



Environmental DNA (eDNA) Analysis for Detection of Invasive Species

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The Federation of Cottage Associations (FOCA) invited the White Lake Property Owners Association to participate in a series of experiments in an effort to apply eDNA techniques to the early detection of the presence of aquatic invasive species.

Below you will find the full report for the 2024 sampling program which screened for the presence of zebra mussels and the spiny waterflea. For this sampling round, only five lakes were invited to participate.

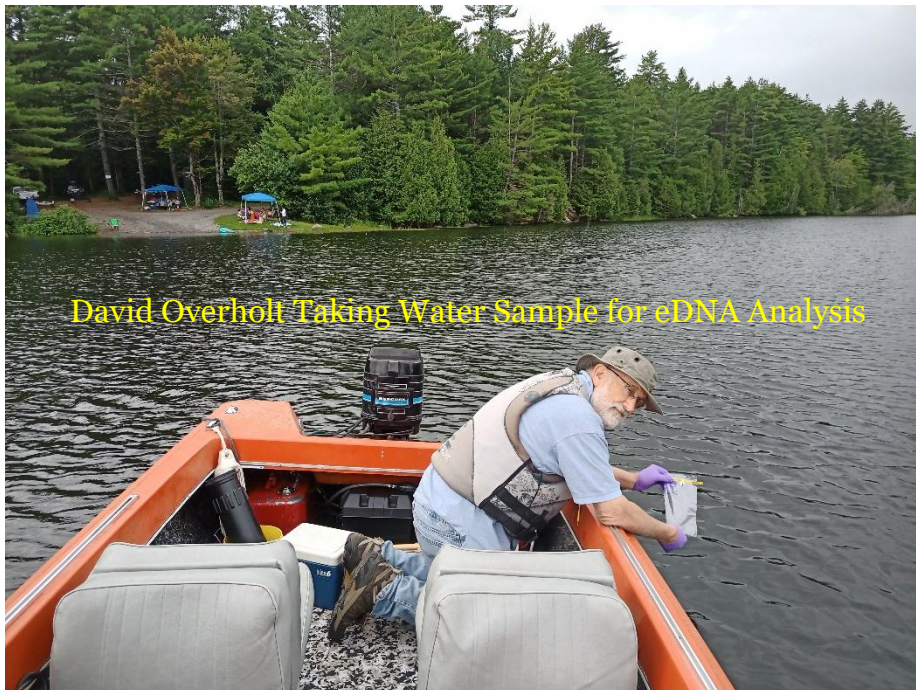
As expected, White Lake tested positive for zebra mussels as this invasive species has been well established in the lake since 2015. White Lake tested negative for spiny waterflea. We have not received any reports of the presence of this invasive nor have we observed them in the field.

We also participated in a 2023 sampling round along with 75 other lakes. This sampling focussed on the detection of water soldier, a very aggressive aquatic plant. The results from this study are not reproduced here because the results were non-conclusive for the three lakes testing positive, White Lake being one of them. Laboratory contamination was cited for the inconclusive results. We have not observed the presence of this plant in White Lake.

For the 2024 study, water and plankton net samples were taken at three separate locations on the lake. The photos below show the array of sampling and sample processing equipment used in this study.



eDNA Sampling and Processing Equipment



David Overholt Taking Water Sample for eDNA Analysis

Invasive Species Centre:

eDNA and Zooplankton Sampling Results 2024-2025

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Background

The IsampleON Project, a collaboration between the Invasive Species Centre (ISC), and the Federation of Ontario Cottagers' Associations (FOCA), was piloted in 2021 to help prevent zebra mussels, spiny waterflea, and other aquatic invasive species from becoming established in inland lakes and gain a better understanding of distribution. The main project focus is the collection and analysis of water samples by FOCA volunteers through the Lake Partner Program, while raising awareness about the importance of AIS prevention. This year's focus was to study the accuracy of different sampling methods in detecting the presence of zebra mussels in Ontario lakes. The two sampling methods compared were traditional plankton towing nets and eDNA analysis.

Traditional Zooplankton Monitoring

Each lake was sampled in three locations: boat launch, windward, and deep water, and each of the 3 samples is tested 3 times to increase accuracy.

Densities for positively tested lakes are measured based on the average number of individual species present within each sample taken and labelled accordingly as low, medium, or high density. Zebra mussel (ZM) densities were determined as such: 1-10 zebra mussels = low density; 10> = medium density; 20> = high density. Spiny waterflea (SWF) densities were determined as such: 1-4 spiny waterfleas = low density; 4> = medium density; 7> = high density.

eDNA Monitoring

Environmental DNA (eDNA) comes from cellular material that is released by living or dead organisms into the environment. This can include tissue, mucus, hair, reproductive cells, feces, and/or urine. eDNA monitoring is a method of sampling which can detect genetic material shed by organisms into the environment. Using eDNA monitoring allows us to detect invasive species early on in the invasion process, from even small amounts of genetic material. This is a game-changing tool for early detection of aquatic invasive species. eDNA monitoring is quick, easy, and cost efficient. However, it does have limitations such as being unable to quantify the number of organisms present, it cannot indicate the life stage of a species or distinguish between hybrids and non-hybrids, or closely related species and it is unable to determine whether that organism remains in the waterbody. For example, the organism may have passed through or died. Further reading on eDNA monitoring can be found on our website.

For eDNA sample processing, Nature Metrics Laboratory was used to analyze samples for the presence of zebra mussels. Spiny waterflea presence was visually identified and therefore not part of the eDNA component of the study. If a positive eDNA sample for zebra mussels was identified, this means that this invasive species might have been in your lake.

See the sections below about *Reading the Results* and *Interpreting Positive/Negative Results*.

Reading the Results

There are two results tables included in this report. Table 1 is a summary of the traditional zooplankton monitoring results for zebra mussel veligers and spiny waterflea, and Table 2 is a summary of eDNA monitoring for zebra mussels.

Find your lake name listed in the first column.

In Table 1 the two columns on the far right show the results for:

- **Zebra Mussel Veliger** shows whether there were larva present from zebra mussels in your sample. This method does not detect genetic material like eDNA.
- **Spiny Waterflea Visual** shows whether there were spiny waterflea present in your sample. This method does not detect genetic material like eDNA.

In Table 2 the right column shows the results for:

- **Zebra Mussel eDNA** shows whether there was zebra mussel genetic material present in your sample.

Negative means the sample did not have one of the tested species in it.

Positive means the sample did have one of the tested species in it, and the density found was indicated for traditional sampling.

Different sampling types provide different forms of information. For example, traditional zooplankton monitoring for zebra mussel veligers only picks up a specific life stage (e.g. larval stage), whereas eDNA sampling picks up all stages of life. However, eDNA sampling will only detect genetic material before it falls apart. It is possible that zebra mussels could shed genetic material into your lake, but it fell apart before you had the chance to capture it in your eDNA sample. Zebra mussel veligers are detectable over a longer period of time.

Additionally, zebra mussel veliger and eDNA results come from different samples of water, therefore different parts of the lake. While a positive result means that the sample had signs of an invasive species in it, that doesn't mean that every sample taken from the lake will have the same result. This is why two different types of samples taken from the same lake may not have the same result. A positive sample from any sampling type is reason enough to take precautions.

Interpreting Positive Results

A positive sample does not mean that an invasive species has established in your lake.

To verify whether the organism is present, we recommend follow-up sampling to visually confirm the extent of invasion. Substrate sampling with dock hangers is one way to do so for invasive zebra mussels and we recommend a wider distribution of sampling throughout the lake.

Only once you have visually confirmed zebra mussels and/or spiny waterflea, we recommend reporting your sighting through one of the sources below.

As always, we advise preparing for possible negative impacts and we strongly encourage you to address possible sources of introduction and spread.

Interpreting Negative Results

Negative samples do not mean invasive zebra mussels and/or spiny waterflea are absent either. This result only means that they were not captured in the samples. Invasive species could be elsewhere in your lake or too low in abundance to detect.

Monitoring is an ongoing responsibility. Continue to keep a watchful eye for signs of invasive species and practice preventative measures like **Clean, Drain, Dry**.

Report invasive species to:

- [EDDMapS](#) App or Website
- [iNaturalist](#) App or Website
- Ontario’s Invading Species Awareness Hotline 1 (800) 563-7711

Zooplankton Results Summary

All samples were processed in-house by the Invasive Species Centre. Five lakes were surveyed, and from this our results found:

- 1 lake was positive for zebra mussels
- 0 lakes were positive for spiny waterflea
- 0 lakes were positive for both zebra mussels and spiny waterflea
- 4 lakes were negative for both species

Zooplankton Results Table

Table 1: Traditional zooplankton monitoring results from the 2024 IsampleON Program.	Zebra Mussel Veliger	Spiny Waterflea Visual
Sample ID		
Mary Lake	NEGATIVE	NEGATIVE

White Lake	POSITIVE (HIGH DENSITY)	NEGATIVE
Lake Wahnapiatae	NEGATIVE	NEGATIVE
Big Gull Lake	NEGATIVE	NEGATIVE
Billings Lake	NEGATIVE	NEGATIVE

eDNA Results Summary

All samples were processed by Nature Metrics Laboratory. Five lakes were surveyed, and from this the results were:

- 1 lake was positive for zebra mussels

eDNA Results Table

Table 2: eDNA monitoring results for zebra mussels from the 2024 IsampleON Program. Sample ID	Zebra Mussel eDNA
Mary Lake	NEGATIVE
White Lake	POSITIVE
Lake Wahnapiatae	NEGATIVE
Big Gull Lake	NEGATIVE
Billings Lake	NEGATIVE

Comparison of eDNA and Traditional Zooplankton Monitoring Techniques

This year's focus was to study the accuracy of different sampling methods in detecting the presence of zebra mussels in Ontario lakes. The two sampling methods compared were traditional plankton towing nets and eDNA analysis. While traditional sampling can provide a positive visual identification at the larval stage, eDNA is able to detect genetic material at all life stages and can detect species that are present in low numbers.

Both eDNA and traditional zooplankton monitoring provided a positive result for White Lake and were negative for the other four lakes. A positive eDNA detection means that DNA from the species of interest was present at the time of sampling. A negative eDNA detection means that the species of interest could be absent from the location and/or there were inadequate amounts of genetic material shed in the environment, or it had broken down before sampling. In this report, both methods had the same result, which demonstrates that they may be used together to provide an additional metric of validation.

Positive: Now What?

Now that you know invasive zebra mussels are present the most important actions are to prevent the spread to neighbouring lakes. Make other cottagers on the lake and visitors to

the lake aware of the presence of invasive aquatic species. Add signage at public boat launches that state the presence of invasive zebra mussels and/or spiny waterflea and the importance of **cleaning, draining and drying** your boat and equipment. Consider setting up for boat washing at your public boat launches. Knowledge is key.

Generally, when zebra mussel veligers are first detected in a lake, there is about a two-year period before adult mussels become visible. Therefore, if veligers are found in your lake, it will give you a chance to prepare for the negative impacts of zebra mussels.

However, it cannot be overemphasized that a negative result is NOT a guarantee that zebra mussels, spiny waterflea and other invading species do not exist in your lake, proper precautions should always be taken in order to prevent the potential spread of invading aquatic species.

The presence of invasive zebra mussels in your lake may also mean some extra work on your part. Zebra mussels can attach to many hard surfaces that can cause clogging of water intake pipes and mechanical issues with boat motors. The good news is there are some tools and strategies you can use to help mitigate some of these consequences.

- **Intake lines and foot valve maintenance:** Draining and drying water intake lines and foot valves can help reduce the risk of blockages. Freezing lines and foot valves after draining is even more effective than simply drying.
- **Water intake pipe filter:** Consider a filter for your intake pipes that feed your cottage water supply to prevent mussels from attaching to the inside of the pipe. This works well for year-round cottage use and with planned maintenance.
- **Lift motor or boat out of water:** After each use, lift your boat motor, propellers and all, up out of the water to decrease the chances of invasive mussels attaching themselves to the motor. If possible, consider investing in a boat lift or ramp to completely remove the boat from the water.
- **Flush boat motor regularly:** Microscopic veligers can be drawn up through the water inlet of the motor and settle inside, causing blockages as it matures. Frequently using a motor flusher or motor muffs can decrease the risk of this occurring.
- **Wear water shoes:** Mussels can have very sharp shells that hurt to step on, especially for children. Invasive mussel populations can grow in such abundance they become difficult to avoid. Consider wearing protection on your feet such as water shoes to avoid injury.

Although there are no recommended management options available in Ontario at this time, there is some research and pilot projects being done in the United States. Check out the

[Invasive Mussel Collaborative](#) to learn more about the projects that are underway and potential future management options.

We thank IsampleON for the opportunity to collaborate on this important diagnostic work. Your role helps protect Ontario lakes from aquatic invasive species.

For questions on anything represented in this report please contact:

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