

The Canadian Field-Naturalist

SUPPLEMENTARY MATERIALS:

A field survey of anuran species in two boreal lakes at the Experimental Lakes Area, Ontario, Canada

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TABLE S1. Fish assemblages in in two boreal lakes in the Experimental Lakes Area, Ontario, Canada, listed in descending order of most to least abundant species. Abundance is based on records of trap-netted adult fish by researchers at the International Institute of Sustainable Development-Experimental Lakes Area (IISD-ELA) as part of their long-term fish monitoring program (L. Hayhurst pers. obs.; IISD-ELA unpubl. data).

Lake 378	Lake 373
Yellow Perch (<i>Perca flavescens</i>)	Northern Redbelly Dace (<i>Chrosomus eos</i>)
Spottail Shiner (<i>Hudsonius hudsonius</i>)	Northern Pearl Dace (<i>Margariscus nachtriebi</i>)
Blacknose Shiner (<i>Notropis heterolepis</i>)	Finescale Dace (<i>Chrosomus neogaeus</i>)
White Sucker (<i>Catostomus commersonii</i>)	White Sucker (<i>Catostomus commersonii</i>)
Northern Pearl Dace (<i>Margariscus nachtriebi</i>)	Lake Trout (<i>Salvelinus namaycush</i>)
Lake Trout (<i>Salvelinus namaycush</i>)	Slimy Sculpin (<i>Cottus cognatus</i>)
Burbot (<i>Lota lota</i>)	
Brook Stickleback (<i>Culaea inconstans</i>)	
Fathead Minnow (<i>Pimephales promelas</i>)	
Slimy Sculpin (<i>Cottus cognatus</i>)	
Longnose Dace (<i>Rhinichthys cataractae</i>)	
Finescale Dace (<i>Chrosomus neogaeus</i>)	

TABLE S2. Primary substrate of anuran survey larval sampling stations in two boreal lakes in the Experimental Lakes Area, Ontario, Canada. Substrate type was qualitatively assessed based on visual observations. If two substrate types were roughly evenly observed at the station, both were listed. Aquatic vegetation was also noted if there was a prominent presence of aquatic vegetation within a 1-m radius of the station. Cobbles are small rocks between 64–256 mm, as defined on the Udden–Wentworth scale. Otherwise, any larger rocks were classified as substrate type “Rocks”. “Bedrock” is defined as a thin layer of sediment atop solid rock. Substrate was specified as “Sand” if sand grains were visible, otherwise “Fine sediment” includes any other fine particle substrate such as mud and silt.

Lake	Station	Substrate	Lake	Station	Substrate
373	1	Sand	378	1	Fine sediment, aquatic vegetation
373	2	Rocks, fine sediment	378	2	Fine sediment, aquatic vegetation
373	3	Cobbles	378	3	Rocks, aquatic vegetation
373	4	Sand	378	4	Rocks, aquatic vegetation
373	5	Sand	378	5	Rocks
373	6	Fine sediment, aquatic vegetation	378	6	Rocks, aquatic vegetation
373	7	Fine sediment, aquatic vegetation	378	7	Rocks, aquatic vegetation
373	8	Sand, aquatic vegetation	378	8	Rocks, aquatic vegetation
373	9	Sand, aquatic vegetation	378	9	Rocks, aquatic vegetation
373	10	Rocks, fine sediment	378	10	Fine sediment, aquatic vegetation
373	11	Sand	378	11	Sand
373	12	Cobbles	378	12	Rocks, aquatic vegetation
373	13	Rocks	378	13	Rocks, aquatic vegetation
373	14	Sand	378	14	Rocks
373	15	Sand	378	15	Rocks, aquatic vegetation
373	16	Rocks	378	16	Rocks, fine sediment
373	17	Sand, aquatic vegetation	378	17	Rocks
373	18	Rocks	378	18	Rocks
373	19	Fine sediment	378	19	Rocks, aquatic vegetation
373	20	Boulders	378	20	Rocks, aquatic vegetation
373	21	Boulders	378	21	Rocks
373	22	Fine sediment	378	22	Rocks, aquatic vegetation
373	23	Fine sediment, aquatic vegetation	378	23	Rocks, aquatic vegetation
373	24	Fine sediment	378	24	Rocks
373	25	Fine sediment	378	25	Bedrock
373	MARSH-A	Fine sediment, aquatic vegetation	378	MARSH-B-dock	Fine sediment, aquatic vegetation

TABLE S3. Date of all anuran egg-mass and larval surveys conducted in the Experimental Lakes Area, Ontario, Canada in 2022.

Date	Lake	Survey	Date	Lake	Survey
31 May	378	Wading	20 June	378	Wading
1 June	378	Snorkelling	21 June	373	Snorkelling
3 June	378	Larval bottle traps	21 June	373	Wading
4 June	378	Larval bottle traps	21 June	378	Larval bottle traps
6 June	378	Snorkelling	22 June	378	Larval bottle traps
6 June	378	Wading	24 June	373	Larval bottle traps
7 June	373	Snorkelling	27 June	378	Snorkelling
7 June	373	Wading	27 June	378	Wading
8 June	373	Larval bottle traps	28 June	373	Larval bottle traps
9 June	373	Larval bottle traps	28 June	373	Snorkelling
13 June	378	Snorkelling	28 June	373	Wading
13 June	378	Wading	18 July	378	Larval bottle traps
15 June	373	Snorkelling	18 July	378	Wading
15 June	373	Wading	4 August	378	Larval bottle traps
20 June	378	Snorkelling	4 August	378	Wading

TEXT S1. Methods and results of DNA barcoding of anuran eggs from two boreal lakes in the Experimental Lakes Area, Ontario, Canada

This supplement contains the methods and results (Table S4) of the Sanger sequencing DNA barcoding with the Folmer *et al.* (1994) COI primer set on amphibian eggs collected in Lake 378 at the International Institute of Sustainable Development-Experimental Lakes Area, northwestern Ontario, Canada in 2022.

Methods

Samples from egg masses were thawed in sterile glass petri dishes and embryos manually excised from the jelly using sterile forceps and a scalpel blade. The embryos were placed in a 1.5 mL microfuge tube containing 180 µL of buffer ATL used in the DNeasy Blood and Tissue Extraction Kit (Qiagen, cat. no. 69506, Venlo, Hulsterweg, Netherlands). The DNA was extracted according to the manufacturer’s instructions with the following modifications: (1) following the 1 h at 56°C incubation of the tissue in ATL buffer and proteinase K solution, with intermittent vortexing, 200 µL of AL buffer was added, the samples vortexed, and then incubated at 56°C for an additional 10 min; (2) the centrifugation step to remove the AW2 buffer was modified such that an initial 1 min spin at 17 000 ×g was performed, and the flow through decanted from the collection tube prior to a final 2 min spin at 17,000 ×g; and (3) the DNA was eluted in 100 µL of elution buffer and the eluate passed over the filter in the spin column a second time to improve DNA recovery. DNA was quantified using a QFX+ (DeNovix, Wilmington, Delaware, USA) or Qubit 4

(Invitrogen, Waltham, Massachusetts, USA) fluorometer and the dsDNA HS Assay Kit (Invitrogen, cat. no. Q32852, Waltham, Massachusetts, USA) and was calibrated with a two-point standard curve, as per the manufacturer’s instructions.

Purified DNA from the amphibian embryo samples was used in separate PCR reactions to amplify the COI region of the mitochondrial genome using the LCO1490/HCO2198 primer set (Folmer *et al.* 1994). Reactions contained 5 µL of 10× PCR buffer, 1 µL of 10 mM dNTPs, 5 µL of each primer (10 µM stock), 0.5 µL of 5 U/µL *Taq* polymerase (*Taq* DNA polymerase, FroggaBio, cat. no. T-500, Concord, Ontario, Canada), 1 µL of template DNA, and 32.5 µL of molecular biology-grade water. Thermocycling conditions were as follows: 94°C for 3 min, 40 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s, and a final elongation step of 72°C for 10 min. PCR products were electrophoresed in a 1.5% agarose 1× Tris-Acetate-EDTA gel at 100 V for 42 min. Bands were excised from the gel with a clean scalpel blade and purified using the EZ-10 Spin Column DNA Gel Extraction Kit (BioBasic, cat. no. BS654, Markham, Ontario, Canada) following the manufacturer’s protocol. Purified PCR products were quantified with a QFX+ or Qubit 4 fluorometer prior to being sent to The Centre for Applied Genomics (Toronto, Ontario, Canada) for Sanger sequencing. Resulting chromatograms were manually edited. Sanger sequencing reads from the forward and reverse primer were merged when possible and NCBI BLAST was used to search for the consensus region sequence

in the NCBI nucleotide collection database to determine species identity.

Literature Cited

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochond-

rial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.

Huang, X., and A. Madan. 1999. CAP3: a DNA sequence assembly program. *Genome Research* 9: 868–877. <https://doi.org/10.1101/gr.9.9.868>

TABLE S4. Results of Sanger sequencing DNA barcoding of eggs collected during egg-mass surveys at Lake L378 in the Experimental Lakes Area, Ontario, Canada in 2022. “Sample ID”: Sample identifier assigned at time of collection; “Date”: date of sample collection; “Morphological observations”: visual observations made at time of sample collection; “Top BLAST hit”: details on top NCBI Nucleotide BLAST hit with default settings. F/R/M indicates whether the forward, reverse, or merged sequence had the top hit; “*Anaxyrus americanus* top BLAST hit”: if the top BLAST hit was not American Toad, details on top American Toad BLAST hit. N/A if top blast hit was American Toad. F/R/M indicates whether the forward, reverse, or merged sequence had the top hit. More detailed data including: forward Sanger read (sequence of forward primer Sanger read after manual trimming of 5’ and 3’ ends by considering chromatograms and quality scores), reverse Sanger read (sequence of reverse primer Sanger read after manual trimming of 5’ and 3’ ends by considering chromatograms and quality scores), and merged sequence (sequence of merged contig as reported by the CAP3 (Huang and Madan 1999) algorithm accessed through the SnapGene software (www.snapgene.com), N/A if reads could not be merged) are all available online with manuscript data in data repository.

Sample ID	Date	Morphological observations	Top BLAST hit	<i>Anaxyrus americanus</i> top BLAST hit
378-22-001	31 May	stranded clutch, clear jelly, attached to vegetation	M: <i>Anaxyrus americanus</i> (NC_047224.1), 99% query cover, 98.38% identity	N/A
378-22-003	31 May	stranded clutch, clear jelly, attached to vegetation	F: <i>Anaxyrus fowleri</i> (MN135421.1), 100% query cover, 99.22% identity	F: MG423325.1, 100% query cover, 98.82% identity
378-22-004	6 June	stranded clutch, clear jelly, attached to vegetation	M: <i>Anaxyrus americanus</i> (NC_047224.1), 99% query cover, 98.38% identity	N/A
378-22-005	6 June	stranded clutch, clear jelly, attached to vegetation	M: <i>Anaxyrus fowleri</i> (MN135421.1), 100% query cover, 99.49% identity	M: MG423325.1, 100% query cover, 99.32% identity
378-22-006	6 June	stranded clutch, clear jelly, attached to vegetation	R: <i>Anaxyrus americanus</i> (NC_047224.1), 100% query cover, 98.59% identity	N/A
378-22-010	13 June	stranded clutch, clear jelly, attached to vegetation	F/M: <i>Anaxyrus americanus</i> (MG422960.1), 99% query cover, 99.52% identity	N/A
378-22-011	13 June	stranded clutch, clear jelly, attached to vegetation	F: <i>Anaxyrus fowleri</i> (MN135421.1), 100% query cover, 98.07% identity	F: MG423325.1, 100% query cover, 97.80% identity
378-22-013	13 June	stranded clutch, clear jelly, attached to vegetation	R/M: <i>Anaxyrus americanus</i> (NC_047224.1), 100% query cover, 99.22% identity	N/A

TABLE S5. Total number of anuran larvae by species detected in two boreal lakes in the Experimental Lakes Area, Ontario, Canada using light baited bottle traps, bottle traps, and dip-netting larval surveys in 2022. All bottle traps (light baited and non-light baited) contained three to four pieces of dry dog food bait. Numbers are totalled within date and lake.

Lake	Date	Survey method	Species	Total number detected
L373	9 June	Dip net	Green Frog (<i>Lithobates clamitans</i>)	3
L373	10 June	Bottle trap	Green Frog (<i>Lithobates clamitans</i>)	6
L373	11 June	Dip net	Green Frog (<i>Lithobates clamitans</i>)	3
L373	25 June	Bottle trap	American Toad (<i>Anaxyrus americanus</i>)	1
L373	25 June	Bottle trap	Green Frog (<i>Lithobates clamitans</i>)	1
L373	25 June	Light-baited bottle trap	Green Frog (<i>Lithobates clamitans</i>)	1
L373	29 June	Bottle trap	Green Frog (<i>Lithobates clamitans</i>)	10
L373	29 June	Bottle trap	Wood Frog (<i>Lithobates sylvaticus</i>)	2
L378	4 June	Bottle trap	Green Frog (<i>Lithobates clamitans</i>)	5
L378	4 June	Dip net	Green Frog (<i>Lithobates clamitans</i>)	3
L378	4 June	Dip net	Mink Frog (<i>Lithobates septentrionalis</i>)	1
L378	5 June	Bottle trap	Green Frog (<i>Lithobates clamitans</i>)	5
L378	22 June	Bottle trap	American Toad (<i>Anaxyrus americanus</i>)	263
L378	22 June	Light-baited bottle trap	American Toad (<i>Anaxyrus americanus</i>)	1584
L378	22 June	Bottle trap	Green Frog (<i>Lithobates clamitans</i>)	4
L378	22 June	Light-baited bottle trap	Green Frog (<i>Lithobates clamitans</i>)	1
L378	22 June	Dip net	Green Frog (<i>Lithobates clamitans</i>)	1
L378	23 June	Bottle trap	American Toad (<i>Anaxyrus americanus</i>)	85
L378	23 June	Light-baited bottle trap	American Toad (<i>Anaxyrus americanus</i>)	204
L378	5 August	Light-baited bottle trap	Gray Treefrog (<i>Dryophytes versicolor</i>)	1
L378	5 August	Light-baited bottle trap	Green Frog (<i>Lithobates clamitans</i>)	2

TABLE S6. Incidental detections of adult Central Newt (*Notophthalmus viridescens louisianensis*) during larval trap surveys in Lake 378 in the Experimental Lakes Area, Ontario, Canada in 2022. No newts were captured in Lake 373.

Date	Survey method	Total number detected
4 June	Bottle trap	1
5 June	Bottle trap	2
22 June	Bottle trap	1
23 June	Light-baited bottle trap	1
23 June	Bottle trap	1
5 July	Light-baited bottle trap	1