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

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

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

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

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

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

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

MATERIAL AND METHODS

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

tail-end areas (Ivanov et al., 2006 ; Ovsyanyi et al., 2000). The choice of sampling stations in our study corresponded to that zoning: st. 2, 5 were located in the zone of "weak" pollution, st. 3 – in the zone of "strong" one, and st. 4 – in the zone of "very strong" one (Fig. 1B).

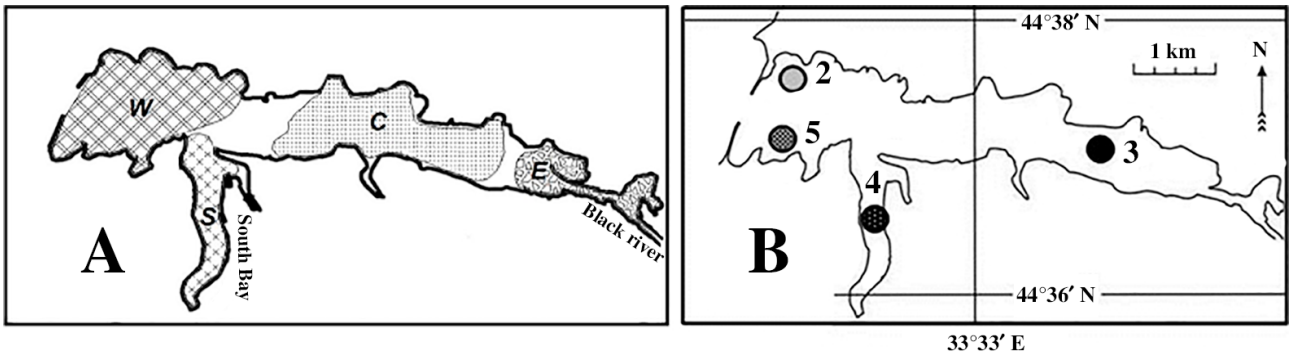

Fig. 1. Map of the Sevastopol Bay: A – zoning according to the distribution of hydrological and hydrochemical indicators [W – western area, zone of "weak" pollution; E – eastern area, "moderate" pollution; C – central area, "strong" pollution; S – southern area, "very strong" pollution (Ivanov et al.,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

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

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

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

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

of the cells, stained with acridine orange, was introduced. In the lack of data on the cell sizes, the biomass was determined by taking the carbon content in one bacterial cell equal to 20 fg ($2 \cdot 10^{-14}$ g C·cell^{-1}) (Lee & Furman, 1987).

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

RESULTS

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

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

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

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

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

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

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

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

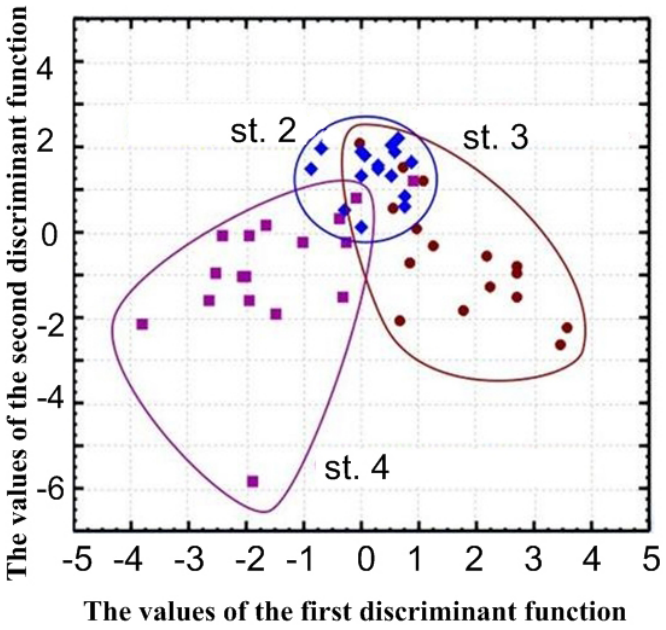

Fig. 2. Values of the first and second discriminant functions in the analysis of physical, hydrochemical, and biological variables (for more information, see the text) for stations 2, 3, and 4 in the Sevastopol Bay in 1998–1999. Sets of indicators for all the stations were significantly different ($p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

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

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

years (1998, 2002), water temperature in the bay reached maximum values (higher than +28 °C), and the density of microorganisms increased up to $8.1 \cdot 10^6$–$10 \cdot 10^6$ cells·mL^{-1}. The maximum variability in abundance was observed at st. 3 (Table 1).

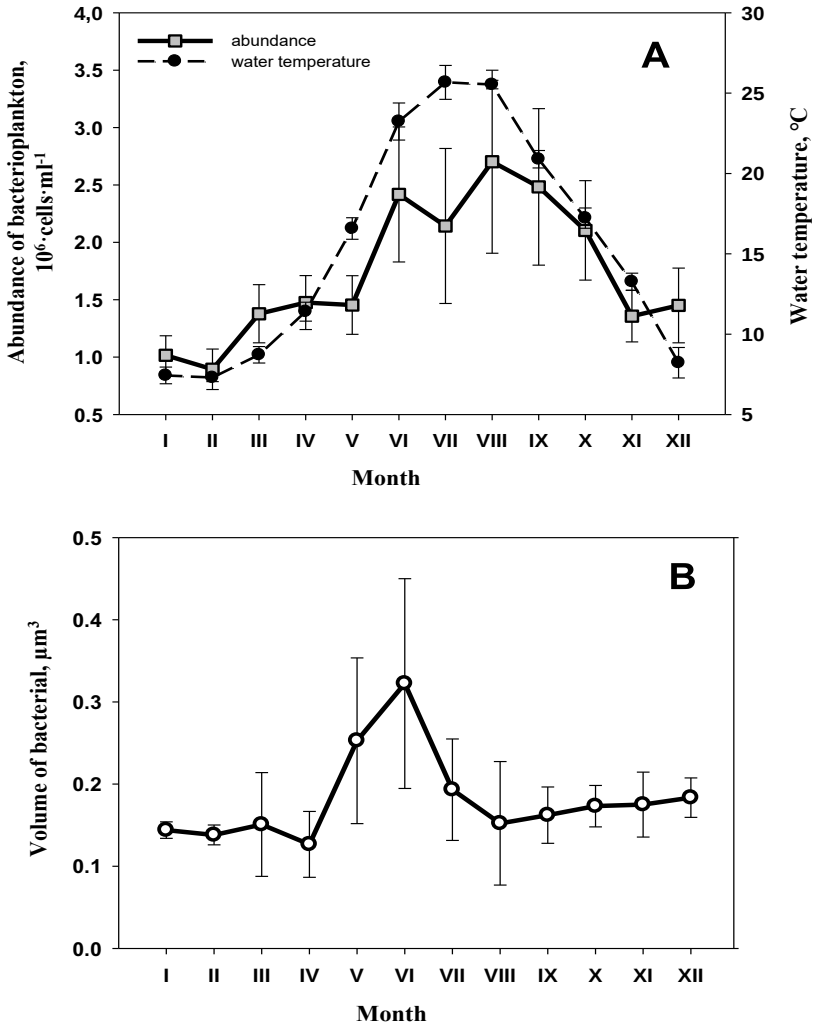

Fig. 3. Seasonal changes in bacterioplankton structural indicators, averaged for the entire water area of the Sevastopol Bay: A – bacterioplankton abundance (·10^6 cells·mL^{-1}) and water temperature (°C) (1992–2005); B – average bacterial cell volume (μm^3) (2004–2005). Data are presented with 95 %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

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

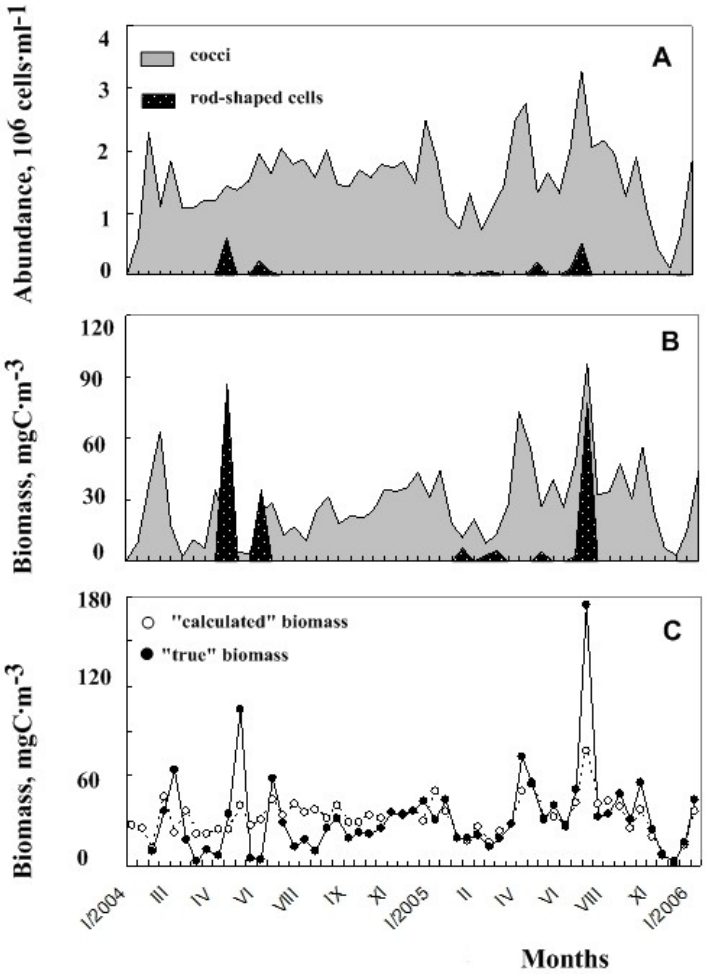

Fig. 4. Bacterioplankton quantitative indicators in the Sevastopol Bay (station 3, 2004–2005):
A – bacterioplankton abundance, considering two groups of cells (cocci and rod-shaped cells);
B – bacterioplankton biomass, considering two groups of cells;
C – ratio of the values of "true" bacterioplankton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

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

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

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

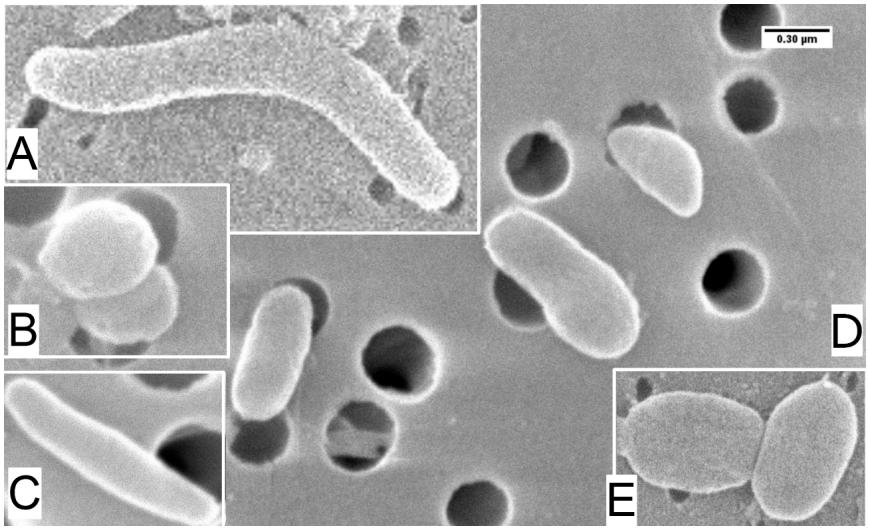

Fig. 5. Morphotypes of bacterioplankton cells of the Sevastopol Bay by electron microscopy:
A – convoluted forms (spirilla);
B – rounded forms (cocci);
C – rod-shaped forms (thin rods);
D and E – rod-shaped forms (thick rods)

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

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

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

DISCUSSION

In the coastal sea areas, experiencing maximum anthropogenic load (bays, lagoons, and estuaries), indicators of the total bacterioplankton abundance are characterized by high values with their pronounced variability, regardless of geographic location and climatic conditions (Kopylov & Kosolapov, 2011 ; Prakticheskaya ekologiya pribrezhnykh regionov…, 1990 ; Heidelberg et al., 2002). As a rule, at the end of unicellular algae bloom and their subsequent die-off, the bacterioplankton abundance

increases significantly (Myrzov et al., 1999). Its maximum values are recorded in summer and autumn months, when water temperature is high. With increasing distance from the coast, bacterial content decreases (Kopylov & Kosolapov, 2011 ; Prakticheskaya ekologiya pribrezhnykh regionov…, 1990 ; Heidelberg et al., 2002). For example, in the Chesapeake Bay (North America) (the estuary of the Susquehanna River, flowing into the Atlantic Ocean), abundance of microorganisms varied within the range of $1 \cdot 10^6$–$20 \cdot 10^6$ cells·mL^{-1} (Heidelberg et al., 2002). For the Gulf of Burgas (the western Black Sea), the density of the bacterial community was of $1 \cdot 10^6$–$12 \cdot 10^6$ cells·mL^{-1} (Prakticheskaya ekologiya pribrezhnykh regionov…, 1990). Close *N* values were recorded in the Gelendzhik and Anapa bays (the northeastern Black Sea): microorganisms' content in summer period reached $12.7 \cdot 10^6$ and $14.2 \cdot 10^6$ cells·mL^{-1}, respectively. These values correspond to the level of hypereutrophic water (Selifonova, 2015 ; Sorokin, 1973). For the estuaries of the Cananéia River (Brazil coast, the Atlantic Ocean) and the Elbe River (North Sea coast), the density of the planktonic microbial population was lower; the values corresponded to the levels of mesotrophic and eutrophic water: $0.2 \cdot 10^6$–$3.8 \cdot 10^6$ and $1.8 \cdot 10^6$–$4.8 \cdot 10^6$ cells·mL^{-1}, respectively (Barrera-Alba et al., 2009 ; Karrasch et al., 2003).

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

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

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

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

The main natural inhabitants of aquatic bacteriocenoses in marine areas are normally coccoid forms, which carry out the final stages of decomposition of more persistent organic compounds (Bogdanova, 2015 ; Ponomareva, 1978). Thus, in relatively low-contaminated areas of the Kola Bay, in bacterial communities gram-positive bacteria predominated, more often coccoid forms; in more eutrophic areas, rod-shaped bacteria prevailed (Bogdanova, 2015 ; Ponomareva, 1978). In the northeastern Black Sea (Golubaya Bay), small coccoid forms predominated; regardless of the season, their ratio was maximum

at coastal stations (up to 83 %) (Mosharova & Sazhin, 2007). Our data on the ratio of morphotypes (cocci prevalence, 69–96 %) and bacterial cell volume (0.02–0.27 μm^3 for cocci; 0.50–0.65 μm^3 for rods) in the Sevastopol Bay are consistent with the results of other authors. Predominance of coccoid forms in all the sites of the bay water area indirectly indicates its relatively stable state.

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

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

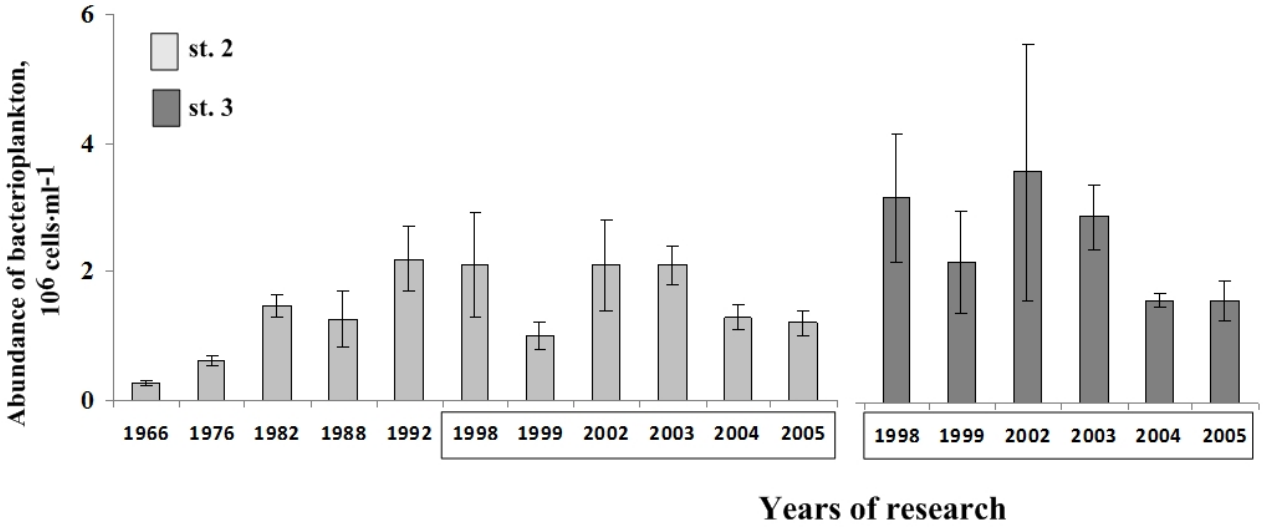

Fig. 6. Interannual dynamics of average annual bacterioplankton abundance in the open (st. 2) and tail-end areas (st. 3) of the Sevastopol Bay (data for 1966–1988 – according to (Gorbenko, 1977 ; Chepurnova et al., 1993 ; Shumakova, 1980); data for 1992 provided by V. A. Ponomarenko)

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

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

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

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

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

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

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

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

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

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

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

One of the main reasons for the decrease in the bacterioplankton content in marine and freshwater ecosystems is its grazing by ic flagellates and ciliates (Sherr et al., 1992). During the warm period of the year, high values of the phagotrophs abundance were noted in various coastal ecosystems; the ranges of their annual fluctuations can be 6–10 times or more, while seasonal values of the bacterial abundance are more static (Tsai et al., 2013). The effect of consumers on the development

of the microbial community can be confirmed by a change in the size structure of bacteria (Golubkov, 2013). Experiments have shown that ciliates and colorless flagellates prefer large bacteria, which are usually actively growing cells or dividing ones. Thus, the bacterial community, with active grazing by phagotrophs, is often represented by small, slowly growing cells. In the absence of consumers, large bacteria become more abundant (Gonzalez et al., 1990 ; Simek & Chrzanowski, 1992).

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

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

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

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

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

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

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

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

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

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

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

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

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