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Resting Stages of Crustaceans in the Crimean Hypersaline Lakes (Ukraine) and Their Ecological Role

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1 Introduction

A presence of resting stages in various aquatic organisms is a long-known phenomenon as a mechanism of species adaptation to exist in unstable aquatic habitats, surviving adverse conditions in the "sleeping" state (Radzikowski, 2013). Bank of resting stages of planktonic organisms is an important component of the plankton community, without which we cannot understand community dynamics (Brendonck and De Meester, 2003). "Sleeping" biodiversity of lakes is one of the memory elements of ecosystems, which contributes to a large extent the choice of a new ecosystem alternative stable state under strong salinity changes (Shadrin, 2013).

Crimea is the largest (nearly 26.5 thousands km^2) peninsula in the Black Sea. The hypersaline water bodies constitute a very characteristic and peculiar habitat type in the region: fifty relatively large lakes and numerous small (from several to hundreds meters long) hypersaline water bodies are in the peninsula (Shadrin, 2009). The Crimean hypersaline lakes are divided into two types: of marine origin (thalassohaline); and of continental origin (athalassohaline – sulfate; the athalassohaline lakes were formed in the calderas of ancient mud volcanoes). Salinity in the lakes fluctuates in wide range, as example, in Lake Kirkoyashskoye – from 20 to 410‰. Crustacea is a most abundant and diverse animal group in these lakes. Species diversity of active animals in extreme strongly changing water bodies is usually less diverse, than in "sleeping" part of community (Brendonck and De Meester, 2003; Shadrin, 2013). *To identify total crustacean species diversity in zooplankton we should study also its "sleeping" component.* At the same time only one small study on a resting egg bank of animals in the sediments of hypersaline lakes in the Crimea was done (Moscatello and Belmonte, 2009). In that study the resting eggs of Turbellaria and Rotifera as well as crustaceans - Anostraca (*Artemia parthenogenetica* Bowen and Sterling, 1978, *A. urmiana* Günther, 1899 and *Phallocryptus spinosa* (Milne-Edwards, 1840)), Cladocera (*Moina salina* Daday, 1888), and Copepoda (*Arctodiaptomus salinus* (Daday, 1885)) were isolated from bottom sediment. The objective of this study was to examine the presence of viable crustacean resting stages in sediments of dried up sites of hypersaline lakes in the Crimea.

2 Material and methods

Sediment samples were taken in dried up parts of the lakes (Table 1, Figure 1), collecting the top 5 cm. 14 sediment samples and one sample of sedimented salt from the Crimean hypersaline lakes were used in the experiments. 50-90 g of bottom sediments of each sample was placed evenly on the bottom of the vessel, the distilled water (100-400 ml) was poured, and all was stirred. In experiments with salt 5 g of salt was placed into an experimental vessel. For each sample 2 versions of experiments with different salinity were done. Almost daily we measured salinity, temperature, and visually searched the presence of living organisms, and measured the length of crustaceans, if they were in the vessel. On average, the experiments lasted one month. Alive specimens were caught and putted into other glass vessels to grow them to adult.

We delivered also experiments under the same conditions with the cysts collected from the surface of

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Lake Dzharylhatch in August 2009; before experiments cysts were stored in water with a high concentration of hydrogen sulfide in the refrigerator.

Table 1 Data on experiments with the Crimean lakes sediments

Lake	Coordinates	DS	DE	SE, ‰	
				1	2
Chokrak 1	45°27'51"N-36°18'34"E	01.06.12	14.09.12	40-45	-
Chokrak 2	45°27'51"N-36°18'34"E	01.06.12	14.09.12	70-100	-
Achi	45°09'23"N-35°25'00"E	08.08.12	15.08.12	40-45	25-35
Shimakhanskoye	45°06'12"N-36°14'59"E	04.08.12	16.08.12	55-70	40-45
Uzunlarskoye1	45°02'43"N-36°06'55"E	16.08.10	21.08.12	65-75	-
Uzunlarskoye 2	45°02'50"N-36°06'41"E	04.08.12	22.08.12	50-75	35-60
Marfovskoye	45°12'11"N-36°06'07"E	04.08.12	22.08.12	70-95	50-85
Kirkoyashskoye	45°04'33"N-36°12'34"E	04.08.12	18.09.12	20-25	30-35
Tobechikskoye 1	45°09'10"N-36°22'39"E	05.08.12	21.09.12	50-70	50-60
Tobechikskoye 2	45°10'55"N-36°17'53"E	05.08.12	25.09.12	70-75	60-65
Tobechikskoye 3	45°11'16"N-36°19'16"E	05.08.12	01.10.12	70-75	35-45
Aktashskoye 1	45°22'50"N-35°51'41"E	06.08.12	17.09.12	40-42	35-40
Aktashskoye 2	45°21'59"N-35°49'29"E	06.08.12	17.09.12	40-50	35-40
Koyashskoye	45°03'03"N-36°11'22"E	06.08.12	01.10.12	90-100	85-150
Tobechikskoye, mud volcano	45°09'10"N-36°22'39"E	06.08.13	20.09.13	40-45	-

Note: DS-date of bottom sediment sampling; DE-date of beginning of the experiment; SE-salinity in the experiment

Fig. 1. Studied lakes in the Crimea (● – lakes where sediment samples were taken) .

3 Results and discussion

Totally in all experiments hatching of *Artemia* (*A. parthenogenetica*, *A. urmiana*), ostracods (*Eucypris mareotica* (Fischer, 1855)), harpacticoids (*Cletocamptus retrogressus* Schmankevitsch, 1875) and cladocerans (*M. salina*) was observed.

Artemia. Hatching of nauplii was observed in 80% of all trials under salinity 35-100‰. In 2 cases, it was *A. urmiana*, in others - parthenogenetic females. *A. urmiana* hatched from the sediment of Lake Marfovskoye and from salt of Lake Koyashskoye (Figure 2). It should be noted that adult *A. urmiana* was not found in Lake Marfovskoye, so this is the first evidence of the presence of this species in the lake. On average the time of the appearance of the first nauplii from sediment was 14.9 days (CV = 0.45) and varied widely - from 10 to 31 days. The results (31 days) of two experiments with sediment taken in the dry bed of Lake Tobechikskoye near siphons of active mud volcano near the village Kostyrino stand out sharply against other results. In this part of the lake we did not observe development of *Artemia* during a number of last years (salinity above 340‰, drying). If we exclude these results, the average time of the occurrence of the first nauplii from sediments was 12.4 days (CV = 0.13). First nauplii appeared faster from salt collected on the Lake Koyashskoye - on day 6 under salinity 100‰. Hatching of nauplii was quite synchronous. In the case of salinity 150‰ no one nauplii hatched for the first 10 days. After this solution was diluted to 80‰, there were first nauplii (21 ind.) on day 6. On average 8.9 nauplii hatched from 1 g of salt during experiments. The duration of the release of nauplii from sediments of different lakes (from the registration of the first to the last registration of new nauplii) varied from 2-3 days to 14 -15 days.

Ostracods. Ostracods (*E. mareotica*) from sediments of Lakes Achi and Aktashskoye hatched in 6 experiments at salinities 35-50‰. In 4 cases, they appeared after the brine shrimp, for 20-22 days from the beginning of the experience. In two cases from sediments of the Lake Aktashskoye only ostracods hatched on 11 and 16 days from the beginning of the experience. On average first ostracods appeared on 18.5 day. Emerged from sediments ostracods had size 0.25-0.30 mm. Based on this, we can assume that there were resting ostracod eggs in sediments.

In two experiments with *Artemia* cysts collected in August 2009 in Lake Dzharylhatch and 3 years stored in water with hydrogen sulfide, during 12 and 22 days we did not observe the appearance of nauplii.

Considering all available data, we can assume that in the hypersaline lakes of Crimea all marked in the plankton crustaceans, except Malacostraca, have resting eggs that can accumulate and persist long after drying of water bodies. This is a common feature of all water bodies, which are subject to rapid fluctuations in salinity and/or dry up. Our results showed that nauplii from separated cysts hatched much faster than from the sediments. Early similar was shown for resting eggs of cladocerans and copepods - nauplii from extracted cysts came out more quickly and synchronously than from sediments (Vandekerkhove et al., 2004). The sediments of different lakes inhibit the output of juveniles from artemia and ostracod resting eggs in varying degrees. The nonsynchronous output of juveniles from resting eggs, which are in the bottom sediments, is one of the adaptations that allow them to exist in the extreme and unpredictably changing environment; this reduces risk that all to hatch under unsuitable conditions (Brendonck and De Meester, 2003).

Fig. 2. Lake Koyashskoye, Kerch peninsula, Crimea (from http://maks-kutashev.livejournal.com/10015.html).

Resting eggs of crustaceans can survive in lake sediments at least decades - hundreds of years (Hairston et al., 1995; Radzikowski, 2013). The presence of the bank of crustacean resting stages in sediments, accumulated over decades - centuries, is a memory of crustacean taxocene about past environmental changes. Genetic diversity of a resting stage bank of crustaceans is significantly higher than that of the active part of taxocene. This is one of the important mechanisms for the maintenance of genetic diversity of crustacean populations and their existence in the extreme changing environment. It was hypothesized about the possibility of selective activation of a resting stage bank - the availability of alternative metagenome expressions of taxocene and populations in ecosystem (Shadrin, 2012). However, experimental data, which show this, are not available yet.

Resting eggs of different groups of crustaceans have high tolerance to different factors including to digestive enzymes of birds (Radzikowski, 2013; Vandekerkhove et al., 2013). Hence high tolerance of resting eggs may also be considered as an adaptation to rapid colonization of temporary and new water bodies (Frisch et al., 2007).

In our experiments nauplii did not come out from cysts stored 3 years in an environment without oxygen, but with hydrogen sulfide. Previously it was shown that the nauplii hatched from cysts, stored for four years in the environment without oxygen and hydrogen sulfide (Clegg, 1997). We can assume that in our case a factor, which led to the death of the cysts, was the presence of hydrogen sulfide. However, there is another difference between our and Clegg's experiments; he at first dried cysts, and then put them into the aquatic environment without oxygen; we did not dry cysts preliminarily. Further research on artemia cyst resistance to anoxic condition and hydrogen sulfide are needed.

To better understand ecosystem dynamics of saline fluctuating lakes we need to pay more attention to study resting stages of different organisms in their bottom sediments.

Key words: saline lakes, resting eggs, Crustacea, Crimea

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References

Brendonck, L. and De Meester. L., 2003. Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. In: Van Donk, E., Spaak, P. and Boersma, M. (eds.), Recent developments in fundamental and applied plankton research. *Hydrobiologia,* 491: 65-84.

Clegg, J.S., 1997. Embryos of *Artemia franciscana* survive four years of continuous anoxia: the case for complete metabolic rate depression. *The Journal of Experimental Biology*, 200: 467–475.

Frisch, D., Green, A.J. and Figuerola, J., 2007. High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. *Aquatic Sciences*, 69: 568-574.

Hairston, N.G.Jr., van Brunt, N.A., Kearns, C.M. and Engstrom, D.R., 1995. Age and survivorship of diapausing eggs in a sediment egg bank. *Ecology,* 76: 1706-1711.

Moscatello, S. and Belmonte, G., 2004. Active and resting stages of zooplankton and its seasonal evolution in a hypersaline temporary pond of the Mediterranean coast (the Vecchia Salina, SE Italy). *Scientia Marina*, 68: 491-500.

Radzikowski, J., 2013. Resistance of dormant stages of planktonic invertebrates to adverse environmental conditions. *Journal of Plankton Research*, 35: 707-723.

Shadrin, N.V., 2009. The Crimean hypersaline lakes: towards development of scientific basis of integrated sustainable management. Wuhan, China, 1-5 November, 2009: *Proceedings of 13th World Lake Conference*. http://www.ilec.or.jp/eg/wlc/wlc13/ wlc13papers1.html; http://wldb.ilec.or.jp/data/ilec/ WLC13_Papers/S12/s12-1.pdf

Shadrin, N.V., 2012. Crustaceans in hypersaline water bodies: specificity of existence and adaptations. In: *Proceedings of*

Int. Conference-school "Actual problems of crustacean study in continental waters", 5-9 November, 2012. Kostroma: Kostroma Publishing House, 2012, 316-319. (in Russian)

Shadrin, N.V., 2013. Alternative stable states of lake ecosystems and critical salinities: is there a rigid connection? *Proceedings of the Zoological Institute, Supplement* 3: 214-221. (in Russian)

Vandekerkhove, J., Niessen, B., Declerck, S., Jeppesen, E., Porcuna, J.M.C., Brendonck, L. and De Meester, L., 2004. Hatching rate and hatching success with and without isolation of zooplankton resting stages. *Hydrobiologia,* 526: 235-241.

Vandekerkhove, J., Martens, K., Rossetti, G., Mesquita-Joanes, F. and Namiotko, T., 2013. Extreme tolerance to environmental stress of sexual and parthenogenetic resting eggs of *Eucypris virens* (Crustacea, Ostracoda). *Freshwater Biology, 58:* 237-247.