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Environmental DNA surveys help to identify winter hibernacula of a temperate freshwater turtle

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Abstract

Background and aims: Overwintering is a critical part of the annual cycle of animals living at high latitudes, and selection of overwintering sites (hibernacula) is important to population persistence. Identifying the overwintering sites of aquatic species is challenging in areas where water bodies are frozen for significant parts of the year. We tested whether environmental DNA (eDNA) approaches could help to locate them.

Materials and methods: We conducted environmental DNA surveys of underwater overwintering sites of the northern map turtle (*Graptemys geographica*), a species of conservation concern in Canada. We collected water samples under the ice in winter across a mid-sized temperate lake and used quantitative PCR with a species-specific probe to quantify concentrations of map turtle eDNA.

Results and discussion: We found localized eDNA signals consistent with known overwintering sites and one previously suspected site. The latter was further confirmed using underwater remote operated vehicle (ROV) visual surveys.

Conclusions: Our study confirms that eDNA can offer insights on a critical part of the annual cycle of aquatic species, for which we know very little.

KEYWORDS

environmental DNA, *Graptemys geographica*, Northern map turtle, overwintering, quantitative real-time PCR

1 | INTRODUCTION

To survive cold winter temperatures, animals living in high latitudes have adapted in various ways to escape the lethal consequences of freezing (e.g., cellular damage) through a critical part of the life cycle known as "overwintering" (Storey and Storey 2011). While homeotherms like birds and mammals can physiologically maintain their body temperatures above the freezing point, vertebrate ectotherms, such as squamates and turtles, cannot. As a consequence, many ectotherms avoid freezing temperatures by selecting microhabitats in which temperatures remain above the freezing point (Greaves and Litzgus 2007; Prior and Weatherhead 1996; Storey and Storey 2011).

In temperate regions, the adults of most freshwater turtle species escape lethally cold temperatures by overwintering under the water where the ambient temperature is well buffered and above freezing point (Ultsch 2006). In northern hemisphere, some species of turtles at their northern range limits spend up to half their lives overwintering (Edge et al. 2009; Litzgus, Costanzo, Brooks, Lee, & Richard, 1999), including several months during which they are trapped under the ice without access to atmospheric oxygen (Jackson and Ultsch 2010). The selection of suitable overwintering

sites presumably plays a central role in the overwintering success of temperate turtles. Many species rely or aerobic metabolism in the winter and will not survive an entire winter in anoxic water (Jackson and Ultsch 2010; Ultsch 2006). Moreover, overwintering turtles are sluggish due to low body temperature and vulnerable to predation by winter active predators such as river otters (Brooks, Brown, & Galbraith, 1991). The selection of overwintering sites by turtles is presumably critical to the persistence of populations at northern latitudes (Ultsch 2006). Thus, identifying where turtles overwinter could provide key insights into the environmental and climatological factors that limit their distribution and to inform their conservation through the protection of critical overwintering habitat.

The northern map turtle (*Graptemys geographica*) is a medium-sized freshwater turtle widely distributed in eastern North America with a northern range limit of approximately 45°N in the provinces of Québec and Ontario, Canada. At these latitudes, map turtles aggregate to overwinter under water in October and typically reemerge when the lake becomes ice-free in early April (Vogt 1980). Northern map turtles are anoxia intolerant (Maginniss, Ekelund, & Ultsch, 2004; Reese, Crocker, Carwile, Jackson, & Ultsch, 2001) and are thus expected to select overwintering sites with sufficient dissolved oxygen to sustain aerobic metabolism for several months. Northern map turtles overwinter communally (Carrière, Bulté, & Blouin-Demers, 2009; Graham and Graham 1992; Pluto and Bellis 1988; Vogt 1980), suggesting that suitable overwintering sites are limited. However, the physicochemical attributes of overwintering sites and the process of habitat selection of overwinter sites remain undocumented.

Researchers typically locate overwintering sites using radio-telemetry (Carrière et al. 2009; Graham, Graham, Crocker, & Ultsch, 2000; Ryan, Conner, Douthitt, Sterrett, & Salsbury, 2008). This approach, while effective, is spatially limited and would fail to identify sites not used by turtles fitted with transmitters. Moreover, in large lakes and rivers, turtles can migrate long distances to reach their overwintering sites (Vogt 1980; Graham et al. 2000), increasing the likelihood of losing individuals with transmitters. Finally, the animals captured and equipped with transmitters may experience some stress. These limitations might be overcome by recent advancements in environmental DNA (eDNA) technology, which is noninvasive, robust, and sensitive in various aquatic environments at different spatial scales. This technique has been used in aquatic environments for biodiversity monitoring (Lacoursière-Roussel et al. 2018), early detection of invasive species (Xia et al. 2018), and testing for the presence and distribution of elusive or endangered species (Eiler et al. 2018; Raemy and Ursenbacher 2018).

Surveys using eDNA are commonly conducted during the active season (e.g., spring and summer) of the focal species, when eDNA is generally more abundant and thus more readily detected (Buxton, Groombridge, Zakaria, & Griffiths, 2017; de Souza, Godwin, Renshaw, & Larson, 2016; Eiler, Lofgren, Hjerne, Norden, & Saetre, 2018; Ostberg, Chase, Hayes, & Duda, 2018). However, eDNA surveys have

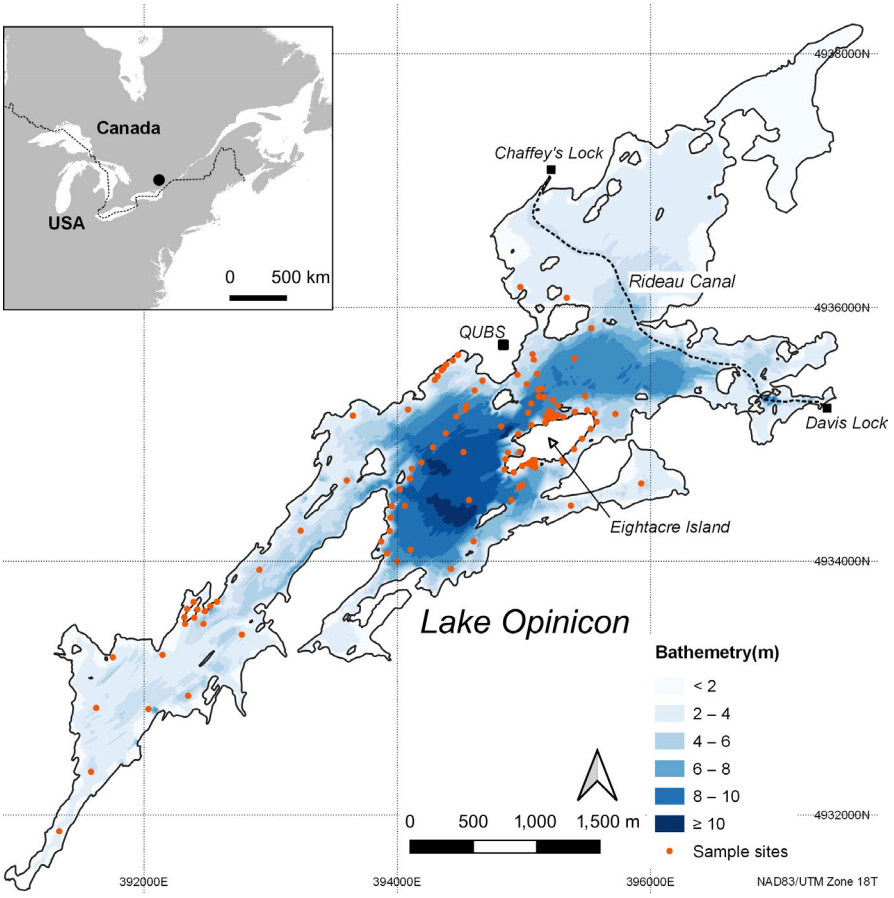

FIGURE 1 Overview of Lake Opinicon showing all sampling sites. The location of Lake Opinicon (solid dot) is shown in the inset map. Eightacre Island, where map turtles are known to overwinter near the shore, is indicated by arrow. Dotted line indicates transit corridor for the Rideau Canal (flowing from Chaffey's Lock to Davis Lock)

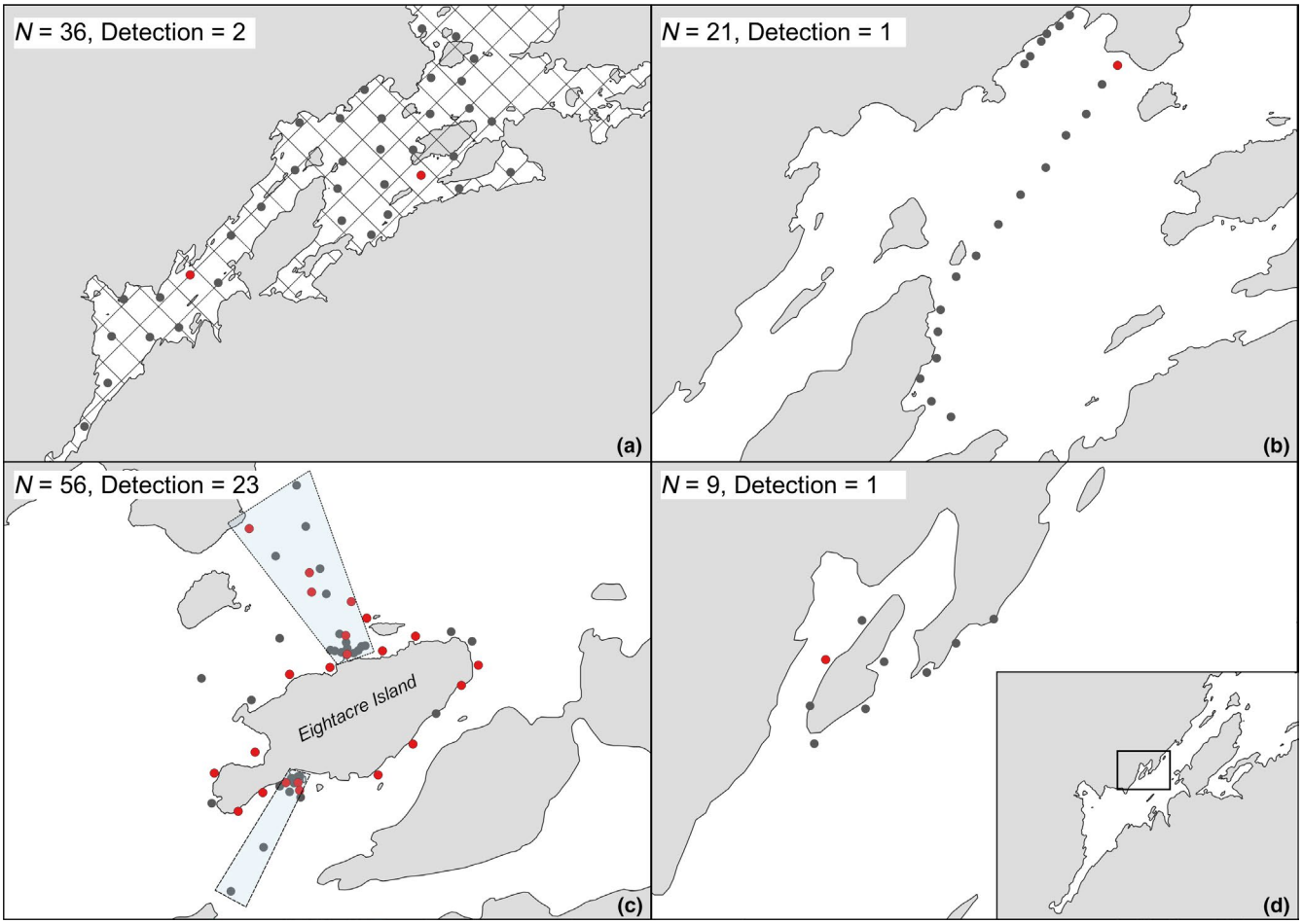

FIGURE 2 Details of all sampling sites and detections. Positive detections are indicated by red dots while dark gray dots represent nondetection. (a) Sites from 2017 comprising a broad survey of the lake. A 400-m grid was laid out to guide the sampling regime. (b) Sites on northern and western shore of the central basin as well as a transect across the basin. (c) Sites near the shore of Eightacre Island and two transects (within the shaded polygons) extending from the known hibernacula. (d) Sampling sites near the shore of the suspected overwintering area. Relative location of this area on Lake Opinicon is shown in the inset map. Sites shown in b—d were sampled during the 2018 winter

also been successful at revealing winter habitats of fish and aquatic invertebrates (Minamoto, Hayami, Sakata, & Imamura, 2019; Wu et al. 2018). The northern map turtle is an excellent candidate to assess the usefulness of eDNA surveys for detecting overwintering sites because of its tendency to overwinter communally. Communal overwintering should result in concentrated sources of eDNA that may compensate for reduced rates of eDNA release because individuals are more quiescent during winter. When covered with ice, lakes experience less mixing (see review by Kirilin et al. 2012), which should lead to less eDNA dispersion and closer correspondence between the location of eDNA signals and actual overwintering locations. Cold water in the winter and low UV radiation under the ice might also cause less DNA degradation (Barnes et al. 2014). Thus, eDNA surveys may be particularly well suited to locate turtle overwintering sites.

Here, we assess the potential for eDNA surveys to locate overwintering sites of northern map turtles in a medium-sized temperate lake in eastern Ontario, Canada. We combine geographically intensive water sampling and a species-specific quantitative real-time PCR (qPCR) assay to test if we could detect overwintering sites. We show the effectiveness of this eDNA-qPCR technique and discuss its potential for studying aquatic species during the winter.

2 | MATERIALS AND METHODS

2.1 | Study site and species

We conducted this study in Lake Opinicon, a medium-sized, shallow lake with average depth of 2.5 m, maximum depth around 11 m, and total area of 7.9 km^2 located in southeastern Ontario, Canada (Figure 1). Lake Opinicon is typically frozen between late December and early April, except for some open water along the Rideau Canal at the lakes' eastern terminus, and near two creeks on its southeastern shore. The estimated population size of northern map turtle is 1,529 individuals (1.9 turtles/ha; Bulté, Carriere, & Blouin-Demers, 2010). Overwintering sites along the shoreline of one island (Eightacre Island; Figure 1) have been identified using radiotelemetry (Carrière et al. 2009) and monitored since 2004 as part of a mark-recapture

study. On the tip of a small peninsula toward the southwest end of the lake, we captured turtles (n = 34) between April 21 and May 12 in 2005, 2006, 2007, and 2018 suggesting the existence of another overwintering site nearby (Figure 1) but were never able to confirm the presence of turtles in the winter using radiotelemetry.

2.2 | Water sampling and eDNA extraction

Water samples were collected during the 2017 and 2018 winters (Figure 1). On 22 February 2017, we sampled a grid of 36 sites spaced approximately 400 m apart across the southwestern portion of the lake (Figure 2a). This sampling was done by author WF without prior knowledge of the location of suspected or confirmed turtle overwintering sites and was designed to serve as an unbiased test of whether systematic sampling of eDNA could reveal the presence of overwintering map turtles.

Between mid-February and mid-March of 2018, we surveyed an additional 85 sites (Figure 2b–d) over 7 days (Table 1) with two goals: (a) To examine the relationship between eDNA concentration and distance from its source (known overwintering sites at the shoreline), where we expect an inverse relationship between distance from the shoreline and eDNA concentration. (b) To assess eDNA detectability around the central basin of the lake and at the aforementioned suspected site where positive detections might reveal an unconfirmed overwintering site. Our sampling focused on four areas: (a) the shoreline of Eightacre Island; (b) one transect extending from each of the two sites where turtle presence was visually confirmed via underwater cameras during sampling; (c) shoreline segments in and around the central basin of the lake; and 4) the neighboring shoreline of the suspected overwintering site in the southwestern portion of the lake. All shoreline samples were collected 5 m away from the shoreline.

At each sampling site, we drilled a hole through the ice using an 8-inch (~20 cm) ice auger (Eskimo) and sampled 1 L of surface water by dipping a 1L sterile plastic bottle (Nalgene) ~50 cm beneath the ice. The bottle was clamped to the end of a 1.5 m long pole and a different sterile bottle was used for each sample. We filtered the water samples on site using Isopore™ polycarbonic membranes (pore size: 1.2 μm; diameter: 47mm, from Millipore) housed in a 47mm in-line filter holder (Pall) using a portable peristaltic pump (Wattera). After filtration, the filters were folded and stored in 2 ml tubes containing 500 μL 2% (w/v) cetrimonium bromide extraction buffer (CTAB). All filters were transported to Queen's University on ice and extracted on the same day of sampling. The extractions were conducted in a dedicated laboratory space using a chloroform-based method adapted from Turner et al. (2014) (details in Supporting Information).

Throughout study, we took precautions to avoid cross-sample contamination. Prior to each sampling day, all equipments, including water sampler, sample bottles, filter holder, and pump tubing, were submerged in 10% commercial bleach overnight and thoroughly rinsed using reverse osmosis water. The filter holder and tubing were rinsed thoroughly using 2 L reverse osmosis water between sampling events. We assessed the possibility of cross-contamination daily by filtering two field blank control samples using reverse osmosis water: one chosen at a random time between two sampling events, and the other at the end of all filtrations. In total, 16 field blank controls were collected along with 121 field samples during the 8 survey days in this study (Table 1).

2.3 | Quantitative PCR assay detection

To detect map turtle environmental DNA, we used a probe-based qPCR assay to maximize specificity for single species detection (Goldberg et al. 2016). We designed a map turtle-specific cytochrome *b* (cyt*b*) primer pair and a TaqMan™ MGB probe (Table 2) using DNA sequences retrieved from GenBank (Table S1). The primers and probe were designed to maximize mismatches with other co-occurring turtles (Table 1). A 693 bp fragment of cyt*b* containing the target 99 bp amplicon (details in Supporting Information) was amplified from blood extracts of one individual map turtle and inserted into a pMiniT 2.0 vector (NEB). DNA concentration of the resulting plasmid was then quantified using a Qubit 3.0 fluorometer (Thermo Fisher) and used as a standard in all qPCR assays. The qPCR

TABLE 1 Survey scheme in this study by date

Survey date	Number of samples	Number of blanks	Number of detections
2017 02 21	36	2	2
2018 02 17	14	2	10
2018 02 18	16	2	10
2018 02 24	23	2	4
2018 02 25	6	2	0
2018 03 21	7	2	1
2018 03 22	8	2	0
2018 03 24	11	2	0
Total	121	16	27

Note: On each survey day, two blank controls were filtered: one at the end of filtrations and one at random between two filtration events. See supporting information for detailed sampling time sequence of each survey day.

TABLE 2 DNA sequences of species-specific northern map turtle primer and probe set compared with sequences of other co-occurring turtles in the area

Species	Region	Sequence (5' – 3')	Mismatches
Northern map turtle	Forward Primer (1-22)	CGCCTACGCAATTCTACGATCT	-
Snapping turtle		TGCCTACGCAATCCTACGATCA	2
Blanding's turtle		TGCCTACGCAATCCTACGATCA	2
Painted turtle		CGCTTACGCAATYCTACGATCT	2
Common musk turtle		TGCCTATGCAATCCTACGATCA	3
Northern map turtle	Probe (27-47)	CAAACAAGTTAGGTGGAGTAC	-
Snapping turtle		CAAACAA TAGG GG GTA	5
Blanding's turtle		CAAACAA TAGG GG GTAC	4
Painted turtle		CAAACAA TAGG GG GTA	5
Common musk turtle		CAAA AA TAGG GG GTAC	5
Northern map turtle	Reverse Primer (78-99)	TCCTAATGCCCACCCTACACAC	-
Snapping turtle		T TAAT CCCA CCT CACAC	5
Blanding's turtle		T CTAAT CCCACCCTACA AC	3
Painted turtle		TC TAAT CCC CCCTACACAC	3
Common musk turtle		T TA T CC A C ACACAC	8

Note: The 3′ end of the MGB probe was modified with a FAM reporter dye. Sequences on the sense strand are displayed from 5′ to 3′.

amplifications were performed on CFX96 Touch™ Real-Time PCR platform (Bio-Rad) with reaction cocktails contained the following: 10 μL SensiFAST™ probe NO-ROX mix (Bioline), 400 nM forward and reverse primer, 200 nM TaqMan probe, 10 μg Bovine Serum Albumin (BSA), 2 μL DNA template with reverse osmosis H_2O added to a final volume of 20 μL. The qPCR cycling profile was as follows: 2 min of polymerase activation at 95°C, 45 cycles of two-step amplification with 10 s of denaturation at 95°C and 20 s of annealing/extension at 60°C. The 137 working samples were analyzed in seven separate 96-well PCR plates (BioRad), and for each plate, we established the standard curve using a seven-point tenfold dilution series from 3×10^6 to 3 copies of template per reaction with laboratory blank controls where the DNA template was replaced by ultrapure water. We performed all qPCR amplifications in triplicate.

We processed the raw qPCR readings using Bio-Rad CFX manager (version 3.1, Bio-Rad, USA). Efficiency, linear dynamic range and limit of detection (LOD) of the qPCR assays were calculated with automatically determined threshold cycle values (C_T), following the MIQE guidelines (Bustin et al. 2009). An eDNA sample was considered to be positive when at least two out of three replicates showed signals within the linear dynamic range (see Supporting Information for details). The quantity of map turtle eDNA within each sample was then calculated based on the standard curve.

2.4 | Statistical analysis

The binary outcomes of qPCR results (i.e., detection or nondetection) from both winters were combined and used in subsequent statistical analyses. We considered each sampling event as a Bernoulli trial with the probability of detection related to each site. We tested whether detection probabilities varied across the lake, corresponding to the distance from known overwintering sites, against the null hypothesis where eDNA is assumed to be evenly distributed across the lake with a *constant detection probability* at all sites (i.e., the observed spatial pattern of detections is simply the result of random sampling among the surveyed sites). We conducted nearest neighbor analysis (Clark and Evans 1954) to measure the degree to which the distribution of detections departs from that predicted from a random distribution. In this analysis, the nearest neighbor distance is defined as the distance between a given site and its nearest neighboring site, and the average nearest neighbor (ANN) distance is the mean of all such distances across all sites. *Observed* and *expected* ANN distance was calculated for the *actual* sites of detection and for a random distribution of detections among surveyed sites with simulated constant detection probability (p), respectively. p was generated using values from .2 to .5 incremented by .01, and for each value of p, we simulated 1,000 random distributions of detections. Additionally, for the subset of sites within 1,500 m of the shoreline of Eightacre Island where we know turtles overwinter, we used a simple logistic regression to model detections/nondetections as a function of distance to shoreline.

All spatial data were analyzed using QGIS (QGIS Development Team 2018; version 3.2.0), and all statistical analyses were conducted in R (R Core Development Team 2018; version 3.5.2).

3 | RESULTS

3.1 | eDNA assay validation and efficiency

Our cyt*b* primer and probe set were specific to map turtle with no signal for qPCR tests using genomic DNA samples from other

TABLE 3 qPCR standard curve summary

Plate	Starting C_q	Ending C_q	NTC C_q	R^2	Slope	Intercept	Efficiency (%)
1	18.39	37.10	38.05	0.997	−3.36	38.44	108.60
2	18.98	37.90	38.14	0.995	−3.15	38.31	107.70
3	18.99	37.22	39.56	0.992	−3.07	37.96	111.70
4	19.11	38.59	38.89	0.996	−3.23	38.80	104.10
5	19.03	38.04	39.86	0.994	−3.20	38.59	105.60
6	18.94	37.74	39.21	0.992	−3.12	38.10	109.30
7	18.74	36.60	40.05	0.991	−3.16	38.16	107.10

Note: eDNA samples were run in seven separate plates that each included a 7-fold standard dilution series and nontemplate control (NTC) in triplicate. Average C_q values are shown for starting (10^7 copies) and ending (10 copies) points of the curve as well as for NTCs.

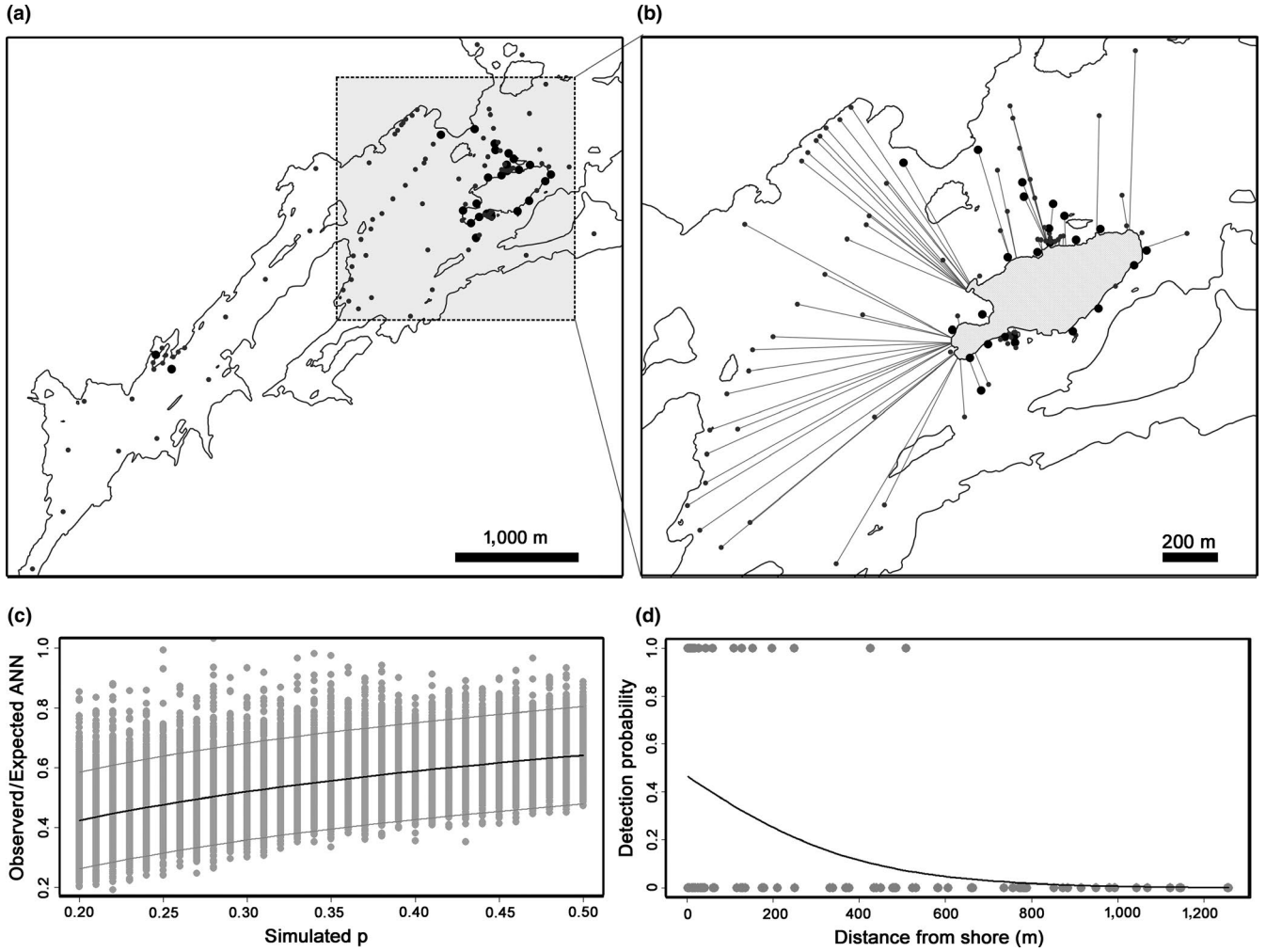

FIGURE 3 (a) eDNA signals across Lake Opinicon from 2017 to 2018 winter sampling. Sites detected/not detected with map turtle eDNA are indicated by black/gray dots, respectively. (b) Sampling sites around Eightacre Island showing shortest distance to shore (lines), detections (black dots), and nondetections (gray dots). (c) Ratio of the observed/expected average nearest neighbor distances calculated from Monte Carlo simulations with increasing global detection probability (*p*) from .2 to .5. Each point represents the ratio from a single simulation. All points lie below a value of 1 suggesting that the distribution of actual detections significantly departs from that expected from random detections. (d) Log logistic regression of the ratios of detection probability as a function of distance from the Eightacre Island shoreline

co-occurring turtles. The efficiency of the qPCR assays ranged from 104% to 110%, with r^2 of the standard curves varying between 0.992 and 0.996 (Table 3). The LOD value of our qPCR assays was 10 copies per reaction, or 500 copies per liter of water. The quantity of target eDNA in the samples with positive detections ranged from 311 to 2,253 copies per reaction, equivalent to 15, 550 to 112, 590

copies per liter of water. We did not detect DNA signals in either field (N = 16) or laboratory negative controls (N = 7), suggesting no field and laboratory contamination (see Supporting Information for details).

3.2 | eDNA detection pattern across the lake

The one-day survey in 2017 winter showed two detections among 36 sites across the lake, with one detection to the southwest of Eightacre Island and another detection near the suspected overwintering site (Figure 2a). This result was combined with known information of map turtle overwintering sites from observations and radiotelemetry, and informed the design of 2018 winter survey scheme.

For our 2018 winter surveys, contrary to our expectations, we did not find eDNA concentration gradients along the two transects extending from known overwintering sites at Eightacre Island shoreline. Instead, the eDNA detections around Eightacre Island and environs presented a mosaic pattern (Figures 2c and 3b). Our logistic regression model showed that detection probability decreases with distance from the shoreline of Eightacre Island, dropping to no detections at approximately 600 m (Figure 3d).

With data from both winter surveys combined, map turtle eDNA was detected in 27 out of 121 sampled sites (22.3%), with localized distribution near known or suspected overwintering sites (Figure 3a). In the nearest neighbor analysis, the *observed* ANN distance was calculated to be 110 m, and the ratios of *observed* to *expected* ANN were less than one across all simulated constant global detection probabilities p (Figure 3c), implying that map turtle eDNA was not homogenously distributed and that the clustering of detections (i.e., higher detection probability around Eightacre Island) is real rather than an artifact of sampling bias.

4 | DISCUSSION

Overwintering is a critical, but generally poorly understood, part of the annual cycle of northern animals. This is particularly true for aquatic animals such as northern freshwater turtles because identifying overwintering sites can be challenging. Locating overwintering sites is essential for understanding the ecology of turtles and may be important for their conservation if suitable sites are limited or under threat from human development. We assessed eDNA as a tool for locating communal overwintering sites of a species of turtle that is at risk in Canada. We show that the eDNA detection pattern broadly matched the known locations of overwintering map turtles previously identified by radiotelemetry. Thus, despite the fact that turtles have low metabolism in the winter and would be expected to release less eDNA than during their active season, the signatures of their presence were sufficient for us to detect them using probe-based qPCR assays.

4.1 | Using eDNA to detect northern map turtle overwintering sites

At the outset of our study, we knew from radiotelemetry data that northern map turtles overwintered near the shoreline of Eightacre Island. Turtles have been observed repeatedly under the ice at two sites using underwater cameras. A third site was suspected at the western end of Lake Opinicon based on observations of basking individuals in early spring. Our eDNA detections showed clustered signals around Eightacre Island reflecting the presence of overwintering turtles (Figure 3a). The suspected overwintering site in the western portion of Lake Opinicon has similar features to those of the known site, with a rocky slope quickly descending to the approximately 4-m-deep lake bottom. To confirm the presence of map turtles at the two known sites, we deployed a Trident remotely controlled underwater drone (OpenROV) in February 2019. Footage from searches at the known overwintering site north of Eightacre Island revealed multiple map turtles. To confirm the presence of turtles at the suspected overwintering sites, we searched with the drone a 150 m long transect parallel to the shoreline. We drilled six holes along this transect and searched a ~20 m radius centered on each hole. We observed one female map turtle, confirming our prediction of another overwintering site although the size of the aggregation of turtles in this area is probably smaller compared with Eightacre Island. The detection probability of eDNA signals in this area was also lower (2 out of 10, see Figure 2) comparing to that around the shoreline of Eightacre Island. More ROV surveys are needed to characterize this hibernaculum. Details and video footage are presented in the Supporting Information.

4.2 | Spatial variation of eDNA detection of overwintering turtles

Instead of a simple eDNA concentration gradient extending from the Eightacre Island overwintering site as we had expected, we found that eDNA detections were patchily distributed (Figures 2c and 3b), with the probability of detection unevenly distributed across sampling sites. This is likely attributable to the overall low concentration and uneven dispersion of the target DNA molecules in water (Furlan, Gleeson, Hardy, & Duncan, 2015), as a result of reduced physiological activity of overwintering map turtles combined with presumed limited water movement under the ice. In our simulation analysis, we varied the hypothetical constant detection probability (p) from 0.2 to 0.5, while the actual overall detection rate across the lake was 0.24 with the highest rate around overwintering site of 0.47. For a given number of sampling sites, we expect the number of simulated detections to increase with higher p, resulting in higher ANN ratios. ANN ratios never surpassed the threshold of 1. In the extreme case when p reaches 100% (i.e., every site yields a positive signal), the ANN distance was estimated to be 123.6 m, reflecting the spatial clustering of sampling sites with turtles. This simulated ANN distance is still larger than the observed ANN of 100.2 m, suggesting that the

distribution of map turtle eDNA detections is unlikely to be an artifact of sampling error. Results from our logistic regression indicated a decreasing detection probability with distance from the shoreline of Eightacre Island, supporting the notion that eDNA signals reflect the physical presence of overwintering turtles. This patchiness of eDNA distribution echoes other studies that show spatial variation and patchiness of eDNA within a single water body (e.g., Eichmiller et al. 2014; Minamoto et al. 2017).

4.3 | Why is detecting overwintering sites important?

Turtles are now among the most endangered vertebrate groups with approximately 58% of all extant species threatened or endangered, with 2% now extinct (van Dijk, Iverson, Rhodin, Shaffer, & Bour, 2014). All turtle species in Ontario, except the eastern painted turtle (*Chrysemys picta picta*), are now considered to be at risk with evidence of decline in abundances across taxa (O. Reg. 2018). The northern map turtle is listed as being of Special Concern on Schedule 1 of the Canadian Species at Risk Act (SARA), and as S2 (Imperilled) and S3 (Vulnerable) in Ontario and Quebec, respectively (Environment Canada 2016). Threats to map turtles and other turtle species are multifold, and include road mortality, increased nest predation, the illegal and commercial wildlife trade, and habitat destruction and alteration (COSEWIC 2012). Coupled with this, long generation time and low recruitment rates make population recovery slow (Keevil, Brooks, & Litzgus, 2018). Critical to the long-term persistence of these turtle species is identifying landscape features that are important for their reproduction and survival, including overwintering sites. Northern map turtles appear to be highly faithful to these sites (Graham et al. 2000). High fidelity to overwintering sites combined with communal overwintering strongly suggests that suitable overwintering sites are limiting and warrant legal protection.

5 | CONCLUSION AND FUTURE DIRECTIONS

We showed the effectiveness of eDNA approaches in a unique scenario where two key factors that would be predicted to confound our ability to detect a species using eDNA were minimized: (a) the movement of the target species for foraging, mating, or dispersal; and (b) water movement that increases eDNA transportation and dispersion. Some eDNA studies seeking to confirm the presence or absence of a particular taxon seem to tacitly assume wide distribution of eDNA during "active seasons" (e.g., within a water body). We took advantage of the opposite phenomenon—spatially concentrated eDNA signals beneath the ice of a frozen temperate lake. We show that species-specific qPCR detection can assist in locating overwintering sites of northern map turtles. Environmental DNA approaches are not replacements for methods like radiotelemetry but rather are complementarity and can provide quick, coarse baseline information before conducting finer scale studies. For example, to survey a lake without prior knowledge of overwintering site, one could use eDNA signals to locate possible areas and conduct more focused surveys with an ROV. The usefulness of this approach is not limited to turtles but could be applied to other taxa that overwinter in freshwater lakes and streams (e.g., fish, neotenic salamanders, and invertebrates) to unravel their life under the ice.

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AUTHOR CONTRIBUTIONS

WF and SCL designed the experiment and methodology; WF collected and processed the samples and performed qPCR assays. WF and SCL analyzed the data. GB provided key information on the overwinter ecology of northern map turtles in Lake Opinicon and contributed to the study design. WF wrote initial drafts of the manuscript, and GB and SCL critically reviewed and edited the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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