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## **Hatch and Die: The Effect of Salinity on the Performance of *Moina macrocopa* (Straus, 1820) (Cladocera: Moinidae) Populations Initiated by Post-Diapause Females**

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**Abstract.** Freshwater salinization may have important implications for the abundance and diversity of zooplankton, which play a key role in the functioning of aquatic ecosystems. Many of these animals undergo developmental arrest in the embryonic stage, which allows them to survive harsh periods and cope with seasonal unfavorable environmental conditions. We studied the effects of salinity in the experiments with individuals and populations of the cladoceran *Moina macrocopa* on the life history traits and population parameters important for cyclical development of animals: the hatching success of resting eggs; the survival of hatchlings; individual performance of post-diapause females and performance of populations initiated by post-diapause females. We observed a decreasing sequence of salinities critical for traits and parameters studied in this work: from 8 g L<sup>-1</sup>, critical for hatching of resting eggs, to 1 g L<sup>-1</sup>, critical for the ability of a population initiated by post-diapause females to produce resting eggs. We conclude that the relatively low salinity (1 g L<sup>-1</sup>) that does not suppress the hatching of resting eggs reduces the ecologically significant ability of the *M. macrocopa* populations to replenish the bank of resting eggs. Thus, freshwater salinization, in the long term, will prevent the re-establishment of *M. macrocopa* and most probably other freshwater cladoceran populations from the banks of resting eggs.

**Keywords:** *Cladocera*, freshwater salinization, life history traits, population dynamics, resting eggs, post-diapause females.

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**Проснись и погибни: влияние солёности  
на продуктивность популяций  
*Moina macrocopa* (Straus, 1820) (Cladocera: Moinidae),  
инициированных самками,  
вышедшими из покоящихся яиц**

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**Аннотация.** Засоление пресноводных экосистем может привести к значимым последствиям для численности и разнообразия зоопланктона, который играет ключевую роль в функционировании водных экосистем. Многие представители зоопланктона обладают способностью к задержке развития на стадии эмбриона, что позволяет им переживать суровые периоды существования и справляться с сезонными неблагоприятными условиями окружающей среды. Мы изучили влияние солёности в экспериментах с отдельными особями и популяциями ветвистоусого рачка *Moina macrocopa* на параметры их жизненного цикла и популяционные характеристики, обладающие значимостью для циклического развития животных: успешность реактивации покоящихся яиц; выживаемость вылупившихся рачков; продуктивность отдельных самок, вышедших из покоящихся яиц и продуктивность популяций, инициированных самками после диапаузы. Мы обнаружили повышение порога чувствительности изучаемых параметров к солёности: от 8 г/л – критической солёности для вылупления покоящихся яиц, до 1 г/л – критической солёности для способности популяции, инициированной самками, вышедшими из яиц после диапаузы, производить покоящиеся яйца. Мы заключили, что сравнительно низкая солёность (1 г/л), которая не влияла на эффективность реактивации покоящихся яиц, негативно воздействует на экологически значимую способность популяций *M. macrocopa* пополнять банк покоящихся яиц. Таким образом, засоление пресных вод в долгосрочной перспективе будет препятствовать восстановлению популяций рачков *M. macrocopa* и, скорее всего, других популяций пресноводных ветвистоусых ракообразных из банков покоящихся яиц.

**Ключевые слова:** ветвистоусые ракообразные, засоление пресноводных экосистем, параметры жизненного цикла, популяционная динамика, покоящиеся яйца, самки рачков, реактивированные из яиц.

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## Introduction

Freshwater salinization is a worldwide environmental problem (Zadereev et al., 2020; Cunillera-Montcusí et al., 2022) caused by such natural and anthropogenic factors as urbanization, water intake, resource extraction, agriculture, long-term use of de-icing road salts, and climate change (Williams, 2001; Velasco et al., 2019). Salinity is a factor that affects population dynamics and shapes community structure (Santangelo et al., 2008). Salinization leads to the degradation of the water quality, contributing to the loss of biodiversity and taxonomic replacement by species (Vengosh, 2003; Huang et al., 2022).

Alternations between the active and resting stages in the life cycle are vital for species encountering alternating optimal and suboptimal environments (Alekseev, Starobogatov, 1996; Hand et al., 2016). Many zooplankton species are capable of a programmed arrest of development (diapause) in the embryonic stage (Stross, Hill, 1965; Alekseev, Starobogatov, 1996). These animals can survive harsh periods (freezing, drying, and other hostile conditions) and cope with seasonal unfavorable environmental conditions by producing resting eggs (Alekseev et al., 2007). The store of such eggs (the bank of resting eggs) in the bottom sediments is formed by actively reproducing females that produce resting eggs with the onset of either seasonal or occasional unfavorable conditions.

The number of resting eggs in the egg bank and their hatching success determine the efficiency of the reestablishment of zooplankton populations after a period of disappearance of the animals from the water column. Several studies estimated the effects of increased salinity on hatching success of resting eggs (Bailey et al., 2004; Santangelo et al., 2014; Lopatina et al., 2021). It was shown that hatching success is almost unaffected by salinity below a threshold value (which varies between 7.6–32 g L<sup>-1</sup> for different species), and resting eggs exposed to saline sediments are able to hatch when transferred to freshwater. In general, resting eggs from egg banks usually have low hatching rates, no higher than 50 % (Hairston et al., 1995), as hatching success of resting eggs is declining with the age (Amsinck et al., 2007). In our previous study (Lopatina et al., 2024), we observed a slight age-related reduction in salinity tolerance of eggs.

The number of resting eggs deposited to the egg bank depends on population size. Although the production of resting eggs is a key event, which is critically important for the ecological success of species with the diapause, there are scarcely any studies on the effect of salinity on the production of resting eggs by cladoceran populations. Several studies demonstrated salinity induced negative changes in population dynamics (Huang et al., 2022; Sarma et al., 2006) but none of those studies estimated an ability of populations exposed to salinity to produce resting eggs.

Thus, there are two key events in the population cycle of a zooplankton species producing resting eggs that can be affected by salinity – the production of resting eggs and reactivation of resting eggs. Taking into account the growing threat of freshwater salinization we focused our study on the effect of salinity on the hatching success of resting eggs after exposure to saline sediments and on the ability of population started by post-diapause females to produce resting eggs.

## Methods

### *General information*

We used one-year-old resting eggs from a laboratory culture of the cladoceran *Moina macrocopa* (Straus, 1820) (Cladocera: Moinidae), which has been maintained at the Institute of Biophysics (Krasnoyarsk, Russia) in cyclical mode for more than 10 years. To obtain resting eggs, we used the previously described protocol (Oskina et al., 2019). The effect of salinity on hatching success of resting eggs and performance of post-diapause females was estimated in acute and chronic tests with individuals and in population experiments.

Tap water (pH – 7.3; total permanent hardness – 62.9 mg equivalent of CaCO<sub>3</sub> L<sup>-1</sup>; total content of cations (macro- and trace elements) – 26.4 mg L<sup>-1</sup>) aged for at least 72 h was used as a culture medium in all experiments. We used road de-icing salt “Bionord” to create salinity treatments (Instructions for using..., 2007; Organization standard..., 2016). The dominant chemical components of “Bionord” are sodium and calcium chlorides (up to 85 % of the total content) (Lopatina et al., 2021). Previously, we demonstrated that toxic effects of solutes of “Bionord” are similar to the effects of solutes of sodium chloride (Lopatina et al., 2020). The stock solution of “Bionord” (200 g L<sup>-1</sup>) was used to prepare salinity treatments. Salinity was

measured as specific electrical conductivity of water with a STARTER ST300C conductometer “Ohaus Corporation” (U.S.A.).

In experiments, we used only undamaged ephippia with 2 fertilized eggs. Prior to the experiments, ephippial eggs were stored in the dark in the refrigerator at 4 °C. All experiments were performed in climate chambers under a controlled photoperiod (light 16 h: dark 8 h) and the temperature favorable for hatching of resting eggs and parthenogenetic reproduction of *M. macrocopa* (for acute and chronic tests, the temperature was maintained at 25 °C, for population experiments at 21–23 °C) (Zadereev, Gubanov, 1996).

### *Acute and chronic tests with resting eggs and post-diapause females*

In the acute test, we placed ephippia individually into vessels with 40 mL of either tap water (control, salinity = 0.1 g L<sup>-1</sup>) or saline tap water. We tested the following salinity treatments: 2, 4, 5, 6, and 7 g L<sup>-1</sup> of “Bionord”, which is equal to salinities: 1.9, 3.5, 4.2, 5.1, and 5.9 g L<sup>-1</sup>, respectively. For each salinity treatment and control, 30 ephippial eggs were tested. We did not feed animals during the acute test. The water in vessels during the acute test was changed daily. The duration of the acute test was 5 days. After 48 hours, we counted live and dead females hatched from the eggs every 24 hours during three consecutive days. Based on these data, the median lethal concentration (LC<sub>50</sub> – the salinity resulting in a survival of hatched females 50 % below the upper limit) was calculated.

For the chronic test, females were hatched from 25–30 resting eggs in vessels containing 500 mL of either tap water (control) or two saline treatments (2 and 4 g L<sup>-1</sup> of “Bionord”, which is equal to salinity of 1.9 and 3.5 g L<sup>-1</sup>, respectively, hatching salinity). Previously, we demonstrated that these concentrations are below

the lethal salinity for *M. macrocopa* resting eggs and females (Lopatina et al., 2020, 2021). After hatching, 3–5-hour-old females were placed individually into vessels with 20 mL of the medium (either control tap water or water with salinities 1.9 and 3.5 g L<sup>-1</sup>, raising salinity). For each hatching salinity treatment and for the control we tested 20 females. We performed three consecutive chronic tests (one test for each hatching salinity) with post-diapause females placed into aged tap water (control), water with salinity of 1.9 g L<sup>-1</sup>, and water with salinity of 3.5 g L<sup>-1</sup> (raising salinities). The animals were fed daily with the non-axenic green alga *Chlorella vulgaris* (Chlorellales: Chlorellaceae) (Oskina et al., 2019) at a concentration of 200 thousand cells mL<sup>-1</sup> (ca. 2.24 µg C mL<sup>-1</sup>). The water in chronic tests, which were run until all animals died, was changed daily.

We measured the body length of each female at 16× magnification on the first day of the experiment and the day before it produced the first clutch. The body lengths were used to calculate the specific growth rate of juvenile

females (Zadereev et al., 2017). For each female, we counted the number of offspring in each clutch and recorded the time of death. These data were used to calculate the average lifespan (days) and fecundity (hatchlings per female) of females.

*Population experiments with post-diapause females*

Resting eggs were placed in 500 mL vessels (30 ephippia in each vessel) containing 100 g of control sediments (calculated salinity 0.33 g kg<sup>-1</sup>) or sediments enriched with the “Bionord” in concentrations 2, 4, and 5 g kg<sup>-1</sup>, with the calculated salinity 2.1, 3.8, and 4.6 g kg<sup>-1</sup>, respectively (3 vessels with control sediment and 9 vessels with saline sediments). Five sets of experimental vessels were filled sequentially at an interval of three weeks (3 vessels with control sediment and 9 vessels with the saline sediments for each set) (Fig. 1).

Artificial sediments were created according to the standard protocol for the production of artificial bottom sediments (OECD, 1984; OECD, 2023). Artificial sediment was prepared

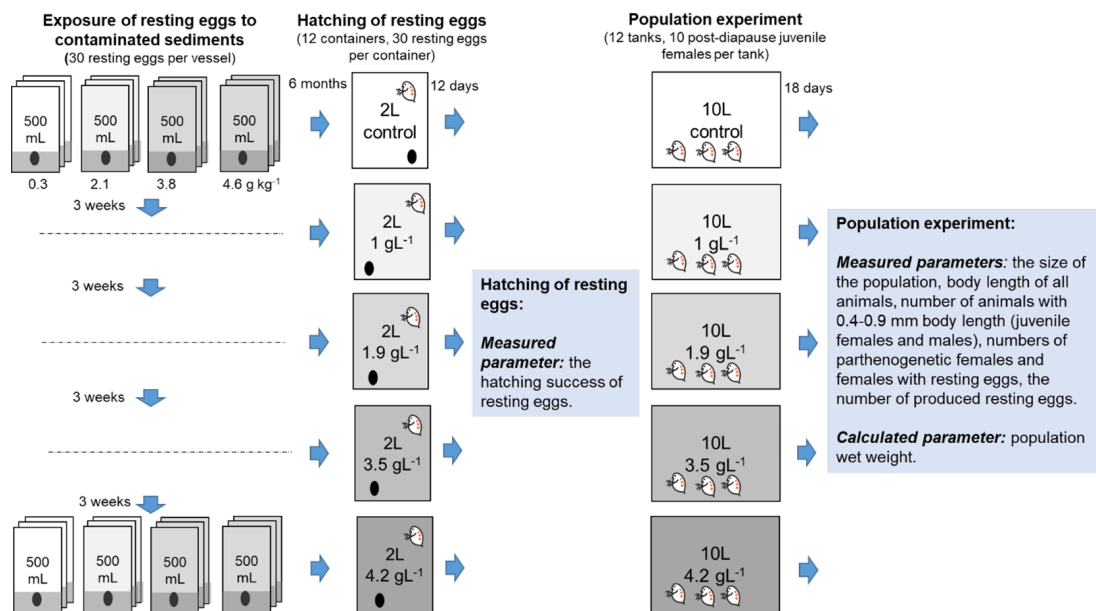


Fig. 1. Design of population experiments with *M. macrocopa* post-diapause females

by mixing the components in the required proportions. The peat, reduced to a powder by a knife-type grinder (5–10 %, dry weight), was moistened with deionized water and infused for 1–2 days to stabilize the pH and microbial component. Afterwards, kaolin (20 %, dry weight) and quartz sand (70–75 %, dry weight) were mixed into the peat suspension. After thorough mixing, the resulting mixture of components was moistened with deionized water, mixed to homogeneity and kept for 7 days under conditions later used as experimental soil storage conditions (darkness, temperature 4 °C). After that, the pH of the artificial sediments was measured (7.0±0.5). To increase the salinity in the artificial sediments, stock solutions of “Bionord” were prepared.

Saline water was added to the sediment with the concentrations of “Bionord” required to achieve the experimental concentrations of “Bionord” in the sediment: 2, 4, and 5 g kg<sup>-1</sup> (salinities 2.1, 3.8, and 4.6 g kg<sup>-1</sup>, respectively). The amount of saline water added to each sample of sediment was calculated to achieve sediment moisture content of 35–40 %. After thorough mixing, the sediment was stored for 24 hours. Then, samples were taken from each experimental sediment to control moisture content and salinity. To control salinity, elutriates were prepared, in which electrical conductivity was measured and the calculated salinity was determined. Each ephippium was carefully placed, using a pipette, into sediments to a depth of about 8–10 mm. All experimental vessels with resting eggs immersed in the sediment were kept in the darkness at 4 °C for 6 months.

After 6 months, resting eggs were removed from 12 storage vessels to 12 containers with 2 L of water. We performed five consecutive experiments: one with control water (calculated salinity 0.1 g L<sup>-1</sup>) and four with saline water (calculated salinities 1.0 g L<sup>-1</sup>, 1.9 g L<sup>-1</sup>, 3.5 g L<sup>-1</sup>,

and 4.2 g L<sup>-1</sup>). Resting eggs were reactivated at a constant temperature of 24 °C and a photoperiod of 16 hours light: 8 hours dark. The alga *Chlorella vulgaris* was added as food to the reactivation containers at a concentration of 200 thousand cells mL<sup>-1</sup>. The hatching of animals from resting eggs began on the second day of reactivation and was monitored over the following 12 days. The hatching success of resting eggs was determined as the number of hatched resting eggs/number of resting eggs in the reactivation container.

To start populations, 10 same-sized (0.5–0.6 mm) post-diapause females were selected from each container. Animals were placed in 12 tanks containing 10 L of tap water with the salinity corresponding to the salinity during the reactivation of resting eggs. *C. vulgaris* was added as food to the water at a concentration of 200 thousand cells mL<sup>-1</sup>. The tanks were kept in a climate room at a temperature of 21–23 °C and a constant photoperiod of 16 h light: 8 h dark. Every day, after thorough but gentle mixing of the water in the tank, the amount of *Chlorella* cells consumed by the population was measured. To determine the amount of the consumed *Chlorella* cells we subtracted the amount of measured cells in the tank (volume of water in the tank in mL \* measured *Chlorella* cell concentration per mL) from the initial amount of algal cells in the tank (volume of water in the tank in mL \* 200 thousand *Chlorella* cells mL<sup>-1</sup>). For the rapid determination of the algal cell concentrations in the samples, we used a previously obtained linear regression between the concentration of *C. vulgaris* cells in the sample determined by the direct microscopic count and the value of fluorescence of chlorophyll “a” measured at wavelength 470–665 with Fluorescence Multimodal Microplate Reader SpectraMax® M series (Molecular Devices SpectraMax M5, U.S.A.). Then, 1 liter of water from each tank

was pumped and filtered through plankton mesh (45 µm), animals were returned to the tank, and 1 L of water was added to the tank. Concentration of *Chlorella* cells was adjusted daily to be equal to 200 thousand cells mL<sup>-1</sup>.

Every third day of the experiment, animals filtered from 1 L of sampled water were not returned to the tank but fixed with 90 % ethanol to count the total number of animals and determine the length of all animals and the type of adult females – parthenogenetic ones or females bearing resting eggs.

For each observation date, we determined the following characteristics of the population in each tank: the size of the populations, calculated population wet weight, numbers of animals with 0.4–0.9 mm body length (juvenile females and males), and numbers of parthenogenetic females and females with resting eggs. The length of counted animals was measured under the light microscope (magnification: 16x). Population wet weight was calculated using the wet weight of animals as  $W=0.114L^{3.027}$ , where W is the animal wet weight (mg) and L is the animal's length (mm).

The experiment lasted 18 days. Samples of animals were taken on Days 3, 6, 9, 12, 15, and 18. At Day 18, the total number of resting eggs produced in each tank was counted.

#### *Statistical analysis*

The LC<sub>50</sub> values (the salinity resulting in survival of females hatched from resting eggs 50 % below the upper limit) in the acute toxicity test were determined in “drc” package for R (Ritz, Streibig, 2005). The differences between hatching success of 1-year-old and 1.5-year-old resting eggs were compared using Mann–Whitney U Test.

The effects of the hatching salinity and raising salinity on the life history traits of the females (life span, specific growth rate of juvenile

females, and the number of produced neonates) in the chronic test were estimated with two-way ANOVA. As the effect of hatching salinity on life history traits of post-diapause females was non-significant, we averaged the values of life history traits of females hatched in the control and the salinity treatments.

The effects of water and sediment salinity on hatching success of resting eggs exposed for 6 months to saline sediments and population characteristics were estimated with two-way ANOVA. The population size, the number of animals with 0.4–0.9 mm body length, the number of parthenogenetic females and females with resting eggs, the intensity of grazing on *Chlorella* cells in the treatments and control, and the total number of resting eggs produced in the treatments and control were compared with Post-hoc Fisher LSD test. All statistical calculations were performed in STATISTICA 8.0.

## **Results**

### *Acute and chronic tests with 1-year-old resting eggs and post-diapause offspring*

Hatching success of resting eggs in the 72-hour acute test was comparable in the control and the salinity treatments (Table 1). The proportion of survivor hatchlings from resting eggs depended on the salinity. The survival of animals abruptly decreased at salinity of 5.1 g L<sup>-1</sup> and was lower by a factor of 3.7 compared to the control. The lowest survival (3.8 %) was at the highest salinity – 5.9 g L<sup>-1</sup> (Table 1).

Except juvenile somatic growth rate, other life history traits (survival and the number of produced offspring) decreased with an increase in the salinity. The lifespans of females and the numbers of neonates produced by females in treatments with salinities (1.9 and 3.5 g L<sup>-1</sup>) were lower by a factor of 1.2–1.8 compared to the control (Fig. 2).

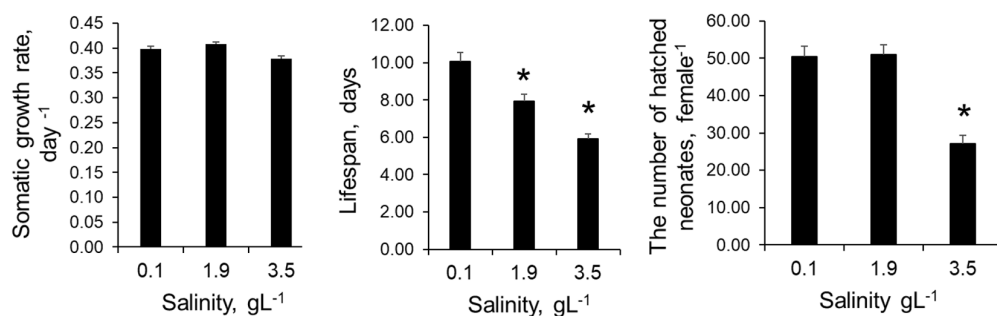


Fig. 2. The effect of the salinity on the life history traits of post-diapause females of *M. macrocopa*. Bars display the mean values  $\pm$  S.E. \*— the difference between control and treatment is significant at  $p < 0.0001$  (two-factor analysis of variance (ANOVA))

*Population experiments initiated with post-diapause females hatched from resting eggs after 6 months of exposure to saline sediments*

We observed only a slight, non-significant decrease in the hatching success of resting eggs of *M. macrocopa* after the 6-month exposure to saline sediments compared with the control sediments (Table 1). However, the time of exposure (6 months) had a significant effect on the hatching success of resting eggs. Hatching success of resting eggs after the 6-month exposure (71 %, 1.5-year-old resting eggs) was lower by a factor of 1.3 than the hatching success

of resting eggs in the acute test (95 %, 1-year-old resting eggs) (Table 1).

The development of populations strongly depended on the water salinity (Fig. 3). We observed the effect of salinity on all measured characteristics of the populations. Populations that developed in saline water had smaller size and wet weight; there were lower numbers of animals with 0.4–0.9 mm body length, parthenogenetic females, and females with resting eggs than in the control populations. The values of population characteristics in salinity treatments also tended to decrease with an increase in salinity (Fig. 3).

Table 1. The effect of salinity on the hatching success and survival of females of *Moina macrocopa* hatched from resting eggs in the 72-h acute test and hatching success of resting eggs of *M. macrocopa* exposed to the saline sediments for 6 months

Acute 72-hour test with 1-year-old resting eggs	Salinity, g L <sup>-1</sup>					
	0.1	1.9	3.5	4.2	5.1	5.9
Hatching success of resting eggs, %	98.3	98.3	93.5	96.7	95.0	88.3
Survival of hatchlings from resting eggs, %	84.8	94.9	88.9	74.1	22.8	3.8
LC <sub>50</sub> for survival of hatchlings (mean value $\pm$ SE)	4.7 $\pm$ 0.1					
Population experiment with 1.5-year-old resting eggs	The salinity of sediments during the 6 month exposure of resting eggs, g Kg <sup>-1</sup>					
	0.33	2.1	3.8	4.6		
Hatching success of resting eggs, % (mean value $\pm$ SE)	73.3 $\pm$ 2.1	73.7 $\pm$ 4.5	72.7 $\pm$ 2.8	64.8 $\pm$ 2.5		

Note: the difference between hatching success of 1-year-old and 1.5-year-old resting eggs is significant at  $p < 0.03$  (Mann-Whitney U Test).



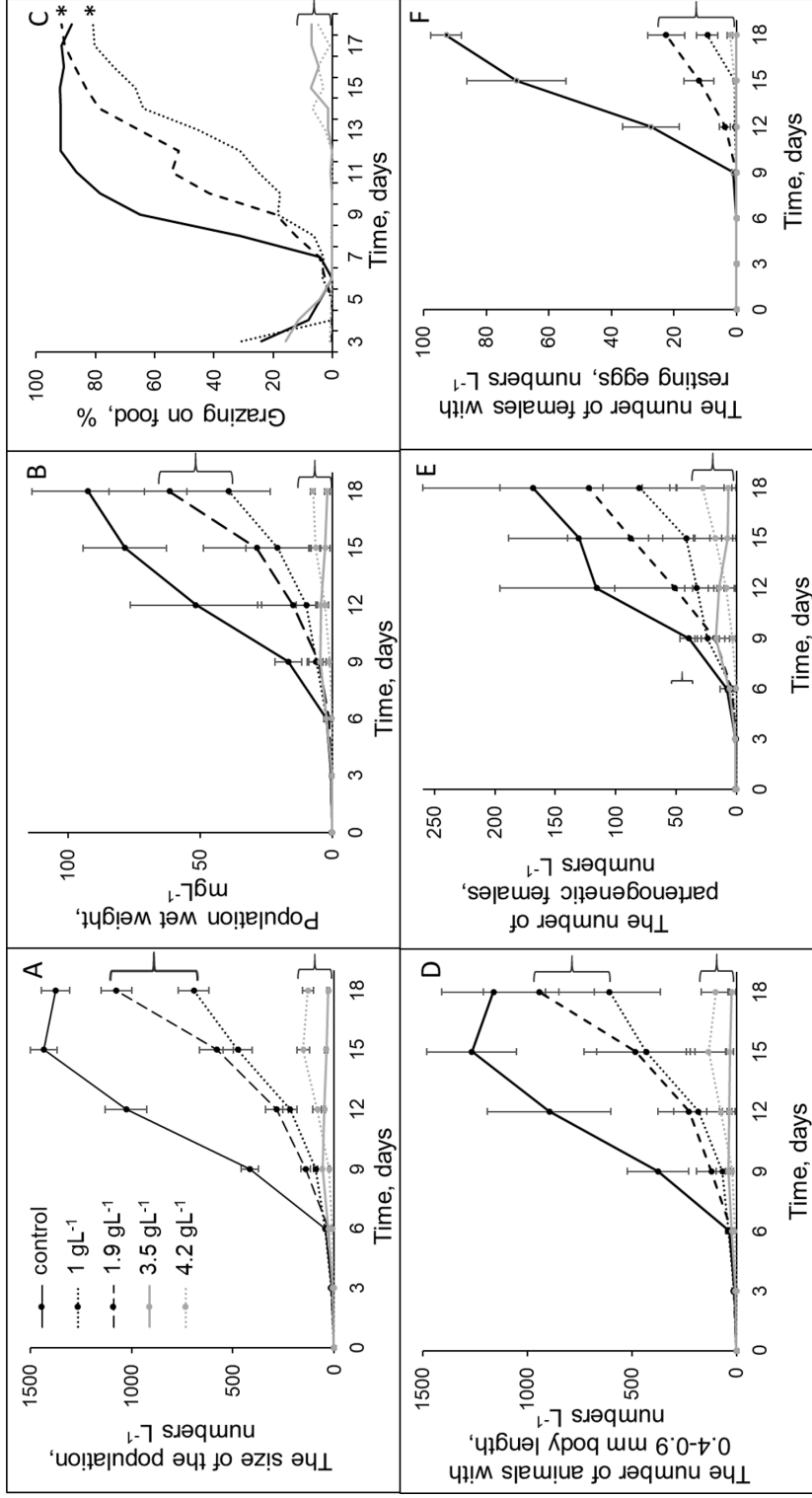


Fig. 3. Development of experimental populations of *M. macrocopa* initiated by post-diapause females hatched from resting eggs exposed for 6 months to saline sediments. The size of the population (A), population wet weight (B), the proportion of grazed food (C), the number of animals with 0.4–0.9 mm body length (D), the number of parthenogenetic females (E) and the number of females with resting eggs (F). Dots – mean values  $\pm$  S.E. A-F – the difference between control and treatments united by brackets is significant at  $p < 0.0001$ ; \* – the difference in intensity of grazing on food of populations affected by salinities 1 g L<sup>-1</sup> (Days 8–17 of the experiments) and 2 g L<sup>-1</sup> (Days 8–14 of the experiments) and the control population is significant at  $p < 0.02$  (Post-hoc Fisher LSD test; ANOVA)

Grazing of cladocerans on *Chlorella* cells, which were used as food, strongly depended on the salinity. At salinities of 1 and 2 g L<sup>-1</sup>, a dramatic decrease in the intensity of grazing of algal cells was observed compared to the control from the Day 8 to Day 14 of the experiments. At salinities of 3.5 and 4.2 g L<sup>-1</sup> practically no algal cells were grazed (Fig. 3).

Salinity had a negative effect on the ability of the *M. macrocopa* populations to produce resting eggs (ANOVA  $p < 0.001$ ). Even at a salinity of 1 g L<sup>-1</sup>, populations produced significantly fewer resting eggs than the control populations. At salinities of 3.5 and 4.2 g L<sup>-1</sup>, resting eggs were critically rare (Fig. 4).

## Discussion

### *Hatching success and survival of hatchlings*

Resting eggs are quite resistant to various toxicants and stressors (for example, Oskina et al., 2019; Zadereev et al., 2017), including salinity. Previously, we demonstrated that hatching success of resting eggs of *M. macrocopa* was sharply reduced at a salinity above 8.5 g L<sup>-1</sup> (Lopatina et al., 2021); for other cladocerans,

higher threshold salinities were detected (above 7.6–32 g L<sup>-1</sup>) (Mungenge et al., 2024; Mabidi et al., 2018; Nielsen et al., 2012; Santangelo et al., 2014). The present study confirmed this observation, as hatching success of resting eggs was not significantly reduced by their exposure to the tested salinities (up to 6 g L<sup>-1</sup> in the acute test and 4.6 g kg<sup>-1</sup> in the 6-month exposure to sediments test).

However, we demonstrated the effect of lower salinity values (within the range of 0.1–5.9 g L<sup>-1</sup>) on the hatching success of eggs of different ages. Previously, we compared hatching success of 4-year-old and 1-year-old resting eggs of *M. macrocopa* and observed a 12.5 % per year decline of hatching success (Lopatina et al., 2024). In the experiment conducted in the current study, we also observed the reduction in the hatching success of resting eggs that can be related to age. The hatching success of 1.5-year-old resting eggs (71 %) was 25 % lower than the hatching success of 1-year-old eggs (95 %). We do not know whether this decline is related to the age of resting eggs or it is associated with the exposure of eggs to saline sediments.

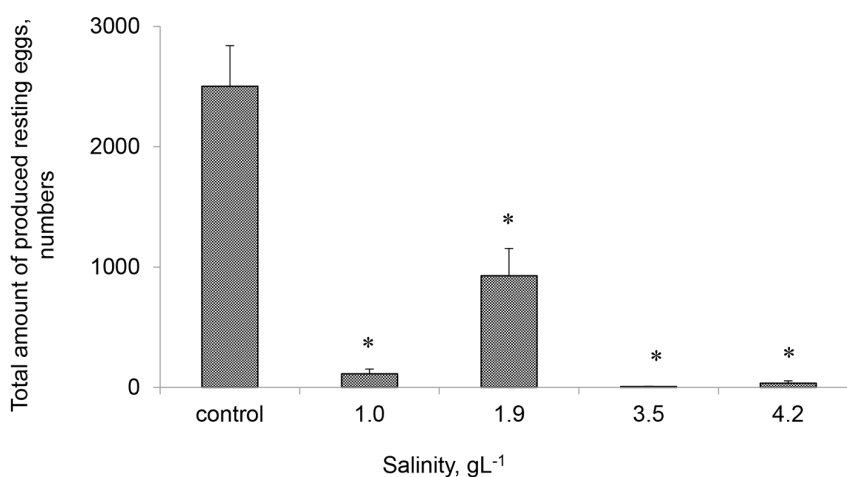


Fig. 4. The effect of salinity on the production of resting eggs by populations of *M. macrocopa* initiated by post-diapause females hatched from resting eggs exposed to saline sediments. Bars – mean values  $\pm$  S.E. \* – the difference between control and treatment is significant at  $p < 0.0001$  (Post-hoc Fisher LSD test: ANOVA)

*Individual performance*

The effect of salinity on freshwater species is generally observed starting from the critical salinity (5–8 ‰) (Khlebovich, 1974). Previously, we studied the effect of salinity on *M. macrocopa* and observed that lifespan, growth rate, and fecundity were dramatically lower when the salinity was above the threshold of 5 g L<sup>-1</sup> and noted that the data on values of NaCl that had an effect on life history traits of other cladoceran species (4.5–6.6 g L<sup>-1</sup>) were comparable with our data (Lopatina et al., 2021). In this study, we investigated the effect of salinity that was close to but not exceeding critical values for the survival of animals (Lopatina et al., 2021). In the chronic test with individual animals, the negative effect of salinity was observed at 3.5 g L<sup>-1</sup>. While the rate of body length growth of juvenile females was not affected, the lifespan and fecundity of parthenogenetic females were reduced, which may be important for population development.

*Population performance*

In population experiments, salinity above 3.5 g L<sup>-1</sup> dramatically reduced the size of the population, the population wet weight, the intensity of grazing, the numbers of small animals and females, and the number of produced resting eggs. However, a significant effect of salinity on the development of populations was observed at lower values (starting from 1.0 g L<sup>-1</sup>). The difference between values of salinity critical for the development of individuals (3.5 g L<sup>-1</sup>) and populations (1.0 g L<sup>-1</sup>) demonstrates that the population is more vulnerable to salinity fluctuations.

Similar results were observed for two cladocerans, *Daphnia magna* and *M. macrocopa* (Huang et al., 2022): their populations were more sensitive to salinity stress than individuals. On the other hand, Sarma et al. (2006) studied the effect of different salinities (from fresh water

to 4.5 g L<sup>-1</sup>) on the population dynamics of ten freshwater rotifer and cladoceran species. The development of populations of all tested species was adversely affected by 1.5–3.0 g L<sup>-1</sup> NaCl. However, salinity of 1 g L<sup>-1</sup> had little effect on the population growth of *M. macrocopa*, which was more resistant to NaCl than other tested Cladocera species (Sarma et al., 2006). Previously, we demonstrated that depending on the pre-adaptation, similar species could differ in their response to the salinity (Zadereev et al., 2022). We assume that populations used in this study and the study that demonstrated higher salinity tolerance of *M. macrocopa* (Sarma et al., 2006) had their origins in habitats with different salinities.

Field data on the effect of salinity on the abundance of *Daphnia magna*, *D. pulex*, *D. longispina*, *Simocephalus exspinosus*, and *Moina brachiata* demonstrated that species richness of Cladocera was highest in those seasons and lakes where salinity was the lowest and in those seasons and lakes where cladoceran abundance was highest (Green et al., 2005). For *Moina micrura*, salinity had direct negative physiological effects on individuals, but indirectly it benefited the population development by reduced invertebrate predation and interspecific competition (Santangelo et al., 2008).

In the current study, the exposure to moderate salinity (1 and 1.9 g L<sup>-1</sup>) resulted in populations sizes that were significantly larger than the sizes of the populations affected by higher salinities (3.5 and 4.2 g L<sup>-1</sup>). However at all salinities studied in this work, populations produced comparable numbers of resting eggs, which were considerably lower compared to the control populations. As we assumed that the survival and success of cladoceran populations with cyclic reproduction critically depends on their ability to produce resting eggs, we focused our study on the evaluation of that reproductive

parameter. At salinity of  $1.0 \text{ g L}^{-1}$ , we observed a critical reduction in the numbers of females with resting eggs and the number of produced resting eggs. Thus, our results support the well-recognized concern about detrimental consequences of freshwater salinization for inland waters (Cunillera-Montcusí et al., 2022) and call into question the ability of resting egg banks to serve as a refuge against temporary salinization.

#### *Relevance of the study to natural habitats*

*M. macrocopa* (Straus, 1819) is widespread in Europe, North Africa, Middle East, Eastern Asia, and North America. The basic type of the habitat preferred by *M. macrocopa* is highly eutrophic ponds with abundant food supply (Petrusek, 2002). *M. macrocopa* was also recently recorded in South America (Vignatti et al., 2013). *M. macrocopa* prefers warm waters. It is expected that this stress-tolerant species will expand its range and invade temporary waters globally because of climate change (Macêdo et al., 2022).

As *M. macrocopa*, like most cladocerans, reproduces by the cyclic parthenogenesis and inhabits warm and food-rich habitats, its populations grow rapidly. After food is depleted or population reaches a high density, it switches to sexual reproduction (Zadereev, Gubanov, 1996). Sexual eggs and ephippia are produced by freshly matured females (Goulden, 1968). The sexual females of *M. macrocopa* continue to either produce ephippia throughout their life or irreversibly switch to parthenogenetic reproduction (Petrusek, 2002). Ephippia production ensures the survival of the population, especially in such ephemeral habitats as drying ponds and lakes (Nandini, Sarma, 2019).

Even though our single species/population study is mostly relevant for *M. macrocopa* inhabiting freshwater ponds and lakes, we consider it has wider applications. Salinity tolerance is a relatively conservative physiological trait, as it depends on the osmotic regulation type (hyperosmotic, hypoosmotic or amphiosmotic) (Smirnov, 2017). Few species (e.g. *Moina mongolica*) can use more than one osmotic regulation type to live in habitats with highly variable salinity (Aladin, 1982). It was demonstrated that with both sudden and gradual increases in salinity above  $1 \text{ g L}^{-1}$ , freshwater taxa are progressively lost and communities become less diverse in outdoor mesocosms with natural zooplankton communities (Nielsen et al., 2008). Thus, for the majority of freshwater Cladocera species, especially those inhabiting temporary lakes and ponds, salinization may pose similar “hatch and die” threat as discovered in our study for *M. macrocopa*.

#### **Conclusion**

We observed a decreasing sequence of salinities critical for the hatching of resting eggs: survival of hatchlings: individual performance of post-diapause females: performance of populations initiated by post-diapause females – above  $8 \text{ g L}^{-1}$ :  $5 \text{ g L}^{-1}$ :  $3.5 \text{ g L}^{-1}$ :  $1 \text{ g L}^{-1}$ . We conclude that salinity values critical for the long-term survival and development of zooplankton populations should be defined based on long-term population experiments. The dissolved ions at the concentrations that do not prevent hatching of resting eggs reduce the ability of *M. macrocopa* and most probably other freshwater zooplankton populations to replenish the bank of resting eggs. Thus, in the long term, this will prevent the re-establishment of population from the depleted egg bank.

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