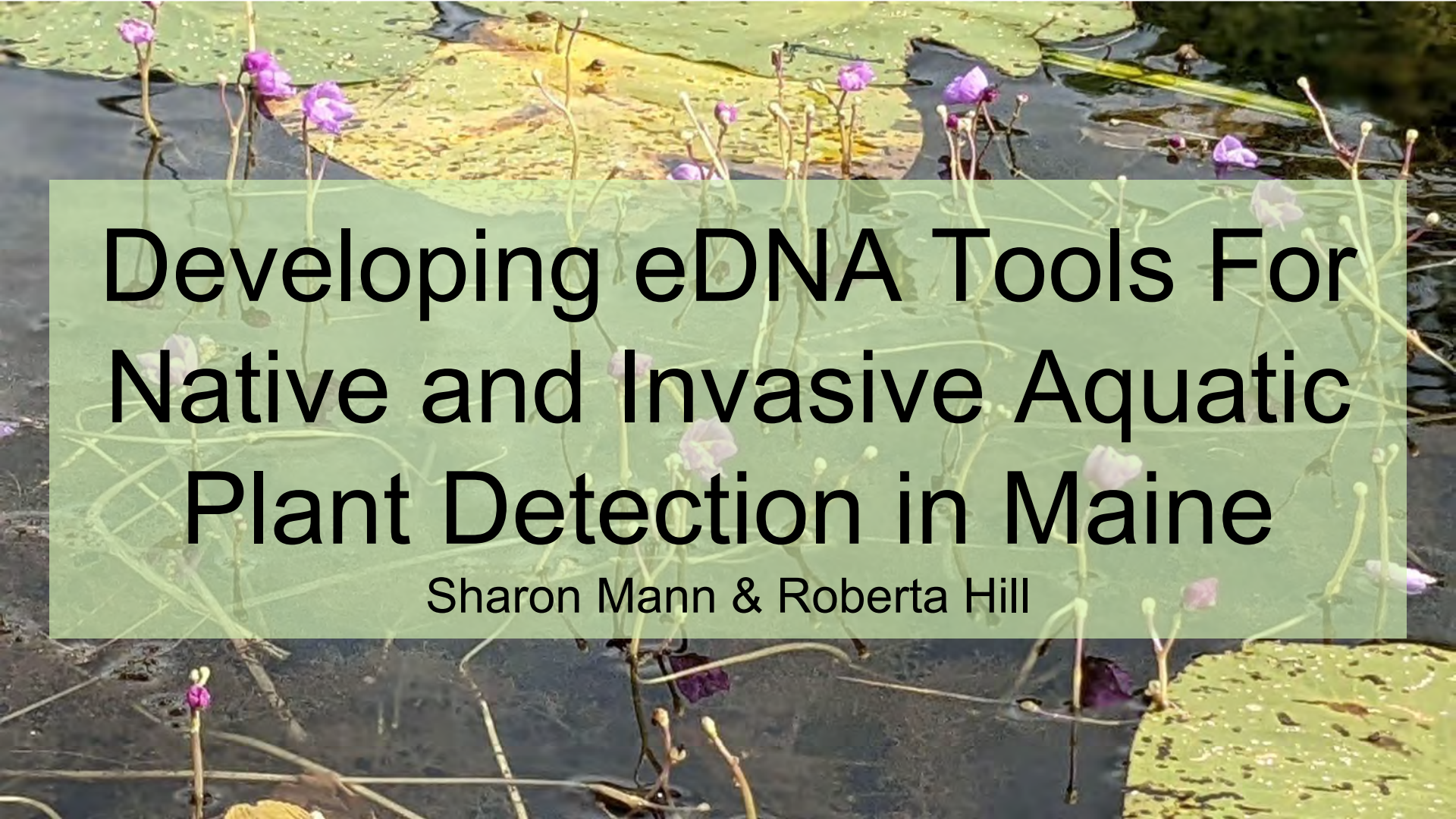


Developing eDNA Tools For Native and Invasive Aquatic Plant Detection in Maine

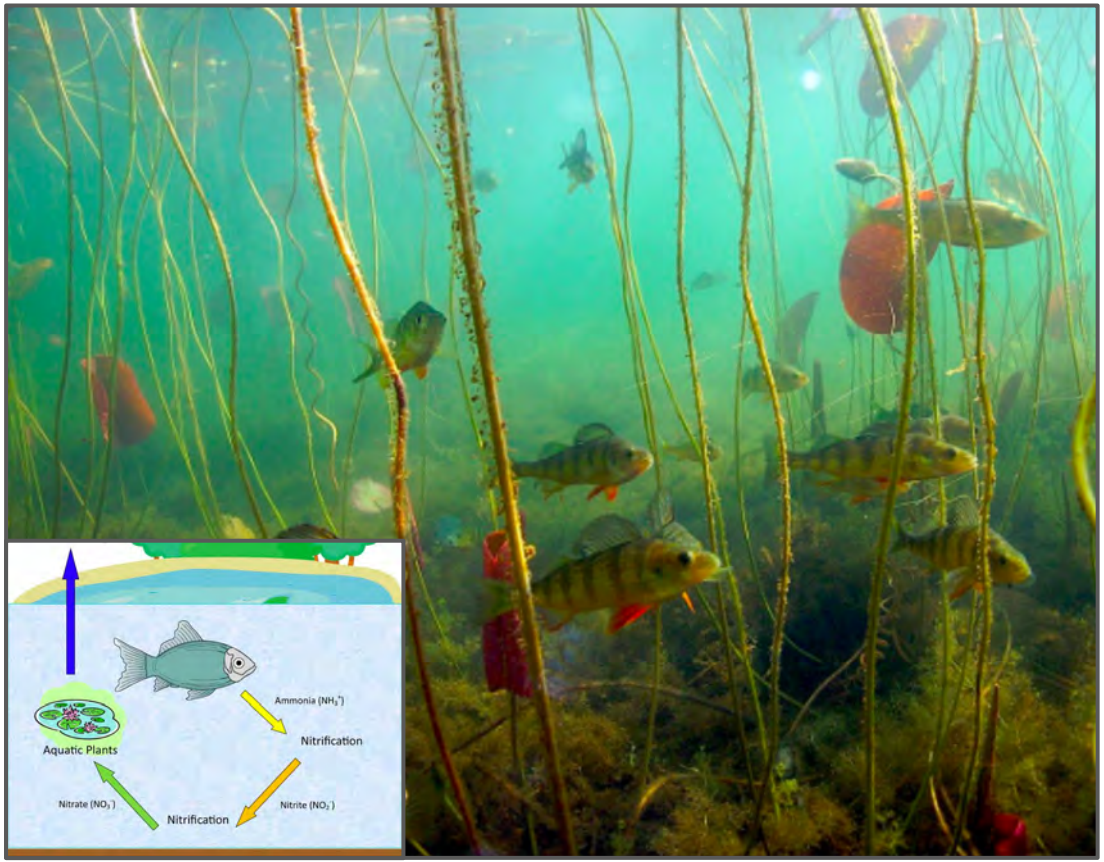
Sharon Mann & Roberta Hill

Environmental Consultant, LWA
Bioregion Regeneration Coordinator CEBE
Former Invasive Species Director, LSM
2robertahill@gmail.com

Invasive Aquatics Program Director, 7 LA
PhD candidate, UMaine
sharon.mann@maine.edu

Aquatic plants are important to wildlife & water quality

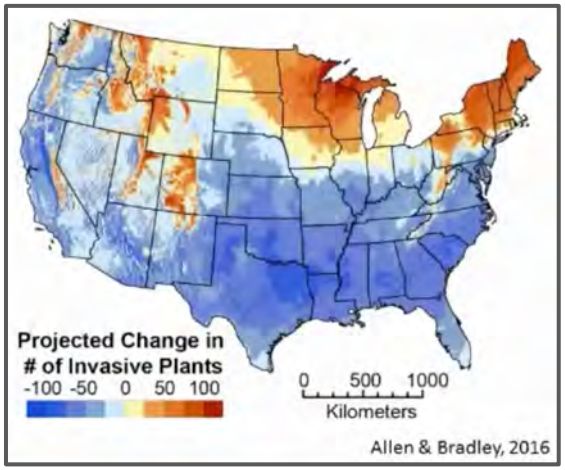
- Protect water quality
- Stabilize shoreline and sediments
- Provide oxygen and play key role in nitrogen cycle
- Enhance biodiversity
- Provide food and shelter for wildlife
- Structure for dragonflies and midges (fish food)



Anthropogenic threats to aquatic plants and those that depend on them

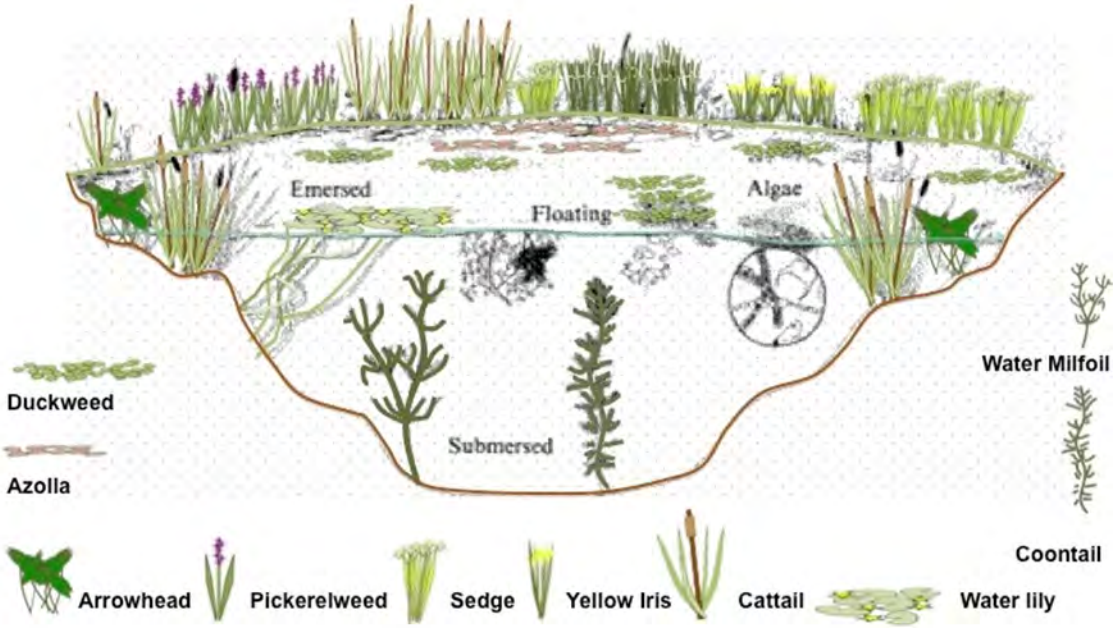
Desire for “clean” swimming areas

Shoreline development



Climate-driven spread of invasive species

Diverse plant communities are more resilient to environmental stress

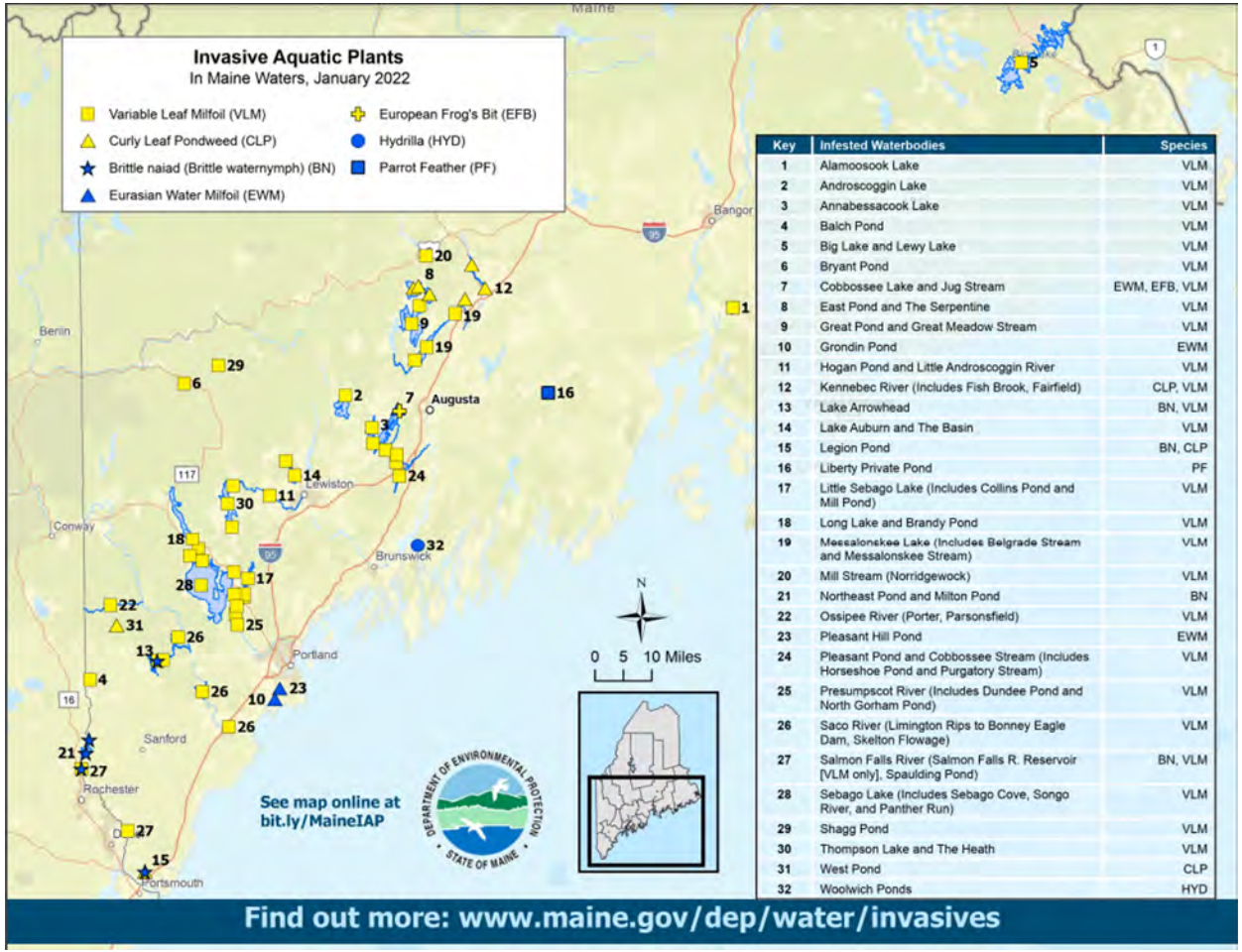


Invasive milfoil in Great Pond

Less than 1% of Maine's lakes are infested with one of "the 11-most unwanted invasive aquatic plants"

Since 2011, the number of infestations has DOUBLED

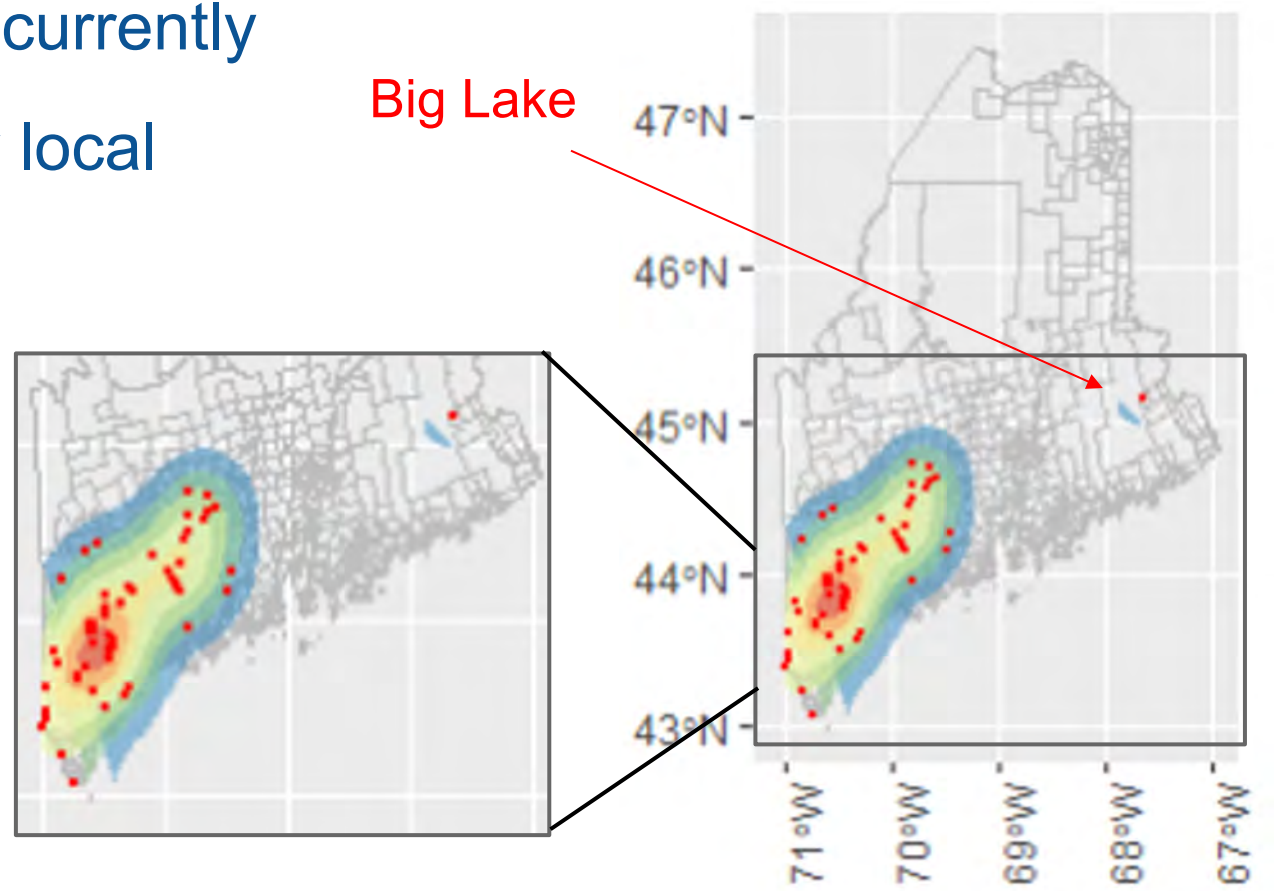
Most of the infested water bodies are popular tourist destinations are are close to major highways



Key	Infested Waterbodies	Species
1	Alamoosook Lake	VLM
2	Androscoggin Lake	VLM
3	Annabessacook Lake	VLM
4	Balch Pond	VLM
5	Big Lake and Lewy Lake	VLM
6	Bryant Pond	VLM
7	Cobbossee Lake and Jug Stream	EWM, EFB, VLM
8	East Pond and The Serpentine	VLM
9	Great Pond and Great Meadow Stream	VLM
10	Grondin Pond	EWM
11	Hogan Pond and Little Androscoggin River	VLM
12	Kennebec River (Includes Fish Brook, Fairfield)	CLP, VLM
13	Lake Arrowhead	BN, VLM
14	Lake Auburn and The Basin	VLM
15	Legion Pond	BN, CLP
16	Liberty Private Pond	PF
17	Little Sebago Lake (Includes Collins Pond and Mill Pond)	VLM
18	Long Lake and Brandy Pond	VLM
19	Messalonskee Lake (Includes Belgrade Stream and Messalonskee Stream)	VLM
20	Mill Stream (Norridgewock)	VLM
21	Northeast Pond and Milton Pond	BN
22	Ossipee River (Porter, Parsonsfield)	VLM
23	Pleasant Hill Pond	EWM
24	Pleasant Pond and Cobbossee Stream (Includes Horseshoe Pond and Purgatory Stream)	VLM
25	Presumpscot River (Includes Dundee Pond and North Gorham Pond)	VLM
26	Saco River (Limington Rips to Bonney Eagle Dam, Skelton Flowage)	VLM
27	Salmon Falls River (Salmon Falls R. Reservoir [VLM only], Spaulding Pond)	BN, VLM
28	Sebago Lake (Includes Sebago Cove, Songo River, and Panther Run)	VLM
29	Shagg Pond	VLM
30	Thompson Lake and The Heath	VLM
31	West Pond	CLP
32	Woolwich Ponds	HYD

Many lakes are currently unstewarded by local groups due to:

- Low population
- Limited funding
- Inaccessibility



Big Lake, Washington County

- Grand Lake Stream area, close to NB, Canada
- Large lake (10,444 AC)
- Part of 17,000 AC system (west branch of Saint Croix River)
- Known worldwide for wilderness beauty & extraordinary fishing.

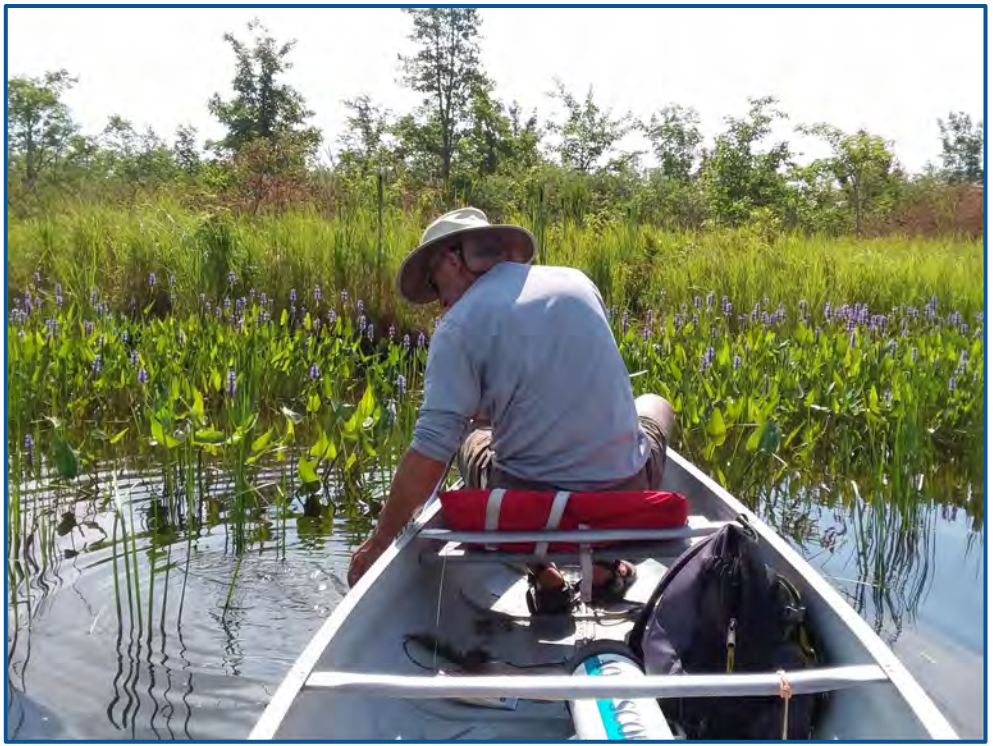
Early detection is KEY! Need a way to survey aquatic plant communities that is:

(1) Inexpensive

(2) Easy

(3) Informative

(4) Adaptable



Genetic material obtained directly from environmental samples without any obvious signs of the biological source material (Thomsen and Willerslev, 2015)

Where does eDNA come from?

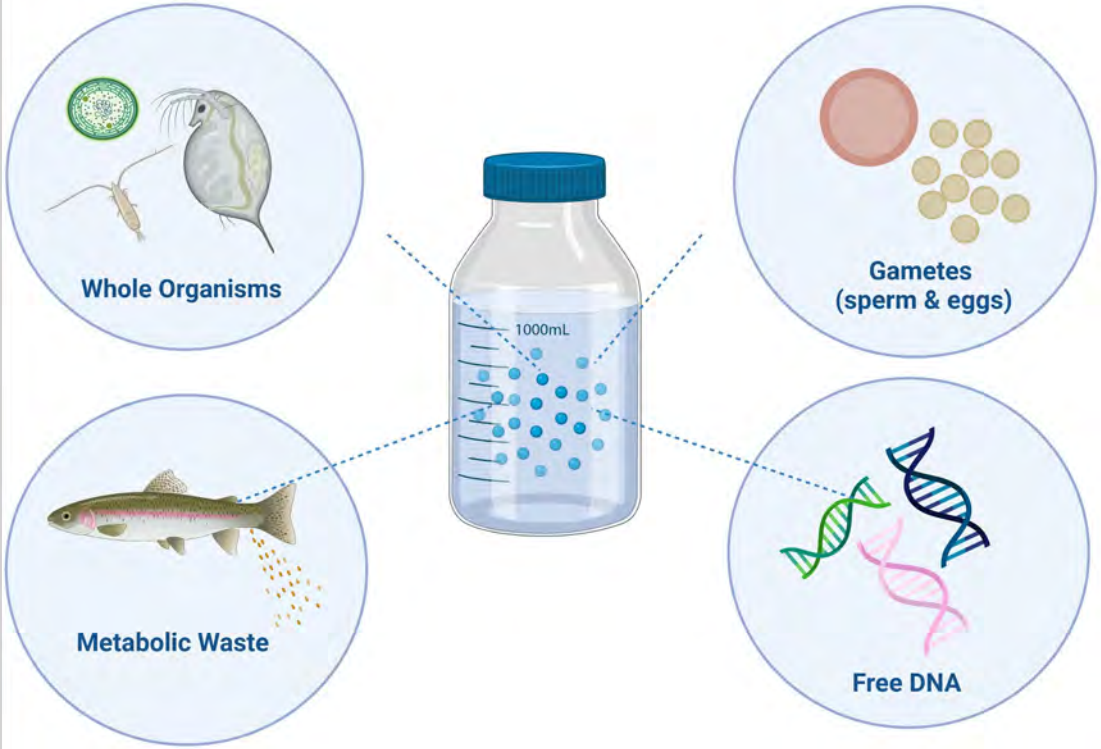
- Cellular decomposition
- Whole shed cells
- Whole microorganisms

Where is eDNA found?

- Water
- Soils
- Air

How is eDNA used?

- Community characterization (metabarcoding i.e. general primers)
- Targeted detection & quantification (qPCR)

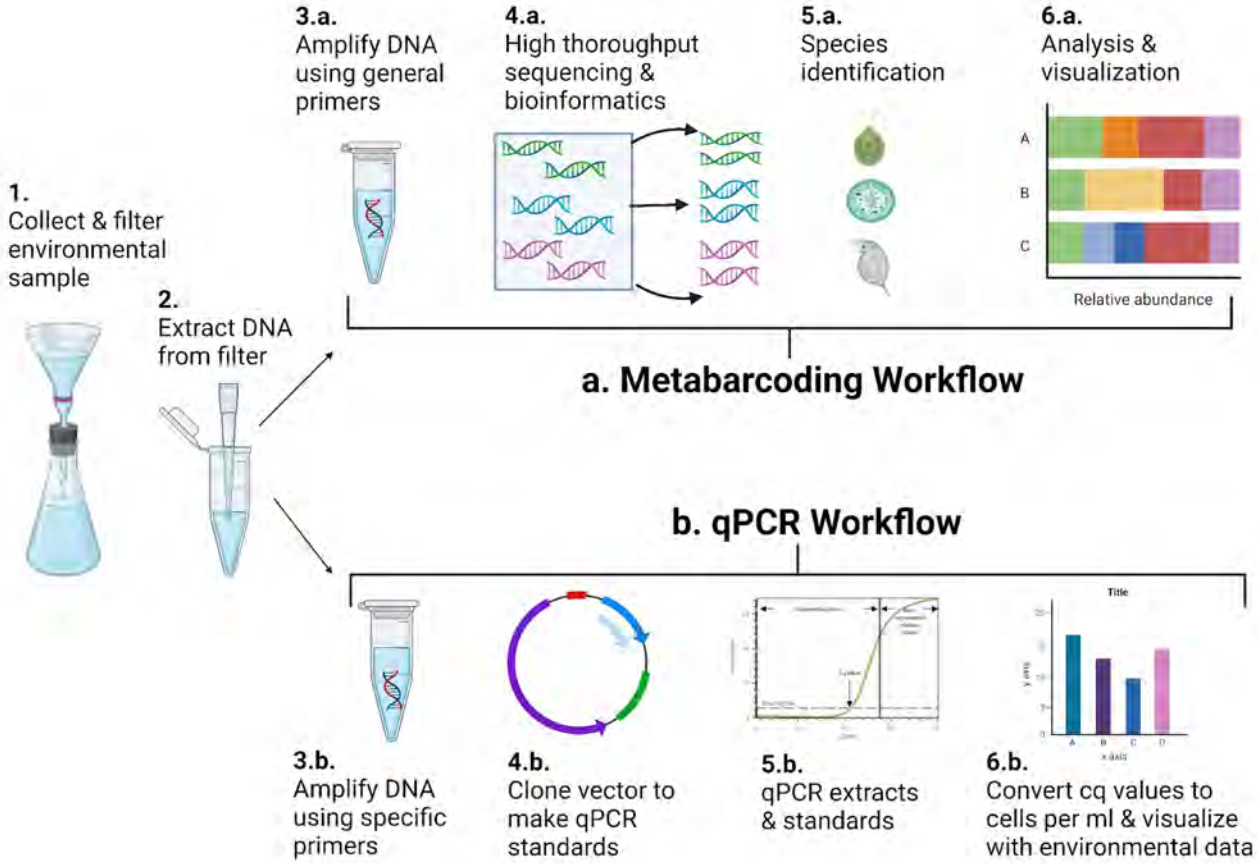


Using a two-tiered approach with eDNA tools

Metabarcoding for whole community composition

qPCR for targeted detection

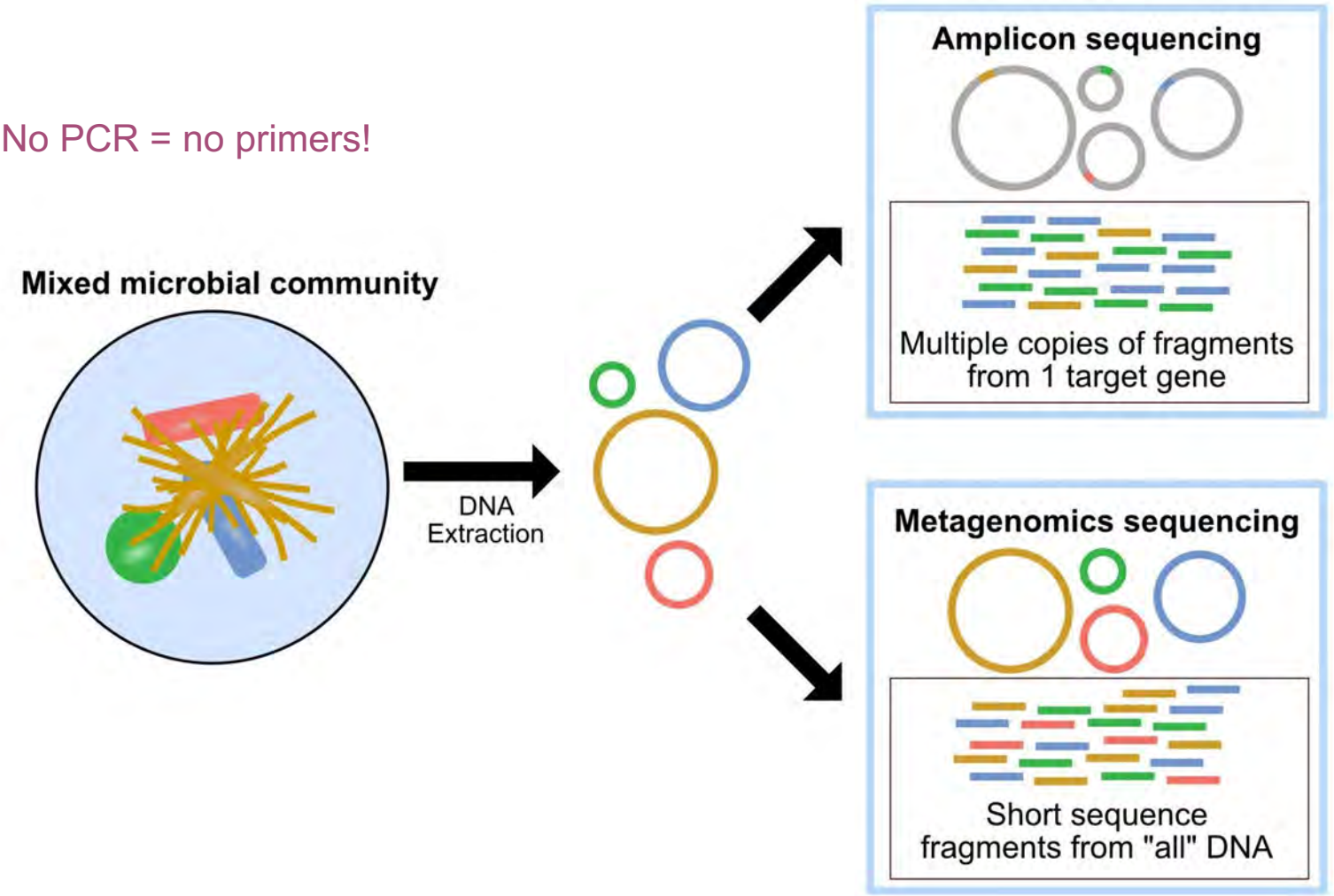
Using a two-tiered approach with eDNA tools



General primers

Specific primers

No PCR = no primers!



1

Develop targeted qPCR assays for single-species detection

2

Build a Maine metabarcoding library of native and invasive aquatic plants for long-term monitoring

3

Inventory population diversity statewide

Half of “the 11 most unwanted” have published targeted qPCR primers

Will add new invasives as they come up

Metabarcoding is all about the library

- Published libraries are not great for aquatic plants
- Aquatic plant sequences from around the globe

- Need to fill our library with our own plant sequences
- Both **native** and **invasive**

Development of an environmental DNA metabarcoding assay for aquatic vascular plant communities

Stephanie A. Coghlan[1] | Aaron B. A. Shafer[1,2] | Joanna R. Freeland[1,3]

[1]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, ON, Canada

[2]Forensics Program, Trent University, Peterborough, ON, Canada

[3]Biology Department, Trent University, Peterborough, ON, Canada

Correspondence
Joanna R. Freeland, Environmental and Life Sciences Graduate Program, Trent

Abstract

Environmental DNA (eDNA) metabarcodes allow for the simultaneous detection of multiple taxa if the barcode regions meet several key requirements including conserved primer-binding sites, interspecific variability that exceeds intraspecific variability, and relatively short amplicons. Currently, there are no established metabarcoding assays for aquatic vascular plants, which could limit biodiversity assessments and the early detection of alien species. We used a combination of novel and

New research has shown that aquatic plant eDNA pairs well with traditional surveys

In some cases, eDNA identified more rare species than identified using traditional methods!

ELSEVIER journal homepage: www.elsevier.com/locate/envres

Estimating aquatic plant diversity and distribution in rivers from Jingjinji region, China, using environmental DNA metabarcoding and a traditional survey method

Fenfen Ji [a,b,c,d], Liang Yan [a,b,c], Saihong Yan [a,b,c], Tianlong Qin [d], Jianzhong Shen [d,**], Jinmiao Zha [a,b,c,*]

[a] Key Laboratory of Drinking Water Science and Technology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China
[b] Beijing Key Laboratory of Industrial Wastewater Treatment and Reuse, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China
[c] University of Chinese Academy of Sciences, Beijing, 100049, China
[d] Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture, College of Fisheries, Huazhong Agricultural University, Wuhan, 430070, China

ARTICLE INFO

Keywords:
Aquatic plant diversity
Environmental DNA metabarcoding
Traditional survey method

ABSTRACT

Traditional survey methods (TSMs) are difficult to use to perform a census of aquatic plant diversity completely in river ecosystems, and improved aquatic plant community monitoring programs are becoming increasingly crucial with a continuous decline in diversity. Although environmental DNA (eDNA) metabarcoding has been applied successfully to assess aquatic biodiversity, limited work has been reported regarding aquatic plant di-

A Metagenomics approach to fingerprinting

Population 1

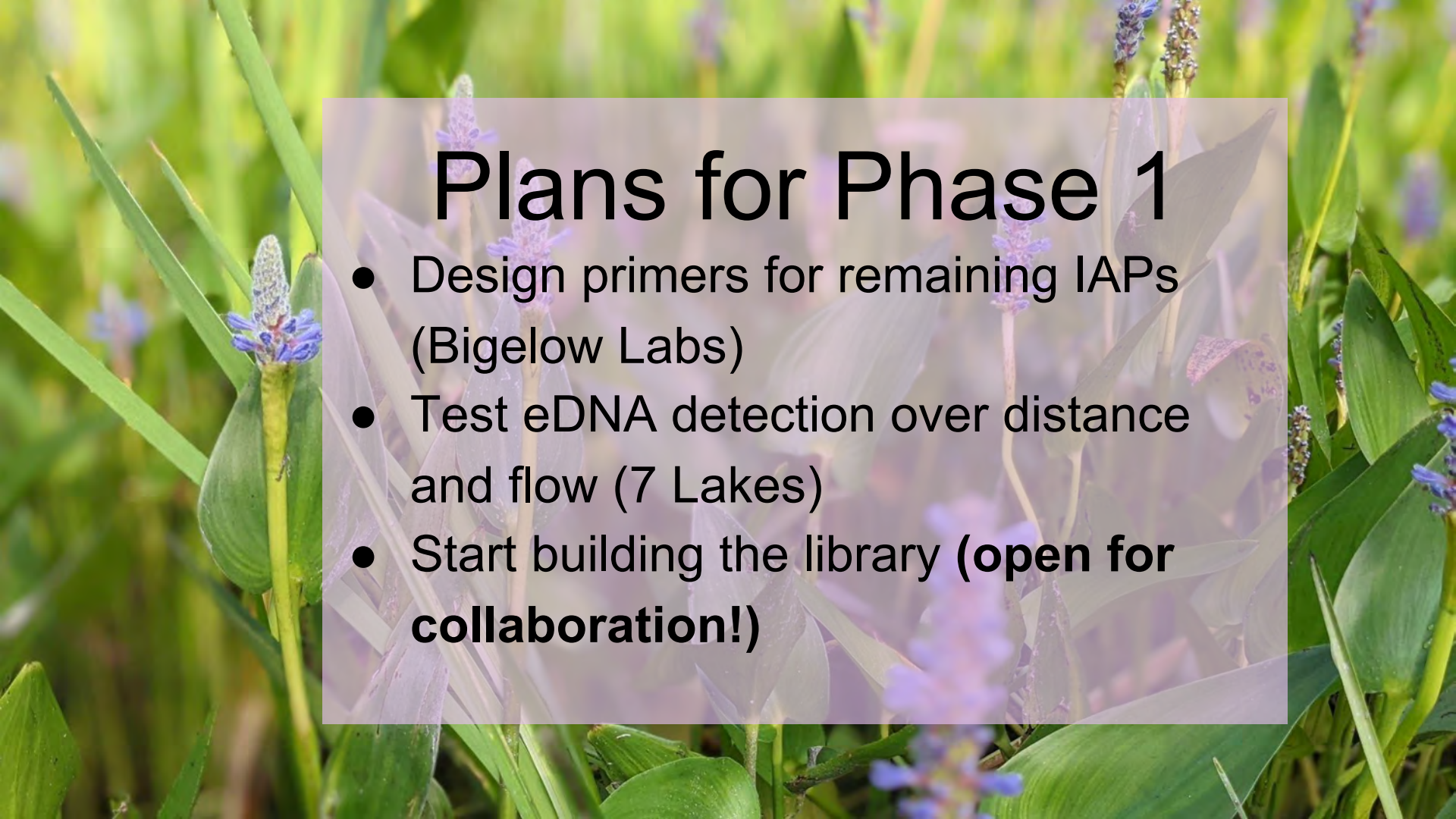
Population 1

Population 2

How will the data be used?

- Faster response to new infestations
- Conservation of rare or threatened native species
- To monitor long-term community shifts
- Invasive species monitoring

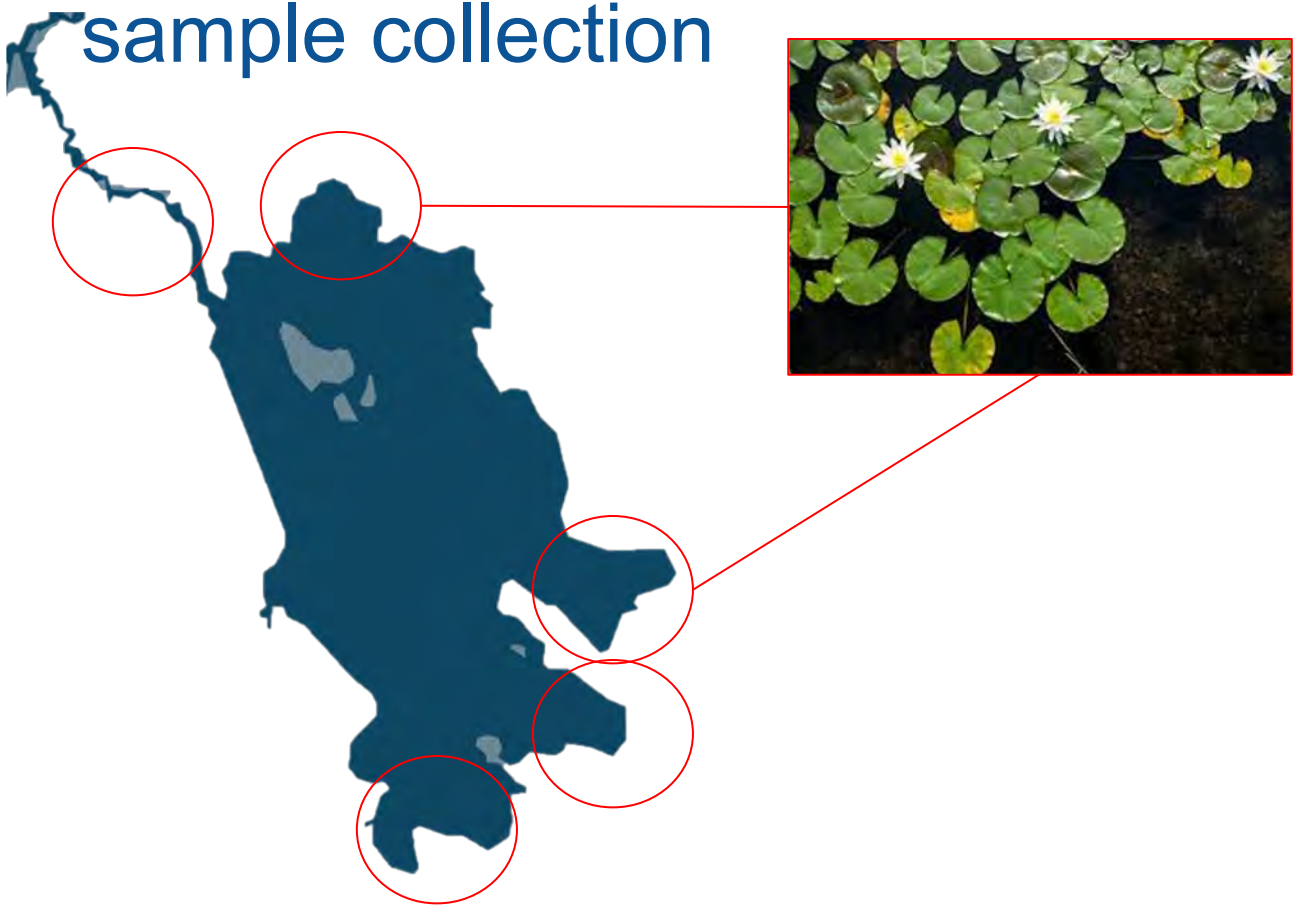
Image credit: Jen Smith Mayo, UMO



Plans for Phase 1

- Design primers for remaining IAPs (Bigelow Labs)
- Test eDNA detection over distance and flow (7 Lakes)
- Start building the library **(open for collaboration!)**

Volunteers will be needed to help with data & sample collection



Step 1.

Identify fertile pockets in the waterbody

Step 2. Inventory all aquatic plants in fertile pockets

Waterbody _____ **Date/s** _____ **Page** _____ **of** _____

Section 3: Native Plant Inventory **For inventory:** ✓ = presence; D = dominant; U = uncommon

…e Plant Checklist **	Sector				Common Native Plant Checklist (con't)	Sector			
					pondweed, small *Potamogeton pusilloid spp.*				
…aria latifolia					pondweed, spiral-fruited *Potamogeton spirillus*				
…ttaria graminea					pondweed, spp. *Potamogeton spp.*				
…agittaria spp.					pondweed, variable *Potamogeton gramineus*				
…ia vulgaris					quillwort spp. *Isoetes spp.*				
… radiata					rush, bayonet *Juncus militaris*				
… gibba					rush, brown-fruited *Juncus pelocarpus*				
…aria purpurea					spatterdock *Nuphar variegata*				
…inor					spikerush, needle *Eleocharis acicularis*				
…ia intermedia					spikerush, Robbin's *Eleocharis robbinsii*				
… spp.					sponge, freshwater spp.				
…lectus tabernaemontanii					stonewort, spp *Nitella spp.*				
…ctus subterminalis					three-way sedge *Dulichium arundinaceum*				
… *Sparganium fluctuans*					water buttercup *Ranunculus spp.*				
…oating-leaf *Sparganium angustifolium*					water lily, fragrant *Nymphaea odorata*				
… *Sparganium spp.*					water lobelia *Lobelia dortmanna*				
…common *Typha latifolia*					water marigold *Bidens beckii*				
cattail, spp. *Typha spp.*					water parsnip *Sium suave*				
coontail *Ceratophyllum demersum*					water starwort, spp. *Callitriche spp.*				
duckweed, common *Lemna minor*					water-milfoil, alternate-flowered *Myriophyllum alterniflorum*				
duckweed, giant *Spirodela polyrhiza*					water-milfoil, dwarf *Myriophyllum tenellum*				
golden pert *Gratiola aurea*					water-milfoil, Farwell's *Myriophyllum farwellii*				

Step 3.

- Collect plant samples (alcohol and herbarium presses)
- Collect water for eDNA outside each fertile pocket
- Take pictures
- GPS locate
- Share information

https://forms.gle/x92PnEgtwYPZWT8E6

Thank you to all of our partners

LAKE & WATERSHED
Associates

Extra Slides

Environmental DNA

Manual for Volunteers

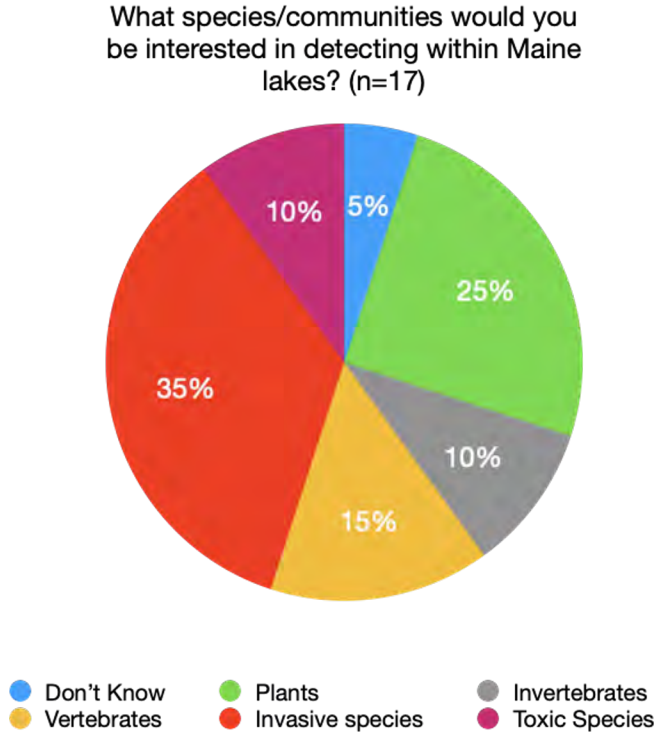
MAINE eDNA

LAKE STEWARDS OF MAINE

Edited in February 2022

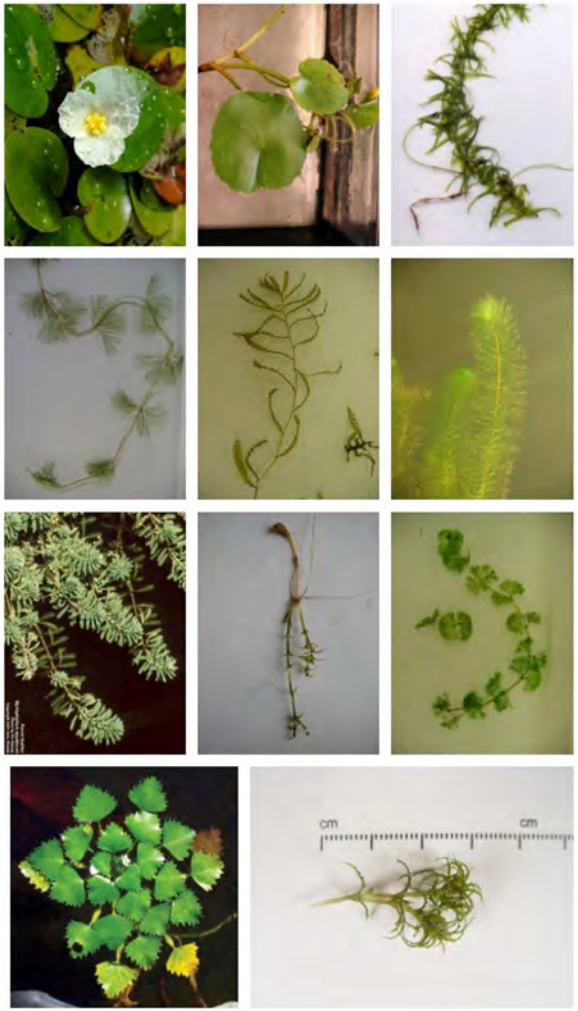
CONTENTS

Question:
What can eDNA methods do for Maine Lakes?



Your Answers:
"Invasive Species" and "Help"

How Can IPPers Help?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