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# THE INTERANNUAL VARIABILITY OF THE PHYTOPLANKTON STRUCTURAL CHARACTERISTICS, ITS SPECIFIC GROWTH RATE AND MICROZOOPLANKTON GRAZING IN THE SURFACE LAYER OF THE SEVASTOPOL BAY (BLACK SEA)

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**Abstract.** The phytoplankton biomass interannual variability, its taxonomic and species composition in the surface layer of the Sevastopol Bay waters during the period of 2000-2014 were analysed. Gradual decrease in the total algae biomass and relative contribution of the diatoms, as well as an increase in the dinoflagellates and coccolitophores contribution, was detected. During the research period relative diatoms biomass decreased on average from 83 % to 44 %, and relative dinoflagellates biomass increased on average from 14% to 38 %. The remaining phytoplankton biomass was generated mainly by coccolitophores, where *Emiliania huxleyi* (Lohmann) W.W.Hay & H.P.Mohler was dominating. According to our calculations, their relative contribution was increasing on average from 2 % to 11 %. The restructuring of the phytoplankton taxonomic composition was occurring mainly as a result of water temperature increase and, to a lesser extent, due to decreasing in the nutrients concentration. For the entire 15-year observation period the positive trend of average annual temperatures values variations was detected with a sufficiently high correlation coefficient ($R^2 = 0.66$). The water temperature increase, especially in a summer, adversely impacts functioning of the most diatoms species. This, probably, is the main reason for the restructuring of their species composition, what is largely pronounced in the summer. In the Sevastopol Bay in the summer 2010-2014, representatives of the *Chaetoceros* genus were not observed among the dominated species, although they caused algae blooms in the previous years. However, other species were detected: *Nitzschia tenuirostris* Mer., *Striatella interrupta* (Ehrenberg) Heiberg, *Cyclotella caspia* Grunow и *Pseudosolenia calcar-avis* (Schultze) B.G.Sundström. The composition of the dinoflagellates dominant species was not varied over the entire observation period. Among them, the representatives of *Prorocentrum*, *Gymnodinium* and *Ceratium* genus were dominating. Variation of the phytoplankton taxonomic and species composition caused a decrease on average annual phytoplankton growth rate, microzooplankton grazing and, also, net primary production. It indicates that the matter and energy flow from phytoplankton to microzooplankton, as well as to the upper trophic levels, is decreasing.

**Keywords**: Black Sea, phytoplankton biomass, growth rate, microzooplankton grazing, nutrients, temperature.

## 1 INTRODUCTION

One of the main ecological problems is an assessment of current state and possible evolution paths of the marine ecosystems in condition of global climate changes and constantly growing anthropogenic pressure. Primary component of any aquatic ecosystem is a phytoplankton, which variability determines the development and dynamic of all subsequent trophic levels. The main primary production consumer is a microzooplankton (Schmoker et al., 2013). Therefore, the amount of organic matter transferred from phytoplankton to subsequent trophic levels depends on its functional state and trophic activity.

Over the last century due to the climate changes, sea water temperature globally raised by approximately 1ºC (Häder and Gao 2015). In surface layer of deep water part of the Black Sea, since the middle of 1990s, the temperature change has been characterized by a positive trend (Oguz and Gliber 2007). As a result of temperature stratification increasing of the water column, the nutrients supply from the depths to the zone of photosynthesis gradually decreases (Mikaelyan et al., 2018). Therefore, phytoplankton biomass as well as the matter and energy flow from the phytoplankton to upper trophic levels is significantly lower, than previously. In the coastal waters in the Crimea peninsula area also multiannual unidirectional positive trend in the water temperature of the surface layer was observed (Repetin 2012). Therefore, the studies which directed at identifying changes occurring in the primary trophic links of these water areas ecosystems due to the warming, are extremely relevant.

The objective of this study was to analyze the interannual variability of phytoplankton biomass, its species taxonomic composition, specific growth rate, microzooplankton grazing and the net primary production in the surface layer of the Sevastopol Bay.

## 2 MATERIALS AND METHODS

The study is based on the results of the authors' own research carried out in the Sevastopol Bay (Figure 1) in the period from 2000 to 2014. Water samples (5 – 8 l) were collected from the 0 – 0.5 m layer, usually, monthly. The exception was the research conducted in 2014, which was carried out on a weekly basis.

For determination of phytoplankton abundance, biomass and species composition, 2 – 3 l samples of sea water were concentrated using the track membranes (1 μm pore size) in an inverse filtering funnel. The condensed to 50 ml samples were fixed with neutralized 1% formaldehyde (final concentration in the sample). The abundance and linear dimensions of algae cells were determined in a 0.1 ml drop, placed into a Naujotte counting chamber, with three replications under light microscope ZEISS Primo Star. Linear measurements were converted to cell volume using appropriate geometric formulas.

Phytoplankton organic carbon concentrations were calculated from the average cell volume for each species of diatoms and dinoflagellates using the equations presented in the work of Menden-Deuer and Lessard (2000), in case of other algae – using the equation (Strathmann 1967). Only in 2014 the phytoplankton biomass was calculated based on the concentration of chlorophyll *a* and the ratio between organic carbon and chlorophyll *a* (C/Chl *a*), the data, which were obtained by us in 2000–2010 for the coastal waters of the Black Sea in the Sevastopol area (Stelmakh and Gorbunova 2018). Phytoplankton species identification was carried out using the manual (Tomas 1997).

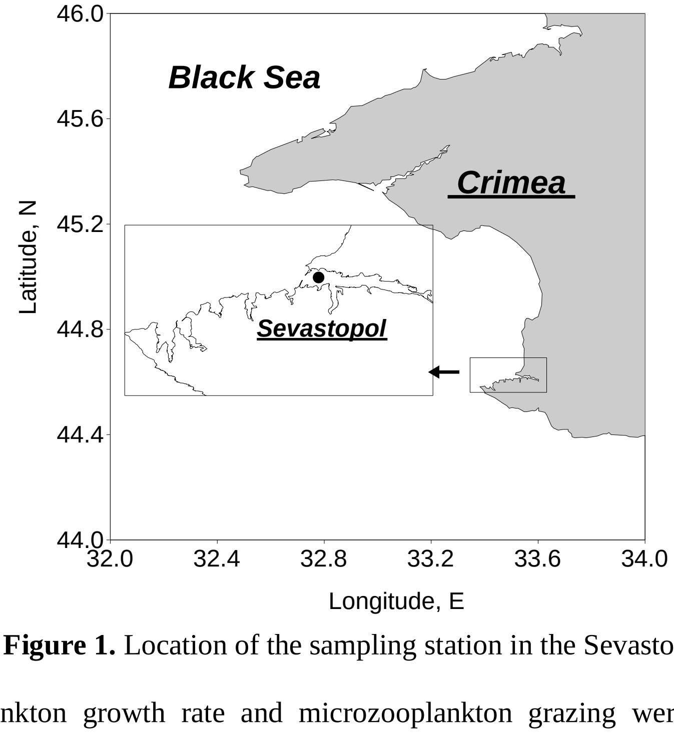

**Figure 1.** Location of the sampling station in the Sevastopol Bay

The phytoplankton growth rate and microzooplankton grazing were calculated by the dilution method (Landry and Hasset 1982) with daily increase of chlorophyll *a* concentration (Chl *a*) in the experimental bottles, which were exposed in a flow – type incubator with natural luminance. Five dilutions of the original sample were used (0.1; 0.25; 0.50; 0.75; 1.0). The initial concentration of Chl *a* was determined only for the undiluted samples, while, for the diluted samples it was calculated using the dilution factor (DF). The observed daily phytoplankton growth rate for each of the 5 dilutions ($\mu_{DF}$) was calculated as:

$$\mu_{DF} = \ln(\mathrm{Chl}a_{\mathrm{final}} / \mathrm{Chl}a_{\mathrm{initial}}) \qquad (1).$$

The linear regression equations were calculated to estimate the interrelations between the observed phytoplankton growth rate ($\mu_{DF}$) and the dilution factor (DF) as:

$$\mu_{DF} = -g \cdot DF + \mu \ , \qquad (2)$$

where μ is true phytoplankton growth rate ($day^{-1}$) and g – the zooplankton grazing rate ($day^{-1}$).

The nutrients concentrations in the water were determined by the methods, described in the previous studies (Stelmakh and Gorbunova 2019). Chlorophyll *a* concentration was measured in the acetone extracts by the fluorimetric method и calculated according to the equation, presented in the following work (JGOFS Protocols 1994).

Net primary production of phytoplankton was calculated based on the phytoplankton biomass and growth rate parameters, as well as on the microzooplankton grazing rate (Moigis and Gocke 2003).

Statistical treatment of the data and regression analysis were carried out using the software Excel 2007 for Windows. The graphs were built using the Grafer 7 program. Map construction was carried out using the program Surfer 8.

## 3 RESULTS AND DISCUSSION

It was determined that phytoplankton biomass and chlorophyll *a* concentration in the Black Sea undergo distinct seasonal and interannual variability both in the deep-water area (Finenko, Kovaleva, Suslin, 2014) and in coastal areas (Stelmakh and Gorbunova 2018) This is caused by the combined influence of abiotic and biotic environmental factors, among which light, temperature and nutrients play a major role. In the surface layer (0–1m) the light, usually, is not limit phytoplankton growth and the role of temperature and nutrients is essential.

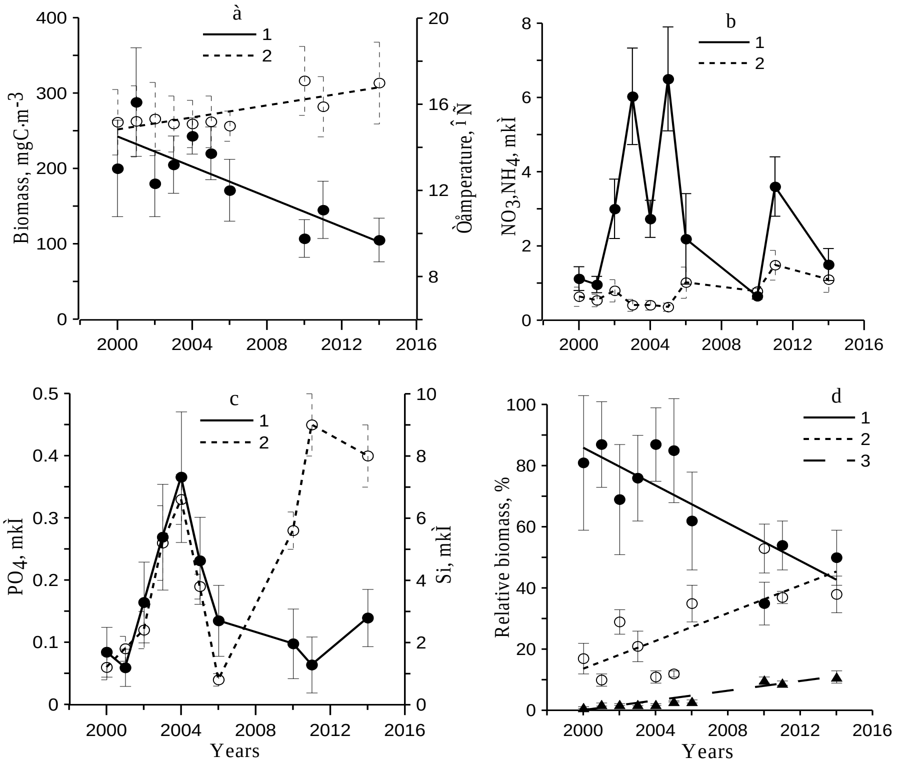

**Figure 2.** Multiannual dynamics of average annual phytoplankton biomass values, relative contribution of its main taxonomic groups, nutrient concentrations and water temperature in the surface layer of the Sevastopol Bay: a – phytoplankton biomass (1) and water temperature (2); b – nitrates (1) and ammonium (2); c – silicon (1) and phosphates (2); d – relative biomass of diatoms (1), dinoflagellates (2) and coccolithophorids (3)

Analysis of the results obtained in the Sevastopol Bay surface water layer from 2000 to 2014, demonstrated that the average annual water temperature values in 2000–2006 varied slightly and were in the range of 15 to 15.3 °C, while in 2010–2014 they increased to 15.9–17.1 °C (Figure 2, a). For the entire 15-

year period of observations, a positive trend in the the average annual temperature values variations was identified with a sufficiently high determination coefficient ($R^2 = 0.66$).

Unidirectional changes in the nutrients concentrations were not observed, but some variations in their concentration were obtained for two time intervals. In 2002–2006 nitrates and silicon concentrations were significantly higher, than in 2010–2014 (Figure 2, b, c). However, phosphates concentration in 2010–2014 was higher, than in the previous period of time. Water temperature increase and changes in nutrients concentrations caused significant restructure of the phytoplankton taxonomic composition (Figure 2, d). A decrease in the relative biomass of diatoms on average from 82 % to 42% ($R^2= 0.62$) and increase in the relative biomass of dinoflagellates on average from 15 % to 38% ($R^2= 0.50$) were identified.

Among the dinoflagellates in all years the representatives of the genus Gynmodinium, *Prorocentrum cordatum* (Ostf.) Dodge, *Prorocentrum micans* Ehrenberg, *Prorocentrum lima* (Ehrenberg) Stein, *Prorocentrum compressum* (Bailey) Dodge, *Ceratium tripos* (O.F.Müll.) Nitzsch., *Ceratium furca* (Ehr.) Clap. Et Lachm and *Scrippsiella trochoidea* (Stein) Balech dominated. The remaining insignificant phytoplankton biomass was constituted mainly of coccolithophorids, among which *E. huxleyi* dominated. According to our calculations, their relative contribution was increasing on average from 2 to 11 % (Figure 2, d).

Water temperature increase, especially in a summer period, adversely affects the diatoms functioning, because their temperature optimum for growth is significantly lower than that of dinoflagellates species. As studies on the Black Sea microalgae cultures indicated, an optimal temperature for diatoms growth is 18–21 ºC, but for dinoflagellates it is – 22–26 ºC (Akimov, Solomonova 2019).

Not only silicon, but also nitrates are essential for the diatoms growth. At the same time dinoflagellates and coccolithophorids grow equally well on both the nitrates and the ammonium (Glibert et al., 2016). A decrease in the nitrates and silicates concentration and an increase in the water temperature led not only to a decrease in the diatoms contribution in the total phytoplankton biomass, but also to a restructuring of their species composition, which is most pronounced in the summer. Thus from 2000 to 2006–2007, during June – August, representatives of the genus *Chaetoceros* predominated among diatoms, primarily *Chaetoceros socialis* H.S.Lauder, and *Coscinodiscus sp.*, *Proboscia alata* (Brightwell), *Pseudo-nitzschia delicatissima* (Cleve), *Licmophora ehrenbergii* (Kützing) Grunow, *Thalassionema nitzshioides* Grun. и *Cerataulina pelagica* (Cleve) Hendey. At that time, as in 2010–2014, representitives of the genus *Chaetoceros* were not observed among the dominating species. However, were observed such species as *Nitzschia tenuirostris* Mer., *Striatella interrupta* (Ehrenberg) Heiberg, *Cyclotella caspia* Grunow and *Pseudosolenia calcar-avis* (Schultze) B.G.Sundström. As a result of changes in species composition of diatoms, which are one of the main nutrition source for microzooplankton, its quality was decreased. It is established that such a widespread in recent years diatom species as *P. calcar-avis* is not grazed by microzooplankton. However representitives of the genus *Chaetoceros* are most preferential for the protozoa nutrition. Therefore, it is no coincidence that the specific rate of microzooplankton grazing on phytoplankton in 2010 and 2014 was approximately 1.5–2 times lower than in 2006–2007 (Table 1).

The increase in the dinoflagellates contribution in 2010 and 2014 was the main reason for the decrease in the phytoplankton specific growth rate (Table 1), because the dinoflagellates growth rate is 2–3 times lower than that of diatoms, even under optimal environmental conditions.

The annual value of the net primary production of phytoplankton was the maximal (69 mg $C \cdot м^{-3} \cdot year^{-1}$) in 2006–2007, when the average annual water temperature was 15 °C. At the same time its increase on average to 16.7 ºC in 2010 and to 17 ºC in 2014 caused the decline in primary production of approximately 1.4–2 times (Figure 3). The absolute contribution of phytoplankton primary production grazed by microzooplankton also decreased.

In consequence of the described above changes of the environment and the phytoplankton taxonomic composition restructuring in the Sevastopol Bay, a negative trend in the phytoplankton biomass variation was identified over the entire observations period with a determination coefficient 0.65 (Figure 2, a). This means that as a result of warming, the flow of matter and energy from phytoplankton to the upper trophic levels decreases.

## 4 CONCLUSION

The main reasons for the decrease in phytoplankton biomass and the restructuring of its taxonomic composition in the surface layer of the Sevastopol Bay waters in the period 2000–2014 are an increase in water temperature and a decrease in the nitrates and silicon concentration in the environment. The restructuring of the phytoplankton taxonomic composition was expressed in a decrease in the relative contribution of diatoms in its total biomass and a synchronous increase in the contribution of dinoflagellates and coccolithophorids. This led to a decrease in the phytoplankton specific growth rate, the rate of its microzooplankton grazing, as well as the net primary production values. As a result, the flow of matter and energy from phytoplankton to the upper trophic levels decreased.

**Table 1.** Seasonal and interannual variability of the phytoplankton biomass (B), relative contribution of diatoms and dinoflagellates, specific phytoplankton growth rate (μ) and microzooplankton grazing rate (g) in the surface layer of the Sevastopol Bay under conditions of increased temperatures

| Seasons | T, ºC | μ, day$^{-1}$ | g, day$^{-1}$ | B, mg C·m$^{-3}$ | Bacillario-phyta, % | Dinophyta, % |
|---|---|---|---|---|---|---|
| 2006–2007 | | | | | | |
| Winter | 9.2±2.2 | 0.23± 0.22 | 0.15±0.13 | 35±30 | 79±16 | 20±4 |
| Spring | 11.8±3.8 | 1.21± 0.81 | 0.77±0.54 | 143±81 | 81±9 | 18±4 |
| Summer | 22.7 ±2.4 | 1.19± 0.80 | 1.05±0.68 | 168±68 | 59±12 | 36±9 |
| Autumn | 16.2±1.4 | 0.53 ±0.32 | 0.34±0.07 | 217±160 | 46±13 | 53±14 |
| An. average | **15.0±0.7** | **0.94±0.18** | **0.68±0.12** | **141±28** | **62±16** | **35±6** |
| 2010 | | | | | | |
| Winter | 9.4±2.1 | 0.42±0.16 | 0.23±0.08 | 55±42 | 62±9 | 36±8 |
| Spring | 12.7±3.3 | 0.34±0.25 | 0.32±0.09 | 82±37 | 56±11 | 39±6 |
| Summer | 24.6±2.5 | 0.80±0.27 | 0.66±0.31 | 112±55 | 14±3 | 78±12 |
| Autumn | 17.9±1.6 | 0.70±0.20 | 0.55±0.18 | 76±27 | 27±7 | 68±10 |
| An. average | **16.7±1.6** | **0.61±0.07** | **0.43±0.17** | **81±40** | **35±7** | **53±9** |
| 2014 | | | | | | |
| Winter | 8.9±2.2 | 0.50±0.21 | 0.36±0.08 | 33±24 | 66±8 | 16±4 |
| Spring | 14.9±2.5 | 0.55±0.32 | 0.29±0.07 | 85±36 | 52±11 | 40±9 |
| Summer | 25.0±2.6 | 1.18±0.63 | 0.43±0.12 | 165±59 | 41±9 | 50±13 |
| Autumn | 16.0±1.2 | 0.58±0.28 | 0.35±0.08 | 155±48 | 40±12 | 54±14 |
| An. average | **17.0±2.2** | **0.70±0.08** | **0.36±0.15** | **105±36** | **50±9** | **38±6** |

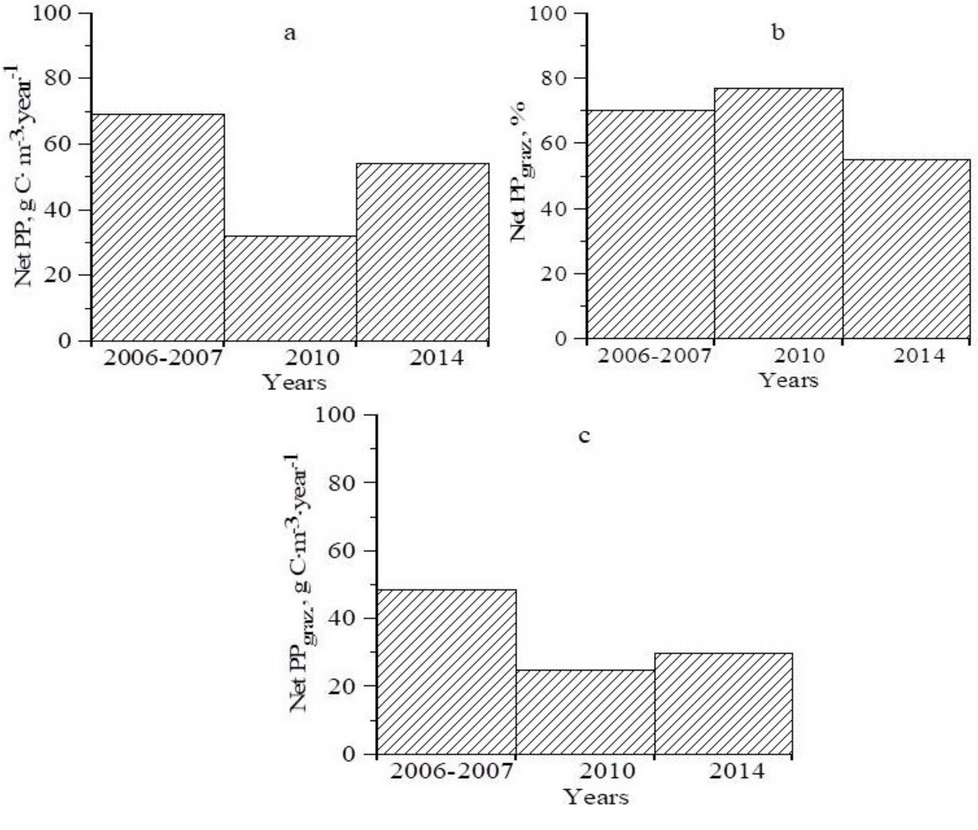

**Figure 3.** Interannual variability of net primary production (Net PP), and its contribution grazed by microzooplankton (Net PP$_{graz.}$) in the surface layer of the Sevastopol Bay

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