Received: 3 August 2021 | Accepted: 31 January 2022

DOI: 10.1111/1365-2656.13678

Journal of Animal Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Parasite dynamics in North American monarchs predicted by host density and seasonal migratory culling

Ania A. Majewska[1] | Andrew K. Davis[2] | Sonia Altizer[2] | Jacobus C. de Roode[1]

[1]Department of Biology, Emory University, Atlanta, GA, USA

[2]Odum School of Ecology, University of Georgia, Athens, GA, USA

Correspondence
Ania A. Majewska
Email: ania.majewska@emory.edu

Funding information
National Science Foundation Directorate for Biological Sciences DEB-1754392 DEB-1754431 Department of Defense SERDP-RC2700; National Institute of General Medical Sciences, Grant/Award Number: 5K12GM000680-19

Handling Editor: Elise Zipkin

Abstract

1. Insect–pathogen dynamics can show seasonal and inter-annual variations that covary with fluctuations in insect abundance and climate. Long-term analyses are especially needed to track parasite dynamics in migratory insects, in part because their vast habitat ranges and high mobility might dampen local effects of density and climate on infection prevalence.
2. Monarch butterflies *Danaus plexippus* are commonly infected with the protozoan *Ophryocystis elektroscirrha* (*OE*). Because this parasite lowers monarch survival and flight performance, and because migratory monarchs have experienced declines in recent decades, it is important to understand the patterns and drivers of infection.
3. Here we compiled data on *OE* infection spanning 50 years, from wild monarchs sampled in the United States, Canada and Mexico during summer breeding, fall migrating and overwintering periods. We examined eastern versus western North American monarchs separately, to ask how abundance estimates, resource availability, climate and breeding season length impact infection trends. We further assessed the intensity of migratory culling, which occurs when infected individuals are removed from the population during migration.
4. Average infection prevalence was four times higher in western compared to eastern subpopulations. In eastern North America, the proportion of infected monarchs increased threefold since the mid-2000s. In the western region, the proportion of infected monarchs declined sharply from 2000 to 2015, and increased thereafter. For both eastern and western subpopulations, years with greater summer adult abundance predicted greater infection prevalence, indicating that transmission increases with host breeding density. Environmental variables (temperature and NDVI) were not associated with changes in the proportion of infected adults. We found evidence for migratory culling of infected butterflies, based on declines in parasitism during fall migration. We estimated that tens of millions fewer monarchs reach overwintering sites in Mexico as a result of *OE*, highlighting the need to consider the parasite as a potential threat to the monarch population.
5. Increases in infection among eastern North American monarchs post-2002 suggest that changes to the host's ecology or environment have intensified parasite

transmission. Further work is needed to examine the degree to which human practices, such as mass caterpillar rearing and the widespread planting of exotic milkweed, have contributed to this trend.

KEYWORDS
animal migration, *Danaus plexippus*, environmental change, milkweed butterfly, neogregarine, *Ophryocystis elektroscirrha*, population abundance, seasonality

1 | INTRODUCTION

Parasites are ubiquitous in natural insect populations, yet most knowledge of insect–parasite dynamics is derived from studies of managed species (e.g. European honeybees; Mondet et al., 2014; Wilfert et al., 2016) and pest insects (e.g. western tent caterpillars and gypsy moths; Elderd et al., 2008; Cory & Myers, 2009). Because insect host abundance can fluctuate dramatically over time, through seasonal cycles and inter-annual variation (Dwyer et al., 2000; Haynes et al., 2009; Myers & Cory, 2013), studies of insect–pathogen interactions are especially useful for uncovering forces affecting pathogen transmission and examining pathogen impacts in natural populations. Numerous studies show that insect–pathogen dynamics can depend strongly on host abundance or density (Bartel et al., 2011; Majewska, Sims, et al., 2019; Ryder et al., 2005), and abiotic factors (including habitat configuration, temperature and rainfall; Saunders et al., 2018). Temperature, in particular, can drive changes in insect abundance and also determine the rates of within-host replication (Mohamed et al., 1985; Wada & Kobayashi, 1980), decay of environmental transmission stages (Kolman et al., 2015; Paaijmans et al., 2012) and host susceptibility to infection (Laughton et al., 2017; Thomas & Blanford, 2003).

Long-term changes in parasite prevalence could cause host population declines (Pedersen et al., 2007; Skerratt et al., 2007; Smith et al., 2006). Anthropogenic activities can intensify pathogen-mediated declines through environmental alterations such as large-scale loss of habitat and climate change (Brearley et al., 2013; Gottdenker et al., 2014; Rohr et al., 2011). Among insects, declines in native bees and other pollinators have been caused by pathogens introduced through human movement (e.g. commercially bred bees) and pathogen spillover from managed to wild insect populations (Goulson & Hughes, 2015). Moreover, habitat loss and degradation can increase host contact rates by crowding individuals into smaller habitats, an effect suggested to increase parasite transmission in bumblebees and butterflies inhabiting urban areas (Goulson et al., 2012; Majewska, Satterfield, et al., 2019). Climate warming might increase the transmission of some insect pathogens through milder winters (increasing the survival of environmental stages) and longer growing seasons over which transmission occurs. Insects exposed to environmental stressors such as pesticides or thermal stress could become more susceptible to pathogen-mediated declines, making efforts to detect and characterize changes in the prevalence of insect pathogens increasingly important for insect conservation (Bartel & Altizer, 2012).

Hosts have evolved several behavioural mechanisms that result in avoidance of parasites and reduced prevalence in the population. Seasonal long-distance migration in particular can reduce parasite transmission, when migrants periodically leave behind habitats that have accumulated infectious stages (i.e. migratory escape; Loehle, 1995; Altizer et al., 2011). Similarly, migratory culling occurs when infected hosts migrate less well and are more likely to perish during the journey than healthy animals, thus lowering infection prevalence (Bartel et al., 2011; Bradley & Altizer, 2005). For some migratory species, the recolonization of breeding habitats also reduces host density by dispersing animals across the landscape. This could lower the risk of infection for pathogens with density-dependent transmission (Ezenwa, 2004; Loehle, 1995), and distribute hosts more evenly across diverse habitats. To counter host behavioural defences, some parasites have evolved transmission via multiple routes, increasing the likelihood of persistence in the host population (Loehle, 1995; Majewska, Sims, et al., 2019).

In this study, we examined temporal infection dynamics in monarch butterflies *Danaus plexippus* as a widespread migratory species. We focus on North American monarchs, which migrate annually from breeding grounds in the northern United States and southern Canada to wintering sites in central Mexico (Brower, 1995). West of the continental divide, a smaller monarch population migrates shorter distances to wintering sites along the coast of California (Leong et al., 2004; Figure 1). Monarchs lay eggs on milkweed (family Asclepiadaceae) during the spring and summer. After several breeding generations, monarchs that emerge in late summer and fall enter a state of reproductive diapause, which facilitates energy conservation during their migration and long overwintering period (Goehring & Oberhauser, 2002). Throughout the winter, adults form clusters and remain in a non-reproductive state until the following spring, when the same adults and their progeny recolonize their summer breeding range (Flockhart et al., 2013). In recent decades, monarch numbers at overwintering sites in eastern and western North America have declined (Pelton et al., 2019; Thogmartin, Wiederholt, et al., 2017), prompting concern for the future of their spectacular migration.

Monarchs have several parasites including the specialist protozoan *Ophryocystis elektroscirrha* (*OE* hereafter; Apicomplexa: Neogregarinida) (McLaughlin & Myers, 1970), which occurs in all monarch populations examined to date (Altizer et al., 2000; Altizer

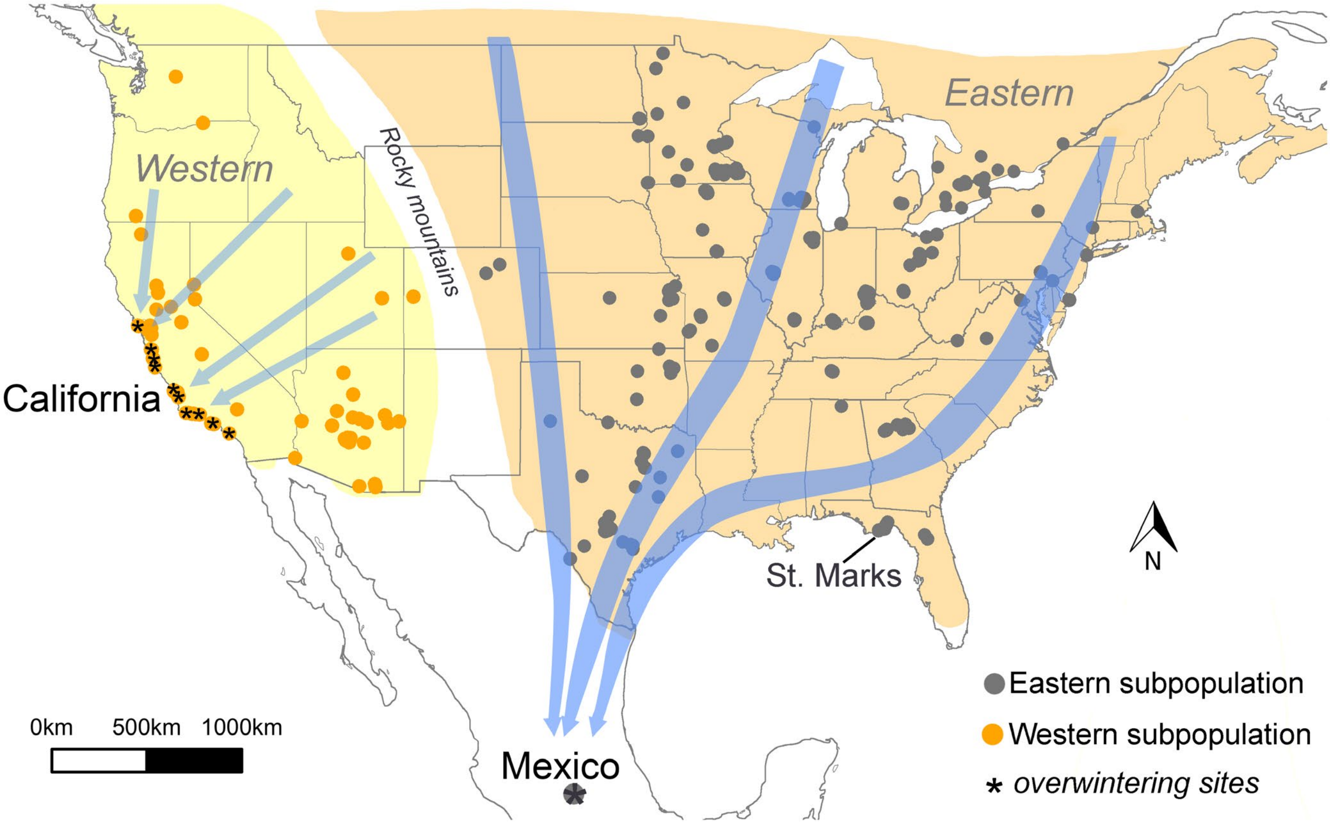


FIGURE 1 Map of North America indicating the eastern (light orange area) and western (yellow area) regions where monarch subpopulations breed. Grey and orange symbols indicate sampling sites. Light blue arrows show approximate migration pathways monarchs undertake during the fall to overwintering sites (star symbols)

& de Roode, 2015). This parasite forms spores on the outside of the body of adults, and infection occurs when caterpillars ingest parasite spores. Parasites replicate internally in caterpillars and pupae, and infected adults emerge covered in dormant parasite spores (de Roode et al., 2006). Parasite spores can be transmitted from females to their offspring during oviposition. In addition, adults can scatter spores onto milkweed leaves which are later consumed by unrelated caterpillars, and males can transfer spores to females during mating, which are later ingested by their offspring (Altizer et al., 2004; de Roode et al., 2009; Majewska, Sims, et al., 2019). In the latter case, females that do not emerge as infected themselves can act as carriers of spores.

Long-distance migration in monarchs is known to reduce parasite prevalence through migratory culling and migratory escape (Altizer et al., 2011; Bartel et al., 2011). Infected monarchs fly less well, have shorter adult life spans and are less likely to survive the long journey to wintering sites (Altizer & Oberhauser, 1999; Bartel et al., 2011; Bradley & Altizer, 2005). Moreover, parasite spores are known to build up on milkweed plants throughout the breeding season (Bartel et al., 2011; Majewska, Sims, et al., 2019). Fall migration allows monarchs to periodically escape contaminated plants and habitats. Because some infected adults successfully migrate and survive overwintering, and because healthy adults can become carriers of the parasite via contacts with infected adults, the parasite persists in migratory populations (Altizer, Hobson, et al., 2015).

Here we compiled previously published and unpublished data on protozoan infection in wild monarchs across North America to examine temporal trends in infection, ask whether eastern and western monarch subpopulations differ in infection rates, and test whether fall migration leads to lower prevalence of infection. We also investigated whether the loss of monarchs during the fall migration (from late summer to arrival at the wintering sites) is associated with a greater proportion of infected adults. In our analysis of temporal trends in infection, we tested whether abiotic (spring and summer temperatures, and breeding season length) and biotic (monarch abundance and resource availability) variables predict changes in parasite prevalence. We chose spring and summer temperatures because conditions during these seasons have been shown to predict the number of monarch generations per year and overall adult abundance (Saunders et al., 2018; Zipkin et al., 2012) which might increase transmission. Warm temperatures increase monarch's developmental rates (Zalucki, 1982) and lower parasite survival in the environment (Nail et al., 2015; Sánchez et al., 2021), potentially impacting infection risk. Finally, we examined a subset of data collected each fall at a single migratory stopover site over 14 years by the same researcher (JCdR), to assess finer scale trends in infection and contamination of fall migrants, and ask how these relate to annual environmental predictors.

2 | MATERIALS AND METHODS

2.1 | Infection data

We compiled samples from our previous field studies of wild North American monarchs (and from samples provided by L.P. Brower) during periods of summer breeding, fall migrating and overwintering. Samples span the time frame of 1968–2019 (*N* = 58,926 adults sampled; details provided in Table S1). For each monarch, we recorded the date and location of sampling, phase of monarch's annual cycle (summer breeding, fall migrating and overwintering) and infection status as described below. We used standard aerial nets to capture adults in flight, or while nectaring on flowers. At overwintering sites, expandable poles were used to capture butterflies in clusters (see Table S2 for locations and details on sampling overwintering monarchs). Monarchs in Mexico were sampled under permits issued from SEMARNAT (Secretaria de Medio Ambiente y Recursos Naturales, including permit numbers 0015, 07840, 08202, 00217).

Field samples were combined with published data from the primary literature on *OE* infection in wild-caught monarchs (see Table S3). From the five published studies that met our criteria, we extracted the date, location (city, state and country) of sampling, phase of monarch's annual cycle (summer breeding, fall migrating and overwintering) along with the total numbers of wild-caught adults, and the proportions of contaminated and heavily infected adults (based on criteria defined below). When the phase of the annual cycle during which monarchs were sampled was unclear, we assumed that adult monarchs sampled in the breeding range in spring and summer months (April–August) were breeding, and that monarchs sampled in fall months (September–November) were fall migrating.

Monarch infection status was determined via non-destructive sampling by pressing a clear sticker (ranging from 1 to 2 cm^2) to the adult's abdomen. Stickers were transferred to an index card or glass slide and scored under a microscope at 50× magnification for *OE* spores. Samples with more than 100 spores were scored as *heavily infected* (infection was acquired at the larval stage following Bartel et al. (2011)), while samples with fewer than 100 spores were scored as *contaminated* (spore transfer between adult monarchs, Altizer et al., 2004; de Roode et al., 2009; Majewska, Sims, et al., 2019). Contaminated adults are temporary, asymptomatic carriers of the parasite spores, most of which are lost within a week of acquisition (Altizer et al., 2004). In contaminated adults, spores cause no harm to the individual, yet the carriers play an important role in the spread and persistence of the parasite in the host population (Majewska, Sims, et al., 2019). This study did not require ethical approval.

For both previously published and newly assembled data, we used information on wild-caught adults only, avoiding possible non-independence of multiple caterpillars collected at a site or plant, and potential effects of captive rearing on infection. We excluded data from sites where adults were identified as year-round breeding monarchs (e.g. south Florida population) that are known to have very high infection rates associated with continual use of non-native milkweed plants and lack of migratory behaviour (Satterfield et al., 2015).

Given the differences in climate, migration distance and routes, and overwintering locations between eastern and western monarchs, we assigned each sampled adult in the United States and Canada east of 108.5°W (east of the continental divide) to the eastern subpopulation and west of 108.5°W to western subpopulation (Figure 1; eastern vs. western region). The subset of data collected at St. Marks, Florida, USA (within the eastern subpopulation) includes fall migrating adults only.

Because some studies did not include exact dates of sampling and other studies sampled over several weeks to months, we aggregated data by migration phase, year, and subpopulation. The sample sizes tended to be large (mean = 583.4 adults; median = 282 adults; Table S1), and we excluded records with fewer than five individuals sampled per year–phase combination. We calculated the proportion of infected adults (scored as heavily infected, >100 spores) and the proportion of contaminated adults (<100 spores) relative to the total number of sampled adults (Table S1). For analyses in which proportions were examined (see below), each year–phase–population combination was treated as the sample unit (*N* = 101 lines of data).

2.2 | Monarch abundance estimates

We estimated eastern monarch subpopulation size in two ways: (a) based on summer adult counts coordinated and archived by the North American Butterfly Association (NABA, naba.org), and (b) from immature monarch density (egg and larva counts) reported by the Monarch Larva Monitoring Project (MLMP). NABA summer monarch counts for June–August were collected across the United States by volunteers from 1977 to 2019 (described further in Ries et al., 2015). Counts are performed in 1 day within a 15-mile (24-km) diameter buffer of a designated site (total of 450 sites), and corrected for number of volunteers and the number of hours volunteers dedicated to counting. We summed NABA butterfly counts for each year and subpopulation to estimate monarch summer population size. Immature monarch (egg and larva) density counts were available from the monarch larva monitoring project (MLMP; Prysby & Oberhauser, 2004) for 1992–2019. The MLMP monitors sites with milkweed plants on a weekly basis for immature stages, reporting the number of eggs and larval instars seen relative to the number of milkweed stalks surveyed (for more details, see Prysby & Oberhauser, 2004). From these counts we calculated a mean density for each year and subpopulation as a second estimate of the monarch summer population size. Preliminary analyses indicated that NABA monarch counts and MLMP monarch density were strongly positively correlated (see Figure S1) and therefore we excluded immature monarch density from further analyses.

We used overwintering colony size estimates to understand the relative change in monarch population size between summer and overwintering seasons, to ask how that relates to the proportion of infected adults. Specifically, we tested the extent to which parasite-related migratory culling reduces overwintering colony size. For the eastern subpopulation, we acquired available overwintering estimates (1993–2019) in Mexico, which are measured by the World Wildlife Fund Mexico and the Monarch Butterfly Biosphere Reserve (Vidal & Rendón-Salinas, 2014). These estimates provide a snapshot in time of forest hectares occupied by clusters of overwintering monarchs (Thogmartin, Diffendorfer, et al., 2017; Vidal & Rendón-Salinas, 2014). Previous work indicates that the overwintering population size in Mexico is affected by summer breeding abundance and processes that occur during the fall migration (Agrawal & Inamine, 2018; Saunders et al., 2019). For the western subpopulation, we used available estimates (1997–2019), from the Xerces Society (www.westernmonarchcount.org; Pelton et al., 2019). For each subpopulation, we divided the overwintering population estimate by the monarch population estimated during the prior summer breeding season (i.e. NABA monarch counts) for each year available, to acquire a ratio of the two estimates. Because the units of these two measures are vastly different (colony area vs. counts of monarchs corrected by the number of volunteers and hours), the absolute values of the ratios are not meaningful. Instead, we focus on the relative difference in the two metrics; higher ratios suggest less change in population size from summer to winter (and therefore less migratory culling), whereas lower values suggest greater loss of monarchs from summer to overwintering seasons, as might occur with high mortality from infection.

2.3 | Environmental correlates

We acquired climate data from PRISM (https://www.prism.oregonstate.edu) using the R package 'PRISM' (Hart & Bell, 2015). We used minimum, mean and maximum temperatures from 1968 to 2019, and averaged these values across the western and eastern regions (as defined above, Figure 1). Averages were obtained for each year, separated by spring months (April–May) and summer months (June–August). For the western region, we restricted the environmental data to west of 114°W and between latitudes 32.5° and 42°N to avoid underestimating values for this region, which occurs when including high elevation sites unsuitable for monarch breeding. We chose spring temperatures because past work showed that they predict monarch abundance later in the summer, and summer temperatures impact the number of generations per year and total adult abundance (Barker & Herman, 1976; Saunders et al., 2018; Zipkin et al., 2012), all of which could impact crowding of monarchs and parasite transmission (Lindsey et al., 2009). Similarly, hot summer temperatures might negatively affect *OE* survival in the environment based on experimental work in captivity (Sánchez et al., 2021). Preliminary analyses revealed that minimum, mean and maximum summer temperatures are highly positively correlated (correlation coefficient > 0.7) and therefore we only include mean temperature in our analyses.

2.4 | Resource availability

To estimate resource availability (milkweed and nectar plants), we used the normalized difference vegetation index (NDVI; https://www.ncei.noaa.gov; Vermote, 2019) derived from satellite imagery, following Saunders et al. (2019). NDVI quantifies live green vegetation and is assumed to indicate the availability of plant resources, including the abundance of caterpillar host plants and adult nectar plants (e.g. Graham et al., 2016; Hu et al., 2021; Saunders et al., 2019). We acquired NDVI values for all years available (1980–2019) and averaged the values for year and region (as defined above) for the month of July, when vegetation greenness is expected to be high. While systematic milkweed (*Asclepias* spp.) counts across North America would be an appropriate estimate of resource availability to include in this study, such estimates are lacking.

2.5 | Breeding season length

We included an estimate of the breeding season length each year, since a longer growing season is expected to provide more opportunities for parasite transmission. We estimated the breeding season length by calculating the cumulative number of days for which average temperature was greater than 12°C between April and October, as this temperature threshold permits development in monarchs (Zalucki, 1982). We used the minimum daily temperature from PRISM climate data to calculate the daily average minimum temperature for each region and counted the number of days with temperature greater than 12°C. This approach provided us with a conservative estimate of the breeding season length for each subpopulation and year.

2.6 | St. Marks migratory monarchs

The subset of data from St. Marks, Florida, USA yielded 14 consecutive years of data collected at the end of October each year, 2009–2019, during the peak of migration. The continuity of the data from the same location provided us with the opportunity to examine finer scale (spatial and temporal) impacts of abiotic and biotic factors on infection. We acquired NABA monarch counts, average spring and summer temperatures, breeding length season and NDVI, as before. Given that the monarchs passing through northeastern region of Florida during migration most likely originate from the Atlantic coastal regions of the United States, we calculated the abiotic and biotic variables for a portion of the eastern region (see Figure S2).

2.7 | Statistical approach

We used R programming for all analyses (R Core Team, 2021). We first asked whether infection and contamination of adults differed between the eastern and western subpopulations. We used logistic regression with probability of infection (or contamination) modelled as the number of healthy versus infected individuals (binary response) in a year–phase–population combination. Next, we used logistic regression to examine whether the probability of infection and contamination differed between years and migration phases (summer breeding, fall migrating and overwintering), with separate analyses for eastern and western subpopulations. To compare infection probability by migration phases, we used Tukey's contrasts with the *glht* function in the 'MULTICOMP' package in R (Hothorn et al., 2017). We also tested whether proportions of contaminated adults were predicted by the proportion of heavily infected adults using Pearson's correlation.

To evaluate migratory culling, we used a simple linear regression model to test for an association between infection prevalence in a given year-phase (predictor variable) and the ratio of estimated overwintering monarch population size to summer breeding population size (response variable). Because the visual inspection of the relationship between infection prevalence versus the ratio of population sizes suggested the presence of outliers, we used Rosner's test (function *rosnerTest*; ENVSTATS package in R) to check for influential data points (Millard et al., 2020). Six outliers were identified in eastern subpopulation (ratios = 11.4, 17.9 and 21.8) and three in the western subpopulation (ratios = 163.9). We present results of statistical analyses without the outliers but include the outliers in figures of raw data (Figure 4). We further explored the estimated impact of infection on overwintering colony size as described in Supporting Information.

To examine how the proportions of heavily infected and contaminated monarchs varied over time (years), we used generalized additive models (GAMs) using the R package 'MGCV' (Wood, 2015); these models were able to characterize the nonlinear relationships we noted upon visual inspection of time-series infection data (Figure 2C–F). We analysed the two subpopulations separately, and included year as a smooth term using the default thin plate regression splines with smoothing parameter selection set to restricted maximum likelihood (RELM; Wood, 2011).

We also examined the extent to which the proportion of heavily infected adults was predicted by monarch population size (i.e. NABA monarch count), average spring and summer temperatures, breeding length season, and NDVI. We restricted the infection data to monarchs collected during migrating and overwintering phases because these data offer aggregate population-wide prevalence estimates. We employed generalized additive mixed effects models (GAMMs) using the R package 'MGCV' (Wood, 2015) to capture nonlinear relationships and temporal correlation structure, which GAMM allows (Wood, 2006). We included the proportion of infected adults as the response variable, and NABA counts, average spring and summer temperatures, breeding length season and NDVI as smoothing terms. We also included year as a first-order autoregressive correlation term to account for temporal autocorrelation (Zuur et al., 2009). We set the basis dimension (*k*) for the smooths equal to 5 to allow

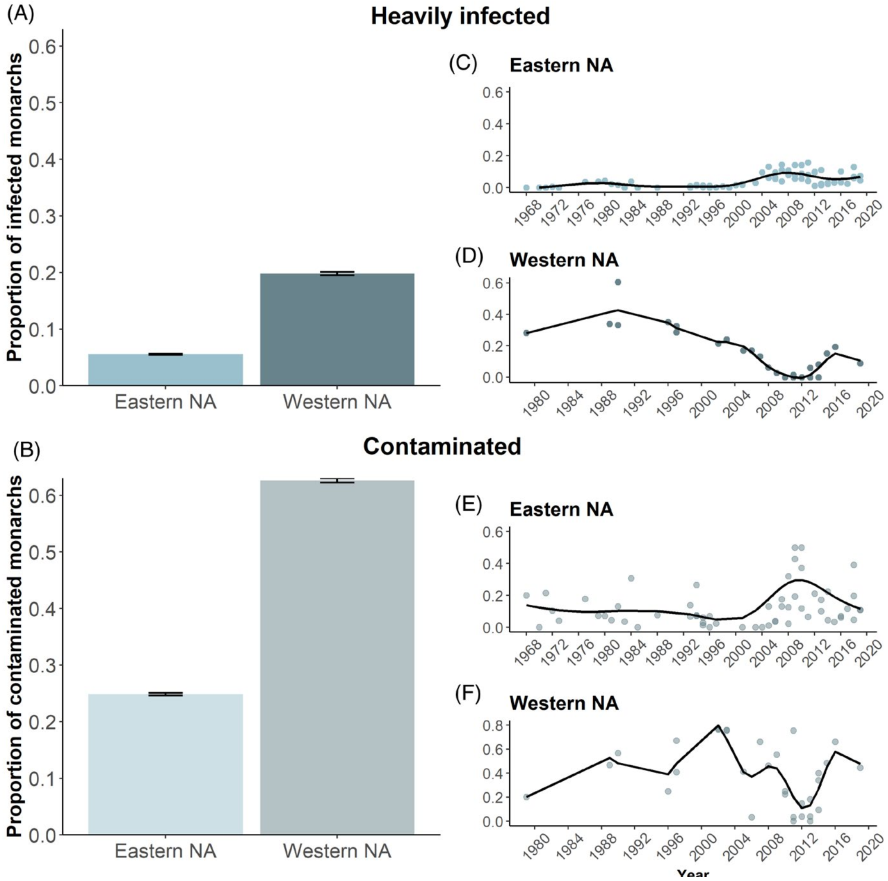


FIGURE 2 Proportion of heavily infected (A, C, D) and contaminated (B, E, F) monarchs in eastern and western subpopulations averaged for: all years (A, B) and for the years sampled (C, D, E, F), where each point represents a proportion of infected or contaminated adults in migration phase and year. Western North America has a higher proportion of infected and contaminated adults. Error bars represent standard error of the mean. Sample sizes and locations are indicated in Table S1 and Figure 1

sufficient degrees of freedom to run the model and assure we set adequate basis dimension with function *k.check* ('MGCV' package; Wood, 2015). We used thin plate regression splines and RELM for smoothing parameters. We used the same approach to examine trends in the proportion of contaminated adults in relation to NABA counts, average spring and summer temperatures, breeding length season and NDVI: we employed GAMMs as before with smoothing terms (k = 5) and a first-order autoregressive correlation term for year.

Finally, we characterized trends in infection and contamination within the St. Marks, Florida, USA dataset. We examined the relationship between the proportion of heavily infected adults and year using GAMM. In a separate analysis, we applied GAMM and included NABA monarch abundance estimates, average spring and summer temperatures, breeding length season and NDVI as smoothing terms and year as a first-order autoregressive correlation term.

3 | RESULTS

The average prevalence of heavily infected monarchs in North America was 10.0% across the entire dataset, including western and eastern subpopulations and all years that monarchs were sampled. For the eastern subpopulation, the prevalence of heavily infected monarchs across all years was 5.5% (N = 40,542 adults sampled from 1968 to 2019) compared to 19.8% (N = 18,384 adults sampled from 1979 to 2019) in the western subpopulation (Figure 2A). Logistic regression indicated that the probability of infection was significantly higher in the western than the eastern subpopulation (z = 50.4, df = 100, $p < 0.001$). For the subset of data which contained information on contamination status, the prevalence of contaminated adults was 37.1% (N = 55,820). The probability of adult contamination was significantly lower (24.8%) in the eastern (N = 37,608) compared to the western subpopulation (62.6%; N = 18,184; Figure 2B; z = 83.5, df = 92, $p < 0.001$).

The proportions of both infected and contaminated adults varied significantly over time in both subpopulations (Figure 2C–F). Year was a significant smooth term in the GAMMs for both the proportions of infected monarchs (eastern: F = 11.1, *edf (effective degrees of freedom)* = 7.2, $p < 0.001$; western: F = 26.1, edf = 8.0, $p < 0.001$) and contaminated monarchs (eastern: F = 2.3, edf = 5.8, p = 0.04; western: F = 4.0, edf = 8.2, $p < 0.01$). We observed a long-term trend towards greater parasite prevalence in eastern subpopulation starting around 2002 (increasing threefold; Figure 2C). In contrast, the proportion of infected monarchs in western region showed a historical high prevalence from 1979 to 2002, followed by a drop in prevalence from 1996 to 2012, and a more recent rise (Figure 2D).

The proportion of heavily infected monarchs differed between migration phases (Figure 3A). Tukey's contrasts indicted that in the eastern subpopulation, infection probability was highest for migrating adults and lower for overwintering and summer breeding adults (overwintering vs. migrating: z = −8.5, $p < 0.001$; breeding vs. migrating: z = −3.2, $p < 0.01$; breeding vs. overwintering: z = 2.2, p = 0.06). In the western subpopulation, infection probability was highest for summer breeding monarchs (breeding vs. migrating: z = 7.6, $p < 0.001$; breeding vs. overwintering: z = 8.9, $p < 0.001$; Figure 3A), but did not differ between overwintering and migrating monarchs (z = −0.3, p = 0.96). The proportion of contaminated adults was positively correlated with the proportion of infected adults in both subpopulations (eastern: r = 0.5, t = 4.1, df = 60, $p < 0.001$; western: r = 0.5, t = 3.0, df = 29, p = 0.01; Figure S3), and varied among migration phases in the subpopulations (Figure 3B). Tukey's contrasts showed that in the eastern subpopulation, contamination probability was lowest among summer breeding individuals (breeding vs. migrating: z = −10.9, $p < 0.001$; breeding vs. overwintering: z = −20.7, $p < 0.001$), and highest for overwintering monarchs (overwintering vs. migrating: z = 32.9, $p < 0.001$). In the western subpopulation, summer breeding monarchs also showed lower probability of contamination compared to migrating (z = −16.0, $p < 0.001$) and overwintering monarchs (z = −14.3, $p < 0.001$), with overwintering monarchs having lower contamination probability than migrating monarchs (z = −6.1, $p < 0.001$; Figure 3B).

The ratio of overwintering to summer population estimates, an inverse indicator of migratory culling, decreased with the proportion of infected adults within both eastern (t = −2.3, df = 47, p = 0.03) and western (t = −3.0, df = 22, p = 0.01) subpopulations. In other words, the strength of migratory culling increased during years with higher infection prevalence (Figure 4).

The analyses of infection prevalence in relation to NABA counts, average spring and summer temperatures, breeding length season and NDVI indicated that in both eastern and western subpopulations, summer adult abundance (i.e. NABA count) predicted greater parasite prevalence (Table 1; Figure 5A,C). In the eastern subpopulation, no other variable was significantly associated with infection prevalence. In the western subpopulation, breeding season length predicted lower infection prevalence (Figure 5C). The analyses of the prevalence of contaminated adults in the eastern subpopulation showed significant effect of summer adult abundance and summer temperatures (Table 1; Figure 5B). In the western subpopulation, spring temperature was found to be a significant predictor of the proportion of contaminated adults, while summer adult abundance had a marginally significant effect (p = 0.08; Table 1; Figure 5D).

Average infection prevalence among fall migratory monarchs captured at St. Marks, Florida, USA across all years (2006–2019) was 7.7% (N = 4,909), and the average prevalence of contaminated monarchs was 20.8% (N = 4,891). GAM analysis of St. Marks data suggested that infection did not differ significantly across years (F = 0.5, df = 1, p = 0.5; Figure 6). Further GAMM analysis of infection prevalence in relation to NABA counts, average spring and summer temperatures, breeding length season and NDVI indicated that only summer temperatures had a marginal positive effect on infection (p = 0.09; Table 1; Figure 6A). For the proportion of contaminated adults, we found nonlinear effects of summer temperatures on contamination (p = 0.05; Table 1; Figure 6B).

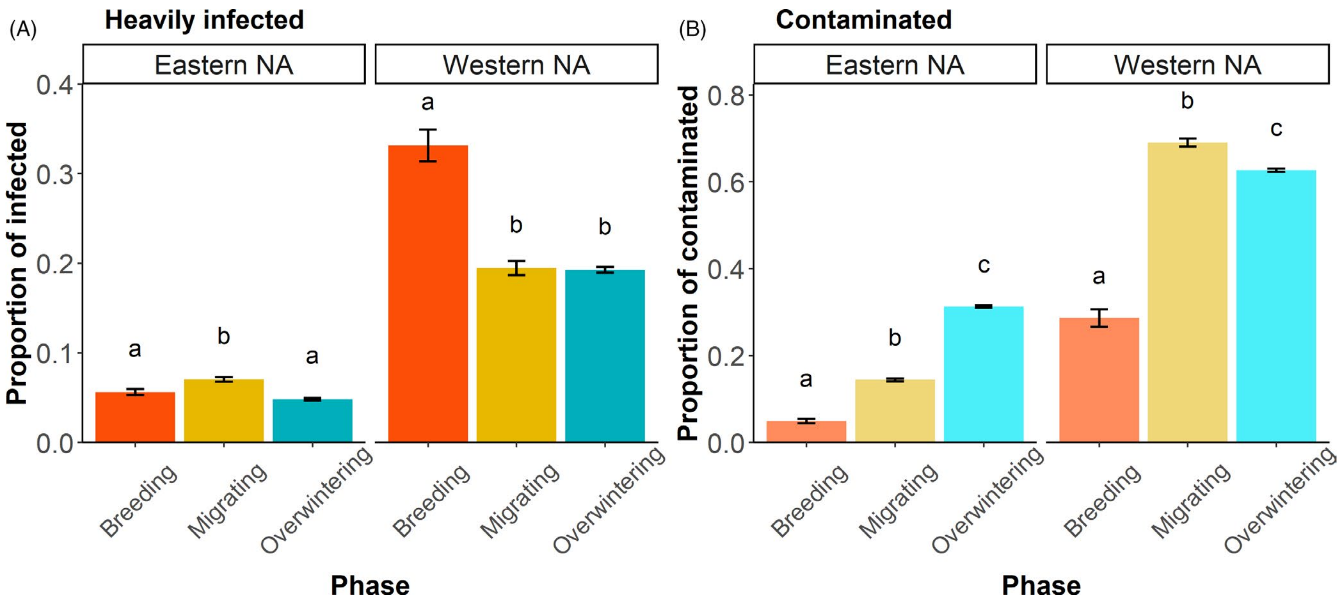
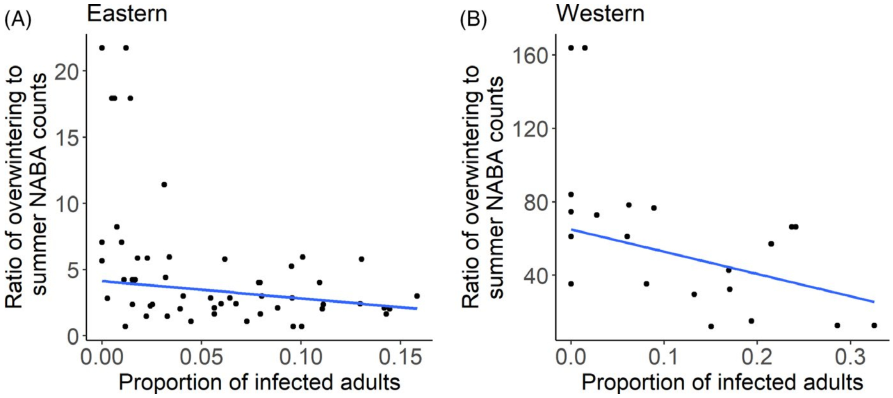


FIGURE 3 Proportion of (A) heavily infected and (B) contaminated monarchs in eastern and western subpopulations in relation to monarch's annual cycle: summer breeding, fall migrating and overwintering. Letters above bars indicate significant differences ($\alpha < 0.05$) among migration phases within subpopulations. Note that *y*-axis scales for the two panels are different

FIGURE 4 Proportion of heavily infected adults and the ratio of overwintering estimates to adult monarch counts (NABA) in a given year and migratory phase for the (A) eastern and (B) western subpopulations. A declining relationship indicates that higher parasite prevalence is associated with lower migration success (i.e. migratory culling)



4 | DISCUSSION

Our study showed significant differences in infection rates and temporal infection trends by a protozoan parasite in eastern and western North American monarchs. We found that the western monarch subpopulation had nearly four times greater prevalence compared to the eastern subpopulation, suggesting that host–parasite dynamics within each region differ, with higher transmission rates in the west. Trends over time in the proportion of infected monarchs differed between the subpopulations: infection rates generally increased in the eastern region for the last two decades, whereas in the west, infection rates declined prior to 2012 and then increased.

Despite the opposing temporal infection trends in eastern and western subpopulations, we found that adult monarch abundance predicted greater infection rates in both subpopulations, highlighting the importance of host density for the transmission of this parasite. Past experimental work suggested that higher density of immature stages may increase susceptibility to *OE* (Lindsey et al., 2009) and theory suggests that high host density can amplify transmission (Begon et al., 2002; Swinton et al., 2002). Besides density effects, infection trends in the western region were impacted by breeding season length, with longer growing seasons associated with lower infection rates. This was counter to our expectation that longer breeding intervals would provide more time for environmental build up and transmission of the protozoan, and for additional contacts between infected and healthy adults, yielding higher overall prevalence. One possible explanation for the unexpected trend might be that longer breeding seasons result in further dispersion of individuals across the landscape, effectively lowering host density. At the same time, longer breeding seasons for monarchs might also correlate with greater availability of milkweed plants for reproduction, further diluting the risk of infection.

We did not find significant effects of spring or summer temperature on infection trends, despite evidence that temperatures can influence *OE* persistence as well as monarch development and abundance (Sánchez et al., 2021; Zalucki, 1982; Zalucki &

TABLE 1 Results of generalized additive mixed effects models for eastern subpopulation, western subpopulation and St. Marks subset of data examining proportion of (a) heavily infected, (b) contaminated monarchs in relation to temperatures (spring and summer), NABA monarch count, NDVI and breeding season length. Models include year as a first-order autoregressive term. Bold font indicates significant effects (p-value < 0.05) and edf reflects the effective degrees of freedom

	Eastern subpopulation			Western subpopulation			St. Marks migrating		
Variable	*F*-value	*edf*	*p*-value	*F*-value	*edf*	*p*-value	*F*-value	*edf*	*p*-value
(a) Heavily infected									
Spring temperatures	0.69	1.00	0.41	0.03	1.00	0.87	0.24	1.00	0.64
Summer temperatures	0.74	1.58	0.59	1.58	1.00	0.23	3.85	1.00	0.09
NABA count	**17.49**	**1.00**	**<0.001**	**5.81**	**1.00**	**0.03**	1.13	1.00	0.32
NDVI	0.02	1.00	0.90	0.39	1.26	0.73	0.54	1.00	0.48
Breeding season length	1.58	1.00	0.22	**5.27**	**1.00**	**0.03**	0.14	1.00	0.72
	$R^2 = 0.54$			$R^2 = 0.33$			$R^2 = 0.39$		
(b) Contaminated									
Spring temperatures	0.89	1.00	0.35	**9.75**	**3.56**	**0.01**	0.15	1.00	0.71
Summer temperatures	**9.15**	**3.12**	**<0.001**	1.49	1.00	0.25	**4.79**	**1.91**	**0.05**
NABA count	**20.12**	**1.00**	**<0.001**	2.85	1.94	0.08	1.42	1.10	0.24
NDVI	2.22	2.07	0.12	1.90	2.97	0.16	0.20	1.00	0.67
Breeding season length	4.19	1.60	0.07	0.65	1.00	0.44	0.15	1.00	0.71
	$R^2 = 0.26$			$R^2 = 0.48$			$R^2 = 0.45$		

Rochester, 1999). However, when we examined a single site, St. Marks, Florida, we found some evidence, although marginally significant, for the impact of summer temperature on infection, suggesting the scale at which we test the relationships is important. We noted nonlinear impacts of temperature (summer for eastern and St. Marks, spring for western) on contamination trends, indicating that temperature might be more important for this route of transmission on the regional scale.

Although direct associations between infection prevalence and temperature were not detected by our analyses, temperature likely has pervasive effects on *OE*–monarch dynamics by affecting both milkweed and host abundance, and through possible effects on monarch migratory behaviour. Deciphering the impact of climate change on monarchs and other insects is difficult given a multitude of simultaneous environmental changes (e.g. habitat loss and degradation). Recent work on over 50 butterfly species of the western United States showed that higher summer temperatures predicted butterfly population growth, whereas higher fall temperature predicted population declines (Forister et al., 2021). The global decline in butterflies and other insects (Forister et al., 2021; Hallmann et al., 2017) is expected to continue with anthropogenic activities and environmental changes, including global climate change.

In both eastern and western North America, the proportion of contaminated adults increased from summer breeding to the overwintering season. One possible explanation for this pattern is that contact rates between infected and healthy adults increase when monarchs aggregate during migration and overwintering. Monarchs are generally solitary during the breeding season, although contacts occur during mating events. Adults become more social during fall migration and form roosts at stopover sites, and massive clusters of thousands or more individuals upon reaching overwintering sites (Brower, 1995; Davis et al., 2012). The shift to a social lifestyle is thought to be necessary for monarchs to survive the overwintering period, yet it might also allow the parasite to persist in the host population. Contamination could also be caused by observer sampling methods, as multiple monarchs are often caught using the same nets (e.g. during fall migration tagging efforts). How much contamination occurs ‘naturally’ as opposed to being an artefact of sampling or capture methods remains an open question and warrants further investigation.

It is important to note that other human activities, which are difficult to estimate, might have influenced monarch infection rates over the last two decades in both subpopulations. For instance, captive rearing and mass breeding of monarchs by companies for sale and release at events (e.g. weddings), as well as butterfly enthusiasts raising caterpillars at home, can result in high transmission rates, with the release of infected adults to the population (Altizer, Lewandowski, et al., 2015). Recent work on tagging efforts of eastern migratory monarchs revealed that an increasing proportion of tagged monarchs are reared by volunteers (Taylor Jr et al., 2020; see Figure S4), suggesting the practice of captive rearing has become more common. Yet, the extent of captive-rearing practices across seasons and regions, and the effect on parasite infection at the population scale remain largely unknown.

We found evidence for migratory culling in the monarch–*OE* system, particularly in the eastern North America subpopulation, where the proportion of infected adults collected during the overwintering season was lower compared to summer breeding adults. This is in agreement with past work showing that *OE* prevalence declines during long-distance migration (Altizer, Hobson, et al., 2015;

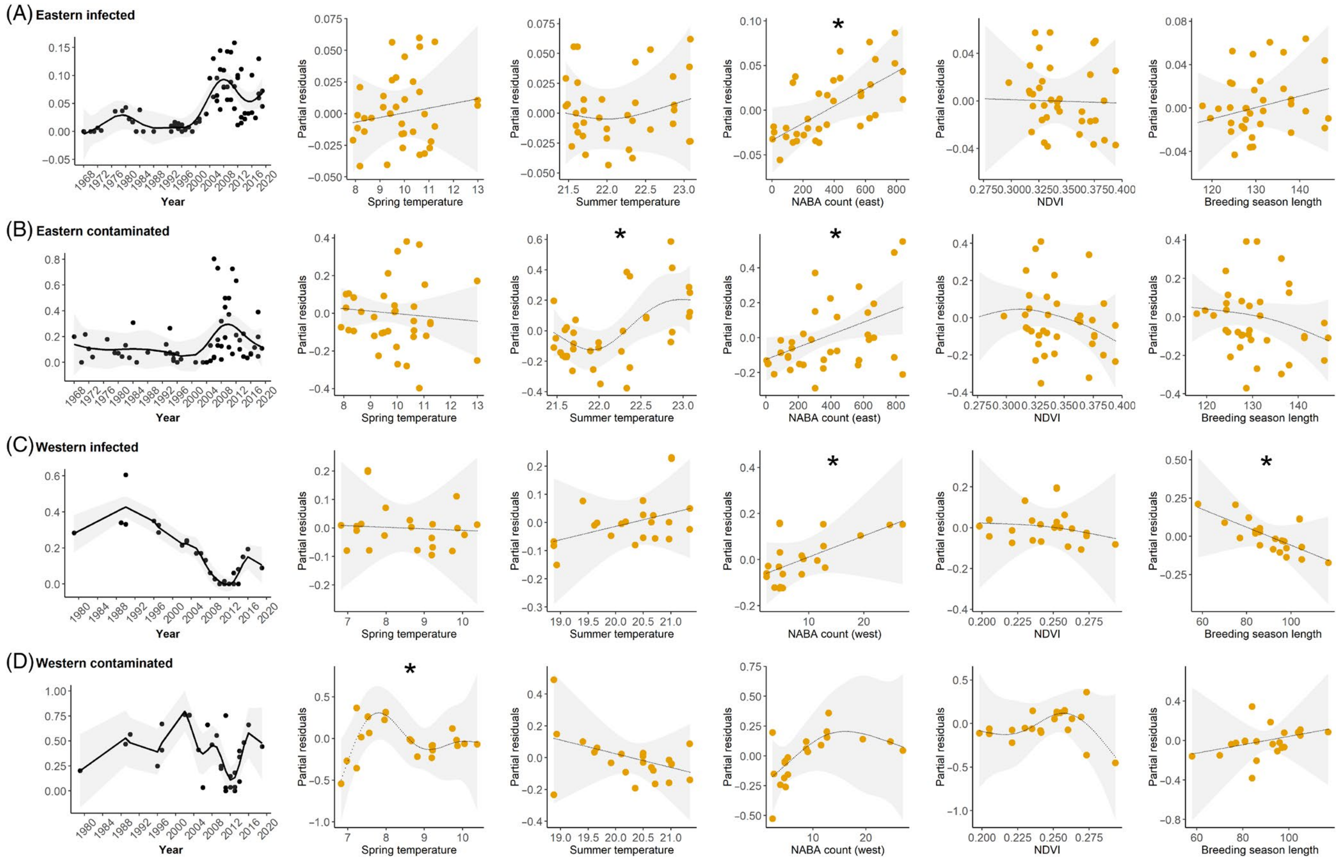


FIGURE 5 (A) Proportion of heavily infected and (B) contaminated eastern monarchs (first panels, solid black line) along with generalized additive model response curves (dotted black lines) with 95% confidence intervals (grey shading) presented for each of the variables (from left to right: spring temperature, summer temperature, NABA monarch counts, NDVI and breeding season length) included in the models (Table 1). (C) Proportion of heavily infected and (B) contaminated western monarch (first panels, solid black line) with the response curves. First panel plots show raw data with each point representing the proportion of heavily infected or contaminated adults in migration phase and year. Significant associations are indicated by * above the plot

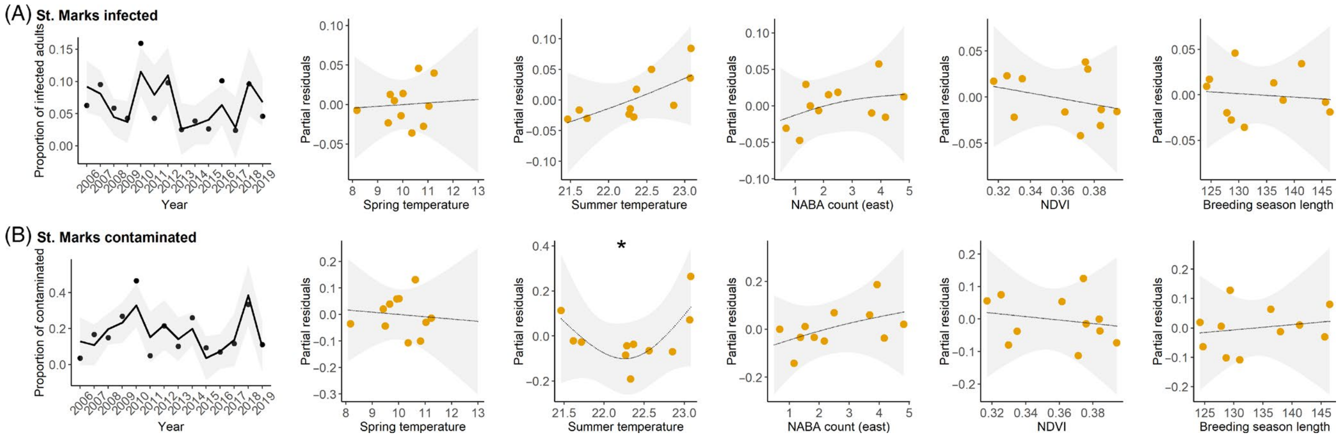


FIGURE 6 (A) Proportion of heavily infected and (B) contaminated migratory monarchs sampled in St. Marks, Florida, USA from 2006 to 2019 along with generalized additive model response curves (solid lines) with 95% confidence intervals (grey shading) presented for each of the variables (from left to right: spring temperature, summer temperature, NABA monarch counts, NDVI and breeding season length) included in the St. Marks model (Table 1). The first plot shows raw data with each point representing the proportion of heavily infected or contaminated monarchs each year

Bartel et al., 2011). For western North America, we also observed a signal of migratory culling; however, the proportion of infected adults sampled during the overwintering season was similar to that of migrating adults. The lack of a detectable difference between migrating and overwintering adults might be due to the fact that western migration is about a third of the distance of the eastern migration (Solensky, 2004), and possibly too short to cull more infected migrants.

Additional factors might be interfering with monarch migration and migratory culling in the western subpopulation. We suspect that the increase in abundance of exotic *A. curassavica* over the last two decades (see Satterfield et al., 2015 supplemental materials, and Figure S5), and the presence of non-migratory populations breeding year-round on this exotic milkweed have increased parasite transmission over time. Previous studies indicate that the monarch's use of exotic milkweed for reproduction is associated with high infection rates (50%–100%; Satterfield et al., 2015; Satterfield et al., 2016). Evidence also suggests that exotic milkweed impacts migratory adults' reproductive diapause, encouraging the formation of non-migratory populations that breed year-round (Batalden & Oberhauser, 2015; Majewska & Altizer, 2019). Because migrating monarchs can interact with non-migratory monarchs in places such as Texas and Florida, this could increase exposure to infection among migrants (Satterfield et al., 2018). Indeed, it has been suggested that western monarchs are changing from a migrating to a non-migratory population (Crone & Schultz, 2021; James, 2021), which we expect will lead to future increases in parasite prevalence.

Host regulation by parasites is common in wildlife (Thompson et al., 2010) and often best recognized following outbreaks of novel pathogens that trigger declines in host abundance (e.g. phocine distemper virus in harbour seals (Rijks et al., 2005) and bacterial conjunctivitis in house finches (Hochachka & Dhondt, 2000)). Negative effects of pathogens on host abundance can be devastating and even cause local extirpation (e.g. ape population declines following outbreaks of Ebola virus; Walsh et al., 2003). Although previously not considered a threat, we suspect that the cryptic negative fitness effects of *OE* have long affected the monarch population, potentially contributing to density-dependent regulation. In more recent years, rising infection rates in eastern North America could contribute to substantial loss of monarchs during fall migration, and will likely continue to do so. Indeed, our calculations for the eastern subpopulation, taking into consideration the estimates of total monarch population size, overwintering population size and the degree of migratory culling, indicate that the increases in parasite prevalence over time might be killing tens of millions of migratory monarchs on a yearly basis (see Table S4).

In conclusion, our study provides evidence for temporal changes in infection prevalence in eastern North American monarchs, shows effects of host density on infection and indicates the loss of monarchs due to infection via migratory culling. Surprisingly, we did not find evidence for large-scale climate variables (temperature) affecting infection. Identifying drivers of monarch–*OE* dynamics is complicated by human activities such as mass rearing of caterpillars, widespread planting of exotic milkweed (*A. curassavica*) and the capture of large numbers of monarchs during migration, which could contribute to changing infection trends. Our results emphasize the need to consider the effects of infection when formulating conservation strategies for monarchs, and to reconsider some of the current human practices (such as caterpillar rearing) which likely amplify transmission.

ACKNOWLEDGEMENTS

The authors thank the members of the Gerardo and de Roode laboratories for constructive feedback on the project. They are grateful to Alfonso Alonso, Paola Barriga, Lincoln Brower, Bill Calvert, David Cook, Paul Cherubini, Linda Fink, Dennis Frey, Bobby Gendron, Pablo Jaramillo, Michael Maudsley, Alexa McKay, Gail Morris, Lori Nicholson, Karen Oberhauser, Terry Peacock, Amanda Pierce, Isabel Ramirez, Rachel Rarick, Eduardo Rendon, Dara Satterfield, Cheryl Shultz, Shawna Stevens, Cameron Thomas, Francis Villablanca, and Robin Will for help with sample collection and permitting. They thank the numerous field assistants who aided in field sampling. They also thank Michael Cerbone, Jim Springer and David Mudd for facilitating data acquisition from NABA as well as Karen Oberhauser for MLMP data. A.A.M. was supported by NIH/NIGMS K12 Postdoctoral Fellowship at Emory University (5K12GM000680-19). This work was supported by NSF grant DEB-1754431 to J.C.d.R., and by SERDP-RC2700 and NSF DEB-1754392 to S.A.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHORS' CONTRIBUTIONS

A.A.M. compiled and analysed the data and wrote the manuscript; A.A.M., A.K.D., S.A. and J.C.d.R. sampled the monarchs, recorded the data, conceived of the study and interpreted the results; A.K.D., S.A. and J.C.d.R. revised and edited the manuscript. All authors contributed to the drafts and approved the final version for publication.

DATA AVAILABILITY STATEMENT

Data used in this study are available on Figshare https://doi.org/10.6084/m9.figshare.17929721.v1 (Majewska et al., 2022).

ORCID

Ania A. Majewska https://orcid.org/0000-0002-0965-3177
Andrew K. Davis https://orcid.org/0000-0002-1945-1819
Sonia Altizer https://orcid.org/0000-0001-9966-2773
Jacobus C. de Roode https://orcid.org/0000-0002-8423-8918

REFERENCES

Agrawal, A. A., & Inamine, H. (2018). Mechanisms behind the monarch's decline. *Science*, *360*, 1294–1296.

Altizer, S., Bartel, R., & Han, B. A. (2011). Animal migration and infectious disease risk. *Science*, *331*, 296–302.

Altizer, S., & de Roode, J. C. (2015). Monarchs and their debilitating parasites: Immunity, migration and medicinal plant use. In K. S. Oberhauser, K. R. Nail, & S. Altizer (Eds.), *Monarchs in a changing* *world: Biology and conservation of an iconic butterfly* (pp. 83–93). Cornell University Press.

Altizer, S., Hobson, K. A., Davis, A. K., De Roode, J. C., & Wassenaar, L. I. (2015). Do healthy monarchs migrate farther? Tracking natal origins of parasitized vs. uninfected monarch butterflies overwintering in Mexico. *PLoS One*, *10*, e0141371.

Altizer, S., Lewandowski, E., Brower, L.P., Morris, G., Howard, E., Nail, K.R., James, D.G., Oberhauser, K., Jepsen, S. & Pyle, R. (2015) Journey North: Captive breeding and releasing monarchs.

Altizer, S., & Oberhauser, K. S. (1999). Effects of the protozoan parasite *Ophryocystis elektroscirrha* on the fitness of monarch butterflies (*Danaus plexippus*). *Journal of Invertebrate Pathology*, *74*, 76–88.

Altizer, S., Oberhauser, K. S., & Brower, L. P. (2000). Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. *Ecological Entomology*, *25*, 125–139.

Altizer, S., Oberhauser, K. S., & Geurts, K. A. (2004). Transmission of the protozoan parasite, *Ophryocystis elektroscirrha*, in monarch butterfly populations: Implications for prevalence and population-level impacts. In K. S. Oberhauser & M. Solensky (Eds.), *The monarch butterfly: Biology and conservation* (pp. 203–218). Cornell University Press.

Barker, J. F., & Herman, W. S. (1976). Effect of photoperiod and temperature on reproduction of the monarch butterfly, *Danaus plexippus*. *Journal of Insect Physiology*, *22*, 1565–1568.

Bartel, R.A. & Altizer, S. (2012). From protozoan infection in monarch butterflies to colony collapse disorder in bees: Are emerging infectious diseases proliferating in the insect world? In A. Aguirre, P. Daszak & R. Ostfeld (Eds.), *Conservation medicine: Applied cases of ecosystem health* (pp. 284–301). Oxford University Press.

Bartel, R. A., Oberhauser, K. S., de Roode, J. C., & Altizer, S. M. (2011). Monarch butterfly migration and parasite transmission in eastern North America. *Ecology*, *92*, 342–351.

Batalden, R. V., & Oberhauser, K. S. (2015). Potential changes in eastern North American monarch migration in response to an introduced milkweed, *Asclepias curassavica*. In K. Oberhauser, K. Nail, & S. Altizer (Eds.), *Monarchs in a changing world: Biology and conservation of an iconic butterfly* (pp. 215–224). Cornell University Press.

Begon, M., Bennett, M., Bowers, R. G., French, N. P., Hazel, S., & Turner, J. (2002). A clarification of transmission terms in host-microparasite models: Numbers, densities and areas. *Epidemiology & Infection*, *129*, 147–153.

Bradley, C. A., & Altizer, S. (2005). Parasites hinder monarch butterfly flight: Implications for disease spread in migratory hosts. *Ecology Letters*, *8*, 290–300.

Brearley, G., Rhodes, J., Bradley, A., Baxter, G., Seabrook, L., Lunney, D., Liu, Y., & McAlpine, C. (2013). Wildlife disease prevalence in human-modified landscapes. *Biological Reviews of the Cambridge Philosophical Society*, *88*, 427–442.

Brower, L. P. (1995). Understanding and misunderstanding the migration of the monarch butterfly (Nymphalidae) in North America: 1857–1995. *Journal of the Lepidopterists Society*, *49*, 304–385.

Cory, J. S., & Myers, J. H. (2009). Within and between population variation in disease resistance in cyclic populations of western tent caterpillars: A test of the disease defence hypothesis. *Journal of Animal Ecology*, *78*, 646–655.

Crone, E. E., & Schultz, C. B. (2021). Resilience or catastrophe? A possible state change for monarch butterflies in western North America. *Ecology Letters*, *24*, 1533–1538.

Davis, A. K., Nibbelink, N. P., & Howard, E. (2012). Identifying large-and small-scale habitat characteristics of monarch butterfly migratory roost sites with citizen science observations. *International Journal of Zoology*, *2012*, 1–9.

de Roode, J. C., Chi, J., Rarick, R. M., & Altizer, S. (2009). Strength in numbers: High parasite burdens increase transmission of a protozoan parasite of monarch butterflies (*Danaus plexippus*). *Oecologia*, *161*, 67–75.

de Roode, J. C., Gold, L., & Altizer, S. (2006). Virulence determinants in a natural butterfly-parasite system. *Parasitology*, *134*, 657–668.

Dwyer, G., Dushoff, J., Elkinton, J. S., & Levin, S. A. (2000). Pathogen-driven outbreaks in forest defoliators revisited: Building models from experimental data. *The American Naturalist*, *156*, 105–120.

Elderd, B. D., Dushoff, J., & Dwyer, G. (2008). Host-pathogen interactions, insect outbreaks, and natural selection for disease resistance. *The American Naturalist*, *172*, 829–842.

Ezenwa, V. O. (2004). Host social behavior and parasitic infection: A multifactorial approach. *Behavioral Ecology*, *15*, 446–454.

Flockhart, D. T., Wassenaar, L. I., Martin, T. G., Hobson, K. A., Wunder, M. B., & Norris, D. R. (2013). Tracking multi-generational colonization of the breeding grounds by monarch butterflies in eastern North America. *Proceedings of the Royal Society B*, *280*, 20131087.

Forister, M., Halsch, C., Nice, C., Fordyce, J., Dilts, T., Oliver, J., Prudic, K., Shapiro, A., Wilson, J., & Glassberg, J. (2021). Fewer butterflies seen by community scientists across the warming and drying landscapes of the American West. *Science*, *371*, 1042–1045.

Goehring, L., & Oberhauser, K. S. (2002). Effects of photoperiod, temperature, and host plant age on induction of reproductive diapause and development time in *Danaus plexippus*. *Ecological Entomology*, *27*, 674–685.

Gottdenker, N. L., Streicker, D. G., Faust, C. L., & Carroll, C. (2014). Anthropogenic land use change and infectious diseases: A review of the evidence. *EcoHealth*, *11*, 619–632.

Goulson, D., & Hughes, W. O. H. (2015). Mitigating the anthropogenic spread of bee parasites to protect wild pollinators. *Biological Conservation*, *191*, 10–19.

Goulson, D., Whitehorn, P., & Fowley, M. (2012). Influence of urbanisation on the prevalence of protozoan parasites of bumblebees. *Ecological Entomology*, *37*, 83–89.

Graham, C. H., Supp, S. R., Powers, D. R., Beck, P., Lim, M. C., Shankar, A., Cormier, T., Goetz, S., & Wethington, S. M. (2016). Winter conditions influence biological responses of migrating hummingbirds. *Ecosphere*, *7*, e01470.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., & Hörren, T. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE*, *12*, e0185809.

Hart, E., & Bell, K. (2015). *R package 'prism': Download data from the Oregon prism project*. Version 0.1.0.

Haynes, K. J., Liebhold, A. M., & Johnson, D. M. (2009). Spatial analysis of harmonic oscillation of gypsy moth outbreak intensity. *Oecologia*, *159*, 249–256.

Hochachka, W. M., & Dhondt, A. A. (2000). Density-dependent decline of host abundance resulting from a new infectious disease. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 5303–5306.

Hothorn, T., Bretz, F., Westfall, P., Heiberger, R. M., Schuetzenmeister, A., & Scheibe, S. (2017). *R package 'multcomp': Simultaneous inference in general parametric models*. v 1.4-8.

Hu, G., Stefanescu, C., Oliver, T. H., Roy, D. B., Brereton, T., Van Swaay, C., Reynolds, D. R., & Chapman, J. W. (2021). Environmental drivers of annual population fluctuations in a trans-Saharan insect migrant. *Proceedings of the National Academy of Sciences of the United States of America*, *118*, e2102762118.

James, D. G. (2021). Western North American monarchs: Spiraling into oblivion or adapting to a changing environment? *Animal Migration*, *8*, 19–26.

Kolman, J. A., Clopton, R. E., & Clopton, D. T. (2015). Effects of developmental temperature on gametocysts and oocysts of two species of gregarines *Blabericola migrator* and *Blabericola cubensis* (Apicomplexa: Eugregarinida: Blabericolidae) parasitizing blaberid cockroaches (Dictyoptera: Blaberidae). *Journal of Parasitology*, *101*, 651–657.

Laughton, A. M., O'Connor, C. O., & Knell, R. J. (2017). Responses to a warming world: Integrating life history, immune investment, and pathogen resistance in a model insect species. *Ecology and Evolution*, *7*, 9699–9710.

Leong, K. L., Sakai, W. H., Bremer, W., Feuerstein, D., & Yoshimura, G. (2004). Analysis of the pattern of distribution and abundance of monarch overwintering sites along the California coastline. In K. S. Oberhauser & M. Solensky (Eds.), *The monarch butterfly: Biology and conservation* (pp. 177–185). Cornell University Press.

Lindsey, E., Mehta, M., Dhulipala, V., Oberhauser, K., & Altizer, S. (2009). Crowding and disease: Effects of host density on response to infection in a butterfly–parasite interaction. *Ecological Entomology*, *34*, 551–561.

Loehle, C. (1995). Social barriers to pathogen transmission in wild animal populations. *Ecology*, *76*, 326–335.

Majewska, A. A., & Altizer, S. (2019). Exposure to non-native tropical milkweed promotes reproductive development in migratory monarch butterflies. *Insects*, *10*, 253.

Majewska, A. A., Davis, A., Altizer, S., & de Roode, J. (2022). Monarch infection data. *Figshare*, https://doi.org/10.6084/m9.figshare.17929721.v1

Majewska, A. A., Satterfield, D. A., Harrison, R. B., Altizer, S., & Hepinstall-Cymerman, J. (2019). Urbanization predicts infection risk by a protozoan parasite in non-migratory populations of monarch butterflies from the southern coastal US and Hawaii. *Landscape Ecology*, *34*, 649–661.

Majewska, A. A., Sims, S., Schneider, A., Altizer, S., & Hall, R. J. (2019). Multiple transmission routes sustain high prevalence of a virulent parasite in a butterfly host. *Proceedings of the Royal Society B*, *286*, 20191630.

McLaughlin, R., & Myers, J. (1970). *Ophryocystis elektroscirrha* sp. n., a neogregarine pathogen of the monarch butterfly *Danaus plexippus* (L.) and the Florida queen butterfly *D. gilippus berenice* Cramer. *Journal of Eukaryotic Microbiology*, *17*, 300–305.

Millard, S. P., Kowarik, A., & Kowarik, M. A. (2020). *R package 'EnvStats': An R package for environmental statistics*. Version 2.4.0.

Mohamed, M. A., Coppel, H. C., & Podgwaite, J. D. (1985). Temperature and crowding effects on virus manifestation in *Neodiprion sertifer* (Hymenoptera, Diprionidae) larvae. *Great Lakes Entomologist*, *18*, 115–118.

Mondet, F., de Miranda, J. R., Kretzschmar, A., Le Conte, Y., & Mercer, A. R. (2014). On the front line: Quantitative virus dynamics in honeybee (*Apis mellifera* L.) colonies along a new expansion front of the parasite *varroa destructor*. *PLoS Pathogens*, *10*, e1004323.

Myers, J. H., & Cory, J. S. (2013). Population cycles in forest lepidoptera revisited. *Annual Review of Ecology, Evolution, and Systematics*, *44*, 565–592.

Nail, K. R., Batalden, R. V., & Oberhauser, K. S. (2015). What's too hot and what's too cold. In K. Oberhauser, K. Nail, & S. Altizer (Eds.), *Monarchs in a changing world: Biology and conservation of an iconic butterfly* (pp. 99–108). Cornell University Press.

Paaijmans, K. P., Blanford, S., Chan, B. H. K., & Thomas, M. B. (2012). Warmer temperatures reduce the vectorial capacity of malaria mosquitoes. *Biology Letters*, *8*, 465–468.

Pedersen, A. B., Jones, K. E., Nunn, C. L., & Altizer, S. (2007). Infectious diseases and extinction risk in wild mammals. *Conservation Biology*, *21*, 1269–1279.

Pelton, E. M., Schultz, C. B., Jepsen, S. J., Black, S. H., & Crone, E. E. (2019). Western monarch population plummets: Status, probable causes, and recommended conservation actions. *Frontiers in Ecology and Evolution*, *7*, 258.

Prysby, M. D., & Oberhauser, K. S. (2004). Temporal and geographic variation in monarch densities: Citizen scientists document monarch population patterns. In K. Oberhauser & M. Solensky (Eds.), *The monarch butterfly: Biology and conservation* (pp. 9–20). Cornell University Press.

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Version 4.0.4.

Ries, L., Oberhauser, K., Taron, D., Battin, J., Rendon-Salinas, E., Altizer, S., & Nail, K. (2015). Connecting eastern monarch population dynamics across their migratory cycle. In K. Oberhauser, K. Nail, & S. Altizer (Eds.), *Monarchs in a changing world: Biology and conservation of an iconic butterfly* (pp. 268–281). Cornell University Press.

Rijks, J. M., Van de Bildt, M. W., Jensen, T., Philippa, J. D., Osterhaus, A. D., & Kuiken, T. (2005). Phocine distemper outbreak, The Netherlands, 2002. *Emerging Infectious Diseases*, *11*, 1945.

Rohr, J. R., Dobson, A. P., Johnson, P. T., Kilpatrick, A. M., Paull, S. H., Raffel, T. R., Ruiz-Moreno, D., & Thomas, M. B. (2011). Frontiers in climate change–disease research. *Trends in Ecology & Evolution*, *26*, 270–277.

Ryder, J. J., Webberley, K. M., Boots, M., & Knell, R. J. (2005). Measuring the transmission dynamics of a sexually transmitted disease. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 15140–15143.

Sánchez, C. A., Ragonese, I. G., de Roode, J. C., & Altizer, S. (2021). Thermal tolerance and environmental persistence of a protozoan parasite in monarch butterflies. *Journal of Invertebrate Pathology*, *183*, 107544.

Satterfield, D. A., Maerz, J. C., & Altizer, S. (2015). Loss of migratory behaviour increases infection risk for a butterfly host. *Proceedings of the Royal Society B*, *282*, 20141734.

Satterfield, D. A., Maerz, J. C., Hunter, M. D., Flockhart, D. T., Hobson, K. A., Norris, D. R., Streit, H., de Roode, J. C., & Altizer, S. (2018). Migratory monarchs that encounter resident monarchs show life-history differences and higher rates of parasite infection. *Ecology Letters*, *21*, 1670–1680.

Satterfield, D. A., Villablanca, F. X., Maerz, J. C., & Altizer, S. (2016). Migratory monarchs wintering in California experience low infection risk compared to monarchs breeding year-round on non-native milkweed. *Integrative and Comparative Biology*, *56*, 343–352.

Saunders, S. P., Ries, L., Neupane, N., Ramírez, M. I., García-Serrano, E., Rendón-Salinas, E., & Zipkin, E. F. (2019). Multiscale seasonal factors drive the size of winter monarch colonies. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 8609–8614.

Saunders, S. P., Ries, L., Oberhauser, K. S., Thogmartin, W. E., & Zipkin, E. F. (2018). Local and cross-seasonal associations of climate and land use with abundance of monarch butterflies *Danaus plexippus*. *Ecography*, *41*, 278–290.

Skerratt, L. F., Berger, L., Speare, R., Cashins, S., McDonald, K. R., Phillott, A. D., Hines, H. B., & Kenyon, N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth*, *4*, 125–134.

Smith, K. F., Sax, D. F., & Lafferty, K. D. (2006). Evidence for the role of infectious disease in species extinction and endangerment. *Conservation Biology*, *20*, 1349–1357.

Solensky, M. J. (2004). Overview of monarch migration. In K. S. Oberhauser & M. Solensky (Eds.), *The monarch butterfly: Biology and conservation* (pp. 79–83). Cornell University Press.

Swinton, J., Woolhouse, M., Begon, M., Dobson, A., Ferroglio, E., Grenfell, B., Guberti, V., Hails, R., Heesterbeek, J., Lavazza, A., Roberts, M., White, P., & Wilson, K. (2002). Microparasite transmission and persistence. In P. J. Hudson, A. Rizzoli, B. Grenfell, H. Heesterbeek, & A. Dobson (Eds.), *The ecology of wildlife diseases* (pp. 83–101). Oxford University Press.

Taylor, O. R., Jr., Pleasants, J. M., Grundel, R., Pecoraro, S. D., Lovett, J. P., & Ryan, A. (2020). Evaluating the migration mortality hypothesis using monarch tagging data. *Frontiers in Ecology and Evolution*, *8*, 264.

Thogmartin, W. E., Diffendorfer, J. E., López-Hoffman, L., Oberhauser, K., Pleasants, J., Semmens, B. X., Semmens, D., Taylor, O. R., & Wiederholt, R. (2017). Density estimates of monarch butterflies overwintering in Central Mexico. *PeerJ*, *5*, e3221.

Thogmartin, W. E., Wiederholt, R., Oberhauser, K., Drum, R. G., Diffendorfer, J. E., Altizer, S., Taylor, O. R., Pleasants, J., Semmens, D., & Semmens, B. (2017). Monarch butterfly population decline in North America: Identifying the threatening processes. *Royal Society Open Science*, *4*, 170760.

Thomas, M. B., & Blanford, S. (2003). Thermal biology in insect-parasite interactions. *Trends in Ecology & Evolution*, *18*, 344–350.

Thompson, R., Lymbery, A., & Smith, A. (2010). Parasites, emerging disease and wildlife conservation. *International Journal for Parasitology*, *40*, 1163–1170.

Vermote, E. (2019). NOAA climate data record (CDR) of AVHRR normalized difference vegetation index (NDVI), version 5. NOAA CDR Program, NOAA National Centers for Environmental Information.

Vidal, O., & Rendón-Salinas, E. (2014). Dynamics and trends of overwintering colonies of the monarch butterfly in Mexico. *Biological Conservation*, *180*, 165–175.

Wada, T., & Kobayashi, M. (1980). Effects of temperature on development of the rice leaf-roller, *Cnaphalocrocis medinalis* Guenee (Lepidoptera, Pyralidae). *Applied Entomology and Zoology*, *15*, 207–214.

Walsh, P. D., Abernethy, K. A., Bermejo, M., Beyers, R., De Wachter, P., Akou, M. E., Huijbregts, B., Mambounga, D. I., Toham, A. K., & Kilbourn, A. M. (2003). Catastrophic ape decline in western equatorial Africa. *Nature*, *422*, 611–614.

Wilfert, L., Long, G., Leggett, H. C., Schmid-Hempel, P., Butlin, R., Martin, S. J. M., & Boots, M. (2016). Deformed wing virus is a recent global epidemic in honeybees driven by varroa mites. *Science*, *351*, 594–597.

Wood, S. N. (2006). *Generalized additive models: An introduction with R*. Chapman and Hall/CRC Press.

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *73*, 3–36.

Wood, S. N. (2015). *R package 'mgcv': Mixed GAM computation vehicle with automatic smoothness estimation*. Version 1.8-33.

Zalucki, M., & Rochester, W. (1999). Estimating the effect of climate on the distribution and abundance of *Danaus plexippus*: A tale of two continents. In J. Hoth, L. Merino, K. Oberhauser, I. Pisantry, S. Price, & T. Wilkinson (Eds.), *1997 North American Conference on the Monarch Butterfly* (p. 151). Commission for Environmental Cooperation, Canada.

Zalucki, M. P. (1982). Temperature and rate of development in *Danaus plexippus* L. and *D. chrysippus* L. (Lepidoptera: Nymphalidae). *Australian Journal of Entomology*, *21*, 241–246.

Zipkin, E. F., Ries, L., Reeves, R., Regetz, J., & Oberhauser, K. S. (2012). Tracking climate impacts on the migratory monarch butterfly. *Global Change Biology*, *18*, 3039–3049.

Zuur, A. F., Ieno, E., Walker, N., Saveliev, A., & Smith, G. (2009). *Mixed effects models and extensions in ecology*. Spring Science and Business Media.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Majewska, A. A., Davis, A. K., Altizer, S. & de Roode, J. C. (2022). Parasite dynamics in North American monarchs predicted by host density and seasonal migratory culling. *Journal of Animal Ecology*, 91, 780–793. https://doi.org/10.1111/1365-2656.13678