

A photograph of a pond filled with numerous green lily pads. Several white water lilies are in bloom, scattered across the pond. The water is dark and reflects the surrounding greenery.

Building eDNA Tools for Aquatic Species Detection to Supplement Monitoring Programs

Sharon Mann, 7 Lakes Alliance

Robin Sleith, Bigelow Laboratory for Ocean Sciences

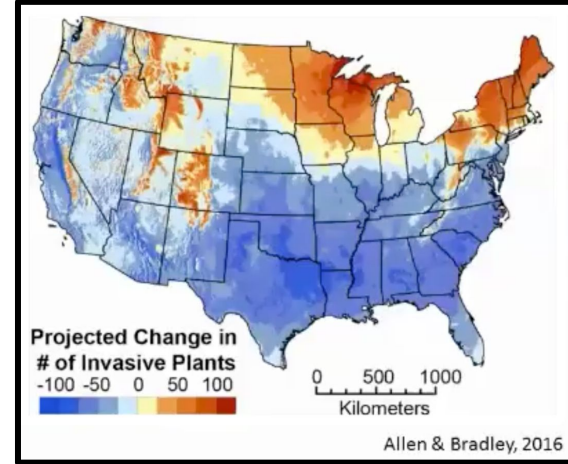
Anthropogenic threats to aquatic plants and those that depend on them



Desire for “clean” swimming areas

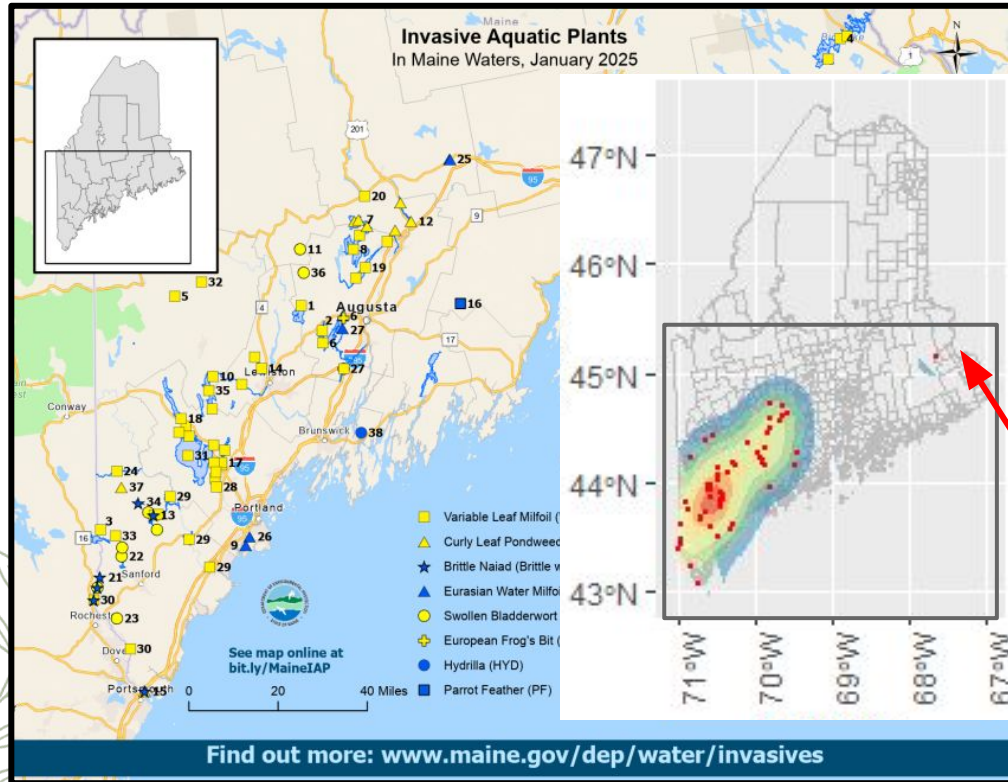


Shoreline development



Climate change and invasive species

There are hundreds of under surveyed lakes in Maine

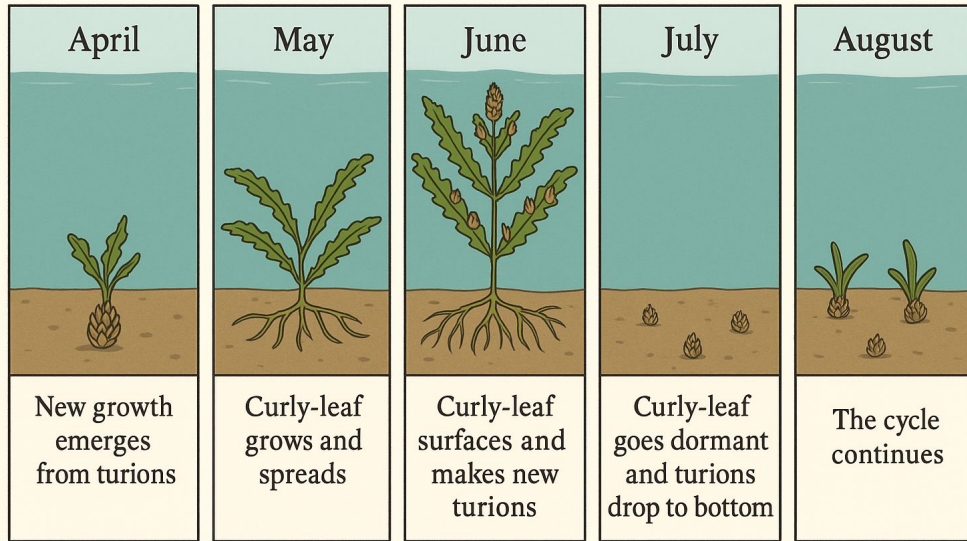


- Low population
- Limited funding
- Inaccessibility

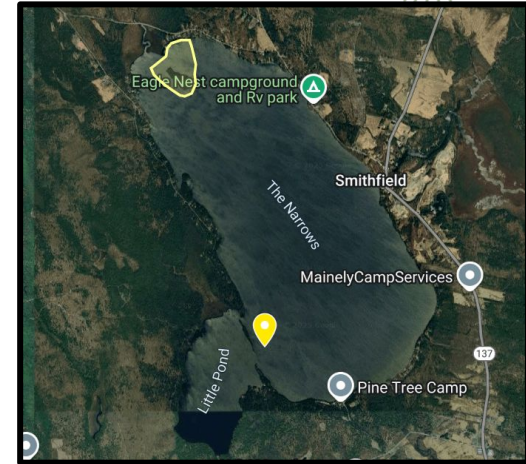
Big Lake

Even if a lake is monitored, invasive species can be sneaky

Curly-leaf pondweed growth in North Pond



IPPs typically are in July & August



Very sneaky



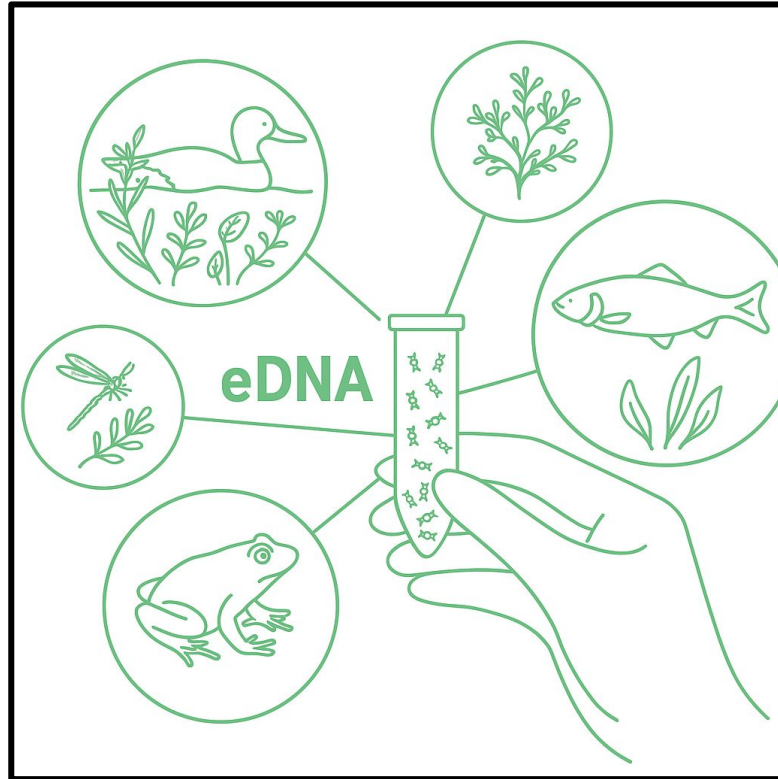
Microscopic life stages and/or native look-alikes

Early detection is KEY! Need a way to survey aquatic communities that is:

- (1) Inexpensive
- (2) Easy
- (3) Informative
- (4) Adaptable



Environmental DNA- genetic material left in an organism's environment



All survey methods
have limitations

- Different shedding rates
- eDNA degradation and dilution
- Design flaws

Common eDNA tools used to answer different questions

Metabarcoding for whole community composition



qPCR/dPCR/LAMP for targeted detection

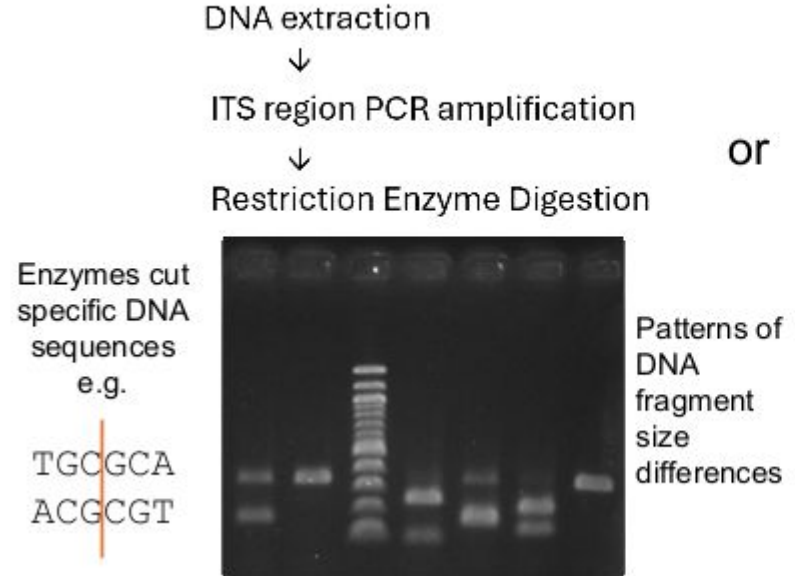


Molecular identification of milfoil tissue samples when reproductive structures are absent

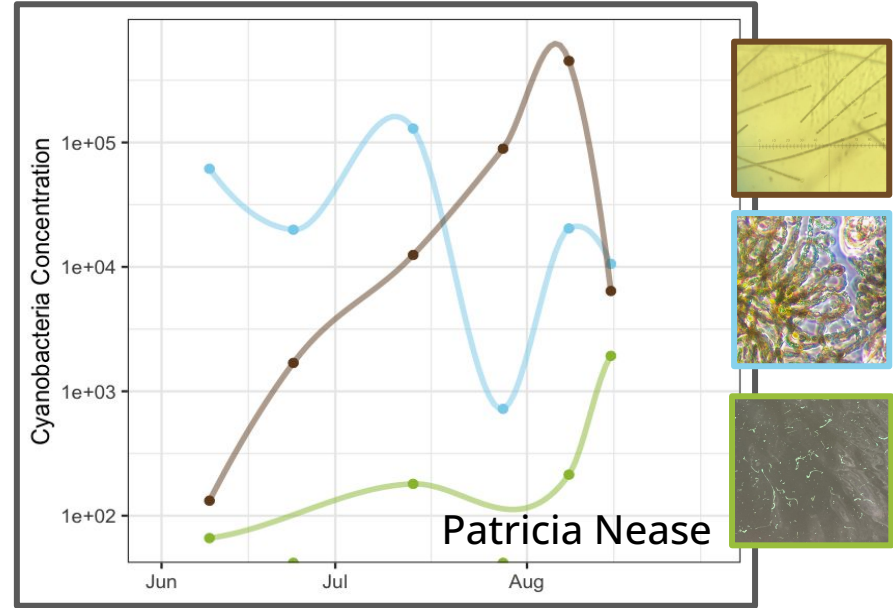
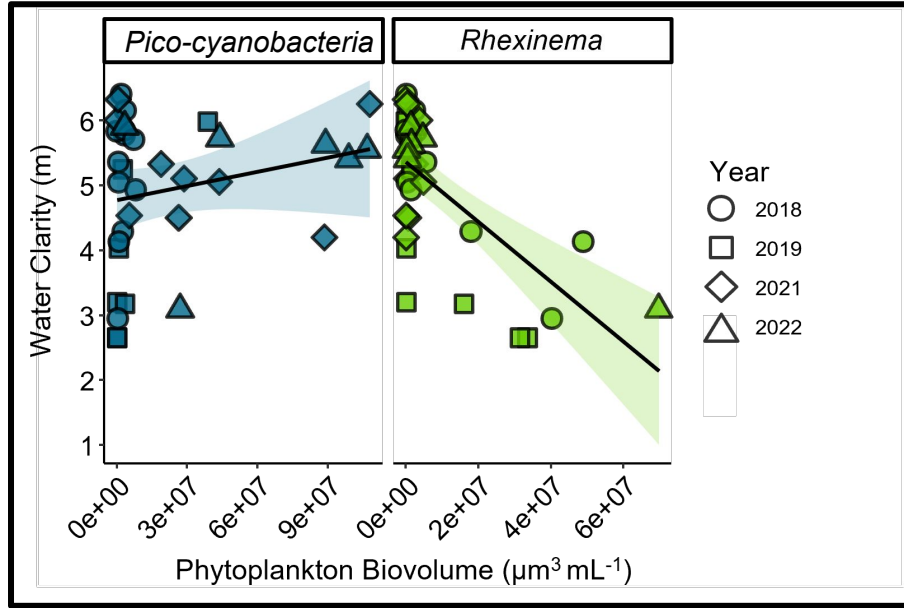


ibernacki@sjcme.edu
Rapid results to
distinguishing native
from non-native milfoils

PCR-RFLP (or sequencing) at St. Joseph's College



Examples of how eDNA enhances existing monitoring programs: algae

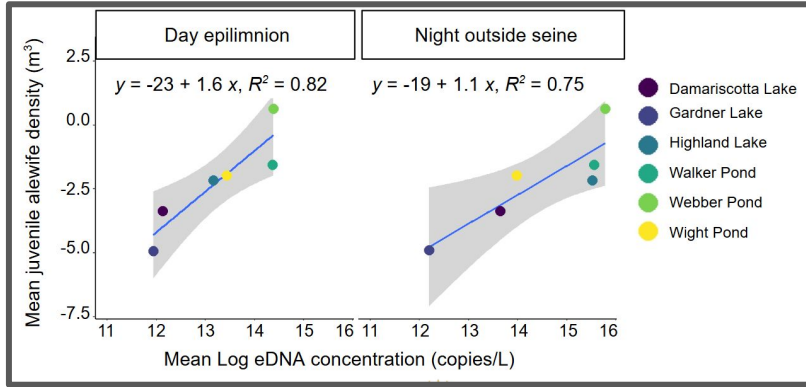


Solved Highland Lake's mystery bloom



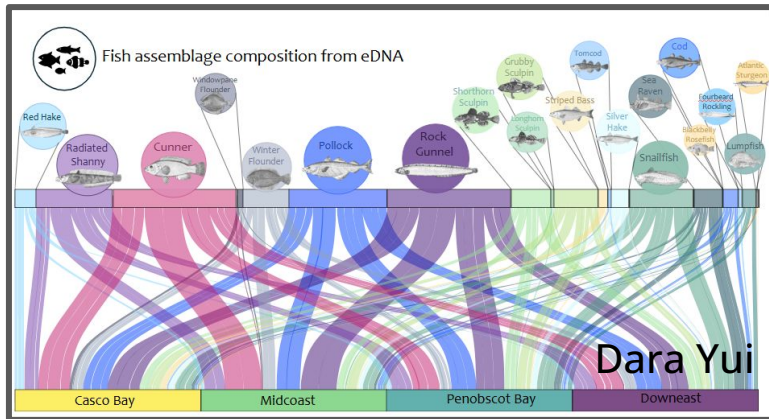
Track cyanobacteria in Damariscotta Lake

Examples of how eDNA enhances existing monitoring programs: fish



Estimate juvenile alewife abundance in lakes

- Strong correlation between fish density and eDNA copies
- Juvenile alewife are hard to count in lakes



Characterize the reef fishes in maine kelp forests

- eDNA found all species found in visual surveys
- eDNA found more species



**Build a DNA library
of Maine's native
and invasive
aquatic plants for
long-term
monitoring**



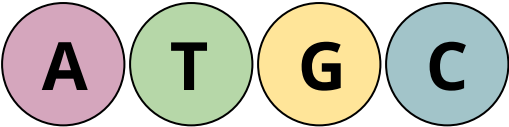
**Develop
targeted qPCR
assays for
single-species
detection**



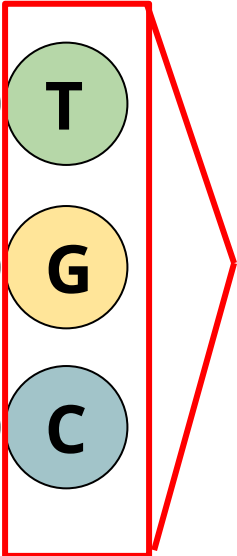
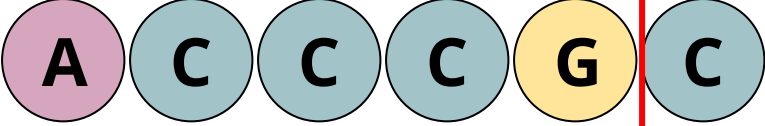
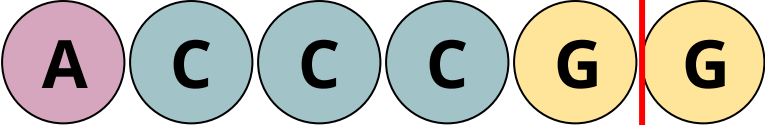
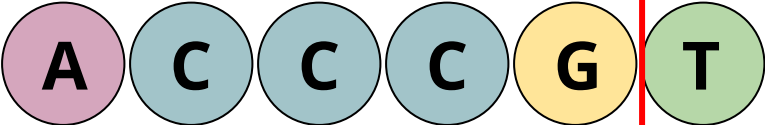
eDNA methods are limited by: primer specificity and incomplete reference libraries



Every individual has a unique genetic code.
We use differences in that those codes to detect species in their environment.



Nucleotide building blocks



Different base pairs





Building a Library that everyone can use!

Unique ID	Scientific Name	Common Name	Priority	Who's Collecting	State	County	Lake name
eDNA-001	<i>M. heterophyllum</i>	variable leaf milfoil	***	Sharon	ME	Kennebec	Great Pond
eDNA-002	<i>M. heterophyllum</i>	variable leaf milfoil	***	All	ME	York	Lake Arrowhead
eDNA-003	<i>M. heterophyllum</i>	variable leaf milfoil	***	Sharon/Cliff	ME	Washington	Big Lake
eDNA-004	<i>M. heterophyllum</i>	variable leaf milfoil	***	Sharon/Cliff	ME	Cumberland	Jordan Pond
eDNA-005	<i>M. heterophyllum</i>	variable leaf milfoil	***	Sharon	ME	Kennebec	Messalonskee Lake
eDNA-006	<i>M. heterophyllum</i>	variable leaf milfoil	***	Roberta	ME	Cumberland	Long Lake
eDNA-007	<i>P. crispus</i>	curly-leaf pondweed	***	Sharon	ME	Kennebec	East Pond
eDNA-008	<i>P. crispus</i>	curly-leaf pondweed	***	Sharon	ME	York	Legion Pond
eDNA-009	<i>P. crispus</i>	curly-leaf pondweed	***	Sharon	ME	Kennebec	Kennebec River
eDNA-010	<i>P. crispus</i>	curly-leaf pondweed	***	Roberta	ME	York	West Pond
eDNA-011	<i>P. crispus</i>	curly-leaf pondweed	***	Laurie Callahan	NH	Cheshire	Connecticut River
eDNA-012	<i>P. crispus</i>	curly-leaf pondweed	***	Laurie Callahan	MA	Franklin	Connecticut River
eDNA-013	Hybrid Milfoil	Hybrid Milfoil	***	Roberta	ME	Cumberland	Little Sebago (Upper Ba
eDNA-014	Hybrid Milfoil	Hybrid Milfoil	***	Roberta	ME	Cumberland	Little Sebago (Lower Ba
eDNA-015	Hybrid Milfoil	Hybrid Milfoil	***	Roberta	ME	Cumberland	Mill Stream
eDNA-016	Hybrid Milfoil	Hybrid Milfoil	***	Roberta	ME	Cumberland	Collins Pond
eDNA-017	Hybrid Milfoil	Hybrid Milfoil	***				
eDNA-018	Hybrid Milfoil	Hybrid Milfoil	***				
eDNA-019	<i>Trapa natans</i>	water chestnut	**	Laurie Callahan	NH	Cheshire	Connetctic River
eDNA-020	<i>Trapa natans</i>	water chestnut	**	Laurie Callahan	VT	Windham	Connetctic River



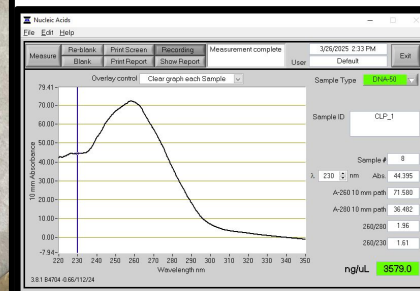
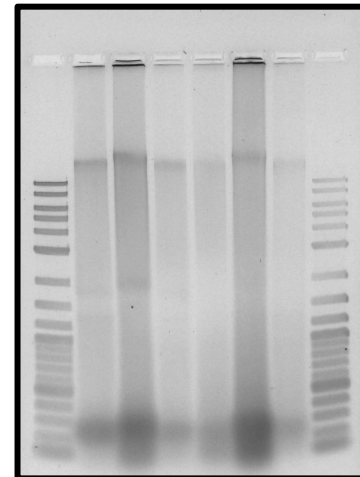
Every specimen has pressed and frozen tissue samples

***Special thanks to
Roberta Hill and
Laurie Callahan!!***



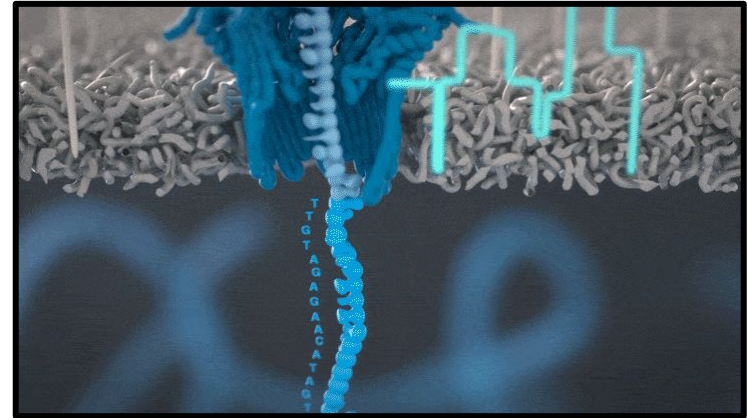
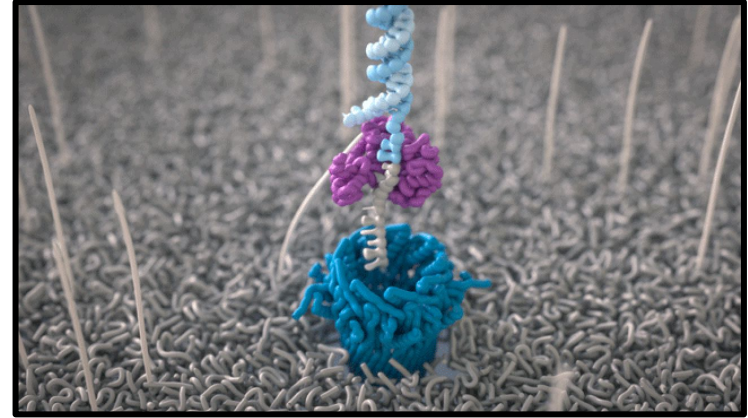


Curly Leaf Pondweed: DNA Extraction





Curly Leaf Pondweed: Nanopore Sequencing



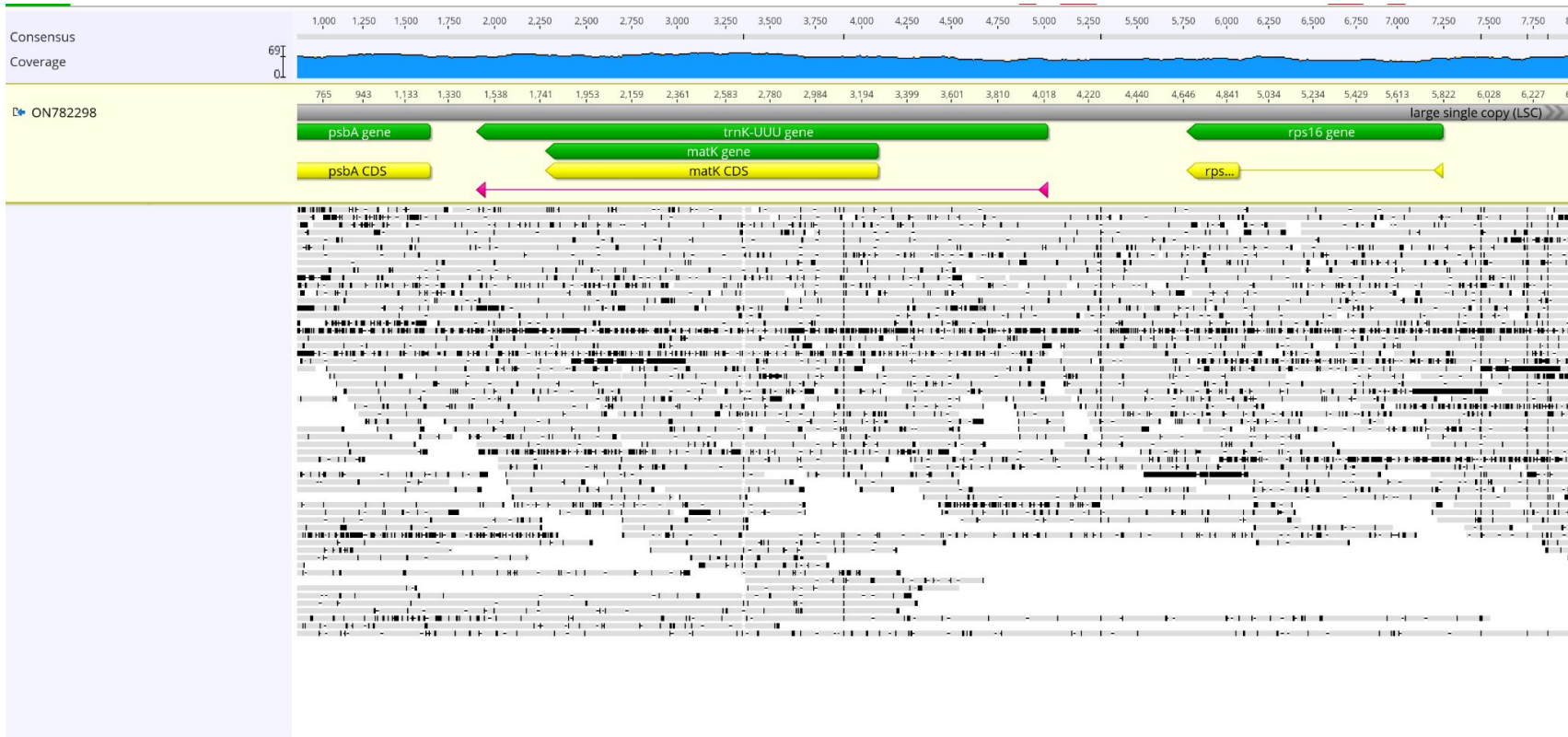


Curly Leaf Pondweed: chloroplast coverage



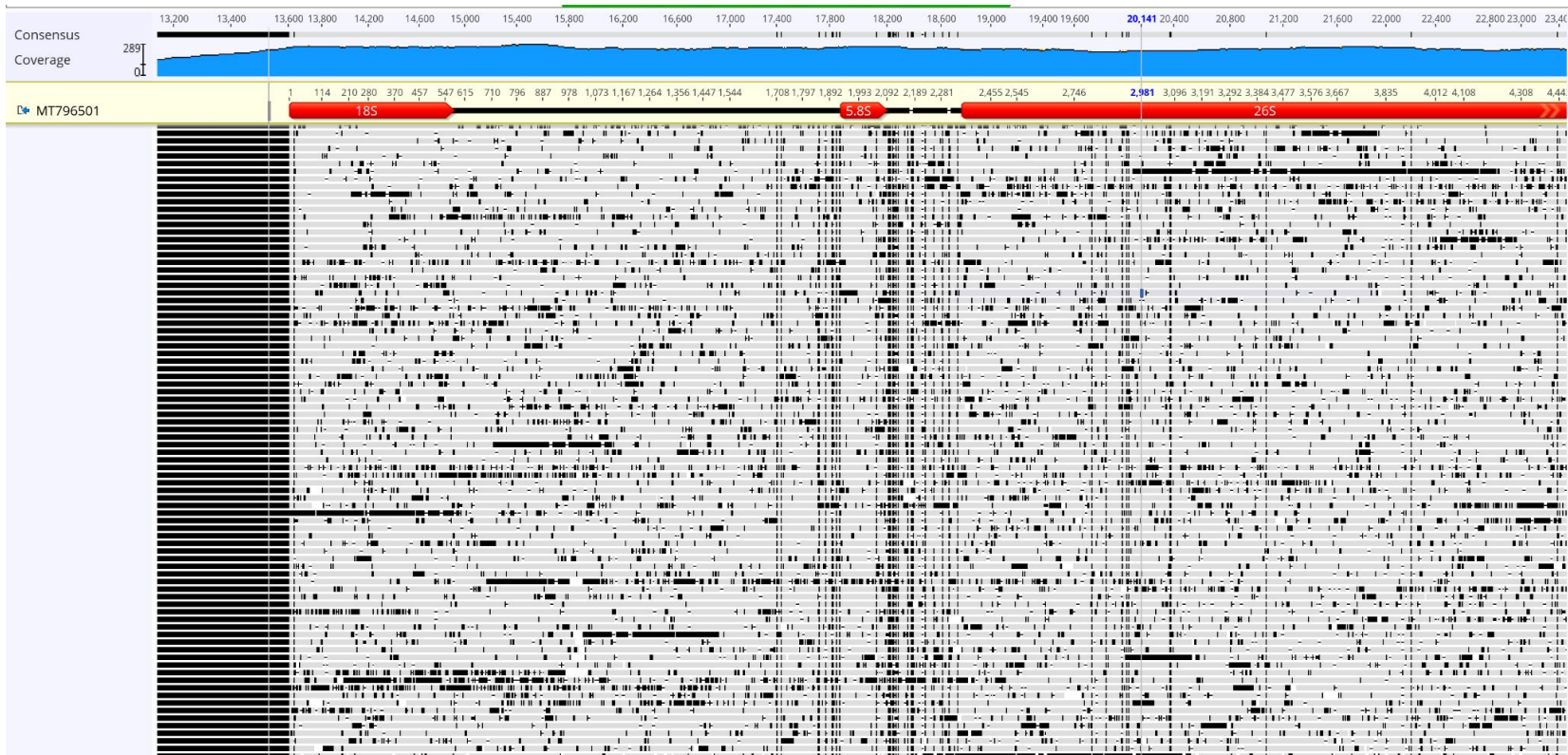


Curly Leaf Pondweed: chloroplast coverage





Curly Leaf Pondweed: ITS coverage





Curly Leaf Pondweed: qPCR assay design

Chloroplast

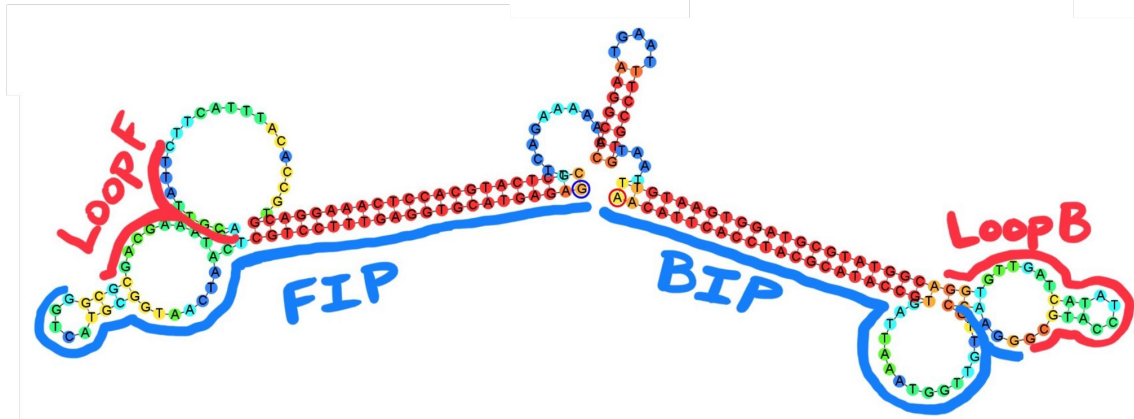


ITS





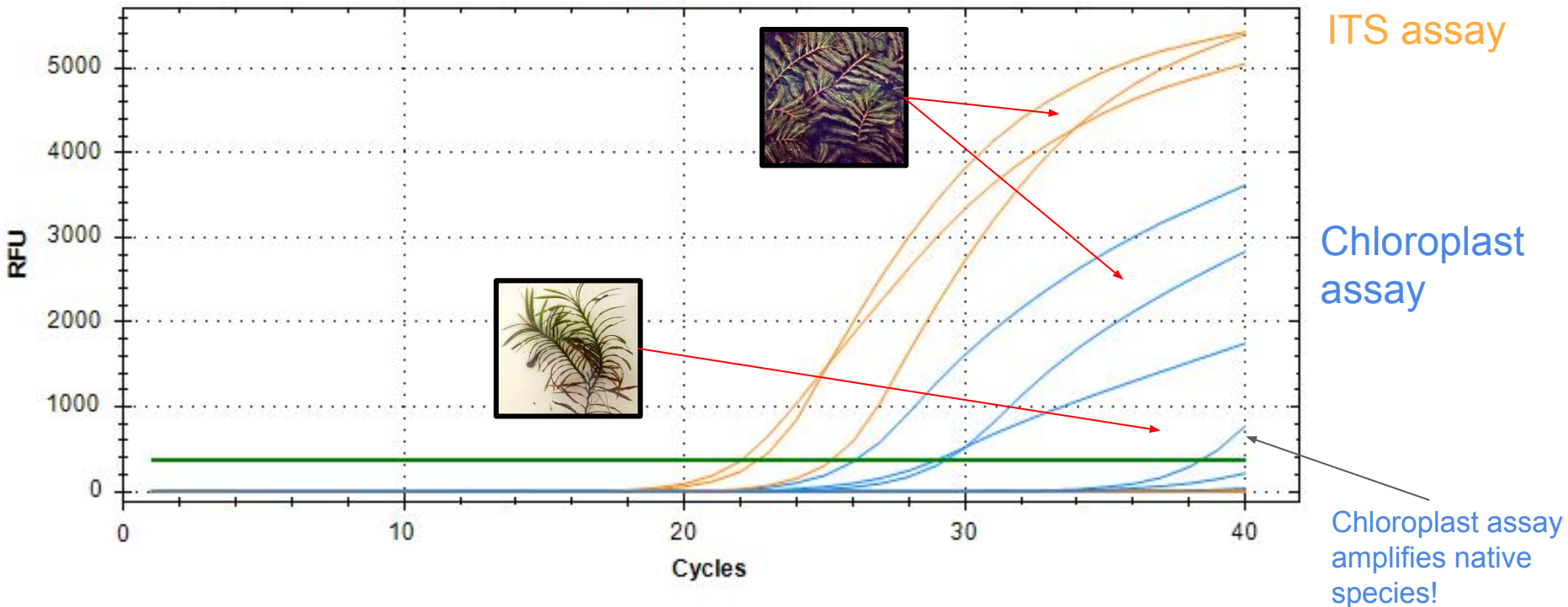
Curly Leaf Pondweed: LAMP assay design





Curly Leaf Pondweed: qPCR testing

Amplification





Curly Leaf Pondweed: qPCR testing



ITS assay

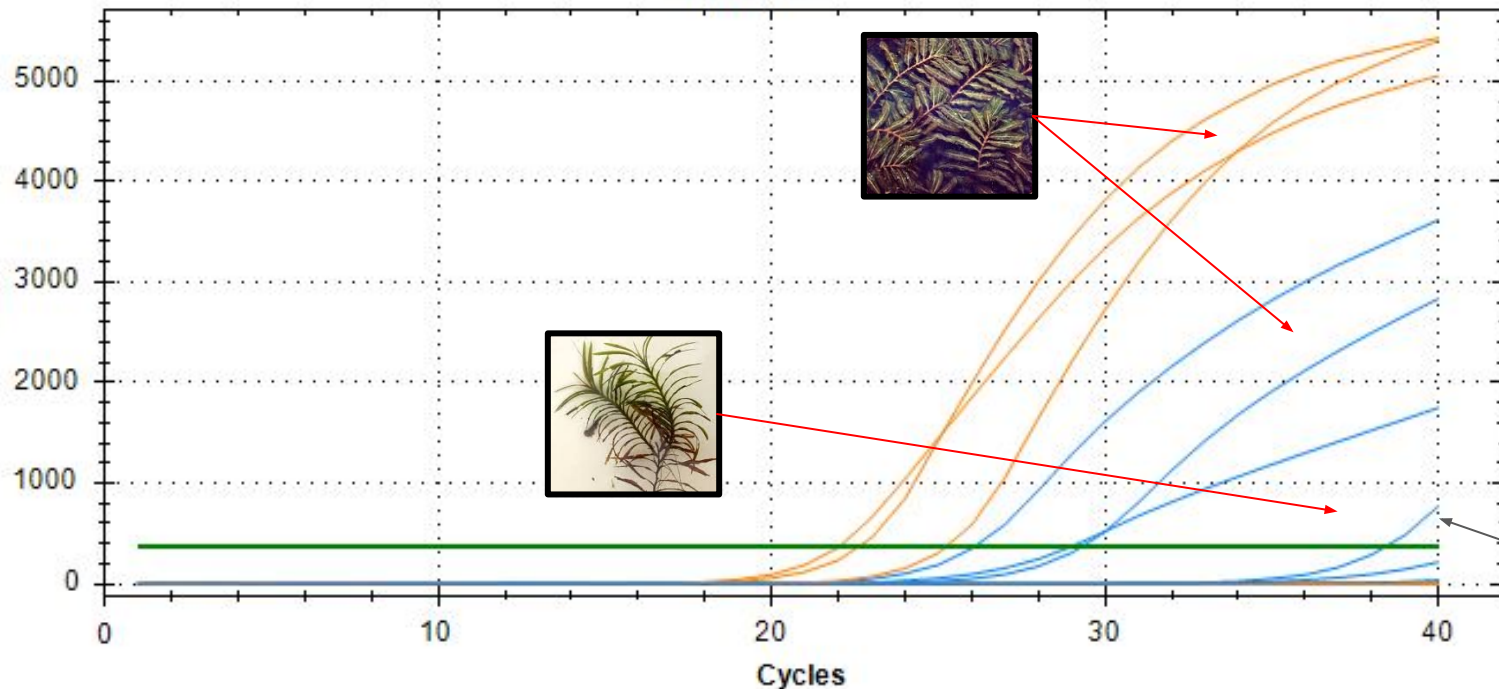


Chloroplast assay



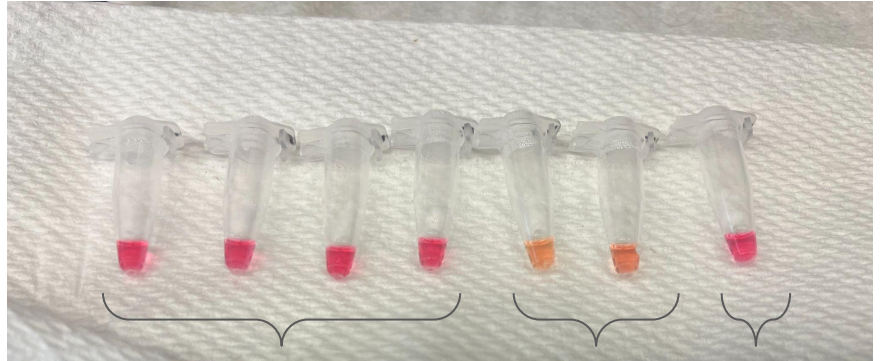
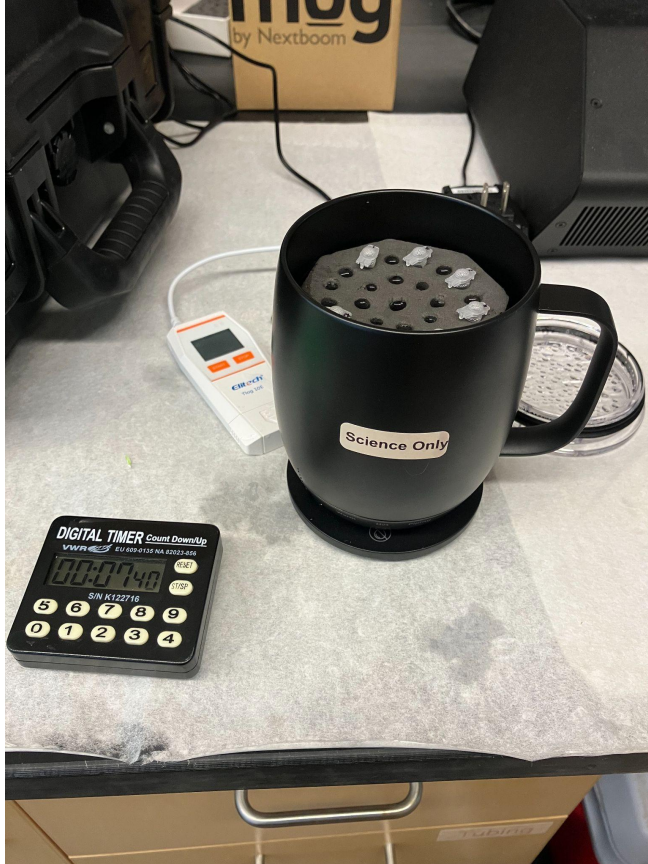
Chloroplast assay
amplifies
species

Amplification





Curly Leaf Pondweed: LAMP testing



Native species

CLP

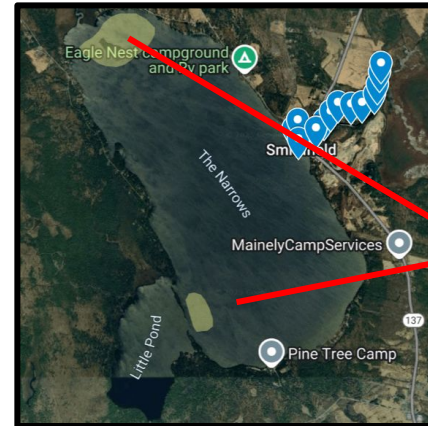
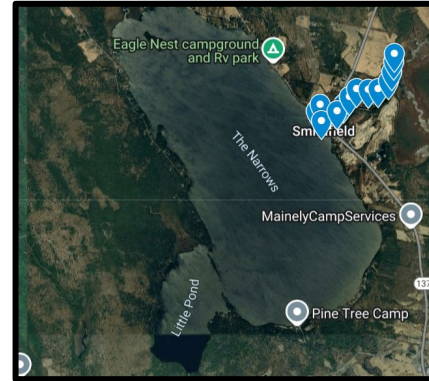
Control





Curly Leaf Pondweed: Next steps

- Test cross reactivity with more native species
- Test sensitivity with both dilutions and eDNA samples
 - eDNA samples foiled by North Pond detections
- Test time of year, volume filtered, extraction protocols to maximize detection capabilities

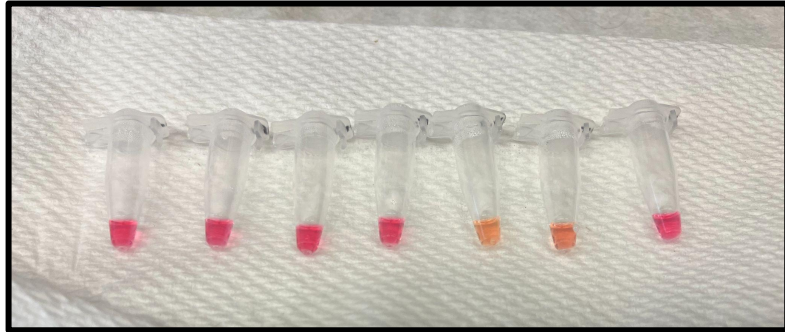


- eDNA water collected along transect
 - CLP counted and weighed
- Unexpected CLP patches**



LAMP at boat launches

Potential tool for rapid detection of invasive invertebrates in bilge water and live wells?



Thank you!

Contact us:

rsleith@bigelow.org

sharon@7lakes.org



Bigelow | Laboratory for
Ocean Sciences

Water Health and Humans Initiative

Thriving communities,
economies, and ecosystems
start with healthy water.

Center for Quantitative Molecular Ecology (CeQME)

Advancing environmental discovery,
one gene at a time



Building a library of Maine aquatic plant species

- Steps in the process (with example coming up)
 - Genome skimming gives us chloroplast, mitochondria, ITS, all at once for taxonomic identification and design of assays - flexible since if an assay changes it is still likely to be in one of these regions!