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Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs

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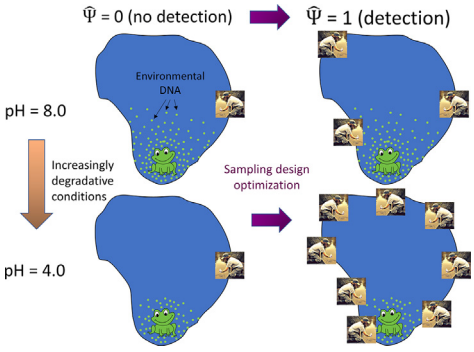
HIGHLIGHTS

- Ecological, hydrological, and physiological processes affect eDNA detection.
- We modeled eDNA detection with matched field surveys for six wetland amphibians.
- Dispersion and degradation had the strongest evidence for impacting detection.
- Adapting sampling designs to account for these processes increased detection rates.
- Pilot studies of biophysical factors influencing eDNA can improve sampling designs.

GRAPHICAL ABSTRACT



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ABSTRACT

The detection of rare macroorganisms using environmental DNA (eDNA) is a powerful new method for conservation and management; the efficacy of this method is affected by physiological, ecological, and hydrological processes. Understanding the processes limiting eDNA detection and accounting for those factors with optimized sampling designs is critical for realizing the potential of this tool. Amphibians are a focus of conservation programs globally and are often difficult to detect, presenting a challenge for effective action. To increase the ability of eDNA techniques to inform conservation and management programs, we investigated the eDNA detection of amphibians compared with field surveys for six species across a gradient of environmental factors expected to affect eDNA detection in three different systems: perennial wetlands, intermittent wetlands, and acidic intermittent wetlands. We applied a baseline sampling design in each wetland and used an occupancy modeling approach to evaluate evidence for processes limiting detection for each species given the presence of the target species. Evidence weights indicated that limiting processes varied across systems and included those associated with increased degradation (pH < 5, temperature > 25 °C) and limited dispersion (wetland area > 1200 m^2, sample volume < 200 mL). Optimized sampling protocols based on model results included an increased number of sampling locations in large and highly degradative (acidic) wetlands and increased filter pore size in high-particulate systems. These improved designs compensated for the previously limiting factors and yielded average detection rates of 0.62–0.86 per water sample. Degradation and dispersion processes appear to strongly influence the detection of amphibians in wetlands. Optimized, adaptive sampling designs can greatly increase the efficacy of eDNA monitoring approaches.

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1. Introduction

Accurate knowledge of species presence at a site is critical to understanding the drivers of species distributions and identifying effective management actions; however, this local inference can be difficult for species that are rare or elusive (Chadés et al., 2008; Lahoz-Monfort et al., 2014). Amphibians are a major focus of conservation programs globally; 43% of amphibian species were experiencing some form of population decrease as of the last global survey (Stuart et al., 2004) with declines predicted to accelerate this century (Hof et al., 2011). A major challenge in amphibian conservation and management is that species can be very difficult to detect (Muths et al., 2005), as evidenced by recent rediscoveries of species thought to be extinct (e.g., Abarca et al., 2010; Biton et al., 2013; Lehr and von May, 2004).

An emerging method for detecting presence of aquatic vertebrates using environmental DNA (eDNA) in water samples has recently gained traction as a powerful tool (Rees et al., 2014), providing an important opportunity to improve amphibian conservation and management programs. As with any sampling method, detection of species by eDNA is imperfect, and is thought to be a product of physiological, ecological, and hydrological processes (Goldberg et al., 2016). Recent eDNA studies have applied occupancy modeling to incorporate this uncertainty when predicting occupancy of a species at a site (e.g., Hunter et al., 2015; Schmelzle and Kinziger, 2016; De Souza et al., 2016). In this framework, the probability of detection is modeled simultaneously with the probability of occupancy of a species at a site given model covariates; higher detection probabilities lead to more precise estimates of occupancy (MacKenzie et al., 2006). Therefore, finding ways to optimize sampling designs to maximize detection probability is essential for improving our ability to understand the distribution of rare species.

The probability of detecting a species given that the organism is present using eDNA is likely influenced by several processes, including production, degradation, adsorption, and transport (Barnes and Turner, 2015). Production rate varies greatly across individuals and within individuals through time (Klymus et al., 2015; Wilcox et al., 2016), and may be influenced by water chemistry. For example, amphibians in high conductivity water exhibit increased stress hormones (Chambers, 2011), which could be associated with increased shedding of eDNA. Degradation rate is likely the result of an interaction between the microorganismal community and abiotic conditions, with higher temperatures, ultraviolet light (UV), and acidity associated with higher degradation rates (Barnes et al., 2014; Strickler et al., 2015; Lance et al., 2017; Seymour et al., 2018). In lentic systems, eDNA has been shown to stay local to sources (Takahara et al., 2013; Eichmiller et al., 2014; Yamamoto et al., 2016), with detection dropping off quickly with distance from the source (Dunker et al., 2016), indicating that dispersion is the primary process of transport for eDNA from sedentary individuals. The same pattern from tidal and lotic systems (Port et al., 2016; Wilcox et al., 2016) suggests that eDNA settling out of the water column into the benthos limits the transport of these particles, indicating that larger wetlands could provide additional challenges for detection of sedentary species.

Developing a sampling design for an eDNA study requires numerous decisions. These include collection method (filter or precipitation), filter material and pore size (if using filters), preservation method, sample volume, spatial sampling design, number of field replicates, extraction method, and analysis method (e.g., qPCR, metabarcoding). Some of these are determined by the goals of the study or logistical constraints, but some must be chosen by the researcher. Many filter materials have now been vetted (reviewed in Goldberg et al., 2016), but their effectiveness may vary depending on extraction method (Renshaw et al., 2015). Larger sample volumes may be prohibited by logistical constraints or the pore size of a filter, and small sample volumes may miss eDNA collection (Schultz and Lance, 2015). However, increasing the pore size may cause a loss of the smaller particles of eDNA (Turner et al., 2014). In addition to the logistical constraints, understanding among-site variation in production, degradation, and dispersion that may limit eDNA detection is critical to designing efficient sampling protocols.

To address the issue of how eDNA sampling design can be optimized to maximize detection probabilities, we tested hypotheses of which covariates most affected detection probability for six amphibian species in three wetland systems. These systems presented a gradient of degradative challenges to eDNA persistence (temperature, pH, canopy cover as a proxy for UV exposure), dispersion [area (as a representation of the maximum distance sampling site could be from the organism)], sample volume), and production (conductivity as a proxy for water chemistry). Our objectives were 1) to understand the evidence for factors limiting eDNA detection at occupied sites for each species given environmental conditions and 2) to investigate how sampling designs could be optimized to compensate for those limitations. To accomplish these objectives, we collected eDNA samples during multiple seasons for each species simultaneously with crews conducting standard field detection surveys and analyzed the data in an occupancy framework. We applied these results to understand and improve detection rates for amphibians in wetland systems.

2. Materials & methods

2.1. Study areas

We focused on two systems in southern Arizona (perennial and intermittent wetlands) and one in the acidic wetlands of Florida, U.S.A. In Arizona, the perennial wetland systems were located in the Huachuca and Pajarito Mountains and the San Rafael Valley. Chiricahua leopard frogs (*Rana chiricahuensis*; federally threatened) persist in upland wetlands in this area and valley wetlands provide habitat for endangered Sonoran tiger salamanders (*Ambystoma mavortium stebbinsi*). Wetlands in this area are primarily cattle tanks (ponds) and restoration sites (wetlands with cattle excluded). The invasive American bullfrog (*R. catesbeiana*), a threat to both native species, was also found throughout this area. These wetlands were a range of sizes and temperatures (Table 1), providing a gradient of challenges for eDNA detection.

Intermittent wetlands in Arizona were in the Huachuca Mountains and filled during the summer rainy season, providing habitat for Arizona treefrogs (*Hyla wrightorum*), a species recently considered as a candidate under the Endangered Species Act of 1973 (ESA). This species spends a limited amount of time at temporary wetlands after they fill with summer rains and can be difficult to detect (Mims et al., 2016). These wetlands were small and warm, providing conditions known to increase degradation (Barnes et al., 2014; Strickler et al., 2015; Lance et al., 2017) and therefore a challenge to detection.

The acidic wetlands study site was located at Eglin Air Force Base (AFB) in the long-leaf pine forests of the Florida panhandle. Wetlands on Eglin AFB provide extensive habitat for reticulated flatwoods salamanders (*A. bishopi*), listed as endangered under the ESA, and ornate chorus frogs (*Pseudacris ornata*), a sensitive species. In addition to having high acidity, these wetlands are large and shallow (Palis, 1997), providing highly degradative conditions and therefore multiple challenges for eDNA detection.

2.2. Assay development

We designed and validated species-specific qPCR assays for four target species (Table 2, Appendix A) and applied two previously-published assays (Strickler et al., 2015; McKee et al., 2015a). For assay design, we compiled sequence data for each target species from GenBank (NCBI) and created an inclusive consensus sequence using Sequencher version 5.2.4 (GeneCodes Corp., Ann Arbor, MI, US). We used Primer Express 3.0.1 (Applied Biosystems, Foster City, CA, US) to design potential qPCR assays using that sequence and then tested those assays in silico using the Primer-BLAST algorithm (Ye et al., 2012), set to indicate any sequence matches with <2 base pair changes total with at least 1 located