Environmental DNA detection range for *Hydrilla verticillata*, a prolific invasive plant

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Abstract: Environmental DNA (eDNA) is a powerful conservation tool that has made significant advancements in the past decade. Since its initial application in 2008 to detect invasive tadpoles, eDNA has become popular for finding rare and invasive species that are otherwise difficult to detect through traditional field sampling methods. This study sought to determine a detectable range of eDNA for an aggressively invasive aquatic plant species, *Hydrilla verticillata* (L. f.) Royle, as well as to understand how stream discharge affects eDNA detection distance. To do so, we surveyed downstream of a large reservoir, which is separated from its distributary by a dam. *Hydrilla verticillata* is present in the reservoir but absent in the distributary. We used correlation analysis to understand the relationship between transportation distance and detection. We also built upon a model developed by Pont et al. (2018) to assess the relationships between stream discharge and eDNA detection distance, as well as to compare this relationship for our plant-based eDNA study with previous animal-based studies. By utilizing this natural system and surveying at different points downstream of the known population, this study detected *H. verticillata* eDNA nearly 5 km from the source population. This study, which is the 1st known instance of correlating plant eDNA to animal eDNA detection ranges, suggests the genetic material of both kingdoms are similarly detectable in flowing freshwaters and supports other studies that suggest discharge is a strong predictor of the detectable distance of eDNA.
Key words: environmental DNA, eDNA, *Hydrilla verticillata*, invasive species, transport distance, early detection rapid response, EDRR, stream discharge

The use of environmental DNA (eDNA) for detecting invasive species has become increasingly widespread among conservation biologists and resource managers in recent years. By analyzing environmental samples for traces of genetic material, practitioners can supplement or replace traditional field survey techniques. This technique is becoming increasingly relevant to the management of invasive species, which are often difficult to detect until local populations become well established. eDNA allows for the early detection of just a few individuals and can potentially be used to prevent the establishment and spread of invasive species (Uchii et al. 2016). Although molecular tools to identify species have been available for decades (Kang'ethe et al. 1982), Ficetola et al. (2008) 1st coined the term eDNA and used the technique in a conservation context when they detected the DNA of the invasive American Bullfrog *Lithobates catesbeianus* tadpole in France. Although most efforts have focused on the detection of animal species (Ficetola et al. 2008, Wilcox et al. 2016, Pont et al. 2018) or microbes (e.g., Tessler et al. 2017), representation of the plant kingdom has increased in the last few years with studies detecting *Elodea canadensis* (Gantz et al. 2018, Anglès D'auriac et al. 2019), *Elodea nuttallii* (Gantz et al. 2018), *Myriophyllum spicatum* (Newton et al. 2016, Kuehne et al. 2020), *Egeria densa* (Fujiwara et al. 2016, Chase et al. 2020, Miyazono et al. 2020), and *Hydrilla verticillata* (Matsuhashi et al. 2016, 2019, Gantz et al. 2018). Some work has been done to estimate the abundance of target organisms (Carraro et al. 2017, 2018, Sansom and Sassoubre 2017, Miyazono et al. 2020, Yates et al. 2021), which is important for the control of invasive species.

In recent years, efforts have been made to identify the detectable distance of eDNA, as well as the factors that

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influence that range. Most eDNA studies focused on the detection range of species have used quantitative polymerase chain reaction (qPCR) to detect a single species of interest. However, these studies have almost exclusively been performed on animal species. The earliest published attempt was by Deiner and Altermatt (2014), who determined the transport distance of eDNA of 2 invertebrate species (*Daphnia longispina* and *Unio tumidus*) in a natural river. Other studies quantified the detectable distance of Brook Trout (*Salvelinus fontinalis*) eDNA by placing a cage containing the subject species in a river and sampling at regular intervals downstream of the source (Jane et al. 2015, Wilcox et al. 2016). Civade et al. (2016) used metabarcoding to survey fish biodiversity in lentic and lotic environments and estimated the detection distance as it was related to biotic and abiotic factors. Pont et al. (2018) tried to further understand the factors affecting eDNA detection distance by conducting a metabarcoding analysis and modeling the impacts of the study river's discharge and wetted width on the transport distance of target eDNA, which has consistently been shown to increase with higher velocities (Jo and Yamanaka 2022). They supplemented this model with results from other similar studies, including the results from Jane et al. (2015) and Wilcox et al. (2016), and found detectable distances of eDNA ranging from <100 m in small streams (Wilcox et al. 2016) to >100 km in a large river (Pont et al. 2018). Pont et al. (2018) also produced a model to predict eDNA transport distances in lotic systems based on fine particulate organic matter (FPOM) transport literature. They found that eDNA acts like FPOM in rivers and used deposition velocity and previously published eDNA transport results to simulate detectable distances of eDNA (Pont et al. 2018).

The focus of the present study is *Hydrilla verticillata* (L. f.) Royle, a particularly aggressive invasive aquatic plant, native to southern Asia and Australia (Cook and Lüönd 1982). Many risk assessments of aquatic plants have identified *H. verticillata* as one of the most detrimental aquatic plants in the northeastern United States and have shown that it is likely to become established if introduced (Gordon et al. 2012, Barnes et al. 2014a, Gantz et al. 2015). It has been described as "the perfect aquatic weed" (Langeland 1996) because of its adaptability, which allows it to thrive in a wide range of environmental conditions, alter the ecosystems it colonizes, and displace native species (Posey et al. 1993). It grows preferentially in shallow waters, but it can tolerate low-light conditions (White et al. 1996) and has been found growing at 15-m depths (Yeo et al. 1984). This depth makes the species potentially difficult to detect with traditional sampling methods. *Hydrilla verticillata* is also particularly difficult to manage because it produces overwintering structures, called turions and tubers, that enable the plant to survive harsh conditions. These structures and *H. verticillata*'s ability to produce new plants from loose fragments (Cook and Lüönd 1982, Langeland 1996) enable it to survive short-term control treatments. Because of its ability to thrive in a wide variety of environmental conditions, its range in New York State, USA, is expanding. It is believed that early detection of *H. verticillata* is key to managing the species, making it a prime candidate for eDNA research in this region where it is a relative newcomer but likely to become established (NISC 2022). By focusing on the early detection of invasive species, resource managers can focus on the prevention of species introduction and extirpation of small populations, rather than the sustained and costly battle of long-term management.

Although *H. verticillata* is one of the better-studied plant subjects in eDNA research (Matsuhashi et al. 2016, 2019, Gantz et al. 2018), there are many unanswered questions related to its detection. Of the published studies that address eDNA detection range in lotic systems, the only plant studied was *E. canadensis* (Anglès D'auriac et al. 2019), a United States native relative of *H. verticillata* that is an invasive species in Norway (the study location). All other lotic range studies have been focused on animal eDNA, and no studies have tried to relate plant and animal eDNA. Our primary objective was to identify the eDNA detection range of *H. verticillata* at a species-specific level. As suggested by Pont et al. (2018), we expected to find a relationship between stream discharge and *H. verticillata* eDNA transport distance.

METHODS

To address our research objectives, we conducted an observational field study in which we sampled a natural system for eDNA at 15 sampling sites and analyzed samples by qPCR to identify eDNA from *H. verticillata.* We then assessed how our findings complemented previous findings by Pont et al. (2018) of animal eDNA detection range and its relationship with stream discharge by adding the study river's discharge and the maximum observed distance of detection from this study to their table of results (Table S1), and we used linear regression to relate our results to their findings.

Study site

The New Croton Reservoir is an 883-ha reservoir separated from its 5-km long distributary, the Croton River, by a dam. The reservoir is of particular importance because it supplies drinking water to New York City, New York, USA, and because it connects to the Hudson River. The New Croton Reservoir and the Croton River are managed under the Croton Hydrilla Control Project, a 5-y management plan implemented by the New York State Department of Environmental Conservation (NYSDEC 2018). Under this plan, the river was treated with herbicides, which were added as a steady drip that only stopped when the flow exceeded 500 cfs. The river was also frequently surveyed for *H. verticillata* throughout the system and any stray plants were manually

pulled. This plan was underway during the time of this study, including a survey 2 d after eDNA sampling occurred. This documentation makes this system of particular interest to this study because it establishes *H. verticillata*'s presence in the reservoir and absence in the distributary. The study site includes a lentic source with large quantities of actively growing *H. verticillata* and is dammed off from the lotic distributary, which is free from the invasive plant. This site is, therefore, an excellent candidate for eDNA research because it allows for a known source of *H. verticillata* to be tracked downstream to determine its eDNA detection range.

Sample collection

We collected eDNA samples downstream of the dam on October 27, 2020. Because of the nature of the river's topography and flow, it was not safe or possible to collect samples at regular intervals, especially between 363 and 1132 m downstream of the dam. We collected most samples (mainly from public access points) as close to every 500 m as possible, except for 3 samples that were collected in the first 500 m (Fig. 1). The base of the dam was considered the 0-m mark. We sanitized all equipment with a 10% solution of bleach and water and rinsed before use. We collected one 1-L water sample at each site in PET bottles. Samples were collected just below the water's surface from the riverbank, except for samples collected at 3973, 4348, and 4904 m, which were collected from a kayak. We collected 1 cooler blank as a negative control by filling an empty sample bottle with bottled spring water and then storing the bottle in the cooler on ice with the rest of the sample bottles until filtration (De Souza et al. 2016). We retrieved a single datapoint for stream discharge for the Croton River from United States Geologic Survey gauge 01375000 located ~350 m below the dam separating the reservoir from the river.

Sample preparation

Prior to DNA extraction, we filtered all water samples, including the negative controls. We filtered samples with 47-mm, 0.45-μL gridded nitrocellulose-membrane filters (catalog no. 145 2045, Nalgene®, Rochester, New York), Masterflex L/S 96410-24 hose (Masterflex Group, Gelsenkirchen, Germany), and battery-powered Alexis Peristaltic Pumps (Pegasus™ Pump Company, Dallas, Texas) to a target volume of 1 L or for 30 min, whichever came first. Samples were filtered within 24 h of collection to minimize degradation of eDNA (Hinlo et al. 2017). We then immediately transferred each nitrocellulose-membrane filter into a microcentrifuge tube (T6649, Sigma-Aldrich, Germany) and transferred the storage tubes into a −20°C freezer until DNA extraction.

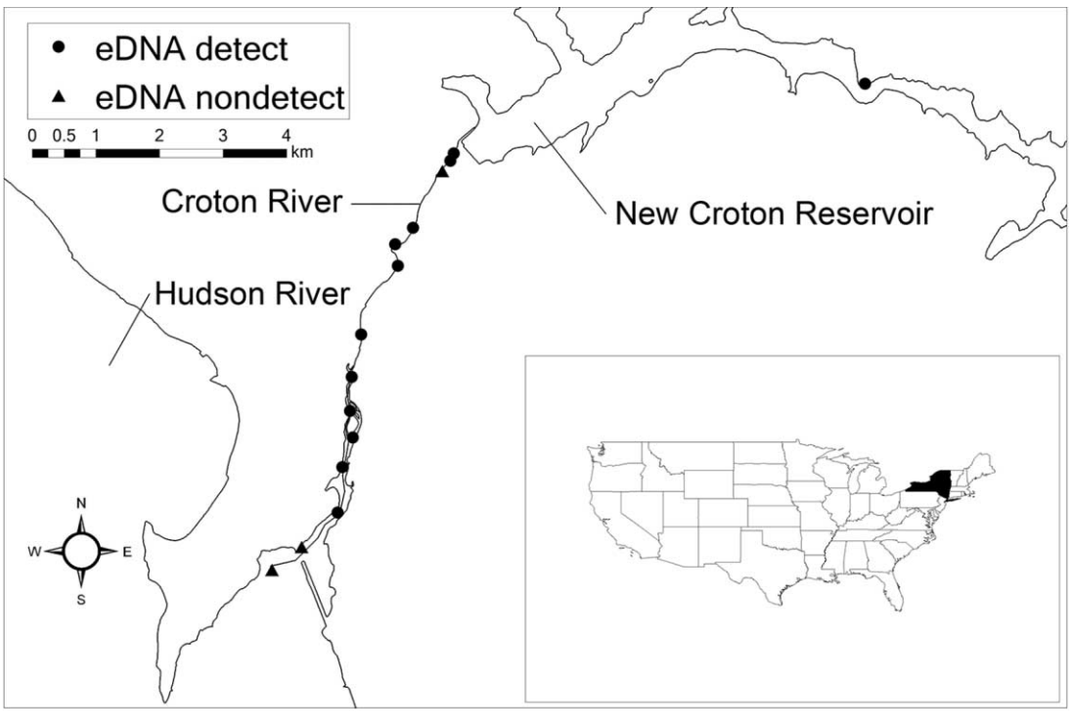

Figure 1. Samples were collected on 27 October 2020 in the Croton River, downstream from the New Croton Reservoir, New York, USA, and later analyzed for the presence of *Hydrilla verticillata* environmental DNA (eDNA). Circles indicate detection of *H. verticillata* eDNA (including the positive field detection at the northern end of the reservoir) and triangles indicate a lack of quantitative polymerase chain reaction amplification at that site. See Table S3 for each sampling site's distance from the dammed reservoir and the number of replicate eDNA detections at each site.

Prior to analyzing field samples, we prepared positive control samples in the laboratory to ensure the efficacy of the primers and the process. We blended sprigs of *H. verticillata* and spring water and filtered out the largest tissue fragments with cheesecloth to prevent the filters from clogging. We then filtered 3 samples (1-L each) of the mixture by using the same methods as described above for sample filtration.

Sample analysis

All DNA extraction and qPCR protocols were performed by the Center for Functional Genomics High Throughput Genomics Facility—University at Albany in Rensselaer, New York. The following protocols were adapted from the work by Matsuhashi et al. (2016) with *H. verticillata*, including the primer set they developed. As in that study, the DNA extraction used here was modeled after the work of Uchii et al. (2016), including the use of the DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany), which has since been found to be the most cost-effective and efficient commercial option for DNA recovery (Hinlo et al. 2017).

The first step to analyzing the samples was for the lab to extract the eDNA from the filters. To extract the eDNA, filters were soaked with 400 μL of Buffer AL (Qiagen) and 40 μL of Proteinase K (Invitrogen™, Waltham, Massachusetts) in an internal container of a standard lab tube and incubated at 56°C for 30 min (Matsuhashi et al. 2016, Uchii et al. 2016). Samples were centrifuged at 5000 *g* for 5 min and then 220 μL of TE Buffer (pH 8.0; Invitrogen) was added to each tube, which were then centrifuged again at 5000 *g* for 5 min. Next, 200 μL of Buffer AL and 600 μL of 100% ethanol were added to each filtrate and mixed by pipetting (Matsuhashi et al. 2016, Uchii et al. 2016). This mixture was then added to a DNeasy Mini spin column (Qiagen) and centrifuged at 6000 *g* for 1 min, which was repeated until the mixture was completely processed. We eluted DNA from the column with 100 μL of Buffer AE (Matsuhashi et al. 2016, Uchii et al. 2016).

Next, the samples were purified prior to qPCR. The OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Irvine, California) was used to remove any inhibitors preventing amplification during PCR. First, a Zymo-Spin™ III-HRC Column (Zymo Research, Irvine, California) was added to a collection tube. Then 600 μL of Zymo Prep-Solution was added to the column, which was centrifuged at 8000 *g* for 3 min. The prepared column was transferred to a clean microcentrifuge tube, and 100 μL of purified DNA solution in AE buffer was added to the column and centrifuged at 16,000 *g* for 3 min. Extracted DNA was then stored in a −20°C freezer for 2 wk before qPCR was performed on these samples.

Next, qPCR was performed with a QuantStudio™ 12K Flex real-time PCR machine (Applied Biosystems™, Waltham, Massachusetts). The primer and probe set used the following *matK* sequences to detect *H. verticillata*: forward primer, 5'-TTTGCGCGAATATGTAGAACTTGT-3'; reverse primer, 5'-GCCAAGGTTTTAGCACAGGAAA-3'; and TaqMan™ MGB probe (Thermo Fisher Scientific, Waltham, Massachusetts), 5'-FAMATTATTGTAGTGGATCTTCA-NFQ-MGB-3' and was developed by Matsuhashi et al. (2016). Each TaqMan reaction contained 900 nM of each primer, 125 nM of the TaqMan probe, 10 μL of TaqMan Environmental Master Mix 2.0, and 3 μL or 25 ng of the DNA solution (see below), bringing the final volume to 20 μL (Matsuhashi et al. 2016). The PCR conditions were 10 min at 95°C, 55 cycles of 15 s at 95°C, and 1 min at 58°C.

We used 2 approaches for standardizing the amount of DNA in solution: a standard volume approach, as was used in Matsuhashi et al. (2016), and a standard concentration approach to account for variability in DNA concentration among the samples. Each TaqMan reaction was prepared as above with 3 μL of DNA solution (standard volume) at first, and replicates were performed in triplicate. Each TaqMan reaction was then prepared as above but with 25 ng of total DNA (standard concentration) and with all reactions in triplicate. To obtain this standard concentration, a NanoDrop™ ND-1000 spectrophotometer (Thermo Fisher Scientific) was used to quantify the amount of DNA in each sample. Samples were each standardized to 25 ng of total DNA to standardize the amount of input DNA. The concentration was standardized to account for variations in concentrations of organic materials found throughout the system (Table S2). The 25-ng concentration was chosen because it approximated the 3-μL concentration from Matsuhashi et al. (2016) for most samples.

The concentration of *H. verticillata* DNA was then based on Matsuhashi et al. (2016), who determined the PCR amplicon to have 373 base pairs, and Puri et al. (2007), who found that 1 pg of *H. verticillata* DNA is 965 million base pairs, and, therefore, 1 ng of total DNA contains ~1000 copies of DNA (as calculated by NEBioCalculator®, https://nebiocalculator.neb.com). To test the efficiency of the qPCR assays, the Qubit® 2.0 (REF# Q32866, Thermo Fisher Scientific) fluorometry concentration of a positive lab control sample was measured at 1.526 ng/μL and was used to prepare a standard curve of eight 10× serial dilutions per Klymus et al. (2019). Serial dilutions with a final concentration from 175 pg/μL to 175 ag/μL were prepared from the 1.526 ng/μL positive control stock and assayed by TaqMan qPCR. We used an R script developed by Klymus et al. (2019) to calculate the limit of detection (LOD).

Relationships between qPCR amplifications and environmental variables

To determine the detection distance of *H. verticillata* eDNA, we assessed presence or absence of qPCR amplification in replicates. To understand the relationship between detection and distance from the source, we used correlation

analysis. Our data were not normally distributed, and we did not assume a linear relationship between detection and distance; therefore, we used Spearman's rank-order correlation to assess the relationship between the percentage of qPCR replicates amplified and distance. We completed this analysis with the *ggpubr* (version 0.2.5; Kassambara 2020), and *ggplot2* (version 3.4.2; Wickham et al. 2023) packages in R (version 1.2.1335; R Project for Statistical Computing, Vienna, Austria).

Finally, we compared our results to a model produced by Pont et al. (2018), which predicted detection distances of eDNA based on discharge of the lotic system in which the data were collected. The data used in this model can be found in Table S1. We confirmed that the residuals of the model met the assumptions for linear regression, so we used the *ggpubr* and *ggplot2* packages in R to fit a simple linear regression model that incorporated our actual results to the discharge (predictor variable) and predicted distances (response variable) from the model produced by Pont et al. (2018).

RESULTS

Performance of qPCR assay

We established the validity of our results through a few key metrics. Matsuhashi et al. (2016) reported that the standard curve (slope $= -3.37$, y-intercept $= 41.003$) had $R^2 = 0.992$ and PCR efficiency of 99.4%, confirmed that the primers and probe set were specific to *H. verticillata* DNA, and confirmed the amplicon by direct sequencing. The authors did not report their LOD. In comparison, the standard curve from this assay had slope of -3.82, y-intercept of 38.969 and R^2 of 0.995, and the LOD was 2 copies/reaction.

eDNA detection with distance

By combining the results of both the 3-μL (standard volume) and the 25-ng (standard concentration) approaches, ≥1 qPCR replicate was able to detect *H. verticillata* eDNA out to 4.9 km from the base of the New Croton Dam (Table S3, Fig. 1). Regardless of approach, we found a moderate negative relationship between the detection of *H. verticillata* eDNA and the distance from the source by accounting for the percentage of qPCR replicates that were amplified (Fig. 2). In the 3-μL samples ($\rho = -0.39$, $p = 0.008$), the relationship was not as pronounced as in the 25-ng samples ($\rho = -0.65$, $p < 0.001$) or for the 2 approaches combined ($\rho = -0.58$, $p < 0.001$). This consistently negative relationship reflects the higher percentage of replicates amplifying closer to the actively growing colonies of *H. verticillata*.

Stream discharge

We found that stream discharge and maximum detection distance for our plant-based eDNA study was comparable to estimates produced by Pont et al. (2018) (Table S2).

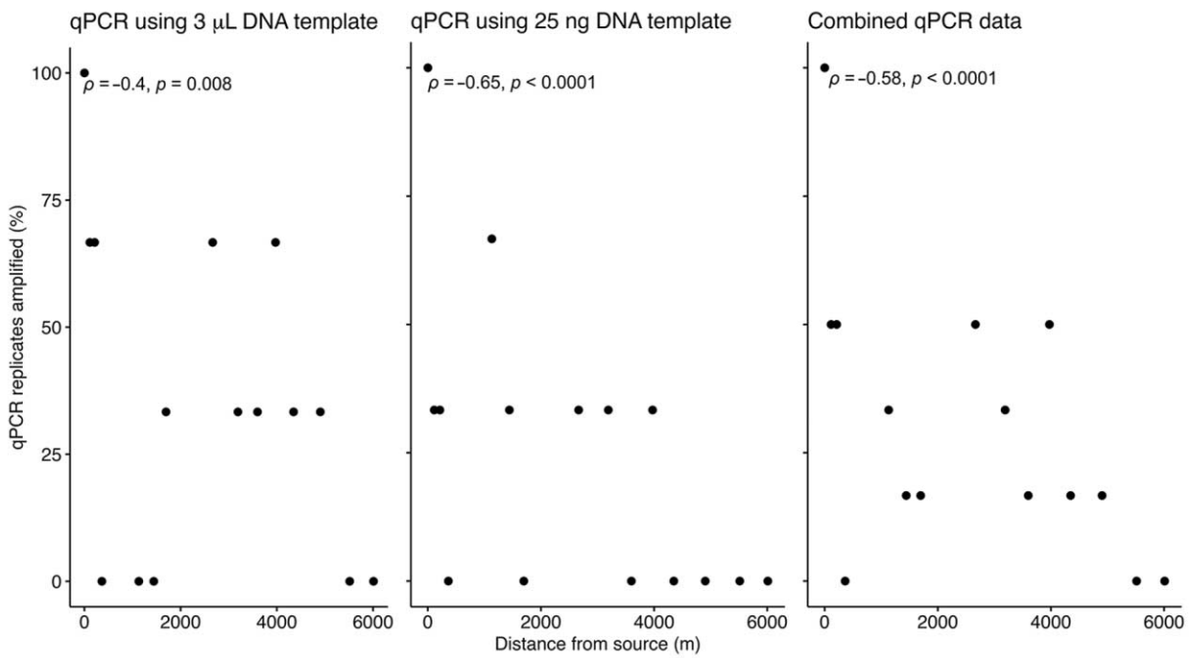

Figure 2. Spearman's correlation of the relationship between the distance from the source at which samples were collected in the Croton River, New York, USA, and the percentage of quantitative polymerase chain reaction (qPCR) replicates amplified for 2 qPCR sample approaches: standard volume (3-μL DNA template), standard concentration (25-ng DNA template), as well as qPCR replicates amplified by both approaches combined.

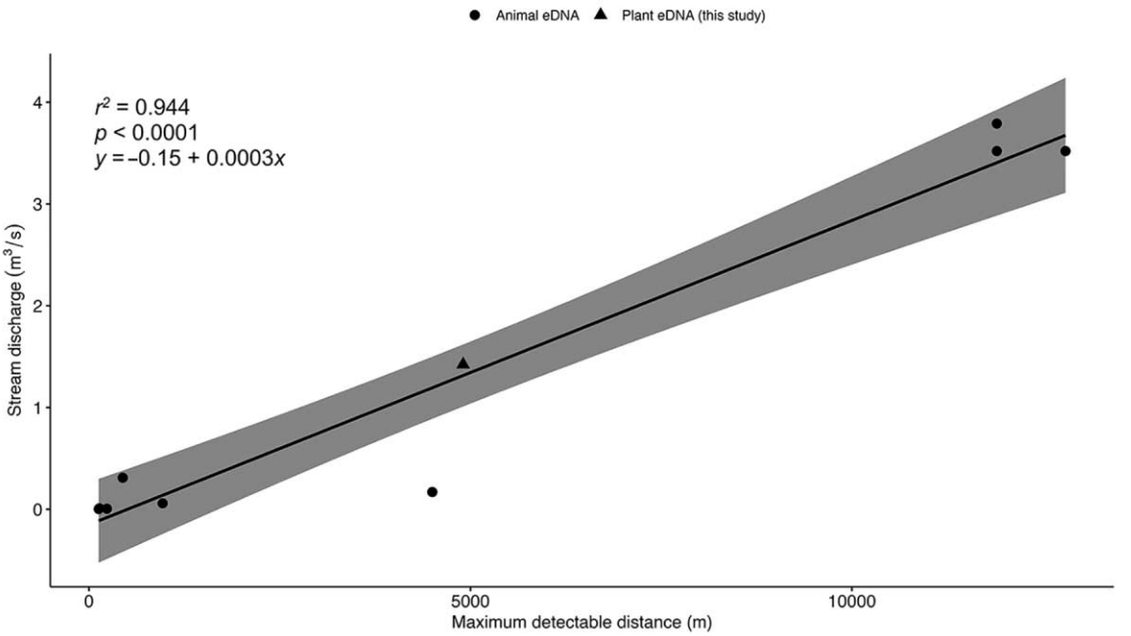

Figure 3. Transportation distance of environmental DNA (eDNA) by stream discharge from a model by Pont et al. (2018). Simple linear regression line and 95% CI (gray ribbon) of predicted detection distances are shown with the maximum detectable distance and stream discharge from this study in the Croton River, New York, USA, added to their data. For this analysis, 1 data point obtained experimentally by Pont et al. (2018) was omitted as an outlier (see Fig. S1).

When our data on maximum detection distance and stream discharge were incorporated into their dataset, there was a strong, positive linear relationship (coefficient = 0.0003, r^2 = 0.94, $p < 0.001$) between the detectable distance of eDNA and the discharge of the river being sampled. In addition, the maximum distance identified in this study (4.9 km from the source) is well within the 95% CI range of the linear model built by Pont et al. (2018) (Fig. 3). For our calculation, we excluded a data point produced by the experimental portion of the study by Pont et al. (2018) as an outlier; however, the trend is still strong with that point included (coefficient = 3.4, $r^2 = 0.99$, $p < 0.0001$; Fig. S1).

DISCUSSION

Stream discharge

Our experimental results aligned strongly with the predictive model based on stream discharge published by Pont et al. (2018). The results of this study support the importance of discharge on the detection distance of eDNA and represent the 1st instance of correlating plant eDNA transportation to discharge, indicating that the model produced is not specific to animal eDNA. Collecting discharge data to inform experimental design and sampling protocols can save time and money by focusing sampling efforts on the approximated detection ranges of eDNA. The relationship between detection range and discharge may then be an important consideration for resource managers (Harrison et al. 2019, Jo and Yamanaka 2022), especially where United States Geologic Survey water current gauges are present.

Although this study was designed to be species specific, our data, which fit well into the Pont et al. (2018) model based on animal eDNA, appear to support stream discharge as being strongly related to eDNA detection distance, regardless of whether the focal species is plant or animal. However, Deiner and Altermatt (2014) found 2 different detection ranges for their 2 target species, both invertebrates, in the same river. Jo and Yamanaka (2022) also noted that the transport distance of Zebra Mussel (*Dreissena polymorpha*) eDNA, identified in Shogren et al. (2019), was detected further downstream than fish eDNA in similar situations. These findings support species-specific differences that could potentially be attributed to something other than genetic material, including behavioral variations, and would be worthy of study in the future.

There are several other potentially important considerations in eDNA detection distance. One is how the density of the eDNA source influences the distance to detection. In their recent review, Jo and Yamanaka (2022) estimated the transport distance of eDNA to be <2 km, but they acknowledge that established populations may be easier to detect further downstream than a handful of recently introduced individuals. The studies referenced in Pont et al. (2018) and

the present study utilize data from established populations (Deiner and Altermatt 2014, Civade et al. 2016, Wilcox et al. 2016, Pont et al. 2018), as well as caged organism studies in rivers otherwise free of the target organism's DNA (Jane et al. 2015, Wilcox et al. 2016), underscoring that differences in population density may be an important consideration for future work. Another consideration is phenology, influenced by plant senescence (Matsuhashi et al. 2019) or animal breeding seasons (Buxton et al. 2017b, Stewart 2019), which has also been found to influence detection rates. Collecting water samples during the peak of these events will likely lead to better detection rates because of the increased availability of genetic material in the environment. Finally, the downstream slope of a river has also been shown to be a strong proxy of eDNA retention in a system, leading to longer transport distances (Fremier et al. 2019). In this study we did not measure slope, but it is conceivable it may have had an impact on the detection range in the Croton River, as evidenced by the steep terrain that prevented establishing evenly distributed sampling sites between 363 and 1132 m.

qPCR amplification and eDNA detection distance

Both the standard concentration (25 ng) and standard volume (3 μL) approaches to eDNA amplification appear to be viable options for qPCR of *H. verticillata*, but the 3 μL of template generally performed better across the range of detection distances. The standard volume approach had a greater number of total positive detections than the standard concentration approach, including 2 points relatively close to the source that the standard concentration approach missed. In addition, the standard volume approach detected *H. verticillata* eDNA ~1 km further than the standard concentration approach was able to detect. Two factors that may have influenced the detection discrepancies are inhibition and the eDNA plume. Jane et al. (2015) found high levels of qPCR inhibitors during times of leaf deposition (i.e., October in New York, when this study took place). However, they also found that using the Environmental Master Mix removed most of those inhibitors (Jane et al. 2015), which, in addition to the Zymo OneStep™ PCR Inhibitor Removal Kit, was used in this study, making it unlikely that inhibition was the main cause for differences between approaches. On the other hand, plumes cause variability in the concentration of eDNA, which can lead to missed detections where eDNA would be expected (Laporte et al. 2020). When possible, it may be worth performing parallel qPCR runs to improve detection rates to account for variations in the concentration of organic materials found throughout the system.

We found that *H. verticillata* eDNA was amplified continuously to the 4.9-km sampling site with 1 early exception. A lack of detection at the 3rd sample point (363 m) followed by continuous subsequent downstream detections likely represents 1 of 2 scenarios. The 1st option is that 363 m is too far for enough *H. verticillata* eDNA to be transported to be detected at that point, which means that other detections came from multiple downstream populations, each spaced ≤350 m apart. The other possibility is that the sample point simply did not contain a sufficient quantity or quality of *H. verticillata* eDNA to be detected, potentially because of the variations in eDNA concentration caused by the eDNA plume at the sample site (Laporte et al. 2020).

There are several reasons why we think that variability in eDNA concentration is likely the reason for imperfect qPCR detection of *H. verticillata* along the distance gradient. First, recent, in-depth, traditional snorkel surveys conducted by the New York State Department of Environmental Conservation, as well as the traditional survey that took place 2 d after our eDNA sampling, did not detect any populations of *H. verticillata* downstream of the dam. Chemical controls in the study area also make it highly unlikely that additional *H. verticillata* populations were present. Still, it is possible it may have been missed because of the inherent uncertainty of traditional and eDNA sampling in natural systems (Jerde 2019), but it would be in very small quantities (especially relative to the reservoir). Second, the near-perfect fit when we added our maximum detection distance and discharge data to the model developed by Pont et al. (2018) supports the finding of only a single source population. Third, the negative correlation between percentage of qPCR replicates amplified and increasing distance from the source suggests that eDNA concentrations declined along the distance of the river. The sample at 363 m was collected from the riverbank where the water and its contents may have a relatively long residual time, depending on the river's flow patterns, leading to the degradation of genetic material. Alternatively, another study (Jane et al. 2015) documented relatively low quantities of eDNA at both the maximum detection range and close to the source, where there were high velocities, which also could have been the case for the sample point at 363 m from the dam. Taken together, we find this body of evidence to strongly suggest that the detectable range from the single population of *H. verticillata* at the dam was ≥4.9 km.

The final detection site of *H. verticillata* eDNA occurred just before the confluence of the Croton and Hudson Rivers. It is possible that *H. verticillata* eDNA was diluted by the waters of the Hudson but could have been detected further downriver had the sampled river been longer. However, based on the correlation between detection and distance (Fig. 2) and the linear fit of the maximum detection point with the Pont et al. (2018) model (Fig. 3), it seems probable that the results of this eDNA study would be relevant in other systems.

Genetic variability

There are at least 2 biotypes of *H. verticillata*. The dominant biotype in the northern United States is the monoecious strain (True-Meadows et al. 2016), which is the biotype

assumed to be present at the study site. A genetically distinct strain was recently discovered less than 200 km away in the Connecticut River (Tippery et al. 2020) and has only been assessed with *trn*L-F, not *matK* as we used here. Although the biotype is unlikely to affect eDNA detection, it is unknown if the new strain is also present in the Croton River. However, we have had success in our work and additional unpublished work (M. Tessler, unpublished data) using *matK* qPCR primer sets in the Croton River. Although there is a chance that an unknown population of the Connecticut River strain was not identified, *matK* is an appropriate primer for this and other similar systems.

Potential impacts of environmental variables on eDNA detection

The degradation of eDNA affects its detectability, and environmental variables can affect the rate at which eDNA degrades (Barnes et al. 2014b, Strickler et al. 2014, Stewart 2019). Both temperature and pH have been experimentally shown to influence the degradation of eDNA (Strickler et al. 2014, Stewart 2019), but it is difficult to differentiate the effects of individual variables on eDNA detection in a natural system. Strickler et al. (2014) found that both lower temperatures and alkaline conditions were associated with lower eDNA degradation rates. In the only published study that focused on plant eDNA detection range, Anglès D'auriac et al. (2019) noted that increased turbidity was associated with high quantities of eDNA. Other environmental factors such as substrate size (Shogren et al. 2017) and type (Buxton et al. 2017a), microbial activity (Barnes et al. 2014b, Shogren et al. 2018), and ultraviolet radiation (Strickler et al. 2014) have also been found to be associated with eDNA detection distance, and these variables are worthy of consideration for future study designs.

Broader implications

In this study, we successfully detected *H. verticillata* eDNA in the Croton River ≥4.9 km from the base of the New Croton Reservoir dam. In addition to identifying the species-specific range of detectability in this river, this study was also the 1st to compare the detectable range of plant and animal eDNA. Our finding that, in this case, the same model that was used to predict animal eDNA based on river discharge can be used for plants opens the door to future eDNA research. These results also imply that stream managers could save time and money by designing sampling protocols with simple stream discharge data. This work further contributes to the growing body of knowledge that supports the use of eDNA as a powerful and cost-effective tool for resource managers to assist with the location and control of invasive species, a necessary fight to support native communities for years to come.

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The authors declare that they have no conflicts of interest to disclose.

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