Received: 13 November 2021 | Revised: 19 January 2022 | Accepted: 19 January 2022

DOI: 10.1002/edn3.283

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences
WILEY

ORIGINAL ARTICLE

Tracking the prevalence of a fungal pathogen, *Batrachochytrium dendrobatidis* (chytrid fungus), using environmental DNA

Megan Congram[1] | Sibelle Torres Vilaça[1] | Chris C. Wilson[2] | Chris J. Kyle[1,3] | David Lesbarrères[4] | Madison J. H. Wikston[1] | Lynne Beaty[1,5] | Dennis L. Murray[1,6]

[1]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, Canada

[2]Aquatic Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Peterborough, Ontario, Canada

[3]Forensic Science Department, Trent University, Peterborough, Ontario, Canada

[4]Genetics and Ecology of Amphibian Research Group (GEARG), Department of Biology, Laurentian University, Sudbury, Ontario, Canada

[5]Department of Biology, School of Science, Penn State Erie, The Behrend College, Erie, Pennsylvania, USA

[6]Biology Department, Trent University, Peterborough, Ontario, Canada

Correspondence
Megan Congram, Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, Canada K9J 0G2.
Email: megancongram@trentu.ca

Present address
Sibelle Torres Vilaça, Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

Funding information
Natural Sciences and Engineering Research Council of Canada, Grant/Award Number: 463602-14

Abstract

Chytridiomycosis, a primary disease driving widespread and unprecedented amphibian declines, is caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). Tracking Bd through space and time requires monitoring protocols that efficiently and reliably assess pathogen prevalence and intensity, which in turn requires an understanding of environment–pathogen dynamics. Environmental DNA (eDNA) was used to track Bd prevalence and intensity in 95 waterbodies in southern Ontario, Canada, and assess zoospore counts relative to biotic, abiotic, and geographic factors. Bd was also monitored on a semi-weekly basis in 10 waterbodies to better understand patterns of temporal variability. Bd showed variable prevalence, with 47% and 29% of waterbodies having zoospores detected in May and July, respectively. Patterns of prevalence were markedly variable both within and across waterbodies, indicating high spatio-temporal heterogeneity. Bd prevalence was not related to environmental factors, geographic variables, or amphibian species richness, but intensity was negatively related to estimated canopy cover. In intensively sampled waterbodies, Bd counts were highly variable through time, with some sites switching from detection to non-detection (and vice versa) across 2-week intervals. We conclude that eDNA can be a useful tool for monitoring Bd zoospores in wetlands but emphasize the need for additional research into environmental and methodological factors affecting zoospore detection and abundance before this method should be widely adopted.

KEYWORDS
amphibian disease, aquatic pathogens, disease monitoring, eDNA, environment-pathogen dynamics, wildlife disease

1 | INTRODUCTION

Amphibian populations worldwide are experiencing unprecedented and rapid declines, many of which are caused by outbreaks of emerging infectious diseases (Daszak et al., 2003; Jones et al., 2008). A primary disease driving these declines is chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (hereafter "Bd," Order Rhizophydiales; Letcher et al., 2006; Longcore et al., 1999; Skerratt et al., 2007). As with many wildlife pathogens, there is currently no effective large-scale treatment for chytridiomycosis

(Geiger et al., 2017; Scheele et al., 2014), and as such, pathogen tracking through space and time is an important tool for assessing status and likely trajectory of vulnerable populations. This is particularly true in areas of high amphibian diversity, or where other stressors like habitat loss or invasive species magnify risk (Fonner et al., 2017; Nichols et al., 2017).

Long-term pathogen tracking requires protocols that can assess pathogen prevalence and intensity with reasonable reliability and efficacy (e.g., Bower et al., 2017; Osnas et al., 2009). The design of such protocols requires an understanding of the effect of abiotic conditions on the pathogen, as well as host–pathogen dynamics (Daszak et al., 2001; Heard et al., 2013). Environment–pathogen dynamics are of particular interest when a pathogen has a free-living stage that is susceptible to abiotic conditions outside of the effect of these conditions on hosts (Anttila et al., 2015; Caraco & Wang, 2008; St-Amour et al., 2008). In the case of Bd, whose infective zoospore stage is free-living in water, current understanding of abiotic influences is limited in large part by the challenges of studying the pathogen, independent of its hosts (Berger et al., 1998; Longcore et al., 1999). As a result of mostly laboratory work, there is evidence to support that Bd can survive across a range of environmental conditions, with zoosporangia growth occurring between 2°C and 27°C. Strain-specific growth peaks for Bd range between 17°C and 23°C (Voyles et al., 2017), while infective free-swimming zoospores can survive short-term freezing and high heat (Piotrowski et al., 2004; Voyles et al., 2017—but see Chestnut, 2015 for evidence that freezing does negatively impact Bd density). Studies of Bd in wild amphibians in both the tropics and temperate zones indicate higher prevalence and intensity of infection in cooler seasons (Gaertner et al., 2009; Korfel, 2012; Pullen et al., 2010), and in cooler wetlands within a season (Muths et al., 2008; Voordouw et al., 2010). Bd zoospores have a broad range of pH tolerance (4.0–10.0; Longcore et al., 1999; Piotrowski et al., 2004; Johnson & Speare, 2005), but appear sensitive to high salinity (Stockwell et al., 2012). Although Bd tolerance for dissolved oxygen (DO) is not known, other Rhizophydiales are obligate aerobes that experience growth inhibition in low-DO environments (Gleason et al., 2008). Abiotic factors such as sunlight and ultraviolet radiation can also negatively impact plankton, including aquatic fungi, directly (Häder et al., 2011), but may also affect these populations by suppressing microfaunal consumer populations higher in the food chain (Day & Neale, 2002; Hamilton et al., 2012). However, these dynamics are complex, and their impacts on Bd prevalence and intensity in the wild likely involve multiple interacting factors and may vary across Bd strains (Hite et al., 2016; Searle et al., 2013).

From a biotic perspective, Bd is known to be more prevalent in amphibian-rich environments (Adams et al., 2017; Bielby et al., 2015), but its propagules are also spread by a variety of other vertebrates (Burrowes & De la Riva, 2017; Johnson & Speare, 2005). Notably, amphibian community composition also influences Bd occurrence, with some species apparently acting as asymptomatic reservoirs and thereby increasing community-level transmission and infection (DiRenzo et al., 2014; Olson et al., 2013; Van Rooij et al., 2015), and these interactions can differ significantly between introduced and endemic Bd strains (Dang et al., 2017; Retallick & Miera, 2007). Accordingly, there remains considerable uncertainty regarding drivers of Bd distribution and abundance in nature, and research efforts should evaluate a suite of candidates across a range of natural conditions.

Environmental DNA (eDNA) can elucidate relationships between Bd and both abiotic and biotic factors. Previous efforts have sought to develop eDNA sampling protocols for a variety of wildlife pathogens (e.g., Huver et al., 2015; Peters et al., 2018; Vilaça et al., 2020), and early attempts to track Bd via eDNA have been promising (Battaglin et al., 2016; Chestnut et al., 2014). Currently, the most prevalent methods for the detection of Bd are host tissue examination or swabbing, and while these methods remain essential for establishing prevalence of infection and disease, they are often challenging to implement for the purpose of monitoring the pathogen itself due both to their impact on sampled animals, and to the logistics of attaining sample sizes large enough to avoid Type II error (i.e., false negative population-level assessment; Skerratt et al., 2008). The use of eDNA to detect and monitor pathogens like Bd in waterbodies can thus allow us to prioritize areas for more intensive disease surveillance, and could potentially act as an early warning system for disease outbreaks or the introduction of new strains into naïve populations.

In this paper, we use eDNA to quantify prevalence and intensity of Bd in waterbodies in southern Ontario, Canada, to better understand relationships between zoospore numbers and environmental conditions. We predicted that Bd zoospores would be more prevalent and have higher intensity: (1) During cooler parts of the spring/summer season, and (2) in wetlands with greater UV exposure and higher water quality (low salinity, neutral pH, and high dissolved oxygen). We also predicted that Bd would be more prevalent in waterbodies having: (3) higher amphibian diversity, and (4) better amphibian habitat in the surrounding area (i.e., lower anthropogenic impact). Ultimately, the goal of this study was to inform the design of eDNA-based Bd pathogen detection and monitoring programs in northern temperate regions, enabling us to more quickly react to potential changes in amphibian disease prevalence in these areas.

2 | METHODS

2.1 | Study area

Our study was conducted in spring and summer of 2017, at 95 waterbodies within a 75-km radius of Peterborough, Ontario, Canada (44.3N, 78.3W; Figure 1). This area is between Georgian Bay and Lake Simcoe-Rideau ecoregions (Crins et al., 2009) and features mixed-wood forests and plains transitioning towards the Canadian Shield to the north. Local permanent wetlands are often open water, dominated by emergent edge vegetation, while ephemeral wetlands are flooded forest bottoms or grass meadows. The local amphibian community is one of the most diverse in Canada, with common

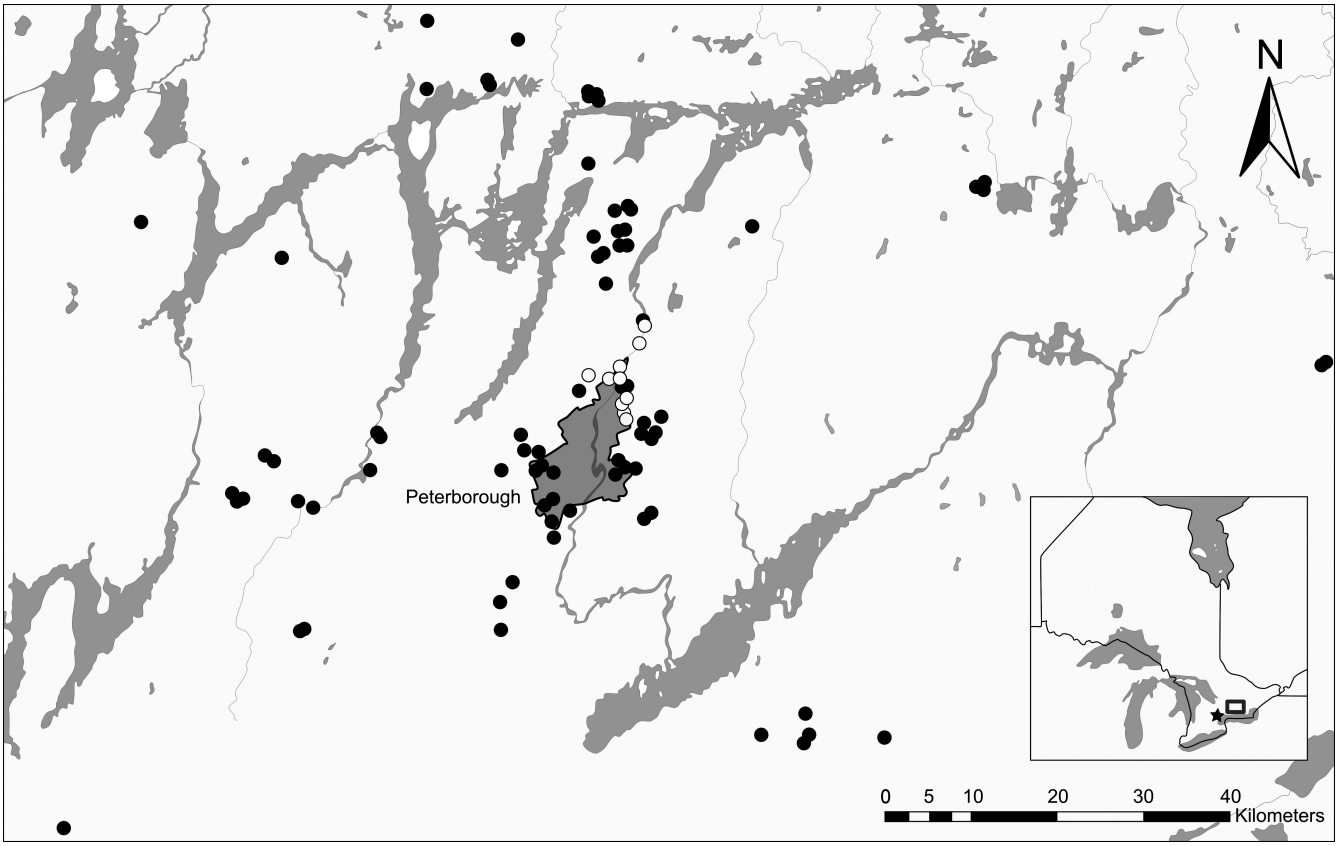

FIGURE 1 Wetlands sampled for *Batrachochytrium dendrobatidis* (chytrid fungus) prevalence and intensity, near Peterborough, Ontario, Canada. Empty circles indicate intensively surveyed sites within the larger sample set. Each wetland was separated by >60 m to establish sample independence. The dark gray area surrounded by a black line delineates the recognized urban area of Peterborough, ON, while the inset map shows the location of this study system in Ontario, Canada, with the large urban centers of Toronto and Ottawa indicated by stars

wetland species including green frog (*Lithobates clamitans*), northern leopard frog (*L. pipiens*), wood frog (*L. sylvaticus*), and spring peeper (*Pseudacris crucifer*; Powell et al., 2016). Bd was previously detected in this region, as was ranavirus (Vilaça et al., 2020), however, only the latter has been linked to local amphibian die-offs (Duffus & Andrews, 2013; Greer et al., 2005). The sampled waterbodies ranged in elevation from 200 to 300 m asl, and occurred across a variety of land-cover types. We selected these waterbodies opportunistically based on access and evidence of prior amphibian occupancy, derived from previous work in the region and preliminary casual surveys.

2.2 | Field methods

We collected five replicate water samples at each waterbody, twice during the active amphibian season (May and July 2017), using field methodology and recommendations described in Chestnut et al. (2014). The replicate samples were separated by at least 10 m in larger waterbodies and evenly spaced around the perimeter of small waterbodies (i.e., those whose perimeters could not accommodate five samples separated by 10 m each). For each replicate, we passed water from the site through a 0.22-μm capsule filter (Sterivex SVGPL10RC; Millipore Sigma) until the filter clogged or a maximum volume of 500 ml (average: 270 ml) had been filtered. After rinsing with phosphate-buffered saline and purged, we filled the capsule filters with preservative lysis buffer and refrigerated them in individual plastic bags until DNA extraction. All field equipment, including boots and waders, was decontaminated between sites (1:10 bleach solution, see NEPARC, 2014) to prevent pathogen transmission, and we used fresh syringes and single-use nitrile gloves for each sampling event to prevent cross-contamination. Due to changes in water level and/or landowner permission between the two sampling periods of the 95 waterbodies sampled in May, only 89 were resampled in July.

For each sampling event, we collected environmental and water quality data at each site. Water temperature and dissolved oxygen (DO) were measured using an ExStik II Dissolved Oxygen meter (ExTech Instruments, New Hampshire, USA), while conductivity, a proxy of salinity (Lewis, 1980), was measured using an ECOTestr conductivity meter (Oakton Instruments, Illinois, USA). We also measured pH using an aquarium pH testing kit (API Aquarium Pharmaceuticals, Pennsylvania, USA). In addition, we approximated percent canopy coverage of the water body using an ocular estimate of sky coverage at each site (see Thornton et al., 2013). Notably, this ocular estimate was then validated in 2018 by using a spherical densiometer (Lemmon, 1956) at a subset of 68 waterbodies, with high correlation between ocular

and spherical densiometer measurements (t_{66} = 20.484, p < 0.001, r = 0.93). Finally, we used satellite imagery (CEC, 2015; Google, 2015) to assess approximate waterbody size, and habitat proportions within a 100-m and 300-m radius of the site. Habitat proportions were based on satellite imagery from the North American Land Change Monitoring System (NALCMS) and consisted of a breakdown of the proportion of each habitat type within a 100-m and 300-m radius of sampling locations at each site. Eight NALCMS cover types were present at our sites, and we further condensed these into four categories: forested cover (trees > 3 m in height), low cover (grasses and shrubs), anthropogenic impact (urban, crops, and bare land), and waterbodies (wetlands and open water).

To further understand the link between water temperature and temporal changes on pathogen infection rates, we also sampled 10 of our sites for Bd on a semi-weekly basis from mid-May to mid-August 2017 (15 weeks and 8 sampling events). This subsample was selected based on likelihood of waterbody persistence through the summer. We fitted each site with two iButton thermal loggers (Maxim Integrated DS1921H) in waterproof housings, stationed ~10 cm below the surface and >10 m apart. These sensors captured water temperature on an hourly basis for the length of the study.

A subset of 29 of our sampling sites were inventoried for amphibian community composition in spring–summer 2016 (Wikston, 2021). These inventories consisted of four different survey techniques, each deployed multiple times per site over the course of the season: visual surveys (for all life stages, including adults, larvae, and egg masses), dip-net sweeps for aquatic amphibians (primarily larvae), call surveys (for breeding adults), and eDNA sampling (for all life stages). Visual surveys encompassed both riparian and littoral zones, with observers overturning movable cover and scanning vegetation for organisms (per Crisafulli, 1997), while dip-net sweeps involved dip-net samples taken at random at several depths along a transect (Wikston, 2021). Amphibian larvae were identified to species (Mills, 2016) and released after the survey (per Shaffer et al., 1994). For call surveys, surveyors identified calling species (i.e., breeding adult anurans) after dark as well an index of abundance (per Bird Studies Canada, 2009). Finally, for eDNA surveys of amphibians, four 1L samples of water were collected from each waterbody, one at each cardinal point where possible or otherwise at least 20 m apart. Samples were refrigerated and filtered through 4.7 cm diameter grade 691 1.5 μm Glass Microfibre Filters (VWR) within 12 h of collection (per Hinlo et al., 2017). Glass filters and remaining residue were frozen at −80°C until analysis. Note that although amphibian species richness was measured in the year prior to sampling for Bd in the same waterbodies, there is no indication of strong inter-annual amphibian community turnover in the study region (D. Murray, pers. obs., see also Martínez-Solano et al., 2003; Werner et al., 2007).

2.3 | Laboratory methods

Timely processing of Bd eDNA samples is important (Chestnut et al., 2014; Spens et al., 2017) and we extracted all samples within 13 weeks of collection. For DNA extraction, we used a DNeasy blood and tissue kit (Qiagen Inc., Hilden, Germany), along with a slightly modified version of the protocol described in Spens et al. (2017) for capsule filters. After removing lysate from the stored capsule filters, we added 720 μl of buffer ATL and 48 units of proteinase K to the filter surface. The capsule filter was then sealed and incubated overnight at 56°C, with regular agitation for the first 3 hours to ensure that filter parts were exposed to proteinase K.

We ran each capsule filter's extract through a quantitative polymerase chain reaction (qPCR) following Kirshtein et al. (2007), with three replicates per sample (15 qPCR replicates per site) in a total reaction volume of 20 μl containing 5 μl sample extract, 10 μl 1X TaqMan Environmental PCR Master Mix 2.0 (Applied Biosystems), 0.6 μl each of the 10 μM forward and reverse primers for the Bd ITS1 gene, which is specific to this pathogen (Boyle et al., 2004), and 0.3 μl of 10 μM fluorescent probe (5′-FAM-CGAGTCGAACAAAAT-MGB-3′; Boyle et al., 2004). Our quantitative PCRs were performed on an Applied Biosystems 7900 detection system, and data analysis was conducted in-system using Applied Biosystems StepOne Plus software. Quantitative PCRs were run with an initial holding stage of 2 min at 50°C and 10 min at 95°C, and then 50 cycles of 15 s at 95°C followed by 1 min at 60°C. We constructed standard curves from a dilution series of synthetic Bd DNA of known concentrations: five 10-fold serial dilutions ranging from 10^5 to 10^1 ITS1 copies. Plates with standard curves with R^2 < 0.90 and efficiency >110% or <90% were rejected and re-run. We tested a subset of samples (n = 25, chosen at random) for inhibition by spiking with synthetic Bd DNA (Biggs et al., 2015). As the recovery consistently exceeded 100% for these samples and multiple methodological steps were taken to reduce inhibition in samples, we opted not to use an internal positive control to reduce our total number of required qPCR runs. While this raises the possibility of false negative detections in waterbodies with particularly low densities of Bd (i.e., <10 copies/L; Wilcox et al., 2018), given the presence of many ITS1 copies in a single zoospore we feel that the likelihood of false negatives is minimal.

The limit of detection (LOD) for the qPCRs was determined to be 0.23 ITS1 copies, based on the cycle numbers of the successful qPCR runs (calculated as per Hunter et al., 2017). However, due to limited reliability of standards with fewer than 10 copies, we chose a limit of quantification (LOQ) of 10 copies—this is, notably, well below the number of ITS1 copies expected in a single zoospore (Longo et al., 2013). Due to this decision, we discarded positive detections from 11 replicates that exceeded LOD but amplified below LOQ. However, in all but one case samples with discarded positive replicates had other positive replicates that were retained, resulting in little impact on detection and quantification of Bd in this study. We also discarded our highest detection (3.2 million copies/L) as an outlier, as it was an order of magnitude higher than any other detection. No extraction or qPCR negative controls (n = 101) tested positive for presence of Bd.

The methods used to detect eDNA in the amphibian species richness subset are fully described in Wikston (2021) and were broadly similar to the above—eDNA was extracted from thawed glass filters per Goldberg et al. (2011) and quantified using qPCR.

Species-specific oligonucleotide controls with synthetic inserts, developed as described in Wilson et al. (2016), reduced false positives, and samples from each waterbody were run in triplicate for each species. Samples were deemed positive for a species if at least one of three replicates produced an amplification above the calculated species-specific LOD.

2.4 | Statistical analysis

Each filter taken at a site during a sampling event was treated as a sub-sample, with the waterbody itself serving as the unit of measure. We averaged the positive qPCR replicates for each filter to estimate Bd copy numbers in that sub-sample (Goldberg et al., 2013; Thomsen et al., 2012), and then the five filters were averaged to obtain an estimate for each wetland per sampling occasion. Detection probability for our sampling method was estimated using the process described by Wilcox et al. (2018). We used Bd copy numbers as the response variable rather than zoospore equivalents because gene copies per zoospore vary between different strains (Longo et al., 2013; Rebollar et al., 2017) and the Bd strain(s) in the area have not been confirmed. These results are thus presented as average number of amplified Bd ITS1 gene copies detected, standardized to a per liter measurement to account for differing sample volumes.

For the large-scale sampling effort, we analyzed the relationship between Bd DNA detection and environmental and geographic variables via hurdle models, a two-part modeling approach where each part describes a different aspect of Bd quantification (Cameron & Trivedi, 1998; Martin et al., 2005). First, Bd prevalence was modeled as a binary outcome using a candidate set of generalized linear mixed models (GLMMs; Table 1) including abiotic and geographic variables. Second, where Bd copies were present, we modeled pathogen intensity using another candidate set of GLMMs (in this instance, Type 2 negative binomial models) based on the same selection of variables. Low correlation between environmental variables in this data set (all $r < 0.70$) justified including each in statistical models; however, three GIS-based geographic variables (forest within 100 m, anthropogenic impact within 100 m, and water within 300 m) were excluded due to high correlation (all $r > 0.75$). Due to the sample size and the inherent complexity of this system, we restricted candidate models to those with two or fewer predictor variables (see Murray et al., 2020); models were also run with the addition of an interaction term where appropriate. This analysis was conducted using the lme4 (Bates et al., 2015) and glmmTMB (Brooks et al., 2017) packages with R Statistical Software (v3.6.2; R Core Team, 2019). Candidate models also included site and, where appropriate, sampling period (May or June) as random factors to account for site-specific variation and seasonal differences.

TABLE 1 Top generalized linear mixed models (ΔAIC < 2.0) assessing Bd prevalence and intensity versus environmental factors at 95 waterbodies near Peterborough, Ontario, Canada

Rank	Model	ΔAICc	Weight	Estimate	Pr (|>z|)	Conditional R^2
Bd prevalence						
1	Conductivity * Low cover (300 m) (interaction term)	0	0.40	−0.45 ± 0.41 −0.51 ± 0.31 −1.48 ± 0.64	0.27 0.10 0.02	0.85
2	Null	4.8	0.04			
Bd intensity						
1	Canopy cover * Forest (300m) (interaction term)	0	0.11	−0.52 ± 0.17 0.18 ± 0.19 0.37 ± 0.18	0.002 0.34 0.046	0.71
2	Canopy cover	0.8	0.07	−0.38 ± 0.16	0.02	0.65
3	Canopy cover + Low cover (300m)	1.0	0.06	−0.38 ± 0.16 −0.24 ± 0.16	0.02 0.13	0.64
4	Canopy cover + Forest (300m)	1.4	0.05	−0.47 ± 0.17 0.25 ± 0.19	0.01 0.18	0.66
5	Canopy cover + Water temperature	1.4	0.05	−0.41 ± 0.17 −0.21 ± 0.15	0.01 0.18	0.66
6	Low cover (300m)	1.6	0.05	−0.23 ± 0.17	0.16	0.65
7	Canopy cover * Human impact (300m) (interaction term)	1.9	0.04	−0.47 ± 0.16 −0.12 ± 0.18 −0.34 ± 0.18	0.005 0.50 0.065	0.68
24	Null	3.4	0.02			

Note: The table includes coefficient estimate (±*SE*) and statistical significance for each parameter in top models and model weight was calculated across all candidate models. Conditional R^2 is a measure of fit. Note that the Low Cover variable reflects the fraction of the landscape within a 300 m radius of the site covered by grass or shrubs, and the Canopy Cover variable represents % obstruction based on visual assessment. Null models are included for reference.

Indicates an interaction model rather than an additive model (vs +).

Amphibian community composition (i.e., species richness) at a subset of 29 waterbodies was determined as the cumulative number of species detected from all survey types in 2016. We then modeled Bd prevalence sampled in the following year using a candidate set of binomial GLMMs that included the random effect of site and the amphibian species richness, along with the above abiotic variables. Due to the small size of the subset and the relatively low number of Bd positives within this subset, only prevalence was modelled. For samples from the 2017 intensively monitored waterbodies, we also used a GLMM to evaluate whether there was a temporal effect on Bd prevalence or intensity.

All candidate models were ranked based on corrected Akaike Information Criteria values (AIC_c), with $\Delta AIC_c < 2.0$ indicating lack of distinction between best-fit and other candidate models (Burnham et al., 2011). We derived likelihood ratio indices (conditional pseudo-R^2s) for top models based on Nakagawa and Schielzeth (2013). A pseudo-$R^2 > 0.40$ was considered to be evidence of reasonable model fit (McFadden, 1978).

3 | RESULTS

The prevalence of Bd varied substantially between sampling periods, with 48.4% ($n = 95$) of sites testing positive in at least one of five capsule filters in May, and 29.1% ($n = 86$) in July. Of these, 33.8% ($n = 71$) tested positive in only one filter during a sampling event, 36.6% were positive in >2 filters, and 2.8% were positive in all five filters. We detected Bd in an average of 2.3 out of five filters (skewness = 0.55, kurtosis = 2.16) at positive sites, and there was generally a positive relationship between average qPCR copies/L for the site and number of positive filters at the site (Figure 2). For all filters containing Bd, we observed a large range of copy numbers, spanning 1360–506,000 copies/L. Within-site Bd intensity also varied widely, with the largest variation during a given sampling event spanning 1240–91,900 copies/L. In both May and July, a single site (1.1%) in each period tested positive in all five capsule filters. Both of these sites had high Bd copy numbers (i.e., >20,000 average copies/L), and each was positive for Bd in both sampling periods.

3.1 | Bd prevalence

Of the sites that were tested twice (90.5% of total), only 22.1% tested positive in both months, with 27.4% testing positive only in May, and 7.0% only in July, with a detection probability of 85% assuming only 25 Bd ITS1 copies per liter of water (Figure 2). Overall, we found that 46.4% of sites tested negative in both sampling periods. In the analysis of Bd prevalence we detected a single statistically distinguishable model, which contained water conductivity, low cover habitat (300 m), and their interaction (Table 1). This model had reasonable overall fit, although only the interaction term had $p < 0.05$. Overall, Bd prevalence generally declined with both conductivity and low cover habitat, with the interaction term indicating that when conductivity is held constant the odds of detection decline by ~8% for every 1% increase in low cover. When low cover is held constant, the odds of detection declined by ~17% for every 100 μS/cm increase in conductivity (Table 1).

3.2 | Bd intensity

The Bd intensity was comparable across sampling periods, with mean Bd concentration being 16,300 copies/L (skewness = 2.19, kurtosis = 3.91) and 19,700 copies/L (skewness = 1.00, kurtosis = −0.23) in May and July, respectively. However, we found that Bd intensity varied widely between positive sites, ranging from 558 copies/L to 200,926 copies/L. For Bd intensity, six of seven top models contained canopy cover as a predictor, with relatively little variability in coefficient estimates between candidates (Table 1). Our top models also included water temperature, dissolved oxygen, or one of three habitat variables (proportion of low cover, forest, or human impact within a 300-m radius). The model containing only the GIS-based estimation of shrub and grass cover (low cover 300 m) at the site was the sole top model to exclude canopy cover. Overall, we found that water bodies with greater canopy cover (i.e., less sun exposure) had lower Bd intensity, with a best-fit model coefficient (−0.52 ± 0.33) indicating that for each estimated 1% increase in canopy cover, Bd intensity declined by ~100 copies/L. The relationship between Bd copy number and canopy cover followed a negative association in both May and July (Figure 3). All top models had reasonable fit (conditional $R^2 > 0.64$, Table 1).

3.3 | Amphibian species richness

Based on amphibian surveys at 29 waterbodies, we estimated average amphibian community richness to be 7.0 ± 0.4 species per site. Green frogs (*L. clamitans*) were present at all sites, and spring peepers (*P. crucifer*), Northern leopard frogs (*L. pipiens*), and gray treefrogs (*Dryophytes versicolor*) were detected at >86% of sites. We detected Bd at 51.7% of these sites, and model selection revealed that species richness was not present in the best-fitting ($\Delta AIC_c < 2.0$) models for this subset. The best-fit model with species richness as a predictor had intermediate plausibility ($\Delta AIC_c = 2.12$), and also contained water temperature. The parameter coefficient for species richness (−0.2 ± 0.46) revealed a trend for the odds of detecting Bd to decrease by ~18.1% for each additional amphibian species detected.

3.4 | Intensive sampling

Waterbodies in the intensively sampled subset (n = 10) were all found to be positive for Bd in at least one of eight biweekly samples taken between May and August. A single waterbody was consistently positive for the entire length of the study, and on average we detected Bd during 56.3% ± 5.6% of sampling events at a given

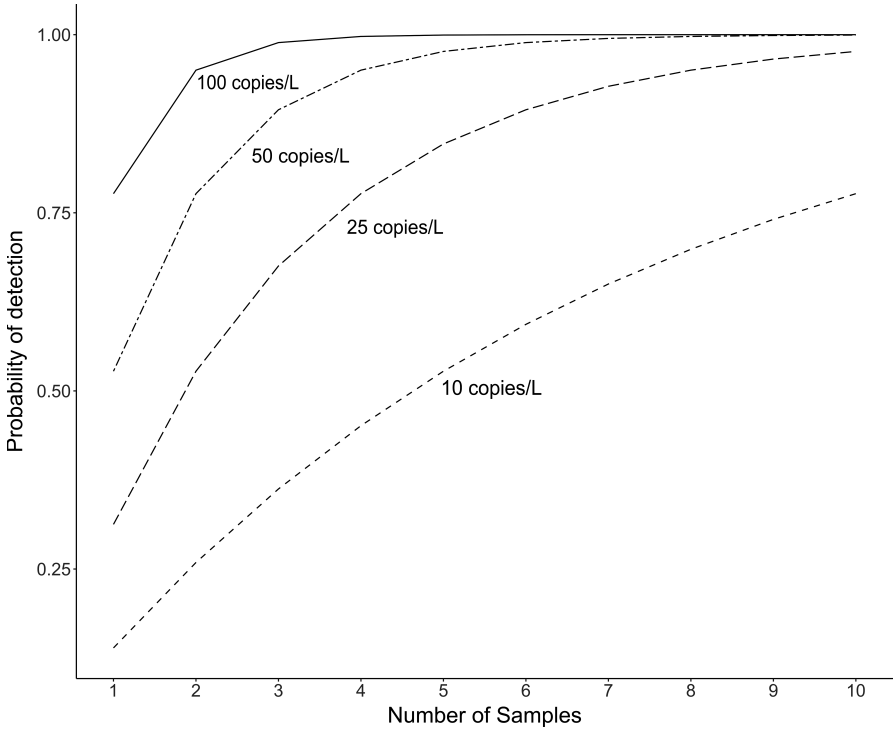

FIGURE 2 Likelihood of detecting *Batrachochytrium dendrobatidis* at a waterbody using our sampling protocol, calculated based on the method from Wilcox et al. (2018). Each line represents the likelihood of detecting Bd in a given number of samples, assuming a certain number of ITS1 copies per liter of water are present at the site, and that 250 ml of water is filtered in each sample (i.e., the average volume sampled per filter in this study)

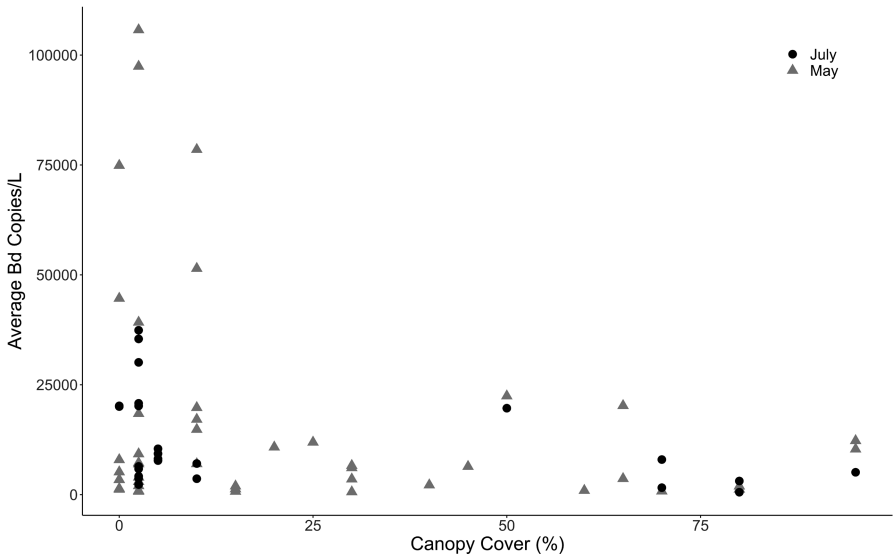

FIGURE 3 Estimated *Batrachochytrium dendrobatidis* intensity in waterbodies near Peterborough, Ontario, Canada, relative to estimated canopy cover at the site

waterbody. We again found that Bd copy number counts varied considerably between sampling events, with within-site change between consecutive positive Bd detections averaging 47.4% (n = 28; skewness = 3.23, kurtosis = 14.27). Bd prevalence tended to decline with time, but qualitatively there was low consistency in prevalence among consecutive sampling periods at a given site (Figure 4). On average, periods of positive detection in a given waterbody lasted ~6 weeks (n = 17; skewness = 0.66, kurtosis = 1.95), with detections ranging from 1500–270,000 copies/L (mean = 23,000 copies/L; skewness = 4.84, kurtosis = 23.32). While mean Bd intensity trended upwards over the course of the study in this subset, as did mean daytime air temperature (min = 6°C, max = 25°C), there was no statistically significant relationship between Bd intensity and time (p = 0.42).

4 | DISCUSSION

This study demonstrates that Bd in natural waterbodies can be detected and tracked through time using eDNA. Bd prevalence and intensity varied widely within and between waterbodies, as well as through time, with only 29% of these waterbodies testing positive in both May and July. Bd prevalence in a given waterbody may have been weakly influenced by the interaction between local water conductivity and low cover habitat, and was not strongly affected by amphibian species richness at the site. Bd intensity, however, was negatively related to canopy cover. Waterbodies sampled every second week exhibited high variability in Bd detection, with low consistency in Bd prevalence between consecutive sampling events. These results highlight that Bd monitoring in

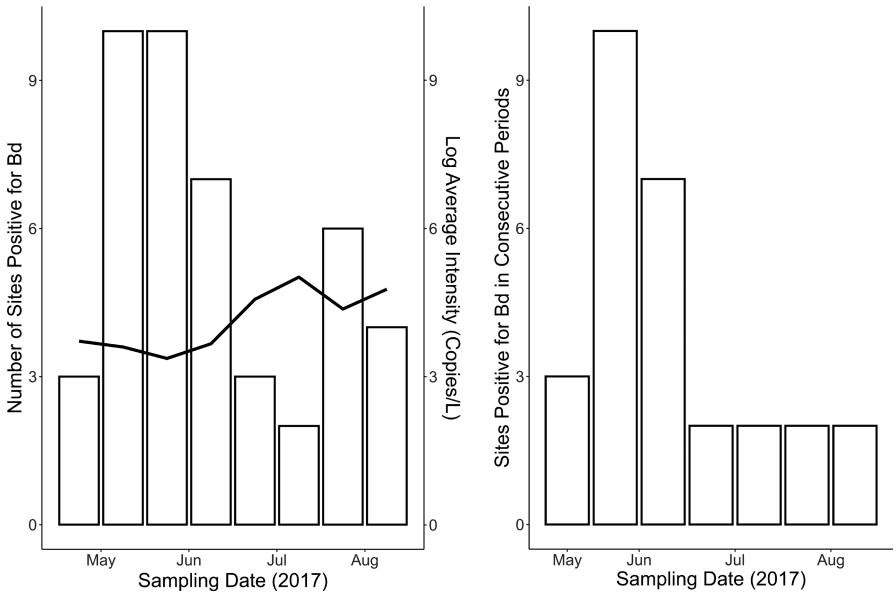

FIGURE 4 Change in *Batrachochytrium dendrobatidis* prevalence at 10 intensively monitored waterbodies near Peterborough, Ontario, Canada. The line on panel A represents log average intensity of Bd detection in that sampling period, while the columns represent the number of sites testing positive. There were two sampling events in each month at each site, once every second week. In panel B, the columns represent the percent of sites positive for Bd in consecutive periods of biweekly sampling

nature is possible via eDNA, but that zoospore detection is highly variable and dependent on sampling intensity, timing, and distribution. While eDNA shows promise for tracking Bd zoospores in waterbodies, further research into Bd–environment dynamics and the dynamics of eDNA detection is needed to optimize sampling design and monitoring protocols to effectively track this pathogen in its free-living stage.

4.1 | Bd variability through space and time

High levels of variability in Bd counts were seen not only between waterbodies tested during the same sampling period, but also between samples taken from the same waterbody at the same time. Bd and other aquatic pathogens are typically dispersed unevenly across waterbodies due to heterogenous distribution of pathogen hosts and vectors in response to prevailing winds and flow patterns (Albery et al., 2022; Chestnut et al., 2014; Walker et al., 2007). Notably, this pattern can be especially pronounced for eDNA in lentic waterbodies (Davison et al., 2017; Eichmiller et al., 2014), as was the case for the majority of the sampled sites. As such, the reported variability illustrates the importance of within-site sampling location and sampling intensity on Bd detection and counts. Within-site variability may also be associated with zoospore chemotaxis in response to spatial dispersion of hosts in a given wetland (Moss et al., 2008; Piotrowski et al., 2004), highlighting the need to also understand local amphibian community structure and distribution when designing Bd survey protocols using eDNA.

The Bd prevalence varied markedly through time and this variability was detected across both May–July and semi-weekly sampling regimes. Temporal variability in Bd detection could be related to a variety of factors, including changes in water temperature and other environmental variables or seasonal differences in habitat use among amphibians (Ruggeri et al., 2018; Voordouw et al., 2010). Notably, high temporal variability in Bd detection between consecutive semi-weekly samples was especially surprising, implying that Bd occurrence (or detection) can be highly stochastic even with fairly intensive within-site sampling. Further, because prevalence and intensity of Bd in waterbodies during the course of the summer follow qualitatively different patterns, sites may experience different processes affecting zoospore detection/occurrence versus abundance. This has important implications for Bd surveillance because it implies that a single sampling event, even if adequately replicated within the wetland, may not yield a representative measure of Bd in the environment. It follows that in general multiple sampling events likely will be needed for robust assessment, and thus designing an appropriate sampling regime for Bd monitoring will require better knowledge of the determinants of prevalence and intensity, including on a regional or local scale. Other eDNA-based surveillance case studies have faced similar challenges (Barnes et al., 2020; Dunn et al., 2017; Eichmiller et al., 2014), and the logical conclusion is that extensive and robust testing of factors affecting detectability of eDNA in aquatic and terrestrial biomes is still broadly needed to fully optimize this technology (Barnes & Turner, 2016; Raemy & Ursenbacher, 2018; Sepulveda et al., 2020). In particular, our results suggest that while our overall sampling regime was likely sufficiently robust to detect Bd zoospores under the conditions present in southern Ontario waterbodies, it is essential to design pathogen monitoring programs to account for local conditions, both biotic and abiotic, that may impact detection probability differently between sites and/or seasons. For example, at sites with large amounts of suspended matter in the water column where filters clog more quickly, it may be necessary to use additional filters to maintain a standard sampling volume, rather than limit the sampling volume to what can easily pass through the standard number of filters. Similar adjustments may be possible and relevant for most steps in the eDNA sampling and analysis process, meaning that site-specific refinement of methods should be prioritized.

4.2 | Environmental factors

While Bd prevalence was associated with the interaction between water conductivity and low cover habitat within 300 m, this relationship was weak and perhaps an artifact of our study design. More importantly, canopy cover was clearly a strong predictor of Bd intensity. Canopy cover affects the amount of ultra-violet radiation (UV-R) reaching the water, which can have a direct, negative effect on Bd zoospores (Hite et al., 2016). However, the relationship between Bd and UV-R is complex and we would be remiss in omitting that UV-R may also have positive effects on Bd infection prevalence in amphibian hosts, through a similar negative impact on microfauna known to graze on free-living Bd zoospores (Hamilton et al., 2012; Schmeller et al., 2014; Searle et al., 2013). Similarly, canopy cover and UV-R in a waterbody can also affect amphibian community composition, with some species preferentially occupying habitat or breeding sites based on site morphology (Schiesari, 2006; Stevens et al., 2006), thereby further influencing the intensity of Bd. The link between UV-R, canopy cover, and Bd in amphibians clearly requires more investigation before these interactions can be properly integrated into a pathogen surveillance program. Likewise, the unclear role of amphibian species on Bd in this study highlights that our ability to track host communities for disease susceptibility remains somewhat uncertain. Population density and amphibian community composition can both be associated with patterns of Bd infection (Adams et al., 2017; Briggs et al., 2010; Horner et al., 2017). Higher population densities lead to quicker infective spread due to greater interaction between individuals, while species may each differ in their susceptibility and ability to transmit the pathogen (Borzée et al., 2017; DiRenzo et al., 2014; Maguire et al., 2016). Our results reinforce that this relationship may not necessarily extend to the detection of Bd in the waterbody. However, we note that, for example, green frogs (*L. clamitans*) were detected at all sites surveyed, and are known to have over-wintering larvae that may act as Bd reservoirs (see Hite et al., 2016). This illustrates that rigorous assessment of the link between free-living Bd zoospore counts and amphibian host communities is especially challenging, and requires efforts beyond the purview of this study.

We conclude that eDNA holds significant promise for monitoring free-living stages of Bd in natural waterbodies, and that an eDNA-based monitoring program could serve as an early warning system for changing disease dynamics in variable environmental conditions. However, we stress that the design of monitoring programs for this, and likely other pathogens will require a strong grasp of both abiotic and biotic conditions, and the factors affecting detection probability in monitored waterbodies. In particular, we highlight our observed high spatio-temporal variability in Bd prevalence and intensity means that eDNA-based surveys for this pathogen will require robust within-site (i.e., adequate spatial spread and high sampling intensity) and temporal (i.e., frequent sampling) sampling design to ensure reliable pathogen detection.

ACKNOWLEDGMENTS

We thank Samantha Grant and Audrey Wilson for their invaluable lab expertise with qPCR analysis, and C-M. Scott, B. Breton, M. Boudreau, and A. Eads for assistance with field and lab work. This work was funded by a Strategic Project grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada (Grant 463602-14).

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

MC – Lead: conception and design of study, collection and analysis of data, writing of manuscript; STV – Supporting: collection and analysis of data, editing of manuscript; CCW – Supporting: design of study, editing of manuscript; Equal: obtainment of funding; CJK – Supporting: editing of manuscript; Equal: obtainment of funding; DL – Supporting: design of study, editing of manuscript; Equal: obtainment of funding; MJHW – Supporting: collection of data (Lead: species richness data), editing of manuscript; LB – Supporting: design of study, analysis of data, editing of manuscript; DLM – Lead: supervision; Supporting: design of study, editing of manuscript; Equal: obtainment of funding.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available from the Trent University Scholar's Portal Dataverse, at https://doi.org/10.5683/SP3/SEPDLR.

ORCID

Megan Congram https://orcid.org/0000-0002-7469-5939
Sibelle Torres Vilaça https://orcid.org/0000-0002-6887-4703
Chris C. Wilson https://orcid.org/0000-0002-9528-0652
David Lesbarrères https://orcid.org/0000-0002-5505-1935
Lynne Beaty https://orcid.org/0000-0003-0778-1922

REFERENCES

Adams, A. J., Kupferberg, S. J., Wilber, M. Q., Pessier, A. P., Grefsrud, M., Bobzien, S., Vredenburg, V. T., & Briggs, C. J. (2017). Extreme drought, host density, sex, and bullfrogs influence fungal pathogen infection in a declining lotic amphibian. *Ecosphere*, *8*(3), e01740. https://doi.org/10.1002/ecs2.1740

Albery, G. F., Sweeny, A. R., Becker, D. J., & Bansal, S. (2022). Fine-scale spatial patterns of wildlife disease are common and understudied. *Functional Ecology*, *36*(1), 214–225. https://doi.org/10.1111/1365-2435.13942

Anttila, J., Kaitala, V., Laakso, J., & Ruokolainen, L. (2015). Environmental variation generates environmental opportunist pathogen outbreaks. *PLoS One*, *10*(12), e0145511. https://doi.org/10.1371/journal.pone.0145511

Barnes, M. A., Brown, A. D., Daum, M. N., de la Garza, K. A., Driskill, J., Garrett, K., Goldstein, M. S., Luk, A., Maguire, J. I., Moke, R., Ostermaier, E. M., Sanders, Y. M., Sandhu, T., Stith, A., & Suresh, V. V. (2020). Detection of the amphibian pathogens chytrid fungus (Batrachochytrium dendrobatidis) and Ranavirus in West Texas,

USA, Using Environmental DNA. *Journal of Wildlife Diseases*, *56*(3), 702–706. https://doi.org/10.7589/2019-08-212

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, *17*(1), 1–17. https://doi.org/10.1007/s10592-015-0775-4

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effect models using lme4. *Journal of Statistical Software*, *67*(1), 1–48, https://doi.org/10.18637/jss.v067.i01

Battaglin, W. A., Smalling, K. L., Anderson, C., Calhoun, D., Chestnut, T., & Muths, E. (2016). Potential interactions among disease, pesticides, water quality and adjacent land cover in amphibian habitats in the United States. *Science of the Total Environment*, *566-567*, 320–332. https://doi.org/10.1016/j.scitotenv.2016.05.062

Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M. A., Hyatt, A. D., McDonald, K. R., Hines, H. B., Lips, K. R., Marantelli, G., & Parkes, H. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences*, *95*(15), 9031–9036. https://doi.org/10.1073/pnas.95.15.9031

Bielby, J., Fisher, M. C., Clare, F. C., Rosa, G. M., & Garner, T. W. J. (2015). Host species vary in infection probability, sub-lethal effects, and costs of immune response when exposed to an amphibian parasite. *Scientific Reports*, *5*, 10828. https://doi.org/10.1038/srep10828

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R. A., Foster, J., Wilkinson, J. W., Arnell, A., Brotherton, P., Williams, P., & Dunn, F. (2015). Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation*, *183*, 19–28. https://doi.org/10.1016/j.biocon.2014.11.029

Bird Studies Canada (2009). *Marsh monitoring program participant's handbook for surveying amphibians*. Bird Studies Canada & Environment Canada.

Borzée, A., Kosch, T. A., Kim, M., & Jang, Y. (2017). Introduced bullfrogs are associated with increased *Batrachochytrium dendrobatidis* prevalence and reduced occurrence of Korean treefrogs. *PLoS One*, *12*(5), e0177860. https://doi.org/10.1371/journal.pone.0177860

Bower, D. S., Lips, K. R., Schwarzkopf, L., Georges, A., & Clulow, S. (2017). Amphibians on the brink. *Science*, *357*(6350), 454–455. https://doi.org/10.1126/science.aao0500

Boyle, D. G., Boyle, D. B., Olsen, V., Morgan, J. A. T., & Hyatt, A. D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms*, *60*(2), 141–148. https://doi.org/10.3354/dao060141

Briggs, C. J., Knapp, R. A., & Vredenburg, V. T. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences*, *107*(21), 9695–9700. https://doi.org/10.1073/pnas.0912886107

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378–400. https://doi.org/10.3929/ethz-b-000240890

Burnham, K. P., Anderson, D. R., & Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: Some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, *65*, 23–35. https://doi.org/10.1007/s00265-010-1029-6

Burrowes, P. A., & De la Riva, I. (2017). Detection of the amphibian chytrid fungus Batrachochytrium dendrobatidis in museum specimens of Andean aquatic birds: Implications for pathogen dispersal. *Journal of Wildlife Diseases*, *53*(2), 349–355. https://doi.org/10.7589/2016-04-074

Cameron, A., & Trivedi, P. (1998). *Regression analysis of count data*. Cambridge University Press.

Caraco, T., & Wang, I.-N. (2008). Free-living pathogens: Life-history constraints and strain competition. *Journal of Theoretical Biology*, *250*(3), 569–579. https://doi.org/10.1016/j.jtbi.2007.10.029

CEC (2015). *North American land change monitoring system*. Available at: http://www.cec.org/north-american-land-change-monitoring-system/ Accessed 25 April 2021

Chestnut, T. (2015). *Emerging infectious disease in lentic environments: The ecology and biogeography of the amphibian chytrid fungus* Batrachochytrium dendrobatidis, *with perspectives on water quality, limnology, and chemical contaminants*. Oregon State University.

Chestnut, T., Anderson, C., Popa, R., Blaustein, A. R., Voytek, M., Olson, D. H., & Kirshtein, J. (2014). Heterogeneous occupancy and density estimates of the pathogenic fungus *Batrachochytrium dendrobatidis* in waters of North America. *PLoS One*, *9*(9), e106790. https://doi.org/10.1371/journal.pone.0106790

Crins, W. J., Gray, P. A., Uhlig, P. W. C., & Wester, M. C. (2009). *The Ecosystems of Ontario, Part 1: Ecozones and Ecoregions*. Ontario Ministry of Natural Resources. Report number: SIB TER IMA TR-01

Crisafulli, C. M. (1997). A habitat-based method for monitoring pond-breeding amphibians. In D. H. Olson, W. P. Leonard, & R. B. Bury (Eds.), *Sampling amphibians in lentic habitats: methods and approaches for the Pacific Northwest (Northwest Fauna 4)* (pp. 83–112). Society for Northwestern Vertebrate Biology.

Dang, T. D., Searle, C. L., & Blaustein, A. R. (2017). Virulence variation among strains of the emerging infectious fungus Batrachochytrium dendrobatidis (Bd) in multiple amphibian host species. *Diseases of Aquatic Organisms*, *124*(3), 233–239. https://doi.org/10.3354/dao03125

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica*, *78*(2), 103–116. https://doi.org/10.1016/S0001-706X(00)00179-0

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2003). Infectious disease and amphibian population declines. *Diversity and Distributions*, *9*(2), 141–150. https://doi.org/10.1046/j.1472-4642.2003.00016.x

Davison, P. I., Copp, G. H., Créach, V., Vilizzi, L., & Britton, J. R. (2017). Application of environmental DNA analysis to inform invasive fish eradication operations. *The Science of Nature*, *104*(3-4), 35. https://doi.org/10.1007/s00114-017-1453-9

Day, T. A., & Neale, P. J. (2002). Effects of UV-B radiation on terrestrial and aquatic primary producers. *Annual Review of Ecology and Systematics*, *33*, 371–396. https://doi.org/10.1146/annurev.ecolsys.33.010802.150434

DiRenzo, G. V., Langhammer, P. F., Zamudio, K. R., & Lips, K. R. (2014). Fungal infection intensity and zoospore output of *Atelopus zeteki*, a potential acute chytrid supershedder. *PLoS One*, *9*(3), e93356. https://doi.org/10.1371/journal.pone.0093356

Duffus, A. L. J., & Andrews, A. M. (2013). Phylogenetic analysis of a frog virus 3–like ranavirus found at a site with recurrent mortality and morbidity events in southeastern Ontario, Canada: Partial major capsid protein sequence alone is not sufficient for fine-scale differentiation. *Journal of Wildlife Diseases*, *49*(2), 464–467. https://doi.org/10.7589/2012-05-147

Dunn, N., Priestley, V., Herraiz, A., Arnold, R., & Savolainen, V. (2017). Behavior and season affect crayfish detection and density inference using environmental DNA. *Ecology and Evolution*, *7*(19), 7777–7785. https://doi.org/10.1002/ece3.3316

Eichmiller, J. J., Bajer, P. G., & Sorensen, P. W. (2014). The relationship between the distribution of common carp and their environmental DNA in a small lake. *PLoS One*, *9*(11), e112611. https://doi.org/10.1371/journal.pone.0112611

Fonner, C., Patel, S., Boord, S., Venesky, M., & Woodley, S. (2017). Effects of corticosterone on infection and disease in salamanders

exposed to the amphibian fungal pathogen *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, *123*(2), 159–171. https://doi.org/10.3354/dao03089

Gaertner, J. P., Gaston, M. A., Spontak, D., Forstner, M. R. J., & Hahn, D. (2009). Seasonal variation in the detection of *Batrachochytrium dendrobatidis* in a Texas population of Blanchard's Cricket Frog (*Acris crepitans blanchardi*). *Herpetological Review*, *40*(2), 184–187.

Geiger, C. C., Bregnard, C., Maluenda, E., Voordouw, M. J., & Schmidt, B. R. (2017). Antifungal treatment of wild amphibian populations caused a transient reduction in the prevalence of the fungal pathogen, Batrachochytrium dendrobatidis. *Scientific Reports*, *7*, 5956. https://doi.org/10.1038/s41598-017-05798-9

Gleason, F. H., Kagami, M., Lefevre, E., & Sime-Ngando, T. (2008). The ecology of chytrids in aquatic ecosystems: Roles in food web dynamics. *Fungal Biology Reviews*, *22*(1), 17–25. https://doi.org/10.1016/j.fbr.2008.02.001

Goldberg, C. S., Pilliod, D. S., Arkle, R. S., & Waits, L. P. (2011). Molecular detection of vertebrates in stream water: A demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLoS One*, *6*(7), e22746. https://doi.org/10.1371/journal.pone.0022746

Goldberg, C. S., Sepulveda, A., Ray, A., Baumgardt, J., & Waits, L. P. (2013). Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshwater Science*, *32*(3), 792–800. https://doi.org/10.1899/13-046.1

Google (2015). *Google Earth Pro 7.3.3.7786*.

Greer, A. L., Berrill, M., & Wilson, P. J. (2005). Five amphibian mortality events associated with ranavirus infection in south central Ontario, Canada. *Diseases of Aquatic Organisms*, *67*, 9–14. https://doi.org/10.3354/dao067009

Häder, D.-P., Helbling, E., Williamson, C., & Worrest, R. (2011). Effects of UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical and Photobiological Sciences*, *10*(2), 242–260. https://doi.org/10.1039/c0pp90040k

Hamilton, P. T., Richardson, J. M. L., & Anholt, B. R. (2012). *Daphnia* in tadpole mesocosms: Trophic links and interactions with *Batrachochytrium dendrobatidis*. *Freshwater Biology*, *57*(4), 676–683. https://doi.org/10.1111/j.1365-2427.2011.02731.x

Heard, M. J., Smith, K. F., Ripp, K. J., Berger, M., Chen, J., Dittmeier, J., Goter, M., Mcgarvey, S. T., & Ryan, E. (2013). The threat of disease increases as species move towards extinction. *Conservation Biology*, *27*(6), 1378–1388. https://doi.org/10.1111/cobi.12143

Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. (2017). Methods to maximise recovery of environmental DNA from water samples. *PLoS One*, *12*(6), e0179251. https://doi.org/10.1371/journal.pone.0179251

Hite, J. L., Bosch, J., Fernández-Beaskoetxea, S., Medina, D., & Hall, S. R. (2016). Joint effects of habitat, zooplankton, host stage structure and diversity on amphibian chytrid. *Proceedings of the Royal Society B*, *283*(1835), 20160832. https://doi.org/10.1098/rspb.2016.0832

Horner, A. A., Hoffman, E. A., Tye, M. R., Hether, T. D., & Savage, A. E. (2017). Cryptic chytridiomycosis linked to climate and genetic variation in amphibian populations of the southeastern United States. *PLoS One*, *12*(4), e0175843. https://doi.org/10.1371/journal.pone.0175843

Hunter, M. E., Dorazio, R. M., Butterfield, J. S. S., Meigs-Friend, G., Nico, L. G., & Ferrante, J. A. (2017). Detection limits of quantitative and digital PCR assays and their influence in presence–absence surveys of environmental DNA. *Molecular Ecology Resources*, *17*(2), 221–229. https://doi.org/10.1111/1755-0998.12619

Huver, J. R., Koprivnikar, J., Johnson, P. T. J., & Whyard, S. (2015). Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecological Applications*, *25*(4), 991–1002. https://doi.org/10.1890/14-1530.1

Johnson, M. L., & Speare, R. (2005). Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. *Diseases of Aquatic Organisms*, *65*, 181–186. https://doi.org/10.3354/dao065181

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, *451*, 990–993. https://doi.org/10.1038/nature06536

Kirshtein, J. D., Anderson, C. W., Wood, J. S., Longcore, J. E., & Voytek, M. A. (2007). Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. *Diseases of Aquatic Organisms*, *77*, 11–15. https://doi.org/10.3354/dao01831

Korfel, C. A. (2012). *Distribution and environmental correlates between amphibians and the fungal pathogen*, Batrachochytrium dendrobatidis. Ohio State University.

Lemmon, P. E. (1956). A spherical densiometer for estimating forest overstory density. *Forest Science*, *2*(4), 314–320. https://doi.org/10.1093/forestscience/2.4.314

Letcher, P. M., Powell, M. J., Churchill, P. F., & Chambers, J. G. (2006). Ultrastructural and molecular phylogenetic delineation of a new order, the Rhizophydiales (Chytridiomycota). *Mycological Research*, *110*(8), 898–915. https://doi.org/10.1016/j.mycres.2006.06.011

Lewis, E. L. (1980). The practical salinity scale 1978 and its antecedents. *IEEE Journal of Oceanic Engineering*, *5*(1), 3–8. https://doi.org/10.1109/JOE.1980.1145448

Longcore, J. E., Pessier, A. P., & Nichols, D. K. (1999). *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia*, *91*(2), 219–227. https://doi.org/10.1080/00275514.1999.12061011

Longo, A. V., Rodriguez, D., da Silva Leite, D., Toledo, L. F., Mendoza Almeralla, C., Burrowes, P. A., & Zamudio, K. R. (2013). ITS1 copy number varies among *Batrachochytrium dendrobatidis* strains: Implications for qPCR estimates of infection intensity from field-collected amphibian skin swabs. *PLoS One*, *8*(3), e59499. https://doi.org/10.1371/journal.pone.0059499

Maguire, C., DiRenzo, G. V., Tunstall, T. S., Muletz, C. R., Zamudio, K. R., & Lips, K. R. (2016). Dead or alive? Viability of chytrid zoospores shed from live amphibian hosts. *Diseases of Aquatic Organisms*, *119*, 179–187. https://doi.org/10.3354/dao02991

Martin, T. G., Wintle, B. A., Rhodes, J. R., Kuhnert, P. M., Field, S. A., Low-Choy, S. J., Tyre, A. J., & Possingham, H. P. (2005). Zero tolerance ecology: Improving ecological inference by modelling the source of zero observations. *Ecology Letters*, *8*(11), 1235–1246. https://doi.org/10.1111/j.1461-0248.2005.00826.x

Martínez-Solano, I., Bosch, J., & García-París, M. (2003). Demographic trends and community stability in a montane amphibian assemblage. *Conservation Biology*, *17*(1), 238–244. https://doi.org/10.1046/j.1523-1739.2003.01096.x

McFadden, D. (1978). Quantitative methods for analyzing travel behaviour of individuals: Some recent developments. In D. Hensher, & P. Stopher (Eds.), *Behavioural travel modelling* (pp. 279–318). Croom Helm.

Mills, P. B. (2016). *Metamorphosis: Ontario's amphibians at all stages of development*. Peter B. Mills (privately published): Printed and bound by SLG Group, Brampton.

Moss, A. S., Reddy, N. S., Dortaj, I. M., & San Francisco, M. J. (2008). Chemotaxis of the amphibian pathogen *Batrachochytrium dendrobatidis* and its response to a variety of attractants. *Mycologia*, *100*(1), 1–5. https://doi.org/10.1080/15572536.2008.11832493

Murray, D. L., Bastille-Rousseau, G., Beaty, L. E., Hornseth, M. L., Row, J. R., & Thornton, D. H. (2020). From research hypothesis to model selection: A strategy for robust inference in population ecology. In D. L. Murray, & B. K. Sandercock (Eds.), *Population ecology in practice* (pp. 17–46). Wiley-Blackwell.

Muths, E., Pilliod, D. S., & Livo, L. J. (2008). Distribution and environmental limitations of an amphibian pathogen in the Rocky Mountains, USA. *Biological Conservation*, *141*(6), 1484–1492. https://doi.org/10.1016/j.biocon.2008.03.011

NEPARC (2014). *Disinfection of Field Equipment to Minimize Risk of Spread of Chytridiomycosis and Ranavirus*. Northeast Partners in Amphibian and Reptile Conservation.

Nichols, J. D., Hollmen, T. E., & Grand, J. B. (2017). Monitoring for the management of disease risk in animal translocation programmes. *EcoHealth*, *14*, S156–S166. https://doi.org/10.1007/s10393-015-1094-4

Olson, D. H., Aanensen, D. M., Ronnenberg, K. L., Powell, C. I., Walker, S. F., Bielby, J., Garner, T. W. J., Weaver, G., & Fisher, M. C. (2013). Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. *PLoS One*, *8*(2), e56802. https://doi.org/10.1371/journal.pone.0056802

Osnas, E. E., Heisey, D. M., Rolley, R. E., & Samuel, M. D. (2009). Spatial and temporal patterns of chronic wasting disease: Fine-scale mapping of a wildlife epidemic in Wisconsin. *Ecological Applications*, *19*(5), 1311–1322. https://doi.org/10.1890/08-0578.1

Peters, L., Spatharis, S., Dario, M. A., Dwyer, T., Roca, I. J. T., Kintner, A., Kanstad-Hanssen, Ø., Llewellyn, M. S., & Praebel, K. (2018). Environmental DNA: A new low-cost monitoring tool for pathogens in salmonid aquaculture. *Frontiers in Microbiology*, *9*, 3009. https://doi.org/10.3389/fmicb.2018.03009

Piotrowski, J. S., Annis, S. L., & Longcore, J. E. (2004). Physiology of batrachochytrium dendrobatidis, a chytrid pathogen of amphibians. *Mycologia*, *96*(1), 9–15. https://doi.org/10.2307/3761981

Powell, R., Conant, R., & Collins, J. T. (2016). *Peterson field guide to reptiles and amphibians of eastern and central North America* (4th ed.). Houghton Mifflin Harcourt.

Pullen, K. D., Best, A. M., & Ware, J. L. (2010). Amphibian pathogen *Batrachochytrium dendrobatidis* prevalence is correlated with season and not urbanization in central Virginia. *Diseases of Aquatic Organisms*, *91*, 9–16. https://doi.org/10.3354/dao02249

R Core Team (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Raemy, M., & Ursenbacher, S. (2018). Detection of the European pond turtle (*Emys orbicularis*) by environmental DNA: Is eDNA adequate for reptiles? *Amphibia Reptilia*, *39*(2), 135–143. https://doi.org/10.1163/15685381-17000025

Rebollar, E. A., Woodhams, D. C., LaBumbard, B., Kielgast, J., & Harris, R. N. (2017). Prevalence and pathogen load estimates for the fungus *Batrachochytrium dendrobatidis* are impacted by ITS DNA copy number variation. *Diseases of Aquatic Organisms*, *123*, 213–226. https://doi.org/10.3354/dao03097

Retallick, R. W., & Miera, V. (2007). Strain differences in the amphibian chytrid *Batrachochytrium dendrobatidis* and non-permanent, sublethal effects of infection. *Diseases of Aquatic Organisms*, *75*, 201–207. https://doi.org/10.3354/dao075201

Ruggeri, J., de Carvalho-e-Silva, S. P., James, T. Y., & Toledo, L. F. (2018). Amphibian chytrid infection is influenced by rainfall seasonality and water availability. *Diseases of Aquatic Organisms*, *127*, 107–115. https://doi.org/10.3354/dao03191

Scheele, B. C., Hunter, D. A., Grogan, L. F., Berger, L., Kolby, J. E., Mcfadden, M. S., Marantelli, G., Skerratt, L. F., & Driscoll, D. A. (2014). Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. *Conservation Biology*, *28*(5), 1195–1205. https://doi.org/10.1111/cobi.12322

Schiesari, L. (2006). Pond canopy cover: A resource gradient for anuran larvae. *Freshwater Biology*, *51*(3), 412–423. https://doi.org/10.1111/j.1365-2427.2005.01497.x

Schmeller, D., Blooi, M., Martel, A. N., Garner, T. J., Fisher, M., Azemar, F., Clare, F., Leclerc, C., Jäger, L., Guevara-Nieto, M., Loyau, A., & Pasmans, F. (2014). Microscopic aquatic predators strongly affect infection dynamics of a globally emerged pathogen. *Current Biology*, *24*(2), 176–180. https://doi.org/10.1016/j.cub.2013.11.032

Searle, C. L., Mendelson, J. R. III, Green, L. E., & Duffy, M. A. (2013). *Daphnia* predation on the amphibian chytrid fungus and its impacts on disease risk in tadpoles. *Ecology and Evolution*, *3*(12), 4129–4138. https://doi.org/10.1002/ece3.777

Sepulveda, A. J., Hutchins, P. R., Jackson, C., Ostberg, C., Laramie, M. B., Amberg, J., Counihan, T., Hoegh, A., & Pilliod, D. S. (2020). A round-robin evaluation of the repeatability and reproducibility of environmental DNA assays for dreissenid mussels. *Environmental DNA*, *2*, 446–459. https://doi.org/10.1002/edn3.68

Shaffer, H. B., Alford, R. A., Woodward, B. D., & Richards, S. J. (1994). Quantitative sampling of amphibian larvae. In W. R. Heyer, M. A. Donelly, R. W. McDiarmid, L. C. Hayek, & M. S. Foster (Eds.). *Measuring and monitoring biological diversity: Standard methods for amphibians* (pp. 130–141). Smithsonian Institution Press.

Skerratt, L. F., Berger, L., Hines, H. B., McDonald, K. R., Mendez, D., & Speare, R. (2008). Survey protocol for detecting chytridiomycosis in all Australian frog populations. *Diseases of Aquatic Organisms*, *80*, 85–94. https://doi.org/10.3354/dao01923

Skerratt, L. F., Berger, L., Speare, R., Cashins, S., McDonald, K. R., Phillott, A. D., Hines, H. B., & Kenyon, N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth*, *4*, 125–134. https://doi.org/10.1007/s10393-007-0093-5

Spens, J., Evans, A. R., Halfmaerten, D., Knudsen, S. W., Sengupta, M. E., Mak, S. S. T., Sigsgaard, E. E., & Hellström, M. (2017). Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: Advantage of enclosed filter. *Methods in Ecology and Evolution*, *8*(5), 635–645. https://doi.org/10.1111/2041-210X.12683

St-Amour, V., Wong, W. M., Garner, T. W. J., & Lesbarrères, D. (2008). Anthropogenic influence on prevalence of 2 amphibian pathogens. *Emerging Infectious Diseases*, *14*(7), 1175–1176. https://doi.org/10.3201/eid1407.070602

Stevens, C. E., Paszkowski, C. A., & Scrimgeour, G. J. (2006). Older is better: Beaver ponds on boreal streams as breeding habitat for the wood frog. *Journal of Wildlife Management*, *70*(5), 1360–1371. https://doi.org/10.2193/0022-541X(2006)70%5B1360:OIBBPO%5D2.0.CO;2

Stockwell, M. P., Clulow, J., & Mahony, M. J. (2012). Sodium chloride inhibits the growth and infective capacity of the amphibian chytrid fungus and increases host survival rates. *PLoS One*, *7*(5), e36942. https://doi.org/10.1371/journal.pone.0036942

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS One*, *7*(8), e41732. https://doi.org/10.1371/journal.pone.0041732

Thornton, D. H., Wirsing, A. J., Roth, J. D., & Murray, D. L. (2013). Habitat quality and population density drive occupancy dynamics of snowshoe hare in variegated landscapes. *Ecography*, *36*, 610–621. https://doi.org/10.1111/j.1600-0587.2012.07737.x

Van Rooij, P., Martel, A., Haesebrouck, F., & Pasmans, F. (2015). Amphibian chytridiomycosis: A review with focus on fungus-host interactions. *Veterinary Research*, *46*, 137. https://doi.org/10.1186/s13567-015-0266-0

Vilaça, S. T., Grant, S. A., Beaty, L., Brunetti, C. R., Congram, M., Murray, D. L., Wilson, C. C., & Kyle, C. J. (2020). Detection of spatiotemporal variation in ranavirus distribution using eDNA. *Environmental DNA*, *2*, 210–220. https://doi.org/10.1002/edn3.59

Voordouw, M. J., Adama, D., Houston, B., Govindarajulu, P., & Robinson, J. (2010). Prevalence of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in an endangered population of northern leopard frogs, Rana pipiens. *BMC Ecology*, *10*, 6. https://doi.org/10.1186/1472-6785-10-6

Voyles, J., Johnson, L. R., Rohr, J., Kelly, R., Barron, C., Miller, D., Minster, J., & Rosenblum, E. B. (2017). Diversity in growth patterns among strains of the lethal fungal pathogen *Batrachochytrium dendrobatidis* across extended thermal optima. *Oecologia*, *184*, 363–373. https://doi.org/10.1007/s00442-017-3866-8

Walker, S. F., Baldi Salas, M., Jenkins, D., Garner, T., Cunningham, A. A., Hyatt, A. D., Bosch, J., & Fisher, M. C. (2007). Environmental detection of *Batrachochytrium dendrobatidis* in a temperate climate. *Diseases of Aquatic Organisms*, *77*, 105–112. https://doi.org/10.3354/dao01850

Werner, E. E., Yurewicz, K. L., Skelly, D. K., & Relyea, R. A. (2007). Turnover in an amphibian metacommunity: The role of local and regional factors. *Oikos*, *116*(10), 1713–1725. https://doi.org/10.1111/j.0030-1299.2007.16039.x

Wikston, M. (2021). *Comparative efficacy of eDNA and conventional methods for monitoring wetland anuran communities*. Trent University.

Wilcox, T. M., Karim, K. J., Young, M. K., McKelvey, K. S., Franklin, T. W., & Schwartz, M. K. (2018). Comment: The importance of sound methodology in environmental DNA sampling. *North American Journal of Fisheries Management*, *38*(3), 592–596. https://doi.org/10.1002/nafm.10055

Wilson, C. C., Wozney, K. M., & Smith, C. M. (2016). Recognizing false positives: Synthetic oligonucleotide controls for environmental DNA surveillance. *Methods in Ecology and Evolution*, *7*, 23–29. https://doi.org/10.1111/2041-210X.12452

How to cite this article: Congram, M., Torres Vilaça, S., Wilson, C. C., Kyle, C. J., Lesbarrères, D., Wikston, M. J. H., Beaty, L., & Murray, D. L. (2022). Tracking the prevalence of a fungal pathogen, *Batrachochytrium dendrobatidis* (chytrid fungus), using environmental DNA. *Environmental DNA*, 4, 687–699. https://doi.org/10.1002/edn3.283