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The effect of extreme climatic events on littorinid snails in two estuarine environments, temperate (NW Spain) and tropical (NE Brazil)

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ABSTRACT

Extreme weather events (e.g., droughts, excessive precipitation) are expected to increase in frequency and severity in the coming decades due to climate change, causing significant impacts on society and ecosystems. Because these events are rare and complex, they have been studied with manipulative experiments. Littorinidae snails inhabit a complex and variable environment in which they must deal with periodic extreme events and are thus considered excellent ecological models for these studies. Therefore, this study aimed to understand the effects of extreme climatic events on the survival and weight of the species *Littorina fabalis* and *Littorina littorea* in Spain and *Littoraria angulifera* and *Littoraria flava* in Brazil. Higher mortality rates and greater weight loss were observed in the desiccation resistance treatment compared to the control treatment in both countries. The results showed dependence on the species' body size. The submergence tolerance treatment indicated that the species from Spain are more susceptible to mortality in response to excessive rainfall and/or coastal flooding. Each species tested for the effect of extreme climatic events using an integrated response strategy with clear latitudinal differences. Understanding the organisms' responses at different latitudes is essential for conservation biology on a global scale.

Keywords: Brazil, Climate change, Desiccation, Littoraria, Littorina, Spain

INTRODUCTION

The phenomenon of climate change has been considered by scientists as the most serious threat to the planet's biodiversity (Wernberg et al., 2012). According to projections, there may be an increase in the global average temperature,

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an increase in the mean sea level, a decrease in marine pH, leading to ocean acidification, and the incidence of extreme events in the next 100 years, all because of greenhouse gas emissions (Stocker et al., 2013).

An extreme event can be understood as an irregularity or deviation in behavior from an average or habitual pattern (Smith, 2011). Extreme weather events can occur as a reduction in cold days, an increase in the duration of heat waves, and an increase in the frequency of heavy rains or severe droughts, causing significant

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impacts on society and ecosystems in general (Easterling et al., 2000; Stocker et al., 2013). As these events are difficult to study due to their rarity and complexity (Smith, 2011), manipulative experiments have been used to test the magnitude and frequency of these events under controlled conditions (Jentsch et al., 2007).

By exceeding the adaptive capacity and physiological limits of many animals and plants, extreme climatic events can result in rapid mortality of species or populations (Sergio et al., 2018). They also influence community structure, ecosystem function, and ecotone boundaries, as they lead to changes in habitat availability and quality, the intensity and duration of ecological interactions, and a spatial shift in niches and thermal stress (Smith, 2011). Thus, these changes may alter the distribution, development, reproduction, and/or survival of organisms (Scheffers et al., 2014; Sergio et al., 2018) with potentially devastating effects in estuarine environments (Wetz and Yoskowitz, 2013; Robins et al., 2016).

Snails of the Littorinidae family are common herbivores in intertidal regions, including estuaries, with an almost pan-global distribution (Mcquaid, 1996a, 1996b; Reid, 1989; Reid et al., 2009; Bosso et al., 2022). These organisms are most frequently found in the supralittoral zone and inhabit a complex and variable environment in which they must deal with extreme periodic events, experiencing highly variable temperatures within a single tidal cycle (Marshall et al., 2010, 2013) and thus are among the most heat resistant metazoans (Liao et al., 2017). Littorinids are considered excellent ecological models for cross-region studies because they are also easily collected, abundant, and widely distributed (Ng et al., 2011; Rolán-Alvarez et al., 2015; Bosso et al., 2022).

The snails of the genus *Littorina* are restricted to the northern hemisphere, and the genus *Littoraria* is essentially tropical (Reid, 1989; Reid, 1996). Recently, there has been an important debate on whether the tropical or temperate species are more vulnerable to climate change, as it holds implications for conservation priorities at a global scale (Vinagre

et al., 2015, 2018; Maia and Troncoso, 2022). However, vulnerability will depend on the organisms' acclimation capacity, which remains largely unknown for most species, especially in the case of extreme climatic events in estuaries (Wetz and Yoskowitz, 2013).

Thus, we tested the hypothesis that extreme climatic events, such as *i*) droughts or water scarcity and *ii*) excessive precipitation or coastal flooding affect the survival and weight of Littorinid gastropods from estuarine environments of different latitudes and that the impacts will be evident sooner in the tropics than in the temperate zone. Hence, this study aimed to evaluate the desiccation resistance and submersion tolerance of *Littorina fabalis* (W. Turton, 1825) and *Littorina littorea* (Linnaeus, 1758) from a temperate estuary in Spain, and of *Littoraria angulifera* (Lamarck, 1822) and *Littoraria flava* (King & Broderip, 1832) from a tropical estuarine area in Brazil.

METHODS

STUDY AREA

This study was carried out at temperate and tropical climate latitudes (Figure 1). The species Littorina littorea and Littorina fabalis were collected from seaweed meadows in the San Simón Inlet, an estuarine area of the Ría de Vigo (Verdugo-Oitavén River System), in Pontevedra, Galicia, Spain. The species Littoraria angulifera and Littoraria flava were collected in the mangrove of Arpoeiras Beach, in the Acaraú River estuary, Ceará, northeastern Brazil (Figure 2). Both areas hold a semidiurnal tidal cycle.

The estuarine area of the Ría de Vigo holds climatic conditions described Temperate Oceanic with Mediterranean influence, characterized by a drastic decrease of precipitation during July and August, and homogenous temperatures throughout the area, ranging from 10 °C to 20 °C, with the months of December, January, and February as the coldest (Perez-Arlucea et al., 2005). Seagrass, seaweed, and soft substrates, mainly muddy, with a high organic matter content, predominate in the San Simón Inlet (Cacabelos et al., 2008).

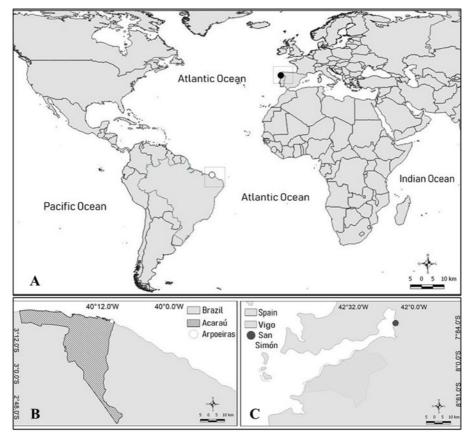


Figure 1. Map of the study areas (A) indicating the collection sites at Arpoeiras Beach (B), Acaraú, Brazil and at the San Simón Inlet, Vigo, Spain (C).

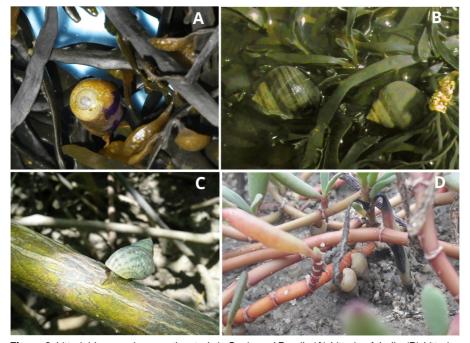


Figure 2. Littorinidae species over the study in Spain and Brazil. (A) *Littorina fabalis*, (B) *Littorina littorea*, (C) *Littoraria angulifera*, and (D) *Littoraria flava*.

Due to Spain's geographic location, the country is very vulnerable to climate change. There will be more extreme events in most regions, with increasing frequency, intensity, and/or amount of heavy precipitation (Stocker et al., 2013).

The Acaraú River originates in the Serra das Matas, in the central-western region of the state of Ceará, in northeastern Brazil. It runs for 315 km in a South-North direction and flows into the Atlantic Ocean in the municipality of Acaraú. According to data obtained by the Cearense Foundation of Meteorology and Water Resources, the region holds a Semi-arid Tropical climate, with an average temperature of 29 °C throughout the year and approximate rainfall of 1,100 mm, with rainfall concentrated from January to June (FUNCEME, 2018). The Acaraú estuary features a predominance of unconsolidated sedimentary substrates and mangrove forests composed of Rhizophora mangle Linnaeus, 1753, Laguncularia racemosa (Gaertner, 1805), Avicennia germinans (Linnaeus) Stearn 1958, and A. shaueriana Stapft & Leechman (Maia and Coutinho, 2012).

The region is also highly vulnerable to extreme events. The scenarios indicate that the Brazilian northeast may suffer a decrease in its water resources with increased precipitation variability and droughts that may result in an increase in the index of consecutive dry days and a decrease in the recharge of groundwater (Stocker et al., 2013).

COLLECTION OF ORGANISMS AND ASSEMBLY OF EXPERIMENTAL UNITS

Sampling was carried out in the spring in both countries (Spain – May/2018 and Brazil – September/2018). All specimens were measured for shell height (distance between the apex and the innermost part of the shell) and shell width (measured perpendicular to the shell height) using a caliper and weighed. Each specimen was labeled for identification at the end of the experiment.

All the experiments were carried out in aquariums (experimental units measuring 43x22x30 cm and with a capacity of 28 liters) (Supplementary Material) in which animals with similar sizes (shell height and live wet weight of the snail) for each species (Littorina fabalis, Littorina littorea, Littoraria angulifera and Littoraria flava) and densities similar to those in the natural environment, calculated from previous observations in the field at each site (NW Spain and NE Brazil) to determinate the sample size in each experimental unit (Table 1).

Table 1. Average shell height $(\pm SD)$ (mm), snail alive wet weight (g) and sample size of Littorinids (individuals per experimental unit) from Spain and Brazil submitted to treatments that simulate extreme events.

		Shell height (mm)	Wet weight (g)	Sample size (ind/unit)
L. fabalis	Spain	13.43 ± 0.70	0.654 ± 0.106	15
L. littorea	Spain	20.91 ± 2.92	3.199 ± 1.160	5
L. angulifera	Brazil	25.99 ± 2.94	2.361 ± 0.548	20
L. flava	Brazil	7.98 ± 0.46	0.181 ± 0.050	25

The temperature in all experiments was kept constant in an isothermal environment, set at 19 C in Spain and 33 °C in Brazil, corresponding to the mean values of water temperature during the three last summer months in Vigo, Spain, and Acaraú, Brazil. The photoperiod was simulated using artificial lighting, used as 16 hours in Spain and 12 hours in Brazil, based on the mean values of natural summer in each country. These data were obtained from the Technologic Institute for the Control of the Marine Environment of Galicia (Intecmat, Rande

Oceanographic Station) in Spain and, from the Network of Monitoring for Benthic Coastal Habitats (Rebentos, subgroup estuaries, Acaraú) in Brazil.

Each experiment (desiccation treatment, submergence treatment, and control) was replicated three times. Thus, 36 experimental units were established to simulate extreme events (4 species x 3 treatments x 3 replications). The studies in Spain were carried out at the Marine Science Station (ECIMAT), Marine Sciences Faculty of the University of Vigo, and the Mangrove Ecology Laboratory

(ECOMANGUE) of the Federal Institute of Ceará, Acaraú campus, in Brazil.

EFFECT OF DROUGHT AND/OR WATER SCARCITY (DESICCATION TREATMENT)

The desiccation resistance test was performed to verify the effect of drought and/or water shortage. After collection, labeling, measurement, and weighing, each individual was placed in the estuary water for an hour to rehydrate (Britton, 1992; Tanaka and Maia, 2006). The specimens were subsequently removed, the excess water dried with a filter paper, and they were placed in the experimental units containing site substrate and abundant feed (≅ 2.8 I of substrate volume) represented by the seaweed Fucus spiralis L. and Ascophyllum nodosum (L.) Le Jolis in Spain, and mangrove leaves of *Rhizophora* mangle, Avicennia spp., and Laguncularia racemosa in Brazil. Seaweed and mangrove leaves were used because the animals were collected from them. They were not replaced during the experiment and were also subjected to desiccation with the animals.

Throughout the experiment, mortality was observed by the absence of foot retraction or closure of the operculum when the animal was touched. All live individuals were weighed daily. This experiment lasted eight days, a period determined by one species reaching 100% mortality.

EFFECT OF EXCESSIVE PRECIPITATION AND/OR COASTAL FLOODING (SUBMERGENCE TREATMENT)

A submersion tolerance test was carried out to evaluate the effect of excessive precipitation and/or coastal flooding. Each individual was subjected to one hour at room temperature in the dry and then placed in the experimental unit containing water from the region where it was collected (Table 2) to remain completely submerged. The water was renewed every six hours to avoid eutrophication and guarantee oxygen support, and the excreta were removed without the animals being emerged. This experiment lasted four days (96 hours), a period determined by one species reaching 100% mortality, with the snails being monitored to assess survival every six hours (tidal frequencies) (Capaldo, 1983). The same substrates described in the previous item were used.

CONTROL EXPERIMENT

In the control experiment, the animals were placed in experimental units with a substrate from their original area containing abundant food and the temperature and photoperiod as described above. The tide effect was simulated by irrigation every 12 hours with water from the estuary where the animals were collected (Table 2), ensuring that they were always moist and had access to the substrate to emerge, simulating natural field conditions. Thus, there were two control treatments (Spain and Brazil). This experiment lasted eight days. The snails were monitored to evaluate survival.

Table 2. Values of water temperature, pH, and salinity at the time of sampling in Vigo, Spain (May/2018) and Acaraú, Brazil (September/2018).

	Spain	Brazil
Water temperature	19 °C	33 °C
рН	7.6	8.3
Salinity	30.6	38

DATA ANALYSIS

The effect of resistance to desiccation and submergence tolerance on survival (%) and weight (differences between final and initial values) of littorinids was evaluated using a two-way Analysis of Variance (ANOVA) test among the different species and treatments by study areas (NW Spain and NE Brazil). When significant differences were found at the 5% significance level (p < 0.05), Tukey's honestly significant difference (HSD) test was used. Percentage data were arcsine square root transformed. The assumptions of homogenous variances and normally distributed residuals were met for all ANOVAs.

RESULTS

EFFECT OF DROUGHT AND/OR WATER SCARCITY (DESICCATION TREATMENT)

SURVIVAL

The survival of Littorinids subjected to the desiccation experiment varied significantly among species and treatments in Spain ($F_{1,8} = 7.4027$, p = 0.026) and in Brazil ($F_{1,8} = 54.270$, p < 0.00008). The results of the analysis indicated that, under

drought conditions and/or water scarcity, the Spanish species *L. fabalis* has a low survival rate (Figure 3A), with mortality beginning to be observed on the third day (Table 3). The data showed that mortality reached 100% after seven days of exposure. For *L. littorea*, although this condition was a limiting factor leading to some mortality, desiccation did not strongly reduce survival; a result evidenced by the similar values observed between the treatment and control experiments with this species (Figure 3A, Table 3).

The results obtained for the Brazilian species indicated that *L. flava* has a low survival rate in the desiccation treatment, with mortality observed from the fourth day onwards. By the end of the eighth day, only around 25% of the organisms remained alive (Figure 3B, Table 3). In the control experiment, the final survival rate was 98.67%. *L. angulifera* showed a similar survival rate between treatment and the control, with only one snail dead at the end of the experiment on the eighth day (Figure 2B). No mortality was observed in the control treatment (Table 3).

Table 3. Survival (%) of Littorinidae species over the experimental days (1 to 8 days) in the control and desiccation treatments in Spain and Brazil.

	Desiccation experiment				Control experiment			
	Spain		Brazil		Spain		Brazil	
Day	L. fabalis	L. littorea	L. angulifera	L. flava	L. fabalis	L. littorea	L. angulifera	L. flava
1	100%	100%	100%	100%	100%	100%	100%	100%
2	100%	100%	100%	100%	100%	100%	100%	100%
3	97.8%	93.3%	100%	100%	100%	100%	100%	100%
4	84.4%	93.3%	100%	98.67%	97.80%	100%	100%	100%
5	64.4%	93.3%	100%	88%	97.80%	100%	100%	100%
6	17.8%	93.3%	100%	74.67%	97.80%	100%	100%	100%
7	6.7%	93.3%	100%	49.33%	97.80%	100%	100%	100%
8	0%	80%	96.67%	25.33%	84.44%	100%	100%	98.67

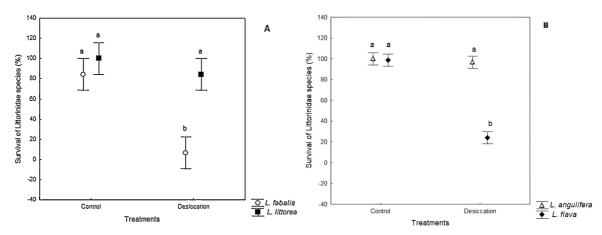


Figure 3. Average survival (± SD) (%) of Littorinidae species in control and desiccation treatments in Spain (A) and Brazil (B). Different letters indicate significant differences according to Tukey's HSD.

WEIGHT

The difference between the initial and final weight of the animals in the desiccation treatment in Spain varied significantly ($F_{1,8} = 343.92$, p < 0.000001); in the experimental groups, a marked loss of weight was observed (mean: – 0.043 g for *L. fabalis* and –0.258 g for *L. littorea*), whereas a slight increase in both *L. fabalis* (average: + 0.0067 g) and *L. littorea* (mean: + 0.0103 g) was observed in the control groups (Figure 4A).

A similar pattern was observed in the Brazilian species. A significant variation in the initial and final weight difference was observed among the species and treatments tested ($F_{1,8} = 7.7423$, p = 0.024). However, the response observed in L. flava between the control (mean: + 0.0086 g) and the desiccation treatment (mean: 0.0067 g) was similar (Figure 3B). The results for L. angulifera showed a significant weight loss in the experimental treatment (mean: - 0.348 g) compared to that observed in the control (mean: - 0.050) (Figure 4B).

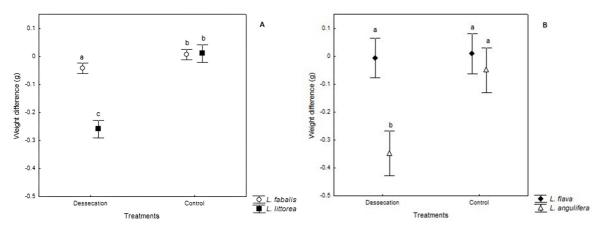


Figure 4. Mean weight difference (final–initial) (± SD) (%) of Littorinidae species between the control and desiccation treatments in Spain (A) and Brazil (B). Different letters indicate significant differences according to Tukey's HSD.

EFFECT OF EXCESSIVE PRECIPITATION AND/ OR COASTAL FLOODING (SUBMERGENCE TREATMENT)

SURVIVAL

The results of the submergence experiment showed a significant difference in survival between species and treatments in Spain ($F_{1,8}$ = 101.833, p = 0.00001). No survival was observed at the end of the experiment with *L. fabalis* (Figure 4A) after 90 hours of exposure (Table 4). The vulnerability of this species is also clearly evident when comparing the final results of the submergence and control treatments. The submergence treatment did not produce the same effect on *L. littorea* because no mortality

was observed throughout the experiment, even in the control group (Figure 5A, Table 4).

The Brazilian species studied showed no significant differences in the survival of the organisms between the submergence and control treatment ($F_{1,8} = 1.7652$, p = 0.22) (Figure 5B). The results expressed in Table 4 indicate the mortality of only one individual of *L. flava* in the control treatment; despite showing mortality in the experimental treatment after 12 hours from the start, the survival rate was 93.33% at the end of the experiment. The experiment with *L. angulifera* showed no deaths in the control group and 90% survival in the experimental group; the first deaths occurred at 42 hours of submergence (Table 4).

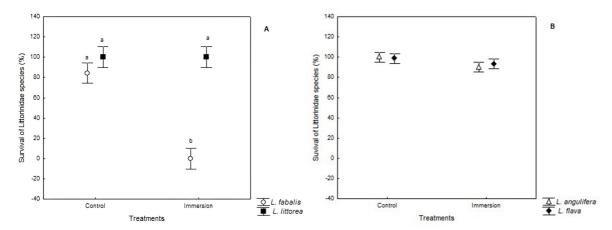


Figure 5. Average final survival (± SD) (%) of Littorinidae species in the control and submergence treatments in Spain (A) and Brazil (B). Different letters indicate significant differences according to Tukey's HSD.

Table 4. Survival (%) of Littorinidae species in the control and submergence treatments in Spain and Brazil.

Submergence experiment					Control experiment			
	Spain		Brazil		Spa	Spain		l
Hour	L. fabalis	L. littorea	L. angulifera	L. flava	L. fabalis	L. littorea	L. angulifera	L. flava
6	100%	100%	100%	100%	100%	100%	100%	100%
12	100%	100%	100%	98.67%	100%	100%	100%	100%
18	100%	100%	100%	98.67%	100%	100%	100%	100%
24	95.6 %	100%	100%	97.33%	100%	100%	100%	100%
30	95.6 %	100%	100%	96%	100%	100%	100%	100%
36	93.3%	100%	100%	93.33%	100%	100%	100%	100%
42	86.7%	100%	98.33%	93.33%	100%	100%	100%	100%
48	86.7%	100%	95%	93.33%	100%	100%	100%	100%
54	84.4%	100%	95%	93.33%	100%	100%	100%	100%
60	77.8%	100%	95%	93.33%	97.80%	100%	100%	100%
66	53.3%	100%	95%	93.33%	97.80%	100%	100%	100%
72	42.2%	100%	95%	93.33%	97.80%	100%	100%	100%
78	35.6%	100%	93.33%	93.33%	97.80%	100%	100%	100%
84	24.4%	100%	93.33%	93.33%	97.80%	100%	100%	100%
90	0%	100%	90%	93.33%	97.80%	100%	100%	100%
96	0%	100%	90%	93.33%	97.80%	100%	100%	98.67%

WEIGHT

No significant differences were observed in the weight of the animals before and after the

submergence treatment, either between species or treatments in Spain ($F_{1,8} = 0.58016$, p = 0.47) (Figure 6A). However, a significant increase in

weight was observed in the Brazilian species L. angulifera (mean: + 0.2353 g) ($F_{1,8}$ = 6.0557, p = 0.04) during the submergence treatment

when compared to the control. The results of this experiment with *L. flava* showed no weight variation between treatments (Figure 6B)

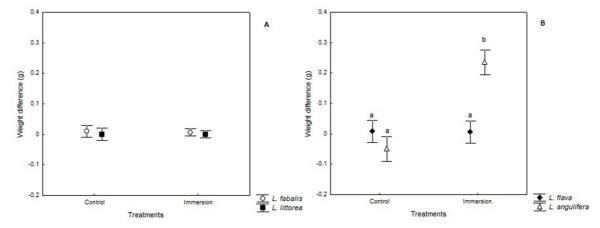


Figure 6. Mean weight difference (final-initial) (± SD) (%) of Littorinidae species between the control and submergence treatments in Spain (A) and Brazil (B). Different letters indicate significant differences according to Tukey's HSD.

DISCUSSION

The results obtained in the experiments simulating environmental conditions in extreme climatic events indicate that estuarine Littorinidae species respond to disturbances. The effect of drought or water scarcity may affect species in both Brazil and Spain in the event of extreme environmental conditions. Higher mortality rates and greater weight loss were observed in the desiccation treatment compared to the control treatment in both countries.

Desiccation is considered an important stress factor, limiting the distribution and abundance of many littorinids in the intertidal region (Chapman, 1997; Rolán-Alvarez et al., 2015; Leeuwis and Gamperl, 2022). Mortality is one of the most substantial pressures in response to this extreme event, which can remove up to 99% of organisms from a population (Moreno and Møller, 2011). These data corroborate those obtained in this study, in which, at the end of eight days under a stressful drought condition, all the species tested responded with mortality; L. fabalis showed a 100% mortality rate at the end of the experiment. Similar results were also reported in *L. angulifera*, with mortality beginning on the third day and few individuals surviving after 15 days without access to water sources under experimental conditions (Tanaka and Maia, 2006).

The data presented in this study also show that littorinids can suffer weight loss when exposed to drought conditions; this effect is probably due to water loss, which accelerates the mortality process. A body reduction of up to 4% caused by water loss in experiments that simulate climate change have been demonstrated in marine invertebrates, affecting weight and survival (Sheridan and Bickford, 2011). Seven Littorinidae species were examinated for body water loss during emersion and obtained similar results (Britton, 1992). Whereas Iacarella and Helmuth (2011), in experimental studies, also related greater weight loss with a decrease in internal water reserves for L. irrotata (Say) and Boehs and Freitas (2022) observed greater protection against desiccation with larger shell sizes of L. angulifera in Brazilian mangroves.

Although this study used individuals of similar body size (Table 1), the Littorinidae species studied obviously have different body sizes. Thus, the desiccation experiment also showed that the snail species with smaller body sizes (*L. fabalis* and *L. flava*) lost more weight than the larger species (*L. littorea* and *L. angulifera*) and experienced higher mortality rates in both

countries. The body size of an animal is a trait that influences fitness, determining its survival ability because the acclimation capacity is better optimized in larger body sizes than in smaller ones (Darnell and Darnell, 2018).

In Littorinidae, the overall larger shells can increase their internal water supply, promoting greater resistance to desiccation (Vermeij, 1972; Chapman, 1997; Moutinho and Alves-Costa, 2000; Tanaka and Maia, 2006; Rolán-Alvarez et al., 2015). The increase in weight observed in this study in *L. angulifera* exposed to the submergence treatment corroborates this previous observation, as the animal possibly has a greater internal water storage capacity than the other species tested, resulting in higher survival values when under desiccation conditions.

The results presented here may be more critical for the Brazilian species than for the Spanish species, based on the scenarios mentioned in the Intergovernmental Panel on Climate Change (IPCC) reports, indicating that the Brazilian northeast may suffer a decrease in its water resources, which is not expected in Spain. Tropical species have higher physiological limits when compared to temperate species, but also exhibit low acclimatization response (Vinagre et al., 2015; Vinagre et al., 2018; Maia and Troncoso, 2022). Thus, tropical Littorinidae may be at risk in environments subject to extreme climatic events that cause desiccation stress (Marshall et al., 2018; Brahim et al., 2018). Differences in shell morphology or size and in survival of littorinids at different latitudes were also observed by Matos et al, (2020) for Echinolittorina lineolata (d'Orbigny, 1840) and Bosso et al, (2022) for L. saxatilis (Olivi, 1792).

According to the data presented here, the effect of excessive precipitation and/or coastal flooding on Littorinidae snails will be more intense in Spain, especially for *L. fabalis*, which has the highest mortality rate (100%) among the species studied. In Brazil, there were no significant differences in survival among individuals in the treatment and control groups.

Hence the fact that the scenarios in the IPCC reports for Spain indicate that the frequency, intensity, and/or amount of heavy precipitation in that country are different from those predicted

for Brazil. Although there is some disagreement about this increase in the frequency of extreme events, experiences of intense rainfall already occur in Spain, with monthly precipitation of almost 1,000 mm in the north (Monjo et al., 2016). Changes in the pluviometric regime and elevations in mean sea level hold the potential to alter existing hydrological and biogeochemical regimes (Smith, 2011), seriously endangering animal biodiversity and its ecological balance (Scheffers et al., 2014; Boersma et al., 2016; Sergio et al., 2018).

During extreme precipitation events, salinity usually decreases in estuaries (Parada et al., 2012), but there are no specific forecasts for these environments. However, increased sea level rise with frequent flooding of high marsh areas is consistent in predictive models (Stocker, 2013) and its effect on snails can be observed without any variation in salinity, modifying primary and secondary productivity that provides resources and habitat for organisms and leads to species extinction or changes in their geographical distribution (Mcmahon, 1988; Ng et al., 2017; Zajac et al., 2017).

The results obtained in this study indicate that Littorinidae snails can respond to these disturbances by exhibiting the aforementioned changes in survival rates and weight loss. Each species reacts to the disturbance by an integrated response strategy with clear latitudinal differences. Understanding animal responses and adaptations to extreme weather events is urgently needed to better estimate potential future impacts.

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AUTHOR CONTRIBUTIONS

- R.C.M.: Conceptualization; Investigation; Writing original draft; Writing review & editing.
- J.S.T.: Methodology; Supervision; Analysis; Investigation; Writing review & editing.

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