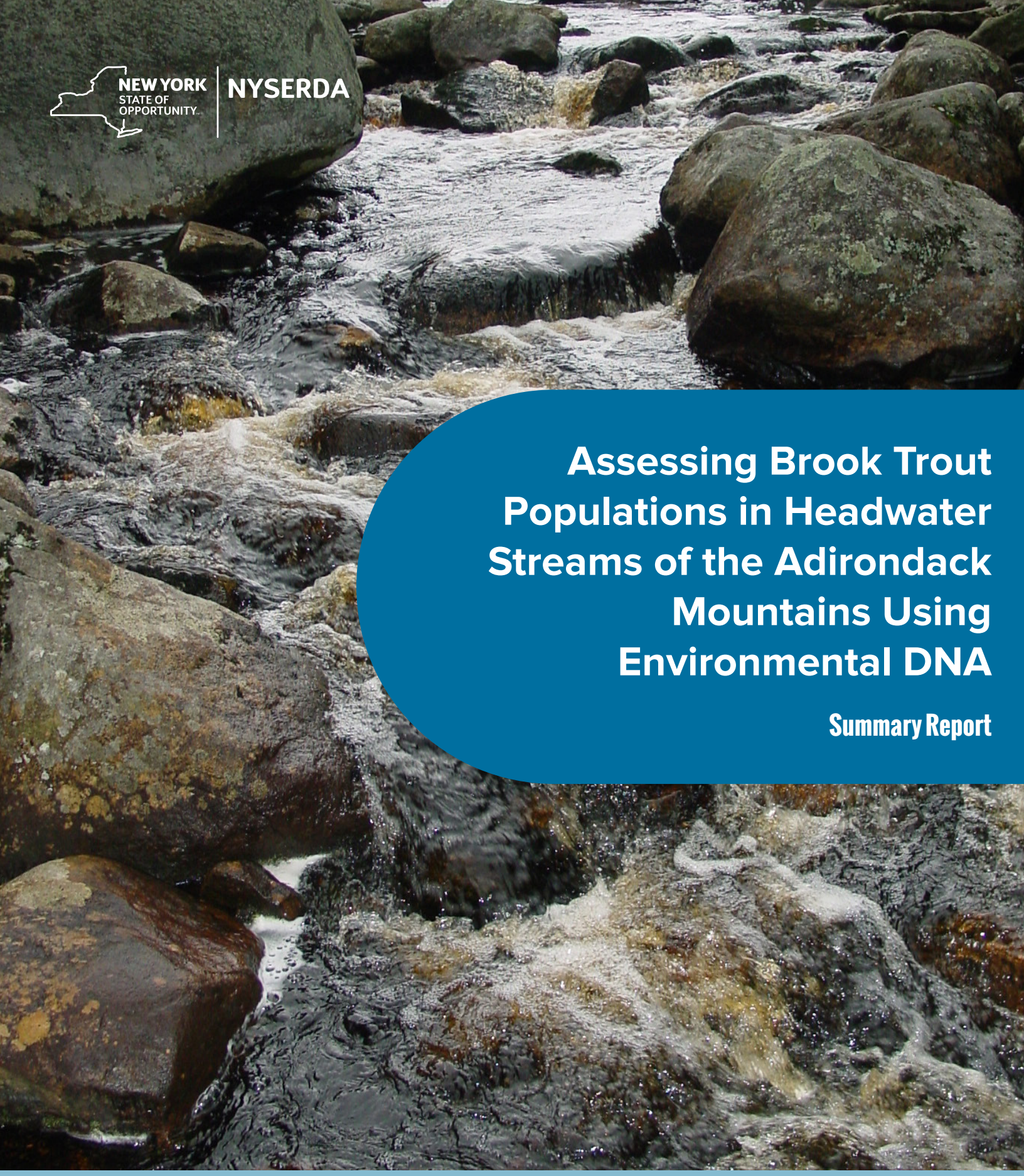

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Assessing Brook Trout Populations in Headwater Streams of the Adirondack Mountains Using Environmental DNA

Summary Report

December 2016

Report Number 17-02

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Cover Image: Inlet to Lake Lila near Nahasane Station, NY. Photo credit to Barry Baldigo

Assessing Brook Trout Populations in Headwater Streams of the Adirondack Mountains Using Environmental DNA

Summary Report

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1 Focus

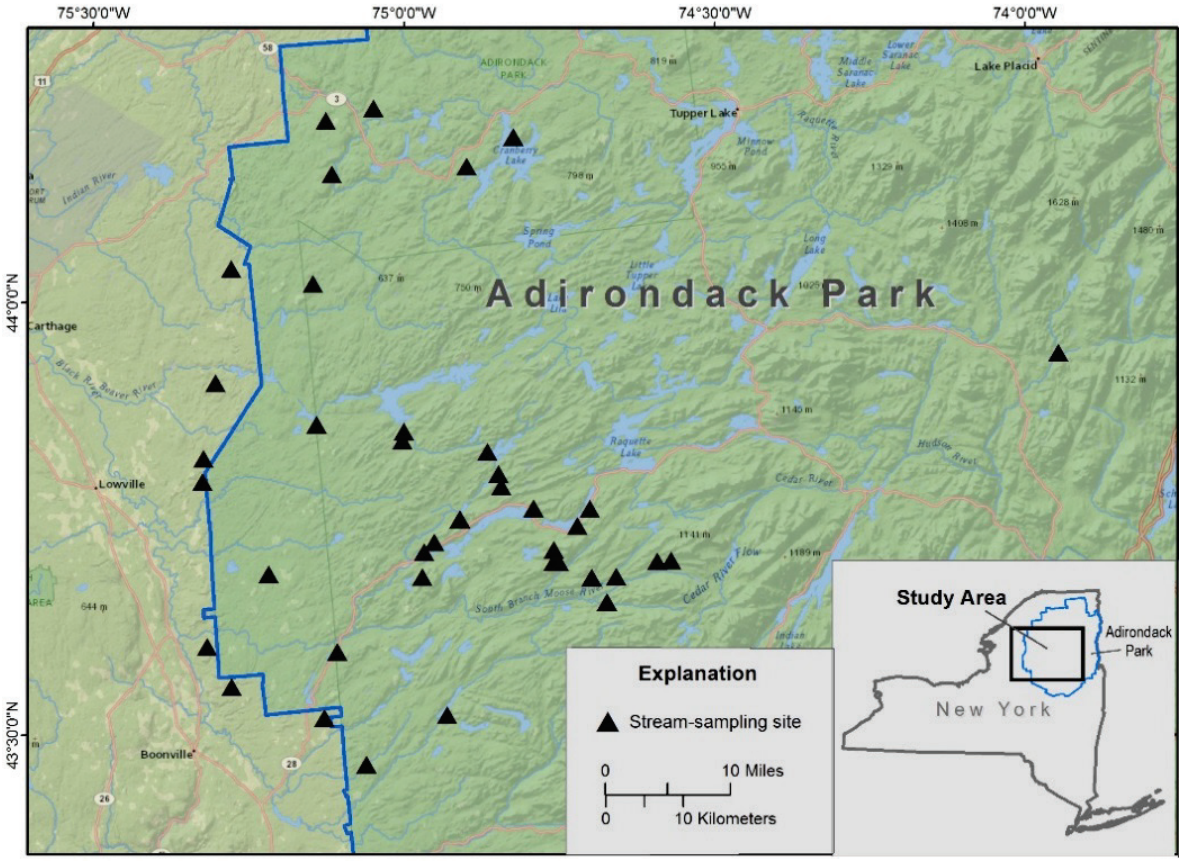
This project evaluated standard fish-survey and environmental DNA (eDNA) sampling methods to determine the ability of eDNA to accurately predict the presence and abundance of resident Brook Trout populations in 40 headwater streams mainly in the western Adirondack Mountains during 2014–2015 (Figure 2). Standard 3-pass electrofishing surveys found that Brook Trout were absent from about 25 percent of study sites, and at low densities in 25 percent of sites, moderate densities in 25 percent of sites, and high densities in 25 percent of sites. Environmental DNA results correctly predicted the presence/absence of Brook Trout in 85.0 to 92.5 percent of study sites and explained 44.0 percent of the variability in density and 24 percent of the variability in biomass of their populations. The findings indicate that eDNA surveys will enable researchers to effectively characterize the presence as well as the abundance of Brook Trout and other species populations in headwater streams across the Adirondack Mountains and elsewhere.

Figure 1. A wild Brook Trout collected from an Adirondack stream.

Photo credit to Barry Baldigo

Figure 2. Location of 40 stream sites, primarily in the western Adirondack Mountains, where fish-community surveys were completed during summers of 2014 and 2015 and Brook Trout eDNA samples were collected during August and September (from Baldigo & others 2017).

Source: Base from National Geographic/Esri; NAD 1983 UTM Zone 18N 1:700,000



2 Context

With advances in genome sequencing, and knowledge of comparative genomics, scientists have identified regions of the genome, termed DNA "barcode" sequences that can serve as unique species identifiers. These DNA signatures (termed environmental DNA or eDNA) may persist in the environment for extended periods of time, sometimes for thousands of years, and can be useful in determining the recent occurrence or presence of a given species within that environment. The advent of polymerase chain reaction (PCR) technology has allowed scientists to detect the presence of a given plant, animal, or microbe simply by sampling soil, water, biofilms or sediments. While it may seem like a novelty that the presence of a species can be known within an environment without actually observing it, the use of eDNA to track invasive species and document the distribution of rare (as well as common) species is gaining ground. eDNA, which likely derives from shed tissue, carcasses, gametes or feces, has been used successfully for both spatial (e.g., surveillance of invasive species) and temporal investigations (reconstructing ecological history).

Detection and monitoring is an important first step in management and conservation efforts. However, the manpower needs and costs involved, particularly across large geographic regions, can be prohibitive. In contrast, collecting and testing a small number of environmental samples (water or sediment) from the site(s) of interest could provide a fast, efficient, and affordable monitoring tool. However, before an eDNA-sampling tool could be accepted widely, it must first be "calibrated" in the field to test or confirm its reliability. With what accuracy can the presence of Brook Trout be detected? What is the sensitivity? Can abundance be determined or merely presence/absence? The USGS recently partnered with the Adirondack Watershed Institute of Paul Smith's College in a project supported by NYSERDA to study the efficacy of eDNA detection for monitoring Brook Trout in small, headwater streams in the Adirondack Mountains of Northern New York.

3 Goals and Objectives

The primary objective of this study was to evaluate the efficacy of eDNA analysis as a rapid and cost-effective tool for assessing the status of Brook Trout populations in headwater streams. Related goals of this study were to test and refine sampling methods, determine the accuracy of presence/absence predictions, and explore the ability of eDNA results to predict the density and biomass of Brook Trout populations.

4 Study Area and Methods

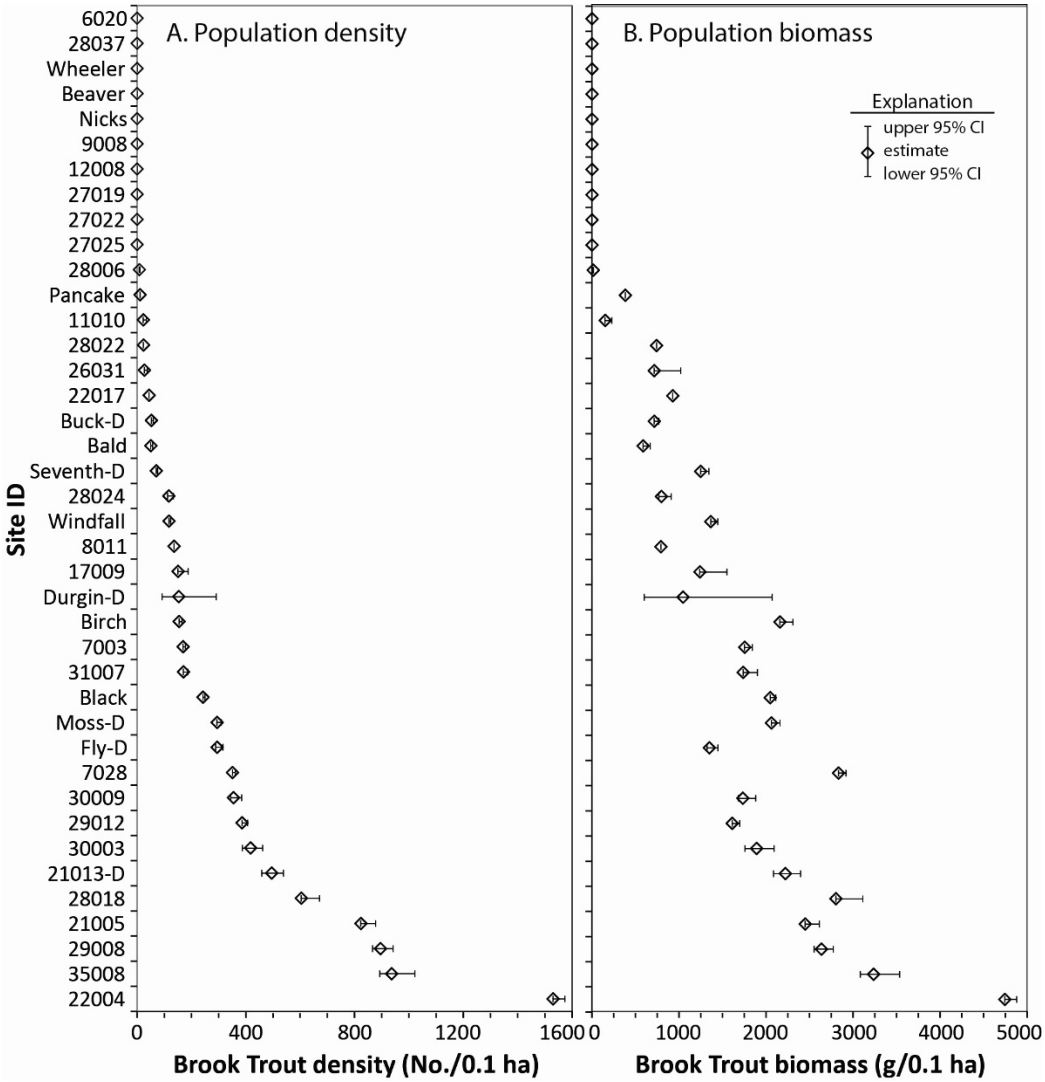
Staff from the USGS and New York State Department of Environmental Conservation conducted 3-pass electrofishing surveys of fish assemblages at 40 stream sites during 2014 and 2015 and estimated the density and biomass of Brook Trout populations using proportional-reduction analysis. The surveys involve passing an electric current through a net-blocked stream segment to stun and capture fish and identify and record the lengths and weights of all fish during three successive passes. After these surveys were completed, the 40 sites were revisited and up to six liters of water were vacuumed through a glass fiber filter and a sediment sample was collected. Samples were frozen on dry ice and transported to Paul Smith's College, where eDNA was extracted and analyzed, generally within 48 hours.

The eDNA extracted from these samples represented a vast mixture, containing DNA remnants of innumerable species (plant, animal, and microbe) that inhabit the stream. PCR was then used to detect the presence of a DNA sequence specific to Brook Trout, by targeting and copying that sequence millions of times. As the number of Brook Trout DNA copies grows, a fluorescent signal is produced, which can be detected by an instrument and used to gauge the starting amount of DNA in the sample. Our test was shown to be highly specific for Brook Trout, and failed to recognize DNA from related species such as Brown Trout and Rainbow Trout.

5 Findings

Results from this study are summarized below and described in greater detail by Baldigo & others (2017). Brook Trout were absent from 10 sites and estimated to be present at low densities (< 100 fish/0.1 ha) at nine sites, moderate densities (100 to 300 fish/0.1 ha) at 11 sites, and high densities (> 300 fish/0.1 ha) at 10 sites (Figure 3A). Estimates of Brook Trout biomass were zero at the E10 sites where they were not collected, low (< 1000 g/0.1 ha) at 10 sites, intermediate (1000 to 2000 g/0.1 ha) at 10 sites, and high (> 2000 g/0.1 ha) at 10 sites (Figure 3B).

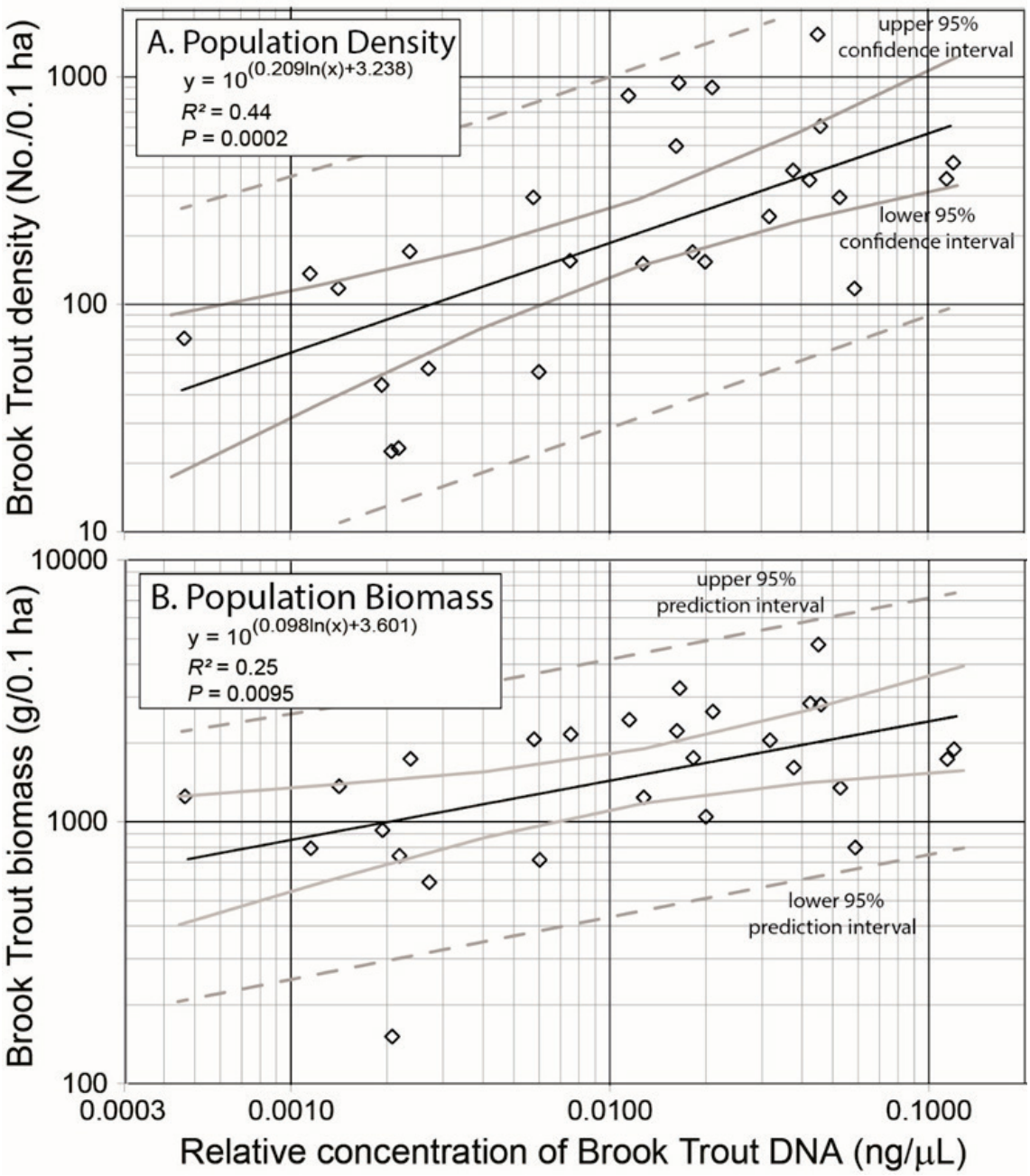
Figure 3. Estimates of Brook Trout population (A) density and (B) biomass from 40 streams surveyed in the Adirondack Mountains during summers of 2014 and 2015 (from Baldigo & others 2017).



The eDNA results from a single water sample collected from each site between 8/25/2015 to 9/2/2015 correctly classified the presence/absence of Brook Trout at 85 percent of the 40 sites. Three of the mis-classified sites were resampled 9/21/15 using multiple field replicates and this effort classified Brook Trout presence/absence at those sites. Following this supplemental sampling effort, eDNA correctly detected the presence of Brook Trout at 27 of 30 sites (90 percent correct classification) where their populations were evident and confirmed the absence of Brook Trout at all 10 sites (100 percent correct classification). The analysis of eDNA from sediment samples failed to detect Brook Trout DNA at 10 out of 10 sites (9 of which were known to contain Brook Trout) and was therefore discontinued.

The relative concentration of Brook Trout DNA could explain a moderate amount of variability (44 percent) in density of local Brook Trout populations (Figure 4A), but only 25 percent of the variability in population biomass (Figure 4B). These differences indicate that the amount of genetic material suspended in the water column corresponded more closely to the number of resident individuals than to the total mass of resident individuals. The resulting models (equations), 95 percent confidence intervals, and 95 percent prediction intervals indicate that eDNA could be used to predict the abundance (with known levels of error) of Brook Trout populations in these and other streams of the region.

Figure 4. Relations between the relative concentration of Brook Trout eDNA and quantitative estimates of (A) density and (B) biomass for Brook Trout populations in 40 streams of the Adirondack Mountains (from Baldigo & others 2017).



6 Project Implications

Our findings have a number of important implications for monitoring and assessing Brook Trout populations (and entire fish assemblages) in streams of the Adirondack Mountains and elsewhere. First, our eDNA results indicate that water is a more effective sampling media than bed sediments in small headwater streams. Whether sediments do not retain genetic material eliminated from Brook Trout or our analytical methods were ineffective at detecting eDNA in sediments, most assessments of Brook Trout presence/absence and abundance in headwater streams should probably avoid using bed sediments until the issue is better understood. Second, the increased accuracy of eDNA results following collection of replicate samples indicates that large sample (filtered) volumes and/or multiple field replicates should be considered where the target species are expected to exist in low numbers or the volume of occupied habitat is very large. Third, the ability of eDNA to accurately detect the presence of Brook Trout at very low densities (1-2 fish/100 m^2) means that the method is well-suited for assessing population distributions across large regions and presence at remote sites where gaining access with large crews and burdensome gear may be problematic. Fourth, the moderately strong relationships between relative concentrations of eDNA and Brook Trout density and biomass make eDNA an effective means to estimate population density and biomass. Though uncertainty (95 percent confidence intervals) around the modeled lines is relatively low, the log scale makes the uncertainty around actual predictions of population densities or biomass for any given relative eDNA concentration quite large. Thus, the use of both models may be most appropriate for inferring the relative abundance (i.e., the absence or low, moderate, or high densities and biomass) of Brook Trout populations in streams of the region. However, quantitative fish surveys are often indispensable for estimating the density and biomass of Brook Trout (and other species) populations when the relations between population (or community) metrics and predictor variables, such as stream discharge, chemistry, temperature, or toxicity need to be accurately characterized. Key objectives of research and monitoring studies or programs will dictate the quality and accuracy of needed fishery data and, thus, determine whether the use of eDNA or traditional survey methods are most appropriate. The scope of the project and funding levels for such efforts will also factor into these decisions. The fifth implication is that eDNA can provide a large cost benefit over traditional fish-survey methods. Though sampling efforts vary widely with stream access, reach area, and fish abundance, the costs for an electrofishing survey at a single stream site might range from $500 to $3,000 USD (2016). In contrast, our experience, and that of other researchers, indicate the costs for analyzing (not collecting

and transporting) an eDNA sample might range from $20 to $50 USD. Even if our per-sample collection and transportation costs (about $90 USD) were factored into the estimate, there would still be a large cost benefit for focusing on eDNA samples, especially for broad regional inventories of a single species at hundreds of sites.

Results from the present study, and those from other investigations, point out a variety of limitations or issues with eDNA surveillance programs, which need further study, development, or improvement. First, the reason behind the absence of (or our inability to detect) Brook Trout DNA in stream sediments is difficult to comprehend and needs to be further explored. Second, current eDNA monitoring efforts typically focus on a single aquatic species due to limitations of the DNA amplification, isolation, and PCR quantification methods. Though it may now be possible to detect eDNA for three or four species from an individual eDNA sample using PCR, and presence/absence information for common species can be obtained using next-generation DNA sequencing, the inability of genomic methods to qualify densities of more than a few species is a major deficiency. Metagenomic methodologies that would enable us to detect and qualify all fish species at a site are needed so they could generate metrics for entire fish assemblages, and other biotic communities from one or only a few eDNA samples. Third, additional investigations are also needed to devise, refine, or standardize effective eDNA sampling methods. Currently, the volumes of water filtered, types of sampling devices (e.g., filters, centrifugation), and time needed to collect eDNA samples can vary widely. More information on the persistence of eDNA under different environmental conditions and types of surface waters could help to standardize sampling and analysis procedures. Fourth, our knowledge of the environmental factors that affect eDNA persistence or degradation in the field and inhibit DNA amplification in the laboratory has increased steadily over the past decade, but is still far from complete. Most of the information gaps and technical challenges that remain, however, should be addressed in the not-so-distant future given the rapid rate at which the field of eDNA research is evolving. Thus, eDNA methods appear to have enormous potential to change the way we monitor and assess the status of species populations, communities, and entire ecosystems.

7 Conclusions

Sampling eDNA in water from the small streams in our study was highly effective in detecting resident Brook Trout populations. Test results from single and replicated water samples accurately predicted the presence/absence of Brook Trout in 92.5 percent of study streams. By filtering relatively large volumes of water, (up to six liters), Brook Trout could be accurately detected even at low abundances. A minimum detection threshold was shown to be about 1 or 2 Brook Trout per 100 square meters of stream habitat.

Water samples contained variable amounts of Brook Trout DNA, and this amount loosely correlated with the density of Brook Trout in the stream. Thus, measurement of eDNA can be used not only to detect presence/absence, but also to roughly predict the abundance of resident Brook Trout populations in streams of the region. For example, detection of a low amount of Brook Trout DNA (0.001 ng) would predict a range from 10 to 350 fish per 0.1 hectare, whereas 100-fold higher Brook Trout DNA (0.1 ng) would predict a range from 90 to 3,000 fish per 0.1 hectare.

Sampling and analysis of eDNA is relatively fast and affordable, and can be much more cost effective than electrofishing surveys when exploring the presence/absence, or the relative abundance, of one or a few species at a large number of study sites. At present, standard electrofishing surveys are still the most effective tool to use when quantitative information on entire fish communities or individual species populations (with known levels of error) are needed for hypothesis-testing studies or long-term ecological monitoring programs in streams.

8 Citations

Baldigo, B. P., L. A. Sporn, S. D. George, and J. A. Ball. 2017. Efficacy of environmental DNA to detect and quantify Brook Trout Populations in Headwater Streams of the Adirondack Mountains, New York. Transactions of the American Fisheries Society 146(1):99-111.

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