# Case Study: Jefferson salamander

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## *Detecting Jefferson salamander on-site*

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

## Background

The Jefferson salamander (*Ambystoma jeffersonianum*) is characterized by a dark grey or brown back with lighter under-parts, with light bluish-grey flecks along the flanks and tail in some individuals (Fig 1). The species is distributed across northeastern U.S. and southern Ontario (Fig 2; MNRF), inhabiting deciduous or mixed upland forest with access to suitable breeding ponds. Breeding ponds are ephemeral or vernal, woodland pools that dry out in late summer/early fall. The species is in serious decline due to habitat loss, pollution of breeding ponds and disturbance to migratory routes. As such, the species is listed as Endangered (Species at Risk Act, SARA). Jefferson salamander also co-occur with *A. laterale* and unisexual forms of *Ambystoma*; the Jefferson salamander-dependent unisexual form is also now protected under the Ontario Endangered Species Act. Note that these two forms are morphologically indistinguishable from each other and other unisexual forms which are not protected, complicating conservation efforts.

Fig 1. Jefferson salamander (*Ambystoma jeffersonianum*).

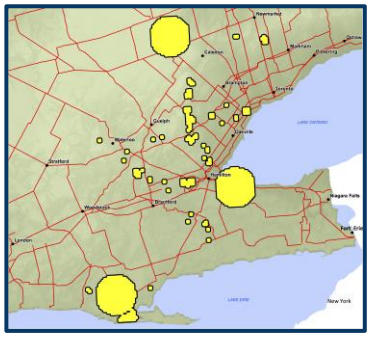

Fig 2. Distribution of Jefferson salamander in Ontario Canada, (Image modified from the Ministry of Natural Resources and Forestry, www.ontario.ca/speciesatrisk).

## How can Precision Biomonitoring help?

Precision Biomonitoring has developed a sensitive assay for the detection of Jefferson salamander 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 Jefferson salamander distributions, as the species can be detected quickly, accurately and in real-time by taking only water samples.

Our triple-lock™ molecular assay, designed for qPCR, have many advantages: a) high specificity to discriminate between Jefferson salamander and closely-related and co-occurring species, including the blue spotted *A. laterale* and unisexual hybrids, and b) extreme sensitivity to detect fewer than ten individual Jefferson salamander gene fragments per sample. Our assay species-specific DNA primers and probes are designed and validated to detect the presence of only Jefferson salamander 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 their life cycles. 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 labor. Hence, chief benefits of this approach include reliability, time-saving and cost effectiveness.

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Our eDNA detection platform is a significant advance over current eDNA detection methods because 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 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 Jefferson salamander assay been validated and applied?

Our team developed a **species-specific eDNA assay** using a mitochondrial marker to identify Jefferson salamander. The assay was first **lab-validated** and tested for specificity using reliable tissue samples from Jefferson salamander and other non-target species provided by Dr. Jim Bogart from the University of Guelph. In collaboration with Stantec Consulting Limited staff in Guelph, Ontario, and conservation and local authorities, we **field validated** the Jefferson salamander assay in ten sites in southern Ontario to indicate whether they were known to be present in the area.

During May, July and September 2017, a total of ten sites were tested for Jefferson salamander eDNA (Table 1). Four sites had historical records (from 2011) of the presence of Jefferson salamander. The remaining six sites did not have historical records of Jefferson salamander. Three 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 the Biomeme two3™ thermocycler. To corroborate the performance of the test, both **positive and negative template controls** were used along with the samples of interest. After using our point-of-need eDNA tool, we detected the target species in all sites with previous historical records of Jefferson salamander (Table 1). In those sites, the presence of Jefferson salamander was further confirmed by non-invasive genetic characterization of empty salamander egg jelly (Sollen et al in prep) using our eDNA marker. Furthermore, we sequenced DNA samples from one pool (marked with an asterisk in Table 1), using Sanger sequencing, to further corroborate the positive results. In addition, we detected the presence of eDNA from Jefferson salamander in one site for which a 2011 historical record did not. This result highlights the sensitivity of our method and the capability to monitor species presence without capture or visual observation. Our **results are overall highly concordant** with previous records of presence/absence of the Jefferson salamander in southern Ontario.

Table 1. Results from Jefferson salamander assay validation.

| Collection site | | Jefferson salamander status | |
|---|---|---|---|
| Authority | Pool | Historical | eDNA |
| 1 | A | YES | YES |
| 1 | B | NO | NO |
| 1 | C | NO | NO |
| 1 | D | NO | NO |
| 2 | E* | YES | YES |
| 2 | F | YES | YES |
| 2 | G | NO | NO |
| 2 | H | NO | NO |
| 2 | I | YES | YES |
| 2 | J | NO | YES |

Fig 3. The Biomeme two3™ thermocycler displaying qPCR results.


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

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