

Morphometric parameters of erythroid hemocytes of alien mollusc *Anadara kagoshimensis* (Bivalvia, Arcidae) under normoxia and anoxia

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ABSTRACT. In the present work we investigated the influence of three days anoxia on hematological parameters, morphological and functional characteristics of erythroid cells of alien bivalve *Anadara kagoshimensis*. Oxygen concentration in seawater was decreased by bubbling with nitrogen gas for 5 h. Temperature was maintained at 20±1°C and photoperiod was 12h day: 12h night. Extrapallial fluids were sampled by a puncture of extrapallial cavity. Three-day exposure to anoxia caused pronounced cellular responses. At the organismic level changes were not observed, as hemoglobin concentration the total number of erythroid cells and mean hemoglobin concentration (*MCH*) remained at the level of normoxia. We observed an increase of cellular anomalies, i.e. shistocytes and erythroid cells with polymorphic nuclei, and cells undergoing reactive amitotic division, which resulted in formation of binuclear cells. Nuclear volume (V_n) increased for more than 40% compared to control level. This increase depended on the duration of anoxia. Changes in cellular volume (V_c) possessed a complicated manner. At the end of experimental period (3rd day of anoxia) nucleocytoplasmic ratio was 36% lower comparing to normoxia. Exposure to anoxia did not cause mortality of erythroid cells. The number of red blood cell shades observed on slides remained at control level.

Морфометрические характеристики эритроидных гемоцитов у моллюска-вселенца *Anadara kagoshimensis* (Bivalvia, Arcidae) в условиях нормоксии и аноксии

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РЕЗЮМЕ. В условиях эксперимента исследовали влияние экспериментальной аноксии на гематологические показатели и морфофункциональные характеристики эритроидных элементов гемолимфы двустворчатого моллюска-вселенца *Anadara kagoshimensis*. Содержание кислорода в воде понижали путем барботажа ее азотом в течение 5

часов. Температуру воды поддерживали на уровне 20±1°C. Фотопериод – 12 ч день : 12 ч ночь. Экспозиция – трое суток. Пробы гемолимфы отбирали пункцией экстрапаллиального пространства. Адаптация анадары к условиям аноксии в течение 3-х суток вызывала изменения в основном на клеточном уровне. Системные процессы не были выражены: концентрация гемоглобина, число эритроцитов, среднеклеточное содержание гемоглобина (*MCH*) сохранялись на уровне контрольных значений (нормоксия). В гемолимфе моллюсков увеличивалось число клеточных аномалий: шистоцитов, а также эритроидных элементов с признаками ядерного полиморфизма, реактивного amitotического деления ядер, приводящего к появлению двухъядерных клеток. Значительно повышался объем ядра (V_n) (более чем 40%). Рост был пропорционален длительности нахождения моллюсков в условиях аноксии. Изменение объема клеток красной крови (V_c) носило более сложный характер: вначале он понижался (1-е сутки эксперимента), затем рос (2-е сутки эксперимента) и стабилизировался на уровне выше контрольных значений (3-и сутки эксперимента). Опережающий рост V_n по отношению к V_c нашел отражение в уменьшении значений ядерно-плазматического отношения (*NCR*). На 3-и сутки экспери-

мента *NCR* было на 36% ниже, чем при нормоксии. Удельная поверхность клеток (SS_c) на протяжении всего эксперимента сохранялась на уровне 1,51-1,57 μm^{-1} . Аноксия не сопровождалась разрушением эритроидных элементов. Число эритроцитарных телей, подсчитанных на гистологических препаратах контрольных и опытных серий, совпадало.

Introduction

Near 53-55 species of bivalve mollusks have been identified in the Azov and Black Sea region [Revkov *et al.*, 2008]. *Cerastoderma glaucum* (Linnaeus, 1758), *Abra segmentum* (Récluz, 1843), *Mya arenaria* (Linnaeus, 1758) and others are the most abundant species in the Azov sea [Studenikina, Frolenko, 2007]; and *Mytilus galloprovincialis* (Lamarck, 1819), *Mytilaster lineatus* (Gmelin, 1791), *Chamelea gallina* (Linnaeus, 1758), *Modiolula phaseolina* (Philippi, 1844), in turn, are numerous for the Black Sea [Revkov *et al.*, 2008]. Functional basis of such a wide distribution of bivalves remains poorly studied except of *M. galloprovincialis*. For mussels qualitative composition of carotenoids [Maoka *et al.*, 2011], antioxidant enzyme complex [Soldatov *et al.*, 2008] and peculiarities of tissue metabolism [Kulikova *et al.*, 2015] have been intensively studied.

Recently, the alien Pacific species, *Anadara kagoshimensis* (Tokunaga, 1906), has become abundant in the Azov and Black Sea region [Revkov, 2016]. *A. kagoshimensis* possesses several unique functional adaptive mechanisms that are fundamentally different from other bivalve species, which allows it to survive in acute hypoxic and anoxic conditions and cope with the exposure to hydrogen sulfide [Zwaan *et al.*, 1991; 1992; Soldatov *et al.*, 2018]. All these features contributed successive invasion of the species in the Azov and Black Sea region. The mollusk has effective anaerobic metabolic pathways [Isani *et al.*, 1986; 1989; Zwaan *et al.*, 1995], which include protein substrates for energy production [Mistri *et al.*, 1988]. The qualitative composition of tissue carotenoids has been investigated in detail [Gostyukhina *et al.*, 2013] and their involvement into the adaptation to the hypoxia has been shown [Borodina, Soldatov, 2019].

Extrapallial fluids of *A. kagoshimensis* contains polymorph hemoglobin (Hb) [Cortesi *et al.*, 1992] which is formed by two components (HbI, HbII) [Chiancone *et al.*, 1981]. Dimer HbI possesses high cooperative effect in oxygen binding [Chiancone *et al.*, 1981; Boffi *et al.*, 1991]. HbII is a tetramer consisting of two polypeptide chains (A and B) [Piro *et al.*, 1996]. HbII is characterized with close to HbI cooperative effect, although its affinity to oxygen is higher [Chiancone *et al.*, 1981; Chiancone *et al.*, 1988; Piro *et al.*, 1996].

Hemoglobin may also contribute the tolerance of

A. kagoshimensis to hypoxia. The Hb concentration and red blood cell number [Koluchkina, Ismailov, 2007] as well as morphology of erythroid hemocytes have been recently determined for the Black Sea population of *A. kagoshimensis* [Novitskaya, Soldatov, 2013]. In the present work we investigated the involvement of extrapallial fluids in the adaptation to anoxia. Therefore, the aim of the study was to determine the influence of *in vivo* experimental anoxia on hematological parameters and morphological and functional characteristics of *A. kagoshimensis* erythroid cells.

Materials and methods

Blood clams, *A. kagoshimensis*, (shell length 30-33 mm) were obtained from the collectors of "Don-Komp" fishing company (Streletskaaya bay, Sevastopol). In an hour after capturing, mollusks were transferred to a laboratory in dry containers, and then acclimated to laboratory conditions in aerated tanks for 2-3 days prior the starting of the experiment.

Experiments were conducted on an original experimental stand, which allows to maintain the given oxygen concentration and temperature for a certain period of time. 30 clams were placed into the tank (13.5 l) (element of experimental stand). The design features of the experimental stand were considered earlier [Soldatov *et al.*, 2018]. Oxygen concentration in the tank was decreased by bubbling of the water with nitrogen gas for 2.5-3.0 h from 8.5-8.7 mg l^{-1} to 0 mg l^{-1} . Dissolved oxygen concentration was measured by oxygen sensor DO Meter ST300D RU ("Ohaus", USA). Water temperature maintained at $20 \pm 1^\circ\text{C}$; photoperiod was 12h day: 12h night. The experimental period for anoxia exposure was 3 days. Control group of mollusks was carried in aerated tanks (dissolved oxygen concentration 8.5-8.7 mg l^{-1}) at similar temperature and photoperiod. Water in control and experimental tanks was completely changed every day to remove metabolic end products.

Hematological parameters

Extrapallial fluids was withdrawn by a puncture of extrapallial cavity. Hemoglobin concentration in probes was estimated using a standard hemoglobin cyanide method ("Agar-med" reagent kit, Russia). The number of erythroid cells in extrapallial fluids was determined on Goryaev chamber [Houston, 1990]. Mean cellular hemoglobin concentration was estimated using the following equation:

$$MCH = Hb/Er$$

where Hb – concentration of hemoglobin (g l^{-1}), Er – the number of erythroid cells ($\text{cells } \mu\text{l}^{-1}$).

Prior to slide preparation, cells were washed 3 times in isotonic NaCl (0.85%) by centrifugation (750 g, 15 min, centrifuge Elmi CM-50). Procedure

of cell washing before microscopic observation is needed due to a high salt content, which, in turn, crystallizes on slides at drying. Oxygen concentration in NaCl washing solution corresponded to that used in experimental protocol. Slides were dyed by combined Pappenheim method [Houston, 1990]. The morphological and cytometric parameters of erythroid cells were examined on a light microscope Biomed PR-2 Lum equipped with camera Levenhuk C NG Series. For each cell, shape, the average diameter and nuclei diameter were assessed.

Morphometric parameters

Linear diameter of cells was estimated on microphotographs (immersion, 1500 \times) using ImageJ 1.44p software [Girish, Vijayalakshmi, 2004]. For each cell, the largest and the smallest cellular diameter (C_1 and C_2 respectively) and the largest and the smallest diameter of nuclei (N_1 and N_2 respectively) were measured (Fig. 1). 100 cells per slide were examined.

On the basis of the data obtained the following morphological parameters were estimated using the equations: cellular volume (V_c) [Houchin *et al.*, 1958] with nuclear volume (V_n) [Tasea, 1976]. Cell width was estimated using equation [Chizhevsky, 1959].

$$V_c = 0.7012 \times ((C_1 + C_2)^2 / 2) \times h + V_n$$

$$V_n = (\pi \times N_1 \times N_2^2) / 6$$

$$h = 1.8 + 0.0915 \times (C_1 - 7.5)$$

where C_1 – large cellular diameter; C_2 – small cellular diameter; N_1 – large nuclear diameter; N_2 – small nuclear diameter; h – cell width.

Also, surface area (S_c) [Tasea, 1976], nucleocytoplasmic ratio (NCR) and specific surface area (SS_c) were determined:

$$S_c = 2\pi a^2 b + (2\pi a b \sin(h^1 e)) / e$$

$$e = (a^2 - b^2)^{-1/2} / a \quad b = 0.67h \quad a = (C_1 + C_2) / 4$$

$$NCR = V_c / V_n \quad SS_c = S_c / V_c$$

Statistical analysis

Statistical and graphical analysis was performed in Grapher software (11.0). The results are expressed as $M \pm SE$. Significance of differences was estimated using Student t-test. Normality of data distribution was assessed using Pearson criteria.

Results

Hematological parameters

Hemoglobin concentration in extrapallial fluids of mollusks carried at normoxic conditions (control group) was 20–22 g l⁻¹ (Fig. 2). The number of erythroid cells reached 55 000 cells μ l⁻¹. Erythroid hemocytes possessed high mean hemoglobin concentration, which was more than 400 pg. Anoxia did

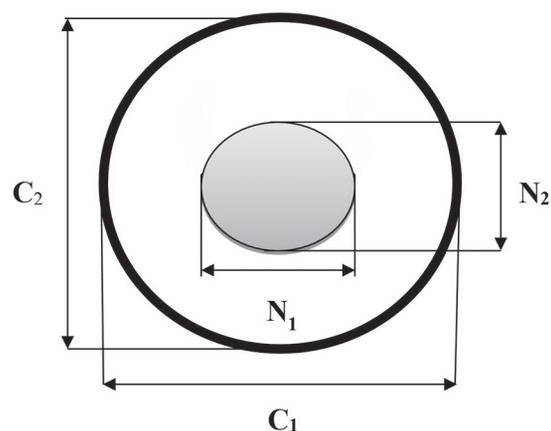


FIG. 1. Linear parameters of extrapallial fluids clam erythroid cells (C_1 – large cellular diameter; C_2 – small cellular diameter; N_1 – large nuclear diameter; N_2 – small nuclear diameter).

РИС. 1. Линейные параметры эритроидных клеток гемолимфы моллюска (C_1 – большой диаметр клетки; C_2 – малый диаметр клетки; N_1 – большой диаметр ядра клетки; N_2 – малый диаметр ядра клетки).

not cause significant changes on these parameters ($p < 0.05$).

Morphological analysis

Mature erythroid hemocytes were large cells possessing nearly round shape; large acentric nuclei situated in acidophilic cytoplasm contained high hemoglobin concentration. Basophilic nuclei possessed high heterochromatin content, indicating low functional activity of the organelle. Cytoplasm of erythroid cells contained numerous dark granules, which were single or associated in aggregates.

Most of the erythroid cells in extrapallial fluids had round nuclei. However, in control specimens we observed several variations of nuclei morphology: (1) kariokinesis, (2) polymorphism of nuclei, (3) atypical position of nuclei within cells, (4) nuclear-free or binuclear cells. The total number of abnormal cells in extrapallial fluids exceeded 10%. After 3 days exposure to anoxia, the number of abnormal cells increased on more than 20%. Kariokinesis, polymorphism of nuclei and binuclear cells were the most abundant anomalies observed in cells after exposure to anoxia. The number of these anomalies in extrapallial fluids of blood clams gradually increased till the 3rd day of experiment ($p \leq 0.05$) (Fig. 3). Kariokinesis was characterized with nuclei division resulting in formation of binuclear cells. Similar type of cell division is usually observed during amitosis. However, this type of cell division excludes equal distribution of chromosomes between daughter nuclei. Polymorphism of nuclei was associated with the changes of its shape.

We also observed various anomalies of shape

Table 1. Linear sizes of erythroid cells of blood clam under anoxia and normoxia.

Табл. 1. Линейные размеры эритроидных клеток моллюска крови при аноксии и нормоксии.

Experimental group	Number of individuals	Number of cells	C_1 , μm	C_2 , μm	N_1 , μm	N_2 , μm
Normoxia (control)	6	600	13.85±0.07	12.77±0.07	3.89±0.03	3.37±0.03
Anoxia (1 day)	5	500	12.67±0.12	11.53±0.11	3.87±0.04	3.39±0.04
Anoxia (2 day)	6	600	15.88±0.17	14.56±0.16	4.20±0.04	3.60±0.04
Anoxia (3 day)	5	500	14.32±0.12	13.12±0.11	4.57±0.03	3.99±0.03

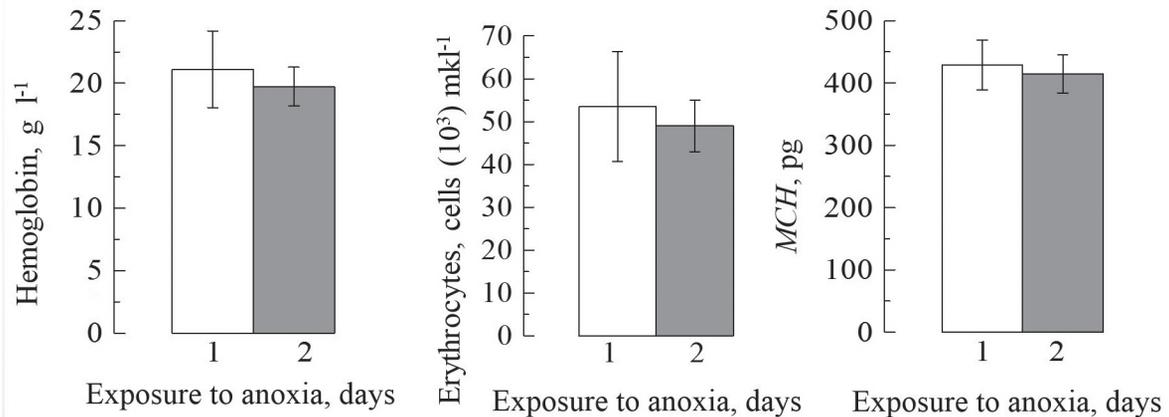
Note: C_1 and C_2 – the large and the small axis of the cell; N_1 and N_2 the large and the small axis of the cell nucleus

FIG. 2. Hematological parameters of clam extrapallial fluids (1 – normoxia; 2 – anoxia at 3 day of experiment).

РИС. 2. Гематологические показатели гемолимфы моллюска (1-нормоксия; 2-аноксия на 3-и сутки эксперимента).

and size of erythroid hemocytes: macrocytes and microcytes, schistocytes, red blood cell shades and poikilocytosis (Fig. 4). In control mollusks, the number of these anomalies exceeded 16% of total cell number and at the end of 3rd day exposure to anoxia the number of abnormal erythroid hemocytes increased up to 32%. Macrocytes and schistocytes gradually increased through the experimental period. Macrocyte number increased from 4% (control group) to 16% ($p \leq 0.05$). The number of schistocytes also increased significantly ($p \leq 0.05$). Macrocytes were relatively large cells, and schistocytes were fragmented parts of cytoplasm of the erythroid cells.

Morphometric parameters of erythroid cells

Erythroid cells of blood clam were large and nearly round. The large and small diameters (C_1 and C_2 respectively) differed on 1 μm (control group) (Table 1). The diameter of oval nuclei was smaller comparing to other cell types in extrapallial fluids and the difference between the values of N_1 and N_2 was 0.5 μm .

After 1 day exposure anoxia did not cause significant changes in linear sizes of cells, however, at the

2nd day of the experiment erythroid cells enlarged on 14-15% ($p \leq 0.05$). These changes were also observed in blood clams at 3rd day of exposure to anoxia. Similarly, in nuclei the most substantial changes in N_1 and N_2 were observed at 3rd day of experiment and consisted about 17-19% ($p \leq 0.05$).

Changes of linear diameters of erythroid cells and their nuclei induced fluctuations of other morphometric parameters (S_c , V_c , SS_c). In control group, the surface area was $520 \pm 6 \mu\text{m}^2$. After 1 day exposure to anoxia S_c decreased on 17-18% ($p \leq 0.05$) (Fig. 5); at the end of 2-days anoxic conditions we observed significant increase of the parameter on 40-41% ($p \leq 0.05$) of control level. At 3rd day of anoxia the surface area of erythroid cells returned to normoxic level. Similar pattern was observed for cellular volume (V_c) (Fig. 5). The relation between changes in S_c and V_c was evidenced by a constant specific surface area of cells during experiment ($1.51-1.57 \mu\text{m}^2$).

Anoxia induced gradual increase of nuclear volume (V_n), which was on 63% ($p \leq 0.05$) of control level ($25.2 \mu\text{m}^3$) at the end of 3rd day of exposure ($40 \mu\text{m}^3$) (Fig. 5). Different influence of anoxia on V_c and V_n of erythroid cells resulted in a specific pattern of

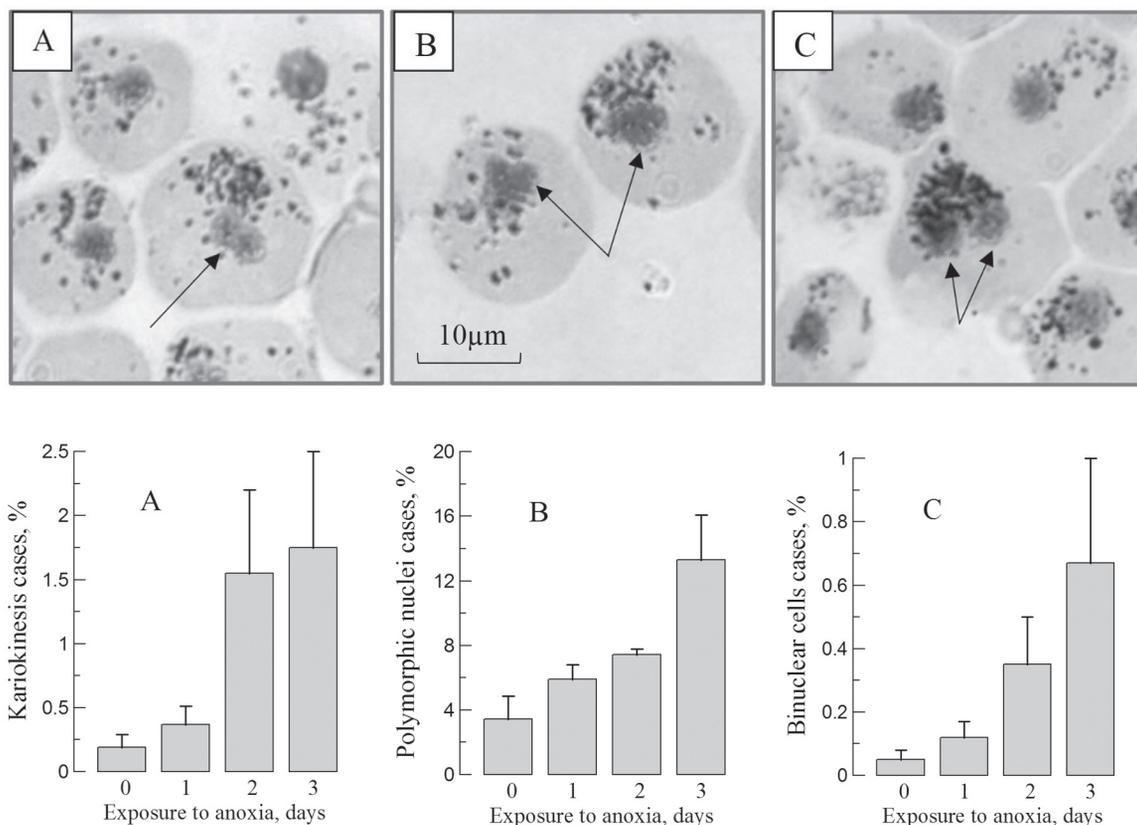


FIG. 3. Morphology of nuclei of extrapallial fluids clam erythroid cells (A – kariokinesis, B – polymorphic nuclei; B – binuclear cells; 0 – normoxia; 1, 2, 3 – days of exposure to anoxia; 2500 \times ; oil immersion).

РИС. 3. Морфология ядер эритроидных клеток гемолимфы моллюска (А – кариокинез, В – полиморфные ядра; С – двуядерные клетки; 0 – нормоксия; 1, 2, 3 – сутки аноксии; 2500 \times ; масляная иммерсия).

changes in NCR. After 3 days exposure to anoxia NCR was 10.5 ± 0.2 , which was on 36% lower comparing to control level (16.5 ± 0.4). The increase of the parameter observed at the 2nd day of experiment was caused by a significant increase of cell volume.

Red blood cell shades

Red blood cell shades were identified as acidophilic spots on slides (Fig. 4B-b). In control mollusks (normoxia) the number of these structures was negligible ($1.38 \pm 0.68\%$). Anoxia was associated with a slight increase of their number and at the end of experimental period (3 day) total number of red blood cell shades was $3.52 \pm 1.61\%$. However, the differences between control and experimental groups were insignificant.

Discussion

Hemoglobin and erythroid cells

Hypoxia (anoxia) is typically accompanied with an increase of blood oxygen capacity [Silkin, Silkina, 2005]. Several processes may cause this effect occurring independently or together [Soldatov, 2005]: (1)

pool of hemocytes into a circulating system (short-term adaptation); (2) activation of erythropoiesis (long-term adaptation); (3) production of erythrocytes with high *MCH* (long-term adaptation); (4) dehydration of blood plasma (increase of red blood cell number).

In the present work 3-day exposure to anoxia was not associated with changes in *MCH* and the number of erythroid cells in extrapallial fluids. Moreover, we did not observe immature erythroid cells with basophilic cytoplasm (high RNA content). These results indicate that blood clam was resistant to lack (or absence) of oxygen. Similar suggestions have been reported previously [Isani *et al.*, 1986; 1989; Zwaan *et al.*, 1991; 1992; 1995]. On the other hand, it is unknown, whether mollusks possess such mechanisms for regulation of extrapallial fluids oxygen capacity.

Size parameters of erythroid cells

Morphometric parameters of erythroid cells changed in proportion to duration of anoxia, as evidenced by a comparative consistency of specific surface area (*SS*). Among the parameters estimated, the most substantial changes were observed in cel-

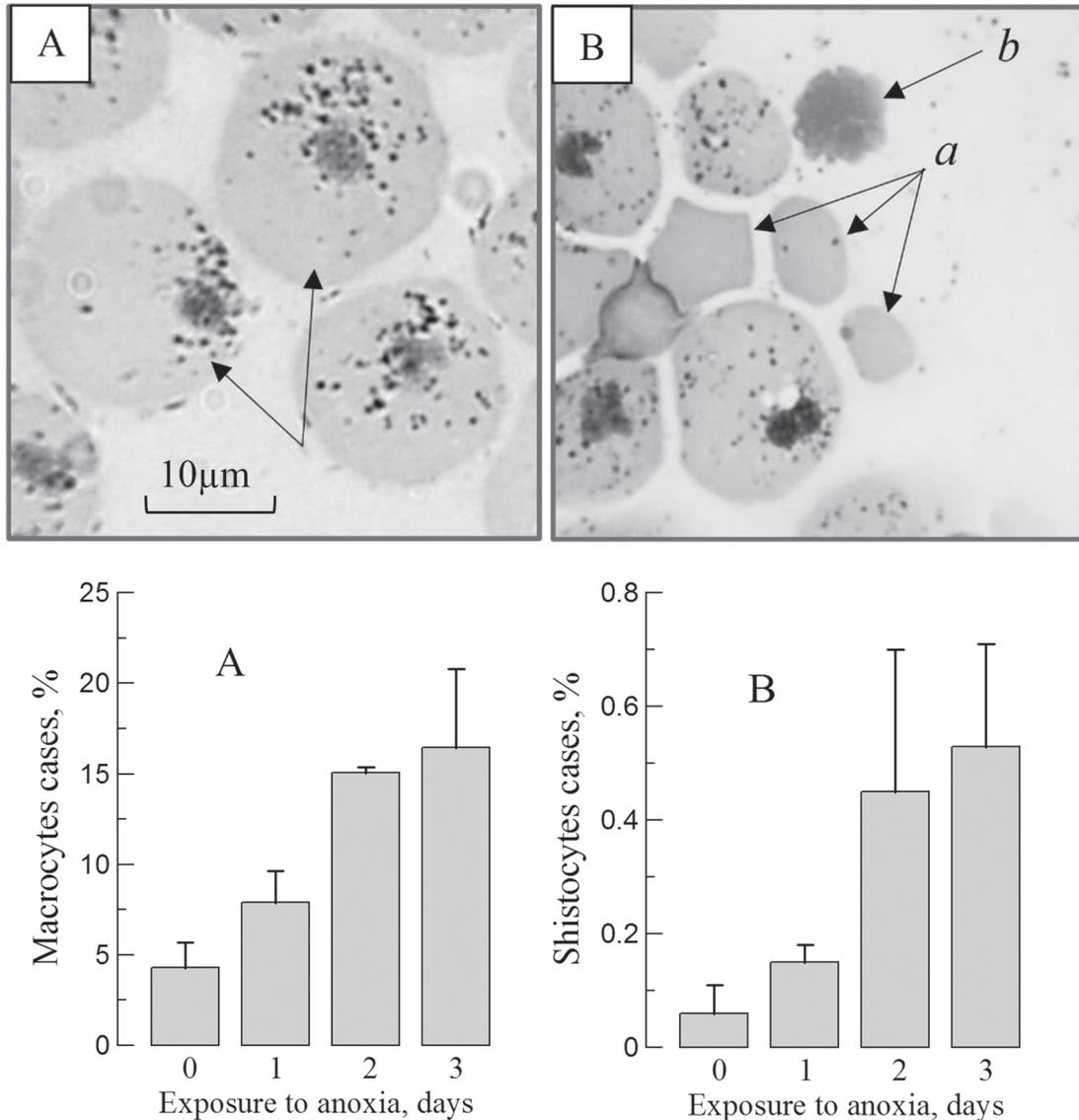


FIG. 4. Macrocytes (A) and shistocytes (B) in clam extrapallial fluids (0 – normoxia; 1, 2, 3 – days of exposure to anoxia; *a* – shistocytes; *b* – red blood cell shades; 2500×; oil immersion).

РИС. 4. Макроциты (А) и шистоциты (В) в гемолимфе моллюсков (0 – нормоксия; 1, 2, 3 – сутки аноксии; *a* – шистоциты; *b* – эритроцитарные тени; 2500×; масляная иммерсия).

lular volume, which increased on more than 40% at the 2nd day of experiment.

Swelling of red blood cells under hypoxic conditions has been intensively studied on lower vertebrates (teleosts) [*z*, 1989]. Increase of cellular volume is caused by an activation of Na-H-exchanger due to binding of plasma catecholamines with β -adrenoreceptors on red cell membrane [Borgese *et al.*, 1987]. Red blood cell swelling is aimed to improve hemoglobin- oxygen binding. On the other hand, Na-H-exchanger may be also activated by a significant acidification of cytoplasm, which is caused by an enhancement of anaerobic metabolism in conditions of anoxia [Walsh *et al.*, 1990; Motais *et al.*, 1992].

At the 1st day of experiment cellular volume of

erythroid cells decreased, which agrees with the results obtained previously on black scorpionfish red blood cells [Andrieieva, Soldatov, 2012]. Shrinkage of erythroid cells may occur due to activation of K^+/Cl^- – cotransporter and the release of organic osmolytes [Jensen, 1995]. The activation of the cotransporter, in turn, may be caused by a slight decrease of intracellular pH (not less than 7.0) [Adragna *et al.*, 2004].

Thus, gradual decrease of intracellular pH is supposed to be a main force driving the changes of the volume of blood clam erythroid cells under anoxia. At initial stages of anoxia, slight acidification caused the activation of K-Cl-cotransporter, and then, finally enhanced ion transport via Na-H-exchanger.

We also observed the increase of shistocyte

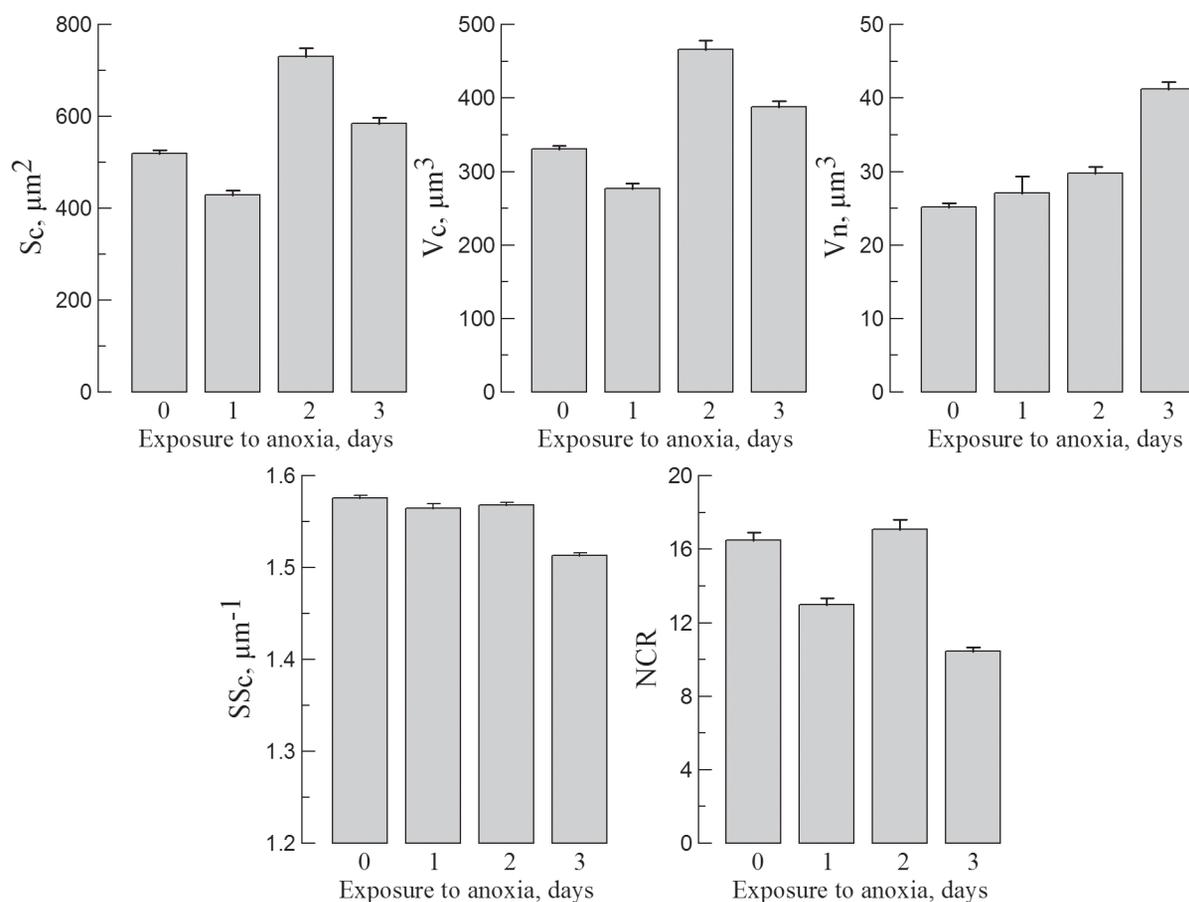


FIG. 5. Functional parameters of erythroid cells in clam extrapallial fluids (S_c – cell surface area; V_c – cellular volume; V_n – nuclear volume; SS_c – specific surface area; NCR – nucleocytoplasmic ratio; 0 – normoxia; 1, 2, 3 – days of exposure to anoxia).

РИС. 5. Функциональные параметры эритроидных клеток в гемолимфе моллюска (S_c – площадь поверхности клетки; V_c – клеточный объем; V_n – объем ядра; SS_c – удельная площадь поверхности клетки; NCR – ядерноплазматическое отношение; 0 – нормоксия; 1, 2, 3 – сутки аноксии).

number in extrapallial fluids of blood clam under anoxia. At normoxic conditions the number of these anomalies did not exceed 0.1%, but after 3 days exposure to anoxia their number significantly increased in more than 5 times (up to 0.6%). The increase of shistocyte number can be observed at anemia [Schiffman, 1998]. Hypothesizing that mollusks are not able to regulate extrapallial fluids oxygen capacity under anoxia, we can suppose “anemia-like” state for blood clams.

Size and shape of nuclei

One of the most substantial cellular responses of blood clam erythroid cells observed in the present work was the increase of nuclear volume (more than 40%). Enlargement of nuclei correlated with the duration of the experiment and was greater than increase of cellular volume, indicating that the process is regulated through the internal nuclear mechanisms. We have previously observed similar responses in black scorpionfish red blood cells under hypoxic conditions [Andrieieva, Soldatov, 2012].

The increase of nuclear volume was accompanied with the enhancement of SYBR Green fluorescence indicating functional activation of red blood cell nuclei. Functional activation of nuclei may be caused by a rearrangement of hemoglobin system, i.e. synthesis of new compartments or changes in the ratio of present elements. As it shown recently, hemoglobin of blood clam is formed by two fractions (HbI, HbII) [Chiancone *et al.*, 1981]. HbI is a dimer, which possesses higher affinity to oxygen and low sensitivity to pH [Chiancone *et al.*, 1981; 1988; Piro *et al.*, 1996]. Therefore, the increase of this component content will be valuable under anoxia. Any changes in protein structure of cells require enhancement of transcription and increase of euchromatin content in cells, which, in turn, is accompanied with nuclei enlargement. This hypothesis is supported by the fact that in teleosts heterogenic system of hemoglobin may rearrange under conditions of hypoxia [Houston, Rupert 1976; Byrne, Houston, 1988; Marinsky *et al.*, 1990].

Anoxia induced cell division in erythroid cells

of blood clam and the kariokinesis was achieved through the reactive amitosis (division of cellular volume excluding equal distribution of chromosomes between daughter cells). Reactive amitosis is not usually accompanied with cytokinesis [David, Uerlings, 1992], which is evidenced by the increase of binuclear cell number in blood clam under anoxia observed in the present work. Many authors consider this process as a compensatory response in conditions of disturbances of cellular metabolism. Similar processes may occur in conditions of anoxia.

Anoxia caused a pronounced increase of number of polymorphic nuclei (irregular shape of nuclei with rough contours) in erythroid hemocytes of blood clam. The number of these anomalies depended on the duration of anoxia. At the 3rd day of experimental period polymorphic nuclei increased up to 16%, which was 3.0-3.5 times larger than in control group. В большинстве случаев подобные клеточные аномалии рассматривают как атипические [Kuzina, 2011]. Most authors considered these changes in cell morphology as atypical [Kuzina, 2011]. However, in the present work we cannot provide evidences for this hypothesis; further investigations of cells using electron microscopy are needed.

Destruction of erythroid cells

The level of cell damage under anoxia was assessed by estimating the number of red blood cell shades on slides. These structures were identified as acidophilic spots. Acidophilic painting of shades is caused by the presence of membrane-bound hemoglobin [Welbourn *et al.*, 2017]. Red blood cell shades appear as a result of lysis of mature red cells, which are typically observed at hemolytic anemia, toxicosis, due to influence of hemolytic poisons, various chemical compounds, low temperature etc. [Habibi *et al.*, 2019]. In the present work we did not observe significant changes in the number of erythroid shades after exposure to anoxia, indicating that these conditions did not influence mortality of erythroid cells in extrapallial fluids of blood clam.

Conclusion

Adaptation of *Anadara* to 3 day-anoxia caused substantial changes which were observed for the most part at cellular level. Organismic responses were absent, as hemoglobin concentration, the number of erythroid cells and MCH remained at control level (normoxia). The number of abnormal cells such as sheistocytes, cells with polymorphic nuclei, and reactive amitosis of nuclei resulting in appearance of binuclear cells increased under anoxia in extrapallial fluids of blood clam. Nuclear volume (V_n) increased for more than 40% and this increase correlated with the duration of experimental period. The volume of erythroid cells (V_e) has changed in complicated

manner decreasing at 1st day of exposure to anoxia and then increasing at the 2nd day and finally stabilizing at 3rd day exposure to anoxia at the higher level comparing to control. Different dynamics of changes in nuclear and cellular volume of erythroid cells caused decrease of NCR under anoxia, which was on 36% lower at 3rd experimental day comparing to normoxic level. Specific surface area did not significantly changed and was 1.51-1.57 μm^{-1} . Anoxia did not cause the increase of the mortality of erythroid cells, which was evidenced by a constant level of red blood cell shades observed on slides. In other words, these changes are not stressful and reflect the natural process of adaptation of mollusks to anoxia conditions.

Acknowledgements

The work is partly supported by State Assignment of FRC IBSS (State registration number AAAA-A 18-118021490093-4) and with the support of Russian Fund for Basic Research (Project 20-04-00037a).

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