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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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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.

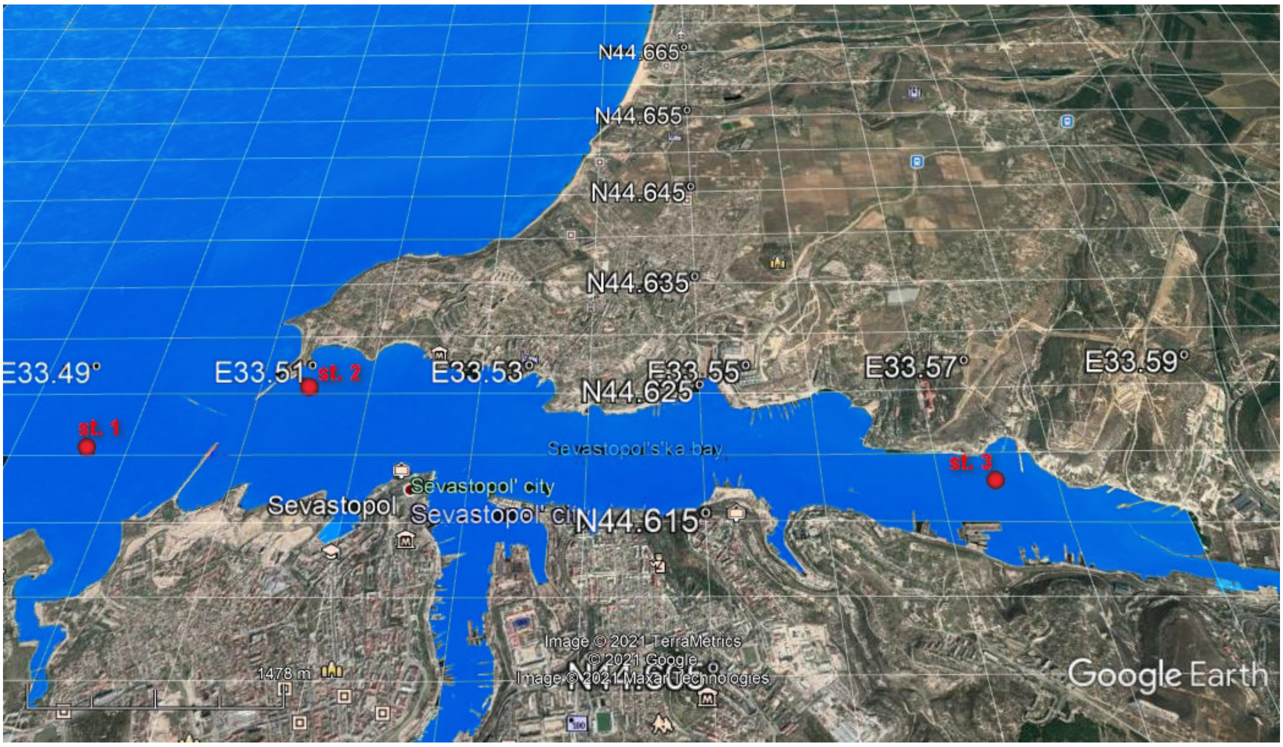

Fig. 1. Schematic map of sampling stations in Sevastopol coastal area: 1, sta. Vekha; 2, sta. Ravelin; 3, sta. Sukharnaya Bay

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):

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

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

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; $i = 1, 2…S$; 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.

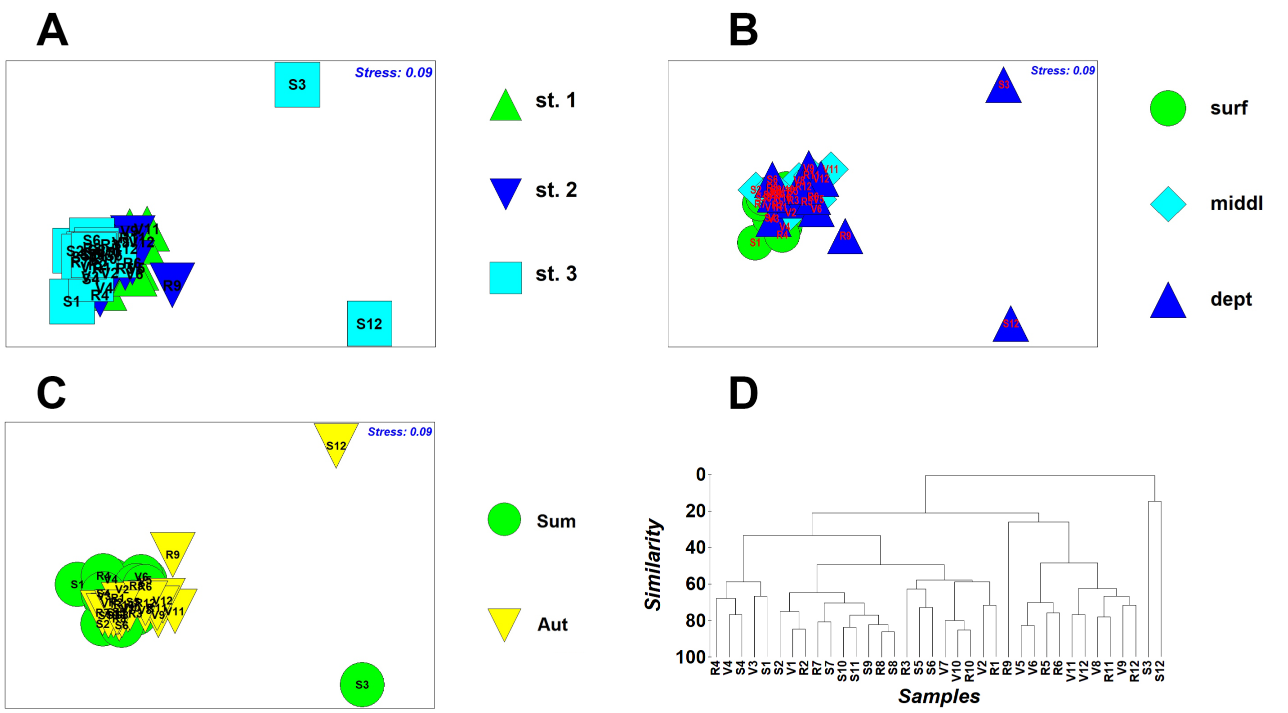

Fig. 2. Graphical (2D) result of MDS analysis of the structure of the metazoan microzooplankton community at different stations (A), at different depths (B), and depending on the season (C); diagram of cluster analysis of the entire data set (D): V, sta. 1; R, sta. 2; S, sta. 3. Numbers 1 to 12 are successive 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.

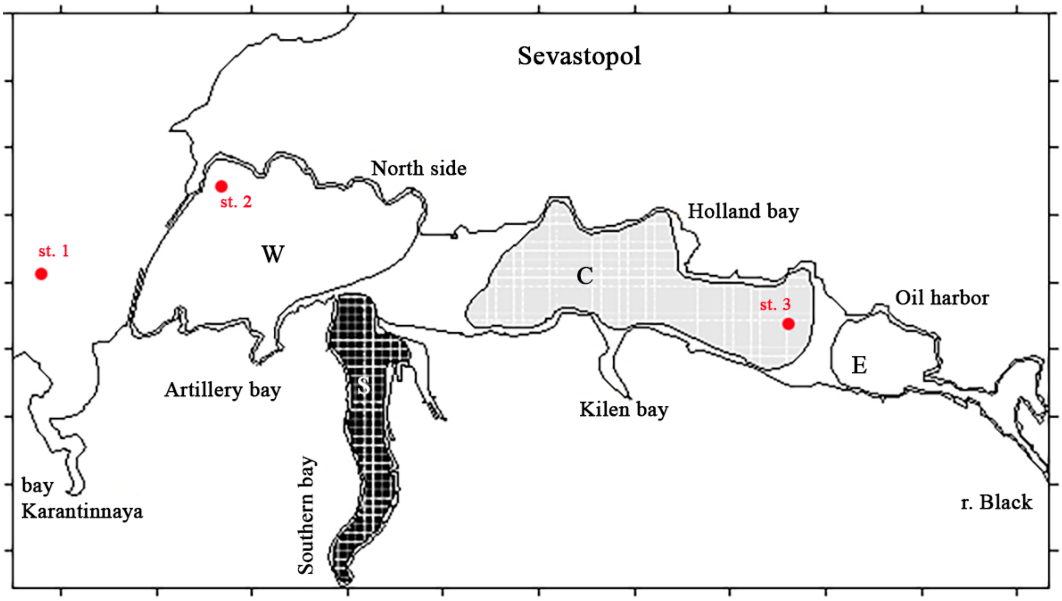

Fig. 3. Zoning of the Sevastopol Bay by the level of water pollution (according to [Ivanov et al., 2006]): areas of low (W), moderate (E), high (C), and very high (S) pollution. Our sampling stations are marked with red circles

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 310×10^3 to 330×10^3 ind.·m^{-3}. Fluctuations in MM abundance at the bay mouth in this layer were more significant: from 140×10^3 to 700×10^3 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.

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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