Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

1 **Title**

2 Low prevalence of the parasite *Ophryocystis elektroscirrha* at the range edge of the eastern

3 North American monarch (*Danaus plexippus*) butterfly population

4 **Authors**

5 Felipe Dargent[1*], Sydney M. Gilmour[1], Emma A. Brown[1], Rees Kassen[1], and Heather M.

6 Kharouba[1]

7 [1]University of Ottawa, Department of Biology, 30 Marie Curie Pvt., K1N 6N5, Ottawa, ON,

8 Canada.

9 *Corresponding author: fdargent@uottawa.ca

10

11

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Abstract

Every year monarch butterflies (*Danaus plexippus* Linnaeus, 1758) from the eastern North American population migrate from Mexico to Southern Canada in the spring. This northward migration has been shown to reduce monarch infection with the host-specific parasite *Ophryocystis elektroscirrha* (OE) (McLaughlin and Myers, 1970); yet, the prevalence of OE at their range limits, and the mechanism(s) responsible, is unknown. We assessed OE infection levels of monarchs at the northern edge of the eastern population distribution around Ottawa, Canada, and found extremely low levels of infection (~1% with upper confidence intervals close to 3%). Low OE infection levels are likely due to low densities of monarchs in this region and/or migratory escape effects, where migrating individuals leave behind areas with high density of conspecifics and high potential for parasite accumulation and transmission. Future work should aim to disentangle the relative contribution of these two mechanisms for governing the decrease in parasitism at the range limits of migratory populations.

Keywords

Monarch butterflies, *Danaus plexippus*, OE, *Ophryocystis elektroscirrha,* range limit, host-specific parasite, prevalence, disease ecology, migration

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

INTRODUCTION

Every spring, monarch butterflies (*Danaus plexippus* Linnaeus, 1758) of the eastern North America population depart their wintering grounds in the highlands of central Mexico (Urquhart and Urquhart 1978) and engage in one of the most striking animal migrations in the world. Individuals born in the previous year leave Mexico in the spring and travel north towards their breeding grounds across the eastern US to Southern Canada (including southern Quebec, Ontario and Manitoba), an area covering over 4000 km (Urquhart and Urquhart 1978; Brower 1995; Flockhart et al. 2019*a*). This northward migration is completed through 3-4 generations. In contrast, the 'return' fall migration southwards is generally completed in a single generation (Prysby and Oberhauser 2004; Solensky 2004).

Importantly, the migration process is influenced by interactions with the host-specific neogregarine parasite of monarchs, *Ophryocystis elektroscirrha* (OE) (McLaughlin and Myers, 1970). Infection with OE can be detrimental to monarchs and may impact population density and persistence (Altizer et al. 2004). For example, infection has been shown to kill pupae, lead to unsuccessful eclosion, shorter female life span (De Roode et al. 2008), wing malformations and decreased flight ability (Bradley and Altizer 2005). It has also been shown to reduce body size and reproductive success (Altizer and Oberhauser 1999).

OE only infects its host when monarch larvae ingest dormant spores (i.e. gregarine oocyte stage) scattered on their egg, which larvae eat upon eclosion, or on *Asclepias* spp., the host plants used by adult butterflies (McLaughlin and Myers 1970; De Roode et al. 2009). After ingestion, OE sporozoites migrate to the hypoderm, reproduce, and spores are deposited in the butterfly cuticle, predominantly on the abdomen (McLaughlin and Myers 1970). Upon emergence, infected hosts may carry millions of oocytes (McLaughlin and Myers 1970; Leong et

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

al. 1992). There are three hypothesized transmission pathways of OE. Maternal (vertical) transmission occurs during oviposition (McLaughlin and Myers 1970) and is thought to account for over 90% of OE transmission (Altizer et al. 2004). Female butterflies transmitting the spores could be infected themselves, and therefore may carry high loads of OE, or might be uninfected but still carry low spore loads following mating or contact with infected adults (i.e., adult transmission (Altizer et al. 2004). Alternatively, infected butterflies resting or nectaring on milkweed can release OE spores that accumulate and are later consumed by monarch larvae (i.e., environmental transmission; Altizer et al. 2004). Although OE is host-specific to monarchs, there is current debate on whether they also infect an species absent in our study area, Queen butterflies (*Danaus gilippus* Cramer, 1775) (Barriga et al. 2016).

While OE infection seems to occur in almost all monarch populations in the world (Altizer and de Roode 2015), the prevalence of the infection (i.e. the percentage of infected individuals) can vary considerably among populations anywhere from 5% up to 100% in some populations (Altizer et al. 2000). It has been noted previously that the eastern North America population has lower OE prevalence than the western North America population, which covers a shorter migratory range from Mexico along the coast of California, and the non-migratory populations in the gulf of Mexico (Altizer et al. 2000).

There are two main theories thought to explain the variation in parasite prevalence within and among migrating monarch populations. The migratory escape hypothesis proposes that as individuals migrate, they leave behind areas where the density of conspecifics has been increasing and thus have higher potential for parasite accumulation and transmission (Folstad et al. 1991; Loehle 1995). Indeed, eastern monarchs travelling north during the breeding season are thought to benefit from migration-facilitated escape from contaminated habitats where OE

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

accumulates over time due to increasing densities of hosts and parasite (Bartel et al. 2011; Flockhart et al. 2018). Alternatively, the migratory culling hypothesis contends that due to the high energetic demands of migration, infected individuals face higher mortality than uninfected individuals during migration and are therefore disproportionally removed from the population (Bartel et al. 2011). This process is thought to occur during the fall migration, when individuals travel longer distances and experience higher energetic demands, leading to lower prevalence in monarchs as they move southward (Bradley and Altizer 2005; Altizer et al. 2011, 2015; Bartel et al. 2011). Thus, northward and southward migration is thought to support different parasite release processes.

Despite extensive study of the eastern North America monarch population (Altizer and de Roode 2015), the prevalence of OE at the northern range limit remains unclear. In particular, additional ecological mechanisms at range limits could influence OE prevalence in their monarch hosts. For example, the low density and decreased connectivity that is typical of subpopulations of organisms at their distribution edge (Sexton et al. 2009) has been shown to be insufficient to support stable parasite populations in other species (Gaston 2009) and can constrain re-colonization of parasites following local extinctions (e.g. Keeling et al. 2004; Kaunisto et al. 2015). Understanding infection patterns at the northern edge of the monarch distribution is important as these are sites predicted to have increased presence of monarchs and milkweed under some climate change scenarios (Lemoine 2015).

We surveyed monarch butterflies and their OE infection rates around Ottawa, Canada, which is at the northern range limit of the eastern North American population. Migrating monarchs consistently reach this region every year, whereas the presence of monarchs in sites further north is less reliable (Flockhart et al. 2019*b*).We predicted that monarchs in this region

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

would have extremely low or no infection by OE based on previous support for the migratory escape hypothesis and the seasonality and length of growing season at this latitude. Monarch residency time is thought to be shorter in this region than further south due to the distance from the overwintering grounds, the shorter growing season, and the earlier defoliation of milkweed in the fall (Bhowmik and Bandeen 1976). These factors reduce the accumulation time for parasites, restrict the host population density, and reduce the accumulation of OE spores through the season and eliminate them from one year to the next.

METHODS

Field collections

We collected eggs, larvae and adults at 29 locations across the city of Ottawa and its surroundings (Figure 1) between June 18th and September 25th 2019 (i.e., covering the monarch breeding season in our region). We collected these samples opportunistically as part of other projects looking into milkweed phenology and milkweed-specialist community diversity. All our collections were done at sites where common milkweed (*Asclepias syriaca* L.), the main host plant for monarch larvae in the region, was present. These sites covered diverse land-use types: urban, suburban, forested and old fields. They spanned an East-West distance of approximately 70 km, and a North-South distance of approximately 23 km (Figure 1). At each site, we inspected at least 60 milkweed plants for eggs and larvae. We noted the instar at the time of collection and transported them to our laboratory at the University of Ottawa to be reared into adults (see below). We also attempted to capture adults that were within 10 m of us, and estimate that our capture success rate was about 30%. We trapped adults with butterfly nets, sampled OE

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

in situ (see below) and then released individuals. In the rare instance when we sampled multiple adults at the same site and day, we kept track of each individual to avoid resampling.

Larval rearing

Eggs and larvae were reared in a controlled environmental chamber (Biochambers Inc., model LTCB-19), with a 12:12 hour light-dark cycle, constant humidity of 60% and a temperature of 28°C during the light cycle and 26°C during the dark cycle, approximating the optimal temperature for larval development (i.e. 27°C - Zalucki 1982; Nail et al. 2015) and OE reproduction (Lindsey 2008). That is, raising hosts and parasite at their optimal temperature increased our chances of detecting whether OE is present in our study area. Larvae were housed individually in 500 ml plastic or glass containers with a fine mesh lid and were fed *ad-libitum* once a day with fresh common milkweed leaves collected from a single site in the field (site of collection varied daily). We checked that leaves were free of dirt but did not wash them with diluted bleach as we were more concerned by the potential negative effects of bleach on the larvae than possible cross infection from OE spores left by infected adults on our milkweed. We deemed the risk of OE accidental infection low because monarch densities in our study area are very low: based on 32 site visits (10 sites visited twice, and 12 with a single visit) between July 9th and September 17th, we found an average number of 0.016 monarch larvae per milkweed stem (no monarchs were found during 17 visits, 266 stems checked on average per site visit) (Dargent et al. *unpublished*). We removed frass and cleaned cages every two days to maintain clean conditions. Upon formation of a chrysalis, we checked the cage daily until an adult emerged. Adults were then sampled for OE infection.

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

145 ***OE sampling***

146 Following the methods of Altizer et al. (2000) we collected OE spores by placing a 1 inch
147 clear envelope sticker (Pop Resin) on the abdomen of each adult and then placing it on a 3x5
148 inch index card. Cards were then inspected under a light optical microscope at 40x and the whole
149 surface was inspected for OE. When OE was detected, we estimated the number of OE spores
150 following Altizer et al. (2000). All laboratory-reared adults were sexed and sampled for OE
151 within a day of eclosion.

152

153 ***Analysis***

154 We estimated 95% confidence intervals for prevalence using the adjusted bootstrap
155 percentile (BCa) method with 1000 resamplings. We performed analyses using the "boot"
156 package (Canty and Ripley 2019) in R (R Core Team 2018).

157 To provide a broader Canadian context to our results, we also calculated prevalence on a
158 subset of the Flockhart et al. (2018) publicly available dataset collected in 2011 (Flockhart et al.
159 2018– Appendix S2). Since we wanted to evaluate prevalence in the northeastern portion of the
160 range (i.e. southeastern Canada), we used data north of 42.5777°N and east of 83.4511°W, which
161 is roughly between the northeast point of Windsor, Ontario and southwest of Ottawa (Figure 1).

162

163 **RESULTS**

164 We collected 140 pre-adult individuals that were reared in the laboratory (n= 65 males,
165 74 females, 1 unidentified) from 28 out of 29 locations surveyed (mean ±SEM individuals per
166 location: males=2.5±0.3; females=2.3±0.3; total=4.8±0.4) (Figure 1, Table S1). We sampled 27
167 adult butterflies in the field (*n*= 12 males, 14 females, 1 unidentified) at 10 locations (mean

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

±SEM individuals per location: males=1.1±0.3; females=1.5±0.3; total=2.7±0.4;) (Figure 1, Table S2).

Out of these 167 individuals, only one female, collected as an egg and reared in the lab, was infected with OE. It had a high infection (>1000 spores). Thus, if we consider our samples to be truly independent (i.e., each offspring laid by a different mother), OE infection prevalence in the city of Ottawa and its surroundings was 0.6% (0, 1.8 95% CI). Alternatively, to account for potential pseudo-independence (each mother lays more than one egg per day and site), we also tested prevalence assuming that on a given sampling day, only samples from adults, and different pre-adult developmental stages from the same site, were independent (n=90). For example, all first instar larvae collected on the same date and at the same location were treated as only one sample. With this conservative approach, we estimated prevalence to be 1.1% (0, 3.33 95% CIs) out of 90 samples.

In comparison, 59.1% of the butterflies (n=98) from the Flockhart et al. (2018) dataset were infected with OE at a low level (i.e. <100 spores) and 14.3% at a high level (i.e. >100 spores). Spore loads of less than 100 may reflect the attachment of spores to an adult, which does not have any effect on the condition of that adult per se (i.e., not a 'true' infection). A 'true' infection follows spore consumption as a larvae (De Roode et al. 2007, 2009).

DISCUSSION

We collected monarch butterflies from 29 locations at the northern edge of the distribution of the eastern North American population and found exceptionally low levels of infection (~1% with upper confidence intervals close to 3%) with the parasite *Ophryocystis elektroscirrha* (OE). Previous assessments of OE infection levels for the entire eastern North

© The Author(s) or their Institution(s)

For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.
Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21

American population report the prevalence of infected individuals (i.e. carrying >100 spores) to be 7% (Altizer and de Roode 2015). However, infection levels range between 2.5% and 18% among years in the northeast part of the population (Bartel et al. 2011). Our OE estimates were also lower than the ones extracted from data collected south of our study area in southeastern Canada (Flockhart et al. 2018). Importantly, the northernmost collection site (45.10318°N, 75.22101°W) for the region sampled by Flockhart et al. (2018) is approximately 35 km south of our study area, and thus closer to the core of the monarch distribution than our samples. This could help explain why their estimate of prevalence Was higher than ours (approximately 10% based on Figure 3 – Flockhart et al. 2018). Although variability in OE infection levels among years and locations can be high (e.g. Bartel et al. 2011), our comparatively low estimate of prevalence suggests that, at least within the year sampled, individuals at the northern edge of their distribution may experience lower infection levels than individuals closer to the core of their distribution.

There are two factors related to migration and edge effects that likely explain the low OE infection levels we found at the northern range edge. First, previous studies have found infection patterns related to the northward migration of the eastern North America population during the spring/summer breeding period consistent with the migratory escape hypothesis. Specifically, that prevalence increases over the season in all areas (as monarch populations grow), southern areas have higher infection rates than northern ones (Bartel et al. 2011; Flockhart et al. 2018), and monarch larval density is positively correlated with OE prevalence (Bartel et al. 2011). Additionally, during the spring/summer migration, regional differences in prevalence are attributable to differences in local infection levels rather than due to differential migration distances between infected and non-infected individuals (i.e. migratory culling) (Flockhart et al.

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

2018). Our findings are consistent with these studies and suggest that the process of migratory escape leads to lower OE prevalence at the limits of the geographic distribution.

Second, the low density of monarchs at their range edge may be insufficient to maintain its host-specific parasite, as seen in other host species (e.g. holly leaf-miners attacked by parasitoids (Brewer and Gaston 2003); Asian house geckos infected by mites (Coates et al. 2017); bumblebees under nest parasitism by *Psythirus spp*. cuckoo bumblebees (Antonovics and Edwards 2011). Models by Flockhart et al. (2019*b*) using community science data show that monarch density is lower in this part of their northern range than in more southern areas. Indeed, we found lower density at our sites than Bartel et al (2011) did in their northeastern Region (>36°30” N). Additionally, since monarch adults cannot become infected (only carry spores if they become exposed) and infection can only occur through ingestion by larvae (McLaughlin and Myers 1970), transmission opportunities might be constrained even further than for other species living at their range edge. In combination, these two mechanisms could explain the exceptionally low OE prevalence at our sites. Future work should aim to disentangle the relative contribution of these two mechanisms for decreasing parasitism at the range limits of migratory populations.

Despite our estimate of prevalence being based on 167 individuals, we do not think our results reflect a limited ability to detect higher prevalence levels (i.e. low detection). While we had a smaller sample than broader-scale studies that rely on community science data (e.g. Bartel et al. 2011; Flockhart et al. 2018), we had a better sampling effort (e.g. number of collected individuals per km^2) than other studies done in nearby regions (e.g. Flockhart et al. 2018). This sampling effort, combined with our sampling during the breeding season in our region, increases

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

our confidence that our prevalence estimate accurately reflects typical OE loads in our study area.

Our study suggests that the current prevalence of OE infection at the northern range edge of the Eastern North American monarch population is very low. As our samples are from a single year in a relatively small area, longer-term studies of OE infection across a larger geographical extent are needed to determine the temporal dynamics of OE infection in this region. Actions to protect monarch populations should integrate disease dynamics across the whole distribution range and evaluate the broader impacts of parasite evasion at the range limits.

Acknowledgements

We thank M. Veselovsky and M. Reich for help with larva collection and rearing, S. Baatnes for access to OE training samples, P Tremblay for help with mapping, and C. Angell for comments on the manuscript. We thank eButterfly and all of their affiliated citizen scientists for collecting, reporting and vetting butterfly observation data used in this publication; the National Capital Commission and the Parc du Gatineau for providing access to their sites; the Ontario Ministry of Natural Resources and Forestry for collection permits. FD was supported by a Frederic Banting Memorial Fellowship (NSERC). HMK was supported by an NSERC Discovery Grant. Equipment used in this study was acquired through support from the Canadian Foundation for Innovation and Ontario Research Fund: Small Infrastructure (to HMK).

References

Agriculture and Agri-Food Canada (2010) Land use for Canada. https://open.canada.ca/data/en/dataset/9e1efe92-e5a3-4f70-b313-68fb1283eadf.

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Accessed 15 Nov 2018

Altizer, S., Bartel, R., and Han, B.A. 2011. Animal migration and infectious disease risk. Science. **331**(6015): 296–302. doi:10.1126/science.1194694.

Altizer, S., Hobson, K.A., Davis, A.K., De Roode, J.C., and Wassenaar, L.I. 2015. Do healthy monarchs migrate farther? Tracking natal origins of parasitized vs. uninfected monarch butterflies overwintering in Mexico. PLoS One **10**(11): 141371. doi:10.1371/journal.pone.0141371.

Altizer, S., and de Roode, J.C. 2015. Monarchs and their debilitating parasites: immunity, migration, and medicinal plant use. *In* Monarchs in a changing world: biology and conservation of an iconic butterfly. *Edited by* S. Altizer, K.S. Oberhauser, and K.R. Nail. Cornell University Press, Ithaca, New York. pp. 83–94.

Altizer, S.M., and Oberhauser, K.S. 1999. Effects of the protozoan parasite *Ophryocystis elektroscirrha* on the fitness of monarch butterflies (*Danaus plexippus*). J. Invertebr. Pathol. **74**(1): 76–88. doi:10.1006/jipa.1999.4853.

Altizer, S.M., Oberhauser, K.S., and Brower, L.P. 2000. Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. Ecol. Entomol. **25**(2): 125–139. doi:10.1046/j.1365-2311.2000.00246.x.

Altizer, S.M., Oberhauser, K.S., and Geurts, K.A. 2004. Transmission of the protozoan parasite, *Ophryocystis elektroscirrha*, in monarch butterfly populations: implications for prevalence and population-level impacts. *In* The monarch butterfly: biology and conservation. *Edited by* K.S. Oberhauser and M.J. Solensky. Cornell University Press, Ithica, New York. pp. 203–218.

Antonovics, J., and Edwards, M. 2011. Spatio-temporal dynamics of bumblebee nest parasites (*Bombus* subgenus *Psythirus* ssp.) and their hosts (*Bombus* spp.). J. Anim. Ecol. **80**(5): 999–1011. doi:10.1111/j.1365-2656.2011.01846.x.

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

282 Barriga, P.A., Sternberg, E.D., Lefèvre, T., de Roode, J.C., and Altizer, S. 2016. Occurrence and host
283 specificity of a neogregarine protozoan in four milkweed butterfly hosts (*Danaus* spp.). J. Invertebr.
284 Pathol. **140**: 75–82. doi:10.1016/j.jip.2016.09.003.
285 Bartel, R.A., Oberhauser, K.S., De Roode, J.C., and Altizer, S.M. 2011. Monarch butterfly migration
286 and parasite transmission in eastern North America. Ecology **92**(2): 342–351. doi:10.1890/10-
287 0489.1.
288 Bhowmik, P.C., and Bandeen, J.D. 1976. The biology of Canadian weeds: 19. *Asclepias syriaca* L. Can.
289 J. Plant Sci. **56**(3): 579–589. doi:10.4141/cjps76-094.
290 Bradley, C.A., and Altizer, S. 2005. Parasites hinder monarch butterfly flight: implications for disease
291 spread in migratory hosts. Ecol. Lett. **8**(3): 290–300. doi:10.1111/j.1461-0248.2005.00722.x.
292 Brewer, A.M., and Gaston, K.J. 2003. The geographical range structure of the holly leaf-miner. II.
293 Demographic rates. J. Anim. Ecol. **72**(1): 82–93. doi:10.1046/j.1365-2656.2003.00682.x.
294 Brower, L.P. 1995. Understanding and misunderstanding the migration of the monarch butterfly
295 (Nymphalidae) in North America: 1857-1995. J. Lepid. Soc. **49**(4): 304–385.
296 Canty, A., and Ripley, B.D. 2019. boot: Bootstrap R (S-Plus) Functions.
297 Coates, A., Barnett, L.K., Hoskin, C., and Phillips, B.L. 2017. Living on the edge: parasite prevalence
298 changes dramatically across a range edge in an invasive gecko. Am. Nat. **189**(2): 178–183.
299 doi:10.1086/689820.
300 Flockhart, D.T.T., Acorn, J.H., Hobson, K.A., and Norris, D.R. 2019*a*. Documenting successful
301 recruitment of monarch butterflies (Lepidoptera: Nymphalidae) at the extreme northern edge of
302 their range. Can. Entomol. **151**(1): 49–57. doi:10.4039/tce.2018.52.
303 Flockhart, D.T.T., Dabydeen, A., Satterfield, D.A., Hobson, K.A., Wassenaar, L.I., and Norris, D.R.
304 2018. Patterns of parasitism in monarch butterflies during the breeding season in eastern North

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

America. Ecol. Entomol. **43**(1): 28–36. doi:10.1111/een.12460.

Flockhart, T.D., Larrivée, M., Prudic, K.L., and Norris, R.D. 2019*b*. Estimating the annual distribution of monarch butterflies in Canada over 16 years using citizen science data. FACETS **4**(1): 238–253. doi:10.1139/facets-2018-0011.

Folstad, I., Nilsseen, A.C., Halvorsen, O., and Anderson, J. 1991. Parasite avoidance: the cause of post-calving migrations in *Rangifer*? Can. J. Zool. **69**(9): 2423–2429. doi:10.1139/z91-340.

Gaston, K.J. 2009. Geographic range limits: achieving synthesis. Proc. R. Soc. B Biol. Sci. **276**(1661): 1395–1406. doi:10.1098/rspb.2008.1480.

Hijmans, R.J. 2020. raster: Geographic Data Analysis and Modeling. R package v. 3.3-7. https://CRAN.R-project.org/package=raster

Kaunisto, K.M., Kaunisto, P., Vahtera, V., and Suhonen, J. 2015. Populations of the damselfly *Coenagrion hastulatum* at the edge of the species range have fewer gregarine and water mite parasites. Freshw. Biol. **60**(4): 794–801. doi:10.1111/fwb.12534.

Keeling, M.J., Bjørnstad, O.N., and Grenfell, B.T. 2004. Metapopulation dynamics of infectious diseases. *In* Ecology, genetics and evolution of metapopulations. *Edited by* I. Hanski and O.E. Gaggiotti. Elsevier Academic Press, Amsterdam. pp. 415–445.

Larrivee, M., Prudic, K.L., McFarland, K., and Kerr, J. 2018*a*. eButterfly: a citizen-based butterfly database in the biological sciences. http://www.e-butterfly.org

Larrivee, M., Prudic, K.L., McFarland, K. and Kerr, J. 2018*b*. eButterfly: a citizen-based butterfly database in the biological sciences. http://www.e-butterfly.org

Lemoine, N.P. 2015. Climate change may alter breeding ground distributions of eastern migratory monarchs (*Danaus plexippus*) via range expansion of *Asclepias* host plants. PLoS One **10**(2). doi:10.1371/journal.pone.0118614.

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

328 Leong, K.L.H., Kaya, H.K., Yoshimura, M.A., and Frey, D.F. 1992. The occurrence and effect of a
329 protozoan parasite, *Ophryocystis elektroscirrha* (Neogregarinida: Ophryocystidae) on
330 overwintering monarch butterflies, *Danaus plexippus* (Lepidoptera: Danaidae) from two California
331 winter sites. Ecol. Entomol. **17**(4): 338–342. doi:10.1111/j.1365-2311.1992.tb01067.x.
332 Lindsey, E.A. 2008. Ecological determinants of host resistance to parasite infection. Doctoral thesis,
333 Emory University, Atlanta, Georgia.
334 Loehle, C. 1995. Social barriers to pathogen transmission in wild animal populations. Ecology **76**(2):
335 326–335. doi:10.2307/1941192.
336 McLaughlin, R.E., and Myers, J. 1970. *Ophryocystis elektroscirrha* sp. n., a neogregarine pathogen of
337 the monarch butterfly *Danaus plexippus* (L.) and the florida queen butterfly *D. gilippus berenice*
338 Cramer. J. Protozool. **17**(2): 300–305. doi:10.1111/j.1550-7408.1970.tb02375.x.
339 Nail, K.R., Batalden, R. V., and Oberhauser, K.S. 2015. What's too hot and what's too cold. *In* Monarchs
340 in a changing world: biology and conservation of an iconic butterfly. *Edited by* S. Oberhauser,
341 Karen, K.R. Nail, and S. Altizer. Cornell University Press, Ithica, New York. pp. 99–108.
342 Prysby, M.D., and Oberhauser, K.S. 2004. Temporal and geographic variation in monarch densities:
343 citizen scientists document monarch population patterns. *In* The monarch butterfly: biology and
344 conservation. *Edited by* K.S. Oberhauser and M.J. Solensky. Cornell University Press, Ithica, New
345 York. pp. 9–20.
346 R Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for
347 Statistical Computing, Vienna, Autria.
348 De Roode, J.C., Chi, J., Rarick, R.M., and Altizer, S. 2009. Strength in numbers: high parasite burdens
349 increase transmission of a protozoan parasite of monarch butterflies (*Danaus plexippus*). Oecologia
350 **161**(1): 67–75. doi:10.1007/s00442-009-1361-6.

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

De Roode, J.C., Gold, L.R., and Altizer, S. 2007. Virulence determinants in a natural butterfly-parasite system. Parasitology **134**(5): 657–668. doi:10.1017/S0031182006002009.

De Roode, J.C., Yates, A.J., and Altizer, S. 2008. Virulence-transmission trade-offs and population divergence in virulence in a naturally occurring butterfly parasite. Proc. Natl. Acad. Sci. U. S. A. **105**(21): 7489–7494. doi:10.1073/pnas.0710909105.

Sexton, J.P., McIntyre, P.J., Angert, A.L., and Rice, K.J. 2009. Evolution and ecology of species range limits. Annu. Rev. Ecol. Evol. Syst. **40**(1): 415–436. doi:10.1146/annurev.ecolsys.110308.120317.

Solensky, M.J. 2004. Overview of monarch migration. *In* The monarch butterfly: biology and conservation. *Edited by* K.S. Oberhauser and M.J. Solensky. Cornell University Press, Ithica, New York. pp. 79–83.

Urquhart, F.A., and Urquhart, N.R. 1978. Vernal migration of the monarch butterfly (*Danaus p. plexippus* L.; Danaidae; Lepidoptera) in North America from the overwintering site in the neo-volcanic plateau of Mexico. Can. J. Zool. **56**(8): 1759–1764. doi:10.4039/Ent11115-1.

Zalucki, M.P. 1982. Temperature and rate of development in *Danaus Plexippus* L. and *D. Chrysippus* L. (Lepidoptera: Nymphalidae). Aust. J. Entomol. **21**(4): 241–246. doi:10.1111/j.1440-6055.1982.tb01803.x.

Figure 1: Eastern Canada monarch sightings, location of collection sites and sample size. Monarch sightings in Eastern Canada reported by citizen scientistis in eButterfly (Larrive et al. 2018*a*) and Mission Monarch (Larrive et al. 2018*b*) from 2016-2018 in red, and samples collected by Flockhart et al. (2018) in yellow. The inset shows our 2019 collection sites (red dots) in the Ottawa region relative to land use types (Agriculture and Agri-Food Canada 2010). The size of the dots is scaled to the number of individuals we collected at each site. This figure was created using in package "raster" version 3.3-7 (Hijmans 2020) in R (R Core Team 2018)

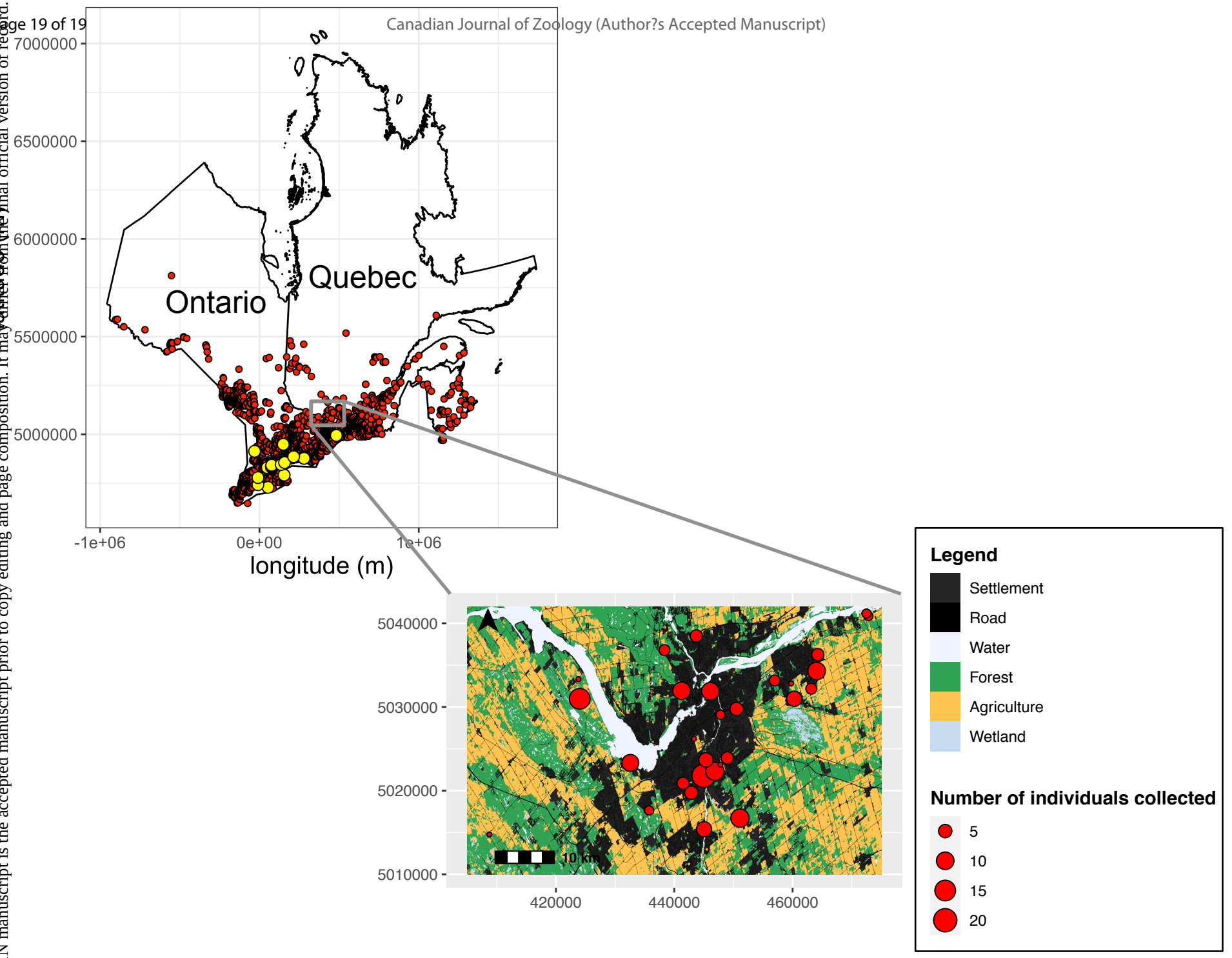
© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

375 version 3.6.3 and assembled from the following data source (raster): Land Use 2010 (Agriculture
376 and Agri-Food Canada 2010).

© The Author(s) or their Institution(s)

Can. J. Zool. Downloaded from cdnsciencepub.com by University of Ottawa on 03/09/21
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.



© The Author(s) or their Institution(s)