



**Effects of environmental variables on the calling behaviour of northern leopard frogs (*Lithobates pipiens*) in Alberta**

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Complete List of Authors:	Sommers, Marcus; Calgary Zoo, Centre for Conservation Research; University of Calgary, Biological Sciences Randall, Lea; Calgary Zoological Society, Centre for Conservation and Research Barclay, Robert M.R.; University of Calgary, Department of Biological Sciences
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M.D. Sommers, L.A. Randall, and R.M.R. Barclay

**M. D. Sommers** (email: marcusdsomm@gmail.com). Centre for Conservation Research, Calgary Zoological Society, 1300 Zoo Rd. N.E., Calgary, Alberta T2E 7V6, Canada. / Biological Sciences, University of Calgary, 2500 University Dr. N.W., Calgary, Alberta, T2N 1N4, Canada

**L.A. Randall** (email: lear@calgaryzoo.com). Centre for Conservation Research, Calgary Zoological Society, 1300 Zoo Rd. N.E., Calgary, Alberta T2E 7V6, Canada.

**R.M.R. Barclay** (email: barclay@ucalgary.ca). Biological Sciences, University of Calgary, 2500 University Dr. N.W., Calgary, Alberta, T2N 1N4, Canada.

**Corresponding author:** Lea Randall (email:lear@calgaryzoo.com) Centre for Conservation Research, Calgary Zoological Society, 1300 Zoo Rd. N.E., Calgary, Alberta T2E 7V6, Canada. Phone:(403) 294-7667

MDS Current affiliation: Centre for Conservation Research, Calgary Zoological Society, 1300 Zoo Rd. N.E., Calgary, Alberta T2E 7V6, Canada.

Effects of environmental variables on the calling behaviour of the northern leopard frog (*Lithobates pipiens*) in Alberta

Marcus D. Sommers, Lea A. Randall, and Robert M.R. Barclay

**Abstract**

Breeding behaviour of temperate-zone amphibians is influenced by environmental variables, but the initiation of calling (phenology) and influences on calling activity may vary with species and region. We investigated the influence of the timing of ice melt, water temperature, and photoperiod on the breeding phenology of *Lithobates pipiens* (Schreber 1782) in southern Alberta, using automated recording units. We also examined the influence of wind speed, relative humidity, water temperature and time of day on calling activity. The initiation of calling varied by 13 days at our three sites, suggesting that calling was influenced more by water temperature and timing of ice melt than photoperiod. Calling was first observed 8-11 days after ice melt at water temperatures of 7.5 - 8 °C at our sites. No calling was detected at water temperature < 5 °C. We recorded nocturnal and diurnal calling at all sites; > 50 % of calling was diurnal, even on days with warm overnight temperatures. Calling activity was influenced by time of day, water temperature, wind, and relative humidity. Our results suggest that date of initiation of calling varies considerably among breeding ponds and that the time of day of peak calling varies with both site and water temperature.

**Keywords:** Auditory surveys; automated recording units; ARUs; breeding; calling; *Lithobates pipiens*; northern leopard frog; phenology; *Rana pipiens*; temperature

## Introduction

Amphibians in the North Temperate Zone experience a relatively short breeding season with seasonal fluctuations in environmental conditions such as temperature, moisture, and photoperiod (Noble 1954; Oseen and Wassersug 2002; Wells 2007; Navas et al. 2008). The northern leopard frog, *Lithobates pipiens* (Schreber, 1782) (formerly *Rana pipiens*), is an example of a species that occupies a broad geographic range in North America, and likely experiences geographic and annual variation in the onset and timing of breeding (Lannoo 2005). Ambient temperature, moisture, and photoperiod play a critical role in the physiology and breeding behaviour of almost all temperate-zone amphibian species (Oseen and Wassersug 2002; Saenz et al. 2006; Both et al. 2008; Canavero et al. 2008), but it is unknown to what degree these environmental factors influence the onset and timing of calling of *L. pipiens*.

The timing of breeding has important implications for reproductive success and survival. Due to colder temperatures at northern latitudes, many anuran species delay breeding until later in the season (Muir et al. 2014) or breed under cooler conditions (Kessel 1965) than conspecifics at southern latitudes. For this reason, anurans may respond to different breeding cues in different parts of their range. For example, in northern Europe, *Rana temporaria* (Linnaeus 1758) and *Rana arvalis* (Nilsson 1842) both use warming spring temperatures as a cue to initiate breeding while further south in their range these species seem to rely more on photoperiod (Loman 2014). An extreme example of cold adaptation occurs in Alaska, where *Lithobates sylvaticus* (LeConte 1825) begins calling before the ice has finished melting (Kessel 1965), something which has not been recorded in the southern reaches of their range (Oseen and Wassersug 2002).

In the northern part of *L. pipiens*' range in Alberta, Canada, the cool climate results in a relatively short summer season and may slow development and compromise the ability to survive and reproduce (Wells 2007). The timing of oviposition depends on water temperature and varies among populations, geographic regions, and elevations (Dole 1967; Corn and Livo 1989). The development rate and

survivorship of *L. pipiens* eggs is also closely tied to water temperature and eggs from populations at northern latitudes demonstrate increased cold tolerance which may allow these populations to reproduce at lower temperatures (Moore 1939, 1949). For example, *L. pipiens* in Quebec only laid eggs at water temperature above 8 °C (Gilbert et al. 1994), whereas *L. pipiens* in the Cypress Hills in Alberta, which is cooler and at higher elevation, laid eggs at temperatures as low as 5.7 °C (Randall et al. 2014). Warming temperatures associated with climate change may also be responsible for advancing the timing of breeding in some locations. For example, there is evidence that *L. pipiens* now breeds almost a month earlier in Ontario than it did only forty years ago (Klaus and Lougheed 2013).

Previous studies have proposed that the phenology and time of year of amphibian breeding calls may be influenced by light levels (time of day), photoperiod (date), and temperature (Oseen and Wassersug 2002; Both et al. 2008; Canavero et al. 2008), but to the best of our knowledge this has been assumed rather than conclusively demonstrated for *L. pipiens* (Hine et al. 1981; AESRD 2013). Most North American species of anurans that call, do so with greatest intensity between sunset and midnight, but *L. pipiens* may call at other times of the day if nights are cool (de Solla et al. 2005; Dorcas et al. 2010). Photoperiod is seasonally variable at high latitudes and may be an important cue for the initiation of breeding activity in some species (Canavero and Arim 2009; Van Sluys and Guido-Castro 2011).

Humidity also influences the activity and calling behaviour of some species of amphibians. Some species call when it is drier and others call more during, or closely following, rain (Bellis 1962; Oseen and Wassersug 2002; Saenz et al. 2006), but in previous studies, humidity and precipitation had no effect on the calling behaviour of *L. pipiens* (Johnson and Batie 2001).

Wind is another variable thought to reduce the frequency of anuran calling, perhaps due to increased evaporative and convective heat loss and moisture loss, or due to acoustic interference through increased noise (Oseen and Wassersug 2002). In anurans, the effect of wind varies among species and is influenced by surrounding habitat and availability of sheltered areas (Oseen and Wassersug 2002; Milne et al. 2013). *Lithobates pipiens* is negatively affected by wind, calling only under calm conditions

(Johnson and Batie 2001). Individuals are also less detectable during visual surveys when it is windy (Smith et al. 2014).

Most studies of *L. pipiens*' breeding behaviour have focused on populations in the southeast parts of the range in the United States and eastern portions of Canada, which typically have a longer summer and wetter climate than the prairie regions of Canada (de Solla et al. 2005; Klaus and Lougheed 2013). There have also been numerous studies which examined breeding behaviour of *L. pipiens* but these have been conducted in an artificial captive setting. To more fully understand the breeding biology of this species in the wild, it is important to determine if environmental cues for breeding and reproduction differ among regions. A better understanding of these cues could improve probability of detection during amphibian surveys and would allow researchers to more effectively time their research. The consequences of failing to detect breeding during surveys prior to landscape modification or development can be devastating to a population if subsequent activities disrupt breeding or result in the loss of breeding habitat. This, combined with limited information regarding breeding of *L. pipiens* in Alberta, despite their schedule 1 (special concern) status under the Species at Risk Act (Government of Canada 2009), necessitates more research into their breeding behaviour in the province. The goal of our study was to help fill this knowledge gap by examining the breeding phenology of *L. pipiens*.

We hypothesized that the initiation of the breeding season of *L. pipiens* is influenced by changes in water temperature and photoperiod. If photoperiod is the dominant influence on the initiation of calling, we would expect that calling would begin on approximately the same date across sites, given that our sites have similar photoperiod (sites were at similar latitude and day length only varied by approximately 5 minutes among sites during the breeding season). If ice melt and water temperature is the dominant driver of the initiation of calling, then we would expect that calling would occur sooner at warmer sites and later at cooler and higher altitude sites. We predicted that calling would begin in mid-April, after ice melt, when there was ~14 hours of daylight and water temperature reached and exceeded 5 °C. In addition, we predicted that wind would negatively influence calling, and that

increased relative humidity would have no effect. We predicted that *L. pipiens* would primarily call after sunset when it was dark (30 minutes after sunset to 0100 h as recommended by AESRD 2013).

## Materials and methods

### *Study organism*

*Lithobates pipiens* is widely distributed across much of North America but has experienced declines in abundance and distribution, particularly in the western portion of its range. For this reason, the western boreal and prairie populations of *L. pipiens* in Canada are designated as “special concern” (COSEWIC 2009) and in Alberta they are listed as “threatened” (AESRD 2012).

The sexual behaviour of *L. pipiens* has been well-described (Noble and Aronson 1942). Individuals migrate to breeding ponds in early spring following emergence from over-wintering sites; although breeding and overwintering may occur in the same waterbody if conditions are suitable (Dole 1968; Wagner 1997). Breeding can occur in a variety of waterbody types, including wet meadows, beaver ponds, shallow marshes, and abandoned gravel pits (Dole 1971; Gilbert et al. 1994; McAlpine 1997). The breeding calls of *L. pipiens* consist of a complex series of snores, grunts, and chuckles<sup>1</sup> (Larson 2004) and males usually call while floating in the water (Russell and Bauer 2000). In Alberta, breeding typically occurs in mid-April to late May and occasionally into early June (Russell and Bauer 2000; Kendell 2002; Randall et al. 2014). Oviposition typically begins 2-3 days after the initiation of calling (Corn and Livo 1989).

### *Study Sites*

We selected three known *L. pipiens* breeding sites in southern Alberta to examine the effect of environmental variables on breeding-call activity<sup>2</sup>. The number of sites surveyed was limited by logistics and equipment availability. Sites were selected that represented various landscapes in which *L. pipiens* exists in Alberta. One site (Bow Gravel; N50.43°, W112.23°, datum WGS 84; 755 m ASL)

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<sup>1</sup> Figure S1 audiovisual file in supplementary material

<sup>2</sup> Figure S2 supplementary material

was located in a former gravel pit and comprised several small, semi-permanent ponds (~5 m wide and ~10 m long) located above a complex of permanent ponds (AESRD 2012). This site was still in somewhat early stages of succession with rocky, exposed soil covering much of the area and little vegetation surrounding the wetlands aside from sparse grass (*Poa compressa* L.) and sagebrush (genus *Artemisia* L.). The second site was a small round pond (20m diameter) on the Alberta side of Cypress Hills Interprovincial Park (Cypress Hills; N49.63°, W110.03°, datum WGS 84; 1462 m ASL). In addition to being at a higher elevation than our other two sites, this site was more sheltered from wind by a mix of aspen poplar (*Populus tremuloides* Michx. 1803), white spruce (*Picea glauca* (Moench) Voss), and lodgepole pine (*Pinus contorta* var *latifolia* Engelm.). The third site (Magrath, N49.42°, W112.87°, datum WGS 84; 978 m ASL) was a crescent-shaped pond (~10 m wide and 50 m long) surrounded by houses, roads and grazing fields; *L. pipiens* had been successfully re-introduced to this site about ten years prior to our study (Romanchuk and Quinlan 2006). All ponds were bordered by cattails (*Typha latifolia* L.) and bull rushes (*Schoenoplectus tabernaemontani* (C.C. Gmel.) Palla).

We used automated recording units (ARUs; Song Meter SM2+, Wildlife Acoustics, Maynard, USA), mounted at a height of approximately 1.5 m, to record *L. pipiens* breeding calls for the first 10 minutes of each hour. A Hygrochron iButton (DS1923, Maxim Integrated, San Jose, USA), attached to the bottom of the ARU, measured ambient air temperature and relative humidity. A HOBO Light Intensity Data Logger Legacy (Onset, Cape Cod, USA) was attached to the top of the ARU to measure illumination. We attached an anemometer (Inspeed.com LLC, Sudbury, USA) about 15 cm above the ARU to record wind speed. It was connected to a Madgetech Pulse 101a Data Logger (Madgetech, Warner, USA), housed in a waterproofed case located below the ARU. We placed three Thermochron iButtons (DS1921G, Maxim Integrated, San Jose, USA) in different locations on the ice surface of each pond to determine when the ice melted and to record water temperature. IButtons were coated in plastidip to waterproof them (Roznik and Alford 2012) and attached to fishing line to help recover them at the end of the season. All monitoring equipment was wrapped in wire mesh to protect it from wildlife. We programmed monitoring equipment

to record for the first 10 minutes of each hour (ARU and anemometer) or to take a single measurement at the beginning of each hour (iButtons and Hobos). We used total number of hours from sunrise to sunset each day as our measure of photoperiod (Government of Canada 2015).

We placed the monitoring equipment at Cypress Hills and Magrath midway along the edge of each pond on the side with the least ambient noise and greatest probability of detecting calls (e.g. north side of the pond and/or side distant from road or other sources of noise). The ARU at Bow Gravel was placed between two nearby ponds (~10 m from each) to maximize the probability of detection from either pond. We set up all monitoring equipment between 5 and 6 April 2014 (prior to ice melt) and collected equipment 44–45 days later. This period extended before and after the suggested dates for conducting amphibian calling surveys in Alberta (AESRD 2013), and successful breeding (indicated by observations of egg masses, tadpoles or young of year) was confirmed at each wetland using visual surveys (unpublished data, 2014).

We analyzed each ten minute recording to determine if calls were present or absent, using a combination of auditory and visual methods<sup>3</sup> (Song Scope Software, Wildlife Acoustics, Maynard, USA). The HOBO light intensity data logger at the Cypress Hills pond failed, and because of this we did not include illumination in our analysis. We did, however, analyze the difference in illumination between Bow Gravel and Magrath. We also lost one ThermoChron iButton at the Bow Gravel pond and at the Cypress Hills pond because the fishing line broke when we attempted to retrieve them. As a result, mean water temperature was calculated at both sites using only two iButtons.

The date of ice melt was determined by examining variation in hourly temperature to see when iButtons on the surface of the ice became submerged in water when ice melted (Anderson et al. 2015). This was easily assessed because daily water temperature was considerably less variable than air temperature. The time of ice melt was confirmed with local residents living on the edge of the Magrath pond (B. Cunningham, personal communication, 2014).

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<sup>3</sup> Figure S1 audiovisual file in supplementary material

*Statistical methods*

We only performed statistical analyses on data recorded after ice melt at each pond as there was no calling before this date, which we attributed to the presence of ice rather than any of the other environmental variables of interest. As a result, the time frame analyzed varied among sites. We tested for differences in water temperature, relative humidity, illumination, and wind speed among sites, as well as differences in relative humidity, water temperature and wind speed by time of day within sites. Because there was unequal sample size and unequal variance in environmental variables among sites, as well as temporal autocorrelation due to hourly sampling, we used generalized least squares (GLS) using restricted maximum likelihood and accounting for order 1 autoregressive error, to analyze our data in R using the nlme package (Pinheiro et al. 2016).

Including strongly correlated terms can result in unstable estimates of regression parameters and inflated confidence intervals (Quinn and Keough 2002; Royston and Sauerbrei 2008). We assessed collinearity of environmental variables using Pearson's tests, with a cutoff of  $R < 0.7$  for omitting a variable (Tabachnick and Fidell 2006) in R v 3.3.1 (R Core Team 2012). This confirmed that date was strongly correlated with photoperiod (Pearson's,  $R = 0.99$ , 3055 df,  $p < 0.0001$ ), thus we used date as a proxy for photoperiod in our models. We did not include air temperature in our analysis because it was positively correlated with water temperature (Pearson's  $R = 0.59$ , 3055 df,  $p < 0.0001$ ) and negatively correlated with relative humidity (Pearson's  $R = -0.81$ , 3055 df,  $p < 0.0001$ ), and because water temperature is likely a better predictor of calling behavior as *L. pipiens* typically call from water (Russell and Bauer 2000).

Our final model examined whether environmental variables were important predictors of the presence or absence of hourly calling activity using a nominal logistic regression, and examined the strength and direction of effects in the model using the beta estimates and the prediction profiler in JMP 11.2.1. (SAS Institute Inc., Cary, North Carolina, USA). We generally did not include interaction terms. We did, however, include a grouping factor that nested date within site to account for temporal

autocorrelation caused by sampling day after day. To assess which model was best supported by the data, we compared the Akaike information criteria adjusted for small sample size (AICc) values for models with all possible combinations of main effects ( $n = 16$ ). We assessed  $\Delta\text{AICc}$  and relative model weights ( $w_i$ ) to determine which models deserved further consideration (Burnham and Anderson 2002). Calling was coded as 0 for no calling and 1 for calling. We did not examine intensity of calling at any one time because it was difficult to accurately assess the number of individuals calling within a recording period. To examine the effect of time of day on calling activity, we divided each day into four periods of equal duration with the overnight period centered on the darkest part of the night (morning: 0400 – 0900 h, midday: 1000 – 1500 h, evening: 1600 – 2100 h, and overnight: 2200 – 0300 h). All continuous variables were standardized. We assessed whether the global model was a good fit to the data or if additional terms needed to be added, using the lack of fit test (JMP 11.2.1 Help).

## Results

The ice went off the breeding ponds at Bow Gravel and Magrath on 6 April but not until 17 April at the Cypress Hills pond. Calling began 11 days after the ice melted at Bow Gravel, 10 days at Cypress Hills and 8 days after ice melt at Magrath. When calling began, water temperature was 7.5 °C at Bow Gravel, 7.8 °C at Magrath and 8.0 °C at Cypress Hills. The lowest water temperature at which calling was observed was 5.0 °C at Magrath, 6.3 °C at Bow Gravel and 6.5 °C at Cypress Hills. The maximum water temperature when calling was observed was 19.3 °C at Magrath, 17.5 °C at Bow Gravel, and 16.3 °C at Cypress Hills.

Calls were detected during 309 out of 1082 intervals at Bow Gravel, 119 out of 801 intervals at Cypress Hills, and 86 out of 1174 intervals at Magrath. Diurnal and nocturnal calling was observed at all sites and approximately 54 % of intervals with calling ( $n = 514$ ) occurred during daylight hours between sunrise and sunset. The majority of intervals with calling occurred between 2200–0200 h at Bow Gravel, 1000–1400 h at Cypress Hills, and 0700–0900 h at Magrath (Fig. 1).

Mean water temperature varied significantly among sites with Magrath being the warmest site

(GLS,  $t = 12.01$ ,  $p < 0.0001$ ), and Cypress Hills being the coldest (GLS,  $t = -4.17$ ,  $p < 0.0001$ ). At Bow Gravel, water temperature was warmer in the evening than midday (GLS,  $t = -4.89$ ,  $p < 0.01$ ) and overnight (GLS,  $t = -6.19$ ,  $p < 0.02$ ), and morning was coolest (GLS,  $t = -11.14$ ,  $p < 0.0001$ ). Similarly, at Magrath, evening was warmer than midday (GLS,  $t = -6.02$ ,  $p < 0.0001$ ) and overnight (GLS,  $t = -5.90$ ,  $p < 0.0001$ ) which did not differ, and morning was coolest (GLS,  $t = -10.96$ ,  $p < 0.0001$ ). Water temperature at Cypress Hills, was significantly warmer in the evening than the rest of the day (GLS,  $t = 5.01$ ,  $p < 0.0001$ ) which did not vary significantly among time periods (GLS,  $t = -2.14$ ,  $p = 0.14$ ). Although water temperature varied significantly with time of day, it only varied by up to 4°C over the course of the day at each site.

Mean wind speed varied significantly among sites, with Bow Gravel being windier than Magrath (GLS,  $t = -9.66$ ,  $p < 0.0001$ ) and Cypress Hills being the calmest (GLS,  $t = -23.36$ ,  $p < 0.0001$ ). Wind speed at Bow Gravel did not differ significantly between midday and evening (GLS,  $t = 1.88$ ,  $p = 0.06$ ), but was least overnight (GLS,  $t = -8.05$ ,  $p < 0.0001$ ) and in morning (GLS,  $t = -7.24$ ,  $p < 0.0001$ ). Cypress Hills was windier in the evening than morning (GLS,  $t = -3.86$ ,  $p < 0.0001$ ) and midday (GLS,  $t = -5.57$ ,  $p < 0.0001$ ), but did not differ from overnight (GLS,  $t = 0.43$ ,  $p = 0.67$ ). Wind at Magrath was stronger at midday than evening (GLS,  $t = 5.21$ ,  $p < 0.0001$ ), and calmer in morning (GLS,  $t = -7.01$ ,  $p < 0.0001$ ) and overnight (GLS,  $t = -8.99$ ,  $p < 0.0001$ ).

Relative humidity was significantly different among sites, with Cypress Hills having significantly higher relative humidity than Bow Gravel (GLS,  $t = 4.18$ ,  $p < 0.0001$ ) and Magrath having significantly lower relative humidity than the other sites (GLS,  $t = -3.44$ ,  $p < 0.0001$ ). There was no difference in illumination between Bow Gravel and Magrath and illumination was not included in the final model (GLS,  $t = -0.06$ ,  $p = 0.95$ ). There was one clear best model to explain calling activity based on the  $\Delta AIC_c$  ( $> 12$ ) and model weight ( $w_i > 0.99$ ) (Table 1). The top calling behaviour model included date[site], site, water temperature, relative humidity, wind speed, and time of day. This model was a good fit to the data ( $R^2(U) = 0.20$ ).

Increasing water temperature had a positive effect on calling, while increasing wind speed and humidity had negative effects. Calling varied with time of day. The most periods with calling present occurred overnight followed by morning and midday, with evening having the least (Table 2). Calling also varied considerably with site, both in total number of periods with calling, as mentioned above, and in time of day of calls (Fig. 1)

## Discussion

Although much is known about the physiology and reproductive biology of *L. pipiens*, little has been reported about the breeding phenology of this species, especially at the northern extent of its range. Because amphibian breeding activity is thought to be heavily influenced by environmental conditions (Lanoo 2005), we expected that variables such as water temperature and photoperiod would influence initiation of breeding activity and that water temperature, wind speed and time of day would influence calling activity. Although photoperiod (day length) was similar at the three sites, the date of first calling differed by 13 days among sites. Based on these results, the initiation of calling activity was more strongly influenced by water temperature and date of ice melt than by photoperiod. However, it is possible that photoperiod is still an important cue to prevent premature breeding during brief warm periods in winter.

The timing of ice melt (06 April) and initiation of calling (14–17 April) was similar for Bow Gravel and Magrath. The timing of ice melt (17 April) and initiation of calling (27 April) was delayed at Cypress Hills. Calling was likely delayed because this site was cooler, a pattern which is consistent with other amphibian studies at high elevations (Corn and Livo 1989; Muir et al. 2014). Calling began 8–11 days after the ice melted at all three ponds, an association which, to the best of our knowledge, has not been reported for *L. pipiens* or any other temperate-zone amphibian species. However, it has been previously reported that the timing of snow melt influences the breeding phenology of some temperate-zone species, as resulting water fills ephemeral breeding ponds (Corn 2003). Future research may reveal whether the timing of ice melt is a consistent predictor of the initiation of calling among temperate-zone anurans.

Despite differences in when calling was initiated, water temperature was remarkably similar among sites when calling was first observed (between 7.5-8.0 °C). Once initiated, calling occasionally (<5 % of intervals with calling) occurred at temperatures <7.5 °C in our study area. The minimum water temperature at which calling was observed was 5 °C, which is cooler than temperature thresholds reported for other parts of the range of *L. pipiens* (Gilbert et al. 1994), but consistent with the minimum breeding temperature (5.7 °C) previously reported for Alberta (Randall et al. 2014). This is also close to the temperature threshold observed for calling in other northern species such as *Rana temporaria* (Muir et al. 2014), *Rana arvalis* (Loman 2014), and *Rana aurora* (Licht 1969). Calling was infrequent at low water temperatures and increased with increased water temperature, and most calling was observed when the mean water temperature was around 11 °C.

Calling by *L. pipiens* was influenced by site, time of day, water temperature, relative humidity, and wind speed. Our data showed that there were significant differences in water temperature, relative humidity, and wind speed among sites which likely contributed to the variation in calling among sites. Our results indicate a positive relationship between water temperature and calling, not only for initiation, but for calling activity throughout the season as well. Water temperature has a strong influence on amphibian function, including calling behaviour, especially in temperate regions, where frogs rely on warm water temperatures to elevate their metabolism enough to call (Dole 1967; Corn and Livo 1989; Lanoo 2005; Saenz et al. 2006).

Wind speed also had the expected effect on calling, increasing wind speeds resulting in decreased calling. It would be energetically wasteful and potentially dangerous for frogs to call in high wind conditions, as increased wind masks the acoustic signals making it ineffective to call in the wind and increases the risk of desiccation (Johnson and Batie 2001; Oseen and Wassersug 2002). Contrary to studies of similar species (Oseen and Wassersug 2002; Yoo and Jang 2012), we found that relative humidity had a negative effect on calling. This relationship is counterintuitive and further research is needed to understand the role humidity plays in calling behaviour for *L. pipiens*.

Calling occurred during all hours of the day throughout our study. Our analysis revealed that although there was significantly more calling overnight (2200 – 0300 h) than at any other individual segment of the day (morning, afternoon, or evening), over half of the periods with calling were during daylight hours (0700 – 2100 h). Diurnal calling has been reported previously for *L. pipiens* when night temperatures were cold (Cummins 1920; Hine et al. 1981; AESRD 2013), but in our study, diurnal calling was not limited to days with cool night temperatures. In addition, there was considerable variation in the time of peak calling among sites. For example, there was more calling at midday in the Cypress Hills, whereas most calling at Bow Gravel occurred overnight. We suggest that frogs called during the day at Cypress Hill because that site was colder but frogs could raise their body temperature by basking in the midday sun. At Bow Gravel, temperatures were warm enough at all times of day that frogs could call at night and avoid the increased predation risk associated with diurnal calling (Oseen and Wassersug 2002). To our knowledge, this apparent preference for diurnal calling, has not been reported before in *L. pipiens*. However, this result does not account for call intensity (number of animals calling), only number of intervals with calling.

Beyond the biological significance of our results, there are important ramifications for researchers and wildlife managers conducting auditory surveys for *L. pipiens*. The breeding period for *L. pipiens* in Alberta is described as occurring between 15 April and 13 May (AESRD 2013) which is consistent with our results. Although the suggested survey dates would correspond with the breeding season at most locations in Alberta, the breeding season may be delayed or protracted in some areas or years if weather conditions are cool or if sites are at high elevation.

The fact that *L. pipiens* rarely called (36 of 328 intervals) during the suggested survey hours (30 minutes after sunset to 0100 h) (AESRD 2013) at two of the three sites, should be a caution for researchers and wildlife managers conducting auditory surveys. *Lithobates pipiens* would not have been detected at two of the three sites (Magrath and Cypress Hills) if surveys had only occurred under the suggested

environmental conditions (wind <20 km/h and air and water temperature >10 °C) and during the recommended survey times and dates (15 April to 12 May) (AESRD 2013). Low rates of detection have been reported for this species in other parts of the range (Crouch and Paton 2002; Shearin et al. 2012). Our study serves to demonstrate the high degree of variability found in northern leopard frog behaviour across a small portion of their range. However, we acknowledge our data were only collected from three breeding sites, and thus may not represent the complete variation among all northern populations.

Our results illustrate that a species-specific and site-specific approach to the timing of auditory surveys may be required to detect some species of amphibians, such as *L. pipiens*, or surveys could result in underestimates of occupancy. We emphasize the importance of conducting multiple surveys at different times of day during the breeding season, particularly when conditions are favourable, to ensure high probability of detection for this species. Additionally, our results suggest that auditory surveys may not be effective at all sites and alternate survey methods should be used (e.g. visual surveys). Furthermore, breeding surveys should be delayed until later in the breeding season at higher elevation sites.

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## Figures

**Figure 1.** Radial plots of proportion of hours with northern leopard frog (*Lithobates pipiens*) calls at three study site in southern Alberta between 06 April and 21 May. Radii of each wedge indicate the proportion of time intervals for each hour of the day in which calling was present. All Sites graph represents the time of day of calls across all three sites. Concentric rings indicate proportion increments of 0.10 for Bow Gravel. Rings indicate increments of 0.05 for All Sites, Cypress Hills, and Magrath.

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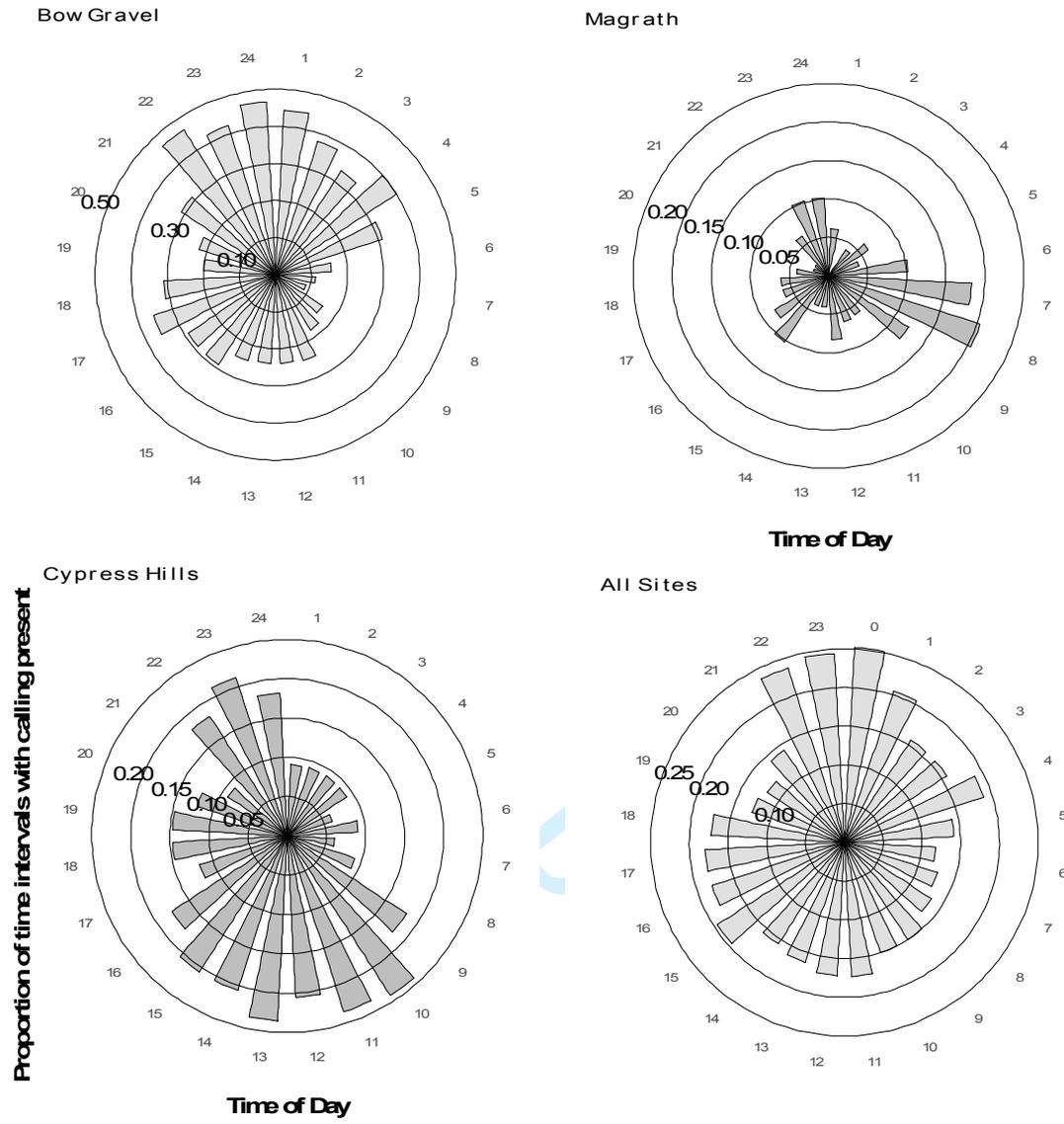


Figure 1.

## Tables

Table 1: Model selection results for nominal logistic regressions of northern leopard frog (*Lithobates pipiens*) calling activity for spring 2014 at three study sites in southern Alberta. Site and date[site] were included in every model to account for repeated measures and all continuous variables were standardized. WT = mean water temperature; HUM = mean relative humidity; KPH = mean wind speed; TOD = time of day; DF = degrees freedom; RSquare (U) = McFadden's pseudo R<sup>2</sup>; AICc is Akaike's Information Criteria corrected for small sample size;  $\Delta i$  is the AICc unit difference from the top model;  $w_i$  represents Akaike's importance weights

Model	DF	RSquare (U)	AICc	$\Delta i$
WT + HUM + KPH + TOD	11	0.20	2252.24	0.00
WT + KPH + TOD	10	0.19	2264.31	12.07
HUM + KPH + TOD	10	0.19	2266.75	14.51
WT + HUM + KPH	8	0.19	2269.61	17.37
WT + KPH	7	0.19	2272.96	20.72
WT + HUM + TOD	10	0.18	2284.07	31.83
HUM + KPH	7	0.18	2284.31	32.07
WT + TOD	9	0.18	2293.74	41.50
HUM + TOD	9	0.18	2296.71	44.47
KPH + TOD	9	0.18	2300.03	47.79
WT	6	0.17	2309.44	57.20
KPH	6	0.17	2310.01	57.77
WT + HUM	7	0.17	2310.36	58.12
HUM	6	0.17	2322.67	70.43
TOD	8	0.17	2324.20	71.96
Intercept	5	0.16	2334.25	82.01

Table 2: Standardized parameter estimates for nominal logistic regression of northern leopard frog (*Lithobates pipiens*) calling activity for spring 2014 at three study sites in southern Alberta. Variables included in analysis were time of day, mean wind speed, mean water temperature, and mean relative humidity. SE = standard error. %95 CI = 95% confidence intervals. Intercept represents the reference level when site = “Magrath” and Time of Day = “Overnight”. Interaction estimates are produced by the nesting of date within site.

Term	Estimate	SE	Lower 95 CI	Upper 95% CI
Intercept	2.32	0.10	2.13	2.51
Site[Bow Gravel]	-1.51	0.11	-1.72	-1.30
Site[Cypress Hills]	0.94	0.18	0.59	1.30
Site[Bow Gravel]:(Date-3.48E+09)	-1.60E-07	9.76E-08	-3.52E-07	-3.11E-08
Site[Cypress Hills]:(Date-3.48E+09)	-1.95E-06	2.29E-07	-2.40E-06	-1.50E-06
Site[Magrath]:(Date-3.48E+09)	5.68E-08	1.26E-07	-1.91E-07	3.05E-07
Water Temperature	-0.44	0.11	-0.66	-0.23
Humidity	0.30	0.08	0.14	0.45
Wind Speed	0.36	0.06	0.23	0.49
Time of Day [Evening]	0.42	0.11	0.2	0.64
Time of Day [Midday]	0.07	0.11	-0.14	0.28
Time of Day [Morning]	-0.07	0.11	-0.30	0.15