Comparison of microplankton heterotrophic-photoautotrophic balance based on the content of ATP and chlorophyll a in the plankton of the northern area of the Black Sea during the autumn and spring seasons

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Abstract
Based on materials on the distribution of microplankton ATP and chlorophyll a concentrations in the euphotic zone of the Black Sea, collected on expeditionary cruises R / V “Professor Vodyanitsky” at October 2016 and at March-April 2017, the heterotrophic photoautotrophic index (HPI) reflecting the ratio of heterotrophic biomass and its photoautotrophic parts of the microplankton community was calculated. The interest in comparing precisely these seasons is due to the fact that they are similar in hydrophysical conditions for the development of the community. Water trophicity was estimated by ATP concentrations as an indicator of the metabolically active microplankton biomass. It has been demonstrated that at the autumn season, the studied waters of the landfill can be estimated as mesotrophic, in the spring they are close to eutrophic. With estimation by HPI, at the autumn season heterotrophic microplankton was dominated in most of the water area, and at spring were parity ratios of heterotrophic and photoautotrophic microplankton biomass.

Key words: The Black Sea, microplankton, ATP (adenosine triphosphate), chlorophyll-a, heterotrophic-photoautotrophic index, seasonal changes.

Introduction
In the practice of hydrobiological study, the productivity assessment of waters under study is one of the most important goals. One of the crucial tasks when studying the ecosystem of pelagial is an assessment of the key trophic link, microplankton (Beers & Stewart 1971). Depending on the type of dominant processes, the biomass can grow or shrink (Vinogradov & Shushkina 1983) consequently changes the feed supply for higher-order consumers. In this regard, it seems appropriate for the study of these processes to apply the complex of biochemical methods taking into account the data on chlorophyll a and ATP ratios in microplankton. Therefore such data reflects the production-destruction processes in the community. The suggested approach is different from the classical one due to its relative simplicity of use and efficiency in data processing.
Material and Methods

The water samples were collected with Sea-Bird CTD-probe with a carousel water sampler. Immediately after sampling 1.5 l of sample water was vacuum filtered through SartoriusTM cellulose nitrate membrane filters, 0.45μm pore size, for ATP and chlorophyll \(a\) analyses. A vacuum pressure of -0.2 - -0.4 atm was used.

Filters with sedimeted samples for chlorophyll \(a\) analyses were dried in dark place. ATP extraction was carried out using the Holm-Hansen method (Holm-Hansen & Booth 1966). Filters with sedimented samples were placed in centrifuge tubes, immersed in 5 ml boiling TRIS buffer (pH 7.75) and kept in a boiling water bath for 5 minutes. Extracts then were poured into plastic tubes. Dried filters for chlorophyll \(a\) analyses and tubes with ATP extracts were stored in a freezer at -18°C until further processing.

For the chlorophyll \(a\) analyses nitrocellulose filters were dissolved with 90% acetone and centrifuged. The extinctions of the eluates were measured in the Specol-11 (Carl Zeiss Jena) spectrophotometer. Chlorophyll \(a\) concentrations were calculated using the formula of Jeffrey and Humphrey (Jeffrey & Humphrey 1974).

Samples for ATP content were analyzed with chemiluminescence method by the firefly luciferase-luciferin technique. Light emission was measured in ATP luminometer 1250 (LKB).

The trophicity status of water bodies was estimated by microplankton ATP and chlorophyll \(a\) concentrations according to D. Karl (Karl 1980).

Heterotrophic-photoautotrophic index (HPI) was calculated by the formula: \(\frac{C_{\text{ATP}}}{C_{\text{Chl}\ a}} \times 100\). HPI values 10-20 means the equal ratio of the biomass of heterotrophic and photoautotrophic organisms in microplankton community. HPI >20 indicates of heterotrophs dominance, < 10 – photoautotrophs, according to criteria developed by Chiaudani and Pagnotta (Chiaudani & Pagnotta 1978).

The previously applied comparison of microplankton triphosphate (ATP) and chlorophyll \(a\) concentration enabled to obtain relatively accurate estimate of production-destruction processes in the waters of the Black Sea and the Antarctic (Sysoev & Sysoeva 2005; Sysoeva et al. 2002).

Results and discussion

At the autumn season, based on the microplankton chlorophyll \(a\) concentrations in surface waters and photic zone, it can be concluded that the most productive waters (communities) were located in the eastern zone of the investigated region. The increased local chlorophyll \(a\) concentrations were registered in the coastal waters adjacent to the eastern part of the Crimean peninsula. In the open sea waters chlorophyll \(a\) concentrations were typical for oligo-mesotrophic waters (0.1-1 mg m\(^{-3}\)), while in the near-shore Crimean waters concentrations were close to values that are associated with eutrophic waters (> 1 mg m\(^{-3}\)) (Fig. 1).

Metabolically active microplankton biomass distribution was less dependent on proximity to the coast and sea depth than the microplankton production potential, according to the ATP concentrations observed. High ATP concentrations values did not have localization patterns. This distribution may be due to the fact that organic component of the food chain did not have a localized source. By ATP concentrations the study area waters can be attributed to mesotrophic (75-250 ng l\(^{-1}\)), with the inclusion of extensive eutrophic zones (> 250 ng l\(^{-1}\)).

At spring, considering the microplankton chlorophyll \(a\) contents in the surface waters and photic zone, its most productive part was located in the eastern zone of the study area, and in the waters adjacent to the Kerch Strait. It green pigment concentrations in this regions reached values typical for eutrophicated waters (> 1 mg m\(^{-3}\)) (Fig. 2).

The distribution of ATP was slightly different from the one of chlorophyll \(a\). Values of surface ATP concentrations were, as a rule, typical of mesotrophic waters. In general, higher ATP concentrations in photic zone were identified in the peripheral regions of the investigated water area at a great distance from the Crimean peninsula and in the southwestern part of the investigated area from the east and west (Fig. 3). Heterotrophs were mainly dominant in the northwestern part of the investigated region, in the region adjacent to the shallow northwestern part of the Black Sea. Distribution trends in photic zone were similar to those in surface water layer. The exception was the significant excess of heterotrophic biomass of microplankton above the photoautotrophic biomass in the middle part of the investigated region (Fig. 4).
Figure 1. Distribution of microplankton chlorophyll \( a \) and ATP concentrations in the Crimean coastal waters and deepwater northern part of the Black Sea at October 2016.

Figure 2. Distribution of microplankton chlorophyll \( a \) and ATP concentrations in the Crimean coastal waters and deepwater northern part of the Black Sea at April 2017.
Figure 3. Distribution of microplankton HPI in the surface waters of the Crimean coastal waters and deep-water northern part of the Black Sea at October 2016.

Figure 4. Distribution of microplankton HPI in the photic zone of the Crimean coastal waters and deep-water northern part of the Black Sea at October 2016.
Figure 5. Distribution of microplankton HPI in the surface waters of the Crimean coastal waters and deep-water northern part of the Black Sea at April 2017.

Figure 6. Distribution of microplankton HPI in the photic zone of the Crimean coastal waters and deep-water northern part of the Black Sea at April 2017.
Estimation of heterotrophic-photoautotroph distribution

At the autumn season, according to identified heterotrophic–photoautotrophic index (HPI), heterotrophic forms of microplankton dominated on a greater part of the investigated region. The balance between heterotrophic and photoautotrophic microplankton was observed in waters adjacent to the Crimean peninsula and in the southwestern part of the investigated area from the east and west (Fig. 3). Heterotrophs were mainly dominant in the northwestern part of the investigated region, in the region adjacent to the shallow northwestern part of the Black Sea. Distribution trends in photic zone were similar to those in surface water layer. The exception was the significant excess of heterotrophic biomass of microplankton above the photoautotrophic biomass in the middle part of the investigated region (Fig. 4). In surface waters, HPI values corresponding to balanced heterotrophic: autotrophic biomass ratios were registered in the waters adjacent to the Crimean peninsula in the northwestern, southern and eastern parts, particularly in the Kerch Strait area (Fig. 5). Heterotrophic biomass was concentrated in the northwestern part of the investigated area, similar to that observed in the autumn season. Differences in the HPI distribution between surface waters and the photic layer, in general, were significantly less than in autumn. It could indicate a much smaller gradient of vertical stratification of water masses in the spring season, compared to the autumn one.

When compared the distribution of metabolically active biomass and HPI, there was a spatial similarity revealed between the high values of the total microplankton biomass and the increased inclusion of the photoautotrophic part of it.

Conclusion

The comparison of microplankton distribution in the Black Sea in spring and autumn time is curiously due to the similarity of hydrophysical parameters of the aquatic environment. In general, if comparisons of distribution of metabolically active biomass and its productive part in the given seasons are made, the similarity in the values can be found in the Crimean shallow near-shore areas: the low ones, with oligomesotrophic value, are in waters at the western part of the Crimean peninsula and the higher ones, with meso-eutrophic value, are in the waters at the eastern shores of the Crimean peninsula, in the waters adjacent to the Kerch Strait. When comparing the distribution of metabolically active biomass and heterotrophic-photoautotrophic index, a spatial similarity in high values of the total microplankton biomass and the production capabilities rate of primary products has become evident during both seasons in the greatest part of the water area under study. Exceptions are the areas with high HPI. The largest local differences in the HPI distribution in terms of season have been identified in deep waters south of the central part of the peninsula: in autumn time HPI values indicated a far exceeding dominance of the heterotrophic forms of microplankton, whereas, in spring season, the equal values of heterotrophic and photoautotrophic microplankton have been registered in the same water area. By and large, the averaged HPI values at autumn turned out to be significantly higher than the ones at springtime. Therefore, it can be concluded that biotic factors played the dominant role in the process of forming a productive–destructive succession stage of microplankton community in the given seasons when the similarity of hydrophysical parameters was not of great significance.

Acknowledgements

This study was carried out under research project IBSS no AAAA-A18-118021490093-4 and AAAA-A18-118020790229-7 and partly supported by the Russian Science Foundation (project no. 18-45-920015). The development of the method was carried out within the framework of the topic “Comprehensive Studies of the current state of the ecosystem of the Atlantic sector in the Antarctic”, No. state registration AAAA-A19-119100290162-0.

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