




Distribution of the boreal chorus frog (*Pseudacris maculata*) in an urban environment using environmental DNA

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Abstract

The boreal chorus frog (*Pseudacris maculata*) is at risk of extinction in parts of its range in Canada. Our objectives were to quantify the influence of local and landscape characteristics on the occurrence of the species in wetlands in southern Québec. We hypothesized that site occupancy depends on local characteristics and landscape characteristics contributing to site connectivity. We developed an environmental DNA (eDNA) method to detect the species and compared the detection probability of this method to traditional call surveys. We collected water samples from a total of 180 sites (90 in 2017, 110 in 2018), whereas we surveyed a subset of 63 sites using both eDNA and call surveys in 2018. Site occupancy varied across years, but was higher in sites where the species had been previously detected during the last 12 years by other studies. Site occupancy did not vary with other local and landscape characteristics, in part due to an apparent decrease in the number of sites occupied by the species since the last 12 years. Detection probability via eDNA (0.81; 95% CI: [0.31; 0.98]) did not differ from that of call surveys (0.62; 95% CI: [0.25; 0.89]). To identify the optimal sampling period for the boreal chorus frog, future studies should estimate the detection probability of eDNA during the breeding season and the larval development period of the species.

KEYWORDS

amphibian, detection probability, landscape, occupancy model, *Pseudacris maculata*, urbanization

1 | INTRODUCTION

The global human population is increasing and is projected to reach 9.2 billion by 2050 (Bongaarts, 2009; United Nations, 2019). This population growth is associated with agricultural and urban development, leading to habitat loss and fragmentation (Foley et al., 2005). Habitat loss and fragmentation are considered major threats to global biodiversity, impacting 86% of threatened mammals, 88%

of threatened amphibians, and 86% of threatened birds (Foley et al., 2005; Sala et al., 2000). Consequently, many scientists suggest that human activities are now responsible for the sixth great mass extinction, especially since current extinction rates of vertebrates are estimated to be 100–1,000 times greater than their natural background extinction rates (Barnosky et al., 2011; Wake & Vredenburg, 2008). Human activities, such as intensive agriculture and urbanization, are predicted to increase dramatically in the future in response

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to human population growth (Ray et al., 2013; Seto et al., 2012). To reduce human impacts on ecosystems, it becomes imperative to identify effective conservation strategies.

Conservation strategies rely on accurate information of species' spatial distribution (Bank et al., 2003; De Wan et al., 2009). Understanding which and how factors affect species distribution allows conservation managers to predict the effect of environmental changes on species presence and abundance, to identify key areas of conservation, and potential reintroduction sites (Klar et al., 2008; De Wan et al., 2009). However, species distribution is determined by factors operating at multiple spatial scales (Bauerfeind et al., 2009; Blevins & With, 2011; Montague-Drake et al., 2009). At the local scale, species occurrence is influenced by abiotic and biotic factors typically associated with individual survival and reproductive success, including resource availability, competition for such resources, parasite loads, and predation risk (Bauerfeind et al., 2009; Blevins & With, 2011; Montague-Drake et al., 2009). At the landscape scale, site occupancy is often affected by factors related to movement among resource patches or local populations (Bauerfeind et al., 2009; Boscolo & Metzger, 2011; Dullinger et al., 2011). The degree to which the landscape facilitates or impedes movement among resource patches is defined as landscape functional connectivity (Taylor et al., 1993). It is determined, among other things, by the behavioral response of individuals to the amount and spatial arrangement of habitats within the landscape (Bélisle, 2005; Tischendorf & Fahrig, 2000). This landscape characteristic is deemed important as it should affect the connectivity of local populations and therefore the exchange rates of individuals among them (Acevedo et al., 2015; Hanski, 1998).

Landscape characteristics as well as local habitat conditions can affect organisms with complex life cycles such as pond-breeding amphibians (Knapp et al., 2003; Mazerolle et al., 2005; Scherer et al., 2012). Pond-breeding amphibians require terrestrial habitats to forage, disperse, and hibernate, as well as aquatic habitats to breed and develop from embryos (Semlitsch, 2008; Wilbur, 1980). Such dual habitat requirements make amphibians particularly vulnerable and contribute indirectly to their population declines worldwide (Alford & Richards, 1999; Stuart et al., 2004). Many factors contribute to amphibian declines, such as climate change, diseases, invasive species, but habitat loss and fragmentation are likely the most important (Alford & Richards, 1999; Cushman, 2006). Amphibians are more threatened of extinction than either birds or mammals, with 41% of species at risk compared to 14% and 25% of bird and mammal species, respectively (International Union for Conservation of Nature, 2019; Stuart et al., 2004). Identifying local and landscape characteristics affecting the distribution of amphibians is thus of utmost importance to ensure their persistence.

Because early life stages of most amphibians are exclusively aquatic, environmental conditions within ponds must be favorable to their survival and development for a pond to be occupied across years (Werner et al., 2009). Pond occupancy and larval development depend on environmental conditions such as aquatic and riparian vegetation cover (Mazerolle et al., 2005; Welch & MacMahon,

2005), canopy cover (Schiesari, 2006; Skelly et al., 2002; Werner et al., 2009), and wetland hydroperiod (Amburgey et al., 2012; Rowe & Dunson, 1995). Furthermore, pond occupancy may also be influenced by landscape structure because juveniles disperse following metamorphosis from their natal pond to terrestrial habitats before ultimately breeding in their natal pond or another pond in the landscape (Gill, 1978; Semlitsch, 2008). Pond-breeding amphibians also migrate regularly through the landscape to access seasonal resources in different habitats (Pilliod et al., 2002; Semlitsch, 2008). Therefore, a pond either isolated by distance or by a landscape structure hindering movements may remain unoccupied despite having favorable local conditions for larval survival and development (Mazerolle & Desrochers, 2005; Rothermel & Semlitsch, 2002).

The habitat composition and configuration of the landscape determine its permeability to amphibian movements (Cline & Hunter, 2014, 2016; Cosentino et al., 2011). For instance, habitat patches can be favorable to movements because they act as stepping stones, reduce the travelling distance, or contain resources (Gibbs, 1993; Saura & Rubio, 2010; Semlitsch & Bodie, 1998). Conversely, anthropic disturbances, such as roads, built-up and intensive row crop areas, can be impermeable or act as barriers to movements (Van Buskirk, 2012; Rothermel & Semlitsch, 2002). These open and disturbed surfaces can be harmful to amphibian movements as they lead individuals to experience high water loss (Mazerolle & Desrochers, 2005; Rothermel & Semlitsch, 2002). Roads can also impede amphibian movements by causing direct mortality through collisions with vehicles, particularly on high-traffic roads (Fahrig et al., 1995; Hels & Buchwald, 2001). Anthropic disturbances surrounding wetlands can also affect negatively amphibian survival, development, and presence because they increase exposure to various contaminants in aquatic habitats such as de-icing salt and pesticides (Karraker et al., 2008; Wagner et al., 2013). The negative impacts of anthropic areas on individual survival, reproduction, and movement are very concerning, especially for species at risk of extinction.

An amphibian particularly at risk of extinction in Canada is the boreal chorus frog (*Pseudacris maculata*) (Committee on the Status of Endangered Wildlife in Canada [COSEWIC], 2008). Populations of the boreal chorus frog from the Great Lakes, St. Lawrence River, and Canadian Shield areas are considered threatened (Committee on the Status of Endangered Wildlife in Canada [COSEWIC], 2008). Within a period of 10 years, the number of its local populations declined by 37% and 30% in southern Québec and Ontario, respectively (Committee on the Status of Endangered Wildlife in Canada [COSEWIC], 2008). Despite conservation concerns about this species, there is limited information on its spatial distribution, making it difficult to implement effective conservation strategies. To fill this gap, the main objective of this study was to quantify the influence of several local and landscape characteristics on the occurrence of boreal chorus frogs in wetlands of the St. Lawrence Lowlands in southern Québec. We hypothesized that site occupancy depends on local characteristics, such as vegetation cover and hydroperiod, and is favored by landscape characteristics contributing to site connectivity. We also hypothesized that site occupancy by the boreal

chorus frog decreases with the amount and proximity of anthropic disturbances. Due to its cryptic nature outside of the breeding season, the boreal chorus frog is mainly inventoried using call surveys (Dodd, 2013; Ouellet et al., 2009; Smith et al., 2014). The breeding season of the boreal chorus frog lasts 2–3 weeks in our study area (Desroches & Rodrigue, 2004; Whiting, 2004), which constrains the time window for call surveys. To overcome this limiting factor, we performed environmental DNA (eDNA) sampling to assess site occupancy. Environmental DNA has been gaining popularity to monitor amphibian populations, given that many species are difficult to detect and that traditional sampling depends on the calling activity or visibility of individuals during specific times and weather conditions (Dejean et al., 2012; Ficetola et al., 2019; Valentini et al., 2016). Indeed, eDNA can expand the window during which species can be detected. However, this method has so far been applied to a limited number of amphibian species and has never been used to detect boreal chorus frogs. Thus, our second objective was to compare the detection probability of boreal chorus frogs via eDNA during the breeding period to that of traditional call surveys.

2 | METHODS

2.1 | Study area and site selection

The study area was located in the administrative region of Montérégie, in southeastern Québec, Canada (Figure 1). Our study area covered 2,250 km² in the form of a 90 km × 25 km corridor along the St. Lawrence River, from Candiac (45°23'00" N, 73°31'00" W) to Contrecoeur (45°51'00" N, 73°14'00" W) (Figure 1). The built-up areas of Montérégie, consisting of residential, commercial, and industrial areas, have been experiencing a rapid growth and harbor a population of >1,550,000 people (~140 persons/km²; Appendix S1). These built-up areas cover 10% of the territory of the Montérégie and are connected by 2,222 km of roads (Appendix S1). More than half (i.e., 54%) of Montérégie is covered by agriculture, 45% of which consists of intensive row crops dominated by maize, soybean, and wheat cultures (Appendix S1). As a consequence, the surface waters of Montérégie are contaminated by many pesticides, notably by glyphosate, atrazine, and S-metolachlor herbicides as well as by thiamethoxam, clothianidin, and imidacloprid (neonicotinoid) insecticides (Montiel-León et al., 2019). With such high anthropic pressure, only 6% and 17% of the territory remains covered by wetlands and forests, respectively (Appendix S1). The study area has an annual mean temperature of 6.2°C and total precipitation of 1010.6 mm (Government of Canada, 2019).

We randomly selected a total of 180 sites in the study area (Figure 1). Sites consisted of ditches and various types of wetlands such as marshes, ponds, and wet meadows. We stratified site selection in two steps. First, we divided the corridor of 90 km × 25 km into six rectangular sections of 15 km × 25 km. Second, within each of these sections, we stratified our selection based on the historical presence of the boreal chorus frog as determined from inventories conducted between 2004 and 2016 by the Ministère des Forêts, de

la Faune et des Parcs du Québec and Ciel et Terre, a local conservation organization. Specifically, we selected 90 sites known to have been occupied by the boreal chorus frog during at least 1 year between 2004 and 2016 and 90 sites without historical information on the species. We selected the 90 ponds without historical information on the species presence based on a combination of databases containing wetland information, as well as satellite imagery and LiDAR. To ensure some level of independence between sites sampled in the same year, we also maintained at least 400 m between selected sites. We considered this distance appropriate, as most *P. maculata* dispersal occurs within 200 m of breeding sites (Dodd, 2013; Whitaker, 1971). During a given year, we thereby sampled between 11 and 17 sites in each of the 15 km × 25 km sections given the availability of sites meeting the above distance criterion.

2.2 | Environmental DNA sampling

We collected water samples for eDNA analyses at 90 sites in 2017 (16 May–19 June) and at 110 sites in 2018 (20 April–8 June). Among the 110 sites sampled in 2018, 20 were also sampled in 2017. Sites were generally visited twice during a given year. However, water samples could not be collected during the second visit at 5 sites in 2017 and 16 sites in 2018 because they had completely dried up. Furthermore, the 20 sites sampled in both years were sampled only once in 2018 because we focused our sampling effort on sites that had not been sampled in 2017. During each visit at a site, we generally collected 8–10 replicates of 0.125 L (i.e., 1 L) of water from different sections of the site (Appendix S2). To avoid contamination, bottles used to collect water were washed beforehand with a 10% bleach solution, rinsed three times with tap water, and rinsed once with site water before collecting the sample. We stored water samples in the dark at 4°C immediately upon collection. We converted sampling date to the number of days elapsed since snowmelt to synchronize dates of each year on a common baseline. Dates of snowmelt were obtained from the Environment and Climate Change Canada climate archives for one weather station in our study area (Government of Canada, 2019).

Following water collection water was filtered within 12 h using a 1.2-μm glass microfiber filter (Whatman GF/C 47 mm, GE Healthcare Life Sciences®) and a peristaltic pump (Masterflex L/S Modular Drive, Cole-Parmer®) in order to recuperate eDNA on the filter. We pooled water sample replicates and, unless the filter clogged, we generally could filter up to 0.75 L of the clearest water obtained after a 30-min decantation period (between 0.125 and 1.25 L of water filtered; Appendix S2). We used a negative control consisting of distilled water to measure potential contamination at the start of each filtration session, for a total of 65 negative filtration controls. Between each sample, we sterilized the equipment with a 10% bleach solution and rinsed it with distilled water. After filtration, we folded the filter in half, wrapped it in aluminum foil and placed it at –20°C until further analyses (<6 months before extraction). The DNA extraction, the amplification, and the design of the quantitative polymerase

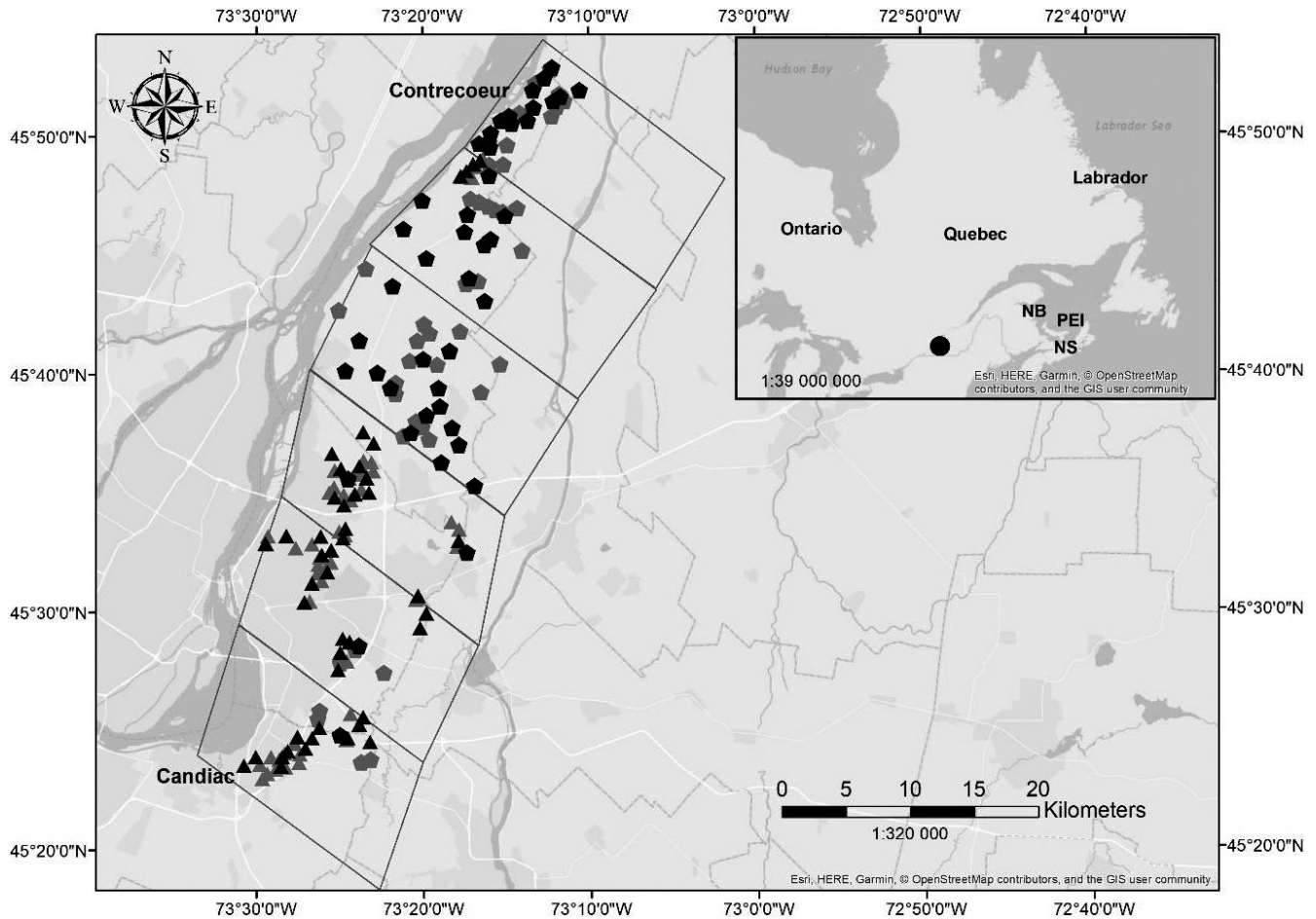


FIGURE 1 The 180 sites sampled to study the distribution of the boreal chorus frog (*Pseudacris maculata*) in southeastern Québec, Canada. Triangles and pentagons denote the 90 sites with and without historical information on the species presence, respectively. Sites sampled in 2017 are shown in black, whereas those sampled in 2018 are in gray. Gray lines show the six rectangular sections of 15 km × 25 km used to stratify the site selection

chain reaction (qPCR) primers and probe followed Goldberg et al. (2011) and Lacoursière-Roussel et al. (2016). Complete details of the protocol are presented in Appendix S3.

2.3 | Call surveys

During the 2018 boreal chorus frog breeding season (20 April–10 May), two listeners conducted call surveys between 11:30 and 20:00 at 63 sites that were simultaneously sampled for eDNA. We conducted 5-min surveys at one to five sampling stations depending on site area after remaining silent and motionless for a 5-min period. When the site area was not available from georeferenced data layers, it was measured in the field using a GPS. The number of sampling stations was, however, fixed at two for ditches. Sampling stations were established randomly at the water edge on the site perimeter with the constraint of being separated by at least 30 m. We combined the data across the sampling stations of a given site to determine whether the species was detected at least once (1) or not (0) during a given visit.

2.4 | Local characteristics

We characterized sites in June and July 2018, which corresponds to the period of metamorphosis in our study area (Whiting, 2004). We estimated vegetation cover in the water and on the shoreline with quadrats of 1 m × 3 m. Each quadrat was centered on a call survey sampling station, so that half of the quadrat was on the shoreline and the other half in the water. The number of sampled quadrats at a given site depended on its area with small (i.e., <0.68 ha), medium (i.e., >0.68 ha to <2.96 ha), and large (i.e., >2.96 ha) sites having one, two, and three quadrats, respectively. In each quadrat, we estimated visually the percentage of canopy closure (i.e., vegetation >3 m in height) above the site. We estimated the cover of five categories of terrestrial vegetation on the shoreline: trees (>3 m), tall shrubs (0.3–3 m), small shrubs (<0.3 m), herbaceous vegetation (>0.05 m), and ground vegetation (<0.05 m). In the aquatic portion of the quadrat, we estimated the cover of three vegetation categories: emerged, submerged, and floating. We estimated the cover of common reed (*Phragmites australis*) in a separate category in the terrestrial and aquatic portion of the quadrat. We also estimated the cover of bare

ground and open water in the terrestrial and aquatic portions of the quadrat, respectively. We quantified vegetation cover with the six following classes: 0%, 1–5%, 5.1–25%, 25.1–50%, 50.1–75%, >75%. For each site, we calculated the mean cover of each vegetation category using the midpoints of the cover classes recorded at the different sampling quadrats. During site characterization, we also recorded whether the site was dry (i.e., 1 = dry and 0 = not dry) as a proxy of the site hydroperiod. Local variables are summarized in Appendix S4.

We summarized vegetation data with a principal component analysis based on a covariance matrix. We used the first principal component scores in the site-occupancy analyses, because it was the only component exceeding the portion of variance explained based on the broken stick distribution (Peres-Neto et al., 2005) (Appendix S5). The first axis (VEG1) explained 64.56% of the variation and was positively associated with canopy cover (Appendix S5).

2.5 | Landscape characteristics

We extracted land-use data from different spatial data bases (Appendix S1). We considered wetlands, forests, and open habitats, such as pastures and wastelands, as landscape elements potentially used by boreal chorus frogs because the species is associated with croplands, old fields, meadows, forest, as well as marshes, and small shallow ponds (Dodd, 2013; Mushet et al., 2012; Whiting, 2004). We also considered roads and human-altered areas, including corn and soybean crops, residential areas, commercial areas, industrial areas, and outdoor recreation areas, such as golf courses, because these landscape elements are potential barriers to the species movement. We measured the relative cover of these landscape elements and the density of main roads (m/ha) in circular buffers of 200, 500, 1,000, and 1,500 m around each site. We used a 200-m buffer because most dispersal movements of the boreal chorus frog occur within 200 m of breeding sites (Dodd, 2013; Whitaker, 1971). We used 500–1,500-m buffers because landscape cover was also found to affect the abundance and occurrence of boreal chorus frogs in buffer zones over 200 m in other studies (i.e., 500–5,000 m) (Browne et al., 2009; Scheffers et al., 2012). Finally, we measured the distance from each site to the nearest anthropic area and main road. Landscape variables are summarized in Appendix S4.

2.6 | Statistical analyses

2.6.1 | Historical presence versus unknown historical presence

We used *t*-tests on the numeric explanatory variables to compare sites where the boreal chorus frog was detected at least once during 2004–2016 and sites without such confirmed past presence. When the variances of the two groups were heteroskedastic, we used generalized least squares implemented in the nlme R package to allow

the variance to vary with the type of wetland (Pinheiro and Bates, 2000; Pinheiro et al., 2018). To avoid inflating the type I error, we applied a Bonferroni correction ($\alpha_{\text{Bonferroni}} = 0.05/10 = 0.005$). For the binary variable of hydroperiod (dry vs. not), we used a chi-square with Yates' continuity correction to compare whether sites with historical information on the species were as likely to dry out than sites without historical information on the species (Yates, 1934). All analyses were performed in R version 3.5.1 (R Core Team, 2018).

2.6.2 | Single-season occupancy models

We used single-season occupancy models to test the relationship between site occupancy as determined by eDNA and habitat or landscape variables, as well as the relationship between detection probability and sampling conditions (MacKenzie et al., 2002). This model type estimates two main parameters: the probability of occupancy of site *i* by the species (ψ_i) and the probability of detecting the species at site *i* during survey *j* (p_{ij}) given that the species is present at the site (MacKenzie et al., 2002). Numerical variables were standardized by subtracting the mean and dividing by the standard deviation. Before standardization, site area was transformed on a logarithm scale because of extreme values. We only included variables that were not strongly correlated in the same model (Pearson $|r| < 0.6$; variance inflation factor < 10). We conducted two series of occupancy analyses. The first was based on the eDNA data collected to assess the influence of local and landscape variables for the two sampling seasons (Appendix S6). The second series of analyses focused on sites that were sampled simultaneously with eDNA and call surveys to compare the detection probability of both methods (Appendix S7).

We estimated model parameters by maximum likelihood using the unmarked R package (Fiske & Chandler, 2011). We performed model selection and multimodel inference using the AICcmodavg R package (Burnham & Anderson, 2002; Mazerolle, 2019). We assessed the goodness-of-fit and overdispersion of the highest ranked model for each of the two series of analyses (local and landscape, eDNA, and call surveys) with a parametric bootstrap approach based on the χ^2 statistic with 10,000 bootstrap samples (MacKenzie & Bailey, 2004). We adjusted the model selection and multimodel inference for overdispersion using the quasi-likelihood Akaike information criteria for small samples (QAIC_c; Burnham & Anderson, 2002). We performed model averaging using the shrinkage estimator for each parameter of interest to make our inferences based on 95% unconditional confidence intervals (95% CI, Burnham & Anderson, 2002).

3 | RESULTS

We detected boreal chorus frogs based on eDNA at 19.4% (35/180) of sampled sites. We detected boreal chorus frogs based on eDNA at 8.9% (8/90) and 26.4% (29/110) of sampled sites in 2017 and 2018, respectively. We detected the species via eDNA in 5.6% and 24.5%

of sampled sites during the first visit, at 6.7% and 7.3% of sampled sites during the second visit, and at 3.3% and 5.5% of sites on both visits in 2017 and 2018, respectively. For sites where we simultaneously conducted call surveys and collected eDNA in 2018, we detected boreal chorus frogs exclusively with call surveys at 6.3% (4/63) of sites, exclusively using eDNA at 15.9% (10/63) of sites, and at 15.9% (10/63) of sites using both sampling methods. We detected boreal chorus frogs at 35.6% (36/101) of the sites where historical presence of the species had been documented in other studies between 2004 and 2016 and at 1% (1/99) of the sites without historical information on the species. We detected boreal chorus frog DNA in at least one replicate with the qPCR assay for 15% (10/65) and 2% (1/48) of the negative filtration and extraction controls, respectively. An average of 1.4 qPCR replicates out of 6 (range: 1–3) contained boreal chorus frog DNA sequences in the negative controls due to contamination. We excluded 12 water samples associated with positive controls. When the species eDNA was detected in site samples, an average of 5.1 qPCR replicates out of 6 were positive. No qPCR inhibition was observed during amplification. The species identity was confirmed in 98% (46/47) of sequenced samples and in the other 2% (1/47), the homology of the sequences was <97% of those of the reference database.

3.1 | Historical presence versus unknown historical presence

Sites with historical information on the presence of boreal chorus frogs differed significantly from sites without information on historical presence of the species in terms of canopy cover, distance to road, and forest cover within 200–1,500-m buffers (Figure 2). Similarly, sites with historical information on the species differed from those without such information regarding the amount of anthropic areas and road density within 1,000- and 1,500-m buffers (Figure 3). Sites in which the species had been detected at least once historically had a lower canopy cover, distance to road, and forest cover within 200–1,500-m buffers than those without such confirmed past presence (Figure 2). The amounts of anthropic areas and road density within 1,000 and 1,500 m were higher for sites with a known historical presence than other sites (Figure 3). Finally, sites with historical information on the species were as likely to dry out than sites without such information on the species ($X^2_1 = 0.09$, $p = 0.76$).

3.2 | Effect of local and landscape characteristics

The model including the effect of historical presence, site dry out, and year on site occupancy, and year on detection probability had the most support (Table 1). This model had an adequate fit with moderate overdispersion ($p = 0.12$, $\hat{c} = 1.80$). The top-ranked model was followed closely by a model with the same structure on occupancy, but that allowed detection probability to vary with days elapsed since snowmelt and volume of water filtered (Table 1). The

probability of boreal chorus frog occurrence was higher in 2018 ($\hat{\beta} : 1.57$, 95% CI: [0.03; 3.10], Figure 4a) than in 2017 and also higher in sites with historical presence of the species than in sites without such information ($\hat{\beta} : 4.26$, 95% CI: [1.49; 7.04], Figure 4b). We found no effect of the other explanatory variables on occupancy. Detection probability did not vary between years ($\hat{\beta} : 0.43$, 95% CI: [-1.46; 2.32]), nor with the volume of water filtered ($\hat{\beta} : 0.07$, 95% CI: [0.73, 0.87]) nor the quadratic effect of days elapsed since snowmelt ($\hat{\beta} : 0.16$, 95% CI: [-0.68, 1]).

3.3 | Sampling with environmental DNA versus call surveys

During visits conducted in 2018 at the 63 sites sampled with both eDNA and call surveys, the boreal chorus frog was only detected at the sites with historically known presence (i.e., detection in 2004–2016). This pattern led to convergence issues when applying the top-ranked model obtained from the analysis of the entire set of 180 sites to the subset of 63 sites sampled with eDNA and call surveys. As a result, we excluded the variable of historical presence from the analysis comparing eDNA and call surveys. The most highly parameterized model for the data using eDNA and call surveys had an adequate fit with moderate overdispersion ($p = 0.16$, $\hat{c} = 1.77$). Among the candidate models considered to compare the performance of eDNA and call surveys to detect the species, the model having the most support allowed detection probability to vary with the sampling method (Table 2). However, this model was only 1.9 times more parsimonious than the null model. There was no evidence for an effect of the sampling method on detection probability ($\hat{\beta} : -0.19$, 95% CI: [-0.65; 0.27]). Indeed, the probability of detection was 0.81 (95% CI: [0.31; 0.98]) with eDNA and 0.62 (95% CI: [0.25; 0.89]) with call surveys.

4 | DISCUSSION

In this study, the probability of occupancy of the boreal chorus frog varied only with year and the historical presence of the species. The probability of occupancy was higher in 2018 than in 2017 and greater in sites where the species had been detected at least once between 2004 and 2016. The higher probability of occupancy in 2018 than 2017 could be due to environmental conditions more favorable to the boreal chorus frog than in 2017. An alternative explanation is that sites sampled in 2018 may have characteristics more favorable for the species presence than sites sampled in 2017. Sites with known historical presence had higher occupancy than sites without such information. A potential interpretation of this result is that sites with known historical presence offer favorable habitat conditions for the species and that these sites have been colonized first in the landscape. Surprisingly, sites with known historical presence generally had less undisturbed habitat and were closer to urban areas than sites without information on the species presence. This apparent contradiction may reflect recent habitat loss and changes

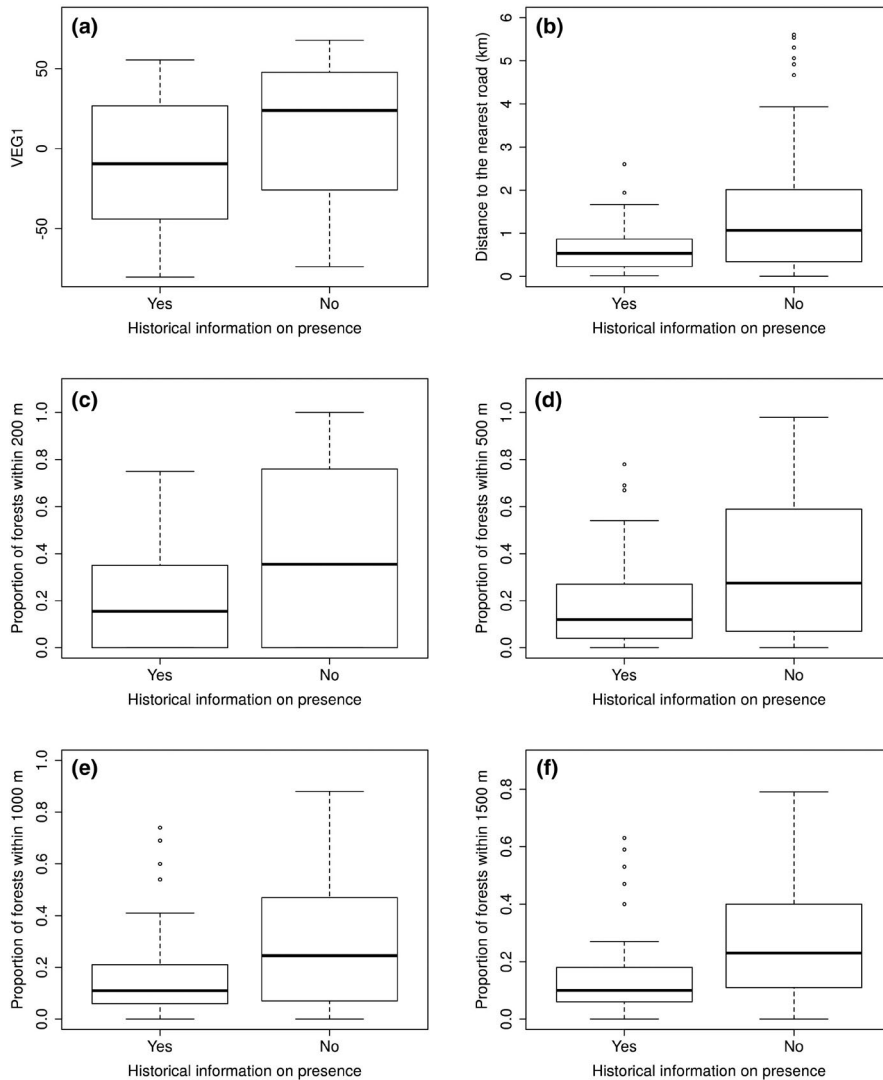


FIGURE 2 Variability of environmental characteristics at 90 sites with information on historical presence of the boreal chorus frog (*Pseudacris maculata*) and 90 sites without such information in southeastern Québec, Canada. Means of all variables presented differed significantly between sites with information on historical presence of the species and sites without such information ($|t_{178}| > 3.72, p < 0.0003$)

in the landscape with increasing isolation of well-established populations. These changes could explain the 48% decline in occupied sites among the 90 sites with known species presence between 2004 and 2016. However, this hypothesis remains speculative and requires a formal assessment.

We did not find any relationship between the probability of occupancy of the species and the local or landscape characteristics. We suspect this result was due to the rarity of the boreal chorus frog in our study area. The species was detected at least once in only 18% of the 180 sites we sampled. Previous studies that found a relationship between site occupancy by amphibians and local or landscape characteristics had at least 40% of sites with one detection (e.g., Hamer, 2018; Mazerolle et al., 2005; Werner et al., 2009; Youngquist et al., 2017). The high proportion of sites unoccupied by boreal chorus frogs but having local and landscape characteristics favorable to the species likely made it difficult to find an effect of these characteristics on site occupancy. Future studies should not only increase sampling effort but also monitor occupancy over several years in a balanced number of sites known to have been occupied in the recent past as well as sites without such information.

4.1 | Local characteristics

Besides information on past detection, no other pond scale variable explained site occupancy by boreal chorus frogs in our study. In contrast to our finding, canopy cover often explains site occupancy by amphibians in other systems (Skelly et al., 1999; Werner et al., 2009). For instance, Skelly et al. (1999) and Werner et al. (2009) observed that canopy cover has a negative impact on site occupancy by the western chorus frog (*Pseudacris triseriata*), a species very similar in habitat requirements to the boreal chorus frog. Although canopy cover did not have an impact on site occupancy in our study, canopy cover was lower in sites where the species had been detected at least once between 2004 and 2016 than in sites without such confirmed historical presence (Figure 2). Ponds with high canopy cover can be detrimental to boreal chorus frogs because closed canopy sites contain less dissolved oxygen, fewer quality nutrients, and are colder than open canopy sites (Schiesari, 2006; Skelly et al., 2002). Therefore, tadpoles grow faster and have higher survival in open canopy sites (Schiesari, 2006; Skelly et al., 2002).

FIGURE 3 Variability of landscape characteristics at 90 sites with information on historical presence of the boreal chorus frog (*Pseudacris maculata*) and 90 sites without such information in southeastern Québec, Canada. Means differed significantly between sites with information on historical presence of the species and sites without such information ($|t_{178}| > 3.42, p < 0.0008$)

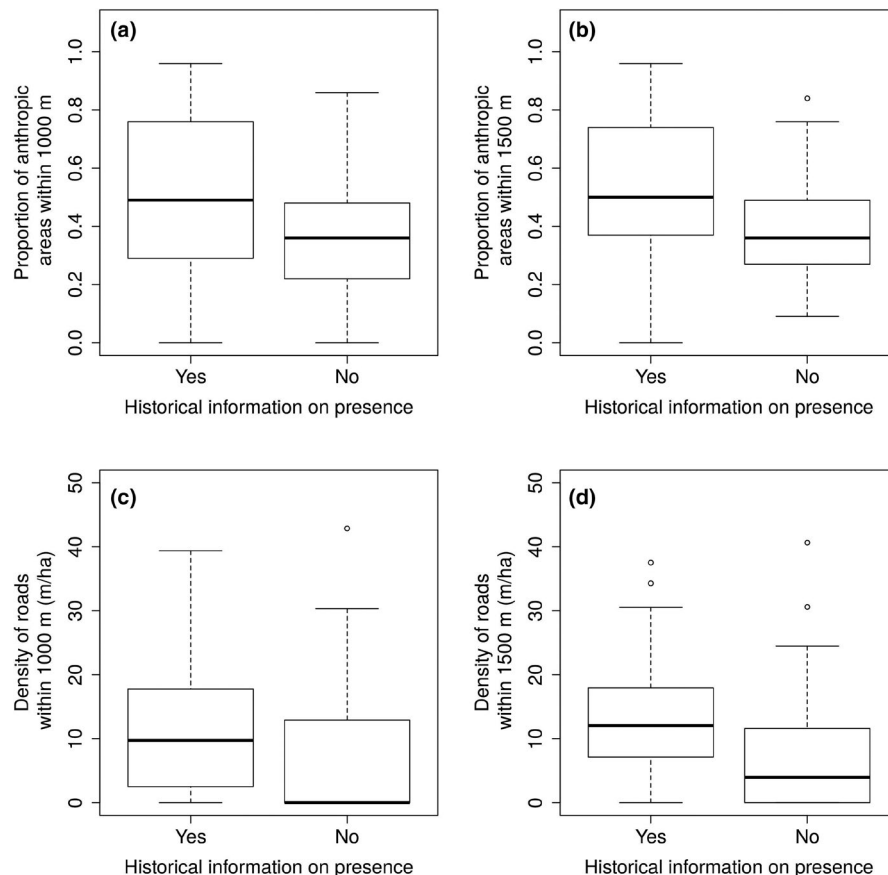


TABLE 1 Model selection among 25 single-season occupancy models assessing the probability of occurrence of boreal chorus frogs (*Pseudacris maculata*) based on eDNA at 180 sites sampled in 2017 or 2018 in southeastern Québec, Canada

Models	Number of parameters	QAIC _c	Δ QAIC _c	Akaike weight
$\psi(\text{Hist.presence} + \text{Dry} + \text{Year}) p(\text{Year})$	7	114.40	0	0.55
$\psi(\text{Hist.presence} + \text{Dry} + \text{Year}) p(\text{JSnow} + \text{JSnow2} + \text{Volume})$	9	114.77	0.37	0.45
$\psi(\text{VEG1} + \text{Area} + \text{Year}) p(\text{Year})$	7	130.70	16.30	0
$\psi(\text{VEG1} + \text{Area} + \text{Year}) p(\text{JSnow} + \text{JSnow2} + \text{Volume})$	9	131.18	16.79	0
$\psi(\text{Wetland.1500} + \text{Forest.1500} + \text{Open.1500} + \text{Year}) p(\text{Year})$	8	134.21	20.81	0

Note: Models were ranked based on quasi-likelihood Akaike information criteria for small samples (QAIC_c) because there was overdispersion in the data ($\hat{c} = 1.80$). Only the first five models are shown for clarity.

Site occupancy by amphibians varies with hydroperiod in different study systems (Green et al., 2013; Hamer et al., 2016), but we found no evidence of such an effect in our study. This result either suggests that the persistence of species is independent of the hydroperiod conditions during larval development or metamorphosis, but more likely, is an artefact of the already low prevalence of the species in our study area. Indeed, sites drying out before metamorphosis negatively impacts boreal chorus frogs because tadpoles cannot not reach the minimum critical development stage and body size to metamorphose to the terrestrial stage (Wilbur, 1980; Wilbur & Collins, 1973). Hydroperiod may have a delayed effect on site occupancy by boreal chorus frogs, because they breed between their first and third year after metamorphosis (Muths et al., 2016;

Whiting, 2004). Environmental conditions from previous years, such as the site hydroperiod, are hence likely to have a greater effect on site occupancy than those prevailing in the year of the survey (Muths et al., 2018). Although this information was not available for our study area, we suggest that future studies consider such carry-over effects.

4.2 | Landscape characteristics

We found no evidence of a relationship between landscape characteristics and site occupancy by boreal chorus frogs. Similarly, Seburn and Gunson (2011) found that the occupancy by boreal chorus frogs

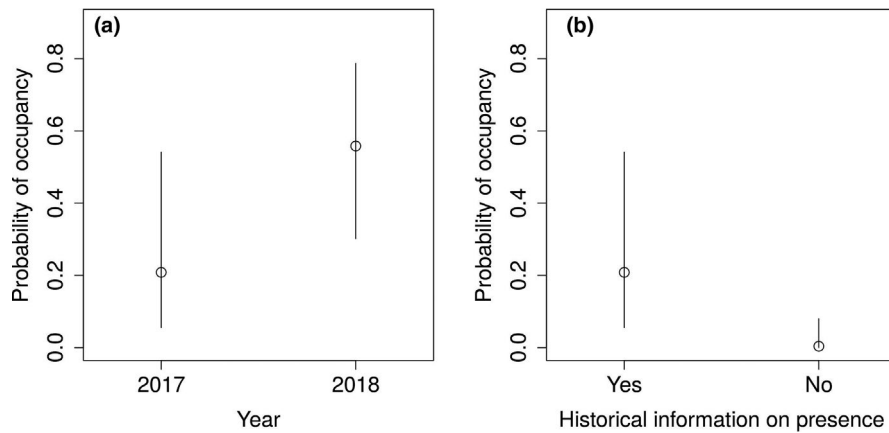


FIGURE 4 Model-averaged estimates of the probability of occupancy of the boreal chorus frog (*Pseudacris maculata*) varying with year (a) and sites with historical information on the presence of the species during 2004–2016 and without such information (b). Error bars denote 95% unconditional confidence intervals

TABLE 2 Model selection of five single-season occupancy models assessing the probability of occurrence of boreal chorus frogs (*Pseudacris maculata*) via eDNA and call surveys at 63 sites sampled in 2018 in southeastern Québec, Canada

Models	Number of parameters	QAIC _c	Δ QAIC _c	Akaike weight
$\psi(\text{Dry}) p(\text{Method})$	4	85.63	0	0.54
$\psi(\text{Dry}) p(.)$	5	86.85	1.22	0.29
$\psi(\text{Dry}) p(\text{Method} + \text{JSnow} + \text{JSnow}^2)$	7	89.11	3.48	0.09
$\psi(\text{Dry}) p(\text{JSnow} + \text{JSnow}^2)$	6	90.46	4.83	0.05
$\psi(\text{Dry}) p(\text{Method} + \text{JSnow} + \text{JSnow}^2 + \text{Method:JSnow}^2)$	8	91.42	5.79	0.03

Note: Models were ranked based on quasi-likelihood Akaike information criteria for small samples (QAIC_c) because data were overdispersed ($\hat{c} = 1.77$).

at historical locations in Ontario, Canada, was independent of several landscape characteristics such as forest cover, built-up, and agricultural areas. Nevertheless, several studies report a negative impact of anthropic disturbances on site occupancy by amphibians (Cayuela et al., 2015; Pellet et al., 2004; Youngquist et al., 2017). The absence of an effect of anthropic disturbances on site occupancy by boreal chorus frogs in our study is even more surprising because agricultural intensification and urbanization are considered the most significant threats to the species' persistence (Committee on the Status of Endangered Wildlife in Canada [COSEWIC], 2008). In contrast, Browne et al. (2009) report a negative and a positive relationship between the abundance of *P. maculata* and urban cover within 500 m and 1,000 m, respectively. Our results suggest that boreal chorus frogs are resilient to a certain amount of disturbance around its reproduction sites. Supporting this hypothesis, Bartelt and Klaver (2017) observed that boreal chorus frogs successfully colonized 100% (22/22) of restored wetlands distributed within a landscape composed of 90% of row crops. Alternatively, the absence of impact of anthropic disturbances in our study could be due to the low amount of anthropic areas or roads (anthropic cover <50% and road density <25 m/ha) surrounding the study sites. Our study area was less anthropized than other studies reporting a negative effect of anthropic disturbances. For instance, Youngquist et al. (2017) indicated an average density of 3,273.8 m/km² of roads within 500 m of sites, a value 4 times larger than in our study area (but see Bartelt & Klaver, 2017).

Results did not suggest a relationship between site occupancy and landscape characteristics. Interestingly, sites with historical information on the species presence were closer to roads and had higher cover of anthropic areas within 1,000 m and 1,500 m than sites without historical information on the species (Figures 2 and 3). For this reason, the higher occupancy we observed in sites with historical information is counterintuitive relative to our initial hypothesis on anthropic disturbance, but consistent with Bartelt and Klaver (2017). Boreal chorus frogs occur in wetlands undergoing anthropic disturbances such as agricultural ponds, roadside ditches, and stock ponds (Dodd, 2013). Thus, the species may have benefited from anthropic wetlands for past dispersal (Committee on the Status of Endangered Wildlife in Canada [COSEWIC], 2008; Dodd, 2013) or showed a spilling or temporary crowding effect as observed in forest passerine birds following clearcutting (Hagan et al., 1996; Schmiegelow et al., 1997). Further studies should investigate the impact of local and landscape characteristics on colonization and extinction patterns of local populations.

4.3 | Sampling with environmental DNA versus call surveys

We developed a new method based on eDNA to detect the presence of boreal chorus frogs from water samples mainly because eDNA allows to sample over a longer time window than traditional call

surveys. Indeed, call surveys started on 20 April and could only detect the boreal chorus frog until 10 May (20 days). In contrast, eDNA allowed detection of the species until 1 June (42 days), which coincided with the end of the sampling season. We hypothesized that eDNA has a higher detection probability than call surveys. In contrast to our hypothesis, both methods had similar probabilities of detecting boreal chorus frog in our study (call surveys: 0.62; eDNA: 0.81), given the precision of their estimation. However, Eiler et al. (2018) report that the detection probability of pool frogs (*Pelophylax lessonae*) using eDNA was higher than visual and call surveys in August, when juveniles and adults occur in the water. Similarly, Dejean et al. (2012) found that the detection probability of American bullfrogs (*Lithobates catesbeianus*) was 0.78 using eDNA comparatively to 0.14 using visual and call surveys. In order to obtain a detection probability with visual and call surveys as high as eDNA sampling, Dejean et al. (2012) estimated that 5.6 times more sites should be visited using visual and call surveys than those sampled with eDNA. These authors also sampled sites when different life stages of bullfrog were simultaneously present in the water with adults, juveniles, and 1–2-year-old tadpoles (Desroches & Rodrigue, 2004). The eDNA does not discriminate between sexes or life stages of individuals in the water and often yields higher detection probabilities than methods focusing on male calls or a specific life stage (Dejean et al., 2012; Ficetola et al., 2019; Valentini et al., 2016). Because boreal chorus frog tadpoles develop within a single season, only adults and eggs were present at sites during the breeding season when we conducted our surveys (Dodd, 2013; Whiting, 2004). This aspect of the species ontogeny may explain the discrepancy with the results of Eiler et al. (2018) and Dejean et al. (2012), who conducted their surveys when tadpoles were also in the water. In support of our argument, Ruso et al. (2019) reported that the detection of the wood frog (*Lithobates sylvaticus*) using eDNA was greater in summer (0.69), when tadpoles were in the water, than in spring (0.35), when only adults and eggs were in the water. In our study area, the peak in the abundance of boreal chorus frog eDNA may also be in summer when tadpoles are in the water. Therefore, we suggest that future studies compare the detection probability obtained from eDNA with that of other sampling methods when different life stages, especially tadpoles, are present in the water.

5 | CONCLUSION

We found that the occupancy of wetlands by boreal chorus frog was higher in sites where the species had been detected during 2004–2016 than in sites without such information. We attribute the lack of relationship between the other local or landscape characteristics and the probability of occupancy of the species to declines in the number of sites occupied by the species and its rarity in our study area. Most local and landscape characteristics varied between sites where *P. maculata* had been detected at least once between 2004 and 2016 and those without such confirmed past presence. The role of local and landscape characteristics should be investigated on the

long-term colonization and extinction dynamics of this threatened species. Finally, we showed that eDNA is a promising tool to detect boreal chorus frogs from water samples, as the detection probability of this method was similar to that of call surveys conducted during the breeding season. However, future studies should estimate the detection probability of eDNA during the breeding season and the one during the larval development period, to identify the optimal sampling window to detect this species at risk with eDNA.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

MJM and YD conceptualized the idea and acquired funding for the project. MPDG, LB, MB, YD, and MJM contributed to the conception and design of the study. MPDG acquired data by conducting field sampling and laboratory work. MPDG and MJM contributed to data analysis and interpretation. MPDG, LB, MB, YD, and MJM contributed to the writing of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on the Dryad data repository at <https://doi.org/10.5061/dryad.8cz8w9gr1>.

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