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USING THE VERTICAL SOUNDING METHOD FOR RECORDING BIOLUMINESCENCE IN THE ANTARCTIC SECTOR OF THE ATLANTIC OCEAN

© 2023 **L. A. Melnik, A. V. Melnik, O. V. Mashukova, and V. V. Melnikov**

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
E-mail: *melniklidi@gmail.com*

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Bioluminescence is an essential element in the functioning of the pelagic community, which is associated with the key ecological role of light in the life of hydrobionts, *inter alia* in the formation of their spatial heterogeneity. The luminescence of marine hydrobionts is a manifestation of their vital activity in the form of electromagnetic radiation in the spectrum visible area, and its kinetic patterns are closely related to mechanism generating their chemical reactions and metabolic processes. Global warming, which undoubtedly has affected the Atlantic sector of Antarctica, caused serious structural and functional alterations in the pelagic community with repercussion on marine bioluminescence, an expressive indicator of environmental conditions. We aimed at studying the possibility of using the method of multiple vertical sounding by the hydrobiophysical complex "Salpa-M," with simultaneous capture of biophysical and hydrological parameters at one station, to investigate the structure and length of fields of luminescence in Antarctic waters. The paper provides the technique for analyzing structural characteristics of bioluminescence, as well as material obtained during the 79th Antarctic expedition onboard the RV "Akademik Mstislav Keldysh." The core of the sounding method is raising (or lowering) the bathyphotometer "Salpa-M" at a constant speed in a given layer [usually, it is the upper productive (0–200 m) or the photic (0–100 m) layer] in the RV's drift. Planktonic bioluminescent organisms, which are the main contributors to the formation of the bioluminescent potential of the pelagial, mostly illuminate when stimulated. Therefore, a bathyphotometer moving at a constant speed creates a standard level of the mechanical stimulation of bioluminescent organisms, and this allows to compare correctly the results of measurements for the vertical structure of the field of bioluminescence carried out in different areas and under various weather conditions (rolling, wind drift, *etc.*). The paper presents a fairly large data set of the integral bioluminescent signal at different horizons. Primary data on bioluminescence intensity, temperature values, electrical conductivity, and photosynthetically active radiation were obtained at 18 hydrographic stations in the studied water area of the Atlantic sector of Antarctica. The article considers an important issue related to the change in seawater bioluminescence in the Atlantic sector of Antarctica studied by the vertical sounding at different levels with a bioluminescent probe. When investigating bioluminescence, its vertical variability in the upper productive layer was determined in relation to features of plankton distribution. As a result, it was found out that the luminescence of Antarctic waters in the photic layer of this area occurs within the range from 8.4×10^{-12} to 104.42×10^{-12} W·cm^{-2}·L^{-1}. Bioluminescence peaks (up to 104×10^{-12} W·cm^{-2}·L^{-1}) were recorded under the thermocline at a 45-m depth in the areas of concentration of the salp *Salpa thompsoni* Foxton, 1961 near the hydrological front, at a distance of about 6–7 miles on either its side. It is shown that the method of vertical sounding in Antarctic waters allows expressing the fields and the structure of aggregations of luminescent organisms.

Keywords: bioluminescence intensity, Atlantic sector of Antarctica, euphotic zone, vertical sounding, plankton

In Antarctic waters, the most important fishery object is the Antarctic krill *Euphausia superba* Dana, 1852, which forms the basis of the diet of numerous consumers. Its reserves in the Southern Ocean amount to hundreds of millions of tons [Samyshev, 1991]. This species is most common in the circumpolar belt between the Antarctica and the polar front [Nicol, Foster, 2016; Nicol et al., 2000]. To date, assessing the state of krill communities is one of the priority areas of research in the Atlantic sector of Antarctica [Spiridonov, Uryupova, 2009; Sprong, Schalk, 1992].

Krill aggregations luminesce due to bioluminescent photophores located on the body of each crustacean: one pair, on the eyestalks; another pair, on the hips of the second and seventh thoracopods; and individual organs, on four segments of the pleon. These organs periodically luminesce for 2–3 s. Bioluminescence is clearly visible in the dark. It is electromagnetic radiation in the visible area of the spectrum, and its kinetic patterns are closely related to mechanism of the chemical reactions and metabolic processes that generate them [Harvey, 1957]. There are many bioluminescent species of hydrobionts: dinoflagellates, radiolarians, and various mobile multicellular animals from polyps, jellyfish, and ctenophores to squids, crustaceans, and fish [Labas, Gordeeva, 2003]. IBSS researchers discovered the ability to luminesce in 364 phyto- and zooplankton species; out of them, 164 turned out to be bioluminescent organisms, and in 137 species, bioluminescence was shown for the first time [Tokarev, 2006]. Bacteria found in seawater at different latitudes, from tropical to polar ones, are bioluminescent organisms as well. In the open ocean, there are on average up to 1,000 cells of luminescent bacteria *per* 1 L of seawater [Gitelson, 1976].

In terms of energy, bioluminescence of zooplankton is obviously higher than that of phytoplankton or bacteria. Various krill species are characterized by the highest intensity and duration of bioluminescence (up to 22 s) [Tokarev, Sokolov, 2001].

The vertical sounding method for determining the level of bioluminescence can be used for rapid assessment of species diversity and spatial distribution of bioluminescent organisms in the analyzed water area.

We aimed at studying the possibility of applying the method of multiple vertical sounding with a hydrobiophysical complex “Salpa-M,” with simultaneous capture of biophysical and hydrological parameters at one station, to investigate structure and length of fields of luminescence in Antarctic waters.

MATERIAL AND METHODS

Data were obtained in February 2020 (122 soundings at 18 stations in the 79th cruise of the RV “Akademik Mstislav Keldysh”) (Fig. 1). Measurements of a field of bioluminescence were carried out day and night with a probe “Salpa-M” [Tokarev et al., 2016]. This biophysical complex has six measuring and service channels:

1) bioluminescence (measurement range from 10^{-12} to 10^{-8} W·cm^{-2}·L^{-1});
2) temperature (measurement range from –2 to +35 °C);
3) pressure (measurement range from 0 to 2 MPa);
4) photosynthetically active radiation;
5) information transmission and remote operation control;
6) control and signaling.

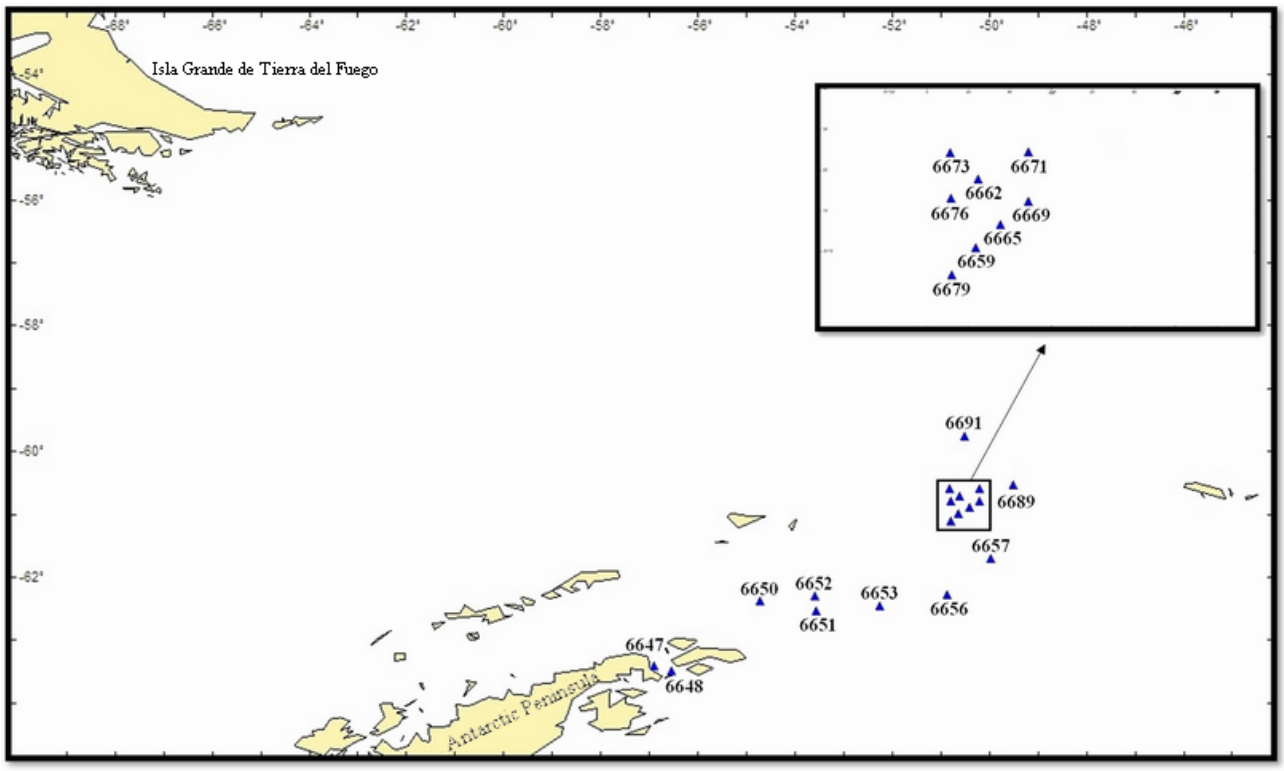

Fig. 1. Map of stations

The measuring channel of bioluminescence intensity. It consists of a measuring chamber, a luminescence collector, a photodetector, a control device, and an interface device, with the latter one used to measure temperature and pressure. The transfer of bioluminescent organisms to an active state in which they luminesce (the irritation) is carried out mechanically; the measuring chamber is used. It consists of eight blade impellers which are located in two groups of four impellers each, spaced apart along the axis.

To reduce the effect of sunlight, four rows of blackened impellers – two groups of rows of mutually perpendicular angles of attack – form a mobile luminescence trap and provide a weakening of light energy by 2×10^7 times with minimal resistance to the incoming water flow. During the axial movement of the bioluminescence probe, the studied incoming water flow enters the measuring chamber. Passing through four rows of blade impellers, water is thoroughly mixed, and bioluminescent organisms it contains are irritated. The latent period (the time before microorganisms begin to luminesce after their irritation) is determined by their species composition. To measure the bioluminescent potential, the time spent by a bioluminescent organism in an active state in the volume of the flow chamber has to exceed the latent period and the duration of its luminescence. Interestingly, the time of occurrence of microorganisms in the measuring chamber after mechanical effect is determined by the flow rate of water through this chamber. When solving the problem of bioluminescence recording, a preliminary analysis of the hydrodynamic flow of water through the measuring chamber was carried out. It showed the need for formation of a turbulent flow in the middle part of the chamber. The discreteness of measurements of characteristics by this complex when probing "down" at a speed of 1.2 $m \cdot s^{-1}$ was 0.25 m. The value was integrated

with software up to 1 m. It is worth noting that the special design of the light pipe forming a conical radiation pattern ensures the transfer of bioluminescence energy of microorganisms from the entire volume of the measuring chamber to the photodetector.

The photodetector. Bioluminescence is low-intensity pulses of light energy. Its measurement is carried out with a photomultiplier tube FEU-71 characterized by a high anode sensitivity (1,000 $A \cdot lm^{-1}$) against the backdrop of a supply voltage of < 1,000 V. The measuring signal of the photomultiplier tube is fed *via* a repeater and subsequent amplification to an interface – an analog-to-digital converter. The information signal is filtered with a constant time of 1–5 s, and this is controlled from a Salpa-T, P shell. The measuring channels for bioluminescence intensity, temperature, electrical conductivity, and hydrostatic pressure are the core ones for the complex.

The habitat of the Antarctic krill is separated from other areas of the ocean by the Antarctic polar front. It is an effective barrier to the spread of marine organisms, and it makes the Southern Ocean a largely isolated ecosystem. The total light effect created by the Antarctic krill is called a field of bioluminescence. Other luminescent hydrobionts make a significant contribution to its formation as well. As a physical field, it is characterized by energy intensity and frequency spectrum. Since this field is formed by biological objects, it is also characterized by biological features: the number of flashes of individual organisms that make this field up and the heterogeneous structure of their distribution in time and space.

When studying bioluminescence of Antarctic waters, we used the method of multiple (5 to 10) sounding of the photic layer of the pelagic zone (1–80 m). Its advantages over other techniques of investigating marine bioluminescence are as follows:

- ability to study the mosaic nature of the spatial distribution of the field of bioluminescence simultaneously with the background characteristics of the environment;
- constant level of effect on the environment and irritation of luminescent organisms;
- possibility to analyze in detail the vertical structure of bioluminescent populations;
- no effect of surface waves on the signal recorded;
- fairly simple solution to the issue of isolating the daily component of the bioluminescence registered.

RESULTS

Hydrological characteristics of the area. At the mesoscale studied site in the Weddell Sea, a hydrological front was detected [Morozov et al., 2020]. It was formed between warmer waters with cold subsurface layer and colder advective waters entering this site from the shallow western Powell Basin (the northwestern Weddell Sea). The research showed that the northern Powell Basin contains relatively warm waters. The frontal zone extended from southwest to northeast in the latitude range from 58° to 61°. The hydrological front was especially pronounced in the photic zone where the temperature difference in the upper layer reached 2 °C [Morozov et al., 2020].

Bioluminescence. The investigation was carried out on a unified grid of stations approved by the general program of scientific research in the 79th Antarctic expedition on the RV “Akademik Mstislav Keldysh.” Bioluminescence was measured day and night. To exclude the phenomenon of photoinhibition, we calculated the coefficients of daily variability for intensity of the field of bioluminescence; with these conversion factors, all data were reduced to those for night time.

In 2020, the analyzed water area was characterized by a seasonal outbreak in abundance of the salp *Salpa thompsoni* Foxton, 1961. This jellyfish-like species absolutely prevailed in zooplankton composition in the area of the southern branch of the Antarctic Circumpolar Current, the Bransfield Strait current, the Antarctic Peninsula coastal waters, and uplifts bordering the Powell Basin from the northwest [Morozov et al., 2020]. The background biomass of euphausiids, including the Antarctic krill, estimated from the Bongo net catches in a 200–0-m layer was two orders of magnitude lower, and the background biomass of other groups was several orders of magnitude lower. These data can be interpreted as a manifestation of the negative effect of the sapl outbreak in abundance on the number of other groups of meso- and macrozooplankton.

Table 1 provides data on the mean amplitude indicators of fields of bioluminescence at 18 stations in the studied area in 2020. The highest level was recorded at sta. 6679 (in the southern Powell Basin) in the area of salp aggregation: the value reached 104.42×10^{-12} W·cm^{-2}·L^{-1}.

Table 1. Volume of sampled material for bioluminescence measurement (2020)

Station No.	Depth of sounding, m	Date	Station start time	Mean bioluminescence, 10^{-12} W·cm^{-2}·L^{-1}	Maximum bioluminescence, 10^{-12} W·cm^{-2}·L^{-1}	Level of maximum bioluminescence, m	Mean temperature, °C	Mean salinity, ‰
6647	35	13.02	14:58	25.93	38.98	11	+0.69	34.29
6648	46	13.02	18:22	5.75	17.52	46	+1.65	35.5
6650	65	14.02	15:54	12.56	25.65	8	+0.22	33.94
6651	65	14.02	22:53	5.14	17.52	35	+0.62	33.72
6652	75	15.02	17:40	4.51	15.99	58	+0.49	33.9
6653	85	16.02	16:05	6.76	25.78	3	+0.39	33.56
6656	65	17.02	14:43	4.61	10.25	65	+0.77	33.25
6657	64	18.02	08:53	4.79	10.25	63	+0.58	33.14
6659	71	19.02	00:09	4.75	10.25	4	+0.69	33.81
6662	70	19.02	06:44	4.61	8.4	67	+1.25	33.7
6665	78	19.02	13:38	6.97	21.03	24	+0.4	33.85
6669	75	19.02	22:11	11.41	103.4	45	+1.15	33.61
6671	75	20.02	03:33	4.41	12.04	61	+1.9	33.79
6673	79	20.02	10:53	8.05	19.49	10	+1.36	34
6676	65	20.02	15:52	11.44	25.66	18	+0.22	33.95
6679	74	20.02	23:15	11.13	104.42	45	+1.15	33.62
6689	75	23.02	16:45	2.71	13.77	26	+1.97	33.24
6691	75	24.02	07:40	1.85	8.4	12	+2.13	33.32

A layer of increased level of bioluminescent potential was registered at a 40–50-m depth, with a single-maximum vertical structure of bioluminescence. Intense outbreaks in this area (against the backdrop of low krill abundance) may be due to high abundance of *S. thompsoni*, since the salp is capable of forming outbreaks of such a potential. As moving north, bioluminescence intensity decreased noticeably. Specifically, at sta. 6676, the level was already 25.66×10^{-12} W·cm^{-2}·L^{-1}. A layer of increased bioluminescent potential was formed at a depth of 15–20 m. At a more northern station, sta. 6673, the value was 19.49×10^{-12} W·cm^{-2}·L^{-1} (Fig. 2).

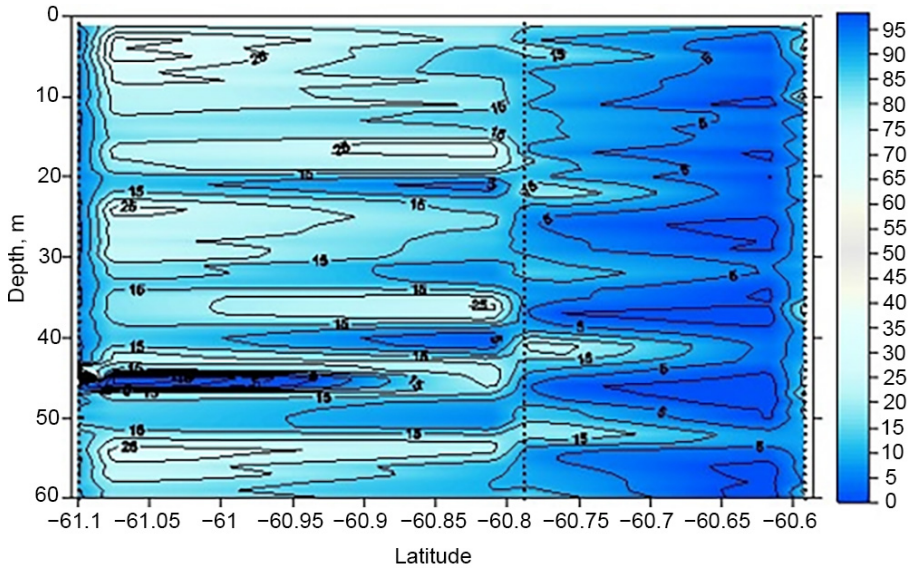

Fig. 2. Bioluminescence: spatial section at stations 6679, 6676, 6673

A layer of increased bioluminescent potential was also recorded at a 10-m depth. The vertical structure of bioluminescence at sta. 6676 and 6673 was similar. Sta. 6647 and 6648 were performed in the Antarctic Sound separating the Joinville Island group from the northeastern tip of the Antarctic Peninsula. In the studied water area, the vertical structure of bioluminescence was characterized by the presence of certain peaks. At sta. 6647, the peak of intensity (25.93×10^{-12} W·cm^{-2}·L^{-1}) occurred at a depth of 11 m. At sta. 6648, the peak of intensity (5.75×10^{-12} W·cm^{-2}·L^{-1}) was registered deeper, at 46 m. The mean level of bioluminescence at sta. 6647 was 38.98×10^{-12} W·cm^{-2}·L^{-1}, and the value was significantly higher than that at sta. 6648 (17.52×10^{-12} W·cm^{-2}·L^{-1}). Sounding at sta. 6651 was carried out at the same time as at sta. 6679 (22:00–23:00), and the mean level of bioluminescence at sta. 6651 did not exceed 5.14×10^{-12} W·cm^{-2}·L^{-1}. Fig. 3 shows mean profiles of bioluminescence, temperature, and salinity values obtained at sta. 6679 and 6651.

In the northwestern Weddell Sea, six stations were performed at different time of the day. The maximum level of bioluminescence (25.65×10^{-12} W·cm^{-2}·L^{-1}) was recorded at sta. 6650 at a 8-m depth. The minimum one (15.99×10^{-12} W·cm^{-2}·L^{-1}) was noted at sta. 6652 at a 58-m depth. The maximum mean level of bioluminescence in the studied water area was registered at sta. 6653 (25.78×10^{-12} W·cm^{-2}·L^{-1}). The vertical structure of bioluminescence at sta. 6650 and 6653 was multi-peaked, and the values were evenly distributed over the entire probing depth. At sta. 6652, a single-maximum vertical structure of bioluminescence was observed.

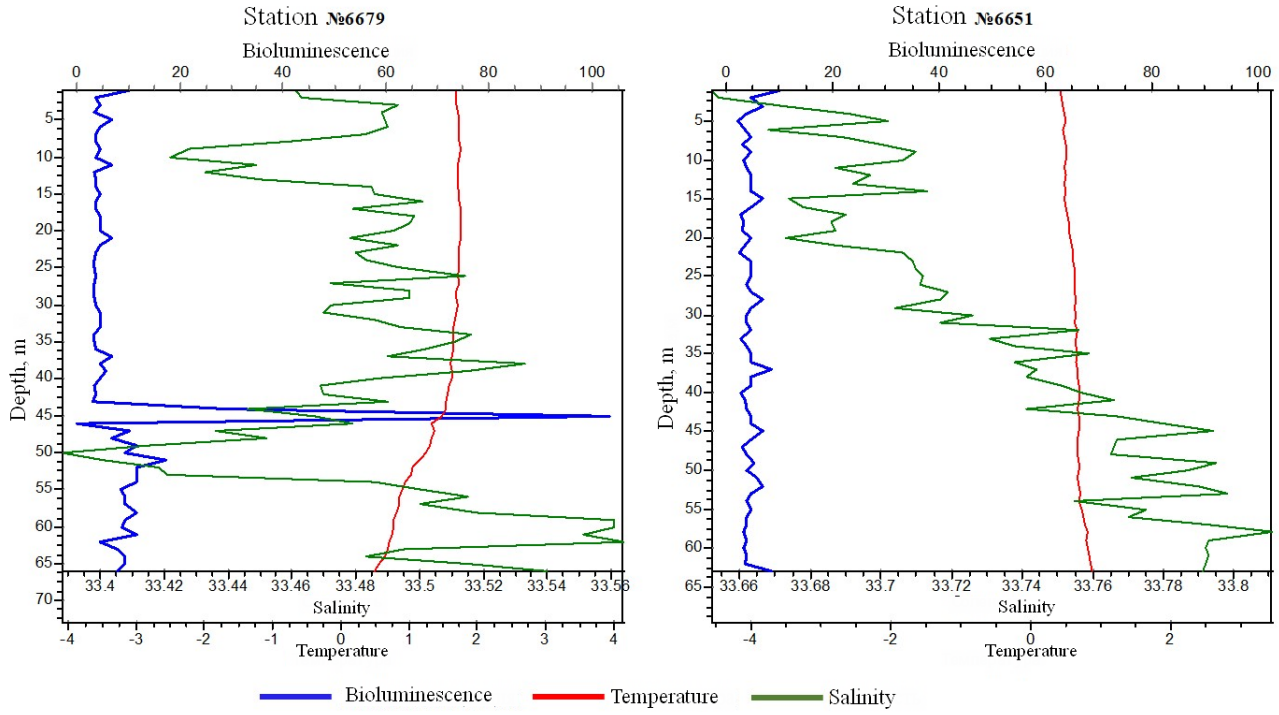

Fig. 3. Vertical profiles of temperature (°C), salinity (‰), and bioluminescence ($\times 10^{-12}$ W·cm^{-2}·L^{-1})

DISCUSSION

The results of this study convincingly showed that the method of multiple sounding with a hydrobiophysical complex "Salpa-M," with simultaneous capture of biophysical and hydrological parameters at one station, is much more effective than other techniques [Kim et al., 2006] for analyzing the structure and extent of fields of luminescence. Among the existing methods for measuring bioluminescence signals in the water column (towing photometers, hanging them on a given horizon, probing certain layers, installing special devices on the bottom, *etc.*), towing and probing are recognized as the most promising and accurate ones [Biolyuminestsentsiya v okeane, 1992]. Their advantage is the registration of bioluminescence by a bathyphotometer moving at a constant speed. Towing can only be used when the RV is moving (at a speed of no more than 4 knots) and on a limited number of horizons, usually in the range of the upper 10 m. The core of the sounding method is raising (or lowering) a bathyphotometer at a constant speed in a given layer (as a rule, the epipelagic or photic one) in the RV's drift.

Thus, applying this method in Antarctic waters is a new opportunity to record the fields and structure of aggregations of krill, salp, and other luminescent organisms.

As found, in Antarctic, one of the main characteristics inherent in the vertical structure of fields of bioluminescent is their stratification which is determined both by the parameters of the pelagic community (species composition, chorological structure, and so on) and the features of water masses. The depth of the layer or layers of maximum luminescence intensity and their number are key characteristics of fields of bioluminescence as well. To date, there is lack of knowledge in the horizontal extent of layers of maximum luminescence intensity for the salp, the mosaic nature of its distribution

at the small-scale level, and the daily, inter-day, seasonal, and inter-annual variability of luminescence intensity. The analysis of this factor, new in the ecology of luminescent hydrobionts, involves carrying out horizontal tows with simultaneous biological sampling and acoustic sounding.

Conclusion. The method of multiple vertical sounding with the hydrobiophysical complex "Salpa-M" turned out to be quite effective in studying the structure and extent of fields of luminescence in Antarctic waters. In 2020, in the Antarctic area investigated, instead of krill fields, salp fields were observed, which may be associated with both climate change and active fishing. The krill creates fields of bioluminescence as continuous flashes of individual crustaceans. The salp forms a completely different field of bioluminescence: its vertical structure is a single-maximum one.

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ИСПОЛЬЗОВАНИЕ МЕТОДА ВЕРТИКАЛЬНОГО ЗОНДИРОВАНИЯ ДЛЯ РЕГИСТРАЦИИ БИОЛЮМИНЕСЦЕНЦИИ В АНТАРКТИЧЕСКОМ СЕКТОРЕ АТЛАНТИЧЕСКОГО ОКЕАНА

Л. А. Мельник, А. В. Мельник, О. В. Машукова, В. В. Мельников

ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН»,
Севастополь, Российская Федерация
E-mail: *melniklidi@gmail.com*

Биолюминесценция — существенный элемент функционирования пелагического сообщества, что связано с важнейшей экологической ролью света в жизни гидробионтов, в том числе в формировании их пространственной неоднородности. Свечение морских гидробионтов — это проявление их жизнедеятельности в форме электромагнитного излучения в видимой области спектра, кинетические закономерности которого тесно связаны с механизмом порождающих их химических реакций и процессов метаболизма. Глобальное потепление, охватившее и Атлантический сектор Антарктики, вызвало серьёзные структурно-функциональные изменения пелагического сообщества, которые отражаются на морской биолюминесценции — экспрессивном показателе состояния среды. Целью работы было изучить возможность применения метода многократного вертикального зондирования гидробиофизическим комплексом «Сальпа-М» с одновременной фиксацией биофизических и гидрологических параметров на одной станции для исследования структуры и протяжённости полей свечения антарктических вод. В статье представлены метод изучения структурных характеристик биолюминесценции и материалы, полученные во время 79-й антарктической экспедиции на НИС «Академик Мстислав Келдыш». Суть метода зондирования состоит в подъёме (или опускании) батифотометра «Сальпа-М» с постоянной скоростью в заданном слое [обычно это верхний продуктивный (0–200 м) или фотический (0–100 м) слой] в дрейфе судна. Планктонные биолюминесценты, вносящие основной вклад в формирование биолюминесцентного потенциала пелагиали, высвечиваются, как правило, только при раздражении. Именно поэтому движущийся с постоянной скоростью батифотометр создаёт стандартный уровень их механического раздражения, что позволяет корректно сравнивать результаты измерений вертикальной структуры поля биолюминесценции, выполняемых в разных регионах и при различных погодных условиях (качка, ветровой снос и т. д.). В работе представлен набор данных об интегральном биолюминесцентном сигнале на разных горизонтах. На 18 гидрографических станциях в исследуемой акватории Атлантического сектора Антарктики были получены первичные данные интенсивности биолюминесценции, значений температуры, электропроводности и фотосинтетически активной радиации. В статье рассмотрен важный вопрос, который связан с изменением биолюминесценции морской воды в Атлантическом секторе Антарктики, изученной методом вертикального зондирования на разных уровнях с помощью биолюминесцентного зонда. При исследовании биолюминесценции выполняли определение вертикальной изменчивости свечения в верхнем продуктивном слое в связи с особенностями распределения планктона. В результате было установлено, что свечение антарктических вод в фотическом слое этого

района происходит в пределах от $8{,}4 \times 10^{-12}$ до $104{,}42 \times 10^{-12}$ Вт·см$^{-2}$·л$^{-1}$. Пики биолюминесценции (до 104×10^{-12} Вт·см$^{-2}$·л$^{-1}$) фиксировали под термоклином на глубине 45 м в зонах концентрации сальп *Salpa thompsoni* Foxton, 1961 вблизи гидрологического фронта, на расстоянии около 6–7 миль по обе стороны от него. Показано, что метод вертикального зондирования в антарктических водах даёт возможность экспресс-регистрации полей и структуры скопления светящихся организмов.

Ключевые слова: интенсивность биолюминесценции, Атлантический сектор Антарктики, фотический слой, вертикальное зондирование, планктон