Climate drives anuran breeding phenology in a continental perspective as revealed by citizen-collected data

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Abstract

Aim: As anuran reproduction is generally linked to the availability of water, frogs and toads are particularly sensitive to climate. We tested the effect of climate on anuran reproductive phenology and daily activity by analysing temporal patterns of reproductive behaviour based on citizen-collected observations.

Location: Brazil.

Methods: We obtained vocalizations and photographs of frogs with inflated air sacs, as well as images of amplexant couples, nests, eggs or tadpoles in initial stages of development from iNaturalist. We analysed hourly patterns, seasonality and duration of the reproductive period using circular statistics in different climate types and tested phylogenetic signals. We analysed data for Bufonidae, Hylidae, Leptodactylidae and Phyllomedusidae in detail. We also reviewed relevant literature.

Results: Among the 8478 (acoustic and photographic) records, 738 (8.7\%) had evidence of reproduction with 284 acoustic records and 454 photographs, representing 184 taxa identified at the species level belonging to 16 families. Climate affected the period and duration of the reproductive season, as well as daily patterns of vocalization. These results were considered phylogenetically independent, as the reconstructions of ancestral character states did not suggest strong phylogenetic signals for temporal patterns of vocalization or reproduction. In Brazil, most frogs reproduce between October and January. Patterns were similar to the results of the literature review, however in the literature data, Bufonidae start reproduction 2 months earlier, and many Hylidae species reproduce until February. In general, frogs from warmer and drier climate regions had shorter and aggregated reproductive seasons, while in tropical monsoon climate they had a uniform temporal pattern.

Main conclusions: At the continental scale, reproductive phenology of anurans and their daily activity is affected by climatic conditions regardless of phylogeny. We found that community science can provide valuable information in Brazil that can be harnessed to monitor effects of climate change on amphibian reproduction.
INTRODUCTION

The availability of food, water and other resources determines the reproductive success of animals, which is usually measured by the number of offspring produced during the reproductive season (Hall et al., 2018; Wikelski et al., 2000). Favourable climate conditions increase access to these fundamental resources, while unfavourable conditions diminish access and therefore decrease reproductive success leading to population declines (Hall et al., 2018; Ogutu et al., 2015). Consequently, strategies for reproduction vary temporally among climate regions, especially considering wet and dry zones (James & Shine, 1985; Shine & Brown, 2008). In dry regions, climatic conditions can vary largely throughout the year, restricting the reproductive period of certain species to a few months or even days, while the relatively stable climatic conditions of tropical rainforests can often support year-round reproduction (Armijos-Ojeda et al., 2021; Stutchbury & Morton, 2001). Considering such patterns, changes in climate will alter when and for how long animals reproduce (Bobrek, 2021; Coppes et al., 2021). Climate change therefore can have negative demographic effects, causing population decline or even species extinction (Cahill et al., 2013; Curtis et al., 2021). In many circumstances, the behavioural responses of the animals to the change can also decrease fitness, resulting in lower population recruitment as a result of a more restricted breeding period (Cappello & Boersma, 2021).

Anurans are particularly sensitive to climate, as their reproduction is tied to the availability of water (Blaustein et al., 2001). Their reproductive season can vary from short to prolonged in arid versus tropical humid regions, respectively (Aichinger, 1987; Bertoluci, 1998), or can be flexible in response to interannual rainfall variation (Gascon, 1991). This strong dependence on climate conditions is a consequence of the physiological characteristics of anurans, including ectothermy and gas exchange through their permeable skin (Duellman & Trueb, 1994). Being ectothermic, anurans are unable to regulate their body temperature internally and face a high risk of dehydration in hot and arid environments, as they use evaporative cooling to stabilize their internal temperature (Feder & Burggren, 1992). In addition, even though anurans can reproduce in terrestrial, semiaquatic and aquatic environments, most species have external fertilization (Wells, 2010) and the survival of eggs and embryos depends on particular macro- and microclimatic conditions (Duellman & Trueb, 1994).

In local communities, the duration of the breeding season can differ among species (Abrunhosa et al., 2006; Canelas & Bertoluci, 2007), however, it is not clear whether such differences result from variation in species traits or represent a strategy to avoid competition for an acoustic niche, calling sites or egg deposition sites (Bourne & York, 2001; Chek et al., 2003; Ernst & Rödel, 2006).

Although some species are better adapted to arid habitats, extreme droughts cause anurans to cease reproduction, reduce their activity and seek refuge in shelters (Denton & Beebee, 1993; Seebacher & Alford, 1999). Empirical evidence shows that the physiological tolerance of geographically separated populations is similar and intraspecific patterns of reproduction may vary spatially (Morrison & Hero, 2003; Wells, 2010). Consequently, widely distributed frog species may exhibit variation in the timing of breeding activity among sites with different climates. For example, the southern population of the wood frog Lithobates sylvaticus reproduces in the winter, while the northern population breeds in the late spring or during the summer (Herreid & Kinney, 1967; Martof & Humphries, 1959). The same pattern can be seen in geographically distinct populations of the Japanese toad Bufo japonicus (Matsui, 1989). Similarly, in Pseudacris species breeding starts later in northern than in southern North American populations (John-Alder et al., 1989). These examples support the idea that climate drives the temporal patterns in breeding activity. Anurans presumably rely on photoperiod, temperature and rainfall cues to guide seasonal allocation of their resources to reproduction (Canavero & Arim, 2009).

During the reproductive season, anurans can also modify daily activity patterns according to short-term variations in humidity and temperature (Guerra et al., 2020; Wells, 2010). While anurans in general vocalize during the night (Duellman & Trueb, 1994; Rocha et al., 2015), many species also call sporadically or continuously during the day (Callaghan & Rowley, 2021). While the environment may be more favourable to being nocturnally active, in ectothermic species cooler temperatures during the night may impede activity. Therefore, in climate regions, where environmental conditions can change enough within a single day to hinder anuran activity, daily activity patterns can vary to a large degree.

Even though the dependence of amphibian reproduction on climate has been well studied, the relation has not been explored from a continental and multi-taxon perspective, and most data originate from studies of local communities. Revisiting this theme and showing the strength of temporal reproductive patterns in the context of climate variation is relevant to infer the potential effects of climate change on threatened amphibian species (Geyle et al., 2022; IUCN, 2021). The recognition of different temporal reproductive patterns in different climate regions can aid in monitoring the response of animals to climate change.

Citizen science (also called community or participatory science) can address many of these demands by allowing scientists to gather a large amount of distributional and natural history data for many taxa, including amphibians (Deviotor et al., 2010; Platenberg et al., 2020; Tulloch et al., 2013). Although citizen science data have their inherent biases, this approach has been helpful to fill knowledge gaps in biodiversity research, and the use of secondary
data can provide opportunities to increase our understanding of ecological patterns (Callaghan et al., 2021). iNaturalist (https://www.inaturalist.org) is an online open-access citizen science platform with a global community of contributors who submit photos, sound recordings and videos of a wide range of organisms. This international platform is highly popular in Brazil, with over 1 million observations of 30,000 species by over 33,000 observers (in April, 2022). Observations are constantly uploaded and updated by a community of over 2 million volunteers (professional or non-professional scientists). These observations are open access and are often verified by taxon experts to provide reliable data to study species ecology or biogeography, or inform conservation (Mesaglio & Callaghan, 2021). Currently, the iNaturalist database contains over 1.3 million amphibian records from around the world.

In this context, we describe the breeding phenology of Brazilian anurans and their daily reproductive activity patterns among different climate regions based on iNaturalist data. We compare temporal patterns of observations among anuran families, assessing potential phylogenetic signals, and contrast climate regions. We expect the climate to be an important factor influencing breeding phenology patterns. As an alternative hypothesis, we test whether these patterns might be a historical consequence of phylogeny, that is, shared patterns by common ancestry. We also discuss the use of citizen-collected data as a source to study and monitor amphibian reproductive patterns in this megadiverse country of the Neotropics.

2  |  METHODS

2.1  |  Citizen science data

We filtered iNaturalist data for anuran records (i.e., frogs and toads) in Brazil, selecting observations that were classified as Research grade. Research grade observations have a voucher photograph (or vocalization), date, latitude and longitude coordinates, and a community-supported identification, with two identifiers or, if more than two, 2/3 of them agreeing on the classification of the taxon. We considered only valid species, that is, species identified at a species level, excluding complexes (Table S1). We downloaded the link of the observation, the geographical coordinates, the hour and date of the observation and the name of the species. Raw data are available in Table S2. We classified the family of each species following Frost (2021). Based on the literature, we classified each species as explosive or prolonged breeders (see Table S1 for references). We also identified species with known larval or direct development using the Amphibio database (Oliveira et al., 2017). We downloaded metadata for frog vocalizations (sound recordings with advertisement calls) from the iNaturalist dataset that were collected in Brazil (Table S1). All of these records were included as evidence of breeding. One of eight researchers (see Acknowledgements) categorized each photograph following the same predetermined guidelines and training based on the presence or absence of reproductive behaviour. Evidence for reproduction included calling male with inflated air sac visible, amplexant couple, nest, eggs, or tadpoles in initial stages of development with no visible limbs (Figure 1). Observations of suspected but not obvious breeding were discussed among the researchers during data collection and removed in case of doubt. We counted each observation only once, even if an observation had more than one photo or a photo and a sound recording of the same event (same observer, same location, same time/day), and we selected the photos with evidence of breeding, when they were present among all photos belonging to one observation. We removed exact image duplicates from multiple observations by the same or different observers. We collected data on 12–28 April, 2021. Finally, we calculated the length of the reproductive season for each family over the year in each climate region by counting the number of months with reproductive events. We also calculated the total number of
(non-consecutive) days with breeding events per year, per family and per climate.

2.2 | Data from the published literature

To compare our results with the published literature, we performed a systematic literature review on the Scopus database between 11 and 17 October, 2021. We used the following keyword combinations: “seasonal AND pattern AND reproduction” OR “temporal AND pattern AND reproduction” OR “breeding AND phenology” OR “pattern OR temporal AND occupancy” AND “Brazil AND Anura”. From the resulting 65 articles, we removed 25 that were not relevant to the aims of this study and one of the two publications by the same authors that were describing different aspects of the same population at the same time. We could not access one of the articles, leaving 39 relevant publications (Table S1). To extend the search, we repeated it on 12–26 March, 2022 using the expression (seasonal AND pattern AND reproduction) OR (temporal AND pattern AND reproduction) OR (breeding AND phenology OR pattern) OR (temporal AND occupancy) AND (Brazil AND Anura AND monthly) using the Publish or Perish ver. 8 software (Harzing, 2007). This search returned 644 articles, with 52 additional relevant publications. Out of the 91 resulting articles, we examined the 80 that we had access to and that contained relevant data (month of confirmed reproduction for different species at a location) based on the title and the abstract.

From each publication, we collected the following information: year(s) and location of the study, such as name of protected area or the nearby city or municipality, with the exact coordinates when available. When the coordinates were not available, we looked them up on Google Maps based on the description of the site. We also obtained the names of frog species in the study and the months with evidence of breeding. From the 760 breeding reports of species at a given location, we obtained 4039 unique species-location-month data points for breeding events. Based on this information, we compared breeding activity patterns at the family level between the published literature and iNaturalist data. We selected four families (Hylidae, Bufonidae, Leptodactylidae, and Phyllomedusidae) with over 20-month-family combinations in both datasets. Another four families (Brachycelphalidae, Hylodidae Microhylidae and Odontophrynidae) had a large enough sample size only in the literature-based dataset.

While the data from iNaturalist represented observations of breeding events for individuals on a given day of the year at a particular coordinate, in the literature-based dataset had a coarser resolution both temporally and spatially, each data point representing a month of reproduction for a member of a particular family in a given geographical area, usually a protected area or near a research station.

2.3 | Data analysis

After excluding observations with erroneous coordinates from the iNaturalist dataset (e.g., outside Brazil), we mapped the location of the observations using QGIS v. 3.18.2 (QGIS Development Team, 2021). We then overlaid the point layer of the frog observations with the climate polygons to obtain a climate classification for each point. We used Köppen-Geiger climate classification at a 1-km resolution as described by Beck et al. (2018) to obtain climate categories corresponding to the geographical location of each observation.

We calculated the number of observations in total and observations with signs of reproduction. From these numbers, we calculated the proportion of observations with signs of reproduction in relation to the total number of observations. To check whether frogs, in general, were more often observed during their reproductive season, we applied a Spearman correlation between the number of observations of reproduction and the total number of anuran observations using the cor.test function in R version 4.0.2 (R Core Development Team, 2020). To quantify the number of observations per family and among climate categories, we created graphs using the “ggplot2” package (Wickham, 2016). We used circular statistics to describe daily and monthly reproductive patterns. We verified the temporal uniformity of breeding behaviour throughout the year (Zar 2010) through the Rayleigh test (Z) of the “circular” package (Agostinelli & Lund, 2017), noting average and standard deviation values. We analysed the seasonality of breeding events per month and vocalizations per hour to identify periods with concentrated occurrence using circular statistics (Morello et al., 2010). First, we converted the month and hour of the observations to angles: 360°/12 months = 30°/month, and 360°/24-h bins = 15°/h. From these values we calculated the mean angle (μ), to obtain the mean date or hour, around which the phenological activity was concentrated and the length of the mean vector (r), indicating the level of aggregation of the phenophase. Higher r values (r > .5) indicate seasonality or aggregation, if the phenophase distribution is not uniform throughout the year or day (p < .005 in the Z test). We analysed monthly breeding and daily activity patterns for Hylidae, Bufonidae, Leptodactylidae, and Phyllomedusidae (families with over 20 breeding observations) using circular statistics, confirming breeding events for members of these four families.

Using breeding-only data, we constructed two generalized linear models (glm) to compare the duration of the reproductive season (in days and months) among Hylidae, Bufonidae, and Leptodactylidae (three families with the highest number of total observations in the iNaturalist dataset, as Phyllomedusidae did not have sufficient data). These glm were fitted with quasipoisson family with days and months as dependent variables, while family was treated as an independent variable. To test the effect of climate region on the duration of the reproductive season, we used two additional glm fitted with quasipoisson family, using days and months as dependent variables and climate region and year as fixed factors. We also calculated hourly vocalization patterns for the iNaturalist data for descriptive purposes.

We used phylogenetic signals and ancestral character state reconstruction to assess the role of phylogeny in the temporal patterns (daily and monthly/phenological) of vocalization. For these analyses, we first removed the 58 observations of tadpoles considering that they might not be precisely associated to the species’ timing of reproduction. Because of the circular nature of the data, we linearized both
variables following the method of Staggemeier et al. (2010). First, we calculated two distance matrices based on the angular distance between each pair of species (the mean angle for peak activity for each species), one matrix for the time over the day (h) and the other for the time over the year (month). These matrices were used to run two Principal Coordinate Analyses (PCoA). We then obtained the scores of the species of the first two components from each PCoA, which represent the species in temporal space (Staggemeier et al., 2010). The observation vocalization time and month are thus represented by two linearized variables each, i.e., the first two components of the PCoA, which we call Time/Month S1 and Time/Month S2).

We calculated the Pagel's $\lambda$ and Blomberg's K phylogenetic signal indices for the four resulting variables (Time S1 and S2, Month S1 and S2) based on 1000 simulations in the randomization test using the “phytools” package (Revell, 2012). If the resulting Blomberg's $K < 1$, it indicates that the temporal patterns of animals vocalizing (vocal activity peaks over the day and year) are less similar to each other for phylogenetically closely related species than expected under the Brownian motion model for trait evolution; in other words, temporal patterns of vocalization have a low phylogenetic signal. Whereas $K = 1$ indicates that the frequency is close to the prediction and $K > 1$ indicates that reproductive patterns are more similar than predicted by the Brownian motion model, that is, the temporal patterns of vocalization have predicted and strong phylogenetic signals, respectively (Blomberg et al., 2003). Pagel's $\lambda$ can vary between 0 and 1, with low values indicating that that trait values are unrelated to phylogenetic relatedness among species and close to 1 indicating a high phylogenetic signal, that is, high congruence between trait evolution and phylogeny under Brownian motion model prediction (Freckleton et al., 2002; Pagel, 1999).

We reconstructed and mapped the ancestral states of these four linearized variables for lineages in our dataset. We used the cont-MAP function of the “phytools” package to estimate the ancestral states through maximum likelihood inference. Phylogenetic analyses were based on the tree of Jetz and Pyron (2018). We kept 256 species in the phylogenetic analyses, removing 27 taxa from the original dataset that were not identified at a species level ($n = 20$) or were not present in Jetz and Pyron's (2018) tree ($n = 7$). All the other 6983 amphibian species and the outgroup were pruned from the tree.

Finally, we produced circular graphs showing monthly distribution of reproductive events in Bufonidae, Hylidae, Leptodactylidae, and Phyllomedusidae based on data from literature to compare with the temporal patterns of reproduction based on the iNaturalist dataset.

3 | RESULTS

3.1 | General temporal pattern in reproduction based on iNaturalist dataset

Among the 8219 photos of Brazilian anurans on iNaturalist (Figure 2), we found evidence of reproduction in 738 observations from 289 species of 16 families. These included 116 observations of amplexant pairs of 58 species, 262 observations with an inflated air sac of 87 species, 26 observations of 17 species with eggs or nests, and 58 observations of 32 species with tadpoles (Table S1). The 283 acoustic records represented vocalization activity of 143 species of 15 families (Table S1). We found positive correlation between the frequency of observations with evidence of reproduction and observations with no evidence of reproduction by month ($r = .972$, $p < 2.2 \times 10^{-16}$; $n = 12$; Figure S8).

Considering all families and climate regions in Brazil, the reproductive season started in October and finished around January, with over 60% of all breeding observations occurring during these 4 months (Figure 3e). Most examples depict vocalizations (Figure 3a) and amplexant couples (Figure 3b). Nevertheless, for some species, eggs and nests were recorded outside this period, an example was the presence of eggs for Dendrobatidae in April, May and August and nests of Leptodactylidae in April (Figure 3c). Most tadpoles were found in April, and the observations belonged to members of Bufonidae, Hylidae and Phyllomedusidae (Figure 3d).

Vocalizing males and amplexant pairs, for all species, were in general recorded between 19h and 21h and between 20h and 21h, respectively (Figure 4).

3.2 | Breeding phenology in Bufonidae, Hylidae, Leptodactylidae and Phyllomedusidae

The anuran families with the highest number of observations in the iNaturalist data were Hylidae ($n = 3211$), Bufonidae ($n = 2394$), Leptodactylidae ($n = 1334$) and Phyllomedusidae ($n = 300$). All four families showed temporally aggregated reproduction between October and January (Table 1; Figure 5). October was the mean month for reproduction for Bufonidae and November for Hylidae, Leptodactylidae, and Phyllomedusidae (Figure 5). We found no difference in the duration of the reproductive season among families (Table 2). Reproductive behaviour also showed convergence and aggregated hourly patterns among families (Table 3). The average time was 19h for Leptodactylidae and Bufonidae and 20h for Hylidae and Phyllomedusidae. All four anuran families had calling males and couples in amplexus observed during the night, with a peak at 19–21h for vocalization and at 20–21h for amplexus.

3.3 | Phylogeny

The phylogenetic signals of most temporal variables (Month S1 and S2, Time S2) were not significant for either Blomberg's $K$ or Pagel's $\lambda$ (Table 4). Indeed, reconstructions of ancestral character states do not suggest any clear phylogenetic signal for temporal pattern of vocalization or reproduction over the year (Figures S1–S5), with the only exception being Time S1, as this variable had a significant signal for Pagel's $\lambda$ ($p = .004$).

Reconstruction of this variable, the first PCoA component (Figure S2) indicates a few lineages with similar calling activity in time, particularly for Melanophryniscus, Hylodes and Phyllomedusa species.
3.4 | Anuran breeding phenology on different climate regions

Although anuran families had not shown differences in breeding phenology, there was a difference among arid and humid climates (Figure 6). In arid hot steppe (BSh, \( r = .88, p = .003 \)), temperate hot summer without dry season (Cfa, \( r = .63, p = .001 \)), temperate dry winter hot summer (Cwa, \( r = .69, p = .001 \)) and temperate dry winter warm summer (Cwb, \( r = .78, p = .001 \)) climates, the reproductive period was concentrated within a few months of the year. On the other
We found differences in the duration of the reproductive season (considering either days or months) among some climate regions, while the year had no significant effect. Frogs in hot and warm climate regions (BSh, Cwa, and Cwb) had significantly shorter reproductive seasons than in other climates (Table 5).

Explosive breeders had a peak in reproduction in October, while prolonged breeders had a continuous reproduction mainly from October to December (Figure S4a). However, the mean month of reproduction was November for both breeding strategies (Table 1). Species with direct development reproduced mainly

**Figure 4** Hourly distribution of frog and toad observations based on iNaturalist data from Brazil illustrating (a) vocalization ($n = 513$), (b) amplexant couples ($n = 112$) and (c) all observations ($n = 7995$). For family colour codes see Figure 3.

**Table 1** Monthly patterns of all observations (ignoring data with missing values; first line, $n_{\text{iNaturalist}} = 8277$) and reproduction events per family and climate based on iNaturalist data

| Subset                             | Climate | Degree (mean ± SD) | Average month | $\rho$  | Pattern   | $p$-Value | $N$ |
|------------------------------------|---------|--------------------|---------------|--------|-----------|-----------|-----|
| All observations                   | All     | 326.3 ± 89.8       | November      | 0.2931 | NU        | 0         | 8277|
| All reproduction                   | All     | 321.6 ± 72.9       | November      | 0.4452 | NU        | 0         | 683 |
| All observations without reproductive cues | All     | 326.7 ± 90.9       | November      | 0.284  | NU        | 0         | 8038|
| B. bufonidae                       | All     | 282.1 ± 91.5       | October       | 0.2792 | NU        | .0021     | 79  |
| H. hyldae                          | All     | 322.8 ± 69.5       | November      | 0.4792 | NU        | 0         | 334 |
| L. leptoactylidae                  | All     | 322.9 ± 68.8       | November      | 0.4897 | NU        | 0         | 127 |
| P. phylomedusidae                  | All     | 307.0 ± 49.2       | November      | 0.6917 | NU        | 0         | 25  |
| All reproduction                   | Af      | 19.0 ± 96.6        | January       | 0.2412 | NU        | .0042     | 94  |
| All reproduction                   | Am      | 309.2 ± 114.3      | November      | 0.1367 | U         | .3138     | 62  |
| All reproduction                   | Aw      | 326.7 ± 66.9       | November      | 0.5059 | NU        | 0         | 203 |
| All reproduction                   | BSh     | 69.4 ± 28.5        | March         | 0.8839 | NU        | .0038     | 6   |
| All reproduction                   | Cfa     | 305.9 ± 54.5       | November      | 0.6365 | NU        | 0         | 159 |
| All reproduction                   | Cfb     | 314.0 ± 71.9       | November      | 0.4546 | NU        | 0         | 98  |
| All reproduction                   | Cwa     | 337.0 ± 49.3       | December      | 0.6905 | NU        | 0         | 37  |
| All reproduction                   | Cwb     | 288.9 ± 39.5       | October       | 0.7885 | NU        | 0         | 18  |
| Explosive reproduction             | All     | 300.8 ± 76.3       | November      | 0.411  | NU        | 0         | 137 |
| Prolonged reproduction             | All     | 322.7 ± 68.2       | November      | 0.492  | NU        | 0         | 390 |
| Direct development – reproduction  | All     | 325.2 ± 69.9       | November      | 0.474  | NU        | 0         | 159 |
| Larval development – reproduction  | All     | 319.6 ± 72.0       | November      | 0.453  | NU        | 0         | 545 |

Note: Climate abbreviations: Af, Tropical Rainforest; Am, Tropical monsoon; Aw, Tropical savannah; BSh, arid hot steppe; Cfa, temperate hot summer without dry season; Cfb, temperate warm summer without dry season; Cwa, temperate dry winter hot summer; Cwb, temperate dry winter warm summer. Categorical results indicate the average months. The pattern was synchronous for all combinations. $\rho$, Rayleigh Test Statistics value. Abbreviations: Doy, day of the year; $N$, number of observations; NU, non-uniform pattern; U, uniform.
In November and December, while species with a larval stage started reproducing and continued until January and February (Figure S4b). Similarly, both development types had the mean month of reproduction in November (Table 1). Species with larval and direct development showed similar general temporal patterns with regard to reproduction (Figure S5). Prolonged breeders were mostly breeding in October to February with a peak in December, while explosive breeders had a peak in October in most climate categories (Figure S6).

### 3.5 Temporal patterns based on the literature review

In general, data from the literature corroborated the temporal reproductive patterns found in the iNaturalist data for Bufonidae, Hylidae, Leptodactylidae and Phyllomedusidae (Figure 7). However, reproduction in Phyllomedusidae and Leptodactylidae was mostly reported between October and December, while in Hylidae it extended to February. On the other hand, Bufonidae...
TABLE 3  Hourly patterns of frog vocalizations (based on inflated air sac and sound recording) per family and climate in iNaturalist data

| Subset | Climate | Degree (mean ± SD) | Average hour | ρ   | Pattern | p-Value | N |
|--------|---------|-------------------|--------------|------|---------|---------|---|
| All    | All     | 295.6 ± 63.4      | 19:00        | 0.5420 | NU      | .0000   | 511 |
| Bufonidae | All     | 291.2 ± 65.1      | 19:00        | 0.5243 | NU      | .0000   | 45  |
| Hylidae | All     | 302.7 ± 48.7      | 20:00        | 0.6971 | NU      | .0000   | 264 |
| Leptodactylidae | All     | 285.8 ± 75.2      | 19:00        | 0.4223 | NU      | .0000   | 101 |
| Phyllomedusidae | All     | 313.3 ± 66.8      | 20:00        | 0.5067 | NU      | .0245   | 14  |
| All    | Af      | 264.3 ± 96.4      | 17:00        | 0.2430 | NU      | .0272   | 61  |
| All    | Am      | 299.6 ± 118.7     | 19:00        | 0.1168 | U       | .5905   | 39  |
| All    | Aw      | 285.0 ± 61.2      | 19:00        | 0.5655 | NU      | .0000   | 159 |
| All    | BSh     | 300.0 ± 67.5      | 20:00        | 0.5000 | U       | .3941   | 4   |
| All    | Cfa     | 298.9 ± 49.6      | 19:00        | 0.6875 | NU      | .0000   | 131 |
| All    | Cfb     | 315.6 ± 54.9      | 21:00        | 0.6317 | NU      | .0000   | 76  |
| All    | Cwa     | 293.2 ± 32.9      | 19:00        | 0.8483 | NU      | .0000   | 31  |
| All    | Cwb     | 310.6 ± 30.4      | 20:00        | 0.8688 | NU      | .0049   | 6   |

Note: Climate abbreviations: Af, Tropical Rainforest; Am, Tropical monsoon; Aw, Tropical savannah; BSh, arid hot steppe; Cfa, temperate hot summer without dry season; Cfb, temperate warm summer without dry season; Cwa, temperate dry winter hot summer; Cwb, temperate dry winter warm summer. Categorical results indicate the average hour. The pattern was synchronous for all combinations. ρ = Rayleigh Test Statistics value.

Abbreviations: N, number of observations; NU, non-uniform pattern; U, uniform.

TABLE 4  The phylogenetic signal tests of Pagel’s λ and Blomberg’s K for the date (month) and time calling patterns with the respective p-values (based on 1000 randomizations)

|                  | Pagel’s λ | Blomberg’s K |
|------------------|-----------|--------------|
|                  | λ         | p            | K             | p             |
| Date Score 1     | 6.611 × 10⁻⁰⁵ | 1 | 0.154 | .240 |
| Date Score 2     | 6.611 × 10⁻⁰⁵ | 1 | 0.150 | .377 |
| Time Score 1     | 0.353     | .004⁺        | 0.145         | .375 |
| Time Score 2     | 6.611 × 10⁻⁰⁵ | 1 | 0.169 | .283 |

Note: Significant values are in bold letters and marked with an asterisk (p < .05). Considering the circular nature of the variables, each one is represented by two linear variables, which correspond to the scores of the first two components of Principal Coordinate Analyses (see text).

starred reproducing in August, with the observations dropping off in January. Other families that did not have enough data in iNaturalist are reported to reproduce between September–January (Brachycephalidae), December–April with an additional peak in August (Hylidae), December–January (Microhylidae) and August–December (Odontophrynidae) (Figure S7). We have not identified large differences in temporal patterns of reproduction in families among different climates, except for Leptodactylidae, which presented a prolonged season in tropical monsoon (Menin et al., 2009; Moreira & Lima, 1991) and a considerable shorter season in arid hot steppe (Madelaire & Ribeiro Gomes, 2016).

4 | DISCUSSION

Our study reinforces the large role climate plays in timing of reproductive phenology of anurans. The timing and duration of the reproductive season are clearly affected by weather conditions that vary regionally in a continental-scale country, such as Brazil. Our results show that the patterns are broadly phylogenetically independent, and the reconstruction of the ancestral states suggested no clear pattern for inclusive lineages. This result agrees with previous empirical observations that show the same species having regionally different phenology (Herreid & Kinney, 1967; John-Alder et al., 1989; Martof & Humphries, 1959; Matsui, 1989). Amphibians depend on water to avoid desiccation and usually also for larval development, and during the drier periods even adults rely on shelters (Duellman & Trueb, 1994; Wells, 2010). Consequently, annual rainfall patterns are known to drive the timing and duration of reproduction in tropical anurans (Duellman, 1978; Kaefer et al., 2012; Prado et al., 2005; Rossa-Feres & Jim, 1994; Ulloa et al., 2019).

While rainfall is a strong determinant of anuran phenology in the tropics, temperature also plays an important role in temperate zones (Hartel et al., 2007; Stebbins & Cohen, 2021), but see Llusia et al. (2013). Temperate-zone anurans in North America have initiated breeding earlier with increasing temperature (Benard, 2015; Blaustein et al., 2001), but the opposite pattern has also been detected in a long-term dataset (Arietta et al., 2020). Nevertheless, this aspect still needs to be studied in anurans from temperate climate regions in Brazil and other regions of the southern hemisphere.

As local temperature and humidity strongly affect calling activity (Guerra et al., 2020; Lannoo & Stiles, 2020), climate change is expected to affect diurnal patterns in calling phenology (Willacy et al., 2015). Unfavourable microclimate caused by global climate change may therefore cause temporal shifts in calling activity (Lannoo & Stiles, 2020). Even though anurans are generally considered to be nocturnal, many frog species may call sporadically or
continuously throughout the day if they are stimulated by favourable conditions and their biological clock enables it (Akmentins et al., 2015; Bridges & Dorcas, 2000; Callaghan & Rowley, 2021; Cui et al., 2011). Therefore, we assume that the uniform pattern (i.e., frogs calling around the clock) we found in tropical monsoon climate is a particular response to strong rainfall events followed by long periods of high humidity and fairly stable temperatures that provide consistently favourable conditions to diurnal vocal activity.

We detected a similar uniform pattern in semiarid hot steppes, where humidity and temperature significantly fluctuate throughout the year, however, this was likely an artefact of the small sample size available for this climate region and we think that more studies are needed to identify a reliable pattern. However, in general, our results corroborated the prevalence of nocturnal activity among Brazilian anurans (Guerra et al., 2020).

We did not detect phylogenetic signals for the phenological patterns of reproduction. This result means that closely related species and lineages on average tend to reproduce in different months, indicating that the observed patterns are not well explained by shared evolutionary history.

To the best of our knowledge, our study was the first to test the impact of phylogeny on phenological and daily breeding activity in anurans systematically. Nonetheless, some authors have discussed the possibility based on anecdotal results (Arias-Balderas & de la Cruz, 2017; Bridges & Dorcas, 2000). For instance, there was no clear phylogenetic pattern related to which species called on a given night during a summer month in a study of nine North American species, with congeneric species calling for different length of time (Bridges & Dorcas, 2000). These results agree with other phenological data for other North American anuran populations. In Canada, Lithobates sylvaticus begins to breed in early spring together with Pseudacris and Anaxyrus species, whereas other Lithobates species (L. clamitans and L. pipiens) begin to breed in late spring together with grey treefrog Dryophytes versicolor, or even later in early summer as observed in American bullfrog (Lithobates catesbeianus) (Klaus & Lougheed, 2013).

On the other hand, a phylogenetic bias may be more common in the phenology of Neotropical frogs and toads (Canavero et al., 2018). In an Atlantic Forest pond, in southeastern Brazil, leptodactylids are the first to breed even before the rainy season, followed by phyllomedusids and hylids with arboreal eggs (Abrunhosa et al., 2006). Although these patterns do not always repeat exactly among different communities (see Aichinger, 1987; Bertoluci, 1998; Bertolucci & Rodrigues, 2002). The phylogenetic pattern observed may be related to shared derived reproductive modes, such as laying eggs in foam nests, underground cavities (Leptodactylidae), or on vegetation above the water (phyllomedusids and some hylids).

These reproductive modes may allow even closely related species to breed in specific seasons even before ponds are filled up (Abrunhosa et al., 2018).
et al., 2006; Gottsberger & Gruber, 2004). Yet, our results indicate that phylogeny is not the driving force behind broad-scale anuran breeding phenology, and, if a phylogenetic pattern is present, it is only detectable at lower taxonomic levels (e.g., genus, species group) similar to reproductive modes (Haddad & Prado, 2005).

Regarding the daily activity pattern, we considered this chronological pattern mostly unrelated to phylogenetic history, even though we identified a significant phylogenetic signal for the first component with Pagel’s λ test. This significant signal was not consistent with the results of Blomberg’s K test and the second
component was nonsignificant in both tests. We assume that this ambiguous result was a consequence of specific phylogenetic patterns present in a few lower-level taxa, including *Melanophryniscus*, *Hylodes*, and *Phyllomedusa* species. Unlike most anurans, the first two taxa are known to be vocally active during the day (Augusto-Alves & Toledo, 2021; Dallagnol Vargas et al., 2020; Vaira, 2005). *Phyllomedusa* species breed during the night, similar to most other members of the order (Wells, 2010). However, unlike other nocturnal taxa, species of this genus seem to reproduce later in the night (Boyle et al., 2021), with the calling activity sometimes peaking after midnight (Bezerra et al., 2021). Similar to the phenological pattern, phylogeny does not seem to explain the hourly distribution of breeding activity at a broad scale. However, it seems to be somewhat more associated with phylogeny at certain less-inclusive taxonomic levels than the phenological pattern. In fact, Bridges and Dorcas (2000) suggested that unlike the calling patterns over the month, the daily calling pattern observed for American species may be conforming to phylogeny, with the peaks occurring before and after the midnight in hylid and ranid species, respectively.

Most Brazilian anuran families were severely underrepresented in the citizen-collected data, the least represented families were Hemiphractidae, Alsodidae and Pipidae with two, eight and 10 observations, respectively. Nonetheless, these families are in general less species diverse compared with others (Frost, 2021) or have relatively inaccessible breeding behaviours, such as some hemiphractids calling in the forest canopy (Folly et al., 2014), microhylids vocalizing underground (Fouquet et al., 2021) or pipids under water (Bünten et al., 1992). In addition, some microhylid species have explosive breeding events that only last a few days or hours (Pombal Jr & Gonçalves Cruz, 2016). As we show, explosive breeders mainly reproduce in the early raining season. Unsurprisingly, most of the iNaturalist data came from high-diverse and abundant taxa, such as Hylidae and Leptodactylidae. Moreover, several species of these families, as well as of Phyllopondemusidae and Bufonidae, have more conspicuous and accessible behaviours, calling near water bodies in peri-urban areas (Ferreira et al., 2010; Menin et al., 2019). They also have remarkable diagnostic visual features related to reproduction. For instance, some phyllopondemusid and hylid species deposit their eggs in very characteristic leaf nests, on surface or the tip of the leaf, or in nests on the ground, while bufonid eggs are deposited in long and characteristic strings in the water (Crum, 2015; Nunes-de-Almeida et al., 2021). The taxonomic biases found citizen platforms likely reflect the relative diversity, abundance, and behavioural conspicuousness and accessibility of Neotropical anurans.

Still, the high consistency between patterns described in the literature and detected in iNaturalist data suggest that citizen science programs can efficiently sample frogs in Brazil. Amphibian sampling through citizen science has already fulfilled its potential in some countries, while for others it is still behind (Oliver et al., 2021; Troudet et al., 2017). In comparison with other megadiverse countries, Brazil has been considered a stronghold for citizen-collected data, due to high taxonomic coverage and survey effort (L. R. Forti & J. K. Szabo, unpublished data). Citizen science can provide long-term data at a continental scale that can serve as a valuable source of information to be harnessed to monitor the effects of climate change on the reproduction of amphibian and other species (Belitz et al., 2020; Mayer, 2010). As the frequency and severity of drought events is predicted to increase (Cook et al., 2014, 2018), this can lead to decreased population recruitment by restricting the reproduction period, especially in regions with tropical mountains (Geyle et al., 2022) and arid climate. Although the severity of the effect will be taxon-dependent, many species that use temporary ponds also may have to congregate in permanent habitats and the increased competition among species by reproductive sites may jeopardize their reproductive success. In this context, species with declining populations should be of special concern and should receive more
attention by volunteers who contribute with occurrence data to iNaturalist and other citizen science platforms.

Unlike for structured surveys, for citizen-collected data the sampling effort is often unknown and data are presence-only, making phenology estimates less reliable (Kelling et al., 2019). Therefore, our results should be interpreted with caution, since the temporal sampling bias originating from volunteer behaviour is not known. However, the similarities in temporal patterns between iNaturalist and literature review data suggest similar temporal biases. The same patterns, average month, and the positive correlation between the number of observations of frogs with no signs of reproduction and the number of observations with reproduction cues shows that frogs in general are more detectable during the reproductive season. In other words, the reproductive season seems to be the cause of the high number of (reproductive or not) observations, as opposed to the idea that there are more observations when the volunteers are more active.

5 | CONCLUSION
Our study brings an innovative regional perspective to study anuran reproductive phenology, previously predominately based on local communities (Plenderleith et al., 2018; Schalk & Saenz, 2016; Walpole et al., 2012). We also highlight the importance of citizen science for data generation to understand patterns on animal biology at such a large scale. We describe amphibian reproductive phenology with regard to climate regions, which is of utmost relevance in a continental-scale country with the highest anuran richness globally.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
All data that support the findings of this study are available in Table S2. Supplementary tables and figures are found at https://zenodo.org/record/6811407#.YshAdRtMK3B.

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**BIOSKETCH**

Members of the research team are from the Federal University of Bahia, Brazil and the Federal University of Rio de Janeiro, Brazil (Fabio Hepp). Our team mainly focuses on biodiversity conservation informed by citizen science data.

Author contributions: J.K.S. and L.R.F. conceived the idea, designed the research, analysed and visualized data and wrote the manuscript draft. F.H. analysed phylogenetic data and contributed to the writing. J.K.S. supervised the research and refined the manuscript. J.M.S. and A.P. collected and categorized data and conducted literature review. All authors participated in revisions and approved the submission.

**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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