Environmental DNA Methods for Detecting Species at Risk Fish

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Latornell Symposium

November 19-21, 2019

PRECISION BIOMONITORING

AZIMUTH ENVIRONMENTAL CONSULTING, INC.

Environmental Assessments & Approvals

National Research Council Canada | Conseil national de recherches Canada

Introduction

- Species at Risk (SAR) detection historically required conventional surveys
- Environmental DNA (eDNA) as a rapid SAR surveillance method is gaining traction in research, but is not widely used in other sectors
- **eDNA:** DNA shed or released in a habitat used to infer species detection/no detection

Introduction

- Advantages for targeted SAR assessments:
  - Time & cost savings (esp. large areas)
  - Improved accuracy & repeatability
  - Covert, rare or patchily distributed species
  - Multi-season monitoring
  - Historic vs. recent signal detection (sediment)
  - Metabarcoding communities

Introduction

- Four-year study to develop eDNA protocols for SAR assessments in environmental consulting

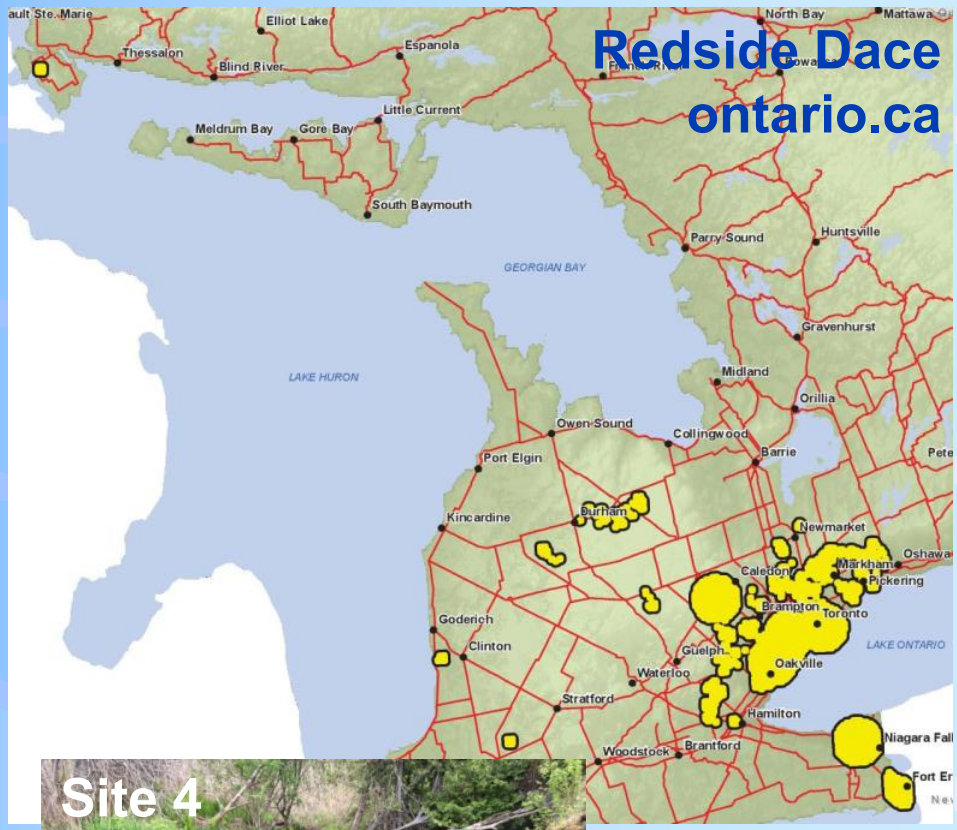
- Five focal SAR in Ontario:
  - Blanding's Turtle (THR)*
  - Little Brown Myotis (END)
  - Silver Shiner (THR)
  - Redside Dace (END)
  - Lake Sturgeon (END)

- Agency engagement (foster support)

***Tarof *et al*. (Submitted to eDNA)**

Introduction

- Occupy pools in shallow streams
- Live to ~4yrs old
- Males spawn annually in gravel riffles at 2-3yrs old (mid May-early June)
- Shed scales



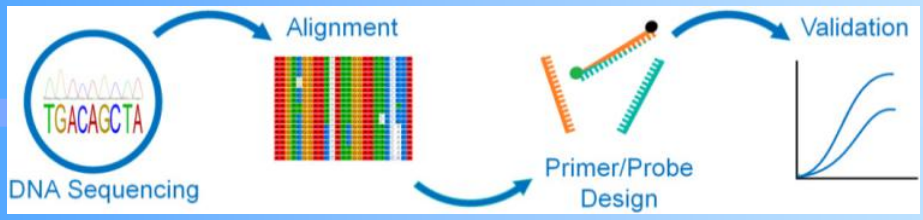
Introduction

- Live at bottom of large rivers and lakes for ≥100yrs
- Males spawn at 12-20yrs old on benthic rock every 2-3yrs (mid May-late June)
- Do not shed scales

Introduction

- **Question:** Do eDNA methods work well for fish with distinctly different ecologies and life histories?

Methods



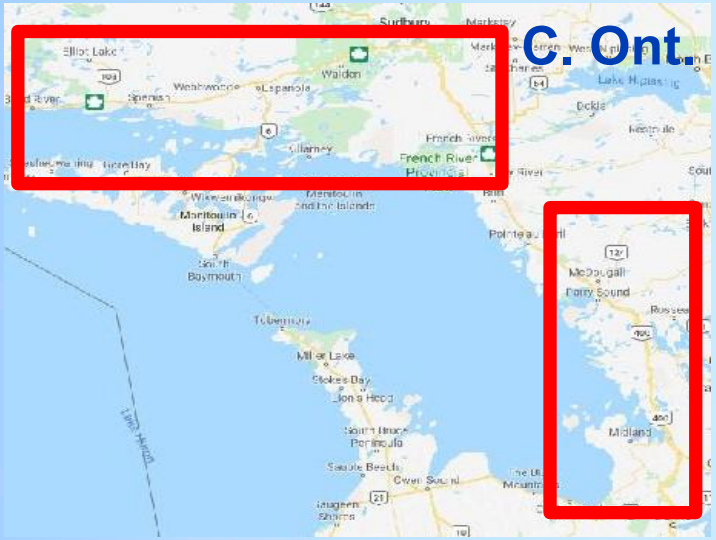
1. Highly specific TripleLock™ mtDNA assay design

2. Sampling regime (year, site & biological replicates)

4. Triplicate qPCRs

3. Fieldwork & eDNA extraction

Methods



- Used published/agency field data to ID pos. & neg. sites
- Sampled fall 2018, spring & summer 2019
- Discrete sampling (2018); equidistant or continuous sampling along 1-5km transects (2019)
- Collected environmental metadata
- Validated markers (low LOD, high qPCR efficiency)

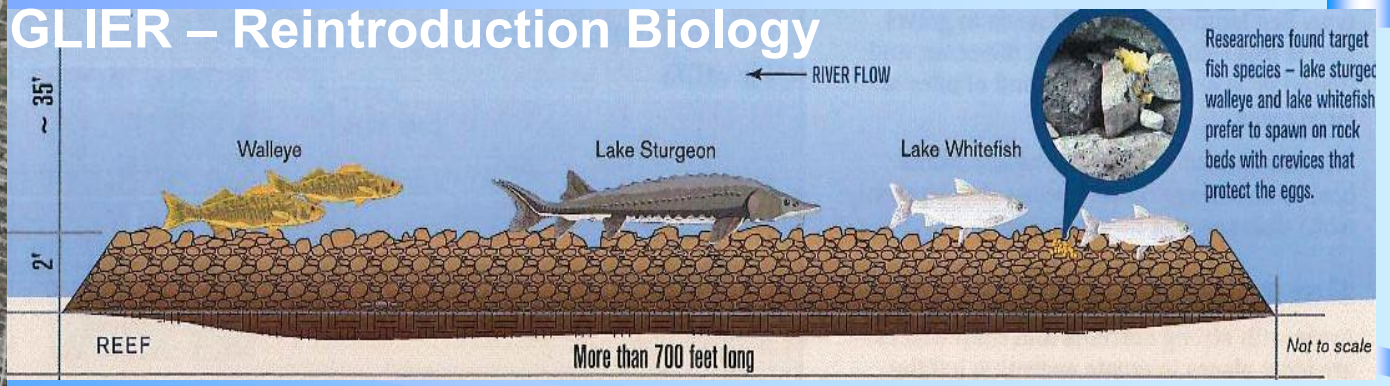
Methods

SAR	Sites	N-Size (Water)	Total Water Volume (L)	N-Size (Benthic)
RD	6	126	374.9	8
LS	7	113	501.5	6
Neg.	2, 4	19	30.3	4
Totals:	**17**	**258**	**906.7L**	**18**

Sampling: Surface/at Depth (& Benthic in 2018 only)
Amplified mtgenome, excluding all other taxa

Methods

Predictive Software™ – Water Volume & Where?



- Predictive analysis:
  - 10L water total (32.9L collected)
  - 5-10m depth
  - 1.2µm pore size
  - Accuracy: 99.5%
  - Survey Power: 99.4%

Results – Redside Dace

Detected from Water at 67% of Sites

Site	Region	Detected 2018	Detected 2019
1	S. Ont.	N	-
2	S. Ont.	N	-
3	S. Ont.	Y	N
4	S. Ont.	Y	Y
5	S. Ont.	-	Y
6[a]	S. Ont.	-	Y
2 Neg. Sites	S. Ont.	N	-

[a]Research Station/Hatchery

Mean per sample detection rate: 0.64 ± 0.35

Results – Redside Dace

	Mean ± SE (n)			
	Positive	**Negative**	**t (χ^2)[a]**	**P**
DO (mg/L)	143.7 ± 46.1 (26)	0.6 ± 0.7 (2)	4.99	**<0.0001***
Temp (° C)	13.2 ± 1.9 (26)	16.5 ± 3.8 (2)	-2.13	**0.04**
pH	8.4 ± 0.3 (28)	8.3 ± 0.3 (2)	0.60	0.55
TDS (ppm)	415.7 ± 63.1 (28)	247.8 ± 84.6 (2)	3.10	**0.004***
Turbidity (NTU)	2.1 ± 0.9 (28)	3.9 ± 4.2 (2)	-0.73	0.47
Depth (cm)	54.6 ± 139.8 (29)	22.8 ± 4.5 (2) (29)	(0.23)	0.63
Flow (m/s)	0.4 ± 0.3 (28)	0.1 ± 0.1 (2)	(2.82)	0.09

Comparisons based on positive sites where species was detected
[a]Non-parametric Mann-Whitney test (non-normal distribution)
*Significant after Bonferroni correction, (Bonferroni P = 0.007)

Results – Lake Sturgeon

Detected from Water at 57% of Sites

Site	Region	Detected 2018	Detected 2019
1	C. Ont.	N	N
2	C. Ont.	Y	N
3	S. Ont.	N	-
4	S. Ont.	N	-
5[a]	S. Ont.	-	Y
6	S. Ont.	-	Y
7[a]	S. Ont.	-	Y
4 Neg. Sites	S. Ont.	N	-

[a]Research Station/Hatchery

Mean per sample detection rate: 0.67 ± 0.36

Results – Lake Sturgeon

	Mean ± SE (n)			
	Positive	**Negative**	**t (χ^2)[a]**	**P**
DO (mg/L)	20.3 ± 39.9 (11)	58.9 ± 69.2 (4)	-0.97	0.35
Temp (º C)	19.3 ± 6.2 (11)	12.9 ± 4.8 (4)	1.39	0.19
pH	8.0 ± 0.3 (11)	7.2 ± 1.4 (4)	1.65[b]	0.29
TDS (ppm)	143.9 ± 66.4 (11)	263.8 ± 53.1 (4)	-3.36	**0.005***
Turbidity (NTU)	5.0 ± 2.6 (11)	2.0 ± 3.4 (4)	0.73[b]	0.55
Depth (cm)	634.9 ± 323.0 (11)	46.6 ± 50.3 (4)	(6.81)	**0.009**
Flow (m/s)	0.4 ± 0.3 (11)	0.1 ± 0.1 (4)	(2.10)	**0.055**

[b]Welch's ANOVA (heteroscedastic variance)
*Significant after Bonferroni correction, (Bonferroni P = 0.007)

Discussion

- Target eDNA was detected from water for both species at ~2/3 of positive sites
- No detection - benthic substrate, negative sites
- Results suggest detection is related to:

	RD	LS
○ Higher DO	√	
○ Cooler water	√	
○ Higher TDS	√	√
○ Water flow rate, depth	?	?

Discussion

- Potential for predictive software™ to refine sampling regime and improve detection

- Remarkable success detecting rare fish species
  - Site 6 (Lake Sturgeon)
    - 51km long
    - Up to 6km wide
    - 3-15m deep
    - Water residence time of 21hrs

Conclusions

- eDNA SAR detection offers exciting new opportunities for environmental consulting

- Sampling regime considerations:
  - Species' life history & ecology of eDNA
  - Equidistant/continuous sampling
  - Metadata to characterize eDNA environment
  - Flow dynamics & bathymetry data may strengthen sampling designs