Environmental Concerns in Rights-of-Way Management

13th International Symposium

Infrastructure projects routinely include biological monitoring surveys for the assessment of rare, keystone, or invasive species to support permitting efforts. Characterizing biodiversity typically requires time-intensive surveys to physically capture organisms of interest, with field crews trained in morphological identification. However, recent genetic technical advancements through the analysis of environmental DNA (eDNA—genetic material released from an organism) has become a promising tool for biomonitoring purposes. This method provides detection of organisms without the need to capture or even see them within the environment, often exhibiting increased sensitivity compared to conventional methodology. Although most progress has occurred for aquatic applications, advancements are focusing on terrestrial environments, including the collection of eDNA from air. While the breadth of eDNA research is promising, current uncertainties and drawbacks have impeded widespread regulatory acceptance of eDNA-based evidence to support permitting and project approvals. We discuss recent advancements for eDNA applications across environments and the path toward incorporating eDNA tools into linear infrastructure projects that require regulatory review. We will provide Stantec case studies and real-world examples for implementing eDNA methodology for biomonitoring surveys, and explore the development of guidelines/standards for eDNA applications to meet environmental mandates by federal and state government agencies.

Biological Monitoring with Environmental DNA: Advancements, Limitations, and Moving Towards Regulatory Acceptance

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INTRODUCTION

Many rights-of-way (ROW) projects involve biological monitoring and surveys to support conservation and/or permitting efforts. These traditional biological surveys typically rely on observations through capture methods and morphological identification. However, many terrestrial and aquatic species are elusive, found in low density, or display morphologically cryptic features, all of which result in difficulties in successful detection. Major advancements over the past decade through the analysis of environmental DNA (eDNA—genetic material released from urine, waste, mucus, or sloughed cells) have considerably improved surveys for a wide range of taxa (Beng and Corlett 2020). The analysis of eDNA has quickly become a powerful tool for improving detection of rare and/or invasive species in freshwater systems (Rojahn et al. 2021).

The applications and implementation of eDNA methodology to address ecological and conservation issues is exponentially growing (Beng and Corlett 2020), with new sampling techniques allowing biologists to gather biodiversity measures from conventional sampling media, such as water (Marshall et al. 2022a), sediment (DiBattista et al. 2019), and soil (Marquina et al. 2019). Additionally, innovative sampling methodologies have been developed to obtain eDNA from unconventional medias, such as air (Clare et al. 2022), salt licks (Ishige et al. 2017), blood meal (Fahmy et al. 2020), snow tracks (Franklin et al. 2019), spiderwebs (Gregorič et al. 2022), and rainfall (Macher et al. 2022). These sampling strategies have proven useful across terrestrial (Leempoel et al. 2020), subterranean (Saccò et al. 2022), marine (Sanchez et al. 2022), estuarine (Hallam et al. 2021), and freshwater systems (Marshall et al. 2022a).

Compared to traditional sampling, eDNA surveys have been found to be more sensitive for detection of species at low densities (Deiner et al. 2021) and are considered less prone to morphological identification biases for species detection at any life stage (Preißler et al. 2019). Because eDNA surveying entails the collection of a mixture of genomic material from many organisms located at or near the site of sampling, this can enable simultaneous biodiversity assessments for a wide range of organisms from a single sample (Compson et al. 2020).

In addition, eDNA surveys tend to be quicker, with lower labor effort, and provide a non-destructive and noninvasive survey tool (Antognazza et al. 2019). Environmental DNA has been used as a means for early detection of biological invasions and for establishing highest probability of eradication success by detecting populations when they are at low densities (Lin et al. 2019). Typically, eDNA is considered a lower cost survey tool compared to traditional methods (Biggs et al. 2015; Qu and Stewart 2019), however cost-effectiveness of eDNA will depend on the overall project size, the sampling region, and the target taxa (Smart et al. 2016).

However, some uncertainties still need to be explored to push eDNA methodology forward. For example, detection of eDNA is largely dependent on both biological and environmental factors, and both are critical components of a proper sampling design. For example, the probability of successfully collecting DNA from the environment is related to the life history (Takeuchi et al. 2019), species behavior (Dunn et al. 2017), and population density of the target species (Baldigo et al. 2017). Thus, an eDNA sampling strategy that targets an optimal sampling season is likely to differ across taxonomic groups and between systems. Additionally, detection of eDNA can be affected by environmental conditions, such as the presence of environmental inhibitors (Lance et al. 2020), distance from source (Goldberg et al. 2016), recent rainfall (Akre et al. 2019), or presence of turbidity and sediment (Barnes et al. 2021). Currently, eDNA sampling is not well suited for addressing population status, such as sex ratios, organism size, or organism/population health (Goldberg et al. 2016), although applications for the collection of eRNA may provide better assessment of this information (Marshall et al. 2021). For some taxa, eDNA has been found to be a weak predictor of abundance or biomass of target taxa (Lamb et al. 2019), however recent work has suggested comparable measures for relative abundance estimates to that of traditional methods may be possible when factoring for allometric scaling (Yates et al. 2022).

Once eDNA samples have been collected, laboratory methodologies can use either a "targeted" species-specific approach or a "broad" community-based approach. Targeted species-specific analysis typically uses quantitative (q)PCR, or more recently digital-droplet (dd)PCR, to detect and quantify a specific DNA fragment for a species of interest. Community-based DNA metabarcoding approaches implement high-throughput sequencing (HTS) technologies (e.g., illumina MiSeq and HiSeq or Oxford Nanopore sequencers), which are capable of simultaneously identifying multiple taxa within a single sample (Compson et al. 2020). Environmental DNA metabarcoding surveys can be implemented for broad taxonomic groups (e.g., as eukaryotes [Stoeck et al. 2010] or vertebrates [Riaz et al. 2011]), or targeted specific groups (e.g., as diatoms [Vasselon et al. 2017], macroinvertebrates [Marshall and Stepien 2020], or fishes [Miya et al.

2015]), providing rapid assessments of biodiversity. Metabarcoding approaches can provide advantages over qPCR/ddPCR by broadly examining biodiversity patterns and allowing the detection of species without the a priori knowledge to test for them (Deiner et al. 2017).

Implementation by Agencies

The first examples for establishing standards for eDNA include a priority conservation species in the United Kingdom, Great Crested Newt (*Triturus cristatus*) (Biggs et al. 2015), and the highly invasive Bighead Carp (*Hypophthalmichthys nobilis*) and Silver Carp (*H. molitrix*) in the U.S. (Amberg et al. 2015). Since then, standards and guidelines have been developed and proposed for steps involved in eDNA collection (CSA 2021), and with qPCR assay development/validation (Thalinger et al. 2021). Within the U.S., eDNA has been proposed and/or implemented as a survey methodology for detection of aquatic invasive species (see review in Morisette et al. 2021). Environmental DNA applications are becoming a priority program across agencies, with the development of eDNA Atlas within the U.S. Department of Agriculture Forest Service (*www.fs.usda.gov/rmrs/projects/aquatic-ednatlas-project*), the 'Omics Strategy and Implementation Plan within National Oceanic and Atmospheric Administration (*sciencecouncil.noaa.gov/NOAA-Science-Technology-Focus-Areas/NOAA-Omics*), eDNA workshops developed by U.S. Fish and Wildlife Service, and the interagency eDNA Working Group (U.S. Geological Survey), just to name a few. For the future success of eDNA programs implemented for ROW-based projects, getting agency support and understanding of applications and potential limitations will be critical.

FRAMEWORK

The use of eDNA provides a fast and cost-effective survey method for complementary biological data that has the potential to improve management of linear projects. We detail four recent applications in which Stantec has implemented eDNA surveys for biological monitoring and discuss the benefits of eDNA applications for future ROW biological/ecological management. These projects span across a range of habitat and target taxa, which includes the detection of aquatic rare and threatened species, aquatic invasive species, terrestrial vertebrates, and the monitoring of pollinator diversity. We discuss these innovative sampling strategies within both terrestrial and aquatic habitats. These eDNA field studies include the use of both qPCR and metabarcoding approaches, and we evaluate eDNA performance with direct comparisons to traditional surveys. Finally, we demonstrate how the use of occupancy modeling and statistical analyses allow practitioners to evaluate probabilities of detection for target taxa, and thereby can elevate eDNA applications to the standards and expectations of traditional methods.

ENVIRONMENTAL DNA APPLICATIONS FOR RIGHT-OF-WAY MANAGEMENT

Aquatic Rare/Threatened/Endangered Species: Evaluating Community-Level Assessments

The greatest diversity of freshwater unionid mussels is found in North America, with ~300 of the 840 global species occurring in the U.S. (Williams et al. 2017). However, of those 300 species, >70% are considered endangered, threatened, or species of concern (Williams et al. 2017). Thus, monitoring and management of mussels is considered a high conservation priority, and eDNA has been demonstrated as a beneficial survey tool for this group (Marshall et al. 2022a).

In 2020, the Six Mile Dam located on the Walhonding River (an Ohio River tributary) in Coshocton County near Warsaw, Ohio, was scheduled for demolition due to structural defects causing risk for failure. The Walhonding River basin was known for extant populations of three federally listed freshwater mussels (*Epioblasma obliquata*, *Plethobasus cyphyus*, and *Theliderma cylindrica*), and thus a mussel relocation was completed within the impacted sections upstream of this dam prior to its demolition. At the same locations of the mussel rescue and relocation, Stantec conducted eDNA sampling to evaluate the effectiveness of the eDNA methodology for detecting a diverse mussel community, which included the presence of federally listed species (Marshall et al. 2022a).

Prior to the demolition of the dam, water samples upstream of the Six Mile Dam were collected for eDNA metabarcoding analysis. In total, 66 water samples were collected from 22 sampling sites across a 1.5 km reach of the river. At each site, triplicate 500 mL water samples were taken from ~10 cm above the substrate and filtered using a 47-mm diameter glass microfiber filter GF/C (nominal pore size 1.2 µm). The collected eDNA was analyzed using a metabarcoding assay capable of detecting all freshwater unionid mussels (Marshall et al. 2022a). At the same 22 sites, rescue surveys were completed using an opportunistic strategy by searching within areas that became dewatered and resulted in exposed river bottom following the dam demolition.

The mussel rescue survey resulted in 363 search hours and found >12,000 mussels across 24 species (Table 1). The

eDNA survey detected the presence of 28 species, which included 22 of the 24 (92%) species found in the rescue survey (Table 1). Both survey methods detected the presence of two federally listed species from multiple sampling sites upstream of the dam (*Plethobasus cyphyus* and *Theliderma cylindrica*). The two species that were not detected with eDNA metabarcoding (*Ptychobranchus fasciolaris* and *Quadrula quadrula*) were the rarest species in the region, each found as only a single individual from the rescue survey (Table 1). Environmental DNA, on the other hand, detected four species not found in the rescue survey (*Alasmidonta viridis*, *Lampsilis ovata*, *Potamilus alatus*, and *Truncilla donaciformis*). Additionally, eDNA revealed hidden cryptic diversity within the genus *Pyganodon*, which was not able to be discerned with morphological characteristics.

To further evaluate the capabilities of eDNA sampling for freshwater mussels, a logistic regression analysis was conducted comparing detection probability comparted to mussel abundance at each of the 22 sites. Through this analysis, it was determined that eDNA displayed a 95% probability of detection when mussel density was >10 individuals per site (site size was ~150 m x ~30 m) (Marshall et al. 2022a). This suggests high sensitivity for mussel detection using eDNA metabarcoding within the Walhonding River. Additionally, by comparing species richness curves between eDNA

Table 1. Freshwater Unionid Mussel Species from the Six Mile Dam Drawdown Detected with a Conventional Rescue Survey (Listed as Mussel Abundance), and with eDNA Metabarcoding. Naming convention follows Williams et al. (2017).

Species	Common Name	Conventional (n)	eDNA
Amblema plicata	Threeridge	6812	X
Actinonaias ligamentina	Mucket	1131	X
Lasmigona costata	Flutedshell	672	X
Lasmigona complanata	White Heelsplitter	641	X
Theliderma cylindrica[a]	Rabbitsfoot	632	X
Lampsilis siliquoidea	Fatmucket	582	X
Tritogonia verrucosa	Pistolgrip	438	X
Lampsilis cardium	Plain Pocketbook	296	X
Fusconaia flava	Wabash Pigtoe	292	X
Pleurobema sintoxia	Round Pigtoe	133	X
Plethobasus cyphyus[a]	Sheepnose	127	X
Strophitus undulatus	Creeper	117	X
Cyclonaias pustulosa	Pimpleback	77	X
Utterbackia imbecillis	Paper Pondshell	76	X
Cyclonaias tuberculata	Purple Wartyback	57	X
Lampsilis fasciola	Wavy Rayed Lampmussel	53	X
Lasmigona compressa	Creek Heelsplitter	31	X
Pyganodon grandis[b]	Giant Floater	17	X
Pyganodon cataracta[b]	Eastern Floater	–	X
Pyganodon sp.[b]	–	–	X
Eurynia dilatata	Spike	5	X
Leptodea fragilis	Fragile Papershell	4	X
Ligumia recta	Black Sandshell	3	X
Villosa iris	Rainbow	1	X
Ptychobranchus fasciolaris	Kidneyshell	1	–
Quadrula quadrula	Mapleleaf	1	–
Alasmidonta viridis	Slippershell	0	X
Lampsilis ovata	Pocketbook	0	X
Potamilus alatus	Pink Heelsplitter	0	X
Truncilla donaciformis	Fawnsfoot	0	X
	Total observed species	*24*	*28*

a. Federally listed freshwater mussel
b. Mussels belonging to the *Pyganodon* genus were identified as *P. grandis* with the rescue survey, while eDNA identified three *Pyganodon* Molecular Operational Taxonomic Units (MOTUs) as *P. grandis, P. cataracta,* and a previously unnamed cryptic *Pyganodon* sp. (Cyr et al. 2007).

sampling, the mussel rescue survey, and a traditional SCUBA survey conducted in 2009, this suggests eDNA provided the highest detection of species richness with relatively low levels of field effort required (Figure 1). These results suggest that eDNA provided similar mussel community composition information to that of traditional surveys and could be completed faster and with less labor. It is important to note that eDNA cannot act as an all-out replacement of traditional methods, as mussel relocations and assessments of organism health/fitness will still require the handing of individuals. However, these eDNA results suggest a preliminary eDNA survey prior to mussel rescues can be advantageous to identify species compositions and locations of interest for presence of threatened and endangered species.

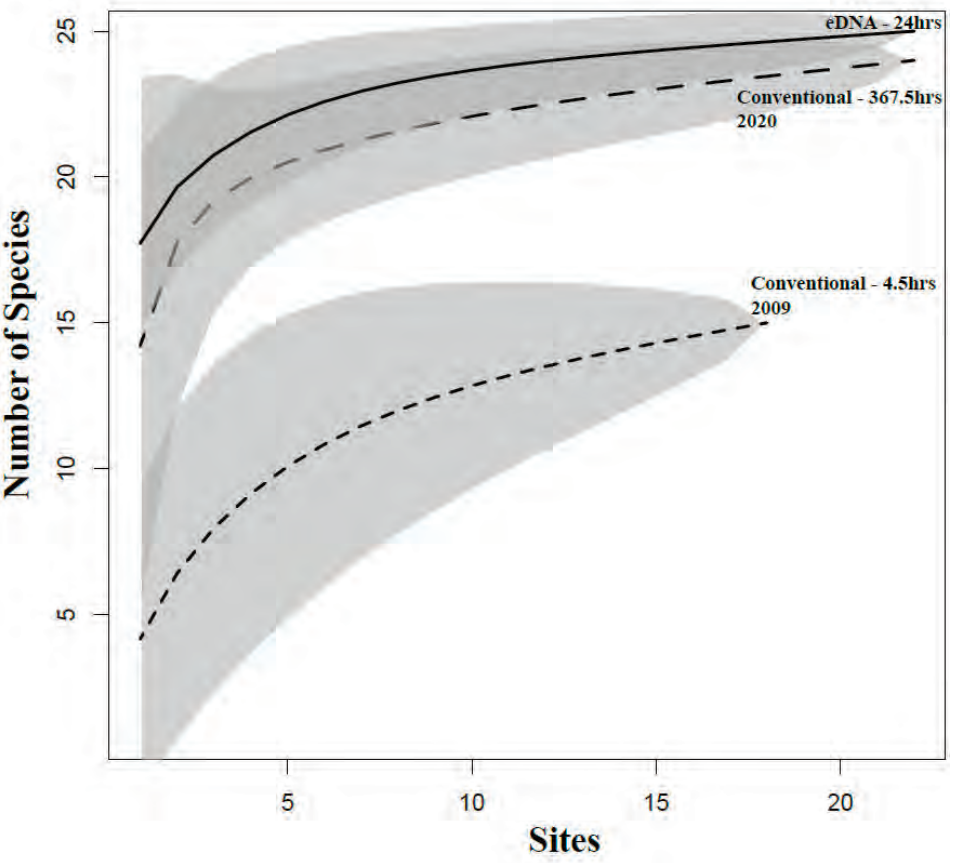

Figure 1. Species accumulation curves for the three sampling methods (2020 eDNA, 2020 mussel rescue and relocation, and a 2009 SCUBA survey). The calculated effort in search hours is listed for each survey. The black line is the estimated number of species, with grey shading representing the 95% confidence interval.

Aquatic Invasive Species: Establishing Probabilities of Detection

Hydrilla is a fast-growing, invasive rooted water plant that was first discovered in the U.S. in Florida in the 1960s. It quickly spread north and, to date, there are known infestations in Maine and Connecticut, including the Connecticut River as well as two known infestations reported in a Cape Cod pond as of 2001. In June and September of 2021, water samples were collected from 10 water bodies in Massachusetts to test for the presence of Hydrilla eDNA. At each of the 10 waterbodies, Stantec collected water samples at three sampling sites using a Niskin-type sampler and/or 1-liter bottle. At each of the three sampling sites, two 1 L samples were collected at different depths (including at the surface and near the sediment) and filtered as a composite sample. Following the analysis for the presence of Hydrilla eDNA using qPCR analysis, occupancy modeling was implemented to compare probability of detection for Hydrilla based on seasonal sampling patterns (i.e., June vs. September) using the R package eDNAoccupancy (Dorazio and Erickson 2018).

Occupancy modeling is often used in ecological surveys to account for imperfect detection of rare and/or elusive animals. For traditional surveys, these models use data collected from repeated surveys at each sampling location to estimate occurrence of a species while accounting for false-negative errors in detection. Considering eDNA is an imperfect sampling method (i.e., detection depends on successful collection of eDNA and successful molecular analysis of samples), occupancy modeling techniques are an ideal analysis to improve understanding of detection probability and estimating species presence.

Environmental DNA surveys typically collect replicate water samples per location and include subsampling within each individual water sample (i.e., qPCR replicates). Therefore, eDNA surveys typically include three nested levels of sampling:

1. Locations (primary sample units) within a study area
2. Water samples (secondary sample units) collected form each location
3. Subsamples (replicate observations) taken from each water sample

Furthermore, a multiscale occupancy model can be implemented to estimate the following:

1. Probability of target species occurrence at the location (Ψ, psi)
2. Conditional probability of target eDNA occurrence in a water sample, given that the target species is present at that location (θ, theta)
3. Conditional probability of positive detection in a qPCR replicate, given that the target eDNA is present in the water sample (*p*)

Based on the framework of a multiscale occupancy model, Stantec compared the probability of eDNA detection within a water sample (*p*) between the two sampling seasons. There was a large overlap in estimated *p* values (Figure 2), suggesting sampling season did not impact the laboratory qPCR analysis. Next, the probability of eDNA collection (θ) was compared between the two sampling seasons. There was a much higher probability of eDNA collection for samples collected in June compared to those from September (Figure 3). In order to reach a 95% probability of eDNA collection, samples collected in June required four total samples per body of water, while samples collected in September required double that sampling effort (Figure 3). When accounting for our sampling design (i.e., three water samples per body of water with six qPCR replicates per eDNA sample), it was calculated June sampling displayed a 94% probability of detection, while September displayed a reduced probability of detection of only 72%. The lower rate of Hydrilla eDNA detection during the fall is likely related to decreased growing rates with lower photosynthetic processing. Similarly, previous Hydrilla eDNA surveys in Japan found that eDNA concentrations changed seasonally, with highest concentrations occurring during the summer growing season (Matsuhashi

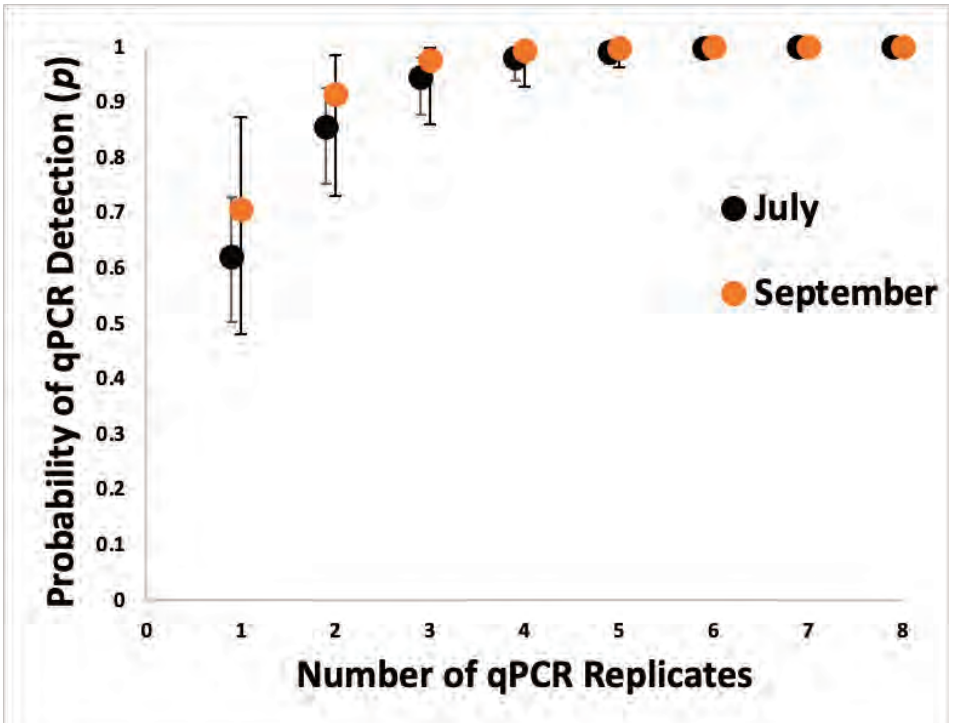

Figure 2. The probability of detection within cumulative qPCR replicates (*p*) from occupancy modeling of Hydrilla eDNA collected in July or September. Error bars represent 95% confidence intervals.

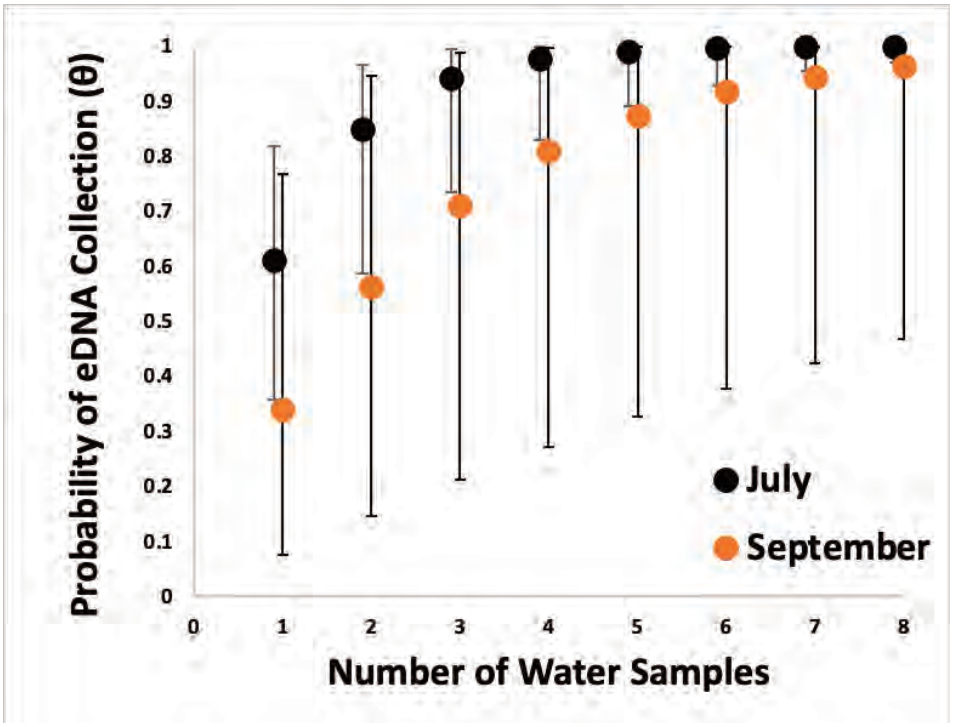

Figure 3. The probability of eDNA collection within cumulative samples (θ) from occupancy modeling of Hydrilla eDNA collected in July or September. Error bars represent 95% confidence intervals.

and Minamoto 2019). The use of occupancy modeling here allowed us to evaluate our sampling design (i.e., number of water samples in addition to the number of qPCR replicates), to provide relevant inferences in seasonality impacts on Hydrilla eDNA detection. Implementing occupancy modeling into eDNA datasets allows end users better interpretation of detection probabilities and evaluation of survey design, to potentially reduce uncertainties associated with eDNA "absence" and help design a more accurate and cost-effective sampling plan.

Moving To Land: Targeting Terrestrial Vertebrates

Biodiversity of North American temperate forest bat populations have rapidly declined, largely due to habitat loss and the lethal White-nose syndrome disease caused by the fungal pathogen *Pseudogymnoascus destructans* (Frick et al. 2020). This decline has increased monitoring efforts of bat populations and species that are protected under the Endangered Species Act (ESA) across the U.S. The analysis of DNA recovered from guano samples has been useful in identifying species and roost locations (Walker et al. 2016), however, not all bat species can be found in large roosts where guano is relatively available for collection. Instead, eDNA that is collected from water sources might provide an easy sampling methodology for the detection of terrestrial organisms relying on drinking water.

Several studies have implemented eDNA surveys for the detection of terrestrial mammals from a source of drinking water. These studies have detected a wide range of species, including coyotes (*Canis latrans*) (Rodgers and Mock 2015), invasive wild boar (*Sus scrofa*) (Davis et al. 2018), elusive jaguar (*Panthera onca*) (Wilcox et al. 2021), and even entire terrestrial mammal communities (Harper et al. 2019). Such an eDNA approach that collects water samples from source drinking water may provide the detection of critically threatened bat populations without relying on a priori knowledge of roost locations, thereby greatly improving bat monitoring and management. Stantec developed and tested a sampling strategy to detect bat eDNA from pools of water found in mixed-mesophytic forests. These pools of water act as an important water resource for bats in the area, and thus bat eDNA (i.e., saliva and hair) may accumulate within these pools following a drinking event.

Forty-seven water samples were collected from 21 pools of water in the forested uplands of the Appalachian Plateau (Marshall et al. 2022b). Environmental DNA from these water samples were analyzed using both species-specific qPCR and community metabarcoding methodologies to test for the detection of two bat species known to be in the region: big brown bat (*Eptesicus fuscus*) and eastern red bat (*Lasiurus borealis*). Through the qPCR analysis, eDNA was successfully detected from big brown bat and eastern red bat within the forested habitat, however the community metabarcoding approach failed to detect bat eDNA across any of the eDNA samples. While the community metabarcoding approach failed to detect bat eDNA, many nontarget amphibians, birds, and mammals were identified (Table 2), suggesting these pools of water can collect eDNA from a wide range of terrestrial taxa. In many regions of the U.S., state and federal agencies design wildlife water holes in strategic locations to maximize wildlife benefits, and thus these water pools provide rare opportunities to measure terrestrial biodiversity

Table 2. Environmental DNA Samples Upland Forests with Positive Detections for Vertebrate Taxa eDNA with Community Metabarcoding Analysis (Source: Marshall et al. 2022b)

Group	Species	Common Name
Amphibians	*Anaxyrus sp.*	American toad, Fowler's toad
	Hylidae sp.	Tree frogs
	Rana sylvatica	Wood frog
	Rana clamitans	Green frog
	Notophthalmus viridescens	Eastern newt
Birds	*Sayornis phoebe*	Eastern phoebe
	Hylocichla mustelina	Wood thrush
	Passeriformes sp.	Possible Turdidae
Mammals	*Didelphidae sp.*	Virginia opossum
	Odocoileus sp.	White-tailed deer
	Prycon sp.	Racoon
	Peromyscus sp.	Deer mice
	Ursus americanus	Black bear

Improving Pollinator Habitat Monitoring: Collecting eDNA on Flowers

Pollinator habitat and natural wildlife growth areas have been recognized as important management priorities to improve insect and arthropod diversity, and many state agencies have begun to provide recommendations for managing pollinator areas (such as the Ohio Pollinator Habitat Initiative). As eDNA applications continue to expand, recent studies have explored the ability to

detect important pollinator species and arthropod diversity patterns from eDNA traces left on flower heads after an insect visitation (Thomsen and Sigsgaard 2018). Stantec tested community metabarcoding methods for the detection of pollinators visiting four different flower species: butterfly milkweed (*Asclepias tuberosa*), wild bergamot (*Monarda fistulosa*), false dandelion (*Pyrrhopappus carolinianus*), and black-eyed susan (*Rudbeckia hirta*). Individual flower heads for each flower species were collected and processed for traces of arthropod eDNA.

Using community metabarcoding, 154 arthropods were detected across the four sampled flower species, which included the detection of 143 insects. Additionally, differences in insect richness were found between flower species, with butterfly milkweed displaying by far the highest species richness (Figure 4). Environmental DNA from false dandelion and black-eyed susan detected far less insect species than that from butterfly milkweed and wild bergamot (Figure 4). Furthermore, there were subtle differences in the insect composition between flowers, suggesting pollinator selectivity for different flower species. This supports previous studies proposing eDNA may be useful in discerning flower-pollinator interactions (Thomsen and Sigsgaard 2018). These results are provided from a preliminary dataset, and future work is underway for analysis of eDNA samples from multiple flower species occurring across varying habitats. Still, this preliminary dataset provides insight into the effectiveness of eDNA sampling to monitor pollinator communities. There was surprisingly high arthropod diversity on just a few individual flowers, with community difference between flower species. Information on flower selection derived from eDNA detection can become an important component for establishing best practices for planning and developing pollinator habitat.

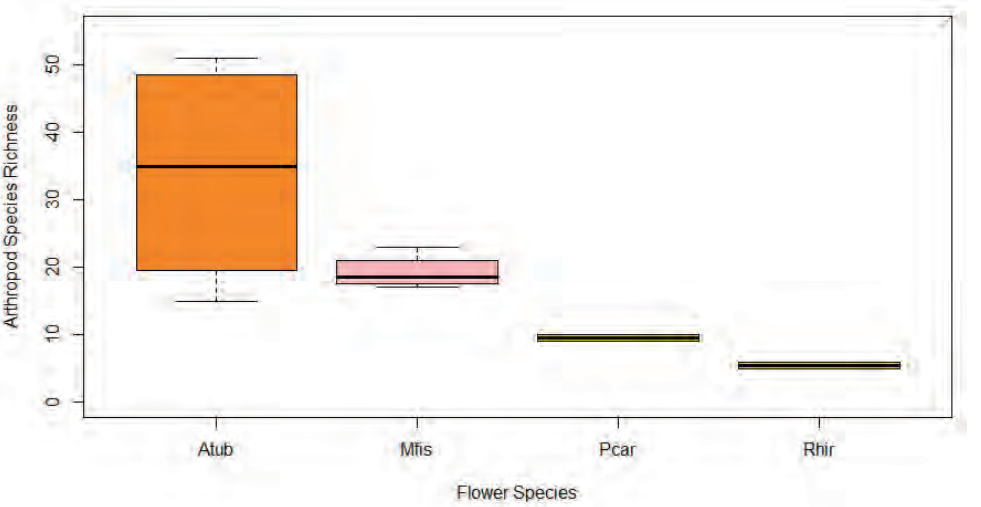

Figure 4. Arthropod species richness detected from eDNA collected on four different flower species. These flowers include butterfly milkweed (*Asclepias tuberosa* - Atub), wild bergamot (*Monarda fistulosa* - Mfis), false dandelion (*Pyrrhopappus carolinianus* - Pcar), and black-eyed susan (*Rudbeckia hirta* - Rhir).

DISCUSSION

Environmental DNA methods have greatly expanded over the past decade, and many studies have demonstrated consistency in detecting biodiversity patterns compared to traditional methods (Fediajevaite et al. 2021; Keck et al. 2022). Here we demonstrate four recent case studies that implement eDNA qPCR or metabarcoding approaches for biological surveys and/or assessments. These applications provide improved or complementary surveys for the detection of invasive species and species of concern (e.g., pollinators or federally listed species) to support conservation and/or permitting linear projects. Furthermore, eDNA methodology has shown tremendous promise for biological monitoring across both aquatic and terrestrial systems. Improved statistical models have been developed to provide better interpretation of eDNA datasets (e.g., the Hydrilla occupancy modeling demonstrated herein) and increase the accuracy and cost effectiveness of eDNA sampling survey design. The case studies presented here demonstrate how eDNA applications continue to grow, and the potential for eDNA surveys within both aquatic and terrestrial landscapes. Standardization and regulator support will continue to expand, allowing eDNA applications to be a complementary survey tool for biodiversity assessment and monitoring programs.

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AUTHOR PROFILES

Nate Marshall, PhD

Nate Marshall, PhD, is an Environmental Scientist with expertise in environmental genomic applications. Marshall has experience developing and implementing eDNA methodology for improving biological surveys. Over the past eight years, he has developed eDNA applications for early detection of aquatic invasive species and has expertise with freshwater macroinvertebrate and fish communities. His current projects include building a basis for eDNA surveys for the detection of freshwater unionid mussels, the most threatened aquatic taxonomic group in North America. This work has involved projects with clients spanning multiple sectors, including transportation, mining, oil and gas, and electric power, and across several states. As the eDNA Technical Lead at Stantec, Marshall seeks to implement genetic-based surveys as a cost-effective and efficient survey tool to aid in biological assessments under Section 7 of the Endangered Species Act.

Jake Riley

Jake Riley is a Project Manager and Certified Ecologist and with over 18 years of fisheries research and ecological experience. Riley is a technical and marketing leader, and part of Stantec's eDNA team and services by managing, executing, and providing technical input on eDNA projects, including study design, sample collection, and analysis. His other recent professional experience includes freshwater fish sampling, fisheries community and population assessments, salmonid spawning and rearing habitat surveys, fish tissue collection, fisheries water quality data analysis and literature reviews, aquatic habitat surveys, federal and state permitting, and biological assessments as consultation under Section 7 of the Endangered Species Act and essential fish habitat preparation. Marshall's prior research experience includes researching predation impediments for lake trout restoration in Lake Champlain and the Great Lakes. Riley holds a Master of Science from the University of Vermont.

Gabe Pelletier

Gabe Pelletier, AWB, is a Wildlife Biologist with five years of professional experience conducting wildlife, fisheries, and aquatic research. Pelletier is part of Stantec's eDNA team where he contributes by creating study design, leading field efforts for eDNA sample collection, and reporting on laboratory analysis. He has a Bachelor of Science in wildlife ecology from the University of Maine. A few examples of his recent project experience include environmental DNA monitoring for early detection of invasive species, studying mercury concentrations in

salmonid species, and radar monitoring of bird and bat movement patterns. Examples of Pelletier's prior experience include mussel identification for a relocation project and raptor surveys to identify and protect nest locations.

Mary Murdoch

Mary Murdoch is a Vice President at Stantec and Technical Leader for Ecosystems Services in Canada. Over the past five years, she has been involved in eDNA studies in North America and Australia, across freshwater and marine habitats. Murdoch completed graduate studies in population ecology and genetics of a freshwater fish species at the University of Guelph. With more than 28 years of environmental consulting experience, Murdoch's focus is on environmental impact assessment and environmental effects monitoring, planning, and permitting for clients across all sectors. She participates in Stantec's innovation program where she is a participant and coach. She routinely presents at national technical conferences and leads Stantec's development and expansion of environmental genomics services globally.