Correlates of Waterbody Characteristics and the Occurrence or Diversity of Larval Amphibians in Central Ontario, Canada

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Abstract

Watershed acidifcation and poor water quality can deleteriously afect amphibian populations. Between 1990 and 2008, we sampled 333 small, permanent (inundated year round) waterbodies that drain forested areas in the Algoma, Muskoka and Sudbury regions of central Ontario, Canada to determine whether water chemistry parameters, fsh presence, and waterbody area and depth predict amphibian presence or diversity. Amphibians were present in some low-pH waterbodies, contrasting earlier studies, and generally water chemistry was not a strong indicator of amphibian presence or diversity in central Ontario. We suspect that other biotic and abiotic factors have a stronger efect on amphibian presence, and that the relationships between chemical and physical attributes and amphibian presence are complex. Future research should focus on long-term habitat change in central Ontario waterbodies to determine how watershed degradation has afected amphibians.

Keywords Acid rain · pH · Water chemistry · Amphibian · Habitat

Widespread concern for amphibian populations has grown recently due to large scale, deleterious anthropogenic impacts on the environment (e.g., development, pollution, climate change), and concomitant amphibian population declines (Beebee and Griffiths [2005;](#page-6-0) Guderyahn et al. [2016](#page-6-1)). Although habitat loss, degradation and fragmentation are signifcant causes of amphibian declines (Ficetola and Bernardi [2004](#page-6-2); Beebee and Grifths [2005](#page-6-0)), other factors also negatively impact amphibian populations (e.g., disease and pathogens; Battaglin et al. [2016\)](#page-6-3).

Parts of North America and Europe have been subject to dramatic alterations to soils and aquatic systems associated with acidic precipitation (Likens et al. [1996](#page-6-4); Jeziorski et al.

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[2008\)](#page-6-5), and consequently the infuence of lake and pond acidifcation on amphibian distribution and survival has received considerable attention (Bradford et al. [1994](#page-6-6)). Studies have focused on identifying species and habitats most vulnerable to acidic deposition by characterizing relationships between the acidifcation of habitats and biological changes, and developing monitoring programs that can track biological responses as acidifying emissions are reduced (e.g., Freda [1986](#page-6-7); McNicol et al. [1995a](#page-6-8), [b\)](#page-6-9). However, the relationships between amphibian occurrence, and chemical and physical characteristics of lakes and wetlands (e.g., water quality, hydrology, morphometry) are complex (Beebee and Grifths [2005](#page-6-0); Battaglin et al. [2016\)](#page-6-3), and the potential for deleterious synergistic impacts of anthropogenic pollution on aquatic food webs, habitat structure and composition, as well as on amphibian reproduction and physiology, suggests that further investigation of these relationships is warranted.

Lakes and wetlands of eastern Canada are of special concern due to the predominance of thin, poorly bufered soils in many watersheds and a history of high levels of acidic deposition (Hecnar and M'Closkey [1996](#page-6-10)), which have had adverse efects on macroinvertebrates, fsh and aquatic birds (McNicol et al. [1995a,](#page-6-8) [b;](#page-6-9) Jefries et al. [2003](#page-6-11)). Among the amphibian species present in Canada, about 50% of their ranges have been historically exposed to acid precipitation (Clark [1992](#page-6-12); Longcore et al. [1993\)](#page-6-13). Watershed acidifcation

can infuence amphibian populations through death of eggs and larvae, changes to embryonic development patterns and malformation of larvae, altered ionic and water exchange across skin and gill membranes, and reduced growth and swimming performance (Freda et al. [1991;](#page-6-14) Jung and Jagoe [1995](#page-6-15)). Despite large reductions in the levels of acidic deposition in eastern Canada and the improvement of acid status for some lakes (e.g., Mallory et al. [1998;](#page-6-16) Keller et al. [2018](#page-6-17)), the majority of monitored waterbodies have shown limited chemical recovery (Jefries et al. [2003;](#page-6-11) Keller et al. [2018](#page-6-17)). Collectively, the suite of environmental stressors that continue to affect water quality in eastern Canadian watersheds may have reduced the suitability of many aquatic habitats for amphibian breeding.

As part of a federal program to evaluate and monitor the efects of acidic precipitation on biota in eastern Canada (McNicol et al. [1995a](#page-6-8), [b\)](#page-6-9), we sampled larval amphibian presence in permanent waterbodies (inundated year round) of central Ontario between 1990 and 2008. Previous studies suggested that chemical changes in water quality due to acid inputs [e.g., reduced pH or alkalinity, increased aluminum (Al)] had a negative impact on amphibian occurrence and numbers in lakes and wetlands (Freda [1986;](#page-6-7) Freda et al. [1991\)](#page-6-14), including research located in our study area (Glooschenko et al. [1992](#page-6-18)). Given that many waterbodies in our study region had low natural bufering capacity and thus were acid-sensitive with high Al (Mallory et al. [1998](#page-6-16)), our overall hypothesis was that water chemistry, and particularly parameters infuenced by local and long-range transport of acidifying pollutants, would limit the suitability of many permanent waterbodies for amphibian habitation. Thus, we predicted that amphibian occurrence in our study sites would be reduced in low pH waterbodies. We assessed whether other chemical and physical waterbody attributes infuenced amphibian occurrence or species richness. We expected that amphibians would more likely be captured in larger waterbodies with higher nutrient concentrations due to increased food resources.

Materials and Methods

Our study was carried out in central Ontario, Canada, in three \sim 100 km \times 100 km study regions: Algoma (45 waterbodies; centre 47° 01′N, 83° 55′W), Muskoka (163 waterbodies; centre 45° 30′N, 79° 06′W), and Sudbury (125 waterbodies; centre 46° 54′N, 80° 41′W; Fig. [1](#page-1-0)). In general, waterbodies in each region cover a similar range of physical and chemical characteristics (McNicol et al. [1995a\)](#page-6-8). We sampled a variety of waterbodies in this study: bogs, fens, marshes, small lakes and ponds, and a few larger, downstream lakes. Most waterbodies drained small, forested watersheds (in Sudbury, the average watershed covered

Fig. 1 Map of study region showing the locations of the central Ontario study sites in Algoma, Muskoka and Sudbury

84 ha; calculated from Mallory et al. [1998\)](#page-6-16), were remote from human activities (e.g., urban, agriculture, roads) other than logging, and could only be accessed by walking, canoeing or helicopter. Water samples were collected 1−8 times from 333 waterbodies across central Ontario between 1990 and 2008 (Table S1 in Supplementary Material); we used mean values from all sampling periods in our analyses. Most waterbodies were relatively small and shallow with short fushing times (Mallory et al. [1998](#page-6-16)), so we collected water samples from mid-lake in early October. We assumed that waters were well mixed in autumn due to winds and turnover, and thus depicted typical water chemistry for all sites (acknowledging that water chemistry can vary spatially within lakes, and that pH may be lower earlier in the year following spring runoff; Wetzel [1983](#page-7-0)). Waterbody areas were measured by geographic information system data while maximum depths were measured using a portable fsh fnder (Mallory et al. [1998\)](#page-6-16).

Thirteen amphibian species may occur in this area (Cook [1984\)](#page-6-19). Our study was focused on permanent waterbodies, somewhat larger than those in Glooschenko et al. ([1992](#page-6-18)), and thus species that principally use quite shallow or ephemeral ponds were not well sampled in our work. Amphibians (principally newts and tadpoles, but rarely adult frogs) were captured in standard wire minnow traps (30 cm \times 70 cm, 6 cm opening, 0.5 cm \times 0.5 cm mesh) baited with \sim 125 mL generic dry dog food, and totally submerged for 24 h near shore at depths < 2 m (McNicol et al. [1995a\)](#page-6-8). These traps were set principally to capture fsh, but also targeted larval amphibians (hence confrming successful breeding in these waterbodies). Six traps were set (occasionally fewer for ponds<1 ha) in June or July around the perimeter of each waterbody. When traps were retrieved, all contents were immediately placed into a jar of MS-222 to be euthanized (as per approved Animal Care protocols and permits), and then transferred to jars with formalin. Back at the laboratory, collections were sorted and amphibians removed. In early years we were confdent of newt identifcations but not confdent of the identifcations of some larval frogs (notably bullfrog *Rana catesbeiana* and green frog *R. clamitans* tadpoles). Thus, we excluded early year identifcations in the analyses for bullfrog and green frog tadpoles, but included early year identifcations in the analyses for newts, which resulted in different sample sizes $(n=216 \text{ vs. } n=333 \text{ respectively}).$ After the early years of sampling, tadpoles were identifed by an expert herpetologist against Canadian national reference collections.

Fish presence/absence was assigned for each waterbody by scoring fsh as "present" if at least one fsh was caught in the site in any year of sampling. If we never caught fsh in a waterbody across all years of sampling, fsh were deemed "absent" for analyses. Few waterbodies sampled in this study supported larger gamefsh. Instead, fsh assemblages (if present) were dominated by small, gape-limited minnows, and some waterbodies supported yellow perch (*Perca favescens*), white sucker (*Catostomus commersoni*) and brook trout (*Salvelinus fontinalis*; McNicol et al. [1995a](#page-6-8)).

Twenty chemical parameters were determined for fltered water samples from each waterbody in most years: pH, conductivity (µS/cm), alkalinity (analogous to acidneutralizing capacity, μ eq/L), calcium (Ca, mg/L), magnesium (Mg, mg/L), sodium (Na, mg/L), potassium (K, mg/L), sulphate (SO_4 , mg/L), silica dioxide (SiO_2 , mg/L), chloride (Cl, mg/L), total nitrogen (TN, mg/L), total phosphorus (TP, µg/L), dissolved organic carbon (DOC, mg/L), total inorganic carbon (TIC, mg/L), total aluminum (Al, µg/L), total iron (Fe, µg/L), total manganese (Mn, µg/L), total zinc (Zn, μ g/L), total nickel (Ni, μ g/L) and total copper (Cu, μ g/L). Chemical analysis procedures are described in Mallory et al. [\(1998\)](#page-6-16).

We examined whether amphibian presence was predicted by acidity, waterbody area and depth, and water column nutrient levels, organic content and solute concentrations. We produced Pearson correlations among all continuous variables to identify collinearity (i.e., high correlations at $|r| > 0.50$ among potential explanatory variables. Calcium, Mg and Na concentrations were highly correlated with alkalinity and conductivity concentrations (*r*>0.66), and TN concentrations were highly correlated with TP concentrations $(r > 0.70)$. Log-alkalinity was highly correlated with pH levels $(r > 0.90)$. Moreover, Al concentrations were highly correlated with pH levels, and Ca, Mg and Na concentrations (Mallory et al. [1998](#page-6-16)). Thus, to minimize collinearity, we did not include Al, Ca, Cl, Mg, Na, TN and alkalinity as explanatory variables in our generalized linear models. We used conductivity as a surrogate for solute concentrations, TP as a surrogate for nutrient status, pH to measure acidity, DOC as a surrogate for organic content, and $SiO₂$ as a surrogate for the degree of weathering inputs from the watershed, and ran models with these chemicals as predictors to assess their biological relevance.

We compared characteristics of the waterbodies with and without each amphibian species using *t* tests to assess which variables infuence the presence of amphibians. We also summed how many amphibian species were found in each waterbody then grouped waterbodies by species richness into four categories based on the number of amphibian species that we found. Then, we compared characteristics of waterbodies among the four categories using MANOVA to assess amphibian species richness. If signifcant efects were found, we explored where that variation came from by conducting univariate ANOVA tests for each parameter.

Subsequently, we created generalized linear models (GLM) with binomial distributions, and we chose the best-ftted models using an information-theoretic approach with R software (RStudio version 0.99.491; as shown in Burnham and Anderson [2002,](#page-6-20) Symonds and Mous-salli [2011](#page-7-1)). We created separate GLMs for the presence of individual species that had adequate presence rates $(>10\%$ of the sites). These models used the mean values of water chemistry and morphometric measurements collected from each of the waterbodies between 1990 and 2008 to determine whether they could explain amphibian occurrence. If amphibians were collected at any time in a waterbody between 1990 and 2008, amphibian use was considered as present. Because there were several well-supported models for each response variable, we used model averaging to identify the strongest explanatory variables, and obtain model average estimates and parameters for competing GLMs (Burnham and Anderson [2002\)](#page-6-20). We defned the global model to include waterbody surface area and maximum depth, and the linear forms of a single chemical parameter chosen to represent acidity, nutrients, organic content, solute concentrations and the degree of weathering inputs. Global models were standardized using the package "*arm*" in R software. We conducted model averaging using the 'top model set' approach (Burnham and Anderson [2002](#page-6-20)). We calculated models at $\Delta AIC_c < 2$ (Burnham and Anderson [2002\)](#page-6-20), and their secondary Akaike Information Criterion (AIC_c) values, delta $AIC_c (\Delta AIC_c)$ values, AIC_c weights (*W_i*), number of fitted parameters (K) , R^2 values, and unconditional model average estimates (with their 95% confdence intervals) were calculated and reported using packages "MuMIn", "AICcmodavg" and "piecewiseSEM" (Symonds et al. [2011](#page-7-1)). We considered explanatory variables as "significant" when confdence limits did not include 0.

Results and Discussion

Amphibians were captured in 76% of the permanent waterbodies in central Ontario (*n*=333) between 1990 and 2008 (Table S1). We captured the following amphibian species: American toad (*Bufo americanus*), bullfrog, eastern newt (*Notophthalmus viridescens*), gray tree frogs (*Hyla versicolor*), green frog, mink frog (*R. septentrionalis*), mole salamander (*Ambystoma* sp.), northern leopard frog (*R. pipiens*), spring peeper (*Pseudacris crucifer*), and wood frog (*R. sylvatica*). American toads, gray tree frogs, northern leopard frogs, spring peepers, and mole salamanders were present $in < 2\%$ of the study sites, and may not have been captured well by our methodology (e.g., younger, small amphibians may have moved through the traps) or in these type of waterbodies (see Glooschenko et al. [1992](#page-6-18)). Thus, we did not further examine characteristics of waterbodies where they occurred, but still included them in the species richness calculations. Wood frogs were found in \sim 5% of the waterbodies. Although we compared waterbody characteristics where wood frogs did and did not occur, data were insufficient to produce reliable models. Green frogs, bullfrogs, mink frogs and eastern newts were present in $>10\%$ of the waterbodies we sampled in central Ontario, and GLMs were created for their presence.

Waterbodies in central Ontario varied considerably in their chemical and physical attributes (Table [1\)](#page-3-0), which have been described previously for Algoma, Muskoka and Sudbury (McNicol et al. [1987](#page-6-21), [1995a](#page-6-8), [b;](#page-6-9) Mallory et al. [1998](#page-6-16)), and had pH measurements low enough to negatively afect amphibian breeding (Dale et al. [1985](#page-6-22); Glooschenko et al. [1992](#page-6-18)). They were typically small $(71\% < 10 \text{ ha})$ and shallow (74% < 10 m maximum depth), and many waterbodies (16%) were highly acidified ($pH < 5.1$). Most conductivity measurements (95%) were \lt 50 μ S/cm, although some waterbodies had relatively higher levels (up to 149 μ S/cm). Most waterbodies (72%) were oligotrophic (TP < 10 μ g/L), with the remainder mesotrophic (17%; 10−20 µg/L TP), and meso-eutrophic and eutrophic $(11\%; > 20 \mu g/L)$ TP;

principally waterbodies in the Muskoka region; Canadian Council of Ministers of the Environment [2004\)](#page-6-23).

Minimum pH levels for waterbodies where bullfrogs, green frogs, mink frogs, wood frogs and eastern newts were captured, respectively, were fairly acidic (generally $pH < 5$). In general, larval amphibians were found in a wide range of waterbodies with varying chemical conditions and physical attributes which in some cases difered from waterbodies where these species were absent (Table [2](#page-4-0)). Waterbodies where we captured bullfrogs were larger, deeper, and had higher pH levels than those where bullfrogs were absent, whereas green frogs were captured in smaller waterbodies with lower pH and lower conductivity levels than those where they were absent. There were no signifcant diferences in pH levels between waterbodies where mink frogs, wood frogs and eastern newts were present or absent.

The minimum pH level where fish were caught ($pH =$ 4.44) was comparable to minimum pH levels where amphibians were caught; however, fsh were captured in only 5 of 44 (11%) waterbodies with pH < 5.0 whereas amphibians were captured in a signifcantly higher proportion of low-pH waterbodies (30 of 44, 68% ; Fisher Exact test, $p < 0.001$). We caught amphibians in a higher proportion of permanent waterbodies lacking fsh (123 of 156; 79%) compared to those where fsh were captured (122 of 177; 69% Fisher Exact test, $p = 0.046$). Thus, amphibians were more commonly found in low pH waterbodies than small fish (also see Lacoul et al. [2011\)](#page-6-24).

All eastern newt and mink frog models were inadequate due to low \mathbb{R}^2 values (<0.10) and/or underdispersion, and were not considered further. For green frogs, pH was not a signifcant predictor of green frog presence in our models. Instead, various combinations of chemical and physical parameters were suitable predictors for their presence in the best-fitted models ($\triangle AIC_c < 2$), but variation explained was low overall (Tables S2, S3). Although pH was a signifcant predictor in the bullfrog models, pH was not a dominant predictor of bullfrog presence (counter to some of the earlier work on amphibian presence in this landscape; e.g., Clark

Table 1 Means (standard deviations; SD) and ranges of physical and chemical parameters of 333 permanent waterbodies sampled in central Ontario, Canada, as well as Pearson correlation coefficients among water chemistry and morphometric variables (correlations in bold were significant $(p < 0.05)$

[1992\)](#page-6-12), and other explanatory variables often had a strong infuence (e.g., conductivity; Tables S4, S5).

We grouped the waterbodies into four categories based on the number of amphibian species that we found: none (*n*=81), one (*n*=93), two (*n*=24), three or more (*n*=18), and compared overall chemical and physical waterbody characteristics by category, which suggested a signifcant diference among richness categories (MANOVA; Wilk's Lambda test criterion 0.8, $F_{1,24} = 1.7$, $p = 0.02$). However, the only parameter that strongly infuenced variation among richness groups was DOC ($F_{3,212} = 3.08$, $p = 0.029$); all other ANOVA univariate comparisons were non-signifcant (all $F_{3,212} \le 1.88$, all $p \ge 0.13$). Amphibian species richness was not well discriminated by waterbody area, depth or chemical characteristics except that richness difered in a non-linear manner among waterbodies with diferent DOC concentrations (Fig. S1), somewhat counter to Glooschenko et al. ([1992](#page-6-18)) who showed reduced amphibian occurrence with higher DOC.

Overall, our regional results suggest that pH and alkalinity may not be the most important factors afecting larval amphibian presence in permanent waterbodies and that some amphibian species can tolerate acidic conditions, which is consistent with work from other areas (Dale et al. [1985](#page-6-22); Hecnar and M'Closkey [1996;](#page-6-10) Lacoul et al. [2011\)](#page-6-24), and consistent among models across and within regions (i.e., Algoma, Muskoka, Sudbury). We cannot discount the possibility that there are synergistic, negative effects of low-pH waterbodies that negatively infuence amphibians, however, such as reduced cations entering watersheds (Jeziorski et al. [2008](#page-6-5)) and increased concentrations of toxic elements like Al (Glooschenko et al. [1992](#page-6-18); Jung and Jagoe [1995](#page-6-15)). Our data support the assertion that the relationship between the presence of amphibians and water chemistry is complex, and that amphibian presence and species richness cannot be predicted on habitat water quality alone due to an array of other biotic and abiotic factors that can directly or indirectly afect habitat requirements (Beebee and Griffiths [2005](#page-6-0); Guderyahn et al. [2016](#page-6-1)). In particular, habitat alteration, degradation and fragmentation, and watershed land-use practices can disrupt aquatic and terrestrial habitat and connectivities for amphibians (e.g., Popescu et al. [2012\)](#page-7-2), and can interfere with critical stages in the amphibian life cycle (Werner et al. [1995](#page-7-3); Guderyahn et al. [2016](#page-6-1)). The watersheds around most of the waterbodies in our study have been subject to some change from forest harvesting, especially around Sudbury where forests were cleared to fuel early metal smelting operations (e.g., Gunn [2012\)](#page-6-25). We also note that the most commonly captured amphibians in the waterbodies we sampled in central Ontario were green frogs and bullfrogs. Both species are habitat generalists, and not highly sensitive to landscape and habitat disturbance (Werner et al. [1995](#page-7-3); Jancowski and Orchard [2013](#page-6-26)), which could allow them to outcompete more sensitive species in our study area, but might also make modelling habitat preferences more difficult.

Our methods employed for capturing amphibians and recording their presence have limitations, including our protocol of using only one trapping technique which was not augmented with other approaches such as calling surveys (Parris [1999](#page-6-27)). Consequently, additional types of sampling might improve the representation of amphibian occurrence (Beebee and Grifths [2005\)](#page-6-0) and increase our somewhat low proportion of occurrence (76% of waterbodies). Furthermore, our analyses focused on whether (principally) larval amphibians were captured in the permanent waterbodies (i.e., present or absent); we did not test whether amphibians inhabited the surrounding habitat and attempted to reproduce in the sampled waterbodies. The latter question could be addressed by a survey for egg masses (e.g., Parris [1999](#page-6-27); Glooschenko et al. [1992\)](#page-6-18), and a subsequent comparison of where masses were observed and whether larvae hatched and survived. However, we undertook considerable ecological research on most of these waterbodies between 1984 and 2008 (Mallory et al. [1994](#page-6-28); McNicol et al. [1995a,](#page-6-8) [b,](#page-6-9) [c\)](#page-6-29), which included focal collections and observations along shorelines. During that research, we did not surmise that we were missing amphibian detections based on observations of egg masses, larvae or adults, nor from listening to amphibian calls. Nonetheless, we recommend additional research to address fner scale issues of possible relationships between environmentally-relevant chemistry and breeding success, as a means of interpreting our results.

Early studies of the central Ontario region clearly linked the presence of various plankton, aquatic macroinvertebrates and fish to changes in waterbody chemistry and acidification (Longcore et al. [1993](#page-6-13); McNicol et al. [1987,](#page-6-21) [1995a,](#page-6-8) [b](#page-6-9); Likens et al. [1996](#page-6-4)), and suggested the same for amphibians (Clark [1992](#page-6-12); Glooschenko et al. [1992\)](#page-6-18). However, the relationships between amphibian occurrence, and chemical and physical waterbody attributes may be more multifaceted than for other aquatic biota because most amphibians can cross terrestrial regions and exploit a variety of habitats. In this sense, drawing linkages between changes in habitat characteristics and amphibian presence is challenging, as it is for waterfowl (McNicol et al. [1995c](#page-6-29)). Our analyses suggest that amphibian distribution among permanent waterbodies in central Ontario is not predominantly infuenced directly by acidity, as pH was not a strong predictor of species occurrence, and factors other than water chemistry likely have a stronger efect. Even if our sampling techniques provided some false negatives (i.e., failed to capture amphibians in lakes where they were present), the distribution of larval amphibians that we did capture were such that most species were found in waterbodies with low bufering capacity (low pH and alkalinity), suggesting that earlier studies in central Ontario overstated the negative relationship between lake acidity and amphibian occurrence. The federal acid rain monitoring program ended in the early 2000s, and no one has returned to resurvey our study sites to date. A current effort is underway to conduct such a survey for common loons (*Gavia immer*) because they are known to be negatively infuenced by acid precipitation (McNicol et al. [1995d\)](#page-6-30). Regardless, a new survey for amphibians would help to better understand factors that strongly infuence amphibian presence in central Ontario. Future research should focus on long-term habitat change (e.g., resource development, road construction) and connectivity in the watersheds around these waterbodies, as we suspect that these factors may have a stronger infuence on amphibian presence or recovery of amphibian populations than water chemistry.

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