THE EUROPEAN FIRE ANT, *MYRMICA RUBRA* (LINNÆUS) (HYMENOPTERA: FORMICIDAE), IN THE CREDIT RIVER WATERSHED

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Abstract

The European fire ant, *Myrmica rubra* (Linneaus) (Hymenoptera: Formicidae), is an invasive species in Canada and the United States. *Myrmica rubra* has proven to be very hard to control once it has invaded an area, and preventing further spread of the species is important for moderating its negative effects. There are limited studies of this species in Ontario. We used pitfall traps to sample *M. rubra* at ten wetlands in the Credit River watershed and explored the relationships between three factors—disturbance, wetness, and proportion of urban land use surrounding the wetlands—and *M. rubra* incidence and abundance. Three of the ten sites had a very high abundance (>30,000 ants) of *M. rubra*. The proportion of surrounding urban cover and disturbance had strong positive relationships with both the incidence and abundance of *M. rubra*. Ecological and land use data predicting *M. rubra* presence and abundance can assist in identifying sites that may be vulnerable to future invasions. We recommend incorporating ant monitoring into existing long-term monitoring efforts in southern Ontario.

Introduction

The European fire ant, *Myrmica rubra* (Linneaus), is an invasive species in North America. First detected in Massachusetts in the early 1900s, it is now found across the northeastern United States and southeastern Canada, as well as in Washington State and British Columbia (Groden et al. 2005; Wetterer and Radchenko 2011; Naumann and Higgins 2015; Chen and Adams 2018). Multiple impacts of the European fire ant in North America have been documented, including on reducing native arthropod biodiversity (Naumann and Higgins 2015; Verble-Pearson and Pearson 2016; Goodman 2018) and slowing growth rates in herring gull (*Larus argentatus* Pontoppidan) chicks (DeFisher and Bonter 2013). Field
and lab studies have also shown that *M. rubra* can increase the abundance of plant-feeding homopterans (McPhee *et al.* 2012), modify seed dispersal processes (Prior *et al.* 2015; Gammans *et al.* 2018), competitively displace native ants (Garnas *et al.* 2014), and disrupt pollination (Cembrowski *et al.* 2014). Their ability to form supercolonies, wherein ants from different nests separated by a certain distance are not aggressive towards each other, provides them with a competitive advantage and allows them to build up very high densities within infested areas (Garnas *et al.* 2007; Naumann *et al.* 2017; Chen *et al.* 2018; Warren *et al.* 2019b).

Although it has been present in Ontario since at least 1975 (Groden *et al.* 2005), the distribution and ecology of *M. rubra* in the province has only begun to be studied. Khan (2018) collected reports of the species from published works, as well as an online survey, and documented its presence across southern Ontario from Oshawa (Durham Region) in the east to Guelph (Wellington County) in the west. All field studies of the European fire ant in Ontario have focused on the Greater Toronto Area and have included investigations of its actual and potential interactions with native and invasive species (Rudmik 2011; Prior *et al.* 2015; Meadley Dunphy *et al.* 2016; Gupta *et al.* 2017; Khan 2018), a description of its population genetics in Toronto (Meadley Dunphy 2016), and a study of the ecological variables related to its presence at conservation areas (Ito 2014).

Ito (2014) found that soil moisture, soil temperature, and elevation were all significant predictors of *M. rubra* presence at 15 conservation areas in the Greater Toronto Area. However, these results may have confounded elevation with habitat type; Ito’s (2014) study sites with higher elevations—and few to no *M. rubra*—were upland habitats such as meadows and dry forests. Previous research has shown soil moisture to be a key factor in determining *M. rubra* presence in New England (Groden *et al.* 2005; Chen and Adams 2018), Ontario (Rudmik 2011), and New York (Warren *et al.* 2019a), as well as for other invasive ant species (Tschinkel 1988; Holway 2005; LeBrun *et al.* 2012). More broadly, the distribution and abundance of invasive ant species is often related to the amount of anthropogenic influence; in their review of the ecology of ant invasions, Holway *et al.* (2002) report that disturbed habitats are more susceptible to invasion by ants than natural habitats.

In combination with their ability to form supercolonies, the European fire ant’s aggressive nature and painful sting makes it a concern for park managers and municipalities where it is established (Groden *et al.* 2005; Wetterer and Radchenko 2011). Control options for *M. rubra* are limited; pesticides can temporarily reduce local populations but have not been effective at completely removing them (e.g. Warren *et al.* 2019a), and research on biological control is in its early stages (Simmons *et al.* 2015). Increased knowledge of the site characteristics associated with the presence and abundance of *M. rubra* in Ontario could assist in predicting its current and future distribution, thereby helping natural resource managers and landowners prevent establishment or locate and eradicate early infestations. In this study, we investigated how soil moisture, disturbance, and degree of urban surroundings were related to *M. rubra* abundance at ten wetland sites. Due to the small sample size in our study and the general paucity of information on *M. rubra* in Ontario, we have chosen to use the data collected in our study in hypothesis generation as opposed to using it to test a particular hypothesis. Our aim is that the results from this work will be used to direct continuing research on this invasive species.
Study sites

European fire ants were sampled at ten wetlands within the Credit River watershed in southern Ontario (Fig. 1; Table 1). The sites were selected from a set of wetlands regularly monitored by Credit Valley Conservation (CVC) based on 1) accessibility; 2) previous anecdotal fire ant observations, ranging from none to infested; and, 3) location in the watershed, with an aim to sample sites throughout the entire jurisdiction. The sites that met these criteria included two types of wetlands—swamps and marshes—as classified by the Canadian Wetlands Classification System. Swamps are dominated by trees or tall shrubs with the water table below a major area of the ground surface whereas marshes are wetter, with shallow levels of standing or slow-moving water, with vegetation primarily composed of aquatic macrophytes (National Wetlands Working Group 1997).
TABLE 1. Characteristics of ten wetland sites sampled for *M. rubra* in 2016: site number and name; geographic coordinates (given as decimal degrees); wetland community type; and the pre-study anecdotal estimates of *M. rubra* abundance.

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Site name</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Wetland community type</th>
<th><em>M. rubra</em> estimates pre-study</th>
</tr>
</thead>
<tbody>
<tr>
<td>W01</td>
<td>Rattray Marsh</td>
<td>43.517</td>
<td>-79.607</td>
<td>marsh</td>
<td>many</td>
</tr>
<tr>
<td>W02</td>
<td>Credit River Marshes</td>
<td>46.554</td>
<td>-79.599</td>
<td>marsh</td>
<td>some</td>
</tr>
<tr>
<td>W37</td>
<td>Cawthra</td>
<td>46.581</td>
<td>-79.576</td>
<td>swamp</td>
<td>none</td>
</tr>
<tr>
<td>W43</td>
<td>Meadowvale South</td>
<td>43.615</td>
<td>-79.715</td>
<td>marsh</td>
<td>some</td>
</tr>
<tr>
<td>W06</td>
<td>Hungry Hollow</td>
<td>43.639</td>
<td>-79.909</td>
<td>marsh</td>
<td>many</td>
</tr>
<tr>
<td>W11</td>
<td>Erin Pine</td>
<td>43.775</td>
<td>-80.053</td>
<td>marsh/swamp</td>
<td>some</td>
</tr>
<tr>
<td>W09</td>
<td>Ken Whillans</td>
<td>43.812</td>
<td>-79.932</td>
<td>thicket/swamp</td>
<td>some</td>
</tr>
<tr>
<td>W31</td>
<td>Alton</td>
<td>43.838</td>
<td>-80.056</td>
<td>swamp</td>
<td>none</td>
</tr>
<tr>
<td>W16</td>
<td>Starr</td>
<td>43.882</td>
<td>-79.955</td>
<td>marsh</td>
<td>none</td>
</tr>
<tr>
<td>W19</td>
<td>Melville</td>
<td>43.925</td>
<td>-80.085</td>
<td>marsh</td>
<td>none</td>
</tr>
</tbody>
</table>

CVC maintains permanent transect lines at each wetland site, to facilitate vegetation surveys as part of a long-term watershed monitoring program (CVC 2013a). Each transect is 40 m long; 1 × 1 m vegetation survey plots were set 5 m out on both sides of the center line at every 10 m along the transect, for a total of 10 vegetation survey plots per transect.

Insect collections

Ants were sampled between 14 and 22 June 2016 using pitfall traps. Ten pitfall traps were placed along the permanent CVC transect; one on either side of the transect line at each 10 m mark, as for the vegetation survey plots described above, but set an additional 10 m from the center of the transect line. Traps (650 g) were filled halfway with diluted propylene glycol, to which a small amount of dish soap was added as a surfactant. Traps were placed within a hand-dug hole so that the upper lip was flush with the ground, and then covered with a raised corrugated plastic roof to prevent rainwater from entering. A chicken-wire fence surrounded each trap to prevent the trapping of non-target organisms, particularly vertebrates. Following Naumann and Higgins (2015), traps were deployed for seven days, after which their contents were strained, rinsed with water, and stored in 95% ethanol prior to processing.

Ant identification and abundance

For samples containing low or intermediate numbers of ants (<1,000), all specimens were counted and identified, using Ellison *et al.* (2012). Samples from three sites that contained very large numbers of *M. rubra* individuals per trap (i.e. >1,000) were scanned for ants of other species, dried in an oven at 65 °C for 24 hours, and weighed to assess the approximate total number of *M. rubra*. A selection of ants from each of the larger samples were removed and identified to ensure that the sample was entirely composed of *M. rubra*. To determine average ant mass, five random samples of 20 ants were removed...
from each sample without out replacement and weighed using an 100G/0.001G B1003T Electronic Balance Laboratory scale. The average was consistent among the samples and used to determine the total number of ants in each weighed sample. While the relationship between biomass and abundance can vary from weak to strong in terrestrial arthropods, it is generally strong for samples collected using pitfall traps and restricted within taxonomic groups (e.g. families, species), as was the case in our study (Saint-Germain et al. 2007).

Environmental variables

As direct measures of site wetness and disturbance were not available, we chose to use proxies for these variables based on the composition of the vegetation community at the survey sites. As one component of CVC’s wetland monitoring protocols, all vascular plants within the ground vegetation and regeneration layers of the monitoring plots are identified to species (CVC 2013a). Each plant species in the database has an associated wetness score in the Floristic Quality Assessment System for Southern Ontario; the value is between −5 and +5, with −5 being an obligate wetland species and +5 an obligate upland species (Oldham et al. 1995; CVC 2010). A mean wetness score can be calculated for a site from the individual scores for each plant species present. This provides a representative value for the wetness of the habitat, with lower and negative numbers representing wetter sites. Values for the plant-based wetness index have been shown to have strong correlations with directly measured values of soil moisture at wetlands in southern Ontario (Francis et al. 2000).

Similarly, we chose to use the proportion of non-native plant species present in the survey plots at each wetland as a measure of anthropogenic disturbance. Our hypothesis was that sites with a higher proportion of non-native species were more likely to have higher levels of anthropogenic disturbance. This is supported by research showing strong correlations between the proportion of non-native plant species at a site and disturbance factors such as agriculture, road building, recreation, as well as housing and industrial development (e.g. Chytrý et al. 2008; McKinney 2008; LaPaix et al. 2009). Data from the last two sample years at each site were used to calculate habitat wetness and disturbance (2013–2016; Table 2). The average over the two years was used as the value for each site.

Finally, the proportion of urban cover in the landscape surrounding each site was taken from a CVC landscape analysis (CVC 2013b). In this analysis, ArcGIS was used to categorize land use in the Credit River watershed as either natural, urban, or agricultural. The amount of each land use type was calculated for an area of 2,000 m surrounding a 30 m buffer around the centre of each monitoring site (CVC 2013b). The total area of urban use was summed and divided by the total land area to determine the proportion of urban land cover surrounding each site.

Data exploration

In the context of hypothesis generation, we used scatterplots to visually assess the relationships between mean wetness, the proportion of non-native plant species, and the proportion of surrounding urban land use with two ant response variables—the proportion of pitfall traps occupied by *M. rubra* (i.e. trap-based incidence) and the log-transformed, standardized abundance of *M. rubra* and at each site.

We focused on incidence as our primary measure of *M. rubra* at each site, acknowledging that the number of individual worker ants collected in each pitfall trap was
TABLE 2. Sampled abundance of *M. rubra* and habitat characteristics of ten wetland sites sampled in 2016: total abundance of *M. rubra* collected (Abd.); the number of pitfall traps in which *M. rubra* was collected; the total number of pitfall traps used for collections; the log-transformed, trap-standardized abundance (Log abd.); mean wetness index; proportion of non-native plant species present in the ground vegetation; proportion of the surrounding land use made up of urban habitats; and the years in which vegetation sampling was conducted and from which the mean wetness index and proportion of non-native plant species were calculated.

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Site name</th>
<th>Abd.</th>
<th>Traps w. <em>M. rubra</em></th>
<th>Total traps</th>
<th>Log abd.</th>
<th>Wetness index</th>
<th>Non-native species</th>
<th>Urban cover</th>
<th>Vegetation sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>W01</td>
<td>Rattray Marsh</td>
<td>56,962</td>
<td>10</td>
<td>10</td>
<td>3.76</td>
<td>-3.49</td>
<td>0.29</td>
<td>0.45</td>
<td>2015, 2016</td>
</tr>
<tr>
<td>W02</td>
<td>Credit River Marshes</td>
<td>30,812</td>
<td>8</td>
<td>8</td>
<td>3.59</td>
<td>-2.41</td>
<td>0.31</td>
<td>0.68</td>
<td>2014, 2016</td>
</tr>
<tr>
<td>W37</td>
<td>Cawthra</td>
<td>517</td>
<td>6</td>
<td>10</td>
<td>1.72</td>
<td>-0.21</td>
<td>0.13</td>
<td>0.80</td>
<td>2015, 2016</td>
</tr>
<tr>
<td>W43</td>
<td>Meadowvale South</td>
<td>25</td>
<td>8</td>
<td>10</td>
<td>0.54</td>
<td>-1.89</td>
<td>0.19</td>
<td>0.55</td>
<td>2014, 2016</td>
</tr>
<tr>
<td>W06</td>
<td>Hungry Hollow</td>
<td>103,615</td>
<td>10</td>
<td>10</td>
<td>4.02</td>
<td>-3.39</td>
<td>0.21</td>
<td>0.56</td>
<td>2013, 2015</td>
</tr>
<tr>
<td>W11</td>
<td>Erin Pine Estates</td>
<td>13</td>
<td>3</td>
<td>10</td>
<td>0.36</td>
<td>-3.61</td>
<td>0.06</td>
<td>0.32</td>
<td>2014, 2016</td>
</tr>
<tr>
<td>W09</td>
<td>Ken Whillans</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0.00</td>
<td>-2.24</td>
<td>0.29</td>
<td>0.13</td>
<td>2015, 2016</td>
</tr>
<tr>
<td>W31</td>
<td>Alton</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>0.18</td>
<td>-2.61</td>
<td>0.07</td>
<td>0.07</td>
<td>2014, 2016</td>
</tr>
<tr>
<td>W16</td>
<td>Starr</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0.00</td>
<td>-3.60</td>
<td>0.10</td>
<td>0.07</td>
<td>2015, 2016</td>
</tr>
<tr>
<td>W19</td>
<td>Melville</td>
<td>47</td>
<td>7</td>
<td>10</td>
<td>0.76</td>
<td>-3.80</td>
<td>0.18</td>
<td>0.46</td>
<td>2013, 2015</td>
</tr>
</tbody>
</table>

*Total abundance of *M. rubra* at these sites was not counted directly but rather estimated using the mass of dried samples (see Methods for details).

*Some sites had fewer than 10 sample traps due to an inability to place traps in areas with standing water or due to traps being disturbed.
likely to be highly dependent on its proximity to one or more colonies and may overestimate the true number of colonies at the site (Longino 2000; Gotelli et al. 2011). Nonetheless, we have also included the total abundance of *M. rubra* at each site as a response variable as it better reflected the differences among sites. For example, we considered that the difference in *M. rubra* populations between Meadowvale South (25 individual ants, 8/10 occupied traps) and Credit River Marshes (estimated 30,812 ants, 8/8 occupied traps) was larger than implied by the difference in incidence alone. Prior to plotting, the total number of *M. rubra* at each site was standardized for sample effort by dividing by the total number of the traps used at the site. Standardized ant data were further log-transformed to account for the large differences in *M. rubra* abundance among sites.

**Results**

Eight of the ten sites sampled had *M. rubra*; three were heavily infested (>30,000 ants collected), one had a relatively moderate presence (>500), and the remaining four sites had very low (<50) presence (Table 2). At Starr and Credit River Marshes, not all traps could be placed along the transect line due to high water levels. At Ken Whillans, one of the traps was overturned. Three of the sites with moderate to heavy *M. rubra* infestations (Rattray Marsh, Cawthra, and Credit River Marshes) were geographically very close together, while one (Hungry Hollow) was more isolated (Fig. 1).

Scatterplots of our data showed positive relationships between both the proportion of invasive plants and the proportion of surrounding urban land use with both ant incidence and ant abundance. By contrast, there was no relationships between mean wetness and both ant response variables indicated by horizontal trendlines (Fig. 2).

**Discussion**

In this study, we estimated the strength of the relationship between three environmental factors—wetness, proportion of non-native plants (as a measure of disturbance), and surrounding urban cover—and *M. rubra* incidence and abundance at wetlands throughout the Credit River watershed in southern Ontario. Both ant incidence and ant abundance increased with increasing disturbance as well as with an increasing amount of urban cover in the surrounding area. Below we discuss some potential explanations of our observations and suggest directions for additional research.

The positive relationship between the proportion of urban cover and *M. rubra* abundance is a new finding for this species. Like other invasive ants, the spread of *M. rubra* in North America is likely largely due to the movement of infested plants, soils, and other materials (Groden et al. 2005; Chen and Adams 2018). For this reason, urban settings and developed areas are often associated with higher abundance and dominance of invasive ant species. The distribution of the Argentine ant (*Linepithema humile* (Mayr)) has been shown to be influenced by human land use both globally (Roura-Pascual et al. 2011) and locally (Carpintero et al. 2004; Bolger 2007; Fitzgerald and Gordon 2012). The establishment of red imported fire ant (*Solenopsis invicta* Buren) colonies also increases
FIGURE 2. Scatterplots showing the relationships between mean wetness (a and b), proportion of non-native plants in the ground vegetation (c and d), and proportion of urban land use in the surrounding 2,000 m (e and f) with the trap-based incidence of *M. rubra* (a, c, e) as well as the log-transformed standardized abundance of *M. rubra* (b, d, f) at ten wetlands in the Credit River watershed, Ontario. The correlation coefficient Pearson’s R is shown on each scatterplot.
near human dwellings (Forys et al. 2002). The relationship between urbanization and invasive ants extends beyond these two well-known species; Lessard and Buddle (2005) found that the relative abundance of the introduced pavement ant, *Tetramorium caespitum* (Linnaeus) increased along a transect from natural to urban sites in Quebec.

Anthropogenic disturbance, in the form of mowing, plowing, and other soil excavations, has been found to be related to increased prevalence and densities of both the Argentine ant (King and Tschinkel 2008) and the red imported fire ant (Tschinkel 1988; LeBrun et al. 2012). It can be difficult to separate the effects of urbanization from those of anthropogenic disturbance; the two are generally related since humans disturb areas that are close to developed areas, either as a result of development itself or because of proximity to development. In our study, Pearson’s R for the variables we used to represent disturbance and urbanization (proportion of non-native plant species present in the ground vegetation and the proportion of the surrounding land use made up of urban habitats, respectively) was estimated to be 0.32. Although this is not an insignificant relationship, we did not feel that it was strong enough to make investigating the relationships of both environmental variables with ant incidence and abundance redundant. It is possible that we would have found a stronger relationship between anthropogenic disturbance and *M. rubra* abundance if we had used a direct measure of disturbance rather than a measure inferred from the proportion of invasive plants. However, finding a variable to provide a direct measure of disturbance can be challenging and using existing monitoring data was parsimonious. In addition, we note that in at least one of the studies cited above, the disturbed areas with high ant abundance also had an increased prevalence of weedy plant species (Tschinkel 1988), lending support to our use of the proportion of invasive species as a proxy for disturbance.

Habitat wetness showed no relationship with *M. rubra* incidence and abundance in our study. We expected that wetness would show a positive relationship with *M. rubra* incidence and abundance based on literature that suggests it is an important factor in the ecology of invasive ant species, including the European fire ant (Gorden et al. 2005; Rudmik 2011; Ito 2014; Chen and Adams 2018; Warren et al. 2019a). It is possible that the use of direct soil moisture measurements instead of a vegetation-based proxy may have led to stronger association between wetness and *M. rubra* abundance, as has been seen in other studies. However, we must also consider that that the small range of variation in wetness in our study may explain the lack of a directional relationship. All of our study sites were wetlands, which seem to be the preferred habitat for the European fire ant. In a large survey of southern New England, Chen and Adams (2018) found that the species was most prevalent in freshwater marshes and wet meadows as well as wetter forests, riparian habitats, and forested wetlands while dry forests, old fields, and mown grass were rarely used.

In terms of identifying other factors affecting *M. rubra* distribution, we suggest that future research might benefit from examining both the landscape scale and microhabitat scale. Although much of the spread of *M. rubra* is due to human-assisted movement, colonies also expand naturally into adjoining areas (Groden et al. 2005; Chen and Adams 2018). Given that many *M. rubra* populations are in wetlands in riparian areas, their spread may be facilitated by the connectivity of suitable habitat in these natural corridors as well as the movement of individuals or even nests by water as with other invasive species (e.g. Hood and Naiman 2000). A landscape assessment of suitable habitat combined with connectivity
to existing populations may help to locate new or potential *M. rubra* nesting infestations. At the microhabitat scale, recent research in the United States has described the general habitat types and abiotic conditions most favoured by *M. rubra* (Chen and Adams 2018; Warren *et al.* 2019a). Gathering more data with respect to the particular soil types and plant species associated with *M. rubra* presence and abundance would assist in understanding the potential distribution, impact and management of this species.

Our study built upon existing monitoring efforts of wetland habitats in the Credit River watershed. We suggest incorporating an ant monitoring program, which could be linked to other biotic wetland monitoring programs such as CVC’s anuran monitoring (CVC 2010), permitting an analysis of the impact of *M. rubra* on amphibian biodiversity. Wetlands provide numerous ecosystem services, including critical habitat for rare and threatened species. The majority of wetlands in southern Ontario have been converted to agricultural or urban uses over the past 200 years (Ducks Unlimited Canada 2010), and the remaining wetlands face numerous threats including ongoing conversion, climate change, and invasive species. Our findings suggest that wetlands within urban landscapes are more likely to have high *M. rubra* abundances, posing another significant threat to the biodiversity in these sensitive communities. Land managers can focus efforts on informing the public of the presence of *M. rubra* at known locations, recommending that users of urban wetlands be on the lookout for new infestations, and restricting movement of soil or other biotic material to reduce the potential spread of this species.

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