



2023 IsampleON – eDNA Results for Water Soldier

BACKGROUND

IsampleON is a project that engages volunteers in monitoring efforts to help prevent the spread of invasive species. Environmental DNA (eDNA) monitoring was piloted during this monitoring program. For more information about eDNA, see the section “*What is eDNA monitoring?*”

Efforts from 2023 helped monitor for the invasive plant “water soldier.” Early detection of this plant is critical to ensure we stop its destructive spread. From the combined effort of volunteers, we collected samples from 76 locations. Thank you to all of the volunteers!

WHAT IS eDNA MONITORING?

eDNA monitoring is a method of sampling to detect genetic material shed by organisms into the environment. Genetic material can be detected from any cells that are shed from organisms. Using eDNA grants us the opportunity to get the jump on invasive species because it can support detection early in the invasion process from even small amounts of genetic material. eDNA slots in well for early detection in part because it is quick, easy and it detects specific species, like invasive species.

However, it does not tell us the number of organisms or whether that organism has persisted in the body of water. For example, the organism may have since passed through or died. Also, due to the advanced nature of detecting trace amounts of DNA, eDNA analysis is an incredibly sensitive process that can easily be contaminated by external sources, such as someone’s gloves or equipment that has been used in another lake.

2023 SAMPLING PROGRAM GOALS

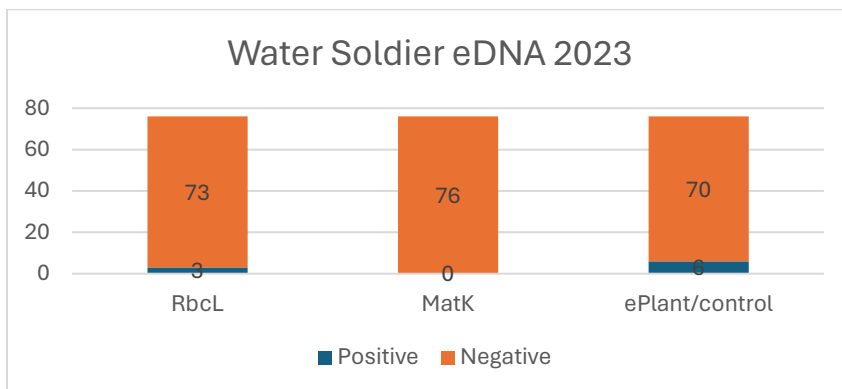
In the case of IsampleON 2023, we looked for all types of eDNA shed by water soldier into the water of your lake. If we found eDNA of one of these species, it means that the invasive species might have been in your lake. See the sections below about “*Reading the Results*” and “*Interpreting Positive/Negative Samples*”.

If you were a volunteer who conducted eDNA sampling, you filtered water from your lake through a filter following the [Aquatic eDNA Participant Guide](#). For more information about eDNA, check out our [Aquatic eDNA Factsheet](#).

LAB REPORT

Purpose and Assay Development: Seventy-six samples were received from FOCA (per attached Table) and tested for the presence of Water Soldier DNA using an eDNA assay developed in-house (Green 2024, undergraduate thesis)*. Briefly, a quantitative polymerase chain reaction (qPCR) assay was developed using sequences for two chloroplast DNA (cpDNA) targets, RbcL and MatK in Water Soldier, in context of controls to test the amount of chloroplast DNA present in the sample (ePlant) and an internal positive control (IPC). These extra control measures help assess for false negative lab results by evaluating the quality of the submitted samples. The assay was tested on known Water Soldier samples and other closely related species to ensure both sensitivity and specificity of assay, where some minor cross-amplification of other species was detected, but not for both target markers, thus suggesting that, holistically, the developed assay was specific for the target species. The assay was also tested on filtered water samples known to contain Water Soldier to ensure that the assay was able to detect this species under the experimental conditions.

Executive Summary of Results: Of the 76 tested FOCA samples, 1/76 was deemed inappropriate for interpretation based on control results, 6/76 samples yielded poor amplification in either the ePlant or IPC control, where results for the target species should be interpreted with caution; 3/76 samples showed trace amplification of the RbcL target only, thus these samples are deemed inconclusive without additional testing. RbcL testing shows reliable amplification down to 5 copies of target material, so values under this are listed as trace amplification. 0/76 samples showed amplification at both targets. In the absence of further testing and samples (as required for clearer interpretation of results and absence/presence assessments of Water Soldier), we take these data to suggest no Water Soldier DNA was detected in the submitted samples above the threshold of detection of the developed assay. More detailed test results are provided in the attached table and summarized in the attached flow chart.



Results: Due to none of the samples testing positive for both the RbcL and MatK marker, none indicate a confirmed positive detection for water soldier. The 6 samples that amplified weakly via the ePlant/IPC control indicate inconclusive results.

* Green, W (2024). *eDNA Detection Assays for the Invasive Aquatic Plant, Water Soldier (Stratiotes aloides): Enabling early detection and intervention with molecular tools. Undergraduate Thesis, BSc FSc. Trent University.*

READING THE RESULTS

A table of the results is found in Table 1 of this document (see pages 3 and 4).

Find your lake name listed in the first column.

The second column lists the results for each lake.

“*Negative*” means the sample analysis did not detect water soldier.

“*Inconclusive*” means the sample analysis determined a small/trace amount of DNA possibly associated with water soldier.

INTERPRETING POSITIVE/ INCONCLUSIVE RESULTS

Positive/inconclusive samples do not indicate whether the 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.

Only once you have visually confirmed water soldier, 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 sources of possible introduction and spread. Monitoring is an ongoing responsibility. Continue to keep a watchful eye out for signs of invasive species and practice preventative measures like [Clean, Drain, Dry](#).

INTERPRETING NEGATIVE RESULTS

Negative samples do not mean water soldier is absent either. This result only means that it was not captured in the samples. Invasive species could be elsewhere in your lake or too low in abundance to detect.

eDNA is a much better tool for presence sampling rather than confirming absence. It is very difficult to get a “false positive” sample but can occur due to contamination in any of the field sampling and lab analysis processes.

Report invasive species to:

- [EDDMapS](#) App or Webpage
(*Early Detection and Distribution Mapping Systems*)
- [iNaturalist](#) App
- Invading Species Awareness Hotline:
1-800-563-7711

Thank you for participating in IsampleON 2023.

Your role helps protect Ontario lakes from aquatic invasive species.

IsampleON 2023 - Table 1: results (over two pages)

Important Reminders about the Results:

- Inconclusive results can be interpreted as having trace amounts of water soldier DNA present, but this can be due to many factors, including contamination. Inconclusive results do not indicate a positive detection of water soldier. Further testing would need to be done to indicate this.
- **In the lakes with inconclusive results (3 of 76) we recommend visual surveying for water soldier.** Although inconclusive results likely reflect lab contamination, increased sampling using alternative sampling is wise in the unlikely event of a true presence of water soldier.
- Negative results do not conclude that water soldier is not present. Some level of visual surveying for water soldier and other priority aquatic invasive species is always recommended to help protect your lake.

Location Lake	Result
Ahmic Lake	Negative
Anstruther Lake	Negative
Ashby Lake	Negative
Bass Lake (Muskoka)	Negative
Basshaunt Lake	Negative
Beaver Lake	Negative
Big Basswood Lake	Negative
Big Cedar Lake	Negative
Big Gull Lake	Negative
Big Hawk Lake / Little Hawk Lake / Halls Lake	Negative
Big Rideau Lake	Negative
Billings Lake	Negative
Black Lake	Negative
Blackstone Lake	Negative
Burnt River	Negative
Canning Lake	Negative
Chandos Lake	Negative
Clear Lake (Perry)	Trace - Inconclusive
Crego Lake	Negative
Crowe Lake	Negative
Duck Lake	Negative
Fawn Lake	Negative

Location Lake	Result
Fortescue Lake	Negative
Glamor Lake	Negative
Gooseneck Lake	Negative
Growler Lake	Negative
Horseshoe L. (Frontenac)	Negative
Horseshoe Lake (Seguin)	Negative
Kennisis Lake	Negative
Lake Clear (Bonnechere)	Negative
Lake Dore	Negative
Lake Healey	Negative
Lake Kashagawigamog	Negative
Lake Mazinaw	Negative
Lake Muskoka	Negative
Lake of Bays	Negative
Lake Rousseau	Negative
Lake St. John	Negative
Lake Talon	Negative
Lake Vernon	Negative
Lake Wahnapiatae	Negative
Lepine Lake	Negative
Limerick Lake	Negative
Little Glamor Lake	Negative
Long Lake East (Perth)	Negative

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Location Lake	Result
Lovesick Lake	Negative
Lower Beverley Lake	Negative
Lower Hay Lake / Hay Lake	Negative
Manitouwabing Lake	Negative
Marl Lake	Negative
Mary Lake	Negative
Mill Lake	Trace - Inconclusive
Mink Lake	Negative
Moore Lake / East Moore / Gull River to Elliot Falls	Negative
Mountain Lake	Negative
Otter Lake / Little Otter	Negative
Pigeon Lake	Negative
Pine Lake	Negative
Ril Lake	Negative
Salerno Lake	Negative

Location Lake	Result
Skeleton Lake	Negative
Skootamatta Lake	Negative
Smith Bay on Dollars Lake	Negative
Sparrow Lake	Negative
St. Nora Lake	Negative
Stormy Lake	Negative
Sturgeon Lake	Negative
Sunny Lake	Negative
Tea Lake	Negative
Trout Lake (Sudbury)	Negative
Upper Buckhorn	Negative
Virgin/Dempsey Lake	Negative
Walker Lake	Negative
White Lake (Lanark)	Trace - Inconclusive
Wolfe Lake	Negative
Wollaston Lake	Negative