ISSN 0026-2617, Microbiology, 2016, Vol. 85, No. 5, pp. 553–561. © Pleiades Publishing, Ltd., 2016.
Original Russian Text © V.S. Mukhanov, O.A. Rylkova, T.Ya. Churilova, E.G. Sakhon, N.V. Pimenov, 2016, published in Mikrobiologiya, 2016, Vol. 85, No. 5, pp. 512–521.

EXPERIMENTAL ARTICLES

Structure and Seasonal Trophodynamics of Picophytoplankton in Sevastopol Bay and Adjacent Waters (the Black Sea)

V. S. Mukhanov[a, 1], O. A. Rylkova[a], T. Ya. Churilova[a], E. G. Sakhon[a], and N. V. Pimenov[b]

[a] *Kovalevsky Institute of Marine Biological Research, Russian Academy of Sciences, Sevastopol, Russia*

[b] *Winogradsky Institute of Microbiology, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia*

1E-mail: v.s.mukhanov@gmail.com

Received February 29, 2016

Abstract—Abundance and seasonal trophodynamics (specific growth rate, daily production, and grazing mortality) of the major picophytoplankton components, *Synechococcus* cyanobacteria (Syn) and picoeukaryotes (Pico-E), were studied at three stations in Sevastopol Bay and adjacent coastal waters (the Black Sea) in 2014 by flow cytometry and the dilution method. Pico-E abundance was shown to increase along the nutrient and pollution gradient from the coastal waters outside the bay (annual average of $7.3 \pm 5.4 \times 10^3$ cells mL^{-1}) to the eastern corner of the bay ($28.7 \pm 11.4 \times 10^3$ cells mL^{-1}), while no relation was found between the water pollution status and Syn abundance ($9.9 \pm 8.7 \times 10^3$ cells mL^{-1}; at all the stations, $n = 27$). Matter flows through the communities (daily production for Syn and Pico-E 0–16.6 and 0–19.3 μg C L^{-1} day^{-1}, respectively; grazing mortality for Syn and PicoE 0–3.6 and 0–21.2 μg C L^{-1} day^{-1}, respectively) were comparable to or even exceeded their biomass stocks (<0.05–6.8 and 0.9–26.5 μg C L^{-1} for Syn and PicoE, respectively), indicating high biomass turnover rates. The highest flow-to-stock ratio (up to 6 for Syn) and a significant imbalance between daily production (P) and grazing mortality (G) were observed in the most polluted and eutrophic waters of the bay in spring (Pico-E: $P/G < 1$) and late summer (Syn: $P/G > 1$). Black River inflow to the bay was hypothesized to be among the mechanisms maintaining this pronounced and long-term imbalance in the open system without any negative consequences for the picophytoplankton assemblages.

Keywords: picophytoplankton, *Synechococcus*, picoeukaryotes, specific growth rate, daily production, grazing mortality, biomass turnover, Black Sea

DOI: 10.1134/S002626171605012X

Understanding of the global role of the smallest photoautotrophs, picophytoplankton (0.2–2.0 μm), in the functioning and structural organization of the pelagic oceanic trophic network (Iturriaga and Mitchell, 1986) and the contribution of this microbial group to the total biomass of the oceanic plankton and to chlorophyll content in oligotrophic waters (Platt et al., 1983; Iturriaga and Mitchell, 1986; Booth, 1988) was achieved relatively recently. These microorganisms were found to possess high specific growth rates (Douglas, 1984). Contribution of *Synechococcus* cyanobacteria to the total photosynthetic carbon fixation in oligotrophic ocean waters may be as high as 25% (Waterbury et al., 1986), while their share in the overall photosynthesis in some areas of the World Ocean may reach 64% (Iturriaga and Mitchell, 1986). In spite of the evident predominance of this group in the open ocean, evidence keeps emerging that picophytoplankton, primarily *Synechococcus*, may be involved in phytoplanktonic blooms in tropical and subtropical coastal ecosystems and (under certain conditions) in moderate latitudes. Outbreaks of small phytoplankton were observed in Florida Bay (Phlips et al., 1999), San Francisco Bay (Ning et al., 2000), the Mediterranean (Agawin et al. 1998; Bec et al., 2005), and the Baltic Sea (Kuosa, 1991).

In spite of the unique hydrological and hydrochemical conditions of the Black Sea and the interest that the ecology of the local microflora produces, picophytoplankton of this basin has been scarcely studied. The data on abundance and spatial distribution of these microorganisms in Black Sea waters, and on their community structure and functional characteristics, are either few and fragmentary (Zaika et al., 1989, 1991; Shalapenok and Shalapenok, 1997; Uysal, 2000, 2001; Feyzioglu et al., 2004; Turkoglu, 2005) or completely lacking, such as the data on production and trophic dynamics. In any case, they are certainly insufficient for the understanding of the role of picophytoplankton in the sea ecosystem.

The goals of the present work were (1) to assess abundance of the two major components of Black Sea picobacterioplankton, *Synechococcus* cyanobacteria and eukaryotic picoalgae, by flow cytometry; (2) to determine their in situ specific production and mor-

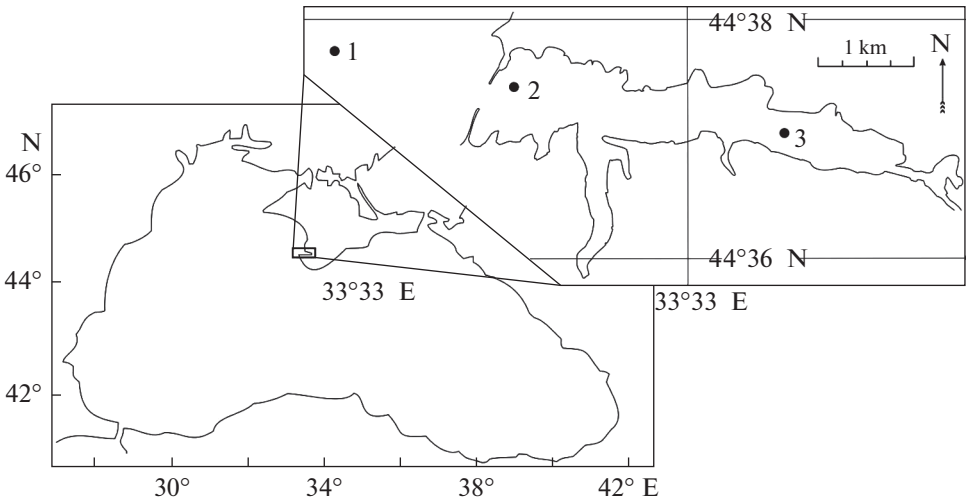

Fig. 1. Sampling stations in the Sevastopol Bay and adjacent waters.

tality associated with trophic processes; and (3) to analyze the seasonal dynamics of these parameters.

MATERIALS AND METHODS

Sampling sites and methods. Picoplankton abundance, biomass, production parameters, and elimination rates were studied in 2014. The samples were collected one to two times a month at three stations in the Sevastopol Bay and in the open sea outside it (Fig. 1). This area is characterized by pronounced gradients of water salinity (increasing) and of water contamination and trophicity (decreasing) from the mouth of the Black River in the apex of the bay (st. 3) to the bay mouth (st. 2) and to the open sea outside the bay (st. 1). The concentration of biogenic elements in the river outflow is 2 to 10 times higher than in the bay (Ovsyanyi et al., 2007), resulting in formation of stable concentration gradients of nitrite nitrogen (from 0.9 μg L^{-1} at st. 1 to 2.3 μg L^{-1} at st. 3), nitrate nitrogen (3.2 and 13.3 μg L^{-1}, respectively), ammonium nitrogen (17.4–23.1 μg L^{-1}), phosphate phosphorus (7.9–8.8 μg L^{-1}), and silicon (57–81 μg L^{-1}) (Gubanov, unpublished data). The average estimates of the E-TRIX eutrophication index, which is a function of concentrations of oxygen, biogenic elements, and photosynthetic pigments (Vollenveider, 1998), are 4.7 (st. 1), 5.1 (st. 2), and 5.5 (st. 3) (Gubanov et al., 2015). Biogenic material arriving with the river flow affect the biological productivity of the water. According to modern radiocarbon assessment of primary production (over 100 mg C m^{-3} day^{-1}), the waters of the bay may be assigned to the hypertrophic type (Egorov et al., 2016). The degree of chronic contamination with petroleum products and heavy metals increases from the open sea outside the bay to the bay center. Thus, the content of oil hydrocarbons in the bottom sediments of st. 1 was ~30 mg 100 g^{-1}, increasing to 80 mg 100 g^{-1} at st. 2 and to 180 mg 100 g^{-1} at st. 3 (Osadchaya et al., 2004). The gradient of the integral index of contaminating/anthropogenic load (Pollution Load Index) is even more pronounced: 8 (st. 1), 2 (st. 2), and 0.08 (st. 3) (Osadchaya et al., 2004).

The samples were collected from the surface water layer using a Niskin bathometer and transferred to the laboratory for analysis. Aliquots were fixed with formaldehyde filtered through <0.2-μm membranes (final concentration 2%) immediately after sampling, dispensed into 5-mL test tubes, frozen at –80°C, and stored on board the ship in dry ice prior to transfer to the laboratory.

Dilution method. Dilution method in its standard version (Landry and Hassett, 1982) was used for experimental assessment of specific rates of growth and picoplankton grazing by consumers. The concept of the method is based on the following assumptions: (1) the grazing press decreases proportionally to the sample dilution (due to decreased probability of predator and prey meeting) and (2) the absence of negative effects of sample dilution on the rate of microbial growth.

Immediately after delivery of native water samples to the laboratory, they were diluted (100, 75, 50, and 20%) with the filtrate (<0.2 μm) of the same sample and dispensed into sterile transparent polycarbonate 0.5-L vials. Aliquots (5 mL) from each vial were fixed and used within a day for quantitative assessment by flow cytometry (see below). The vials with dilutions were incubated for 15–24 h submerged in the sea (at the mouth of the Sevastopol Bay) under conditions of ambient illumination and temperature. Microbial concentrations in the vials were then determined and instantaneous specific growth rate (μ) and specific grazing rate (*g*) were calculated (i) based on the exponential growth model assuming (ii) that in every dilu-

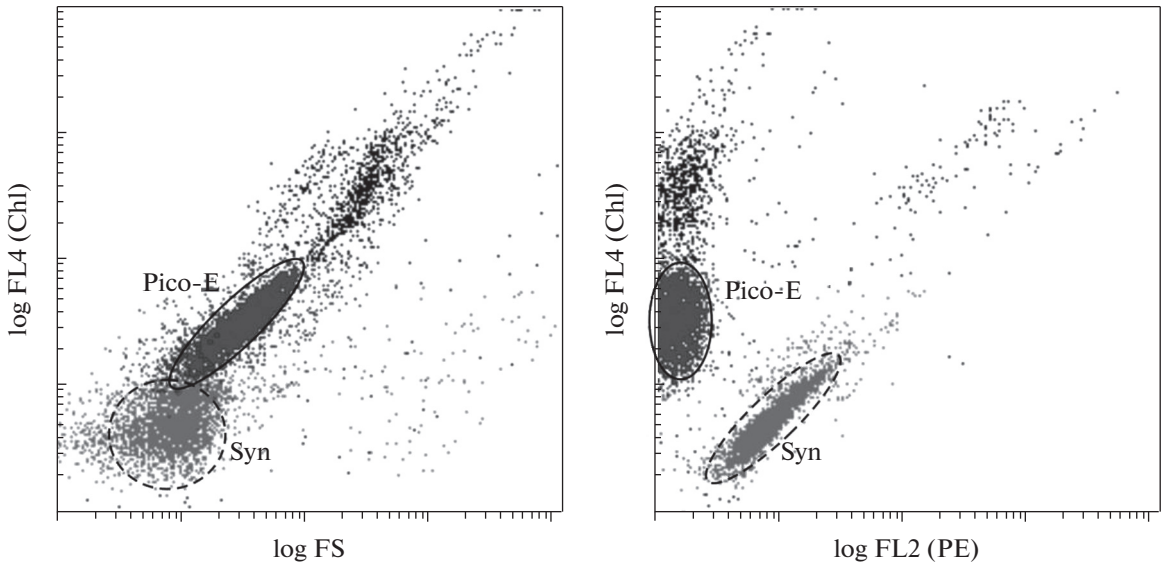

Fig. 2. Identification of the clusters of *Synechococcus* (Syn) and eukaryotic picophytoplankton (Pico-E) in the space of cytometric variables: forward light scattering (FS, cell size) and fluorescence in the red (FL4, Chl) and orange (FL2, PE) spectral regions.

tion apparent specific growth (or extinction) rate $r = \mu - g$, and (3) the μ value is constant for all dilutions, while g depends on dilution degree.

Flow cytometry. The samples collected at sea and during the experiment were analyzed in a stationary laboratory on a Cytomics™ FC 500 flow cytometer (Beckman Coulter, United States) equipped with a 488-nm argon laser, using the CXP software package.

Total abundance of picophytoplankton was determined in unstained samples by gating the cell population on 2-parameter cytograms of direct light scattering (FS channel) and autofluorescence in the red (FL4, 675 nm) and orange (FL2, 575 nm) spectral regions on dimensionless logarithmic scales. Cell size (FS) and orange fluorescence of phycoerythrin (FL2) were used for identification of the clusters of *Synechococcus* picocyanobacteria (Syn) and eukaryotic picoalgae (Pico-E) in the space of cytometric variables as was described previously (Marie et al., 2005). These groups form characteristic clusters on the cytograms, which are characterized by high content of phycoerythrin (Syn) and its absence (Pico-E) (Fig. 2).

The concentration of picophytoplankton cells was calculated from the flow rate (60 μL min^{-1}), counting time (480 s), and the number of cells registered during this time interval (at least 3000). The quality of measurements was controlled using the Flow-Check™ calibration fluorospheres (Beckman Coulter, United States) at a known concentration in the sample. Microbial biomass in carbon units was calculated using coefficients: 0.20 pg C cell^{-1} for *Synechococcus* (Heldal et al., 2003) and 0.53 pg C cell^{-1} for picoeukaryotes (Worden et al., 2004).

Cytometric data were processed using the Flowing Software v. 2.5.0 package (Perttu Terho, Turku Centre for Biotechnology, University of Turku, Finland, www.flowingsoftware.com).

Statistical analysis. Statistical treatment of the data was carried out using a STATISTICA (data analysis software system), v. 10 software package (StatSoft, www.statsoft.com).

RESULTS AND DISCUSSION

The average numbers of *Synechococcus* (*SN*) and picoeukaryotes (*PN*) for the whole area were 9.9 ± 8.7 (±SD) and 16.3 ± 12.4 × 10^3 cells mL^{-1}, respectively (Table 1). Thus, the average contribution of *Synechococcus* to the total abundance of picophytoplankton in the surface layer did not exceed 38%, and its share of the biomass was still lower (19%). While the average specific production of *Synechococcus* (0.70 ± 0.46 day^{-1}) was reliably higher than that of picoeukaryotes (0.20 ± 0.20 day^{-1}), no reliable difference was found in the values of daily production (2.30 ± 3.68 and 2.71 ± 4.22 μg C L^{-1} day^{-1}, respectively) according to the pairwise *t*-test, $p > 0.05$. On the contrary, no reliable difference was found between specific grazing rates (*Sg*, *Pg*) for two groups (0.43 ± 0.34 and 0.34 ± 0.30 day^{-1}, respectively), while daily grazing of picoeukaryotes (*PG* = 4.01 ± 5.84 μg C L^{-1} day^{-1}) was much higher than that of *Synechococcus* (*SG* = 1.05 ± 1.07 μg C L^{-1} day^{-1}). As food objects, picoeukaryotes were probably preferable to picocyanobacteria. Shift of the balance in trophic processes to grazing (negative balance) observed in picoeukaryotes, with the average *Pμ/Pg* ratio being less than one

Table 1. Designations, measuring units, and statistics for the studied variables

Variable		Min	Max	$m \pm SD$
T	Temperature, °C	7.3	28.0	15.7 ± 6.8
SN	*Synechococcus* abundance, 10^3 cells mL^{-1}	0.1	34.1	9.9 ± 8.7
SB	*Synechococcus* biomass, μg C L^{-1}	<0.05	6.8	2.0 ± 1.7
Sg	Grazing rate for *Synechococcus*, day^{-1}	0.00	1.52	0.43 ± 0.34
SG	Daily grazing for *Synechococcus*, μg C L^{-1} day^{-1}	0.00	3.61	1.05 ± 1.07
Sμ	Specific production for *Synechococcus*, day^{-1}	0.00	1.66	0.70 ± 0.46
SP	Daily production for *Synechococcus,* μg C L^{-1} day^{-1}	0.00	16.60	2.30 ± 3.68
PN	Picoeukaryote abundance, 10^3 cells mL^{-1}	1.7	49.9	16.3 ± 12.4
PB	Picoeukaryote biomass, μg C L^{-1}	0.9	26.5	8.6 ± 6.6
Pg	Grazing rate for picoeukaryotes, day^{-1}	0.00	0.94	0.34 ± 0.30
PG	Daily grazing for picoeukaryotes, μg C L^{-1} day^{-1}	0.00	21.19	4.01 ± 5.84
Pμ	Specific production for picoeukaryotes, day^{-1}	0.00	0.62	0.20 ± 0.20
PP	Daily production for picoeukaryotes, μg C L^{-1} day^{-1}	0.00	19.28	2.71 ± 4.22

Table 2. Pairwise correlation coefficients between the studied variables*

	T	*SN*	*SB*	*Sg*	*SG*	*Sμ*	*SP*	*PN*	*PB*	*Pg*	*PG*	*Pμ*	*PP*
T	1	0.03	0.03	−0.22	−0.02	0.53	0.52	−0.21	−0.21	0.34	−0.02	0.18	0.09
SN		1	1	−0.17	0.45	−0.16	0.44	0.09	0.09	−0.14	−0.11	0.29	0.14
SB			1	−0.17	0.45	−0.16	0.44	0.09	0.09	−0.14	−0.11	0.29	0.14
Sg				1	0.69	0.28	0.05	0.39	0.39	0.47	0.75	0.07	0.21
SG					1	0.14	0.36	0.39	0.39	0.33	0.59	0.3	0.32
Sμ						1	0.63	−0.06	−0.06	0.22	0.25	0.17	0.1
SP							1	0.14	0.14	0	0.11	0.24	0.17
PN								1	1	0	0.56	0.37	0.77
PB									1	0	0.56	0.37	0.77
Pg										1	0.64	0.32	0.11
PG											1	0.31	0.43
Pμ												1	0.69
PP													1

* Statistically significant parameters ($p < 0.05$) are underlined.

and negative difference existing between daily production and daily grazing in most cases, is an indirect confirmation of this suggestion. In the case of *Synechococcus*, the balance was positive, probably due to both higher production potential and lower suitability of this microorganisms as a food object (Johnson et al., 1982).

According to the results of pairwise correlation analysis (Table 2), temperature had no effect on abundance and grazing rates of both groups of picophytoplankton. This could be an indication of weakly pronounced seasonality of these parameters or of considerable differences in their seasonal dynamics at different stations. The latter suggestion was confirmed by detailed analysis of the seasonal dynamics of picophytoplankton structure and functions (see below). The absence of relation between abundance of picocyanobacteria and the temperature regime of the water was reported previously (Worden et al., 2004).

Production parameters of picoeukaryotes were also temperature-independent, while *Synechococcus* exhibited statistically significant correlation between the growth parameters and temperature (0.53 and 0.52 for specific and daily production, Table 2). The absence of significant correlation between production parameters of *Synechococcus* and picoeukaryotes indicated the different mechanisms controlling their growth. On the other hand, reliable positive correla-

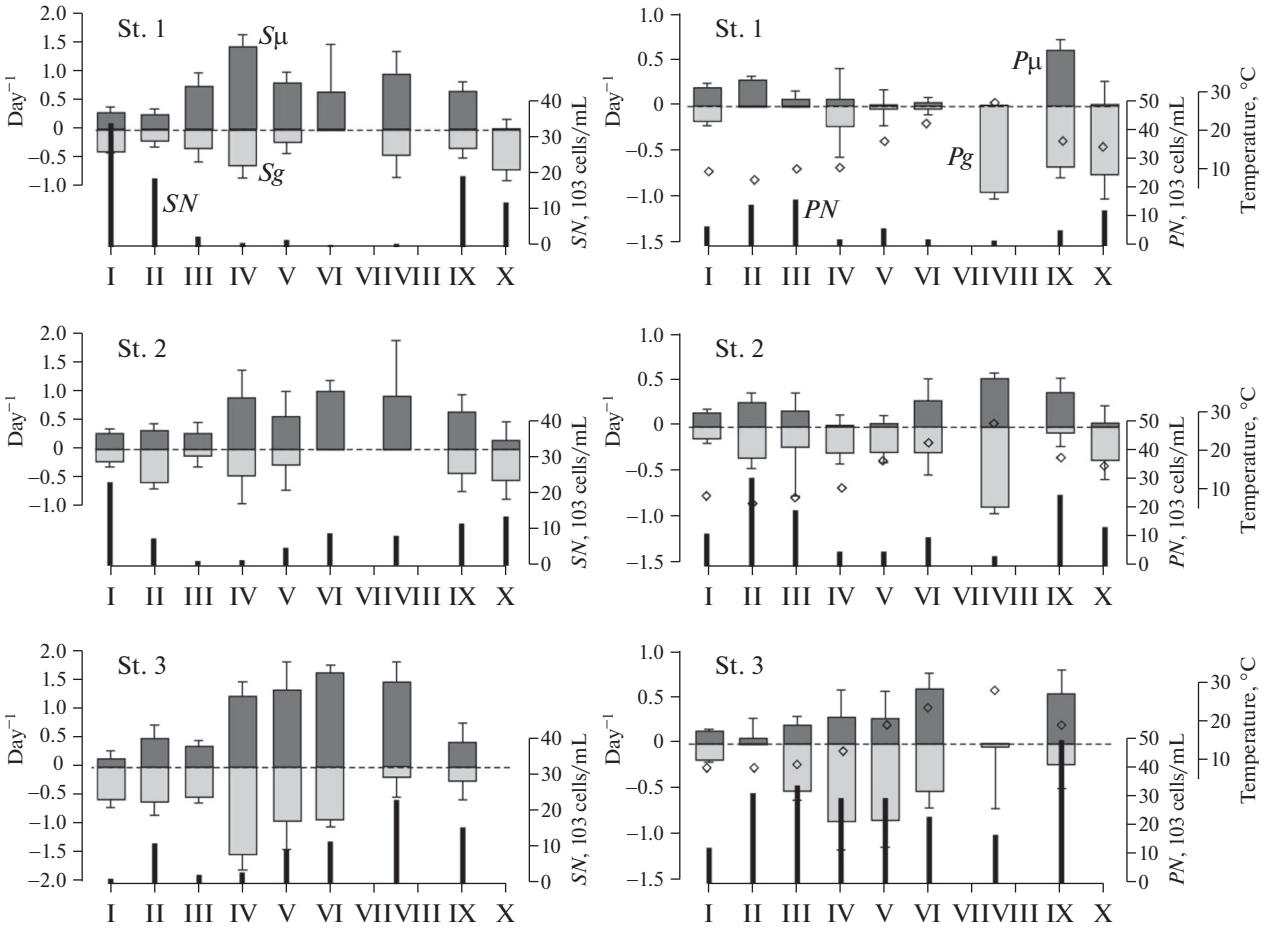

Fig. 3. Seasonal variations of abundance (*SN, PN*), specific production (*S*μ, *P*μ), and grazing rate (*Sg, Pg*) for *Synechococcus* picocyanobacteria (left graphs) and eukaryotic picoalgae (right graphs) at three stations in the Sevastopol Bay and adjacent waters in 2014. Specific grazing rates are shown as negative values. Designations are as in Table 1.

tion between these groups in grazing efficiency by phagotrophs (0.47 for *Sg* and *Pg*; 0.53 for *SG* and *PG*, Table 2) indicated that both could act as food objects for the same consumer. Finally, correlation analysis revealed that high consumption rates for both picophytoplankton components (*SG* and *PG*) were associated with high abundance (*PN*) and biomass (*PB*) of picoeukaryotes (see the relevant statistically significant correlations in Table 2). Thus, high numbers and biomass of picoeukaryotes (we assume, the preferred food object) provided for high rates of grazing of both microbial groups.

Analysis of the seasonal dynamics of abundance and rates of the trophic processes in the community revealed pronounced trends (Fig. 3). Abundance of *Synechococcus* at st. 1 outside the bay (up to 3×10^4 cell mL^{-1}) and at st. 2 in the mouth of the Sevastopol Bay (up to 2.5×10^4 cells mL^{-1}) was highest in winter and autumn, while at st. 3 in the apex of the bay it peaked in summer, at the temperature maximum (up to 2.5×10^4 cells mL^{-1}), which may explain a significant correlation between abundance of these microorganisms and temperature. Picoeukaryotes were numerous in February–March (up to 3.5×10^4 cells mL^{-1}) and in September–October, the period of the absolute maximum in their abundance (up to 5.0×10^4 cells mL^{-1}).

At all stations the highest specific production of *Synechococcus* (up to 1.7 day^{-1} at st. 3) was observed during the warm months at the temperature peak, while picoeukaryotes exhibited different dynamics at different stations (Fig. 3): at stations 1 and 2 high values were recorded in winter and autumn, while the absolute maximum in open waters occurred in autumn (0.62 day^{-1}).

The seasonal variation in specific rates of grazing were similar for both microbial groups (*Sg* and *Pg* on Fig. 3), indicating the action of the same grazing press against both groups. This parameter increased in cold months at st. 1 and 2 and in spring and early summer at st. 3 (up to 1.52 and 0.62 day^{-1} for *Synechococcus* and picoeukaryotes, respectively).

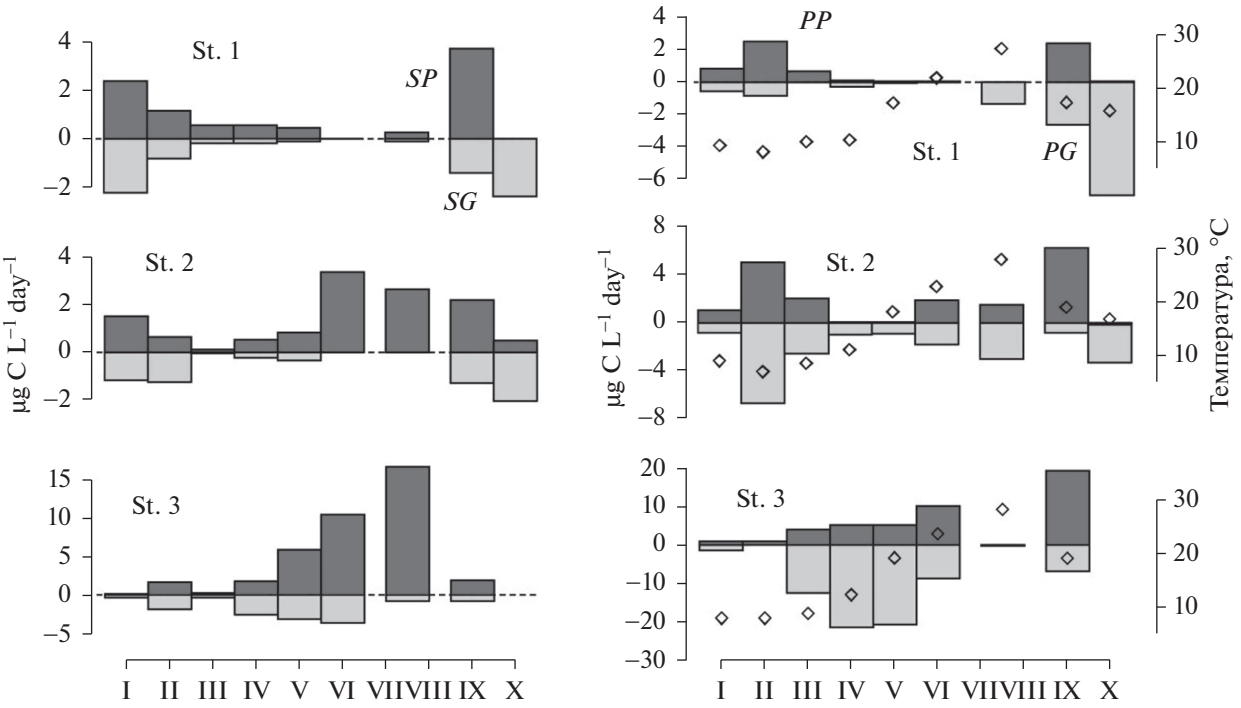

Fig. 4. Seasonal changes in daily production (*SP, PP*) and grazing (*SG, PG*) for *Synechococcus* picocyanobacteria (left graphs) and eukaryotic picoalgae (right graphs) at three stations in the Sevastopol Bay and adjacent waters. Daily grazing is presented as negative values. Designations are as in Table 1.

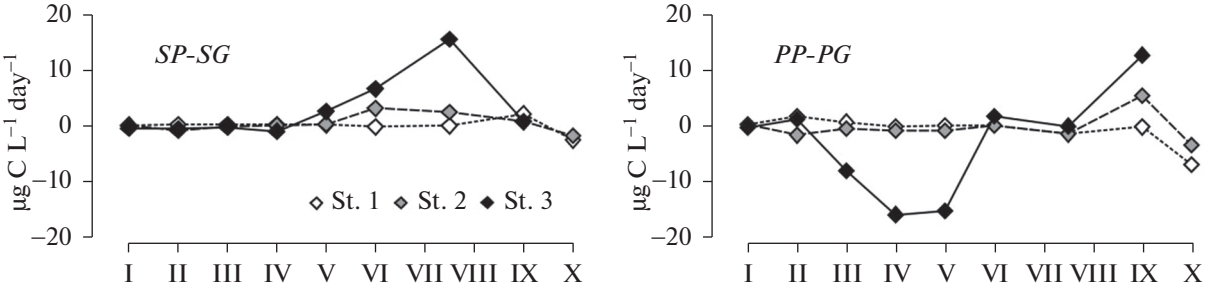

Fig. 5. Seasonal dynamics of the balance of trophic processes (daily production minus daily grazing) for *Synechococcus* picocyanobacteria (left graphs) and eukaryotic picoalgae (right graphs) at three stations in the Sevastopol Bay and adjacent waters. Designations as in Table 1.

The seasonal variations in daily production (*SP*, *NP*) and elimination by grazing (*SG*, *PG*) were more pronounced and differed considerably at different stations (Fig. 4). A gradual shift of the peaks of picophytoplankton daily production and elimination by grazing from autumn–winter to spring–summer occurred in the trophicity gradient from open waters to the bay apex. At st. 3 in the bay apex, a pronounced shift in the balance of trophic processes (calculated as the difference between daily production and grazing) to positive values, i.e., to biomass accumulation, occurred in summer for *Synechococcus* and in autumn for picoeukaryotes (Fig. 5). Shifting of the balance to negative values, i.e., to biomass consumption due to grazing, was observed for picoeukaryotes in spring.

The distribution of abundance and biomass of *Synechococcus* and picoeukaryotes from relatively clean open waters (st. 1) to the most contaminated and desalinated waters in the bay apex (st. 3) was different (Fig. 6). While no statistically significant difference was found in *Synechococcus* abundances between the stations, the biomass stocks of picoeukaryotes increased significantly along the pollution and water eutrophication gradient from 3.9 ± 2.9 (st. 1) to 15.2 ± 6.0 µg C L^{-1} (st. 2). While the first two stations exhibited no significant differences in any *Synechococcus*-related parameter, considerably higher productive and

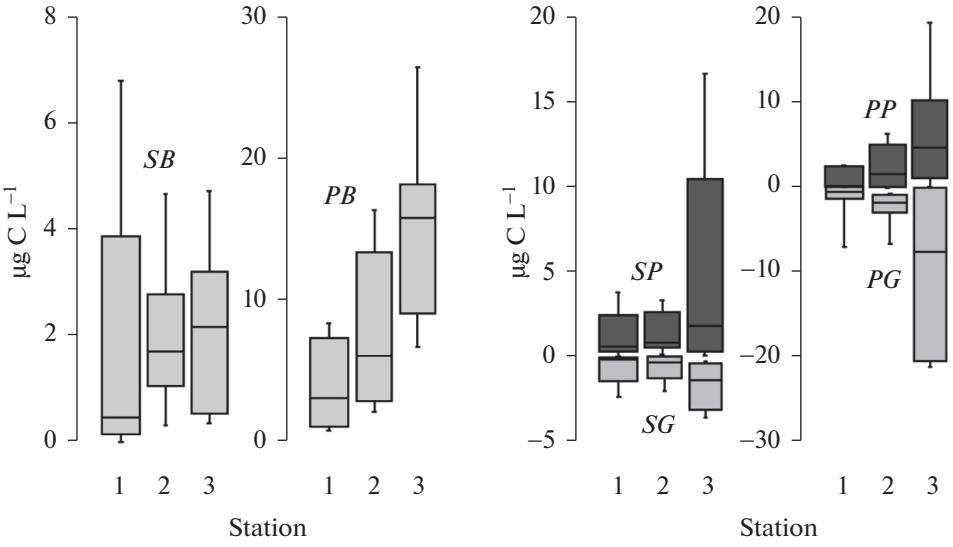

Fig. 6. Box-whisker diagrams of biomass (*SB*, *PB*), daily production (*SP*, *PP*), and grazing (*SG*, *PG*) for *Synechococcus* picocyanobacteria (left graph) and eukaryotic picoalgae (right graph) at three stations in Sevastopol Bay and adjacent waters. Extremes, medians, and the upper and lower quartiles are shown. Designations as in Table 1.

trophic parameters were observed in picocyanobacteria of the bay apex (st. 3) ($p < 0.05$), and the range of their variation was considerably broader. This area was also characterized by significantly higher values of daily production (*PP*, up to 5.8 μg C L^{-1}) and elimination (*PG*, 8.8 μg C L^{-1}) of picoeukaryotes (Fig. 6).

Although the ranges of variation of the quantitative parameters of picophytoplankton obtained in the present work for the Black Sea coastal waters are generally in agreement with the previously published data on various areas of the World Ocean, including the Black Sea (Uysal, 2000, 2001; Feyzioglu et al., 2004; Turkoglu, 2005), the ratio of abundance of *Synechococcus* and picoeukaryotes was considerably lower than the one reported by other authors (e.g., Zaika et al., 1989). Moreover, the previously reported reliable correlation between abundance of *Synechococcus* and picoeukaryotes in oceanic waters (Worden et al., 2004) was not observed, probably indicating a more pronounced ecological isolation of these groups in Black Sea coastal waters.

Picocyanobacteria *Prochlorococcus*, the most abundant and important component of picophytoplankton in oligotrophic oceanic waters (Biller et al., 2015), do not occur in the Black Sea. Nevertheless, the contribution of *Synechococcus* to the overall picophytoplankton abundance proved to be low (<40%). On the contrary, total abundance of eukaryotic picoalgae and their share in the Black Sea picophytoplankton community were high beyond expectation: the pioneering works of Zaika et al. (1989) gave the maximal assessment of picoalgal abundance and biomass in the Black Sea (open areas) not exceeding 0.7×10^3 cells mL^{-1} and 0.78 μg dry wt L^{-1}, respectively.

According to our observations, in the coastal and open Black Sea waters (Mukhanov et al., unpublished data) *Synechococcus* and picoeukaryotes occupy different ecological niches: the former prefer deep layers (40–50 m, below the thermocline during the stratification period) with low levels of temperature and illumination, while the latter inhabit the upper layer (above the thermocline during the stratification period). This spatial isolation is in agreement with the previously reported evidence of cyanobacterial maxima developing in the layers with low (<18°C) temperature (Worden et al., 2004), increased picoalgal abundance in upwelling zones (Shalapyonok et al., 2001) and their decreased abundance in the stratified waters with low content of biogenic elements (Campbell et al. 1997). Quantitative predominance of eukaryotes in the picophytoplankton of the Sevastopol Bay was probably due to sampling in picocyanobacteria-impoverished biotope.

The values of the rates of productive and trophic processes in the Black Sea picophytoplankton community were within the range of variation for these parameters in other areas of the World Ocean (Worden et al., 2004; Tsai et al., 2012, etc.). Comparability of the values of daily production (up to 19 μg C L^{-1} day^{-1}) and grazing of microorganisms by consumers (up to 21 μg C L^{-1} day^{-1}) with their biomass (up to 27 μg C L^{-1}) indicated high rates of biomass turnover in the community. High variability and occasional imbalance of the productive and trophic parameters at different time scales is among the possible consequences of this situation (Worden and Binder, 2003). At st. 3 in the apex of the bay, which is subject to the highest anthropogenic load and desalination by the

Black River water, imbalance in productive and trophic processes was maintained for long periods, which was difficult to explain by short-term fluctuations of the production/mortality ratio. Importantly, the biogeocenosis of this area is an ecotone at the border of the freshwater and marine biomes. This is an open flow system, somewhat resembling a chemostat, with high renewal rates of its components (microbial content in the water column, suspended and dissolved organic matter, biogenic elements, etc.) provided by the river flow-off. The pronounced positive balance, with production significantly exceeding mortality and which was observed in summer in *Synechococcus* population at st. 3 (Fig. 5), probably resulted from dilution of the microorganisms by river flow with low content of cyanobacteria and by their transfer to the mouth of the bay and outside it. Thus, mechanical transfer of excessive *Synechococcus* biomass could create the favorable conditions for growth of the population and simultaneously prevent excessive biomass accumulation. The pronounced negative balance, with mortality significantly exceeding production, which was observed in autumn for picoeukaryotes at st. 3 (Fig. 5), could be maintained for a long time only under condition of biomass inflow with river water. Verification of this hypothesis required further research on quantitative assessment of picophytoplankton in the river flow-off.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research, projects no. 15-04-08768 A and 14-04-90421.

REFERENCES

Agawin, N.S.R. and Agusti, S., Abundance, frequency of dividing cells and growth rates of *Synechococcus* sp. (cyanobacteria) in the stratified Northwest Mediterranean Sea, *J. Plankton Res.*, 1997, vol. 19, pp. 1599–1615.

Agawin, N.S.R., Duarte, C.M., and Agusti, S., Growth and abundance of *Synechococcus* sp. in a Mediterranean Bay: seasonality and relationship with temperature, *Mar. Ecol. Prog. Ser.*, 1998, vol. 170, pp. 45–53.

Bec, B., Husseini-Ratrema, J., Collos, Y., Souchu, P., and Vaquer, A., Phytoplankton seasonal dynamics in a Mediterranean coastal lagoon: emphasis on the picoeukaryote community, *J. Plankton Res.*, 2005, vol. 27, pp. 881–894.

Biller, S.J., Berube, P.M., Lindell, D., and Chisholm, S.W., *Prochlorococcus:* the structure and function of collective diversity, *Nature Rev. Microbiol.*, 2015, vol. 13, no. 1, pp. 13–27.

Booth, B.C., Size classes and major taxonomic groups of phytoplankton at two locations in the subarctic Pacific Ocean in May and August 1984, *Marine Biol.*, 1988, vol. 97, no. 2, pp. 275–286.

Campbell, L., Liu, H., Nolla, H.A., and Vaulot D., Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991–1994 ENSO event, *Deep Sea Res. Part I: Oceanographic Research Papers*, 1997, vol. 44, no. 2, pp. 167–192.

Douglas, D.J., Microautoradiography-based enumeration of photosynthetic picoplankton with estimates of carbon-specific growth rates, *Mar. Ecol. Prog. Ser.*, 1984, vol. 14, no. 2–3, pp. 223–228.

Egorov, V.N., Gulin, S.B., Malakhova, L.V., Mirzoeva, N.Yu., Popovichev, V.N., Tereshchenko, N.N., Lazorenko, G.E., Plotitsyna, O.V., Malakhova, T.V., Proskurnin, V.Yu., Sidorov, I.G., Stetsyuk, A.P., and Gulina, L.V., Standardization of the Sevastopol Bay water quality by the flows of deposition of ^{90}Sr, ^{137}Cs, $^{239,240}Pu$, ^{210}Po, Hg, PCB, and DDT into the bottom sediments, *Water Res.*, 2016 (in press).

Feyzioglu, A.M., Kurt, I., Boran, M., and Sivri, N., Abundance and distribution of cyanobacteria *Synechococcus* spp in the south-eastern Black Sea during 2001 summer, *Indian J. Mar. Sci.*, 2004, vol. 33, pp. 365–368.

Gubanov, V.I., Gubanova, A.D., and Rodionova, H.Yu., Diagnosis of trophicity of the Sevastopol Bay and the Sevastopol coastal water, *Proc. Conf "Aktual'nye problemy akvakul'tury v sovremennyi period"* (Focal Problems of Aquaculture at the Modern Period), Rostov-on-Don, 2015, pp. 64–67.

Heldal, M., Scanlan, D.J., Norland, S., Thingstad, F., and Mann, N.H., Elemental composition of single cells of various strains of marine *Prochlorococcus* and *Synechococcus* using X-ray microanalysis, *Limnol. Oceanogr.*, 2003, vol. 48, pp. 1732–1743.

Iturriaga, R. and Mitchell, B.G., Chroococcoid cyanobacteria: a significant component in the food web dynamics of the open ocean, *Mar. Ecol. Prog. Ser.*, 1986, vol. 28, pp. 291–297.

Johnson, P.W., Huai-shu, X., and Sieburth, J.M., The utilization of chroococcoid cyanobacteria by marine protozooplankters but not by calanoid copepods, *Ann. Inst. Oceanogr. Paris Nouv. Ser.*, 1982, vol. 58, pp. 297–308.

Kuosa, H., Picoplanktonic algae in the northern Baltic Sea: seasonal dynamics and flagellate grazing, *Mar. Ecol. Prog. Ser.*, 1991, vol. 73, pp. 269–276.

Landry, M.R. and Hassett, R.P., Estimating the grazing impact of marine micro-zooplankton, *Mar. Biol.*, 1982, vol. 67, pp. 283–288.

Marie, D., Simon, N., and Vaulot, D., *Phytoplankton cell counting by flow cytometry*, *Algal Cult. Techn.*, 2005, pp. 253–268.

Ning, X., Cloern, J.E., and Cole, B.E., Spatial and temporal variability of picocyanobacteria *Synechococcus* sp. in San Francisco Bay, *Limnol. Oceanogr.*, 2000, vol. 45, pp. 695–702.

Osadchaya, T.S., Alemov, S.V., and Shadrina, T.V., Ecological quality of the Sevastopol Bay bottom sediments: retrospective and modern state, *Ekol. Morya,* 2004, no. 66, pp. 82–87.

Ovsyanyi, E.I., Artemenko, V.M., Romanov, A.S., and Orekhova, N.A., Frlow of the Chernaya river as a factor in formation of the water-salt regime and ecological state of the Sevastopol Bay, in *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon* (Ecological Safety of the Coastal and Shelf Zones), 2007, vol. 15, p. 57.

Phlips, E.J., Badylak, S., and Lynch, T.C., Blooms of the picoplanktonic cyanobacterium *Synechococcus* in Florida

Bay, a subtropical inner-shelf lagoon, *Limnol. Oceanogr.*, 1999, vol. 44, pp. 1166–1175.

Platt, T., Rao, D.V.S., and Irwin, B., Photosynthesis of picoplankton in the oligotrophic ocean, *Nature*, 1983, vol. 301, pp. 702–704.

Shalapenok, L.S. and Shalapenok, A.A., Heterogeneous pigment composition of phycoerythrin-containing picocyanobacteria *Synechococcus* spp. in the Black Sea, *Microbiology* (Moscow). 1997, vol. 66, no. 1, pp. 80–85.

Shalapyonok, A., Olson, R.J., and Shalapyonok, L.S., Arabian Sea phytoplankton during Southwest and Northeast Monsoons 1995: composition, size structure and biomass from individual cell properties measured by flow cytometry, *Deep Sea Res. Part II: Topical Studies in Oceanography*, 2001, vol. 48, no. 6, pp. 1231–1261.

Tsai, A.Y., Gong, G.C., Sanders, R.W., Chiang, K.P., and Chao, C.F., Heterotrophic bacterial and *Synechococcus* spp. growth and mortality along the inshore-offshore in the East China Sea in summer, *J. Oceanogr.*, 2012, vol. 68, pp. 151–162.

Türkoglu, M., Succession of picoplankton (coccoid cyanobacteria) in the Southern Black Sea (Sinop Bay, Turkey), *Pakistan J. Biol. Sci.*, 2005, vol. 8, pp. 1318–1326.

Uysal, Z., Chroococcoid cyanobacteria *Synechococcus* spp. in the Black Sea: pigments, size, distribution, growth and diurnal variability, *J. Plankton Res.*, 2001, vol. 23, pp. 175–190.

Uysal, Z., Pigments, size and distribution of *Synechococcus* spp. in the Black Sea, *J. Marine Systems*, 2000, vol. 24, no. 3, pp. 313–326.

Vollenveider, R., Giovanardi, F., Montanari, G., and Rinaldi, A., Characterization of the trophic conditions of marine coastal waters, with special reference to the NW Adriatic Sea, *Environmetrics*, 1998, no. 9, pp. 329–357.

Waterbury, J.B., Watson, S.W., Valois, F.W., and Franks, D.G., Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus, Can. Bull. Fish Aquat. Sci.*, 1986, vol. 214, pp. 71–120.

Worden, A.Z. and Binder, B.J., Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synechococcus* populations in oligotrophic environments, *Aquat. Microbiol. Ecol.*, 2003, vol. 30, pp. 159–174.

Worden, A.Z., Nolan, J.K., and Palenik, B., Assessing the dynamics and ecology of marine picophytoplankton: the importance of the eukaryotic component, *Limnol. Oceanogr.*, 2004, vol. 49, pp. 168–179.

Zaika, V.E., Shevchenko, V.A., and Bulatov, K.V., *Ekologiya morskogo fototrofnogo pikoplanktona* (Ecology of Phototrophic Marine Picoplankton), Moscow: NTsBI, 1989.

Zaika, V.I., Shalapenok, L.S., and Pokotilov, S.L., *Izmemenie chastoty delyashchikhsya tsianobakterii po glubine b vremeni sutok* (Frequency of dividing cyanobacteria depending on depth and time), Available from VINITI, 1991, Sevastopol, no. 870-B91.

Translated by P. Sigalevich

SPELL: 1. OK