

Short Communication

Tradeoffs of a portable, field-based environmental DNA platform for detecting invasive northern pike (*Esox lucius*) in Alaska

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Abstract

Environmental DNA (eDNA) has improved detection probabilities of aquatic invasive species but lab-based analytical platforms for eDNA analyses slow opportunities for rapid response. Effective approaches that address this analytical bottleneck and improve capacity for rapid response are urgently needed. We tested the sensitivity of a portable, field-based eDNA platform relative to widely used lab-based eDNA approaches for detecting invasive northern pike (*Esox lucius*) in eight lakes on Alaska's Kenai Peninsula. The portable, field-based platform takes ~1 hr from sample collection to final results and uses a field-based DNA extraction kit, a shelf-stable assay, and a portable real-time PCR thermocycler. Lab-based approaches take days to weeks to months for final results and use lab-based DNA extraction kits, lab-bound assays, and benchtop real-time thermocyclers. We found that the portable, field-based approach was less sensitive than lab-based approaches and was more prone to inhibition, thus increasing potential for false-negatives. Until sensitivity and inhibition issues can be resolved, this portable, field-based approach is best viewed as a complement to rather than a replacement of standard eDNA lab-based approaches.

Key words: Biomeme, detection probability, fish, lakes, molecular, occupancy

Introduction

Early detection and rapid response are pillars of most invasive species programs because they increase the probability that invasive species control and containment efforts will be successful (Sepulveda et al. 2012). Over the last decade, environmental DNA (eDNA) approaches have improved detection probabilities for rare and cryptic aquatic invaders (Rees et al. 2014), but the lab-based platforms required for eDNA analyses can slow opportunities for rapid response (Egan et al. 2015). Effective approaches that address this analytical bottleneck and improve capacity for rapid response are urgently needed.

Portable, field-based molecular platforms have been recently developed to help address this bottleneck. Examples of these platforms include light transmission spectroscopy (Egan et al. 2015), loop-mediated iso-

thermal amplification (LAMP; Lee 2017), and nanopore technology (MinION; Laszlo et al. 2014). Though these platforms are portable and provide rapid information, they are difficult to tailor to challenging conditions (e.g., inhibition; McKee et al. 2015) while in the field; whereas lab-based platforms are more easily optimized. Consequently, tradeoffs in detection sensitivity and performance between portable, field-based eDNA platforms and lab-based platforms likely exist. Identifying these tradeoffs is critical prior to implementation by resource managers.

Here, we critically assessed tradeoffs of a new portable, field-based rapid eDNA approach developed by Biomeme Inc.® (Philadelphia, PA) that uses real-time PCR technology relative to lab-based real-time PCR technology eDNA approaches for detecting invasive northern pike (*Esox lucius*; pike) in south-central Alaska. Fisheries managers currently use

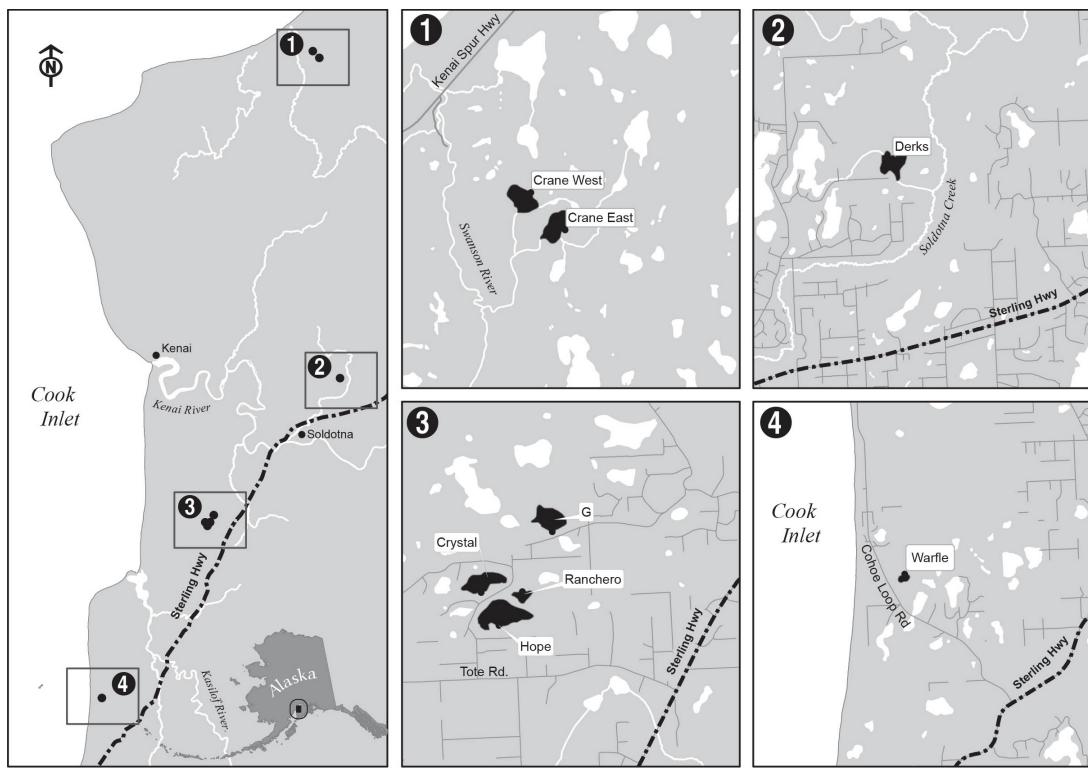


Figure 1. Environmental DNA water samples were collected from eight lakes near Soldotna in southcentral Alaska.

lab-based eDNA approaches for surveillance of invasive pike populations in Alaska (Dunker et al. 2016); however, the turnaround times between water sample collection and eDNA results have varied from weeks to months since samples are transported to molecular labs for DNA extraction and analysis. This analytical bottleneck has limited eDNA surveillance and stymied opportunities for rapid response to invasions, especially when surveying in remote and hard to access waters.

Methods

Study area

We collected water samples from eight lakes near Soldotna, AK in October 2017 (Figure 1; physical characteristics described in Supplementary material Tables S1 and S2). Three of these lakes had higher pike catch-per-unit-effort (CPUE; Crystal, Hope, Ranchero), two lakes had lower pike CPUE (Warfile, G), and three lakes (Derks, Crane East, Crane West) were suspected to contain pike, but pike presence was unknown. The higher CPUE lakes averaged 1.21 pike/hr, (range of 0.41–1.86) and the lower CPUE lakes averaged 0.08 pike/hr (range of 0.03–0.13). Due to recent pike

eradication efforts in this area (Dunker et al. 2016), a third lake with confirmed lower pike densities did not exist.

Water sampling methods

At each water body, we collected ten, two-liter samples in the littoral zone at equidistant locations around each lake according to Dunker et al. (2016). Water samples were individually bagged to prevent cross-contamination, placed on ice inside coolers, and transported back to the Alaska Department Fish and Game (ADFG) lab in Soldotna where samples were immediately filtered. Field blanks of deionized water were collected at each site and one travel blank of deionized water was placed in each cooler.

We removed three, 500-ml subsamples from each 2-L water sample and then performed filtration on each 500-ml subsample. Two of these 500-ml water samples were each filtered through 47 mm, 1.0 μm mixed cellulose ester (MCE) filters (Stereitech Corporation; Kent, WA) and the third sample was filtered through a 47 mm, 1.2 μm Whatman® glass-fiber filter (GE Healthcare) using a peristaltic pump (Geotech Environmental Equipment Inc.). Both MCE and GF filters were used since ADFG currently uses these

filter types for eDNA water samples. Consequently, we compared the Biomeme results to the lab-based results for each of these filter types. For each water sample, one MCE filter was placed into Biomeme's filter sample tube pre-filled with 3 ml of lysis. The remaining two filters were placed in individual sterile Whirl-Pak® (Nasco Corporation) bags and frozen at -20 °C until DNA extraction.

DNA analytical methods

We used the portable, field-based approach (hereafter referred to as Biomeme approach) and the standard lab-based approach (hereafter referred to as lab approach) to analyze filters for pike DNA. The Biomeme approach included a field-capable DNA extraction, a shelf-stable assay, and a portable real-time PCR thermocycler. The lab approach included lab-based DNA extraction, a lab-bound assay, and a benchtop real-time PCR thermocycler.

Biomeme approach

We used the Biomeme Field Test Kit™ for DNA extraction, which is designed for use only with MCE filters. The Biomeme kit utilizes a filtration-based method in which DNA selectively binds to the silica membrane inside Biomeme's proprietary sample column. Subsequent washes through a sequence of specially formulated buffers produce purified DNA upon elution. We followed Biomeme's six-step protocol which takes ~ 5 minutes (see Supplementary material Appendix 1 for details). The purified DNA was then stored in the elution buffer until PCR.

To analyze DNA extract for presence of pike DNA, we used Biomeme's two3™ portable real-time thermocycler. The Biomeme two3™ has two channels (FAM and Cy5) and three wells so duplex reactions can be run for three samples simultaneously. A limitation of only three wells is that standard curves cannot be run with samples, so separate runs cannot be normalized and target DNA amount cannot be accurately quantified.

We pipetted 20 µl of the purified DNA into each well, which was pre-filled with a lyophilized assay that included the *EluCOI* marker specific to pike DNA (Olson et al. 2015). The assay is a proprietary formulation of the *EluCOI* primers and probe, master mix and an internal positive control (IPC) that is reconstituted upon addition of the aqueous DNA extract sample. We followed Biomeme's recommended thermocycler protocol for this assay (see Appendix 1). Results are ready in ~ 45 minutes. Output of the Biomeme two3™ thermocycler is provided via a smartphone interface and includes amplification curves and the cycle number at which fluorescence

increased above background values (C_q) for the pike marker (FAM channel) and for the IPC (Cy5 channel). Samples judged positive for pike DNA were those which amplified. Samples judged inhibited were those for which the IPC failed to amplify. Because this Biomeme approach is intended for rapid field use, we intentionally did not attempt to resolve inhibition issues via dilution or inhibitor removal kits. To provide guidelines for the limit of detection with this assay, we also ran serial dilutions of $5e^1$, $5e^3$, and $5e^5$ template copies using synthesized pike DNA specific to the *EluCOI* marker. The C_q values for $5e^1$ template copies were 38–40.

Lab-based approach

All DNA extractions were performed with Qiagen DNeasy Blood and Tissue Kits (Qiagen, Inc.) according to the manufacturer's instructions and stored at -80 °C. All extractions and plate pipetting were done in rooms reserved for extracting eDNA samples and PCR prep, respectively, where no PCR products or other sources of high concentration DNA are handled. The assay was conducted using a quantitative PCR (qPCR) system; specific details are provided in Appendix 1. Samples were run in triplicate during qPCR. Technical replicates with $C_q < 40$ and efficiencies 1.5–2 were scored as positive for pike DNA. Efficiencies of 2 indicate perfect doubling, while an efficiency of 1 indicates no amplification.

Analysis

We used raw data and modeled probabilities of eDNA detection to assess efficacy of the portable, field-based eDNA approach relative to lab-based approaches. With the raw data, we computed simple summaries of sample and subsample positives for each analytical approach. Sample-level summaries include the number of samples in a water body with ≥ 1 positive technical replicate (liberal criteria) and with ≥ 2 positive technical replicates (conservative criteria).

To model probabilities of eDNA detection, we used the eDNAoccupancy R package to fit Bayesian, multi-scale occupancy models to our data (Dorazio and Erickson 2017). These models are appropriate for eDNA surveys, such as ours, that include three, nested levels of sampling: primary sample units (i.e., a lake) within a study area, secondary sample units (i.e., a water sample) collected from each primary unit, and subsamples (i.e., PCR technical replicates) of each secondary sample unit. We used Bayesian model-selection criteria (posterior-predictive loss criterion [PPLC] and widely acceptable information criteria [WAIC]) to compare support for models fitted with and without covariates. Models with

lower PPLC and WAIC values are favored. Covariates of eDNA occurrence in primary sample units included site and pike density (high, low, unknown). Covariates of eDNA occurrence in samples and eDNA detection in subsamples included site and analytical approach (Biomeme, lab-based glass fiber filters, and lab-based nitrocellulose filters). We then computed estimates of derived parameters for eDNA occurrence and detection probabilities for the most favored model. These estimates and their standard errors were computed using a Markov chain containing 11,000 iterations (1000 burn-in).

Results

The Biomeme approach amplified pike DNA only in the three higher pike CPUE lakes, while the lab-based approach using either filter type amplified pike DNA in the three higher CPUE lakes, in one of the unknown lakes (Derks Lake), and in multiple field, travel, and lab blank samples (Figure 2). These field results were consistent for both liberal (≥ 1 technical replicate positive) and conservative (≥ 2 technical replicates positive) positive criteria. No pike DNA was amplified by any method in the lower pike CPUE lakes and post-study gillnetting (late October) in these lakes failed to capture pike, suggesting that possibility that pike may not have been present.

In higher pike lakes, the Biomeme approach amplified 30–45% of the technical replicates ($n = 30$ per lake; Figure 3); however, 11 of these technical replicates had very late amplification ($C_q > 38$). The lab-based MCE and GF filters amplified 85–97% and 75–85% of technical replicates, respectively, and all had C_q values < 38 (Figure 3). In Derks Lake, the lab-based MCE filter amplified ~80% of technical replicates ($n = 30$) while GF filters only amplified ~30% of technical replicates. For the blanks, the lab-based MCE filters amplified 30% ($n = 33$) of technical replicates, while the GF filters amplified 46% ($n = 33$) of technical replicates, thus providing strong evidence that the source for deionized water had low-level contamination. All non-template controls (NTCs) were negative.

The model with the lowest PPLC and WAIC values included only analytical approach as a covariate for the conditional probabilities of pike DNA occurrence in samples (θ) and of pike DNA detection in subsamples (p). Model fit was not improved by including covariates for occupancy probability at a site (Ψ). Mean Bayesian estimates of θ and p were positive and greater than 0 for both lab-based approaches, while estimates for the Biomeme approach did not differ from 0. Though θ and p mean values were greater for MCE filters than for GF filters, their 95% credible limits overlapped.

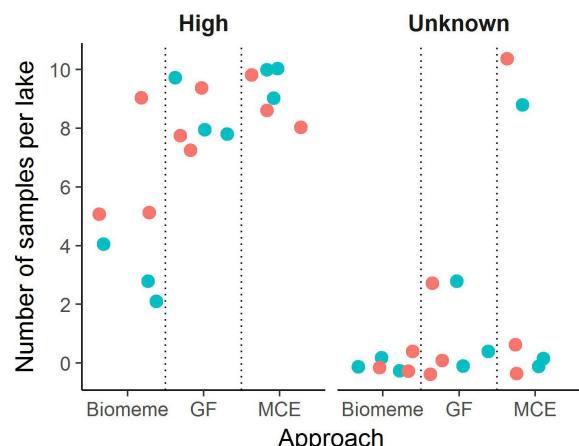


Figure 2. The number of water samples with positive amplification from higher catch-per-unit-effort (CPUE) pike lakes and lakes with unknown pike presence using the Biomeme approach and using the lab-based approach with glass fiber (GF) filters or mixed cellulose ester (MCE) filters. Water samples with ≥ 1 technical replicate that amplified (green dots) and with ≥ 2 technical replicates that amplified (red dots) are displayed. All samples from lower pike CPUE lakes were negative.

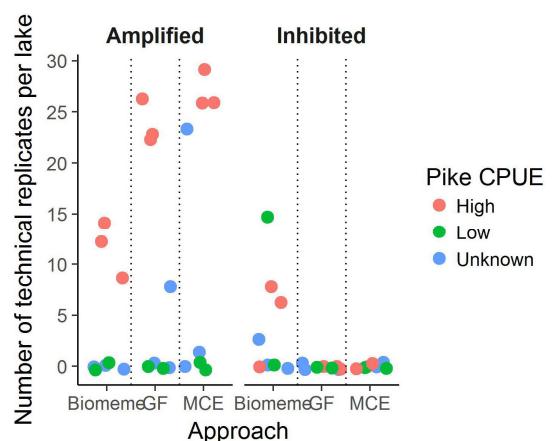


Figure 3. The percent of technical replicates ($n = 30$ per lake) that amplified pike DNA or were inhibited using the Biomeme approach and using the lab-based approach with glass fiber (GF) filters or mixed cellulose ester (MCE filters). Samples were collected from lakes with high and low pike catch-per-unit-effort (CPUE) and lakes with unknown pike presence.

Similarly, mean derived estimates of θ and p of both lab-based approaches were greater than the Biomeme approach (Figure 4). The mean θ (\pm 95% credible limits) was 0.42 (0.28–0.57) for the Biomeme approach, 0.81 (0.69–0.90) for lab-based MCE filters, and 0.66 (0.52–0.78) for GF filters. Thus, \sim 1.9 water samples must be analyzed with the Biomeme approach in order to have the same mean θ as one water sample using the lab-based MCE filters. The mean p (\pm 95% credible limits) was 0.60 (0.45–0.74)

for the Biomeme approach, 0.85 (0.77–0.91) for lab-based MCE, and 0.79 (0.70–0.86) for lab-based GF filters. Thus, ~ 1.4 Biomeme technical replicates must be analyzed in order to have the same mean detection probability as one lab-based MCE filter technical replicate.

Importantly, IPCs in all technical replicates using the lab-based approaches successfully amplified, but 15% (31/208) of all technical replicates analyzed using the Biomeme approach were inhibited (i.e., IPC failed to amplify; Figure 3). All three technical replicates were inhibited for four water samples, each from a different lake, so no information was provided about pike DNA occurrence. An additional 2% of Biomeme technical replicates had amplification curves indicative of partial inhibition (e.g., $C_q > 40$).

Discussion

Environmental DNA has become an important tool for managing invasive species, but slow sampling-to-results pipelines and specialized, expensive equipment limit its potency and widespread use. We found that the Biomeme approach shows promise in addressing these limitations, though it had lower detection probabilities and greater prevalence of inhibition than lab-based approaches. Importantly, the Biomeme approach also failed to detect contamination, which could result in increased potential for false positives and have serious management implications (Darling and Mahon 2011). The Biomeme required nearly twice the number of samples to achieve detection probabilities that were comparable to the lab approach, resulting in an additional hour of analysis time. While this additional time is not ideal, it is important to put into context of the much longer turnaround time (days – months) for lab-based results. As such, portable approaches such as the Biomeme deserve consideration as an addition to the early detection toolbox. However, the lower detection probabilities and inhibition issues we found for the pike assay suggest that the Biomeme approach is best used as a rapid screening tool for pike. Negative results and inhibited samples should be followed up with lab-based eDNA sampling and non-molecular approaches, such as gill netting.

Our findings highlight two areas for additional research to improve the application of portable eDNA platforms to a broad variety of water conditions typically encountered by invasive species managers. First, portable eDNA platforms must be robust to qPCR inhibition since the organic compounds that cause inhibition spatially and temporally vary. With lab approaches, inhibition can be circumvented via dilution, spin-column purification, or modifying the

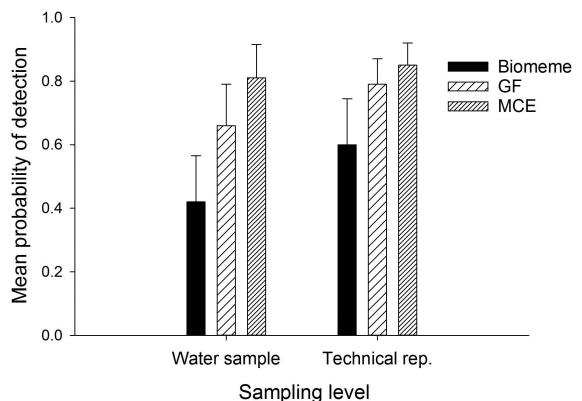


Figure 4. Mean (\pm 95% C.I.) derived estimates of the conditional probabilities of detection for a water sample (θ) and for a technical replicate (p) using the Biomeme approach and the lab-based approaches with either glass fiber (GF) filters or mixed cellulose ester (MCE filters).

qPCR protocol (McKee et al. 2015). With the Biomeme approach, the only field-tenable option is dilution but this requires specialized equipment (e.g., micropipettor) and diluting samples can reduce the sensitivity of the assay. Improving the DNA extraction kit to remove more inhibitors or using a qPCR recipe that is more robust to inhibition may expand the use of this platform to a broader array of waters.

Second, portable eDNA platforms must be capable of sampling large volumes of water. All other things being equal, filtration of larger water volumes increases detection probability of rare DNA (Turner et al. 2014). Filter material directly influences flow rate and GF filters have a faster flow rate than MCE filters, especially when processing challenging samples that result in clogging (Hinlo et al. 2017). In this study, we standardized filter samples to 500 ml since this was the water volume that could be consistently filtered by MCE filters without clogging, even though GF filters were able to filter up to three times this volume before clogging. For 500 ml samples, MCE filters had higher detection rates than GF filters, which is consistent with other eDNA studies comparing filter types (Hinlo et al. 2017) and further highlights that not all filter types produce the same results. Only MCE filters are compatible with the Biomeme extraction kit we assessed, so sampling large water volumes with the Biomeme approach requires increasing filter pore size, processing many small volume samples, or pooling DNA extract from multiple filters. Given that the performance of filter material and pore size can vary by site and target species (Goldberg et al. 2016), portable eDNA approaches that allow users to optimize filter type should result in improved efficiency in a broader array of waters.

The promise of portable, field-based eDNA platforms is attractive to invasive species programs. However, the tradeoffs we identified in this study indicate that new eDNA tools, like the Biomeme approach, must be thoroughly assessed before they are used as an alternative to lab-based eDNA platforms or non-molecular approaches. Since most invasive species programs have limited resources, we underscore the recommendation by Goldberg et al. (2016) to conduct pilot studies prior to investing in new eDNA tools since site characteristics strongly influence detection probabilities.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. Method details.

Appendix 2. Lake physical characteristics.

Table S1. Geospatial location, size, volume and maximum depth of waterbodies included in this study.

Table S2. Water quality near the deepest location of each lake as recorded by Alaska Department of Fish & Game in fall 2017.

Visibility was measured using a secchi disk. No data were recorded for Crane East and West and Warfle lakes.

This material is available as part of online article from:

http://www.reabic.net/journals/mbe/2018/Supplements/MBI_2018_Sepulveda_etal_Appendix_1.pdf

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