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**Author** Kaiser, Kristine

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## Evaluation of a Long-Term Amphibian Monitoring Protocol in Central America

KRISTINE KAISER<sup>1</sup>

### Department of Biological, Geological, and Environmental Sciences, Cleveland State University, Cleveland, Ohio 44115, USA

ABSTRACT.—The Maya Forest Monitoring Project (Mayamon) was established in 1997 as an outgrowth of the Belize working group of the Declining Amphibian Populations Task Force. For nine years, Mayamon volunteers censused anuran populations using a protocol that estimates numbers of individuals on the basis of male vocalization. To date, the protocol has been evaluated only through a series of post hoc power analyses; I performed the first field test to assess the effect of species-specific mating system characteristics, survey length, survey frequency, and pond selection on census results for anuran communities within a tropical moist forest in Belize. Under the current protocol, it would take, on average, 359 months of sampling to detect the 11 species I detected at this site using vocalization surveys. In addition, I introduce a method using ANCOVA to determine ideal survey length. Arbitrarily setting the ideal detection to 90% yields a required sampling protocol of 21 minutes; the current minimum of 15 min yields only an 80% detection rate. This method could be adapted for use with other monitoring programs, allowing both the assessment of current efficacy and the extrapolation of required sampling length to reach a given efficacy. The results of these approaches indicate that the Mayamon protocol methodology should be extended if it is to allow investigators to adequately understand the community dynamics of amphibians.

It is widely recognized that amphibian populations are declining at an alarming rate: over one-third of all amphibian species are now listed as species of concern, with another 6% listed as near threatened (Stuart et al., 2004). In the past several years, great strides have been made in understanding the causes of declines: emerging infectious diseases including the chytrid fungus (Fellers et al., 2001, 2004; Mendelson et al., 2004; Lips et al., 2006), herbicides and pesticides (Sparling et al., 2001; Hayes et al., 2003, 2006; Relyea, 2005), and climate change (Pounds et al., 2006) have all been linked to declines in several countries. However, the factors underlying others still are not well understood (Waldman and Tocher, 1998; Alford and Richards, 1999; Eterovick et al., 2005). Thus, there is a critical need for rapid and accurate methodologies for assessment of amphibian population sizes and community composition.

The Maya Forest Monitoring Project (Mayamon) monitored anurans in the Yucatán Peninsula of Central America for nine years, beginning in 1997 (Herrera-MacBryde, 1998). The Mayamon sampling model depends entirely upon volunteer biologists living in the region. The protocol is a simple calling survey. Observers visit each site once a month, between 1900 and 2300 h for 15–30 min, and rate abundance of each vocalizing anuran species on a four-point scale (1 = 1-5 frogs, 2 = 6-20 frogs, 3 = 21-50 frogs, 4 = >50 frogs). Sampling is conducted from May until October, when the majority of amphibian reproduction occurs in this region. Volunteers choose the sites they will monitor; the guidelines state only that it be a pond, stream, or section of a lakeshore.

Although the Mayamon protocol was in use in three countries for almost a decade, its effectiveness was evaluated only through post hoc power analyses (Arrigoni, 2003), which do not allow for understanding how local factors (e.g., rainfall timing and abundance) affect the data. The monitoring of species with an explosive breeding habit (sensu Wells, 1977) poses another problem: these species vocalize for less than a month and could easily escape detection under the current protocol, although they could be among the most locally abundant species. Therefore, the frequency and duration of the protocol are important aspects that should be evaluated for efficacy; if a protocol misses a species on a given night, what is the likelihood that it will detect it on another night? Can a survey of suboptimal length be ameliorated by frequency of sampling?

The purpose of this project therefore was twofold: (1) to determine how species-specific mating system characteristics, length of calling

<sup>&</sup>lt;sup>1</sup>Present address: Department of Ecology and Evolutionary Biology, University of California Los Angeles, 621 Charles East Young Drive South, P.O. Box 951606, Los Angeles, California 90095-1606, USA; E-mail: kriskaiser@ucla.edu

		Explosive or pro-				
Species	Elegans	Millionario	Tapir	Warree	longed breeder?	
Agalychnis callidryas Cope, 1862	Р	Р	Р	Р	Prolonged	
Agalychnis moreletii Duméril, 1853	Р	Р	Р	Р	Prolonged	
Bufo valliceps Wiegmann, 1833	А	Р	Р	А	Prolonged	
Dendropsophus microcephala Cope, 1886	Р	Р	Р	А	Prolonged	
Gastrophryne elegans Boulenger, 1882	Р	А	А	Р	Explosive	
Rana berlandieri Baird, 1854	А	Р	А	А	Prolonged	
Rhinophrynus dorsalis Duméril and Bibron, 1841	Р	А	Р	Р	Explosive	
Smilisca baudinii Duméril and Bibron, 1841	Р	Р	Р	А	Prolonged	
Smilisca cyanosticta Smith, 1953	Р	А	А	А	Prolonged	
Tlalocohyla loquax Gaige and Stuart, 1934	Р	Р	Р	А	Prolonged	
Tlalocohyla picta Günther, 1901	Р	Р	Р	А	Prolonged	

TABLE 1. Species of frogs detected at each of four pond sites at Las Cuevas Research Station, Belize. P = present at a given pond; A = absent. Explosive or prolonged breeders are defined as in Wells (1977).

survey, frequency of surveys, and site selection affect the efficacy of the Mayamon sampling protocol; and (2) to determine the ideal survey length based on desired detection efficiency.

#### MATERIALS AND METHODS

I conducted this study at Las Cuevas Research Station (LCRS), Chiquibul Forest Reserve, Cayo District, Belize (16°44'N, 88°59'W) between 21 May and 27 August 2002. The station is situated at approximately 500-m elevation on the northern slope of the Maya Mountains in deciduous forest and deciduous/ semievergreen seasonal forest (Penn et al., 2004). Annual rainfall is approximately 1,500 mm. The staff of LCRS became a Mayamon participant in 1998.

I chose four study ponds for this project: two ponds within the forest (Elegans and Warree), and two open ponds (Millionario and Tapir), adjacent to forest but with less than 10% canopy cover. All but Millionario are seasonal ponds, containing no standing water in the dry season; Millionario holds water year round, although it decreases substantially in size. I performed surveys between 1800 and 2300 h. I defined a sampling event as a 15-min listening interval, corresponding to the minimal period specified in the Mayamon protocol and a pond night as the set of four successive sampling events that occurred on a given night at a given pond. During each sampling event, I listened for vocalizing anurans and recorded the species and the number of calling individuals of each species. However, for the purposes of this paper, I will focus only on the presence or absence of species in a given sampling event.

To determine the effect of survey length on sampling efficiency, I tested the difference between 15 and 30 min, the two extremes of the Mayamon protocol. Because the species detected in any two sampling events on a given night would not be independent, I randomly assigned three of the four sampling events within a pond night to 15-min or 30-min periods using a PERL resampling script, and calculated a Detection Efficiency (DE; after Pierce and Gutzwiller, 2004). DE is a proportion, defined as the number of species detected in a sampling event divided by the total number of species detected on that pond night. To make the data linear with respect to time, I used the transform ln [1 - (DE + 0.1)].

I used ANCOVA to determine the ideal survey length, including pond, sampling duration, day of study, and time of start in the model. I fit the data to the curve  $DE = 1 - e^{-kt}$  where *k* was experimentally determined to be 0.11. The transform was selected to linearize the data and was weighted to prevent the DE from reaching 1, as DE should be 1 at  $t = \infty$ . All statistical analyses were carried out in SAS 9.0 (SAS Institute, Inc., Cary, NC, 2002).

#### Results

I surveyed using the Mayamon protocol for a total of 119 pond nights and 484 sampling events. I detected a total of 11 species (Table 1). The number of species at a pond ranged from 4– 9. A total of 13 species are known to breed in the area (unpubl. data).

A Wilcoxon Signed Ranks test yielded a significant difference in detection efficiency between 15- and 30-min sampling periods (W+ = 675, Z = 5.5566, P << 0.001). Based on these data, at 15 min, the Mayamon sampling protocol detects, on average, 80% of all species that would be detected on a given night.

Ideal survey length was determined using ANCOVA. After linearizing the data, I arbitrarily set the desired detection efficiency at 90%, yielding a required survey length of 21 min.



FIG. 1. Species accumulation curve of frog species for four study ponds combined using the Mayamon protocol. Note break in X-axis. A total of 359 actual sampling events were required to detect all species.

Two explosive breeders (*Rhinophrynus dorsalis* and *Gastrophryne elegans*) were detected in the first two weeks of the survey. I detected *G. elegans* on two sampling nights, within four days of each other and *R. dorsalis* on seven nights, all within a period of approximately two weeks. These frogs were not detected again during the study. Only one individual of *Smilisca cyanosticta* was observed on one occasion during the survey, despite the fact that this species was observed throughout the season at nonfocal ponds. All other species were detected throughout the survey.

I created species accumulation curves to visualize when in the study species were detected (Figs. 1, 2). For this analysis, I calculated the number of sampling events required to detect all species detected at a given pond. The range for individual ponds was five sampling events (Warree pond, with four species) to 78 sampling events (Elegans pond, with nine species). For all four ponds combined, the total number of actual sampling events required to detect all 11 species was 359.

Site selection also had an effect on survey results. The ponds in this study had, on average, only seven of 13 (54%) frogs known to be in the area. Only two species were detected at all four study ponds.



FIG. 2. Species accumulation curve of frog species for four study ponds combined using the Mayamon protocol first 10 species only. Exclusion of the last data point allows for closer examination of the pattern of the early data points.

I used a series of two-way contingency tables (Zar, 1999), one per species, to test for differences among study ponds in the frequency with which a given species was detected during my surveys. Pond nights were treated as observations, and each was classified by pond and detection status (present or absent on a given pond night). For each of the seven species for which detection frequencies were sufficiently high for a valid test (N > 5), the Chi-squared procedure returned a highly significant departure from independence (Table 2). For each of these seven species, the probability of detection differs substantially across the four study ponds.

#### DISCUSSION

Despite the fact that the Mayamon protocol is no longer in use, the protocol model is noteworthy in that it continued for nine years with little outside funding or expertise, other than the volunteer biologists themselves. The Mayamon protocol is designed to maximize both the efficiency of the protocol and the participation of volunteers. However, with any survey protocol, the limitations must be understood to appropriately interpret the data it

TABLE 2. Results of two-way contingency table tests of the homogeneity of detection probabilities of frog species across four study ponds. Species showed different probabilities of detection at different ponds, supporting the idea that a suite of ponds would be needed to capture the majority of vocalizing anuran species in a region.  $\alpha = 0.0045$  using a Bonferroni correction. Degrees of freedom = 3 and P < 0.0001 in all cases.

	Species								
	Agalychnis callidryas	Agalychnis moreletii	Bufo valliceps	Dendropsophus microcephala	Rana berlandieri	Smilisca baudinii	Tlalocohyla loquax		
Likelihood ratio	50.73	63.64	39.35	60.66	113.44	27.70	86.44		

generates. Although the Mayamon protocol successfully detects the most common species, it is susceptible to several sources of error. I discuss these issues further below.

Length of Survey.—The current minimal Mayamon protocol (15 min of sampling) is likely to detect, on average, only 80% of the species actually present at a pond on any given sampling night. Adding only 6 min to the protocol brings the detection efficiency to 90%. Shirose et al. (1997) tested the efficacy of the NAAMP protocol, another volunteer-based regional monitoring program, and found that the majority of all species identified at a site were heard in the first minute, with the number of new species detected per minute declining thereafter. Even when comparing 5-min to 3min surveys, the longer survey increased the number of species detected by less than 1%. In addition, Crouch and Paton (2002) determined that the 10-min sampling interval of the survey they were evaluating in Rhode Island was sufficient to ensure a probability of greater than 90% for the detection of any of the seven species studied. In contrast, my results show that even at 15 min, the number of species detected is still increasing. Furthermore, as the number of species detected at a pond increased, so did the time required for calling surveys to detect them. This may represent a fundamental difference in monitoring program needs between the tropics and temperate regions: the more speciose a region is, the more time-intensive a protocol may need to be.

*Frequency of Sampling.*—For prolonged-breeding species, there was no obvious indication that they are more or less likely to be detected in the beginning, middle, or end of the Mayamon sampling season (data not shown). Nevertheless, site-specific patterns of breeding assemblages may affect the efficacy of the protocol elsewhere.

Gastrophryne elegans and R. dorsalis are explosive breeders and reproduce primarily at the onset of the summer rains. Therefore, a more intensive sampling effort early in the rainy season would be more likely to detect explosively breeding species. In addition, in many species, especially in the tropics, a pulse of breeding activity coincides with the onset of the rainy season, even for prolonged breeders (Gascon, 1991). This activity takes place on a few nights as the first rains begin. Icochea et al. (2002) described the males of the anuran species assemblage in Peru as most active in the first month of the rainy season, suggesting that this is an important time to monitor anuran populations.

Considered together with the results presented here, this identifies a specific point for improving the efficacy of the current protocol in accurately assessing community composition. Explosive breeders may go completely undetected; large aggregations of a prolonged breeding species could go undetected early in the season and be heard only in small choruses through the rest of the summer, leading to the mistaken interpretation that that species is not present in large numbers. Such a scenario could seriously skew the resulting estimates of species richness.

Despite the fact that most species in this study called frequently enough that they were detected throughout the monitoring period for the Mayamon protocol, it took 359 sampling events to detect the 11 species encountered with calling surveys at all four ponds. Under the minimal Mayamon protocol, that is almost 60 years (Mayamon samples only for six months each year) of surveying before all species would have even been detected, to say nothing of how long it would take to understand population trends among these species. Ten (91%) of the 11 species detected were recorded within 45 sampling events, but this is still the equivalent of almost seven years of Mayamon sampling. This result indicates that the Mayamon protocol is sensitive to underdetection of species, which do not call for long periods of time, regardless of species abundance (Arrigoni, 2003). When the temporal component of the probability of detecting explosive breeders is considered, the objectives of Mayamon are likely to be better served with a more intensive effort in the beginning of the rainy season.

The fact that it took so long to detect the 11 species may reflect natural variation in calling. Alternatively, it may be indicative of assemblage-level selection or interactions shaping the calling behavior of different species. Species accumulation curves are often log-normal, especially for tropical communities, with common species being detected quickly and rare species being detected slowly after the initial jump in the curve (Smith, 1980). However, the last species detected, *S. cyanosticta*, was encountered on several occasions in forest ponds that were not included in the surveys.

Site Selection.—In this study, ponds had, on average, seven of the 13 species breeding in the area. Only two species were detected at all four study ponds. That different species of frog breed at different ponds is well known; however, it is not considered explicitly in the site selection criteria of the Mayamon protocol.

I detected between 31% and 70% of the frog species known to be breeding in the immediate vicinity of LCRS at the four study ponds. A concurrent study identified 12 of 13 species at one pond not monitored in this study using an automated recording device on only five nights (unpubl. data). These data suggest that, if resources are limited, and a program can only monitor one or very few sites in a region, it is worthwhile to carry out preliminary monitoring to determine which pond(s) will allow the monitoring of the most species. Although this approach introduces bias into the protocol, program managers must weigh the goals of a project carefully: if the objective is to collect population data on as many amphibian species as possible, then careful pond selection should be employed. However, the stated goal of the Mayamon protocol was to detect population trends; as such, random or stratified random site selection should be used. By sampling different habitats, researchers have a better chance at detecting species common to either but not both.

*Improving Sampling Protocols.*—The Mayamon protocol exhibits several weaknesses inherent to many rapid assessment protocols. Some are basic shortcomings that are easily corrected, by increasing the minimal sampling length and the frequency of sampling. Some flaws require somewhat more involved solutions.

The inability of the protocol to reliably detect certain species could be partly ameliorated by the compilation of a species list for the site (e.g., as in Roberts et al. 2007), based not only on what is found in standardized surveys but also on anecdotal sightings and other sampling methods. With this method, there would at least be a "checklist" of species, and special attention could be given to species that are not being detected through vocalization surveys.

The inclusion of other methods would also improve our understanding of amphibian dynamics and species assemblages. The placement of "Frogloggers" in the field (Peterson and Dorcas, 1992; unpubl. data) would allow monitoring to take place over greater periods of time with fewer person-hours in the field. Visual encounter surveys or surveys for tadpoles or eggs would be feasible in some regions, such as Belize, where larvae and/or egg masses are distinct enough to allow species identification.

For protocols already in place, addition of new methods pose a challenge for comparing data before and after protocol modification. However, the decision to incorporate new methodology or change a protocol should be based on the objective of the protocol. If the goal is better served through a modified protocol, is it not better to change the protocol than to be constrained to a protocol that is not sufficient?

The need for these additional data invariably must be balanced against the constraints of data collection, primarily the dearth of funding apportioned to monitoring programs and the need for skilled volunteers over long periods of time.

*Conclusion.*—Amphibian populations undergo natural, and in some cases extreme, population fluctuations (Bragg, 1954, 1960; Pechmann et al., 1991). Therefore, baseline population data are critical for assessing whether observed changes in population size represent natural fluctuations or something that exceeds normal background population fluctuations.

There are few regional monitoring programs in place globally, although the number is increasing. Most programs are in temperate regions and are carried out by volunteers (e.g., Shirose et al., 1997; Crouch and Paton, 2002). Given the scarcity of resources for monitoring efforts, particularly in the tropics where so much natural history information is lacking for many species, it is important to establish long-term monitoring efforts to help detect population trends and to understand how effective a protocol is. There seem to be differences between tropical and temperate monitoring in terms of the effort required to detect all or nearly all species within a community; although more studies on tropical monitoring would help elucidate these differences, few such programs exist.

However, the fundamental problem lies with the all-too-familiar paradox that there is no one protocol that will generate large return for little work and the lack of funding for institution of more intensive protocols. With protocols requiring few person-hours, it is difficult to obtain results that are sufficient for long-term management of populations or understanding of population dynamics. Moreover, some species thought to be rare have been shown to be abundant but not well detected by standard techniques (Gibbons, 1983). In short, to fully understand amphibian population dynamics, or the dynamics of most longer-lived taxa, a more time-intensive protocol needs to be implemented over time and over larger geographical scales, standardized, to make data from various studies comparable. Gibbons et al. (1997) suggest a balance between opportunistic surveys for rare species and standardized methods for comparative data. However, the species that were poorly detected in this study were not rare species: they were readily encountered either at other sites around LCRS or for short, intense periods at monitored sites. The Mayamon protocol simply underdetected them.

The continued lack of understanding of the causes underlying some amphibian declines, or methods to mitigate them, underscores the need for protocols which yield reliable population data. Although the Mayamon protocol is a reasonable first step for a long-term monitoring program, it alone is not sufficient to allow for

detection of population declines. Although the paucity of data available on amphibian populations in some regions may cause some to argue that some data are better than none at all, perhaps the establishment of a minimum standard should be considered by the herpetological community to maximize the data acquired from long-term monitoring projects. The establishment of such a standard of data necessary to determine population trends could give direction to fledgling long-term protocols and allow resources to be put to the most effective use. If no other methodology is to be implemented, then at the very least, tremendous care should be taken in the interpretation of data collected under the Mayamon protocol and protocols like it. I show here that a data-based model exists for determining the ideal length of survey for a given target efficacy, whatever that threshold level may be.

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