Specific features of the daily dynamics and vertical distribution of bioluminescence in the period of algae bloom in the coastal waters of the Sea of Japan

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In this paper, we consider specific features of the daily dynamics and vertical bioluminescence distribution of the luminous phytoplankton in the coastal zone of the Sea of Japan in the period of algae bloom. For estimation of algae luminescence intensity, we apply the method based on measurement of the integrated bioluminescence signal of plankton netting samples at luminescence stimulation with ultrasound. It is shown, that this method allows one to perform a clear separation of the bioluminescence signals from phyto- and zooplankton. It has allowed us to establish daily rhythm of the luminous phytoplankton division, as well as to obtain the characteristics of its vertical distribution and to show that no daily vertical migration observed in the period of full algae bloom. It is shown that modification of their vertical distribution is caused by a significant survival of the young-grown cells.

Introduction

Inhomogeneity of the spatial distribution of phytoplankton occurs not because of random fluctuations of abiotic factors, but it is an inherent feature of the plankton communities, the nature and specific features of which are not yet properly investigated.[1,2] The existence of large-scale inhomogeneities of the plankton distributions (population and subpopulation) has been shown in a number of papers.[3,4] However, the regularities of a smaller scale (from tens kilometers and down to their minimum dimensions caused by the local turbulence) are known very poorly. It is explained by the fact that researches aimed at biological mapping on such scales are too laborious and very complicated. In addition, they require highly skilled personnel and methods using research vessels. The inhomogeneities of the phytoplankton distribution are most pronounced in the period of algae vegetation.

In the environment, there are two kinds of water bloom: seasonal, natural outbreaks of the phytoplankton growth (annual cycles) and incidental, i.e. red tides. The red tide is a conditional, widespread term for intense water bloom in the sea. At intense growth of microorganisms (bacteria, algae, and infusorians), the seawater changes its color and becomes yellow, rubiginous or pink. This circumstance has also served the basis for choosing the name for this phenomenon known since the most ancient times. The red tides by its shape can vary from the local spots up to the strips several miles long lasting from 2 days to months.

Most frequently, plankton algae[5] cause the mass "bloom". The fact that usual seasonal phytoplankton growth will grow into a flash of the red tide depends on a number of factors like abundance of the necessary biogenic elements, as well as favorable hydrological and meteorological conditions. The intense pollution of internal seas and coastal waters on the global scale led to a significant change of the environment. It has served the cause of more frequent occurrence of local zones of the mass algae bloom. Moreover, in many cases the vegetation of toxic species accompanies it.

During the past decades, the red tides have left the category of phenomena and became of natural disasters causing huge damage, including the human victims. The mass vegetation of toxic species leads to the diseases and deaths of the fish, toxicity of mollusks, which can accumulate toxins and store them for some time without any obvious damage for themselves. Using these products in food may cause poisoning, including lethal cases. Especially frequently, this phenomenon is observed on the Pacific and Atlantic coasts of the USA, in Canada, England, Japan, and nearby the coast of Kamchatka.[5–7] The increasing occurrence of these phenomena, unpredictability and grave consequences demand more and more attention to their studies.

Mostly the toxic tides are caused by the dinophyte algae (peridineans, dinoflagellates). One of the features of these algae including the species producing toxins is their ability to luminesce. The cells respond to the external mechanical, electric or chemical actions by the light signal. This circumstance enabled creating the instruments and techniques for measuring vertical and spatial luminescence distribution in the sea and using these data for estimation of the phytoplankton distributions.[2]

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In this paper, we consider the features of daily dynamics and the vertical bioluminescence distribution of the luminous phytoplankton species in a coastal zone in the period of algae bloom. The data have been obtained during the long-term study of the phytoplankton bioluminescence dynamics in the coastal waters of the Sea of Japan. The observations have covered the periods of the mass algae vegetation to full seasonal bloom close to the red tide. For estimation of algae luminescence intensity, we used the method based on measurement of the integrated bioluminescence signal from the plankton netting samples at the luminescence stimulation by ultrasound. We used a small-size Juday net (the cell size of 40 μm) to sample the plankton. The algae cell concentration in the netting sample is increased by 150 times. The luminescence stimulation was performed with the ultrasound at 880 kHz frequency. Radiation intensity at the luminescence stimulation in a sample made up 2 W/cm^2 that provided the cell preservation after the stimulation and its ability to withstand multiple excitation. Both duration and intensity of the stimulation were calibrated. The photometer calibration was carried out with the help of light standard containing C^{14}. The measurement time for each sample was 5 to 10 minutes. In analyzing of simultaneously collected samples, the differences in the species composition of plankton communities have been followed up.

In recording the plankton's bioluminescence starting from some depth and up to the surface directly in the sea with the instruments that enable one to observe the luminescence signals from plankton organisms in the mode of continuous sounding using mechanical stimulation of the luminescence the recorded images of the light flashes are distorted. The distortions happen due to both the nature of light signals themselves and due to the number of instrumental characteristics. The same refers to the instruments with an active circulation of the water samples. Thus, for separating the signals from phyto- and zooplankton, we use special computation methods, which allow one to obtain approximate characteristics of plankton vertical distribution[2] conditionally, from the shape of earlier received signals.

The method of studying the luminescence of plankton samples at ultrasonic stimulation has significant advantages over the methods that use mechanical stimulation of the luminescence. It allows obtaining the signals, which have not been distorted by the instrument, separating the signals of bioluminescence from phyto- and zooplankton and, using the plankton property of recovering after the stimulation, which makes it possible to repeatedly investigate the same sample. In Fig. 1, the bioluminescence signals from samples containing only the phytoplankton, zooplankton, and phyto- and zooplankton are depicted.

Since the time delay of light pulse from the beginning of the irritating effect for the zooplankton is always longer, than for the phytoplankton, the initial part of the phytoplankton flash is always clearly distinguished in the signal. Taking into account the exponential behavior of the phytoplankton flash decay it is possible to reconstruct its shape precisely enough in spite of the zooplankton luminescence superposed on it at later moments.

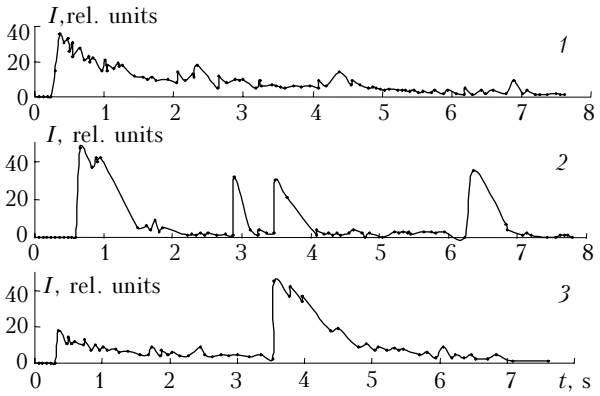

Fig. 1. Records of integrated signals of plankton samples containing: (*1*) only phytoplankton; (*2*) only zooplankton; (*3*) mixture of phyto- and zooplankton.

As seen from the figures, separation of light signals from phyto- and zooplankton is possible even in the case of samples containing a mixture of both species. Since the phytoplankton luminescence energy at its constant species composition is proportional to its population, the population can be estimated from measured intensity of the bioluminescence as it has been shown in our earlier paper.[8]

As known, there exist endogenous and exogenous daily rhythms of the plankton luminescence. In estimating the population of the luminous phytoplankton species directly in the sea, it is necessary to take into account possible presence of the luminescence rhythms determined by the intracellular processes. The vertical phytoplankton migration can also be the cause of changes in the vertical distribution of its population.[9] In order to estimate the influence of daily rhythms of the plankton luminescence and migration on the change of bioluminescence intensity, a modeling experiment has been carried out. A polyethylene reservoir $3\times3\times3$ m^3 volume has been submerged in the surf zone with the ports of 1×1m^2 size made from wattled material with the cell size of 40 μm, sewn in all sides and bottom of the "aquarium" that was filled in through the top in the daytime, when the population of the plankton luminous species is minimum. The initial population of the bioluminescent species in the "aquarium" was estimated to be in the limits from 20 to 40 cells/l. The observation results are presented in Fig. 2.

The population of the bioluminescent species was expected to increase monotonically within several days. However the obtained pattern of the

bioluminescence dynamics in the "aquarium" and in parallel in a control space outside of the "aquarium," turned out to be qualitatively identical, with an insignificantly lower population of the luminous phytoplankton in the "aquarium." The bioluminescence intensity and, correspondingly, the number of luminous cells start to increase sharply with the beginning of dark time of the day. It achieves the maximum value by 23 hours and after that sharply falls off to the minimum value by the midday when the amount of luminous species decreases down to the several cells in 1 l.

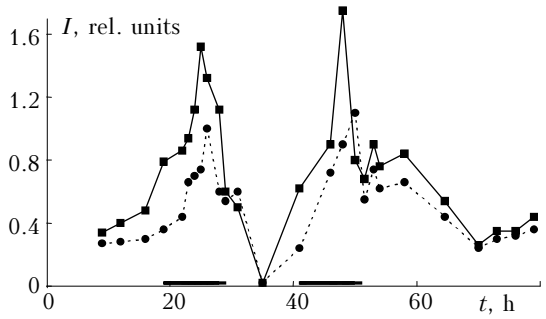

Fig. 2. The phytoplankton bioluminescence intensity in a coastal zone (solid line); in the experimental "aquarium," submerged in the same zone (dashed line).

In laboratory experiments with samples it was shown, that in the luminous phytoplankton species, widely growing in the period of fall bloom, the endogenous rhythm of the luminescence is not observed and the inhibiting effect of the daylight on bioluminescence is clearly seen. Therefore, for measuring the luminescence intensity at daytime, the inhibiting effect of the luminescence by the daylight was removed by keeping the investigated sample in darkness during ten minutes. It is groundless to assume, that at night, shown in Fig. 2 by the dark lines along the abscissa axis, the cell bioluminescence in the "aquarium" was inhibited by light (night illumination in the experiment zone was missing) or that cells of the luminous phytoplankton were eaten by the zooplankton or mollusks. The zooplankton population in the "aquarium" was minimum, and mollusks were missing. In the morning hours and in the daytime, the agglomerations of dinoflagellate cells at the bottom of the "aquarium" were not observed, which could be connected with the vertical migration of cells and this explains the morning recession of luminescence intensity.

All the above-mentioned allows one to explain the result obtained in the experiment by synchronous cell fission in the nighttime with the loss of bioluminescence ability by the juvenile cells which can freely penetrate inside the "aquarium" because of their small size through the screen filters in its sides and bottom. It also explains the course identity of the luminescence daily rhythms in the sea and inside the "aquarium." This conclusion is proved by the investigations showing the presence of dinoflagellate synchronous fission under natural conditions.[10] The researchers of the Institute of Oceanology of the Russian Academy of Sciences carried out these investigations in the Black Sea.

For estimation of the vertical migration capability of the luminous phytoplankton species directly in the sea, the characteristic measurements of the vertical luminescence distribution in different bays of the Possiet gulf of the Sea of Japan have been carried out during the nighttime both in the initial stage of luminescence growth, and at lowering of its intensity. The typical characteristics of vertical distribution of the bioluminescence intensity in different bays are depicted in Fig. 3.

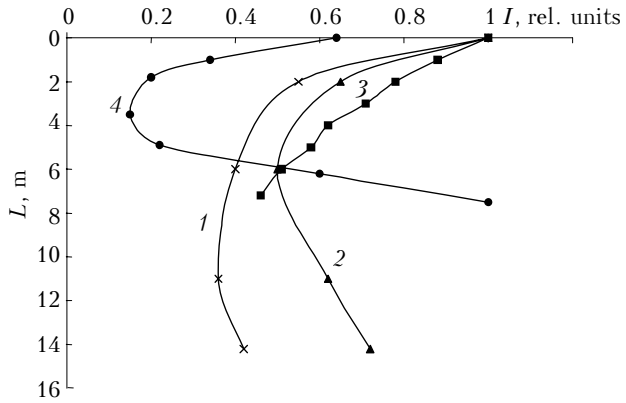

Fig. 3. Vertical distribution of bioluminescence intensity in bays of the Gulf of Peter the Great, reduced to unity: in the center of the bay (*1*); in the zone of 20-m depth (*2*); in the surf zone (*3*); abnormal distribution in the surf zone (*4*).

In order to remove the effect of significant change of luminescence intensity during the nighttime as it has been shown earlier, the maximum values of the luminescence intensity in the figure were reduced to unity. The maximum luminescence intensity and, consequently, the maximum population of the luminous phytoplankton are concentrated on the surface (Fig. 3, curves *1–3*). The luminescence intensity falls equally with the depth. Sometimes we observed an insignificant increase of the luminescence intensity in the bottom layers.

Thus, the conducted experiments have not shown the presence of daily vertical migration of the luminous phytoplankton in the sea under regular conditions. At the same time, an anomalous vertical distribution of the luminous phytoplankton (Fig. 3, curve *4*) has been recorded. Such a vertical distribution of the luminescence intensity lasted during 2 days in the places where the fish scrap was buried on the bay bottom. In this case, high luminescence intensity was also recorded in the surface water layer.

Because of a rather short life of the adult cells of the luminous phytoplankton after beginning of dark time of the day, since 21 until 23 hours, the

grown cells simply have no time to migrate to areas where conditions are more favorable for their existence. After this time, the period of intensive cell fission begins. This is proved by the results obtained at studying vertical bioluminescence distributions directly in the sea and in the experimental "aquarium." Therefore, the recorded anomalous shape of the vertical distribution of the phytoplankton luminescence intensity can be a consequence of a higher survival probability of young-grown cells under favorable conditions at modification of the vertical distribution of their food resources.

Such conditions keep in the surface water layers and appear in the bottom layers due to the diffusion of biogenic elements from the buried scraps. At the division of the adult cells in two, four or eight, and in the case of favorable conditions of the juvenile cells, which are carried by the current and turbulence in the surface waters, the algae cells rapidly occupy a newly appeared suitable niche, intensively growing in the favorable environment.

At research of spatial algae distribution, especially in the areas of algae vegetation, the question arises on determination of the place and moment of the change in the phytoplankton species composition. Such observations are of special importance in the presence of toxic species. In the course of this study, we have tested the method based on studying the characteristics of the luminescence intensity fall off at multiple excitation of a plankton sample. This method enables one to estimate the light energy that aliquot with the given plankton community can release in response to the external pulse excitation. The experiments have been carried out at various species composition of the luminous phytoplankton. The obtained characteristics are shown to be different for the netting samples with various species composition of luminous cells.

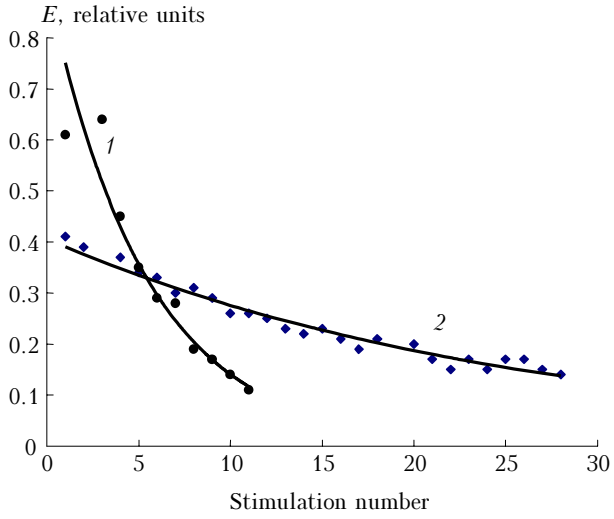

Fig. 4. Characteristics of the energy decrease of the bioluminescence pulses at multiple excitation of the plankton sample: (*1*) in summer, (*2*) in fall.

The characteristics of the luminescence energy decrease ($E=\oint I(t)\mathrm{d}t$) in summer and fall season samples at multiple excitation of the luminescence are presented in Fig. 4.

The obtained characteristics are well approximated by the exponential functions. The square of the correlation coefficient for both curves is about 0.96. For summer season, the parameter of the luminescence fall off is equal to 0.186, for fall it is 0.0387. High speed and accuracy of this parameter measurement allow using it for the express testing of the species composition changes in the luminous phytoplankton.

Thus, the conducted experiments on studying the bioluminescence in the period of fall growth of the phytoplankton have shown, that bioluminescence intensity and, correspondingly, the population of adult species of the luminous, potentially toxic plankton species, have a well-defined daily rhythm and sharply change in the early night hours. This circumstance significantly complicates the estimation of their population using standard biological methods, when sampling of plankton is carried out with the four-hour intervals. The estimation of juvenile cells population in the daytime also does not allow determining the future number of adult species because of unknown survival factor for young-grown cells. For the corrected estimation of the dinoflagellate population in early night hours sampling with an interval of about tens of minutes is necessary.

The method of recording the phytoplankton bioluminescence with the use of ultrasonic luminescence stimulation allows estimating rapidly the luminescence change, establishing the moment of its maximum growth, i.e. the maximum dinoflagellate population, and significantly simplifies the solution of this problem. The capability of distinctly separating the phyto- and zooplankton light signals is used for obtaining the characteristics of vertical distribution of the phytoplankton luminescence. In nature, the vertical migration of the luminous phytoplankton was not observed in the investigated coastal waters.

The conducted experiments allow one to conclude, that modification of the vertical distribution of the luminous phytoplankton species can be determined by the change of the survival degree of the juvenile cells. This phenomenon takes place depending on the environmental characteristics, in particular, on feeding conditions at various depths, instead of being the compulsory consequence of the phytoplankton migration. It was established, that the parameters of luminescence decrease in the case of multiple stimulation of the plankton sample considerably change depending on the species composition of the plankton. Therefore, it is possible to observe the moments of the species composition change in the luminous species community, in studying spatial algae distribution or its variability in time.

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