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Preliminary estimate of a Gray Treefrog (*Hyla versicolor*) population at a protected site in New Brunswick using photo identification and community science

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Abstract
We provide a preliminary population estimate (n = 120, 95% CI 65–722) of (male) Gray Treefrog (*Hyla versicolor*) at the Hyla Park Nature Preserve, New Brunswick, Canada's first amphibian conservation site. As proof of concept, we also demonstrate the efficacy of a minimally invasive photographic identification method (PIM) that uses pattern recognition software for estimating the population of a visually cryptic amphibian that is subject to physiological colour change. Finally, we validate the use of PIM data collected by community participants and the opportunity it provides to engage and educate the local community about amphibian conservation.

Key words: Amphibian conservation; citizen scientists; community scientists; *Dryophytes*; pattern recognition software; urban parks

Introduction
Mark–recapture studies of amphibian species often rely on invasive tagging or marking techniques (e.g., implanting passive integrated transponders or toe clipping; Sullivan and Hinshaw 1992; Donnelly *et al.* 1994). These methods can be expensive and require experienced personnel. Toe clipping can be problematic because amphibians can grow back the appendages, making it difficult to distinguish recaptures (Ritke and Semlitsch 1991; Sullivan and Hinshaw 1992; Donnelly *et al.* 1994). Furthermore, there is evidence that toe-clipping may reduce survival or alter the behaviour of some species of frogs (McCarthy and Parris 2004; Ginnan *et al.* 2014). To avoid invasive techniques for marking individuals, some amphibian research has used photographic identification methods (PIMs) to identify individuals (Donnelly *et al.* 1994; Schoen *et al.* 2015; Romiti *et al.* 2017). Although the technique is not suitable for all species, PIMs can be used for the re-identification of species that have individually unique and unchanging epidermal patterns. Such methods can be cost-effective, require fewer permits than other wildlife sampling techniques, and may allow surveyors with a range of experience to assist with data collection and analyses (Morrison *et al.* 2011; Deutsch *et al.* 2017). In studies with large photo libraries, matching photos can become time consuming and increase the probability of visual errors, but with pattern recognition software, this technique can become cost-effective (Gamble *et al.* 2008).

Successful mark–recapture studies require many personnel hours, especially if the survey window is short. Studies that require large study areas or many surveys have used community scientists (also referred to as citizen scientists) to help collect meaningful data (Bonney *et al.* 2009; Weber *et al.* 2016). Studies that take advantage of community science can acquire more data in a shorter time, provide education, and foster communication between researchers and the community (Cooper 2007; Bonney *et al.* 2009). A negative perception by the public of some amphibian species can present conservation challenges (Prokop and Fančovičová 2012; Weber *et al.* 2016; Vergara-Ríos *et al.* 2021); using community science at the local level can help educate and engage the public about the importance of amphibians (Weber *et al.* 2016).

Many protected areas lack basic population data that are critical for tracking population changes to help direct management practices (Busby and Parmelee 1996). Protected zones in urban areas can be subject to threats, such as roads, climate change, pollution, and invasive species, which can alter the distribution of species of interest (Weber *et al.* 2016). Hyla Park Nature Preserve in Barker's Point, New Brunswick, is a small, protected wetland surrounded by urban development. The presence of Gray Treefrog (*Hyla* (*Dryophytes*) *versicolor*; taxonomy according to AmphiaWeb 2023) resulted in the park being protected in 1995, when it became Canada's first amphibian conservation park (McAlpine and Vail 2005). At the time, evidence suggested that Gray Treefrog had a very restricted range in Maritime Canada, although currently it appears to be undergoing a dramatic range expansion in the region (McAlpine *et al.* 2009; McAlpine 2023). The nature preserve is surrounded by housing, invasive species, road networks, and a metal recycling facility (McAlpine and Vail 2009). Because of these outside negative pressures, a baseline study was initiated to monitor the Gray Treefrog population and help with future management decisions. McAlpine *et al.* (1980) emphasized the need for a population estimate of the Hyla Park population.

Our study sought to demonstrate the efficacy of a minimally invasive sampling method for a visually cryptic species subject to diel colour change, validate the use of data collected by community scientists, and provide a preliminary estimate of the population of Gray Treefrogs at Hyla Park Nature Preserve.

Methods

Study area and community scientist training

We conducted our study in a 7.3-ha area in Hyla Park Nature Preserve (45.951720°N, 66.609181°W) in Barker's Point, New Brunswick, Canada (Figure 1). The nature preserve consists of a provincially

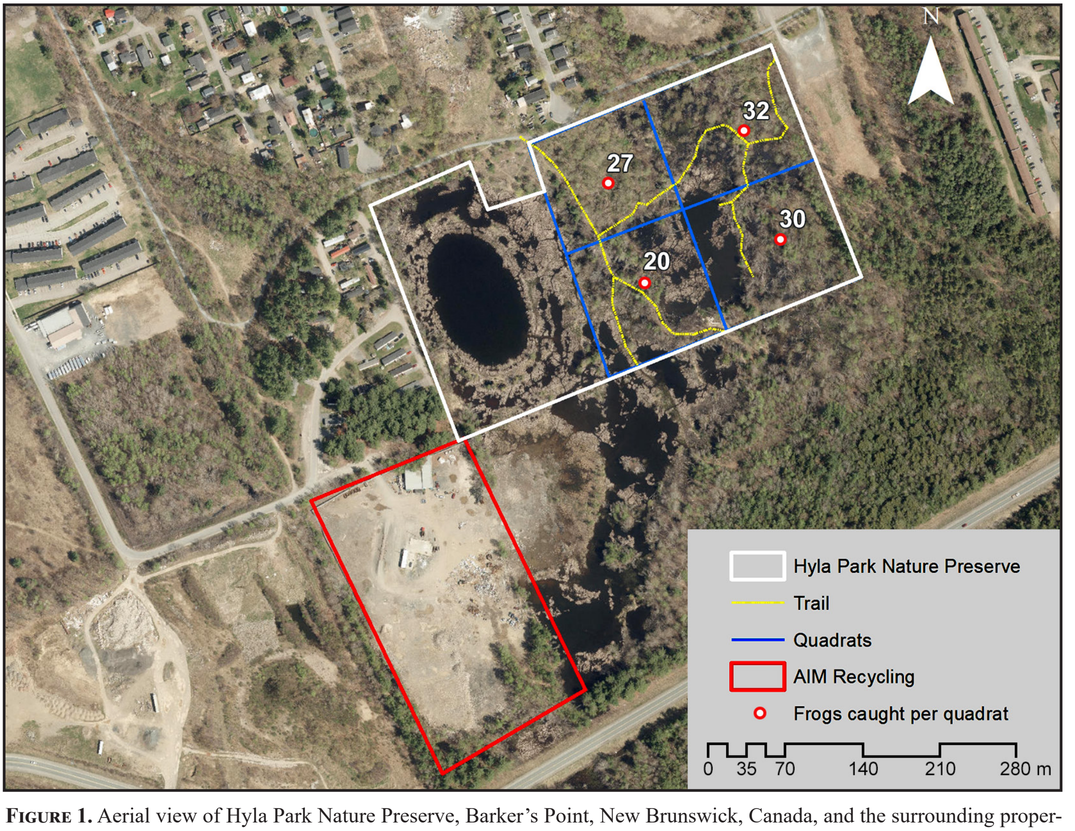

FIGURE 1. Aerial view of Hyla Park Nature Preserve, Barker's Point, New Brunswick, Canada, and the surrounding properties, including a metal recycling facility and residential development.

significant wetland, three ponds (~0.49 ha, ~0.22 ha, and ~1 ha, each <2 m in depth), and a young deciduous forest (<50 years). The ponds are mostly accessible by a system of walking trails.

Before the field season, community scientist participants attended a presentation by S.W. on the nature of the Hyla Park site, survey methods, and basic life history of Gray Treefrogs. Participants were taught how to identify a Gray Treefrog (by sight and call), how to capture, handle, and release Gray Treefrogs without injury to the animals and with minimal disturbance, safety precautions required when working the site, and general information about why the study was being undertaken. They were also taught how to use a global positioning system (GPS) free mobile app called Avenza (Avenza Systems Inc., Toronto, Ontario, Canada), which is capable of taking accurate waypoints, and they were encouraged to use this app when they located a Gray Treefrog.

Field surveys

During spring 2019, we monitored weekly to pinpoint the onset of the Gray Treefrog breeding season in New Brunswick. Typically, male Gray Treefrogs begin calling in late May to early June at Hyla Park (McAlpine *et al.* 1980). Once calling was detected, we surveyed the site four times from 12 June to 3 July. We conducted surveys when weather conditions were judged favourable for calling by Gray Treefrogs and also for both visual and aural detection by investigators. Conditions included minimal wind (<20 km/h), no precipitation, and an air temperature >20°C. Community scientists ($n = 7–10$) began capturing treefrogs 30 min after sunset (typically around 2100) and surveyed for 1.5 h. The area was split into four quadrats and observers were divided evenly. Participants listened for Gray Treefrogs calling, homed in on a calling frog, and then captured what they believed were calling individuals by hand or with a net. Unfortunately, sex of captured treefrogs was not confirmed, but we believe most or all were males. Participants placed individual treefrogs in translucent plastic containers with air holes. Each container had an identification card that included: participant name, a unique identification code, quadrat, coordinates (from GPS signals received on cell phones) where available, and the type of vegetation or substrate the frog was captured on. Participants then transported the frogs to a photography station established at the field site.

An experienced herpetological researcher was responsible for taking a photo of the dorsal pattern of each Gray Treefrog captured. All individuals were photographed at least twice. Standardized photographs were taken with a Canon EOS Rebel T5i/700D camera (Canon Canada, Brampton Ontario, Canada) using a 0.03 m^3 lightbox photo studio (Amzdeal-US). The camera was stationed on a tripod, ~1 m over the photo studio. Individual frogs that were dark in colouration at capture (i.e., with dispersed chromatophores) were placed in light blue containers for 2–5 min before being photographed. This prompted pigment in chromatophores to contract, lightening the epidermis and enhancing dorsal pattern recognition when images were later compared (Figure 2). To avoid recaptures, treefrogs were kept in their individual containers until each nightly survey was completed and then released at their capture locations.

To avoid the spread of any potential pathogens among treefrogs, participants washed nets with a 10% bleach solution after each capture and wore latex gloves when handling treefrogs. A clean paper liner was placed on the bottom of the lightbox for each photo, and fresh latex gloves were used before handling each frog for photography. After each nightly survey was completed, frog storage containers were disinfected for 10 min in a bleach solution and participant community scientists never reused a container during a survey.

Photographic identification method

We chose the highest quality image to represent captured individuals and cropped each image to

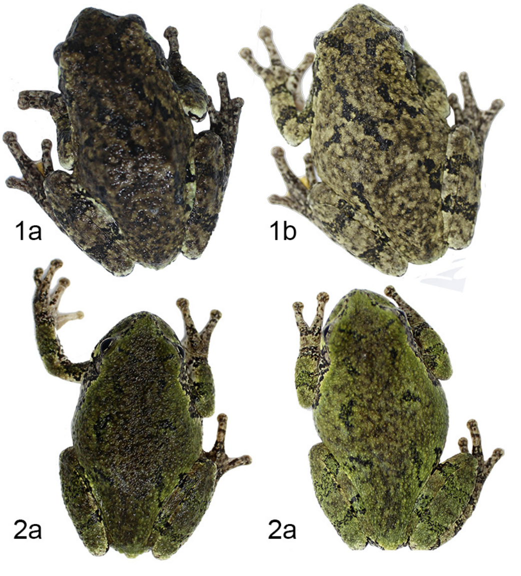

FIGURE 2. Photographs demonstrating physiological colour change in Gray Treefrog (*Hyla versicolor*) collected at Hyla Park Nature Preserve, Barker's Point, New Brunswick. Photos 1a and 2a show two different treefrogs with dark epidermis (melanin dispersed in chromatophores) and patterning obscured while the corresponding b photos show the same treefrogs after spending 2–5 min in a light-coloured container. The epidermis is lightened (melanin concentrated in chromatophores) and individually unique patterning is emphasized. Photos: Shaylyn Wallace.

treefrog body length and width. We compared and matched photos using the program Wild-ID (Bolger *et al.* 2012), a graphical interface that automatically presents users with a subset of 20 similar images for comparison. We confirmed recognition of a single individual from this subset, or rejected the entire subset, by visually inspecting the series of contending images. To calculate the accuracy of the matching program, six community scientist participants each analyzed the entire dataset using Wild-ID. Subsequently, we calculated the false acceptance rate (FAR: the probability of matching two images of different individuals) and false rejection rate (FRR: the probability of failing to match two images of the same individual) for each observer to assess error rate (Bendik *et al.* 2013; Cruickshank and Schmidt 2017). The FAR is calculated as the number of false matches divided by the total number of identification attempts. The FRR is calculated as the number of correct matches missed divided by the total number of matching pairs. For comparison, the most experienced participant matched all the images manually and calculated the total time to sort the entire sample without using matching software.

Statistical methods

We conducted population estimates in R (v. 4.0.2; R Core Team 2020) using the Schumacher-Eschmeyer (S-E) method in the package "fishmethods" (v. 1.12-1; Nelson 2023) with α set to 0.05. A Poisson distribution was used to set CIs for Schnabel and a *t* distribution for S-E. Abundances were rounded down to the nearest integer.

Results

A total of 109 captures were made during the three sampling periods with 80 individual frogs identified (Table 1). From the 109 captures, 24 individuals were captured during two surveys and five were captured during all three surveys (Table 1). Because community scientists located treefrogs largely by call, the population estimates presented here are for males only. An additional 35 treefrog captures were made during the first survey on 12 June, but were excluded from our population estimates and PIM analysis because treefrogs were not subjected to a period of epidermal lightening (i.e., chromatophore contraction) before photography.

TABLE 1. Gray Treefrog (*Hyla versicolor*) captures from Hyla Park, Barker's Point, New Brunswick, in 2019.

Date	New captures	Recaptures	Total captures
18 June	33	0	33
25 June	35	15	50
3 July	12	14	26
Totals	80	29	109

Observer errors using PIMs were rare. While five of the six community scientists each misidentified one pair, yielding a FRR (false rejection rate) of 2.4% (12 mismatched pairs/510 matching pairs), the false acceptance rate (FAR) was zero (0 false pair matches/480 matching attempts). The Wild-ID program scored the recapture photos from 0.005 to 0.25 (mean 0.069; scores range from 0 to 1.0). Using Wild-ID reduced the identification of recaptures to 1.5–2 h per reviewer for our sample; without the software, we estimated ~30 h per reviewer.

The Schumacher and Eschmeyer (1943) population estimator method yielded an abundance of 120 (male) Gray Treefrogs (95% CI 65–722); however, lower and upper CI varied considerably. The inverse SE was 0.0005.

Discussion

We used PIMs successfully as a non-invasive mark–recapture technique for male Gray Treefrogs at a site in New Brunswick. Wild-ID software dramatically reduced the number of hours spent identifying recaptures compared with traditional methods that involve searching for matching patterns across all captures manually, which is time-consuming. False acceptance and false rejection rates using the software were low (<2.4%), which validated its use in future Gray Treefrog population studies.

Identifying individuals with PIMs based on their patterns has been used in past amphibian studies (Bendik *et al.* 2013; Elgue *et al.* 2014; Bradley and Eason 2018). However, to our knowledge, PIM has never been tested with a visually cryptic amphibian species capable of diel physiological colour change. Because PIMs require a consistent pattern for identification, software may struggle to recognize individuals among those species that rely on physiological colour change to enhance crypsis. Physiological colour change of individual treefrogs proved to be a problem at the outset of the fieldwork, as some individuals arrived at the imaging station with a heavily pigmented epidermis (i.e., melanin dispersed in chromatophores), making it difficult to distinguish the dorsal pattern. However, we found that we could easily and quickly (<5 min) stimulate the contraction of melanin in chromatophores in individual treefrogs by transferring them to a light blue container. Using this technique, we were able to lighten the epidermal pattern and easily identify individual treefrogs. Note that we did not warm the frogs; the light blue container resulted in the frogs changing their colour.

The sampling effort by community scientists varied across the sampling period. The first survey had the most participants (10), whereas the last survey had the fewest (seven). Although limiting the number of community scientists is an option, it reduces community engagement. However, unequal sampling effort also should be considered. Community scientists were not canvassed, but many stated that they volunteered because they were excited to capture treefrogs while contributing to amphibian conservation efforts, and survey dates fit their personal schedules. Vergara-Ríos *et al.* (2021) noted that community science activities that involve a field component are important for developing positive perceptions about amphibians. Providing field opportunities for community scientists is likely to encourage ongoing involvement in projects. By pairing an experienced herpetological researcher with responsibility for Gray Treefrog photography with community scientists who captured treefrogs, we were able to obtain a sufficiently large sample for a preliminary population estimate. We were able to do this on a small budget, while also engaging and educating the local community about amphibian conservation, as well as the value of Hyla Park Nature Reserve and its associated species and wetlands.

Our study provides the first population estimate for male Gray Treefrogs in Hyla Park Nature Preserve, New Brunswick, Canada's first amphibian conservation site. We also present evidence for the utility of PIMs for population estimates of cryptically coloured amphibians subject to physiological colour change and highlight the value of community science for data collection and engagement. One of our objectives was to test the feasibility of using community scientists in monitoring a Gray Treefrog population in an urban park. We successfully maximized our data collection by involving the local community, and we avoided costly or invasive sampling techniques. Although we have demonstrated proof of concept for use of PIMs with cryptic amphibians, we suggest that improvements to future population estimates could be made by confirming the sex of each individual photographed, as well as ensuring the exact geographic coordinates for each individual captured.

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