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RESEARCH PAPER

Study of Parameters of Biological Rhythms of Plankton Communities in Natural Conditions

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Abstract

The change in the intensity of bioluminescence fields in the coastal area of the western shelf of the Crimea in the dark time of day was discussed. It is noted, that the biomass of luminous organisms closely correlate with the biomass of plankton and other pelagic creatures, including commercial pelagic fish. The parameters of the basic biological rhythms of plankton communities are found using method of Fourier series. These rhythms leads to change of intensity of the bioluminescence field. It is shown, that the change of the intensity of bioluminescence field with a 14 hours period due to duration of photo- and dark- periods. Changes in the intensity of the bioluminescence fields with periods of 4.7 and 2.8 hours due to endogenous circadian rhythms of plankton community. An original method for evaluation of errors of periods found of biological rhythms was proposed. The correlation coefficient between measured and calculated values of the intensity of the bioluminescence fields was estimated, taking into account the influence of three main biological rhythms, was r = 0.906, that confirms the correctness of the assumptions made.

Keywords: Bioluminescence fields, biological rhythms, transform fourier, periods spectral components, measuring window.

Introduction

The important element of Black Sea planktonic community functioning is the intensity of bioluminescence field originated by luminous organisms. The intensity of the bioluminescence field changes periodically (Ugarova and Brovko, 1981; Gitelzon *et al*., 1992; Widder, 2001; Cherepanov *et al*., 2007; Haddock *et al*., 2010; Widder, 2010; Kratasyuk and Esimbekova, 2012; Melnikova, 2014). This is due to the fact that the majority of physiological and biochemical processes of living organisms vary rhythmically during the day. The biomass of luminous organisms is closely associated with the biomass of plankton and other pelagic inhabitants, including commercial pelagic fish species. Therefore, informative characteristics of processes of vital activity of marine communities are very important (Kideys *et al*., 2000; Koray, 2001; Kudryasheva *et al*., 2002; Rudneva *et al*., 2008; Bat *et al*., 2011).

The analysis of literature data has showed that the life cycles of most species of luminous aquatic organisms can be consisted of several repeating at regular intervals processes, called biological rhythms.

In particular, many physiological processes of microalgae have the endogenous circadian rhythms: at nutrition, breathing, growth, pigments formation and other. It is known that the majority of physiological and biochemical processes vary during the day. Some of the processes activate in the dark period of day, others in the photoperiod.

Daily change of daytime and night allow to divide light-dependent and dark process (such as growth and reproduction) of phytoplankton in time. As a result, processes at darkness are active when the sun sets and periodically repeated every dark period of day (Melnikova *et al*., 2013).

In recent years, more and more methods are widely used for studying life processes, occurring in biological systems and based on measurement of parameters of physical fields arising during life of biological systems (including marine communities). One such physical field is bioluminescence field produced in the Black Sea by luminous plankton. The use of high-speed devices that measure the intensity of bioluminescence field, can be applied to the study of migrations, the spatial and temporal distribution and other processes of aquatic communities included bioluminescent organisms (Melnikova and Lyamina, 2014, 2015).

The use of biophysical equipment allows the

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study of the vital processes of pelagic communities in natural conditions in real time without disturbing the structure and interspecific relationships of aquatic organisms.

Not only the opportunity of identification of biological rhythms of pelagic communities, but also evaluation of their parameters and peculiarities of interspecies relationship appears at combination of analytical and mathematical methods of processing of amplitude-temporal variability of the bioluminescence field intensity created by pelagic communities.

The aim of study is identification of parameters of biological rhythms that affect the change in intensity of bioluminescence field generated from plankton communities at their life in the dark, as well as estimation of parameters error of rhythms determining caused by non-multiplicity of rhythm period and duration of temporary of measurement window.

Material and Methods

Data collected in October of 2012 in the dark period of day in coastal area of Sevastopol (Kruglaya bay) were used as the material of investigation (Figure 1). The depth in the study area was nearly 70 m.

The spatial structure of the intensity of bioluminescence field was studied using method of multiple bathymetric sounding of the water column, using hydrobiophysical complex "Salpa-M". The measurements were made at the dark. The first measurement coincided to sunset (17 p.m.), the last - to the sunrise (6 a.m.). Total duration of the measurement time (called time-measuring window) was 14 hours. The duration of measurement equal to one period of reiteration of life processes, taking place in the dark. Ten soundings were done every hours with 2 minutes intervals. Then, the data were averaged for each hour, so discreteness of measurements was 1 hour. The measurement step for depth layer was 1 m.

Measurements of the intensity of bioluminescence field carried out for depth range from 0 to 60 meters. At the analysis of the results obtained the differentiation of range noticed on 5 meters layers of depth was carried done. Then, the 5-meter layer with maximal value of intensity of bioluminescence fields and depths ranged within intensity of bioluminescence exceeded 0.5 P_{max}. It means that the depth range with high level of bioluminescence has been allocated. It was a depth range from 0 to 35 meters. For this layer further analysis has been carried out.

The discrete transform of Fourier was used as the method of determining of the amplitude-temporal characteristics of biological rhythms and periodic changes of the intensity of bioluminescence field.

The Fourier transform allows us to present the original time dependence of the measured intensity of bioluminescence field as a summation of harmonic functions (spectral components) with different periods and amplitudes that characterize the peculiarities of aquatic communities. The spectral components obtained constitute the frequency spectrum of original time series of intensities of the bioluminescence field.

The presentation of Fourier series will be written as (Jenkins and Watts, 1972).

$$I(t) = a_0 + \sum_{n=1}^{N/2} A_n \cos\left(\frac{2\pi n t}{T_0} + \varphi_n\right). \quad (1)$$

$I(t)$ — the intensity of bioluminescence field at time t;

$a_0 = \frac{1}{N}\sum_{j=1}^{N} x_0(j)$ — constant value or zero harmonic;

$x_0(j)$ — initial value of the time series in the j-th time;

N — number of data points of the original time

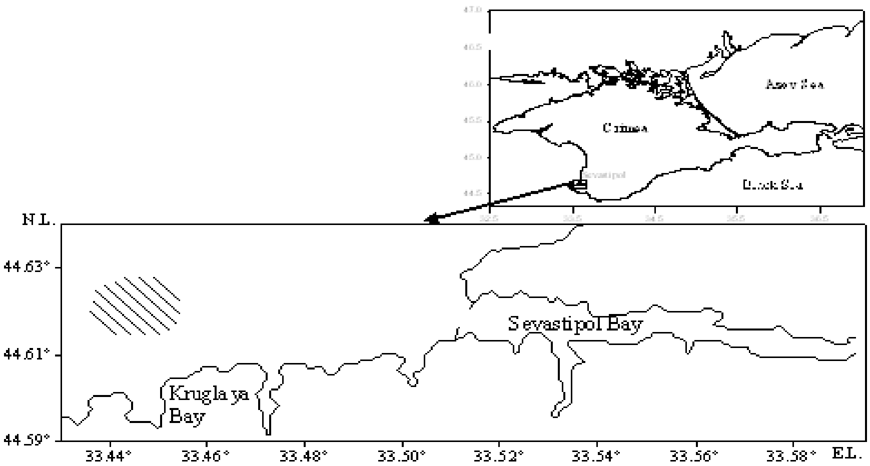

Figure 1. Scheme of the study region of the Kruglaya Bay, Black Sea (costal area near Sevastopol).

series;

$N/2$ — the number of harmonics of a Fourier series; $n = 1, 2, \ldots N/2$ – the number of harmonics; T_o — the period of the first harmonic equal to the duration of temporary measurement window; A_n — the amplitude of the n-th harmonic.

$$A_n = \sqrt{a_n^2 + b_n^2} \qquad (2)$$

a_n, b_n — the coefficients of the Fourier series.

φ_n — the initial phase of the n-th harmonic, which is calculated from:

$$\varphi_n = arctg\,\frac{b_n}{a_n} \quad \ldots\ldots \qquad (3)$$

Periods of spectral components in Fourier series are arranged in a number of periods decreasing and multiples of duration of the measurement window.

The period of n-th harmonic is calculated from:

$$T_n = \frac{T_o}{n}. \qquad (4)$$

where T_n — period of n-th harmonic.

Let´s discuss the influence of unlimited duration of temporal window of sequence of measured values on spectral composition of multiple repetitive process (Krivosheev, 2006).

Limited in time series of measured intensities of bioluminescence field can be presented as multiplication of the original repeated several times signal and the rectangular pulse with duration T_o that is equal to duration of multiplication of measurements:

$$I_o(t) = I(t) \cdot W(t)$$

where $I_o(t)$ — the intensity of bioluminescence field within temporal measurement window that have duration T_o;

$I(t)$ — the intensity of bioluminescence field of oft-recurring process;

$W(t)$ — a rectangular pulse with a constant amplitude with duration equal to duration of the temporal measurement window.

So, the spectrum of periodical process investigated, that is determined by intensity of the bioluminescence field $I(t)$, according to the properties of the Fourier transform (Marple-ml, 1990; Krivosheev, 2006), will be equal to multiplication of the spectra of oft-recurring signal and spectrum of rectangular window with duration T_o.

The spectrum of a rectangular measuring window can be found from the relation (Jenkins and Watts, 1972)

$$W(f) = ET_o \left| \frac{\sin\left(\frac{2\pi f T_o}{2}\right)}{\frac{2\pi f T_o}{2}} \right|. \qquad (5)$$

where E — amplitude factor determined by sensitivity of the measuring device.

If the duration of the biological rhythm corresponds exactly to any of the harmonics of the Fourier series (see the formula (4)), according to (5) this biological process in the Fourier series will contain only a single spectral component equal to biological process duration. The amplitude of intensity of bioluminescence field changes caused by this process is determined by formula (2).

If the duration of real biological process is different from the period of the harmonic components (4), the signal spectrum will be expanded. The additional harmonics components are appeared with amplitudes which can be found using equation (5). This expansion of the spectrum is called the "spectral leakage"(Marple-ml, 1990).

This feature has been used for the development of an original method of finding of errors of biological rhythm period. This error is not a multiple of biological rhythm of measurement window duration.

The gist of the method proposed is as follows.

The amplitudes of the additional spectral components that have emerged due to the deviation of the period of real biological process of the grid of frequencies of the Fourier series, from spectral leakage were determined.

Then, according to values of the amplitude of additional spectral components, the deviation of the real biological process from the period of n-th harmonic of discrete Fourier series was determined (formula (4)). This deviation is an error of period of the biological rhythm determined.

Further, taking into account the deviation of rhythm period calculated according ratio (5), the decreases of amplitude of basic harmonics, which were found using Fourier series and characterized changes in the intensity of the bioluminescence field.

Results and Discussion

In Table 1 the average values measured of intensity of bioluminescence field for each hour of probing during dark time of day are presented.

Table 1 shows that the intensity of bioluminescence field during dark time of day is exposed to fluctuations. The intensity of bioluminescence has been gradually increased to 19 p.m., then slight decreased to 20 p.m., then increasing of bioluminescence field to 23—24 p.m. can be seen, then — it is decreasing to 1 a.m. and new rise to 3 a.m. to fall by 6 a.m. So, the general tendency is increasing of bioluminescence field intensity with the onset of night time and its fall to morning.

The processes of the rise and fall of intensity of bioluminescence field are repeated in the same time every dark period of day. This is due to the fact that the change of photo- and dark time of day is a synchronizing factor that provides start of dark processes. This fact allows us to consider them as a

Table 1. The average data of intensity of bioluminescence field on traverse of Kruglaya bay

Number	Time of day, hour	The intensity of bioluminescence field, pW/(cm^2·L)
1	17	267±17
2	18	2050±199
3	19	3350±253 — max
4	20	2730±218 — min
5	21	3030±130
6	22	3700±94
7	23	4880±214 — max
8	24	4300±228
9	1	3010±91 — min
10	2	3760±122
11	3	4800±94 — max
12	4	4012±182
13	5	3530±245
14	6	2890±124 — min

periodic process, so the discrete Fourier transform can be applied to these processes.

The original time series of values of the intensity of bioluminescence field (see Table 1) was decomposed in a Fourier series.

Table 2 shows the parameters calculated from the spectral components of the change of intensity of bioluminescence field. Table 2 contains all spectral components computed for temporal series measured. Total field of bioluminescence (in accordance with formula (1)) was the sum of the spectral components found. The spectrogram of temporal variability of the bioluminescence field intensity is demonstrated in Figure 2.

It can be seen from the results of spectral analysis (Table 2) that the first, third and fifth spectral components have the largest amplitudes. The amplitudes of these spectral components were: 931, 725 and 656 pW/(cm^2·L) respectively. These three significant spectral components (three biological rhythms) were the main contributors to the change of intensity of bioluminescence fields at night. The rest spectral components will be considered as incidental, emerged as a result of mismatch of real biological rhythms periods of harmonics of a Fourier series, determined according with formula (4). These spectral components will not be taken into account in further analysis relating to identification of biological rhythms. Their impact will be considered only at estimation of error of biological rhythms periods determining.

The graphs of changes in the intensity of bioluminescence field depending on processes characterized by three basic harmonics with considering of the constant component (dotted line) are presented in Figure 3.

It is evident that the values of all three harmonics are increasing with dark time of the day coming. Thus, we can conclude that the conversion of photo- and dark- periods is synchronizing factor, providing the origin of biological processes. Such process are more active in dark period. The processes, forming the first harmonic of spectrum, are responsible for slow changes in the intensity of the bioluminescence fields, and processes that form third and fifth harmonic spectrum - for rapid change.

The first harmonic characterizes the process of growing intensity of the bioluminescence field during the dark time of day and the fall in the morning. This cycle is due to the change and duration of the photo- and dark- periods. Analysis of the literature revealed that one of the factors of the daily rhythm of bioluminescence intensity is sunlight. The intensity of bioluminescence changes 30-100 times because of the intensity of the sun's daily motion. (Krasnow *et al*., 1980; Hamman *et al* 1981; Gitelzon *et al*., 1992; Sullivan and Swift, 1994; Li *et al*, 1996; Akimoto *et al*, 2004; Melnikova, 2014). Analysis of the vertical profiles of day and night bioluminescence of Black Sea plankton showed that the total luminescence in October-November in 60-m water layer was significantly higher at night than during photoperiod.

We found that the amplitude of the first harmonic was 931 pW/(cm^2·L), that characterize its contribution to the change in intensity of bioluminescence field during dark time of day.

A characteristic feature of the high-frequency waves (third and fifth harmonic) with a period of 4.7 and 2.8 hours are processes associated with increasing of quantity of plankton cells during day.

The quantitative development of marine phytoplankton depends primarily on the rate of cell division and the intensity of their consumption by zooplankton. This fact was described in some literature references (Lanskaya, 1967; Piontkovsky and Petipa, 1975; Meeson, 1977; Greze, 1979; White, 1979; Stolbova *et al*., 1982; Hastings. 1983; Vedernikov *et al*., 1985; Kovalev, 1993; Wilson, 1998; Akimoto *et al*, 2004; Titlyanov *et al*., 2004; Hastings, 2007). Paper of S.A. Piontkovsky and T.S. Petipa dedicated to the study of circadian rhythm in nutrition at *Acartia clause* showed that the relation between night and photo periods feeding intensity of crustaceans of different age depend on their different

Table 2. The parameters of spectral components of Fourier series

Number of spectral components	Period, hour	Amplitude of harmonic, pW/(cm^2·L)	Initial phase, radian
0	—	3147	—
basic (1)	14,0	931	–2,959
2	7,0	432	–3,069
3	4,7	725	–2,119
4	3,5	411	2,72
5	2,8	656	–3,096
6	2,3	210	2,246
7	2,0	11	0

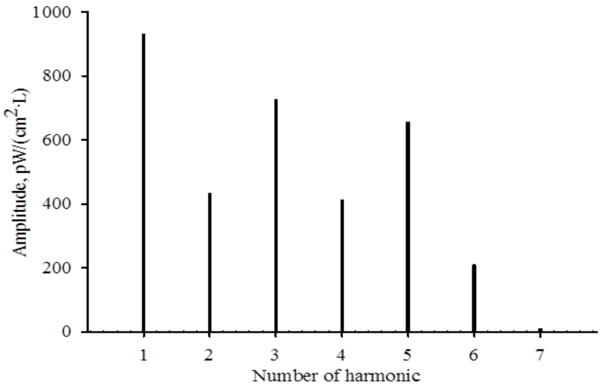

Figure 2. The spectrogram of changes in the intensity of the bioluminescence fields for dark time of day.

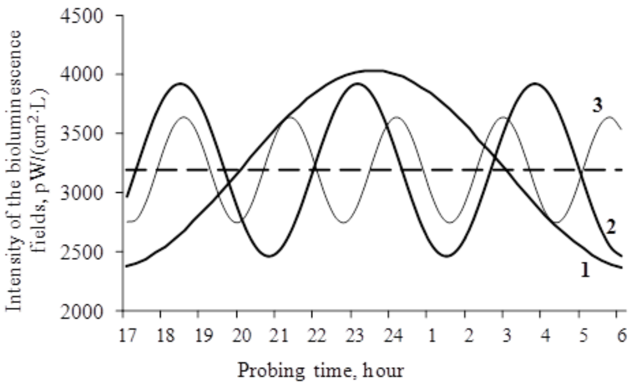

Figure 3. The contribution of the basic biological rhythms in change of intensity of bioluminescence field at dark day of time: 1 — biological rhythm with a period of 14 hours; 2 — biological rhythm with a period of 4.7 hours; 3 — biological rhythm with a period of 2.8 hours; constant component indicated as dotted line.

ability to migrate. If crustaceans migrate intense, they will eat intense also. For example, mature males and females in non-reproduction period and *Acartia clausi* copepodites on phase V migrate more active than being on other stage. They stick deeper water layers in photoperiod. At night, rising to the surface, they are eating with a much greater intensity than during the photoperiod as compared to the other groups. Young copepods and nauplius, in contrast, have smaller amplitudes of migrations and constantly live in the upper water layers, and eat with the greatest intensity during photoperiod of day. The presence of different circadian rhythms of feeding is due to, probably, with different adaptation of age groups of planktonic organisms to light. It is known that males survive at bright light worse than in low light (Piontkovsky and Petipa, 1975) .

The total process of passage of food through the intestine of *Acartia clausi* being on all stages and many other species of copepods in period of relatively intensive feeding on phytoplankton has average duration 3 hours. The duration of digestion increases in average to 5 hours at feeding of additional animal food that influences on circadian rhythm of feeding intensity.

There are some opinions on problem of circadian rhythms of cell division of planktonic algae. L.A. Lanskaya (Lanskaya, 1967) made conclusion, after study of rate of Black Sea phytoplankton cell division in unialgal cultures, that division of most species of dinoflagellates occurs every day, but the maximum number of dividing cells decrease in the evening (18—19 p.m.) and at night. A.V. Kovalev and N.G. Stolbova with coauthors also noticed maximum cell reproduction at night (Stolbova *et al*., 1982; Kovalev, 1993).

The above patterns of phytoplankton cell division during dark time of the day suggest that presence in our research the intensity increased of bioluminescence field at 19, 23-24 p.m. and to 3 a.m. is a result of the prevalence of cell division rate of dinoflagellates and zooplankton grazing in this time.

This character of variability of bioluminescence field intensity indicates that the third and fifth harmonics with periods of 4.7 and 2.8 hours and amplitudes 725 and 656 pW/(cm^2·L), respectively, making a significant contribution to the periodic changes in the intensity of bioluminescence fields at dark period of day, due to endogenous circadian rhythms of plankton community.

Calculations showed that the correlation coefficient between values theoretical calculated for three highest harmonics and measured values of changed intensity of bioluminescence field is r = 0.906. This indicates that the changes described by the combined effect of the first, third and fifth spectral components well characterize the processes that cause the changes of bioluminescence field at dark period of day and confirms the correctness of assumptions.

The estimation of deviation of rhythm periods, found by method of Fourier series from the periods of biological rhythms of plankton communities in nature, has been made by above method.

It is assumed that the error in determining of the first harmonic component is mainly due to the discrete measurements and is equal to half of increment of discreteness of measurements over time, that is, the error is $\Delta T_1 = \pm\ 0.5$ hours.

Deviation of the third and fifth harmonics from discrete in frequency spectral components of the Fourier series values has led to spectral leakage (presence of additional spectral components). It means that the appearance of the second, fourth, sixth and seventh spectral components in the Fourier series due to deviation of the three major significant harmonic components (first, third and fifth) from discrete harmonics of Fourier series, calculated according to expression (4). The analysis of the amplitudes of these harmonics according to formula (5) showed that estimated values of the amplitudes of second (432), fourth (411), sixth (210) and seventh (11) harmonics will be at deviations of first harmonic $\Delta T_1 = \pm\ 0.5$ hours; third harmonic $\Delta T_3 = \pm\ 0.6$ hours and the fifth harmonic $\Delta T_5 = \pm\ 0.2$ hours.

Consequently, the duration of three biological rhythms, making a main contribution to the change of bioluminescence field intensity in the dark period of day and their deviations will be equal to:

$$T_1 = 14 \pm 0.5\ ;$$

$$T_3 = 4{,}7 \pm 0.6\ ;$$

$$T_5 = 2{,}8 \pm 0.2\ \text{[hours]}.$$

Found deviations of periods of basic harmonics lead to a change in the amplitudes of the harmonic components. Calculations carried out in accordance with the formula (5) have allowed to find deviations of basic harmonic amplitude on the curves of intensity of bioluminescence fields:

$$A_1 = 931 \pm 15;$$

$$A_3 = 725 \pm 23;$$

$$A_5 = 656 \pm 40\ [\text{pW/(cm}^2\cdot\text{L)}]$$

Conclusions

It was shown that changes of the intensity of bioluminescence field formed by plankton communities in the dark period of day in the coastal area of Sevastopol can be described by three rhythmic processes with periods of 14 hours, 4.7 hours and 2.8 hours, due to biological rhythms.

Endogenous nature of rhythms was confirmed and the mechanism (use of Fourier series) was proposed for determining of periodicity of the process of reproduction and feeding of phyto and zooplankton at night.

The considered periodic processes lead to a change of intensity of the bioluminescence fields, formed by plankton communities with amplitudes 931, 725 and 656 pW/(cm^2·L). The correlation coefficient between measured and calculated values for three harmonics was equal to r = 0.906, that confirms correctness of the assumptions made.

The estimation of errors of parameters of rhythms, found by Fourier series and due to non-multiple periods of biological rhythms and duration of measurement window, was made. It was showed that the error in rhythm determining which has duration of 14 hours is ± 0.5 hour, 4.7 hours rhythm duration has error ± 0.6 hour, error for rhythm duration of 2.8 hours was± 0.2 hours.

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