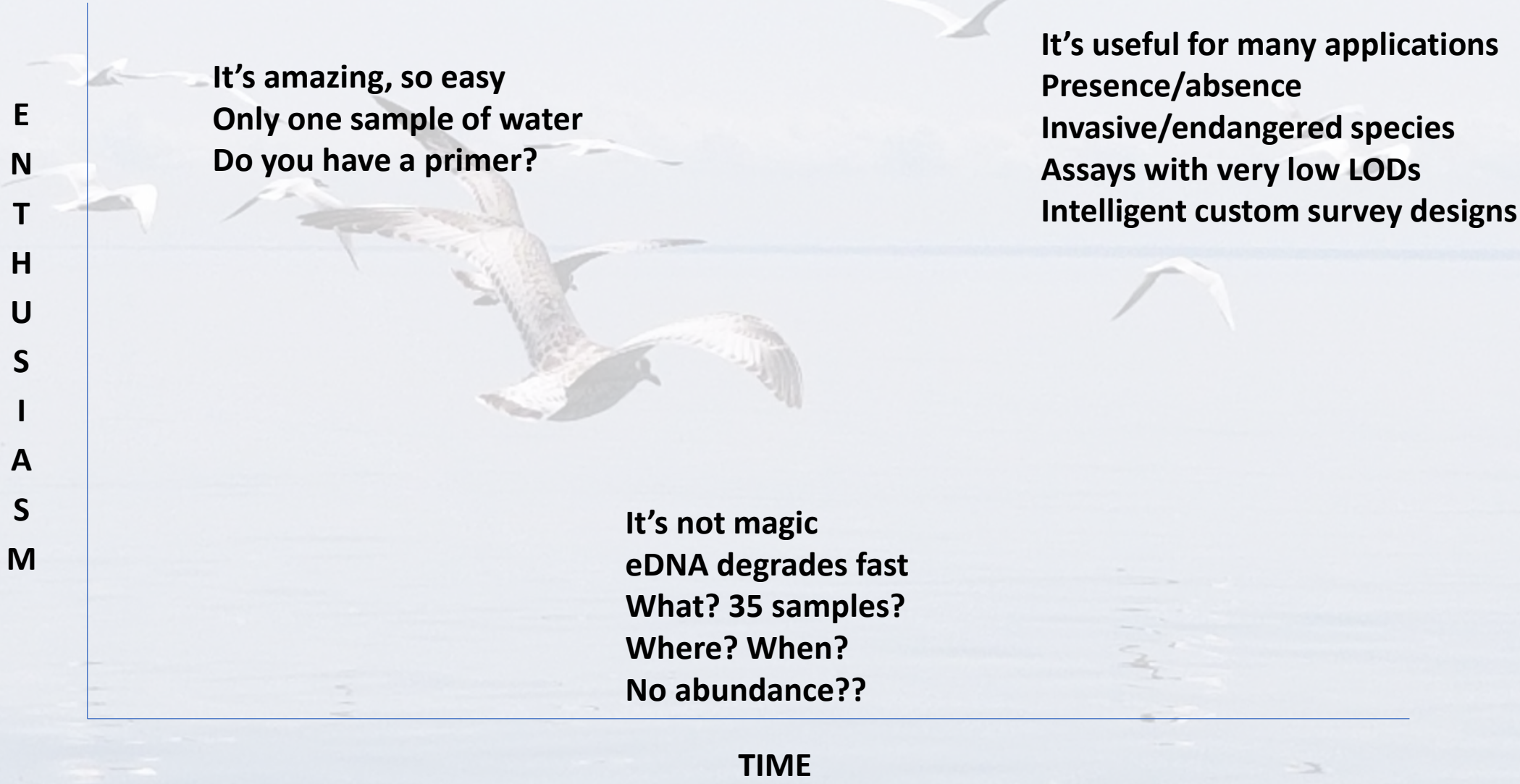
How eDNA may help revive the fortunes of the American eel *Anguilla rostrata* in Ontario

PRECISION BIOMONITORING
—Intelligent DNA Solutions—

Mario Thomas, Ph.D.

Attitude towards eDNA

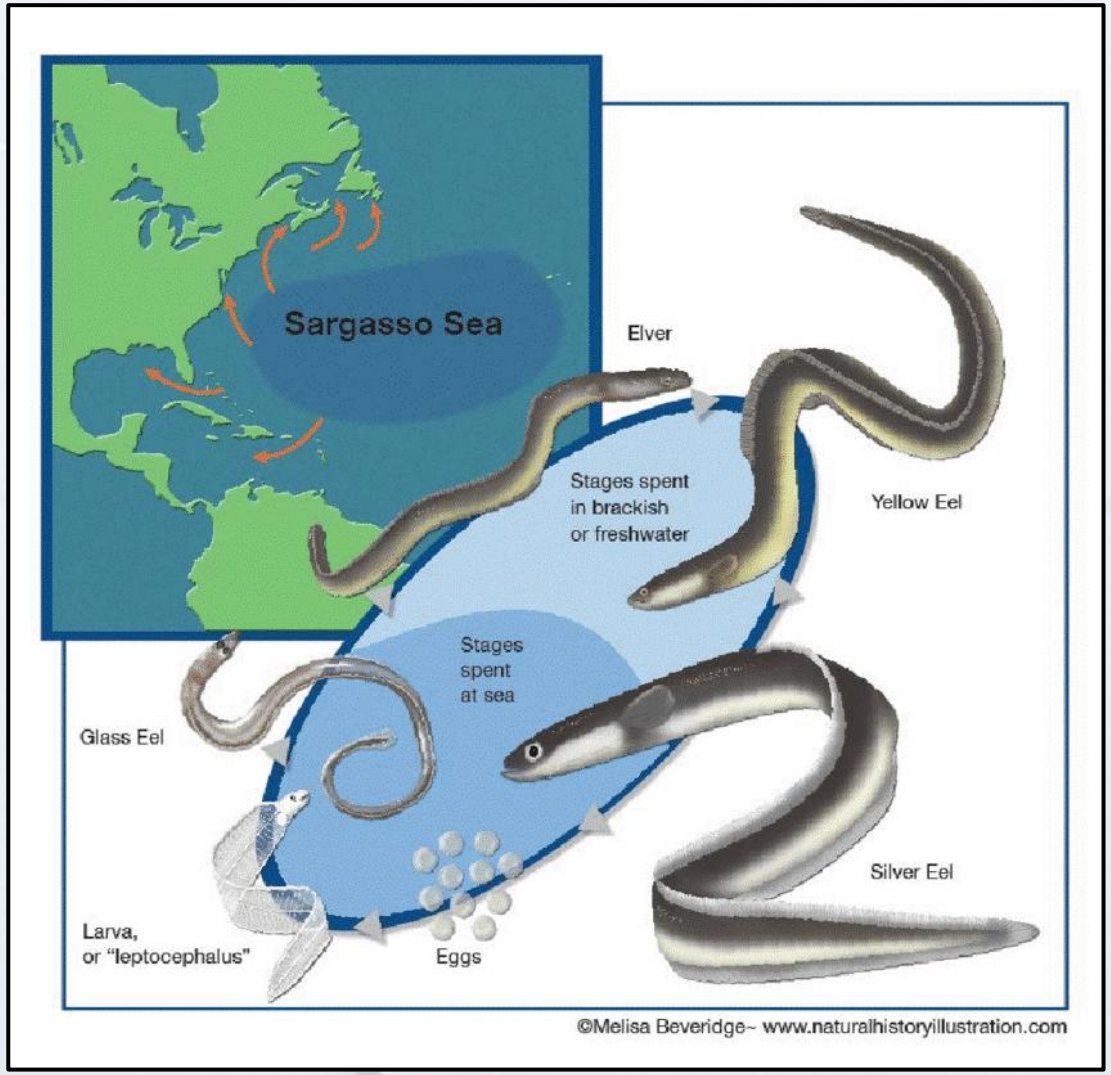


A Potted Natural History of American Eels

- American eel (*Anguilla rostrata*) spends its life in both fresh and saltwater
- Adults migrate from freshwaters across eastern North America to Sargasso Sea to spawn – and die.

- Larvae emerge from eggs, mature into “glass” eels, thence migrate to fresh/brackish waters

- Elvers mature into “yellow eels”, thence full adult “silver” eels

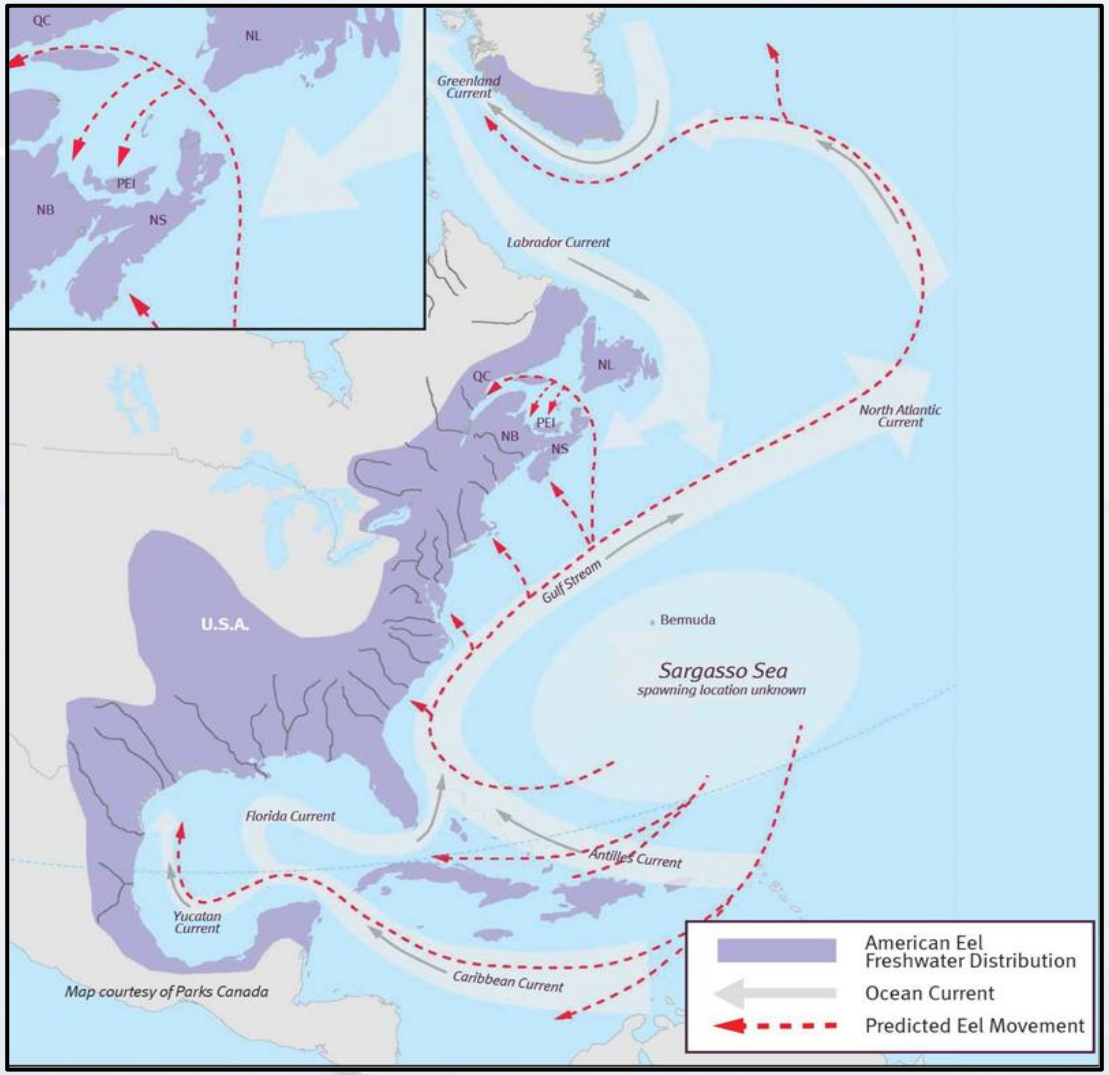


Eels are highly migratory!

Fig: Life cycle of the American eel, *Anguilla rostrata*.
©2013 Melisa Beveridge

Distribution of Canadian American Eels

- Canadian freshwater populations (ON, QC, Maritimes) sourced via Laurentian and Fundy ingress
- Inland Ontario populations most distal from source, and thus more prone to natural and anthropogenic influences on occupancy



Eels are highly migratory!

Fig: Migration routes of the American eel, *Anguilla rostrata*. © DFO, Canada

The Conservation Status of Ontarian Eels

- Once made up half of all biomass (life) in Lake Ontario
- Navigation, dams, overfishing, pollution caused a rapid decline in the 1980's
- Although not SARA listed ("no status"), COSEWIC (2012) recommend "threatened" status.
- In Ontario, species is listed as "Endangered" under the SARO (2008)

Status

Endangered

"Endangered" means the species lives in the wild in Ontario but is facing imminent extinction or extirpation.

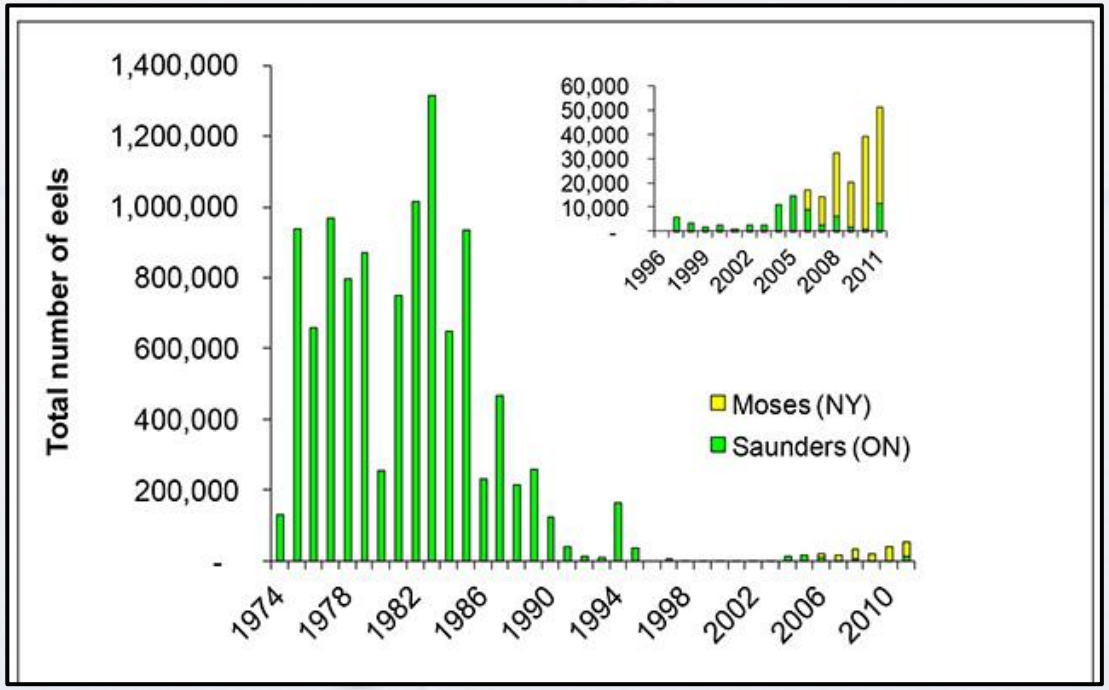


Fig: Eel capture at fish ladders at Moses-Sanders Dam, Cornwall, Ont., on US border. From MacGregor et al. (2013), Ontario Recovery Strategy

Slippery Customers: Monitoring Eels

- Conventional methods of monitoring include electrofishing, dip-netting, fyke netting, pots, bottom-trawling, etc.
- All have varying sensitivities, usually assessed as catch per unit effort (CPUE), which become problematic at low population densities
- Most have potential injurious impacts on local habitat and biota
- Trawling can reduce water quality and physically damage habitat

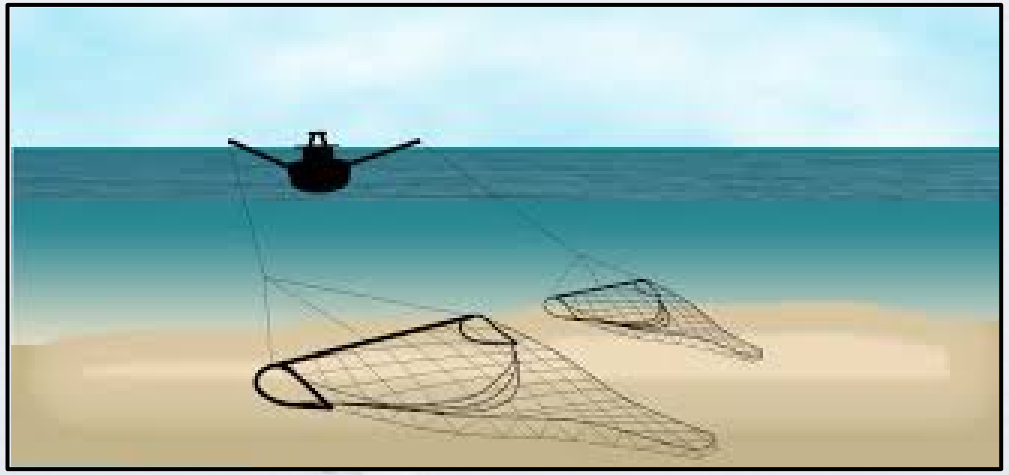


Fig: Schematic of typical bottom-trawling apparatus

eDNA as Eel Management Tool

- eDNA highly sensitive, specific and robust method to non-invasively sample for aquatic targets in all manner of waterbodies
- DNA can be extracted onsite from samples collected anywhere in water-column, optimizing the sampling process
- The potential for long-term monitoring at high resolution is huge, including much of the Laurentian watershed native to eels
- Needs demonstration: proof-of-concept

Fig: Example of onsite eDNA extraction – immediately preserving eDNA, including rare molecules from low abundant species– reducing risk of degradation of filters in transit

Molecular Basis of Detection

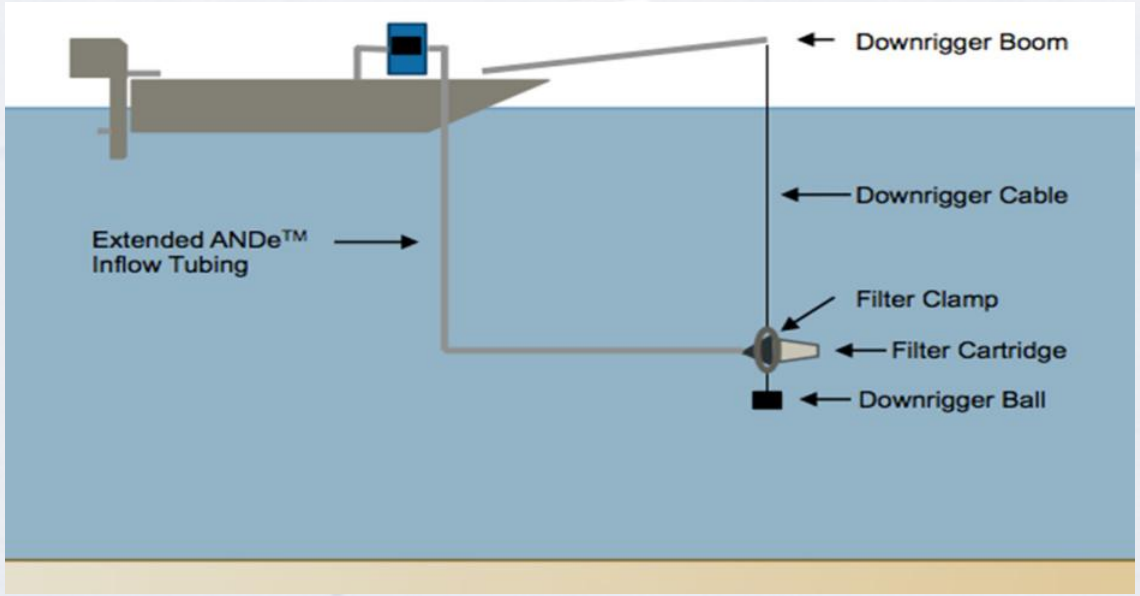
- A molecular genetic assay was developed specifically for the eel (qPCR)
- Developed cognizant of the evolutionary genetics of this species
- Some American eel populations may contain introgressed European eel genes
- To avoid false negative, assay developed to amplify American and European eel DNA
- There are no other remotely closely-related species in Lake Ontario (no risk of false positive)

Species	No. of samples	Detection Result
American eel (*A. rostrata*)	7	Positive
Atlantic salmon (*Salmo salar*)	1	Negative
Brown trout (*Salmo trutta*)	1	Negative
Rainbow trout (*Oncorhynchus mykiss*)	1	Negative
Coho salmon (*Oncorhynchus kisutch*)	1	Negative
Cisco (*Coregonus artedi*)	1	Negative
Round whitefish (*Prosopium cylindraceum*)	1	Negative
Lake whitefish (*Coregonus clupeaformis*)	1	Negative
Sand shiner (*Notropis stramineus*)	1	Negative
Creek chub (*Semotilus atromaculatus*)	1	Negative
W. blacknose dace (*Rhinichthys obtusus*)	1	Negative
Northern pike (*Esox lucius*)	1	Negative
Round goby (*Neogobius melanostomus*)	1	Negative

Table: Specificity table showing qPCR assay only detects American eel amongst other potentially synoptic species.

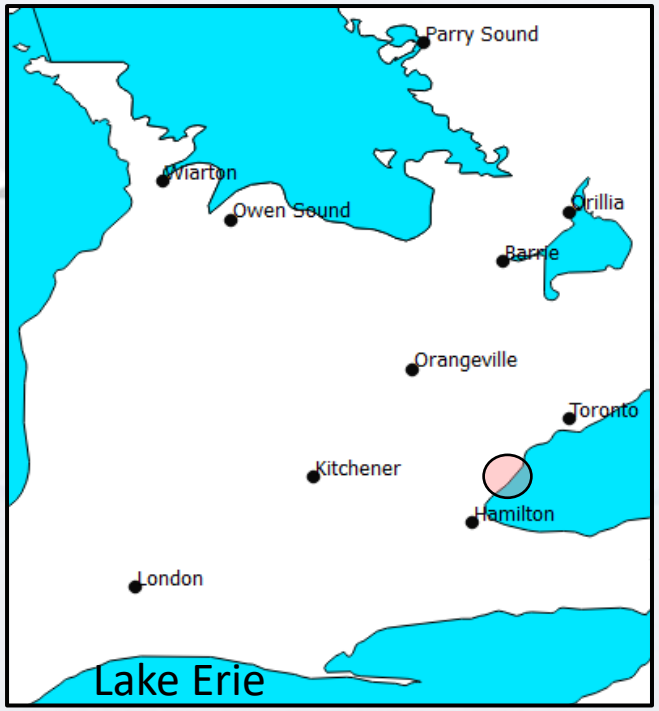
At-Depth Detection

- Eels are largely benthic, with habitat preferences for crevices in which to hide and ambush prey
- Optimal targeted sampling would be at depth, near to the bottom of waterbody
- In house onsite eDNA apparatus = ANDe, for which we developed a bespoke rig to sample at-depth (up to 20 m)
- Rigging attached to boat to allow easy access in large lentic environments, e.g., Lake Ontario. Samples taken 0.5 m above floor



Proof-of-Concept: Testing the Tool

- Collaboration with Town of Oakville and Tarandus Associates Ltd. Extreme western edge of current eel distribution.
- Oakville harbor subject to habitat restoration, including “eel condominiums” to provide refuge
- Comparative approach in a site approx. 300m by 100m : Day 1: eDNA survey; Day 2: conventional electrofishing
- eDNA: 15 sites (3 biological replicates of 3 L) filtered through 1.2 µm filter. DNA extracted onsite. 3 qPCR tests per biological replicate



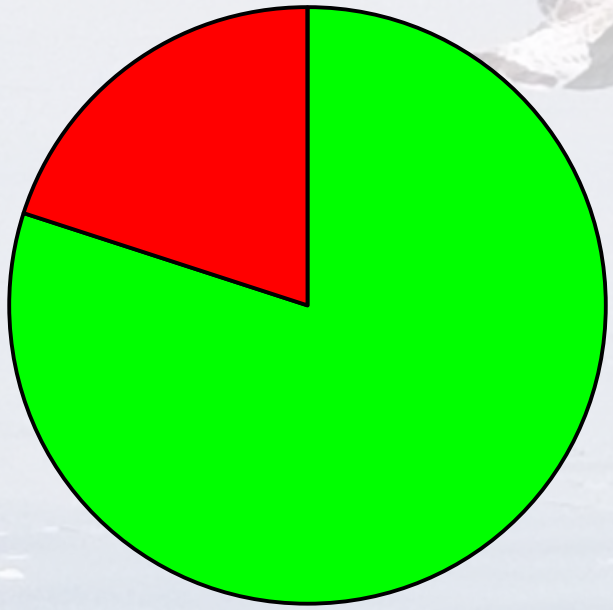
= Oakville Site, Lake Ontario

eDNA Detection of Eel in Lake Ontario

- High levels of American eel eDNA detection
- 80% ‘sites’ = at least one positive detection

Fig: Lake Ontario, north shore looking eastwards.

Proportion of positive sites



Evaluation of eDNA Success and Applicability

- eDNA, taken as proxy of the species itself, shows American eel to be present in the vicinity of the enhanced habitat
- Definitive evidence that eDNA can detect the species when present in a single visit
- 3 conventional methods in 4 visits failed to detect any American eel

Date	Collection Method	# American eels Collected
15/05/19	seine	0
	electrofisher	0
16/05/19	trap nets	0
31/07/19	seine	0
	electrofisher	0
01/08/19	trap nets	0

Table: Results of 3 conventional methods and 4 visits

Future of Eel eDNA Detection

- eDNA sensitive tool to monitor for signs of refugial populations at current distribution edge and track movements in estuarine/marine realms
- eDNA can also monitor reintroductions, monitor *in situ* habitat enhancement and recolonization post dam removal/eel ladder installation

Acknowledgments

- Our thanks are warmly extended to Don Speller of Tarandus Associates Limited who assisted in eDNA surveys and performed electrofishing; and to Rakesh Mistry of the Town of Oakville, Ontario, for greenlighting the project and making all resources available

Tarandus Associates Limited
Environmental Consultants

OAKVILLE

- We also acknowledge the laboratory work and enterprise of Louis Gasparini and Kevin Romanick, respectively

We would love you to contacts us:

Mario Thomas, Co-founder & CEO
mario.thomas@precisionbiomonitoring.com
Tel: +1 226 486 3252
Twitter: @eDNAmonitoring

PRECISION BIOMONITORING
—Intelligent DNA Solutions—