<u>**The Impact and Spread of the European Fire Ant, *Myrmica rubra* (Hymenoptera: Formicidae) in south-Central Ontario**</u>

by

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Completing this paper would not have been possible without the help of many generous individuals.

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Foreword

I wrote my Plan of Study wanting to critically engage with conservation policies affecting protected areas and habitat management practices within them. I wanted to focus on the presence of invasive species within protected areas due to their growing threat to biodiversity worldwide. This led to the creation of my three learning components; management of protected areas, perspectives on invasive species and natural science practice in conservation. Each of these components was explored within my final paper, albeit to varying degrees. I completed my learning objectives within these components through case studies, course work including learning protected area management strategies in Costa Rica, independent studies on protected areas, invasive species and the policies surrounding them, and my research on the European fire ant, *Myrmica rubra*, an understudied invasive species within southern Ontario.

The two components which were the primary focus within my research project were the management of protected areas, and natural science practice in conservation. My research, focusing on the current known range of *M. rubra* within south-central Ontario and its potential impacts on environmentally sensitive anuran species, helped me explore the challenges of managing invasive species within protected areas, and how natural science practice could help bridge some of those gaps. In developing this research project, understanding the fundamental challenges of the management of protected areas was a key aspect of determining the scope of the project, which helped fulfill the objectives of this component. Gaining a better understanding of the tools, literature and methodologies used within the natural sciences to help bridge gaps in ecological understanding was another key aspect of this project which helped to fulfill the objectives of this learning component.

The second component, perspectives on invasive species, is definitely explored within my research, as my final papers present a very particular view of *M. rubra*; one of a negative ecological force. However, there is more nuance within this component than my papers could viably explore. I undertook separate independent studies to understand the varying degrees to which the term "invasive species" is understood within colloquial language, as well as in policies guiding their management. My research required the assistance of the Credit Valley Conservation Authority, and through their help I was able to see the numerous challenges the field of protected area management faces; struggles in population and habitat monitoring, landscape level changes affecting habitats, and the spread of invasive species. Information gathered through collaborative research, such as the one that follows, can be helpful in managing the impacts of invasive species within this multivariate field.

Abstract

The European fire ant, *Myrmica rubra* (Linneaus; Hymenoptera: Formicidae) is a species native to Europe that has an invaded regions from the palearctic to the temperate zones of United States and Canada. It is a species that expands rapidly once introduced, and has proven hard to manage within its invaded range. Understanding its current spread and impact is a key factor in limiting the species' negative effects. In this study a combination of pitfall traps within wetland sites managed by the Credit Valley Conservation Authority (CVC), qualitative responses from a survey of naturalists and ecologists, and news reports were used to determine the current range of *M. rubra* within south-central Ontario. A second aspect of the study incorporated a qualitative analysis of the potential impact of *M. rubra* on anuran species within wetlands based on each species' life history traits. These effects were then quantified by analyzing the changes in anuran populations on wetlands with high and low *M. rubra* abundances.

Project Introduction

The Ontario Invasive Species Act of 2015 defines an invasive species as "a species not native to Ontario…," and one that "… is harming the natural environment of Ontario, or of the part of Ontario in which it is present, or is likely to harm the natural environment of Ontario or of a part of Ontario, regardless of whether it is present in Ontario or in a part of Ontario" (Ontario Invasive Species Act, 2015). Within this definition lie some of the major elements of habitat management, conservation policy, and the view on invasive species, that have spurred my interest within this field. Although the Ontario Invasive Species Act defines what is considered to be an invasive species, the terms 'natural environment,' and 'harm,' are not defined. This leaves a gap in the understanding of what activities of an invasive species are considered harmful, and hence, which invasive species are matters of concern, and which are not. Some naturalists on the other hand, have a much broader definition of invasive species that defines them as any species that has established itself outside of its native range (Richardson, et al, 2000).

In a cross-jurisdictional and cross-habitat study on the impacts of invasive species within protected spaces created for the World Bank, Poorter et al. (2007) identified invasive species to be among the top five biggest challenges facing protected area management strategies. Furthermore, in the International Union for the Conservation of Nature's (IUCN) Worlds Parks Congress held in 2005, invasive species were already identified as being a significant threat to protected areas, above infrastructure development and agricultural encroachment (IUCN, 2005). Invasive species, such as European Fire Ants (*Myrmica rubra*), pose multiple threats to protected areas. They can often out-compete native flora and fauna for resources, cause harm to any public that may interact with these protected spaces, can present a direct and indirect threat to the survival of protected species, and can often be a large economic cost if left unchecked and unmanaged (IUCN, 2005; Poorter et al. 2007). However, one of the key challenges for invasive species management within protected spaces is that in most jurisdictions, protected areas, by definition, are regions generally left unmanaged so as to help develop and regenerate naturally balanced ecosystems (Poorter et al. 2007).

Myrmica rubra are small (avg. 3mm) highly competitive ants which sting (Groden et al. 2005). *Myrmica rubra* have had negative impacts on bird reproduction rates (DeFisher et al. 2013), arthropod biodiversity (Naumann and Higgins 2015) and pollination processes (Cembrowski et. al 2014). They can also cause anaphylactic shock in people with bee sting allergies (Arevalo and Gorden 2007). Sites with high abundance of *M. rubra* ("infestations") in British Columbia were found to have reduced arthropod biodiversity compared to non-infested sites (Naumann and Higgins 2015). Infested sites can also impact human use and enjoyment of protected areas; Wetterer and Radchenko (2011) described a protected area in New York which occasionally closed due to *M. rubra* activity.

In a preliminary survey targeting naturalists in southern ON, 60% of the survey participants (n=71) were stung by *M. rubra*, and almost 70% of respondents' experienced infestation levels of *M. rubra* within their most visited parks and public spaces (Junaid Khan and Gail Fraser unpublished data). These results identify an opportunity to better understand the spread of *M. rubra*, their impact on human use of protected areas, and biodiversity within these regions.

Anurans (the order for frogs and toads) are widely considered to be biological indicators of ecological changes within a region (Allen et al. 2004; Czechowski et al. 2000; Badzinski et al.

2008) . This is due to the fact that many anuran species have traits that are finely tuned to their environments. Short reproduction seasons, temperature-dependant hibernation cycles, sensitivity to pathogens and specific habitat requirements are some of the ways in which anuran populations' help in understanding ecological changes within a region (Badzinski et al. 2008).

Of the 13 anuran species identified in Ontario, 10 have been recorded within the Credit River watershed since 2003 (CVC, 2011). Due to the unique life history strategies of each of these species, using reproduction periods, transformation and maturation timelines, habitat preferences, and overall species behavior, it is possible to develop species-specific predictions on the possible impacts of *Myrmica rubra* on each species. The following two papers analyze the geographic range of *M. rubra* within southern Ontario, and its impacts on anuran species within the region.

New distribution record of *Myrmica rubra* (Hymenoptera: Formicidae) for south-central Ontario

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Abstract: We examined the distribution of the invasive *Myrmica rubra* in south central Ontario. The species presence was originally noted in Ontario in 1975. Using news reports, online surveys and pitfall traps, we provide detailed records on its distribution within the province.

Key words: *Myrmica rubra*, southern Ontario, invasive species

Introduction

Myrmica rubra are small (avg. 3mm), polygynous, generalist ants which sting (Groden et al. 2005). In their native range of Europe and northern Asia, *M. rubra* serve as an important host species to rare butterflies belonging to the genus *Maculinea*, and the near-threatened species *Phengaris nausithos* (Arevalo and Groden, 2007; Ellison et al. 2012; Elmes et al. 1998). In their invaded range in North America, where abundant, *M. rubra* can negatively impact arthropod biodiversity (Naumann and Higgins 2015), disrupt pollination processes (Cembrowski et. al 2014) and may negatively impact ground-nesting gull chick growth rates (DeFisher et al. 2013). They can cause anaphylactic shock in people with bee sting allergies (Arevalo and Groden 2007). Sites with high densities can also impact human use and enjoyment of protected areas; Wetterer and Radchenko (2011) describe a protected area in New York which occasionally closed due to *M. rubra* activity.

The earliest reliable record of *M. rubra* in Canada is 1915 in Quebec; the species has subsequently been reported in Ontario (1975), Nova Scotia (1998), New Brunswick (2005), Prince Edward Island (2008), and British Columbia (~2010) (Naumann and Higgins, 2015; Wetterer and Radchenko, 2011). Localized records of this species in each province are lacking.

Methods

We used three approaches to create a distribution map for *M. rubra* in south-central Ontario. The first involved a collation of news reports, community bulletins, and governmental announcements relating to the presence of *M. rubra* in south-central Ontario. The second was based on field samples identified with taxonomic keys (Ellison et al. 2012; Fisher and Cover 2007; Holldobler and Wilson, 1990), and the third was a survey of naturalists and ecologists in southern Ontario. Locations identified within news reports were used to cross check data within survey reports of *M. rubra*.

News Reports

Utilizing Google, all news on the presence of *M. rubra* within southern Ontario was collected and geo-tagged using WGS84 (World Geodetic System 1984) coordinates, where applicable. Reports without any geographical locations were not included in the final range map. Reports included community bulletins, informational flyers, and mainstream news reports on the presence of *M. rubra* within regions of south-central Ontario.

Field samples

In June 14-22 2016 and June 13-20 2017 a total of 13 wetland sites within the Credit Valley watershed were sampled using pitfall traps (Table 1, Figure 1). Each site had 10 traps placed in small hand-dug holes along existing transects in place for tracking micro-habitats (Duthie, 2016). Each pitfall trap consisted of a 650 gram container filled halfway with soap, water, and propylene glycol. Small corrugated plastic covers were placed over the containers. Chicken wire fencing were placed around each trap to inhibit larger animals from falling in. Traps were deployed for 7 days, after which contents were stored in alcohol. Ants samples were sorted, counted and identified under a microscope (Nikon SMZ 1000 with magnification 0.8 – 8x) using (Ellison et al. 2012). Samples containing more than 1000 *M. rubra* were dried in a drying oven at 65^{o} C for 24 hours and weighed (100G/0.001G B1003T Electronic Balance Laboratory scale) for total ant biomass. A random subsample of 100 ants, removed 20 at a time, was weighed from each sample to determine the average ant biomass. As some traps were disturbed and contents lost, total ant numbers were divided by the number of traps set at each site to calculate the number of *M. rubra* found per trap. *Myrmica rubra* abundance across all sites was classified as low (1-50 ants per trap), moderate (51-500 ants per trap), high (501-3000 ants per trap), and very high (>3000 ants per trap).

Survey

We used a survey developed using Survey Monkey, targeting naturalists and ecologists in south-central Ontario in order to gain more widespread *M. rubra* distribution data. The survey was emailed out once in June 2016 and July 2017. The survey included true/false, ratings-based, open-ended, and multiple choice questions designed to ascertain if survey participants had been stung by *M. rubra* in south-central Ontario and the locations of the encounter. Two other common species of ants in south-central Ontario were used within the survey (*Camponotus pennsylvanicus* and *Formica rubicunda*) to ensure ants identified as *M. rubra* were correctly identified. *Camponotus pennsylvanicus* has been known to sting human beings, but its distinct size and black coloration (Ellison et al., 2012; Holldobler and Wilson, 1990) differentiates it from *M. rubra*. *Formica rubicunda* is a common species of red ant that co-occurs with *M. rubra* in south central Ontario, but is not aggressive, and is not known for stinging human beings (Ellison et al. 2012; Fisher and Cover, 2007). WGS84 coordinates were identified using Google

Maps for all named locations. Using ArcGIS, all coordinate data was transposed onto a topographical map of south-central Ontario. All locations identified within the survey were cross-referenced with news reports identifying locations where *M. rubra* had previously been reported.

Identification

Distinguishing *M. rubra* features are a scape pointing gently upward from the small and thin frontal lobes. A propodeum level with the pronotum, a pair of propodeal spines, and a miniature size (average 3mm; Figure 2) help distinguish the species (Ellison et al. 2012) from other reddish-brown ants in south-central Ontario.

Results

There were 8 reports of *M. rubra* in the news in south central Ontario (Figure 1). Locations identified as having *M. rubra* presence within news reports were Richmond Hill, Doncrest Valley, Oak Ridges, McKenzie Wetlands/Aurora, Toronto Islands, Tommy Thompson Park, Cherry Beach and the Don Valley Ravine. Only one of the news reports (Banner, 2010; McKenzie Wetlands/Aurora) identified *M. rubra* as having stung municipal workers within a local park. All other locations were identified as having high abundances of *M. rubra*.

The field samples showed very high abundances of *M. rubra* in three sites (>3000 per site), high abundance (51-500 per site) at one site, and low abundances (1-50 per site) were found in six locations (Figure 1). We had 83 survey responses, 59 (71.1%) of which indicated being stung by *M. rubra*. Of those 19 were from one site (Tommy Thompson Park, Figure 1). Five of the locations identified in news reports were also identified within the survey (Richmond Hill, Tommy Thompson Park, Cherry Beach, Don Valley Ravine, Toronto Island).

Discussion

This study shows that *M. rubra* is not uncommon in south central Ontario and while some areas show very high abundance, there is unevenness in their abundance. Many of the sites of occurrence were protected areas. One area with very high abundance, Tommy Thompson Park, is a human made site created by the dumping of construction debris (Foster and Fraser, 2014). *Myrmica rubra* at this site also shows unevenness in its distribution (Gupta et al. in press), but the very high abundance observed there is likely due to the history of the site. Like other invasive ant species, *M. rubra* are spread through human activities (Holway et al., 2002; McGlynn 1999). Similarly, urban cover around the conservation areas sampled amount appears to be an important predictor of *M. rubra* presence and abundance (Duthie et al. 2016).

Myrmica rubra colonies exhibit logistical growth (Elmes, 1973; Hochberg et al., 1994) and can out-compete most other ant and arthropod species (Naumann and Higgins, 2015). With a polygynous colony structure, *M. rubra* colony densities can reach three times the densities of other ant species in south-central Ontario (Allen et al. 2004). Sites with low abundance of *M. rubra* could translate into areas with high or very high abundance and thus should be monitored. Further work could examine the spatial distribution of this species with respect to waterways.

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Tables and Figures

Table 1: *M. rubra* abundance data from 13 sites within the Credit Valley Watershed, sampled in 2016 and 2017

Site Name	**Latitude**	**Longitude**	**Year(s) Sampled**	**Average *M. rubra* found per site**	**Abundance**
Ken Whillans	43.811	-79.932	2016	0	None
Robert Baker	43.768	-80.007	2017	0	None
Starr	43.882	-79.955	2016	0	None
Speersville	43.906	-80.016	2017	2	Low
Alton	43.838	-80.056	2016	5	Low
Erin Pine	43.775	-80.053	2016	13	Low
Warwick	43.821	-79.903	2017	16	Low
Meadowvale South	43.615	-79.715	2016	25	Low
Melville	43.924	-80.084	2016	47	Low
Cawthra	43.580	-79.576	2016	517	High
Credit River	43.554	-79.598	2016	30812	Very High
Rattray Marsh	43.517	-79.606	2016	56962	Very High
Hungry Hollow	43.639	-79.908	2016	103615	Very High

Figure 1. Map of *Myrmica rubra* distribution in south-central Ontario.


Figure 2. A worker *M. rubra* specimen

Impact of an invasive ant species (*Myrmica rubra*) on anuran populations in wetland habitats in southern Ontario

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Abstract: We examined primary habitat preferences at three life- stages, seasonally active times, and current geographic ranges for 10 anuran species found within the Credit River Watershed in southern Ontario. We compared this information with that of an invasive species of ant, *Myrmica rubra* (Linnaeus; Hymenoptera, Formicidae) to determine each anuran species' likelihood of interaction with *M. rubra*. To quantitatively test the impact of *M. rubra* on anuran populations, we collected *M. rubra* abundance from seven wetlands within the Credit River Watershed using pitfall traps. We compared the percentage of years six anuran species were present on wetlands with high and low *M. rubra* abundance. Results showed that all species qualitatively tested were likely to have some interaction with *M. rubra*. Quantitative results identified one species, *Pseudacris crucifer*, as having significant occurrence changes based on *M. rubra* abundance.

Key words: *Myrmica rubra*, southern Ontario, invasive species, anurans

Introduction

Frogs and toads (anurans) are often considered indicators of ecological conditions due to their sensitivity to anthropogenic stressors (Beebee and Griffiths, 2005; Price et al., 2007; Welsh and Ollivier, 1998) and steep declines in amphibian populations worldwide (Beebee and Griffiths, 2005; Collins and Storfer, 2003; Lips, 1999) are a cause for concern for conservationists, policy makers, and the public at large. Factors such as climate change, disease, habitat destruction, and the effects of environmental contaminants have historically been identified as key factors in this global decline (Beebee and Griffiths, 2005; DeGarady and Halbrook, 2006; Daszak et al., 2003; Pounds et al., 1999). However, there is a paucity of data on how invasive species may impact anuran populations (Beebee and Grffiths, 2005; Gibbon et al., 2000; Riley et al., 2005). A prolific invasive species – a small (approx. 3mm) red ant native to the Palearctic regions of Europe – *Myrmica rubra* (GISD, 2018) is largely unstudied for its potential impacts on anurans.

Myrmica rubra colonies exist across a wide range of habitats in their invaded range (Ito, 2014, Horton 2011, Chen 2016), but in Maine appear more often in moist soil conditions (Groden et al. 2005). They have the capacity to survive in colder climates compared to other invasive ant species (Czechowski et al. 2000). The species is most active between May and August, and enters pre-hibernation in September (Ellison et al., 2012; Groden et al., 2005). Their foraging behavior is highly generalist; they are omnivores documented to prey upon a variety of invertebrates including beetles, springtails, and various arthropods (Muller and Hilker, 1998; Naumann and Higgins, 2015; Reznikova and Penteleeva, 2001). Their presence negatively impacted herring gull (*Larus agentatus*) chick growth in Maine (DeFisher and Bonter, 2013) and qualitative reports from a survey of naturalists and ecologists in southern Ontario show some evidence of *M. rubra* preying upon small vertebrates, including salamanders and frogs (Khan et al., 2018).

Wetland dwelling anuran populations may be negatively impacted by *M. rubra*. In Canada, *M. rubra* has established populations in seven provinces (Newfoundland and Labrador, New Brunswick, Nova Scotia Quebec, Prince Edward Island, Ontario and British Columbia; Wetterer and Radchenko 2011, Naumann and Higgins 2015). An assessment of anuran populations in Ontario from 1995-2006 show declining trends in American Toads (*Bufo americanus*), Chorus Frogs (*Pseudacris triseriata*/*maculata*), Wood Frogs (*Lithobates sylvaticus*), and Northern

Leopard Frogs (*Lithobates pipiens*) (Badzinski et al., 2008). In southern Ontario, *M. rubra* was first observed within the Credit River watershed in 2001. This area hosts ten anuran species (Table 1). Following FrogWatch protocols (Badzinzki et al., 2008), the Credit Valley Conservation Authority (CVC) has data on anuran populations collected on an annual basis from 2003 to 2017. Although temporal trends between 2003 and 2008 suggest no overall changes in anuran species occurrence within the wetlands, spatial differences in trends suggest that anuran species occurrences were much lower in the lower physiographic zone than in the middle and upper physiographic zones (CVC, 2011). Green Frogs (*Lithobates clamitans*) and Spring Peepers (*Pseudacris crucifer*) were identified as the most commonly occurring species throughout the watershed, however Spring Peepers were consistently absent from the lower physiographic zone (CVC, 2011). In the Credit River Watershed urban cover and disturbance is higher in the lower zone compared to the higher zone and was also associated with increased *Myrmica rubra* abundance (Duthie 2016).

In this study, we used a qualitative and quantitative approach to consider how anurans in the Credit River watershed may be impacted by *M. rubra*. The qualitative approach considered three key metrics of interaction, for ten anuran species: 1) does a species' primary habitat overlap with *M. rubra's* primary habitat; 2) does a species' known geographic range in southern Ontario overlap with that of *M. rubra*; and 3) does a species' non-hibernation period overlap with *M. rubra's* most active times. For example, species that lay their eggs within water bodies (*B. americanus, L. sylvaticus,* etc.) likely have no overlap with *M. rubra* at this life-stage because *M. rubra* is not found directly in water (Table 1). Whereas, species that lay eggs on grasses closer to wetland edges (*L. clamitans*) likely have overlap with *M. rubra*, based on its presence in moist habitats (Table 1; Groden et al. 2005).

We quantified *M. rubra* abundance at seven CVC wetlands that also had between 12 and 15 years of anuran trends from Frog Watch efforts. Using presence/absence trends for six anuran species, we predicted that wetlands with high *M. rubra* abundance would show fewer years of a species being present compared to wetlands with low *M. rubra* abundance.

Methods

Study site

Conservation authorities in Ontario are delineated by watersheds, and the sampling for this study was done on seven wetlands within the Credit River Watershed in southwestern Ontario, managed by the Credit Valley Conservation Authority (Table 2). The CVC divides their managed wetlands into three categories, based on morphological, geological, and geographical features. The Lower physiographic zone contains over 85% of the human population within the watershed, also making it the most urbanized zone. The Middle and Upper physiographic zones lie on and above the Niagara Escarpment respectively (CVC, 2011). Wetlands sampled spanned all three physiographic zones (See Latitude/Longitude and Physiographic Zone in Table 2).

Swamps and marshes were the two types of wetlands sampled within this study, as classified by the Canadian Wetlands Classification System. Swamps have water tables at or just below the surface, are comprised of highly decomposed wood and organic material, and are dominated by trees and tall shrubs. Marshes on the other have shallow, fluctuating, surface water, have lower levels of decomposing material, and are primarily comprised of aquatic macrophytes (National Wetlands Working Group 1997).

Ant abundance

In June 14-22, 2016, all seven wetlands within the Credit River watershed were sampled using pitfall traps (Duthie, 2016; Andersen, 1991; Garnas, 2004; Naumann & Higgins 2015; Table 2). Each site had 10 traps placed in small hand-dug holes following existing transects designed by the CVC (Duthie, 2016; Khan et al., 2018). Each pitfall trap (a 650 g container) was filled halfway with soap, water, and propylene glycol. Small corrugated plastic covers were placed over the containers. Chicken wire fencing was placed around each trap to prevent larger animals from falling in. Traps were deployed for 7 days, after which contents were stored in alcohol.

Ants samples were sorted, counted and identified under a microscope (Nikon SMZ 1000 with magnification 0.8 – 8x) using Ellison et al. (2012). Samples containing more than 1000 *M. rubra* were dried in a drying oven at 65^{o} C for 24 hours and weighed (100G/0.001G B1003T Electronic Balance Laboratory scale) for total ant biomass. A random subsample of 100 ants, removed 20 at a time, was weighed from each sample to determine the average ant biomass. Wetlands were ranked by ant abundance: sites with 0-50 *M. rubra* were ranked as having no or low abundance, and sites with more than 3,000 *M. rubra* were ranked as high abundance.

Interaction Matrix – qualitative assessment of anuran interaction with M. rubra

In Maine *M. rubra* is most active, hunting for food and territory, and reproducing, between mid-May to late August (Ellison et al. 2012; Groden et al., 2005). Their current known range within southern Ontario goes east from the Credit River watershed to the shores of Lake Ontario close to the towns of Ajax and Pickering, and south from the township of Aurora to the southern-most tip of Toronto, Tommy Thompson Park (Khan et al., 2018).

For ten anuran species occurring in the Credit River watershed, we compiled the literature on: a) niche, based on primary habitat(s) use; b) the time they are seasonally active and c) their geographic range in southern Ontario (Table 1). Each category was scored based on the likelihood of interaction (Low = 1, Moderate =2 or High =3) and scores across categories were tallied, for a maximum total of nine. Species with a total score from 0 - 3 were identified as having a low likelihood of interaction with *M. rubra*, those between 4 - 6 were ranked as moderate, and those between 7 - 9 as high. Species with high or moderate scores of vulnerabilities were predicted to decline on wetlands with high *M. rubra* abundance. The primary habitat metric was split into three sub-categories, egg, early development (tadpole/toadlet/froglet) and adult, to address the often-unique habitats of each life-stage (Conant, 1998; Harding, 1997; Nature, 2013).

Anuran presence/absence trends & M. rubra

Wetlands sampled for anurans for a minimum of 12 years and maximum of 15 years between 2003 and 2017 were used in this study. Annual presence of a species was determined if a species called during any sampling period (Badzinski et al., 2008). To be included in the analysis a species had to have been present at least once across the study years. We compared the percentage of years a species was present or absent, between 2003 and 2017, for wetlands with high and low *M. rubra* abundances. There was no trend data for *M. rubra* and ants were only sampled once for abundance. We predicted that anuran species with medium or high interaction score based on the interaction matrix would have a significant difference in the proportion of years they were present within wetlands with a high abundance of *M. rubra*.

Statistical analysis

Due to the small sample sizes of wetlands being tested we used Mann-Whitney U tests to compare the percentage of years a species was present or absent for wetlands with high and low *M. rubra* abundance.

Results

Ant abundance

Three wetlands had high *M. rubra* abundance (total ants trapped per site); Hungry Hollow Wetland (103,615), Rattray Marsh (56,962), and Credit River Marsh (30,812). Four wetlands had no or low *M. rubra* abundance Ken Whillans (0), Starr (0), Melville (47), and Erin Pine (13) (Table 2).

Interaction Matrix

All ten anuran species overlapped with *M. rubra* for seasonal activity, with seven of them earning a score of 3, but species that ended hibernation in earlier spring months (March-May; *P. crucifer, L. palustris, P. triseriata*) had a lower overall overlap, and received scores of 2 (Arnold, 2000; Department of Natural Resources, 2017; Landry, 2018). Nine species overlapped with the current known range of *M. rubra* in southern Ontario (Table 1). All but one species overlapped with *M. rubra* as adults (Table 1). Two species overlapped at the tadpole/froglet/toadlet stage (*B. americanus,* and *L. sylvaticus*; Grossman, 2002; Keihl, 2015). Range overlaps varied across the species; *B. americanus* and *L. clamitans* are found ubiquitously throughout *M. rubra's* range, receiving scores of 3. *Lithobates sylvaticus*, *L. pipiens, P. crucifer,* and *L. catesbeianus* were found in more than half of *M. rubra's* range, receiving scores of 2. *Hyla versicolor*, *P. triseriata*, and *L. septentrionali* were found in less than half of *M. rubra's* current known range, receiving scores of 1. *Lithobates palustris* has not been reported in any of *M. rubra's* current known range, receiving a score of 0 for this category (Nature, 2013). Three species ranked as high for possible interaction with *M. rubra,* six ranked as moderate and one species ranked as low (Table 1).

Anuran presence/absence trends and M. rubra

Of the 10 species of anurans detected within the Credit River watershed, six were found to have been present on the seven wetlands sampled for ant abundance (Table 3). *P. crucifer* presence was negatively associated with *M. rubra* abundance (p = 0.002); the remaining five anurans showed no association (Table 3).

Discussion

In this study our aim was to determine whether the abundance of *M. rubra* on wetland habitats in southern Ontario was a determinant factor in occurrence changes for anurans using two approaches. Qualitatively, the interaction matrix showed that three species (*B. americanus*, *L. clamitans*, and *L. sylvaticus*; Table 1) have significant overlap with *M. rubra* and six with moderate interaction scores. The qualitative ranking did not match the quantitative assessment; anurans with the highest interaction scores showed no difference, and only one of the moderately ranked species showed a difference in annual presence between wetlands with high and low *M. rubra* abundance (Table 3).

The qualitative ranking revealed that primary habitat overlap with *M. rubra* in early developmental stages was not found across all species, as many anurans lay eggs within water bodies; a habitat type not known to be accessed by *M. rubra* for prey (Anderson, 2002). Similarly, species that overlap in their overall range with *M. rubra* were not always found to overlap in their adult stages. For example, *L. septentrionali* currently has a low range overlap with *M. rubra*, but due to the species spending most of its life under water (Kauzlarich, 2000), an overlap within primary habitats at any life stage is unlikely. Whereas *L. palustris* currently has no overlap with *M. rubra's* range (Nature, 2013), but the species is known to wander far from its hibernation ponds in the summer months (Arnold, 2000), making its overlap with *M. rubra* during this time more likely. Overlap in food sources was not specifically considered within this study, but *M. rubra* is a highly generalist species which feeds on vertebrates and invertebrates alike (Ellison et al., 2012; Groden et al., 2005; Czechowski et al., 2000), making competition for food sources with anurans likely. All of the species ranked with moderate or high interaction with *M. rubra* show population declines across southern Ontario *(*Badzinski et al. 2008). CVC's data trends from 2003 to 2008 also reported much lower anuran occurrences within the physiographic zone where *M. rubra* abundances were found to be the highest (CVC, 2011; Duthie, 2016).

One of the most commonly occurring species *P. crucifer* has declined in southern Ontario (Badinski et al. 2008) and was the single species showing lower annual presence in wetlands with high *M. rubra* abundance. Their relatively ubiquitous distribution and sensitivity to a variety of environmental stressors relating to habitat quality has identified *P. crucifer* as a key indicator species for environmental changes within the Great Lakes region and beyond (Knutson et al. 1999; Knutson et al., 2000; Price et al., 2007). Knutson et al. (1999, 2000) identified *P. crucifer* as an indicator of forest health within the Midwestern United States, as the species population reduced with increased deforestation. The species was also found to be an indicator of wetland health, with it being present on wetlands with better habitat quality, as quantified by Price et al. (2007). As our sample sizes were small, further research is needed to understand how *M. rubra* factors into the many drivers (Badinski et al. 2008) causing the decline of *P. crucifer* and other anurans identified as vulnerable to *M. rubra* in southern Ontario.

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Tables and Figures

Table 1: Ten anuran species qualitatively ranked for potential for interactions with *M. rubra* based on life primary habitat requirements, the timing of seasonal activities and their range overlap in southern Ontario in the Credit River watershed.

Species	Primary Habitat Overlap[a] (0-1)			Time Active Overlap[b] (1-3)	Range Overlap[c] (1-3)	Total interac-tion score (0-9) and ranking	References
	Egg	Early Development (Tadpole/Froglet/Toadlet)	Adult				
American Toad (*Bufo americanus*)	0	1	1	3	3	8 High	Grossman (2002)
Green Frog (*Lithobates clamitans*)	1	0	1	3	3	8 High	Gillilland (2000)
Wood Frog (*L. sylvaticus*)	0	1	1	3	2	7 High	Environment and Parks (2009); Kiehl (2015); Nature (2013)
Northern Leopard Frog (*L. pipiens*)	0	0	1	3	2	6 Moderate	Environment and Parks (2013); Nature (2013)
Gray Tree-Frog (*Hyla versicolor*)	0	0	1	3	1	5 Moderate	Mueller (2006); Nature (2013); NatureWorks (2017)
Spring Peeper (*Pseudacris crucifer*)	0	0	1	2	2	5 Moderate	Department of Natural Resources (2017); Nature (2013)
American Bullfrog (*L. catesbeianus*)	0	0	0	3	2	5 Moderate	Bruening, (2002); Nature (2013)
Western Chorus Frog (*P. triseriata*)	0	0	1	2	1	4 Moderate	Landry (2018); Nature (2013)
Mink Frog (*L. septentrionali*)	0	0	0	3	1	4 Moderate	Kauzlarich (2000); Nature (2013)
Pickerel Frog (*L. palustris*)	0	0	1	2	0	3 Low	Arnold (2000); Nature (2013)

[a]Primary habitat category ranking totalled to 3. Each subcategory was based on a binary response variable (Yes; 1, No; 0). The questions asked to determine score(s) were as follows:

Are the eggs found in primary habitats where *M. rubra* has be found?
Are tadpoles/froglets/toadlets found in primary habitats where *M. rubra* has been found?
Are adults found in primary habitats where *M. rubra* has been found?
[b]The ranking was based on whether the anuran species in its active/non-hibernation period (reproduction, active feeding, calling, etc) during the time of year when *M. rubra* is known to be active:
Species is active during the entirety of *M. rubra's* active period (3)
Species is active during some of *M. rubra's* active period (2)
Species is active during very little of *M. rubra's* active period (1)
Species is active during none of *M. rubra's* active period (0)
[c]The ranking was based The questions asked to determine score(s) were as follows: Is the anuran species found in the same geographic range as *M. rubra's* current known geographic range in southern Ontario? Score of 1-3 based on the following:
Species is found throughout the current known range of *M. rubra* (3)
Species is found in more than half of the current known range of *M. rubra* (2)
Species is found in less than half of the current known range of *M. rubra* (1)
Species is found in none of the current known range of *M. rubra* (0)

Table 2: *Mymica rubra* abundance data from 7 sites within the Credit Valley Watershed, sampled in 2016

Site Name	Latitude	Longitude	Physiographic Zone	*M. rubra* abundance per site	Abundance (None, Low, High)
Ken Whillans	43.811	-79.932	Middle	0	None
Starr	43.882	-79.955	Upper	0	None
Melville	43.924	-80.084	Upper	47	Low
Erin Pine	43.775	-80.053	Upper	13	Low
Credit River	43.554	-79.598	Lower	30812	High
Rattray Marsh	43.517	-79.606	Lower	56962	High
Hungry Hollow	43.639	-79.908	Lower	103615	High

Table 3: Proportions of six focal anuran species present on seven wetlands with low and high *M. rubra* abundance between 2003 and 2017 in the Credit River Watershed in Ontario.

Wetland site	**Proportion of years present (%)[c]**					
Sites with high[a] *M. rubra* abundance	*B. americanus*	*L. clamitans*	*L. sylvaticus*	*L. pipiens*	*H. versicolor*	*P. crucifer*
Hungry Hollow	3/12 (25%)	3/12 (25%)	2/12 (16.7%)	0/12 (0%)	2/12 (16.7%)	0/12 (0%)
Rattray Marsh	7/15 (46.7%)	6/15 (40%)	1/15 (6.7%)	1/15 (6.7%)	0/15 (0%)	0/15 (0%)
Credit River Marsh	3/13 (23.1%)	1/13 (7.7%)	0/13 (0%)	1/13 (7.7%)	0/13 (0%)	0/13 (0%)
Average of % ± standard deviation across sites with high *M. rubra*	31.6 ± 13.1	24.2 ± 16.2	7.8% ± 8.4	4.8% ± 4.2	5.6% ± 9.6	0.0% ± 0.0
Sites with low[b] or no *M. rubra* abundance						
Melville Marsh	7/13 (53.8%)	13/13 (100%)	0/13 (0%)	3/13 (23.1%)	1/13 (7.7%)	10/13 (77%)
Erin Pine Estates	3/12 (25%)	3/12 (25%)	3/12 (25%)	1/12 (8.3%)	3/12 (25%)	8/12 (66.7%)
Ken Whillans Wetland	1/15 (6.7%)	4/15 (26.7%)	8/15 (26.7%)	2/15 (13.3%)	2/15 (13.3%)	14/15 (93.3%)
Starr Wetland	4/15 (26.7%)	2/15 (13.3%)	0/15 (0%)	2/15 (13.3%)	7/15 (46.7%)	9/15 (60%)
Average of % ± standard deviation across sites with low or no *M. rubra*	28% ± 19.4	41.3% ±40	14% ±15	14.5% ± 6.2	23.2% ± 17.2	74.2% ± 14.5
Mann Whitney U test comparing percent years present for wetlands with	0.30	-0.80	-0.60 (p	-2.50 (p=0.06	-1.71	-10.2

high and low *M. rubra* abundance; test statistic (p value)	(p=0.80)	(p=0.50)	= 0.60)	)	(p=0.15)	(p=0.002)

[a]High abundance was defined as >3,000 ants per wetland.
[b]No or low abundance was defined as 0-50 ants per wetland.
[c]Presence based on call levels (see Badzinski et al., 2008 and Credit Valley Conservation, 2011)