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Rapid detection of amphibian chytrid fungus *Batrachochytrium dendrobatidis* using *in situ* DNA extraction and a handheld mobile thermocycler

Colleen Kamoroff[1,3,*], Caren S. Goldberg[2], Robert L. Grasso[1]

[1]Resources Management and Science, Yosemite National Park, CA 95318, USA
[2]School of the Environment, Washington State University, Pullman, WA 99164, USA

[3]*Present address:* Stillwater Science, Davis, CA 95618, USA

ABSTRACT: The amphibian chytrid fungus (*Bd*) has caused declines and some extinctions of amphibian populations worldwide. Early and accurate *Bd* detection is essential for management of susceptible anurans. We analyzed the effectiveness of *in situ* DNA extraction with a handheld mobile quantitative PCR (qPCR) thermocycler to detect *Bd* on frog skin swabs and in water samples using environmental DNA (eDNA). We collected duplicate eDNA samples and skin swabs from 3 *Bd*-positive *Rana sierrae* populations. We processed one set of samples using a field protocol (a handheld thermocycler) and the other half using a standard lab protocol. We detected *Bd* DNA in all *R. sierrae* swabbed using both the field and lab protocols. We also detected *Bd* DNA in eDNA samples at all sites, although the field and lab protocols failed to detect *Bd* eDNA at separate singular sites; results from the field and lab eDNA protocol did not match. The probability of detecting *Bd* DNA in the technical replicates was lower for the field protocol than the lab protocol, suggesting the field protocol has lower sensitivity and may not detect low quantities of DNA. Our results suggest that the field extraction protocol using a handheld qPCR platform is a promising tool for rapid detection of *Bd* in susceptible amphibian populations, yielding accurate results in less than 60 min. However, the applied field protocol may be prone to false negatives when analyzing low-quantity DNA samples such as eDNA water samples or frog swabs with low pathogen loads.

KEY WORDS: Disease surveillance · Environmental DNA · eDNA · Amphibian chytrid fungus · Mountain yellow-legged frogs · Adaptive management · Yosemite National Park

1. INTRODUCTION

Emerging fungal and fungal-like diseases have caused severe die-offs and extinctions in wild animal and plant species and are a threat to ecosystem health and function (Fisher et al. 2012). One highly virulent disease is chytridiomycosis, a skin disease caused by the invasive amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*; Longcore et al. 1999). *Bd* is linked to at least 500 amphibian species declines over the last half-century and 90 presumed extinctions (Berger et al. 1998, Stuart et al. 2004, Skerratt et al. 2007, Scheele et al. 2019, see also Fisher & Garner 2020). In the California Sierra Nevada, historical records show that *Bd* has been present since the 1970s (Fellers et al. 2001). *Bd* has been linked to the precipitous declines of 2 endemic and endangered species of yellow-legged frogs, Sierra Nevada yellow-legged frog *Rana sierrae* and southern mountain yellow-legged frog *R. muscosa*, collectively referred to as the mountain yellow-legged frog complex (Rachowicz et al. 2005, Vre-

*Corresponding author: cdkamoroff@gmail.com

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denburg et al. 2010) and referred to hereafter as MYLF.

Prior to the arrival of *Bd,* MYLF populations were already diminished due to the introduction of non-native trout which prey on tadpoles and adults (Knapp & Matthews 2000). Non-native trout also fragment and isolate MYLF populations by occupying and barring dispersal corridors as well as adjacent water bodies (Bradford & Graber 1993). *Bd* has caused declines and localized extirpations of many persisting populations in the few remaining fish-free habitats in the Sierra Nevada (Vredenburg et al. 2010, Russell et al. 2019). Given these 2 prominent causes for decline, MYLF species are listed as Endangered by the IUCN (IUCN 2020), the state of California (California Department of Fish and Wildlife 2003), and the US Fish and Wildlife Service (US Fish and Wildlife Service 2014).

There are few remaining MYLF populations on protected public lands (e.g. National Parks) where *Bd* has not been detected. Once *Bd* becomes detectable, the remaining populations are likely susceptible to mass die-off events (Rachowicz et al. 2005, Vredenburg et al. 2010, Knapp et al. 2016). *Bd*-positive MYLF populations persisting with the disease are thought to have an adaptive immune response (Knapp et al. 2016), but both population types (where *Bd* is undetectable and where *Bd* is persisting) require monitoring for adaptive management (e.g. translocation and augmentation) and treatment options such as intervention with antifungal agents at the onset of mass die-off events (Harris et al. 2009).

Established techniques for *Bd* detection include swabbing keratinized skin on frogs and mouthparts of tadpoles, followed by analyzing the swabs for *Bd* zoospores using quantitative polymerase chain reaction (qPCR) techniques (Boyle et al. 2004). More recently, *Bd* has been detected using environmental DNA (eDNA) techniques (Kirshtein et al. 2007, Walker et al. 2007, Hyman & Collins 2012, Chestnut et al. 2014). eDNA is a non-invasive alternative survey tool that is not dependent on finding and handling a host organism and can potentially increase detection of aquatic pathogens when few individuals are infected. Species detection using eDNA methods is accomplished by collection and identification of live and dead microbes and trace DNA particles that are extracted from water samples (Taberlet et al. 2012). A recent study in the Sierra Nevada detected *Bd* in water samples prior to an MYLF chytridiomycosis die-off event (Kamoroff & Goldberg 2017).

The National Park Service (NPS) is actively monitoring and managing populations of endangered MYLF in Yosemite National Park (YNP). The NPS and their partners are currently collecting swabs from MYLF populations to determine if an outbreak is imminent as well as collecting samples from persisting *Bd*-positive populations to determine *Bd* load and epizootic disease dynamics in park populations. Early and accurate detection as well as reliable quantification of *Bd* is a critical component in managing overall MYLF recovery, a task compounded by the difficulty of reaching occupied sites, as MYLF populations inhabit high elevation (>1830 m) alpine lakes often in remote wilderness or wilderness-like settings.

A new wave of rapid *in situ* DNA extraction and qPCR analysis methods is being developed and utilized in conservation and wildlife management with promising results (Sepulveda et at. 2018, Thomas et al. 2020). The NPS currently uses lab-based DNA and eDNA extraction/analysis methods for surveillance of *Bd* across YNP (YNP unpubl. data). However, lab-based approaches require hiking samples ≥10 miles (~16 km) out of the field, followed by additional transport time to a temporary storage facility, and further delays during shipping and lab processing which can result in a minimum turnaround time of weeks to months. Our goal was to improve this process by rapidly detecting and quantifying *Bd* DNA using extracted samples collected and analyzed directly in the field. In this study, we analyzed the effectiveness of using an *in situ* DNA extraction method combined with a handheld mobile thermocycler for real-time qPCR analysis in the field. The field-based platform for DNA extraction and mobile real-time qPCR analysis yields results in less than 60 min and does not require hiking samples out of the field for lab analysis. We compared the results of the *in situ* DNA extraction and analysis approach with lab-based extraction and analysis using 2 sampling strategies: frog skin swabs and eDNA filtered water samples.

2. MATERIALS AND METHODS

2.1. Study area

We collected eDNA samples as well as MYLF skin swabs from 3 different known *Bd*-positive populations across YNP (Fig. 1). All 3 sites were part of YNP's long-term MYLF population and disease monitoring program, ranging in elevation from 3069 to 3202 m and size from 0.3 to 4.7 ha. To prevent the spread of disease, we disinfected all field gear (e.g.

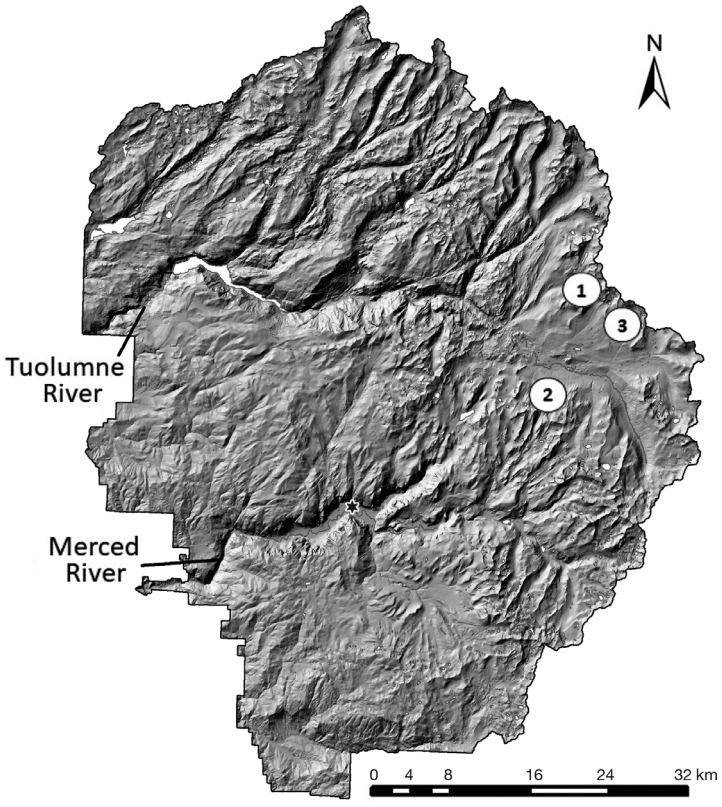

Fig. 1. Yosemite National Park, California, USA. Numbers in circles indicate approximate locations of Sites 1, 2, and 3; only general locations are shown to protect sensitive Sierra Nevada yellowlegged frog populations. *: Yosemite Valley Visitor Center (37.7488° N, 119.5872° W)

boots, nets, etc.) in a 0.016% solution of quaternary ammonia between sample sites (Johnson et al. 2003). We did not enter the water with gear prior to eDNA sample collection.

2.2. Sample collection

2.2.1. eDNA samples

We collected 500 ml of surface water in a clean (soaked in a 10% bleach solution and rinsed in distilled water) polypropylene grab bottle or a sterile DNA-free Whirl-Pak® every 40 m around the perimeter of each site. Due to varying lake sizes, the amount of water collected varied from 1500 to 2380 ml between sites (see Table 1). We split each 500 ml water sample in half and filtered duplicate 250 ml samples using separate filter cups for comparison testing (Fig. 2). There was no method for determining which treatment received the first 250 ml from the 500 ml sample. We mixed each 500 ml sample in the field prior to filtering by gently inverting the Whirl-Pak or grab bottle to ensure even mixture of DNA throughout the sample. The amount of water collected was 5× the amount of water used for detection of *Bd* at a site in 2015 (Kamoroff & Goldberg 2017). At the end of each sample collection, we filtered duplicate 200–250 ml samples of distilled water for a negative control (Fig. 2). We filtered all water samples using a single-use 47 mm diameter 0.45 µm cellulous nitrate filter membrane (AF045W50; Sterlitech) and a polypropylene vacuum flask with a rubber stopper fixed to a hand peristaltic pump or motorized pump. To remove the filter membrane, we used single-use forceps or forceps soaked in a 50% commercial bleach solution and then rinsed in distilled water. All personnel wore single-use disposable latex gloves during sample collection and changed gloves prior to handling filter membranes. We either extracted DNA from the filters on-site immediately (<10 min) following collection and filtration, or we stored the filters in 95% ethanol at room temperature away from any light source (Minamoto et al. 2016) and extracted the samples in a lab within 6 mo following collection (Fig. 2).

2.2.2. Frog skin swabs

We collected *Bd* skin swabs from all adult or subadult MYLF found at all 3 sites (1–3 frogs site^{-1}; see Table 1). We swabbed 10× on the frog's side, 10× upper arm, 10× lower leg, and between the toes using a polyurethane cloth-tipped swab (Biomeme). We used separate swabs for each side of the frog (i.e. right and left side) for comparison testing (Fig. 2). We either extracted DNA from the swabs on-site immediately (<10 min) following collection and filtration or stored the swabs in 95% ethanol at room temperature away from any light source (Minamoto et al. 2016) and extracted the samples in a lab within 6 mo of collection (Fig. 2).

All accessible MYLF populations in YNP were known to be *Bd*-positive. In order to swab potential *Bd*-negative anurans, we opportunistically swabbed 4 California red-legged frogs *Rana draytonii* recently introduced to Yosemite Valley, YNP, and one American bullfrog *Lithobates catesbeianus* in Catalina Island, Catalina National Park, California. At the time of swabbing, it was unknown whether the *R. draytonii* or *L. catesbeianus* were *Bd*-positive. None of the frogs were treated prior to release.

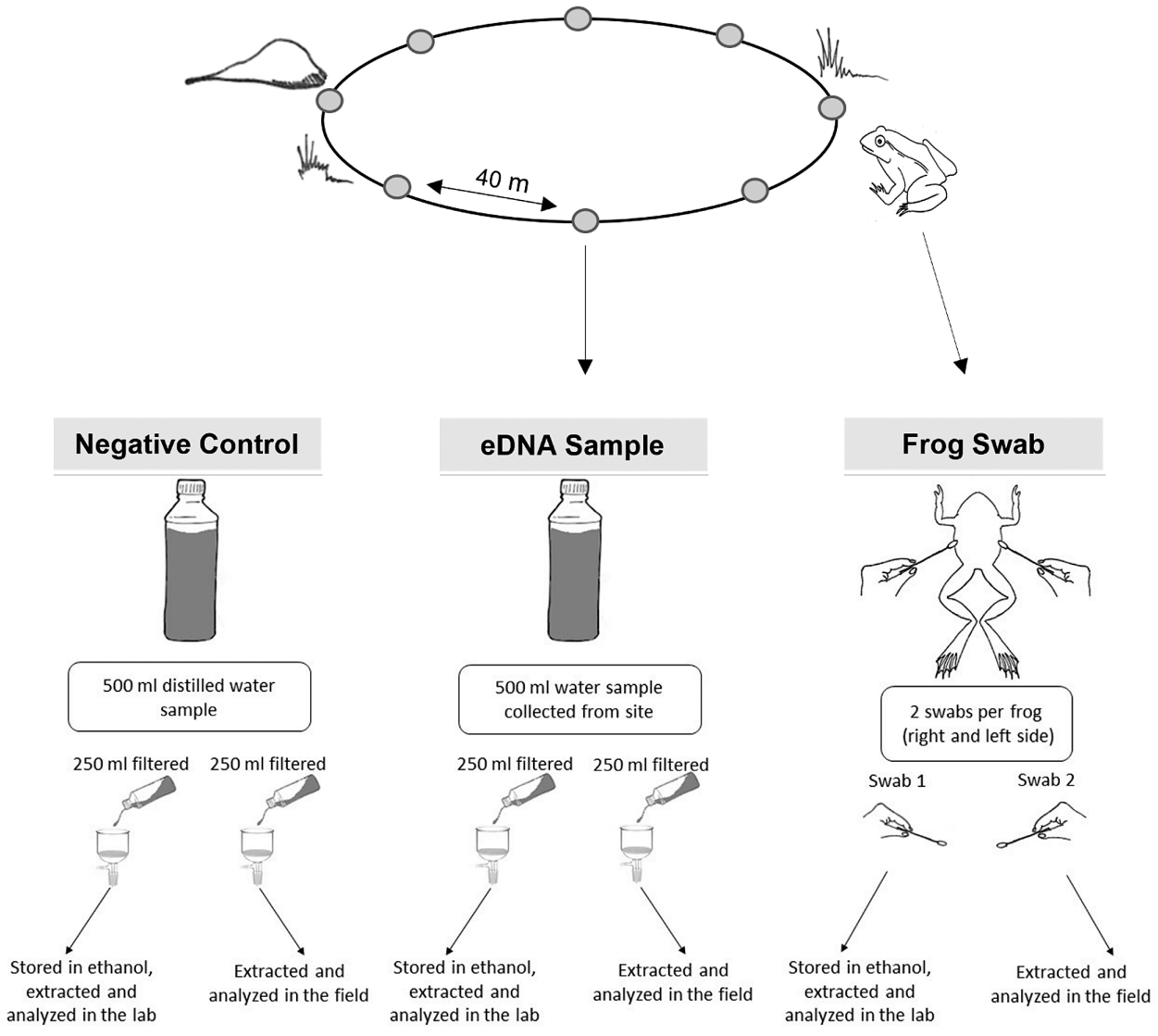

Fig. 2. Experimental design for lab and field comparison testing of environmental DNA (eDNA), frog swab, and blank samples collected at 3 sites across Yosemite National Park and analyzed for the presence of *Batrachochytrium dendrobatidis* (*Bd*) DNA

2.2.3. Lab protocol for DNA analysis

We stored the filters and swabs in 95% ethanol at room temperature away from any light source (Minamoto et al. 2016) and extracted the samples at Washington State University lab within 6 mo following collection. For eDNA filters, we cut each filter in half using a scalpel that was decontaminated with a 50% bleach solution and rinsed in distilled water, used half the filter for DNA extraction, and stored the other half in 95% ethanol as a reserve. We used the QIAshredder/Qiagen DNeasy Blood and Tissue DNA extraction protocol (Goldberg et al. 2011) in a limited-access room, where no high-quality DNA (blood or tissue samples) or qPCR products are processed, using best practices for eDNA (Goldberg et al. 2016). For frog swabs, we extracted DNA from the entire swab using Qiagen DNeasy Blood and Tissue DNA extraction protocol without QIAshredder in a tissue lab. We analyzed the samples using a previously published *Bd* qPCR assay (Boyle et al. 2004), with the substitution of Environmental Master Mix (ThermoFisher), 3 µl of DNA extract in triplicate reactions, and running for 50 cycles on a BioRad qPCR machine (BioRad Laboratories). To quantify initial DNA copy number of *Bd* in the eDNA and swab samples, we created a standard curve by using a 4-point serial dilution (10–10 000 copies) of a synthesized gene (gBlocks; Integrated DNA) in duplicate on each plate. We can detect quantities of DNA outside the

range set by the standard curve, but exact quantities cannot be determined if they are outside the set range. All wells included an exogenous positive control to ensure no qPCR inhibition had occurred (IPC; ThermoFisher). We created and analyzed negative extraction and qPCR controls with every extraction batch and plate.

2.2.4. Field protocol for DNA analysis

We used reagents, assay, handheld qPCR, and protocols from Biomeme Inc. We extracted all samples in the field within 10 min of collection using the M1 Sample Prep Kit and protocol for eDNA or DNA respectively, and we extracted DNA from the entire swab or filter. We first mixed the samples (either swabs or filters) in the Biomeme Lysis Buffer by adding the samples to the buffer solution provided in 2 ml screw-cap tubes and then shaking the tube vigorously by hand for 1–2 min. The samples were then bound to a silica membrane by pumping the lysed samples through the Biomeme's Sample Prep Column using a single-use 1 ml syringe. Using the syringe, we removed unwanted material by washing the sample with Biomeme Protein Wash and Biomeme Wash Buffer (also provided in 2 ml screw-cap tubes). Following the wash, we air-dried the samples by repeatedly pumping air through the column with the syringe. Lastly, we eluted the purified nucleic acids from the column using the Biomeme Elution Buffer. The final elution solution (1 ml) was stored in the 2 ml screw-cap tubes. The entire extraction process took 2–5 min to complete. The extracted DNA is stable in cool environments away from UV light. Due to the limited size of the handheld qPCR device, we could only run one sample at a time. Therefore, we stored the extracted DNA out of the sun (or in a fridge when available) and analyzed the samples within 0–24 h following extraction. We analyzed the samples using Go-Strips™, a custom shelf-stable assay developed from a previously published *Bd* qPCR assay (Boyle et al. 2004). The Go-Strips contained primer, probe, master mix, and an internal positive control and only required the addition of extracted DNA (the eluted DNA from the field extraction). The exception to this process was at Site 1, where we added pre-mixed primer and probe stored on ice to the Go-Strips that contained master mix and internal positive control. We ran our samples on a two3™ mobile real-time PCR machine (~2 lb ≈ 1 kg). To quantify the initial DNA copy number of *Bd* in the samples, we created a standard curve by using a 3-point serial dilution (100–10 000 copies) of a synthesized gene (gBlocks; Integrated DNA) prior to running reactions. We used 20–40 µl of DNA extract in each reaction (20 µl at Site 1; 40 µl at Sites 2 and 3). We increased the amount of DNA extract for Sites 2 and 3 after consulting with Biomeme personnel to increase the chances of DNA detection. The two3™ contains 3 wells and can run 2 channels. We ran each sample in triplicate (3 technical replicates using all available wells) with an internal positive control (duplex reaction using both channels). As a result, we could not run a standard curve or field negative simultaneously (as described in Sepulveda et al. 2018). For analysis, we used the cycles and temperatures for DNA and eDNA analysis respectively as recommended by Biomeme. To run all skin swab reactions, we used a cycle of 15 min at 95°C followed by 45 cycles at 94°C for 60 s and 60°C for 60 s. To run all eDNA reactions, we used cycles of 15 min at 95°C followed by 50 cycles at 94°C for 60 s and 60°C for 60 s. If inhibition occurred, we diluted samples 1:1 using molecular-grade water stored in a clean (washed in a 10% bleach solution and rinsed in distilled water) polypropylene grab bottle and reanalyzed the sample. We diluted inhibited samples in the field using a 20 µl pipette with disposable filter tips. We analyzed the eDNA negative control samples collected on-site as the negative control for field PCR. Due to the limited size of the two3™, we analyzed the negative control samples after all water samples and swabs were analyzed.

2.2.5. Data analysis

We considered *Bd* to have been detected in a sample if ≥1 PCR technical replicate tested positive for frog skin swabs. For eDNA samples, we considered *Bd* to have been detected at the site if ≥1 technical replicate was positive in ≥1 eDNA sample collected at the site. We considered a technical replicate to be positive if an exponential increase occurred at any point during the qPCR cycles (as described in Goldberg et al. 2013, see also Ellison et al. 2006).

To model *Bd* DNA detection probability, we used multi-scale occupancy models in R v.3.6.0 (R Core Team 2019) and the package 'eDNAoccupancy' (as described in Sepulveda et al. 2018, see also Dorazio & Erickson 2018). We compared a null model to models fitted with covariates that affected the occurrence of *Bd* DNA in the sample (θ; sample type [swab vs. eDNA]) and covariates that affected the detection of *Bd* DNA in the technical replicate or sub-sample (p;

Table 1. Sample collection meta-data and results across all 3 sites and sample types: control sample (blank), environmental DNA sample (eDNA), and frog swab (swab). 'Samples collected' refers to number of frogs swabbed, eDNA samples collected, and eDNA blanks collected for PCR analysis of *Batrachochytrium dendrobatidis* (*Bd*) DNA. *Bd* DNA detection and quantification results for field and lab methods are indicated by 'Field' or 'Lab' respectively. Mean *Bd* DNA quantification is the average number of *Bd* DNA copies found across all samples and technical replicates with standard deviation (SD) across all samples. Note that lab quantities listed in the last column have been scaled up to represent total quantities in the sample because only a fraction of the DNA extracted from each sample is quantified during PCR analysis. (–) not applicable. DNA below the level of the standard curve is marked by '<100'.

Site	Sample type	Total filtered (ml)	Samples collected	DNA detected		DNA quantification (mean ± SD)	
				Field	Lab	Field	Lab
Site 1	eDNA	2380	4	Yes	Yes	<100	129133.3 ± 138905.3
Site 2	eDNA	3150	3	Yes	No	<100	–
Site 3	eDNA	1500	3	No	Yes	–	30068.67 ± 10606.67
Site 1	Swab	–	2	Yes	Yes	<100	24886.67 ± 7379.33
Site 2	Swab	–	1	Yes	Yes	<100	4820500.00
Site 3	Swab	–	3	Yes	Yes	<100	696138.00 ± 613085.00
Site 1	Blank	250	1	No	No	–	–
Site 2	Blank	200	1	No	No	–	–
Site 3	Blank	250	1	No	No	–	–

analysis approach [field vs. lab]). We assessed the models using the posterior-predictive loss criterion (PPLC) and widely acceptable information criteria (WAIC). We calculated detection probability and their standard errors using a Markov chain containing 11 000 iterations (1000 burn-in).

3. RESULTS

We detected *Bd* DNA on all Sierra Nevada yellow-legged frogs (n = 6) swabbed for comparison testing across all 3 sites using the field and lab protocols (Table 1, Fig. 3). Of the anuran populations with unknown *Bd* status, we detected *Bd* DNA on 3 swabs collected from *Rana draytonii* (n = 4) as well as 1 swab from *Lithobates catesbeianus* (n = 1) using the field and lab protocols. We did not detect *Bd* on one *R. draytonii* swab using the field and lab protocol. Additional lab analysis of the *R. draytonii* verified the negative results.

We detected *Bd* DNA in eDNA samples collected at 2 out of the 3 sites using both the lab and field protocols but not from the same sites. We did not detect *Bd* DNA at Site 3 using the field protocol and we did not detect *Bd* DNA at Site 2 using the lab protocol (Table 1, Fig. 3).

We were unable to quantify DNA from both the eDNA and swab samples using the field protocol because all DNA levels were below the standard curve (<100 copies), suggesting that an adjusted standard curve (1–100) would have been more appropriate for the field methods. All positive *Bd* eDNA and swab samples extracted and analyzed using the lab protocol were >100 copies and within

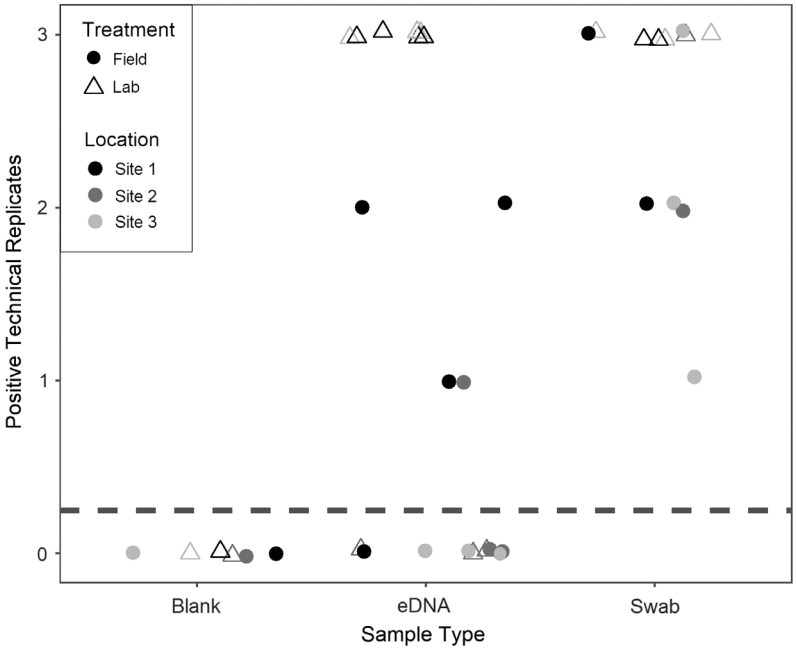

Fig. 3. Number of technical replicates that detected *Batrachochytrium dendrobatidis* (*Bd*) DNA for each sample across all 3 sites and sample types: control sample (blank), environmental DNA sample (eDNA), and frog swab (swab). We detected *Bd* DNA in at ≥1 technical replicate for icons above dashed line; we did not detect *Bd* DNA in any technical replicate for icons below the dashed line

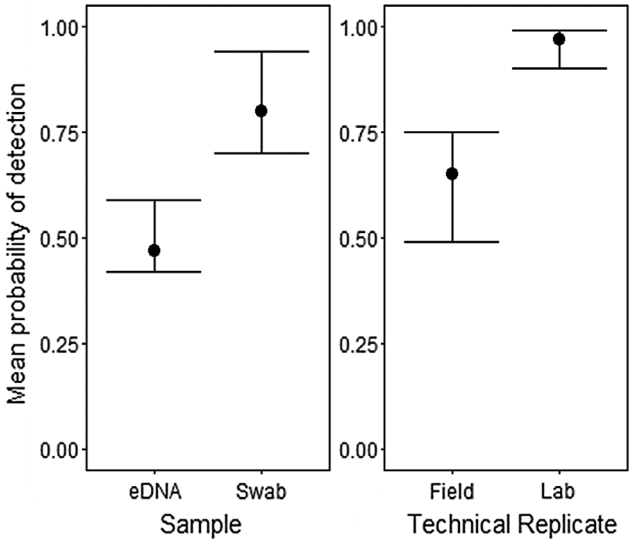

Fig. 4. Mean conditional detection probability (±95% CI) of *Batrachochytrium dendrobatidis* (*Bd*) DNA occurrence in (left panel) eDNA or swab samples and (right panel) technical replicates (sub-samples) using the field lab protocols

the limits of quantification (Table 1). All quantification-cycle values for samples extracted in the lab were <40.

We detected *Bd* in more technical replicates using the lab protocol than the field protocol (Figs. 3 & 4). The best fit detection probability model with the lowest PPLC and WAIC included sample type (swab vs. eDNA) and analysis approach (field vs. lab) as covariates for the sample (θ) and sub-sample (*p*) respectively. The mean derived estimated of θ was higher for swab samples compared to eDNA samples, as expected, and the mean derived estimated of *p* was higher for the lab protocol compared to the field protocol (Fig. 4). The mean θ (±95% credible limits) was 0.47 (0.42–0.59) for the eDNA samples and 0.80 (0.70–0.94) for skin swabs. The mean *p* (±95% credible limits) was 0.65 (0.49–0.75) for the field protocol and 0.97 (0.90–0.99) for the lab protocol. Approximately 1.5 technical replicates would have to be analyzed using the field protocol in order to have the same mean detection probability as one technical replicate using the lab protocol. Reduced detection probability of the field-based approach is compounded when collected as eDNA samples, where 1.7 technical replicates would need to be analyzed in order to have the same mean detection probability as one technical replicate from a frog swab.

While using the field protocol, one skin swab and one eDNA sample experienced inhibition at Site 3. To resolve this problem, we removed the inhibition in the skin swab and eDNA sample through 2 serial dilutions (1:1) with molecular-grade water (final dilution: 10 µl DNA and 30 µl H_2O). We detected *Bd* DNA in the skin swab but failed to detect *Bd* DNA in the eDNA sample. However, we did not detect *Bd* DNA in any of the uninhibited eDNA samples collected at Site 3 using field analysis techniques. There was no issue with inhibition for all samples extracted and analyzed using the lab protocol. All negative control samples, extraction negatives, and qPCR negatives tested negative for both the lab and field protocols.

4. DISCUSSION

4.1. Rapid and effective detection of *Bd*

Mobile handheld real-time PCR thermocyclers are promising tools for rapidly detecting *Bd* in susceptible amphibian populations, and our work advances the development of non-invasive protocols that can be applied to global aquatic disease surveillance programs. We detected *Bd* DNA in all positive frog skin swabs collected across multiple sampling locations and amphibian species using rapid in-the-field methods, demonstrating the effectiveness of our rapid protocol for detecting *Bd* presence in the field. Previously, to detect *Bd* in individuals, amphibian skin swabs had to be stored to prevent DNA degradation, transferred out of the field, and processed in a lab. The difficulty involved with collecting and processing swabs in a lab is compounded when sampling species that inhabit remote wilderness or locations that are otherwise difficult to access. Although the sample size was small (n = 11), which limits inferences, the rapid, *in situ* method we applied yielded accurate results for all amphibians swabbed in less than 60 min and did not require the transfer of samples out of the field for lab analysis. Rapid detection of *Bd* is a critical tool in disease surveillance and initiating rapid intervention and treatment options such as salvaging animals for captive rearing efforts or on-site anti-fungal treatments (Harris et al. 2009). Rapid detection of *Bd* can also help in monitoring individual frogs during translocation or population augmentation events as well as the monitoring of captively reared individuals' post-reintroduction events. The ability to detect *Bd* presence in an individual in less than 60 min could aid in the disease surveillance of MYLF and other *Bd*-susceptible amphibians where high loads are expected if *Bd* is present.

We also detected *Bd* DNA in eDNA samples at the same number of sites using the rapid in-the-field method compared to traditional lab methods. We did not detect *Bd* DNA at Site 3 using field analysis and

we did not detect *Bd* DNA at Site 2 using lab analysis (Table 1); swabs from both sites tested positive as swab samples have a higher probability of detecting *Bd* DNA on amphibians than in eDNA samples from the water (Fig. 4). The false negative result in both the lab and field methods suggest that a more sensitive eDNA surveillance strategy should be used (i.e. increase number and quantity of water samples collected and/or increase number of technical replicates), as generally recommended for eDNA surveys (Goldberg et al. 2016).

4.2. Technical limitation and sensitivity

We detected *Bd* DNA in more technical replicates for the samples processed in the lab than samples processed in the field, suggesting that our field-based methods may not be as sensitive. Approximately 1.5 technical replicates would have to be analyzed using the field protocol in order to have the same mean detection probability of 1 technical replicate using the traditional lab protocol. Our findings are consistent with Sepulveda et al. (2018), who found lower detection of northern pike in eDNA samples and subsamples processed with a field protocol compared to traditional lab techniques. As a result, the field extraction approach failed to detect DNA in areas collected with low densities of northern pike (Sepulveda et al. 2018). Additionally, we used a liberal positive criterion, where a sample was considered positive if ≥1 technical replicate detected *Bd* DNA. A more conservative approach, where a sample is considered positive if ≥2 or 3 technical replicates detect the target DNA, as is typically applied to eDNA analysis, would likely increase false negative rates of the field-based protocol.

All quantities of DNA detected using rapid in-the-field techniques were below the standard curve, further evidence that in-the-field methods lack sensitivity. Binary detection (i.e. presence/absence) of *Bd* DNA is an important metric for understanding disease dynamics and host risk. However, DNA quantification of both eDNA and swab samples is critical to the ecological interpretation of the results. Vredenburg et al. (2010) found *Bd* prevalence increased rapidly and infection intensity increased exponentially with declines of MYLF evident after average infection intensity of ~10 000 zoospores per swab. Determining when *Bd* levels and infection intensities experience rapid/exponential growth before lethal threshold levels is critical for management to implement conservation strategies. Such determination can only be accomplished through accurate quantification of *Bd* load on skin swabs and potentially eDNA samples, and the current in-the-field methods do not appear sensitive enough to accurately quantify DNA at this time. Changing the serial dilutions (e.g. 10–1000 or 1–100) could better quantify low densities for in-the field extraction. However, our work suggests that current extraction or analysis methods are not as sensitive as traditional lab techniques.

The field methods used in this study quickly and accurately detected *Bd* in skin swab DNA and eDNA samples; however, there are limitations, and we suggest cautious implementation. The in-the-field methods detected significantly lower DNA copy numbers compared to the lab methods, suggesting that the in-the-field methods may not detect DNA at low densities and may not be appropriate for detecting low-level infections. Similarly, eDNA is typically used as a surveillance tool for rare or elusive species (Rees et al. 2014) or for early detection/monitoring of invasive species (Jerde et al. 2011, Hunter et al. 2015, Kamoroff et al. 2020). Being able to detect trace amounts of DNA in a water sample is critical for successful use of eDNA techniques. Prior to the use of rapid field techniques, further assessment should be made to ensure that target DNA can be detected at low quantities. Our study design was to determine how the rapid in-the-field techniques (DNA extraction and qPCR) compared to traditional lab processes. As a result, we cannot comment on which part of the field techniques — the DNA extraction or the hand-held qPCR platform — is responsible for the reduction in *Bd* detection. Since conducting the research, Biomeme has updated their DNA extraction procedure to increase sensitivity. We suggest additional research to determine how the in-the-field DNA extraction process compares to traditional lab processes. For example, a future study should extract the same sample using in-the-field techniques as well as traditional lab techniques and then run the results on the same qPCR platform. Similarly, it is imperative to understand the limitation of detection for the field technique. We suggest a future study to extract known amounts of eDNA or DNA that has been serially diluted to determine what DNA quantities are detectable using rapid in-the-field techniques.

4.3. Costs analysis

Bd detection for conservation and management projects needs to be reliable as well as able to meet

budget and time constraints. Typical costs (in US$) for lab extraction and analysis of swabs and eDNA samples are ~$10–35 and ~$50–150 respectively, depending on type of lab, extraction method, and number of samples processed. Typical qPCR machines used in lab analysis have a 96 well capacity and can multiplex up to 5 targets well^{-1}, resulting in a high-volume throughput per run. The M1 sample prep kit for field-based DNA and eDNA extraction cost was $15 sample^{-1} and the custom Go-Strips™ were $10 well^{-1} (Biomeme). Total cost of analysis using the Biomeme field methods was $45 for both eDNA and DNA extractions run in triplicate wells. The two3™ mobile real-time PCR machine has a 3 well capacity and can multiplex 2 targets well^{-1} (the target species and an internal positive control). The limited wells inherent in a small handheld qPCR machine will take much longer to run a large number of samples than a lab-based machine. As a result, for projects requiring high numbers of samples, lab-based extraction and analysis may be more cost- and time-efficient. Additionally, the limited wells prevent running negatives and standard curves at the same time. Although we found no evidence of contamination, eDNA detection techniques are prone to false positives, and best practices suggest running standard curves and extraction negatives with every batch and plate (Goldberg et al. 2016).

5. CONCLUSIONS

We demonstrated that rapid and accurate detection of *Bd* in frog skin swabs can be accomplished using field extraction and analysis techniques with a mobile real-time PCR device. The methods applied worked for presence/absence detection data of high-quality (swab) samples. Field-based DNA extraction and qPCR analysis is a promising management tool to aid in the recovery of declining amphibian species. However, there are tradeoffs to using the rapid in-the-field methods, as traditional lab methods are more sensitive to low quantities of DNA (Sepulveda et al. 2018). Currently, our methods could be used to accurately detect presence but could not be used for accurate DNA quantification and should be used cautiously when detecting low-quality DNA samples (i.e. eDNA) or low quantities of DNA, especially when the target specimen is present at low densities. Additionally, scope of operation should be considered, as it may be more cost- and time-efficient to run high volumes of samples using lab-based approaches.

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