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# Bioluminescence of the Black Sea Ctenophores-Aliens as an Index of their Physiological State

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<http://dx.doi.org/10.5772/65063>

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## Abstract

Three experiment series on the ctenophores *Mnemiopsis leidyi* and *Beroe ovata* bioluminescence variability investigation were conducted: (1) depending on ctenophores size and ontogeny stage; (2) depending on temperature conditions and (3) depending on season. The ctenophores luminescence was registered using the laboratory complex "Svet" by methods of mechanical and chemical stimulation. Ctenophores light-emission characteristics are changing in the process of ontogenesis and rising proportionally to the organism mass growth. Seasonal dynamics of the ctenophore-aliens light-emission characteristics has been revealed: the highest indices of *M. leidyi* and *B. ovata* bioluminescence are observed in the summer period and minimal indices for both species were registered in the winter-spring period. Environment temperature affects considerably at the amplitude-temporal characteristics of the ctenophores light-emission. The bioluminescence reaction optimum for *M. leidyi* is achieved under the temperature of  $26 \pm 1^\circ\text{C}$ , and for *B. ovate*—under the temperature of  $22 \pm 1^\circ\text{C}$ , while its minimum for both ctenophores was registered under the temperature of  $10 \pm 1^\circ\text{C}$ . Thus, results of the investigations have detected the opportunity to use ctenophores *M. leidyi* and *B. ovata* light-emission characteristics as an index for their physiological state estimation.

**Keywords:** light-emission characteristics, ecological-physiological indices, *Mnemiopsis leidyi*, *Beroe ovata*, the Black Sea

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## 1. Introduction

Bioluminescence as a manifestation of an organism life activity in a form of electric-magnetic radiation in the visible region of spectrum is the most important ecological factor of marine environment [1]. Quite recently, they considered that microplankton—bacteria and dinoflagellates—makes the main contribution into formation of the Black Sea bioluminescence

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field [2–5]. But for a number of the World ocean regions, another fraction of plankton community makes the major contribution into bioluminescence field formation, in particular jellyfish macroplankton [3, 6, 7]. For instance, ctenophores *Mnemiopsis leidyi* and *Beroe ovata*, which quite recently inhabited the Black Sea are luminescent organisms, bioluminescent intensity of which exceeds hundreds of thousands to million times light-emission of the majority of the microplankton representatives.

There are totally about 150 species of ctenophores, of them in 46 species, living in wide range of temperatures, bioluminescent ability has been registered reliably [8, 9]. For the past 30 years, the Black Sea ctenophore fauna became considerably more rich: Until 1980, it has been presented by one species of pleurobrachia (*Pleurobrachia pileus* (O.F. Muller, 1776)), from 1980-1990-th two species from genus mnemiopsis (*Mnemiopsis leidyi* A. Agassiz, 1865) and beroe (*Beroe ovata* Mayer, 1912) were added and in 2007 near the Turkish and Bulgarian shores bolinopsis (*Bolinopsis vitrea* (L. Agassiz, 1860)) was also found. Now it is not yet clear whether this species inhabiting Mediterranean sea will be able to naturalize in the Black Sea,, but it was met already in 2010 [10]. That is why our work will be devoted exclusively to the parameters of life activity of only two alien ctenophores: *Mnemiopsis leidyi* and *Beroe ovata*.

Ctenophores—aliens not only reached the list of the Black Sea macroplankton but they also considerably influenced structure dynamics of its ecosystem, thus attracting great attention to them. The climate warming and increasing of the anthropogenic eutrophication led in a number of cases to considerable growth of not only ctenophores populations but jelly-fish as well, which influenced condition of the marine communities and effected human economic activity: fishing nets and water canals were blocked, obstacles for marine bathing were created, and in the Black Sea anchovy fishing sharply decreased with the first flash of the mnemiopsis mass development [11–13].

At present time, there is quite great number of works devoted to physiology and ecology of different ctenophores species, including the Black Sea populations [12, 14–20]. From 1980, they conduct intensive studies of the ctenophores—aliens in the Black Sea: they reveal features of their distribution by the sea regions in connection with depth, temperature and salinity; they also study peculiarities of nutrition, breathing and reproduction. As for mnemiopsis, they revealed effect of the environment temperature on such characteristics as population vertical distribution in pelagial [13, 21–23], reproduction rate [24], metabolism intensity [25, 26] and some peculiarities of luminescence under experimental conditions [27, 28]. The same data were received for beroe as well [28–30].

But such important ecological characteristic of the ctenophores as bioluminescence still remains to be not much studied. In particular, the studies of the light-emission parameters in the Black Sea populations of *M. leidyi* and *B. ovata* by present time were conducted exclusively in the Department of Biophysical Ecology, IBSS NASU (now—IMBR RAS). Such indices of the ctenophores bioluminescent as a change of intensity and duration of the light-emission in ontogenesis are studied not enough, influence of different environment factors on the bioluminescence parameters is studied insufficiently, still unclear is connection between the organism physiological indices and its luminescence. Nevertheless, it is known that on the base of the amplitude-time characteristics of bioluminescence we can make a conclusion about the organism functional condition [27, 28, 31, 32].

In connection with the above mentioned, we consider it to be extremely important to continue investigation of the light-emission in the Black Sea alien ctenophores, to reveal an influence of different factors on them and to evaluate accordance of their functional state with variability of the bioluminescence parameters.

## 2. Materials and methods

Experimental investigations were conducted in the Biophysical Ecology Department of the A.O. Kovalevsky Institute of Marine Biological Research (IMBR) from 2007 to 2012. Ctenophores with sizes of 35–40 mm (oral-aboral length for *M. leidyi* and total for *B. ovata*) were collected by Judy net in the Crimea coastal zone at the layer of 0–50 m. Not damaged samples without content in the gastrovascular cavity were chosen for experiments. Three experiment series were conducted: (1) depending on ctenophore size and ontogeny stage; (2) depending on temperature conditions and (3) depending on season. The freshly caught animals were left for 2–3 h to adapt to the conditions similar to *in situ*.

The investigation of *B. ovata* bioluminescence parameters in ontogenesis was carried out in September–November 2007–2009 in three experiment series: (1) depending on ctenophore size; (2) depending on their physiological state and (3) on ontogeny stage. *B. ovata* individuals with wet weight from 0.06 to 19.53 g were taken for the first experiment series. Unbroken individuals were placed into 5 l containers with filtered marine water (membrane filters pore diameter is 35  $\mu\text{m}$ ) at a temperature of  $21 \pm 2^\circ\text{C}$  [33]. For estimation of *B. ovata* luminescence variability in relation to reproduction stage specimens were separated into four groups: (1) 50-mm long—just-caught individuals before gonada formation; (2) 50-mm long individuals with mature gonads; (3) ctenophore eggs spawned by the second group; (4) ctenophore larvae grown from eggs of the third group. Adult just-caught *B. ovata* organisms were put in 20 l aquariums with filtered water under temperature  $20 \pm 2^\circ\text{C}$  with feeding *M. leidyi* ( $L = 40$  mm). Experiments of *B. ovata* light signal registration by ontogeny stages were carried out by the methodology [33, 34].

The investigation of *M. leidyi* bioluminescence parameters in ontogenesis was carried out in July–August 2007–2010. Ctenophores of 3–65 mm size (oral-aboral length) were selected from the plankton samples, taken by Judy net in the upper 10 m layer. Ctenophores wet weight was calculated by the volume of displaced water in measuring cylinder with further weighing of each specimen on the microanalytic weighs AN 50 with accuracy up to 0.01 g. In experiments on the ctenophores size influence on their bioluminescence characteristics, fresh-caught specimens were separated to six size groups: (1)  $0.0073 \pm 0.00036$  g; (2)  $0.52 \pm 0.026$  g; (3)  $3.69 \pm 0.18$  g; (4)  $12.77 \pm 0.63$  g; (5)  $35.06 \pm 1.75$  g and (6)  $42.03 \pm 2.10$  g. To avoid photoinhibition of the ctenophores bioluminescence, they were kept before measuring for 2 h in darkness with  $24 \pm 2^\circ\text{C}$  temperature. Ctenophores were kept in vessels with 3–5 l volume marine water, filtered through the membrane filters with 35  $\mu\text{m}$  pores diameter. Proper filtration of the ctenophores medium was necessary for exclusion of the by-catches of another luminescent organisms (first of all dinoflagellates), which could distort the results of experiments when studying

ctenophores bioluminescence at the initial ontogenesis stages (eggs and larvae). To study an influence of the ctenophores reproductive system condition on the characteristics of their bioluminescence, the caught in the sea adult specimens (40 mm length) were divided to three experimental groups: (1) ctenophores freshly caught in the sea (with gonads at early stage of development, which served as a control after 2-h adaptation in the filtrated water); (2) ctenophores with eggs clutches, formed in the laboratory conditions after experimental feeding; (3) specimens after eggs spawning out. Such ctenophores were preliminary kept under conditions analogous to those of the second group during 6 h; during this period, they produced new eggs clutches and they spawned eggs out. The third group ctenophores bioluminescence characteristics were registered directly after their spawning.

It is known that at natural conditions calanoid copepods, dominating in mesozooplankton composition in the second half of the summer season make the main part of the Black Sea mnemiopsis feeding [35]. That is why we used calanoid copepods *Calanipeda aquaductes*, grown in the laboratory for fish cultivation for nutrition of the ctenophores in the experimental conditions. Before measuring bioluminescent characteristics, the second group ctenophores were kept isolated during 5 h in 5 l vessels with concentration of the late copepodite stages of copepods at the level of  $60 \text{ ex} \cdot \text{l}^{-1}$  (with food supply of 300 ex for one specimen of ctenophores). Copepods concentration in the experimental vessels was determined before the beginning of experiment, counting specimens in an aliquota of volume in the Bogorov camera. In 3 h after beginning of exposition, concentration of food was corrected to initial volumes. With such food supply, ctenophores reproduce actively and in 5 h of nutrition they form ready for spawning eggs clutches [18]. This group of ctenophores was lighted directly after formation of clutch in them.

For estimation of variability of luminescence biophysical characteristics in the ctenophores in ontogenesis, they were divided into four groups: (1) freshly caught in the sea specimens of 40 mm length before gonads formation, adapted to the conditions of experiment under complete darkness during 2 h; (2) ctenophores of 40 mm length with matured gonads, formed as a result of experimental nutrition during 5–6 h after catching; (3) eggs, spawned out by the second group ctenophores, 0.40–0.50 mm diameter; (4) developed from the ctenophores eggs larvae, 0.25–0.30 mm diameter. To receive eggs and then larvae, the freshly caught adult ctenophores were isolated in 5 l vessels with filtered water, where they were fed by copepods. Eggs clutched by ctenophores were collected by filtration of all the water volume through 100  $\mu\text{m}$  sieve. Eggs collected on the sieve were washed into 200 ml glass cylinder, and the number of eggs was calculated in all the volume under microscope. Size of eggs and larvae were measured with accuracy of 0.01 mm under microscope. The measurements of the bioluminescence characteristics were conducted in 15–20 specimens of each experimental group and repeated three times. Before light-emission stimulation ctenophores were kept in the filtered marine water with  $24 \pm 2^\circ\text{C}$  temperature. The given temperature conditions are optimal for quick eggs spawning by ctenophores and further larvae development [25].

For investigation of temperature variability, uni-sized (35–40 mm length) ctenophores were divided in the laboratory into five groups and contained in different temperature conditions: (1)  $10 \pm 1^\circ\text{C}$ ; (2)  $16 \pm 1^\circ\text{C}$ ; (3)  $22 \pm 1^\circ\text{C}$ ; (4)  $26 \pm 1^\circ\text{C}$  and (5)  $30 \pm 1^\circ\text{C}$ . *M. leidyi* and *B. ovata* were

kept in the temperature-controlled aquariums (50 l) with the filtered marine water, being adapted during 6–8 h to the temperature close to such in the sea in the given period [36].

The main parameters: amplitude, energy and bioluminescence duration of the alien-ctenophore under the different temperature conditions were compared. For research of seasonal dynamics, bioluminescence uniform-sized samples group (40 mm) of ctenophores were taken. The adaptive period before experiments on ctenophore bioluminescence was 2 h. Experiments on ctenophore bioluminescence characteristics registration on the laboratory complex—luminescope “Svet” [31] were conducted after the adaptive period. Special cuvette for mechanical, chemical and electrical stimulation of the plankton organisms, made of transparent organic glass, in which experimental organisms were placed, was set into the luminescope dark chamber. Biophysical characteristics of the ctenophore light-emission were investigated by mechanical and chemical stimulation in our experiments. Mechanical stimulation method, the most adequate to the natural stimuli, chemical stimulation by ethyl alcohol give more prolonged and bright signals with maximal values [32, 33].

### 3. Results

#### 3.1. Seasonal dynamics of the *Mnemiopsis leidyi* bioluminescence

The studies conducted had revealed in *M. leidyi* bioluminescence intensity considerable seasonal fluctuations for its amplitude characteristic as well as for temporal one (Figure 1).

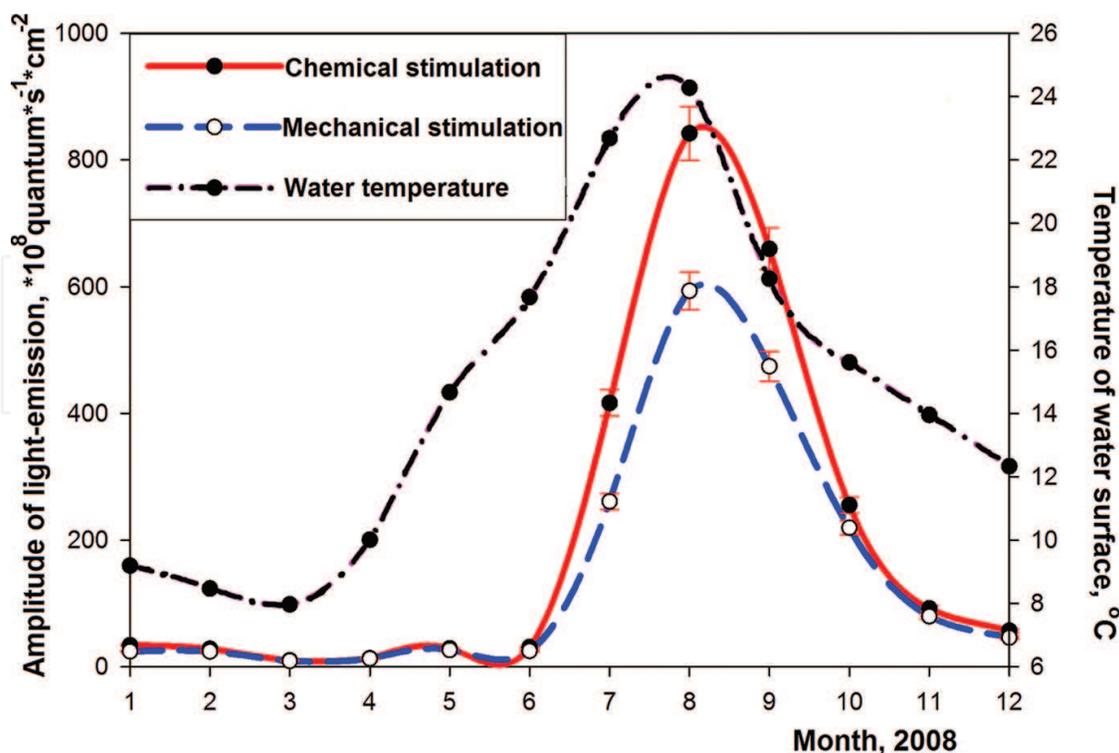


Figure 1. *Mnemiopsis leidyi* light-emission amplitude seasonal dynamics under different stimulation types [31].

Thus, the ctenophores in the winter period gave not very intensive flash with the amplitude until  $70.0 \pm 3.4 \cdot 10^8$  quantum $\cdot$ s $^{-1}$  $\cdot$ cm $^{-2}$  and duration about 2.0 s. Low bioluminescence values were observed in the spring period with minimum on March ( $9.93 \pm 0.49 \cdot 10^8$  quantum $\cdot$ s $^{-1}$  $\cdot$ cm $^{-2}$  and  $9.21 \pm 0.46 \cdot 10^8$  quantum $\cdot$ s $^{-1}$  $\cdot$ cm $^{-2}$ ) under the chemical and mechanical stimulation correspondingly [31].

The average luminescence amplitude ( $260.94 \pm 13.04 \cdot 10^8$  quantum $\cdot$ s $^{-1}$  $\cdot$ cm $^{-2}$ ) was registered in June. The light-emission characteristics rise with peak in August and make  $841.97 \pm 42.09 \cdot 10^8$  quantum $\cdot$ s $^{-1}$  $\cdot$ cm $^{-2}$ . It is related with ctenophores reproduction in July–August. *M. leidyi* luminescence intensity under the chemical stimulation is 2–2.5 times greater ( $p < 0.05$ ) than under the mechanical one [31].

The luminescence amplitude of *M. leidyi* decreases almost 11 times in the middle of November, if compared with the summer period. Light-emission energy of ctenophores depending on season changes analogically with their amplitude indices (**Figure 2**) [31].

Thus, minimal energy values of *M. leidyi* were registered in February and maximal in August, making  $659.97 \pm 32.98 \cdot 10^8$  quantum $\cdot$ cm $^{-2}$  and  $393.39 \pm 19.66 \cdot 10^8$  quantum $\cdot$ cm $^{-2}$  under the chemical and the mechanical stimulation correspondingly. The *M. leidyi* luminescence

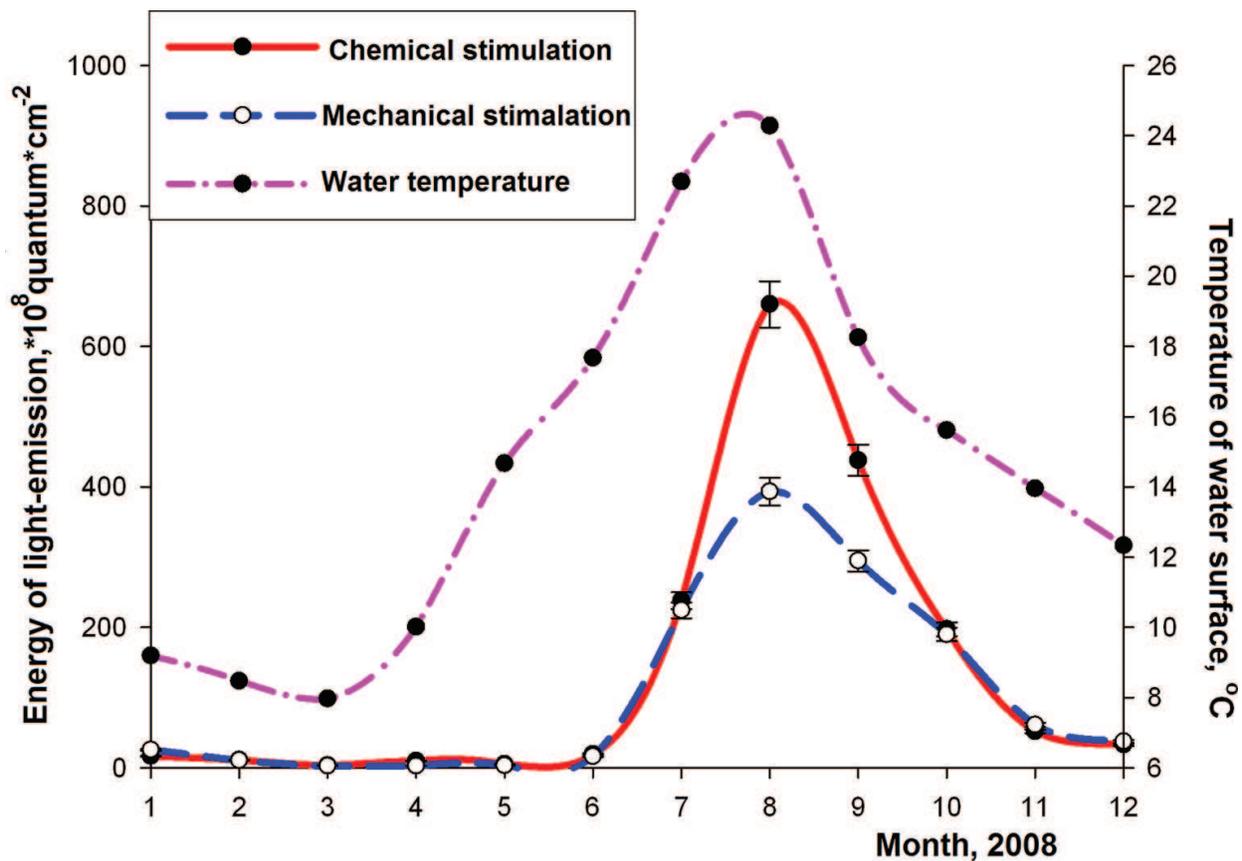


Figure 2. *Mnemiopsis leidy* light-emission energy seasonal dynamics under different stimulation types [31].

energy reduces during the following period and decreases 12 times in November if compared with July.

*M. leidy* light-emission duration changes considerably depending on season (Figure 3). Thus, the shortest flashes are registered in February–March, making 0.79–1.32 s and more prolonged luminescence duration is observed in August–September and it achieves 2.77–3.46 s ( $p < 0.05$ ) [31].

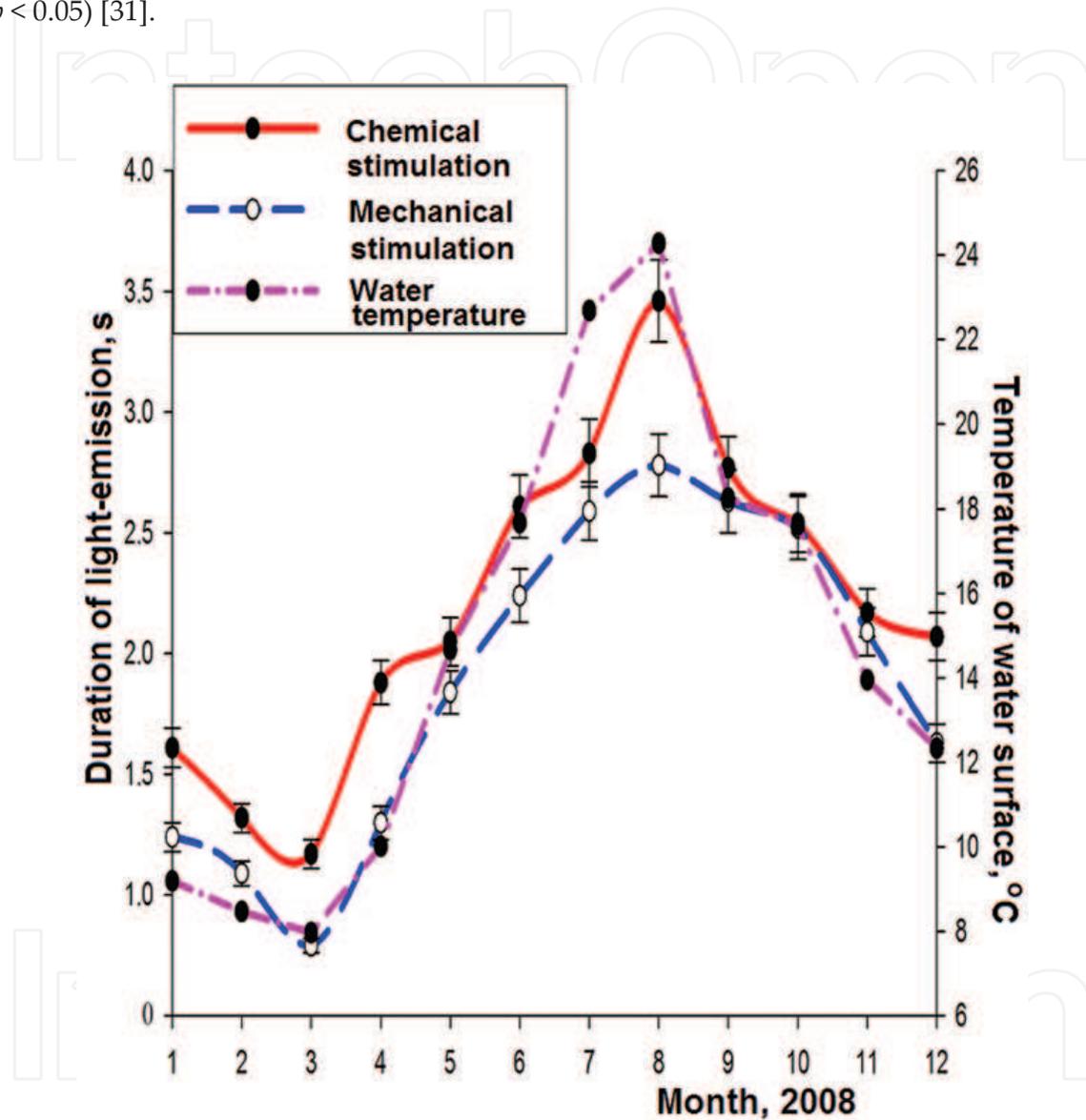


Figure 3. *Mnemiopsis leidy* light-emission duration seasonal dynamics under different stimulation types [31].

### 3.2. Seasonal variability of the *B. ovata* bioluminescence characteristics

The typical luminescence signal of *B. ovata* is represented by a number of flashes, superimposing one on another, with several amplitude peaks with sharp increasing background and the same damping decrement.

The bioluminescence has significant seasonal differences [31]. Thus, several weak signals may be observed for ctenophore, luminous in the winter period (Figure 4), followed by the flash of negligible intensity with the greatest amplitude ( $56.7 \pm 2.83 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$ ).

The ctenophore bioluminescence is depressed even more in the spring period, with the minimal values in May: one to two weak signals are observed with the amplitude up to  $35.96 \pm 1.79 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$ . The bioluminescence intensity increases up to  $537.6 \pm 26.88 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$  which is registered in summer. *B. ovata* maximal bioluminescence is registered in July, their intensity achieves  $1382.25 \pm 69.11 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$  and duration up to  $2.86 \pm 0.14$  s. Ctenophore light-emission intensity is 1.5 times higher under the mechanical stimulation than under the chemical one ( $p < 0.05$ ) [31]. *B. ovata* luminescence characteristics decrease up to  $98.75 \pm 4.93 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$  in August. The second peak of light-emission intensity is observed in September, achieving  $852.56 \pm 42.62 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$ . Luminescence amplitude reduces 15 times by December, if compared with the autumn peak and makes  $56.7 \pm 2.83 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$  and  $27.01 \pm 1.35 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$  under the mechanical and chemical stimulation correspondingly. Ctenophore luminescence energy seasonal changes are the same (Figure 5).

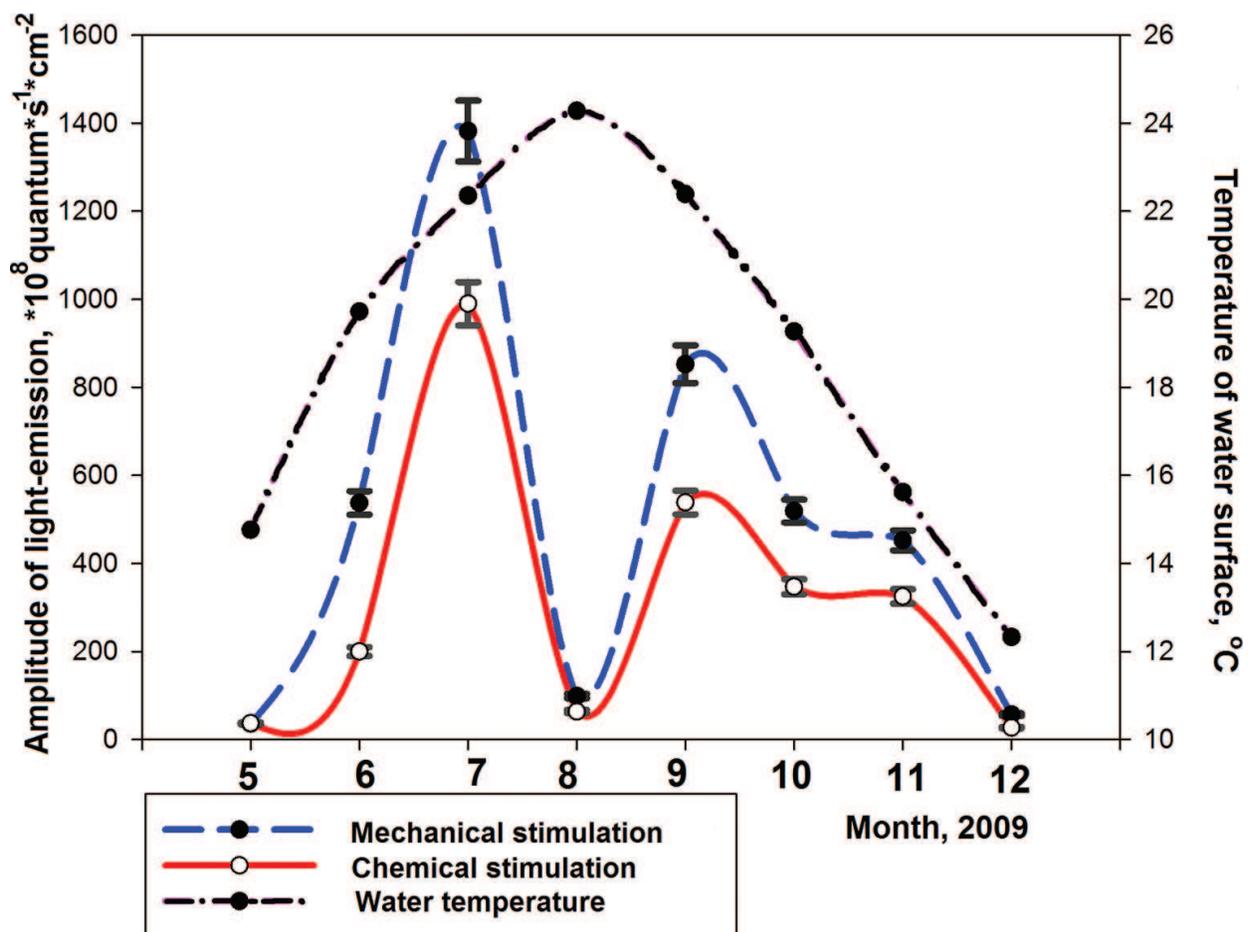


Figure 4. *Beroe ovata* light-emission amplitude seasonal dynamics under different stimulation types [31].

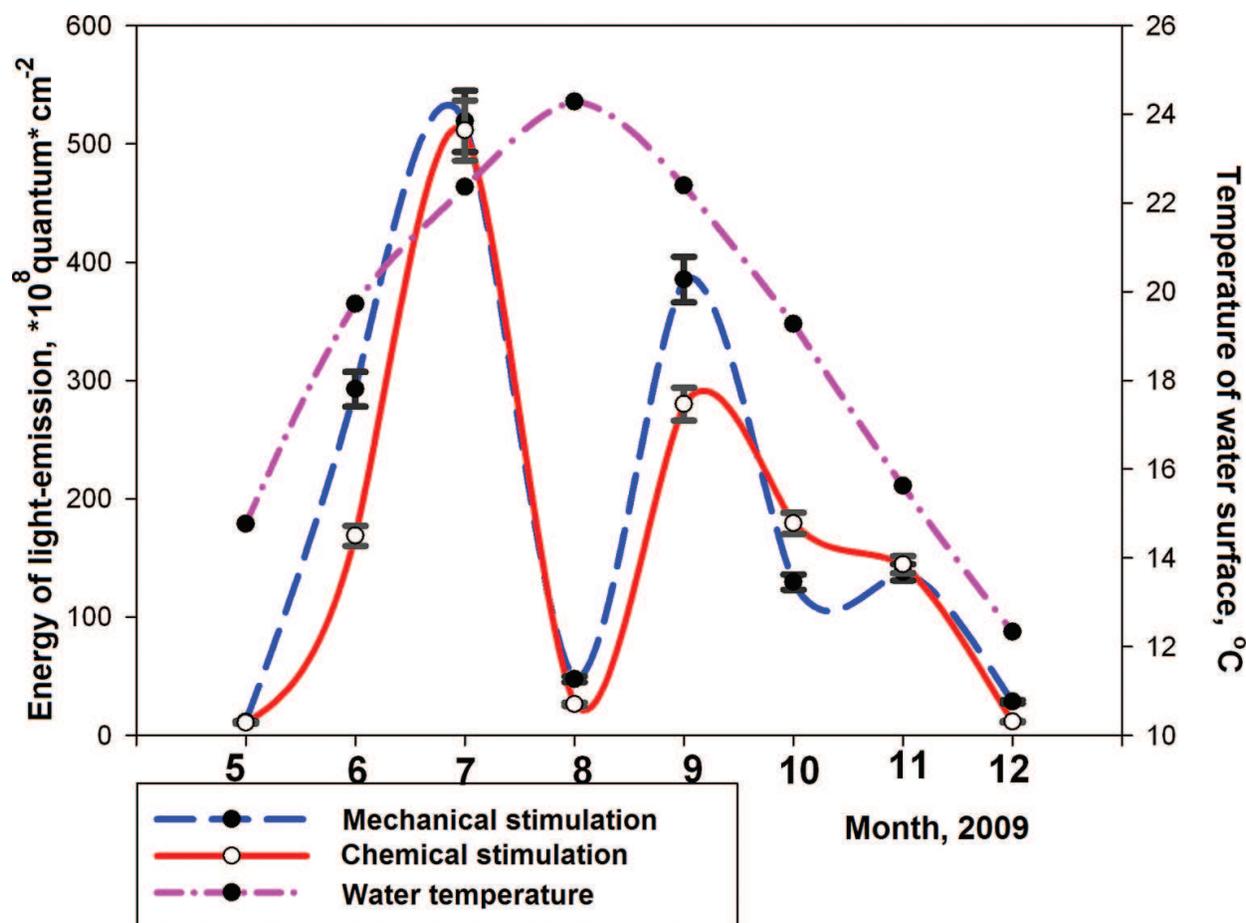


Figure 5. *Beroe ovata* light-emission energy seasonal dynamics under different stimulation types [31].

Low energy values of ctenophores are observed in winter-spring period with minimum in May. *B. ovata* bioluminescence energy is maximal in July, making  $518.94 \pm 25.94 \cdot 10^8$  quantum $\cdot\text{cm}^{-2}$  under the mechanical stimulation and  $511.88 \pm 25.59 \cdot 10^8$  quantum $\cdot\text{cm}^{-2}$  under the chemical one. Decrease of the luminescence energy indices is observed in August, if compared with the previous month. *B. ovata* light-emission amplitude rises again in autumn with maximum in September and decreases 1.5 times if compare with the summer period ( $p < 0.05$ ). Light-emission energy decreases 11 times ( $p < 0.05$ ) by December [31]. Light-emission duration of ctenophore like its intensity in the different seasons change considerably (Figure 6).

More prolonged signals are registered in July and September, making 2.54–2.86 s, the shortest luminescent signals of *B. ovata* are observed in May (1.06 s) and in December (0.9 s) ( $p < 0.05$ ). *B. ovata* trophic state like this of *M. leidyi* is depressed in the winter-spring period [31, 37], and it reveals itself in reducing its luminescence amplitude-temporal characteristics. But *B. ovata* nutritive conditions are the most favorable in early autumn period, in September especially [37], which affects the ctenophore bioluminescence activity increase in the given period.

*B. ovata*, if compared to other jelly-fish, is the species sensitive to the temperature swings more than others [38]. The temperature rise in the Black Sea in May up to 16°C leads to *B. ovata* early

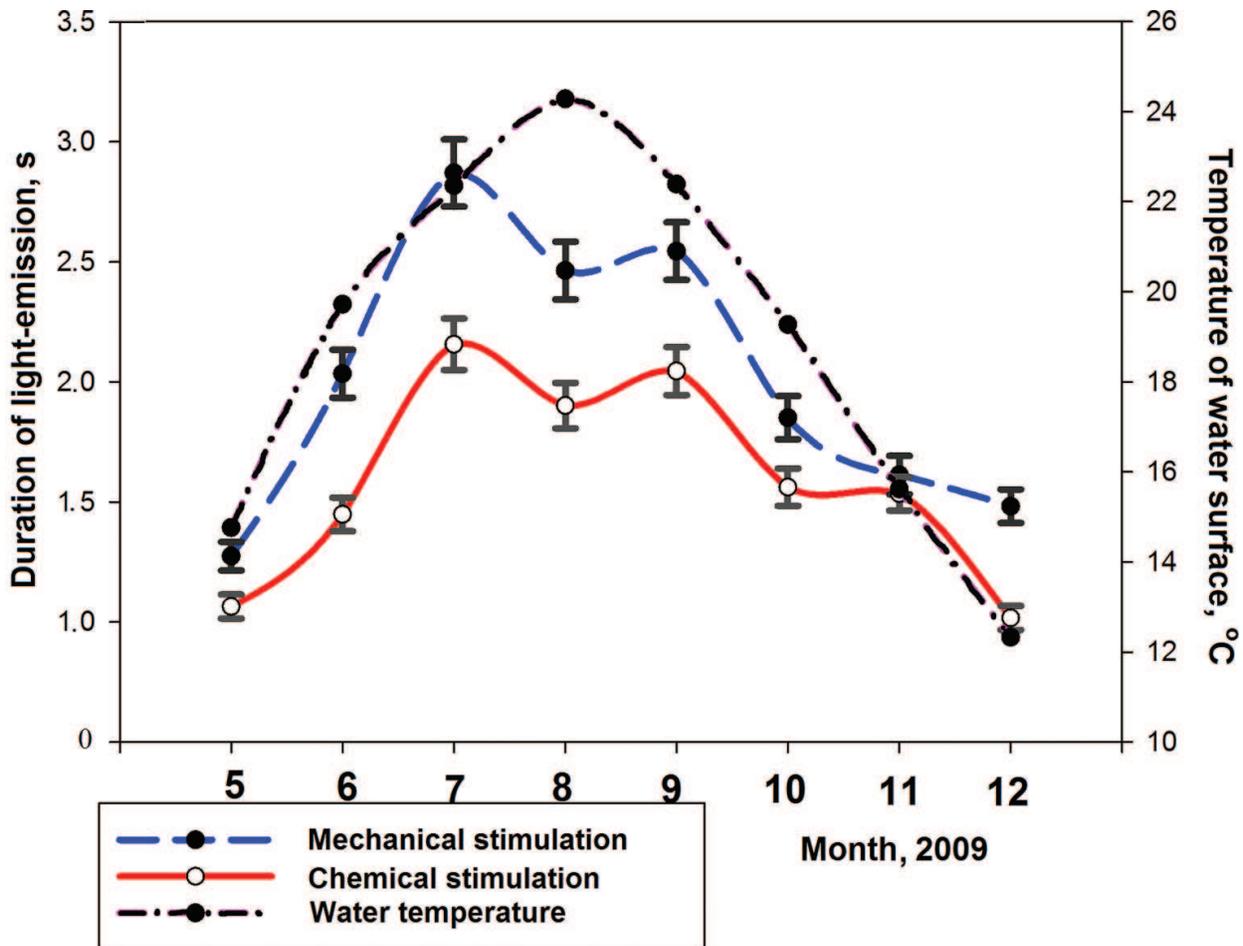


Figure 6. *Beroe ovata* light-emission duration seasonal dynamics under different stimulation types [31].

appearance, but ctenophore luminescence values are low in the spring period. The first ctenophore bioluminescence peak is observed in July. The water temperature rise in August up to  $26^{\circ}\text{C}$  leads to *B. ovata* abundance decrease in the given period. The ctenophore second maximum was found in September while the water temperature falls to  $20 \pm 2^{\circ}\text{C}$ . From October till March, the ctenophore state is depressed. Food supplies and mass spawning reduce unfavorably the *B. ovata* functional state as well as its bioluminescence indices [31, 39].

Thus, seasonal variability of ctenophores light-emission parameters was established. Our investigations showed that maximal bioluminescence values for mnemiopsis are registered in August, whereas beroe maximal bioluminescence is observed twice—in July and in September. Light-emission minimal values for both ctenophores were observed in the winter-spring period [31].

### 3.3. Influence of the temperature on the *M. leidyi* bioluminescence

The investigation results have shown considerable changes of the *M. leidyi* bioluminescence intensity, connected with temperature changes (Table 1). Thus, maximal indices of the ctenophore signals amplitude were observed under the temperature of  $26 \pm 1^{\circ}\text{C}$ . *M. leidyi*

bioluminescence intensity under chemical stimulation was 1.5 times higher, than under the mechanical one, making  $1432.94 \pm 71.64 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$ .

The temperature increases up to 30°C leads to four times decrease of ctenophore luminescence intensity, making  $322.34 \pm 16.1 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$ . *M. leidyi* light-emission intensity decreased two times ( $p < 0.05$ ) comparing with optimum under the temperature decrease down to 22°C. The temperature decreases down to  $10 \pm 1^\circ\text{C}$  leads to more considerable bioluminescence intensity change, up to its minimal values  $17.32 \pm 0.83 \cdot 10^8$  under mechanical and  $17.93 \pm 0.89 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$  under the chemical stimulation correspondingly [36].

Characteristics of light-emission	Amplitude of light-emission, quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$		Energy of light-emission, quantum $\cdot$ cm $^{-2}$		Duration of light-emission, s	
	1	2	1	2	1	2
10 $\pm$ 1°C	$29.52 \pm 1.47 \cdot 10^8$	$33.52 \pm 1.67 \cdot 10^8$	$12.47 \pm 0.62 \cdot 10^8$	$15.51 \pm 0.77 \cdot 10^8$	$1.82 \pm 0.09$	$1.94 \pm 0.097$
16 $\pm$ 1°C	$219.45 \pm 10.97 \cdot 10^8$	$332.33 \pm 16.61 \cdot 10^8$	$197.43 \pm 9.87 \cdot 10^8$	$283.97 \pm 14.19 \cdot 10^8$	$2.51 \pm 0.12$	$2.70 \pm 0.13$
22 $\pm$ 1°C	$545.75 \pm 27.28 \cdot 10^8$	$632.95 \pm 31.64 \cdot 10^8$	$407.19 \pm 20.35 \cdot 10^8$	$417.65 \pm 20.388 \cdot 10^8$	$2.89 \pm 0.14$	$3.48 \pm 0.17$
26 $\pm$ 1°C	$910.81 \pm 45.54 \cdot 10^8$	$1432.94 \pm 71.64 \cdot 10^8$	$725.33 \pm 36.26 \cdot 10^8$	$894.64 \pm 44.73 \cdot 10^8$	$3.14 \pm 0.16$	$3.53 \pm 0.17$
30 $\pm$ 1°C	$322.34 \pm 16.12 \cdot 10^8$	$488.43 \pm 24.42 \cdot 10^8$	$294.89 \pm 14.74 \cdot 10^8$	$265.15 \pm 13.25 \cdot 10^8$	$2.54 \pm 0.12$	$2.67 \pm 0.17$

Remark: 1, mechanical stimulation; 2, chemical stimulation.

**Table 1.** Light-emission characteristics of *M. leidyi* under different temperatures.

*M. leidyi* light-emission energy changes under different temperatures (**Table 1**). Thus, maximal values of ctenophore luminescence energy were registered under 26°C, making  $894.64 \pm 44.7 \cdot 10^8$  and  $725.33 \pm 36.2 \cdot 10^8$  quantum $\cdot$ cm $^{-2}$ —under the chemical and mechanical stimulation correspondingly.

Bioluminescence energy decreases two times ( $p < 0.05$ ) under the temperature of 22°C. *M. leidyi* light-emission energy minimal indices were observed under the temperature of 10°C [36]. Temperature fluctuations affected the *M. leidyi* light-emission duration change with minimal indices under the temperature 10°C, under its rise up to 30°C making 1.94 and 2.67 s correspondingly. The most continuous signals were registered under the temperature of 26°C,  $3.54 \pm 0.15$  s, under the chemical stimulation especially.

### 3.4. Influence of the temperature on the *B. ovata* bioluminescence

Amplitude and light-emission energy considerable changes, connected with the environment temperature change, were revealed in ctenophore *B. ovata* (**Table 2**). Thus, *B. ovata* flashes in amplitude had the maximal indices under the temperature of  $22 \pm 1^\circ\text{C}$  regardless the type of stimulation, having achieved  $1150 \pm 57.51 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$  under the mechanical and  $822.03 \pm 41.10 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$  under the chemical stimulation correspondingly.

Ctenophore reacts with more low light-emission amplitude indices with the temperature rise up to 26°C, but minimal values of the luminescence amplitude are registered under the temperature of 30°C, achieving  $49.01 \pm 2.4 \cdot 10^8$  under the mechanical stimulation and

Characteristics of light-emission	Amplitude of light-emission, quantum·s <sup>-1</sup> ·cm <sup>-2</sup>		Energy of light-emission, quantum·cm <sup>-2</sup>		Duration of light-emission, s	
	1	2	1	2	1	2
10 ± 1°C	4.92 ± 0.24·10 <sup>8</sup>	3.42 ± 0.16·10 <sup>8</sup>	2.95 ± 0.12·10 <sup>8</sup>	1.67 ± 0.08·10 <sup>8</sup>	1.03 ± 0.05	1.02 ± 0.05
16 ± 1°C	551.14 ± 27.55·10 <sup>8</sup>	482.89 ± 24.14·10 <sup>8</sup>	262.22 ± 13.11·10 <sup>8</sup>	156.12 ± 7.8·10 <sup>8</sup>	1.91 ± 0.09	1.76 ± 0.08
22 ± 1°C	1150.36 ± 57.51·10 <sup>8</sup>	822.03 ± 41.10·10 <sup>8</sup>	530.19 ± 26.51·10 <sup>8</sup>	482.65 ± 24.13·10 <sup>8</sup>	3.03 ± 0.15	2.47 ± 0.12
26 ± 1°C	577.06 ± 28.85·10 <sup>8</sup>	268.81 ± 13.44·10 <sup>8</sup>	166.97 ± 8.34·10 <sup>8</sup>	148.63 ± 7.43·10 <sup>8</sup>	2.12 ± 0.10	2.08 ± 0.10
30 ± 1°C	49.01 ± 2.45·10 <sup>8</sup>	29.23 ± 1.46·10 <sup>8</sup>	14.73 ± 0.73·10 <sup>8</sup>	13.84 ± 0.69·10 <sup>8</sup>	1.53 ± 0.07	1.49 ± 0.07

Remark: 1, mechanical stimulation; 2, chemical stimulation.

**Table 2.** Light-emission characteristics of *B. ovata* under different temperatures.

29.23 ± 1.46·10<sup>8</sup> quantum·s<sup>-1</sup>·cm<sup>-2</sup> under the chemical one. The *B. ovata* functional state is negative under low temperature also. Thus, temperature decreases down to 10 ± 1°C weakens ctenophore moving activity and lowers their luminescence intensity values: down to 3.42 ± 0.16·10<sup>8</sup> quantum·s<sup>-1</sup>·cm<sup>-2</sup> and 4.92 ± 0.22·10<sup>8</sup> quantum·s<sup>-1</sup>·cm<sup>-2</sup> under the chemical and mechanical stimulation correspondingly. bioluminescence energy of beroe change as well as its amplitude depending on different temperatures. Thus, maximal values are registered under the temperature of 22°C (530.19 ± 26.5·10<sup>8</sup> quantum·cm<sup>-2</sup>) and minimal—under the temperature of 10°C, making 2.95 ± 0.12·10<sup>8</sup> quantum·cm<sup>-2</sup>. *B. ovata* light-emission duration varied considerably under the temperature changes [36].

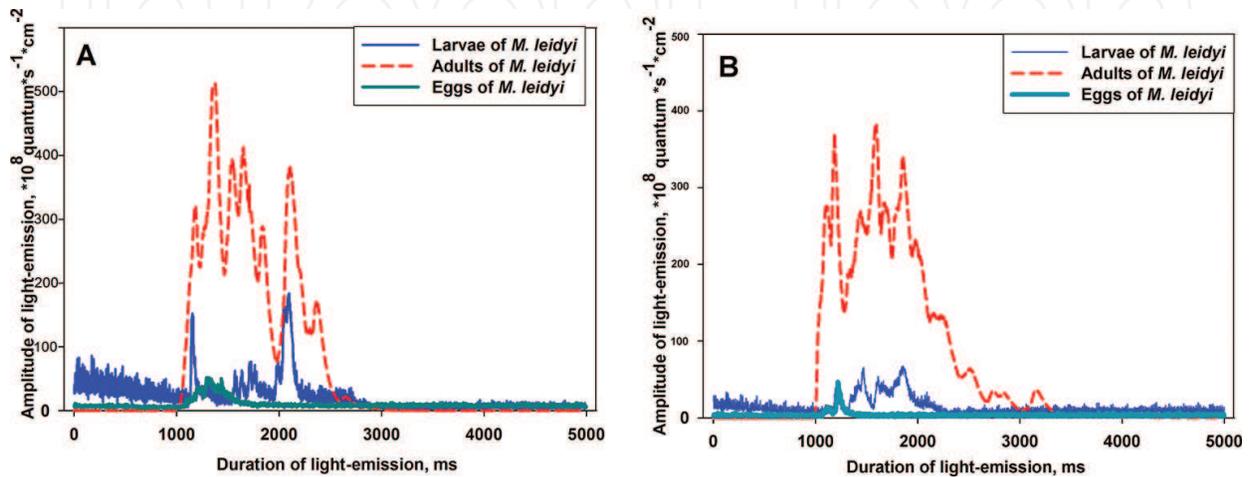
The shortest bioluminescent signals were observed under the temperature of 10°C, making 1.02 ± 0.05 s, and the most continuous under 22°C, achieving 3.03 ± 0.15 s. Ctenophore light-emission characteristics changes, under different temperature conditions, can be explained, we believe, by these organisms physiological adaptations to the environment temperature oscillations point of view. Indeed, the most intensive *M. leidyi* luminescence is observed under the temperature of 26 ± 1°C, and *B. ovata*—under 22 ± 1°C, which are the most favorable for their functional state. Thus, according to the data of Anninsky with co-authors, *M. leidyi* [25] breeding peak is observed under the temperature of 24–26°C in August, and ctenophore *B. ovata* [12, 19] autumn abundance growth under the temperature 20–22°C. Ctenophores under the temperature 22°C are actively breeding, and their metabolism is considerably higher than under lower temperatures. Ctenophores light-emission amplitude decreases for several orders under the temperature to 10°C can be explained by their populations abundance sharp reduction reduces in the autumn-winter period [23, 36].

Maximal activity of the enzyme-substrate complex, basic for the ctenophores luminescence was observed under the temperature of 30°C *in vivo* [36, 40, 41]. Thus, light-emission amplitude maximum was observed in our investigations under following temperatures: under 26°C for *M. leidyi* and under 22°C for *B. ovata* [36].

### 3.5. Bioluminescence characteristics changes in the *M. leidyi* ontogenesis

After 5–6 h of experimental feeding ctenophores of 40 mm length produced from 3.0 to 4.5 thousands of viable. The spawning peak was observed at night (23–24 h), which

corresponds to the data of other researchers [24]. Duration of development from eggs spawning to larvae getting out in our investigations was of 16–19 h. Typical bioluminescent signals of ctenophores *M. leidyi* under mechanical and chemical stimulations at different stages of ontogenesis are presented in **Figure 7a** and **b**. As one can see in **Figure 7** and **Table 3**, ctenophores luminescence characteristics change considerably depending on the development stage.



**Figure 7.** The typical bioluminescence signals of *M. leidyi* at the different ontogenesis stages: (A) under mechanical stimulation and (B) under chemical stimulation.

The most intensive bioluminescence is observed in adult specimens (with matured gonads), in which amplitude-time characteristics reach maximum magnitudes: amplitudes up to

Ontogenesis stages of <i>M. leidyi</i>	N	L (mm)	Amplitude of light-emission (quantum·s <sup>-1</sup> ·cm <sup>-2</sup> )		Energy of light-emission (quantum·cm <sup>-2</sup> )		Duration of light-emission, s	
			1	2	1	2	1	2
Stimulation types								
Just-caught individuals (control)	43	40	(112.16 ± 5.61) · 10 <sup>8</sup>	(144.18 ± 7.20) · 10 <sup>8</sup>	(109.68 ± 5.48) · 10 <sup>8</sup>	(143.36 ± 7.16) · 10 <sup>8</sup>	2.39 ± 0.12	2.75 ± 0.13
Reproductive ctenophores	38	40	(424.46 ± 21.22) · 10 <sup>8</sup>	(470.98 ± 23.54) · 10 <sup>8</sup>	(284.76 ± 14.23) · 10 <sup>8</sup>	(311.24 ± 15.56) · 10 <sup>8</sup>	3.28 ± 0.16	3.93 ± 0.19
Ctenophore eggs	25	0.40–0.50	(0.39 ± 0.019) · 10 <sup>8</sup>	(0.89 ± 0.04) · 10 <sup>8</sup>	(0.23 ± 0.012) · 10 <sup>8</sup>	(0.52 ± 0.026) · 10 <sup>8</sup>	0.45 ± 0.02	0.76 ± 0.03
Ctenophore larvae	30	0.25–0.30	(1.44 ± 0.08) · 10 <sup>8</sup>	(3.13 ± 0.15) · 10 <sup>8</sup>	(0.48 ± 0.022) · 10 <sup>8</sup>	(1.07 ± 0.05) · 10 <sup>8</sup>	1.33 ± 0.067	1.86 ± 0.11

Remark: 1, mechanical stimulation; 2, chemical stimulation.

**Table 3.** The bioluminescence characteristics of ctenophore *M. leidyi* at the ontogenesis.

$(470.98 \pm 23.54) \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$  and duration of signal—up to  $3.93 \pm 0.19$  s. Light-emission amplitude in the adult specimens three times and signal energy two times ( $p < 0.05$ ) exceeds analogous characteristics of the control group ctenophores.

Luminescence durations in the given ctenophore groups also differ considerably. For example, luminescence duration in the adult specimens for 1.18 s exceeds the same in control. Signal duration in the control group ctenophores three to four times exceeded those in their eggs and larvae. The weakest luminescence was registered in ctenophores eggs (Table 3), expressed in low amplitudes (less than  $0.39 \pm 0.019 \cdot 10^8$  quantum $\cdot$ s $^{-1}\cdot$ cm $^{-2}$ ) and light-emission energy (less than  $0.23 \pm 0.012 \cdot 10^8$  quantum $\cdot$ cm $^{-2}$ ), as well as small duration of the bioluminescent signal—up to  $0.45 \pm 0.02$  s. Comparing bioluminescence of the ctenophore eggs and larvae, we stated that the larval stage luminescence amplitude was 3.5 and energy two to three times higher than analogous characteristics of the eggs bioluminescence. Signal durations of ctenophore larvae also two to three times exceeded analogous parameters in eggs ( $p < 0.05$ ). The results of correlation of the light-emission in *M. leidyi*

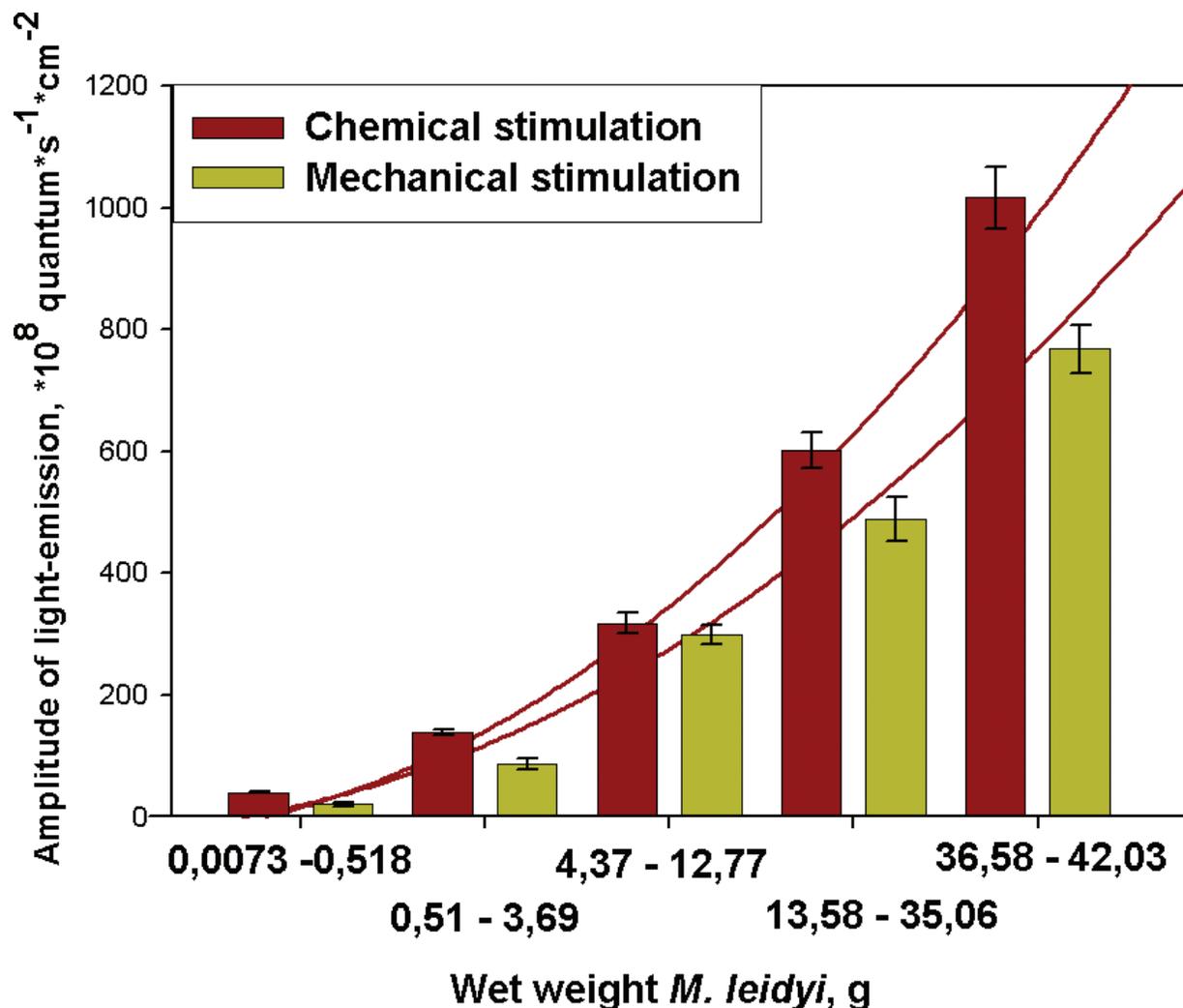
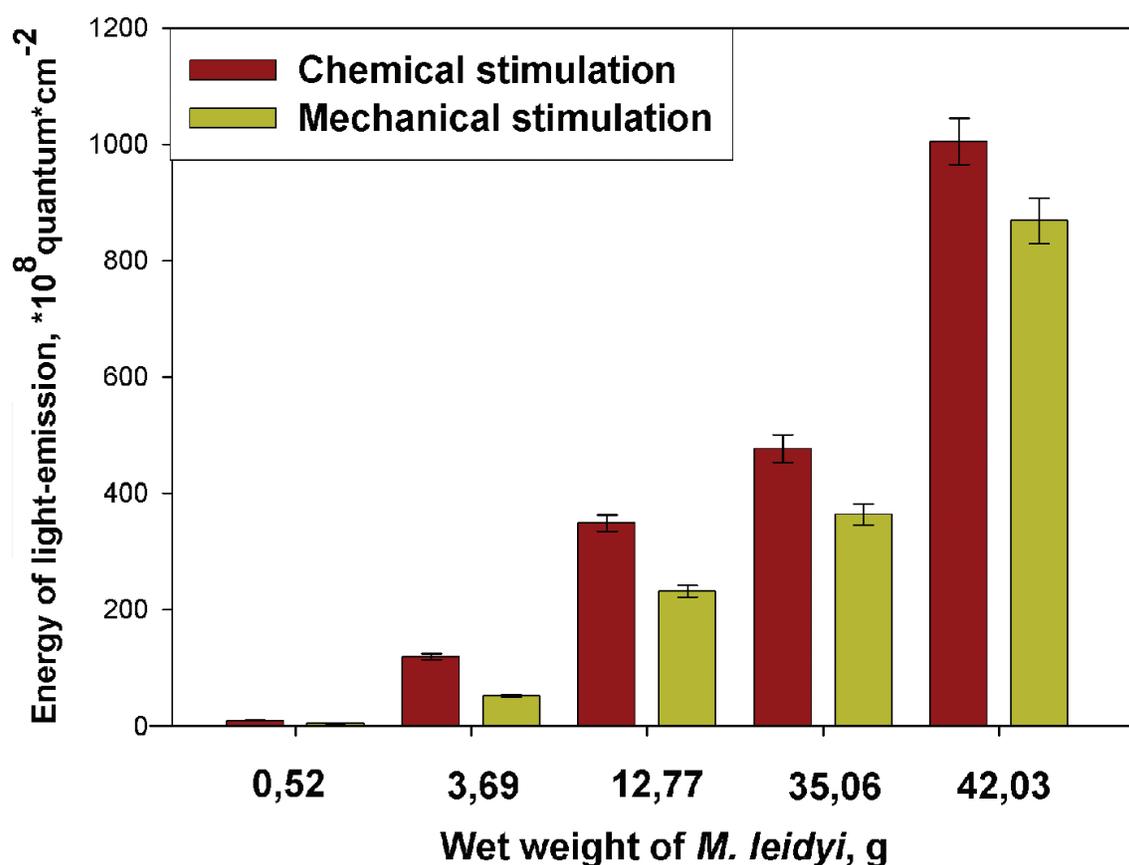


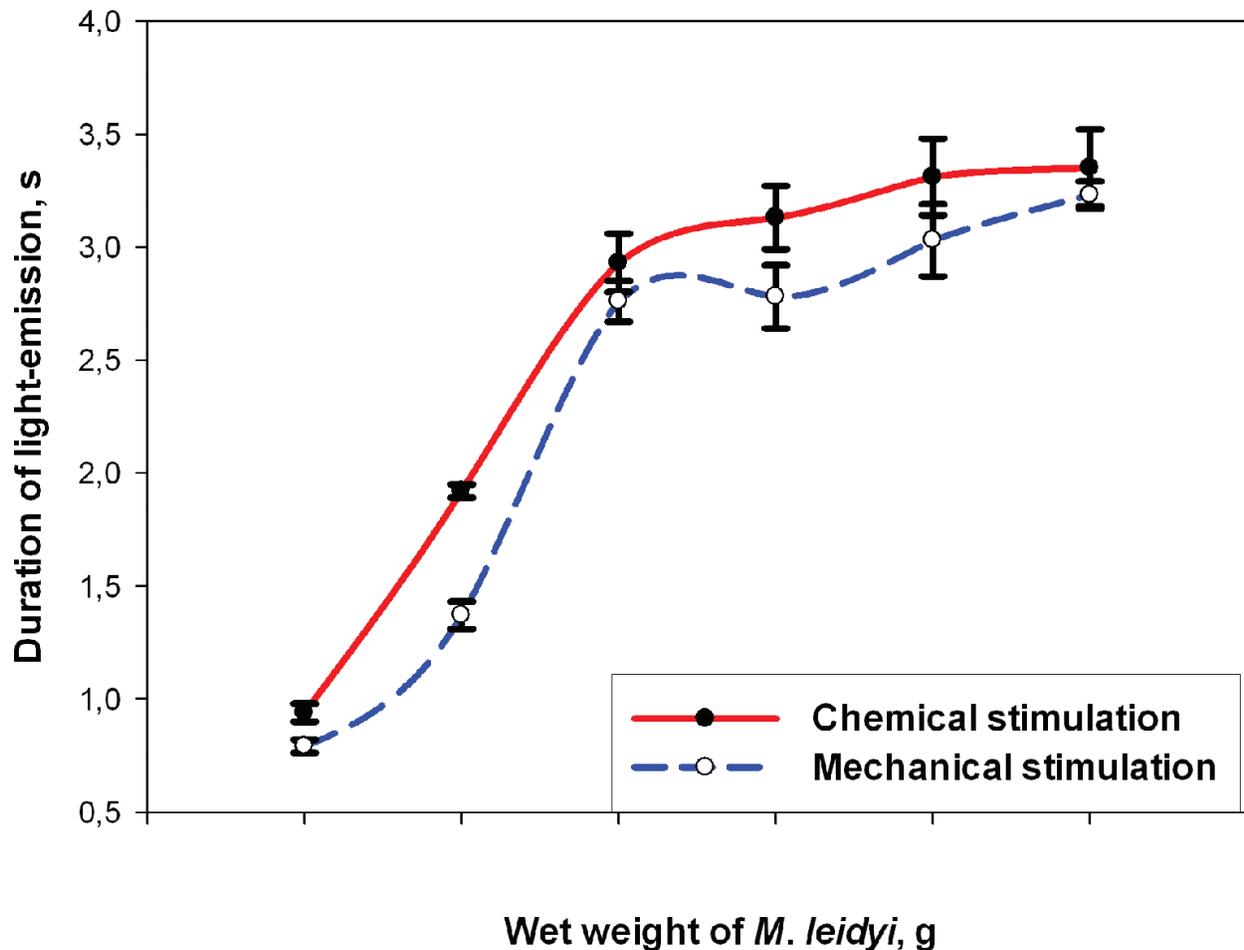
Figure 8. Variability of the bioluminescence amplitude ctenophore *M. leidyi* depending on wet weight of the individuals under mechanical and chemical stimulation.

ctenophores depending on specimen size under the mechanical and chemical stimulation are given in **Figures 8–10**.

It has been revealed that magnitudes of amplitude, energy and duration of the bioluminescent signals in the freshly caught ctenophores depended directly on their size. For example, luminescence intensity in *M. leidyi* with wet weight  $0.52 \pm 0.026$  g makes under mechanical stimulation  $1.32 \cdot 10^8$  quantum $\cdot$ s $^{-1}$  $\cdot$ cm $^{-2}$  and under chemical— $3.55 \cdot 10^8$  quantum $\cdot$ s $^{-1}$  $\cdot$ cm $^{-2}$  (**Figure 8**), while in big specimens (with wet weight 42.03 g) its intensity makes  $(767.56 \pm 42.21) \cdot 10^8$  quantum $\cdot$ s $^{-1}$  $\cdot$ cm $^{-2}$  under mechanical stimulation and  $(1016.93 \pm 50.84) \cdot 10^8$  quantum $\cdot$ s $^{-1}$  $\cdot$ cm $^{-2}$  under chemical one. Analogous situation is observed at the bioluminescence energetic indices (**Figure 9**), which grow with an increase of the organism size (from  $(0.89 \pm 0.035) \cdot 10^8$  to  $(1004.28 \pm 40.17) \cdot 10^8$  quantum $\cdot$ cm $^{-2}$  under chemical stimulation and from  $(0.29 \pm 0.01) \cdot 10^8$  quantum $\cdot$ cm $^{-2}$  to  $(868.26 \pm 39.07) \cdot 10^8$  quantum $\cdot$ cm $^{-2}$  under mechanical one). Luminescence duration (**Figure 10**) of less in size organisms (with wet weight  $0.0073 \pm 0.00036$  g) made under mechanical stimulation  $0.79 \pm 0.03$  and under chemical— $1.37 \pm 0.06$  s, but in the second size group (with wet weight  $0.52 \pm 0.026$  g) ctenophores bioluminescence duration under both types of stimulation increased 2–2.5 times. Further on with an increase of the specimen size in groups from 10 to 65 mm ctenophore luminescence duration practically did not change,



**Figure 9.** Variability of the bioluminescence energy ctenophore *M. leidyi* depending on wet weight of the individuals under mechanical and chemical stimulation.



**Figure 10.** Variability of the bioluminescence duration ctenophore *M. leidyi* depending on wet weight of the individuals under mechanical and chemical stimulation.

reaching in the biggest specimens  $3.35 \pm 0.16$  s under mechanical stimulation and  $2.78 \pm 0.13$  s under chemical one.

**Figure 11** represents variability of the biophysical characteristics of *M. leidyi* light-emission depending on the stage of the organism's reproduction. As it can be seen at the illustrative material presented amplitude of light signals appeared to be the most sensitive index of the bioluminescence (**Figure 11**), its maximum magnitudes were registered in a group of ctenophores with eggs clutches, where they two to three times ( $p < 0.05$ ) exceeded luminescent intensity in the freshly caught specimens (control).

Having compared ctenophore bioluminescence after spawning and those in control we found that luminescence amplitude in the control group 14 times exceeded amplitude in the spawned specimens. Light-emission energy in the spawning ctenophores with clutch reached if compared with other groups of organisms maximum magnitudes up to  $(139.46 \pm 8.36) \cdot 10^8$  quantum $\cdot$ cm $^{-2}$ , which 1.5 times exceeded analogous indices in specimens from the control group and 53 times ( $p < 0.05$ ) the same indices in the spawned ctenophores, showing the lowest energetic indices to  $(2.62 \pm 0.13) \cdot 10^8$  quantum $\cdot$ cm $^{-2}$ . The signals duration in ctenophores

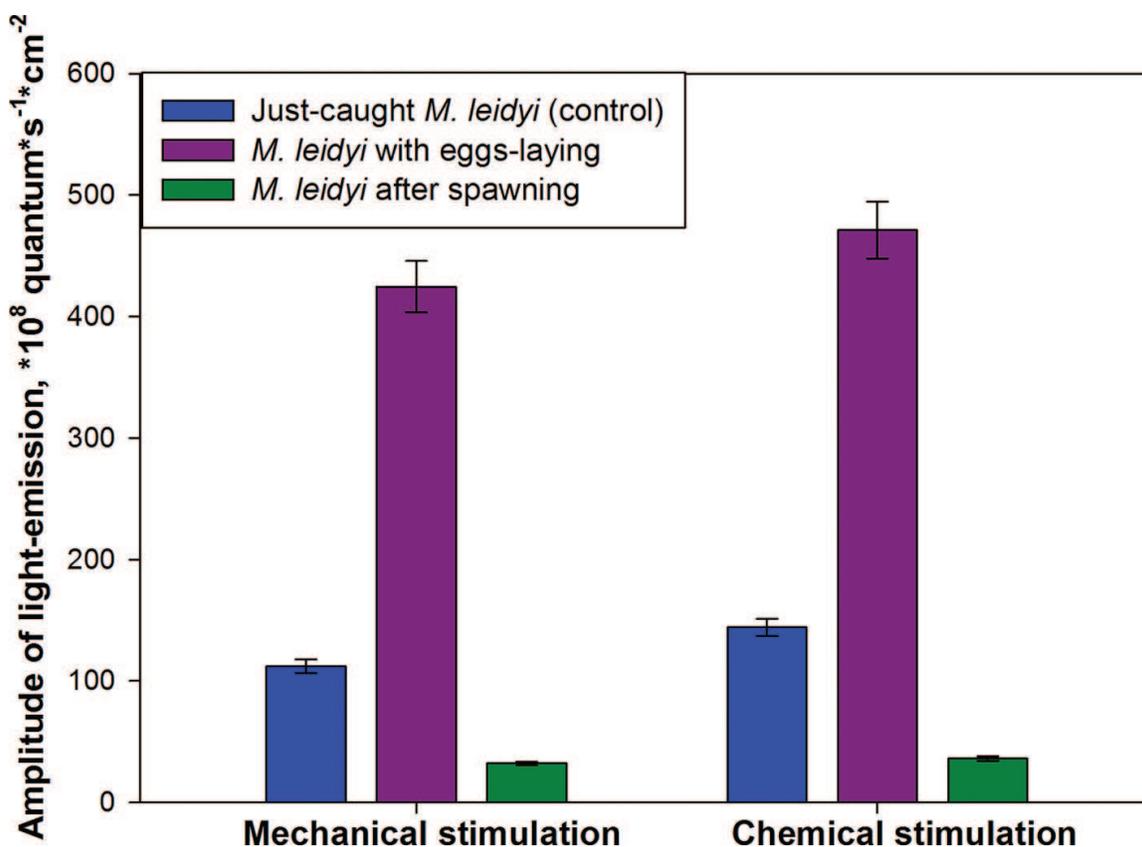


Figure 11. Variability of the bioluminescence amplitude ctenophore *M. leidyi* at the reproduction period.

with clutch and control exemplars practically did not differ, making from 3.28 to 3.62 s, but 3.5 times they exceeded such signals in the spawned specimens, which gave the least time of luminescence to  $0.90 \pm 0.045$  s.

### 3.6. Bioluminescence variability in the *B. ovata* ontogenesis

Bioluminescence energy values depend on quantity of secret, produced in the time of organism irritation. So with the increase of the ctenophore age and body mass growth, the more is secret content. Thus, luminescence intensity is a function of organism's mass, that is,  $A = f(W)$ . Amplitude and bioluminescent signal duration of newly caught ctenophores directly depend on dimension, that is, on wet weight of the investigated organism (Figure 12) [33].

*B. ovata* light-emission amplitude of organisms with body mass till  $0.06 \pm 0.003$  g under mechanical stimulation was two times more than the one under chemical stimulation, achieving  $(11.39 \pm 0.56) \cdot 10^8 \text{ quantum} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}$ . Light-emission intensity grows when *B. ovata* body mass increases from 0.06 to 19.53 g, achieving  $(925.74 \pm 45.27) \cdot 10^8 \text{ quantum} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}$ . The shortest luminescent signals (0.46–0.94 s) were produced by small-sized ctenophores (Figure 13).

Beroe light-emission duration increased, achieving from 1.44 to 2.37 s, as body mass raised [33]. The organisms with body mass  $19.53 \pm 0.97$  g produce 2–2.5 times more prolonged

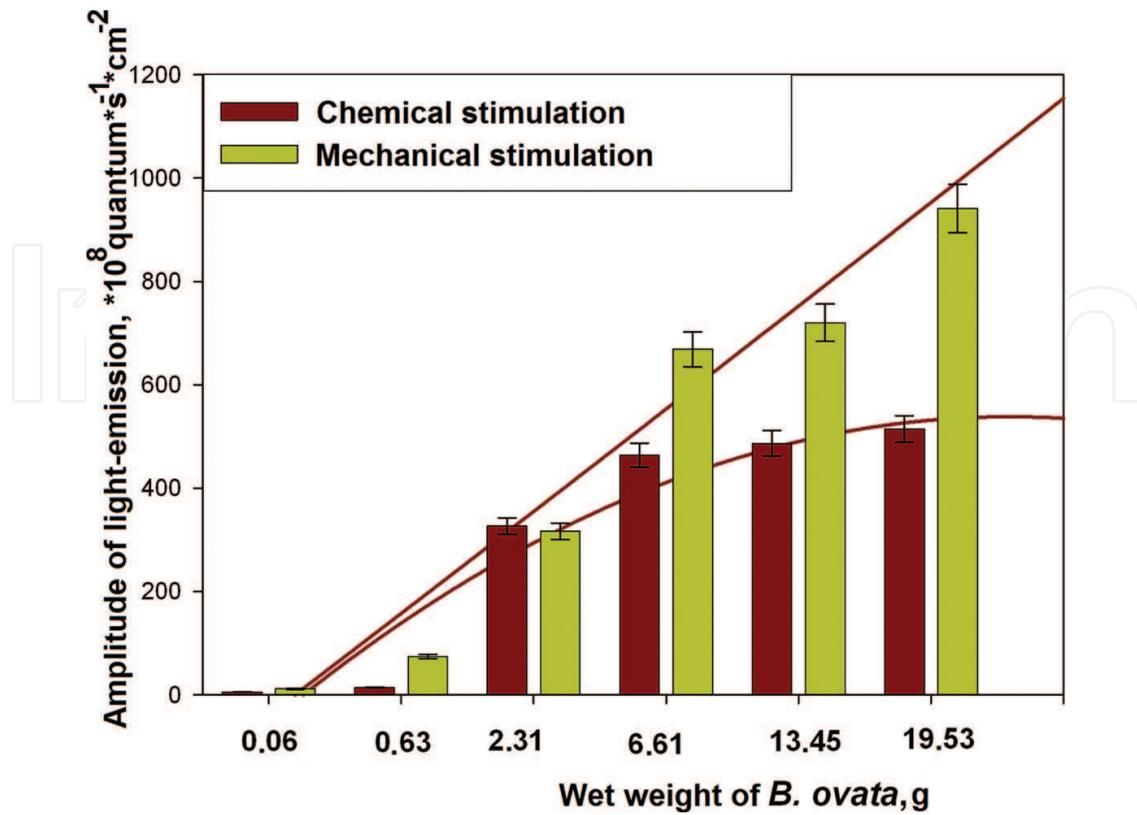


Figure 12. *B. ovata* light-emission amplitude in terms of organism wet weight under mechanical and chemical stimulation [33].

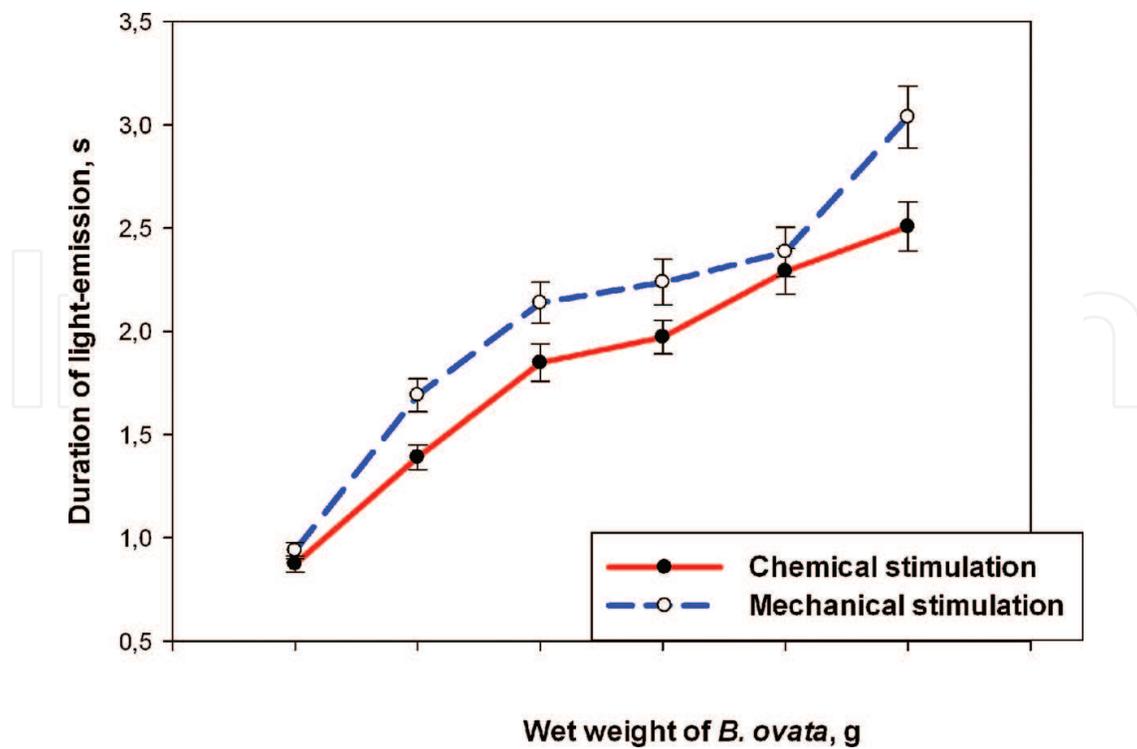


Figure 13. *B. ovata* bioluminescent signal duration depending on body mass wet weight under mechanical and chemical stimulations.

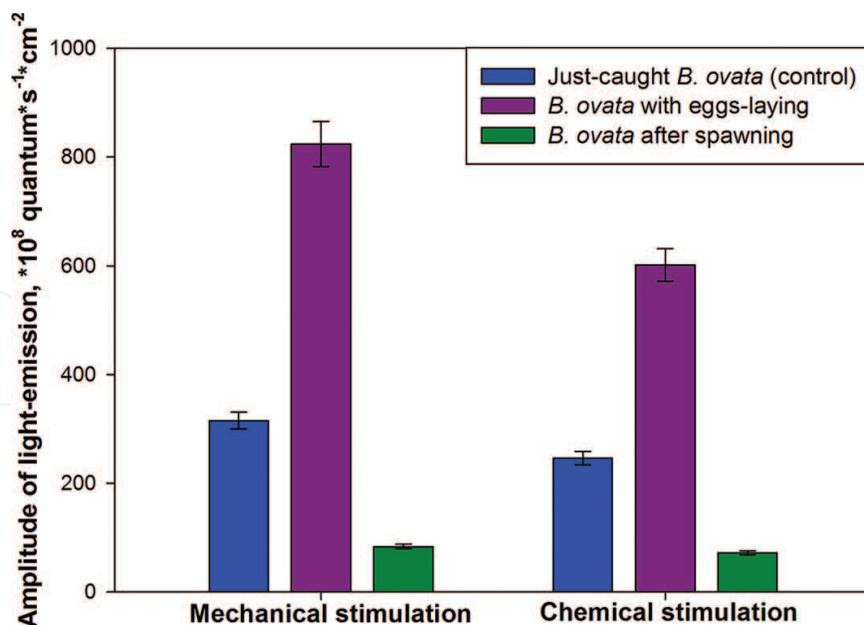


Figure 14. *B. ovata* bioluminescent signal amplitude in reproduction period [33].

light-emission signals than small-sized ctenophores. Experiment's results of the ctenophore reproduction system investigations detected that bioluminescence amplitudes were maximum in ctenophores with egg clutches (Figure 14), being two to three times more ( $p < 0.05$ ) than in the control group. However, bioluminescence indices were three to four times more in the control organisms than in the post-spawning group. Light-emission energy of spawning individuals if compared with other groups achieved maximal indices until  $(434.41 \pm 21.7) \cdot 10^8$  quantum $\cdot$ cm $^{-2}$  [33].

The post-spawning group gave the lowest energy indices until  $(56.77 \pm 2.83) \cdot 10^8$  quantum $\cdot$ cm $^{-2}$ . Light-emission durations in ctenophores with eggs clutches were the same as in the control

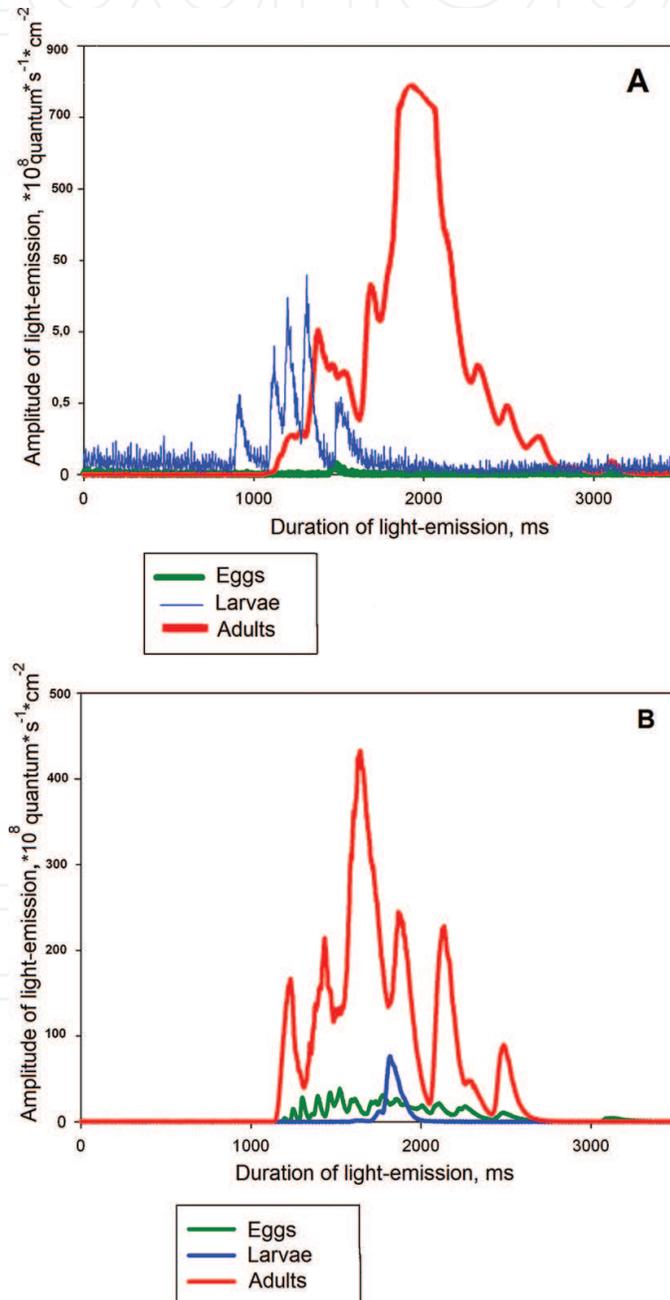
Ontogenesis stages of <i>B. ovata</i>	L (MM)	Amplitude of light-emission (quantum $\cdot$ s $^{-1}$ $\cdot$ cm $^{-2}$ )		Energy of light-emission (quantum $\cdot$ cm $^{-2}$ )		Duration of light-emission, s	
		1	2	1	2	1	2
Just-caught individuals (control)	50	$(315.36 \pm 15.76) \cdot 10^8$	$(246.23 \pm 12.31) \cdot 10^8$	$(331.09 \pm 16.55) \cdot 10^8$	$(177.60 \pm 8.88) \cdot 10^8$	$2.27 \pm 0.11$	$1.39 \pm 0.06$
Reproductive ctenophores	50	$(823.91 \pm 41.18) \cdot 10^8$	$(601.72 \pm 30.08) \cdot 10^8$	$(434.41 \pm 21.72) \cdot 10^8$	$(259.75 \pm 12.98) \cdot 10^8$	$2.49 \pm 0.12$	$1.86 \pm 0.09$
Ctenophore eggs	0.80–0.85	$(0.76 \pm 0.03) \cdot 10^8$	$(0.28 \pm 0.01) \cdot 10^8$	$(0.53 \pm 0.02) \cdot 10^8$	$(0.21 \pm 0.01) \cdot 10^8$	$0.89 \pm 0.04$	$0.33 \pm 0.016$
Ctenophore larvae	0.4–0.5	$(6.07 \pm 0.3) \cdot 10^8$	$(2.26 \pm 0.1) \cdot 10^8$	$(3.71 \pm 0.17) \cdot 10^8$	$(1.49 \pm 0.06) \cdot 10^8$	$1.64 \pm 0.08$	$1.08 \pm 0.05$

Remark: 1, mechanical stimulation; 2, chemical stimulation.

Table 4. The bioluminescence characteristics of ctenophore *B. ovata* at the ontogenesis [33].

group. The lowest light-emission time was in the post-spawned group up to  $1.51 \pm 0.07$  s. The *B. ovata* clutch contained from 2.0 till 7.0 thousands of eggs with size up to 0.80–0.85 mm. Free-swimming larvae with body length of 0.4–0.5 mm appeared on the third day after spawning.

Ctenophore eggs have low luminescence indices with intensity peaks up to  $(0.76 \pm 0.03) \cdot 10^8$  quantum $\cdot$ s $^{-1}$  $\cdot$ cm $^{-2}$ , light-emission energy values—up to  $(0.53 \pm 0.02) \cdot 10^8$  quantum $\cdot$ cm $^{-2}$  and short bioluminescent signal—up to  $0.89 \pm 0.048$  s. It was shown also that larvae biolumi-



**Figure 15.** Typical *B. ovata* bioluminescent signals at different ontogeny stages under mechanical (A) and chemical (B) stimulations [33].

nescence intensity was eight times and energy—seven times more than eggs had ( $p < 0.05$ ) (Table 4, Figure 15).

The same situation was observed for eggs and larvae light-emission durations. Thus, larvae luminescence duration was two to three times more than the eggs' one. *B. ovata* light-emission amplitude rises with ctenophore growth. Adult individual's light-emission amplitude exceeded the larvae one. Luminescence duration in the control organisms is 0.63 s more than in the larvae. Consequently, present research detected that *B. ovata* light-emission characteristics significantly change in ontogeny, at the reproductive stages and rise proportionally with body mass growth [33].

#### 4. Discussion

*M. leidyi* and *B. ovata* light-emission seasonal variability can be conditioned by specificities of ctenophores chemical composition seasonal dynamics. Thus, different food supply is the main reason of the organism's biochemical composition changes. Ctenophores physiological state in the winter-spring periods is depressed, that is connected with food concentration deficit [27]. That is why light-emission intensity and energy have the lowest values in these periods. Food conditions of ctenophore are most favorable in the summer, which is connected with rise of glycogen and waxes concentrations in the *M. leidyi* [42]. Other maximal light-emission amplitude values are registered in August as well. Other reason of the bioluminescence seasonal changes is the Black Sea water temperature variability. Thus, water temperature lowering to  $8 \pm 2^\circ\text{C}$  in winter-spring leads to decrease in amplitude-temporal indices of ctenophores bioluminescence. Low temperatures are unfavorable for the *M. leidyi* vital activity, their motion function and metabolic processes and negatively influence reproduction condition [39, 43, 44]. That is why the ctenophore light-emission characteristics decrease in this period. Ctenophores light-emission seasonal dynamics can be explained by the seasonal changes of their biochemical composition, connected with the food supply [31, 39].

That allows using our experiments results in different variants of the ecological monitoring of the coastal water area. Environment temperature affects considerably the amplitude-temporal characteristics of the Black Sea alien-ctenophore light-emission. It was revealed that bioluminescence reaction optimum for *M. leidyi* is achieved under the temperature of  $26 \pm 1^\circ\text{C}$  and for *B. ovata*—under the temperature of  $22 \pm 1^\circ\text{C}$ , while its minimum for both ctenophore species was registered under the temperature of  $10 \pm 1^\circ\text{C}$ . As it follows from the results of the experiments conducted, bioluminescence is characteristic for *M. leidyi* ctenophores at all the stages of individual development, but with considerable changes in its parameters during ontogenesis. It is necessary to underline that our investigations were conducted at the period of the ctenophores reproduction from July to September, maximum of which in *M. leidyi* takes place in August [43].

But at the period of intensive growth and reproduction, when fodder zooplankton biomass cannot supply needs for support and reproduction of the population, ctenophores are under

deficit of food [18, 20]. That is why increase in the mnemiopsis abundance during reproduction is accompanied with a decrease of its average individual mass, first of all due to a decrease of the big size individuals share in the population [33, 39]. During the given investigations, we also observed in the zooplankton samples domination of fry, eggs and larvae of the ctenophores and to a less extent availability of matured specimens.

Undoubtedly flashes intensity depends on a number of photoprotein in photocytes and maturation of the photocytes themselves. Its content increases with age and consequently with an increase of linear sizes and body mass. Thus, light-emission energy is a function of the organism mass, that is,  $E=f(W)$ . It is also known that trophic factor effects considerably life activity and bioluminescent characteristics of ctenophores [27]. For example, according to our data, freshly caught ctenophores with full stomachs gave eggs and germs averagely in 6 h. But without feeding germs did not develop and they perished, having not reached the larval stage. In the laboratory conditions with satisfactory supply of food ctenophores are close to the conditions of the specimens *in situ* [18, 20]. At the before-spawning period, ctenophore is getting ready for the reproduction, accumulates necessary for this organic substances and contains quite great energetic potential, equal to the sum of its own one and this of eggs. That is why just at the given period, we observe the highest amplitude-energetic parameters of their bioluminescence. Visual observations of the ctenophores behavior at the period of their spawning have shown that individuals after ovulation feel themselves worse, become less mobile, some of them fall to the bottom. Such behavior is identical to this *in situ*, when ctenophores spawning not only influences their moving activity but in some cases also causes specimens death [33]. According to some researchers, organic losses in the ctenophores after spawning can make 6.9% of body. But the full-day losses for exchange in ctenophore with body mass of 25 g at 26°C are estimated as 5.6% of body [43].

In other words, the loss of substance with sex products is quite comparable with losses of an organism for breathing. This points to domination of the generative metabolism strategy in ctenophores and explains accompanying slowing of its growing at the reproductive period [14]. As bioluminescence is closely connected with the breathing chain of organism [3], it is quite understandable that considerable change in the functional condition and metabolism in ctenophores during reproduction are reflected in the observed low indices of the bioluminescence in the spawned individuals if compared with the control. Differences in the ctenophores bioluminescence parameters we revealed at different ontogenesis stages can be also explained by changes in their biochemical composition during their individual development. For instance, according to Finenko and Anninsky, organic substance composition differs considerably in eggs and larvae from the same in the matured specimens. In particular, content of organic substance in *M. leidyi* eggs makes only  $0.25 \mu\text{g}\cdot\text{mg}^{-1}$ , but in the body of two-day larvae of *M. leidyi* of 0.26–0.30 mm size  $25.1 \pm 8.3 \mu\text{g}\cdot\text{mg}^{-1}$  of wet substance [14].

Due to the fact that the organic substances stock provide early survival of larvae and maximum growth rate parallel to minimum exchange more bright lighting of larvae if compared with the ctenophore eggs can be explained, as we think, by great content of organic substance in larvae. Together with this, specific content of organic substance in the ctenophore early larvae is 20–30 times higher than the corresponding magnitudes for adult specimens. Change in number of photocytes in developing individuals can present one more reason of the regis-

tered by us variability of the luminescence characteristics on the ctenophores in ontogenesis. For example at early stages of the ctenophores development, when growth of twinkling rowing plates begins in organisms we observe an increase of the photocytes cytological maturity. At more late stages, when embryo begins to feed itself we observe an increase of the photocytes number. And at last with development of the organisms, we register an increase of the photoprotein number in the photocytes tissues of the adult specimens [40].

That is why it is quite explainable that quantum issue of the ctenophores bioluminescence is minimal at early stages of the organism's development and it is maximal at those late. Besides differences in the ctenophores bioluminescence parameters can be conditioned, according to our opinion, by peculiarities of the ctenophores biochemical composition, determined by their dependence on nutrition quantity and spectrum. According to the data of Anninsky et al. [14], concentration of organic substance in the ctenophore body depends considerably on their size. Protein in the ctenophore body is dominating oxidized substrate and its share in the ctenophore organic substance is of 80–85%. Correlation of concentrations of free amino acids and protein is maximal in small individuals with highly active metabolism and minimal in big organisms. There is domination in lipids of fractions, characteristic for the cell membranes: phospholipids make  $35.7 \pm 9.6\%$  of general lipids. But in bigger organisms, they observe a tendency to increase number of waxes and sterine ethers. For example their content was of  $4.0 \pm 3.6$ ;  $5.5 \pm 3.2$  и  $7.1 \pm 4.0\%$  in ctenophores with the size 10–20, 21–30 and 31–50 mm, correspondingly. In carbohydrates, glycogen dominated; its content grew a bit with an increase of ctenophores size and made  $25 \pm 4$ ;  $28 \pm 5$ ; and  $36 \pm 12 \mu\text{g}\cdot\text{g}^{-1}$ , when body length was 10, 11–20 and 31–50 mm correspondingly [42].

And at last with organisms growing hydration increases and individuals motility decreases. Thus, protein-lipid and carbohydrate exchange effect changes in the ctenophores bioluminescence parameters. But, as it has been already marked with development of organisms' quantity of photoprotein in the ctenophores photocytes and concentration of the substrate of the bioluminescent reaction—luciferin increase, which influence reinforcement of the bioluminescent activity in adult ctenophores [40]. Taking into consideration fermentative nature of the bioluminescent reaction, we can presume that change in the rate of fermentative processes affects duration of the bioluminescent signals. Really maximal bioluminescence is observed in small specimens with higher fermentative activity and shorter signal duration. In adult individuals, we observed decrease in metabolism and connected with this decrease in luciferase fermentative activity, which facilitates more long light-emission [45]. Thus, development of organisms along the way of increasing body hydration and decrease of the active exchange in more big specimens, lowering of their motility and maneuver is compensated by the most important ecological characteristic: less access for predators due to more developed luminescent organs and correspondingly maximal yield of the bioluminescence energy. It gives grounds for supposition that bioluminescence protective function is the most important component in the ctenophores ecology.

Our investigations with *Beroe* larvae were conducted in the period of ctenophore reproduction—from September to November. *B. ovata* spawning peak is observed in October [19, 33, 34]. Juveniles, eggs and larvae predominated in the zooplankton samples from mid-September till October. Similar to *M. leidyi Beroe* prepared for reproduction in prespawning

period. Composition of ctenophores changed. Probably, for this reason, high bioluminescent amplitude-energetic parameters of *B. ovata* were observed. Recording beroe behavior in the spawning period has shown that the organisms after fertilization became less mobile. The *B. ovata* eggs clutch *in situ* was similar to our laboratory experiments, but varied from  $4500 \pm 250$  until 28,000 eggs per day. Distinction in clutch sizes was determined by different organisms' sizes, temperature conditions and availability of nutrition budget [14, 33, 46]. Adult *B. ovata* organisms lose large amount of organic material with reproductive products [14]. Ctenophores with body mass 15.4 g loose from 6% to 8% of organic matter per day, from different data, at temperature conditions of 19–21°C [14, 33, 38].

Accordingly, the fact that bioluminescence is closely related to biochemical processes in organism and to its physiological state [28, 47] is well substantiated by our data that the lowest ctenophore bioluminescent parameters are produced by post-spawned individuals at reproduction period. It is revealed by us that ctenophore bioluminescent parameters dissimilarity at different reproductive stages are explained by changes of their biochemical composition in ontogenesis. The eggs and larvae composition of organic matter differed much from the adult individuals [14]. Beroe light-emission parameters changeability in ontogenesis can be related with photocyte quantitative variability of growing individuals and their cytological maturity [40]. We suppose that ctenophore light-emission characteristics changeability with body mass growth can be determined by specialty of their biochemical composition depending on sizes [23].

At the same time, as the organism develops along the way of body growing hydration and active metabolism [14, 23], decrease of great individuals' mobility and maneuverability is compensated by one of highly important qualities: the lowered survival capability due to more developed bioluminescent organs and, consequently, maximum bioluminescent energy discharge.

## Acknowledgments

The authors are grateful for the valuable advices during the given work conduction to Khanaichenko A.N., Finenko G.A. the scientists of KIMBR, Russia; to Juk V.F., Belogurova Yu.B. and M.I. Silakov, leading engineers of KIMBR, Russia, for the help in work with laboratory equipment and making the program of its verification.

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