ISSN 2075-1117, Russian Journal of Biological Invasions, 2023, Vol. 14, No. 4, pp. 581–587. © Pleiades Publishing, Ltd., 2023.
Russian Text © The Author(s), 2023, published in Rossiiskii Zhurnal Biologicheskikh Invazii, 2023, No. 3, pp. 117–125.

Adaptation of *Anadara kagoshimensis* (Tokunaga, 1906) to Hypo- and Hyperosmotic Environment: Hemocyte Response

T. A. Kukhareva[a], *, V. N. Rychkova[a], **, A. A. Soldatov[a], *, A. Yu. Andreeva[a], ****, and E. S. Kladchenko[a], *******

[a] *Kovalevsky Institute of Biology of the Southern Seas, Russian Academy of Sciences, Sevastopol, 299011 Russia*

**e-mail: altali@yandex.ru*

***e-mail: novitsky_valya@mail.ru*

****e-mail: alekssoldatov@yandex.ru*

*****e-mail: andreevaal@gmail.com*

******e-mail: kladchenko_ekaterina@bk.ru*

Received December 13, 2022; revised August 28, 2023; accepted September 1, 2023

Abstract—*Anadara kagoshimensis* (Tokunaga, 1906) is an alien bivalve mollusk that has successfully colonized the Black Sea and the Sea of Azov despite a significantly lower salinity level of these waters compared to its native region. The morphological and morphometric characteristics of erythrocytes from *A. kagoshimensis* during adaptation to hypo- and hyperosmotic experimental conditions were analyzed by light microscopy. The control group of mollusks was maintained at 18‰ salinity. Experimental groups were maintained at the salinity levels of 8, 14, 35, and 45‰. A decreased salinity level was obtained by diluting seawater with distilled water at a rate of 1.5 ± 0.5‰ per day. An increased salinity was obtained by addition of sea salt to an aquarium at a rate of 2.5 ± 0.5‰ per day. The exposure period was 2 days. The natural salinity range of *A. kagoshimensis* was found to fall within 14—35‰. No significant cell morphology changes were observed under such conditions. At the same time, exposure of the mollusks to the environmental salinity of 8 and 45‰ caused an obvious stress expressed via appearance of cell anomalies and changes in the linear characteristics of erythrocytes. At the same time, no cell lysis was observed, and the values of the specific surface area and the nuclear-cytoplasmic ratio remained unchanged. The results of the study indicate the ability of the mollusk to survive for some time in an aquatic environment with extremely low or high salinity.

Keywords: *Anadara*, erythrocytes, hypo- and hyperosmotic stress, light microscopy, morphometry

DOI: 10.1134/S2075111723040094

INTRODUCTION

Anadara kagoshimensis (Tokunaga, 1906) is a bivalve mollusk common for the malacofauna of the Indo-Pacific region, including the coastal waters of India, Sri Lanka, Indonesia, Japan, and Australia (Poutiers, 1998). This species invaded the Black Sea and the Sea of Azov with the ballast water of ships (Shiganova, 2009). It was first discovered near the Caucasian coast in 1968 (Kiseleva, 1992). In the subsequent years, it colonized the coastal waters of both seas and became one of the dominating benthos species (Revkov et al., 2008) owing to its high eurybiontic nature. *A. kagoshimensis* is tolerant to acute hypoxia (anoxia; Andreenko et al., 2009; Soldatov et al., 2021) and hydrogen sulfide (Soldatov et al., 2018). The spreading of this typical oceanic species into the Azov-Black Sea region assumes its ability to survive in a hypoosmotic environment, especially in the Sea of Azov, where *A. kagoshimensis* became a common species (Zhivoglyadova et al., 2021). However, the limits of its osmotic tolerance still remain poorly studied.

Marine bivalve mollusks are known as typical osmoconformers (McFarland et al., 2013; Solan and Whiteley, 2016). The osmolarity of their internal medium (hemolymph) corresponds to their environment. In this case, the ability of the osmoconformer's cell systems to compensate a hypo- or hyperosmotic load is of critical importance. Hemolymph cells (hemocytes) represent suitable objects to study osmoregulation processes. In the case of *A. kagoshimensis*, they are represented mainly by erythroid forms, which distinguishes this species from other bivalves (Holden et al., 1994; Morello et al., 2004). The reduction of water salinity was shown to cause a decrease in the total number of hemocytes in *A. kagoshimensis* living near the coast of China. At the same time, their phagocytic activity and the ability to generate reactive oxygen species increased (Zhang et al., 2019). The ability of hemocytes of the Black Sea *Anadara* to

respond to the oxygen deficiency and hydrogen sulfide contamination of the aquatic environment was studied under experimental conditions. Among registered changes were changes in the form and size of cells, the number of granular inclusions, formation of typical anomalies, etc. (Soldatov et al., 2018, 2021). Such changes mean hemocytes are sensitive to the environmental conditions and can be used as markers of the state of the mollusk organism as a whole.

In this study, we tried to determine the range of the osmotic tolerance of *A. kagoshimensis* under in vivo conditions. To do this, we studied erythroid elements of the mollusk hemolymph and analyzed their morphological and morphometric characteristics under hypo- and hyperosmotic loads.

MATERIALS AND METHODS

The objects of the study were adult bivalve mollusks *A. kagoshimensis* (Tokunaga, 1906). The total number of specimens was 50; the average weight of mollusks including the shell was 15.6 ± 1.5 g, and the average shell valve diameter was 35.5 ± 1.1 mm. Mollusks were collected in autumn 2020 in the Black Sea near Sevastopol (44°60′ N, 33°44′ E); the site of collection was characterized by the water temperature of 20°C, salinity level of 18.3‰, and oxygen content of 8.5 mg/L. The collected mollusks were transported to a laboratory in plastic containers without water. In the laboratory, they were placed in aquariums with the density of one mollusk per 3–5 L of water and maintained at conditions similar to those at the site of collection (23.3 ± 0.1°C, salinity level of 18.2 ± 0.02‰, pH 8.1 ± 0.01, oxygen content of 7.7 ± 0.1 mg/L).

The oxygen content and water temperature were measured by a portable oximeter equipped with a ST300D temperature sensor (Ohaus, United States). The water salinity and pH were controlled by a portable sensION 5 HACH conductometer-salinometer (Cole Parmer, United States) and a ST2100-F pH meter (Ohaus, United States).

To evaluate the range of salinity adaptation, the mollusks were divided into five groups each containing ten individuals. The control group was maintained at the salinity level of 18‰, while experimental groups were maintained at 8, 14, 35, and 45‰ salinity. A decreased salinity level (14 and 8‰) was obtained by diluting seawater with distilled water at a rate of 1.5 ± 0.5‰ per day. After reaching the required salinity level, the mollusks were incubated under these experimental conditions for 2 days. An increased salinity (35 and 45‰) was obtained by a gradual addition of salt (Red Sea salt, France) to an aquarium at a rate of 2.5 ± 0.5‰ per day. After 6 or 10 days (without taking into account the acclimatization period), when the salinity level reached 35 or 45‰, respectively, the further exposure under experimental conditions was 48 h. To remove metabolites, the water in aquariums was refreshed every day with the maintenance of the same salinity level in the course of the whole experiment. The mollusks were fed a *Tetraselmis viridis* (strain IBSS-25) microalgae mix provided by the working collection of the Department of Biotechnology and Phytoresources of the Kovalevsky Institute of Biology of the Southern Seas. The microalgal suspension was added at a ratio of 5–10 mL per each 50 L of aquarium water. The water temperature, pH, and oxygen content were maintained at the control values for the whole experimental period.

Hemolymph was collected from the extrapallial space by a sterile syringe, then washed in sterile seawater three times for 5 min (500 g), and filtered through a 20-μm filter to remove aggregates. After washing, cells were concentrated and used for preparation of smears. The smears were stained according to a combined Pappenheim method (Zolotnitskaya, 1987) and analyzed using a Biomed PR-2 Lum light microscope equipped with a Levenhuk C NG Series camera. The large (C_1) and small (C_2) cell diameters (without pseudopodia) and diameters of their nuclei (N_1, N_2) were measured from photographs using an ImageJ 1.44 program.

On the basis of the data obtained, the volume and surface area of individual cells (V_c, S_c) and their nuclei (V_n, S_n) were calculated (Novitskaya and Soldatov, 2013); then the specific cell surface area (S_c/V_c) and the nucleocytoplasmic ratio (V_n/V_c) were determined. At least 1000 cells were analyzed for each smear.

The normalcy of the distribution was examined by the Kolmogorov–Smirnov test. The differences between groups were analyzed using the RStudio v. 4.1.0 program package (R Core Team, 2021). The data were analyzed by ANOVA; the significance of results was checked by the Tukey's criterion with a 95% confidence interval. The results were presented as mean ± standard error.

RESULTS

Survivability of mollusks. Both hypo- and hyperosmotic load did not cause death of *A. kagoshimensis*. During the whole duration of the experiment, shell valves remained half-opened.

Erythrocyte morphology. The study of smears revealed *A. kagoshimensis* erythrocytes representing large slightly elongated and rounded cells; the average diameter measured along the major and minor axes was 16.2 ± 0.1 and 13.3 ± 0.1 μm, respectively (Fig. 1c). In some cases, the formation of pseudopodia was observed. The cytoplasm contained numerous granular inclusions. The nuclei were rather small and acentric, and the diameters measured along their axes were 5.0 ± 0.03 and 3.7 ± 0.03 μm. They have a basophilic staining type and highly concentrated chromatin.

The studied hypo- and hyperosmotic stresses caused some changes in the morphological traits of

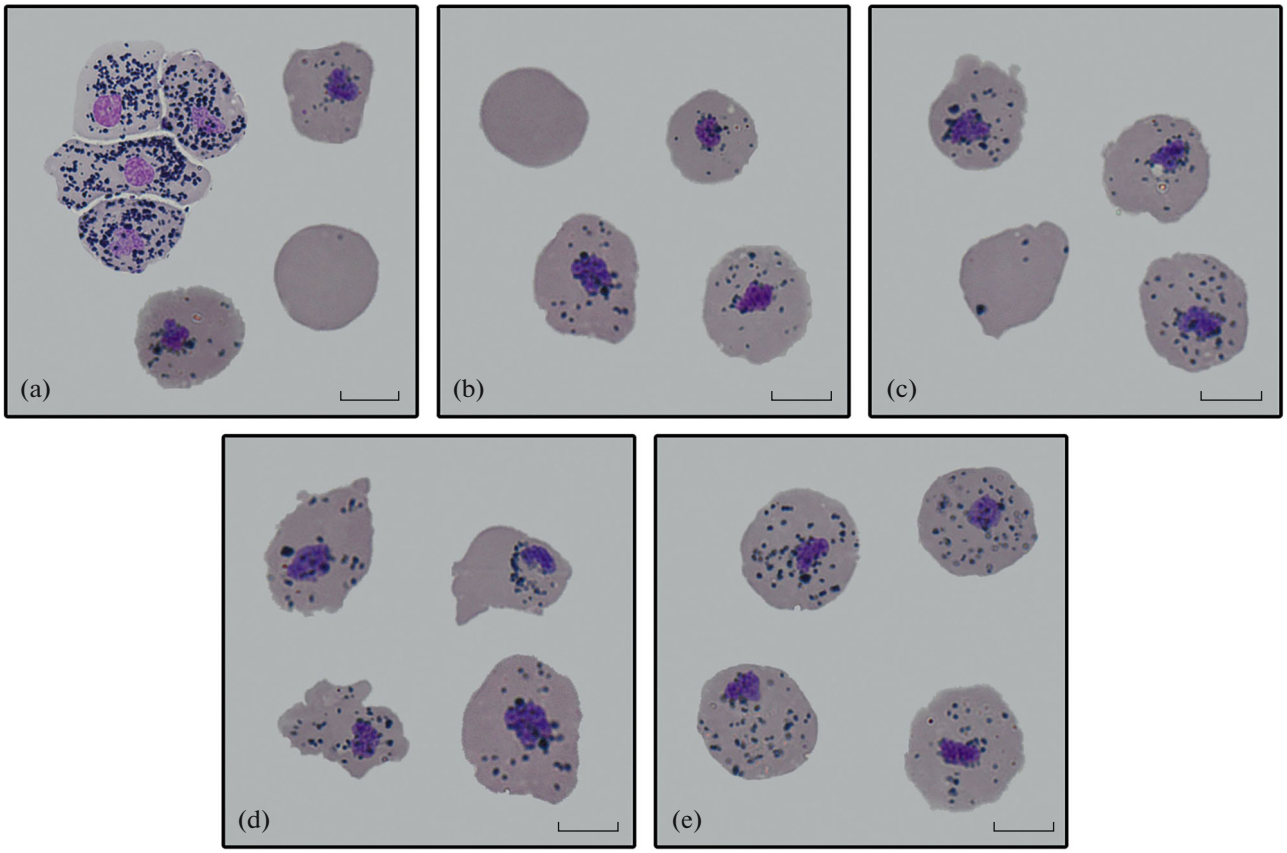

Fig. 1. Effect of the salinity level on the morphological characteristics of *Anadara kagoshimensis* erythrocytes. Salinity levels: (a) 8‰; (b) 14‰; (c) 18‰ (control); (d) 35‰; (e) 45‰. The scale bar corresponds to 10 μm.

erythrocytes. When the salinity level decreased to 14‰, the form of erythrocytes and their nuclei became irregular, and grains shifted toward nuclei. The number of pseudopodia in cells decreased, and some acaryocytes were observed in a hemolymph (Fig. 1b). In the case of the further salinity decrease to 8‰, the above-described morphological changes were still present; moreover, the cells swelled and formed aggregates, which were observed across the whole area of the smear (Fig. 1a). However, no increase in the number of erythrocyte shadows, which reflect a cell lysis, was observed.

An increase of the salinity to 35‰ also caused abnormalities in the form of cells and nuclei; the number of pseudopodia in cells increased. Cytoplasmic grains were concentrated near nuclei (Fig. 1d). However, in the case of the further salinity increase to 45‰, the cells became rounded again, and cytoplasmic grain became evenly distributed around the nucleus. Almost a complete absence of pseudopodia was observed (Fig. 1e). No growth in the number of erythrocyte shadows was observed.

Linear size of cells. Variations in the seawater salinity significantly influenced the cell size parameters (Figs. 2a, 2b). Decreased salinity resulted in an increase in the length of both major and minor cell axes to 17.1 ± 0.1 and 14.2 ± 0.1 μm, respectively, at the salinity level of 8‰. The increase in salinity to 35‰ caused a certain reduction of the major axis length (15.5 ± 0.1 μm) and an increase in the minor axis length (13.4 ± 0.1 μm); however, the further salinity increase to 45‰ caused an increase of both axes to 17.2 ± 0.2 and 14.5 ± 0.1 μm, respectively. The values obtained for the extreme salinity values (8 and 45‰) exceeded the control values by 6–8% ($p < 0.05$). The C_1/C_2 ratio did not significantly change for all variants and varied between 1.1–1.3 (Fig. 2c).

Linear size of nuclei. Changes in the level of salinity also provided some influence on the size of erythrocyte nuclei (Figs. 2d, 2e). The decrease in salinity to 14‰ caused swelling of nuclei with an increase of the length of the major and minor axes to 5.5 ± 0.04 and 4.1 ± 0.04 μm, respectively. The further desalination to 8‰ was accompanied by a certain decrease in these parameters to 5.15 ± 0.03 and 4.1 ± 0.02 μm. Increased salination also caused an increase in the length of both the major and minor axes; at 45‰, their lengths were 5.3 ± 0.1 and 4.1 ± 0.04 μm, respectively. At the extreme points corresponding to the salinity of 8 and 45‰, the sizes of the major and

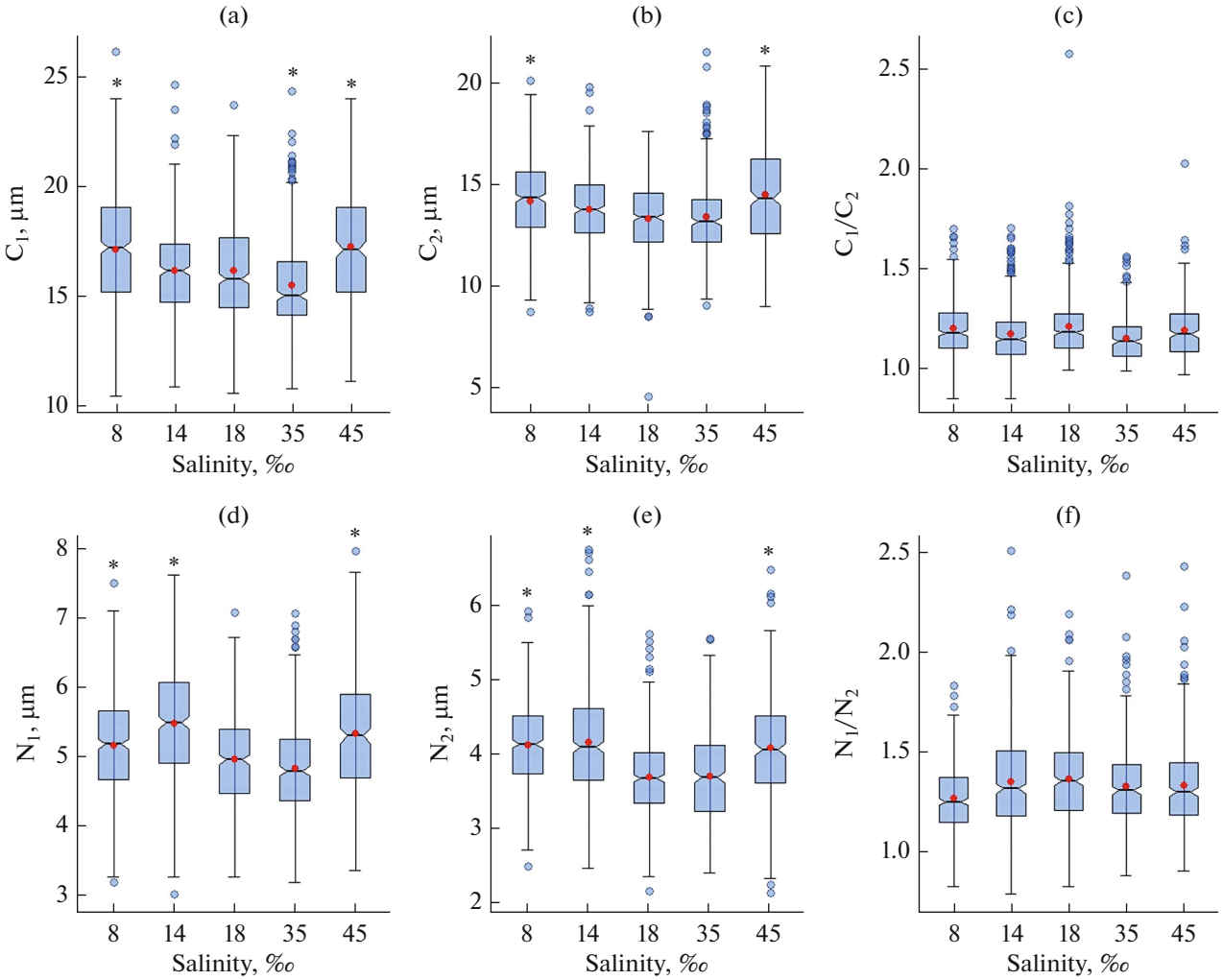

Fig. 2. Effect of the salinity level on the size of erythrocytes and their nuclei in *Anadara kagoshimensis*: (a) major cell axis length; (b) minor cell axis length; (c) ratio between the lengths of the major and minor cell axes; (d) major nucleus axis length; (e) minor nucleus axis length; (f) ratio between the lengths of the major and minor nucleus axes. Significant differences between the control and experimental groups are indicated with an asterisk ($p < 0.05$).

minor axes of a nucleus (N_1, N_2) were greater than the control values by 4–7% ($p < 0.05$). For all experimental variants, the N_1/N_2 ratio remained at the level of 1.2–1.5 (Fig. 2f).

Volumetric characteristics of erythrocytes and their nuclei. The growth of the diameters of both cell axes under hypoosmotic load resulted in a corresponding increase in cell volume (V_c) (Fig. 3a). At the salinity level of 8‰, V_c was 529.4 ± 8.4 μm^3, which exceeded the control by 19% ($p < 0.05$). A hyperosmotic load resulted first in a decrease in cell volume to 420.4 ± 7.6 μm^3 at 35‰ and then in its growth to 547.3 ± 12.1 μm^3 at 45‰, which was 23% higher than in the control variant ($p < 0.05$). The nucleus volume (V_n) increased by one-third under minimal and maximal salinity, reaching 47.3 ± 0.8 and 48.9 ± 1.3 μm^3 for the salinity levels of 8 and 45‰, respectively ($p < 0.05$; Fig. 3b). The maximum nucleus volume (52.2 ± 1.2 μm^3) was observed at the water salinity level of 14‰. Quantitative changes in the nucleus volume were comparable with cell volume changes, which was confirmed by the lack of significant differences between the V_n/V_c values in their control and experimental groups (Fig. 3c).

Total and specific surface area of erythrocytes. The total surface area of erythrocytes (S_c) increased under hypoosmotic conditions and reached 804.2 ± 12.9 μm^2 at 8‰. Under hyperosmotic conditions, S_c first slightly decreased (at 35‰) and then increased to 829.9 ± 17.9 μm^2 at 45‰ (Fig. 3d). At the extreme values (8 and 45‰), a 1.2-fold increase in the total surface area of erythrocytes was observed compared to the control ($p < 0.05$). The specific cell surface area

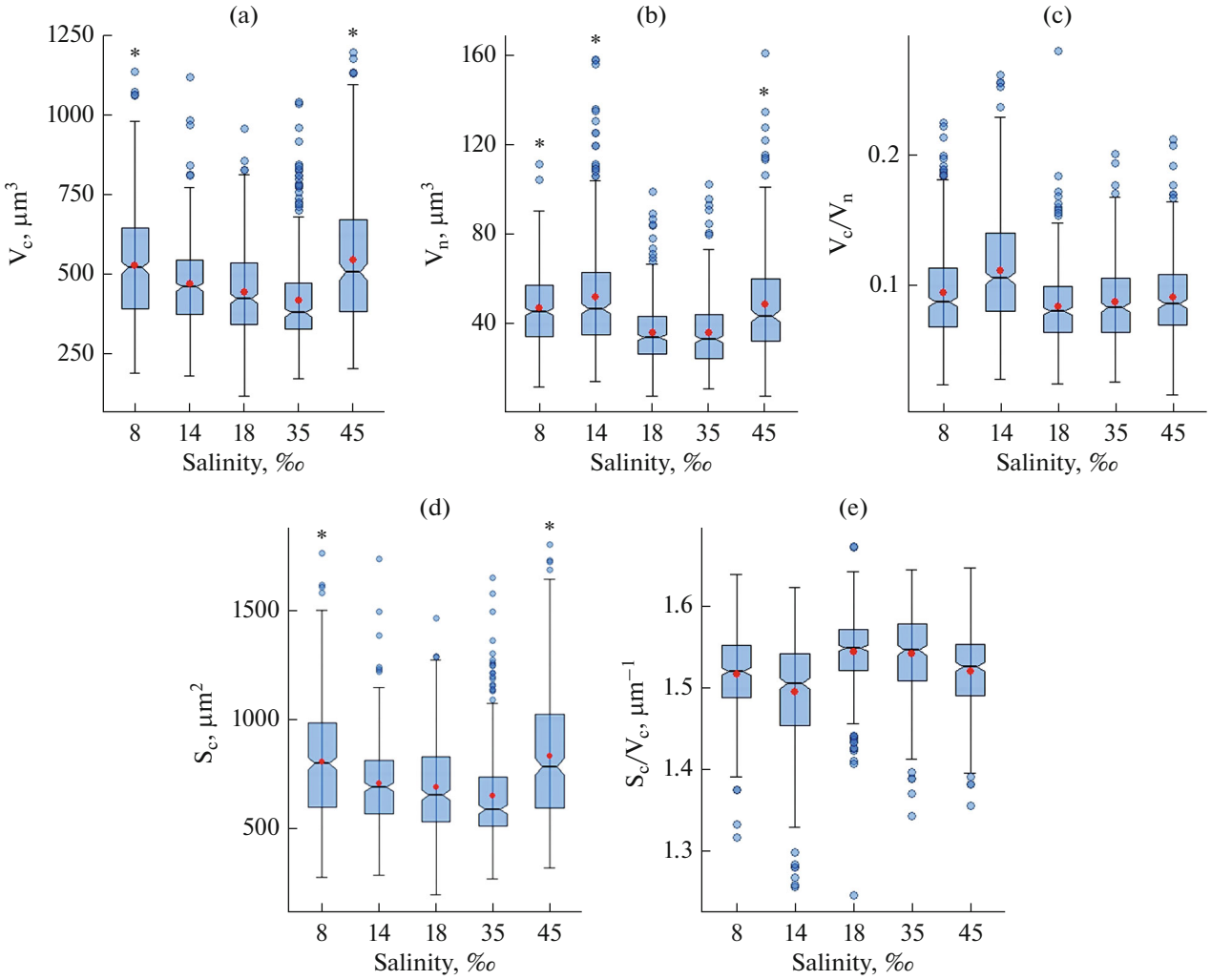

Fig. 3. Effect of the salinity level on the calculated characteristics of erythrocytes and their nuclei in *Anadara kagoshimensis*: (a) erythrocyte volume; (b) erythrocyte nuclear volume; (c) nuclear-cytoplasmic ratio; (d) total erythrocyte surface area; (e) specific erythrocyte surface area. Significant differences between the control and experimental groups are indicated with an asterisk ($p < 0.05$).

S_c/V_c remained at the level of 1.5 μm^{-1} in all experimental variants (Fig. 3e).

DISCUSSION

On the basis of the above-presented information, our attention should be focused on the following moments, which should be discussed:

— A hypoosmotic load was accompanied by a stable growth of the average cell volume of red blood cells and their nuclei.

— Similar changes were also observed under a hyperosmotic load; in this case, the cell volume increase was more pronounced (up to 23%).

— Morphological changes in erythroid forms were rather insignificant and included mainly changes in the form of cell nuclei, dislocation of grain inclusions, and changes in the number of pseudopodia; cell aggregation was observed only at the salinity level of 8‰, and no erythrocyte shadow formation was registered.

An increase in the volume of erythrocytes in a hemolymph of *A. kagoshimensis* under a hypoosmotic load was quite expectable because of the hydration of the hemolymph and cell cytoplasm usually occurring in osmoconformers (Bregante et al., 2016). However, it did not exceed 19%, which allowed the development of processes intended to reduce the cell volume (regulatory volume decrease, RVD). This process may be based on a cation-anion exchange at the level of cell membranes by the following scheme: K^+–Cl^- symport and (or) K^+–H^+ antiport (Cossins and Gibson, 1997). In both cases, osmotically bound water is removed from cells. In relation to mussel hemocytes, the first process is assumed (Bregante et al., 2016). One should also keep in mind the removal of organic

osmolytes, such as taurine and betaine (Jackson et al., 1994; Torre et al., 2013). In the case of a hyperosmotic load, mollusk organisms provide more complex responses. An average osmotic load (35‰) was accompanied by an insignificant reduction of a cell volume of hemocytes without any further correction. This response is quite natural because of a hyperosmolarity of the hemolymph. However, mollusk exposure to a 45‰ salinity was accompanied by a significant increase in the volume of red blood cells, which can be explained only by the processes of a regulatory volume increase (RVI) and can be based on the entry of hydrated ions into a cell, namely, the Na^+/H^+ exchange and the cotransport of Na^+, K^+, and Cl^- (Cossins and Gibson, 1997).

Osmoregulation processes in *A. kagoshimensis* erythrocytes during adaptation to the hypo- and hyperosmotic environmental conditions are possibly developed by the RVD and RVI type. In this case, however, these processes are far from being perfect, because cell volumes do not return to initial values. At the same time, no significant cell anomalies are developed. Note that the number of erythrocyte shadows (i.e., destroyed cells) in a hemolymph do not increase, which can be explained only by a high osmotic tolerance of red blood cells that was shown for *A. kagoshimensis* erythrocytes in the earlier studies (Novitskaya and Soldatov, 2011). The last fact reflects a high elasticity of cytoplasmic membranes of mollusk cells, which is rather a consequence of the peculiarities of their phospholipid composition.

The results of this study show that the salinity range of 14–35‰ falls within the limits of the osmotic tolerance of *A. kagoshimensis*. This is evidenced by the lack of well-manifested changes in the morphology and morphometry of red blood cells. Exposure of the mollusks to an environment with the salinity of 8 or 45‰ results in some stress manifested via an increased volume of red blood cells, which indicates imperfect osmoregulation processes occurring by the RVD and RVI type. This is substantially compensated by a high osmotic tolerance of erythroid cells. At the same time, the specific surface area of red blood cells remains almost the same, which is important for the appropriate realization of the respiratory function of the hemolymph. All these adaptive mechanisms seem to provide a successful colonization of hypoosmotic waters of the Black Sea and the Sea of Azov by *A. kagoshimensis* (Revkov et al., 2008; Zhivoglyadova et al., 2021), as well as the ability of this mollusk to withstand extremely low (8‰) and high (45‰) water salinity levels for some time.

CONCLUSIONS

The water salinity range of 14–35‰ represents the zone of a functional comfort for *A. kagoshimensis*, which is confirmed by the lack of significant changes in the red blood cell morphology and morphometry. The threshold of osmotic tolerance of this species seems to be water salinity of 8 and 45‰. At these salinity levels, significant changes in the linear and volumetric characteristics of erythroid cells are observed, as well as the appearance of a number of cell anomalies (form of nuclei, localization of granular inclusions, changes in the number of pseudopodia) and a significant increase in cell volume. However, no cell destruction was observed, which indicates their high osmotic tolerance. No changes were observed in the specific surface area of erythrocytes. These facts mean that *A. kagoshimensis* is able to withstand extreme hypo- and hyperosmotic loads for a certain time.

FUNDING

The study of the effect of a hypoosmotic load on the hemocytes of *A. kagoshimensis* was carried out within the framework of the State Assignment of the Federal Research Center Institute of Biology of the Southern Seas "Principles of the Immune System Organization in Food Hydrobionts and the Study of the Effect of Environmental Factors on the Functioning of Their Defense Systems" (theme no. 121102500161-4).

The study of the effect of a hyperosmotic load on the hemocytes of *A. kagoshimensis* was carried out within the framework of the State Assignment of the Federal Research Center Institute of Biology of the Southern Seas "Functional, Metabolic, and Toxicological Aspects of the Occurrence of Hydrobionts and Their Populations in Biotopes Differing in Their Physicochemical Regimes" (theme no. 121041400077-1).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experimental protocols were performed in accordance with the EU guidelines on the use of animals for experimental and other scientific purposes (86/609/CEE) and in compliance with the rules approved by the Order of the Presidium of the USSR Academy of Sciences no. 12000-496 (April 2, 1980) and the Order of the USSR Ministry of Higher Education no. 22 (September 13, 1984). All efforts were made to use the minimum number of animals required to obtain reliable scientific data.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

Andreenko, T.I., Soldatov, A.A., and Golovina, I.V., Adaptive reorganization of metabolism in the bivalve mollusk *Anadara inaequivalvis* Bruguiere under experimen-

tal anoxia, *Doklady Natsional'noi Akademii Nauk Ukrainy*, 2009, no. 7, pp. 155–160.

Bregante, M., Carpaneto, A., Piazza, V., Sbrana, F., Vassalli, M., Faimali, M., and Gambale, F., Osmoregulated chloride currents in Hemocytes from *Mytilus galloprovincialis, PLoS One*, 2016, vol. 11, no. 12, p. e0167972.
https://doi.org/10.1371/journal.pone.0167972

Cossins, A.R., and Gibson, J.S., Volume-sensitive transport systems and volume homeostasis in vertebrate red blood cells, *J. Exp. Biol.*, 1997, vol. 200, no. 2, pp. 343–352.
https://doi.org/10.1242/jeb.200.2.343

Holden, J.A., Pipe, R.K., Quaglia, A., and Ciani, G., Blood cells of the arcid clam, *Scapharca inaequivalvis, J. Mar. Biol. Assoc. U. K.*, 1994, vol. 74, no. 2, pp. 287–299.
https://doi.org/10.1017/S0025315400039333

Jackson, P.S., Morrison, R., and Strange, K., The volume-sensitive organic osmolyte-anion channel VSOAC is regulated by nonhydrolytic ATP binding, *Am. J. Physiol.: Cell Physiol.*, 1994, vol. 267, no. 5, pp. C1203–C1209.
https://doi.org/10.1152/ajpcell.1994.267.5.C1203

Kiseleva, M.I., Comparative characteristics of benthic communities off the Caucasus coasts, in *Mnogoletnie izmeneniya zoobentosa Chernogo morya* (Long-Term Changes in the Zoobenthos of the Black Sea), Kiev: Naukova Dumka, 1992, pp. 84–99.

McFarland, K., Donaghy, L., and Volety, A.K., Effect of acute salinity changes on hemolymph osmolality and clearance rate of the non-native mussel, *Perna viridis*, and the native oyster, *Crassostrea virginica*, in Southwest Florida, *Aquatic Invasions*, 2013, vol. 8, no. 3, pp. 299–310.
https://doi.org/10.3391/ai.2013.8.3.06

Morello, E.B., Solustri, C., and Froglia, C., The alien bivalve *Anadara demiri* (Arcidae): A new invader of the Adriatic Sea, Italy, *J. Mar. Biol. Assoc. U. K.*, 2004, vol. 84, no. 5, pp. 1057–1064.
https://doi.org/10.1017/S0025315404010410h

Novitskaya, V.N. and Soldatov, A.A., Erythroid elements of the hemolymph in *Anadara inaequivalvis* (Mollusca: Arcidae) under experimental anoxia: Functional and morphometric characteristics, *Morskoi Ekologicheskii Zhurnal*, 2011, vol. 10, no. 1, pp. 56–64.

Novitskaya, V.N., and Soldatov, A.A., Features of the functional morphology of erythroid elements of the hemolymph of the bivalve mollusk *Anadara inaequivalvis*, Black Sea, *Gidrobiol. Zh.*, 2013, vol. 49, no. 4, pp. 69–77.

Poutiers, J.M., Gastropods, in *FAO Species Identification Guide for Fishery Purposes. The Living Marine Resources of the Western Central Pacific*, 1998, vol. 1, pp. 363–648.

Revkov, N.K., Abaza, V., Dumitrache, C., Todorova, V., Konsulova, T., Mickashavidze, E., et al., The state of zoobenthos, *Commission on the Protection of the Black Sea Against Pollution*, 2008, pp. 243–290.

Shiganova, T.A., Alien species in the ecosystems of the southern inland seas of Eurasia, *Extended Abstract of Doctoral (Biol.) Dissertation*, Moscow, 2009.

Solan, M., and Whiteley, N., *Stressors in the Marine Environment: Physiological and Ecological Responses; Societal Implications,* Oxford University Press, 2016.
https://doi.org/10.1093/acprof:oso/9780198718826.001.0001

Soldatov, A., Kukhareva, T., Morozova, V., Richkova, V., Andreeva, A., and Bashmakova, A., Morphometric parameters of erythroid hemocytes of alien mollusc *Anadara kagoshimensis* under normoxia and anoxia, *Ruthenica*, 2021, vol. 31, no. 2, pp. 77–86.
https://doi.org/10.35885/ruthenica.2021.31(2).3

Soldatov, A.A., Kukhareva, T.A., Andreeva, A.Y., and Efremova, E.S., Erythroid elements of hemolymph in *Anadara kagoshimensis* (Tokunaga, 1906) under conditions of the combined action of hypoxia and hydrogen sulfide contamination, *Russ. J. Mar. Biol.*, 2018, vol. 44, no. 6, pp. 452–457.
https://doi.org/10.1134/S1063074018060111

Torre, A., Trischitta, F., Corsaro, C., Mallamace, D., and Faggio, C., Digestive cells from *Mytilus galloprovincialis* show a partial regulatory volume decrease following acute hypotonic stress through mechanisms involving inorganic ions, *Cell Biochem. Funct.*, 2013, vol. 31, no. 6, pp. 489–495.
https://doi.org/10.1002/cbf.2925

Zhang, M., Li, L., Liu, Y., and Gao, X., Effects of a sudden drop in salinity on immune response mechanisms of *Anadara kagoshimensis, Int. J. Mol. Sci.*, 2019, vol. 20, no. 18, p. 4365.

Zhivoglyadova, L.A., Revkov, N.K., Frolenko, L.N., Afanas'ev, D.F., The expansion of the bivalve mollusk *Anadara kagoshimensis* (Tokunaga, 1906) in the Sea of Azov, *Russ. J. Biol. Invasions*, 2021, vol. 12, no. 2, pp. 192–202.
https://doi.org/10.1134/S2075111721020120

Zolotnitskaya, R.P., Methods of hematological research, in *Laboratornye Metody Issledovaniya v Klinike: Spravochnik* (Laboratory Research Methods in the Clinic: Handbook), Moscow: Meditsina, 1987, pp. 106–148.

Translated by N. Statsyuk