# Case Study: Brook Trout

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## *Detecting brook trout on-site*

*using our point-of-need environmental DNA (eDNA) detection platform*

## Background

The brook trout (*Salvelinus fontinalis*) is a common cold-water fish native to Ontario. Brook trout are dark olive-green dorsally, with pale spots and red dots surrounded by blue halos along the flanks. Their fins are red with distinctive white-leading edges (Fig 1).

The species is distributed all around Ontario, from small brooks of southern farmlands to the larger rivers, ponds and lakes of the north. However, brook trout live and thrive only in very clean and cold waters. Biologically, they are considered an indicator species due to their exceptional sensitivity to environmental changes, thus revealing the overall health of the ecosystem. Major threats to the species include habitat loss, introduced species, and the increase in water temperature due to climate change.

Fig 1. Brook trout (*Salvelinus fontinalis*)

## How can Precision Biomonitoring help?

Precision Biomonitoring has developed a sensitive assay for the detection of brook trout DNA from water samples. Using our point-of-need eDNA tool, we can provide real-time confirmation of the presence of DNA from this species within two hours including water sampling. Our point-of-need platform will expedite efforts to delimit brook trout distributions, as the species can be detected quickly, accurately and in real-time by taking only water samples.

Our Triple-Lock™ molecular assays, designed for qPCR, have many advantages: a) high specificity to discriminate between brook trout and other, closely-related and co-occurring species and b) extreme sensitivity to detect fewer than ten individual brook trout gene fragments per sample. Our assay species-specific DNA primers and probes are designed and validated to detect the presence of only brook trout DNA fragments present in the water column. Sampling eDNA—DNA that is shed by organisms through daily physiological processes—is advantageous because it can be used to monitor species presence without capture or visual observation, across populations, during different seasons and at varying stages of the life cycle. eDNA sampling can be applied in lake, river and marine environments, and is highly sensitive relative to conventional methods (e.g., netting, electrofishing) and requires less labour. Hence, chief benefits of this approach include reliability, time-saving and cost effectiveness.

Our eDNA detection platform is a significant advance other the status quo in eDNA detection methods, in that results can be achieved in hours rather than weeks. The system includes use of a positive control and negative template to guard against false positives and false negatives. It can be widely and synchronously implemented, with a facility for

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cloud—based sharing of data. Our point-of-need platform features highly portable, battery-charged handheld thermocyclers that perform the thermomechanics of the molecular biological assay. These machines display the result graphically in real-time and transfer the data immediately to a host data portal. Precision Biomonitoring can facilitate monitoring programs by using extremely sensitive molecular-based assays to increase the scalability of ongoing and future surveillance efforts, while also allowing for more resource effective management plans to be enacted.

## How has the brook trout assay been validated and applied?

Our team developed a **species-specific eDNA assay** using a mitochondrial gene to identify brook trout. The assay was first **lab-validated** and tested for specificity using reliable tissue samples from brook trout and other non-target species collected via conventional electrofishing.

In collaboration with conservation and local authorities, we **field validated** the brook trout assay in a two stream-system in Northern Ontario with contemporary records of the presence or absence of Brook trout.

During September 2017, a total of 14 sites were tested for brook trout eDNA (Table 1). For 11 sites, there are contemporary records of the presence of brook trout by conventional fishing methods (Stream 1, sites 1-10). One site located downstream (Stream 1, site 11) is considered unsuitable for the species, and brook trout have not been caught there. Three additional negative control sites were also sampled (Stream 2, sites 12-14). Two 1L water samples were taken per site using the Smith-Root ANDe™ water sampling system, and three eDNA assay replicates per water sample were analyzed using qPCR. To corroborate the performance of the test, both **positive and negative template controls** were used along with the samples of interest. Additionally, immediately after taking eDNA samples in the field, a two-pass electrofishing approach was taken to capture all fishes in both stream-systems. At each site, a ten-metre section of stream was isolated via the placement of upstream and downstream blocking nets to prevent escape. Sampling progressed in a downstream-upstream trajectory to ensure no translocation of 'foreign' eDNA to sites that had not been sampled. Fishes were then identified to species-level. After completion, all fish were released back into the section in which they were caught.

After using our eDNA point-of-need tool, we detected Brook trout in 2.5 times more sites than observations using conventional electrofishing (Table 1). No brook trout was detected in Stream 2 as expected. Our results agree with the literature suggesting that eDNA has a higher sensitivity of detection compared with conventional methods; thus, supporting the use of eDNA as an appropriate tool for monitoring aquatic diversity.

Table 1. Results from brook trout assay validation.

| Collection site | | Brook trout status | |
|---|---|---|---|
| Stream | Site | Electrofishing | eDNA |
| 1 | 1 | NO | NO |
| 1 | 2 | YES | YES |
| 1 | 3 | NO | NO |
| 1 | 4 | NO | NO |
| 1 | 5 | NO | YES |
| 1 | 6 | NO | NO |
| 1 | 7 | NO | YES |
| 1 | 8 | YES | YES |
| 1 | 9 | NO | NO |
| 1 | 10 | NO | NO |
| 1 | 11 | NO | YES |
| 2 | 12 | NO | NO |
| 2 | 13 | NO | NO |
| 2 | 14 | NO | NO |

**For more information on our eDNA platform or for interest in detection of other species contact us**

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