50 m, *S. sprattus* spawned; maximum abundance of its eggs, 22 ind.·m$^{-2}$, was recorded near the Cape Khersones above a depth of 92 m. In addition to *M. merlangus* (a common representative of the family Gadidae), eggs and larvae were found, which we identified as *Trisopterus luscus* (Linnaeus, 1758) (Table 2). This cod species has been described for the North and Mediterranean Seas [Alonso-Fernández et al., 2010; D'Ancona, 1933; Russell, 1976]; there, its eggs and larvae are noted throughout the year with maximum abundance from October to May.

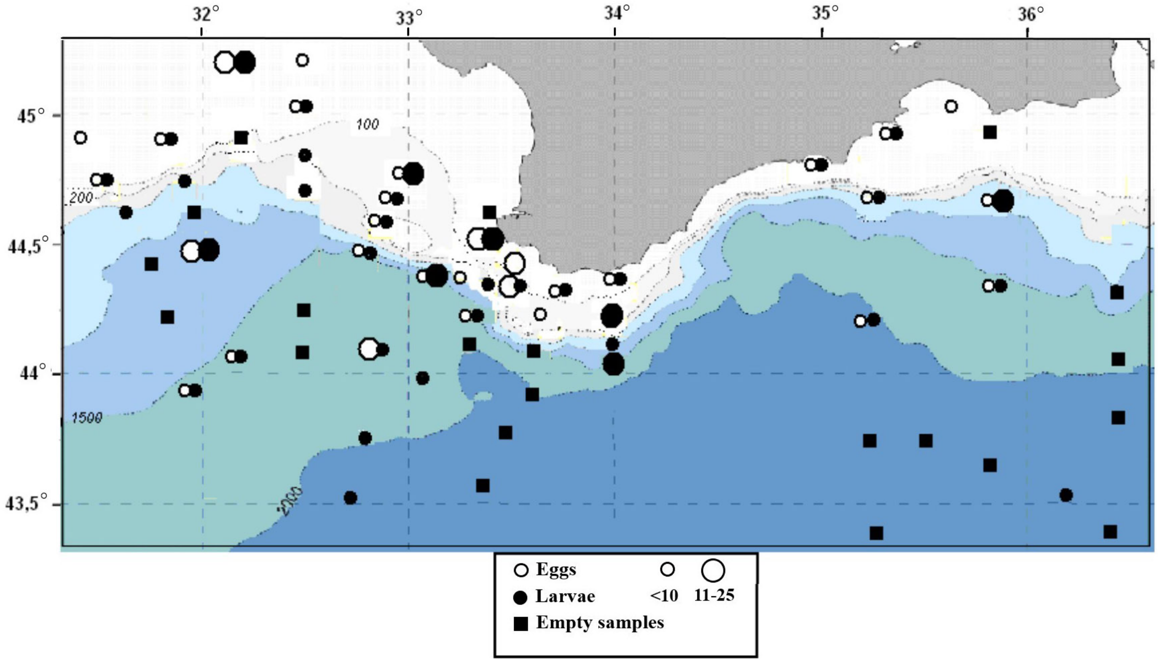

**Fig. 5.** Schematic map of ichthyoplankton distribution (ind.·m$^{-2}$) in October 2016

**Table 2.** Ichthyoplankton species composition and mean abundance (ind.·m$^{-2}$) in several areas of the Black Sea (2016)

| Species | Cape Tarkhankut – the city of Alupka | | | | Cape Meganom – the Kerch Strait | |
|---|---|---|---|---|---|---|
| | 30.09–09.10 | | 19.10 | | 10.10–17.10 | |
| | Eggs | Larvae | Eggs | Larvae | Eggs | Larvae |
| *Engraulis encrasicolus* | 1.2 | 2.1 | – | – | – | – |
| *Sprattus sprattus* | 1.4 | – | 2.0 | – | 0.6 | 0.1 |
| *Merlangius merlangus* | 0.5 | – | 0.5 | – | 0.9 | 0.7 |
| *Trisopterus luscus* | 0.2 | 0.5 | – | 1.0 | – | – |
| Gadidae sp. | – | 0.2 | – | – | – | – |
| *Gaidropsarus mediterraneus* | – | – | – | – | 0.5 | 0.1 |
| *Molva macrophthalma* | – | – | – | 0.5 | – | – |
| *Mugil cephalus* | 0.1 | 0.1 | – | – | – | 0.1 |
| *Syngnathus schmidti* | – | 1.5 | – | 0.5 | – | 0.4 |
| *Scorpaena porcus* | – | 0.1 | – | – | – | – |
| *Pomatoschistus marmoratus* | – | – | – | – | – | 0.1 |
| *Pomatoschistus minutus* | – | 0.1 | – | – | – | 0.1 |
| *Pomatoschistus pictus* | – | 0.1 | – | – | – | 0.1 |
| *Gobius* sp. | – | – | – | 0.5 | – | – |
| *Arnoglossus kessleri* | – | – | – | – | – | 0.1 |
| Mean abundance | 3.4 | 4.7 | 2.5 | 2.5 | 2.0 | 1.8 |

Out of warm-water species, the European anchovy *E. encrasicolus* continued its spawning. It was productive, as evidenced by the presence in samples of live eggs at different stages of development and larvae on yolk, mixed, and external nutrition. The proportion of dead eggs in samples did not exceed 30%. Maximum abundance of the European anchovy eggs (18.0 ind.·m$^{-2}$) was registered on the Cape Khersones traverse; of larvae (14 ind.·m$^{-2}$), at Cape Tarkhankut. In addition to the anchovy, samples contained larvae of gobies of the genus *Pomatoschistus* (*P. minutus* and *P. pictus*), flathead grey mullet *Mugil cephalus* Linnaeus, 1758, pelagic pipefish *S. schmidti*, and black scorpionfish *Scorpaena porcus* Linnaeus, 1758 (Table 2, Fig. 5).

In the second 10-day period of October, ichthyoplankton was sampled at 17 stations in the eastern sector of the survey (Cape Meganom – the Kerch Strait) after a sharp decrease in SST values (Fig. 5). There, eggs and larvae of 9 fish species were identified. The mean abundance of eggs was 2.0, and the mean abundance of larvae was 1.8 ind.·m$^{-2}$ (Table 2). At coastal stations, in ichthyoplankton, in addition to eggs of the temperate-water *Gaidropsarus mediterraneus* (Linnaeus, 1758), larvae of warm-water species were registered – gobies of the genus *Pomatoschistus* and scaldback *Arnoglossus kessleri* Schmidt, 1915. At stations on the slope of depths, ichthyoplankton was mainly represented by eggs and larvae of fish species common for the winter spawning season – *M. merlangus*, *S. sprattus*, and *G. mediterraneus*. In ichthyoplankton, there were no European anchovy eggs and larvae. Out of warm-water species, single *S. schmidti* juveniles were caught, as well as larvae of gobies, which were probably brought there by the current along the eastern periphery of the CrAC due to increased northeast winds [Marine Portal of the Marine Hydrophysical Institute, 2020]. Above depths of more than 2,000 m, only at 1 out of 8 surveyed stations, *S. schmidti* larva was recorded.

At the end of the second 10-day period (on 19 October, 2016), 4 stations were surveyed from the city of Sevastopol to Cape Sarych in the depth range 73–314 m. There, in contrast to the SST in the first 10-day period of October, the SST no longer exceeded +17 °C. In ichthyoplankton, eggs and larvae of only 6 fish species were registered (with the mean abundance of ~ 2.5 ind.·m$^{-2}$) (Table 2). Out of warm-water species, single specimens of *Gobius* sp. larvae were found, and *S. schmidti* juveniles were noted. Temperate-water species were represented by eggs of *S. sprattus* and *M. merlangus*, as well as by larvae of Mediterranean predators that have recently invaded the Black Sea – *T. luscus* and *Molva macrophthalma* (the family Lotidae) [D'Ancona, 1933; Russell, 1976]. Those were caught above a depth of ~ 300 m seaward of the Laspi Bay (Table 2). In recent years, single larvae of these species are regularly recorded off the Crimean Peninsula from Cape Tarkhankut to the Kerch Strait [Klimova, Podrezova, 2018].

The nutrition of different-sized larvae of three fish species prevailing in ichthyoplankton was studied – two warm-water (*E. encrasicolus*, 21 specimens; *S. schmidti*, 22 specimens) and one temperate-water (*T. luscus*, 6 specimens) (Table 1).

The analysis of the size composition of *E. encrasicolus* larvae showed as follows: 9% were on yolk nutrition; 27%, on mixed; and 64%, on external. In *E. encrasicolus* larvae with a total length (TL) from 6.2 to 14.7 mm, which were on external nutrition, food objects in intestines were recorded mainly as an amorphous mass, and it could not be identified. Only two larvae had copepod nauplii, 0.15 to 0.2 mm in size, which retained chitinous membranes; in the intestine of a 14.7-mm larva, a copepodite *Acartia clausi* Giesbrecht, 1889 was identified (0.75 mm).

Juvenile stages of Copepoda (Calanoida) were predominantly found in intestines of the pelagic pipefish 13.2 to 87 mm in size. In certain cases, Cladocera and eggs of hydrobionts were noted. In the nutrition of *S. schmidti* of size group II, the proportion of small food items (up to 0.25 mm) did not exceed 8%; in larvae of size group III, organisms 0.4–0.7 mm long prevailed in intestines. Pelagic *S. schmidti* mostly consumes species predominating in plankton, and it is clear that the prevalence of larger food items in its intestines is related to their abundance in the sea.

In larvae, identified by us as *T. luscus*, with a length (TL) of 2.6–4.0 mm, there were juvenile stages of copepods (Calanidae and Oithonidae) ranging 0.175–0.375 mm – from 2 to 8 specimens *per* intestine.

In general, during the study period (October 2016), low abundance of ichthyoplankton was observed, with a rather large number of species for open waters (15). For entire sample, the species richness index was 11.5. Due to a lack of pronounced prevalence in ichthyoplankton (dominance index was 0.2), we recorded rather high values of evenness index (0.7) and species diversity index (2.8) for the off-season [Odum, 1986; Pielou, 1966; Shannon, Weaver, 1949; Simpson, 1949].

**Mesoplankton.** Mesozooplankton was dominated by *A. clausi*, *C. euxinus*, *Paracalanus parvus* (Claus, 1863), *Penilia avirostris* Dana, 1849, and *Parasagitta setosa* (J. Müller, 1847), usually having a maximum or close to it biomass in autumn months. The only exception is *Pseudocalanus elongatus* (Brady, 1865): its biomass, as a rule, reaches the highest values in March–April. On the outer shelf and in the deep-sea areas, mesozooplankton composition differed somewhat. There was a regular increase in the proportion of cold-water species with distance from the coast. In descending order by biomass, on the outer shelf, the following species prevailed: *P. setosa* (20.4%), *P. parvus* (18.3%), *A. clausi* (12.2%), and *P. avirostris* (12.1%). At greater depths, the prevailing species were *C. euxinus* (32.1%), *P. elongatus* (21.5%), *P. parvus* (12.0%), and *P. setosa* (11.3%).

At 39 stations of the deep-sea epipelagic zone, the biomass of the dominant copepod *C. euxinus* varied depending on the position of the lower boundary of the oxygen zone ($\sigma_t = 16.2$). Specifically, with its shift from 100–125 to 126–150 m, the biomass of this crustacean increased from (6,200 ± 800) to (9,020 ± 1,020) mg·m$^{-2}$. In the deep-sea range of the lower boundary of the oxygen zone, 151–180 m, the value decreased to (7,300 ± 1,900) mg·m$^{-2}$. In total, the abundance and biomass of common and fodder (without *Noctiluca* species) zooplankton were twice as high in the open pelagic zone than on the shelf (Table 3).

**Table 3.** Mesozooplankton composition, abundance (ind.·m$^{-2}$), and biomass (mg·m$^{-2}$) on two stations of the Cape Khersones traverse (above the depth of 92 and 1,800 m) in October 2016

| Species | Outer shelf (50–200 m) | | | | Deep-sea areas (> 200 m) | | | |
|---|---|---|---|---|---|---|---|---|
| | ind.·m$^{-2}$ | % | mg·m$^{-2}$ | % | ind.·m$^{-2}$ | % | mg·m$^{-2}$ | % |
| Total mesozooplankton | 332,153 | – | 7,570 | – | 569,524 | – | 14,840 | – |
| Fodder mesozooplankton | 315,599 | – | 6,700 | – | 552,134 | – | 13,950 | – |
| Mesozooplankton < 0.5 mm | 134,592 | 100 | 1,022 | 100 | 222,244 | 100 | 983 | 100 |
| *Paracalanus parvus* | 44,082 | 32.8 | 249 | 24.4 | 83,225 | 37.4 | 352 | 35.8 |
| *Acartia* spp. | 24,004 | 17.8 | 77 | 7.5 | 38,615 | 17.4 | 126 | 12.8 |
| *Pseudocalanus elongatus* | 4,885 | 3.6 | 22 | 2.2 | 23,174 | 10.4 | 109 | 11.1 |
| *Centropages ponticus* | 7,424 | 5.5 | 33 | 3.2 | 9,662 | 4.3 | 42 | 4.3 |
| Copepoda nauplii | 19,000 | 14.1 | 14 | 1.4 | 32,000 | 14.4 | 24 | 2.4 |
| *Penilia avirostris* (< 0.5 mm) | 2,315 | 1.7 | 23 | 2.3 | 625 | 0.3 | 6 | 0.1 |
| *Oikopleura dioica* | 14,250 | 10.6 | 377 | 36.8 | 3,750 | 1.7 | 108 | 11.0 |
| Other organisms | 18,632 | 13.8 | 227 | 22.2 | 31,193 | 14.0 | 200 | 20.3 |

This pattern was not observed for small fodder zooplankton (organisms < 0.5 mm), which is the main food of fish larvae. Its abundance on the outer shelf (134,592 ind.·m$^{-2}$) was inferior to the abundance in the deep-sea epipelagic zone (222,244 ind.·m$^{-2}$), but the opposite trend was noted for the values of the biomass of small organisms: 1,022 mg·m$^{-2}$ on the shelf and 983 mg·m$^{-2}$ at a deeper station. Out of small species and forms, copepodites *P. parvus* prevailed everywhere in terms of abundance (32.8 and 37.4%). In terms of biomass, *Oikopleura (Vexillaria) dioica* Fol, 1872 dominated on the outer shelf (36.8%), while *P. parvus* dominated in the deep-sea areas (35.8%).

In total, in the deep-sea areas, the biomass of copepods and, apparently, crustacean zooplankton was associated with the abundance and biomass of *Pleurobrachia pileus* (O. F. Müller, 1776) ($p < 0.01$), as well as with the abundance of *A. aurita* ($p < 0.05$).

**Gelatinous macroplankton** was mainly represented by the scyphomedusa *A. aurita* and three species of ctenophores (*M. leidyi*, *P. pileus*, and *B. ovata*), occurring together in more than 90% of samples (Fig. 6).

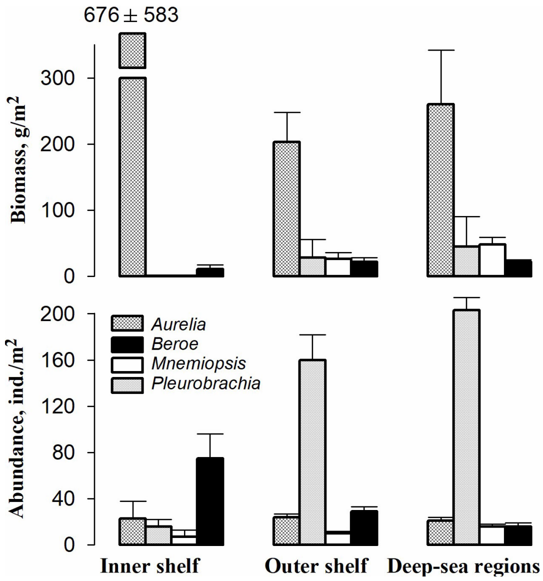

**Fig. 6.** Gelatinous macroplankton abundance (ind.·m$^{-2}$) and biomass (g·m$^{-2}$) in the central Black Sea in October 2016 (mean values ± standard errors)

The mean biomass of *A. aurita* in areas with depths > 200 m was 203–260 g·m$^{-2}$, and the abundance was 21–24 ind.·m$^{-2}$. High biomass values were observed on the coastal shelf: (676 + 583) g·m$^{-2}$. The absence of significant differences in the biomass and abundance of the scyphomedusa in various areas of the epipelagic zone can be traced according to the geometric mean estimates (79–157 g·m$^{-2}$ and 10–21 ind.·m$^{-2}$) and is confirmed statistically ($p > 0.05$). For this species, the maximum biomass (2,611 g·m$^{-2}$) and abundance (92 ind.·m$^{-2}$) were noted on the periphery of the eastern core of the MCG (sta. 79).

On the shelf and in the deep-sea areas, ctenophores *P. pileus* and *M. leidyi* competing with the jellyfish had the same mean biomass, but *P. pileus* prevailed in terms of abundance and distribution. There were two areas of the largest accumulations of this ctenophore – the periphery of the eastern core of the MCG (up to 161 g·m$^{-2}$ and 444 ind.·m$^{-2}$) and border areas of the SevAC (up to 93 g·m$^{-2}$ and 331 ind.·m$^{-2}$).

*M. leidyi* was found in macroplankton at 58 stations. In total, this ctenophore occurred more often and in higher abundance in the direction from the inner shelf towards the deep-sea epipelagic zone. Despite its local concentrations (up to 325 $g \cdot m^{-2}$ at sta. 79), its biomass usually did not exceed 50 $g \cdot m^{-2}$; in open waters, the value averaged 26–48 $g \cdot m^{-2}$. *M. leidyi* abundance increased closer to the eastern sea area, where, in rare cases, the value reached 70 $ind. \cdot m^{-2}$ (sta. 63).

The third species, *B. ovata*, was found almost everywhere in the sea. Its mean biomass ranged 11–22 $g \cdot m^{-2}$, and the values were higher in the same areas, as the biomass values for *M. leidyi* ($p < 0.01$). *A. aurita* reached higher abundance there as well ($p < 0.05$). The abundance of ctenophores was maximum in the Feodosiya Gulf area (108–112 $ind. \cdot m^{-2}$) and decreased with distance from the Crimean coast. These data give grounds to suggest that *B. ovata* population initially developed in the coastal zone, and its spreading to open waters occurred later.

The total abundance of ichthyoplankton, as well as fish eggs and larvae separately, was higher ($p < 0.05$) in areas with higher abundance of a ctenophore *B. ovata*. Moreover, the abundance of fish larvae turned out to be positively related to the abundance of the scyphomedusa *A. aurita* ($p < 0.05$) and, apparently, to the temperature of the sea upper layer ($p < 0.07$). The occurrence of particular ichthyoplankton representatives could be associated with various biotic and abiotic conditions. The abundance of *E. encrasicolus* eggs and larvae was related to the abundance of *B. ovata* alone ($p < 0.05$). The abundance of *S. schmidti* larvae and juveniles mainly correlated with the abundance and biomass of the copepod *C. euxinus* ($p < 0.05$), the biomass of all mesoplankton (taking that on average its value is 1.6 times higher than that of *C. euxinus* [Anninsky, Timofte, 2009]) ($p < 0.05$), and the abundance of the jellyfish *A. aurita* ($p < 0.05$). For eggs of *S. sprattus* and *M. merlangus*, no relationship with the habitat conditions of these species was found ($p > 0.05$).

## DISCUSSION

Ichthyoplankton species composition, abundance, and spatial distribution in various areas of the Black Sea are mostly limited by the hydrological regime, primarily by water temperature as a factor determining the rhythm of biological processes; also, those are limited by the food supply and the impact of predators [Dekhnik, Pavlovskaya, 1979].

The period of plankton studies corresponded to the initial stage of the autumn hydrological season with a rather smooth decrease in the SST during the survey in the western sector in the first 10-day period of October 2016 and with a sharp drop in SST values in the eastern sector in the second 10-day period, after a strong storm.

In October, warm-water fish species usually complete their spawning, while temperate-water ones only begin to spawn. Therefore, ichthyoplankton, as a rule, is dominated by larvae of older age groups of warm-water fish and eggs of temperate-water fish [Dekhnik, 1973]. In the first 10-day period of October 2016, in the western sector of the survey, 10 species were identified in ichthyoplankton, and a relatively high mean abundance of fish eggs and larvae was observed. Out of larvae, the warm-water *E. encrasicolus* prevailed; in terms of abundance of eggs, temperate-water *S. sprattus* dominated. However, eggs of the European anchovy accounted for a rather significant proportion in samples, 35.3%. Moreover, in 2016, unlike the situation in previous years [Dekhnik, 1973; Klimova, Podrezova, 2018; Klimova et al., 2010], *E. encrasicolus* spawning was productive, as evidenced by the presence of larvae in samples (44.7% of the total abundance of all species) of all age groups. At the same time, eggs of 3 species and larvae of 2 species of temperate-water fish were recorded (see Table 2). On 1–14 October, 2005, when the SST was on average 2 °C lower than in 2016, eggs and larvae of 11 fish species were found in ichthyoplankton [Klimova et al., 2010]. The mean abundance of eggs was twice as low as in 2016. Only common temperate-water species spawned: *S. sprattus*, *M. merlangus*, and *G. mediterraneus*. Spawning was productive for *M. merlangus* alone. Larvae of warm-water fish were represented by 8 species. *E. encrasicolus* completed its spawning; its larvae of older age groups prevailed in samples. This corresponded to the data of ichthyoplankton studies of the 1950s–1970s [Dekhnik, 1973].

A sharp change in the parameters of thermal stratification after the storm on 9–10 October, 2016, altered the structure of species composition of ichthyoplankton on the shelf of the southwestern Crimea (from Cape Sarych to the city of Sevastopol): the proportion of temperate-water fish increased. The abundance of ichthyoplankton species at the end of the second 10-day period of October has almost halved; the mean abundance of fish eggs and larvae did not exceed 2.5 ind.·m$^{-2}$ (Table 2). Eggs of temperate-water *S. sprattus* and *M. merlangus* were registered, but their spawning was unproductive. Samples contained only larvae of temperate-water invaders *T. luscus* and *M. macrophthalma*. In catches, there were no *E. encrasicolus* eggs and larvae. Warm-water species were represented only by single larvae of gobies and *S. schmidti* juveniles.

In the eastern sector of the survey, ichthyoplankton was sampled in the second 10-day period of October 2016. After the storm, background SST values dropped to +16…+16.5 °C. On the shelf and on the slope of the northeastern water area (from Cape Meganom to the Kerch Strait), eggs and larvae of 9 fish species were noted, and this is almost the same number of species as in the western sector of the survey before the storm. However, the absence of *E. encrasicolus* eggs and larvae in samples (those prevailed in samples of the western sector of the survey in the first 10-day period of October) led to a decrease in the mean abundance of eggs to 2.0 ind.·m$^{-2}$ and larvae to 1.8 ind.·m$^{-2}$ (see Table 2). Temperate-water species were represented by eggs and larvae of fish common to winter ichthyoplankton (*S. sprattus*, *M. merlangus*, and *G. mediterraneus*), while warm-water ones were represented only by single larvae of *Pomatoschistus* gobies and *A. kessleri*, as well as by *S. schmidti* juveniles. Southward, at deep-sea stations, samples were practically empty (as mentioned earlier, at 8 surveyed stations, just 1 specimen of the pelagic pipefish was caught).

Both in October 2016 and October 2005, favorable conditions were observed for the survival of larvae of warm-water and temperate-water fish [Klimova et al., 2010]. Larvae on mixed and external nutrition with empty intestines were not found during the daytime. In October 2016, the food of *S. sprattus* larvae on external nutrition was mostly in the form of an amorphous mass; in the largest specimens, juvenile stages of copepods were identified. This corresponds to the data of [Duka, Sinyukova, 1976] on feeding habits and intestinal structure of larvae of this species. A detailed analysis of *S. schmidti* feeding of various size groups is given based on material sampled in September 1987, when the SST was comparable to that in October 2016 [Gordina et al., 1991]. In larvae of size group III, both in September 1987 and October 2016, large food organisms (0.4–0.7 mm long) prevailed in intestines. In the feeding of *S. schmidti* larvae of size group II, small food objects prevailed in September 1987; their proportion did not exceed 8% in October 2016. Previously, small *Oithona nana* Giesbrecht, 1893 accounted for 18–51% of the total abundance of consumed organisms in intestines of the pelagic pipefish of size group II [Gordina et al., 1991]. Currently, this species is not recorded in the Black Sea zooplankton.

High amount of food objects in intestines of fish larvae indicated a good food supply; this was confirmed by mesoplankton studies. In the initial phase of the autumn hydrological season in 2016, the sea cooling occurred more slowly than in 2005, and favorable temperature conditions for the development of warm-water zooplankton were maintained for longer [Klimova et al., 2010]. Mostly due to the growth in populations of warm-water species (*P. parvus*, *Centropages ponticus* Karavaev, 1895, *P. avirostris*, *etc.*), in 2016, the abundance and biomass of fodder zooplankton, both on the outer shelf and in the deep-sea areas, reached the level of the upper limit of the corresponding data of 2005 [Klimova et al., 2010]. A small number of samples in 2016 does not allow us to estimate interannual differences in the abundance and biomass of small-sized zooplankton statistically. However, during this period, the abundance of food organisms < 0.5 mm in size increased by 4–6 times, and their biomass rose by 7–8 times. This is quite consistent with the trend towards a rise in the abundance of all crustacean plankton that has emerged in recent years [Anninsky et al., 2016]. An increase in its abundance and biomass indicates certain (possibly temporary) rise in trophicity of the Black Sea water, which is also confirmed by data on predatory jellyfish [Anninsky, Timofte, 2009]. In the deep-sea areas, the biomass of the jellyfish *A. aurita* was significantly higher in 2016 ($p < 0.01$) than in 2005. *P. pileus* biomass doubled over this period ($p < 0.001$). *M. leidyi* biomass (~ 250 $g \cdot m^{-2}$ *prior* to a seasonal outbreak of *B. ovata*) increased by 2–3 times. All this indicates that there were more gelatinous macroplankton in 2016. Potentially, it could have a stronger effect on entire mesozooplankton, including ichthyoplankton complexes, than in 2005. However, considering the abundance of mesozooplankton and that of fish eggs and larvae, the predatory pressure of gelatinous species did not cause significant damage to the populations of prey. Apparently, this is also indicated by the positive relationship between the total abundance of fish larvae and the abundance of *A. aurita*. Such a dependence, as well as a similar one (between the abundance of early age stages of the European anchovy and a ctenophore *B. ovata*), may indicate obvious trophic vectors of ichthyoplankton distribution in the sea. In the first case, such a vector could be the abundance of microplankton; in the second case, it could be greater development of small feeding zooplankton under a weak predatory pressure of plankton-feeding ctenophores. It is worth noting that *B. ovata* has a stronger effect on *M. leidyi* population than in previous years. It appears earlier in plankton and adapts more quickly to seasonal shredding of individuals in the prey population, forming its own, small-sized generation *via* larval reproduction [Finenko, Datzyk, 2016]. Moreover, *B. ovata* penetrates the deep-sea epipelagic zone, where *M. leidyi* used to escaped complete grazing [Anninsky et al., 2013].

On the one hand, relatively high SST values in the first 10-day period of October 2016 contributed to the prolongation of spawning of the European anchovy and other warm-water fish species. On the other hand, those prevented full expansion of the sea surface layer in October by cold-loving gelatinous species (*A. aurita* and *P. pileus*), which prefer lower temperature [Anninsky, 2009]; this could lead to the displacement of both species into deeper horizons. High abundance of *A. aurita* and *P. pileus* intensified their competition and intraspecific food rivalry at these depths. Apparently, this caused a decrease in both mean umbrella diameter of jellyfish and growth rate of its generation. Specifically, in October 2016, the growth rate of *A. aurita* generation was one of the lowest over the past 15 years.

**Conclusions:**

1. In October 2016, against the backdrop of climate change, in the hydrological regime of the Black Sea, eggs and larvae of 15 species representing 9 families of both temperate-water and warm-water complexes were registered in shelf and deep-sea areas off the Crimean Peninsula. The mean abundance of eggs was 2.92, and larvae, 3.56 $ind. \cdot m^{-2}$. Relatively high indices of species diversity, a wide size range of larvae, and the presence of food organisms in their intestines indicated favorable conditions for ichthyoplankton survival during the study period.

2. Shifts in the abundance and species structure of ichthyoplankton were mediated by the characteristics of the hydrological regime during the research. In the first 10-day period of October (the initial phase of the autumn hydrological season, when the surface water circulation system in the study area was still consistent with the summer one, and the water temperature exceeded +21 °C), eggs and larvae of 10 fish species were noted in the western sector of the survey. Their mean abundance was 3.4 and 4.7 ind.·m$^{-2}$, respectively. In the sea, productive spawning of the warm-water *Engraulis encrasicolus* still occurred, as evidenced by a low proportion of dead eggs (30%) and the presence of larvae of all age groups in samples – on yolk (9%), mixed (27%), and external (64%) nutrition. The spawning of temperate-water *Sprattus sprattus* and *Merlangius merlangus* was unproductive. Single larvae of two Gadidae species were recorded.

In the second 10-day period of October, with a sharp drop in the sea surface temperature to +16.5…+17 °C against the backdrop of increased wind-wave mixing, in the eastern sector of the survey, eggs and larvae of 9 more fish species were registered, but their mean abundance decreased to 2.0 and 1.8 ind.·m$^{-2}$, respectively. Species structure was already typical for the autumn spawning season. In samples, there was no *E. encrasicolus*. Productive spawning of temperate-water *Gaidropsarus mediterraneus*, *S. sprattus*, and *M. merlangus* was noted. Warm-water species were represented only by larvae of older age groups.

At the end of the survey, on the shelf of the southwestern Crimea, from the city of Sevastopol to Cape Sarych, ichthyoplankton was represented only by 6 species: single specimens of warm-water larvae of *Gobius* sp. and *Syngnathus schmidti*, eggs of temperate-water *S. sprattus* and *M. merlangus*, and larvae of Mediterranean invaders *Trisopterus luscus* and *Molva macrophthalma*.

3. Despite the fact that gelatinous predators were abundant in 2016, they did not cause significant damage to meso- and ichthyoplankton populations. In 2016, early appearance (in April) of a ctenophore *Beroe ovata* and its penetration into the deep-sea epipelagic zone weakened the predatory pressure of *Mnemiopsis leidyi* on zooplankton of the upper quasi-homogeneous layer, where eggs and larvae of most fish species develop. The relatively high sea surface temperature (more than +20 °C) prevented full expansion of this biotope by cold-loving gelatinous species (*Aurelia aurita* and *Pleurobrachia pileus*) and contributed to their displacement into deeper horizons, where their competition and intraspecific food rivalry intensified.

*This work was carried out within the framework of IBSS state research assignment "Regularities of formation and anthropogenic transformation of biodiversity and biological resources of the Sea of Azov–Black Sea basin and other areas of the World Ocean" (No. 121030100028-0), "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. 121030300149-0), and "Functional, metabolic, and toxicological aspects of hydrobionts and their populations existence in biotopes with different physical and chemical regimes" (No. 121041400077-1). The research was carried out at the core facility RV "Professor Vodyanitsky" (IBSS).*

**Acknowledgement.** The authors express their sincere gratitude to Yu. Zagorodnyaya, PhD, for her help in identifying food objects in intestines of fish larvae.

## REFERENCES

1. Anninsky B. E., Timofte F. The distribution of zooplankton in the western Black Sea in October 2005. *Morskoj ekologicheskij zhurnal*, 2009, vol. 8, no. 1, pp. 17–31. (in Russ.). https://repository.marine-research.ru/handle/299011/996
2. Anninsky B. E., Finenko G. A., Datsyk N. A., Ignatyev S. M. Gelatinous macroplankton in the Black Sea in the autumn of 2010. *Okeanologiya*, 2013, vol. 53, no. 6, pp. 758–768. (in Russ.). https://doi.org/10.7868/S0030157413060014
3. Anninsky B. E., Finenko G. A., Datsyk N. A. The trophodynamic role of gelatinous predators in planktonic communities of the coastal regions 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. 221–224. (in Russ.). https://repository.marine-research.ru/handle/299011/3169
4. Artamonov Yu. V., Alekseev D. V., Skripaleva E. A., Shutov S. A., Deriushkin D. V., Zavyalov D. D., Kolmak R. V., Shapovalov R. O., Shapovalov Yu. I., Fedirko A. V., Shcherbachenko S. V. Peculiarities of seasonal and synoptic variability of water structure in the zone of the Rim Current at autumn and winter 2016. *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon morya*, 2018, no. 1, pp. 32–43. (in Russ.). https://doi.org/10.22449/2413-5577-2018-1-32-43
5. Gordina A. D., Oven L. S., Tkach A. V., Klimova T. N. Distribution, reproduction and feeding of the pelagic pipe-fish, *Syngnathus schmidti*, in the Black Sea. *Voprosy ikhtiologii*, 1991, vol. 31, no. 1, pp. 107–114. (in Russ.)
6. Dekhnik T. V. Pokazateli eliminatsii v embrional'nyi i lichinochnyi periody razvitiya chernomorskoi khamsy. *Trudy Sevastopol'skoi biologicheskoi stantsii*, 1960, vol. 13, pp. 216–244. (in Russ.). https://repository.marine-research.ru/handle/299011/5474
7. Dekhnik T. V. *Ikhtioplankton Chernogo morya*. Kyiv : Naukova dumka, 1973, 235 p. (in Russ.). https://repository.marine-research.ru/handle/299011/5654
8. Dekhnik T. V., Pavlovskaya R. M. Zakonomernosti raspredeleniya, dinamiki chislennosti i vyzhivaniya ryb na rannikh etapakh ontogeneza. *Productivity of the Black Sea* / V. N. Greze (Ed.). Kyiv : Naukova dumka, 1979, pp. 268–272. (in Russ.). https://repository.marine-research.ru/handle/299011/8059
9. Duka L. A., Sinyukova V. I. *Rukovodstvo po izucheniyu pitaniya lichinok i mal'kov morskikh ryb v estestvennykh i eksperimental'nykh usloviyakh*. Kyiv : Naukova dumka, 1976, 134 p. (in Russ.). https://repository.marine-research.ru/handle/299011/7900
10. Ivanov V. A., Belokopytov V. N. *Oceanography of the Black Sea*. Sevastopol : EKOSI-Gidrofizika, 2011, 212 p. (in Russ.)
11. Klimova T. N., Vdodovich I. V., Anninskii B. E. Ichthyoplankton in the plankton community of the western sector of the Black Sea in October 2005. *Voprosy ikhtiologii*, 2010, vol. 50, no. 3, pp. 349–355. (in Russ.)
12. Klimova T. N., Subbotin A. A., Melnikov V. V., Serebrennikov A. N., Podrezova P. S. Spatial distribution of ichthyoplankton near the Crimean Peninsula in the summer spawning season 2013. *Morskoj biologicheskij zhurnal*, 2019, vol. 4, no. 1, pp. 63–80. (in Russ.). https://doi.org/10.21072/mbj.2019.04.1.06
13. *Marine Portal of the Marine Hydrophysical Institute* : site. (in Russ.). URL: http://dvs.net.ru/mp/index_ru.shtml [accessed: 16.06.2020].
14. Odum Yu. *Ekologiya* : in 2 vols / transl. from English / V. E. Sokolov (Ed.). Moscow : Mir, 1986, vol. 2, 376 p. (in Russ.)
15. Troshchenko O. A., Subbotin A. A. Abioticheskie faktory i usloviya obitaniya gidrobiontov pribrezhnoi zony Yugo-Vostochnogo Kryma. Gidrologicheskie osobennosti. In: *The Biology of the Black Sea Offshore Area at the South-Eastern Crimea*

/ N. S. Kostenko (Ed.). Simferopol : ARIAL, 2018, pp. 46–59. (in Russ.). https://repository.marine-research.ru/handle/299011/5150

16. Troshchenko O. A., Subbotin A. A., Eremin I. Yu. Variability of main limiting environmental factors in the process of bivalve mollusk cultivation at the mussel farm in Sevastopol. *Uchenye zapiski Krymskogo federal'nogo universiteta imeni V. I. Vernadskogo. Geografiya. Geologiya*, 2019, vol. 5 (71), no. 2, pp. 308–321. (in Russ.)
17. Finenko G. A., Datzyk N. A. The reproductive features of ctenophore *Mnemiopsis leidyi* in inshore zone 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. 1, pp. 317–319. (in Russ.). https://repository.marine-research.ru/handle/299011/3048
18. Alonso-Fernández A., Vergara A., Saborido-Rey F. Embryonic development and spawning pattern of *Trisopterus luscus* (Teleostei: Gadidae) under controlled conditions. *Journal of the Marine Biological Association of the United Kingdom*, 2010, vol. 91, spec. iss. 6, pp. 1281–1287. https://doi.org/10.1017/S0025315410000147
19. Anninsky B. E. Organic composition and ecological energetics of the jellyfish *Aurelia aurita* L. (Cnidaria, Scyphozoa) under Black Sea conditions. In: *Trophic Relationships and Food Supply of Heterotrophic Animals in the Pelagic Ecosystem of the Black Sea* / G. E. Shulman, B. Öztürk, A. E. Kideys, G. A. Finenko, L. Bat (Eds). Istanbul, Turkey : Black Sea Commission Publications, 2009, pp. 99–160.
20. Auth T. D., Daly E. A., Brodeur R. D., Fisher J. L. Phenological and distributional shifts in ichthyoplankton associated with recent warming in the northeast Pacific Ocean. *Global Change Biology*, 2017, vol. 24, iss. 1, pp. 259–272. https://doi.org/10.1111/gcb.13872
21. *Copernicus Marine Service* : [site]. URL: https://data.marine.copernicus.eu/products [accessed: 02.11.2019].
22. D'Ancona U. Order: Acanthini. Family 1: Gadidae. In: *Fauna and Flora of the Bay of Naples*. Monograph No. 38: Eggs, larvae and juvenile stages of Teleostei / S. Lo Bianco (Ed.). 1933, pt. II, pp. 188–261, plates XII–XV. (English transl. by Israel Program for Scientific Translations, 1969).
23. Finenko G. A., Romanova Z. A., Abolmasova G. I., Anninsky B. E., Svetlichny L. S., Hubareva E. S., Bat L., Kideys A. E. Population dynamics, ingestion, growth and reproduction rates of the invader *Beroe ovata* and its impact on plankton community in Sevastopol Bay, the Black Sea. *Journal of Plankton Research*, 2003, vol. 25, iss. 5, pp. 539–549. https://doi.org/10.1093/plankt/25.5.539
24. Fincham J. I., Rijnsdorp A. D., Engelhard G. H. Shifts in the timing of spawning in sole linked to warming sea temperatures. *Journal of Sea Research*, 2013, vol. 75, pp. 69–76. https://doi.org/10.1016/j.seares.2012.07.004
25. Klimova T. N., Podrezova P. S. Seasonal distribution of the Black Sea ichthyoplankton near the Crimean Peninsula. *Regional Studies in Marine Science*, 2018, vol. 24, pp. 260–269. https://doi.org/10.1016/j.rsma.2018.08.013
26. Margalef R. Information theory in ecology. *General Systems*, 1958, vol. 3, pp. 36–71.
27. McQueen K., Marshall T. C. Shifts in spawning phenology of cod linked to rising sea temperatures. *ICES Journal of Marine Science*, 2017, vol. 74, iss. 6, pp. 1561–1573. https://doi.org/10.1093/icesjms/fsx025
28. *National Center for Environmental Information* / National Oceanic and Atmospheric Administration (NOAA) : [site]. URL: https://www.nodc.noaa.gov/SatelliteData/pathfinder4km [accessed: 22.04.2018].
29. Parsons T. R., Lalli C. M. Jellyfish population explosions: Revisiting a hypothesis of possible causes. *La Mer*, 2002, vol. 40, pp. 111–121. http://www.sfjo-lamer.org/la_mer/40-3/40-3-2.pdf
30. Pielou E. C. Species-diversity and pattern-diversity in the study of ecological succession. *Journal of Theoretical Biology*, 1966, vol. 10, iss. 2, pp. 370–383. https://doi.org/10.1016/0022-5193(66)90133-0

31. Purcell J. E., Uye S. I., Lo W. T. Anthropogenic causes of jellyfish blooms and direct consequences for humans: A review. *Marine Ecology Progress Series*, 2007, vol. 350, pp. 153–174. https://doi.org/10.3354/meps07093
32. Richardson A. J., Bakun A., Hays G. C., Gibbons M. J. The jellyfish joyride: Causes, consequences and management responses to more gelatinous future. *Trends in Ecology & Evolution*, 2009, vol. 24, iss. 6, pp. 312–322. https://doi.org/10.1016/j.tree.2009.01.010
33. Robinson K. L., Ruzicka J. J., Decker M. B., Brodeur R. D., Hernandez F. J., Quiñones J., Acha E. M., Uye S., Mianzan H., Graham W. M. Jellyfish, forage fish, and the world's major fisheries. *Oceanography*, 2014, vol. 27, no. 4, pp. 104–115. https://doi.org/10.5670/oceanog.2014.90
34. Russell F. S. *The Eggs and Planktonic Stages of British Marine Fishes*. London ; New York ; San Francisco : Academic Press, 1976, 524 p.
35. Shannon C. E., Weaver W. *The Mathematical Theory of Communication*. Urbana, IL : University of Illinois Press, 1949, 117 p.
36. Simpson E. H. Measurement of diversity. *Nature*, 1949, vol. 163, pp. 688. https://doi.org/10.1038/163688a0
37. Turan C., Erguden D., Gürlek M. Climate change and biodiversity effects in Turkish seas. *Natural and Engineering Sciences*, 2016, vol. 1, iss. 2, pp. 15–24. http://dx.doi.org/10.28978/nesciences.286240
38. Vinogradov M. E., Shushkina E. A., Mikaelyan A. S., Nezlin N. P. Temporal (seasonal and interannual) changes of ecosystem of the open waters of the Black Sea. *Environmental Degradation of the Black Sea: Challenges and Remedies* / S. Beşiktepe, Ü. Ünlüata, A. S. Bologa (Eds). Dordrecht : Kluwer Acad. Publ., 1999, pp. 109–129. (NATO Science Series: 2. Environmental Security ; vol. 56).

# СОСТОЯНИЕ ИХТИО-, МЕЗО- И МАКРОПЛАНКТОННЫХ КОМПЛЕКСОВ У КРЫМСКОГО ПОЛУОСТРОВА (ЧЁРНОЕ МОРЕ) В СВЯЗИ С ОСОБЕННОСТЯМИ ГИДРОЛОГИЧЕСКОГО РЕЖИМА В ОКТЯБРЕ 2016 Г.

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Климатические изменения в гидрологическом режиме Чёрного моря, отмечаемые с 1990-х гг., отразились на состоянии эпипелагических комплексов морских организмов, прежде всего на сезонной изменчивости их биологических циклов. Это оказало существенное влияние на фенологию нереста природных популяций рыб, видовое разнообразие и пространственное распределение ихтиопланктона, а также на устоявшиеся трофические взаимоотношения в планктонном сообществе. В конечном итоге характер взаимодействия между различными звеньями трофической цепи в эпипелагических комплексах, их сезонная и межгодовая изменчивость влияют на эффективность нереста рыб, прежде всего массовых промысловых видов, и в значительной степени определяют успех пополнения их будущих поколений. С целью изучения видового состава, численности и пространственного распределения ихтиопланктона в октябре 2016 г. (89-й рейс НИС «Профессор Водяницкий», 30 сентября — 19 октября) были проведены исследования в шельфовых и открытых водах Чёрного моря у Крымского полуострова, проанализированы не только икра и личинки рыб, но и биомасса мезо- и макропланктона. Пробы ихтио- и макропланктона отбирали сетью Богорова — Расса (площадь входного отверстия — 0,5 м$^2$; ячея — 300 мкм) методом тотальных вертикальных ловов от дна до поверхности моря в области шельфа и от нижней границы кислородной зоны до поверхности моря в глубоководной части. Ихтиопланктон фиксировали 4%-ным раствором формалина и анализировали позже под микроскопом, определяя таксономический состав организмов и по возможности — наличие и состав пищи в кишечниках личинок рыб. Проанализированы данные о видовом составе и пространственном распределении ихтио-, мезо- и макропланктона, а также о питании личинок рыб Чёрного моря у Крымского полуострова в октябре 2016 г. Период съёмки соответствовал начальной фазе осеннего гидрологического сезона. Ихтиопланктон был представлен икрой и личинками 9 видов тепловодных и 6 видов умеренноводных рыб. Средняя численность икры рыб составляла 2,92, а личинок — 3,56 экз.·м$^{-2}$. Низкая доля (30 %) мёртвой икры тепловодной хамсы *Engraulis encrasicolus*, а также наличие её разноразмерных личинок в море свидетельствовали о продолжении результативного нереста. Биомасса зоопланктона возрастала в направлении от шельфа к глубоководным районам. Мелкоразмерные фракции планктонных организмов преобладали на шельфе, обеспечивая здесь лучшие кормовые условия для выживания личинок рыб. Несмотря на значительную биомассу желетелых-планктофагов в октябре 2016 г., их влияние на ихтиопланктонные комплексы Чёрного моря, по-видимому, оставалось несущественным.

**Ключевые слова:** ихтиопланктон, мезопланктон, желетелый макропланктон, питание личинок рыб, видовое разнообразие, пространственное распределение, Чёрное море

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 2, pp. 74–90
https://marine-biology.ru

UDC 574.583.033(262.5.04)

# ABUNDANCE, SPECIES DIVERSITY, AND STRUCTURE OF THE METAZOAN MICROZOOPLANKTON COMMUNITY IN THE BAY–SEASIDE GRADIENT (ON THE EXAMPLE OF THE SEVASTOPOL BAY)

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Received by the Editor 13.02.2021; after reviewing 22.04.2021;
accepted for publication 16.02.2023; published online 31.05.2023.

The aim of the research is to identify regular changes in the abundance, species diversity, and structure of metazoan microzooplankton (MM) communities under gradient conditions of the sea coast on a relatively small spatial scale. The relevance of the work lies in the paucity of such studies, which allow to assess indirectly the anthropogenic load (pollution, eutrophication) on marine biota and to apply species diversity indices to evaluate the trophic status of local water areas. The investigation covered three coastal areas of the city of Sevastopol: the open seaside, the mouth of the Sevastopol Bay, and its inner area. The localization of sampling stations reflects the gradient of environmental conditions, which is characterized by various degrees of the effect of natural and anthropogenic factors on the biota. The study was carried out in summer and autumn seasons. MM was sampled on three horizons of the water column: surface, 0–5-m, and 0–10-m layers. In the open seaside and at the bay mouth, MM abundance along the vertical was characterized by a greater evenness; in the inner bay area, the differences between the layers could reach 5–700 times. Maximum MM abundance (1,837.1 thousand ind.·m$^{-3}$) was registered in early August in the surface layer in the inner bay area. Since the late summer, the abundance decreased in all the studied water areas. The species diversity of the MM community, which was assessed applying the Shannon, Simpson, Pielou, and other indices, decreases from the open seashore towards the inner bay area. This pattern persisted in both seasons. The most informative indices were the Shannon, Simpson, and Pielou ones. Those reflected well both seasonal changes in species diversity and direction of changes in the trophic gradient of the local bay waters. Applying multivariate analysis, cases of significant alterations in the MM community structure were revealed for the bottom water layer in the inner bay area. The main probable cause of these local changes is the occurrence of hypoxic conditions in the lower water horizons of the polluted bay areas, which leads to the degradation of the abundance and species composition of the studied zooplankton community.

**Keywords:** metazoan microzooplankton, abundance, species diversity, community structure, bay–seaside gradient, trophic status of the water area

Species diversity can serve as one of the indicators of the ecosystem well-being. With an increased level of pollution of the natural environment, species diversity decreases: there are a drop in species richness and a change in species distribution in terms of abundance [Magurran, 1992]. At the same time, the extinction of species affects key processes that are important for productivity and sustainability of ecosystems. Thus, a decrease in biodiversity itself becomes an active factor affecting the ecosystem functioning, and it is comparable with such factors (drivers), as global warming, elevated $CO_2$ level, eutrophication, *etc.* [Cardinale et al., 2012; Hooper et al., 2012]. Therefore, one of key tasks of ecology is to study causes of degradation and ways to preserve and restore the natural level of biodiversity in ecosystems subject to significant anthropogenic load.

The Sevastopol Bay belongs to marine areas intensively used by man for several centuries. It is a semi-enclosed water area, oriented in the latitudinal direction from east to west and characterized by hindered water exchange with the open (and less polluted) part of the sea. The bay is shallow, and anthropogenic load is high there, which is due to several factors, *inter alia* the Chernaya River inflow. Having a significant length from the apex at the river confluence to the bay mouth and exit (about 7 km), its water area is characterized by the presence of a natural gradient of ecological and anthropogenic factors.

For many years, monitoring studies of hydrological, hydrochemical, and biological characteristics of the bay waters are carried out. As a result, several characteristic zones with different thermohaline and hydrochemical water structure were revealed, and their seasonal changes were determined [Ivanov et al., 2006]. The trophic indices for various areas of the Sevastopol Bay were calculated [Sovga et al., 2020]. As shown, differences in zooplankton abundance and mortality are related to the intensity of water exchange, surge winds, and seasonal changes in the sea level [Repetin et al., 2003]. Microzooplankton is one of the biological research objects in the bay [Seregin, Popova, 2016, 2017, 2019]. It is one of the key components of marine food webs; it transfers a significant part of matter and energy from lower trophic levels (phyto- and bacterioplankton) to higher ones [Calbet, 2008]. Specifically, metazoan microzooplankton (hereinafter MM) plays an important role in feeding of juvenile fish in the Black Sea [Klimova et al., 2017; Melnikov et al., 2015]. Its crustacean fraction is especially valuable: it is the most significant link in the diet of fish larvae, providing conditions for the reproduction of fish resources in the sea and its local water areas. A detailed study of MM composition, ecological features, and quantitative characteristics is of great importance, in particular, in terms of the problem of anthropogenic load on coastal waters. The MM community, formed mainly by short-lived species, can quickly respond to changes in environmental conditions, which makes these organisms a convenient object for diagnosing such transformations, including those under the effect of anthropogenic factors [Dyatlov, 2000]. For example, earlier we recorded an increased proportion of rotifers in the MM composition when coastal seawater is polluted with domestic sewage [Seregin, Popova, 2012].

We aimed at determining possible differences in the abundance, species diversity, and structure of the metazoan microzooplankton community on a relatively small spatial scale of the gradient open coast – bay mouth – inner bay area, which is characterized by varying degrees of natural and anthropogenic load. Also, we tried to assess the applicability of some indices of the species diversity to analyze its changes in several areas with different water trophicity.

## MATERIAL AND METHODS

In August (07.08 and 29.08), late October (24.10), and early November (06.11) of 2019, metazooplankton was sampled in three coastal areas of the city of Sevastopol: in the open seaside, abeam the Karantinaya Bay mouth (sta. 1); at the mouth of the Sevastopol Bay, opposite the Konstantinovskaya Battery (sta. 2); and in the inner bay area, abeam the Sukharnaya Bay (sta. 3) (Fig. 1). These stations correspond to long-term monitoring stations in Sevastopol coastal waters and belong to local water areas with varying degrees of effect of natural and anthropogenic environmental factors on the biota. At each station, samples were taken from the surface (S) ~ 10-cm water layer and from the layers of 0–5 and 0–10 m. In summer and autumn, 18 samples were taken; in total, 36 samples. From the surface, water was simply scooped up with a plastic sampler; in the water column, it was sampled with the Apstein plankton net (an inlet area of 0.025 $m^2$) fitted with a nylon sieve with 30-µm mesh. According to the data of our previous investigations [Seregin, Popova, 2016] and the conclusions made by other authors [Svetlichny et al., 2016], the use of different sampling techniques for the studied community did not cause significant differences in the results obtained. Simultaneously with plankton, water was sampled to determine salinity; water temperature was measured in the surface and at a depth of 5 and 10 m with a hydrological thermometer; and wind speed and direction were recorded.

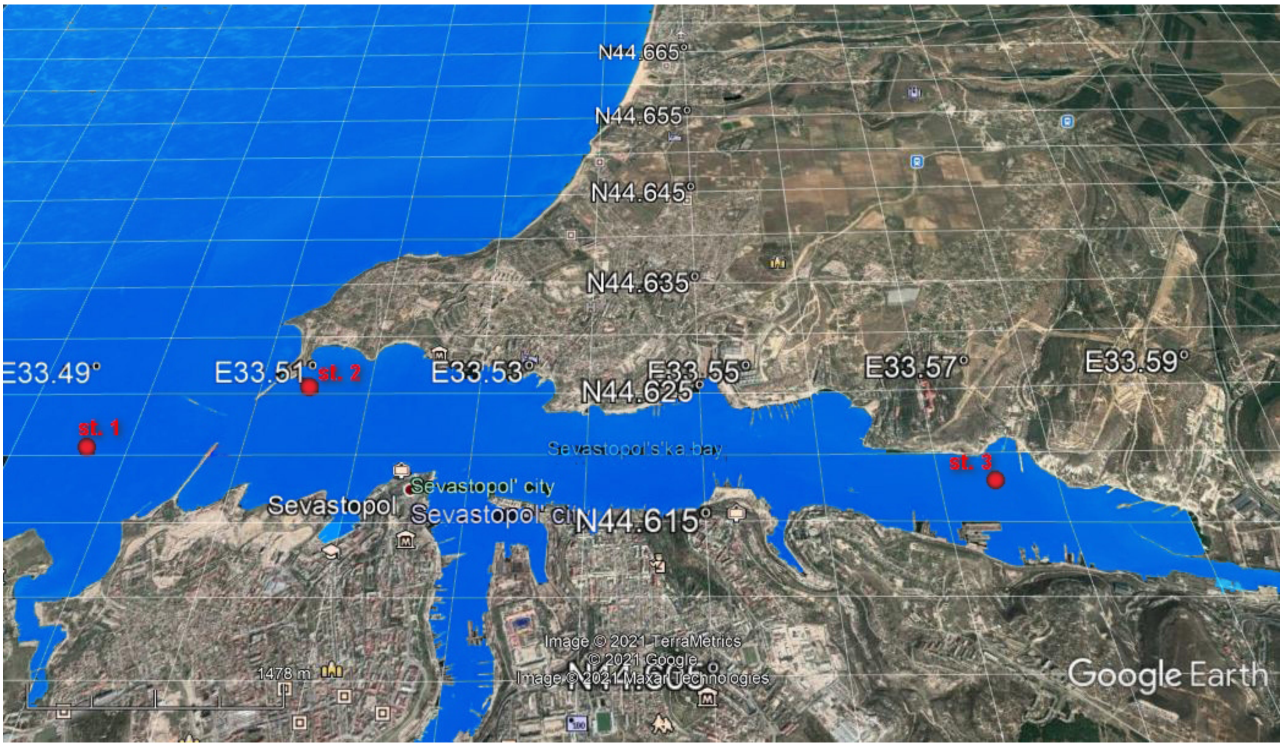

**Fig. 1.** Schematic map of sampling stations in Sevastopol coastal area: 1, sta. Vekha; 2, sta. Ravelin; 3, sta. Sukharnaya Bay

Samples were prepared and treated in accordance with [ICES Zooplankton Methodology Manual, 2000]. All surface and net samples were delivered to a laboratory, concentrated using a reverse filtration funnel, and fixed in 40% formaldehyde down to a final content of 4% in a sample. All samples were analyzed totally in a Bogorov chamber under an MBS-9 microscope at 32-fold magnification. Copepoda nauplii were identified according to [Sazhina, 1985]. The procedure was described in detail in [Seregin, Popova, 2016].

The results of quantitative sample treatment were given as the concentration of organisms (ind.·$m^{-3}$) in the surface, 0.1–5-m ("middle", Nm), and 5–10-m ("bottom", Nb) layers. MM abundance (thousand ind.·$m^{-3}$) in the 0.1–5-m (Nm) and 5–10-m (Nb) layers was calculated according to formulas (1, 2):

$$Nm = (N_5 \times 5 - N_0 \times 0.1)/(h_5 - h_0)\,, \qquad (1)$$

$$Nb = (N_{10} \times 10 - N_5 \times 5)/(h_{10} - h_5)\ , \tag{2}$$

where $N_0$ is the abundance of metazoan microzooplankton in the surface layer;

$N_5$ is the abundance in the 0–5-m layer;

$N_{10}$ is the abundance in the 0–10-m layer;

h is the vertical extent of the layer.

The obtained data were processed in PRIMER v5 program [Clarke et al., 2014]. To include the initial data on MM abundance in the general matrix of results, those were preliminarily √-transformed to reduce a possible effect of a significantly higher abundance of mass species. The program makes it possible to calculate, in particular, species diversity indices. We used several of the most common ones [Magurran, 1992; Shitikov et al., 2003]:

1. Shannon index, $H' = -\Sigma p_i \ln p_i$, where $H'$ is the numerical value of the Shannon index; $i = 1, 2 \ldots S$; S is the number of species; $p_i$ is the proportion of individuals of the $i$-th species in a sample; $p_i = n_i / N$; $n_i$ is the abundance of the $i$-th species; N is the total abundance.
2. Simpson index, $D$, in two forms:

2.1. Probability of interspecific encounters, $PIE = 1 - D = 1 - \Sigma(p_i^2)$, where $D$ is the probability that two in a row taken individuals belong to the same species (other designations are the same as for the Shannon index).

2.2. Polydominance index, $1 / D$.

3. Pielou evenness index, $E = H' / H_{max} = H' / \ln S$.
4. Margalef species richness index, $D_{Mg} = (S - 1) / \ln N$.
5. Brillouin index, $HB = (\ln N! - \Sigma \ln n_i!) / N$.

A relatively large number of used indices is due to the following considerations. Despite the fact that estimates of different indices for the same communities often correlate with each other, some researchers consider it incorrect to apply one of them (richness, diversity, or evenness) without parallel data on other indices, even those that are close in meaning [Pesenko, 1982]. In particular, we have shown earlier that the Shannon index turned out to be more informative for assessing the short-term dynamics of changes in the species diversity of the MM community: its value reacted to changes in the species structure several days earlier than the value of the Simpson index [Seregin, Popova, 2017].

The similarity/dissimilarity in the structure of the MM community at different stations and depths, as well as at various points in time, was assessed by the Bray–Curtis index. To present the similarity/dissimilarity graphically, we applied cluster analysis (according to the group average method) and multidimensional scaling (MDS). The contribution of different species to the similarity/dissimilarity in the MM structure at different stations and sampling horizons at various points in time was assessed using SIMPER program (PRIMER v5).

## RESULTS

**Characteristics of weather and hydrological conditions at the sampling time.** On 7 and 29 August, sampling almost did not differ in terms of weather conditions. Air temperature (night…day) was +19…+29 and +20.5…+30.5 °C, respectively (http://www.sevmeteo.info). On both days, the wind was of a northwestern direction and of low speed: less than 1 m·s$^{-1}$ at the beginning of the month and 1–2 m·s$^{-1}$ at the end. At the same time, calm conditions were recorded at the station in the inner bay area (abeam the Sukharnaya Bay) on both dates. In early August, the temperature of the upper 10-m water layer was characterized by the following indicators (at a depth of 10 m and on the surface): +21.3 and +25.7 °C in the open seaside; +18.0 and +25.6 °C at the bay mouth; and +24.0 and +26.1 °C in the inner bay area. The salinity of the surface layer varied slightly: 18.00–18.03 and 18.06‰. In late August, the temperature was +24.2 and +24.4 °C; +24.1 and +24.5 °C; and +23.8 and +24.6 °C, respectively.

In October, the wind speed slightly increased (up to 2–4 m·s$^{-1}$), and its direction was almost the same (north-north-western). The air temperature varied from +13.0 °C at night to +25.0 °C during the day. The water temperature at a depth of 10 m and on the surface dropped noticeably and amounted to +17.4 and +18.1 °C in the open seaside; +17.2 and +17.8 °C at the bay mouth; and +17.4 and +17.7 °C in the inner bay area. The salinity of the surface water layer was 18.28–18.09 and 17.89‰, respectively.

Early November was marked by an increase in southward winds up to 6–7 m·s$^{-1}$ and a further decrease in water temperature. The values were +16.0 and +17.0 °C for the open seaside; +16.5 and +16.8 °C for the bay mouth; and +14.3 and +15.2 °C for the inner bay area. The air temperature varied from +16.0 to +22.0 °C.

**Estimates of metazoan microzooplankton abundance.** In early August, the total abundance of MM in the open seaside was 331.7 thousand ind.·m$^{-3}$ in the surface layer; 110.9 thousand ind.·m$^{-3}$ in the 0.1–5-m layer; and 312.5 thousand ind.·m$^{-3}$ in the 5–10-m layer. At the bay mouth, the values were as follows: 138.1; 216.1; and 103.0 thousand ind.·m$^{-3}$, respectively. In the inner bay area, MM abundance reached 1,837.1; 340.2; and 2.6 thousand ind.·m$^{-3}$, respectively. Thus, in the open seaside and at the bay mouth, MM abundance along the vertical was more even, while in the inner bay area, the differences between the layers reached 5–700 times. The maximum abundance was recorded in the inner area in the surface layer, and the value was the highest for a 10-year observation period in the Sevastopol Bay [Seregin, Popova, 2019].

At the end of the month, MM abundance changed significantly in terms of both stations and depths. Only the abundance in the surface layer of the open seaside remained relatively the same: 311.5 thousand ind.·m$^{-3}$. In the underlying layers, the values decreased by an order of magnitude and amounted to 24.4 and 24.7 thousand ind.·m$^{-3}$. As a result, the weighted average abundance in the water column dropped by more than 7 times. At the bay mouth, MM abundance on the surface increased several times compared to the value in early August (up to 708.4 thousand ind.·m$^{-3}$); in the underlying layers, it was 20 times lower than on the surface. The weighted average abundance of MM decreased compared to that for the beginning of the month by about 4 times. In the inner bay area, the abundance on the surface dropped significantly; for the entire studied layer, the weighted average value increased by about 6 times, primarily due to its rise in the bottom layer. In general, the distribution of abundance along the vertical became more even (Table 1).

In autumn, a further decrease in the total abundance of MM in the water column occurred at each studied station. In most cases, the vertical structure of the microplankton community was characterized by a drop in abundance from the surface to deeper water layers. In November at sta. 3, the situation was the same as in early August: MM concentration in the lower layer was very low, more than 2 orders of magnitude lower than in the surface layer (Table 1).

**Table 1.** Metazoan microzooplankton total abundance (× $10^3$ ind.·$m^{-3}$) in Sevastopol coastal area by sampling dates

| Station | Layer, m | Summer | | Autumn | |
|---|---|---|---|---|---|
| | | 07.08.2019 | 29.08.2019 | 24.10.2019 | 06.11.2019 |
| Sta. 1 (the open seaside) | Surface | 331.7 | 311.5 | 132.5 | 110.3 |
| | 0.1–5 | 111.0 | 24.4 | 55.9 | 14.5 |
| | 5–10 | 306.8 | 24.7 | 37.5 | 18.2 |
| Sta. 2 (the Sevastopol Bay mouth) | Surface | 138.1 | 708.4 | 420.0 | 136.6 |
| | 0.1–5 | 215.7 | 30.7 | 185.5 | 28.1 |
| | 5–10 | 88.3 | 29.8 | 9.4 | 46.3 |
| Sta. 3 (the inner bay area) | Surface | 1,830.2 | 291.1 | 274.0 | 309.1 |
| | 0.1–5 | 314.4 | 78.2 | 182.6 | 182.6 |
| | 5–10 | 2.6 | 125.8 | 171.7 | 1.2 |

**Characteristics of the species diversity.** In MM composition in the analyzed period, a crustacean and a non-crustaceous fractions were distinguished. The first one consisted of nauplii and copepodite stages of Black Sea copepods: species of the genus *Acartia* Dana, 1846, *Paracalanus parvus* (Claus, 1863), *Pseudocalanus elongatus* (Boeck, 1865), *Centropages ponticus* Karavaev, 1895, *Oithona davisae* Ferrari F. D. & Orsi, 1984, *Oithona similis* Claus, 1866, *Pseudodiaptomus marinus* Sato, 1913, *Calanus euxinus* Hulsemann, 1991, and Harpacticoida representatives. Moreover, the crustacean fraction included nauplii and cypris larvae of Cirripedia, cladoceran *Pleopis polyphemoides* (Leuckart, 1859), and, very rarely, small-sized *Penilia avirostris* Dana, 1849. In the non-crustaceous fraction, Bivalvia and Gastropoda veligers prevailed, as well as Polychaeta larvae. In small abundance, rotifers were recorded; larvae of appendicularian *Oikopleura dioica* Fol, 1872, ctenophores *Beroe ovata* Bruguière, 1789 and *Pleurobrachia pileus* (O. F. Müller, 1776), and phoronids, and some other organisms were found as well.

In early August, 22–34 species and life forms of MM were identified in the open seaside, with noticeably fewer MM on the surface than in deeper layers. A similar dependence of the MM distribution was revealed at the station at the bay mouth: 19–35 species and stages were recorded, with the maximum number of species in the middle layer. At sta. 3 (in the inner bay area), the diversity varied within 15–30 species, and the distribution over the horizons was directly opposite compared to that at sta. 1 and 2: the maximum number of species was noted in the surface layer, and the number of species decreased with depth. In the lower layer, 2 times less species were found than in the upper layers. In the lower layer, crustacean plankton was represented by younger stages of copepods *O. similis*, *P. parvus*, and *P. elongatus* (all species are relatively cold-water ones), Harpacticoida nauplii, and cladoceran *P. polyphemoides*. Out of non-crustaceans, *O. dioica*, ctenophores *B. ovata* and *P. pileus*, and turbellarians were recorded.

In late August, a more even pattern was observed in terms of species distribution by both stations and depths. The situation with a sharp decrease in the number of species in the bottom layer repeated itself in late October at sta. 2 and in early November at sta. 3. In Table 2, seasonally averaged species diversity indices are given, which were calculated in PRIMER considering all dates, stations, and layers of the water column.

**Table 2.** Seasonal mean values of species diversity indices

| Season | Station | Layer, m | Seasonally averaged indices of species richness and diversity | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Margalef, $D_{Mg}$ | Shannon, $H'$ | Simpson, *PIE* | Simpson, $1 / D$ | Brillouin, $HB$ | Pielou, $E$ |
| Late summer | 1 | 0 | 1.85 | 1.17 | 0.45 | 1.83 | 1.17 | 0.36 |
| | | 0.1–5 | 2.72 | 2.09 | 0.79 | 4.64 | 2.09 | 0.61 |
| | | 5–10 | 2.74 | 2.21 | 0.82 | 6.20 | 2.21 | 0.64 |
| | 2 | 0 | 1.58 | 0.97 | 0.38 | 1.78 | 0.96 | 0.33 |
| | | 0.1–5 | 2.49 | 1.90 | 0.70 | 4.18 | 1.9 | 0.58 |
| | | 5–10 | 2.01 | 1.60 | 0.64 | 2.90 | 1.59 | 0.51 |
| | 3 | 0 | 1.96 | 1.27 | 0.49 | 2.54 | 1.26 | 0.39 |
| | | 0.1–5 | 2.27 | 1.49 | 0.59 | 2.46 | 1.49 | 0.45 |
| | | 5–10 | 1.78 | 1.56 | 0.62 | 3.36 | 1.54 | 0.55 |
| Autumn | 1 | 0 | 1.80 | 1.81 | 0.74 | 3.91 | 1.81 | 0.59 |
| | | 0.1–5 | 2.83 | 1.75 | 0.71 | 3.53 | 1.74 | 0.52 |
| | | 5–10 | 2.24 | 1.77 | 0.72 | 4.17 | 1.76 | 0.56 |
| | 2 | 0 | 1.94 | 1.90 | 0.77 | 4.28 | 1.90 | 0.59 |
| | | 0.1–5 | 2.28 | 1.80 | 0.71 | 3.63 | 1.80 | 0.57 |
| | | 5–10 | 1.93 | 1.73 | 0.74 | 3.85 | 1.73 | 0.59 |
| | 3 | 0 | 1.59 | 1.72 | 0.70 | 3.48 | 1.73 | 0.57 |
| | | 0.1–5 | 2.22 | 1.68 | 0.70 | 3.45 | 1.68 | 0.51 |
| | | 5–10 | 1.50 | 1.37 | 0.58 | 2.4 | 1.36 | 0.53 |

The Margalef index reflects species richness in a certain habitat according to the ratio of the number of species and their abundance: the higher the value, the greater the species richness of a given biotope [Shitikov et al., 2003]. Accordingly, the maximum species richness in summer was observed at sta. 1 (the open seaside): the weighted average Margalef index in the water column was 2.71. The values for the bay mouth and its inner area were 2.23 and 2.02, respectively. In autumn, there was a slight decrease in species richness upon maintaining the regularity in the gradient open coast – bay mouth – inner bay area: the weighted average values of the Margalef index were 2.52 – 2.10 – 1.85, respectively.

The Shannon index (see Table 2) combines species richness and evenness into one value and quantitatively differentiates systems with the same species richness but with varying degrees of dominance of certain species [Shitikov et al., 2003]. For summer season, the weighted average values of the index in the studied 10-m water layer in the gradient open coast – bay mouth – inner bay area were 2.14 – 1.74 – 1.52; this confirmed that the highest species diversity is characteristic of seawater outside the bay, and with moving towards its inner area, species richness and evenness decrease. In autumn, the Shannon index values were 1.76 – 1.77 – 1.52, which indicated the *status quo* of biodiversity in the inner area and its higher (and similar) state in the geographically nearest water areas of the bay mouth and the open seaside. At the same time, the level of the species diversity in open waters slightly decreased compared to that for summer season. The same result was obtained when calculating the Simpson index, *PIE*. In summer, its weighted average values amounted to 0.80 – 0.67 – 0.60; in autumn, to 0.72 – 0.72 – 0.64.

As a control, we applied the Brillouin index, which is used if random selection of objects is not guaranteed or not all species of the community are taken into account. Its values almost completely coincide with the values of the Shannon index and reflect already identified patterns of changes in biodiversity in the studied water areas (Table 2).

To assess the evenness of species distribution, we additionally used the Pielou index, *E*. Its higher value corresponds to a greater evenness of species distribution in terms of their relative abundance. In summer, the weighted average values of the Pielou index for stations in the gradient open coast – bay mouth – inner bay area were 0.62 – 0.54 – 0.50, respectively; in autumn, 0.54 – 0.58 – 0.52. Thus, in summer, species distribution in the open seaside is more even compared to that at the bay mouth, and even more so in its inner area. In autumn, greater evenness is characteristic of the bay mouth; it remained minimum in the inner area.

**Similarity/dissimilarity in the community structure.** At first glance, the calculated values of the Bray–Curtis index point to a fairly significant variation in the level of similarity of the MM community at different stations and horizons in various time periods. In general, according to all data, the similarity coefficient varied from 0.5 to 85.1%. Similarity coefficient values up to 50.0% accounted for 43% of all cases; other data indicated a higher level of similarity. Cases of low similarity (not higher than 24%) accounted for about 10% of all pairwise comparisons. These exceptions were the data for the lower layer at sta. 3 in early August and in November, as well as for the same layer at sta. 2 in late October. The results obtained using cluster analysis and MDS are graphically presented in Fig. 2.

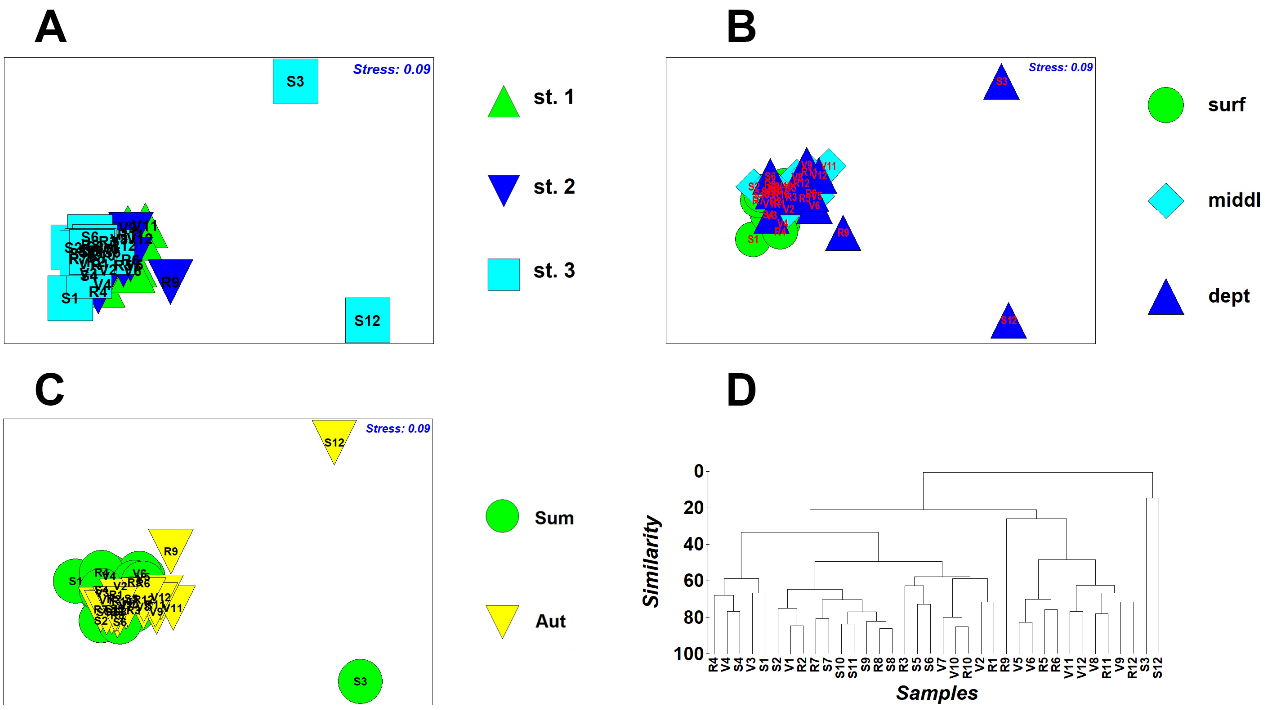

**Fig. 2.** Graphical (2D) result of MDS analysis of the structure of the metazoan microzooplankton community at different stations (A), at different depths (B), and depending on the season (C); diagram of cluster analysis of the entire data set (D): V, sta. 1; R, sta. 2; S, sta. 3. Numbers 1 to 12 are successive sample numbers by horizons and dates: 1–6, for summer season; 7–12, for autumn season

The mean level of similarity for stations (including all horizons and sampling times) was 34.4% for sta. 1; 35.8% for sta. 2; and 35.0% for sta. 3. The average level of dissimilarities between the stations varied within 63.2–72.1%. The mean level of similarity for the sampling layers was as follows: 41.9% for the surface layer; 41.2% for the middle layer; and only 26.7% for the bottom layer. The level of dissimilarities ranged from 63.8 to 74.4%. The similarity by seasons (for all stations and depths) was 30.8% for summer and 39.3% for autumn; the dissimilarities were much higher: 67.9%.

Let us consider the cases of the lowest similarity. On 7 August at sta. 3 (in the inner bay area), the level of similarity in the bottom layer compared to that in the surface and middle layers was only 8.2 and 4.4%, respectively. In terms of other stations, horizons, and seasons, it was lower than 24.0%. As shown above, the abundance and species composition at this station in the bottom layer were significantly lower than in the upper layers and at other stations. A similar situation occurred for the same habitat in early November (06.11.2019): the level of similarity of the MM community was 5.2 and 6.7% in relation to the upper layers (between them, the level of similarity according to Bray–Curtis was 83.7%) and did not exceed 23.0% in terms of other stations, horizons, and seasons. Quantitative and qualitative indicators of the MM community were also much lower in comparison with those for other stations and sampling depths. Indicators of abundance and species richness of the community at sta. 2 in the 5–10-m layer on 24.10.2019 occupy an intermediate position between the main data array and the above MM indicators for the inner bay area. The level of similarity with the surface and middle layers (the Bray–Curtis index between them is 72.9%) was 33.3 and 29.9%, respectively; with other indicators, it varied from 19 to 45%.

The results of the MM species composition analysis using SIMPER program indicate that the most abundant species have the greatest effect on the similarity/dissimilarity in the structure of the studied community. In the crustacean fraction, those are various age stages of copepod *O. davisae*, *Acartia* naupliar stages, and, occasionally, *P. parvus* nauplii. In the non-crustaceous fraction, the most significant ones were Bivalvia veligers and Polychaeta larvae; less significant ones were Gastropoda veligers and *O. dioica* larvae. Interestingly, changes in *O. davisae* abundance determined 73.7–84.3% in the proportion of station similarities (for all depths and sampling times); 65.5–81.7% in the proportion of depth similarities (for all stations and times); and 69.2–82.2% in the proportion of season similarities (for all stations and depths).

## DISCUSSION

**Species diversity and trophic conditions of water areas.** According to the data of long-term monitoring by researchers of the Marine Hydrophysical Institute of RAS, the open sea area in the immediate vicinity of the bay mouth is less polluted than the bay waters due to hindered water exchange between the bay and the sea [Ivanov et al., 2006; Kondratev, Vidnichuk, 2020; Slepchuk et al., 2017; Sovga et al., 2020]. While the water area near sta. 1 (in the open seaside) is regarded as conditionally clean, the water area of sta. 2 (at the Sevastopol Bay mouth) is characterized by low pollution, and the water area of sta. 3 is characterized by high pollution (Fig. 3).

It should be taken into account that the vertical structure of currents in the Sevastopol Bay, especially in its deeper area, has a pronounced two-layer structure: in the surface layer, the current is directed inside the bay, to the east, and in the bottom layer, it is directed towards the bay mouth, to the west [Lemeshko et al., 2014]. Accordingly, the flow of conditionally unpolluted waters (under appropriate hydrodynamic and meteorological conditions) is directed inside the bay along the surface, while the distribution of polluted and more trophic waters from the apex and center bay areas towards its mouth occurs in the lower horizon.

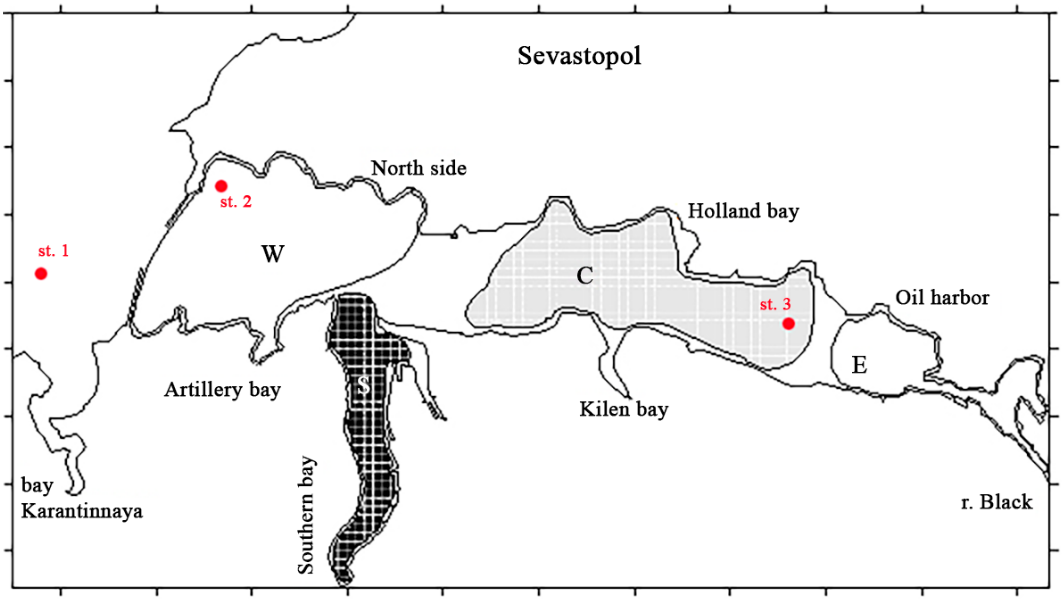

**Fig. 3.** Zoning of the Sevastopol Bay by the level of water pollution (according to [Ivanov et al., 2006]): areas of low (W), moderate (E), high (C), and very high (S) pollution. Our sampling stations are marked with red circles

Usually, eutrophication of water bodies results from an excessive supply of biogenic elements and easily oxidizable organic matter, the main sources of which are river runoff and industrial wastewater. In the Sevastopol Bay, it is caused by the Chernaya River inflow, untreated or conditionally treated sewage, and storm water from the drainage basin. Because of their effect, there are differences in the level of primary production of organic matter in various spots of the bay [Ivanov et al., 2006]. Considering the main sources of pollution and eutrophication, the trophic level of the bay waters is higher in its apex and especially in its center area and much lower in the mouth area. In general, the trophic level of the Sevastopol Bay waters is characterized as transitional from low to medium, and the main determining factor is inorganic forms of nitrogen [Slepchuk et al., 2017; Sovga et al., 2020].

The use of alpha diversity indices for aquatic ecosystems allows not only to determine the species diversity of the biota in a water body or its part (water area), but also to indirectly assess their trophic status. This approach is implemented in the schemes for the integrated use and protection of water resources when analyzing the state of aquatic ecosystems [Metod otsenki, 2021]. The results obtained by us on the biodiversity of the MM community on a relatively small spatial scale of the studied water area are in complete agreement with the general provisions of this approach. A greater biodiversity of the community was registered for less polluted water areas, and *vice versa*: a decrease in biodiversity was recorded under higher anthropogenic load on the water area. Considering the similarity of the systematic composition of MM within the investigated area, we assume that the dissimilarities in biodiversity and community structure between the stations are mainly due to differences in the abundance and degree of dominance of the most abundant species.

Based on the schemes for the integrated use and protection of water resources, the trophic status of the studied water areas in terms of the obtained values of the Shannon index can be assessed as follows. In summer season, the open seaside was characterized by the oligotrophic status of waters; the bay mouth, by the mesotrophic status; and the inner area, by transitional from mesotrophic to hypertrophic. In autumn, the trophic status in the gradient open coast – bay mouth – inner bay area was characterized as mesotrophic – mesotrophic – transitional to hypertrophic. Apparently, a certain rise in the trophic level of open waters results from an increase in the phytoplankton abundance in the Black Sea in October–November [Finenko et al., 2019] and a concomitant increase in the concentration of organic matter in water. An assessment of the trophic level of Sevastopol coastal waters applying E-TRIX [Vollenweider et al., 1998] also showed that seasonal maximum trophic levels coincide with the peaks of phytoplankton blooms – autumn and spring ones. The trophic level of the Sevastopol Bay waters was assessed as transitional from low to medium [Slepchuk et al., 2017]. The Shannon index is often used in monitoring environmental pollution and assessing the trophic status of aquatic ecosystems. For example, it was shown as follows: when analyzing fish diversity, the value of the index sharply increases with distance from the spot of waste discharges into water [Magurran, 1992]. In the study of zooplankton in the lakes of the Chebarkul group, a clear dependence of the Shannon index (in terms of the abundance of different species) on the trophic status of the water body was revealed [Puznetskite, Marushkina, 2005]. According to the results of our investigations of the species diversity in May–June 2013 in the Sevastopol Bay mouth, its waters were characterized as mesotrophic [Seregin, Popova, 2017]. A similar result was obtained earlier when applying pollution indices and E-TRIX [Gubanov et al., 2002].

The Simpson index, *PIE*, characterized the species diversity of the MM community as high in summer and autumn in the open seaside and in autumn in the bay mouth. In the bay, the diversity of MM was assessed as moderate. Some inconvenience of using this index seems to be its insufficient visibility and “resolution”: its values have a narrow range of variation even with great changes in habitat conditions and community characteristics, as do the values of the Pielou evenness index [Puznetskite, Marushkina, 2005]. Moreover, as shown in our study of short-term MM variability, *PIE* may respond to alterations in species composition of the community with a delay compared to the Shannon index [Seregin, Popova, 2017]. Many researchers consider the Simpson polydominance index the best measure of alpha diversity assessment [Shitikov et al., 2003]. Its weighted average values for the water column showed more obvious dissimilarities in the species diversity in the gradient open coast – bay mouth – inner bay area. For summer season, those were 5.46 – 3.51 – 2.90, respectively; for autumn season, 3.85 – 3.78 – 2.92. Thus, the values reflected both differences in the station location in the trophic gradient and, partly, seasonal changes in the species diversity.

Low values of the Pielou evenness index generally indicated a pronounced dominance of abundant species in the MM community of Sevastopol coastal area. Indeed, in recent years, in summer and autumn zooplankton of the studied waters, a neritic Copepoda species *O. davisae* prevails – a recent invader in the Black Sea. The abundance of its various age stages, especially within the bay, can account for 90% or more of the total abundance of MM, strongly affecting the evenness of the community species composition [Seregin, Popova, 2016, 2019]. Earlier, for the Mediterranean Sea coast, it was shown that high abundance of Oithonidae representatives is often related to an increased level of anthropogenic load on coastal marine areas, and these species can serve as bioindicators of anthropogenic pollution [Drira et al., 2018; Serranito et al., 2016]. In our case, the proportion of *O. davisae* effect on the similarity of the community structure in the investigated station gradient increased from 73.7% at sta. 1 (in the open seaside) to 79.3% at sta. 2 (at the bay mouth) and 84.3% at sta. 3 (in the inner bay area). This species has bioindicator features, since the parameters of its abundance can reflect an increased trophic level for water areas. Interestingly, simultaneously with a rise in *O. davisae* contribution along the gradient open coast – bay mouth – inner bay area, the proportion of *Acartia* effect decreased (7.9 – 6.7 – 4.3%, respectively), as well as that of Bivalvia veligers (6.5 – 5.6 – 2.6%).

**Dissimilarities in the community structure and the effect of environmental factors on it.** The results of the study showed that the most significant changes in the structure of the MM community occurred in the lower water layer in the inner bay area (sta. 3). Those were accompanied by both a noticeable decrease in the total abundance and a reduction in the species numbers.

The situation at the bay mouth (sta. 2) in the 5–10-m layer on 24.10.2019 occupies an intermediate position between the state of the MM community in the inner bay area and in open waters. The species composition at sta. 2 was richer than at sta. 3 in November, but poorer than in summer. The crustacean fraction included small nauplii and copepodites of stage II of *P. parvus*, late nauplii and copepodites II–III of *O. davisae*, copepodites II of *A. clausi*, junior copepodites of *P. marinus*, Harpacticoida nauplii, cladoceran *P. polyphemoides*, and Isopoda. In the non-crustaceous fraction, small Bivalvia veligers prevailed. Compared to the upper layers, there were no Cirripedia, copepodite stages of Harpacticoida, a full range of stages of *P. parvus*, *O. davisae*, *A. clausi*, and *P. marinus*. Moreover, out of the non-crustaceous plankton, there were no *O. dioica*, *Pleurobrachia*, polychaetes, and hydromedusae. The dissimilarity coefficients with the overlying layers were 66.7% with the surface one and 70.1% with the middle one; those were lower than the corresponding coefficients at sta. 3 (91.7 and 95.6% in summer; 94.8 and 93.3% in autumn). This fact can be due to lower intensity of the negative effect of unfavorable environmental factors on the MM at the bay mouth compared to its inner area.

Analysis of weather and hydrological conditions during sampling showed that at sta. 3 in early August, the highest temperature (+24…+26.1 °C) and calm wind were recorded. Such conditions, with a developed summer vertical stratification of the bay waters, can result in a lower oxygen content in deeper water layers. "…In years with hot, low-wind summer seasons, due to the weakening of dynamic processes, adverse effects on the ecosystem increase. Specifically, hypoxia phenomena were observed in the bottom layers of the bay, when the water saturation with oxygen was lower than 50%" [Ivanov et al., 2006, p. 24]. This results from a discharge of untreated domestic wastewater and a so-called summer peak in phytoplankton development, which is not characteristic of open sea areas but characteristic only of polluted waters [Ivanov et al., 2006]. The process of deoxygenation of marine coastal waters because of human activities is a challenge for the entire World Ocean [Rabalais et al., 2014]. Analysis of summer survey data for the Sevastopol Bay over the past 10 years revealed regular occurrence of hypoxic conditions and formation of hydrogen sulfide near the bottom at sta. 8 neighboring sta. 3 [Kondratev, Vidnichuk, 2020]. According to their information, relative oxygen content in the bottom layer at sta. 3 in August 2019 was also reduced and accounted for only 56% of saturation. Apparently, the situation with a decrease in the abundance and species richness of MM at sta. 2 (in the bay mouth) in October is related to spreading of polluted waters from the center area of the bay with the bottom current there [Lemeshko et al., 2014]. In the direction towards the bay mouth, the concentration of contaminants dropped due to dilution; accordingly, the degrading effect on the structure of the community decreased. Such negative phenomena in the MM community in autumn can be caused by seasonal changes in the Black Sea level as well, which play the greatest role in the processes of water exchange in the Sevastopol Bay. Long-term studies have shown that minimum sea levels are most often observed in October–November [Goryachkin, Ivanov, 2006]. At this time, the inflow of cleaner waters from the open sea into the bay decreases, and this can lead to a general drop in biomass and abundance of zooplankton organisms [Repetin et al., 2003].

**Conclusion.** In summer, in the surface water layer of the open seaside, the abundance of metazoan microzooplankton (MM) was characterized by relative stability, and the values were from $310 \times 10^3$ to $330 \times 10^3$ ind.·m$^{-3}$. Fluctuations in MM abundance at the bay mouth in this layer were more significant: from $140 \times 10^3$ to $700 \times 10^3$ ind.·m$^{-3}$. The maximum abundance was recorded on the surface in the inner area of the Sevastopol Bay and was the highest for a 10-year observation period there. MM abundance along the vertical in the open seaside and at the bay mouth was more even, while in the inner area, the differences between the layers could reach 5–700 times. In autumn season, a decrease in the total abundance of MM in the water column was registered for all studied stations. In most cases, the vertical structure was characterized by a drop in abundance from the surface to deeper water layers.

The direction of changes in the species diversity of the MM community was characterized by a decrease in the gradient open coast – bay mouth – inner bay area in accordance with increasing pollution and trophicity of the bay waters due to an increase in anthropogenic load on the biota. This pattern persisted in both summer and autumn seasons. The most informative of the indices used were the Shannon index, the Simpson polydominance index, and the Pielou evenness index. They reflected seasonal changes in the species diversity and the direction in the trophic gradient of the local water areas of the bay. The weighted average indicators of the biodiversity index for the entire water column showed that the status of open waters was most often characterized as oligotrophic; of the bay mouth, as mesotrophic; and of the inner bay area, as transitional from meso- to hypertrophic.

Low values of the Pielou evenness index generally indicated a pronounced degree of dominance of mass species in the MM community of the coastal area of the city of Sevastopol, in particular, copepod *Oithona davisae* – a recent invader in the Black Sea. The species has bioindicator features; its quantitative characteristics can be used in assessing the trophic status of water areas. Its contribution to the index of community structure similarity in the studied gradient from open waters to the inner bay area increased from 73.7% at sta. 1 (in the open seaside) to 79.3% at sta. 2 (at the bay mouth) and 84.3% at sta. 3 (in the inner bay area). At the same time, the proportion of *Acartia* effect decreased along the gradient (7.9 – 6.7 – 4.3%, respectively), as well as that of Bivalvia veligers (6.5 – 5.6 – 2.6%).

Situations of significant changes in the structure of the MM community are typical for the bottom water layer in the inner bay area and at the mouth. The main factor determining the periodic degradation of the species composition and MM abundance in these habitats seems to be low water saturation with oxygen, which is caused by increased trophicity of waters and disturbances in the process of water exchange in the Sevastopol Bay.

*This work was carried out within the framework of IBSS state research assignment “Functional, metabolic, and toxicological aspects of hydrobionts and their populations existence in biotopes with different physical and chemical regimes” (No. 121041400077-1) and “Regularities of formation and anthropogenic transformation of biodiversity and biological resources of the Sea of Azov–Black Sea basin and other areas of the World Ocean” (No. 121030100028-0).*

**Acknowledgement.** We express our sincere gratitude to T. Bogdanova for determining salinity in seawater samples.

## REFERENCES

1. Goryachkin Yu. N., Ivanov V. A. *Uroven' Chernogo morya: proshloe, nastoyashchee i budushchee* / V. N. Eremeev (Ed.) ; NAS of Ukraine, Marine Hydrophysical Institute. Sevastopol : [EKOSI-Gidrofizika], 2006, 210 p. (in Russ.)
2. Gubanov V. I., Stelmah L. V., Klimenko N. P. Complex assessments of the Sevastopol offshore water quality (the Black Sea). *Ekologiya morya*, 2002, iss. 62, pp. 76–80. (in Russ.). https://repository.marine-research.ru/handle/299011/4565
3. Dyatlov S. Ye. Role and position of bioassays in complex monitoring of pollution of marine environment. *Ekologiya morya*, 2000, iss. 51, pp. 83–87. (in Russ.). https://repository.marine-research.ru/handle/299011/4306
4. Ivanov V. A., Ovsyany E. I., Repetin L. N., Romanov A. S., Ignatyeva O. G. *Hydrological and Hydrochemical Regime of the Sebastopol Bay and Its Changing Under Influence of Climatic and Anthropogenic Factors* / NAS of Ukraine, Marine Hydrophysical Institute. Sevastopol, 2006, 90 p. (in Russ.)
5. Klimova T. N., Vdodovich I. V., Ignatiev S. M., Seregin S. A., Kuzminova N. S., Pustovarova N. I., Popova E. V. The ichthyoplankton state in the Sevastopol Bay mouth (Black Sea). *Zhurnal Sibirskogo federal'nogo universiteta. Biologiya*, 2017, vol. 10, no. 1, pp. 74–86. (in Russ.). https://doi.org/10.17516/1997-1389-0009
6. Kondratev S. I., Vidnichuk A. V. Local seasonal hypoxia and hydrogen sulphide formation in the bottom waters of the Sevastopol Bay in 2009–2019. *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon i kompleksnoe ispol'zovanie resursov shel'fa*, 2014, no. 28, pp. 25–30. (in Russ.)
8. Melnikov V. V., Klimova T. N., Ignatiev S. M., Vdodovich I. V., Popova E. V., Seregin S. A. Conditions of the ichthyo-, microzoo-, and macroplanktonic complexes in the Crimean Peninsula area in July 2013. *Sistemy kontrolya okruzhayushchei sredy*, 2015, no. 1 (21), pp. 94–102. (in Russ.)
9. *Metod otsenki kachestva vod i sostoyaniya vodnykh ekosistem v skhemakh KIOVR* : presentation. (in Russ.). URL: https://ppt4web.ru/ehkologija/metod-ocenki-kachestva-vod-i-sostojanija-vodnykh-ehkosistem-v-skhemakh-kiovr.html [accessed: 09.02.2021].
10. Magurran A. E. *Ecological Diversity and Its Measurement* : transl. from English. Moscow : Mir, 1992, 181 p. (in Russ.)
11. Pesenko Yu. A. *Printsipy i metody kolichestvennogo analiza v faunisticheskikh issledovaniyakh.* Moscow : Nauka, 1982, 288 p. (in Russ.)
12. Puznetskite K. S., Marushkina E. V. Primenenie indeksov al'fa-raznoobraziya zooplanktonnykh soobshchestv dlya otsenki troficheskogo statusa vodoemov (na primere nekotorykh ozer chebarkul'skoi gruppy). *Vestnik Chelyabinskogo gosudarstvennogo universiteta. Seriya 12. Ekologiya. Prirodopol'zovanie*, 2005, vol. 12, no. 1, pp. 22–25. (in Russ.)

13. Repetin L. N., Gordina A. D., Pavlova E. V., Romanov A. S., Ovsyany E. I. Effect of oceanographic factors upon the ecological state of the Sevastopol Bay (the Black Sea). *Morskoi gidrofizicheskii zhurnal*, 2003, no. 2, pp. 66–80. (in Russ.)
14. Sazhina L. I. *Naupliusy massovykh vidov pelagicheskikh copepod Mirovogo okeana*. Kyiv : Naukova dumka, 1985, 238 p. (in Russ.). https://repository.marine-research.ru/handle/299011/5670
15. Seregin S. A., Popova E. V. Bacterioplankton and metazoan microzooplankton in the Black Sea waters near Crimea coast in summer 2010. *Morskoj ekologicheskij zhurnal*, 2012, vol. 11, no. 2, pp. 65–74. (in Russ.). https://repository.marine-research.ru/handle/299011/1214
16. Seregin S. A., Popova E. V. Short-term variation of metazoan microzooplankton in a coastal area of the Black Sea: The regularities and determining factors. *Morskoj biologicheskij zhurnal*, 2017, vol. 2, no. 1, pp. 56–65. (in Russ.). https://doi.org/10.21072/mbj.2017.02.1.06
17. Slepchuk K. A., Khmara T. V., Man'kovskaya E. V. Comparative assessment of the trophic level of the Sevastopol and Yuzhnaya bays using E-TRIX index. *Morskoi gidrofizicheskii zhurnal*, 2017, no. 5 (197), pp. 67–78. (in Russ.). https://doi.org/10.22449/0233-7584-2017-5-67-78
18. Sovga E. E., Mezentseva I. V., Slepchuk K. A. Comparison of assimilative capacity and trophic index for various parts of the Sevastopol Bay water area. *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon morya*, 2020, no. 3, pp. 63–76. (in Russ.). https://doi.org/10.22449/2413-5577-2020-3-63-76
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
20. Shitikov V. K., Rozenberg G. S., Zinchenko T. D. *Kolichestvennaya gidroekologiya: metody sistemnoi identifikatsii*. Tolyatti : IEBV RAN, 2003, 463 p. (in Russ.)
21. Calbet A. The trophic roles of microzooplankton in marine systems. *ICES Journal of Marine Science*, 2008, vol. 65, iss. 3, pp. 325–331. https://doi.org/10.1093/icesjms/fsn013
22. Cardinale B. J., Duffy J. E., Gonzalez A., Hooper D. U., Perrings Ch., Venail P., Narwani A., Mace G. M., Tilman D., Wardle D. A., Kinzig A. P., Daily G. C., Loreau M., Grace J. B., Larigauderie A., Srivastava D. S., Naeem Sh. Biodiversity loss and its impact on humanity. *Nature*, 2012, vol. 486, pp. 59–67. https://doi.org/10.1038/nature11148
23. Clarke K. R., Gorley R. N., Somerfield P. J., Warwick R. M. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*. 3rd ed. Plymouth : PRIMER-E, 2014, 260 p.
24. Drira Z., Kmiha-Megdiche S., Sahnoun H., Tedetti M., Pagano M., Ayadi H. Copepod assemblages as a bioindicator of environmental quality in three coastal areas under contrasted anthropogenic inputs (Gulf of Gabes, Tunisia). *Journal of the Marine Biological Association of the United Kingdom*, 2018, vol. 98, spec. iss. 8, pp. 1889–1905. https://dx.doi.org/10.1017/S0025315417001515
25. *ICES Zooplankton Methodology Manual* / R. P. Harris, P. H. Wiebe, J. Lenz, H. R. Skjoldal, M. Huntley (Eds). Bodmin,

Cornwall : Academic Press, 2000, 684 p. https://doi.org/10.1016/B978-0-12-327645-2.X5000-2

26. Hooper D. U., Adair E. C., Cardinale B. J., Byrnes J. E. K., Hungate B. A., Matulich K. L., Gonzalez A., Duffy J. E., Gamfeldt L., O'Connor M. I. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, 2012, vol. 486, pp. 105–108. https://doi.org/10.1038/nature11118
27. Rabalais N. N., Cai W.-J., Carstensen J., Conley D. J., Fry B., Hu X., Quiñones-Rivera Z., Rosenberg R., Slomp C. P., Turner R. E., Voss M., Wissel B., Zhang J. Eutrophication-driven deoxygenation in the coastal ocean. *Oceanography*, 2014, vol. 27, no. 1, pp. 172–183. http://dx.doi.org/10.5670/oceanog.2014.21
28. Seregin S. A., Popova E. V. Different-scale variations in the abundance and species diversity of metazoan microzooplankton in the coastal zone of the Black Sea. *Water Resources*, 2019, vol. 46, no. 5, pp. 769–779. https://doi.org/10.1134/S009780781905018X
29. Seregin S. A., Popova E. V. Long-term dynamics of abundance of the copepod-invader, *Oithona davisae*, in the coastal waters of the Black Sea. *Russian Journal of Biological Invasions*, 2016, vol. 7, no. 4, pp. 374–382. https://doi.org/10.1134/S207511171604007X
30. Serranito B., Aubert A., Stemmann L., Rossi N., Jamet J. L. Proposition of indicators of anthropogenic pressure in the Bay of Toulon (Mediterranean Sea) based on zooplankton time-series. *Continental Shelf Research*, 2016, vol. 121, pp. 3–12. https://doi.org/10.1016/j.csr.2016.01.016
31. Svetlichny L. S., Hubareva E. S., Khanaychenko A. N., Gubanova A. D., Altukhov D. A., Beşiktepe S. Adaptive strategy of thermophilic *Oithona davisae* in the cold Black Sea environment. *Turkish Journal of Fisheries and Aquatic Sciences*, 2016, vol. 16, pp. 77–90. https://doi.org/10.4194/1303-2712-v16_1_09
32. Vollenweider R. A., 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

# ОБИЛИЕ, ВИДОВОЕ РАЗНООБРАЗИЕ И СТРУКТУРА СООБЩЕСТВА МЕТАЗОЙНОГО МИКРОЗООПЛАНКТОНА В ГРАДИЕНТЕ БУХТА — ВЗМОРЬЕ (НА ПРИМЕРЕ СЕВАСТОПОЛЬСКОЙ БУХТЫ, ЧЁРНОЕ МОРЕ)

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Цель исследования — выявить закономерные изменения в обилии, видовом разнообразии и структуре сообществ метазойного микрозоопланктона (ММ) в градиентных условиях морского прибрежья на относительно небольших пространственных масштабах. Актуальность работы определяется малочисленностью подобных исследований, позволяющих опосредованно оценить степень антропогенного воздействия на морскую биоту и установить трофический статус локальных акваторий с помощью индексов видового разнообразия. Проанализированы три акватории прибрежья города Севастополя: открытое взморье, устье Севастопольской бухты и внутренняя её часть. Локализация станций отбора проб отражает градиент условий среды, характеризующийся разной степенью воздействия на биоту природных и антропогенных факторов. Исследования проведены в летний и осенний периоды года. Пробы ММ отбирали из трёх горизонтов водного столба — поверхностного, 0–5-метрового и 0–10-метрового слоёв. В открытом взморье и устье бухты численность ММ по вертикали была более выравненной, тогда как в глубине бухты различия между слоями могли достигать 5–700 раз. Максимальная численность ММ (1837,1 тыс. экз.·м$^{-3}$) отмечена в начале августа в поверхностном слое во внутренней части бухты. С конца лета происходило снижение обилия во всех исследованных акваториях. Видовое разнообразие сообщества ММ, оценённое с помощью индексов Шеннона, Симпсона, Пиелу и др., уменьшалось в направлении от открытого взморья вглубь бухты. Эта закономерность сохранялась в оба сезона. Наиболее информативными оказались индекс Шеннона, индекс полидоминантности Симпсона и индекс выравненности Пиелу. Они хорошо отражали как сезонные изменения видового разнообразия, так и направленность изменений в градиенте трофности локальных акваторий бухты. С помощью многомерного анализа выявлены случаи существенной трансформации в структуре сообщества ММ придонного слоя вод в глубине бухты. Основной вероятной причиной этих локальных изменений является возникновение в загрязнённых участках бухты гипоксийных условий в нижних горизонтах вод, приводящее к деградации численности и видового состава исследуемого зоопланктонного сообщества.

**Ключевые слова:** метазойный микрозоопланктон, обилие, видовое разнообразие, структура сообщества, градиент бухта — взморье, трофический статус акватории

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 2, pp. 91–103
https://marine-biology.ru

UDC [582.26/.27:57.013]:542.943’78

# ASSESSMENT OF ANTIOXIDANT ACTIVITY OF SEAWEED EXTRACTS FROM THE SEA OF JAPAN *IN VITRO* AND *IN VIVO*

© 2023 **S. E. Fomenko, N. F. Kushnerova, V. G. Sprygin, E. S. Drugova, L. N. Lesnikova, and V. Yu. Merzlyakov**

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Received by the Editor 12.01.2022; after reviewing 25.05.2022;
accepted for publication 16.02.2023; published online 31.05.2023.

Seaweeds are a source of important biologically active substances: lipids, amino acids, phenolic compounds, polycarbohydrates, *etc.* Polyphenolic compounds are one of the perspective groups of constituents of marine origin with high antioxidant activity; those play a key role in the life of marine macrophytes, allowing them to quickly respond to external stress and to perform protective functions. At the same time, the multicomponent composition of the phenolic fraction of the seaweed extract provides a wide spectrum of its pharmacological activity, *inter alia* a regulatory effect on numerous homeostasis disorders occurring during pathological processes in humans and animals. Wherein, the available opportunities for the practical use of seaweed extracts have not yet been depleted, and this is of undoubted interest for modern science. The aim of the work was to carry out a comparative assessment of the antioxidant activity of hydroalcoholic extracts isolated from the thalli of three classes of algae [brown (*Sargassum pallidum*), green (*Ulva lactuca*), and red (*Ahnfeltia fastigiata* var. *tobuchiensis*)] and to analyze their effect on indices of the endogenous antioxidant system of liver and blood in mice under experimental stress. Seaweeds were sampled in summer in the coastal waters of the Peter the Great Bay (the Sea of Japan). Sampled seaweeds were dried at a temperature of about +50 °C, grinded in a laboratory mill to particles 0.5–1 mm in size, and extracted with 70% ethanol *via* repercolation. In the extract of the brown alga *S. pallidum*, the highest content of polyphenols was recorded – (218.2 ± 20.3) mg-Eq GA·g$^{-1}$ dry weight. In the extract of the green alga *U. lactuca*, the value was (16.2 ± 1.8) mg-Eq GA·g$^{-1}$ dry weight; in the extract of the red alga *A. fastigiata* var. *tobuchiensis*, (9.1 ± 1.6) mg-Eq GA·g$^{-1}$ dry weight. Accordingly, the antiradical activity of *S. pallidum* extract towards the cation radical ABTS$^{+}$ and the alkyl peroxyl radical was significantly higher than that of *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts. The effect of these seaweed extracts on the antioxidant defense indices of liver and plasma in mice under acute stress was studied experimentally. Weight indicators (weight of animals and weight coefficients of their internal organs) and biochemical indices (level of antiradical activity, malondialdehyde and reduced glutathione content, and activity of antioxidant enzymes) were established. The experiment was carried out on white outbred male mice (weight of 20–30 g). To model conditions of acute stress, mice were fixed vertically by the dorsal neck crease for 24 h. Alcohol-free seaweed extracts were injected into mice stomachs as an aqueous suspension (a dose of 100 mg of total polyphenols *per* kg of body weight) through a tube twice: right before vertical fixation and in 6 h. Into stomachs of the animals of the control and the “stress” groups, distilled water was injected in a volume equal to that of the injected extracts. In this model, all the attributes of stress manifested themselves: adrenal hypertrophy, involution of the thymus and spleen, and ulceration of the gastric and intestinal mucosa. Moreover, disturbances of the antioxidant defense system were recorded: a decrease of antioxidant enzymes activity in blood plasma, a drop in reduced glutathione content in liver, and an increase of the malondialdehyde level. Under the effect of the extracts, in all the groups of animals under stress, a tendency to stabilization of the studied antioxidant defense indices was observed. Interestingly, the values in mice receiving *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts were inferior to those in the group of animals receiving *S. pallidum* extract. In the latter group of mice, there were no significant differences from the control values in terms of antioxidant defense indices. This is due to the fact the main components of the polyphenolic fractions of green and red algae are monomeric flavonoids, while brown algae contain high molecular weight phlorotannins. The latter ones are characterized by higher antioxidant activity than low molecular weight polyphenolic fractions of green and red algae.

**Keywords:** seaweeds, polyphenols, antioxidant activity, stress, mice

An important component of marine ecosystems and a key link in food chains of many species of marine organisms are seaweeds serving as a source of organic matter and energy. Due to their diverse composition, they are used as a raw material for production of several substances with beneficial properties. Specifically, seaweeds contain easily digestible proteins, amino acids, lipids, polysaccharides, carotenoids, minerals, polyphenolic compounds, *etc.* [Michalak, Chojnacka, 2015].

Among secondary metabolites that make up seaweeds, an important group of substances are polyphenolic compounds with pronounced antioxidant properties. These compounds are produced by seaweeds and seagrasses to perform protective, structural, and reproductive functions [Pradhan et al., 2021]. Polyphenols are involved in growth and reproduction processes of seaweed cells and in formation and early development of cell walls, forming a complex with alginic acid, a structural polysaccharide of a cell wall [Imbs, Zvyagintseva, 2018]. Polyphenols are capable of protecting macrophytes from damage by pathogenic bacteria, grazing by herbivores, and UV exposure. According to international classification, polyphenolic compounds include various subclasses: phenolic acids, flavonoids, lignans, stilbenes, and so on [Zhong et al., 2020]. In composition of brown algae and some species of red algae, a special group of phenolic compounds was found – phlorotannins, which are oligomers of phloroglucinol (1,3,5-trihydroxybenzene) [Ragan, Glombitza, 1986]. Phlorotannins are the main cytoplasmic components of seaweeds; those are contained in specific organelles – physodes [Ragan, Glombitza, 1986]. Unlike other polyphenolic compounds, phlorotannins are characterized by the fact that about 90% of their total amount is in free form [Bogolitsyn et al., 2018]. These compounds are accumulated mainly in outer layers of the epidermis and in cortical layer of the thallus [Shibata et al., 2004], which allows them to quickly respond to external stress and to perform protective functions.

A rising interest in seaweeds is due to the content of bioactive components in them, which can be used as pharmaceuticals, nutraceuticals, and food additives. As known, preparations derived from seaweeds exhibit a wide range of pharmacological properties: antibacterial, antiviral, antitumor, antimicrobial, hepatoprotective, *etc.* [Cotas et al., 2020; Manach et al., 2004].

Health benefits of seaweeds for humans and animals are largely due to the ability of their polyphenolic compounds to scavenge free radicals, which may help in reducing oxidative stress [Zhong et al., 2020]. The mechanism of active binding of free radicals involved in the development of several pathological processes in the body is based on the presence of a branched structure of conjugated double bonds of high mobility and a large number of free hydroxyl groups in macrophyte polyphenols.

In previous studies, we found that extracts isolated from a number of marine macrophytes representing different classes [brown, *Sargassum pallidum* (Turner) C. Agardh, 1820; green, *Ulva lactuca* Linnaeus, 1753; and red, *Ahnfeltia fastigiata* var. *tobuchiensis* (Kanno & Matsubara) Skriptsova

& Zhigadlova, 2022] have shown a pronounced protective effect in various experimental models. Thus, an extract from the brown alga *S. pallidum*, enriched with polyphenolic compounds, had a hepatoprotective effect in modelling hepatosis in rats [Sprygin et al., 2017]. The lipid fraction of an extract from the green alga *U. lactuca* showed a preventive effect under acute stress, which manifested itself in the preservation of carbohydrate–lipid metabolism in liver and a decrease in the level of lipid peroxidation [Fomenko et al., 2019]. The pharmacological effect of an extract from the red alga *A. fastigiata* var. *tobuchiensis* was expressed in the ability to restore the lipid composition of blood and the ratio of phospholipid fractions in erythrocyte membranes [Kushnerova et al., 2020]. As a continuation of the studies carried out, it seems relevant to acquire new knowledge on the biological activity of the investigated seaweed extracts and to clarify the prospects for their use as antioxidant agents.

The aim of the work is to compare antioxidant activity of hydroalcoholic extracts isolated from thalli of the brown alga *Sargassum pallidum*, green alga *Ulva lactuca*, and red alga *Ahnfeltia fastigiata* var. *tobuchiensis* and to determine their effect on antioxidant defense indices of liver and blood plasma of mice under experimental stress.

## MATERIAL AND METHODS

The objects of the study were seaweeds:

- *Ulva lactuca* [= *Ulva fenestrata*], division Chlorophyta, class Ulvophyceae, order Ulvales, family Ulvaceae;
- *Sargassum pallidum*, division Phaeophyta, class Cyclosporophyceae, order Fucales, family Sargassaceae;
- *Ahnfeltia fastigiata* var. *tobuchiensis* [= *Ahnfeltia tobuchiensis*], division Rhodophyta, class Florideophyceae, order Ahnfeltiales, family Ahnfeltiaceae [Skriptsova, Zhigadlova, 2022].

The selected seaweeds are the most widespread in the seas of the Far East and are the main, mass species.

Seaweeds were sampled in August–September 2021 in the Peter the Great Bay coastal waters (the Sea of Japan). A sample included 20 thalli of each species. All thalli were cleaned of epiphytes and zoobenthos, washed with seawater and then distilled water, and dried. A thallus in the air-dried state was grinded using a laboratory mill to 0.5–1-mm particles and extracted with 70% ethanol *via* repercolation. The extract yield was 1 L *per* 1 kg of dry raw material. Extraction with ethanol is an effective method of seaweed processing: during it, most mineral and organic substances exhibiting biological activity are extracted, and ethanol, due to its low toxicity, is the most preferable for the extraction of phenolic compounds among all solvents [Cotas et al., 2020].

Seaweed extracts were evaporated in a vacuum until ethanol was completely removed; then, those were extracted with chloroform to scavenge lipophilic substances and pigments – in accordance with the technique described earlier [Sprygin et al., 2013]. The resulting aqueous fraction containing polyphenols was evaporated to dryness in a vacuum and resuspended in water to obtain stock solutions (10 $mg \cdot mL^{-1}$), in which total polyphenols (hereinafter PP) and antiradical activity (hereinafter ARA) were preliminarily determined. All biochemical studies were carried out on a Shimadzu UV-2550 spectrophotometer (Japan). Total PP were determined using the Folin–Ciocalteu reagent at a wavelength of 765 nm [Parys et al., 2007]. Gallic acid (GA) was used as a reference standard; total PP were expressed in mg-Eq $GA \cdot g^{-1}$ dry extract. The level of ARA was also assessed spectrophotometrically towards the cation radical $ABTS^{+}$ ($\lambda$ = 734 nm) [Re et al., 1999] and the alkyl peroxyl radical (λ = 414 nm) [Bartosz et al., 1998]. When determining ARA, Trolox (a water-soluble analog of vitamin E) was used as a reference standard. ARA was expressed in μmol Trolox·mg$^{-1}$ PP. The results were statistically processed with Instat 3.0 software package and GraphPad Prism program, with the second one including the function of checking a sample compliance with the normal distribution law. To determine the statistical significance of differences depending on the distribution parameters, the parametric Student's $t$-test or the nonparametric Mann–Whitney $U$-test were used.

The experiment was carried out on white outbred male mice (weight of 20–30 g). Animals were kept under vivarium conditions in cages of 5 individuals on a standard diet, with natural light, and at a constant air temperature of +20…+22 °C.

To model conditions of acute stress, mice were fixed vertically by the dorsal neck crease for 24 h. This stress model is used in laboratory animals in experimental studies [Kushnerova et al., 2005]. Alcohol-free seaweed extracts were injected into mice stomachs as an aqueous suspension (a dose of 100 mg of total PP *per* kg of body weight) through a tube twice: right before vertical fixation and in 6 h. This concentration corresponds to a known therapeutic dose for polyphenolic hepatoprotectors [Vengerovsky et al., 1999]. Into stomachs of the animals of control and "stress" groups, distilled water was injected in a volume equal to that of the injected extracts.

In the course of the study, there were five groups of animals, 10 mice each: 1, control (intact); 2, "stress" (vertical fixation by the dorsal neck crease); 3, "stress + *Sargassum* extract"; 4, "stress + *Ulva* extract"; and 5, "stress + *Ahnfeltia* extract." Animals were taken out of the experiment by decapitation under light ether anesthesia in compliance with the rules and international recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [1986].

After exposure to acute stress, weight of animal, the mass index of internal organs (mg of organ weight *per* 100 g of body weight), and the number of ulcerations of the gastric mucosa were determined. The latter ones were counted visually: we determined the number of formed ulcerative lesions. The design of the study was approved by the ethics committee of V. I. Il'ichev Pacific Oceanological Institute FEB RAS.

Blood for research was sampled from neck vein of animals into vacuettes with 1% heparin solution. To separate plasma, blood was centrifuged for 10 min at 3,000 rpm. Then, plasma samples were frozen at a temperature of −80 °C for further determination of biochemical parameters. After extraction, liver was washed in physiological saline and frozen in a refrigerator at −80 °C as well. The state of the antioxidant system was assessed in blood plasma of animals by a spectrophotometric method by the value of total ARA (λ = 734 nm) [Re et al., 1999], the level of malonic dialdehyde (hereinafter MDA) (λ = 532 nm) [Buege, Aust, 1978], activity of superoxide dismutase (hereinafter SOD) (λ = 550 nm) [Paoletti et al., 1986], and glutathione enzymes – glutathione reductase (hereinafter GR) [Goldberg, Spoone, 1983] and glutathione peroxidase (hereinafter GPx) (λ = 340 nm) [Burk et al., 1980], as well as by the value of the level of reduced glutathione (hereinafter G-SH) in liver tissue (λ = 412 nm) [Ellman, 1959].

## RESULTS

*Prior* to conducting the experiment aimed at analyzing the state of the antioxidant defense system of an animal body under stress, total PP and ARA were determined in samples of extracts of the macrophytes studied. Evaluation of the quantitative composition of PP in them showed that their content varies significantly in different macrophyte species (Table 1). The highest amount of PP was registered in the extract of the brown alga *S. pallidum*, and the value was 13.5 times higher than in *U. lactuca* and 24 times higher than in *A. fastigiata* var. *tobuchiensis*.

**Table 1.** Polyphenol content and antiradical activity of the seaweed extracts ($M \pm m$)

| Seaweed | Total polyphenols, mg-Eq GA·g$^{-1}$ dry extract | Antiradical activity towards ABTS$^+$, μmol Trolox·mg$^{-1}$ PP | Antiradical activity towards alkyl peroxyl radicals, μmol Trolox·mg$^{-1}$ PP |
|---|---|---|---|
| *Sargassum pallidum* | 218.2 ± 20.3 | 1.62 ± 0.04 | 0.64 ± 0.02 |
| *Ulva lactuca* | 16.2 ± 1.8 | 0.32 ± 0.03 | 0.15 ± 0.02 |
| *Ahnfeltia fastigiata* var. *tobuchiensis* | 9.1 ± 1.6 | 0.13 ± 0.03 | 0.06 ± 0.01 |

An important aspect of investigating the antioxidant potential of the studied seaweed extracts is the assessment of their ARA towards the cation radical ABTS$^+$ and the alkyl peroxyl radical. The level of ARA in the extracts of the macrophytes varied significantly, as well as PP content. Specifically, *S. pallidum* extract was characterized by a higher level of ARA towards ABTS$^+$: the value was 5 times higher than the corresponding value in *U. lactuca* and 12.5 times higher than in *A. fastigiata* var. *tobuchiensis*. A similar trend was observed for ARA indices towards the alkyl peroxyl radical. Alkyl peroxyl radicals are formed in the body during lipid peroxidation and are one of the main initiators of free radical reactions. In *S. pallidum* extract, this index was 4 times higher than the corresponding value for *U. lactuca*. An even lower level of APA towards the alkyl peroxyl radical was recorded in *A. fastigiata* var. *tobuchiensis*: its value was 2.5 times less than that for *U. lactuca* and 10 times less than that for *S. pallidum*.

The next stage of the experimental studies was the investigation of the effect of the seaweed extracts on the state of the antioxidant defense system in animals under stress. Their vertical fixation by the dorsal neck crease for 24 h was accompanied by the manifestation of all known attributes of stress, such as adrenal hypertrophy, involution of the thymus and spleen, and ulceration of the gastric and intestinal mucosa. These alterations were recorded in all animals subjected to stress (the groups 2–5). However, there were significant differences between the groups in terms of severity. Thus, in the group 2 (“stress”), weight of animals decreased by 23% ($p < 0.01$) with a simultaneous decrease in the mass index of internal organs (liver and spleen) by an average of 19–23% ($p < 0.05$) (Fig. 1). In the group 2, under stress, the number of recorded ulcerations of the gastric mucosa was (2.6 ± 0.1) pcs *per* animal; in the control, it was 0.

When assessing the state of the antioxidant system of animals under stress, a drop in ARA value of blood plasma by 46% was revealed ($p < 0.001$) compared to the value in the control. At the same time, there was a decrease in the activity of one of key enzymes of the antioxidant defense system, SOD, by 40% ($p < 0.001$) (Fig. 2). The level of G-SH in liver dropped by almost 2 times (Fig. 3), while the activity of GR, an enzyme which plays the main role in maintaining a certain concentration of G-SH inside the cell, decreased by 26% ($p < 0.001$). The activity of another key enzyme of the glutathione unit, GPx, which catalyzes the reduction of hydrogen peroxide ($H_2O_2$) and organic peroxides in the presence of G-SH, was reduced by 35% ($p < 0.001$). Such changes in the antioxidant defense indices can be defined as its weakening. Disturbances in the functioning of the antioxidant defense system under stress conditions also manifested themselves in an increase in MDA content by 68% ($p < 0.001$) (Fig. 3), which is a biomarker of oxidative stress.

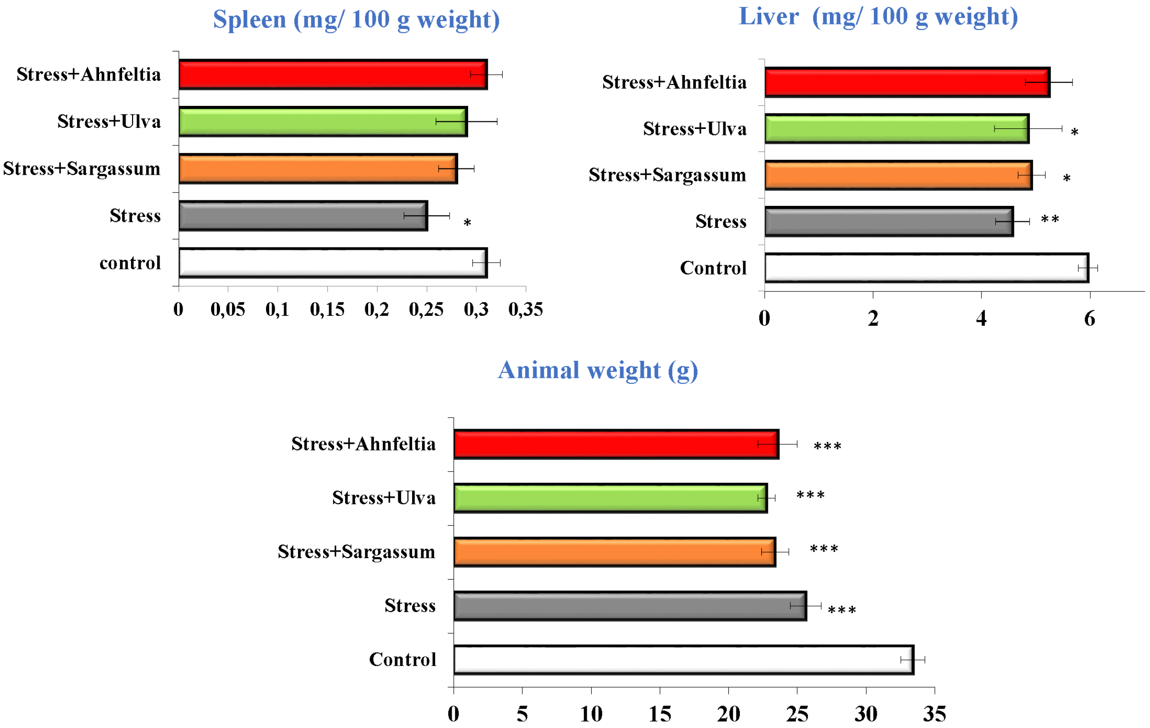

**Fig. 1.** The effect of the seaweed extracts on the total weight of mice and weight coefficients of their internal organs under stress. Differences are statistically significant compared to the control: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***)

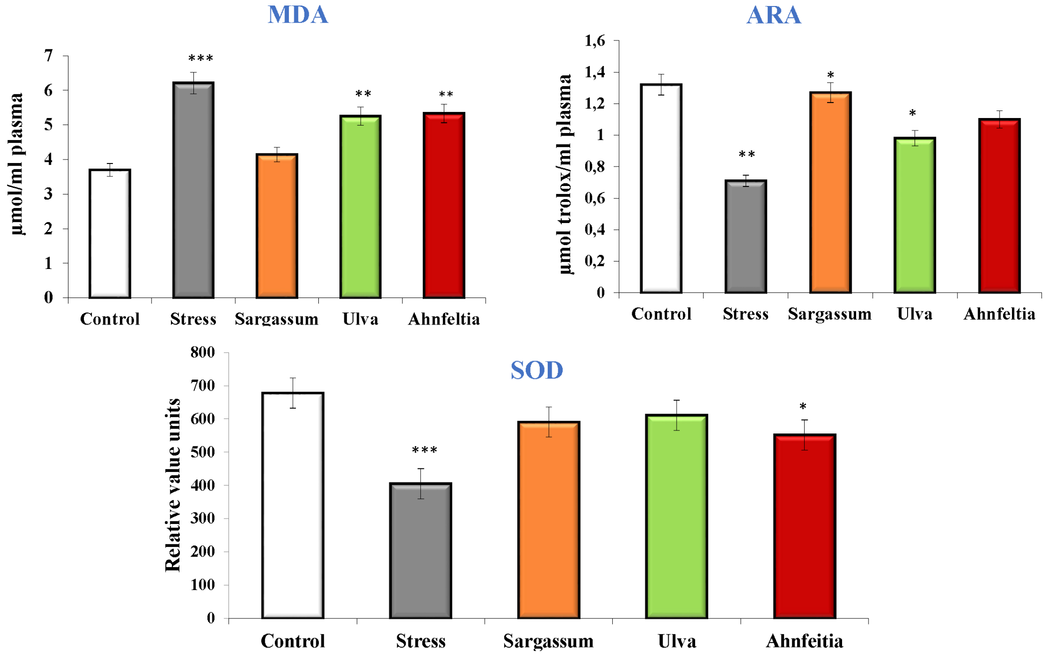

**Fig. 2.** The effect of the seaweed extracts on the antioxidant defense indices of mice under stress. MDA, malondialdehyde; ARA, antiradical activity; SOD, superoxide dismutase. Differences are statistically significant compared to the control: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***)

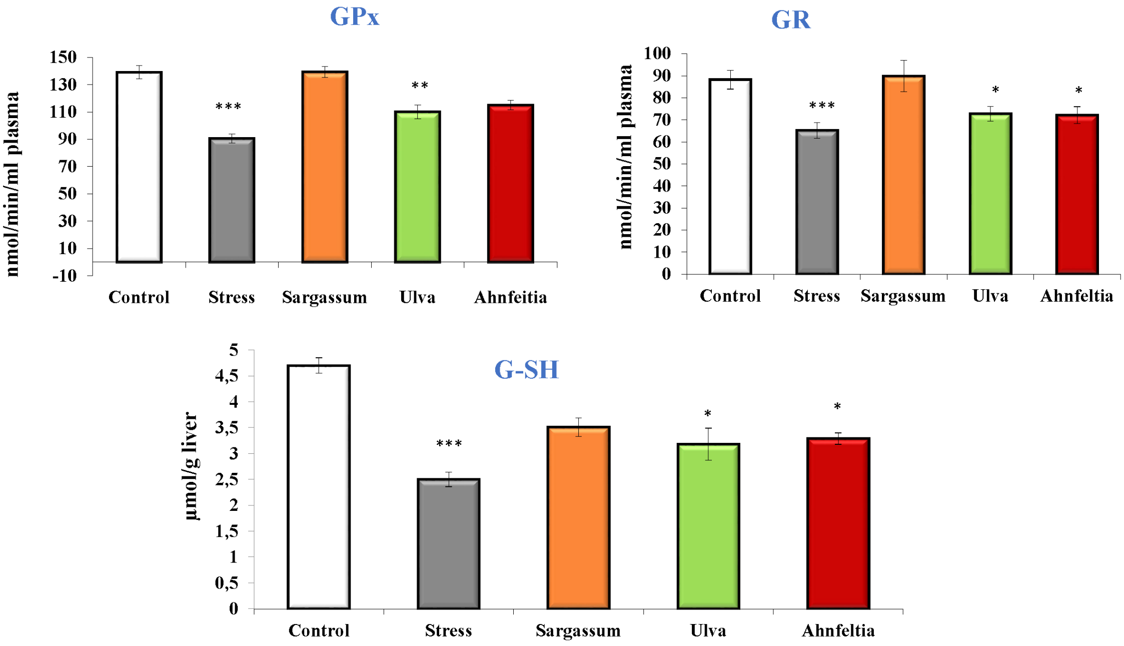

**Fig. 3.** The effect of the seaweed extracts on the glutathione system indices in mice under stress. GPx, glutathione peroxidase; GR, glutathione reductase; G-SH, reduced glutathione. Differences are statistically significant compared to the control: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***)

The administration of the seaweed extracts against the backdrop of acute stress (the groups 3–5) was accompanied by a tendency to reduce the severity of involutional alterations in internal organs compared with their severity in the group 2 (“stress”). Specifically, in the groups of mice treated with *U. lactuca* and *S. pallidum* extracts, the relative mass of liver increased by an average of 6–8% ($p < 0.05$), while in the group 5, under the effect of *A. fastigiata* var. *tobuchiensis* extract, the value was 15% higher ($p < 0.05$). Moreover, in mice receiving the seaweed extracts, the relative mass of spleen increased by an average of 12–24% ($p < 0.001$). As noted, the injection of the seaweed extracts did not lead to the complete restoration of the relative mass of internal organs, but contributed to a significant increase in these indicators compared to the group 2 (see Fig. 1). In terms of the body weight parameters of mice in the groups 3–5, these indicators significantly differed from the control. Importantly, these animals had no ulcerations of the gastric mucosa.

In all groups of animals treated with the seaweed extracts, against the backdrop of stress, there was a tendency to stabilize the studied antioxidant defense indices (Figs 2, 3). Thus, in the group 3 (*S. pallidum* extract), the values corresponded to the control ones. The comparison with the group 2 (“stress”) revealed as follows: in these mice, the level of MDA in blood plasma decreased by 33% ($p < 0.001$), ARA value increased by 1.8 times ($p < 0.001$), and the activity of SOD rose by 46% ($p < 0.001$). Under the effect of *S. pallidum* extract, there was also an increase in the level of G-SH in liver tissue by 40% ($p < 0.001$), while the activity of antioxidant enzymes, GPx and GR, in blood plasma rose by an average of 38–54% ($p < 0.001$).

The state of the antioxidant system in animals of the groups 4 and 5 (mice treated with *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts under acute stress) was characterized by positive dynamics as well. However, the studied biochemical parameters still differed significantly from the control. At the same time, when compared with the group 2 (“stress”), it was recorded as follows: in blood plasma of mice of the groups 4 and 5, there was an increase in the level of ARA by 37% ($p < 0.001$)

and 54% ($p < 0.001$), respectively. The activity of SOD in animals of the group 4 (*U. lactuca*) rose by 51% ($p < 0.001$); in mice of the group 5 (*A. fastigiata* var. *tobuchiensis*), it rose by 36% ($p < 0.001$). Meanwhile, MDA in blood plasma of these animals decreased on average by 14–16% ($p < 0.001$). In terms of the level of G-SH in liver tissue and the activity of glutathione enzymes, there was positive dynamics as well. Specifically, the use of *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts was accompanied by an increase in G-SH content by 27 and 32%, respectively ($p < 0.05$). In its turn, the activity of GPx in animals in these groups rose on average by 22–27% ($p < 0.05$), and the activity of GR, by 12–20% ($p < 0.05$).

## DISCUSSION

From the results obtained, it follows that under conditions of acute stress, the complex functional balance of organs and systems of the whole organism is disturbed. This is evidenced by a drop in the relative mass of internal organs (liver and spleen). Interestingly, a significant decrease in the spleen mass index results from the involution of the lymphatic system, which is associated with increased secretion of steroid hormones by the adrenal cortex causing the breakdown of lymphocytes and inhibition of metabolic processes in cells [Chrousos, 2009]. The antioxidant defense system is stressed due to overproduction of free radicals under certain exposure [Şahın, Gümüşlü, 2007]. As a result, the antioxidant system of the body is incapable of coping with their excessive production, while the activity of antioxidant enzymes (SOD, GPx, and GR) and G-SH content decrease. This phenomenon underlies the violations of many metabolic reactions in the body. Evidence of increased generation of free radicals is a significant decrease in ARA value along with a rise in the level of MDA in blood plasma of mice; it is characterized by high activity of peroxidation of fatty acids making up membrane lipids and is accompanied by a rise in the permeability of cell membranes in various tissues [Şahın, Gümüşlü, 2007]. Subsequently, the lack of antioxidant defense factors leads to an uncontrolled increase in lipid peroxidation processes and to development of oxidative stress.

The administration of the seaweed extracts against the backdrop of stress was accompanied by a rise in the activity of antioxidant enzymes and G-SH content with a simultaneous drop in the level of MDA. However, in the groups 4 and 5, as it was noted earlier, the values of the antioxidant defense indices (MDA, G-SH, GPx, and GR) still differed from the control ones. At the same time, the values of the antioxidant defense indices in mice treated with *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts were inferior to those in animals of the group 3 (*S. pallidum*). This fact is confirmed by the calculation of statistical significance between the values of the studied biochemical parameters in blood plasma and liver tissue of mice of the groups 3–5. Thus, the values of the activity of GPx and GR for blood plasma in animals treated with *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts (the groups 4 and 5, respectively) were lower on average by 17–21% ($p < 0.05$) compared with the values in the group 3 (*S. pallidum* extract). Significant differences between these groups were also revealed for other indices: the level of MDA was higher by 27–28% ($p < 0.01$); G-SH content was lower by 7–9% ($p < 0.05$); and ARA was lower by 13–23% ($p < 0.001$).

In our opinion, this effect is driven by the fact that metabolic activity of polyphenols in the extract of the brown alga *S. pallidum* is noticeably higher than in the extracts of *U. lactuca* and *A. fastigiata* var. *tobuchiensis*. Accordingly, *S. pallidum* extract has a higher level of ARA, and this is confirmed by the data obtained (see Table 1). As known, the main components of the polyphenolic fractions of green and red algae are monomeric flavonoids [Alagan et al., 2017; de Quirós et al., 2010]. In turn, high molecular weight phlorotannins of brown algae and their extracts enriched with phlorotannins exhibit high antioxidant activity [Ferreres et al., 2012; Wang et al., 2012], in contrast to low molecular weight polyphenolic fractions of green and red algae. According to the researchers [Agregán et al., 2018], seaweed extracts with high content of polyphenolic compounds have a pronounced antioxidant potential.

Based on the data obtained, it can be concluded as follows: under stress, the administration of seaweed extracts contributed to the restoration of the indices of the antioxidant defense system, which plays a key role in the course of most vital processes.

**Conclusions:**

1. In mice, under conditions of experimental acute stress, a violation of metabolic reactions of the body was registered, which was accompanied by involution of the lymphatic system, appearance of ulcerations of the gastric mucosa, decrease in the weight of internal organs, stress in the antioxidant defense system, and activation of lipid peroxidation reactions.
2. The administration of seaweed extracts contributed to the stabilization of the antioxidant defense system, which is involved in the course of most vital processes.
3. A prophylactic use of seaweed extracts enriched with polyphenolic compounds under stress conditions contributed to the restoration of weight coefficients of internal organs of animals (liver and spleen) and the absence of ulcerations of the gastric mucosa.
4. Seaweeds *Sargassum pallidum*, *Ulva lactuca*, and *Ahnfeltia fastigiata* var. *tobuchiensis* are a promising raw material for the production of drugs capable of increasing the potential of the endogenous antioxidant defense system of the body under conditions of stress-induced disorders.
5. The predominant effect of the extract of the brown alga *S. pallidum* under stress is determined by the high-molecular structure of phlorotannins, which provides higher antioxidant activity compared to that of monomeric flavonoids in *U. lactuca* and *A. fastigiata* var. *tobuchiensis* extracts.

*This work was carried out within the framework of the state research assignment of V. I. Il'ichev Pacific Oceanological Institute FEB RAS "Ecological and biogeochemical processes in marine ecosystems: The role of natural and anthropogenic factors" (0211-2021-0014, registration number 121-21500052-9).*

## REFERENCES

1. Bogolitsyn K. G., Druzhinina A. S., Ovchinnikov D. V., Kaplitsyn P. A., Shulgin E. V., Parshina A. E. Polyphenols of brown algae. *Khimiya rastitel'nogo syr'ya*, 2018, no. 3, pp. 5–21. (in Russ.). https://doi.org/10.14258/jcprm.2018031898
2. Vengerovsky A. I., Markova I. V., Saratikov A. S. Doklinicheskoe izuchenie gepatozashchitnykh sredstv. *Vedomosti farmakologicheskogo komiteta*, 1999, no. 2, pp. 9–12. (in Russ.)
3. Imbs T. I., Zvyagintseva T. N. Phlorotannins are polyphenolic metabolites of brown algae. *Biologiya morya*, 2018, vol. 44, no. 4, pp. 217–227. (in Russ.). https://doi.org/10.1134/S0134347518040010
4. Kushnerova N. F., Sprygin V. G., Fomenko S. Ye., Rakhmanin Yu. A. Impact of stress on hepatic lipid and carbohydrate metabolism, prevention. *Gigiena i sanitariya*, 2005, no. 5, pp. 17–21. (in Russ.)
5. Kushnerova N. F., Fomenko S. E., Sprygin V. G., Momot T. V. The effects of the lipid complex of extract from the marine red alga *Ahnfeltia tobuchiensis* (Kanno et Matsubara) Makienko on the biochemical parameters of blood plasma and erythrocyte membranes during experimental

stress exposure. *Biologiya morya*, 2020, vol. 46, no. 4, pp. 269–276. (in Russ.). https://doi.org/10.31857/S0134347520040051

6. Sprygin V. G., Kushnerova N. F., Fomenko S. E., Sizova L. A., Momot T. V. The hepatoprotective properties of an extract from the brown alga *Saccharina japonica*. *Biologiya morya*, 2013, vol. 39, no. 1, pp. 50–54. (in Russ.)
7. Sprygin V. G., Kushnerova N. F., Fomenko S. E., Drugova E. S., Lesnikova L. N., Merzlyakov V. Y., Momot T. V. The influence of an extract from the marine brown alga *Sargassum pallidum* on the metabolic reactions in the liver under experimental toxic hepatitis. *Biologiya morya*, 2017, vol. 43, no. 6, pp. 444–449. (in Russ.)
8. Fomenko S. E., Kushnerova N. F., Sprygin V. G., Drugova E. S., Lesnikova L. N., Merzlyakov V. Yu. Lipid composition and membranoprotective action of extract from marine green algae *Ulva lactuca* (L.). *Khimiya rastitel'nogo syr'ya*, 2019, no. 3, pp. 41–51. (in Russ.). https://doi.org/10.14258/jcprm.2019035116
9. Agregán R., Munekata P. E. S., Franco D., Carballo J., Barba F. J., Lorenzo J. M. Antioxidant potential of extracts obtained from macro- (*Ascophyllum nodosum*, *Fucus vesiculosus* and *Bifurcaria bifurcata*) and micro-algae (*Chlorella vulgaris* and *Spirulina platensis*) assisted by ultrasound. *Medicines*, 2018, vol. 5, iss. 2, art. no. 33 (9 p.). https://doi.org/10.3390/medicines5020033
10. Alagan V. T., Valsala R. N., Rajesh K. D. Bioactive chemical constituent analysis, *in vitro* antioxidant and antimicrobial activity of whole plant methanol extracts of *Ulva lactuca* Linn. *British Journal of Pharmaceutical Research*, 2017, vol. 15, no. 1, pp. 1–14. https://doi.org/10.9734/BJPR/2017/31818
11. Bartosz G., Janaszewska A., Ertel D., Bartosz M. Simple determination of peroxyl radical-trapping capacity. *Biochemistry and Molecular Biology International*, 1998, vol. 46, iss. 3, pp. 519–528. https://doi.org/10.1080/15216549800204042
12. Buege J. A., Aust S. D. Microsomal lipid peroxidation. In: *Biomembranes. Part C, Biological Oxidants, Microsomal, Cytochrome P-450, and Other Hemoprotein Systems* / F. Sidney, P. Lester (Eds). New York : Academic Press, 1978, pp. 302–310. (Methods in Enzymology ; vol. 52). https://doi.org/10.1016/s0076-6879(78)52032-6
13. Burk R. F., Lawrence R. A., Lane J. M. Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration: Effect of selenium deficiency. *The Journal of Clinical Investigation*, 1980, vol. 65, iss. 5, pp. 1024–1031. https://doi.org/10.1172/JCI109754
14. Chrousos G. P. Stress and disorders of the stress system. *Nature Reviews Endocrinology*, 2009, no. 5, pp. 374–381. https://doi.org/10.1038/nrendo.2009.106
15. Cotas J., Leandro A., Monteiro P., Pacheco D., Figueirinha A., Gonçalves A. M. M., da Silva G. J., Pereira L. Seaweed phenolics: From extraction to applications. *Marine Drugs*, 2020, vol. 18, iss. 8, pp. 384–431. https://doi.org/10.3390/md18080384
16. de Quirós A. R.-B., Lage-Yusty M. A., López-Hernández J. Determination of phenolic compounds in macroalgae for human consumption. *Food Chemistry*, 2010, vol. 121, iss. 2, pp. 634–638. https://doi.org/10.1016/j.foodchem.2009.12.078
17. Ellman G. L. Tissue sulfhydryl group. *Archives of Biochemistry and Biophysics*, 1959, vol. 82, iss. 1, pp. 70–77. https://doi.org/10.1016/0003-9861(59)90090-6

18. *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.* Strasbourg : Council of Europe, 1986, 11 p. (European Treaty Series ; no. 123). URL: https://rm.coe.int/168007a67b [accessed: 28.12.2021].
19. Ferreres F., Lopes G., Gil-Izquierdo A., Andrade P. B., Sousa C., Mouga T., Valentão P. Phlorotannin extracts from Fucales characterized by HPLC-DAD-ESI-MS*n*: Approaches to hyaluronidase inhibitory capacity and antioxidant properties. *Marine Drugs*, 2012, vol. 10, iss. 12, pp. 2766–2781. https://doi.org/10.3390/md10122766
20. Goldberg D. M., Spoone R. J. Assay of glutathione reductase. In: *Methods of Enzymatic Analysis.* Vol. 3: Enzymes 1. Oxidoreductases, transferases. 3rd edition / H. U. Bergmeyer (Ed.). Weinheim : Verlag Chemie, 1983, pp. 258–265.
21. Manach C., Scalbert A., Morand C., Rémésy C., Jiménez L. Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition*, 2004, vol. 79, iss. 5, pp. 727–747. https://doi.org/10.1093/ajcn/79.5.727
22. Michalak I., Chojnacka K. Algae as production systems of bioactive compounds. *Engineering in Life Sciences*, 2015, vol. 15, iss. 2, pp. 160–176. https://doi.org/10.1002/elsc.201400191
23. Paoletti F., Aldinucci D., Mocali A., Caparrini A. A sensitive spectrophotometric method for the determination of superoxide-dismutase activity in tissue extracts. *Analytical Biochemistry*, 1986, vol. 154, iss. 2, pp. 536–541. https://doi.org/10.1016/0003-2697(86)90026-6
24. Parys S., Rosenbaum A., Kehraus S., Reher G., Glombitza K.-W., König G. M. Evaluation of quantitative methods for the determination of polyphenols in algal extracts. *Journal of Natural Products*, 2007, vol. 70, iss. 12, pp. 1865–1870. https://doi.org/10.1021/np070302f
25. Pradhan B., Patra S., Behera C., Nayak R., Jit B. P., Ragusa A., Jena M. Preliminary investigation of the antioxidant, anti-diabetic, and anti-inflammatory activity of *Enteromorpha intestinalis* extracts. *Molecules*, 2021, vol. 26, iss. 4, pp. 1171–1187. https://doi.org/10.3390/molecules26041171
26. Ragan M. A., Glombitza K. W. Phlorotannins, brown algal polyphenols. In: *Progress in Phycological Research*. Bristol : Biopress Ltd, 1986, vol. 4, pp. 129–241.
27. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 1999, vol. 26, iss. 9–10, pp. 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3
28. Şahın E., Gümüşlü S. Stress-dependent induction of protein oxidation, lipid peroxidation and anti-oxidants in peripheral tissues of rats: Comparison of three stress models (immobilization, cold and immobilization–cold). *Clinical and Experimental Pharmacology and Physiology*, 2007, vol. 34, iss. 5–6, pp. 425–431. https://doi.org/10.1111/j.1440-1681.2007.04584.x
29. Shibata T., Kawaguchi S., Hama Y., Inagaki M., Yamaguchi K., Nakamura T. Local and chemical distribution of phlorotannins in brown algae. *Journal of Applied Phycology*, 2004, vol. 16, pp. 291–296. https://doi.org/10.1023/B:JAPH.0000047781.24993.0a
30. Skriptsova A. V., Zhigadlova G. G. A revision of the red algal genus *Ahnfeltia* on the Russian coast of the North Pacific. *Phycologia*, 2022, vol. 61, iss. 4, pp. 396–402. https://doi.org/10.1080/00318884.2022.2061154

31. Wang T., Jónsdóttir R., Liu H., Gu L., Kristinsson H. G., Raghavan S., Ólafsdóttir G. Antioxidant capacities of phlorotannins extracted from the brown algae *Fucus vesiculosus*. *Journal of Agricultural and Food Chemistry*, 2012, vol. 60, iss. 23, pp. 5874–5883. https://doi.org/10.1021/jf3003653
32. Zhong B., Robinson N. A., Warner R. D., Barrow C. J., Dunshea F. R., Suleria H. A. R. LC-ESI-QTOF-MS/MS characterization of seaweed phenolics and their antioxidant potential. *Marine Drugs*, 2020, vol. 18, iss. 6, pp. 331–352. https://doi.org/10.3390/md18060331

# ОЦЕНКА АНТИОКСИДАНТНОЙ АКТИВНОСТИ ЭКСТРАКТОВ ИЗ МОРСКИХ ВОДОРОСЛЕЙ ЯПОНСКОГО МОРЯ *IN VITRO* И *IN VIVO*

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Морские водоросли являются источником важных биологически активных соединений — липидов, аминокислот, фенолов, полисахаридов и др. Перспективную группу веществ морского происхождения составляют полифенольные соединения, обладающие высокой антиоксидантной активностью, которые играют ключевую роль в жизнедеятельности морских макрофитов, что позволяет им быстро реагировать на внешний стресс и выполнять защитные функции. В то же время многокомпонентный состав фенольной фракции экстракта из водорослей обусловливает широкий спектр её фармакологической активности, включающей регулирующее влияние на многочисленные нарушения гомеостаза при патологических процессах в организме животных и человека. При этом имеющиеся возможности практического использования экстрактов из водорослей ещё не исчерпаны, что представляет несомненный интерес для современной науки. Цель работы — выполнить сравнительную оценку антиоксидантной активности водно-спиртовых экстрактов, выделенных из талломов представителей трёх классов водорослей [бурых (*Sargassum pallidum*), зелёных (*Ulva lactuca*) и красных (*Ahnfeltia fastigiata* var. *tobuchiensis*)], а также проанализировать их влияние на показатели антиоксидантной защиты печени и плазмы крови мышей при экспериментальном стрессе. Водоросли собирали в летние месяцы в прибрежных водах залива Петра Великого Японского моря, затем сушили при температуре около +50 °С, измельчали на лабораторной мельнице до частиц размером 0,5–1 мм и экстрагировали 70%-ным этиловым спиртом методом реперколяции. Наибольшее количество полифенолов отмечено в экстракте бурой водоросли *S. pallidum* — (218,2 ± 20,3) мг-экв ГК$\cdot$г$^{-1}$ сухого веса. В экстракте зелёной водоросли *U. lactuca* значение этого показателя составляло (16,2 ± 1,8) мг-экв ГК$\cdot$г$^{-1}$ сухого веса, в экстракте красной водоросли *A. fastigiata* var. *tobuchiensis* — (9,1 ± 1,6) мг-экв ГК$\cdot$г$^{-1}$ сухого веса. Соответственно, антирадикальная активность экстракта *S. pallidum* по отношению к катион-радикалу 2,2'-азино-бис(3-этилбензотиазолин-6-сульфоновой кислоты) (ABTS$^+$) и алкилпероксильному радикалу была существенно выше, чем таковая экстрактов *U. lactuca* и *A. fastigiata* var. *tobuchiensis*. Проведена экспериментальная проверка с целью определить влияние исследуемых экстрактов водорослей на показатели антиоксидантной защиты печени и плазмы мышей в условиях острого стресса. В задачи эксперимента входило установление весовых показателей (вес животных, индекс массы внутренних органов) и биохимических параметров (уровень антирадикальной активности, содержание малонового диальдегида и восстановленного глутатиона, активность антиоксидантных ферментов). Эксперимент по стрессовому воздействию проводили на белых беспородных мышах-самцах массой 20–30 г. Острый стресс моделировали путём вертикальной фиксации животных за дорсальную шейную складку на 24 ч. Освобождённые от спирта экстракты водорослей вводили в виде водной взвеси в дозе

100 мг общих полифенолов на кг массы тела в желудок мышам через зонд дважды — непосредственно перед вертикальной фиксацией и спустя 6 ч. Животным контрольной группы и группы «стресс» вводили дистиллированную воду в объёме, равном объёму вводимых препаратов. В данной модели проявились все атрибуты стресса: гипертрофия надпочечников, инволюция тимуса и селезёнки, изъязвления слизистой желудка и кишечника. Также были отмечены нарушения системы антиоксидантной защиты, которые выражались в снижении активности антиоксидантных ферментов в плазме крови, уменьшении содержания восстановленного глутатиона в печени и увеличении уровня малонового диальдегида. Под действием экстрактов во всех группах животных на фоне стресса прослежена тенденция к стабилизации исследуемых показателей антиоксидантной защиты. При этом показатели у мышей, получавших экстракты из *U. lactuca* и *A. fastigiata* var. *tobuchiensis*, уступали аналогичным параметрам в группе животных, получавших экстракт *S. pallidum*. В группе животных, получавших экстракт *S. pallidum*, в показателях антиоксидантной защиты не было выявлено достоверных отличий от контрольных значений. Данный факт обусловлен тем, что основными компонентами полифенольных фракций зелёных и красных водорослей являются мономерные флавоноиды, тогда как в бурых водорослях присутствуют высокомолекулярные флоротаннины, которые проявляют более высокую антиоксидантную активность, чем низкомолекулярные полифенольные фракции зелёных и красных водорослей.

**Ключевые слова:** морские водоросли, полифенолы, антиоксидантная активность, стресс, мыши

Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 2, pp. 104–106
https://marine-biology.ru

NOTES

UDC [597.556.31-14+575.21](262.5)

# VARIABILITY OF THE BLACK SCORPIONFISH, *SCORPAENA PORCUS* LINNAEUS, 1758 (SCORPAENIDAE), FROM TWO BLACK SEA LOCALITIES

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Received by the Editor 19.10.2020; after reviewing 22.03.2022;
accepted for publication 16.02.2023; published online 31.05.2023.

Comparative investigation of morphological variability of the black scorpionfish, *Scorpaena porcus* Linnaeus, 1758, from two distant Black Sea localities – Foros village (Crimean Peninsula) and Malyi Utrish village (Krasnodar Krai) – was carried out. Due to sexual dimorphism in the black scorpionfish and small abundance of males in the samples, only mature females were analyzed. A total of 54 individuals were examined (29 from Foros and 25 from Malyi Utrish). We used 5 meristic and 26 morphometric characters. In the meristic characters, there were no region-related differences between black scorpionfish females from Foros and Malyi Utrish; in the morphometric characters (maximum body height, length of the first dorsal fin, distance between pectoral and abdominal fin, length of snout, eye diameter, and length of lower jaw), the differences were statistically significant. The canonical discriminant analysis showed that *S. porcus* females from Foros were correctly classified with an accuracy of 97%, while females from Malyi Utrish – with an accuracy of 100%. It was suggested that the obtained results are a manifestation of modification variability. However, there were no significant differences between black scorpionfish females from two localities in the total length and body mass, which could result from differences in trophic conditions and fish abundance. This may indicate the existence of a complex of modification and interpopulation variability in *S. porcus* from the investigated Black Sea localities. Though black scorpionfish pelagic eggs can be transported *via* sea currents, spatial isolation and limited migrations may lead to the formation of local populations of *S. porcus*. Nevertheless, analysis of genetic markers is required to test the hypothesis.

**Keywords:** *Scorpaena porcus*, morphological variability, interpopulation variability, isolation, Black Sea

The aim of the study is to carry out a comparative morphological analysis of the black scorpionfish *Scorpaena porcus* Linnaeus, 1758 from two distant Black Sea localities – the coastal zone off Foros village (Crimean Peninsula) and the coastal zone off Malyi Utrish village (Krasnodar Krai).

*S. porcus* were caught with hook fishing gear (spinning rods with different equipment) in the coastal zone off Foros village (44°38′82.88″N, 33°78′17.94″E) in July–August 2019 and in the vicinity of Malyi Utrish village (44°70′48.25″N, 37°47′04.52″E) in September 2019. Due to small abundance of males in the samples and possible sexual dimorphism in size characteristics [Peskov, Manilo, 2016], only females of the black scorpionfish were examined.

Morphometric measurements were carried out according to the scheme by I. Pravdin [1966], with 5 meristic and 26 morphometric characters being used. Body mass (m) was registered as well.

## RESULTS

In the samples, the absolute length of females ranged from 14 to 26 cm; mass, from 46 to 304 g. The studied individuals of the black scorpionfish from the Foros and Malyi Utrish villages did not differ in meristic characters, while the differences in several morphometric characters were significant (Table 1). Interestingly, in all cases, the mean values of the characters were higher in fish caught in the coastal zone off Malyi Utrish village than in *S. porcus* caught in the coastal zone off Foros village.

**Table 1.** Significantly different measurement means, standard deviations, and *t*-test results for *Scorpaena porcus* females from two Black Sea localities

| Character | Locality | | | | | | *t*-test |
|---|---|---|---|---|---|---|---|
| | Foros (*n* = 29) | | | Malyi Utrish (*n* = 25) | | | |
| | Mean | Standard deviation | Standard error | Mean | Standard deviation | Standard error | |
| gh | 4.56 | 0.89 | 0.17 | 5.13 | 0.73 | 0.15 | 2.56 |
| tu | 2.15 | 0.35 | 0.06 | 3.12 | 1.61 | 0.32 | 3.17 |
| vz | 1.99 | 0.46 | 0.09 | 2.27 | 0.39 | 0.08 | 2.37 |
| an | 1.39 | 0.31 | 0.06 | 1.62 | 0.23 | 0.05 | 3.04 |
| np | 1.37 | 0.18 | 0.03 | 1.53 | 0.16 | 0.03 | 3.26 |
| k111 | 1.99 | 0.38 | 0.07 | 2.24 | 0.36 | 0.07 | 2.53 |

**Note:** gh, maximum body height; tu, maximum height of dorsal fin; vz, distance between pectoral and abdominal fin; an, length of snout; np, eye diameter; k111, length of lower jaw.

Moreover, according to the results of the discriminant analysis, which was carried out for the entire complex of morphometric characters, we obtained 98% of correct classifications of the black scorpionfish individuals by the localities. At the same time, 28 fish caught off the coast of Foros were in their own group (97%); only 1 *S. porcus* was classified by the value of the discriminant function with the individuals caught off the coast of Malyi Utrish. As for the individuals from Malyi Utrish, all 25 females (100%) were classified in their own group by the values of the discriminant function.

It is worth noting that observed high degree of discrimination of the black scorpionfish from Foros and Malyi Utrish villages was provided by the characters, the differences between the mean values of which were statistically significant. It cannot be ruled out that this is a manifestation of modification variability. However, it should be noted as follows: according to our data, there are no statistically significant differences in the body length and mass of female *S. porcus* from the coastal zones of Foros and Malyi Utrish, which could primarily be related to differences in trophic conditions and species population density in these localities.

*S. porcus* is known to lead a sedentary lifestyle [Smirnov, 1986]. Despite the presence of pelagic eggs in this species [Smirnov, 1986], which can be transported *via* sea currents, we believe that spatial isolation can play a significant role in the formation of local populations of the black scorpionfish. This is partly confirmed by the morphological differences revealed. Apparently, in this case, we deal with a combination of modification and interpopulation variability. However, to verify this assumption, analysis of genetic markers is required.

*This work was carried out within the framework of IBSS state research assignment "Fundamental studies of population biology of marine animals, their morphological and genetic diversity" (No. 121040500247-0).*

## REFERENCES

1. Pravdin I. F. *Rukovodstvo po izucheniyu ryb.* Moscow : Pishchevaya promyshlennost', 1966, 370 p. (in Russ.)
2. Smirnov A. I. *Fauna Ukrainy.* Vol. 8. *Ryby.* Iss. 5. *Okuneobraznye (bychkovidnye), skorpenoobraznye, kambaloobraznye, prisoskoperoobraznye, udil'shchikoobraznye.* Kyiv : Naukova dumka, 1986, 320 p. (in Russ.)
3. Peskov V. N., Manilo L. G. Sex differences and sex identification in the small-scaled scorpionfish, *Scorpaena porcus* (Scorpaenidae, Scorpaeniformes). *Vestnik zoologii*, 2016, vol. 50, no. 4, pp. 355–362.

# ИЗМЕНЧИВОСТЬ МОРСКОГО ЕРША *SCORPAENA PORCUS* LINNAEUS, 1758 (SCORPAENIDAE) ИЗ ДВУХ МЕСТООБИТАНИЙ В ЧЁРНОМ МОРЕ

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Проведено сравнительное исследование морфологической изменчивости скорпены *Scorpaena porcus* Linnaeus, 1758 из двух отдалённых черноморских местообитаний — посёлков Форос (Крымский полуостров) и Малый Утриш (Краснодарский край). Из-за полового диморфизма у морского ерша и малого количества самцов в пробах для анализа использовали только половозрелых самок. Всего обследовано 54 скорпены (29 из Фороса и 25 из Малого Утриша). Использованы 5 меристических и 26 морфометрических признаков. По меристическим признакам региональные различия между самками скорпены из Фороса и Малого Утриша не отмечены, тогда как по морфометрическим (максимальная высота тела, длина первого спинного плавника, расстояние между грудными и брюшными плавниками, длина рыла, диаметр глаза и длина нижней челюсти) различия были статистически значимы. Канонический дискриминантный анализ показал, что самки *S. porcus* из Фороса правильно классифицируются с точностью 97 %, а самки из Малого Утриша — 100 %. Высказано предположение, что полученные результаты являются проявлением модификационной изменчивости. Между тем существенных различий между самками морского ерша из двух местонахождений по общей длине и массе тела не зарегистрировано, что могло быть обусловлено различиями в трофических условиях и в численности рыб. Это обстоятельство может свидетельствовать о наличии комплекса модификационной и межпопуляционной изменчивости у скорпены из исследованных черноморских биотопов. Несмотря на то, что пелагическая икра *S. porcus* может переноситься морскими течениями, пространственная изоляция и ограниченность миграций способны привести к формированию локальных популяций морского ерша. Однако для проверки предположения необходим анализ генетических маркеров.

**Ключевые слова:** *Scorpaena porcus*, морфологическая изменчивость, межпопуляционная изменчивость, изоляция, Чёрное море

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 2, pp. 107–110
https://marine-biology.ru

UDC 597.556.333.1(262.5)

# FINDING OF THE GOLDEN GOBY *GOBIUS XANTHOCEPHALUS* (GOBIIDAE) OFF THE SOUTHEASTERN COAST OF CRIMEA (THE BLACK SEA)

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Received by the Editor 08.02.2021; after reviewing 04.07.2021;
accepted for publication 16.02.2023;

Information is given on the finding of the golden goby *Gobius xanthocephalus* Heymer & Zander, 1992 (Gobiidae, Perciformes) off the southeastern coast of Crimea (the Black Sea), where this species was not previously recorded. An individual was registered during underwater ichthyological studies in the Dvuyakornaya Bay water area, in the vicinity of the northeastern tip of the Kiik-Atlama Peninsula (44°57′N, 35°23′E). The golden goby was recorded at a depth of 8.5 m at the foot of the underwater part of the rocky slope, in a shaded niche among a cluster of boulders. The finding of this species in the Southeastern Crimea indicates the expansion of its range in polyhaline water areas of the northern Black Sea.

**Keywords:** range, Dvuyakornaya Bay, Kiik-Atlama Peninsula

The golden goby *Gobius xanthocephalus* Heymer & Zander, 1992 (Gobiidae, Perciformes) is a representative of the East Atlantic–Mediterranean faunistic complex [Manilo, 2014; Manilo et al., 2013]. This fish is distributed in the Eastern Atlantic along the coast of Portugal and the Canary Islands. In the Mediterranean Sea, it is common off the coast of France [Manilo, 2014]. In the Black Sea, the golden goby was recorded for the first time in 1967 in the Kruglaya Bay water area (Sevastopol); there, 6 individuals were caught. Initially, this species was mistakenly identified as *Cabotia schmidti* de Buen, 1930; then, as *Gobius auratus* Risso, 1810 [Gordina, 1976]. Later, a reidentification showed that those individuals belong to the species *Gobius xanthocephalus* [Boltachev et al., 2009; Vasilyeva, Bogorodskii, 2004]. Subsequently, the golden goby was found in the Black Sea off the southeastern [Karpova, Saksaganskii, 2011] and western [Boltachev et al., 2009; Manilo et al., 2013] coast of Crimea, as well as off the coast of the Caucasus in the vicinity of Abkhazia [Vasilyeva, Bogorodskii, 2004]. In the waters of the Southeastern Crimea, this species has not been previously registered.

On 14 August, 2020, in the Dvuyakornaya Bay water area, in the vicinity of the northeastern tip of the Kiik-Atlama Peninsula (44°57′N, 35°23′E) (Fig. 1), during underwater ichthyological observations, we detected a single individual of this species. The golden goby was found at a depth of 8.5 m at the foot of the underwater part of the rocky slope, in a shaded niche among a cluster of boulders. The inner area of the niche was covered with sand and shells of large mussels; on the periphery, there were boulders with sparse vegetation.

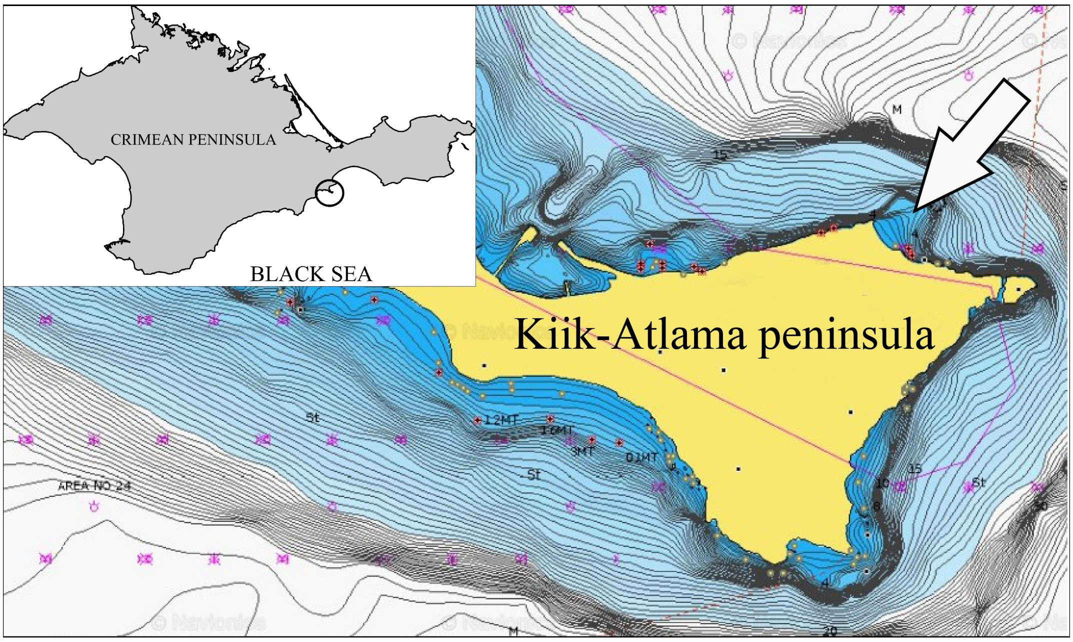

**Fig. 1.** Spot of finding (marked with →) of the golden goby *Gobius xanthocephalus* in the Southeastern Crimea

The golden goby is characterized by a bright color distinguishing it from other gobies in the area. This fact served as the basis for initial underwater species identification of the individual and for subsequent analysis of the photos taken. The general color background of the fish is pale yellow, lighter on the belly. Along the entire body, there are narrow longitudinal stripes formed by small, dotted, reddish spots. The head to its back is golden yellow. Two parallel red stripes run along the eyes: the first one along the upper vertical of the eye, while the second one through the pupil. There is a V-shaped strip from the anterior vertical of the eye to the middle of the upper jaw. The pectoral fins are transparent, with black spots at the base. On the dorsal fins, the spots form parallel dashed lines. The anal and caudal fins are bluish at their base (Fig. 2). In general, the coloration of the individual corresponds to that described earlier [Manilo, 2014; Vasil'eva, 2007; Villegas-Ríos, Bañón, 2010].

**Fig. 2.** The golden goby *Gobius xanthocephalus*; the Dvuyakornaya Bay, the Kiik-Atlama Peninsula (original photo by P. Donchik)

The fish stayed near the bottom and moved in short and sharp movements. While the diver was taking photos, the golden goby was very cautious not to let him get close. As the diver approached, the fish gradually disappeared into the inner area of the niche.

Based on the fact of the golden goby finding off the southeastern coast of Crimea, one can conclude on the expansion of the range of this species in polyhaline water areas of the northern Black Sea.

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

## REFERENCES

1. Boltachev A. R., Karpova E. P., Danilyuk O. N. Findings of new and rare fish species in the coastal zone of the Crimea (the Black Sea). *Voprosy ikhtiologii*, 2009, vol. 49, no. 3, pp. 318–332. (in Russ.)
2. Vasil'eva E. D. *Ryby Chernogo morya. Opredelitel' morskikh, solonovatovodnykh, evrigalinnykh i prokhodnykh vidov s tsvetnymi illyustratsiyami, sobrannymi S. V. Bogorodskim.* Moscow : VNIRO, 2007, 238 p. (in Russ.)
3. Vasilyeva E. D., Bogorodskii S. V. Two new species of gobies (Gobiidae) in the ichthyofauna of the Black Sea. *Voprosy ikhtiologii*, 2004, vol. 44, no. 5, pp. 599–606. (in Russ.)
4. Gordina A. D. Raspredelenie i sezonnye izmeneniya chislennosti vzroslykh ryb v zaroslevykh biotsenozakh Chernogo morya. *Biologiya morya*, 1976, iss. 39, pp. 78–92. (in Russ.). https://repository.marine-research.ru/handle/299011/2153
5. Karpova E. P., Saksaganskii V. V. Raspredelenie ryb semeistva bychkovykh (Gobiidae) u chernomorskogo poberezh'ya Kryma. In: *Suchasni problemy teoretychnoi ta praktychnoi ikhtiolohii* : tezy IV Mizhnar. ikhtiol. nauk.-prakt. konf., Odesa, 7–11 September, 2011. Odesa : Odes'k. nats. un-t im. I. I. Mecnikova, 2011, pp. 117–118. (in Russ.)
6. Manilo L. G. *Ryby semeistva Bychkovye (Perciformes, Gobiidae) morskikh i solonovatykh vod Ukrainy*. Kyiv : Naukova dumka, 2014, 243 p. (in Russ.)
7. Manilo L. G., Boltachev A. R., Karpova E. P. Gobiidae invasive species of the Crimean marine waters. *Zbirnyk prats Zoolohichnoho muzeiu*, 2013, no. 44, pp. 50–69. (in Russ.)
8. Villegas-Ríos D., Bañón R. First record and new meristic data of *Gobius xanthocephalus* (Gobiidae) from Galician waters (NW Spain). *Cybium*, 2010, vol. 34, no. 3, pp. 311–314. https://doi.org/10.26028/cybium/2010-343-010

# ОБНАРУЖЕНИЕ ЗОЛОТИСТОГО БЫЧКА *GOBIUS XANTHOCEPHALUS* (GOBIIDAE) В РАЙОНЕ ЮГО-ВОСТОЧНОГО ПОБЕРЕЖЬЯ КРЫМА (ЧЁРНОЕ МОРЕ)

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Приведены сведения об обнаружении золотистого бычка *Gobius xanthocephalus* Heymer & Zander, 1992 (Gobiidae, Perciformes) в районе юго-восточного побережья Крыма (Чёрное море), где ранее данный вид не находили. Особь этого вида отмечена при проведении подводных ихтиологических исследований в акватории Двуякорной бухты, у северо-восточной оконечности полуострова Киик-Атлама (44°57′ с. ш., 35°23′ в. д.). Бычок зарегистрирован на глубине 8,5 м у подножия подводной части скалистого склона, в затенённой нише среди скопления глыб. Обнаружение золотистого бычка в районе Юго-Восточного Крыма свидетельствует о расширении его ареала в полигалинных акваториях северной части Чёрного моря.

**Ключевые слова:** ареал, Двуякорная бухта, полуостров Киик-Атлама

1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 2, pp. 111–112
https://marine-biology.ru

CHRONICLE AND INFORMATION

# ON THE 90th ANNIVERSARY OF PROFESSOR OLEG MIRONOV (1933–2022)

9 March, 2023, marks the 90th anniversary of the birth of Oleg Mironov – a well-known hydrobiologist, D. Sc. (1971), Professor, and organizer of the first marine sanitary hydrobiology laboratory in the USSR (1964), which was later transformed into the department (1971).

Almost all his scientific activity was connected with the Sevastopol Biological Station (until 1963) and A. O. Kovalevsky Institute of Biology of the Southern Seas. He worked as a librarian at IBSS even *prior* to going to university. In 1952, he entered Kirov Military Medical Academy in Leningrad and began specializing in marine sanitary ecology. Thus, he carried out a range of scientific works aimed at solving medical and environmental issues for the Black Sea Fleet. When teaching at the general hygiene department at the Grodno State Medical Institute, O. Mironov started active research on the radioactivity levels of environmental objects in the Grodno vicinity. His studies on radioactivity were continued at IBSS. Here, he carried out a series of works on the biomigration of artificial radionuclides from sea to land (in particular, on strontium-90 and cesium-137 transport into human body by commercial hydrobionts).

In 1963, after defending his PhD thesis in medicine, he began working at IBSS of the Academy of Sciences of the Ukrainian SSR as a junior researcher. In 1964, IBSS scientific council decided to develop research on marine sanitary biology, and Oleg Mironov organized and headed the laboratory. In 1970, after defending his D. Sc. dissertation in biology, he launched experimental and field studies, the results of which are still of great practical and scientific interest.

He concentrated on investigating dangerous pollutants of seas and oceans – oil and oil products. By the mid-1970s, he substantiated the fundamental concept of *interaction of marine organisms and their communities with pollution as a part of the general natural process of matter transformation and energy transfer to the marine environment*. This concept determined the main research trends of IBSS marine sanitary biology department for decades. Moreover, it formed the basis of the international program on oil pollution biomonitoring in the Mediterranean basin. His investigations were of high relevance. This was confirmed by the fact that his team was involved in carrying out key state programs and projects, including “The World Ocean”, GIZM, “Environment”, and GESAMP (Joint group of experts on the scientific aspects of marine environmental protection). Our colleagues studied the water areas of the Black, Caspian, and Barents seas, *inter alia* certain spots of the Pacific Ocean.

O. Mironov was the first one to analyze the effect of oil and oil products on mass species of the Black Sea hydrobionts: representatives of phyto- and zooplankton, fish, and benthos. Under expeditionary conditions, new data were obtained on the patterns of distribution, abundance, biochemical characteristics, and species composition of oil-oxidizing microorganisms in the Black, Mediterranean, and Red seas, as well as in various areas of the Pacific, Atlantic, and Indian oceans. Extensive studies of the destructive activity of oil-oxidizing microflora made it possible to calculate the potential ability of the Black Sea to self-purify from oil pollution.

Oleg Mironov authored the experimentally substantiated concept of the purposeful use of marine organisms and their communities for the purification of oil-containing (polluted) seawater and for sanitation of coastal water areas. The concept was implemented: technical hydrobiological systems were placed in several anthropogenically stressed areas off Sevastopol. This repeatedly confirmed the applied significance of fundamental research of the marine sanitary hydrobiology department. Currently, scientific trends laid down by O. Mironov are developed, and the effect of oil and oil products on the environment is studied in the chemoecology laboratory of IBSS radiation and chemical biology department.

His contribution to the organization of long-term monitoring studies in Sevastopol coastal and marine areas is invaluable. In 1973, he was the first to organize complex chemical and biological surveys of almost all the city bays. The investigations are carried out till now.

The results of his research were summarized in several monographs edited by him: *Interaction Between Sea Organisms and Oil Hydrocarbons* (1985), *Sanitary-Biological Aspects of the Sevastopol Bays Ecology in XX Century* (2003), and *Sanitary-Biological Investigations in Coastal Area of Sevastopol Region* (2009). Those represent a unique and scientifically based *anthropogenic history* of the region. Oleg Mironov published more than 400 scientific papers. His works are widely recognized not only in Russia, but worldwide.

A talented scientist and an excellent organizer, Oleg Mironov created a school of marine sanitary hydrobiologists–ecologists. Under his scientific supervision, more than 20 PhD theses were defended, as well as 1 D. Sc. dissertation. He was an active science communicator – he repeatedly appeared on television and gave comments in other media on actual environmental problems of our city and country. His articles were published in several newspapers and popular science magazines: *Native Nature*, *Chemistry and Life*, *Nature*, *Man and the Element*, and so on.

The bright memory of a remarkable scientist and an amazing person will forever remain in the hearts of his students, colleagues, and friends.

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## К 90-ЛЕТНЕМУ ЮБИЛЕЮ ПРОФЕССОРА ОЛЕГА ГЛЕБОВИЧА МИРОНОВА (1933–2022)

9 марта 2023 г. исполнилось 90 лет со дня рождения Олега Глебовича Миронова — кандидата медицинских и доктора биологических наук, профессора, организатора первой в СССР лаборатории морской санитарной гидробиологии, а также автора более чем 400 публикаций.

Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 2, pp. 113–114
https://marine-biology.ru

# ON THE 80th ANNIVERSARY OF PROFESSOR YURIY TOKAREV

On 19 August, 2023, Yuriy Tokarev would have turned 80. He was deputy director for research at the Institute of Biology of the Southern Seas, scientific supervisor of IBSS, laureate of the State Prize of Ukraine in Science and Technology, Honored Worker of Science and Technology of the Republic of Crimea, D. Sc., Professor, and head of the biophysical ecology department.

Yu. Tokarev was born in the city of Poti (Georgian SSR) into a military family. In 1945, they moved to the city of Sevastopol. There, he entered school in 1950 and graduated with a silver medal in 1960. The same year, he became a student of the radio electronics faculty at the Sevastopol Branch of the Odessa Polytechnic Institute (since 1963, the Sevastopol Instrument Engineering Institute). In 1965, he graduated with honors and received the qualification of a radio engineer. In 1967, Yuriy Tokarev was invited to the bioluminescence laboratory of the plankton department at IBSS of the Academy of Sciences of the Ukrainian SSR to work as a senior engineer. In 1972, he was elected to the position of a junior researcher in this department; in 1976, he was appointed deputy director for general affairs. Since 2002, he headed the biophysical ecology department; since 2015, he was also scientific supervisor at our institute.

In 1990, Yu. Tokarev defended his PhD thesis, and in 2001, his D. Sc. dissertation. Both scientific works covered biophysical aspects of pelagic ecology.

He published over 250 scientific papers in prestigious domestic and foreign journals, as well as 8 monographs. He was involved in dozens of scientific projects, from regional to international ones, and headed some of them. His activities were marked by the award *For Scientific Achievements* of the National Academy of Sciences of Ukraine (2006) and the State Prize of Ukraine in Science and Technology (2007). Moreover, he was awarded the title of Honored Worker of Science and Technology of the Republic of Crimea (2011).

Since 1969, participating in numerous scientific expeditions on research vessels and making presentations at international conferences, forums, and symposiums, Yuriy Tokarev visited more than 50 countries. He worked in the Pacific, Indian, and Atlantic oceans as a head of a scientific group and expedition scientific secretary, deputy head, and head.

Yu. Tokarev proposed a new direction in marine research – biophysical ecology, *i. e.*, study of environmental issues using biophysical techniques. It is difficult to enumerate all the achievements of this scientist and the staff of his department in an important and promising direction of modern hydrobiology – in biophysical ecology of hydrobionts. The main ones are: establishing patterns of formation of the biological field structure at a small-scale level, which play a key role in increasing the efficiency of fishing; development of recommendations and new approaches for the assessment and rational use of the World Ocean biological resources; and elaboration of modern hydrobiological terminology and new ideas about non-trophic interactions of pelagic communities having great general biological and practical significance.

High demands on himself and others, adherence to principles in investigations, benevolence, erudition, intelligence, decency, outstanding talent as a scientist, teacher, and leader, and exceptional human qualities earned Yuriy Tokarev the well-deserved respect of his colleagues, graduate students, and junior researchers. Under his supervision, a number of PhD theses were defended, and several more works were prepared for defense. Being one of the most respected and experienced professors at IBSS, he taught a course in biophysics at the Sevastopol Technical University, was involved in various educational and pedagogical activities, and curated the Junior Academy of Sciences in Sevastopol.

Yu. Tokarev was a talented person with a subtle sense of humor and a profound knowledge in history, art, literature, music, and sports. He was very passionate about poetry and wrote himself; his poems always reflected an insatiable thirst for life, optimism, and love for everything beautiful.

## ПАМЯТИ УЧЁНОГО: К 80-ЛЕТИЮ СО ДНЯ РОЖДЕНИЯ ПРОФЕССОРА ЮРИЯ НИКОЛАЕВИЧА ТОКАРЕВА

19 августа 2023 г. исполнилось бы 80 лет заместителю директора по научной работе и заведующему отделом биофизической экологии ИнБЮМ Юрию Николаевичу Токареву. Он стал автором более чем 260 публикаций и был активным участником научных экспедиций.

ISSN 2499-9768
9 772499 976005 23002 >

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