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SEASONAL AND DIEL VOCAL BEHAVIOR OF THE NORTHERN RED-LEGGED FROG, *RANA AURORA*

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ABSTRACT—Males of most anuran species use acoustic communication to attract females. *Rana aurora* (Northern Red-legged Frog) is one of a few anuran species that calls underwater, making it difficult to survey using traditional visual and auditory methods. *R. aurora* are experiencing significant population declines and are listed as a sensitive species in Oregon. Lack of information on basic life-history strategies may limit conservation efforts. This study explored calling behavior and breeding phenology of *R. aurora* by quantifying seasonal and diel calling patterns. An audio recorder with an underwater microphone (called a hydrophone) was used to capture underwater vocalizations at Finley National Wildlife Refuge near Corvallis, Oregon, USA. Results suggest that male *R. aurora* chorus from January until March for up to 14 h per day at a time. This is a significantly longer duration than previously recorded. This study shows that passive acoustic surveys can improve the detection and monitoring of a cryptic species, which allows for better monitoring and surveying of this species.

Key words: breeding, detection, phenology, *Rana aurora*

Many species of conservation concern live in areas that are difficult or impossible to survey using conventional visual methods. Without accurate monitoring techniques and current information on distribution, phenology, and abundance, effective management can be challenging. Amphibians are the most threatened group of vertebrates and are often very difficult to monitor accurately because their active behavior is usually limited to certain parts of the day or season (Bridges and Dorcas 2000). Amphibians play key roles and account for a considerable amount of biomass in terrestrial and aquatic ecosystems (Blaustein and others 1994; Stuart and others 2004; Adams and others 2013). Identifying accurate and efficient amphibian monitoring strategies is essential for their conservation.

Males of many anuran species vocalize to attract females and to establish territories within a breeding area (Gerhardt and Huber 2002). Acoustic advertisement can be energetically costly, with some males losing up to 40% of their body weight during the course of a breeding season (Gerhardt and Huber 2002). Females use vocal cues to initiate breeding and to find mates (Gerhardt and Huber 2002; Bee and Schwartz 2013; Velez and others 2013). These vocalizations are useful to amphibian monitoring and are accurate indicators of both seasonal and diel breeding phenology.

Rana aurora (Northern Red-legged Frog), which ranges from northern California to British Columbia and has a primarily coastal distribution (Jones and others 2005), is a semiaquatic species that vocalizes underwater. Because of this characteristic, traditional visual and acoustic survey methods are ineffective for this species. It is a species of conservation concern in Oregon, where its populations have been in decline due to habitat loss, land-use change, and invasive species (Kiesecker and Blaustein 1997, 1998; Kiesecker and others 2001). Although breeding behavior and life history of *R. aurora* have been described previously (Storm 1960; Licht 1969), little is known about its calling behavior or breeding phenology.

Information on call dynamics and breeding phenology in *R. aurora* is scarce, and has not been revisited since the late 1960s. Both Licht (1969) and Storm (1960) described male vocalization as a very quiet call which is heard over a 2-wk breeding season. Licht (1969) determined that *R. aurora* produces its mating calls underwater, a calling strategy that is relatively rare and restricted to a closely related ranid group (*R. aurora, R. cascadae, and R. boylii*) in western North America, and some *Xenopus* species (Hayes and Miyamoto 1984; Yager 1992; Given 2008).

The predominant *R. aurora* call has been previously described (Licht 1969) and contains 2 to 5 individual syllables of increasing amplitude, with a dominant frequency between 450 and 1300 Hz. Males vocalize primarily at night and begin their calling after ambient air temperature has been >5°C for several days (Licht 1969). *R. aurora* mating calls have not been thoroughly examined for structure or diel and seasonal patterns.

Understanding the communication behavior and breeding phenology of this species can allow for more thorough surveying and monitoring, which provides more information to manage habitat for *R. aurora*. Passive acoustic monitoring has been used across a wide variety of taxa to determine phenology, occupancy, and communication behavior. This technique is particularly useful in habitats where the species is cryptic (Marques and others 2013), dispersed over large areas (Stafford and others 1998), or when other complications make it difficult to monitor visually. Passive acoustic recording allows for continuous monitoring during the breeding season, even when individuals are calling underwater.

We conducted a pilot study to examine the seasonal and diel calling behavior of *R. aurora* using passive acoustic recordings. Specifically, we determined when vocalizations started and ended each day of the breeding season, and thus the total length of that season.

Methods

Data Collection

We installed a commercially available passive acoustic monitoring device (Song Meter SM2+, Wildlife Acoustics Inc., Maynard, MA) at the edge of a freshwater pond at Finley National Wildlife Refuge near Corvallis, Oregon (44°25′3.58″N, 123°19′32.32″W). The pond is a permanent water body with an average depth of 1.5 m that is surrounded by agricultural lands, primarily grass-seed crops. We equipped the recorder with an omni-directional and pre-amplified HTI 96-min hydrophone (High Tech Inc., Long Beach, MS) featuring a sensitivity of –165 dB re 1V/μPa to quantify the aquatic soundscape of the pond. The incoming analog signal was high-pass filtered at 180 Hz, amplified with 36 dB, digitized at 96 kHz and 16-bit resolution, and stored on 4x 128 GB SD memory cards. The system had a flat response (3dB) in the relevant frequency range of 200 Hz to 3000Hz.

The hydrophone was located approximately 2 m from the shoreline at a water depth of 1 m. We left this recorder in place to record continuously from 31 January to 9 May 2014 to encompass the previously determined *R. aurora* breeding season. We downloaded data and exchanged batteries in the recorder every 11 d. In addition, we recorded air temperature using an iButton temperature logger (Maxim Integrated, San Jose, CA) programmed to take 1 reading every hour for the duration of the breeding season.

Data Analysis

Long-term spectral average (LTSA) plots were created from the hydrophone data using the software Triton (Wiggins and others 2010). These plots were examined visually and aurally for evidence of *R. aurora* calls as described in Licht (1969). Once identified, all instances of long-term chorusing were logged. Daily sunrise and sunset times as well as hourly air temperature (°C) were also recorded. Data were analyzed in R (R Core Team 2015) for patterns of vocalization, including diel calling behavior, influence of air temperature on overall calling time, and seasonal calling behavior. Frequency and duration of calling per 24 h was measured from 12:00 (noon) to 12:00 (noon) local time every day because of the nocturnal calling behavior of this species (Licht 1969).

Results

Over the 90-d monitoring period, *R. aurora* calls were recorded on 32 d, between 31 January

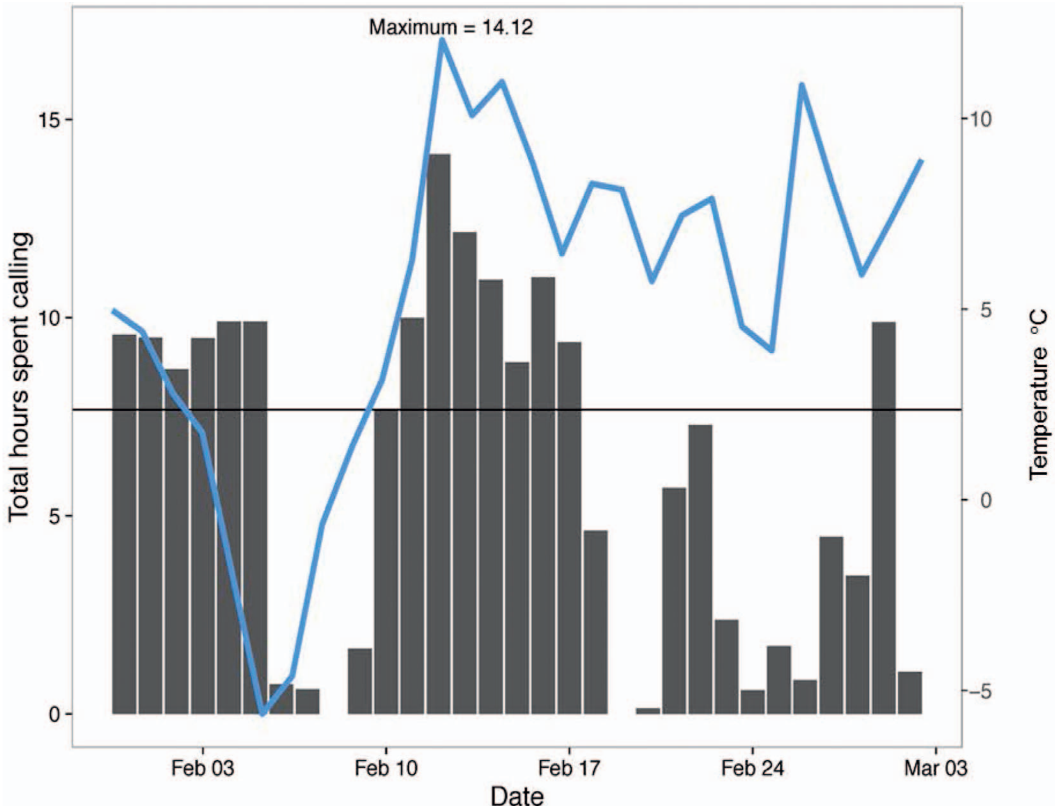

FIGURE 1. Total daily hours spent calling, 12:00–12:00. Line indicates the median, 7.675 h per 24-h period. Blue line is hourly temperature in °C.

and 3 March 2014. Median chorus length was 7.675 h (IQR = 8.169 h), and maximum of time spent chorusing in one 24-h period was 14.12 h (Fig. 1).

Chorus start time appeared to be related to sunset, starting on average 40 min after sunset each day (Fig. 2). Chorus end time did not appear to be strongly related to sunrise. An example long-term spectral average (Fig. 3) shows an approximately 9-h chorus from 5–6 February 2014.

Chorusing was already occurring on 31 January and continued until 3 March, a total of 32 d, suggesting that the breeding season lasts significantly longer than previously described in the literature (2-wk period; Licht 1969). Calling duration and start time were correlated with air temperature; colder temperatures coincided with shorter chorus duration and later start times (Fig. 2).

DISCUSSION

We have demonstrated that there is a considerable amount of novel information gained by passive acoustic monitoring techniques. *R. aurora* were previously thought to be explosive breeders with a relatively short 2-wk breeding period (Briggs 1987). Storm (1960) documented the *R. aurora* breeding season as approximately 2 wk, while Licht (1969) found the breeding season at his 2 study sites to be 15 to 27 d. Here, we recorded 32 d of mating calls, but failed to determine the initiation of breeding in this population. Therefore, it is likely that the breeding season lasts longer than 32 d at this site. While the duration of breeding activity may be specific to the population and year (Licht 1969), our results suggest that the breeding season may be more prolonged in some years and some populations than originally documented for this species. Additionally, given the energetic costs of calling for males, a maximum

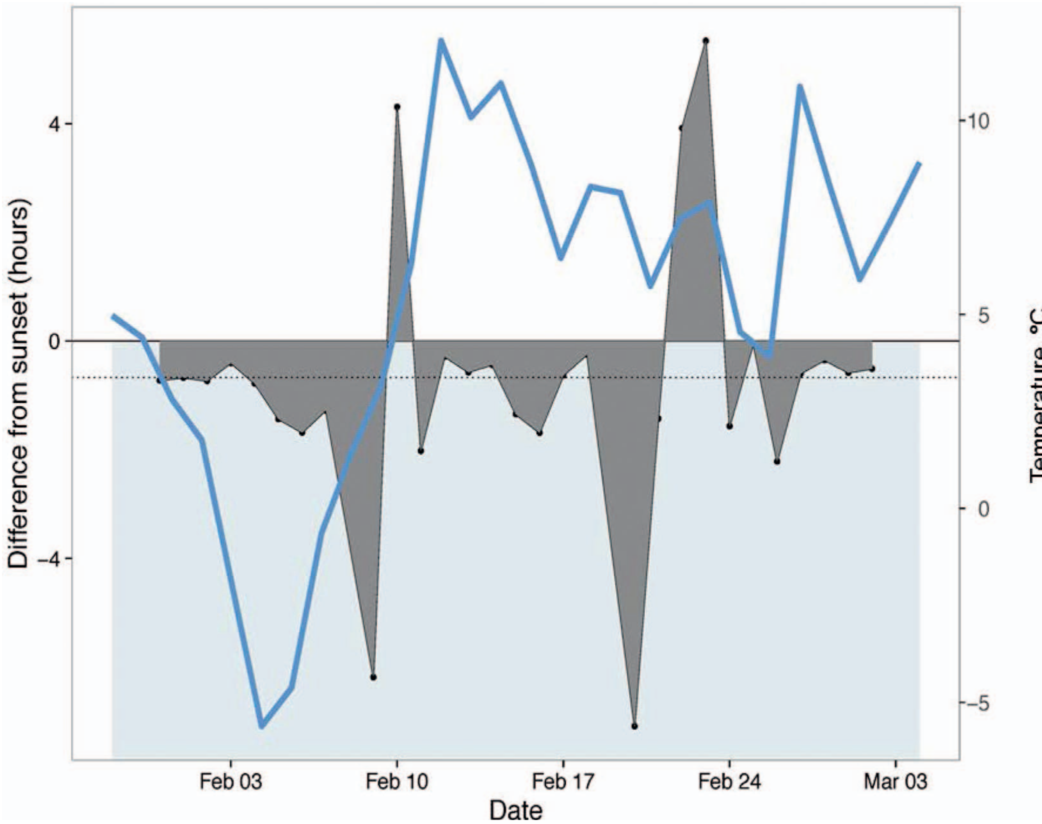

FIGURE 2. Time of chorus start as difference from sunset (median = 0.67 h, dotted line). Shaded area (lower half of figure) is hours after sunset; unshaded area (upper half of figure) shows daylight hours. Blue line is hourly temperature in °C.

chorus duration of 14 h is long in comparison to other species (Gerhardt and Huber 2002).

Interestingly, we recorded 2 distinct periods of reduced calling (Fig. 1). These periods corresponded closely with 2 cold snaps during the winter of 2014, from 9–11 February and 15–18 February, when temperatures reached well below 0°C. This corroborates Licht's (1969) assessment of the response of this species to low air temperatures. We also found that *R. aurora* chorusing appears to be predominantly nocturnal. Most of the chorusing hours occurred after sunset and continued throughout the night. Only very rarely was a chorus recorded during daylight hours, and this occurred primarily when nighttime temperatures were very low, which further demonstrates the relationship between chorusing and temperature.

Aquatic amphibians are challenging to monitor since they are often cryptic and difficult to detect via in-air acoustics. As is often the case, species that are difficult to monitor are those for whom monitoring data are most important, and conservation status may be inaccurate for data-deficient species. Our case study shows that passive acoustic monitoring can provide valuable information on breeding behavior, vocalization, and phenology in aquatic and semiaquatic species such as *R. aurora*. This technique has the potential to provide information about behavior, phenology, and habitat occupancy to monitor populations in the face of threats such as habitat loss, global climate change, and resulting range shifts. We found that *R. aurora*, a species of conservation concern, has a longer breeding season than previously thought, and that vocalizations are strongly tied to temperature and time of day. This information may help managers better limit disturbances during breeding, such as agricultural runoff or

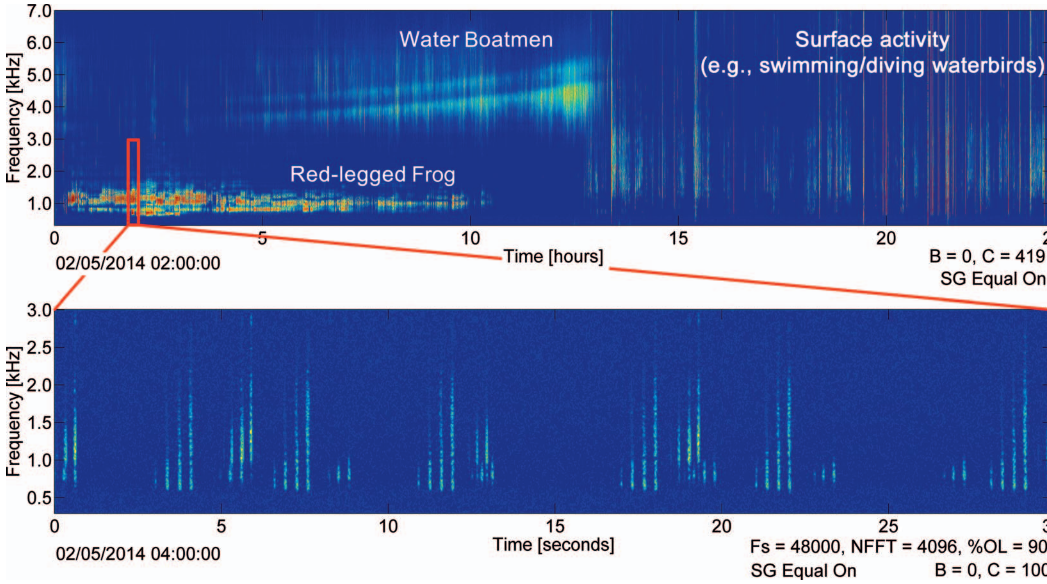

FIGURE 3. Long-term spectral average (LTSA) plot of Finley Office Pond underwater soundscape recorded on 5 February 2014 at 02:00 UTC (upper panel). Spectrogram showing individual *R. aurora* calls (lower panel).

grazing by cattle, which may compromise water quality (DeSolla and others 2002).

Passive acoustic monitoring is a valuable tool for determining phenology and occupancy of *R. aurora*. Further study is needed, however, to determine if these findings apply across populations and habitats. Further research could focus on call types and functions, and variation among individual frogs. This type of monitoring could be combined with detection methods, such as eDNA sampling (Lodge and others 2012), to gain an overall view of population structure in breeding ponds without the need for visual surveying.

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