Comparison of eDNA and electrofishing survey methods for management purposes

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Abstract

The use of environmental DNA (eDNA) for detection of species is rapidly increasing. However, little research has been done examining the efficacy of eDNA surveys for use in management compared to traditional methods such as electrofishing. We utilized a previously developed eDNA survey for federally endangered Roanoke Logperch *Percina rex* to directly compare eDNA and backpack electrofishing surveys at 23 sites (8 presumed positive, 8 presumed negative, and 7 exploratory). Detection rates, person hours, and total cost were evaluated for each method. Electrofishing detected Roanoke Logperch at one site while eDNA detected Roanoke Logperch at nine sites. Additionally, eDNA survey methodology required approximately 57% fewer person hours and reduced cost by 31%, even when molecular troubleshooting was included in the calculations. With optimized protocols, eDNA surveys are expected to reduce person hours by 77% and total cost by 54% over traditional electrofishing while simultaneously increasing detection rates. While eDNA cannot currently address questions of age class structure, population estimates, or health, these rapid and cost-effective surveys can provide information that will help management agencies streamline and prioritize future intensive electrofishing surveys.

Introduction

Species surveys are a vital component of conservation and management efforts. Routine monitoring is critical to assess presence/absence in streams, population expansion or contraction, recruitment, health, and other demographic data. Electrofishing is one of the most widely used methods for such studies, but requires several hours per site with multiple personnel, resulting in a labor, time, and cost intensive effort. For low density populations, detection rates are low, despite the resources committed to the surveys. Environmental DNA (eDNA) is an increasingly accepted method for detecting species in both aquatic and terrestrial systems (Olson, Briggler, & Williams, 2012; Schill & Galbraith, 2019; Leempoel, Hebert, & Hadly, 2020). This method utilizes DNA amplification to detect shed cells from species of interest within an environmental sample. Samples may consist of water, soil, fecal material, and even air (Baker, Steel, Nieukirk, & Klinck, 2018; Schure et al., 2021; Serrao, Weckworth, McKelvey, Dysthe, & Schwartz, 2021). Environmental DNA assays can be used for a variety of purposes including detection of species of interest, diet studies, ecosystem assemblages, and assessment of ecosystem health (Staley et al., 2018; Ruppert, Kline, & Rahman, 2019). While this utility of eDNA in answering research questions is generally acknowledged, use of eDNA for management survey purposes requires extensive validation and has been less intensively studied. Management agencies generally seek answers to a particular species of interest in a specific region, and a separate eDNA assay must therefore be developed for each species and validated in each ecosystem. Before any assay can be adopted for routine monitoring, each assay must be rigorously field tested for reliability, repeatability, and robustness.

While several papers have demonstrated that eDNA detection rates outperform those for electrofishing (McColl-Gausden et al., 2021; Penaluna et al., 2021; Gouette et al., 2020), we have found only one paper presenting a cost comparison of the two methodologies. (Evans, Shirey, Wieringa, Mahon, & Lamberti, 2017). The authors concluded that while less sampling effort was required for brook trout eDNA analysis than for electrofishing, the overall cost for electrofishing to determine presence/absence of brook trout was 33% less expensive than eDNA processing. However, this study compared the two methods using a common species, and thus did not reflect the increased effort expected for electrofishing of low biomass species.

Roanoke Logperch *Percina rex* was listed as endangered by the United States Fish and Wildlife Service (USFWS) in 1989 (54 FR 34468). Roanoke Logperch is considered endemic to the Roanoke and Chowan River basins in North Carolina and Virginia (Wood and Nichols 2009). In Virginia, the species is located throughout segments of the Roanoke, Pigg, Smith, Chowan, and Nottoway rivers (Rosenberger 2007). In North Carolina, one large Roanoke Logperch metapopulation is found in the Dan, Smith, and Mayo rivers, including the tributaries of Big Beaver Island, Cascade, and Wolf Island creeks (Roberts and Strickland 2017). Jenkins and Burkhead (1994) hypothesized that all populations of Roanoke Logperch within the Roanoke drainage were historically well connected (Rosenberger 2007). However, recent years have seen a decline throughout the species' range, most likely due to population fragmentation from dams and other barriers in combination with a deterioration in water quality resulting from sedimentation and pollutants (Barbarossa et al. 2020, Malik et al. 2020, Verhelst et al. 2021). Critically endangered species such as Roanoke Logperch require routine monitoring to assess presence/absence in streams, population expansion or contraction, recruitment, and other demographic data necessary for effective conservation management. However, current survey methods using standard electrofishing techniques are often ineffective with a 30-40% detection rate (NCWRC, personal comm).

Strickland and Roberts (2019) recently developed an eDNA assay for Roanoke Logperch. They surveyed 12 historically occupied habitats in Virginia and detected Roanoke Logperch in 11 of those sites. No detections were made from 4 assumed unoccupied sites. The Strickland and Roberts study (2019) presents a strong case for the ability to use eDNA to detect presence/absence of Roanoke Logperch. However, additional studies would be beneficial before adoption of this method for routine monitoring. First, to assess fidelity of their primer set specifically for Roanoke Logperch, Strickland and Roberts tested their primers *in vitro* for most co-occurring darter species, the exceptions being Johnny darter *Etheostoma nigrum* and tessellated darter *Etheostoma olmstedi.* Vouchered specimens of these species are available at the North Carolina Museum of Natural Sciences and were tested in this study for cross-amplification. Our study examined the utility of this assay in all historically occupied North Carolina habitats, thus extending the assessment of this assay to encompass the full Roanoke Logperch distribution range. Finally, Strickland and Roberts (2019) used historical survey data to presume presence/absence in their study. We conducted side-by-side eDNA and electrofishing surveys in both historical and non-assessed habitats, thus allowing for direct comparison of positive detections, cost, and effort between eDNA and electrofishing monitoring. This study can therefore serve as a model to analyze the cost-benefit of eDNA surveys for low biomass species in comparison to electrofishing. This data will be crucial for management agencies as they consider the cost-benefit of traditional versus eDNA methodologies

Methods

Sampling – Twenty-three sites were chosen for survey based on historical data: 8 presumed positive sites, 8 presumed negative sites, and 7 exploratory sites that have not previously been surveyed but where suitable habitat occurs (Figure 1). Sampling occurred from May through September of 2023. Traditional surveys using electrofishing to collect Roanoke Logperch were conducted on the same days as eDNA collection for each site. Each site was surveyed using backpack-electrofishing by kicking the water vigorously into a 15 ft. seine. This process was repeated throughout riffle and run habitats at each site and total number of seine hauls was recorded. One liter water samples for eDNA analysis were taken in triplicate (one from the left bank, one from the center of the stream, and one from the right bank) at each site before electroshocking commenced. One milliliter of 10% Benzalkonium chloride (BAC) was added to water samples to preserve DNA (Yamanaka et al., 2017). Samples were kept on ice and frozen within 12 hours of collection. Flow rate, temperature, and turbidity measurements were taken at each site to allow for analysis of the effect of these environmental covariates on eDNA detection.

Molecular protocols – Tissue samples were extracted with Macherey-Nagel NucleoSpin Tissue Kit (Allentown, PA). Water samples were filtered using 0.45 µM polyethersulfone membranes and extracted using the Omega E.Z.N.A. Water DNA Kit (Norcross, GA) according to manufacturer's directions. DNA was eluted using 100 µL elution buffer. Quantitative PCR (qPCR) was conducted in triplicate for each sample using primer and probe sequences published by Strickland and Roberts (2019) and PrimeTime Gene Expression Master Mix (IDT, Coralville, IA). Reactions were carried out using 4 µL DNA and $MgCl_2$ at a final concentration of 1.2 mM. Any sites that did not have positive detections after the initial round of PCR were repeated using 4 µL DNA that had been doubled in concentration using a vacufuge. Detection rates are calculated for positive sites as the number of positive PCR amplifications divided by the number of PCR replicates per site. Detection rates were also analyzed by pooling 10 µL of extract from each of the three samples collected per site and conducting qPCR using the pooled sample. Each plate included two negatives and a series of 6 serial 1:10 dilutions starting with 1 ng/µL of genomic

Roanoke Logperch DNA used to quantify the amount of starting material (Roanoke Logperch DNA) in each sample.

Environmental covariates – The effect of temperature (degrees Celsius, °C), turbidity (Nephelometric Turbidity Units; NTU), and flow rate (cubic feet per second, cf/s) on eDNA detection was evaluated using the EDNAOCCUPANCY (Dorazio & Erickson, 2018) package in R. This program estimates posterior summaries of occupancy and detection using Bayesian models. Model fitting was tested running 20,000 iterations using Posterior Predictive Loss Criterion (PPLC) and Widely Applicable Informative Criterion (WAIC). The best fit model was run with 40,000 iterations to examine the effects of covariates on probability of detection within a site (Ψ), within a sample (θ), and within a PCR replicate (p).

Cost Analysis – All person hours used for completion of this project were tracked and included permanent staff, temporary staff, and volunteers. In order to calculate person hours under the assumption that only eDNA sampling would have occurred, a total of 60 hours was added to lab time to account for 3 collection days at 10 hours using 2 people. Cost was determined using applicable North Carolina Wildlife Resources Commission (NCWRC) agency hourly rates.

Results

Cross-amplification and Limits of Detection– qPCR using 1:10 serial dilutions of Roanoke Logperch, Johnny Darter, Tesselated Darter, Roanoke Darter consistently and exclusively amplified Roanoke Logperch DNA. No amplification was seen in the three co-occurring species tested, even at volumes as high as 1 ng/µL. The assay routinely detected Roanoke Logperch DNA at concentrations at or above $1x10^{-3}$ ng/µL, with inconsistent amplification at $1x10^{-4}$ ng/µL and $1x10^{-5}$ ng/µL. Quantification indicated that starting DNA in our samples ranged from $4.56x10^{-5}$ ng/µL to $1.92x10^{-4}$ ng/µL and detection rates per site ranged from 0.11 to 0.33. Amplification of non-pooled samples in triplicate resulted in the identification of 9 positive detections sites, while amplification of pooled samples resulted in seven positive identification sites. Of the positive PCR amplifications, 5 came from samples collected from the left bank, 8 from the right bank, and 2 from mid-stream.

Survey Results – Electrofishing surveys resulted in one positive detection of Roanoke Logperch. This detection occurred at a presumed positive site on the Mayo River downstream of Washington Mill Dam. Positive eDNA detections occurred at 9 sites: 6 presumed positive, 2 exploratory, and one presumed negative (Figure 1, Table 1).

Environmental Covariates – The best fit model was one that indicated effects of flow, temperature, and turbidity on the probability of detection in PCR replicates (Table 2). Bayesian estimates with this model indicated that increasing turbidity and flow had positive effects on eDNA detection while increasing temperature had a negative effect on eDNA detection (Table 3). However, plots of the effect of turbidity and flow on detection probability did not demonstrate a clear positive relationship (Figure 2 and Figure 3). Plots of the effect of temperature on eDNA detection did suggest an inverse relationship. However, confidence intervals were wide (Figure 4). The second best fit model included flow as an indicator of probability of eDNA occurrence at a site, and Bayesian estimates for that model were similar to those obtained from Model 1 (Table 3).

Cost Analysis - A total of 569.5 person hours were used to conduct electrofishing surveys for the 23 sites in this study at a total cost of $14,302. In comparison, a total of 245.5 hours and $9,972 were used to conduct eDNA surveys. Time for eDNA included sampling hours, lab processing, and analysis.(Table 4).

Discussion

The Roanoke Logperch eDNA assay developed by Strickland and Roberts has now been evaluated throughout the known range of occupation. As with the previous paper, we found good detection rates, with 6 of 8 presumed historically occupied sites positive for eDNA detection. Two presumed positive sites, Wolf Island and Dan River at Lindsay Bridge, did not have positive detections with either electrofishing or eDNA. Wolf Island has not had a positive electrofishing detection since 2010 and may not harbor a resident population. The Lindsay Bridge Dam was removed in 2020 at the second site in question and replaced with a weir. This habitat alteration makes it unlikely that fish will congregate below the weir, but rather move upstream or downstream of this altered site to better suited habitat. That theory is supported by multiple positive eDNA detections both upstream and downstream of this site, including the two exploratory sites located just upstream of the dam removal, indicating that fish have migrated into newly accessible habitat. We also had one detection in a presumed negative site. This site was the first location sampled for the study, so positive detection via contaminated equipment is unlikely. The location is approximately 34 miles upstream of the removed Lindsey Bridge Dam, and fish had ample opportunity to traverse this distance in the three years between dam removal and our survey efforts, leading us to conclude that this detection is a true positive.

Quantification and detection rates indicated that the starting material in our samples exists at the limit of detection for this assay. Therefore, PCR amplification will most likely be somewhat stochastic, leading to the need for multiple samples and multiple PCR reactions to improve chances of detection. Many labs follow similar methods as the one outlined in this study, with 3 samples taken per site and each sample amplified in triplicate to increase chances of positive detection (Dorazio & Erickson, 2018). The use of this method is supported by the fact that positive qPCR amplifications decreased when samples were pooled, thus decreasing the chances for detection. Furthermore, we found that chances of detection were higher when samples were collected near the banks as opposed to mid-stream. This effect could be caused by decreased flow along the banks as well as detritus collection that tends to occur on stream edges. Such detritus would include cellular debris, thereby increasing the amount of DNA available for detection. Therefore, we recommend that future studies for this system seek to improve detection rates by pulling samples only from stream sides, with 2 collected on the left bank and 2 collected from the right bank. The increase of samples per site from 3 to 4 will also improve chances for positive detection of eDNA.

Temperature, flow, and turbidity were tested for effects on eDNA detection probability. Positive detections occurred through the range of values for all covariates, and modeling with EDNAOCCUPANCY indicated that neither flow nor turbidity affected probability of detection with this assay. Modeling did indicate a possible positive relationship between temperature and probability of detection. However, confidence intervals were wide, indicating a need for further data points to elucidate the effect of temperature on the probability of Roanoke Logperch eDNA detection.

We used this assay as a model system for performing a cost-benefit analysis for electrofishing versus eDNA surveys for a low biomass fish. Positive detections using eDNA greatly outperformed electrofishing detections. Additionally, eDNA analysis took 57% less time at a cost savings of 31% (Table 1). Both survey methods could be optimized for future surveys. For future electrofishing surveys, we anticipate being able to reduce person hours in the field by

optimizing the number of staff used to 4 for small reaches and 6 for large reaches. This would result in a reduction of person hours needed to 532.5 and a total cost of $12,738. The eDNA hours used in this project included time for cross-amplification studies and qPCR condition optimizations. Now that the assay has been optimized, we anticipate that the same survey could be repeated in future with 120 person hours at a cost of $5,816, resulting in a time savings of 77% and a cost savings of 54%.

Cost incursions will be agency specific, and the numbers presented here reflect surveys conducted in-house for the NCWRC. It should also be noted that the per sample cost for both electrofishing surveys and eDNA surveys will decrease the more sites that are included. For example, optimized cost to repeat this study of 23 sites would be $554 for electrofishing and $252 for eDNA per site. However, calculations using the same hourly rates to conduct an electrofishing survey for one site would be $1,500 and $800 for an eDNA survey, which assumes reagents have already been purchased. This increase comes due to the inability to cost share between sites for everything from fuel to lab reagents.

The use of eDNA in comparison to electrofishing for management surveys does result in the loss of information such as age class structure and specimen health. However, we feel that the increased detection rates coupled with significant time and cost savings make this method a valuable tool for management agencies. We suggest that eDNA surveys can be used to quickly survey species' ranges for presence/absence in a time and cost-effective manner. Management agencies can then use this information to focus more intensive electrofishing efforts. For example, the eDNA detections obtained in this study indicate three new potential occupancy sites that could be selected for further electroshocking efforts to confirm presence and examine questions of recruitment, age class structure, relative abundance, and health in these new habitats.

Environmental DNA can serve as a valuable tool for low biomass species' surveys. In addition to increased detection power and cost savings, eDNA does not require direct handling of animals, eliminating stress to imperiled organisms. Additionally, eDNA can be used in sites that are difficult to reach by either backpack or boat electrofishing, but where water samples may still be taken. The detection capability of eDNA surveys can also allow biologists to readily identify new populations or range expansions more readily. Coupling eDNA methods with more traditional survey methods such as electrofishing will allow managers to increase their capacity in performing critical surveys for listed species or species under review and provide critical information for conservation decisions.

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Table 1. Detection comparison for electrofishing versus eDNA surveys.

Collection site	***Historical Occupancy***	**Electroshocking Detection?**	**eDNA Detection?**
Big Beaver Island Creek	Yes	No	Yes
Mayo River at Hwy 135	Yes	Yes	Yes
Dan River at Lindsay Bridge	Yes	No	No
Smith River at River Dr Access	Yes	No	Yes
Mayo River at North Water St	Yes	No	Yes
Cascade Creek at Highway 311	Yes	No	Yes
Wolf Island Creek	Yes	No	No
Smith River at Kings Hwy	Yes	No	Yes
Big Creek at Hwy 268 and Hwy 89	No	No	Yes
Snow Creek at Sheppard Mill Rd	No	No	No
Dan River at Collinstown Rd. Bridge	No	No	No
Dan River at Flippin Road	No	No	No
Dan River at Seven Island Rd.	No	No	No
Mayo River at Anglin Mill Rd	No	No	No
Belews Creek, Goodwill Church Rd	No	No	No
Mayo River at Avalon Dam	No	No	No
Town Fork Creek at Hwy 311?	??	No	No
Jacobs Creek at Hwy 704	??	No	No
Buffalo Creek at Hwy 311	??	No	No
Matrimony Creek at Hwy 770	??	No	No
Dan River at Bent Plantation Rd	??	No	Yes
Dan River at Hwy 311	??	No	Yes
Dan River at Settle Bridge	??	No	No

Table 2. Models were tested to evaluate the best fit for environmental covariates of temperature (temp), tubidity (turb), and flow on the probability of eDNA occruence at a site (Ψ), within a sample occupancy(θ), or within a PCR replicate (p) using posterior predictive loss criterion (PPLC) and widely accepted informative criterion (WAIC). The best fit model was one in which temperature, flow, and turbidity all affect the probability of detection within a PCR replicate, but do not affect probability of eDNA occurrence at a site or within a sample.

Model	**PPLC**	**WAIC**
Ψ(-), θ (-), p (-)	21.74	23.59
Ψ(temp), θ (-), p (-)	21.71	23.84
Ψ(turb), θ (-), p (-)	21.57	23.58
Ψ(flow), θ (-), p (-)	21.65	23.63
Ψ(), θ (temp), p (-)	22.83	25.29
Ψ(), θ (turb), p (-)	22.83	25.21
Ψ(), θ (flow), p (-)	22.28	24.59
Ψ(), θ (-), p (temp)	17.57	21.07
Ψ(), θ (-), p (turb)	21.45	23.95
Ψ(), θ (-), p (flow)	21.44	24
Ψ(), θ (-), p (temp, turb)	17.524	21.21
Ψ(), θ (-), p (temp, turb, flow)	16.69	20.63
Ψ(turb), θ (-), p (temp, turb, flow)	16.74	20.83
Ψ(flow), θ (-), p (temp, turb, flow)	16.7	20.8
Ψ(turb, flow), θ (-), p (temp, turb, flow)	16.79	20.8
Ψ(-), θ (flow), p (temp, turb, flow)	17.48	21.8

Table 3. Bayesian estimates of model parameters for 1) Ψ(), θ (-), p (temp, turb, flow) and 2) Ψ(flow), θ (-), p (temp, turb, flow). Negative values indicate inverse relationships with detection probablity and positive values indicate positive relationships with detection probability.

Model 1	Mean	50%	2.50%	97.50%
beta.(Intercept)	0.559	0.467	-0.457	2.078
alpha.(Intercept)	-0.491	-0.535	-1.147	0.395
delta.(Intercept)	-0.174	-0.175	-0.82	0.468
delta.temp	-0.753	-0.731	-1.667	0.039
delta.turb	0.31	0.318	-0.449	1.03
delta.flow	0.255	0.259	-0.37	0.854

Model 2	Mean	50%	2.50%	97.50%
beta.(Intercept)	0.714	0.63	-0.404	2.227
beta.flow	0.224	0.214	-0.896	1.403
alpha.(Intercept)	-0.53	-0.571	-1.152	0.336
delta.(Intercept)	-0.185	-0.183	-0.849	0.462
delta.temp	-0.719	-0.696	-1.664	0.071
delta.turb	0.311	0.319	-0.427	1.038
delta.flow	0.224	0.23	-0.419	0.831

Table 4. Cost comparison for electrofishing versus eDNA surveys.

	Electrofishing	**eDNA**	**% Difference**
Actual Person Hours	569.5	245.5	57%
Actual Cost	$14302	$9872	31%
Optimized Person Hours	532.5	120	77%
Optimized Cost	$12738	$5816	54%

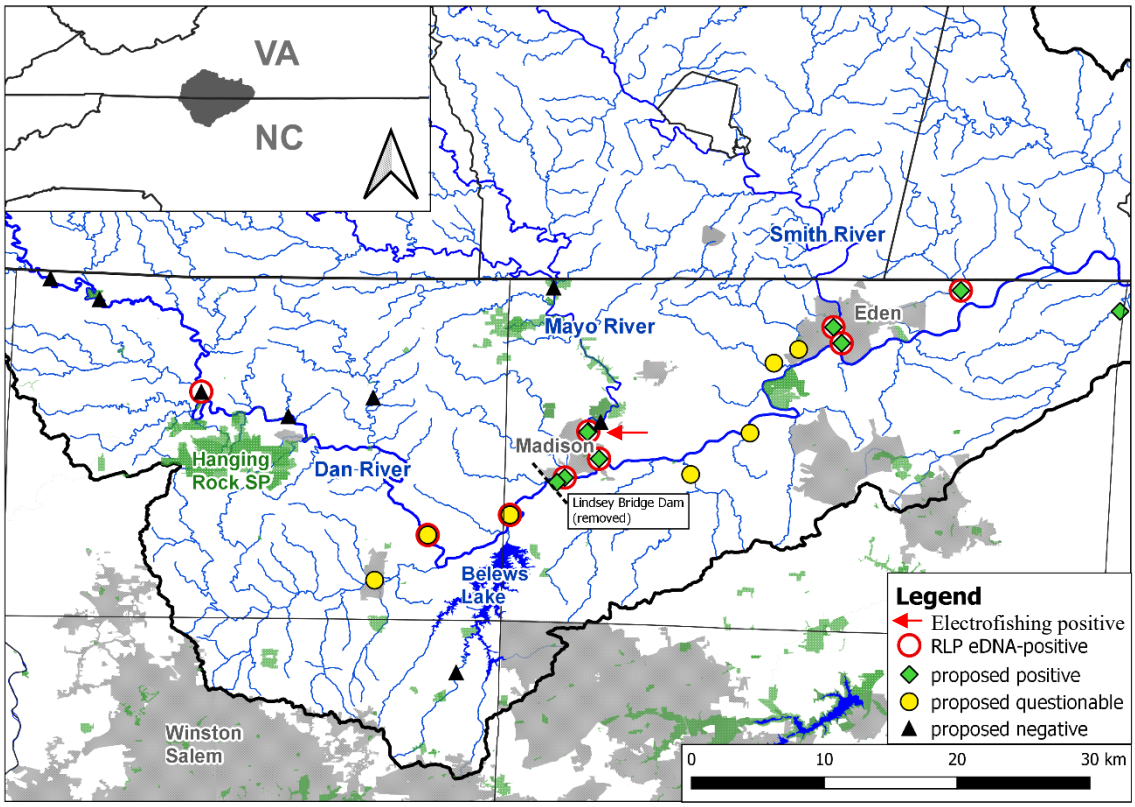

Figure 1. Roanoke Logperch survey results. 23 sites were chosen for survey based on historical occupancy data. One positive site was identified via electrofishing while 9 positive sites were identified via eDNA.

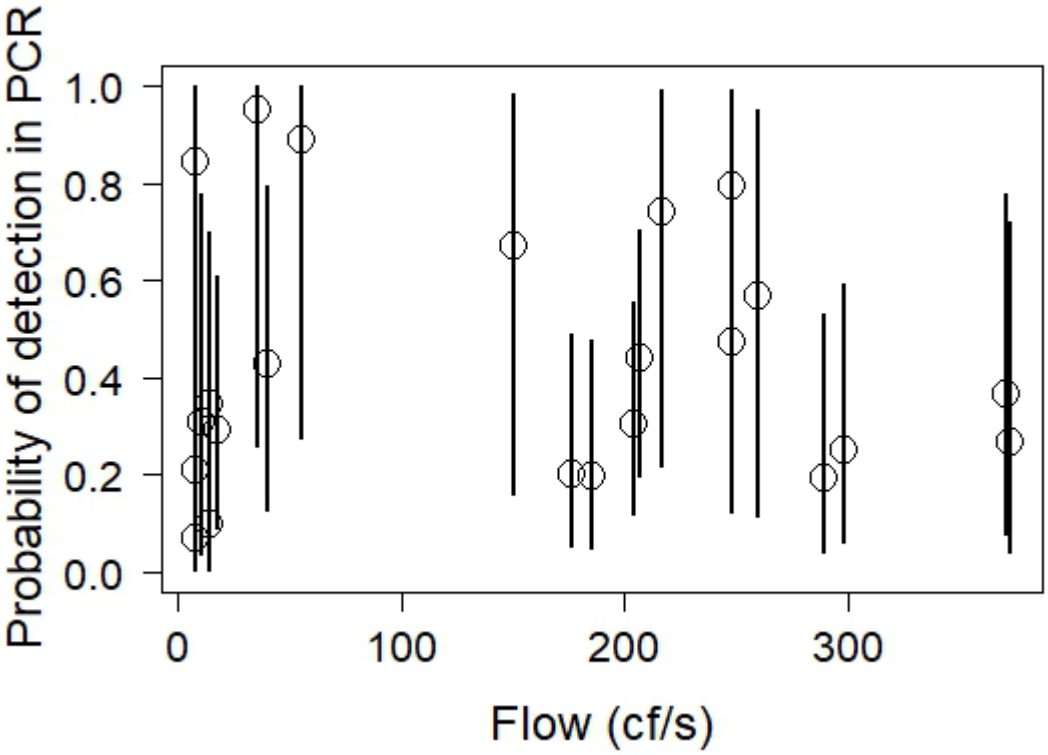

Figure 2. No effect of flow rate on probability of occurrence for Roanoke Logperch eDNA was detected.

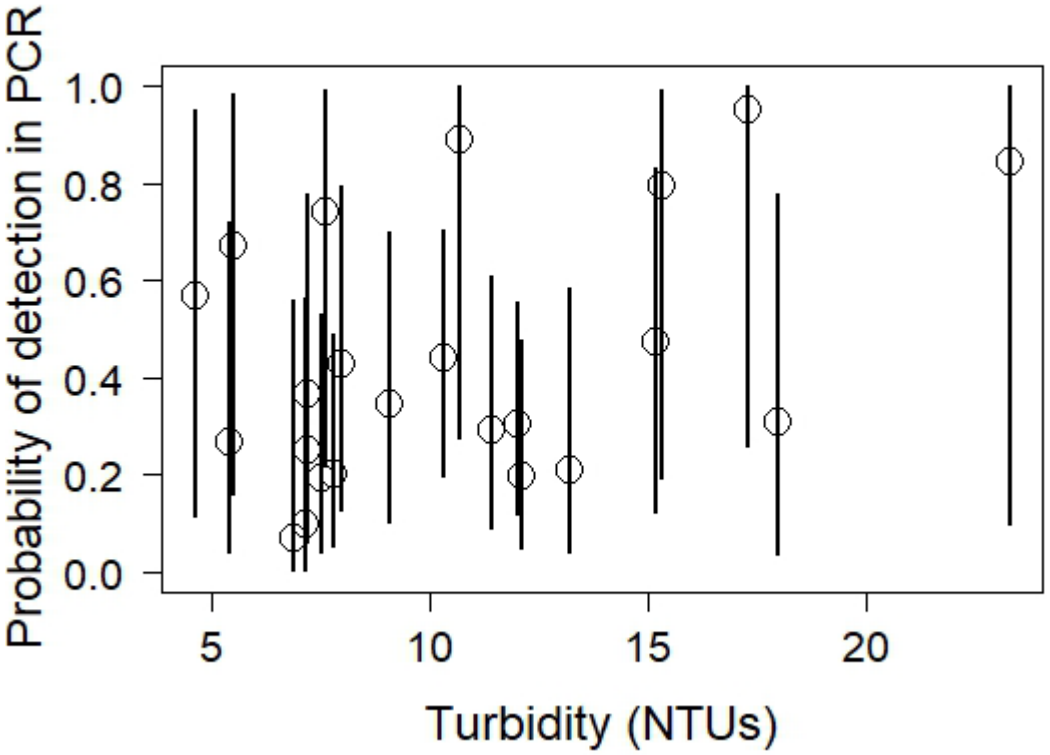

Figure 3. No effect of turbidity on probability of occurrence for Roanoke Logperch eDNA was detected.

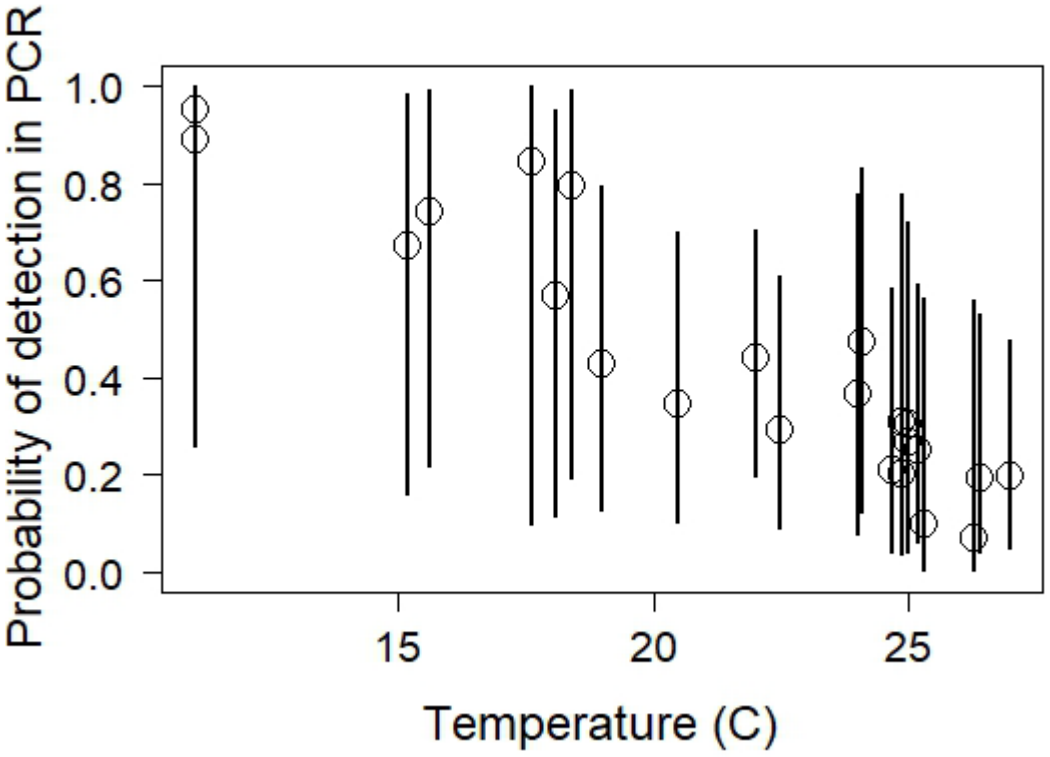

Figure 4. An inverse relationship between probability of eDNA detection and temperature is suggested, indicating that increasing temperatures may decrease chances for eDNA detection. However, confidence intervals are wide, demonstrating the need for further data points and exploration of this environmental covariate.