Introduction

Understanding the mechanisms that generate and maintain the temporal distribution patterns of organisms is one of the main challenges of ecology. Over narrow temporal scales (e.g., annual), variations in the distributions of populations and, consequently, of assemblages are influenced by both biotic and abiotic factors (Sicilia et al., 2006; Shurin, 2007), such as climate conditions (Pombal Jr., 1997; Adler and Levine, 2007), competition (Toft, 1985), and the availability of water bodies (Rodrigues et al., 2010). Despite an increase in the numbers of studies conducted, the volume of knowledge regarding temporal patterns is much lower than that of spatial patterns of biodiversity. Thus, studies analysing temporal distributions are essential for understanding and predicting future changes in the structure and functioning of assemblages that result from human activities.

Anuran reproductive activity begins with the male’s calling activity upon which the female chooses her partner (Wells, 2007). This calling activity is the first step in reproduction; however, vocalisation does not always indicate reproductive activity (Toledo et al., 2015). Amphibian species in tropical and temperate regions with seasonal climate conditions have interrupted reproductive cycles, whereas anuran species in tropical wet regions tend to have long reproductive periods (Wells, 2007), although exceptions exist (see Ximenez and Tozetti, 2015). Climatic factors (e.g., relative humidity, rainfall, and temperature) are considered the main factors that can regulate the activities of ectothermic organisms such as anurans (Pombal Jr. et al., 1994; Hartel et al., 2007; Wells, 2007).

In tropical and subtropical regions, rainfall is expected to be one of the main abiotic factors that determine the calling and reproductive activities of anurans (e.g., in Bolivian anurans, Schalk and Saenz, 2016), while in temperate regions, the main factor seems to be temperature (e.g., Lithobates pipiens in Canada, Sommers et al., 2018). In the subtropical region, the
climates with hot summers, cold winters, and no dry
a transitional region between tropical and temperate
pond-breeding anurans in the Pampa biome, which is
the temporal distribution of the calling activities of
change affects amphibians (e.g., Zank et al., 2014).
In regions with seasonal climates, the combined effects of temperature and precipitation appear to influence activity patterns (e.g., Guerra and Araóz, 2015; Lion et al., 2019) because anurans usually limit their reproductive activities to the warm and humid time of the year (e.g., Heyer et al., 1990; Pombal Jr., 1997; Hartel et al., 2007). In addition to these climatic factors, recent studies have linked the seasonal activities of anurans to the photoperiod, particularly in subtropical regions (Both et al., 2008; Canaverio et al., 2008; Canaverio and Arim, 2009).

Photoperiod is an important environmental variable for ectotherms because it provides information about the time of year that is not influenced by climate variations (Gotthard, 2001). Recognition of the season is important because it dictates the physiological rhythms of organisms and thus supports the recognition of favourable reproductive periods. Furthermore, the complex interaction between photoperiod and temperature seems to control the biological rhythms of anurans by triggering hormonal stimuli related to reproduction, including gametogenesis (Saidapur and Hoque, 1995; Huang et al., 1997) and calling activity (Hatano et al., 2002; Both et al., 2008; Kaefer et al., 2009). In the scenario of global climate change in which there is evidence of climate change promoting infectious disease, anuran biodiversity is being eroded (Pounds et al., 2006), and climate change could reduce the climatic areas that are suitable for the presence of various species (Zank et al., 2014). Given these conditions, it is possible to achieve insights into how climate change influences amphibians. There is evidence that climate change affects amphibians (e.g., Zank et al., 2014); thus, there is a need to understand the factors that interfere with the temporal distributions of anuran assemblages in different geographic zones. This information will enable the achievement of insights into how climate change affects amphibians (e.g., Zank et al., 2014).

In this manuscript, we present the factors that influence the temporal distribution of the calling activities of pond-breeding anurans in the Pampa biome, which is a transitional region between tropical and temperate climates with hot summers, cold winters, and no dry season (Overbeck et al., 2009). We hypothesised that climatic variation and photoperiod shape the temporal distribution of anuran activity. Our predictions are the following: (i) there is seasonality in the calling activity of anurans; and (ii) climate variables, especially temperature and photoperiod, modulate the temporal variation in the calling activity of anurans, i.e., higher temperatures and longer photoperiods elicit greater numbers of species in the breeding chorus.

Materials and Methods

Sampling was performed in the Fundação Estadual de Pesquisa Agropecuária (Fepagro: 30º 20’16.03” S; 54º 15’46.68” W, 126 m above sea level) in the municipality of São Gabriel, state of Rio Grande do Sul, southern Brazil. The study area is covered mainly by open savannah vegetation (i.e., the “Pampa biome”) with a predominance of grassland, shrubs, and, to lesser extents, arboreal and cultivated areas (Hasenack et al., 2010).

The southern portion of Brazil occupies a transitional region between tropical and temperate climates (Overbeck et al., 2009). The climate is classified as sub-tropical and has a mean annual temperature of 18.1°C and a mean annual rainfall of 1,162 mm (Maluf, 2000). The rainfall is unevenly distributed throughout the year (the total was 1,802 mm during the study period), without a marked dry season. The Pampas exhibit marked climate seasonality, particularly in temperature (Rossato, 2011). During the study period, the mean monthly temperature ranged from 11.1°C in the coldest month to 26.5°C in the warmest month. In the study area, the temperature and photoperiod are cyclical (Both et al., 2008), and it is possible to observe two distinct seasons that are determined mainly by temperature and photoperiod (the warm season has a longer photoperiod, and the cold season has a shorter photoperiod).

To reduce the variations in the anuran detection probabilities that are likely associated with species-specific differences in calling activities, breeding strategies, and abundances, we sampled the pond-breeding anurans in four ponds from March 2009 to February 2010 during three consecutive days per month with the exception of July when no sampling was performed due to logistical difficulties. We considered the species activities and compositions (dependent variables) based on the sums of the anuran records over the three sampling days for each pond and for each month. We sampled four ponds as follows: Pond 1 (30º 20’30.48” S, 54º 15’35.76” W) is...
a permanent, anthropised pond with grasses (Poaceae) and Bromelia sp. (common names penguin and gravatá; Bromeliaceae) along part of the margin and an area of 1471.6 m²; Pond 2 (30° 20’28.12” S, 54° 15’35.76” W) is a semi-permanent pond that dries out after long periods without rain and has grasses and Bromelia sp. plants in the surrounding area and an area of 1613.2 m²; Pond 3 (30° 20’27.72” S, 54° 15’46.74” W) is a permanent pond with predominantly grasses growing around and within it and an area of 1951.5 m²; and Pond 4 (30° 20’20.95” S, 54° 15’31.52” W) is a temporary pond that accumulates water only after heavy rainfall, has grasses and some trees in the surrounding area, and has an area of 575.8 m².

We sampled the calling activities of the anuran species at the breeding sites of each pond (Scott Jr. and Woodward, 1994) using auditory search methods (Crump and Scott Jr., 1994). At each pond, we recorded the number of males calling and the composition of anurans comprising the calling activity. Samples were collected by three researchers beginning in the late afternoon (18:00 hrs - 19:00 hrs) and stopping at approximately 23:00 hrs; thus, the sampling effort totalled 432 hrs. The samples were collected along the edges of the ponds, and the sampling efforts were proportional to the sizes of the environments (Scott Jr. and Woodward, 1994). Voucher specimens were deposited in the Scientific Collection of the Zoology Sector, Department of Biology, Universidade Federal de Santa Maria (ZUFSM) (license SISBIO/IBAMA 14596-1), Santa Maria, Brazil. The monthly climatic variables of the mean minimum and maximum temperature, minimum and maximum humidity, precipitation, photoperiod, and atmospheric pressure were obtained from the Instituto Nacional de Meteorologia (INMET, http://www.inmet.gov.b) with reference to the meteorological station of São Gabriel, which is 4.5 km away from the study area.

The existence of seasonality was evaluated with a sinusoidal equation (Canavero et al., 2008) in which the presence of seasonality was verified with the adjusted data, which, in this case, was the variation in the number of species engaging in calling activity (dependent variable) fit to the predicted sinusoidal wave model using an alpha equal to 5%. This analysis has been shown to be very consistent for examinations of temporal patterns in the southern region of Brazil (Canavero and Arim, 2009). The existence of variation in the compositions between the warm (from October to March) and cold (from April to September) seasons (see Figure 1) was verified with an analysis of similarity (ANOSIM) with 999 permutations that was used to test for differences between the treatments (seasons) based on a similarity matrix that was constructed by applying the Bray-Curtis dissimilarity index (Legendre and Legendre, 2012).

We applied generalised linear models to identify the model that best explained the temporal variation in species calling activity (dependent variable) using the following abiotic monthly (independent) variables: mean maximum and minimum temperature, maximum and minimum humidity, rainfall, atmospheric pressure, and photoperiod. To remove the effect of the measurement scales, all variables were transformed such that the mean was equal to zero, and the standard deviation was equal to one. Subsequently, we used the variance inflation factor (VIF) to analyse the presence of multicollinear variables. The variables with VIF values > 3 were eliminated to reduce the multicollinearity (Quinn and Keough, 2002). Accordingly, the first variable to be eliminated was the mean maximum temperature. Subsequently, the VIF was recalculated, which resulted in the elimination of the minimum humidity, atmospheric pressure, and, finally, the photoperiod. With the remaining noncollinear variables, we elaborated a global model of the variation in the calling activities of the males (dependent variable) in relation to the remaining independent variables (i.e., mean minimum temperature, maximum humidity, and precipitation). Based on the expected influence of each of the abiotic variables, a priori models were constructed from the

Figure 1. Mean monthly temperatures (maximums and minimums) and monthly precipitations in the municipality of São Gabriel, state of Rio Grande do Sul, southern Brazil between March 2009 and February 2010.
global model using all combinations of the variables. For each model, we calculated the Akaike’s information criterion with correction for small sample sizes (AICc). We calculated the percentage of the deviation explained (%DE) as a measure of the goodness-of-fit of each model. We used the Akaike weights to evaluate the model-selection uncertainties. To select the best model, we considered only the models that met the following criteria: (i) a $\Delta$AICc of less than 2.0, (ii) inclusion in the set of the best-supported models with combined Akaike weights of 0.70 (i.e., the 70% confidence set), and (iii) an evidence ratio relative to the best-supported model below 3 (Burnham and Anderson, 2002; Burnham et al., 2011). We built scatter plots with linear adjustments to evaluate the directions of the effects of the variables from the best model on the species involved in calling activity. These analyses were implemented using the R program version 2.15.1.

Results

We recorded 19 species of anurans distributed across five families and ten genera (Table 1). The greatest numbers of calling species were recorded in the warmer months, especially January and February (i.e., the austral summer), and the lowest numbers of calling species were recorded from April to June (Table 1; i.e., autumn and winter). The compositions of the anuran species varied between the warmer (October to March) and colder (April to September) months (ANOSIM: $r = 0.589$, $P = 0.006$). Only one species (Scinax squalirostris) was found to call in all samples. Three species (Hypsiboas pulchellus, Scinax squalirostris, and Odontophrynus americanus) were heard only in the coldest and/or driest of the sampled months (April to June). Seven species were recorded only in the warmest months of the year (Table 1).

The calling activity exhibited a strong fit to the sinusoidal model; the sinusoidal distribution explained 70% of the seasonal variation in the presence of the calls of anuran species ($r^2 = 0.70; F = 43.53; P < 0.001$; Figure 2). This result evidenced the seasonality in the activities of the anurans. Based on the generalised linear models, the most parsimonious model for explaining the temporal variation in the species engaged in calling activity comprised only the variable of the mean minimum temperature (AICc = 58.7), which alone explained 60% of the variation in calling activity (Table 2). An increase in the monthly mean minimum temperature was related to an increase in the anuran calling activity (Figure 3).

Discussion

We verified the existence of seasonality in the species composition and calling activity of pond-breeding anurans in a region with temperatures that display marked seasonality. The results confirmed the existence of a seasonal pattern of pond-breeding anuran assemblages in the Pampa biome in which greater male anuran calling activity occurred from October to March, and reduced activity was observed from April...
The seasonal distribution of the anuran assemblages was influenced by the variation in the minimum temperature; this finding differs somewhat from the findings of previous studies conducted in the same region. Both et al. (2008) determined that anuran calling activity was explained only by photoperiod, but this climatic factor was closely correlated with the maximum temperature. Additionally, in this study, only the maximum temperature was considered because the temperatures were themselves correlated. Ximenez and Tozetti (2015) found that anuran calling activity did not display a significant relationship with any of the examined environmental variables, but captures during

to September (e.g., Both et al., 2008; Canavero et al., 2008; Santos et al., 2008; Canavero and Arim, 2009; Ximenez and Tozetti, 2015).

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Table 1. Monthly occurrences of the calling activities of 19 anuran species from March 2009 to February 2010 in the Fundação Estadual de Pesquisa Agropecuária, municipality of São Gabriel, state of Rio Grande do Sul, southern Brazil.

<table>
<thead>
<tr>
<th>Year</th>
<th>Family/Species</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>Bifurcata clavata (Cope, 1862)</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
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<td>✈</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
</tr>
<tr>
<td>2010</td>
<td>Leptodactylus latrans (Steffen, 1815)</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
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<td>✈</td>
<td>✈</td>
</tr>
<tr>
<td></td>
<td>Physalaemus viviparum (Boettger, 1883)</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
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<td></td>
<td>Physalaemus riograndensis Milstead, 1960</td>
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<tr>
<td></td>
<td>Pseudopaludicola falcipes (Hensel, 1867)</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
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<td>✈</td>
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<tr>
<td></td>
<td>Microhylidae Elachistocleis bicolor (Guérin-Méneville, 1838)</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
<td>✈</td>
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<tr>
<td></td>
<td>Odontophrynidae Odontophrynus americanus (Duméril and Bibron, 1841)</td>
<td>✈</td>
<td>✈</td>
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<td>✈</td>
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</tbody>
</table>

Table 2. The five best abiotic variable models that were generated to explain the temporal variation in the anuran species calling activities. \( k \) = number of parameters; \( \text{AICc} \) = corrected Akaike’s information criteria; \( \Delta \text{AICc} \) = difference in the corrected Akaike’s information criteria; \( w \text{AICc} \) = weight of the corrected Akaike’s information criteria; \( \%\text{DE} \) = percent of the deviation in the response variable explained by the model under consideration.

<table>
<thead>
<tr>
<th>Model</th>
<th>( k )</th>
<th>( \text{AICc} )</th>
<th>( \Delta \text{AICc} )</th>
<th>( w \text{AICc} )</th>
<th>Deviance</th>
<th>( %\text{DE} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calling activity + minimum temperature</td>
<td>2</td>
<td>58.7</td>
<td>0.00</td>
<td>0.60</td>
<td>6.06</td>
<td>59.98</td>
</tr>
<tr>
<td>Calling activity + rainfall + minimum temperature</td>
<td>3</td>
<td>61.9</td>
<td>3.18</td>
<td>0.14</td>
<td>5.31</td>
<td>94.93</td>
</tr>
<tr>
<td>Calling activity + minimum temperature + maximum humidity</td>
<td>3</td>
<td>62.7</td>
<td>2.62</td>
<td>0.10</td>
<td>6.05</td>
<td>60.06</td>
</tr>
<tr>
<td>Calling activity – rainfall</td>
<td>2</td>
<td>63.3</td>
<td>4.58</td>
<td>0.06</td>
<td>10.64</td>
<td>29.70</td>
</tr>
<tr>
<td>Calling activity – rainfall + maximum humidity</td>
<td>3</td>
<td>64.0</td>
<td>5.29</td>
<td>0.05</td>
<td>7.42</td>
<td>50.96</td>
</tr>
</tbody>
</table>
cold months were more numerous on days with higher minimum temperatures. These findings indicate that further studies of the factors that influence the activities of anurans in different climates are needed.

Higher temperatures favour metabolic functioning (Wells, 2007) and are consequently more favourable for calling activity and, therefore, reproduction (Both et al., 2008). In some species, higher temperatures can promote spermatogenesis (Rastogi, 1976), gametogenesis (Saidapur and Hoque, 1995), locomotion (Rome et al., 1992), and embryonic and larval development (Wells, 2007). In regions in which the monthly mean minimum temperature can often reach values lower than 10 °C in the winter, this variation is extremely important in the regulation of the activities of ectothermic animals and can limit the breeding activities of local species. The relationship between the number of species of calling males and temperature is nonlinear because high temperatures adversely affect metabolism via the inactivation of enzymes and the acceleration of water loss (Wells, 2007). Therefore, the existence of seasonality in terms of the variation in minimum temperatures in the environments of pond-breeding anurans is related to the action of this climatic variable as a limiting factor in the colder months and as a factor that stimulates calling activity and reproduction in the warmer months.

We found that the compositions of calling anuran species differed between the warmer (October to March) and colder months (April to September) because of the greater numbers of species engaged in calling activity in the months with higher minimum temperatures. The temporal variation in the anuran species compositions over time is considered to be among the main dimensions of niche partitioning among species (Schoener, 1974; Pianka, 1981), but this is not perceived as a common mechanism of coexistence (Kronfeld-Schor and Dayan, 2003). The temporal change in species composition is related to breeding phenology and reproductive strategy, which vary from explosive to prolonged breeders (Wells, 2007). Despite the evident seasonality in the anuran assemblage, this pattern is not reflected in all species (Both et al., 2008; Ximenez and Tozetti, 2015; present study). Most species engage in calling during warmer periods; however, a few species engage in calling activity throughout the year (e.g., *Scinax squalirostris* and *Hypsiboas pulchellus*) or even specifically during the period of the coldest weather and a shorter photoperiod (e.g., *Odontophrynus americanus*). The seasonal changes in species composition observed in the present study and in other anuran assemblages in the Pampa (e.g., Both et al., 2008; Canavero et al., 2008; Ximenez and Tozetti, 2015) are probably due to variation in the thermal tolerance of each species. Thus, the influences of abiotic factors, particularly climatic factors, on calling activity correspond with the reproductive strategies of the various species (Oseen and Wassersug, 2002) and, consequently, with the anuran assemblage. The limitation on anuran calling activity imposed by lower minimum temperatures affects the phenology of pond-breeding species and consequently influences the dynamics of the assemblage.

Our results present a mismatch when compared to other studies on anuran assemblages in temperate areas in South America regarding the major abiotic driver of temporal variation in calling activity (see Both et al., 2008; Canavero et al., 2008; Santos et al., 2008; Canavero and Arim, 2009; Ximenez and Tozetti, 2015). The influence of photoperiod on the calling activities of Pampa anurans has been frequently observed (e.g., Both et al., 2008; Canavero and Arim, 2009). In addition to the fact that photoperiod is correlated with minimum temperature, we observed a major influence of the minimum temperature on the species composition of the calling activity of pond-breeding anurans. Clearly, assemblage-level interpretations of how abiotic factors drive the calling activities of anurans are complex due to the interactions between abiotic factors, and these factors most likely act synergistically on the seasonal distribution of anurans.

We corroborated our hypothesis that the seasonality in the calling activity of pond-breeding in austral anurans is strongly influenced by climatic features. This result emphasises the role of physiological constraints imposed by environmental conditions in shaping the calling activities of anurans. In the present scenario of global climate change, anurans of areas with seasonal climates, such as the Pampa biome, will probably alter their temporal distributions (e.g., Beebee, 1995), which will consequently affect all of the dynamics of the interactions and co-occurrences of species (see Blaustein et al., 2001). For example, an increasing minimum temperature has been found to be associated with demographic increases and expanded ranges possibly due to increases in the available time for larval growth and development (Bosh et al., 2018) and because an earlier onset of spring could influence both the survival and reproductive output of females (Reading, 1998). Therefore, elucidating temporal distribution patterns and understanding the mechanisms that affect seasonal distributions are extremely important for predicting the
effects of environmental change on anuran assemblages in ponds.

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