Heavy metals influence on the ctenophores *Mnemiopsis leidyi* and *Beroe ovata* bioluminescence

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**Abstract**

Investigations were conducted in the Department of biophysical ecology of Kovalevsky IMBR of RAS in September - October of 2013 and 2015. The body length of the gathered for experiments ctenophores was 35 – 40 mm. Characteristics of the ctenophores light emission were studied under the mechanical and chemical stimulations, with the usage of laboratory complex “Svet”. The following HM salts: Cu₂S0₄, ZnCl₂, PbCl₂ and HgCl₂ in different concentrations were used in our experiments. The just-caught samples, contained in the clean marine water were used as a control. The exposition time was 1, 3 and 24 hours under the temperature of 21 ± 2°C. The investigations results have shown considerable variability of the ctenophore luminosity characteristics in dependence of metal concentration and exposition duration. It was stated that minimal concentrations of cooper, zinc and mercury stimulates ctenophores bioluminescence and the high ones inhibit. The alien ctenophore luminescence inhibition was registered under the lead activity under all investigated concentrations. We can place investigated metals as following: Zn < Cu < Hg < Pb, according to the force of the toxic influence on the ctenophore bioluminescence. Thus, alien ctenophore bioluminescence parameters can serve as a sensitive express-indicator of the resistance degree to the heavy metals impact and be the expressive index of the marine environment regional pollution.

**Key words:** pollutants, light emission parameters, ctenophore, Black sea environment.

**Introduction**

Most contaminants entering the marine environment become local and regional pollution sources disrupting the normal biological processes (Tokarev 2006). Toxic elements actively affect reproduction and metabolic activities of plankton organisms representing the bottom levels of a food chain (Marine report 2009; Luk’yandenko & Cherkashin 1987; Finenko et al. 2003; Tokarev & Shulman 2007). Accumulation of heavy metals in organisms of different taxonomic groups, as well as behavioral responses of hydrobionts to the presence of heavy metal ions, have been quite well investigated (Vasil’kov 2005; Klishko et al. 2007; Mosharov et al. 2009). Years of field and experimental research showed that toxic heavy metals, such as cadmium, lead, mercury, copper and zinc are among the most significant pollution actors in marine environments and should be a priority for further investigation (Vasil’kov 2005; Nikitina et al. 2009; Rudneva 1995).
Being one of the enzyme-substrate components of the internal biophysical cycles, bioluminescence system of the plankton can be affected by exposure to environmental toxicants (Tokarev et al. 2016). In a number of cases, changes of the functional characteristics of organisms, suppression or shifting of the phase and period of circadian rhythms of the bioluminescence and its characteristics under the influence of certain chemical and physical stressors are shown (Yevstigneyev & Yevstigneyev 2005; Heimann et al. 2002; Tokarev et al. 2007). In light of the foregoing, one of objectives of this research was to investigate the influence of heavy metals on the bioluminescence of ctenophores. These studies have not been carried out so far.

Material and Methods

Research was conducted at the Biophysical ecology department of A.O. Kovalevsky Institute of Marine Biological Research (IMBR) Russian Academy of Sciences (RAS) in September – October 2013 and 2015. Sampling of ctenophore mesozooplankton species was performed in water column 0-50 m in the nearshore area of Sevastopol at a distance from the coast up to 2 miles. Ctenophore individuals of the same size (35-40mm) were selected and placed in 3-5 L containers with filtered marine water (35 μm membrane filter) at a temperature of 21 ± 2 °C and were adapted to the experimental conditions. Ctenophores were divided into 4 groups: 1) individuals exposed to heavy metals (HM) at concentration of 1/10 MAC (Maximum Allowed Concentration) (List 1995); 2) individuals exposed to HM concentration of MAC; 3) individuals exposed to HM concentrations exceed MAC by 10 times 4) control group – freshly-caught ctenophore individuals kept in clean marine water. Undamaged individuals without contents in gastrovascular cavity were taken for investigations. The exposure time for ctenophores specimens of the control and experimental groups (40 individuals in each experimental group) was 1, 3 and 24 hours.

The following metals salts: CuSO₄, ZnCl₂, PbCl₂ and HgCl₂ were used in experiments, as a number of Black Sea water zones are feared to be exposed to considerable pressure by these metal salts (Omelchenko et al. 2007; Marine report 2009; List 1995). The bioluminescence signals of ctenophores were registered using laboratory unit “Svet” (Tokarev et al. 2016). At the same time, bioluminescence signals produced by the individuals from the control group were registered. Mechanical and chemical stimulations were applied to individuals from each group. In order to obtain a reliable picture closest to the natural stimulation, the mechanical stimulation of the ctenophores was carried out by creating a flow of water in a reservoir with a bioluminescence producer by means of electromechanical pump (Tokarev et al. 2016). The chemical stimulation was used to obtain information on the maximum ctenophore bioluminescence potential. 96% ethyl alcohol at concentration of 10% was tested as a chemical reagent. It was injected with a syringe into a cuvette (Tokarev et al. 2016).

Results

The effect of copper on M. leidyi and B. ovata bioluminescence

The luminous intensity of the M. leidyi individuals exposed to low concentrations of copper in the first hours of exposure period was almost the same as in the control group: (331.89 ± 16.59)·10⁸ quantum·s⁻¹·cm⁻² under chemical and (242.29 ± 12.11)·10⁸ quantum·s⁻¹·cm⁻² under mechanical stimulation (Table 1). After 3 hours of exposition the amplitude of signals in first experimental group reached values 1.5 – 2 times higher than in control (p < 0.05). After a 24-hour exposition bioluminescence signal amplitude of ctenophores exposed to HM concentration at 0.1 MAC decreases, remaining, however, higher than the control ones and amounting to (46.08 ± 2.3)·10⁸ quantum·s⁻¹·cm⁻² under chemical and (42.86 ± 2.13)·10⁸ quantum·s⁻¹·cm⁻² under mechanical stimulation. Ctenophores that were exposed to the copper at concentration of MAC during the first exposition hours had luminescence intensity 6 times lower than ones in the control group (p < 0.05) under both mechanical and chemical stimulations. This trend continues with increase in exposure time. Thus, after 3 and 24 hours of exposure, ctenophores from this groups had luminescence intensity 2 times lower than the control ones (p < 0.05).
The lowest luminescence amplitudes were registered when *M. leidyi* individuals were exposed to copper at concentration of 10 MAC. At the same time, the luminescence intensity of *M. leidyi* individuals decreases by almost a factor of 10 with the increase of the exposure time from 1 to 24 hours. Thus, the ctenophores luminescence amplitude after 1 hour exposure equals to \((28.84 \pm 1.3) \times 10^8 \text{ quantum} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}\) under chemical stimulation and \((20.83 \pm 1.03) \times 10^9 \text{ quantum} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}\) under mechanical one. This is 20 times lower than those obtained in the control group. After 24 hours period of exposure the luminescence intensity of *M. leidyi* from this group reached its minimum: \((2.75 \pm 0.13) \times 10^8 \text{ quantum} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}\) under chemical and \((2.61 \pm 0.12) \times 10^9 \text{ quantum} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}\) under mechanical stimulation, that is 11 times lower than the control values. Even more significant variability depending on the concentration of the reagent can be observed in the bioluminescence energy (Table 1).

Exposure of ctenophores *M. leidyi* at various copper concentrations has significant effect on the duration of their light emission (Table 1). Thus, the longest duration of signals – up to 3.58 s under chemical and 3.06 s under mechanical stimulation were recorded in ctenophores kept at low concentrations of copper and these values were lower than registered in the control group. Signal duration decreases with increase in exposure time in all experimental groups. The shortest signals were recorded in the ctenophores chemically stimulated at 10 MAC of copper: the duration of the signal in the first hours of exposure was 2.56 s, in 24 hours – only 1.44 s.

**The effect of copper on the variability in the luminescence characteristics of *B. ovata***

Exposing ctenophores even to copper concentrations of MAC reduces energy parameters of luminescence under mechanical stimulation in the first hour of exposure by a factor of 2, amounting to no more than 50% of the control (Table 1). After 3 hours of exposure at a given metal concentration, the luminescence intensity of ctenophores decreases by a factor of 3 (p < 0.05) compared to the control. At low concentrations of copper, the intensity of the light emission of the organisms under mechanical stimulation (Table 1) approaches the control in the first hours, amounting to 97.69% of it, but after 3 hours the amplitude of the signal increases, reaching the maximum value amounted to 251.6%. After 24 hours of exposure, the signal intensity decreases and does not differ greatly from the control. The amplitude of the signals observed in organisms under chemical stimulation at a copper concentration of 0.1 MAC in the first hour of exposure was equal to 162.61% of the control and after 24 hours of exposure it had maximum value – up to 424.74%, which is 4 times higher than the values in the control.

At copper concentrations of 10 MAC, the amplitude of the light splashes detected in the ctenophores during the first hours of exposure was only 2.25% under mechanical stimulation, and under chemical stimulation – 7.74% of the control group values. At the same time, the increase of the exposure time of individuals in solution with a high copper concentration does not affect the light emission amplitude. It remains greatly lower than the light emission amplitude of individuals in the control group: 13.86% of the control values under mechanical stimulation and 9.5% of the ones under chemical stimulation (p < 0.05). A similar situation is observed when the effect of copper ions on the energy and duration of ctenophores luminescence is investigated (Table 1).

The longest signals of the ctenophores light emission during the first hours of the exposure are observed at the lowest copper concentrations, remaining slightly below control, under mechanical stimulation 86.28% of the control, and under chemical stimulation – 98.07% (Table 1). The shortest signals were recorded in individuals exposed to copper concentration of 10 MAC.

**The effect of zinc on *M. leidyi* and *B. ovata* light emission**

**The effect of zinc on *M. leidyi* bioluminescence**

The maximum light emission amplitudes were observed in ctenophore *M. leidyi* exposed to the minimal reagent concentrations (Table 1). The intensity of the light emission of this experimental group with a short exposure time (1 hour) is 2 to 3 times higher than in the control, and after 3 hours of exposure it increases, exceeding the control values by 6 times (p < 0.05). A longer exposure time (24 hours) of the same group showed that the intensity of the light emission reached maximum under chemical stimulation: \((1383.06 \pm 69.15) \times 10^8 \text{ quantum} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}\). With an increase in the concentration of zinc the intensity of the ctenophore light emission decreases reaching a minimum at 24 hours exposition of the ctenophores at HM concentration
of 10 MAC: \((8.97 \pm 0.43) \times 10^8\) quantum\(\cdot\)s\(^{-1}\)\(\cdot\)cm\(^{-2}\) under chemical and \((2.75 \pm 0.12) \times 10^8\) quantum\(\cdot\)s\(^{-1}\)\(\cdot\)cm\(^{-2}\) under mechanical stimulations.

Bioluminescence signal duration also varies depending on the reagent concentration and exposure time. Longer signals were recorded in ctenophores, contained at 0.1 MAC. However, in the first exposure hours the duration of the signals registered were by 0.57 s, and at a 24 hours exposure – by 1.5 times (p < 0.05) lower than those in the control. The shortest signals were recorded during 24 hours exposure of ctenophores to zinc concentration of 10 MAC: \(1.67 \pm 0.07\) s under chemical and \(1.16 \pm 0.04\) s under mechanical stimulations.

Thus, the response of the \(B.\) \(ovata\) bioluminescence system under the action of zinc is similar to the response of \(M.\) \(leidy\) under the action of copper. However, the stimulating effect of zinc on \(M.\) \(leidy\) bioluminescence is stronger. As a result, the luminescence intensity of ctenophores exposed at a low concentration of zinc by 3.5 times under mechanical and 5.5 times (p < 0.05) under chemical stimulation is higher than luminescence intensity of ctenophores under the action of copper (Table 1).

**The effect of zinc on \(B.\) \(ovata\) bioluminescence**

The effect of zinc chloride on \(B.\) \(ovata\) bioluminescence revealed that the intensity of the light of the ctenophores exposed to the minimum zinc concentration (0.1 MAC) did not differ from the control in the first hours of exposure, reaching \((757.40 \pm 37.87)\times 10^8\) quantum\(\cdot\)s\(^{-1}\)\(\cdot\)cm\(^{-2}\) – under mechanical stimulation and \((502.13 \pm 24.10)\times 10^8\) quantum\(\cdot\)s\(^{-1}\)\(\cdot\)cm\(^{-2}\) – under chemical stimulation (Table 1). With an increase in exposure time the intensity of the light emission of the ctenophores from this group was increasing and after 3 hours of exposure exceeded 3 times the control. And after 24 hours of exposure the intensity of the light emission exceeded 8 times (p < 0.05) the control. The light intensity of the ctenophores exposed to zinc at concentration of MAC does not differ from the control, particular under mechanical stimulation. However, with increasing exposure time the intensity of the luminescence decreases, remaining at the same time 2 times higher than the amplitude values in the control (p < 0.05). An increase in the zinc concentration up to 0.5 mg\(\cdot\)L\(^{-1}\) leads to a twofold decrease in the luminescence of the ctenophores compared to the control (p < 0.05). This pattern is continued throughout the entire exposure time. Therefore, at a 24 hours exposure, the intensity of light of ctenophores of this experimental group reaches its minimum values - up to \((9.22 \pm 0.43)\times 10^8\) quantum\(\cdot\)s\(^{-1}\)\(\cdot\)cm\(^{-2}\).

The duration of the flashes (Table 1), as well as in experiments with copper, showed significant deviation from the control values. However, on the whole, it decreases with increasing exposure time in all experimental groups. The longest signals were recorded at low zinc concentrations: \(2.63 \pm 0.12\) s during the first exposure hours under chemical and \(2.28 \pm 0.11\) s under mechanical stimulation. After 3 hours of exposure, the duration of the light emission of the ctenophores of this group increased, amounting to \(2.98 \pm 0.14\) s under chemical and \(2.81 \pm 0.14\) s under mechanical stimulation, which exceeded the control values by 0.52 and 0.59 s respectively.

Chemically stimulated ctenophores exposed to zinc at concentration of MAC had light emission duration that exceeded the control values by 0.43 s already after 1 hour of exposure, but it decreased in 24 hours, remained 1.5 times higher than the control. The shortest light emission signals were registered in the ctenophores exposed to zinc concentration of 10 MAC: \(1.17 \pm 0.05\) s under chemical and \(0.46 \pm 0.02\) s under mechanical stimulation in the first exposure hours and they further decreased by 3 – 4 times (24 hours exposure).

The nature of the effect of zinc on \(B.\) \(ovata\) bioluminescence variability is similar to that observed in \(M.\) \(leidy\) species exposed to the copper. Thus, luminescence energy parameters of ctenophores have maximum values when species are exposed to low zinc concentrations but have minimum ones with increasing metal concentrations and time of exposure. However, in comparison with copper, ctenophores' luminescence is less inhibited by zinc. Thus, the intensity of luminescence of \(B.\) \(ovata\) ctenophores, exposed to low concentrations of zinc, is 2 times higher under mechanical and 4 times higher under chemical stimulation than of those ones exposed to minimal concentrations of copper (Table 1). Resistance of ctenophores species to the action of zinc was also expressed in changes in the motor activity of organisms. Thus, the ctenophores individuals exposed to concentration of zinc even of 10 MAC for 10-12 hours, moved actively and only at a longer exposure (24 hours) settled down to the bottom.
Table 1. Heavy metals influence at the *M. leidyi* and *B. ovata* bioluminescence.

| Metal, exposition duration | Concentration | *Mnemiopsis leidyi* | *Beroe ovata* |
|---------------------------|---------------|---------------------|---------------|
|                           |               | Amplitude | Duration | Amplitude | Duration |
| Zn (24 h)                 | 0.001 mg·l⁻¹ | 650%      | 76%      | 850%      | 160%     |
|                           | 0.01 mg·l⁻¹  | 50%       | 71%      | 190%      | 110%     |
|                           | 0.1 mg·l⁻¹   | 25%       | 65%      | 50%       | 45%      |
| Cu (24 h)                 | 0.01 mg·l⁻¹  | 200%      | 73%      | 430%      | 62%      |
|                           | 0.1 mg·l⁻¹   | 48%       | 45%      | 55%       | 70%      |
|                           | 1.0 mg·l⁻¹   | 23%       | 30%      | 50%       | 50%      |
| Pb (24 h)                 | 0.001 mg·l⁻¹ | 18%       | 73%      | 18%       | 82%      |
|                           | 0.01 mg·l⁻¹  | 12%       | 52%      | 16%       | 80%      |
|                           | 0.1 mg·l⁻¹   | 10%       | 20%      | 5%        | 72%      |
| Hg (24 h)                 | 0.001 mg·l⁻¹ | 90%       | 100%     | 350%      | 75%      |
|                           | 0.01 mg·l⁻¹  | 20%       | 62%      | 60%       | 67%      |
|                           | 0.1 mg·l⁻¹   | 10%       | 12%      | 50%       | 50%      |

- all data in percent of similar control parameters.

The effect of lead on *M. leidyi* and *B. ovata* light emission

The effect of lead on the light emission of the ctenophore *M. leidyi* differs significantly from that of other metals. The intensity of luminescence of ctenophores (Table 1) decreases dramatically with increasing exposure time in all experimental groups and is proportional to the concentration of the metal in solution.

Thus, the ctenophores exposed to the lead concentration of 0.1 MAC produce weak bioluminescence signals – up to \((76.5 ± 3.7) \times 10^8\) quantum·s⁻¹·cm⁻² under mechanical and \((97.16 ± 4.7) \times 10^8\) quantum·s⁻¹·cm⁻² under chemical stimulation, which is 5 times lower than that in the control group. Individuals exposed to lead at concentration of 1 MAC reduced the intensity of light emission under chemical stimulation by 6.5 times in comparison with the control during the first hours of exposure and by 5 times under mechanical ones (p < 0.05). The luminescence amplitude of the ctenophores of this group decreased with increasing exposure time, reached the minimum values after 24 hours of exposure: \((34.16 ± 1.6) \times 10^8\) quantum·s⁻¹·cm⁻² under mechanical and \((42.19 ± 1.9) \times 10^8\) quantum·s⁻¹·cm⁻² under chemical stimulation. The lowest values of luminescence intensity were recorded in ctenophores exposed to the lead concentration of 10 MAC, amounting to 11.76% of the control under chemical and 4.6% under mechanical stimulation.

The duration of *M. leidyi* bioluminescence events also shows inhibition pattern at all given concentration of reagent (Table 1). Thus, the duration of the light emission of the mechanically stimulated ctenophores exposed to the concentration at 0.1 MAC during the first hours of exposure comes close to the control group values and chemically stimulated ctenophores produce bioluminescence signals with duration amounted to 70% of that in the control.

With an increase in the exposure time, the duration of the luminescence signals of mechanically stimulated *M. leidyi* individuals decreases gradually. In the case of chemical stimulation, it gradually increases and afterwards, after 3 hours of exposure, decreases, reaching its minimum values in 24 hours. The shortest signals are recorded in the ctenophores exposed to a high concentration of lead. At the same time in
the first hours of exposure they make up 35.19% of the control, and 16.12% of the control after 24 hours of exposure.

Thus, the amplitude-time parameters of the ctenophore *M. leidyi* luminescence during the entire exposure period decreases in all experimental groups. At the same time, the expected stimulating effect (registered during the exposure of the ctenophores to low concentrations of copper and zinc) of lead at concentration of 0.1 MAC on the *M. leidyi* was not observed.

**The effect of lead on *B. ovata* bioluminescence**

The result of the investigations of the *B. ovata* luminescence have shown that the ctenophore luminescence essentially inhibited at all concentrations of lead ions used and these results are similar to those obtained for *M. leidyi*. The amplitude of the *B. ovata* bioluminescence has values close to minimal at all given concentrations.

Even when ctenophores exposed to a lead concentration of 0.1 MAC, already in the first hours of exposure, the light emitted by ctenophores had low intensity: 6 – 7 times lower than the control values (p < 0.05). Exposition of the ctenophores to lead concentrations of MAC resulted in even lower values of the emission amplitude: (41.01 ± 2.05)·10^8 quantum·s^{-1}·cm^{-2} – under chemical and (66.15 ± 3.3)·10^8 quantum·s^{-1}·cm^{-2} under mechanical stimulations respectively.

The minimal ctenophore light emission was recorded in individuals exposed to a concentration of lead at 10 MAC. The amplitude of the light emission during the first hours of exposure is 34 times lower than that in the control group. The increasing of exposure time to 24 hours resulted in the minimal values of the amplitude as well: (1.54 ± 0.06)·10^8 quantum·s^{-1}·cm^{-2} under mechanical and (1.66 ± 0.07)·10^8 quantum·s^{-1}·cm^{-2} under chemical stimulation.

The ctenophore light-emission duration varies significantly (Table 1), depending on the concentration of lead used and the duration of exposure. Longer signals are recorded in the group of ctenophores exposed to the lead concentration of 0.1 MAC: in the first hours of exposure from 1.72 ± 0.08 s under chemical and up to 2.17 ± 0.1 s under mechanical stimulation. The light emission duration decreases after 3 and 24 hours of exposure, however, remained maximal: 1.21 ± 0.06 s under chemical and 1.71 ± 0.08 s under mechanical stimulation.

In the other experimental groups, the duration of the light emission is significantly lower than that in the control and is reduced with increasing of exposure time. The shortest signals, especially at a 24 hours exposure, are recorded in individuals exposed to lead concentrations of 10 MAC. The duration of the light emission in this group reaches: 0.88 ± 0.03 s under mechanical and 1.09 ± 0.05 s under chemical stimulation, which amounts to 50 % of the control. Thus, the inhibition of luminescence of *B. ovata* ctenophores, as well as *M. leidyi* ones, under the influence of lead ions increases both with an increase in the concentration of a given metal and with an increase in the exposure time.

**The effect of mercury on *M. leidyi* and *B. ovata* bioluminescence**

**The effect of mercury on *M. leidyi* bioluminescence**

The results of the experiments showed that there are no significant changes in the luminescence of *M. leidyi* under the action of mercury ions (Table 1). Thus, at a mercury concentrations of 0.1 MAC, the amplitude of luminescence of the ctenophores after 1 hour of exposure is 1.5 to 2 times lower than in the control, but after 3 hours an increase in the luminescence intensity is observed. It increased in such a way that the luminescence amplitude in this group after 24 hours exposition do not differ significantly from the control and amounted to (67.55 ± 3.36)·10^8 quantum·s^{-1}·cm^{-2} under chemical and (46.08 ± 2.3)·10^8 quantum·s^{-1}·cm^{-2} under mechanical stimulation. When ctenophores are exposed to the mercury concentration of MAC the amplitude of luminescence of ctenophores is 2 to 2.5 times lower in the first hours of exposure, and 24 hours later is 5 times lower than the control (p < 0.05).

The lowest intensity of *M. leidyi* luminescence was recorded in ctenophores exposed to mercury concentration of 10 MAC. Thus, the bioluminescence amplitude in this group after 1 hour exposure was (1.76 ± 0.07)·10^8 quantum·s^{-1}·cm^{-2} under chemical and (1.51 ± 0.06)·10^8 quantum·s^{-1}·cm^{-2} under mechanical stimulation. After 3 hours exposure to mercury, the luminescence amplitude decreased to the minimum values: (0.40 ± 0.02)·10^8 quantum·s^{-1}·cm^{-2} under chemical and (0.26 ± 0.013)·10^8 quantum·s^{-1}·cm^{-2} under mechanical stimulation. The 24 hour exposure to concentration of mercury exceeding MAC by a factor of 10 caused stopping of the light emission in ctenophores.
Observations of the ctenophores behavior showed that at high concentrations of mercury in the first hours of exposure, the motor activity of ctenophores is disturbed. It can be observed by slowing down of comb plate beating. After 3 hours of exposure the ctenophores practically do not rise to the surface, and after 24 hour they settle down to the bottom, losing up to 80% of their wet weight.

The duration of the ctenophore luminescence (Table 1) also varies significantly in proportion to the concentration of the reagent. The duration of light emission of individuals exposed to high mercury concentrations in the first hours was $1.46 \pm 0.06$ s – under mechanical stimulation and $1.57 \pm 0.07$ s – under chemical stimulation. After 24 hours of exposure the duration of luminescence was minimal – 0.87 and 1.11 s respectively. The longest light emission event, especially during the first hours of exposure (up to $2.21 \pm 0.11$ s – under chemical stimulation and $1.82 \pm 0.08$ s – under mechanical stimulation), is registered in ctenophores exposed to the lowest mercury concentrations. However, after 24 hours exposure the light emission duration in this group is decreased.

The effect of mercury on B. ovata bioluminescence

The effect of mercury on the B. ovata bioluminescence variability resembles that of copper and zinc cations, but the sensitivity of the bioluminescence system to mercury ions is significantly higher. Thus, the exposure of the ctenophores to a concentration of mercury equal to the MAC leads to a light emission with intensity reaching $(537.6 \pm 26.88) \cdot 10^8$ quantum·s$^{-1}$·cm$^{-2}$, which is close to the amplitude values in the control. However, after 24 hour exposure at a given mercury concentration, the amplitude of the light emission of the ctenophores, especially of those chemically stimulated, decreases by a factor of 2 ($p < 0.05$).

The intensity of the light emission of ctenophores exposed at low mercury concentrations in the first hours of exposure, especially of those that are chemically stimulated, is 1.5 times higher than that in the control. After 3 hour exposure, the luminescence activity of the ctenophores increases. The light emission amplitude is 5 times higher under mechanical stimulation and 8 times higher under chemical stimulation than that of individuals in the control ($p < 0.05$).

With an increase in the exposure time to 24 hours, the amplitude of the light emission of individuals decreases, remaining 4 times higher than the values in the control ($p < 0.05$). At high concentrations of mercury (0.001 mg · L$^{-1}$), the amplitude of the light emission of the ctenophores decreases by 2.5 times in the first few hours under mechanical stimulation and by 4.5 times when chemical stimulation used ($p < 0.05$). After a 3-hour exposure of the ctenophores at a given mercury concentration, the intensity of bioluminescence is reduced by 4 times ($p < 0.05$) both under mechanical and chemical stimulations. Further exposure of the ctenophores for up to 24 hours leads to a sharp decrease in the amplitude of their bioluminescence – by a factor of 7 compared with the control ($p < 0.05$).

The duration of luminescence of ctenophores in all experimental groups decreased with increasing exposure time (Table 1). The longest signals were recorded in individuals during the first hours of exposure at low mercury concentrations and reached $2.54 \pm 0.12$ s – under mechanical and $2.66 \pm 0.3$ s – under chemical stimulation. The shortest signals were registered in the ctenophores exposed at mercury concentrations of 10 MAC for 24 hours and amounted to $1.21 \pm 0.06$ s under mechanical and $0.75 \pm 0.037$ s under chemical stimulation.

Discussion

It is known that the entry of copper and mercury ions into the external environment leads to degenerative changes in the membranes responsible for the energy processes in the organisms (Kutsenko 2002; Rudneva et al. 2011), which negatively affects their physiological state. Our investigations, however, have shown stimulating effect of low concentrations of copper, zinc and mercury on the ctenophore luminescence which is replaced by inhibition of their light emission with an increase in the concentration of HM and the time of their exposure. A similar effect of the copper compounds on the biological processes occurring in other hydrobionts and plankton communities has been described in a number of publications (Vasil’kov 2005; Tokarev et al. 2016; Mosharov et al. 2009). On the other hand it is known that in various living organisms (arthropods, worms, protozoa, bacteria, etc.) heavy metals inhibit the enzymatic activity (Kutsenko 2002). And in some cases toxic effects of HM can occur when hydrobionts are exposed to even small concentrations of the compounds (Tokarev et al. 2007).
The results of our studies unexpectedly showed higher toxicity of copper compared to zinc. In our opinion, it could be explained by the fact that zinc can have a double effect on hydrobionts. Thus, studies of the effect of zinc ions in aquatic environments on the hemocytes of the gastropod mollusc Planorbarius purpura reported a reduction in the number of prohemocytes in the hemolymph of mollusks infected with trematodes and as a result deterioration of hemolymph immune abilities (Kirichuk & Stadnichenko 2010). For uninfected organisms, including ctenophores, this metal is vital as zinc is an essential constituent of over 200 metalloenzymes participating in proteins and lipid metabolism, nucleic acid synthesis (Davydov & Tagasov 2002). At the same time high HM concentrations lead to disturbances in the functional development of hydrobionts. It is for this reason that maximum of the light emission of ctenophores registered at a low zinc concentrations and minimum values registered at a high zinc concentrations, indicates the activation of enzymatic processes in the ctenophores organism at zinc concentration of 0.1 MAC and their inhibition at zinc concentration of 10 MAC (Kutsenko 2002).

Among the HM we investigated, mercury and lead compounds are considered to be the most dangerous (Linnik 2010; Stancheva et al. 2010). Differences in bioluminescence responses of ctenophores to lead and mercury can be explained by different toxicological effects produced by these HM. According to the hygienic classification, adopted in Russia, mercury belongs to the first class danger contaminant (an extremely dangerous chemical substance). Our investigations showed the same effect of mercury on ctenophore bioluminescence (stimulated at 0.1 MAC, inhibited at 10 MAC), but the 24 hours exposure of M. leidyi at mercury concentration of 10 MAC leads to the loss of bioluminescence activity. Ctenophore B. ovata, in contrast, was more resistant to high concentration of mercury, retaining the luminescent capability at 24 hours exposure. It could be connected with the peculiarities of the B. ovata morphology (a higher percentage of the protein in total organic matter) – reduced membrane permeability. B. ovata has higher luminescent intensity. In terms of toxic impact on the ctenophores M. leidyi and B. ovata, as well as on bioluminescence in general, investigated metals can be arranged as follows: Zn < Cu < Hg < Pb.

Generally, inorganic mercury compounds are less toxic than organic ones (methylmercury etc.) due to its lipophilicity and reduced ability to affect components of enzyme systems. Perhaps this is one of the reasons why the HgCl₂ (mercuric chloride) used in our studies did not cause such a pronounced toxic effect on the ctenophores. However, in experiments with Noctiluca scintillans Sur., Yu.N. Tokarev group had obtained other results and metals in terms of their toxicity had been arranged as follows: Fe < Zn < Pb < Cu < Hg (Tokarev et al. 2016). The studies of Evstigneev P. V. and Bityukov E. P. had showed that increasing degree of inhibition of the copepod Pleurobrachia gracilis Claus, 1863 bioluminescence by the toxic substances could be expressed as follows: phenol < PbCl₂< petroleum < CuSO₄< surfactants < HgCl₂ (Tokarev et al. 2016).

Lead's effect on the bioluminescence of the ctenophores was entirely different from the effect produced by other heavy metals investigated. Complete inhibition of bioluminescence by low lead concentration as well as by high ones was registered. This toxicant belongs to a group of highly cumulative substances with polytropic actions and it can lead to various metabolic immune disorders, depending on the degree of intoxication.

Lead binds with the greatest affinity to sulfhydryl groups but also interacts with a diverse array of nucleophilic ligands, including amine, phosphate, and carboxyl groups, inhibits protein synthesis and the activity of the enzymes, leading to the formation of modified proteins (Rudneva et al. 2011). It was mentioned earlier that protein forms about 80% of the ctenophore total organic content (Anninsky et al. 2005; Clarke et al. 1992). That is why the inhibition of luminescence in ctenophores during exposure to lead can be explained primarily by the structural and functional changes of luciferin-luciferase system that is of protein nature as well as by possible metabolism inhibition.

Thus, the universality of the relationship between bioluminescence of the organisms and their bioenergy is obvious. The high sensitivity of the ctenophore bioluminescence revealed during our investigation allows to use ctenophores as bio-indicators for quality assessments of the marine environment. The possibility to estimate the accumulation of heavy metals in ctenophores and its threshold limits at different environmental gradients is an important area for further research.
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