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Threshold Concentrations of Cations in the Water Necessary for Maintaining the Ionic Balance Between Organism of *Chironomus balatonicus* Devai et al. Larvae and Environment

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Abstract—Threshold concentrations of sodium, potassium, calcium, and magnesium in water determining the borders of the *Chironomus balatonicus* range in waterbodies are 0.6–0.62, 0.11–0.12, 0.071–0.073, and 0.022–0.028 mmol/L, respectively. In the freshwater section of the Curonian Lagoon of the Baltic Sea and other waterbodies with weakly mineralized water where the content of ions is lower than the threshold values, larval *Ch. balatonicus* cannot dwell because of the impossibility of maintaining the ionic balance between the organism and environment. The threshold concentrations and rates of loss of ions from an organism in various species of aquatic organisms are comparatively analyzed.

Keywords: Chironomus balatonicus, range, aquatic organisms, sodium, potassium, calcium, magnesium

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INTRODUCTION

In the Curonian Lagoon of the Baltic Sea, a gradient from fresh to brackish water of certain salinity is well expressed. The larvae of *Chironomus balatonicus* Devai et al. dwell in the brackish water but are absent from the freshwater areas [5]. The reason for such a different distribution of the larvae is still unclear.

Water mineral content is a very important ecological factor strongly influencing distribution, tolerance, growth and many other physiological processes in the aquatic biota. The ions of Na, K, Ca, and Mg are essential elements necessary for the vital activity of animals and plants. These salts enter the aquatic organisms not only with food, but also via transport directly from water [1, 8–12].

The contents of ions in the hemolymph in the larvae of various chironomid species are considerably higher than in the environment [7]. This leads to the diffusion of electrolytes from organism to environments. The active absorption of ions from water through the body cover compensates the losses of ions [1, 4]. As a result, the body content of ions is maintained at constant levels. Minimal concentrations of certain ions in the environment at which extreme capabilities of pumps to fully compensate losses of electrolytes from an organism may be treated as “threshold concentrations.” If the concentrations of waterborne ions are lower than the threshold values, the transporting systems fail to fully compensate the losses of ions that result in the death of an organism due to desalination. This is why those minimal contents of various ions in the water at which maintaining the ionic balance between organism and environment is still possible determine the borders of a species range in waterbodies (i.e., the state of full compensation of losses of ions due to their transport through the covers of chironomids).

The goal of the present paper is to determine the threshold concentrations of Na, K, Ca, and Mg in water necessary for maintaining of ionic balance between the organism of *Chironomus balatonicus* larvae and the environment.

MATERIALS AND METHODS

The larvae of *Ch. balatonicus* living in the Vistula Bay of the Baltic Sea were studied. The larvae were sampled on June 16, 2015, in the central part of the bay using a small rectangular drag (opening of 40 × 20 cm). The sampling was performed from a vessel at depth of 4.5 m. The initial location of sampling was at the point of 54°30′91″ N, 19°51′62″ E; the final one was at 54°31′34″ N, 19°52′32″ E. The chironomids were shipped to the laboratory of Marine ecology of the Atlantic branch of the Shirshov Institute of Oceanology, Russian Academy of Sciences, where they were

Table 1. Rates of losses of cations to distilled water from organisms of various aquatic animals

Species	Losses of ions form organisms, mmol per 100 g wet weight per 1 h				Reference
	Na	K	Ca	Mg	
Chironomus balatonicus Devai et al.	37.7	6.5	5	1.2	Original data
C. salinarius Kieffer	6–8	–	–	–	[4]
C. riparius Meigen	0.02–0.04	–	–	–	[4]
C. agilis Shobanov et Djom	0.02–0.04	–	–	–	[4]
Rutilus rutilus L.	0.07 ± 0.02	0.01 ± 0.002	0.02 ± 0.01	0.003 ± 0.001	[6]
Perca fluviatilis L.	0.1 ± 0.01	0.02 ± 0.01	0.004 ± 0.0006	0.0011 ± 0.0001	[6]
Sphaerium suecicum Lamarck	0.012 ± 0.002	0.0028 ± 0.0011	0.0108 ± 0.0014	–	[2]
Lymnaea peregra Müller	0.012 ± 0.001	0.0017 ± 0.0008	0.029 ± 0.003	–	[2]
Lithoglyphus naticoides Pfeiffer	0.014 ± 0.0003	0.005 ± 0.0008	0.04 ± 0.007	0.008 ± 0.0009	[6]
Astacus astacus L.	0.014 ± 0.003	0.0021 ± 0.0002	0.02 ± 0.01	0.005 ± 0.001	[6]
A. pallipes Lereboullet	0.015 ± 0.002	–	–	–	[14]
Potamon niloticus Edw.	0.099 ± 0.015	0.0062 ± 0.0006	–	–	[13]

acclimated to new conditions for 5 days. Following this, the larvae were washed with distilled water and groups of eight individuals each were placed in five glass containers with 10 mL of distilled water. A total of 40 larvae were examined. After the larvae were placed into containers, 5 mL of water was taken from the containers to determine the initial content of cations. Following this, 5 mL of distilled water was added to containers. One day later, 5 mL of water was sampled from each container. After this, 75 mL of distilled water was added to each container in three steps for 8 h, giving a final volume of 80 mL. Further on, 5 mL of water was sampled day after a day without the addition of distilled water. Prior to the experiments, distilled water was aerated for 2 to 3 days to neutralize acid reaction and to reach pH 6.5. The water was aerated during the whole span of the experiment. The water temperature during the study fluctuated within an interval of 23–24°C. The contents of ions were measured on a Saturn (OKBA NPO Khimavtomatika, Severodonetsk, Ukraine) atomic absorption photometer. The concentrations of cations in water samples were expressed as mmol/L. The data are given as means and standard errors of means.

RESULTS

Placing *Ch. balatonicus* into distilled water stimulated pupation in a certain amount of specimens. En course of the experiment, 19 pupae were formed, 8 of which reaching the stage of imago while the rest died. Thirty minutes after placing the larvae in distilled water, the concentrations of Na, K, Ca, and Mg were 0.61, 0.11, 0.08 and 0.02 mmol/L, respectively (Fig. 1). Due to additions of distilled water into the experimental containers, the concentrations of waterborne ions was decreased by a factor of two. One day later, the contents of electrolytes in water increased due to the loss of ions from the larvae and became higher than the initial values: Na, K, Ca, and Mg, 0.72, 0.2, 0.09, and 0.03 mmol/L, respectively. Immediately after collecting the daily samples, owing to three-step additions of distilled water in 1 day (75 mL of distilled water was added to 5 mL of the initial water), the concentrations of Na, K, Ca, and Mg ions in the experimental medium decreased to 0.045, 0.0125, 0.0056, and 0.0019 mmol/L, respectively. By the end of day two of the experiment, the concentrations of these ions rose again to the initial values recorded at the beginning of the experiment due to a loss of ions from larvae.

The rates of losses of Na, K, Ca, and Mg from *Ch. balatonicus* considerably differed (Table 1). The highest loss was a characteristic of Na: 5.8, 7.6, and 31.7 times higher than of K, Ca, and Mg, respectively.

By the second to fourth days of the experiment, the concentrations of waterborne cations was maintained within a narrow diapason of concentrations (in Fig. 1 this diapason is marked by solid lines relative to the abscissa). After 4 days of the experiment, the contents of ions in water were steadily increasing, indicating the inability of the larvae to maintain stable ionic balance.

DISCUSSION

During the first to second days after placing various aquatic animals in distilled water, the concentrations of ions in the water increased, with certain rates indicating a loss of these ions from the animal organisms (Table 1). Later, the contents of cations in distilled water stabilize at some levels remaining within a narrow zone of concentrations during the whole span of the experiment.

After placing *Ch. balatonicus* larvae in distilled water, a sharp increase in the concentrations of various

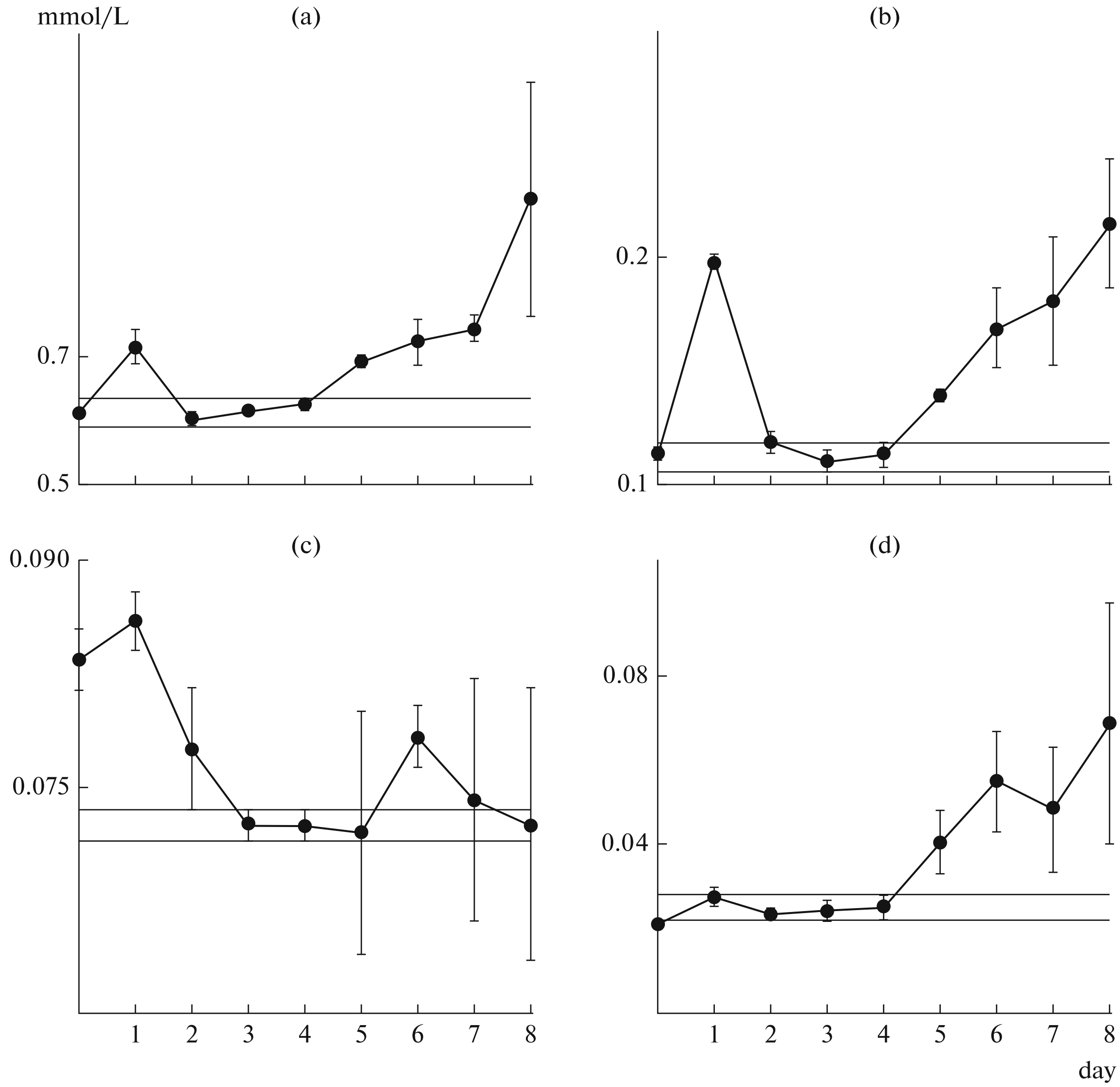

Fig. 1. Dynamics of content (mmol/L) of cations Na (a), K (b), Ca (c), and Mg (d) in the medium following the placement of *Chironomus balatonicus* larvae into distilled water. The threshold concentrations of electrolytes in water at which the ionic balance between larvae and the environment is achieved are marked by horizontal lines.

ions took place in 1 day (Fig. 1). Due to the addition of distilled water, the concentrations of ions in the experimental medium dropped to minimal values that remained within narrow zone of concentrations during the following 2–4 days.

Stabile levels of concentrations of ions in the water during the trial indicate an ionic balance between the organism and environment: the state of full compensation of losses of ions by their transport. These concentrations of ions are the threshold (extreme) concentrations necessary for the survival of *Ch. balatonicus* larvae. At concentrations of electrolytes in water below the threshold levels, the larvae would die due to desalination. At threshold concentrations of electrolytes in the environment, an organism is able only to survive because growth and development demand additional intake of ions. Hence, the dwelling of *Ch. balatonicus* larvae is only possible in waterbodies where the concentrations of various ions in the water are above the threshold values.

At threshold concentrations, the larvae of *Ch. balatonicus* are able to maintain its ionic balance with the environment in a period of 3 days (Fig. 1). Then temporal increase in concentrations of ions in the medium takes place. This indicates the desalination of larvae due to an inability to fully compensate the losses of ions from the organism. Larvae of *Ch. balatonicus* die due to desalination in 8 days.

In experiments with a duration of to ≥2 weeks, other studied species of aquatic animals are able to survive and maintain an ionic balance between the organism and environment. Analysis shows that, com-

Table 2. Concentrations of cations in the water of various areas in the Vistula and Curonian bays of the Baltic Sea

Date	Geographic coordinates		Concentrations of ions, mmol/L			
	N	E	Na	K	Ca	Mg
	Vistula Bay					
2011, 17.X	54°40′50″	20°20′00″	40.9	0.86	1.20	0.92
	54°39′20″	20°13′30″	47.8	0.83	1.08	1.09
	54°37′60″	20°07′20″	57.4	1.15	1.32	1.33
	54°40′80″	20°01′75″	57.4	1.09	1.21	1.37
	54°34′80″	20°04′30″	98.3	2.31	1.68	2.41
	54°34′40″	19°54′80″	67.1	1.24	1.20	1.62
	54°31′50″	19°51′70″	62.6	1.15	1.07	1.50
	54°36′88″	19°55′52″	67.8	1.15	1.08	1.59
	54°37′50″	20°01′50″	67.8	1.19	1.20	1.62
2013, VI						
8	54°39′53″	19°56′21″	40.9	0.74	5.06	4.77
13			35.7	0.64	3.37	4.17
17			31.3	0.54	3.56	4.32
	Curonian Bay					
8	55°13′47″	20°55′22″	0.73	0.11	1.15	0.57
8	55°01′34″	20°38′09″	0.57	0.08	1.46	0.96
8	54°58′48″	20°33′58″	0.50	0.08	1.74	0.62

pared to other studied aquatic species, *Ch. balatonicus* larvae in similar conditions (experiments in distilled water) are unable to maintain an ionic balance with the environment for a long time. In addition, such conditions accelerate the switch of some specimens to other stages of development.

Such a response of *Ch. balatonicus* larvae to exposure in distilled water may relate to a higher permeability of body covers determining the excessive rate of loss of ions from an organism (Table 1). Compared to other chironomid species, in *Ch. balatonicus* larvae the rate of loss of Na ions was on average five times higher than in the marine species *Ch. salinarius* living in the White Sea and 1267 times higher than in the larvae of freshwater *Ch. riparius* and *Ch. agilis*. In other freshwater animals, the rates of losses of Na, K, Ca, and Mg ions on average are 380–543, 6–3824, 59–1250, and 20–1091 times lower than in *Ch. balatonicus* (Table 1). Aquatic organisms compensate the loss of ions from an organism by their transport from an environment that is accompanied by expenditures of energy. Consequently, when compared to other aquatic species, the larvae of *Ch. balatonicus* use a system of ionic transport that is less efficient and more demanding of energy to compensate for extremely high loses of electrolytes to maintain ionic homeostasis. Presumably, the inability of *Ch. balatonicus* larvae to maintain ionic balance with environment at threshold concentrations relates to a deficiency (fast exhaustion) of energetic resources.

The levels of Na, K, Ca and Mg in the water of Vistula Bay inhabited by *Ch. balatonicus* larvae (Table 2) are higher than the threshold values revealed for this species (Table 3) on average by 59, 6, 61, and 184 times, respectively. It is likely that in this waterbody *Ch. balatonicus* larvae face no problems with maintaining an ionic balance with environment.

In terms of water salinity, the Curonian Lagoon splits into two zones. The northern part of the lagoon is subject to the effect of brackish water of the Baltic Sea: wind-driven seawater enters the lagoon via a narrow strait near Klaipeda and mixes with freshwater. As a result, a certain gradient of salinity and levels of Na and K ions is present in the lagoon. In the northern part of the Curonian Lagoon the concentrations of waterborne ions are higher than the threshold values, which enables the larvae to maintain an osmotic and ionic balance with environment. The larvae of *Ch. balatonicus* occur only in the brackish water of the northern part of the lagoon, from Klaipeda to the village of Juodkrante [5].

In the central part of the bay (near the village of Morskoy), the levels of monovalent ions in water approach threshold values, declining in the southward direction (Table 2). This is why the larvae of *Ch. balatonicus*, at Na and K concentrations below the threshold, are unable to maintain an ionic balance between the organism and environment. This is why *Ch. balatonicus* is unable to establish itself in the freshwater areas of the Curonian Lagoon.

Table 3. Threshold environmental concentrations of cations for various species of aquatic biota

Species	Threshold environmental concentrations of cations, mmol/L				Literature source
	Na	K	Ca	Mg	
Chironomus balatonicus Devai et al.	0.6–0.62	0.11–0.12	0.071–0.073	0.022–0.028	Data of the Authors
Ch. salinarius Kieffer	0.4–0.6	–	–	–	[4]
Ch. riparius Meigen	0.2	–	–	–	[4]
Ch. entis Shobanov	0.1	–	–	–	[4]
Ch. plumosus L.	0.07–0.08	–	–	–	[4]
Ch. agalis Shobanov et Djom	0.04	–	–	–	[4]
Ch. borokensis Kerkis et al.	0.04	–	–	–	[4]
Rutilus rutilus L.	0.015–0.019	0.012–0.015	0.006–0.009	0.002–0.003	[6]
Perca fluviatilis L.	0.0045–0.0051	0.0099–0.0112	0.0005–0.0007	0.0004–0.0005	[6]
Carassius auratus L.	0.02–0.03	0.008–0.015	0.05–0.06	0.05	[3]
Astacus astacus L.	0.0087–0.0174	0.0046–0.0087	0.0105–0.0222	0.0012–0.0033	[6]
Lithoglyphus naticoides Pfeiffer	0.0024–0.0047	0.0014–0.0025	0.025–0.038	0.0023–0.0032	[6]
Dreissena polymorpha Pallas	0.07	0.0015	0.30	0.01	[6]
Sphaerium suecicum Lamarck	0.10	0.0049	0.05	–	[2]
Spirogyra sp. Link	0.003–0.007	0.002–0.003	0.0017–0.0022	0.0012–0.0018	[6]
Elodea canadensis Michaux	0.0014–0.0021	0.00013–0.00024	0.12–0.16	0.0056–0.0075	[6]

The population of *Ch. balatonicus* occurs sporadically in Lake Balaton [15]. Presumably, the population of this species in the lake lives at the border of the range and the fluctuation of environmental conditions in some years may cross the borders of the tolerance zone, resulting in a lack of species generations. The data of the present study suggest that a lack of generations of *Ch. balatonicus* in Lake Balaton may relate to the periods when concentrations of waterborne Na and K are insufficient for survival, development, and growth. Further studies are necessary to elucidate this issue.

The efficiency of the establishment of aquatic biota in freshwaters is assessed by the values of threshold concentrations: the lower these values are, the better the ability of an organism to survive in the low-mineralized water is (Table 3). In regards to the decreasing efficiency of ionic regulations (i.e., increase in threshold concentrations) relative to sodium ion, the descending order of the studied species is as follows: *Elodea canadensis* → *Lithoglyphus naticoides* → *Spirogyra* sp. → *Perca fluviatilis* → *Astacus astacus* → *Rutilus rutilus* → *Carassius auratus* → *Chironomus agalis*, *Ch. borokensis* → *Dreissena polymorpha*, *Ch. plumosus* → *Spharerium suecicum*, *Chironomus entis* → *Ch. salinarius* → *Ch. balatonicus*. In many waterbodies worldwide, the concentrations of Na ions ranges 0.06–0.42 mmol/L. The analysis shows that, in freshwater waterbodies with such a content of Na ions, two chironomid species, *Ch. salinarius* and *Ch. balatonicus*, would not survive and develop due to their inability to maintain a balance between the organism and environment.

In regards to a decreasing ability to maintain the potassium balance between organism and water, the order of studied species is as follows: *Elodea canadensis* → *Lithoglyphus naticoides* → *Dreissena polymorpha* → *Spirogyra* sp. → *Spharerium suecicum* → *Astacus astacus* → *Carassius auratus* → *Perca fluviatilis* → *Rutilus rutilus* → *Chironomus balatonicus*. In most freshwater bodies, the concentration of K ions fluctuates within the limits of 0.008–0.079 mmol/L. Only for *Ch. balatonicus* are the threshold concentrations of K higher than the concentrations of this ion in freshwater. In such waterbodies, *Ch. balatonicus* larvae cannot live successfully due to their inability to maintain a potassium balance between the organism and environment. Elodea possesses the best ability to maintain a potassium balance with the environment. It is likely that such an ability enables this species to survive and establish itself in small ponds, where the level of waterborne potassium may decrease to an extremely low value of 0.0017 mmol/L due to the mass development of algae [6].

For the studied species, the increasing order of threshold concentration of calcium is as follows: *Perca fluviatilis* → *Spirogyra* sp. → *Rutilus rutilus* → *Astacus astacus* → *Lithoglyphus naticoides* → *Sphaerium suecicum* → *Carassius auratus* → *Chironomus balatonicus* → *Elodea canadensis* → *Dreissena polymorpha*. Perch exhibits the best ability to remove calcium from the environment. The threshold concentrations of waterborne Ca ions for the crucian carp and fingernail clam do not differ, but are considerably higher than in roach and crayfish. Elodea and, especially, zebra mussel have the highest threshold concentrations of Ca ions in water. The larvae of *Chironomus balatonicus* would not be able to maintain a calcium balance with the environment in those waterbodies where contents of Ca ions in waters <0.067–0.073 mmol/L. Waterbodies with such a low level of calcium ion are most common in northern latitudes.

The threshold concentrations of magnesium for the studied aquatic species rise in the following order: *Perca fluviatilis* → *Spirogyra* sp. → *Astacus astacus* → *Rutilus rutilus* → *Lithoglyphus naticoides* → *Elodea canadensis* → *Dreissena polymorpha* → *Chironomus balatonicus* → *Carassius auratus*. The threshold concentrations of Mg ion in water necessary to maintain a balance between *Dreissena polymorpha*, *Chironomus balatonicus*, and *Carassius auratus* and the environment are close to each other and are an order higher than those ones for other species of freshwater animals (Table 3). No freshwater waterbodies where the content of Mg ions in water is lower than the threshold values for studied species were found [2–4, 6].

Thus, the permeability of body covers of *Chironomus balatonicus* larvae and threshold concentrations of Na and K in water necessary for maintaining an ionic balance with the environment are higher than in other studied species of aquatic animals.

Threshold concentrations of waterborne cations reflect environmental conditions in which the initial maternal population of *Ch. balatonicus* was formed. If this species may have dwelled for some time in conditions of low water mineralization, it should preserve the ability to adapt to such an environment. Living in a highly mineralized environment, aquatic species are unable to gain adaptive capabilities to survive in low-mineralized water.

CONCLUSIONS

Threshold concentrations of waterborne Na, K, Ca, and Mg determining the borders of *Ch. balatonicus* range in waterbodies are as follows: 0.6–0.62, 0.11–0.12, 0.071–0.073, and 0.022–0.028 mmol/L, respectively. The contents of Na and K ions in the freshwater areas of the Curonian Bay and other low-mineralized waterbodies are insufficient for maintaining an ionic balance between *Ch. balatonicus* and the environment, which determines conditions inappropriate for living for this species. The data on threshold concentrations of ions in the environment allow for forecasting the borders in which various species (including invasive species) can spread in fresh waterbodies, the conditions of formation of the initial maternal population of a species, and assessing the extent of the effects of decreasing concentrations of electrolytes in water owing to floods and other reasons on aquatic biota.

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