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TOLERANCE OF FRESHWATER BIVALVE *Cyanocyclas brasiliana* (Deshayes, 1854) TO THE VARIATION OF SALINITY IN LABORATORY CONDITIONS

Tolerância do bivalve dulcícola *Cyanocyclas brasiliana* (Deshayes, 1854) à variação de salinidade em condições de laboratório

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ABSTRACT

The species *Cyanocyclas brasiliana* (Deshayes, 1854) is endemic to South America and occurs in northern Brazil, in the state of Amazonas, Pará and Piauí. The present study was carried out in the Parnaíba River estuary, Piauí, Brazil. The objective of this study was to verify the tolerance limit and survival of the freshwater bivalve *Cyanocyclas brasiliana* submitted to an increasing salinity gradient under laboratory conditions. The specimens (19.49 to 26.47 mm) were kept in containers with 2 liters of water with constant aeration and at a density of 2.5 animals per liter. 7 treatments (salinities of 0, 1, 2, 3, 4, 5 and 6) were performed with 5 replicates per treatment. In the first 36 hours there was no death of any individual; mortality started after 48 hours at 3, 4, 5 and 6‰; it was more significant from 72 to 96 hours for treatments ranging from 2 to 6‰ and every specimen for treatments of 2 and 3‰ died from 120 to 144 hours. LC50 ranges from 3.1 at 72 h and 3.3 at 84 h, so the limiting average salinity was 3.2‰ and this species is characterized as stenohaline. Lethal death time (LT50) was: 81.8, 82.3, 78.5, 61.1 and 63.5 h for salinities of 2, 3, 4, 5 and 6‰ respectively. This species does not support great variations in salinity under experimental conditions, being well adapted to survive in salinities between 0 and 1.

Keywords: endemic, mortality, survival.

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RESUMO

A espécie Cyanocyclas brasiliana *(Deshayes, 1854) é endêmica da América do Sul e ocorre no norte do Brasil, nos estados do Amazonas, Pará e Piauí. O presente estudo foi realizado no estuário do Rio Parnaíba, Piauí, Brasil. O limite de tolerância e a sobrevivência do bivalve de água doce* Cyanocyclas brasiliana*, submetido a um gradiente de salinidade crescente, foi avaliado em condições de laboratório. Os espécimes (19,49 a 26,47 mm) foram mantidos em recipientes com 2 litros de água com aeração constante e densidade de 2,5 animais por litro. Foram realizados 7 tratamentos (salinidades de 0, 1, 2, 3, 4, 5 e 6) com 5 repetições por tratamento. Nas primeiras 36 horas não houve óbito de nenhum indivíduo; a mortalidade iniciou após 48 horas em 3, 4, 5 e 6‰; foi mais significativo de 72 a 96 horas para tratamentos variando de 2 a 6‰ e todos os espécimes para tratamentos de 2 e 3‰ morreram de 120 a 144 horas. A CL50 varia de 3,1 às 72 h e 3,3 às 84 h, portanto a salinidade média limitante foi de 3,2‰ e essa espécie é caracterizada como estenohalina. O tempo letal de morte (LT50) foi: 81,8; 82,3; 78,5; 61,1 e 63,5 h para salinidades de 2, 3, 4, 5 e 6‰, respectivamente. Essa espécie não suporta grandes variações de salinidade em condições experimentais, estando bem adaptada para sobreviver em salinidades entre 0 e 1.*

Palavras-chave*: endemia, mortalidade, sobrevivência.*

INTRODUCTION

The estuary can be defined as an aquatic ecosystem in which the waters of a river mix with marine waters, producing measurable gradients of salinity (Tundisi & Tundisi, 2008). The effect of tides and variable salinity are factors that make the dynamics of estuarine environments quite complex, having effects on the structure of the biological communities that colonize these ecosystems. In fact, salinity is an abiotic factor key in estuaries, changing daily, seasonally and annually, in which the precipitation/evaporation/tide relationship changes (Tundisi & Tundisi, 2008). The effect of tidal variations on the shallow areas under their influence, where periods of full water coverage and exposure of the substrate, as a result of the organisms living there, alternate (Lima *et al*., 2010).

The estuarine organisms are always susceptible to salinity variations, being a fundamental characteristic that organisms living in this habitat present physiological adaptations in order to have great tolerances to this environmental condition (Odum, 1961). Due to the salinity variation, the animals have two physiological strategies: osmoconformation and osmoregulation. Osmoconformer animals alter the osmotic concentration of their body fluids according to the variation of the external environment. Osmoregulatory animals have the ability to regulate the osmotic concentration of their fluids, that is, even in the presence of environmental variation they are able to maintain an osmotic gradient between the extracellular medium and the external environment (Klôh, 2011). However, this osmoregulation usually entails large energy costs.

In estuarine environments, the animals can be exposed to oscillations in salinity, temperature, pH, oxygen, among others. Even in relation to this environmental dynamism, both osmoconformer and osmoregulatory animals must maintain the osmotic concentrations of their extracellular fluids stable, as alterations of this medium bring variations in the cellular volume and, in extreme cases, cause the loss of cellular function and in even more

extreme cases the death. In order to adjust to events with salinity variations, the cells try to maintain the cellular volume within the proper conformations (Veiga, 2013).

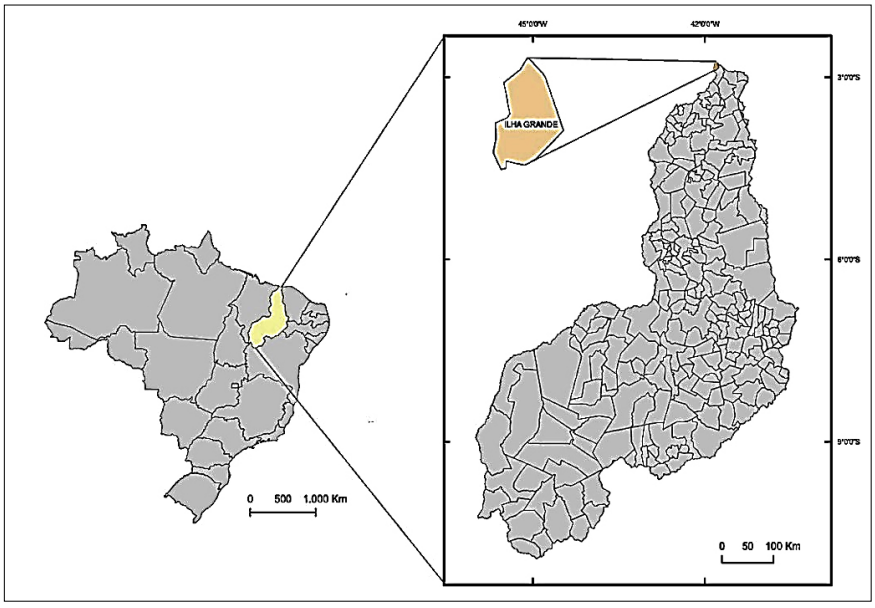
The bivalves of the genus *Cyanocyclas* Blainville, 1818, are endemic to the easternmost part of South America with only sweet representatives (Parodiz, 1996). The species *Cyanocyclas brasiliana* occurs in northern Brazil, in the states of Amazonas (Prime, 1870), Pará (Deshayes, 1854) and in the Northeast in the state of Piauí (Brito; Mansur & Rocha-Barreira, 2015). Known popularly in the Parnaíba river delta region as "marisco", this bivalve is used as food and source of income for the local community that sells it mainly to the larger urban centers such as São Luis (Maranhão state) and São Paulo (São Paulo state). Although this species occurs in great density in the limnic portion and in the upper estuary of the Parnaíba delta, Piauí state, there is no information about its tolerance to salinity variation. Thus, the objective of this study was to verify the tolerance limit and survival of the freshwater bivalve *Cyanocyclas brasiliana* submitted to an increasing salinity gradient under laboratory conditions.

MATERIAL AND METHODS

Study area

The present study was carried out in the upper estuary of the Parnaíba river, located in the Environmental Protection Area of the Parnaíba Delta near Ilha Grande, in the municipality of Parnaíba, Piauí state, Brazil (Figure 1).

Figure 1 – Map of study area in the municipality of Ilha Grande, Piauí state, Brazil



Preparation of the experiment

Specimens of *Cyanocyclas brasiliana* were collected in July 2019, in the Parnaíba river delta in the Piauí state, Brazil (2º48'49,6" S, 041º50'13,2" W).

The specimens were collected in plastic buckets of 20 liters, the water of the collection site being conditioned in plastic bottles of 2 liters.

The experiment was carried out at the Laboratory of Limnology of the Federal University of Piauí (UFPI), Parnaíba Campus. The specimens were kept in plastic containers

with 2 liters of water with constant aeration. Organisms were acclimatized for 24 hours at a maximum density of 2.5 animals per liter in containers with water from the collection site, recording the temperature and salinity of the water (Clesceri; Greenberg & Trussel, 1989).

In the natural environment the mean water temperature was 29.2° C and the salinity did not exceed 1. Thus, the animals were subjected to 7 different treatments were performed corresponding to salinities of 0, 1, 2, 3, 4, 5, and 6 with 5 replicates per treatment. Each replicate contained 5 animals of similar sizes ranging from 19.49 to 26.47 mm. Survival was assessed in 35 experimental units from 0 to 144 hours.

The experiment started with the recording of the initial salinity in each of the containers. Every 12 hours, the salinity was checked using a manual refractometer and the water temperature with a digital thermometer. The salinity elevations of the water used in the containers were carried out using a mixture of sea water with water from the river itself until the salinity was increased by 1. No feed was provided to the bivalves, since the water of the aquariums were changed daily and considering that the renewed water itself provided the necessary food for this filter feeders. This process was repeated until the end of the experiment, determined by the total mortality of the animals every 12 hours up to a total of 144 hours. The condition of death of the animals was evaluated by the partial or total opening of the valves and absence of muscular contraction to the touch. In all treatments, the procedure was the same, differing that in the control aquaria, with salinity 0, the renewed water maintained the same salinity as initially recorded.

Data analysis

The number of dead organisms has been recorded and tabulated in Microsoft® Excel® spreadsheets. To determine lethal concentration (LC50) and lethal time (LT50), which guarantees the death of 50% of the animals, the statistical package GraphPad Prism 6 was used.

The effect of saline concentration over time was compared using a Student's t-test. A One-Way ANOVA and Tukey post-hoc test were used to analyze the lethal time in saline concentrations both at a significance level of $\alpha = 0.05$.

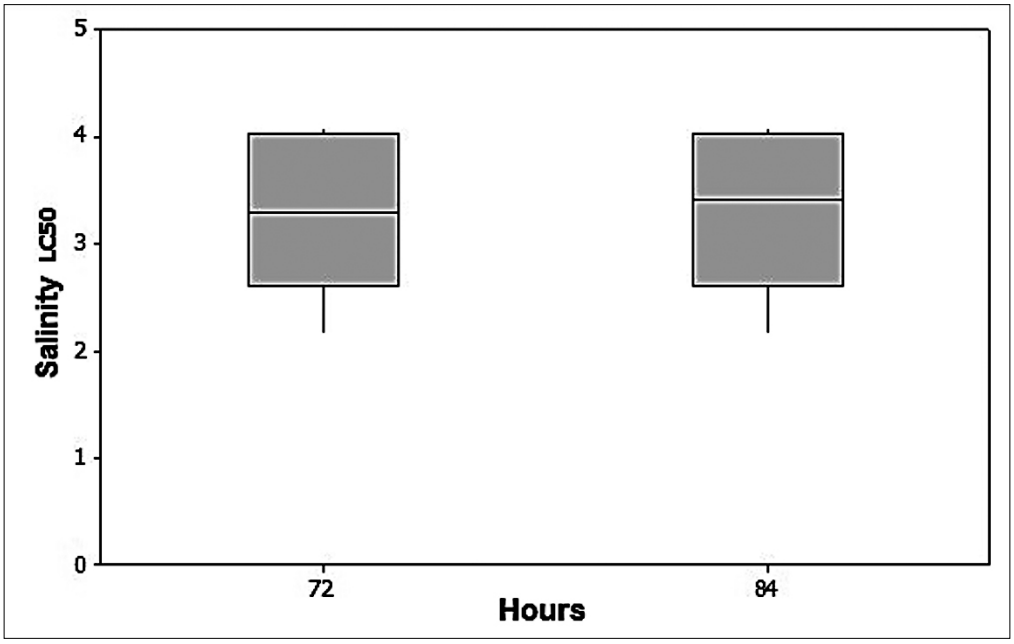
RESULTS

In the first 36 hours there was no mortality for any treatment. At the salinity of 0 and 1, the animals had no behavioral changes or other signs of perturbation, with no mortality occurring at these salinity concentrations during the experiment. At salinity concentrations 3, 4, 5 and 6, survival started to decrease after 48 hours of experimentation. Between 72 and 96 hours, the effects were felt for most specimens at salinities from 2 to 6‰. From 120 to 144 hours, the remaining specimens of treatments of 2 and 3‰ died.

From 24 and 60 hours, there was a mortality lower than 50%. From 96 hours, the mortality of more than 50% of the animals was observed. From 72 to 84 hours intervals, the percentage of mortality varied above and below 50%, indicating that the exposure time to these salinity concentrations affects the survival of bivalves differently. At intervals longer than 96 hours, mortality was very close to 100%. By Anova test it was verified that there was no statistical difference in the mortality of the bivalves kept in the salinity concentrations 3, 4, 5 and 6 by 72 and 84 hours. This finding shows that the animals support this variation in salinity within this time interval, but after 84 hours the organisms are significantly affected (Figure 2).

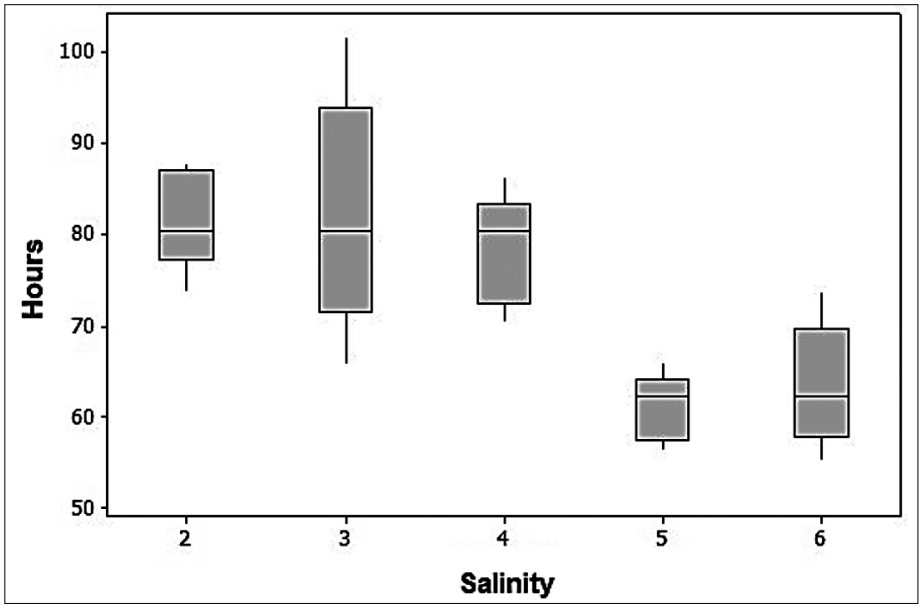
The mean Lethal Concentration of salinity (LC50) observed was 3.1‰ at 72 hour and 3.3‰ at 84 hours with standard deviation 0.349 and 0.350 respectively. Thus, it is possible to state that salinity concentration from 3.2 limits the survival of the species under experimental conditions.

Figure 2 – Lethal Concentration of salinity (LC50) at 72 and 84 hours in the tolerance experiment of the bivalve *Cyanocyclas brasiliana* submitted to an increased salinity gradient



The exposure time did not affect the survival of the animals at concentrations of salinity 0 and 1. The one-way ANOVA test showed significant differences in the lethal time of the bivalve at concentrations of 2 to 6. By the test of post-hoc comparison Tukey was verified the lethal time was similar in salinities 2, 3 and 4, however it was different from the salinity concentrations 5 and 6 (Figure 3).

Figure 3 – Lethal Time (LT50) of *Cyanocyclas brasiliana* at salinity concentrations ranging from 2 to 6‰



DISCUSSION

The factors that limit and control the distribution of organisms in estuarine systems are salinity, sediment type, temperature, food supply, colonization capacity and biological interactions such as competition, predation, parasitism and facilitation, among others (Sousa; Dias & Antunes, 2006; Sousa *et al.*, 2008). According to Kinne (1964), tolerance limits to salinity variation are generally higher in animals existing in brackish, hypersaline or brine waters than in those organisms living in fresh or marine water. Castagna and Chanley (1973), with 29 species of bivalves, confirm tolerance to salinity variation, corroborating Kinne's assertion regarding the estuarine and marine species analyzed (Leonel & Silva, 1988). In the present research, however, has demonstrated that the freshwater bivalve *Cyanocyclas brasiliana*, from the Parnaíba river delta, under experimental conditions, does not support large variations of salinity, being typically a freshwater species.

Experimental studies carried out with other bivalves, such as the zebra mussel *Dreissena polymorpha* and the gastropod *Melanoides tuberculatus*, performed by several authors (Farani, 2013; Bolaji *et al.*, 2011; Mackie & Kilgour, 1992; Baker *et al.*, 1993) showered at the survival and tolerance to salinity variation can be determined in relation to the different stages of the life cycle, as well as the time in which they are exposed to the highest salinities. Such condition guarantees the adaptive plasticity of these species. *C. brasiliana* seems to present a tolerance restricted to the salinity variation, presenting a LC50 varying from salinity 3.1 to 3.3, while *Melanoides tuberculatus* showed values of LC50 ranging from 21.56 to 24.42 (Bolaji *et al.*, 2011; Farani, 2013) and *Dreissena polymorpha* ranging from 2.7 to 7.6 (Baker *et al.*, 1993; Barber, 1992; Fong *et al.*, 1995; Mackie & Kilgour, 1992).

Comparing with other bivalves of the Cyrenidae family registered in Brazil, Mansur *et al.* (2004) reported that species of invasive exotic bivalves in the São Francisco river, *Corbicula fluminea* supports salinity concentration up to 13, being considered as freshwater bivalve, and *Corbicula fluminalis* supports salinity concentration between 15 and 50, a brackish water bivalve and being able to invade estuarine areas. However, Calazans (2014) tested *C. fluminea* specimens in sea water with salinity concentration 20 and all individuals died in 24 hours. These results may indicate that the upper limits of tolerance are only reached when the organisms are subjected to gradual acclimatization to elevation of salinity.

The survival time may also be associated with the temperatures at which the bivalves are exposed. Baker *et al.* (1993) reported a LC50/96h of 7.6 for adults not acclimatized to zebra mussel *Dreissena polymorpha* at 19° C, surviving up to 42 days, when acclimatised slowly at 8.0 salinity at 4 or 10° C. For *C. brasiliana*, due to the tropical condition, with high and constant environmental temperatures throughout the year, the synergic effect of temperature and salinity variations was not considered in the experiment. The TL50 observed for *C. brasiliana* can be considered low, varying from 88.8 to 91.1 hours for lower salinities, up to 63.5 hours for salinities of 5 and 6.

After this research, the question is: what factors contribute to the survival and establishment of the dense population of *C. brasiliana* in the upper estuary of the Parnaíba river delta, since these organisms do not support large salinity variations? It is probable that the water flow of the river itself and the geology of the estuarine outlet are determinants of this freshwater condition in the area. Although this area is located only 8 km upstream of the mouth of estuarine delta, the entrance of the seawater in the upper part of the estuary

due to the tides is relatively limited. Thus, the sampling site is located at the limit of salinity influence, which defines the limit of distribution of this species in the estuary. Downstream, the salinity is too high for its survival. The presence of *C. brasiliana* in other upstream areas of the river still needs to be investigated in the future. Although there are no supporting studies, the proximity of dunes to the area of *C. brasiliana* can promote an additional source of freshwater through percolation and underground drainage into the river, which would also contribute to a reduction of the local salinity concentration.

CONCLUSION

In this work, it was verified that the species of bivalve *Cyanocyclas brasiliana* collected in the upper estuary of the Parnaiba river delta does not support large variations of salinity under experimental conditions, being well adapted to survive in salinities between 0 and 1. After 72 hours (TL50%) and with salinity concentration greater than 2 the survival decreased. The salinity lethal concentration (LC50%) was 3.2. *C. brasiliana* presented characteristics of freshwater stenoaline. Concentration salinity was shown to be a determinant of *C. brasiliana* survival under experimental conditions, thus explaining the limited distribution of this species in the upper estuary of the Parnaíba river delta.

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