Case Study: Redside Dace

Precision Biomonitoring Inc. precisionbiomonitoring.com info@precisionbiomonitoring.com

Detecting redside dace on-site

Using our mobile eDNA detection platform

Background

The redside dace (*Clinostomus elongatus)* is a species of minnow-like fish that is found in small streams in a few isolated watersheds in Ontario. Its appearance is characterized by a half-body length lateral red stripe below a full length yellow streak (Fig 1). Approximately 80% of the Canadian populations are located in the 'golden horseshoe region' of southwestern Ontario (Fig 2; MNRF). The species is in serious decline with extirpations recorded in historic locations. As such, the species is listed as Endangered (Species at risk act, SARA). The redside dace is especially vulnerable to changes in habitat. Its current distribution is at risk of further diminishment due to the continuing urbanization and development in the 'golden horseshoe'.

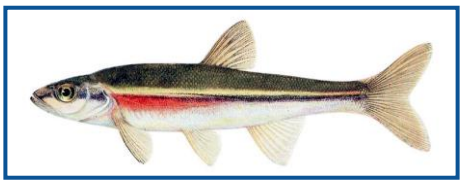

Fig 1. Redside Dace (*Clinostomus elongatus)*

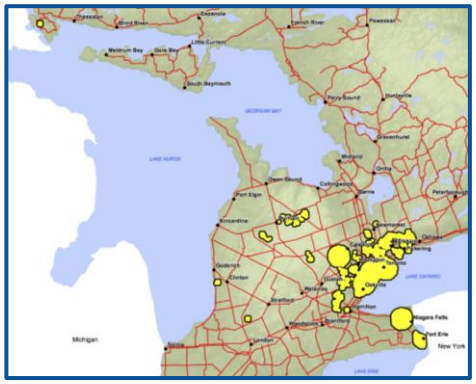

Fig 2. Distribution of Redside Dace in Ontario, Canada (Image modified from the Ministry of Natural Resources and Forestry, www.ontario.ca/speciesatrisk).

How can Precision Biomonitoring assist?

Precision Biomonitoring has developed a sensitive assay for the detection of redside dace DNA from water samples. Using our eDNA point-of-need tool, we can provide in-the-field confirmation of the presence of DNA from this species within two hours after a water sample has been taken. Thus, our on-site platform will expedite efforts to delimit redside dace 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, possess: a) high specificity to discriminate between redside dace and other, closely-related and co-occurring species and b) extreme sensitivity to detect fewer than ten individual redside dace gene fragments per reaction. Our assay is predicated on the design of species-specific DNA primers and probes that will detect the presence of only redside dace DNA fragments present in the water column (environmental DNA). Sampling environmental DNA (eDNA)—DNA that is shed by organisms through daily physiological processes—is advantageous because it can be used to monitor species throughout the year, across populations, during different seasons and at varying stages of their life cycles. It can be equally applied in lake, river and marine environments, and is highly sensitive relative to conventional methods (e.g., netting, electrofishing) and potentially much more cost-effective. Hence, chief benefits of this approach include indirect sampling of the target species, reducing environmental impacts of surveys as no individual fish is caught, zero bycatch mortality, and remote and difficult-to-access environments can be sampled.

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 is

PRECISION BIOMONITORING

less prone to false results and can be widely and synchronously implemented, with a facility for cloud—based sharing of data. Our on-site platform features highly portable, battery-charged thermocycling machines that perform the thermomechanics of the molecular biological assay. These machines will display the result graphically in real-time and also be able to 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 redside dace assay been validated and applied?

Our team developed a **species-specific eDNA assay** using a mitochondrial gene to identify redside dace. The assay was first **lab-validated** and tested for specificity using reliable tissue samples from redside dace and other non-target species provided by the Royal Ontario Museum (ROM).

In collaboration with conservationists and local authorities, we **field validated** the redside dace assay in three watersheds in southwestern Ontario that had recent catch data (in the last twenty years) to indicate whether they were present in the area.

During August 2017, a total of 13 sites were tested for redside dace eDNA (Table 1). For seven sites, there are historical records of the presence of redside dace by conventionalmethods (watershed 2). 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 on the Biomeme two3™ thermocycler . To corroborate the performance of the test, both **positive and no template controls** were used along with the samples of interest. After using our eDNA point-of-need tool, five of the sseven sites with historical records located near Lake Ontario detected the target species (Table 1). There are not historical records of the presence of redside dace in the six sites near Lake Erie (watershed 3) and eDNA testing confirmed this. There are several potential reasons why redside dace was not detected using eDNA in two sites near Lake Ontario (watershed 1): a) redside dace had gone locally extinct in those sites, b) there is potential for DNA inhibition due to local physical/chemical characteristics of the sites, and c) more sampling effort would be necessary to detect very rare DNA fragments. Thus, our **results are largely concordant** with previous records of presence/absence of the redside dace in southwestern Ontario.

Table 1. Results from redside dace assay validation.

Collection site		Redside dace status	
Watershed	Site	Historical	eDNA
1	1	YES	YES
1	2	YES	YES
1	3	YES	YES
1	4	YES	NO
1	5	YES	NO
2	6	YES	YES
2	7	YES	YES
3	8	NO	NO
3	9	NO	NO
3	10	NO	NO
3	11	NO	NO
3	12	NO	NO
3	13	NO	NO

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

Precision Biomonitoring Inc.

1-647-466-8008
info@precisionbiomonitoring.com
www.precisionbiomonitoring.com
Twiiter: @eDNAmonitoring

PRECISION BIOMONITORING