

The effect of survey date on assessing calling activity of Hylidae

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In response to global amphibian decline the scientific community initiated the development of large-scale amphibian inventory and monitoring programs. One such program is the North American Amphibian Monitoring Program (NAAMP). Implementation and maintenance of a protocol that adequately characterizes amphibian calling activity across a continent is challenging. Several previous studies demonstrated that the NAAMP survey protocol can introduce biases into the program's data set. We conducted call surveys using sound files from automated recording systems to determine (i) if the NAAMP protocol misses peak calling activity of late season Hylidae species (*Acris crepitans*, *Hyla chrysoscelis*, and *Hyla cinerea*) in the Piedmont of South Carolina and (ii) if changing the NAAMP sampling regime for the Piedmont would impact detection of the focal species in the region. Our results showed that 2 of the 3 species (i.e., *H. chrysoscelis* and *H. cinerea*) reached peak calling activity outside of the NAAMP's third and final sampling period. We suggest that the addition of a fourth sampling period for the Piedmont may better characterize calling activity of Hylidae in the region (e.g., capture peak in calling activity and length of calling season). However, since a fourth sampling window encompassed the end of calling activity for the focal species, the likelihood of detecting the focal species during a fourth sampling window decreased. Addition of a later sampling window would need to be implemented cautiously, as we also determined that call survey noise indices were significantly higher during the late summer months due to insect activity. The higher noise indices may negatively impact call survey accuracy in a later sampling window.

Introduction

Global amphibian declines have concerned scientists for many years^{1,2}. Alarming, current rates of amphibian decline exceed those of other faunal groups, including mammals and birds³. Part of the concern surrounding amphibian decline stems from the view that amphibians are reliable bioindicators of environmental degradation, as amphibians have highly permeable skin and a life cycle that allows for exposure to terrestrial and aquatic stressors^{4,5}. Many of the leading factors that contribute to amphibian decline are caused by anthropogenic activities and include habitat loss, climate change, pollution, and disease^{3,6}. The factors implicated in the decline of amphibian populations also stand to jeopardize ecosystem services human populations depend on (e.g., water and air quality)^{7,8}.

In response to global amphibian decline, the scientific community initiated the development of large-scale amphibian inventory and monitoring programs. One such program is the North American Amphibian Monitoring Program (NAAMP), which was designed by the United States Geological Survey. Male anurans (i.e., frogs and toads) have species-specific breeding vocalizations that make them obvious in the environment during breeding events. The NAAMP takes advantage of the calling activity of male anurans by using standardized call surveys to score anuran presence in a region. While a standardized protocol is necessary for evaluating long-term trends in amphibian presence and persistence in an area, implementation and maintenance of a protocol that adequately captures and characterizes anuran calling activity across a continent is challenging.

Several previous studies demonstrated that the NAAMP survey protocol can introduce biases into the program's data set. The biases may impact assessment of anuran presence and activity in an area. Two studies found that the NAAMP protocol requirement to conduct one survey per site during a sampling period is inadequate for detecting all species in a wetland due to the complexity of interspecific differences in breeding phenologies^{11,12}. Work by Hayes and colleagues indicated that the NAAMP protocol stipulation requiring an observer to conduct

call surveys at stops along a route in sequential order introduces a temporal bias to call survey data¹³. Cochran and colleagues demonstrated that the third and final sampling window for the NAAMP in the Piedmont region of South Carolina did not capture peak calling activity of late season Hylidae species (i.e., Eastern Cricket Frog/*Acris crepitans*, Cope's Gray Treefrog/*Hyla chrysoscelis*, and Green Treefrog/*Hyla cinerea*)¹⁴. Thus, the breeding activity of these species in the Piedmont region of South Carolina may be underestimated. The objectives of the current study take the work of Cochran and colleagues a step further by evaluating whether a fourth sampling window would (1) better capture the peak calling activity of *A. crepitans*, *H. chrysoscelis*, and *H. cinerea* in the Piedmont region, and (2) impact the number of call surveys needed to detect a species.

Materials and Methods

To evaluate anuran calling activity during a hypothetical fourth NAAMP sampling window, we conducted call surveys using sound files from four automated recording systems (ARSs) located at four wetlands (Cleveland, Ludwick, Patterson, and Scotsgrove) in Spartanburg County, South Carolina. We conducted call surveys following the NAAMP protocol, and using ARS sound files collected from July 15th, 2012 through August 30th, 2012 (dates that would correspond to a fourth sampling window in the Piedmont). We chose 2012 as our focal year as Hayes and colleagues conducted call surveys using ARS sound files from the same wetlands during dates corresponding to the NAAMP sampling window three (May 15th through June 30th)¹³. By combining their data with our call survey data, we could evaluate if a fourth sampling window would capture the peak in calling activity of our three focal species (i.e., *A. crepitans*, *H. chrysoscelis*, and *H. cinerea*) and impact the probability of detecting each species. As in the previous study, we conducted call surveys using the 5 minute ARS sound files recorded at 20:30, 21:30, 22:30, and 23:30 (times that meet the NAAMP protocol stipulation that surveys must be conducted between half an hour after sunset and one in the morning). A potentially confounding factor impacting call surveys conducted

during a fourth sampling window is insect noise. Calling by insects (e.g., cicadas and grasshoppers) is correlated with increasing temperatures and peak insect calling often occurs in the warmest months¹⁵. Thus, we evaluated whether noise indices of call surveys conducted during the two sampling windows differed.

Data Summary and Analysis

We used frequency of occurrence to estimate the calling activity of the three focal Hylidae species. We calculated frequency of occurrence as the total number of surveys in which a species was scored as present divided by the total number of surveys conducted. When calculating frequency of occurrence, we only used call survey data from wetlands where a species was detected at least once. To visualize the effect of time on calling activity, we plotted frequency of occurrence for each species by week, as well as by sampling window.

In order to determine the minimum number of surveys needed to detect a species with 95% confidence during each sampling window, we used the equation $0.95 = (1 - \text{Frequency of Occurrence})^n$, transformed to $n = \ln(0.05) / \ln(1 - \text{Frequency of Occurrence})$.

We used a one-tailed t-test to determine if the average noise index of call surveys conducted during the fourth window was significantly higher than the average noise index of call surveys conducted during the third sampling window.

Results

We did not detect all 3 species at each of the 4 wetlands; *H. cinerea* was absent at Patterson and *H. chrysoscelis* was absent from Cleveland. Calling activity of *H. chrysoscelis* and *H. cinerea* peaked in late July, while calling activity of *A. crepitans* gradually declined as the sampling period progressed (Figure 1). For all species, calling activity ceased after August 24th (Figure 1). The frequency of occurrence of the focal species decreased during sampling window four (Figure 2), which increased the minimum number of surveys needed to detect a species in sampling window four (Figure 3). The average noise index level for the hypothetical fourth window was significantly higher (44%) than the average noise index level for NAAMP sampling window 3 (t-stat= 17.4, df=1306, p-level<0.001, Figure 4).

Discussion

The results of our study suggest that the addition of a fourth NAAMP sampling window would better capture peak calling activity of Hylidae species in the Piedmont region of South Carolina. As Cochran and colleagues suspected¹⁴, peak calling activity of *H. cinerea* and *H. chrysoscelis* was not captured by the current NAAMP protocol. Our study was limited to ARS data from the year 2012. If the findings of this study are found to be consistent in subsequent years, scientists interested in evaluating the breeding phenology of late season Hylidae in the Piedmont would want to sample through August. Interestingly, the breeding assemblages at two of the four wetlands (Ludwick and Patterson) differed between our study and that of Hayes and colleagues, in which *A. crepitans* was not detected at Ludwick and *H. chrysoscelis* was detected at Patterson¹³. Temporal differences in breeding assemblages at wetlands could indicate partitioning of breeding activity among coexisting species¹⁶.

Additionally, the minimum number of surveys needed to detect each focal species increased during our hypothetical fourth

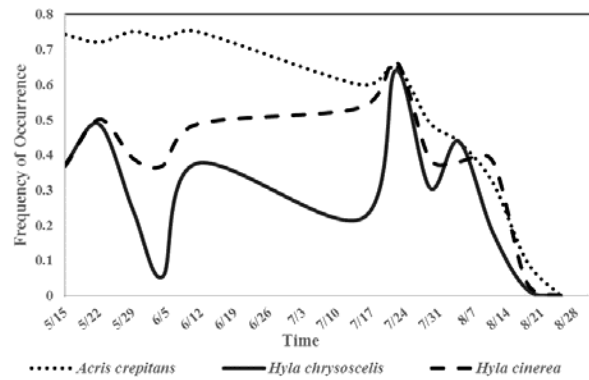


Figure 1. The effect of time on the calling activity of *Acris crepitans*, *Hyla chrysoscelis*, and *Hyla cinerea* in Spartanburg County, South Carolina.

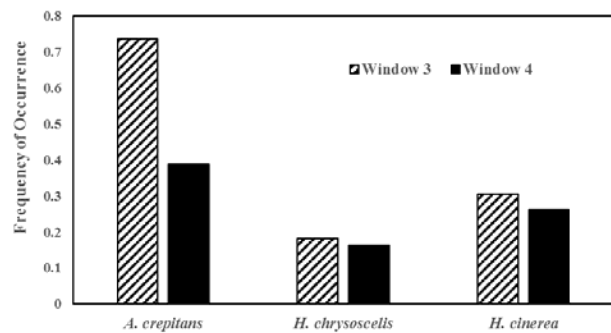


Figure 2. The effect of sampling window on the occurrence of *A. crepitans*, *H. chrysoscelis*, and *H. cinerea* in call surveys during NAAMP Window 3 and our hypothetical 4th window during the year 2012.

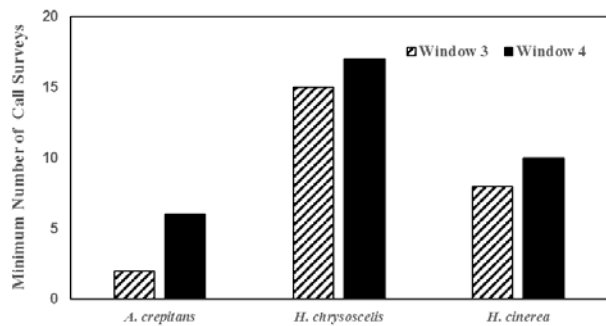


Figure 3. The effect of sampling window on the minimum number of call surveys needed to have a 95% probability of detection for *A. crepitans*, *H. chrysoscelis*, and *H. cinerea*.

sampling window. The increase in the number of surveys needed was due to each species lower frequency of occurrence in call surveys during the fourth sampling window. The drop off in frequency of occurrence was related to capturing the end of the calling season for all three species (i.e., no calling was detected past August 24th). Overall, our results suggest that an observer would have a lower probability of detecting these species when conducting a call survey in our hypothetical fourth window relative to NAAMP's current third sampling window. Thus, if a

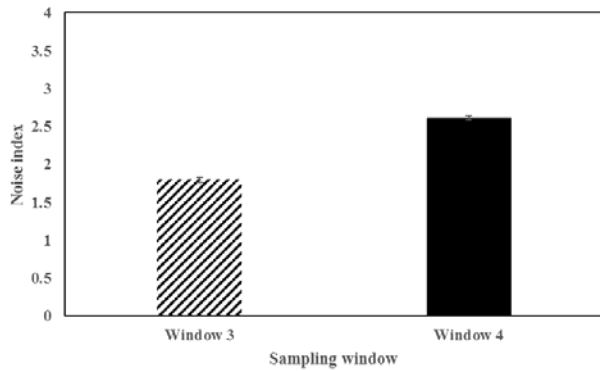


Figure 4. The effect of sampling window on average noise index.

scientist is simply interested in detecting the focal species, the current NAAMP sampling window three worked better than an hypothetical sampling window four. It would be interesting to see if our results would hold for other species in the Piedmont, as a previous study conducted in the state of Rhode Island found that four sampling windows were necessary to have a high probability of detecting all species within the region¹⁷.

We found noise index levels associated with the ARS call surveys to be significantly higher during our hypothetical window four, mostly due to insect activity. This result was not surprising, as several NAAMP volunteers in our region have noted problems in detecting anurans calling over the deafening insect choruses as early as sampling window 3 (Pilgrim, unpublished data). High noise index levels could present a problem in implementing a fourth sampling window. Specifically, high noise indices can inhibit an observer's ability to hear calling anurans, or increase the occurrence of false-positive detections by the observer².

The results of our study yielded mixed results for supporting the addition of a fourth sampling window for the Piedmont region of South Carolina. We were able to capture peak calling activity in Hylidae species in July and early August, but the overall detection probabilities for each species went down in the hypothetical fourth window. In addition, noise levels during late summer were near or above noise index thresholds allowed by NAAMP protocol. A middle ground might be extending the third sampling window, rather than implementing an entirely new fourth sampling window. Interestingly, at the end of the current study, the NAAMP state coordinator for South Carolina notified all volunteers that sampling windows for the Piedmont would be extended during the 2016 season. Thus, our next step is to conduct call surveys using the ARS sound files that correspond to the extended third sampling window and evaluate the impact of the extended window on detection probabilities for Hylidae in the Piedmont.

Acknowledgements

We would like to thank Jarad Cochran for project suggestions and discussion. In addition, we would like to thank the Spartanburg Area Conservancy and the generous landowners who allow us to use their properties as sites for our ARSs. We are grateful for financial support provided by the Office of Sponsored Awards and Research Support at USC Upstate, and the Magellan Scholars program at USC Columbia.

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