From fresh to salty: exploring water salinity tolerance in *Rhinella ornata* (Anura, Bufonidae) tadpoles on Brazilian Atlantic Forest

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Abstract

Changes in salinity levels of aquatic environments may significantly affect the distribution and abundance of many species, including amphibians. Typically, amphibians inhabit environments with low salinity. However, tadpoles of *Rhinella ornata* can often be found in environments in which stochastic events, such as high tides, can dramatically modify the water salinity of ponds in coastal regions. Here, we test the hypothesis that elevated salinity negatively affects the survival and latency of tadpoles of *Rhinella ornata*. We exposed 32 tadpoles to different salinity levels (0, 10, 18, 30 parts per thousand or ppt) and measured the time of death and latency time in individuals of two body size classes. Mortality was significantly different between intermediate and high salinity levels (18 and 30 ppt) but did not depend on body size. These results show that tadpoles of *Rhinella ornata* seem to be intolerant of high salinity, regardless of size. However, there was no mortality at lower salinity levels (0 and 10 ppt) and no difference in latency period between the two lower levels, implying that these tadpoles can survive in low water salinity for a short period, enabling them to swim to less brackish environments. Overall, this trait seems to be important for *Rhinella ornata* to tolerate shoreline environments, which are vulnerable to stochastic events that dramatically increase pond salinities.

Keywords: amphibian, brackish environments, latency, mortality, salt concentration, salt tolerance.

Do doce ao salgado: explorando a tolerância à salinidade da água em girinos de *Rhinella ornata* (Anura, Bufonidae) na Mata Atlântica brasileira

Resumo

Mudanças nos níveis de salinidade de ambientes aquáticos podem desempenhar um papel significativo na distribuição e abundância de muitas espécies, incluindo anfíbios. Normalmente, os anfíbios habitam ambientes com baixa salinidade. No entanto, girinos de *Rhinella ornata* podem frequentemente ser encontrados em ambientes nos quais eventos estocásticos, como marés altas, podem modificar drasticamente a salinidade da água de lagoas em regiões costeiras. Aqui, testamos a hipótese de que a salinidade elevada afeta negativamente a sobrevivência e a latência de girinos de *Rhinella ornata*. Expusemos 32 girinos a diferentes níveis de salinidade (0, 10, 18, 30 partes por mil – em inglês, *parts per thousand ou ppt*) e medimos o tempo de morte e o tempo de latência em indivíduos de duas classes de tamanho corporal. A mortalidade foi significativamente diferente entre os níveis intermediários e altos de salinidade (18 e 30 ppt), mas não dependeu do tamanho corporal. Esses resultados mostram que os girinos de *Rhinella ornata* parecem ser intolerantes à alta salinidade, independentemente do tamanho. No entanto, não houve mortalidade em níveis de salinidade mais baixos (0 e 10 ppt) e nenhuma diferença no período de latência entre os dois níveis mais baixos, o que implica que esses girinos podem sobreviver em baixa salinidade da água por um curto período, permitindo que nadem para ambientes menos salobros. No geral, essa característica parece ser importante para *Rhinella ornata* tolerar ambientes costeiros, que são vulneráveis a eventos estocásticos que aumentam drasticamente as salinidades das lagoas.

Palavras-chave: anfíbio, ambiente salobro, latência, mortalidade, concentração de salinidade, tolerância a salinidade.

1. Introduction

Aquatic environments that undergo large changes in salinity may have had a significant role in determining the distributions and abundances of many species of vertebrates, which can be induced to disperse or migrate in response to these changes (Sabat, 2000; Bentley, 2002; Greenberg, 2012). This is particularly true in places like estuaries, where there is a well-defined gradient between habitats that are truly marine and freshwater (Elliott; McLusky, 2002). Furthermore, for many of these organisms, osmotic regulation is a vital physiological process and is frequently energetically expensive (Bentley, 2002; Karraker; Gobbs, 2011). For example, Peña-Villalobos et al. (2016) showed that females of *Xenopus laevis,* in a hyper-osmotic environment, showed a significant positive correlation between metabolic rates, plasma urea, and osmoregulatory adjustments, with an 80% increase in total and mass-specific standard metabolic rates, supporting the idea that osmoregulation incurs a metabolic cost common across species.

Typically, amphibians are found in low-salinity environments such as wetlands, ponds, and streams (McDiarmid; Altig 1999; Wells, 2007). When exposed to high salinity conditions, there is the potential for losing water through the permeable skins, and death may occur from desiccation (Wells, 2007). Tadpoles generally have a lower tolerance for high salinity than adults (Wells, 2007). As a result, most field studies on amphibians have focused on the distribution of adults associated with salinity in aquatic habitats (Hopkins; Brodie, 2015). Previous study indicates that an increase in salt concentration may hurt tadpoles' survivorship, metamorphosis, weight, and activity (Collins; Russell 2009; Langhans et al., 2009).

Particularly, Karraker et al. (2008) showed that embryos and the larval stage are vulnerable to the effects of increased salinity, which may have important impacts on some anuran populations, even leading to local extinction. Thus, the high salinity of ponds may affect the community structure by influencing the development and performance or excluding salt-intolerant species (Collins; Russell 2009; Langhans et al., 2009; Brown; Walls, 2013). However, several amphibian species have evolved physiological adaptations that allow them to tolerate brackish environments, which allow them to survive in these environments, e.g., *Bufotes viridis*, *Lemnonectes cancrivora*, *Rhinella marina*, *Thoropa taophora*, among many other taxa (McDiarmid; Altig, 1999; Hopkins; Brodie, 2015). Other species can survive and move for a period in water with relatively high salinity, which may allow the tadpoles to seek more favorable environments (less salinity) (McDiarmid; Altig 1999; Wells, 2007).

The genus *Rhinella* belongs to the family Bufonidae, a diverse group of toads found predominantly in the Americas. Bufonidae is one of the most widespread amphibian families, encompassing approximately 55 genera and over 650 species globally (Frost, 2024). Within this family, *Rhinella* stands out as one of the most species-rich genera, comprising around 97 recognized species, many of which are distributed across Central and South America (AmphibiaWeb, 2025; Frost, 2024). These toads are found in brackish pools, streams, estuaries, as well as beaches, mangroves, among other brackish habitats (Hopkins; Brodie 2015). *Rhinella ornata*, commonly known as the "*Cururu*" toad, is a semi-terrestrial anuran in the family Bufonidae, endemic to the Atlantic Forest of southeastern Brazil (Baldissera Jr. et al., 2004; Brasileiro et al., 2005; Frost, 2024).

This species is characterized by its distinctive color patterns, which include a mix of dark and light markings that provide effective camouflage in its forest floor habitat (Brasileiro et al., 2005; Frost, 2024). *Rhinella ornata* is primarily nocturnal and terrestrial, feeding on a variety of small invertebrates, and is known for its toxic parotoid glands that deter predators (Toledo et al., 2011). The species is highly sensitive to environmental modification, particularly habitat degradation caused by deforestation and urban expansion, which has led to its classification as Near Threatened by the IUCN Red List (IUCN, 2025).

The tadpoles of *Rhinella ornata* are typically found in temporary or permanent water bodies, such as puddles, streams, and ponds, within their Atlantic Forest habitat., but can also be found in aquatic environments where stochastic events, such as abnormally high tides or marine storms, can dramatically alter water salinity (authors' pers. obs.). These environments include streams, ponds, and small lakes near beaches. The tadpoles of this species are benthic, where they feed on organic detritus, algae, and decomposing plant material, playing an important role in nutrient cycling (Altig; McDiarmid, 1999)

In this context, this study aims to investigate the variation in salinity tolerance in *Rhinella ornata* tadpoles. Objectively. we tested the hypothesis that water salinity influences both survival (Hypothesis 1) and latency— the time during which the animals remained motionless before swimming (Hypothesis 2)—of *Rhinella ornata* tadpoles. We expect higher water salinity to be associated with higher mortality, and intermediate salinity levels to be associated with increased latency time, at short-term exposure

2. Materials and Methods

2.1 Study site

The study was conducted in a small tributary of the Picinguaba River, located on the "Praia da Fazenda, Núcleo Picinguaba", Parque Estadual da Serra do Mar - PESM, Ubatuba, São Paulo state, Southeastern Brazil (Assis, 1999). The "Núcleo Picinguaba" covers an area of 47,500 ha, covering 80% of the total territory of the municipality of Ubatuba. Located in the hydrographic region of the coastal slope, it has landscapes that range from the seacoast to the escarpments of the Serra do Mar, covering various phytophysiognomies, such as the Dense Ombrophilous Forest, the Restinga forests, and the Mangrove.

The study area is in a Restinga forest, a vegetation formation that occurs on the coastal sandbanks, at altitudes ranging from 0 to 50 m (Nóbrega et al., 2011). The regional climate is humid tropical, without a dry season, with an average annual precipitation of over 2,200 mm (Peel et al., 2007). The study site is located on "Praia da Fazenda", close to the base of the "Núcleo Picinguaba", at an approximate altitude of 10 m, and it can be subject to the seasonal influence of the water table, and so, the typical tidal regime of the region (Nóbrega et al., 2011).

2.2 Study species

Tadpoles of *Rhinella ornata* (For more details about the taxon, see the Introduction section).

2.3 Experimental Design

Specimens of *R. ornata* tadpoles were captured in the study area, using a handheld dipnet on October 20, 2012, from a stream pool adjacent to the coastline at "Núcleo Picinguaba/PESM". The characteristics of the water collection site were 0 ppt (0 parts per thousand or ppt) of salinity and a temperature of 16 °C. This study was conducted at the site to less stress for the animal. The tadpoles collected were sorted into two size classes based on body size (excluding the tail), using a handheld analog caliper: Small [S]: mean SVL = 6.9 ± 0.39 mm and Large [L]: mean SVL = 8.1 ± 0.24 mm. They were then separated into two plastic containers (50 mL), with salinity water levels described above. Individuals from each size class were randomly assigned to four treatments with different salinity levels: control (0% seawater – 0 ppt), 20% seawater (10 ppt), 50% seawater (18 ppt), and 100% seawater (30 ppt). Salinity was measured using a handheld refractometer. The salinity treatments were created by diluting seawater with freshwater. Each tadpole was placed in a plastic container (50 mL), which already contained water with salinity levels described above. Each treatment had four tadpoles (replicates) for each tadpole size class, and n = 32.

For each tadpole, we recorded the time to death (in seconds), a well-established toxicological method for determining relative tolerances (Newman, 2013). For the individuals that did not die (tadpoles exposed to 0 and 10 ppt), we recorded the latency time to investigate if tadpoles can move to lower salinity water, right after exposure. We left tadpoles in the container for a period (5 minutes), for adaptation to address the stress associated with movement into a new container. After this period, we started to observe tadpoles until they stopped swimming, and then recorded the time in seconds until they began moving again, and then we added the total latency time. Each individual was tested three times, and the median latency time was used as the standard measurement for each replicate.

2.4 Data Analysis

Each experiment was analyzed separately. In the first experiment, we applied a Generalized Linear Model (GLM) with a quasipoisson (mortality time) and poison (latency time) distributions to test the effect of salinity on the tadpoles. For the first hypothesis, the response variable was time to death (in seconds), while for the second hypothesis, it was latency time (in seconds). The predictor variables for both hypotheses were tadpole body size ([S] and [L]) and salinity levels (0 ppt (control), 10 ppt, 18 ppt, and 30 ppt). Additionally, we tested whether there was a significant difference between the time to death between the 18 ppt and 30 ppt groups and the latency time between the control group (0 ppt) and 10 ppt of salinity concentration, applying an ANOVA analysis. All statistical analyses, tools, and visualizations were conducted in the R environment (R Core Team, 2024).

3. Results

We found that higher salinity was associated with higher mortality, with significant difference between 18 ppt and

30 ppt ($p < 0.001$, Table 1), with mean shorter time of survival in 30 ppt group: 40.6 ± 9.4 s (mean ± SD), I relation to18ppt group: 149.0 ± 43.2 s (mean ± SD) (Appendices Table S1 and S2). In this study, 16 of 32 tadpoles that were exposed to salinity died (50% mortality), and all individuals who died had been exposed to high salinity levels (18 and 30 ppt).

Table 1. GLM (quasipoisson family) table comparing the mortality time of the individuals sampled tadpoles *R. ornata* by saline water (18 and 30 ppt) and body size classes ([S] e [L]) of tadpoles (italic values mean significant values of *p*).

	Mortality Time		
Predictor	Residual Deviance	F	P
Salinity	529.50	69.641	*<0.001*
Size	0.07	0.0087	0.9274
Salinity*Size	12.82	1.6867	0.2184

Note: F represents the *F-statistic* or the ratio of explained variance to unexplained variance, testing the overall significance of the model; and P is the *p-value* or the probability that the observed result occurred by chance under the null hypothesis. Source: Authors, 2025.

Table 2. GLM (Poisson family) table comparing the latency of individuals sampled tadpoles *R. ornata* with salinity (0 and 10 ppt) and body size classes ([S] e [L]) of tadpoles (* means significant *p* values).

	Latency Time		
Predictor	Residual Deviance	F	*P*
Salinity	1415.64	1.7993	0.1849
Size	19.14	0.0243	0.8766
Salinity*Size	0.14	0.0002	0.9894

Note: F represents the *F-statistic* or the ratio of explained variance to unexplained variance, testing the overall significance of the model; and P is the *p-value* or the probability that the observed result occurred by chance under the null hypothesis. Source: Authors, 2025.

However, there was no effect of tadpole body size on mortality time in either 18 or 30 ppt groups ($p = 0.94$, Table 1) or the interaction effect of salinity with size on mortality time ($p = 0.2184$, Table 1). Additionally, we did not find a significant difference in latency period between tadpoles' body size ($p = 0.8766$, Table 2), different salinity levels groups tested (0 ppt and 10 ppt; $p = 0.1849$, Table 2), or their interactions ($p = 0.9894$, Table 2, Figure 2).

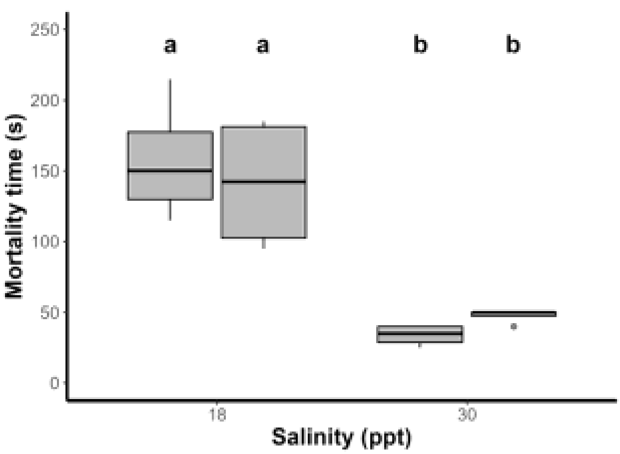

Figure 1. Boxplots of mortality time about water salinity (18 and 30 ppt) and body size classes of tadpoles of *Rhinella ornata*. Distinct letters denote significant differences. Source: Authors, 2025.

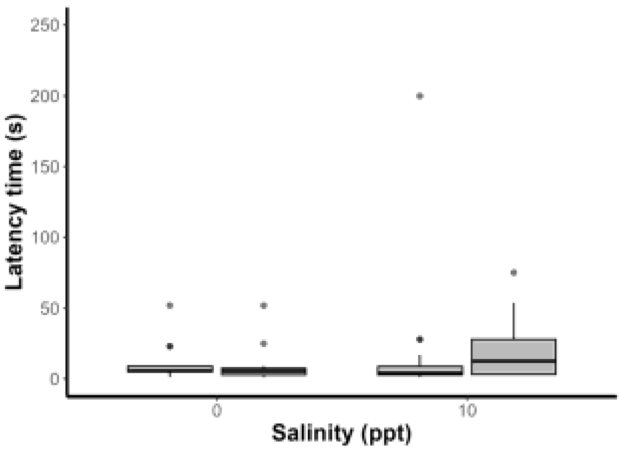

Figure 2. Boxplots of latency time about water salinity (0 and 10 ppt) and body size classes of tadpoles of *Rhinella ornata*. Source: Authors, 2025.

4. Discussion

In this study, we found that high salinity in water was related to higher mortality of tadpoles, with 100% death of individuals in 30 and 18 ppt. However, our results also indicate that *R. ornata* tadpoles possibly can survive for at least a few hours at a salinity of 10 ppt (20% seawater), as there was no death recorded in this experiment in 10 ppt as well as in the control group (0 ppt). In caudates, richness and abundance declined sharply with even small increases in salinity, highlighting their limited salt tolerance (Izzo et al., 2021). Conversely, anurans (all life stages) showed peak richness and abundance at moderate salinity levels (around 5–6 g/L^{-1}), with some species tolerating surprisingly high salinity, as demonstrated by Lorrain-Soligon et al. (2023). Several tadpole species are known to be osmoregulatory organisms, regulating internal solute concentration regardless of the external concentration (McDiarmid; Altig 1999; Wells, 2007). As a result, if there is an increase in salinity from zero to 100% (which represents a greater than ten-fold increase in osmotic pressure), the osmotic pressure in the plasma of the animal only doubles (Gordon; Tucker, 1965).

On the other hand, many species of amphibians are intolerant of high concentrations of salt. Chinathamby et al. (2006) showed that only 39% of *Litoria ewingii* tadpoles survived the treatment group (16% seawater); while in the group control (0%), there was 92% survival. Rios-López (2008) showed that in 22–25% seawater, *Leptodactylus albilabris* exhibited 100% mortality. Szeligowski et al. (2022) demonstrate that chronic exposure of *Rana sylvatica* larvae to road salt alters gill morphology, potentially impairing gill functions such as osmoregulation, gas exchange, and foraging.

Also, *Bufo spinosus* tadpoles showed altered behavior in response to salinity, with negative impacts on growth, mortality, and behavioral complexity. For instance, medium-term exposure to salinity reduced activity, decreased growth, and increased mortality rates, while abrupt changes in salinity led to an increase in tadpoles' escape distance when faced with a predation threat, but their overall activity decreased (Lorrain-Soligon et al., 2024a). Thus, exposure to high salt concentrations can negatively impact the development and survival of tadpoles, leading to local extinction, thus negatively affecting species richness and community structure by excluding salt-sensitive species.

However, several species of the family Bufonidae (e.g., *Rhinella marina*, *Bufotes viridis*, *Bufo bufo*, *Anaxyrus fowleri*), which live preferably in forest areas, are occasionally found adjacent to coastlines, beaches, and mangroves (Hopkins; Brodie, 2015; Wells, 2007). Physiological adaptations probably allow these species to tolerate up to 60% salinity (McDiarmid; Altig, 1999; Wells, 2007). For example, Lorrain-Soligon et al. (2024b) showed that, even though environmental salinity can negatively impact reproduction and early development in *Bufo spinosus* tadpoles exhibited partial recovery when returned to freshwater, demonstrating resilience to salinity stress.

This tolerance pattern is to some degree consistent with the results obtained in the present study, in which we found that mortality of *R. ornata* was only observed in 18 ppt (50% of seawater) and 30 ppt (100% seawater). Moreover, Rios-López (2008) showed *Rhinella marina* tadpoles exhibited 60% mortality, which is notably very similar to that obtained for a congeneric species, *R. ornata*, in the present study (50% mortality). Despite some tadpoles being capable of osmoregulation, previous works suggest there may be a limit to the period of survival even in intermediate and low salinity (Christy; Dickman, 2002; Alexander et al., 2012; Hua; Pierce, 2013).

However, several species within the *Rhinella* genus (e.g., *R. marinus*, *R. viridis*, *R. bufus*, *R. fowleri*) primarily inhabit tropical forests but are also found near beaches and mangroves (Hopkins; Brodie, 2015; Wells, 2007). These species can tolerate salinity levels up to 60% seawater, a tolerance consistent with the findings of this study, where mortality was only observed at salinity levels of 18 ppt (approximately 50% seawater). At high concentrations (30 ppt), the time to death was much faster (mean 30 s) compared to intermediate concentrations (18 ppt, mean 95 s). These results indicate that these animals were considerably more sensitive to higher salinity compared to lower salinity. However, contrary to our prediction, there was no difference in latency between individuals in 0 ppt (control) and 10 ppt salinity groups, demonstrating a relative tolerance of tadpoles to low salinity concentration, limited to short-term exposures (~4 h).

5. Conclusions

Although *Rhinella ornata* is seemingly very susceptible to high salinity, the mortality of individuals was not always as immediate, with survival up to 215 seconds in 18 ppt and up to 50 seconds in 30 ppt (Appendix, Table S1). Moreover, like other Bufonidae species, this species may frequently be observed in ponds along the coastline, at least in the coastal plains of São Paulo state (author's pers. obs.). Although limited to a small population sample, these findings suggest that in stochastic events with extreme increases in salinity (e.g., high tides and marine storms), tadpoles of *R. ornata* may be able to resist a short period of exposure to high water salinity, possibly seeking refuge in areas with lower salinity.

In addition, we verified that low salinity did not significantly affect the survival or mobility of tadpoles tested, indicating that the tadpoles of *R. ornata* may tolerate low levels of salinity for short-term exposures, being then able to migrate to environments with less brackish water. Accordingly, these findings, supported by discussed similar results in the *Rhinella* genus among other anuran species, suggest that this tolerance may be a key trait to the survival and occurrence of tadpoles in ponds along the coastline, habitats that are susceptible to occasional increases of salinity.

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7. Authors' Contributions

Thiago Leão Pires, *Micael Eiji Nagai*, *Raissa Furtado* and *Sandra Benavides-Gordillo* equally contributed with the conceptualization, *Thiago Leão Pires*, *Micael Eiji Nagai*, *Raissa Furtado* and *Sandra Benavides-Gordillo* contributed with the investigation and data curation; *Thiago Leão Pires* led the formal analysis and visualization, with contributions from *Micael Eiji Nagai*; *Thiago Leão Pires* led the writing – original draft; *Micael Eiji Nagai*, *Raissa Furtado* and *Sandra Benavides-Gordillo* equally contributed with the writing – review & editing.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Collection permits were provided by ICMBio (#31554-1).

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Informed Consent Statement

Not applicable.