

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

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Abstract

With over 60% of Canada's amphibian species at-risk, field surveys and studies can contribute important knowledge to better inform assessments and conservation efforts. The diversity of amphibians inhabiting lakes, specifically semi-remote boreal lakes, has been relatively understudied compared to wetlands and temporary waterbodies. The goal of our study was to survey the anuran species associated with freshwater boreal lakes in a historically undocumented area in northwestern Ontario, Canada. In May–August 2022, we conducted egg surveys, larval surveys, and automated call recording surveys at two boreal lakes at the International Institute of Sustainable Development-Experimental Lakes Area. We detected seven anurans: American Toad (*Anaxyrus americanus*), Gray Treefrog (*Hyla versicolor*), Green Frog (*Lithobates clamitans*), Mink Frog (*Lithobates septentrionalis*), Northern Leopard Frog (*Lithobates pipiens*), Spring Peeper (*Pseudacris crucifer*), and Wood Frog (*Lithobates sylvaticus*). We also incidentally detected adult Central Newt (*Notophthalmus viridescens louisianensis*). American Toad was the most extensively detected species among our survey methods, with all life stages detected. In contrast, Northern Leopard Frog was infrequently detected in calling surveys at one lake. Although anuran populations can vary annually and within relatively small spatial scales, our results suggest that freshwater boreal lakes harbour a diversity of anurans and should not be overlooked as valuable amphibian habitat. As anthropogenic impacts continue to threaten boreal ecosystems, our surveys provide baseline knowledge of anurans in these semi-remote boreal lakes of Canada, critical for further studies and amphibian conservation efforts.

Key words: Anuran; field survey; freshwater lakes; boreal; amphibian community; frog; toad; Ontario

Introduction

The vulnerability and ecological importance of amphibians underscore the need to better understand their ecology and distribution for effective conservation. As of December 2025, over 60% of Canadian amphibian species assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2025) were designated as at-risk, where they face threats such as habitat loss and changes, human development, and pollution (Lesbarrères *et al.* 2014; Luedtke *et al.* 2023). These threats can alter amphibian species distributions (Seaborn *et al.* 2021) and community structure (Lehtinen *et al.* 1999; Gagné and Fahrig 2007), leading to potential impacts on whole ecosystems (Whiles *et al.* 2013; Alonso *et al.* 2024). As such, a lack of knowledge of amphibian

species distributions in many areas of Canada continues to pose a challenge for informing proper status assessments and conservation efforts (Lesbarrères *et al.* 2014). Field surveys and monitoring can therefore be valuable in documenting species diversity, distribution, and their trends (Desroches *et al.* 2010) to guide conservation efforts.

Amphibian survey and monitoring efforts in North America are unevenly distributed among habitat types and geographic regions. Within peer-reviewed literature, amphibians are relatively well studied in wetlands and ephemeral water bodies such as vernal pools and ponds (Hecnar and M'Closkey 1998; Garretson *et al.* 2020; Luymes and Chow-Fraser 2022; Ray *et al.* 2022). However, they remain understudied in permanent lakes (i.e., lentic bodies of freshwater;

Walls 2014), even though they are known to make use of these valuable lake habitats (Eaton *et al.* 2005; Finlay and Vredenburg 2007; Dodd 2023). Many of the lakes in Canada are in the boreal biome: an estimated 1.5 million (Schindler 1998). With roughly 25 freshwater-dependent amphibian species in Canada (Desforges *et al.* 2022), amphibian use of these many boreal lake habitats remains unknown. Geographically, the remoteness of many of these boreal lakes further limits accessibility for survey and monitoring. Unsurprisingly, this trend can also be seen from large-scale monitoring efforts, which tend to be concentrated along roads and more accessible areas (Weir *et al.* 2009; Petrovan *et al.* 2020).

Boreal lakes provide many important ecosystem services and host a diversity of species but are increasingly threatened by climate change and other anthropogenic impacts (Schindler 2001; Ruckstuhl *et al.* 2007). Because of its latitude, the boreal biome is predicted to be one of the most threatened by climate warming (Ruckstuhl *et al.* 2007; Berner and Goetz 2022). Climate change is impacting the physical, chemical, and biological processes within these boreal lakes (Schindler *et al.* 1996; Schindler 2001; Keller 2007; Guzzo and Blanchfield 2017). A previous study on the effects of climate warming in the boreal lakes of northwestern Ontario found changes in lake temperature, water mixing, and productivity (Schindler *et al.* 1996). With all these threats to Canada's boreal lakes, there is still very limited knowledge on the presence and roles of amphibians in the boreal region and how impacts such as climate change may affect the anurans these lakes support (Lesbarrères *et al.* 2014). Therefore, the boreal lakes in northwestern Ontario, Canada provide a unique opportunity for expanding our knowledge of anuran communities in this remote region and better informing the conservation of these threatened habitats.

There is a disparity between the abundance of boreal lakes in northwestern Ontario, Canada, and surveys of frog and toad (order Anura) species diversity in the lakes. Some anuran surveys have been conducted in the eastern parts of the region (e.g., the Thunder Bay region; Abbott 2004; Robinson 2004) but to our knowledge, there have not been any substantial efforts to monitor or survey anurans in boreal lakes in the region's western part (encompassing the Kenora District of Ontario) documented in peer-reviewed literature. However, we found two published amphibian reports: a new geographical distribution record for Eastern Newt (*Notophthalmus viridescens*) from lakes at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA), in the Kenora District (Hayhurst *et al.* 2017) and a record of Gray Treefrog (*Hyla*

versicolor) observations from the Red Lake area in the Kenora District (Weller 2009). The sparsity of data and observations for anuran species in this region can also be seen in the Ontario Reptile and Amphibian Atlas (Ontario Nature 2023), where there are relatively far fewer observations, even for anuran species with known ranges extending to the northwestern region of Ontario, than in southern Ontario.

Here, we report results from our field surveys of anuran species in two boreal lakes at the IISD-ELA in northwestern Ontario, Canada. We conducted egg, larval, and adult calling surveys to provide a comprehensive survey of the anuran species because different species may be present at various life stages during the survey period. Our goal was to conduct the first field survey of the anuran species inhabiting lakes at the IISD-ELA and to compare our results with our initial assessment of the species expected to be found in the area. We aim to contribute baseline knowledge of the anuran diversity inhabiting semi-remote boreal lakes in Ontario, Canada.

Methods

Survey area: IISD-ELA Lake 373 and Lake 378

The IISD-ELA (49.6603°N, 93.7283°W) consists of 58 freshwater lakes and their catchments that have been set aside for scientific research. Being designated for research, the lakes in the area remain relatively pristine; the lakes are not accessible to the public and there are no recreational activities such as fishing, boating, leisure, nor development on, or directly around, the lakes. The IISD-ELA is in Treaty 3 territory, on the traditional land of the Anishinaabe and the homeland of the Métis Nation. This remote area, ~52 km southeast from the nearest city of Kenora, Ontario, has been the site of long-term monitoring since the 1960s as well as many ecosystem-level experiments and manipulations (Blanchfield *et al.* 2009). The lakes are in the central region of the Boreal Shield Ecozone, where the mixed wood forest grows over the geology of the Precambrian Shield (Brunskill and Schindler 1971; Keller 2007). At the IISD-ELA, all surveys were conducted within and adjacent to two boreal lakes: Lake 373 (L373; 49.7431°N, 93.7984°W) and Lake 378 (L378; 49.7113°N, 93.7743°W). Both lakes are small, oligotrophic, and dimictic, headwater lakes of similar size (L373: 27.4 ha, 21 m maximum depth; L378: 24.7 ha, 16.7 m maximum depth). These lakes host a multitude of aquatic species, with fish populations that have been studied for multiple decades as part of a long-term monitoring program led by the IISD-ELA (datasets can be requested online from the IISD-ELA). Both L378 and L373 host various fish species, with 12 species recorded in L378 and six species in L373

(Table S1; L. Hayhurst pers. obs.; IISD-ELA unpubl. data). Common to both lakes are multiple species of minnows (small-bodied Cyprinidae and Leuciscidae), and larger-bodied fishes such as Lake Trout (*Salvelinus namaycush*) and White Sucker (*Catostomus commersonii*). Notably, L378 also has Burbot (*Lota lota*) and an abundant population of Yellow Perch (*Perca flavescens*), both species not found in L373. The two lakes are ~3.7 km apart and both consist of a variety of shoreline microhabitats that may allow for a diversity of aquatic species to inhabit the lakes.

Survey sites for each of the sampling methods were established around the two lakes (Figure 1). Exact survey location selection was determined by accessibility and microhabitat type. Survey locations needed to be accessible by boat and/or waders. Otherwise, we started by dividing survey locations to be roughly evenly spaced all around the lake shoreline. Then, within those locations, exact survey sites depended on the microhabitat of the area. We avoided steep shores and tall rock banks and gave preference to shallow bays, areas with large fallen trees, and areas with dense aquatic vegetation. The selected survey sites had various substrates, from fine sediment to bedrock (Table S2).

Species expected

We initially determined which anuran species were known to have ranges extending into north-western Ontario (Table 1; Dodd 2023; Ontario Nature 2023). Then we determined which species were expected to be encountered from each of our survey approaches by considering method limitations and timing, and each species' known range, habitat preferences, and life history traits (Harding and Mifsud 2017; Dodd 2023; Ontario Nature 2023). A designation of "maybe" was assigned if, for that life stage, the timing of our surveys was not likely to overlap with their known developmental timing but they were known to breed in permanent lakes with fish, or survey timing was likely to overlap but they were not likely to breed in permanent lakes with fish.

Egg-mass surveys

Egg-mass surveys were conducted from 30 May to 4 August 2022, using two different methods (snorkelling and wading) because of the various types of shoreline habitat around the lakes. Survey effort was concentrated in the first two months to align with peak anuran breeding season for most Ontario species (Harding and Mifsud 2017). Snorkelling and wading surveys were conducted on the same day when possible, and when not, on adjacent days due to time constraints.

Snorkelling surveys were performed along 20 m transects parallel to the lakes' perimeter and ~1–2 m deep (Figure 1). Each of the two lakes had 10 transects

that were roughly evenly distributed around the lake's perimeter, while also considering areas where anuran eggs might likely be present, e.g., areas with fallen trees or submerged vegetation, avoiding steep rocky drops. Snorkelling surveys were conducted between May and June, with L373 surveyed four times and L378 surveyed five times (Table S3).

Wherever snorkelling was not possible, such as very shallow bays around the lake with considerable emergent or shoreline vegetation, egg-mass surveys were conducted by wading. Wading surveys were performed by two people walking around the perimeter of the vegetated area and then in a series of parallel transects through the area, if vegetation density permitted, visually scanning the submerged vegetation for eggs. Wading surveys were conducted four times in L373 and seven times in L378 between May and August (Table S3).

Initial species identification was made using photographs (Nikon COOLPIX W300, Nikon Canada, Mississauga, Ontario) of eggs cross-referenced with visual guides (Parmelee *et al.* 2002; Altig and McDiarmid 2015). When egg masses were encountered, we carefully excised four to five eggs from a representative mass using scissors, placed the eggs in a collection tube on ice, and then stored them at -20°C . These collected eggs were shipped to the University of Waterloo and stored at -80°C until they were DNA barcoded (see Text S1) to verify species identification.

Larval surveys

Larval surveys were conducted between 4 June and 5 August 2022, using bottle traps. Bottle traps were constructed following designs outlined in Wilkinson (2015), using 2 L plastic drink bottles cleaned with a 10% bleach solution. The bottle traps had a small circular opening of ~2.5 cm in diameter, into a ~1 L interior where the larvae were trapped. A small hole was made opposite the trap entrance and angled outside of the water to allow air exchange. Before deploying, four pieces of dry dog food kibble (Country Harvest Pet Food, Spectrum Food Services Ltd., Moorefield, Ontario, Canada) were placed inside every trap. Although previous studies have found variable success of food baited versus non-food baited amphibian traps (Adams *et al.* 1997; Baker 2013), our preliminary trials found food baiting led to slightly higher numbers of trapped anuran larvae without affecting diversity or predator presence (J.O.K. unpubl. data). Each food-baited bottle trap was fixed into place ~0.2 m under water at each station with a flagged metal stake. For each lake, bottle traps were set out in 25 different stations distributed along the entire lake shoreline, with some of these stations concentrated among shallower bays (Figure 1).

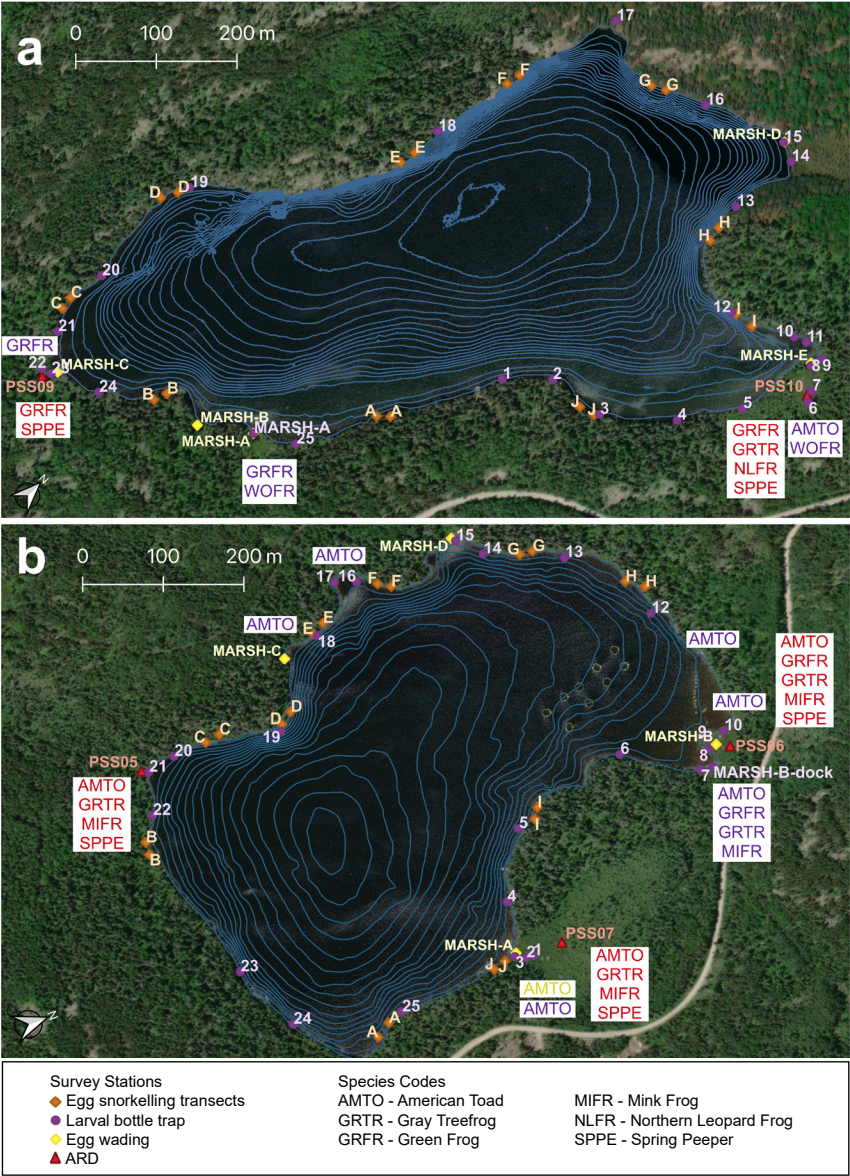


FIGURE 1. Satellite maps of the two boreal lakes, a. Lake 373 and b. Lake 378, at the International Institute of Sustainable Development-Experimental Lakes Area, northwestern Ontario, Canada surveyed for anuran eggs, larvae, and adults in 2022. Survey stations for each survey method are differentiated by object colour: yellow for wading egg surveys, orange for snorkelling egg transects, purple for larval bottle traps, and red for adult automated recording devices (ARD). Each station was assigned an arbitrary identifier, with letters for snorkelling egg surveys, labels “MARSH” A-E for wading egg surveys, and numbers for the larval survey stations (with one larval survey station assigned as “MARSH-B-dock” instead of a number due to its proximity to a defunct dock and to egg survey station “MARSH-B”). All ARD call survey stations have the prefix “PSS”. For the snorkelling egg surveys, there are two stations with the same letter, with one point representing the transect start and the other point represents the transect end. Lake bathymetry (shown in blue) taken from IISD-ELA (2022) are shown for each lake, where each contour line interval represents a depth change of 1 m. Each white text box on the map is associated with the closest station point and lists all the species detected at the station (some lists have been combined with a neighbouring station to reduce space). The colour of the species text corresponds to the colour of the survey method for which the species were detected, for example: purple-coloured text in the box lists all the species found during larval surveys that the nearest larval bottle trap station (represented by a purple dot). These maps were created using QGIS, with data plotted on the ESRI Online World Imagery basemap (www.esri.com) using the EPSG:3857 - WGS 84 / Pseudo-Mercator coordinate reference system.

TABLE 1. Anuran species expected to be found in northwestern Ontario and detected during our 2022 surveys using egg surveys, larval surveys, and adult call surveys. Expectations were based on known species ranges, life history traits, habitat preferences, and survey timing and limitations (further details in Discussion). Each species was assigned a “YES” if highly expected to be detected, a “MAYBE” if it might be detected, or a “NO” if not expected to be detected by each survey method. If the species was detected at least once using the survey method, it was assigned “YES”; otherwise it was assigned “NO”.

Species	Expected/detected		
	Egg survey	Larval survey	Adult call survey
American Toad (<i>Anaxyrus americanus</i>)	YES/YES	YES/YES	YES/YES
Boreal Chorus Frog (<i>Pseudacris maculata</i>)	NO/NO	NO/NO	NO/NO
Gray Treefrog (<i>Hyla versicolor</i>)	MAYBE/NO	MAYBE/YES	YES/YES
Green Frog (<i>Lithobates clamitans</i>)	MAYBE/NO	YES/YES	YES/YES
Mink Frog (<i>Lithobates septentrionalis</i>)	MAYBE/NO	YES/YES	YES/YES
Northern Leopard Frog (<i>Lithobates pipiens</i>)	MAYBE/NO	MAYBE/NO	NO/YES
Spring Peeper (<i>Pseudacris crucifer</i>)	MAYBE/NO	MAYBE/NO	YES/YES
Wood Frog (<i>Lithobates sylvaticus</i>)	NO/NO	NO/YES	NO/NO

Starting in mid-June, an additional light-baited bottle trap was deployed at select stations. These were baited with the same amount of dog food plus a glow stick (Item no. 9202, Coghlan’s Ltd., Winnipeg, Manitoba, Canada) because funnel traps with glow sticks have been found to trap greater numbers of tadpoles than traps without glow sticks (Grayson and Roe 2007; Bennett *et al.* 2012).

Traps were deployed at various times of day, depending on safe weather conditions, but all traps were left overnight and collected between 20 and 24 h after deployment to ensure animal safety and survival. Larval anurans found in the traps were photographed, identified visually, and counted, with visual identifications confirmed after the surveys using the photographs. We also photographed and recorded adult newts (order Caudata) incidentally found in the traps during our surveys to support the only other known record of Eastern Newt observations in this area of northwestern Ontario, Canada (Hayhurst *et al.* 2017). All animals were released at the location of collection as soon as they were photographed and counted.

Full larval bottle trap surveys (at all 25 stations in each lake) were conducted in June a total of four times at L378 and three times at L373 (Table S3). In addition, a subset of the stations (which were in more favourable larval habitat) were visited an additional four times at L378 between June and August and once at L373 in June to survey as many larvae as possible. When checking the bottle traps, larvae were occasionally observed nearby but not captured in the traps. Again, to survey as many larvae as possible, a medium-sized aquarium net was brought during the deployment and collection of bottle traps to capture, identify, and record these individuals using a modified dip-netting technique. These observations were recorded three times in L373 and two times in L378 in June.

Adult call surveys

To determine which species of adult calling anurans were present in the lake communities, SM4 Song Meter ARDs (Wildlife Acoustics, Inc., Maynard, Massachusetts, USA) were used to automatically record calls from 2 June to 1 October 2022. Recording start date was dependent on ice-off timing of the lakes and road accessibility. We extended the recording period into the fall because Green Frog (*Lithobates clamitans*) has been known to call into August in the Great Lakes region (Harding and Mifsud 2017). The ARDs were set with a sample rate of 44.1 kHz (or 48 kHz) on stereo with a gain of 16 dB and preamp of 26 dB. Each ARD recording was programmed to capture representative anuran calling in the region (Schueler 2025 most succinctly highlights the key calling times in anurans specifically for this region) with considerations for optimal memory storage, battery life, and analysis ability. The ARDs recorded a 10 min audio file every hour for 3 h starting 1 h before sunrise, at 6 h after sunrise, and then again every hour for 5 h starting 1 h before sunset (a total of nine 10 min audio files per day).

Devices were set up on land around the perimeter of the lake (Figure 1), within ~2 m on shore or within shallow lake bays, in areas with no running water to minimize background noise. A total of five devices (two around L373 and three around L378) were set up about 1 m above ground-level, mounted onto an artificial post, or natural vegetation if present, that could stabilize the meter with minimal movement.

The recorded audio files were analyzed using the Kaleidoscope Pro v5.6.3 (Wildlife Acoustics) analysis software. To analyze the recordings for anuran calls, software parameters were set to optimally detect anuran calls, i.e., between 50 and 3500 Hz and 0.1–5 s duration (Trowbridge and Litzgus 2022). For species

identification, Kaleidoscope Pro's Cluster Analysis feature was used to automatically scan the recordings for anuran calls and sort the detections into clusters (groups) of similar sounds. A separate cluster analysis was run for every ARD, with the analysis set to detect a maximum 500 clusters from each recording file. For each cluster analysis, all the clusters were separated based on species call identification after manually listening to a minimum of 10 detections from each cluster.

To set cluster analysis parameters, the accuracy of species identification from the clusters was verified by manually listening to a random subset of one of the ARDs and comparing various parameter settings for highest accuracy rates. Cluster identification accuracy was determined using the equation adapted from Larsen *et al.* (2021):

$$\text{Accuracy} = \frac{\left[\frac{(\text{Accurately identified species heard})}{(\text{total number of detections manually verified})} + \frac{(\text{Accurately identified when no species heard})}{(\text{total number of detections manually verified})} \right]}{2}$$

For the parameter 'cluster distance', we found a cluster distance of 0.65 m to have slightly higher detection accuracy than 0.75 m (82% versus 80%). This is a shorter distance than the default setting (1.0 m) and the 0.75 m cluster distance used by Trowbridge and Litzgus (2022), meaning that the detections will be more similar to each other in the clusters, but rare detections may be lost. Therefore, with increased accuracy, there is a possibility we did not detect infrequent or rare species. With the parameters set, the accuracy of the clusters was verified by manually listening to a random 1% subset of cluster analysis detections (631 detections) from a different ARD that had undergone species call identification, with an accuracy of 90%, a false negative detection rate (where no species were detected from cluster analysis but at least one species is heard) of 7%, and a false positive detection rate (where a species was detected from cluster analysis but that species is not heard) of 3%.

All graphs were made using R Studio (version 2023.12.1+402; R Core Team 2023) and the suite of R packages included in 'tidyverse' (Wickham *et al.* 2019).

Results

We detected seven of the eight anuran species known to occur in northwestern Ontario using at least one of our survey methods (Table 1): American Toad (*Anaxyrus americanus*), Gray Treefrog (*Hyla versicolor*), Green Frog, Mink Frog (*Lithobates septentrionalis*), Northern Leopard Frog (*Lithobates pipiens*), Spring Peeper (*Pseudacris crucifer*), and Wood Frog (*Lithobates sylvaticus*). Boreal Chorus Frog (*Pseudacris maculata*) was not expected to be detected

and we failed to detect it. While we did not expect to detect Wood Frog, we did so using larval surveys.

Egg mass surveys

During the 11 wading surveys conducted between 31 May and 4 August 2022, we found hundreds of American Toad egg masses in a shallow, soft bottom bay around L378; the species' eggs were first detected on 31 May and observed during subsequent visits on 6 and 13 June. American Toad egg masses were also observed during one snorkelling survey of L378 on 1 June. Unfortunately, we were unable to accurately count the egg masses to estimate abundance. Because of their explosive breeding, American Toad egg mass strands were clustered throughout the bay in a single area ~10 m along the shallow shoreline, making individual strands indiscernible. We are uncertain how far the adults swam to the egg-laying site but informally have observed toads swimming around the lake and have heard from others who have seen them swimming in the middle of the lake. DNA barcoding identified all sampled egg masses as either American Toad or Fowler's Toad (*Anaxyrus fowleri*). Given what is known about the extremely limited range of Fowler's Toad in Canada (COSEWIC 2010), all barcoded egg masses were determined to be American Toad (Table S4). No other species were detected during our egg mass surveys.

Larval surveys

We detected five species in larval traps: American Toad, Gray Treefrog, Green Frog, Mink Frog, and Wood Frog (Table 2). American Toad larvae were most abundant but only during a brief period, with over 2000 larvae surveyed between 22 and 25 June in bottle traps and light-baited bottle traps; 800 larvae were collected in one light-baited trap. Green Frog larvae were found most frequently from early June to early August. They were captured via bottle traps, light-baited bottle traps, and dip-netting. Gray Treefrog larvae were found in our larval surveys on one day in August, in a light-baited bottle trap. Mink Frog larvae were found one day in June via dip-netting, and Wood Frog larvae were found once on 29 June in bottle traps. The total numbers of larvae detected by survey method and lake are in Table S5.

The larvae were primarily restricted to shallow, bay habitats. In L378, American Toad larvae were found in seven different stations around the lake. However, all other detected species (Green Frog, Gray Treefrog, and Mink Frog) were only found in one area at the north end of the lake (Figure 1)—the widest, shallowest bay in the lake. In L373, larvae were found along the eastern shore of the lake. American Toad and Wood Frog larvae were found in a wider, shallow bay on the northern side of L373.

TABLE 2. Number of larvae of each species detected in Lake 373 and Lake 378 at the International Institute of Sustainable Development-Experimental Lakes Area, northwestern Ontario, Canada between June and August 2022 during larval surveys.

Lake	Date	Species	Number of larvae
L373	9 June	Green Frog (<i>Lithobates clamitans</i>)	3
L373	10 June	Green Frog (<i>Lithobates clamitans</i>)	6
L373	11 June	Green Frog (<i>Lithobates clamitans</i>)	3
L373	25 June	Green Frog (<i>Lithobates clamitans</i>)	2
L373	25 June	American Toad (<i>Anaxyrus americanus</i>)	1
L373	29 June	Green Frog (<i>Lithobates clamitans</i>)	10
L373	29 June	Wood Frog (<i>Lithobates sylvaticus</i>)	2
L378	4 June	Green Frog (<i>Lithobates clamitans</i>)	8
L378	4 June	Mink Frog (<i>Lithobates septentrionalis</i>)	1
L378	5 June	Green Frog (<i>Lithobates clamitans</i>)	5
L378	22 June	Green Frog (<i>Lithobates clamitans</i>)	6
L378	22 June	American Toad (<i>Anaxyrus americanus</i>)	1847
L378	23 June	American Toad (<i>Anaxyrus americanus</i>)	289
L378	5 August	Green Frog (<i>Lithobates clamitans</i>)	2
L378	5 August	Gray Treefrog (<i>Dryophytes versicolor</i>)	1

Green Frog and Wood Frog larvae were found in smaller, but still shallow areas of the lake.

We also incidentally trapped adult Central Newt (*Notophthalmus viridescens louisianensis*), a subspecies of Eastern Newt, in our bottle trap surveys. They were the only other amphibian species encountered or recorded during our surveys. Although they are not anurans, we report them here to supplement the only other known record of this newt species observation in this area (Hayhurst *et al.* 2017). In total, we trapped seven adult newts in L378 (Table S6). No newts were trapped in L373.

Adult call surveys

From the cluster analysis of the ARD data, we detected calls from six anurans: American Toad, Gray Treefrog, Green Frog, Northern Leopard Frog, Mink Frog, and Spring Peeper (Figure 2). Previously, the same six species were detected using a manual listening analysis approach from a subset of recordings from an ARD placed by L378 in 2019 (S. Watson and S. Wong unpubl. data). American Toad and Spring Peeper were detected on recordings from all five ARDs, whereas Northern Leopard Frog was only detected infrequently from one ARD at L373 (Figure 1). The most frequently detected call was Spring Peeper, peaking in detection frequency in June and continuously being heard throughout the season from June to October at both lakes (Figure 2). American Toad and Gray Treefrog calls were also most frequently detected earlier in the season at both lakes, with American Toad detections peaking in early June and Gray Treefrog detections peaking later in June, continuing to be heard into July. Mink Frog calls were only

detected in L378, primarily from late June to August, peaking in July (Figure 2b). Green Frog calls at both lakes were also primarily detected from late June to August, with the highest frequency of detections in July. Northern Leopard Frog calls were rare and only heard in L373, with small detection peaks of five detections in late June and again in mid-July (Figure 2a).

Discussion

We conducted anuran surveys of two boreal lakes at the IISD-ELA to add to the limited knowledge of their breeding in and occupancy of freshwater lakes, focussed in northwestern Ontario, within the central region of the Boreal Shield Ecozone. There have been previous surveys of amphibians in other regions of Ontario (de Solla *et al.* 2006; Desroches *et al.* 2010) as well as surveys in the Canadian boreal region of pond-breeding species (Feldman *et al.* 2023). However, to our knowledge, this is the first survey of anurans in boreal lakes in northwestern Ontario, Canada. In the spring and summer of 2022, we conducted a multi-stage field survey consisting of egg mass, larval, and call surveys. Overall, we detected seven species: American Toad, Gray Treefrog, Green Frog, Mink Frog, Northern Leopard Frog, Spring Peeper, and Wood Frog. We also incidentally detected Central Newt.

American Toad was prominent in one of our study lakes, as they were encountered during all surveyed life stages. Larval American Toad were also the only species we detected around steeper shorelines compared to more gently sloping sites, such as lake bays, suggesting they inhabit more shoreline habitats than

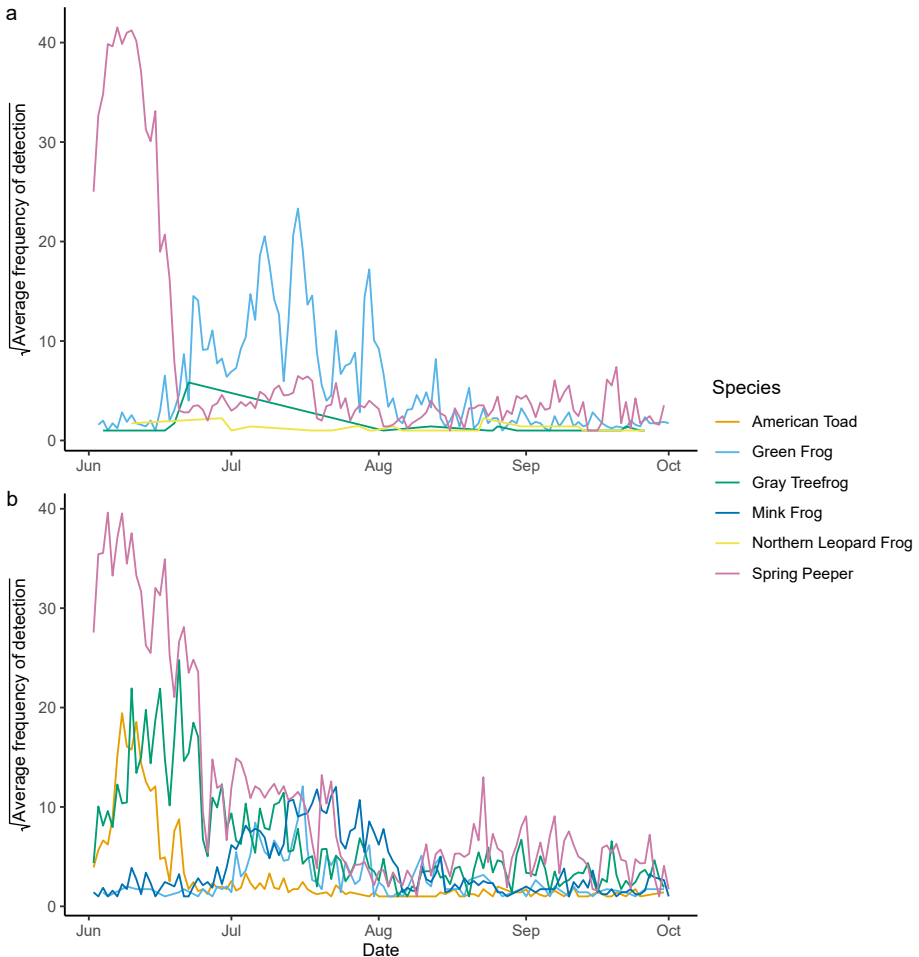


FIGURE 2. The daily frequency of detections (number of calls detected from our cluster analysis) for each species over time averaged over all automatic recording devices from (a) Lake 373 and (b) Lake 378 at the International Institute of Sustainable Development-Experimental Lakes Area, northwestern Ontario, Canada in our 2022 call surveys. The y-axes show square-root transformed values for improved visualization.

other species. Interestingly, American Toad calls were detected on all recording devices around study lake L378, but none around study lake L373. In L373, the only evidence of their presence was one detection of a larva, suggesting more localization between lakes than expected because American Toad is typically considered an aquatic breeding habitat generalist (Buskirk 2003; Desroches *et al.* 2010). Among anurans in southern Ontario, they are relatively tolerant of predatory fish in the same breeding ponds (Hecnar and M'Closkey 1998) but avoid breeding in habitats with predatory amphibians (Petranka *et al.* 1994) such as Wood Frog larvae, which were only found in L373. This may be why we did not find them in L373 where otherwise, their life history traits suggest they are well adapted to breed in lakes (Lannoo

2021). Because there are few studies of the presence of American Toads in Canadian boreal lakes, we also note anecdotally that American Toad has commonly been observed in and around other lakes at the IISD-ELA (J.O.K. pers. obs.).

We also found that Gray Treefrog, Green Frog, and Mink Frog use these boreal lakes for breeding. We encountered all three species in our larval surveys, confirming that lake shorelines and bay habitats are among their preferred breeding habitats (Harding and Mifsud 2017; Dodd 2023). Similarly, Woodford and Meyer (2003) used calling and observational surveys and found all three species around lakes in Wisconsin, USA. Further, we detected these species during our call surveys. Particularly, we detected all three species calling later in the season, which

supports their breeding periods being relatively long (Dodd 2023) and extending into the late summer in Ontario (Ontario Nature 2023). Very few observations have been reported for these species in this region. However, we did find one other published record of Gray Treefrog presence in roadsides, ponds, and wetlands in the Kenora District of northwestern Ontario (Weller 2009); we contribute evidence of their presence in boreal lakes within this region.

We did not find evidence that Spring Peeper and Northern Leopard Frog use these boreal lakes for breeding, but our call surveys show they inhabit the areas around them. For both species, our larval survey timing was expected to overlap their developmental period (Dodd 2023; Ontario Nature 2023). However, Spring Peeper is most likely to breed in fish-free and temporary water bodies (Ethier *et al.* 2021; Ontario Nature 2023) and Northern Leopard Frog also prefers clear waters with no predatory fish (Dodd 2023). Therefore, it is likely they are breeding nearby (as indicated by our call surveys) but not actually using these lakes for breeding. Spring Peeper was the most frequently detected species in our call surveys, being detected by all recording devices. Spring Peeper is not known to travel far from their breeding sites (Ethier *et al.* 2021) and therefore likely breed near the lakes.

We also detected Northern Leopard Frog during our call surveys, despite not expecting to detect them. Calling records in Ontario peak in early May (Ontario Nature 2023), during their short mating season (Harding and Mifsud 2017), earlier in the spring and before we deployed our recording devices. However, infrequent Northern Leopard Frog calls were recorded in June by one device around one of the lakes (L373). Calling may be later in more northern regions, because the atlas is biased to southern and eastern Ontario. Another possible explanation is that many species are known to occasionally call out of breeding season (i.e., 'rain calls'; Miller 2021), including Northern Leopard Frog (Dodd 2023). Northern Leopard Frog may be breeding in areas near the lakes as they can hibernate in permanent water bodies with fish and are known to move overland to breed in fish-poor waters (Dodd 2023; Schueler 2025).

We did find some evidence that Wood Frog may also use boreal lakes for breeding, although they were not initially expected to be found using adult calling, egg, and larvae surveys (Table 1). We captured Wood Frog larvae on one day (29 June) in one of the lakes (L373) using bottle traps. However, this was unexpected because Wood Frog is known to breed from April to May in the Great Lakes region, with offspring completing metamorphosis in 7–15 weeks (Harding and Mifsud 2017). With our study lakes in the northernmost area of the Great Lakes region, it

is possible we encountered larvae developing later than expected, as breeding typically begins later with higher latitude (Guttman *et al.* 1991). Additionally, they are known to prefer breeding in vernal, fishless pools (Dodd 2023), although they have been found to breed in boreal lakes with fish in other regions of Canada (Eaton *et al.* 2005). This detection may be an example of aberrant breeding site selection. Additionally, we did not find other evidence of Wood Frog breeding because of their early spring emergence (i.e., before our ARDs were deployed). However, future survey efforts earlier in the year could provide a better understanding of their presence in the lakes as other surveys have found evidence of breeding populations in a wide range of habitat types (Hokit and Brown 2006) and Wood Frog has an extensive range throughout Canada (Dodd 2023).

Boreal Chorus Frog was not found during our surveys nor did we expect to detect it. While their range extends into our study region, their preferred breeding habitat of open canopy, shallow, fish-free waters, and their early spring breeding season (Harding and Mifsud 2017; Ethier *et al.* 2021), make them highly unlikely to be encountered in any of our surveys. Further, the timing of our call surveys was not optimal—other call surveys for Boreal Chorus Frog in Canada began as early as March (Seburn *et al.* 2014) while we started our recording devices in June. Therefore, there is a chance they are present in this area but were not detected. Further efforts to determine the presence of this species are critical because there is evidence of declines in other regions of Ontario (Seburn *et al.* 2014). Another survey from the northeastern part of their range found them associated with shallow, fishless pools (Ouellet *et al.* 2009). Therefore, they may be in our study region but not in the boreal lakes and, instead, in smaller water bodies.

We also recorded an incidental detection of adult Central Newt. They can inhabit shallow areas of permanent water bodies (Ontario Nature 2023) similar to where our larval traps were deployed. The Ontario Reptile and Amphibian Atlas (Ontario Nature 2023) reports a relative lack of observational data for this species in Ontario, so we include our observations to add insight into the broader amphibian diversity in these boreal lakes, especially in the northwest Ontario region where data for this species are sparse (Hayhurst *et al.* 2017).

We detected later breeding species with our egg-mass surveys. This method is well suited for species such as American Toad because of the abundance of this species in the region (Harding and Mifsud 2017) and the number of eggs produced per female (between 2000 and 20 000 eggs; Harding and Mifsud 2017), making the large clusters of American Toad

egg masses easily visible along the shallow shoreline. Their breeding timing in Ontario (Ontario Nature 2023) also coincided well with the timing of our egg mass surveys. However, other species may have been missed because of the relatively late timing and infrequency of our surveys. For example, we found Gray Treefrog, Green Frog, and Mink Frog larvae, suggesting they use the lakes for breeding, but we did not find their egg masses. These species have a relatively quick development time, typically hatching within one to three weeks (Dodd 2023). Eggs from early spring breeders such as Spring Peeper and Wood Frog (Dodd 2023) may also have hatched before our egg surveys.

We found bottle traps to be useful in surveying large areas such as whole lake shorelines for larvae. In particular, bottle trap surveys were well suited to capture species such as American Toad, which were the most frequently detected larvae, as they tend to aggregate in large groups, in shallow water (Beiswenger 1975). This makes them more likely to be captured in large numbers if traps are placed in locations where they aggregate. However, similar to the egg-mass surveys, starting larval surveys earlier in the season could improve detection, as many Ontario anurans have early spring breeding seasons. Despite the relatively late timing of our surveys, we were still able to capture larvae with longer development times, such as Mink Frog and Green Frog, which can delay metamorphosing into adult forms for up to two or three years, respectively (Dodd 2023). Therefore, the longer larval developmental times increased the potential to overlap with the timing of our larval surveys.

Various factors can affect species detection during our calling surveys, including survey timing and species-specific calling characteristics. The timing of call surveys can affect species detection probabilities (de Solla *et al.* 2006) and we may have missed early spring breeders such as Wood Frog and Spring Peeper because our recording devices were not deployed until June. The time-of-day at which the devices were set to record calls may also affect species detection. Species such as Mink Frog have been found to call intensely during the afternoon in northwestern Ontario (Schueler 2004), whereas our devices concentrated recording around dusk and dawn. Conversely, the calling time for frequently detected species overlapped with these scheduled recording times. In addition, factors such as species call frequency and strategy can affect the accurate detection of calls from further distances (de Solla *et al.* 2005). For example, Spring Peeper has high pitched and frequent calling, with males sometimes calling continuously for over an hour (Forester *et al.* 1989) making them highly likely to be detected if they are breeding

in the vicinity of the recorder. Conversely, Northern Leopard Frog calls do not carry far (de Solla *et al.* 2005) making them less likely to be detected.

There were other limitations in our recording analyses that may have influenced the frequency of species detections. After verification, we found that our species identifications from cluster analysis would typically underestimate species detections, e.g., a detection containing an anuran call was identified as not containing an anuran call. Further, we verified accuracy on a random subset of all detections from an ARD and found that accuracy differed among species. Species-specific responses to conditions such as rainfall (Oseen and Wassersug 2002; Yoo and Jang 2012) could increase calling activity during times with overlapping sounds, such as heavy rainfall or bird calls, which may lead to underestimation and reduced accuracy of detection for these species. We found that the majority of calling activity subsided by the end of August, suggesting that we could have ended our recording efforts at that time, and still adequately achieved our objective.

Finally, we note that our study encompasses two lakes in the northwestern Ontario boreal forest region over one survey year while other studies have found anuran presence and diversity can vary within relatively small spatial (Bosch *et al.* 2004) or temporal scales (Gómez-Rodríguez *et al.* 2010). Variables such as ice cover duration can vary temporally and spatially, even between lakes in close proximity (Higgins *et al.* 2021). Thus, by only surveying two lakes, we gain insight into the diversity of anurans that can inhabit boreal lakes in this region but caution against making broader assumptions without further surveys. Temporally, environmental variables such as annual temperature (McMenamin *et al.* 2008; Sommers *et al.* 2018), rainfall (Yoo and Jang 2012), and annual ice-off timing (Sommers *et al.* 2018) have species-specific effects on behaviour and developmental timing. These variables can affect species detectability. Further, amphibians undergo natural annual population fluctuations (Marsh 2001), which can also affect detectability. Therefore, while 2022 was considered an average year for temperature, precipitation, and ice-off variables at the IISD-ELA (IISD-ELA unpubl. data), we acknowledge that only one year of surveys can limit our ability to detect the full diversity of species at the lakes. Again, further surveys and monitoring efforts are encouraged to provide a comprehensive survey of the anuran diversity in boreal lakes, especially in this region, where records are limited.

Anthropogenic pressures such as climate warming and resource exploitation continue to threaten boreal lakes (Schindler 2001; Ruckstuhl *et al.* 2007).

Boreal lake habitat in our survey region of north-western Ontario, Canada, can be critical for species such as Mink Frog and Green Frog, with ranges that extend this far north and west (Harding and Mifsud 2017; Dodd 2023). Knowing which species are present can be important for predicting effects of long-term changes to distributions and species ranges. Further, knowing that certain species are present can inform broader conservation plans—Spring Peeper is a potential indicator of ecological condition (Price *et al.* 2007).

There is a relative paucity of research and monitoring on amphibians in freshwater lakes compared to other habitats (Walls 2014) and a bias of monitoring efforts in accessible areas (Petrovan *et al.* 2020). We acknowledge that our study provides a one-year snapshot and because of natural annual population fluctuations in amphibians (Marsh 2001), long-term monitoring is needed for accurate diversity estimates. Regardless, this is timely because boreal lakes continue to face pressures from climate warming and resource exploitation (Ruckstuhl *et al.* 2007; Berner and Goetz 2022). As such, we provide evidence that freshwater boreal lakes are important habitats for anurans that should be considered when conducting ecological monitoring and conservation.

Author Contributions

Writing – Original Draft: J.O.K., J.F.P., J.D.T., and B.A.K.; Writing – Review & Editing: J.O.K., J.F.P., D.M.O., J.D.T., B.A.K., and T.H.R.; Data Curation: J.O.K., J.D.T., and T.H.R.; Conceptualization, Investigation, and Methodology: J.O.K., D.M.O., J.D.T., B.A.K., T.H.R., and J.F.P.; Formal Analysis: J.O.K., J.D.T., and J.F.P.; Visualization: J.O.K. and J.F.P.; Project Administration and Funding Acquisition: D.M.O., B.A.K., and J.F.P.

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Supplementary Materials:

TABLE S1. Fish assemblages in two boreal lakes in the Experimental Lakes Area, Ontario, Canada, listed in descending order of most to least abundant species.

TABLE S2. Primary substrate of anuran survey larval sampling stations in two boreal lakes in the Experimental Lakes Area, Ontario, Canada.

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

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

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.

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.

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.