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Surviving in the urban jungle: The role of foam nests as thermal insulator in *Pleurodema borellii* (anura: Leptodactylidae)

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ABSTRACT

Urbanisation stands as a primary driver of biodiversity loss globally, reshaping natural landscapes and imposing novel environmental conditions upon organisms. This ecological novelty poses challenges, and species capable of thriving in urban environments are considered tolerant. Among vertebrates, anurans are the most susceptible to land-use changes, mainly due to their life history and morphological traits. They exhibit a great diversity of reproductive strategies and modes, among which the foam nest plays several important roles, such as moderating or buffering adverse conditions. In this study, we assessed the foam nest of *Pleurodema borellii*, an urban tolerant species, as a potential thermal insulator in the city of San Salvador de Jujuy, Argentina. We carried out our surveys within San Salvador and surrounding areas, focusing on urban and peri-urban environments. We measured the height, length, and internal temperature of 48 foam nests, as well as the temperature of their immediate environment (water and air at one cm above the nest), over consecutive days from the first day until the nest disappeared. Finally, we collected four to five embryos from each foam nest once a day to identify their developmental stage. Our results suggest that foam nests not only mitigate low temperatures but also buffer against the heat of urban environments. These findings suggest that the foam nest could be one of the traits that facilitates the occurrence of this anuran species in the city and therefore may explain its tolerance to urbanisation.

1. Introduction

Urbanisation is a process in continuous growth that transforms the structure, composition, and dynamics of natural landscapes, and it is recognized as one of the main causes of biodiversity loss around the world (McDonnell et al., 1997; McKinney, 2006; Czech et al., 2000). As cities expand, drastic alterations in land cover take place, causing a decrease in native vegetation and an increase in impervious and dry surfaces (Hamer and McDonnell, 2008). These changes, combined with heat emissions from human activities (i. e. transportation and industrial processes), and solar irradiance absorbed and re-radiated by buildings and paved roads, along with the local greenhouse effect caused by pollutants, contribute to the increase in air temperature. As a result, temperatures in urban areas are often higher compared to less urbanised or rural areas, and more noticeable during the night (Pickett et al., 2001; Rizwan et al., 2008; Gaston et al., 2010). This microclimatic phenomenon is known as urban heat island (UHI), and has received much attention from researchers in recent years (Caorsi et al., 2019; Chown and Duffy, 2015; Cunnington and Fahrig, 2010; González del Pliego et al., 2019; Kousis and Pisello, 2020). The intensity of the UHI phenomenon depends on the geographic location, population density, and the specific characteristics of the city (Grimm et al., 2008; Munzi et al., 2014), imposing new and variable environmental conditions that represent an ecological novelty (Barlow et al., 2007). Some species are more or less susceptible to these changes, depending on their environmental tolerance ranges (Hamer and Parris, 2011; Rubbo and Kiesecker, 2005). Those species that manage to persist and thrive in the city through different physiological and behavioural mechanisms are considered tolerant to urbanisation (McKinney, 2002).

Amphibians are currently the most threatened group of vertebrates, showing a dramatic decline in their populations worldwide (Alford and Richards, 1999; Stuart et al., 2004; Wake, 1991; Wyman, 1990). It has been reported that amphibians are particularly vulnerable to the effects of urbanisation due to their life history and morphological traits, which include permeable skin, limited mobility, and a complex life cycle that generally involves an aquatic tadpole stage and terrestrial adults (Navas

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and Otani, 2007; Wake and Koo, 2018). Adults and tadpoles generally occupy different environments, therefore changes in the landscape due to anthropogenic activities will have different consequences for each. While adults can escape adverse thermal conditions by moving for shelter or water to rehydrate, their embryos cannot move from the place they were deposited (Guevara-Molina et al., 2020). Although embryos display varying thermal tolerances and consequently employ diverse strategies to mitigate high-temperature stress, such as early hatching, there is still a substantial risk of mortality if such adverse conditions persist (Anderson and Andrade, 2017; Guevara-Molina et al., 2022).

In the city of San Salvador de Jujuy, located within the Yungas Forest in northwestern Argentina (Burkart et al., 1999), previous research has reported that the degree of urbanisation negatively impacts the taxonomic and functional diversity of anuran species, indicating the existence of a habitat filter that may be restricting the presence of certain anuran species that have complex habitat requirements, while allowing more generalist species to thrive in these altered environments (Pereyra et al., 2020). The study suggested four species -*Physalaemus cuqui*, *Physalaemus biligonigerus*, *Pleurodema borellii*, and *Leptodactylus macrosternum*-as potentially urban-tolerant (Pereyra et al., 2020). One common functional trait shared by these tolerant species is that they all lay their eggs in foam nests that float on the surface of the water. Foam nests in anurans have evolved independently multiple times, primarily, but not exclusively, during significant climatic events like the Paleocene-Eocene thermal maximum, reflecting a complex interplay of behavioural and morphological changes, structural adaptations, and environmental pressures (Faivovich et al., 2012; Pereira et al., 2017). Several studies have reported that foam nests provide multiple adaptive functions, protecting offspring from suboptimal conditions during embryogenesis and in the later stages following hatching (Gould, 2021). For example, foam nests allow for increased variation in egg size within a clutch, serving as a bet-hedging strategy that enhances reproductive success in unstable habitats (Pupin et al., 2018). Additionally, foam nests have been shown to provide insulation for embryos against temperature variations, which is critical for survival in environments with inconsistent thermal conditions (Dobkin and Gettinger, 1985; Kusano et al., 2006; Méndez-Narváez et al., 2015; Rodrigues et al., 2020). The distinctive biochemical properties of the foam, including specific proteins that enhance resilience against environmental stress and protect eggs from fungal infection, further contribute to the survival of eggs and larvae in challenging habitats (Fleming et al., 2009). Collectively, these functions highlight the foam nest's significance as a key innovation that has allowed species to endure and adapt to various environmental challenges, facilitating the colonisation of previously inaccessible areas, promoting diversification and survival in new ecological niches (Pereira et al., 2017). Therefore, understanding the ecology of foam nests can provide valuable insights into how species may cope with future environmental challenges (Fischer, 2023). In the context of urban expansion, foam nests may offer a crucial adaptive advantage that enhance the resilience and survival of anuran species. However, it remains unclear whether foam nests function similarly in urban habitats as they do under natural conditions, given the unpredictability of urban environments, such as increased temperatures and their variability.

The aim of this study was to explore the potential role of the foam nest of *P. borellii* as a thermal insulator in environments with different levels of urbanisation (urban vs peri-urban areas) and sunlight exposure (direct sunlight, cloudy, and under shade). We hypothesise that the foam nest of *P. borellii* contributes to the species tolerance to urbanisation by attenuating the high environmental temperatures found in the city. We predict that the size of the foam nests, characterised by their length and height, will be larger in urban areas than in peri-urban areas and it will correlate with nest temperature. Secondly, we expect that foam nests in urban environments will present higher temperatures and significant thermal differences from their immediate surroundings (water and air at one cm above the nest) compared to those found in peri-urban areas. Lastly, we expect that nest height, duration, and temperature will influence the observed final embryonic development stage, with higher temperatures likely leading to more advanced developmental stages.

2. Materials and methods

2.1. Study species

The rufous four-eyed frog, *Pleurodema borellii* (Peracca 1985) is a small ovoid frog (4–6 cm; Fig. 1 b)), belonging to the Leptodactylidae family (Ferraro, 2009). Its distribution includes Bolivia and the North-western provinces of Argentina (Jujuy, Salta, Tucumán, Catamarca, La Rioja, and Córdoba) between 400 and 3000 m asl (Cabrera et al., 2017; Ferraro and Casagranda, 2009). The species is categorised as "Not threatened" according to the last assessment of the conservation status of amphibians in Argentina (Vaira et al., 2012). The amplectant pair, characterised by a male smaller than the female, reproduces during the rainy season from September to February in shallow and vegetated temporary ponds (Vaira, 2002). The reproductive pair deposits nearly 1500 eggs in a floating foam nest (Fig. 1c)), constructed from the oviductal secretions that the female releases during the amplexus, while the male shakes it using his hindlimbs (Faivovich et al., 2012; Halloy and Fiaño, 2000; Lavilla et al., 2000).

2.2. Study area

The present study was carried out from September to January during the years 2021–2023, in different areas of the city of San Salvador de Jujuy and nearby peri-urban areas, in NW Argentina (24°11′08″S, 65°17′58″W, 1300 m asl) (Fig. 1 a)). The city is located within the Southern Andean Yungas ecoregion, defined as one of the most biodiverse ecosystems in Argentina (Brown et al., 2005). Despite its relatively small size (approximately 19 km2) and a population of around 300.000 inhabitants, according to The National Institute of Statistics and Censuses INDEC, (2022), the city is undergoing significant urban development but still retains numerous green areas and some urban reserves, with remnants of native forest surrounding it, providing shelter to many species.

We classified sites into urban and peri-urban habitats based on the proportion of vegetation, buildings, and paved surface surrounding the water bodies, following the urbanisation index proposed by Pereyra et al. (2020). Urban sites featured extensive paved surfaces and buildings, whereas peri-urban areas represented transitional zones with higher vegetation cover and fewer paved surfaces and buildings. We characterised each environment by recording relative humidity and environmental temperature using a Multifunction Environmental Meter with a precision of 0.1% y 0.1 °C respectively. Measurements were taken in the morning and afternoon each time we sampled the nests throughout the entire sampling period. Within each habitat type, we actively searched for foam nests in ponds exposed to sunlight and under shade. While we located foam nests in urban ponds exposed to sunlight (USun) and under the shade (UShade); we were unable to find foam nests in peri-urban ponds under shade, and therefore we registered only foam nests exposed to sunlight (PUSun). Additionally, during our surveys in USun, we encountered both sunny days with direct sunlight and cloudy days, each presenting distinct thermal environments. Therefore, we decided to divide these data into two categories: nests under sunny (USun_s) and cloudy (USun_c) conditions. Consequently, for the analysis, we considered the four situations mentioned above as different thermal conditions. When identifying a foam nest laid the night before, we proceeded to measure its height and maximum length using a calliper with a precision of 0.01 mm. We determined both, the temperature inside the foam (Tn), and the temperature of its immediate environment by recording the temperatures of the water (Tw) and the air at 1 cm above the nest (T1cm) using a type K thermocouple (Termopar k 0,8 mm 2 TES® 1312). The size and thermal measurements of the foam nests were taken twice a day, once in the morning (between 8

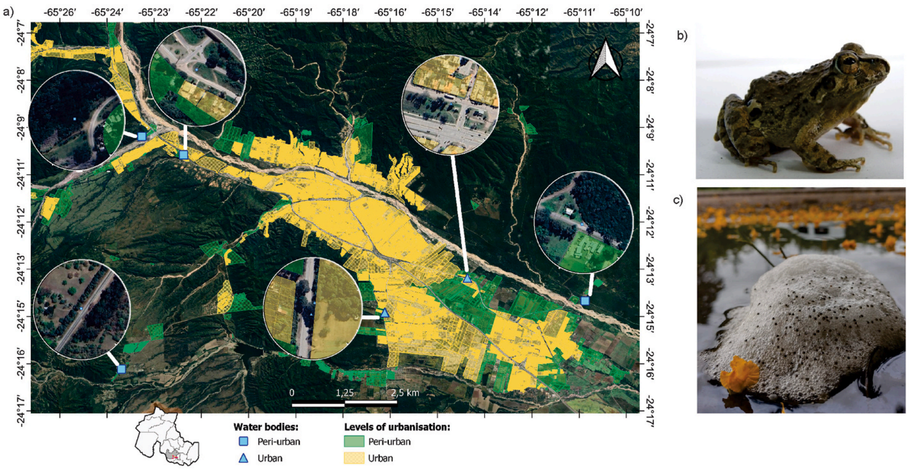

Fig. 1. a) View of the study areas in the city of San Salvador de Jujuy and nearby areas, highlighting the sampled water bodies in urban and peri-urban areas. b) Picture of an adult male of *Pleurodema borellii* from the study area. c) Foam nest made by *P. borellii* in an urban water body.

a.m. and 11 a.m.), and once in the afternoon (between 4 p.m. and 6 p.m.). This procedure was carried out from the first day of the nest in the pond until its disappearance or when no visible embryos were present. Lastly, we carefully extracted four embryos from each foam nest once a day using a pipette and then fixed them with 10% formalin in Eppendorf tubes®, following the protocol proposed by Antoniazzi et al. (2021). In the laboratory, we identified the stages of embryonic development according to Gosner (1960) using a binocular magnifying glass.

2.3. Data analysis

2.3.1. Comparative analysis of thermal conditions and nest size

To confirm that air temperature (Ta) and relative humidity (RH) were different among the four thermal conditions (USun_s, USun_c, UShade, and PUSun), we performed an analysis of variance (ANOVA), including time of the day (morning-afternoon) as a cofactor. We also compared the environmental values between morning and afternoon within each situation, separately using an ANOVA. To address the lack of independence in records taken at the same locations during the same times and days, we averaged the data prior to analysis. We assessed and confirmed that the assumptions of normality and homoscedasticity were met for both variables.

To test for differences in nest size across thermal conditions, we first visually explored the variation in nest height and length registered over consecutive days for each thermal condition. We then compared the initial size of the nests with an ANOVA, defining the nest height and length from the morning of the first day of sampling as response variables and the thermal condition as a fixed factor. We performed a DGC *post hoc* test when significant differences were found. To test the relationship between temperature (Tn) and the foam nest size, we use a mixed general linear model. We set Tn as the response variable, the initial height and length of the foam nests as independent variables, and the identity of the nest, days, and thermal conditions as random factors.

2.3.2. Comparative analysis of nest and immediate surrounding temperatures across different thermal conditions

To compare Tn, Tw and T1cm across the four thermal conditions we employed general linear mixed models (GLMM). We included the thermal conditions and nest duration as fixed terms, and time of the day (morning or afternoon) as a random factor. We also compared Tn, T1cm, and Tw between morning and afternoon within each thermal condition separately also using GLMM. For this comparison, we treated the time of the day (morning or afternoon) and days as fixed terms. In the Tn comparison, we also included nest height as a fixed term and nest identity as a random factor. For the comparison of Tw and T1cm, we defined pond identity as a random factor. Additionally, we explored the variability in Tn, T1cm and Tw for each thermal condition by estimating their coefficient of variation (CV) and its 95% confidence intervals through bootstrapping. We estimated the daily CV only for the first and second day of the nest, separately.

We assessed whether the nest attenuates the immediate environment temperatures by determining if the difference between Tn and the immediate environment (Tw and T1cm) were statistically significant using paired tests. We performed the analysis for each thermal condition (USun_s, USun_c, UShade, and PUSun) and time of the day (morning/afternoon) separately. To explore for differences between Tn and T1cm, we used a paired *t*-test; whereas to test for differences between Tn and Tw, we used a non-parametric Wilcoxon paired test. A positive mean value resulting from the difference between Tn and its immediate environment indicates that the foam nest exhibits a higher temperature. Conversely, negative mean values indicate that Tn is lower than the temperature of either immediate environment.

2.3.3. Embryonic developmental stages

To examine the influence of foam nest size, its duration over the days, and its temperature on the final embryonic developmental stage, we first visually explored the mean values and standard deviation of the different stages of embryonic development registered over consecutive days under each thermal condition. We compared the observed final developmental stages across thermal conditions with a Kruskall Wallis test. Then, we examine the relationship between the observed final development stage and the predictor variables: initial nest height, Tn, and nest duration using ordinal regression analysis. Given the small sample size, we transformed the continuous variables into categorical variables to avoid issues of perfect separation. This approach enabled more stable model fitting and clearer interpretation of results.

All model assumptions were explored using graphical methods (Zuur et al., 2009). Analyses were carried out with the InfoStat (2008) and the figures were made with the ggplot2 package (Wickham, 2016) from the free software R program (R Core Team, 2022).

3. Results

3.1. Comparative analysis of thermal conditions and nest size

Urban environments exposed to sunlight (USun_s) presented a significantly higher air temperature (Ta) than the other conditions (Supplementary Fig. 1). In regards to relative humidity (RH), urban environments measured under cloudy days (USun_c) presented significantly higher RH values than USun_s and UShade (Supplementary

Fig. 1). We did not find a significant difference when comparing the values of RH and Ta between morning and afternoon within the three thermal conditions exposed to sunlight. In contrast, UShade exhibited significantly lower Ta and higher RH values in the morning compared to the afternoon (Supplementary Fig. 2).

We sampled a total of 58 foam nests; however some nests (10) were excluded from the analyses due to complete destruction caused by various factors such as heavy rains, vehicular activity, or, in some instances, the construction of new nests atop the existing ones. Consequently, we used 10 nests from USun_s (two ponds), 12 from USun_c (one pond), 10 from UShade (one pond), and 16 from PUSun (four ponds) for the analysis. The foam nests in USun_s had an average duration of one and a half days, with a maximum of two days. Nests from PUSun and USun_c also presented an average duration of one and a half days, with a maximum of three days. Conversely, foam nests in UShade exhibited an average duration of four days with a maximum of five days (Fig. 2 a)).

We found no differences in the initial length of nests among the four conditions ($F = 1.10$, $df = 3$, $p = 0.36$) (Fig. 2 a)). However, we did find significant differences in nest heights ($F = 11.73$, $df = 3$, $p < 0.001$), with nests from PUSun being significantly shorter than those in urban environments. Additionally, nests from USun_s and USun_c were significantly higher than those from UShade (Fig. 2 a)). Regarding variation in height and length of nests throughout the days, we observed a decrease in height, with nests from USun_s and USun_c presenting a relatively more pronounced slope compared to those from PUSun and UShade (Fig. 2 b)). On the other hand, nests from UShade showed a relatively constant decrease in their length, whereas nests from the three thermal conditions exposed to the sun (PUSun, USun_s, and USun_c) showed a marked increase in their length through the days; near the end their length showed an abrupt decrease as the foam started to disintegrate (Fig. 2 b)). With respect to Tn, a positive and significant relationship was found with the nest height ($t = 2.36$, $df = 141$, $p = 0.019$); however, no significant association was observed with nest length ($t = 1.56$, $df = 141$, $p = 0.121$).

3.2. *Comparative analysis of nest and immediate surrounding temperatures across different thermal conditions*

Nests temperatures (Tn) differed significantly across the four thermal conditions ($F = 22.92$, $df = 3$, $p > 0.01$), with nests from USun_s presenting hotter temperatures (mean Tn = 32.16 ± 1.47), followed by nests from PUSun and USun_c (mean Tn = 27.30 ± 1.40; mean Tn = 25.74 ± 1.42, respectively), and lastly, the coldest nests were from UShade (mean Tn = 20.67 ± 1.42) (Fig. 3 a)), b)). We also observed significant differences in T1cm and Tw across thermal conditions (Tw $F = 20.79$, $df = 3$, $p < 0.01$, T1cm $F = 24.60$, $df = 3$, $p < 0.01$), with higher values of Tw and T1cm in USun_s (mean Tw = 31.44 ± 1.66; mean

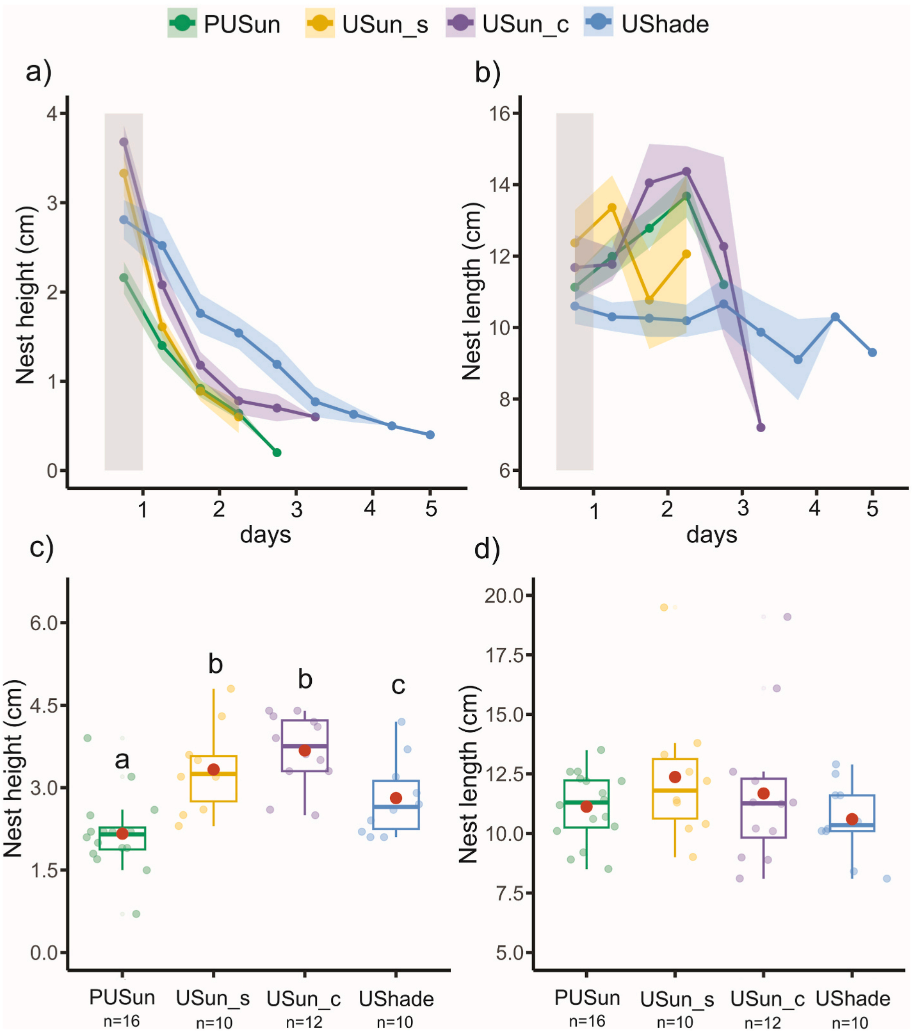

Fig. 2. Mean values and standard deviation in the a) Nest height and b) Nest length in urban (USun_s, USun_c, and UShade) and peri-urban environments (PUSun) throughout their duration. Boxplots showing ANOVA results of the differences in initial c) Nest height and d) Nest length across the four thermal conditions. Red dots indicate mean values. Different letters denote significant differences according to the *post hoc* test.

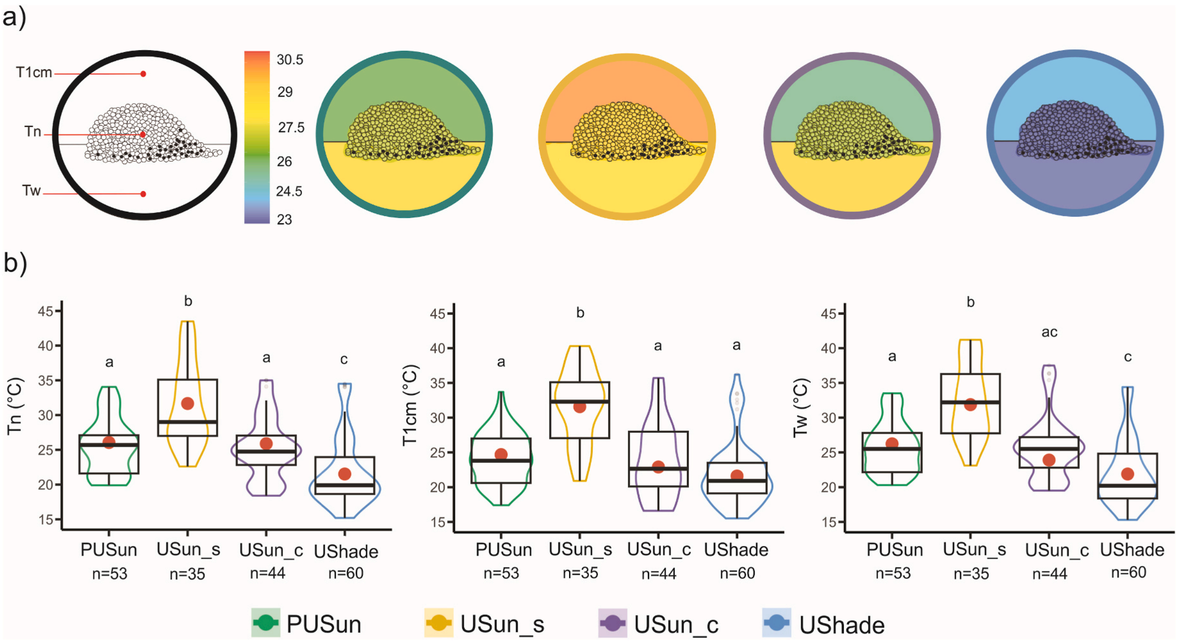

Fig. 3. a) Diagrams summarising the thermal characteristics of the foam nests of *P. borellii* (Tn) and their immediate thermal environment: air temperature at 1 cm (T1cm) and water temperature (Tw), measured in the afternoon of the first day at each thermal condition. b) Violin plots showing the results from the general linear mixed models comparing the thermal characteristics of *Pleurodema borellii* foam nests (Tn) and their immediate surroundings, T1cm and Tw, across the four thermal conditions. The black horizontal line indicates the median, the box represents the interquartile range (IQR), and red points indicate mean values. Different letters correspond to significant differences according to the *post hoc* analysis.

T1cm = 31.35 ± 1.49) compared to the other three thermal conditions (USun_c mean Tw = 22.91 ± 1.84, mean T1cm = 22.38 ± 1.66; UShade mean Tw = 21.89 ± 2.58, mean T1cm = 21.60 ± 2.24; PUSun mean Tw = 26.24 ± 1.43, mean T1cm = 24.78 ± 1.49) (Fig. 3 b)). Tn and its immediate thermal environment showed an increase from morning to afternoon in PUSun, USun_c, and UShade. In contrast, USun_s showed a significant decrease in temperature between these time periods (Supplementary Fig. 2).

On the first day of nests, daily variation in Tn was significantly higher in USun_s nests compared to PUSun (Table 1). Daily variation in T1cm also showed significant differences, with higher estimated CV values in USun_s and USun_c. Tw did not show significant differences in their CV (Table 1). On the second day, nests from UShade showed a higher daily variation in their temperatures compared to the ones from the other situations, this CV value was significantly different when compared to USun_c, which showed the lowest CV. Lastly, daily variation in T1cm in the second day was significantly lower in USun_c, while CV for Tw was similar in all situations (Table 1).

Table 1
Coefficients of variation and 96% confidence intervals (in parenthesis) estimated by bootstrapping. Variables with significant differences across situations are marked in bold, and different letters denote the significant differences.

Days of nests	Variables	PUSun	USun_s	USun_c	UShade
Day 1	**Tn**	13.8 (10.1–16.0) a	20.3 (16.9–23.8) b	15.45 (11.9–19.0) ab	14.82 (9.8–20.6) ab
	T1cm	10.7 (7.6–13.9) a	17.4 (12.2–21.9) b	20.3 (15.3–24.9) b	12.5 (8.9–15.7) a
	Tw	13.3 (8.9–17.0)	14.3 (13.5–21.2)	16.2 (10.8–20.2)	14.7 (10.3–19.6)
Day 2	**Tn**	16.3 (12.5–19.7) ab	16.7 (13.1–19.5) ab	12.4 (9.9–14.1) a	21.4 (13.5–27.1) b
	T1cm	16.5 (12.2–20.1)	15.8 (12.5–18.9)	18.1 (13.9–21.2)	17.7 (11.1–23.2)
	Tw	13.7 (11.0–16.1) a	16.79 (14.3–17.8) b	11.9 (9.8–13.8) a	20.8 (12.7–26.1) ab

The t-tests showed that the differences between Tn and T1cm were positive and significant during the morning of the first day in PUSun and USun_s (Fig. 4). In the afternoon, these thermal differences were positive in PUSun and USun_c, but negative in USun_s, indicating that in the later condition, Tn was significantly lower than T1cm. The nests from UShade did not show significant differences in their temperature compared to T1cm until the morning of the third day. There were no significant differences between Tn and Tw in PUSun, but we observed a positive relationship in the morning of the first day in USun_c, and a negative relationship in the afternoon of the first day in USun_s and in the first and second day in USun_c. In the case of nests from UShade, this difference was significant and negative during the first day of measurements, and during the morning of the third day (Fig. 4).

3.3. Embryonic developmental stages

The embryos from UShade, USun_c, and PUSun exhibited earlier Gosner developmental stages during the initial days of nests, ranging from stages 2–5 (i.e. from after fertilisation until the third cleavage), compared to those from USun_s, which were between stages 8–11 (i.e. from the mid-cleavage, as the blastomeres continue to decrease in size, to the period of the blastopore formation). Further, most of the registries from the final day of nests from USun_c and USun_s were (median and maximum values), at stage 21 (i.e. when the tail fins remain opaque and the embryo's cornea becomes transparent, rendering the eyes clearly visible) and 22 (i.e. when the tail fins become transparent, circulation begins within them, and the operculum covering the external gills starts to form), respectively. Lastly, most embryos registered in the last days of nests from PUSun and UShade were at stage 20 (median values) (i.e. when circulation becomes visible through the external gill filaments, indicating the development of the circulatory system, along with the onset of muscular responses), although the maximum developmental

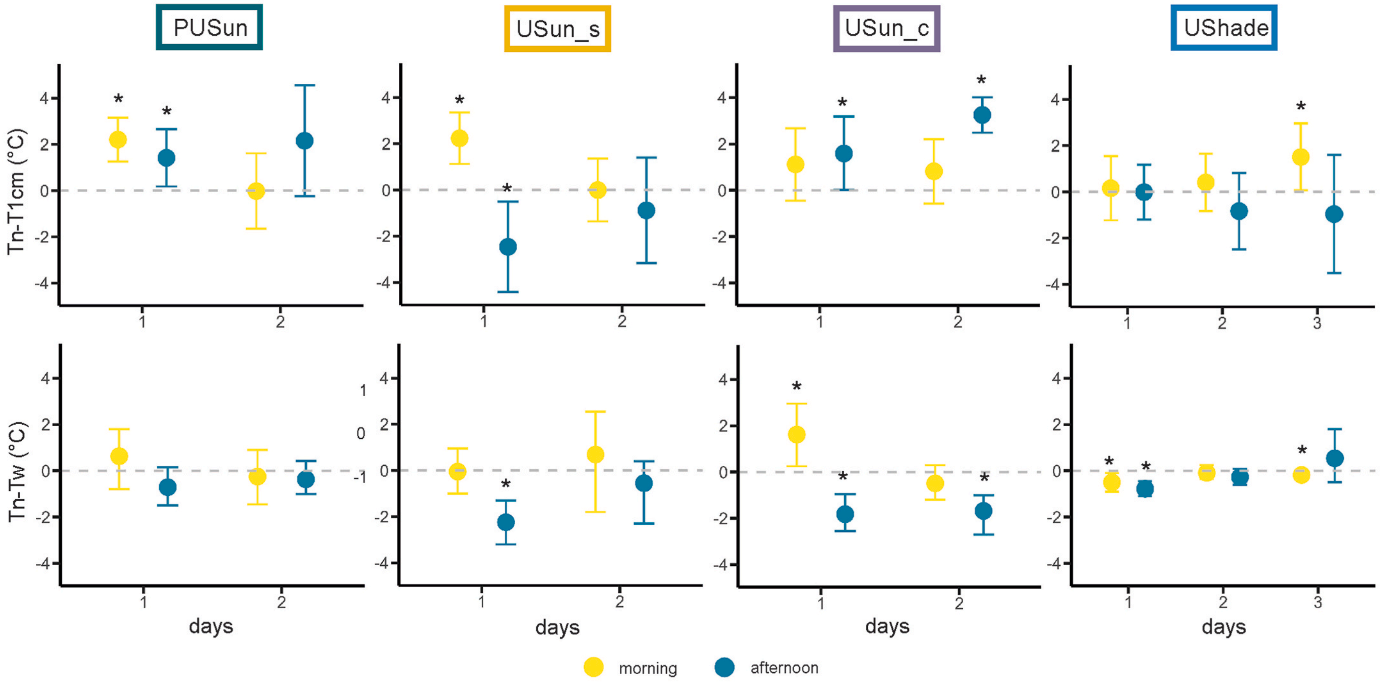

Fig. 4. Results of the paired *t*-test and the non-parametric Wilcoxon paired test exploring the differences between the temperatures of the foam nests (Tn) and their immediate thermal environment (T1cm and Tw) respectively, conducted in the morning and afternoon in the four environmental thermal conditions, throughout the duration. *Asterisks denote significant differences in the paired tests. Dashed grey line highlights the zero.

stages registered in these thermal conditions were stage 23 (i.e. when the operculum continues to develop and the external gills begin to disappear, it marks the transition to the larval phase) (Fig. 5). It is interesting to note that embryos from UShade and USun_c exhibited a high variation in the developmental stages compared to those from PUSun and USun_s. Observed final developmental stages were significantly different across thermal conditions (Kruskal Wallis H = 9.06, p = 0.02), with the final embryonic stages in USun_s being significantly more advanced than those observed in the other thermal conditions, according to the *post hoc* test. The ordinal regression analysis revealed that Tn ($\chi^2 = 4.46$, df = 3, p = 0.02) and duration of nest ($\chi^2 = 7.6$, df = 3, p = 0.05) were significant predictors of the observed final developmental stage of embryos, indicating a positive relationship.

4. Discussion

We demonstrated that the foam nest of *Pleurodema borellii* insulates embryos from low environmental temperatures while also attenuating high environmental temperatures. We found that foam nest temperature is related to nest height and varies across different thermal conditions. Additionally, we found that the nest temperature significantly differs from that of its immediate environment, and that these temperature differences across thermal conditions have a significant impact on embryonic development. Our results provide support to our hypothesis that foam nests might be one of the key traits contributing to this species' tolerance to urbanisation.

The environmental characterisation, based on air temperature (Ta) and relative humidity (RH) measurements across the different thermal

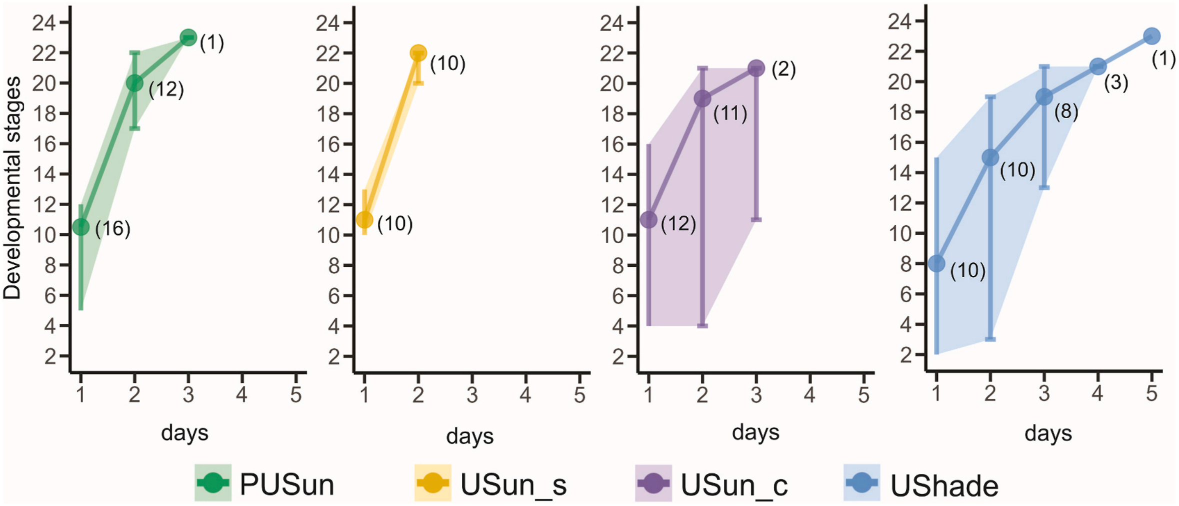

Fig. 5. Developmental stages found in nests of *Pleurodema borellii* in each thermal condition throughout the days. The points represent the mean values, and the shaded areas represent the standard error. The number of nests surveyed each day is indicated in parenthesis.

conditions, confirms the presence of an urban heat island effect. This effect is more evident in urban areas exposed to sunlight, which exhibit higher temperatures and lower humidity levels compared to the peri-urban areas. These findings highlight the diverse thermal conditions experienced by the embryos of this species.

Nests exhibited variations in both size and duration across the different thermal conditions. Urban nests exposed to sunlight were higher than those under the shade and from peri-urban areas, and their duration appeared to be also influenced by the intensity of sunlight exposure (Fig. 2). As nest height is associated, among other factors, with the time spent by the amplectant pair in constructing it (Rodriguez Muñoz et al., 2019), our results suggest that individuals in urban environments may be investing more time in nest construction. Amphibian embryos are susceptible to desiccation since they lack an impermeable and protective membrane (Touchon and Warkentin, 2008), therefore higher foam nests may keep eggs off the surface, preventing them from drying out (Dalgetty and Kennedy, 2010). Although we did not test if higher nests prevent eggs from drying out in our study, this trait could increase their survival probability in drier environments, such as cities. Additionally, a higher nest may imply a greater surface area for heat and oxygen exchange between the nest and its surroundings. This would allow the nest to absorb heat from the sunlight, affecting and even accelerating embryonic development (Dobkin and Gettinger, 1985; Méndez-Narváez et al., 2015). Indeed, we observed a variation in embryonic development time across the three different urban conditions, with faster development recorded in the scenario characterised by higher temperatures and greater sunlight exposure (USun_s). In contrast, in conditions with minimal or no direct sunlight, as seen in USun_c and UShade, respectively, the temperature of the nest was cooler. This led to slower embryonic development compared to USun_s, reflected in the longer duration of nests and relatively earlier developmental stages when released into the water (Fig. 2), even though nests heights were similar. On the other hand, nests in USun_c and PUSun exhibited similar temperatures and durations, despite significant differences in their heights. Given that nest height showed a positive and significant relationship with Tn, suggesting that this trait enhances heat absorption (Dobkin and Gettinger, 1985), one might expect USun_c nests to have at least slightly higher temperatures than PUSun nests, even under similar thermal conditions (i.e. Tw and T1cm). The fact that we did not observed this could be explained by differences in sunlight exposure intensity between the two situations: while PUSun nests are under direct sunlight, USun_c nests are also exposed to sunlight but potentially at a different intensity due to cloud cover (Dobkin and Gettinger, 1985).

The characterisation and comparison of nests across different thermal conditions revealed an interesting variation in their temperatures, which followed a similar pattern to that observed in the immediate thermal environment. Previous research has suggested a correlation between nest temperature and environmental conditions, such as air and substrate temperatures, as well as sunlight exposure (Méndez-Narváez et al., 2015; Seale, 1982). It would be worthy to explore whether the overall thermal variation experienced by the embryos of this species may be related to some level of plasticity in their thermal tolerance, both within the nest and after they are released into the water. This thermal plasticity could represent an early-stage trait that explains the persistence of the species across the diverse thermal conditions surveyed (Westneat et al., 2019; Krist, 2023). Further studies on the thermal tolerance and survival rates of embryos may help to better understand how parental adaptations interact with embryonic responses, enabling survival in urban environments.

The efficiency of the foam nest in regulating temperature relative to T1cm was evident only on the first day in USun_s and until the second day in USun_c and PUSun. After this period, most nests began to disintegrate, likely due to the increasing size and mobility of the embryos (pers. obs.) which could facilitate the dispersion of bubbles, leading to the gradual decomposition of the nest and the subsequent decline in its thermal insulation capacity. This gradual disintegration would facilitate the release of the embryos, allowing them to acclimate to potentially higher environmental temperatures (Gorzula, 1977). The ability of the nests to insulate embryos from low temperatures is evident in PUSun. We observed a significant positive difference between Tn and T1cm during the morning in this condition, and despite a temperature increase from morning to afternoon, this positive difference persisted throughout the afternoon. The absence of difference between Tn and Tw suggests that the water influences the nest temperature in this condition (Méndez-Narváez et al., 2015). The positive difference between Tn and T1cm is also observed in USun_c, which presents a similar immediate thermal environment as PUSun, reaffirming that nests are acting as insulators against low temperatures. Nests from USun_s showed also a positive difference during the morning of the first day, but switched to a negative difference during the afternoon, which was also observed between Tn and Tw. During the afternoon, the nest appears to function as a thermal insulator against high T1cm, which can rise up to 40.3 °C, and remained with no significant changes throughout the day. It is interesting to mention that we observed that nests from USun_s developed a dry and hard surface by the second day, unlike nests from the other thermal conditions that maintained a sticky and moist foam. This particular characteristic has been documented in previous studies, and is thought to contribute to heat retention while preventing overheating (Dobkin and Gettinger, 1985). These results are consistent with those reported by Méndez-Narváez et al. (2015), which conducted their study in a savannah environment from Colombia characterised by significant temperature fluctuations. The authors reported that the foam nests of three species from the Leptodactylidae family - *Physalaemus fischeri*, *Leptodactylus knudseni*, and *Leptodactylus fuscus* - protected the embryos from the diurnal high temperatures and kept them warmer during the night low temperatures. Other studies conducted with *Physalaemus cuvieri*, *Leptodactylus labyrinthicus, Rhancophorus arboreus*, and *Pleurodema tucumanum* have reported lower nest temperature compared to air temperature (Rodrigues et al., 2020; Fernandes et al., 2016; Kusano et al., 2006; Rodriguez Muñoz et al., 2019). These previous results, along with ours, support what was stated by Méndez-Narváez et al. (2015) regarding the thermal function of the foam nest, which is to shield the embryos from extreme high or low temperatures.

UShade exhibited a similar environmental temperature to USun_c and PUSun, but without the exposure to sunlight. This caused the foam nest to have a lower temperature than the surrounding air during the coldest hours of the morning. This result reaffirms that exposure to sunlight enables foam nests to absorb enough heat, thereby maintaining a relatively stable internal temperature even during periods of low immediate environment temperatures (Dobkin and Gettinger, 1985). This phenomenon has also been observed in the foam nests of other species, such as *Engystomops pustulosus*, which showed an increase in nest temperature as a result of exposure to sunlight during the day (Dobkin and Gettinger, 1985). In other cases, such as *Leptodactylus knudseni* and *Leptodactylus labyrinthicus*, foam nests also exhibited temperature fluctuations due to differences in sun exposure at the oviposition site (Shepard and Caldwell, 2005).

Lastly, we observed that in all urban thermal conditions, the nest was warmer than the surrounding water during the afternoon for at least the first day. This may suggest that afternoon water temperatures could be harmful to the embryos and larvae, and therefore this increased thermal insulation might be protecting them from detrimental thermal stress. All together, these findings raise prompt questions regarding the potential impact on the survival rate of the tadpoles within the nest and even post-release.

The discrepancies observed in developmental stages across the different thermal conditions over the days may explain the variation in nest length over time. Embryos in the early stages have limited movement capacity, allowing the nest's height to decrease without completely disintegrating. When the embryos from the four thermal conditions reached Gosner stages 18–20, we observed a reduction in nest length, likely due to more active embryo movements contributing to the nest disintegration. The accelerated embryonic development and disintegration of foam nests in USun_s suggest a temperature-driven influence on morphogenesis. This may lead, along with other factors, to embryos being released into the pond earlier, potentially as a mechanism to avoid adverse thermal conditions, such as high and variable temperatures (Guevera-Molina 2020). The embryos recorded in the last day of nests were between the Gosner stages 20 and 23. These stages exhibit differences in gill development, while at stage 20 gills show a progressive development of filament, at stages 20–23 it shows a complete development (Gosner, 1960). While an early release into the water may expose embryos to predation by their conspecifics (Halloy, 2006), it could represent an adaptive strategy to overcome the unfavourable conditions prevalent in urban environments. Further, the considerable high variation in the Gosner larval stages recorded on the last day in USun_c, with one individual still at very early Gosner stages (i. e. stage 11), may indicate the importance of sunlight exposure intensity. Nonetheless, given the low number of embryos recorded at these stages, further studies are needed to determine whether this represents a consistent pattern or an anomaly. Nests from USun_c and USun_s shared similarities in height and length but differed in the intensity of sunlight the nests received. Therefore, the small variation in Gosner stages observed in USun_s and PUSun suggests that with direct solar sunlight, nests maintain a higher and uniform temperature, leading embryos to develop at the same rate. In the case of USun_c and UShade, reduced sunlight exposure could cause significant temperature variations within the nests. Consequently, embryos may be exposed to differing temperatures within the nest, leading to significant variations in developmental stages.

The challenge encountered in finding foam nests within shaded peri-urban environments during this study may suggest that the species exhibit oviposition site preferences influenced by the thermal landscape. It is plausible that they prefer water bodies with direct sunlight exposure, since it offers enhanced thermal stability and shorter embryo development time (Dobkin and Gettinger, 1985; Licht, 1971; Seale, 1982). Additional research on oviposition site selection is essential to test this hypothesis.

Prior studies on thermal ecology of foam nests were conducted under non-urbanised conditions. Therefore, in these cases the observed thermal variations and properties of nests were mainly given to geographic variation. To our knowledge, this is the first study to investigate thermal properties of foam nests, incorporating urbanisation as a disturbance factor. In our investigation, we found that under natural conditions, the nest provides thermal insulation to keep the embryos warm during the low morning temperatures. However, when considering urban environments, where we registered values of T1cm over 40 °C, the nest serves as a thermal buffer, safeguarding the embryos from potentially harmful high temperatures. It has been documented for other species, such as *Rhacophorus arboreus*, that temperatures exceeding 30 °C can have a negative impact on embryo survival (Kusano et al., 2006). In the case of *Agalychnis callidryas*, it was found that 41 °C was a lethal critical temperature (Guevara-Molina et al., 2020). In the case of *Pleurodema borellii*, a considerable number of unhatched embryos were observed in the nests from USun_s compared to those in the other thermal conditions (*pers obs*). This suggests that foam nests in urban environments may be exposed to critical and potentially lethal temperatures for the embryos. However, further studies on the thermal ecology of embryos and hatching success are needed to confirm this hypothesis.

5. Conclusion

The current expansion of urban environments creates a need to understand how organisms respond, cope, and adapt to urbanisation (Isaksson, 2015). Understanding the ecological and life history traits of anuran species associated with its tolerance or avoidance to urban environments could help improve our knowledge of the relationships between diversity and urbanisation (Callaghan et al., 2019). In this context, identifying not only those species sensitive to anthropogenic disturbances, but also which stage is the most vulnerable, will enable more effective measures for territorial planning and actions for biodiversity conservation (Leong et al., 2016; Socolar et al., 2016).

Our results showed several differences in the nests across the thermal conditions studied, with nests from the urban environment exposed to direct sunlight being higher, having shorter duration and exhibiting higher temperatures than those from the other thermal conditions. Furthermore, we demonstrated that the foam nests of *P. borellii* insulate the embryos from low morning temperatures as well as from the high afternoon temperatures registered in urban areas. We also observed differences in the final embryonic developmental stage, with larvae from urban environments exposed to direct sunlight being released into the water within a shorter period of time and at a significantly more advanced stage of developmental than those from the other thermal conditions. These results support our hypothesis that the foam nest is one of the key traits that facilitates the persistence of *P. borellii* in urban environments. Further studies are needed in order to explore whether the variation in nest height between urban and non-urban environments is due to differences in behaviour (i.e. time spent constructing the nest) or differences in reproductive effort. Additionally, evaluating thermal tolerances and survival rate of all stages (i.e. embryos, larvae, and adults) would help assess whether the stressful conditions of the city could negatively impact the persistence of *P. borellii.*

CRediT authorship contribution statement

Ana Boggio: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Laura Pereyra:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data accessibility statement

The processed data required to reproduce the above findings cannot be shared at this time due to technical limitations. Based on these results, we aim to expand the research and incorporate new topics to further elucidate the presence of anurans in the urban ecosystem and their strategies for surviving in the urban heat island. We have not yet determined which or how much of the data will be used, but given the possibility that sharing the data could hinder future research, we prefer not to disclose it at this time.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2024.104022.

Data availability

Other (please explain: e.g. ‘I have shared the link to my data as an attachment’).

Pleurodema borellii foam nests (Original data) (Mendeley Data) https://data.mendeley.com/datasets/wz6tgyd6n5/1

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