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Impact of Environmental Salinity on Growth and Development of Naked Amoebae in Beach Sands of the Black Sea in the Region of Odesa, Ukraine

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Abstract: The impact of different levels of salinity (17.6 ‰, 12.6 ‰, 7.6 ‰ and 2.6 ‰) on growth and development of naked amoebae was studied in beach sands of the Black Sea in the region of Odesa, Ukraine. The decreasing salinity was followed by reduced fecundity and increased generation time of the naked amoeba cells. *Vannella devonica*, *V. aberdonica*, *V. plurinucleolus*, *Thecamoeba orbis*, *T. hilla*, *Stenamoeba* sp. and *Acanthamoeba griffini* were the most tolerant to gradual salinity decrease, while *Saccamoeba marina*, *Vexillifera armata*, *Mayorella gemmifera* were less tolerant. *Cochliopodium gulosum* survived at a medium salinity of 17.6 ‰. Under the low medium salinities (7.6 ‰ and 2.6 ‰), most amoebae existed as floating forms. After the saline shock (12.6 ‰, 7.6 ‰ and 2.6 ‰), neither growth nor development were observed in *Saccamoeba marina*, *Cochliopodium gulosum* and *Mayorella gemmifera* and their floating forms remained motionless.

Key words: marine amoebae, locomotor and floating forms, salinity tolerance

Introduction

There are many studies dedicated to the impact of environmental factors (temperature, oxygen, pH and organic matter) on the composition of animal-like organisms (naked and testate amoebae, heterotrophic flagellates and ciliates) in natural biotopes (Schaeffer 1926, Alpatova 2010, Konstantinenko & Mikheeva 2017, Patsyuk 2018). Additionally, some studies have been dedicated to the impact of water salinity on the composition of certain animal species, vertebrates and invertebrates (Warwick 1971, Davenport 1972, Barnes 1989 & Zhao & Sun 2006). However, only a few works provide data on salt resistance of microorganisms. Most of the studies deal with the physiological tolerance of microorganisms to hypersalinity (Rodriguez-Valera et al. 1981, Lugo et al. 1998). In these works, the behavioural and physiological reactions of freshwater Protista were documented for increases in environmental salinity and osmotic pressure (e.g., Oshima et al. 1986). The vacuole complexes of freshwater protists are used to control the water intake. In hyperosmotic environments, the compensatory mechanisms of ionic force are also engaged: free amino acids, univalent cations and carbohydrates act as osmolytes to establish osmotic balance (Hausmann et al. 2003). Literature data report that in addition to osmotic stress, the ionic environmental component causes changes in cell morphology, physiology and cell state. For instance, the marine amoeba *Vannella mira* Schaeffer, 1926 does not reproduce in isotonic solutions of certain salts, requiring NaCl and $CaCl_2$ for fast reproduction (Butts 1935). Freshwater and

soil amoebae *Acanthamoeba castellanii* Volkonsky, 1931 and *Naegleria gruberi* Schardinger, 1899 react to the concentration of electrolytes in the medium. Gradually decreasing the concentration of electrolytes reduces the amoebae's motion speed and their ability to attach to substrate (Preston & King 1984).

There are quite few works describing the impact of the water salinity and environmental osmolarity on the naked amoebae development (Cowie 2005, Cowie & Hannah 2006). Various naked amoebae species react to environmental salinity changes in different ways. The marine amoeba *Platyamoeba* (*Vannella*) *pseudovannelida* Hauer et al., 2001, found in the Salton Sea (California), has a wide range of salt resistance, from 0 ‰ to 138 ‰ (Hauer et al. 2001). Hauer & Rogerson (2005) isolated amoebae from a tidal beach in Florida, where they endure salinity ranging from 2‰ to 120‰ (Hauer & Rogerson 2005). Narrower salinity ranges have been observed for other species of naked amoebae:

- 10–35‰ for *Trichamoeba pallida* Schaeffer, 1926 (Schaeffer 1926);
- 17–35‰ for *Trichamoeba schafferi* Radir, 1927 (Radir 1927);
- 3–30‰ for *Flabellula citata* Schaeffer, 1926 (Page 1971a), *Flabellula hoguae* Sawyer, 1975 (Sawyer 1975b), *Thecamoeba hilla* Schaeffer, 1926 and *Thecamoeba orbis* Schaeffer, 1926 (Page 1971b);
- 7.5–30‰ for *Thecamoeba rugosa* Schaeffer 1926 (Schaeffer 1926) and *Clydonella rosenfieldi* Sawyer, 1975 (Sawyer 1975a); *Vannella murchelanoi* Sawyer, 1975 (Sawyer 1975b), *Vannella weinsteini* Sawyer, 1975 and *Stygamoeba polymorpha* Sawyer, 1975 (Sawyer 1975a, 1975b);
- 16–35‰ for *Vexillifera aurea* Schaeffer, 1926 (Schaeffer 1926);
- 15–30‰ for *Clydonella vivax* Sawyer, 1975 (Sawyer 1975a);
- 7–30‰ for *Clydonella sindermanni* Sawyer, 1975 (Sawyer 1975a);
- 22.5–30‰ for *Clydonella wardi* Sawyer, 1975 (Sawyer 1975a);
- 20–32.5 ‰ for *Lingulamoeba leei* Sawyer, 1975 (Sawyer 1975a);
- 3.5–35‰ for *Vannella bursella* Page, 1974, *Vannella plurinucleolus* Page, 1974 (Page 1974); *Vannella crassa* Schaeffer, 1926 (Schaeffer 1926);
- 2.5–25‰ for *Vannella nucleolilateralis* Anderson, Nerad et Cole, 2003 (Anderson et al. 2003);
- 0–32‰ for *Acanthamoeba griffini* Sawyer, 1971 (Sawyer 1971);
- 0–30‰ for *Cochliopodium clarum* Schaeffer, 1926 (Schaeffer 1926, Sawyer 1975a).

The marine protists can get into the estuary ecosystems or their substrate may undergo the salinity change, so these protists may face decreasing environmental salinity (Cowie 2005, Cowie & Hannah 2006).

Species-specific resistance to different salinity level can be an important element determining the population structure of amoebae in a particular biotope (Reid 1930, Smith 1955). For example, precipitation events can decrease the water salinity of beach sands (Reid 1930); salinity of interstitial water can vary during the year from fully marine water to oligohaline (weakly saline) water (Smith 1955). The time of salinity change differs: from gradually increasing or decreasing during an extended period or more abrupt and rapid salinity changes (Smith 1955, Cowie 2005, Cowie & Hannah 2006).

The present work aimed at studying the impact of different environmental salinity on the viability of 12 naked amoeba species collected from beach sand near the Black Sea (Odesa Region, Ukraine). We examined the reaction of amoebae to the salinity changes in laboratory-based growth media, gradually decreasing from 17.6‰ to 2.6‰ with 5‰ stepwise and a more rapid salinity change without acclimatisation.

Materials and Methods

Collection and identification of naked amoebae

The samples of sand soil were collected on the Black Sea shore (Odesa Region, Ukraine) during the warm season in 2019. The naked amoebae were cultivated in laboratory conditions on non-nutrient agar following Page's method (Page 1983). All of the collected amoebae were primarily kept in cultures on Modified Føyns Erdschreiber Medium (MErds) (Page 1983) with 17.6‰ salinity (to match the salinity in the samples collected in the Black Sea) and incubated at +22 °C. The naked amoebae were observed using a Lomo MBR-3 light microscope, taxonomic identification was performed based on descriptions by Page (1983). DNA was extracted from two species (*Vannella simplex* Bovee, 1965 and *A. griffini*) to confirm their taxonomic identification, because morphological data were insufficient for their species identification; genomic DNA was isolated using the guanidine isothiocyanate method (Maniatis et al. 1982). The 18S rRNA gene was amplified using universal eukaryotic primers: RibA 5'-ACCTGGTTGATCCTGCCAGT-3' and RibB 5'-TGATCCTTCTGCAGGTTCACCTAC-3' (Medlin et al. 1988).

Comparison of the obtained DNA sequences with the data of GenBank was performed using BLAST (NCBI; https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Gradual acclimatisation of naked amoebae for different environmental salinity

The impact of salinity on isolated protists was studied in Petri dishes (d = 100 mm) containing 20 ml of modified medium (MErds) and rice grains to provide the nutrition for growth and development of naked amoebae. In experiments, Petri dishes were used to prepare the control and experimental (with different salinity) conditions. Amoebae from the primary cultures with 17.6‰ were seeded in Petri dishes either with full salinity MErds (17.6‰, control dishes) or in dishes with decreased salinity (12.6‰, 7.6‰, 2.6‰; experimental dishes). Each experimental round included five control and five experimental Petri dishes. Media with decreased salinity (from 17.6‰ to 2.6‰ with 5‰ step) were prepared via changing the proportion of studied sterile marine water and distilled water. Since naked amoebae were collected when the temperature of water was from 22 to 25°C, all the experiments were conducted at 22°C. Naked amoebae cultures were kept for 48 hours for adaptation to new conditions. After two days, the control and experimental samples were examined using a Lomo MBR-3 stereomicroscope (×40 magnification) for the first count. Such observations were performed during seven days to obtain sufficient data on growth and generation of the naked amoebae (Cowie 2005, Cowie & Hannah 2006).

During the experiment, we counted protists in ten fields of view every 24 hours. The data from the replicated treatments were transformed and expressed as mean counts per mm^2 and used to calculate the amoebae's growth rate. After each initial enumeration, the amoebae were transferred to Petri dishes containing MErds of salinity decreased by 5‰ concentration intervals. The dilution steps in the experiments were conducted until the salinity in the Petri dishes reached 2.6‰. The entire experiment consisted of the following gradual series of decreasing salinity with 5‰ at each step: 17.6‰ → 12.6‰ → 7.6‰ → 2.6‰.

Salinity stress

Amoebae growing in the 17.6‰ maintenance medium were transferred into the experimental media with gradually decreasing salinity without acclimatisation with previously added rice grains. At each stage, there were ten replicates and ten fields of view were examined every 24 hours.

Characterisation of naked amoebae motile forms

The rate of amoeba motility was measured using a camera mounted on the microscope. The motion of amoebae was recorded via observation of 15 amoebae if they were moving for 60 minutes. The shape of amoeba cells was drawn and the centres of cells were connected with lines to measure the approximate length of cellular motion. The lengths were summed, to calculate the total path and the mean rate of locomotion of each cell (Cowie & Hannah 2006). Under treatment conditions at a salinity of 2.6‰, amoebae did not attach to the substrate. All were in floating forms. Certain amoeba species (floating forms) were transferred from the 2.6 ‰ medium and placed into separate deep-well plates with media of 17.6‰, 12.6‰ and 7.6‰ salinity. These amoebae were kept for five days and then the number of actively moving amoebae was counted. This allowed us to establish the time during which the floating forms could survive in the conditions not supporting the growth and the attached forms. These amoebae were kept for five days and then the number of actively moving amoebae was counted. This allowed us to establish the time during which the floating forms could survive in the conditions not supporting the growth and the attached forms.

Statistical analyses

The number of cells in field of view was transformed into number of cells/mm^2 and the growth curves were drawn. To calculate the slope of the exponential growth phase, we used regression analysis. The cells' productivity was plotted on the graphs. The growth rate constant (K) was calculated by the formula of Stanier et al. (1976):

$$K = (\log_{10} N_t - \log_{10} N_0)/0.301\ t,$$

where N_t was the final number of cells, N_0 was the initial number of cells and t was the time in hours.

The generation time in hours was calculated as 1/k. We examined the data to verify that it satisfied the necessary assumptions required to apply ANOVA. All the data were analysed using one-way ANOVA followed by a post-hoc, pairwise Bonferroni comparison. We used the statistical software Minitab v1 9.1.1.0 for the analyses.

Results

In our experiments, we changed the salinity of media where the naked amoebae reproduced via diluting the seawater by distilled water. Thus, the protists were af-

fected by the osmotic pressure and by the concentration of ionic components (Oshima et al. 1986). Twelve naked amoebae were identified: *Saccamoeba marina* Anderson, Rogerson & Hannah, 1997, *Vexillifera armata* Page, 1979, *Vannella devonica* Page, 1979, *Vannella aberdonica* Page, 1980, *Vannella plurinucleolus* Page, 1974, *Vannella simplex* Bovee, 1965, *Cochliopodium gulosum* Schaefer, 1926, *Mayorella gemmifera* Schaeffer, 1926, *Thecamoeba orbis* Schaeffer, 1926, *Thecamoeba hilla* Schaeffer, 1926, *Stenamoeba* sp. and *Acanthamoeba griffini* Sawyer, 1971. All they reacted differently to the gradually decreasing environmental salinity.

The results of the decrease of environmental salinity under the different environmental salinity treatments (17.6‰, 12.6‰, 7.6‰, 2.6‰) for all studied species are reported here. There were no significant differences compared to the generation time of control populations ($p>0.05$), i.e. the repeated sub-cultivation processes did not cause significant changes of growth rate and generation time in naked amoebae.

Species as *V. devonica, V. aberdonica, V. plurinucleolus, T. orbis, T. hilla, Stenamoeba* sp. and *A. griffini* were the most resistant to gradual salinity decrease. Amoebae reproducing in the media with 7.6‰ and 2.6‰ salinity had lower cell productivity compared to the control populations (Figs. 1-7).

For instance, the cell productivity in *V. devonica* at 7.6‰ salinity was 438±40 cells/mm^2, at 2.6‰ – 128±20 cells/mm^2, for the control population – 7324±845 cells/mm^2. In *V. aberdonica*, it was: at 7.6‰ salinity – 502±63 cells/mm^2, at 2.6‰ – 169±18 cells/mm^2; for the control population – 6894±732 cells/mm^2. In *V. plurinucleolus* at 7.6‰ salinity, it was 624±52 cells/mm^2, at 2.6‰ – 428±25 cells/mm^2 and for the control population – 5328±426 cells/mm^2. In *T. hilla* at 7.6‰ salinity, the productivity was 583±32 cells/mm^2, at 2.6‰ – 283±15 cells/mm^2, and for the control population – 3832±304 cells/mm^2. In *T. orbis* at 7.6‰ salinity, the cell productivity was 728±68 cells/mm^2, at 2.6‰ – 384±62 cells/mm^2 and for the control population – 6832±304 cells/mm^2. In *Stenamoeba* sp. at 7.6‰ salinity, the cell productivity was 384±28 cells/mm^2, at 2.6‰ – 103±15 cells/mm^2, and for the control population – 4402±203 cells/mm^2. For *A. griffini* at 7.6‰ salinity, it was 1834±388 cells/mm^2, at 2.6‰ – 688±298 cells/mm^2 and for the control population – 6003±328 cells/mm^2.

The mean generation time for the control populations of *V. devonica* was constant, 25.3-38.9 hours^{-1}; for *V. aberdonica* 22.8-35.7 hours^{-1}; for *V. plurinucleols* 27.8-39.1 hours^{-1}; for *T. orbis* 28.8-40.5 hours^{-1}; for *T. hilla* 26.5-40.4 hours^{-1}; for *Stenamoeba* sp. – 27.3-39.4 hours^{-1}; for *A. griffini* 25.6-36.4 hours^{-1}. During a gradual decrease in salinity, the mean generation time for naked amoebae increased compared to the control (Table 1).

After the change of environmental salinity from 17.6‰ to 12.6‰, all amoebae remained attached to the substrate with their characteristic cell features. With the gradually decreasing of environmental salinity, the motion speed of naked amoebae changed.

Some of the fan-shaped (*V. devonica, V. aberdonica, V. plurinucleolus*), striate (*T. orbis, T. hilla*)

Table 1. The mean generation time (in hours) of naked amoebae affected by gradually decreasing salinity

No	Species	Environmental salinity, ‰			
		17.6	**12.6**	**7.6**	**2.6**
1	*Saccamoeba marina*	38.34±2.4	100.18±12.6	floating forms	floating forms
2	*Vexillifera armata*	39.33 ± 4.3	122.53±13.8	floating forms	floating forms
3	*Vannella devonica*	36.18±4.2	38.19±4.3	floating forms/46.60 ±3.9	floating forms/112.16 ±12.9
4	*Vannella aberdonica*	37.03±2.8	40.93±2.7	floating forms/49.18±3.2	floating forms/105.9 ±14.5
5	*Vannella plurinucleolus*	38.04±3.2	43.44±3.5	floating forms/48.63 ±3.6	floating forms/128.33 ±9.6
6	*Vannella simplex*	37.74±0.5	142.89±2.8	floating forms	floating forms
7	*Cochliopodium gulosum*	59.84±2.8	floating forms	floating forms	floating forms
8	*Mayorella gemmifera*	34.46±8.5	183.64±18.5	floating forms	floating forms
9	*Thecamoeba orbis*	40.82±2.8	44.25±3.9	floating forms/45.8 ±3.8	floating forms /105.84 ±25.6
10	*Thecamoeba hilla*	42.13±1.8	48.32±2.5	floating forms /46.64 ±3.5	floating forms /106.03 ±18.7
11	*Stenamoeba* sp.	38.84±4.5	44.84±3.6	floating forms /46.83 ±2.8	floating forms/106.85 ±2.8
12	*Acanthamoeba griffini*	36.54±2.4	38.83±2.8	floating forms/43.54±3.5	floating forms/04.8 ±3.5

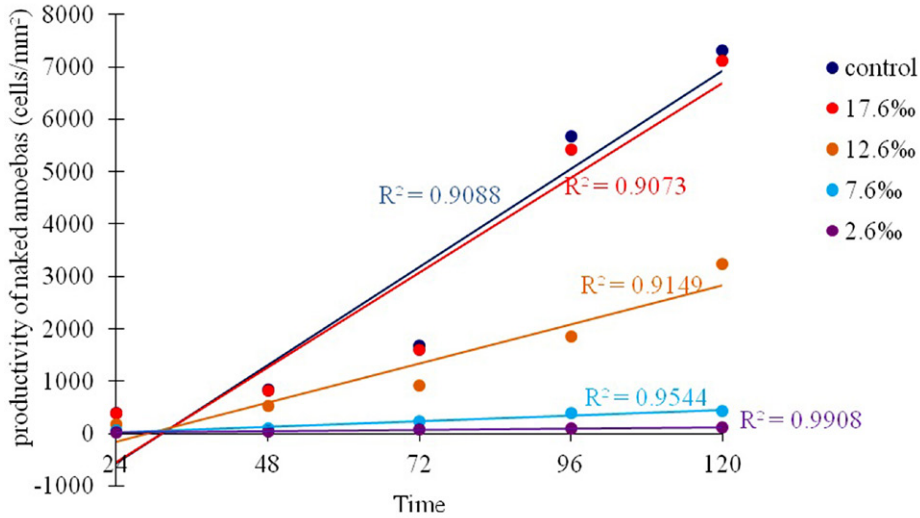

Fig. 1. Productivity of *Vannella devonica* at different environmental salinity.

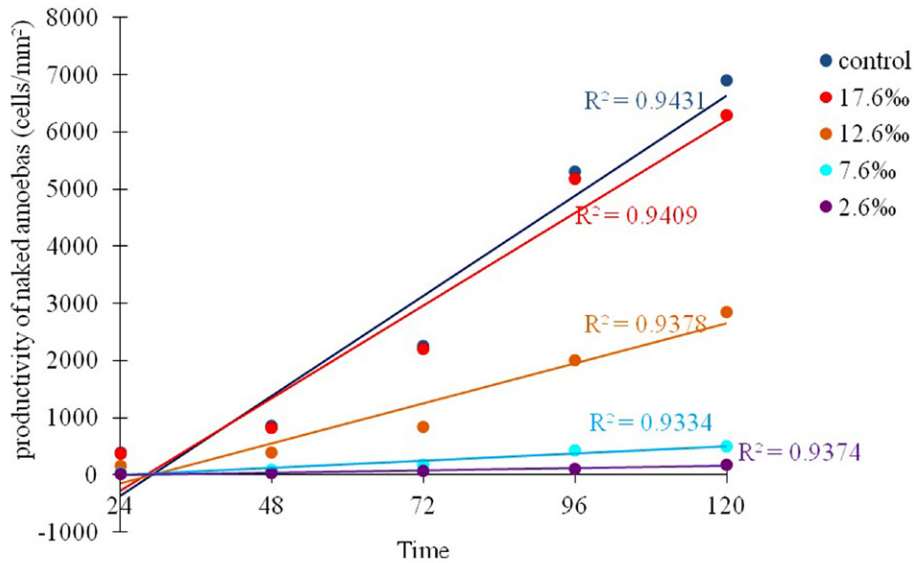

Fig. 2. Productivity of *Vannella aberdonica* at different environmental salinity.

and lingulate (*Stenamoeba* sp.) morphotypes had spherical (floating) shape without cytoplasm outgrowths at 7.6 ‰ and 2.6 ‰ salinity. They were not attached to the substrate. With decreasing salinity, the number of floating forms increased. *Vannella devonica, V. aberdonica, V. plurinucleolus, T. orbis, Stenamoeba* sp. and *A. griffini* demonstrated moderate cell vacuolisation along with decreasing salinity, while the attached cells of *T. hilla* were highly vacuolated at 7.6 ‰ and 2.6 ‰.

Saccamoeba marina, V. armata, C. gulosum and *M. gemmifera* were less tolerant to the decrease in environmental salinity than the aforementioned species. For *C. gulosum* cell growth was not observed at 12.6 ‰, 7.6‰ and 2.6 ‰. Amoebae in these media were floating and of spherical shape.

The generation time and cell productivity in *S. marina, V. armata, V. simplex* and *M. gemmifera* were affected under the environmental salinity of 12.6‰.

The generation time of experimental cells of *S. marina, V. armata, V. simplex* and *M. gemmifera* at 12.6‰ was significantly higher ($p<0.01$) than that in control cells at 17.6‰. Thus, the generation time for *S. marina* at 17.6‰ was 38.34 hours, at 12.6‰ it was 100.18 hours. For *V. armata*, at 17.6‰ salinity, it was 39.33 hours and; at 12.6‰, it was 122.53 hours. For *M. gemmifera* at 17.6‰ salinity, it was 34.46 hours, and at 12.6‰ – 183.64 ‰. For *V. simplex* at 17.6‰ salinity, it was 37.74 hours, and at 12.6‰ – 142.9 hours. Reduced growth rate was followed by low productivity of amoebae cells under 12.6‰ salinity compared to the control.

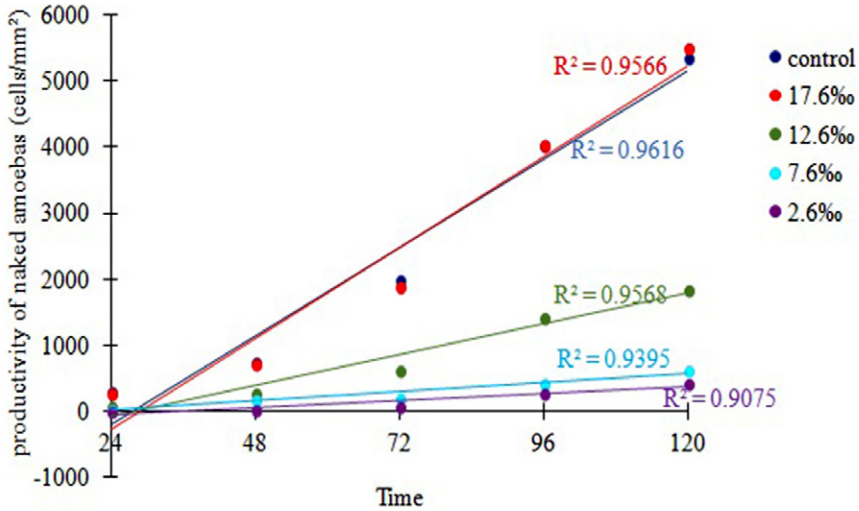

Fig. 3. Productivity of *Vannella plurinucleolus* at different environmental salinity.

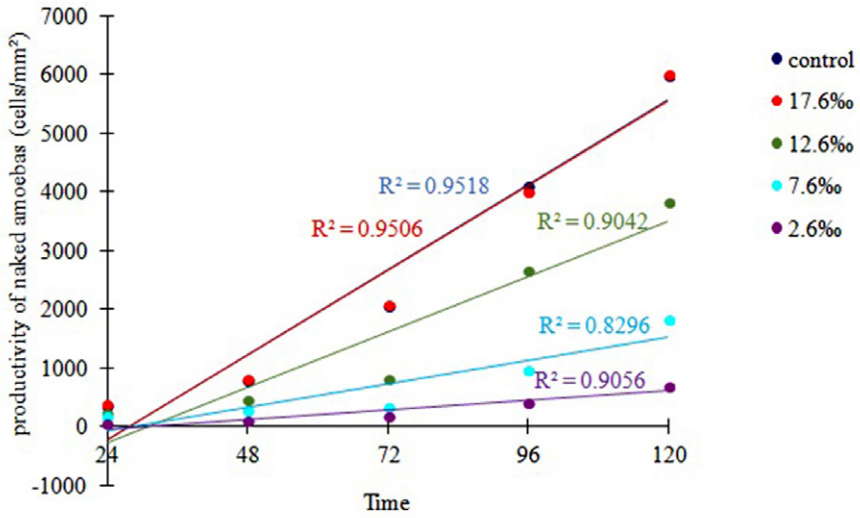

Fig. 4. Productivity of *Acanthamoeba griffini* at different environmental salinity.

The cell productivity for *S. marina* at 17.6‰ was 946±35 cells/mm^2 and at 12.6‰ – 189±13 cells/mm^2. For *V. armata* at 17.6‰, it was 1035±40 cells/mm^2 and at 12.6‰ – 128±24 cells/mm^2. For *V. simplex* at 17.6‰ salinity, the cell productivity was 1036±131 cells/mm^2 and at 12.6‰ – 104±16 cells/mm^2. For *M. gemmifera*, the values were: at 17.6‰ – 896±29 cells/mm^2 and at 12.6‰ – 73±9 cells/mm^2.

We observed no significant morphological changes in the cells of *S. marina, V. simplex, M. gemmifera* at environmental salinity of 12.6 ‰. All of them were attached to the substrate. Only a few cells of *V. armata* had the spherical shape without sub-pseudopodia and the cells of *M. gemmifera* were vacuolated. *Saccamoeba marina, V. armata, V. simplex* and *M. gemmifera* did not attach to the substrate in the media of 2.6‰ and 7.6‰ salinity; all their cells were floating and spherical.

With decreasing salinity, the rate of motility of these amoebae changed. In *S. marina*, it was 4.2±0.8 µm/min compared to 13.1±1.8 µm/min for the control. In *V. simplex* – 2.8±1.0 µm/min at 12.6‰ salinity compared to 12.4 µm/min for the control. In *M. gemmifera*, it was 5.2±1.6 µm/min at 12.6‰, compared to 15.3 µm/min in control. In *V. armata*, it was 3.6±0.9 µm/min at 12.6‰ compared to 11.2±1.3 µm/min for the control.

The generation time and cell productivity of naked amoebae cells affected by salinity stress are presented in Table 2. Fast salinity stress without acclimatisation triggered a decrease in the resistance in all amoebae species.

The cells of naked amoebae placed in medium with 17.6‰ salinity demonstrated growth rates,

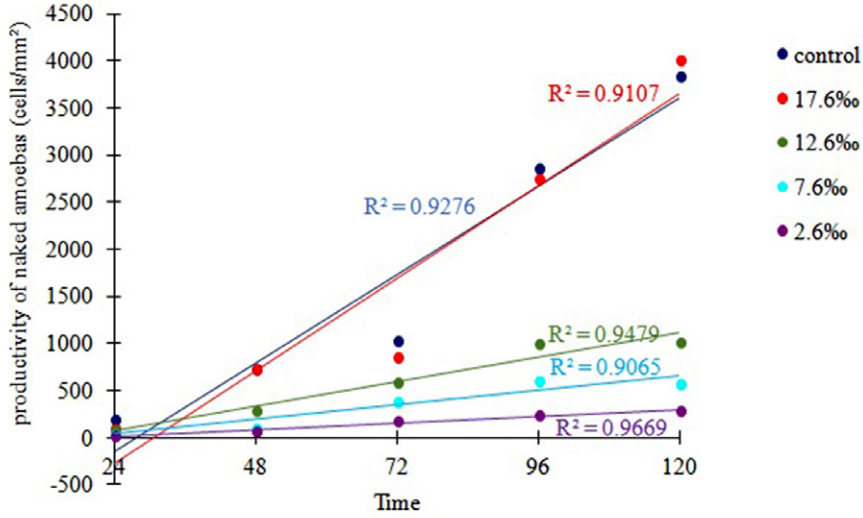

Fig. 5. Productivity of *Thecamoeba hilla* at different environmental salinity.

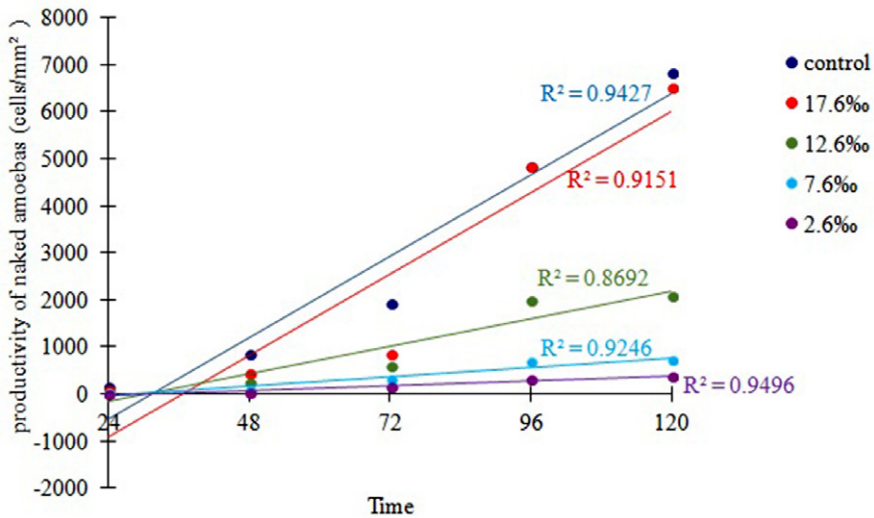

Fig. 6. Productivity of *Thecamoeba orbis* at different environmental salinity.

generation times and cell productivities similar to gradually acclimatised cells.

However, when grown at 12.6 ‰, for taxa such as *V. armata, V. devonica, V. aberdonica, V. plurinucleolus, V. simplex, T. orbis, T. hilla, Stenamoeba* sp. and *A. griffini,* the generation time was significantly higher ($p<0.001$), while the productivity was lower ($p<0.001$) at 12.6‰ than at 17.6 ‰. Some cells of *V. devonica, V. aberdonica, V. plurinucleolus, T. orbis* and *T. hilla* were spherical without cytoplasm outgrowths. A number of these cells, which remained attached at 12.6‰, had high cytoplasm vacuolisation.

Acanthamoeba griffini was observed in the medium with 7.6‰ salinity, while the amoebae of all other species retained floating forms and were scarce. Cells of *A. griffini* were highly vacuolated. Besides, *S. marina, C. gulosum* and *M. gemmifera* were observed as a few small floating forms at environmental salinity of 12.6 ‰, 7.6‰ and 2.6 ‰. There were no morphological changes observed for cells of attached amoebae at the rapid salinity changes.

The rate of motility in naked amoebae also changed. It was:

Vannella armata – 10.3±1.8 μm/min at 17.6‰ and 4.5±0.5 μm/min at 12.6‰;

Vannella devonica – 1.3±0.9 μm/min at 17.6‰ and 3.8±0.8 μm/min 12.6‰;

Vannella aberdonica – 12.2±1.8 μm/min at 17.6‰ and 5.2±1.8 μm/min at 12.6‰;

Vannella plurinucleolus – 11.8±1.3 μm/min at 17.6‰ and 4.8±2.1 μm/min 12.6‰;

Vannella simplex – 12.8±2.1 μm/min at 17.6‰ and 5.4±0.7 μm/min at 12.6‰;

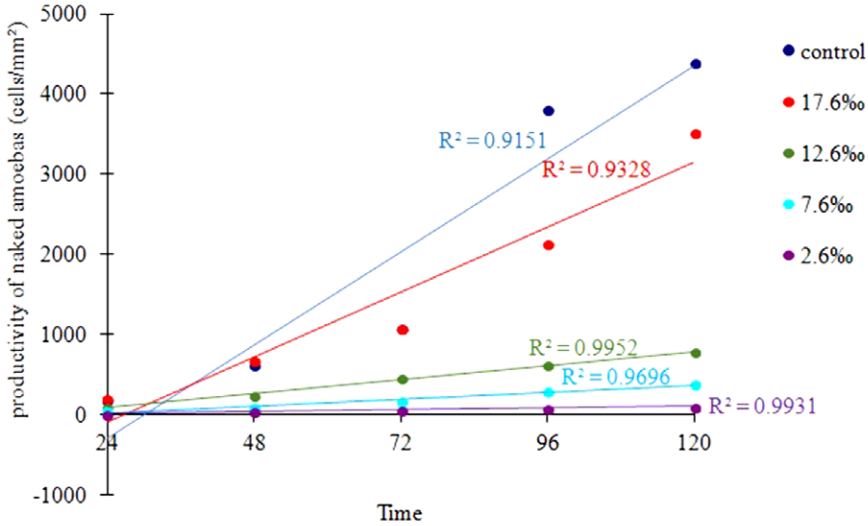

Fig. 7. Productivity of *Stenamoeba* sp. at different environmental salinity.

Table 2. The generation time (GT) (in hours) and cell productivity (CP) (cells/mm^2) of naked amoebae and the effect of rapid environmental salinity changes.

No	Naked amoeba species	Environmental salinity, ‰							
		17.6		12.6		7.6		2.6	
		GT	CP	GT	CP	GT	CP	GT	CP
1	*Saccamoeba marina*	62.18 ±3.5	1328 ±88	floating forms		floating forms		floating forms	
2	*Vexillifera armata*	48.23 ±8.3	1825 ±25	134.21 ±0.8	435 ±84	floating forms		floating forms	
3	*Vannella devonica*	39.84 ±5.3	6823 ±28	128.83 ±2.3	580 ±23	floating forms		floating forms	
4	*Vannella aberdonica*	41.84 ±8.2	6562 ±113	118.23 ±8.4	384 ±110	floating forms		floating forms	
5	*Vannella plurinucleolus*	38.84 ±2.1	5221 ±246	153.84 ±2.1	684 ±108	floating forms		floating forms	
6	*Vannella simplex*	42.32 ±4.1	1241 ±28	128.83 ±8.3	284 ±25	floating forms		floating forms	
7	*Cochliopodium gulosum*	58.34 ±0.9	983 ±105	floating forms		floating forms		floating forms	
8	*Mayorella gemmifera*	68.35 ±2.3	832 ±85	floating forms		floating forms		floating forms	
9	*Thecamoeba orbis*	48.84 ±8.5	5843 ±118	134.15 ±0.9	684 ±63	floating forms		floating forms	
10	*Thecamoeba hilla*	49.28 ±2.3	2948 ±85	152 ±2.4	983 ±63	floating forms		floating forms	
11	*Stenamoeba sp.*	51.15 ±8.4	3644 ±123	128.84 ±3.5	1082 ±59	floating forms		floating forms	
12	*Acanthamoeba griffini*	42.84 ±3.5	5844 ±284	93.55 ±8.4	2834 ±193	183.44±8.4	583 ±43	floating forms	

Thecamoeba orbis – 9.84±1.3 µm/min at 17.6‰ and 3.2±0.6 µm/min at 12.6‰;

Thecamoeba hilla – 10.15±2.1 µm/min at 17.6‰ and 3.8±1.1 µm/min at 12.6‰;

Stenamoeba sp. – 10.13±1.8 µm/min at 17.6‰ and 3.8±1.2 µm/min at 12.6‰;

Acanthamoeba griffini – 9.8±1.1 µm/min at 17.6‰ and 2.4±0.5 µm/min 12.6‰.

The lower level of salinity for *V. devonica, V. aberdonica, V. plurinucleolus, T. orbis, T. hilla* and *Stenamoeba* sp. was 10 ‰ compared to gradually acclimatised amoebae.

According to our study, most of the floating forms of naked amoebae (*S. marina, V. armata, V. devonica, V. aberdonica, V. plurinucleolus, V. simplex, T. orbis, T. hilla, Stenamoeba* sp. and *A. griffini*) survived for a week in the medium with 2.6‰ and 7.6‰ salinity. Such amoebae like *C. gulosum*

and *M. gemmifera* were able to survive with enough amount of floating forms up to 72 hours but, after 80 hours, living amoebae were not found in cultures.

Thecamoeba orbis is known to endure environmental salinities from 3‰ to 30‰ (Page 1971b), *V. plurinucleolus* – 3.5 ‰ to 35‰ (Page 1974) and *A. griffini* – 0‰ and 32‰ (Sawyer 1971). Our research showed that, at salinity of 2.6‰, these amoebae were partially presented as floating forms and partially were attached to substrate, slowly growing and reproducing.

Discussion

In order to assess the role of naked amoebae in microbial food chains in marine ecosystems, it is necessary to elucidate the influence of environmental factors (for example, temperature and water salinity) on the growth and development of these protists. Butler & Rogerson (1996) studied the effect of temperature (from 5°C to 20°C) on the reproduction rate of 11 naked amoeba species isolated from the Clyde Sea area (Scotland). It has been established that the growth rate of naked amoebas increases with temperature (generation time decreases). The average cell proportions also depend on temperature: most naked amoebas have large cell proportions at low temperatures. Cowie & Hannah (2006) studied the salt tolerance of four naked amoeba species to a gradual and sharp decrease in the salinity of the environment. Small amoebae show the greatest resistance to both forms of salinity changes, in addition, with a sharp decrease in salinity, amoebae acquire a floating form and morphological changes in cells are observed. Page (1974) found that *Platyamoeba* Page, 1969 can survive at water salinity from 3.5 ‰ to 35 ‰. *Vexillifera browni* Sawyer, 1975 withstands salinity of 20 ‰ and above, while at salinity below 20 ‰ the species acquires a floating form and dies (Sawyer 1975b).

Vexillifera telmathalassa Bovee, 1956 survives at a salinity of 36-16 ‰, while at a salinity of 12 ‰ the shape of the cells changes and they become immobile (Anderson 1994). *Flabellula mira* (*Vannella mira*) Schaeffer, 1926 grows in seawater at a salinity ‰ of 7 to 52.5 ‰. *Vannella* sp. and *Vahlkampfia* sp., isolated from Florida seawater, can withstand salinities of 12 ‰ to 92 ‰ and 2 ‰ to 72 ‰, respectively (Hauer & Rogerson 2005). According to our data, with a gradual decrease in the salinity of the environment from 17.6 ‰ to 2.6 ‰, *V. aberdonica*, *V. plurinucleolus*, *T. orbis*, *T. hilla*, *Stenamoeba* sp., *A. griffini* withstand the entire range of changes; *S. marina*, *V. armata*, *V. simplex*, *M. gemmifera* – from 17.6 ‰ to 12.6 ‰; *C. gulosum* – only 17.6 ‰.

With a sharp decrease in the salinity of the environment (from 17.6 ‰ to 2.6 ‰), only *A. griffini* can grow and multiply at a salinity of 17.6 ‰ to 7.6 ‰; *V. armata*, *V. devonica*, *V. aberdonica*, *V. plurinucleolus*, *V. simplex*, *T. orbis*, *T. hilla*, *Stenamoeba* sp. – 17.6-12.6 ‰; *S. marina*, *C. gulosum*, *M. gemmifera* – 17.6 ‰.

Our research confirms that marine naked amoeba species tolerate water salinity, which is optimal for their existence and it is difficult to tolerate reduced environmental salinity.

Conclusion

We observed the reaction of 12 naked amoebae species to different environmental salinities and to the resultant salt stress. These amoeba species reacted differently to the changes of environmental salinity; and overall, poorly endured the low environmental salinity. Rapid decreasing of environmental salinity without acclimatisation decreased the generation time and the tolerance of the studied amoebae; species as *S. marina, V. armata, V. devonica, V. aberdonica, V. plurinucleolus, V. simplex, T. orbis, T. hilla, Stenamoeba* sp. and *A. griffini* were able to survive during prolonged period of time, but as floating forms. The growth parameters were normal until the threshold values were reached, after which the growth parameters were impaired and thus the work of the osmoregulatory apparatus was deteriorated.

The small amoebae like *V. devonica, V. aberdonica, V. plurinucleolus, T. orbis, Stenamoeba* sp. and *A. griffini* were more durable to gradual and rapid changes of environmental salinity, they almost did not show morphological changes, unlike t cells of the larger species.

The larger amoebae demonstrated high cell vacuolisation and, under the salinity of 7.6‰ and 2.6‰, these amoebae existed as floating forms and moved slowly. Decreased environmental salinity caused decreased rate of locomotion and increasing numbers of floating forms along with a rise in generation time.

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