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Evaluation of different research methodologies for acoustic monitoring of anuran populations

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Abstract

Amphibians are important for a wide variety of reasons. However, in recent years their global populations have seen a sharp decline. It is therefore increasingly important to conduct research relevant to their protection and success. The main way this is accomplished with anurans is through the monitoring of male vocalizations. This can take the form of Manual Call Surveys, Automated Recording Systems, or some hybridization of the two. However, selection of the proper research method can be complex and time-consuming, and use of the incorrect method can result in wasted funding and useless data. Very few studies have been recently published comparing the different methods in relation to anurans, and none of them have provided a user-friendly guide for selecting methods. This paper aims to both summarize the wide importance of amphibians and research related to them, as well as provide a simple guide and thought-process for selecting the proper anuran research method. This will be done via a dichotomous key-like guide leading to the method most appropriate for the study of interest.

Dedication

This thesis is dedicated to all the friends and family who encouraged me throughout this process, especially my parents Richard and Sarah Schwartz for their love and support along the way. From homeschooling me and teaching me how to write, to encouraging me in difficult times in college, they have played an instrumental role in the production of this thesis.

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"I own that I have endeavoured to offer something on this important subject worth the publick's acceptance; but yet I am sensible, that all this time I have been, as it were, representing with a coal the sun's meridian rays: so that this my little essay can pretend to no merit, on any other account, but that of its conformity to nature, which I hope I shall, in time, be allowed not to have misrepresented."

Jan Swammerdam, "The Book of Nature" (1669)

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I. Introduction

Amphibian Importance to Humans

Amphibians hold a significant role in human society in a variety of ways. First, they have become important for direct human use due to a multitude of factors. For instance, amphibians are used as a human food source worldwide (Crump, 2010). Frog meat is used in markets of West Africa (Mohneke, Onadeko, Petersen, & Rödel, 2010), American diners, gourmet restaurants of Western Europe (Platt, 2011), and a variety of establishments across many other cultures. Amphibians have also become popular components of the global pet trade, with amphibians and reptiles being some of the most commonly traded animals (Herrel & Meijden, 2014).

Perhaps most importantly, amphibians are significant contributors to education and human wellness research. Frogs are frequently used to teach students anatomy and biological processes. Frogs have been used in physiological research to understand topics such as regeneration (Pough 2007), early vertebrate development (Burlibasa, 2011), and human cardiovascular disease (Schefft, 2017), and have been used in the development of a variety of pharmaceuticals. Skin secretions from some frog species have even led to potential treatments of human ailments such as Alzheimer's (Miller, 2000). Amphibians can also be used as model species to understand the negative effects of environmental pollutants on human reproductive health and fertility (Burlibasa, 2011), as well as other human ailments. In addition to the utilitarian uses of amphibians, humans benefit from the esthetic experiences that amphibians give to them. Many people around the world benefit from their nightly choruses, bright coloration, and entertaining demeanors.

In addition to economics and direct human use, amphibians have made a major impact on human culture. Frogs are used in everything from traditional medicine (Narzary & Bordoloi, 2014), to religious worship (Boll, 2004; Dobkin de Rios, 1974; Nepal, 1990) and frequently appear as religious symbols of fertility and good fortune (Chwalkowski, 2016). References to amphibians are found throughout literature, from inspiring Aristophanes' comedy "Batrachoi" ("The Frogs"), to influencing writers and thinkers such as Aristotle, Darwin, Thoreau, and Orwell (Miller, 2000).

Amphibians clearly benefit humanity in a diverse range of ways, but the importance of their continued conservation and protection is also based on their intrinsic value as fellow creatures sharing our planet. Amphibians have existed on Earth for approximately three hundred and fifty million years (Crump, 2010), far shorter than hominids' six to seven million years (Diamond, 1993). While the ethics surrounding the above statements are not objective, they are certainly worth our consideration. As stated in the "World Charter for Nature" of the United Nations 1982 General Assembly: "Every form of life is unique, warranting respect regardless of its worth to man, and, to accord other organisms such recognition, man must be guided by a moral code of action."

Amphibian Ecological Importance

Amphibians play a critical ecological role. Native to virtually all but the most extreme of environments (Crump, 2010), the influence of amphibians is felt worldwide. Being ectothermic, amphibians' energy conversion efficiency is far greater than that of most endotherms (Crump, 2010), thereby contributing greatly to the energy flow of their environment. Amphibians also lead complex lifestyles as aquatic larvae and terrestrial adults, influencing two distinct environments as well as connecting the flow of energy between them (Whiles et al., 2006). Additionally, amphibians' position in the food web as both predator and prey contributes greatly to their role in energy conversion and nutrient cycling (Crump, 2010). Tadpoles, for instance, have been shown to influence organic matter dynamics, primary production patterns, and algal community structure (Whiles et al., 2006) which can lead to the reduction of eutrophication rates (Crump, 2010). Terrestrial adult amphibians keep insect numbers in check, while also serving as a food source for terrestrial predators (Whiles et al., 2006). Lastly, due to their relatively large impact on ecosystem structure, several sources suggest that amphibians are a keystone species (Whiles et al., 2006).

Importance as Indicator Species

Stemming from their ecological significance, amphibians also make excellent indicator species, meaning they can be used to monitor environmental changes and provide early warning signs of ecological changes to come (Siddig et al., 2015). Anurans (frogs and toads) specifically are sensitive to environmental stressors due to their biphasic lifestyle, semipermeable skin (Vitt et al., 1990), and external fertilization and development (Burlibasa, 2011), as well as their highly specialized physiology and specific microhabitat requirements (Welsh & Ollivier, 1998). Additionally, anuran sampling methods are relatively inexpensive, therefore aiding in the practicality of their study (Heyer et al., 1994). Anuran populations can be used to gauge habitat health, as well as provide indicators of environmental contamination (Hamer et al., 2003). The use of anurans as indicator species is significant to the continued health and prosperity of our species. Most factors currently threatening anurans also affect human health (increase in ultraviolet radiation, water pollution, etc.) (Halliday, 2008), and declining anuran populations could easily be an indicator of the decline in the global health of our environment (Halliday, 2008). In the words of Henry David Thoreau: “The frog far away in the wood, that burns no coal nor wood, perceives more surely the general and universal changes....” (1859).

Amphibian Decline

Amphibian populations worldwide are in decline (Halliday, 2008; Crump, 2010; Stuart 2004, etc.). According to the International Union for Conservation of Nature’s (IUCN) criteria, nearly one third of amphibian species (almost two thousand species) are threatened with extinction (Stuart et al., 2004), and over seven percent are considered critically endangered (Stuart et al., 2004). Despite having existed on Earth for about three and a half million years, their current rate of extinction is likely many thousand times higher than their past background rate (McCallum 2007 as cited in Halliday, 2008). There are multiple reasons for global amphibian decline, many working synergistically with one another (Blaustein & Kiesecker, 2002). Loss of adequate habitat is often listed as one of the worst components (Halliday, 2008), but invasive species, climate change, over-exploitation, pollution, disease, and other factors all contribute significantly (Halliday, 2008). The spread of the fungal disease Chytridiomycosis (*Batrachochytrium dendrobatidis*) has drawn recent attention for its quick spread and lethal effects on amphibians around the globe. Some hypothesize its success is linked to the weakening of amphibian immune systems due to environmental changes (Kendrick, 2000).

The loss of this unique group of organisms is a significant problem worthy of greater attention. With the loss of amphibians comes the loss of valuable resources to humanity, as well as the loss of an important symbol to many. Sometimes even being referred to as “canaries in a coal mine,” it is critical for us to take heed of the warnings amphibians are sending us (Roy, 2002).

Behavior and Life History

Amphibians have a few fundamental characteristics that directly affect the research methods used in their study. Amphibians are ectothermic (their body temperature and metabolism are largely affected by the temperature of their external environment), have thin and permeable skin, and produce eggs with thin membranes (Halliday, 2008). All these features make amphibians vulnerable to environmental threats such as climate change and pollution (Halliday, 2008), more so than any other tetrapod (Duellman & Trueb, 1986). Because of these characteristics, amphibians are generally restricted to warm, moist habitats, and are therefore mainly found in temperate and tropical regions (Halliday, 2008). Amphibians are found mostly in forest and fresh water habitats (Stuart et al., 2004) and are primarily nocturnal and dormant throughout most of the winter (Ministry of Environment, 2014). Anurans have well-developed vocal structures for producing loud calls in the attraction of mates (Duellman & Trueb, 1986). Because this is the main mode of communication for anurans, detection of calls is an effective means for evaluating their population (Dorcas et al., 2010).

Current Research

There are a variety of research methods used for monitoring amphibians, but much amphibian research focuses on anuran call detection as the main monitoring technique (Dorcas et al., 2010). There are costs and benefits to this strategy depending on the research foci, but monitoring via call detection can be more practical than intensive survey methods such as capture-recapture (Corn et al., 2000). Additionally, unlike other research methods, some call detection methods allow for the data contribution of volunteers via citizen science programs. Anuran acoustic monitoring mostly takes the form of Manual Call Surveys (hereafter referred to as MCS) and Automated Recording Systems (hereafter referred to as ARS) or some hybrid of the two.

II. Objectives

The aim of this paper is not to analyze the background and history of the two methods (which has already been discussed extensively in multiple studies [Corn et al. 2000; Digby et al., 2013, Dorcas et al., 2010, etc.]), nor is it to declare which of the two methods is best overall (which depends on the specific parameters of the study), but rather to provide researchers with the necessary resources to make informed and structured decisions regarding their choice of methodology. This will start via the summation and description of anuran monitoring research. It will be followed by an in-depth analysis of the differing research methods (MCS vs. ARS). This will culminate in a user-friendly decision key providing a step-by-step guide in selecting which methodology is most appropriate for the researcher's study of interest. Finally, recommendations for use of the decision key and advice for experimental design will be provided.

Minimal research has been conducted comparing and contrasting MCS to ARS methods. Some studies have examined the two methods as they relate to bird calls

(Digby et al., 2013; Hutto & Stutzman, 2009; Celis-Murillo et al., 2008), but bird calls and their research differ significantly from anuran vocalization and therefore comparing them can be difficult. There is a very small percentage of research comparing MCS to ARS in relation to frog calls (Corn et al., 2000), however no sources have been found of studies doing so adequately in the last decade and none have attempted a decision key.

III. MCS and ARS Background

The rapid decline of anurans (and biodiversity in general) together with anurans' inarguable significance, make the study and conservation of anurans more important now than ever before. As the decline of anuran populations has become more apparent, the use of acoustic monitoring protocols has increased (Dorcas et al., 2010). While these strategies are limited in some regards (mostly in their ability to relay population size and relative abundance), and are used solely for anurans, they are very helpful in monitoring the status and trends of anuran populations, as well as their responses to environmental change (Dorcas et al., 2010). Acoustic monitoring for anurans is split into the two distinct categories of MCS and ARS, both of which have their own unique functions and uses.

MCS

Used in detecting anurans for the last several decades (Dorcas et al., 2010), MCS involve human observers listening for male anuran vocalizations in person. In North America, MCS protocols are often based on the North American Amphibian

Monitoring Program (NAAMP), but vary from state to state (Burton et al., 2006). Within the set survey duration, observers record species detection (and often their abundance), with the use of roadside surveys along predetermined routes being most common (Dorcas et al., 2010; Inkley, 2006). Weather permitting, surveys are conducted at night (during peak call time) roughly between dusk and midnight (Dorcas et al., 2010). Used throughout both the United States and many other countries (Dorcas et al., 2010), MCS can be used by large organizations such as the United States Geological Survey's (USGS) Amphibian Research and Monitoring Initiative (ARMI) and the Association of Zoos and Aquariums' (AZA) Frog Watch USA program (Inkley, 2006), by scientists researching independently, or by trained volunteers in citizen science programs (Droege & Eagle, 2005). Citizen science programs are volunteer-based initiatives that utilize trained volunteers to gather data over a wide spatial and temporal scale (Inkley, 2006). While citizen science programs may contain their share of complications, they allow the use of a greater number of personnel and the option to use them is restricted mostly to MCS.

MCS are best used for the monitoring of multiple anuran populations over a large space (Dorcas et al., 2010). In general, they are mostly used to gather inventory data of an area, to take occupancy measurements (determining a species' relative occurrence between populations), or to monitor responses to habitat and climate changes within populations (Dorcas et al., 2010).

ARS

In recent years, the development of ARS methods has increased the variety of anuran vocalization research possibilities (see Xie et al., 2017). ARS involve the placement of recording mechanisms to automatically collect vocalization data without the presence of an observer. The data is later analyzed via either human inspection (e.g. spectrograms) or automatic detection software (Digby et al., 2013). There are multiple types of ARS available for purchase (many of which originally designed for bird calls), or they might be custom built for a given study (Dorcas et al., 2010). Regardless of the origin, ARS typically consist of a recorder, timer or controller, microphone, power supply, and housing (Peterson and Dorcas 1994, as cited in Dorcas et al., 2010). ARS are typically mounted adjacent to anuran breeding habitats and can be set to record at specific times of day (such as peak of calls) (Dorcas et al., 2010). They can be left at the study site for long periods of time and provide a permanent data record (Xie et al., 2016).

Generally, ARS are best used for the intensive collection of data at a single or a few study sites and are usually used to monitor fluctuations in a population, or for detection of species presence (Dorcas et al., 2010). ARS are sometimes used for detection of rare species or species with unpredictable calling patterns (Dorcas et al., 2010).

IV. Discussion

Complications

MCS and ARS methods both have their share of complications. Deciding which is most appropriate for the project of interest can be challenging and time consuming.

Before deciding which of the two acoustic monitoring methods to use, one must keep some important factors in mind in order to properly direct the focus of the study. Researchers must have a clear idea of the hypothesis or question they wish to answer, as this will directly affect which method they choose. They must also know the level of resources and funding they have available, as well as the amount of time and manpower they have at their disposal, as these will also determine which method is most appropriate. Additionally, the experience level of the observers, the species type and number, the location of the study site(s), and other factors, must all be kept in mind in order to properly select the methodology that best conveys the proper data and most accurately answers the study's question or hypothesis.

Cogalniceanu and Miaud (2010) list the steps of scientific inquiry as "(1) perceive that a problem/question exists; (2) formulate a possible explanation (i.e. devise a hypothesis); (3) formulate alternative hypotheses; (4) identify the best approach to test the hypothesis (i.e. theoretical models, experiments, or field observations); (5) collect and analyze data; (6) support or reject the hypothesis; and (7) understand the meaning and implications of the results. The original hypothesis can then be modified, experiments repeated and, with time, conceptual understanding attained." This paper's analysis and decision key is constructed under the assumption that 1-3 have been determined, and its aim is therefore to provide the support to determine steps 4 and 5, which will then allow the researcher to decide 6 and 7.

Analysis

Before beginning any research study, the methodology must be carefully chosen as it will determine the outcome of the entire process. Poor planning can result in countless wasted hours, dollars, and data samples. There are many pros and cons to both MCS and ARS methods (see Table 1). Deciding which is most appropriate for the study of interest can be difficult and time consuming, and different factors will necessitate the use of one method over the other (or possibly some hybridization of the two).

MCS has plenty of its own strengths, many of which are related to the weaknesses of ARS. Some of these are practical, fundamental factors that should be considered first when choosing between methodologies. For instance, typically ARS tend to be more expensive than MCS (Corn et al., 2000), and therefore the level of funding received for the study should be carefully considered. MCS require little to no expenses other than fuel to and from sites (and can often be conducted via the enlistment of volunteers). ARS, however, require expensive recording equipment, software for data analysis, and the possible pay of research assistants (Corn et al., 2000). In addition to expenses, ARS tend to require a higher amount of researcher experience and training time compared to MCS (Corn et al., 2000). ARS users need to learn the operation and maintenance (and possibly design and assembly) of their acoustic hardware, as well as the use of the data analysis software (Dorcas et al., 2010; Digby et al., 2013). Additionally, this equipment can be damaged (by natural or artificial causes) or stolen, posing a risk to the sole reliance on automatic methods (Corn et al., 2000). Additionally, given that MCS allows for the recruitment of volunteers, MCS is likely the best choice if more personnel is needed for the study (Dorcas et al., 2010).

Similarly, if education is one of the goals of the program, MCS's ability to incorporate volunteer citizen scientists is the better option due to its accessibility and easy learning curve.

Similarly, ARS also has its own strengths, many of which are related to the weaknesses of MCS. While ARS may require more experience with equipment and time needed to train personnel, MCS relies heavily on the experience of observers and their ability to properly identify calls (Corn et al., 2000), which can potentially lead to inaccurate recording of data. Similarly, MCS can be more affected by inter-observer bias compared to ARS, given the reliance on individuals to make quick judgements on what calls are heard. ARS, however, provides the chance for other personnel to check questionable call identification (Corn et al., 2000; Dorcas et al., 2010).

MCS can also be restricted to use with only certain anuran species. While, MCS can be used to detect common species, there is a smaller chance of catching vocalizations of rarer species (Corn et al., 2000). ARS, however, can be used to detect rare species as well as ones with unpredictable calling patterns (Corn et al., 2000; Dorcas et al., 2010). Species that call quietly or do not call, call infrequently, have specific calling periods in response to environmental conditions (such as heavy rain), or have short breeding seasons, are better monitored using ARS over MCS (Dorcas et al., 2010). MCS could miss these calls due to its short observation window (Genet & Sargent, 2003), while ARS allows for a large collection of data over a larger temporal scale (Wimmer et al., 2012). Droege & Eagle have suggested that only about fifty-five of the one hundred and three North American anuran species are suitable for MCS monitoring (as cited in Dorcas et al., 2010), leaving MCS best suited for species that vocalize clearly during predictable sessions.

ARS can also be good to use in difficult to access locations, minimizing the number of trips observers need to take to access the study site (Dorcas et al., 2010). ARS only entail the initial placement of the devices (and possibly the occasional maintenance), whereas MCS requires a new trip for each session. Lastly, MCS can also cause disturbances to study sites, resulting in lower calling activity for part of the listening period, while ARS minimizes this issue (Dorcas et al., 2010).

Table 1: This table is constructed to provide a quick, generalized overview comparing the strengths and weaknesses of MCS and ARS methods. It is solely meant as a helpful, organizational tool and therefore some exceptions may apply.

MCS		**ARS**	
Pros	Less expensive	Cons	More expensive
	Less personnel experience & training time		More personnel experience & training time
	Little to no equipment needed		Equipment subject to failures, damage, and theft
	Allows use of more personnel via volunteers		Use of volunteers is difficult
	Can be used for education of citizens		Steeper learning curve
Cons	Heavy reliance on observer accuracy	Pros	Less room for human error
	Inter-observer bias		Allows other personnel to check questionable data
	Restricted to common or easy to monitor species		Can be used for most species
	Can be restricted to easily accessible locations		Can be used in locations difficult to access
	Can create local disturbance		Minimizes potential disturbance

In addition to the different pros and cons found between the two methods, there are certain basic criteria that should be considered before selecting a method. According to Corn et al. (2000), different regions of North America are often better suited for one method over the other. In Western North America where there are fewer roads (i.e. less accessibility), fewer habitats, lower frequency of anuran calls, and other restricting factors, the use of MCS can be more challenging and ARS may be the only viable option. Additionally, the Southeast United States is often a better setting for ARS due to its high anuran diversity and longer breeding season that can make the use of volunteers for a MCS-like method more difficult. Manual, therefore, is often best for Northeastern and Central North America (Corn et al, 2000).

The breadth of the study is also an important factor to keep in mind. Selection of the most appropriate method is largely influenced by the geographic and temporal scales of the project, as well as the quantity of populations and species being studied. Generally, MCS is well-suited for studies with multiple species or populations over a large spatial scale (Corn et al., 2000; Dorcas et al., 2010). ARS, however, is most appropriate for studies collecting intensive data on one or a few locations (Dorcas et al., 2010). ARS can be used for multiple species within a small geographic area, or studies on the ecology and natural history of single species (Corn et al., 2000). Additionally, due to its ability to monitor consistently over a long time period, ARS is better suited for the collection of large volumes of data over a large temporal scale (Wimmer, et al., 2012).

Lastly, another important factor for consideration is the various risks posed by different sites. A location far away from human development could be unsafe for a researcher (Hutto and Stutzman, 2009), especially if they are observing calls alone. A study site close to human development, however, could also pose risks to observers, as well as to ARS equipment that could become subject to theft or damage (Corn et al., 2000). Traveling in groups, properly communicating to local residents and authorities, and other precautions may need to be taken to decrease safety risks when using either research method. Deciding which method is most appropriate will therefore depend largely on the situation and the careful judgement of the observer.

The above background information provides basic criteria for the initial considerations of a study. The ultimate purpose or end goal of the research is also a critical aspect for determining the proper methodology. While both methods can be appropriate in some cases, in most scenarios there are clear distinctions in which method should be used.

The purposes of MCS include inventory and occupancy measurements (Dorcas et al., 2010), while ARS is well-suited for determining presence/absence data and the conducting of surveys of single species (Dorcas et. al., 2010; Wimmer, et al., 2012). Additionally, both methods can serve the purpose of monitoring fluctuations in a population over time (Dorcas et al., 2010). ARS is better suited for monitoring of relationships between environmental conditions and aspects such as anuran calling activity through the dual-use of ARS and “dataloggers” (Corn et al., 2000; Dorcas et al., 2010), whereas MCS can be used to monitor the response of species to habitat and climate change at a population or community level (Dorcas et al., 2010). ARS also functions to help understand anuran phenology and behavior, whereas MCS is not recommended for these purposes (Corn et al. 2000). In summary, there are a multitude of various factors needing consideration when choosing research methodology. In general, MCS seem to be better for overarching data regarding large scale projects, whereas ARS tend to be better for more specific data collection.

Given the numerous contrasts between these two techniques, rather than using one single method over the other, it is often best to use them both congruently or to form a hybrid of the two. For a single survey, one might want to use a single technique (Corn et al., 2000). However, as the temporal scale, spatial scale, species number, and other factors increase, the need for multiple methods increases as well (Corn et al., 2000).

Hybrids could simply take the form of ARS and MCS methods being fully used together. This would allow researchers to collect large amounts of data covering multiple topics, as well as ensure their data's accuracy. Additionally, observers could simply bring recording devices with them on their call surveys in order to later verify the accuracy of their observations. Another simple hybrid would be the use of ARS to record calls, but with human observers occasionally visiting the sites to conduct call surveys in order to check the sensitivity of the recording devices. Additionally, a more complex integration of the two methods could be the use of MCS, but with the annual implementation of ARS for "quality control" (Corn et al., 2000). Meaning ARS systems could track the breeding, phenology, and other factors in order to ensure that year's MCS surveys were being conducted at the appropriate times (Corn et al., 2000).

However, the combination of both methods into a hybrid would likely increase the financial costs of the study, and so its use would depend on the funding available. Additionally, hybrid methods would increase the complexity of the study, and so care would need to be taken to ensure the method is appropriately addressing the goals of the study.

Given the multitude of components needing consideration, how does one decide which method is most appropriate to use? It is obviously very important for researchers to be well informed on their subject of interest, as well as its various research methodologies. However, equally important is the ability to bridge the gap between research subject and research method. Knowing how to select and apply the proper methodology is a critical step in conducting a successful study. Little research has been done comparing anuran vocalization research methods, and no recent work has been found providing a user-friendly guide for choosing between them. Even for experienced researchers, taking into account all of a study's various factors can be difficult and time-consuming. This study is therefore meant to collect and summarize all of these factors, while also providing a guided thought-process for working through the study's methodology design.

Audience

Providing such a tool is critical in the assistance of a variety of individuals and studies and can be used by stakeholders of many different backgrounds and experience levels. This range could include researchers with extensive experience in the acoustic monitoring field, undergraduate students conducting research for the first time, researchers coming from an unrelated field, government employees tasked with designing a program for citizen scientists, and other categories. The availability of a guide such as this will now allow the adoption of anuran vocalization monitoring by a greater variety of people, places, and organizations, many of whom may not have had the tools for designing a research study before.

How to Use Key

For those looking to conduct a study and needing to choose the proper methodology, a helpful guide has been provided below in the form of a decision key. This key should clarify any confusions regarding the various methods and help users properly select between them. The decision key is meant to be used much like a dichotomous key, with the steps being followed in numerical order, each one presenting a question followed by a pair of answers. The user should choose the answer that best relates to the nature of their study and from there move on to the indicated next question. Once the user has reached the end of the decision key, they will be presented with the research method that should best apply to their project of interest. If the answer to a question falls somewhere in the middle, or both answers seem equally appropriate, then both options should be followed to see where they lead. If they lead to different methods, then either more information should be gathered to determine the most appropriate method, or both should be tested in the study.

The guide focuses primarily on MCS and ARS methods. However, in most cases a hybrid of the two methods is often better, given that it can provide greater monitoring accuracy and the reviewing of any uncertain results (Corn et al., 2000). If a study needs to be especially precise, and enough time and funding is available, integration of both methods is usually the best option. Therefore, many of the answers below reading “MCS” or “ARS” could be replaced with “Hybrid” if enough time and funding are available. Additionally, the first two couplets discuss the breadth of the project, indicating that an especially broad or complicated study should be done via a hybridized method.

This paper and its decision key is meant to provide helpful guidance. It should not be allowed to become restrictive, rather the user should use it for the information it contains and the suggestions that it presents. Those using the guide should have a thorough understanding of their research focus and the question they wish to answer. From there, the guide can be of most assistance.

It is important to note that this guide is written based solely on studies related to anuran research and conservation, and is therefore intended to be used strictly for these purposes. It therefore cannot guarantee accuracy when used for other purposes such as impact surveys before land development, etc. Additionally, before conducting research at a new site, it is necessary to first assess the legal accessibility of the site and determine if a permit is required. Finally, after selecting which category of method should be used, it is the responsibility of the user to properly educate themselves on the design and implementation of such methods based on their research into relevant studies and protocol (such as NAAMP protocol, etc.).

V. Decision Key

1 Study is large and complex, being conducted on many species over very large temporal and geographic scales (i.e. across multiple states over a period of years)...Hybrid

1 Study is smaller and less complex..2

2 Project integrates multiple different topics/surveys at once (i.e. examines occupancy as well as phenology and behavioral data)..Hybrid

2 Project focuses on only one or two topics..3

3 Education or integration of a citizen science program is the purpose of project...MCS

3 Education or integration of a citizen science program is not the project's purpose...4

4 Not enough funding is available to purchase expensive recording equipment and analysis software (overall costs can be up to tens of thousands of dollars depending on equipment used)..MCS

4 Enough funding is available to purchase expensive recording equipment and analysis software, or equipment and software is already available......................5

5 Study takes place in Western United States or other region where habitat quality, number of roads, frequency of calls, etc. are low...........................ARS

5 Study takes place in other region where there is high quality of habitat, number of roads, calling frequency, etc...6

6 Researcher(s) has/have no previous experience working with recording equipment and digital vocalization analysis/software and is/are not capable of receiving relevant training..MCS

6 Researcher(s) has/have had previous experience or is/are capable of receiving relevant training..7

7 Species of interest are locally considered rare or infrequent (check appropriate sources and documentation for local species

occurrence)..ARS

7 Species of interest are locally common..8

8 Some species do not vocalize loudly in predictable calling sessions (do not have a consistent calling time or season)...............ARS

8 All species vocalize loudly during predictable calling sessions...............9

9 Study sites are difficult to access relative to the researcher's ability (require extensive time and/or risk researcher safety).....................ARS

9 Study sites are relatively easy to access..10

10 Study necessitates a long temporal scale (over a year)..............ARS

10 Study can be conducted within short timeframe.............................11

11 Study examines single species ecology/natural history.........ARS

11 Study focuses on multiple species..12

12 Study takes place over a large spatial scale (i.e. across an entire U.S. state or more)...MCS

12 Study takes place within a small spatial scale......................13

13 Research takes place across several sites.................MCS

13 Research takes place at one or a few sites.....................14

14 Study aims to gather inventory and/or occupancy data..MCS

14 Aim of study does not include inventory and/or occupancy data..15

15 Study aims to gather data on anuran phenology and/or behavior...ARS

15 Aim of study does not include data on anuran phenology and/or behavior....................................16

16 Study aims to monitor population and/or community response to habitat or climate change...MCS

16 Aim of study does not include monitoring of population and/or community response to habitat or climate change.................................17

17 Study aims to monitor relationship between environmental conditions and anuran calling activity..ARS

17 Aim of study does not include monitoring of relationship between environmental conditions and calling activity.................MCS

VI. Suggestions

In order to best carry out future research with anuran vocalizations, I highly recommend that researchers take the time to properly analyze their project and consider all factors (such as those presented in this paper) relating to their subject. In doing so, they will not only save both time and finances, but will have a much better understanding of their subject matter. Additionally, I suggest that this decision key be used to help researchers save the time and frustration often associated with research design.

Additionally, anyone designing a research study should be open to modifying their methods as new information surfaces. Research methods are meant to give the study focus and direction, not restrict the research in one direction. This guide can be continually reused in order to properly update the choice in methodology as situations change.

While this paper has attempted to adequately cover the basics of anuran acoustic monitoring methods, future research could expand the guide to include questions regarding specific localities, recorder equipment, and other factors beyond the scope of this paper. Additionally, given the temporal restrictions that influenced the breadth of this study, future studies could be conducted using this decision key in real situations to test its accuracy and make any changes accordingly.

VII. Conclusion

The need to study and conserve anurans has never been greater. As their populations decline worldwide, it is critical that research be conducted efficiently and accurately in order to provide for them a safe future. This paper potentially provides a needed service to anuran conservation. It is notable both in its unique summary of the wide impact and importance of anurans, as well as the potential increase in efficiency it lends to the process of anuran conservation. It attempts to both summarize anuran acoustic research methods as well as provide a user-friendly guide on how to properly apply them to specific projects. It is critical that studies like this continue to be conducted with the purpose of saving this critical group of organisms for their sake and for ours.

Literature Cited

Blaustein, A. R. & Kiesecker, J. M. (2002). Complexity in conservation: Lessons from the global decline of amphibian populations. *Ecology Letters, 5*(4). https://doi.org/10.1046/j.1461-0248.2002.00352.x

Boll, V. (2004). The distribution and ethnozoology of frogs (and toad) in north-eastern Arnhem Land (Australia). Retrieved from http://sciencepress.mnhn.fr/en/periodiques/anthropozoologica/39/2/la-distribution-et-l-ethnozoologie-des-grenouilles-et-crapaud-dans-le-nord-est-dela-terre-d-arnhem-australie

Burlibasa, L. (2011). Amphibians as model organisms for study environmental genotoxicity. doi:10.15666/aeer/0901_001015

Burton, E. C., Gray, M. J., Schmutzer, A. C. (2006) Comparison of anuran call survey durations in Tennessee wetlands. Retrieved from:

https://www.researchgate.net/publication/267698844_Comparison_of_Anuran_Call_Survey_Durations_in_Tennessee_Wetlands

Celis-Murillo, A., Deppe, J. L., & Allen, M. F. (2008). Using soundscape recordings to estimate bird species abundance, richness, and composition. *Journal of Field Ornithology, 80*(1). doi:10.1111/j.1557-9263.2009.00206.x

Chwalkowski, F. (2016). *Symbols in arts, religion and culture: The soul of nature.* Retrieved from https://books.google.com/books?id=W-22DQAAQBAJ&pg=PA445&lpg=PA445&dq=%22frog%22+religious+symbolism&source=bl&ots=vJXU5fq_Mu&sig=ACfU3U09tz7rXEBQU8nBdxN8oRCkVxmKvA&hl=en&sa=X&ved=2ahUKEwi-m92D0dDgAhWCpoMKHQAhBJc4ChDoATADegQIBRAB#v=onepage&q&f=false

Cogalniceanu, D. & Miaud, C. (2010). Setting objectives in field studies. In E. K. Dodd Jr. (Ed.), *Amphibian ecology and conservation: A handbook of techniques* (pp. 21-35). Oxford, NY: Oxford University Press.

Corn, P. S., Muths, E., & Iko, W., M., (2000). A comparison in Colorado of three methods to monitor breeding amphibians. *Northwestern Naturalist, (81)*1. doi: 10.2307/3536896

Crump, M. L. (2010). Amphibian diversity and life history. In E. K. Dodd Jr. (Ed.), *Amphibian ecology and conservation: A handbook of techniques* (pp. 3-19). Oxford, NY: Oxford University Press.

Diamond, J. (1993). *The third chimpanzee: The evolution and future of the human animal.* New York, NY: Harper Perennial.

Digby, A., Towsey, M., Bell, B. D., Teal, P. D. (2013). A practical comparison of manual and autonomous methods for acoustic monitoring. *British Ecological Society, 4*(7). https://doi.org/10.1111/2041-210X.12060

Dobkin de Rios, M. (1974). *The influence of psychotropic flora and fauna on Maya Religion.* Retrieved from https://www-jstor-org.proxy.lib.utc.edu/stable/2740991?seq=1#metadata_info_tab_contents

Dorcas, M. E., Price, S. J., Walls, S. C., & Barichivich, W. J. (2010). Auditory monitoring of anuran populations. In E. K. Dodd Jr. (Ed.), *Amphibian ecology and conservation: A handbook of techniques* (pp. 281-298). Oxford, NY: Oxford University Press.

Droege, S. & Eagle, P. (2005). Evaluating calling surveys. In M. Lannoo (Ed.), *Amphibian declines: The conservation status of United States species* (pp.314-319). London, England: University of California Press.

Duellman, E. W. & Trueb, L, (1986). *Biology of Amphibians.* McGraw-Hill, Inc.

Genet, K. S. & Sargent, L. G. (2003). Evaluation of methods and data quality from a volunteer-based amphibian call survey. *The Wildlife Society 31*(3), 703-714. Retrieved from https://www.jstor.org/stable/3784590?seq=1#page_scan_tab_contents

Halliday, T. R. (2008). Why amphibians are important. *Zoological Society of London, 42*(1). Retrieved from https://zslpublications.onlinelibrary.wiley.com/doi/full/10.1111/j.1748-1090.2007.00037.x

Hamer, A. J., Makings, J. A., Lane, S. J., & Mahony, M. J. (2003). Amphibian decline and fertilizers used on agricultural land in south-eastern Australia. *Agriculture, Ecosystems & Environment, 102*(3). Retrieved from https://www-sciencedirect-com.proxy.lib.utc.edu/science/article/pii/S0167880903003608

Herrel, A. & Meijden, A. V. (2014). An analysis of the live reptile and amphibian trade in the USA compared to the global trade in endangered species. Retrieved from https://www.researchgate.net/publication/259762005_An_analysis_of_the_live_reptile_and_amphibian_trade_in_the_USA_compared_to_the_global_trade_in_endangered_species

Heyer, W. R., Donnelly, M. A., McDiarmid, R. W., Hayek, L. -A. C., & Foster, M. S., (1994). *Measuring and monitoring biological diversity: Standard methods for amphibians.* The Smithsonian Institute.

Hutto, R. L. & Stutzman, R. J. (2009). Humans versus autonomous recording units: A comparison of point-count results. *Journal of Field Ornithology, (80)*4. doi:10.1111/j.1557-9263.2009.00245.x

Inkley, D. B., (2006). Final Report Assessment of Utility of Frogwatch USA Data 1998-2005 [PDF file]. Retrieved from https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=2ahUKEwik2JCwn-LgAhXH5YMKHTeUBFIQFjAAegQIBhAC&url=https%3A%2F%2Fwww.speakcdn.com%2Fassets%2F2332%2F1998-2005_fw_data_assessment.pdf&usg=AOvVaw2XdaCNq-K1JIcXk4ml9HkW

Kendrick, B. (2000). *The Fifth Kingdom* (3rd ed.). Newburyport, MA: Focus Publishing.

Miller, G. L. (2000). *Nature's fading chorus: Classic and contemporary writings on amphibians*. Washington, D.C.: Island Press.

Ministry of Environment. (2014). *Amphibian Auditory Survey Protocol.* (Fish and Wildlife Branch Technical Report No. 2014-1.0). Regina, Saskatchewan. 3211 Albert Street

Mohneke, M., Onadeko, A. B., Petersen, M., & Rödel, M.-O. (2010). Dried or fried: amphibians in local and regional food markets in West Africa. Retrieved from https://www.researchgate.net/publication/264351401_Dried_or_fried_Amphibians_in_Local_and_Regional_Food_Markets_in_West_Africa

Narzary, J., & Bordoloi, S. (2014). Ethnozoological practices on frogs of Bodo tribe from Kokrajhar district, Assam, India. *American Journal of Ethnomedicine, 1*(6). Retrieved from http://www.imedpub.com/articles/ethnozoological-practices-on-frogs-of-bodotribe-from-kokrajhar-district-assam-india.php?aid=10911

Nepal, N. (1990). Frog worship: A unique culture. Retrieved from http://www.digitalhimalaya.com/collections/journals/ancientnepal/index.php?selection=115

Platt, J. (2011). How eating frog legs is causing frog extinctions. Retrieved from https://blogs.scientificamerican.com/extinction-countdown/how-eating-frog-legs-is-causing-frog-extinctions/

Pough, F. H. (2007). Amphibian biology and husbandry. Retrieved from https://www.semanticscholar.org/paper/Amphibian-biology-and-husbandry.-Pough/abee12f5b9d36d58a0fedbfdd6579abab512bbf1

Roy, D. (2002). Amphibians as environmental sentinels. *Journal of Biosciences 27*(3). doi:10.1007/BF02704906

Schefft, M. (2017). Research makes leap with frog models. Retrieved from https://medicalxpress.com/news/2017-01-frog.html

Siddig, A. A. H., Ellison, A. M., Ochs, A., Villar-Leeman, C., & Lau, M. K. (2016). How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in *Ecological Indicators. Ecological Indicators, 60.* Retrieved from https://www.sciencedirect.com/science/article/pii/S1470160X15003696

Stuart, S. M., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L., Waller, R. W. (2004). Status and Trends of Amphibian Declines and Extinctions Worldwide. *Science, 306*(5). Retrieved from https://www.jstor.org/stable/3839782?seq=1#page_scan_tab_contents

Swammerdam, J. (1669). *The book of nature; or The history of insects: A particular treatise on the generation of frogs.* London: C.G. Seyffert.

Thoreau, H. D. (1906). Journal. In *The Writings of Henry David Thoreau.* Boston and New York: Houghton Mifflin Company.

United Nations (1982). World charter for nature. Retrieved from http://www.un.org/documents/ga/res/37/a37r007.htm

Vitt, L. J., Caldwell, J. P., Wilbur, H. M., & Smith, D. C. (1990). Amphibians as harbingers of decay. *Bioscience, 40*(6). Retrieved from https://www-jstor-org.proxy.lib.utc.edu/stable/1311379?seq=1#metadata_info_tab_contents

Welsh Jr., H. H., Ollivier, L. M., (1998). Stream amphibians as indicators of ecosystem stress: A case study from California's redwoods. *Ecological Society of America, 8*(4). Retrieved from https://esajournals.onlinelibrary.wiley.com/doi/10.1890/1051-0761%281998%29008%5B1118%3ASAAIOE%5D2.0.CO%3B2

Whiles, M. R., Lips, K. R., Pringle, C. M., Kilham, S. S., Bixby, Brenes, R., Connelly, S., Colon-Gaud, J. C., Hunte-Brown, M., Huryn, A., D., Montgomery, C., & Peterson, S. (2006). The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. *Ecological Society of America, 4*(1). Retrieved from https://esajournals.onlinelibrary.wiley.com/doi/10.1890/1540-9295%282006%29004%5B0027%3ATEOAPD%5D2.0.CO%3B2

Xie, J., Towsey, M., Zhang, J., & Roe, P. (2016). Frog call classification: a survey. *Artificial Intelligence Review*. Retrieved from https://www.researchgate.net/publication/310845457_Frog_call_classification_a_survey

Xie, J., Towsey, M., Zhu, M., Zhang, J., Roe, P. (2017). An intelligent system for estimating frog community calling activity and species richness. *Ecological Indicators, 82*(p.13-22). https://doi.org/10.1016/j.ecolind.2017.06.015

Vita

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