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# Vocal analysis of he newly described Atlantic Coast leopard frog

Carrie J. Kosiba

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### Abstract
Urbanization alters animal behaviors and especially impacts amphibians due to their sensitivity to environmental change, small home ranges, and reliance on acoustic communication to locate mates. A newly discovered leopard frog species, Rana kauffeldi, was recently described from the New York City metro area. This species was previously characterized as R. sphenocephala due to their nearly identical morphology, but the two species are distinguished based on genetics and calling characteristics. The goal of this project was to document calling intensity and phenology (timing) differences between urban and rural populations of R. kauffeldi, as well as describe variation across latitude, and document temperature conditions. We hypothesized that rural locations would have larger choruses because they are less fragmented and thus more likely to support larger populations. We also predicted that light pollution would cause urban populations to begin calling later in the evening. Finally, we predicted that warmer temperatures would allow southern sites to begin calling earlier in the year, and we performed descriptive analyses of temperature trends throughout the season. We found significantly larger choruses at rural sites, a non-significant trend of calling beginning later in the evening at urban locations, calling began significantly earlier in the year at southern sites, and a non-significant pattern of temperatures increasing throughout the season. It is important to further understand the impacts of urbanization on animals because it can alter essential breeding behaviors like calling, and to accurately define species to formulate appropriate conservation efforts.

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VOCAL ANALYSIS OF THE NEWLY DESCRIBED ATLANTIC COAST LEOPARD FROG

By

Carrie Kosiba

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**Abstract**

Urbanization alters animal behaviors and especially impacts amphibians due to their sensitivity to environmental change, small home ranges, and reliance on acoustic communication to locate mates. A newly discovered leopard frog species, *Rana kauffeldi*, was recently described from the New York City metro area. This species was previously characterized as *R. sphenocephala* due to their nearly identical morphology, but the two species are distinguished based on genetics and calling characteristics. The goal of this project was to document calling intensity and phenology (timing) differences between urban and rural populations of *R. kauffeldi*, as well as describe variation across latitude, and document temperature conditions. We hypothesized that rural locations would have larger choruses because they are less fragmented and thus more likely to support larger populations. We also predicted that light pollution would cause urban populations to begin calling later in the evening. Finally, we predicted that warmer temperatures would allow southern sites to begin calling earlier in the year, and we performed descriptive analyses of temperature trends throughout the season. We found significantly larger choruses at rural sites, a non-significant trend of calling beginning later in the evening at urban locations, calling began significantly earlier in the year at southern sites, and a non-significant pattern of temperatures increasing throughout the season. It is important to further understand the impacts of urbanization on animals because it can alter essential breeding behaviors like calling, and to accurately define species to formulate appropriate conservation efforts.

## Introduction

Habitat loss due to development, agriculture, and other types of land conversion is occurring on a worldwide scale and is a severe threat to biodiversity (Hanski 2011). Habitat loss leads to fragmented populations, that are smaller in size and more isolated, which are significant factors contributing to species' extinction (Fahrig 1997). A variety of organisms are impacted by the loss of habitat including vascular plants, amphibians, reptiles, birds and mammals (Brooks et al. 2002). Rapid habitat loss is occurring on a global scale; for example, an estimated 33% of wetlands have been lost as of the year 2009 and the most significant contributor is human development (Hu et al. 2017).

Urbanization, the conversion of land to primarily impervious surfaces dominated by roads and buildings, is a particularly dramatic form of landscape change. Urbanization is detrimental to many species because of the new and typically unfavorable environmental conditions that are introduced, such as novel predators, noises, light pollution, temperature shifts, and habitat loss (Bonnington, Gaston, and Evans 2013; Long et al. 2017; Marini et al. 2017; Miller et al. 2017; Pyke 2004). For example, urban feral cats decrease the nest survival rate of urban bird species through predation (Oppel et al. 2014), and some bird species decrease their calling distance to accommodate for the frequent noise interruptions, leading to more dense and fragmented urban populations (Parris et al. 2013). Urbanization decreases invertebrate species richness and alters aquatic environmental conditions including oxygen concentrations, temperature, pH, nitrogen, and phosphorus which are important variables for aquatic invertebrates (Martins et al. 2017). These urbanized environments are very different from natural rural habitats, and it is important to understand how urbanization impacts species' distribution and behavior.

Amphibians are particularly sensitive to environmental changes such as urbanization, which can alter their breeding behavior and population size, increase exposure to toxins and noise pollution, and create fragmented populations (Brühl, Pieper, and Weber 2011; Guzy et al. 2012). Frog ponds surrounded by developed human land are associated with lower amphibian species richness and abundance indicating that many species are negatively impacted by urban conditions (Hamer and Parris 2011; Gagné and Fahrig 2010). Frogs' aquatic life stages are very susceptible to toxin and chemical exposures, through skin absorption, in areas with urban waste (Brühl, Pieper, and Weber 2011). Perhaps the greatest impact of urbanization is direct habitat destruction. For example, the loss of urban wetlands has led to the extirpation of many amphibian species (Guzy et al. 2012), but amphibians are also impacted by fragmented populations and light pollution in urban areas. Fragmentation caused by urban landscape can decrease the genetic diversity of amphibian populations and result in a lower reproductive success rate and population decline (Okamiya and Kusano 2018).

Calling is used to attract females and signal a males' presence to other males and is essential for amphibian reproductive success (Pröhl 2003), but this aspect of anuran biology is also disrupted by urbanization. The frequency of anthropogenic noise overlaps with frog calls and often triggers calling, which can reduce fitness because of the large amount of energy expended for calling (Engbrecht et al. 2015). Frogs may call at a higher pitch with greater traffic noise (K. Parris, Velik-Lord, and North 2009) or may increase their calling rate in the presence of anthropogenic noise (Kaiser et al. 2011). Anthropogenic noise alters female frogs' response to calling by increasing their frequency of choosing unattractive male frog calls over normally attractive calls (Schou, Levengood, and Potvin 2021). Unnatural urban surfaces are associated with a reduction in calling from male frogs due to lack of suitable land or human disturbances as well as a decreased frog population density (Pellet, Guisan, and Perrin 2004; Pellet, Hoehn, and Perrin 2004). Urbanization is associated with smaller and more fragmented frog populations and therefore quieter calling choruses are more likely in these environments (Pellet, Guisan, and Perrin 2004). Frogs primarily begin calling at dusk and light cues impact the onset of chorusing (Oseen and Wassersug 2002). Human caused light pollution also has numerous effects on frog populations and especially can impact their calling; for example, anthropogenic light pollution present in the evening may delay the onset of chorusing (Oseen and Wassersug 2002).

Environmental factors including temperature also impact the calling behaviors of frog species, especially in areas of urban heat islands which are warmer than surrounding rural areas (Nwakaire et al. 2020). Frog breeding activity often begins after a minimum water temperature is reached in the environment (Wheeler et al. 2018), and as temperatures extend above or below the peak temperature, calling can decline and cease (Oseen and Wassersug 2002). There is a positive relationship between temperature and the number of individuals calling (Hatano, Rocha, and Van Sluys 2002) and temperatures are expected to increase during the calling season. Temperatures at the beginning of the calling season can more strongly predict the onset of calling (Oseen and Wassersug 2002) and frogs in warmer areas near the equator are likely to begin calling earlier in the year.

Bioacoustics technology is useful to study amphibians because it can recognize slight distinctions and variations among species call parameters (Feinberg et al. 2014). Such studies typically consist of deploying audio recorders then using analysis software to look at characteristics such as calling intensity, frequency, interruptions, length, and more. Bioacoustic data can capture natural calling characteristics with much less disruption than a traditional survey and distinctions within the same species among environments can be detected more easily. It also allows for permanent storage of vocal recordings that can be used in future studies to compare calling activity, track changes in breeding phenology, or identify other species presence. The recordings document the species present in a specific location at a specific time; these data can then be compared in the future to track the rate and distance that certain species move in response to the changing climate.

Study System

A new leopard frog species, *Rana kauffeldi*, was recently discovered in the New York City metro region, a well-studied area with dense human populations (Feinberg et al. 2014). Incredibly, this cryptic species was able to survive in the densely populated New York City metro area without being formally described until just a few years ago (Feinberg et al. 2014). Subsequent surveys determined that the species' range extends south to North Carolina (Schlesinger et al. 2018). *R. kauffeldi* was previously incorrectly classified as the Southern Leopard Frog *R. sphenocephala* (Newman et al. 2012) because both species begin breeding around the same time of year and overlap in their ranges (Feinberg et al. 2014). *R. kauffeldi* and *R. sphenocephala* are nearly morphologically identical but can be differentiated by genetics, morphology, and call characteristics (Feinberg et al. 2014). Genetic comparisons revealed that Pickerel frog (*R. palustris*) is most closely related to *R. kauffeldi* and morphological evidence showed that *R. kauffeldi* had the smallest eye-to-naris, thigh, and shank measurements in comparison to similar frogs in the area (Feinberg et al. 2014). *R. kauffeldi* also sounds very similar to the Wood frog (*Lithobates sylvaticus*), though distinctions exist, therefore *R. kauffeldi* is doubly cryptic with both *R. sphenocephala* and *L. sylvaticus* (Feinberg et al. 2014). *R. kauffeldi* calling characteristics of pulse rate and number, duration, duty cycle, and call rate differ significantly from *R. sphenocephala* and *L. sylvaticus* (Feinberg et al. 2014). There has not been extensive research focusing on the impact of the urban environment on the calling behavior and population size of *R. kauffeldi*. With the discovery of a new species, it is important to conduct further research to learn about how their calling varies in urban and rural environments and across geographic range.

The goal of this study was to assess the impacts of urbanization and environmental variation on the calling patterns of *R. kauffeldi* and to determine how their breeding phenology changed with latitude. We hypothesized that 1) rural populations of *R. kauffeldi* would produce larger and more frequent choruses because of less fragmented populations; 2) urban populations would begin calling later in the evening because of more light pollution earlier in the evening; and 3) southern populations would begin calling earlier in the year because of warmer temperatures (Hatano, Rocha, and Van Sluys 2002). We also documented temperature trends throughout the calling season for descriptive analyses (Figure 1). To test the hypotheses, we analyzed vocal recordings of urban and rural *R. kauffeldi* populations to compare the chorus rating and phenology of the calling season. In this study, we compared the chorus level, estimated the timing of the calling season of *R. kauffeldi* in both urban and rural areas, and performed descriptive analyses of temperature patterns throughout the calling season to assess the types of conditions that are amenable to the largest breeding congregations.

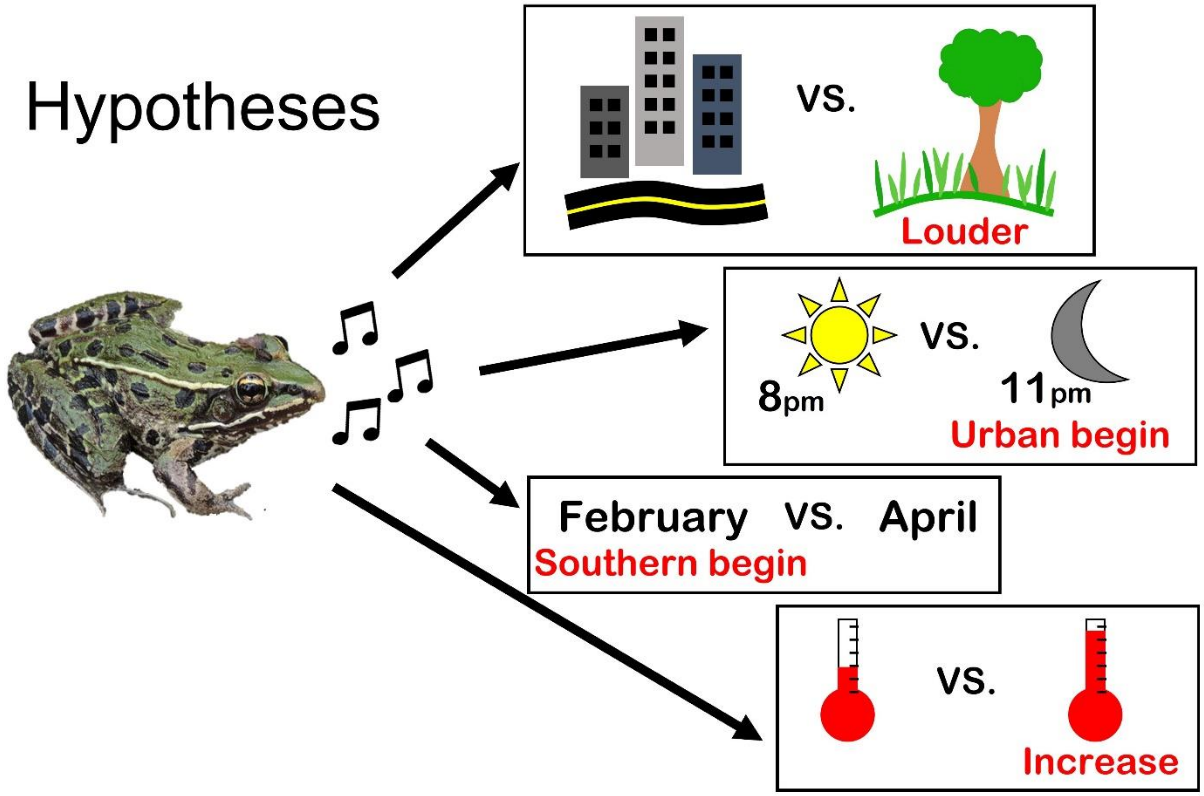


**Figure 1**. Hypotheses.

**Methods**

Acoustic recording collection

To compare calling characteristics among urban and rural environments, acoustic recorders were placed at 12 rural sites and 11 urban sites, with latitudes ranging from 36.913° to 41.469°. Data from the National Land Cover Database (U.S. Geological Survey 2019) was used to determine the percent of developed land within a one kilometer radius from the recorder placement to quantify level of urbanization. The radius of one kilometer is an appropriate home range size for many frog species (Martof 1953). Developed land percentage was calculated as the percent of developed land (including high, medium, and low intensity developed land and open impervious surface) out of the total land cover, excluding areas of open water. Frog vocalizations were recorded from February 21, 2017 to July 12, 2017 by J. Feinberg at various locations along the Atlantic coast of the United States (Figure 2). Vocal recordings were collected using an Olympus DS-40 digital voice recorder and Sennheiser MKE 400 directional microphone. Five-minute recordings were made at 8:00pm, 9:30pm, and 11:00pm each night of deployment, when the frogs were most actively calling. In total there were 4,477 recordings captured across all three times of day. Primary calls of *R. kauffeldi* were present at thirteen of the sites: six urban and seven rural.

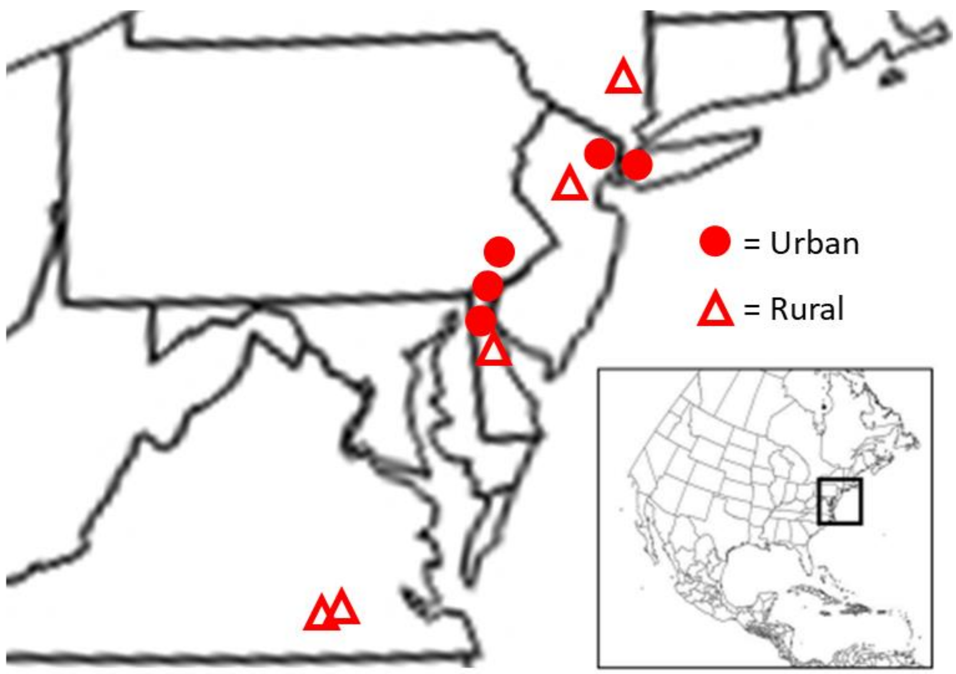


**Figure 2**. Locations of audio recorders.

Calling intensity

To test the first prediction that rural populations produce louder choruses more frequently, the chorus level was determined for each recording at all urban and rural locations. Acoustic recording analysis software, Kaleidoscope Pro (*Kaleidoscope Pro Analysis Software* (version 5) 2018), was used for the recording analyses with the following settings: spectrogram FFT length 2048 and Hanning window size 1024, for best visualization of the spectrogram. Using this software, the chorus rating for both urban and rural populations of *R. kauffeldi* were determined for all recordings. The standard North American Amphibian Monitoring Program chorus ranking index (Eastern Ecological Science Center 2016) described below was used to identify the chorus level of *R. kauffeldi* in each recording (Figure 3):

0 = no individuals calling

1 = individuals can be counted; there is space between calls

2 = calls of individuals can be distinguished but there is some overlapping of calls

3 = full chorus, calls are constant, continuous, and overlapping.

Only primary frog calls were considered in the chorus ranking, secondary and hybridized calls were not included, and the highest chorus level produced at any point in each recording served as the rating for that recording.

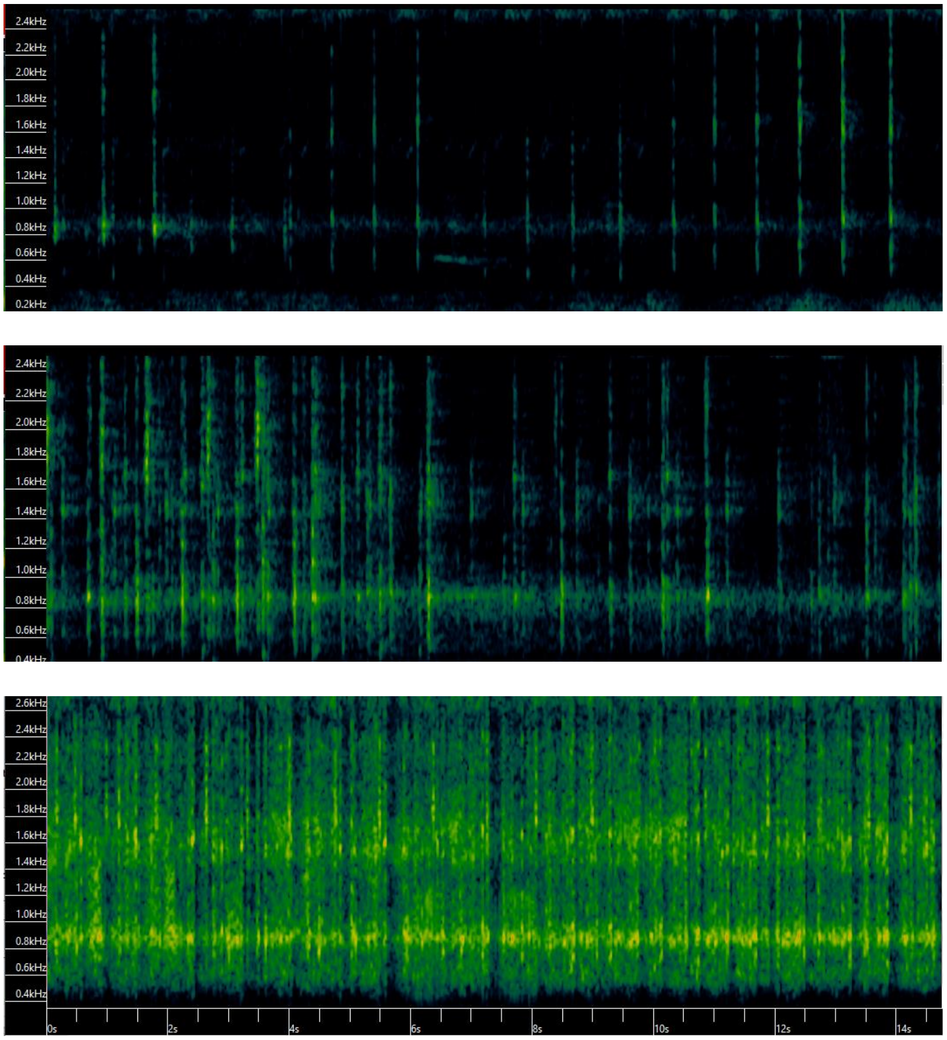


**Figure 3**. Spectrograms containing green vertical lines indicating *R. kauffeldi* calls. 1 chorus rating (top), 2 chorus rating (middle), and 3 chorus rating (bottom).

Nightly calling phenology

To test the second prediction that calling will begin later in the evening at urban locations, the time of day (8:00pm, 9:30pm or 11:00pm) that calling was first heard was recorded for each day.

Seasonal calling phenology

To test the third prediction that southern populations will begin calling earlier in the year, the earliest and latest dates that calling occurred were documented to outline the calling phenology. 11 recording sites captured the beginning of the calling season consisting of six northern populations (three urban and three rural) and five southern locations (two urban and three rural) with latitudes ranging from 36.91° to 41.47°. These ranges of the calling season were plotted against the latitude of each site for comparison among northern and southern locations.

Temperature patterns

To capture temperature patterns throughout the calling season, water and air temperature data were recorded using the iButton hygrochron temperature device. The iButton's were deployed simultaneously for the duration of the acoustic recording collection and recorded parameters every hour. Water temperatures were captured throughout the vocal recorder deployment at four urban and four rural populations and air temperatures were captured at three urban and two rural populations. Water temperature descriptive analyses were conducted on nine populations and air temperature descriptive analyses on six populations in which the iButton captured data from the first to the last calling days at each site. The maximum daily water and air temperatures were determined for the first, peak, and last day that calling was heard at a single site. The peak of the calling season was defined as the day in the middle of consecutive days with the highest chorus rating at each site.

Statistical analyses

To test if rural populations have more frequent louder choruses and begin calling earlier in the evening, regression analyses were conducted. Using JMP pro analysis software (*JMP Pro* (version 15), 2021), logistical regression models examined if chorus intensity differs between urban and rural populations. The response variable was the number of days with a two or three chorus rating and the predictor variables were urban or rural categories and latitude. To examine the relationship between the beginning of calling at night in urban and rural locations, a logistical regression model was generated. The predictor was the number of days that calling began at 8pm or 11pm and the response variables were urban or rural categories and latitude. To test if southern sites began calling earlier in the year and have longer calling seasons, a regression model with latitude as the predictor and the start dates of calling and the length of the calling season were the response variables. Consecutive days without *R. kauffeldi* calling before and after the season were excluded from all statistical analyses and one urban and two rural sites were also excluded from subsequent analyses due to sample sizes of fewer than 13 recordings with *R. kauffeldi* present. Using these methods, relationships of calling in both urban and rural populations of *R. kauffeldi* were examined to determine how the different environmental conditions impact their calling behavior.

## Results

### Vocal recordings

The three recording sites with the greatest number of recordings containing *R. kauffeldi* calling were rural (sites 2, 6 and 7) (Figure 4). For nearly all sites, calling occurred at all three times in the night, although some sites with the smallest sample sizes did not have calling documented at all three times. The three sites with the greatest number of recordings with calling at 8:00pm were all rural. Most rural sites have the largest number of recordings with calling at

8:00pm and have fewer recordings as time progresses whereas some urban locations have more recordings at the later times in the evening.

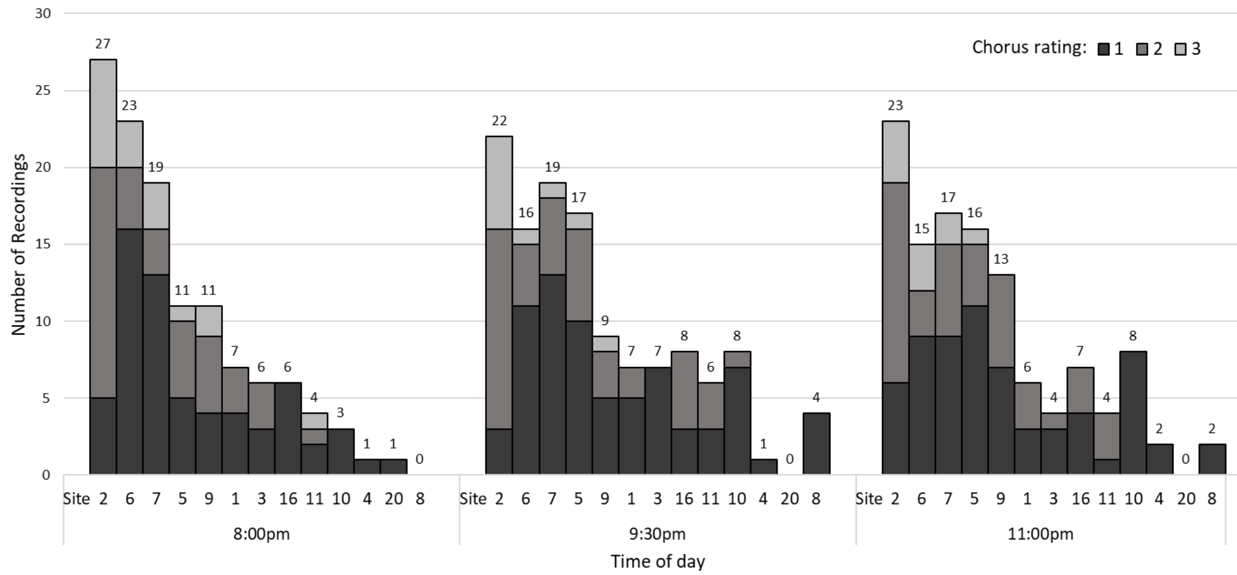


**Figure 4.** Number of recordings with a chorus rating of 1, 2, or 3 at each site per time of night.

Rural locations have the most recordings with calling at 8:00pm and fewer recordings when time approaches 11:00pm (Figure 5). Urban locations have a larger number of recordings containing choruses at 9:30pm and 11:00pm.

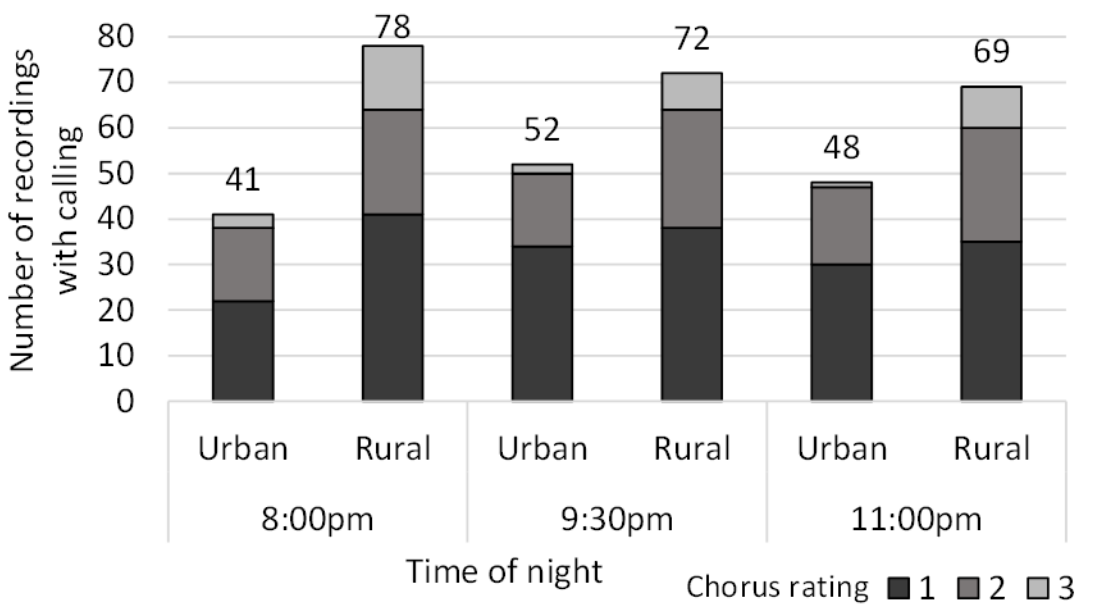


**Figure 5.** Number of recordings with *R. kauffeldi* calling for urban and rural sites at each time of night.

Calling intensity

The regression analysis of the number of days with a 2 or 3 chorus rating indicated that rural locations had significantly more frequent larger choruses than urban populations ($p = 0.021$; Figure 6). Locations with a smaller percent of developed land had significantly larger choruses and choruses were larger, but not significantly, as latitude increased ($p = 0.491$).

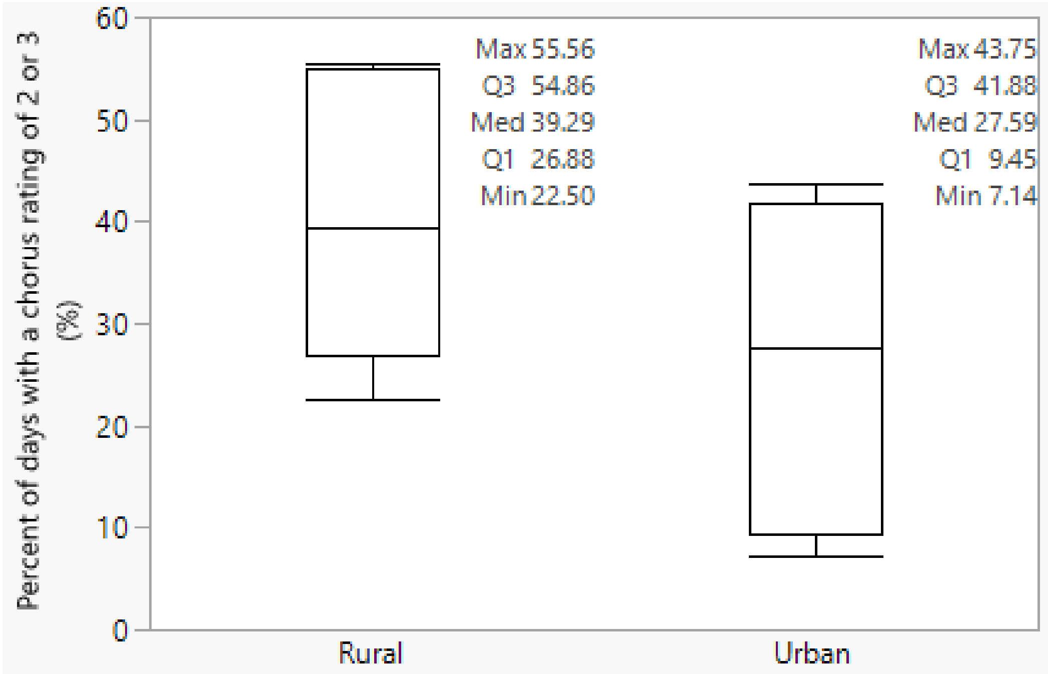


**Figure 6.** Percent of days with a chorus rating of 2 or 3 for urban and rural populations.

Nightly calling phenology

Rural populations began calling three hours earlier than urban populations, although the difference between urban and rural sites was not significant. Rural sites had a 26% larger median percent of all recordings with calling beginning at 8:00pm (p = 0.177; Figure 7) than urban sites. Locations at higher latitudes began calling at 8:00pm more often than lower-latitude sites but the difference was not significant (p = 0.434).

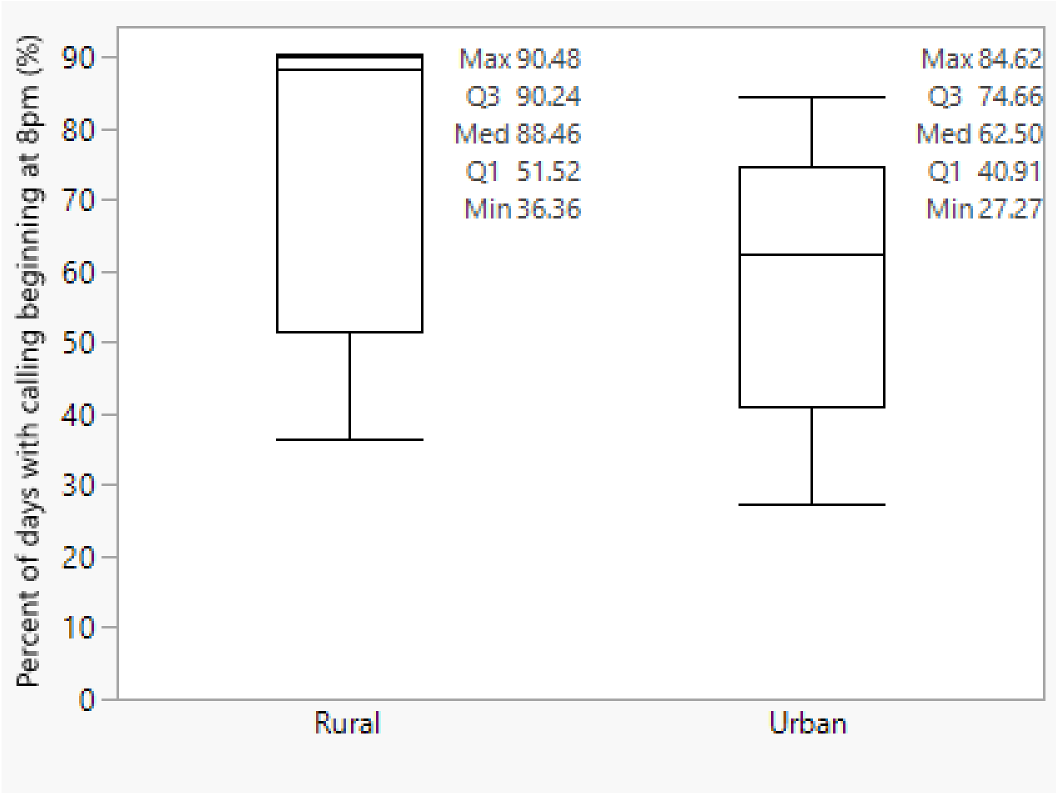


**Figure 7.** Percent of days with calling beginning at 8:00 pm for urban and rural populations.

Calling began more often at 11:00pm at urban sites versus rural sites, but this trend was not statistically significant (p = 0.821; Figure 8). Although calling beginning at 11:00pm was rare across the board, urban sites had a 5% higher median of days with calling starting at 11:00pm than rural sites (urban = 9%; rural= 4%). Sites with higher latitudes also began calling later in the evening than lower-latitude sites, but this difference was not significant either (p = 0.678).

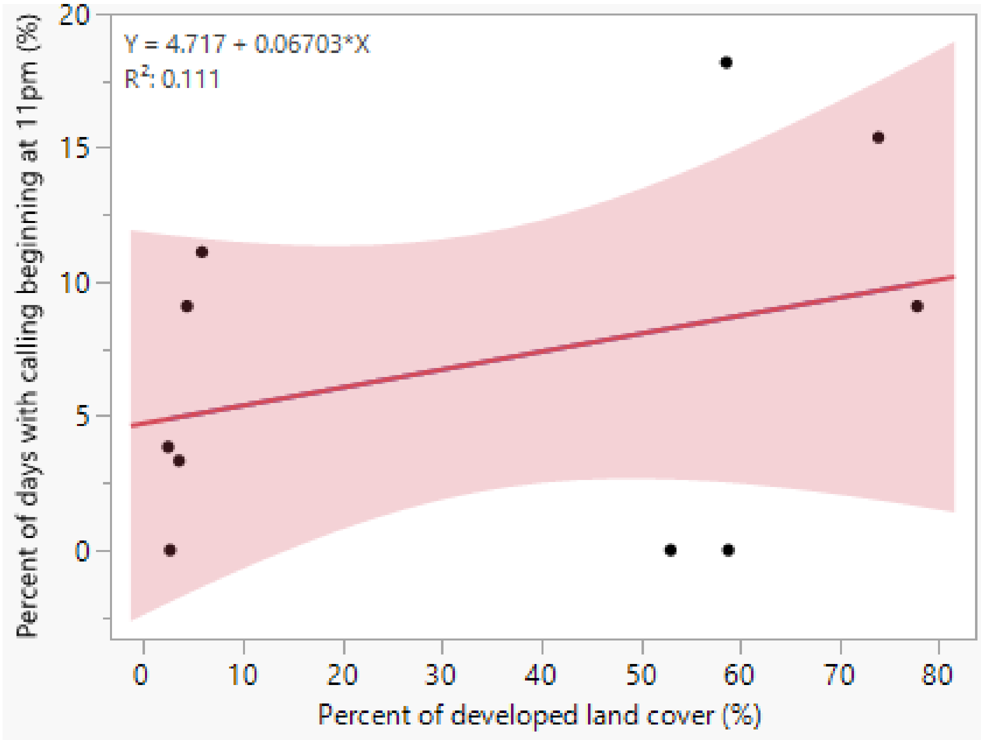


**Figure 8.** Percent of days with calling beginning at 11:00 pm vs. Percent developed land cover.

Seasonal calling phenology

Southern locations began calling significantly earlier in the year ($p = 0.002$) and had significantly longer calling periods ($p = 0.049$; Figure 9) than northern sites. Southern populations began calling 36 days earlier than northern sites on average. The average date that calling began for southern locations (latitude < 39.80°) was February 25, 2017 and for northern locations was April 1, 2017. The range of the beginning dates of the calling season at southern populations was from February 21, 2017 to March 6, 2017 and the beginning date range for northern populations was March 28, 2017 to April 4, 2017. Most northern locations had an overall significantly shorter calling season, average season length 16 days, than the southern sites, average season length 37 days.

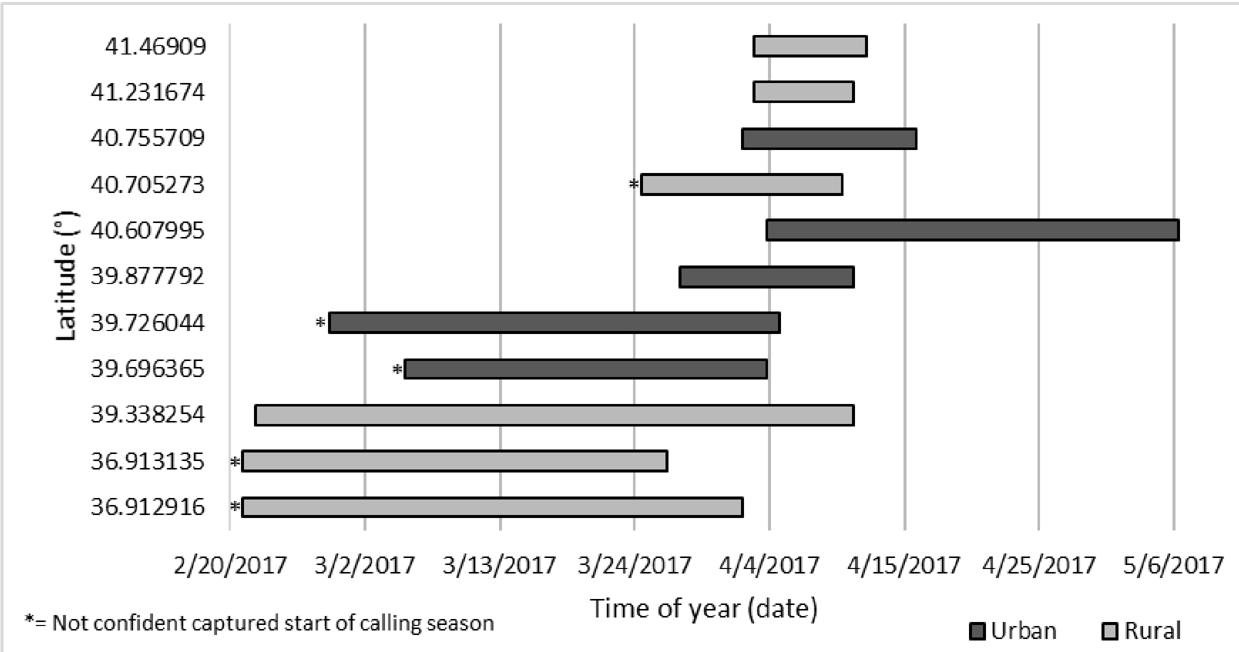


**Figure 9.** Beginning and end dates of calling for urban and rural sites vs. latitude.

Temperature patterns

Maximum water temperature increased overall, on average, throughout the calling season (Figure 10). The average daily maximum water temperature at the start of the calling season for all sites was 15.8 ± 7.1°C, the average maximum temperature at the peak of calling was 16.9 ± 5.8°C and the average maximum end temperature was 18.5 ± 3.8°C. Rural sites had a wider range of temperatures across the sites and three rural sites had the warmest maximum temperatures at each point in the season.

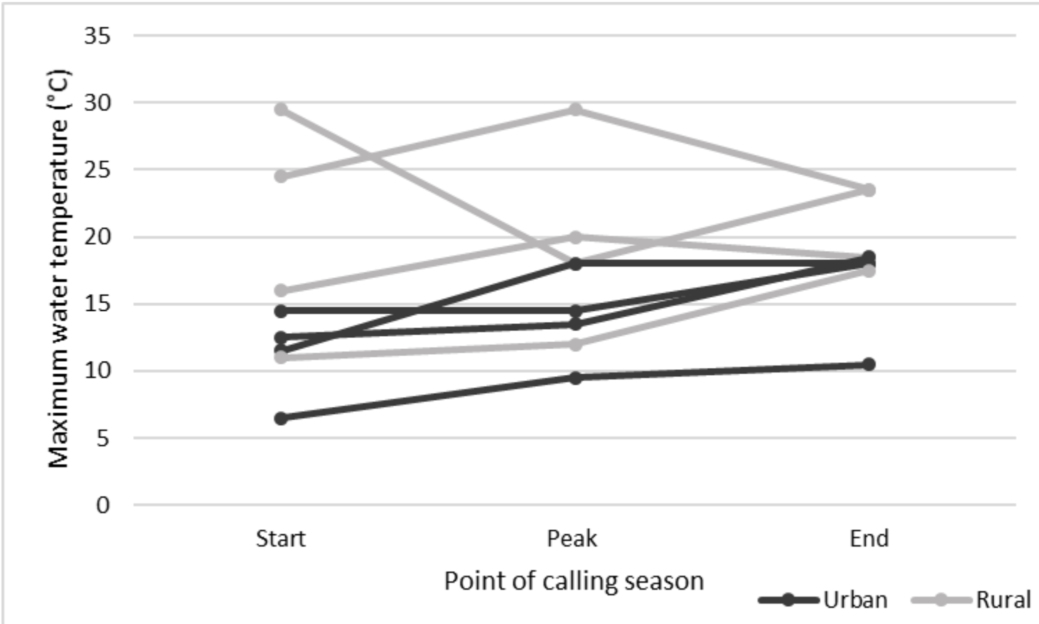


**Figure 10.** Average maximum water temperature at the beginning, peak, and end of calling per site.

Maximum air temperatures increased overall on average throughout the calling season. The average maximum air temperature at the start of the calling season for all sites was 18.7 ± 4.6°C, the average temperature at the peak of calling was 24.3 ± 8.8°C and the average end temperature was 29.3 ± 4.9°C.

## Discussion

We documented differences in calling between urban and rural *R. kauffeldi* populations and across latitude. The results show that rural populations produced larger choruses for a greater overall percentage of days than urban populations. Although the trend was not significant, urban populations more frequently began calling three hours later in the day than rural populations. We found differences in calling across latitude; on average, southern populations began calling 36 days earlier in the year and had a calling season that was 21 days longer than at northern locations. Both water and air temperatures increased, on average, throughout the duration of the calling season.

The hypothesis that there would be more frequent, larger choruses in rural environments was supported. However, this may not necessarily indicate larger population sizes; chorus ratings have not been confirmed to be an accurate predictor of population size (Pellet, Helfer, and Yannic 2007; Corn et al. 2011) because choruses do not include non-calling males, females, and juveniles. Other studies have found frog choruses occurred more frequently in rural locations, which is similar to our results (Green, Govindarajulu, and Higgs 2021).

The hypothesis that calling would begin later in the evening at urban locations was supported, but the difference was not significant. The small sample size of five urban and five rural sites that captured *R. kauffeldi* calling is a possible explanation for the non-significant statistical difference. This trend is similar to other studies and is likely due to anthropogenic light exposure from vehicles and buildings (Dias et al. 2019; Baker and Richardson 2006). Human light sources are normally present hours after dusk which can delay the perception of dusk and cause calling to begin later in the day.

Urban locations experience more anthropogenic light which often interrupts calling and can explain the quieter choruses at urban locations (Dias et al. 2019). Anuran species often begin calling in low ambient light settings which serves as an indication of a decreased risk of predation from diurnal animals and therefore light absence can predict the time of day that chorusing begins (Oseen and Wassersug 2002). Smaller and less dense populations in urban areas due to fragmentation may also contribute to quieter choruses (Okamiya and Kusano 2018). Urban environments with light pollution and fragmented populations likely result in less optimal conditions for breeding behaviors. These factors may explain the occurrences of quieter choruses at urban locations, suggesting that this environment type is less ideal for larger choruses and breeding.

The hypothesis that calling would begin earlier in the year at southern locations was supported. The difference in the onset of calling was explained by the warmer temperatures at southern sites which allowed for an earlier and longer season (Larsen et al. 2021). Temperatures at the beginning of the calling season can more strongly predict the onset of calling in the year, so the warmer southern sites were expected to start calling earlier (Larsen et al. 2021; Oseen and Wassersug 2002). The true temperature at the onset of calling may have been missed at some sites because the recorders were deployed when calling was predicted to begin, but calling may have begun before some of the recorders were deployed. There is possibility that calling began a few days earlier than captured at one of the northern sites, which likely would not significantly influence the results since the five other sites captured the beginning of calling. The longer calling season at southern sites allows for a greater number of calling nights and more chances for breeding. This may lead to larger populations in these areas and more successful breeding. Frog calling activity often declines and ceases as temperatures extend above their range (Oseen and Wassersug 2002), and rapidly increasing temperatures due to climate change may alter the optimal timing and duration of the frog breeding season.

*R. kauffeldi* is a recently discovered species and therefore a lot is unknown about its behavior and interactions with other species and its environment. This study provided important information about *R. kauffeldi's* interactions with its environment and basic natural history to aid with conservation. Because conservation lists rely on research and population surveys to accurately categorize a species' status, some species like amphibians, reptiles, and plants appear to be more threatened by extinction than is currently understood due to lack of research (Brooks et al. 2002) which is an issue for effectively protecting these species. Also, many animal species are yet to be discovered, especially organisms with similar morphologies, those located in places like the deep ocean, or microscopic organisms. Even *R. kauffeldi* was more recently discovered in a highly urbanized and densely human populated area, New York (Feinberg et al. 2014). Data deficiency is detrimental for proper conservation and species classification, and it is important to study novel species to protect them.

Examining the calling phenology of sympatric frog species could support that anthropogenic conditions lead to changes in calling behavior. The Pickerel frog *R. palustris*, which is a close relative of *R. kauffeldi*, often begins calling around 8:00pm and the calling intensity increases afterwards, which is similar to *R. kauffeldi* (Todd, Cocklin and Dorcas 2003). Perhaps *R. palustris* responds similarly to urbanization. The Southern Leopard frog *R. sphenocephala* has been found to begin calling after midnight; this may be due to anthropogenic light or other factors which could also influence *R. kauffeldi* calling phenology (Bridges and Dorcas 2000). Our recorders did not capture audio past 11:00pm and it may be beneficial for future studies to compare calling intensities at later times. Population surveys are another method to gather demographic information and identify environment types that support larger choruses (Pellet, Helfer, and Yannic 2007).

Studies like this are important because the results show differences in calling behavior of *R. kauffeldi* populations between urban and rural environments and across latitude. Appropriate breeding conditions are essential for species' success and urban advancements may negatively impact species breeding and therefore survival. Variations among latitude reveal that amphibians are likely to be susceptible to changes in environmental conditions due to climate change, which may influence their survival and persistence at southern latitudes. Future studies aimed to understand variables that impact breeding and survival success of animal species will identify areas of concern for conservation. Proper species identification of new or existing cryptic species is also important to determine future preservation mechanisms. It is important to develop a deeper understanding of animal and environmental interactions and the impacts of urbanization through research to formulate conservation efforts for species survival.

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