

ECOGRAPHY

Research Article

The macroecology of immunity: predominant influence of climate on invertebrate immune response

Adam Z. Hasik¹ , Maggie Blondeau², Jacob Harvey², Tania Groleau², Tonia de Bellis^{2,3}, Eric J. Pedersen², Alex Córdoba-Aguilar⁴, Katie E. Marshall⁵, Laura Ferguson⁶ and Jean-Philippe Lessard² 

¹Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK

²Department of Biology, Concordia University, Montreal, QC, Canada

³Department of Biology, Dawson College, Montreal, QC, Canada

⁴Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

⁵Department of Zoology, University of British Columbia, Vancouver, BC, Canada

⁶Department of Biology, Acadia University, Wolfville, NS, Canada

Correspondence: Adam Z. Hasik (adamzhasik@gmail.com)

Ecography

2026: e08253

doi: [10.1002/ecog.08253](https://doi.org/10.1002/ecog.08253)

Subject Editor:

Jean-François Guégan

Editor-in-Chief: Miguel Araújo

Accepted 15 January 2026



The immune system is the primary defense against parasites. With the ever-increasing rate of disease, epidemiologic models considering geographic variation in immune responses could prove useful. Despite increasing interest in the macroecology of parasitism and infectious diseases, we know little about the macroecology of immune responses (i.e. macroimmunology). Host characteristics, parasite exposure, and environmental factors can all affect immunity, but how these factors shape spatial variation in the strength of immune responses remains underexplored. We captured odonates (dragonflies and damselflies) and their conspicuous ectoparasitic mites from 42 sites spread across a geographic area spanning the temperate and boreal forest biomes in eastern Canada. We then conducted immune response bioassays on 1237 individuals from 63 odonate species. We used generalized additive models and structural equation models to relate immune responses to host body size, parasite load, pH, temperature and precipitation while accounting for spatial autocorrelation in immune ability and evolutionary relationships among host species. We found significant differences in the strength of immune response among host individuals, and this variation was best explained by climatic conditions, specifically strongly decreasing with precipitation. While host species significantly differed in immune response strength, we found no effect of host body size, evolutionary relationships among hosts, or parasitism on immune response. Our study investigating the drivers of immune response across dozens of species spread across two biomes is the most comprehensive to date. Climatic conditions have a strong influence on host immune response, regardless of host characteristics or parasitism rates. Strong immune responses were associated with low levels of annual precipitation, which could relate to the role of cuticular melanin content in desiccation resistance, and the melanin-based encapsulation response being



www.ecography.org

© 2026 The Author(s). Ecography published by John Wiley & Sons Ltd on behalf of Nordic Society Oikos

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

a byproduct of this adaptation. A spatially explicit understanding of the biological processes affecting immunity could improve epidemiological models of disease risk that inform disease management globally.

Keywords: immune defenses, macroecology, odonate, parasite, precipitation, temperature

Introduction

Predicting parasite and pathogen spread is increasingly relevant and challenging in a highly connected world (Tsiotas and Tselios 2022), and an animal's immune system is the first line of defense against attack by parasites and pathogens. Yet, the factors driving variation in immunity among individuals, populations, and species are poorly studied and rarely factored into epidemiologic models (Becker et al. 2019). Characteristics of the host, exposure to parasites or pathogens, and the abiotic environment can interact in complex ways to affect immunity (Sweeny and Albery 2022), but their interactions are challenging to elucidate (Johnson et al. 2019).

As the immune system is the primary line of defense against infection by parasites, pathogens, and disease, it is assumed to be costly in terms of fitness and should therefore lead to tradeoffs with life-history traits (e.g. fecundity, fertility, Albery et al. 2021). Although a plethora of studies have provided key evidence of immune variation due to such tradeoffs, most studies emphasize the role of biotic factors such as predation (Duong and McCauley 2016) and resource availability (Hasik et al. 2025a) without considering that of abiotic factors (Lazzaro and Little 2008). A relationship between immune response and temperature is expected in both invertebrate ectotherms (Mastore et al. 2019) and vertebrate endotherms (Butler et al. 2013), due to the thermal sensitivity of the enzymes involved in immune responses (Catalán et al. 2012). When one scales this temperature-dependent immunity to explore the effect of climate (specifically, temperature and humidity), then climate is expected to be a clear driver of geographic variation in immunity (Li et al. 2024).

Parasites are a leading cause of disease and death around the world and thus are drivers of life-history evolution via their effects on host fitness (Hasik and Siepielski 2022a) that have the potential to affect host macroevolutionary dynamics (Hasik et al. 2025b). The majority of organisms on earth are infected by at least one parasite (Price 1980), and yet, we have a very limited understanding of the multifarious factors governing the intensity of infection and, therefore, the health cost. Immune responses are necessary to defend organisms from the deleterious and fitness-reducing effects of parasites (and disease in general, Hasik and Siepielski 2022a). Although there is increasing interest in the macroecology of parasites and infectious diseases (Stephens et al. 2016), we know very little about macroimmunology (Becker et al. 2020). Both among-individual and interspecific variation in immune response surely plays a central role, but the factors regulating immunity in natural settings are poorly understood, which can interfere with the accuracy of predictive epidemiologic models. Environmental factors and local parasite pressure can

independently drive differences in immunity across space, but they could also act in concert (Becker et al. 2020). Parasitism varies among host populations distributed across large-scale environmental gradients (LoScerbo et al. 2020, Hasik and Siepielski 2022b) and at fine spatial scales, within populations (Albery et al. 2019, Hasik et al. 2025a). To date, however, the focus on a limited set of taxa, specifically vertebrates (Becker et al. 2020), limits our ability to identify generalities regarding the relative influence of environmental conditions and parasitism on immune defenses that would apply across host-parasite systems (Rolff and Siva-Jothy 2003).

Here, we use data from a large-scale field sampling of communities of dragonflies and damselflies and their larval water mite ectoparasites to assess the relative influence of the abiotic environment, parasite pressure, and host characteristics on host immunity. We quantified the magnitude of melanin encapsulation, which is a well-studied aspect of invertebrate immunity, and related among-host individual variation in this immunological trait to variation in host body size, parasite load, and the abiotic environment (pH, temperature, precipitation). As both the local environment experienced and the immune response of a given individual will depend on species-specific traits which may be phylogenetically constrained, we also tested for evidence of phylogenetic signal in the predictors and in immune response, to rule out phylogenetic dependence leading to spurious environment-immune response relationships. Finally, we used structural equation models (SEMs) to test a hypothesized causal model of the relationship between environmental and biotic variables and immune response; we hypothesized that the local abiotic environment and parasitism (itself influenced by the local environment) would act together to drive variation in immune defenses.

Material and methods

Study system

For our hosts we collected odonates (e.g. dragonflies and damselflies); invertebrates that spend the majority of their lives in the water column as aquatic larvae. Adult odonates are frequently parasitized by larval *Arrenurus* water mites (Acari: Hydrachnida) which leave the host when the latter returns to water to reproduce as adults (Smith et al. 2010, Hasik et al. 2023a). Larval water mites are opportunistic parasites, and a single species can parasitize multiple odonate species (Zawal and Buczyński 2013), while a single odonate species can be simultaneously parasitized by several mite species (Smith and Oliver 1986). Water mite parasitism is affected by both host traits such as body weight and environmental factors such as pH, conductivity, dissolved oxygen,

and air temperature (LoScerbo et al. 2020, Hasik et al. 2024). There is also substantial variation in mite prevalence between and within species (Ilvonen et al. 2018, Hasik and Siepielski 2022b, Hasik et al. 2024) and water mites reduce host survival (Forbes and Baker 1991), although there appears to be no general trend toward either sex being more infected (Ilvonen et al. 2016). Both climatic variables and water chemistry influence not only odonate community composition in aquatic systems, but also parasite prevalence and intensity (Arrowsmith et al. 2018, LoScerbo et al. 2020, Hasik and Siepielski 2022b, Hasik et al. 2024).

Odonates defend themselves from water mites via a general immune defense shared among invertebrates: the phenoloxidase cascade (González-Santoyo and Córdoba-Aguilar 2012). In short, the phenoloxidase cascade produces melanin as its end product, which encases water mite feeding tubes and prevents the mites from gorging on the host's haemolymph (Hasik et al. 2023a), and parasite infection in this system is

known to reduce fat reserves that affect immune defenses (Siva-Jothy and Plaistow 1999). Though it is a general defensive mechanism, resistance to water mites is known to vary intra- and inter-specifically. Resistance is greater when the host is locally rare (Lajeunesse et al. 2004), and increases with both the prevalence and intensity of parasitism (Nagel et al. 2010, Kaunisto et al. 2017), the degree of local adaptation to parasites (Gómez-Llano et al. 2020), and resource availability (Hasik et al. 2023b).

Odonate sampling

We opportunistically sampled odonates using a butterfly net in the summers of 2019 and 2020. We standardized the time of day for all sampling events and found no evidence for an effect of temperature at time of collection or date (i.e. seasonal effects) on immune responses (Supporting information). We collected animals near water bodies at 42 sites ranging from the northern temperate forest region to the middle of the

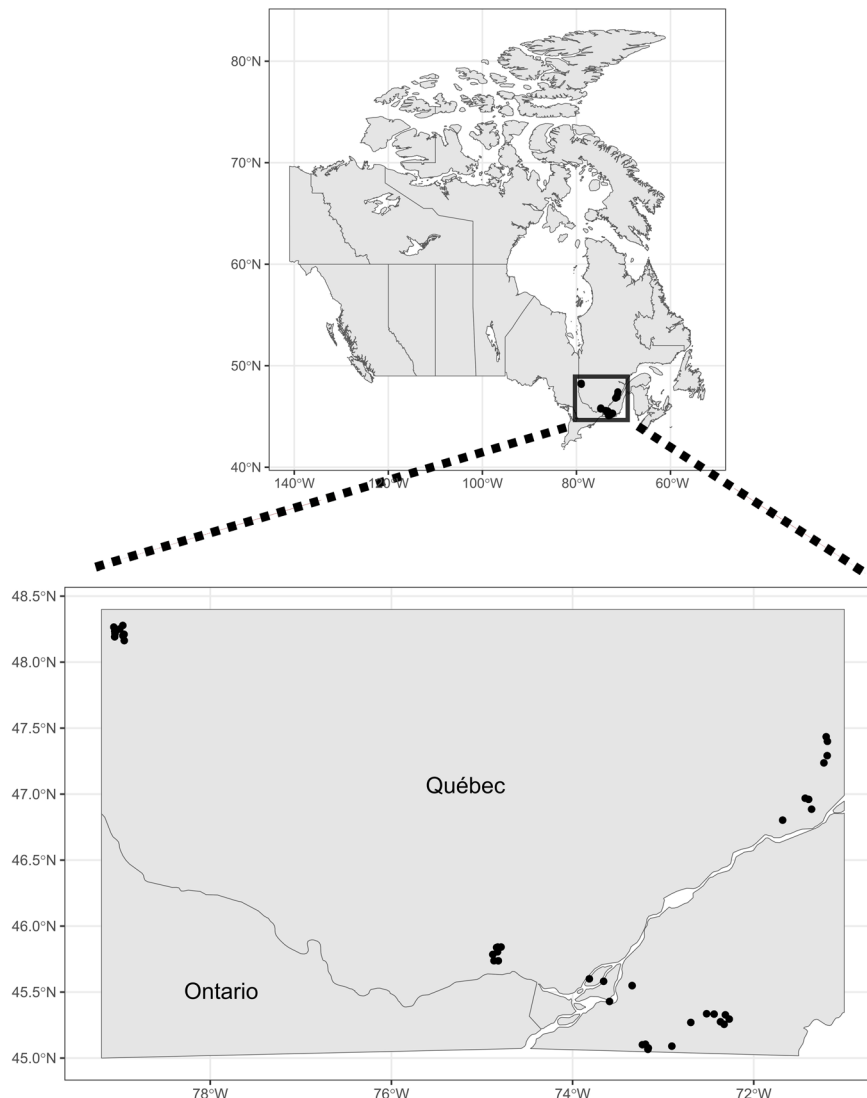


Figure 1. Map of the study sites across southeastern Canada, with the lower panel showing a zoomed-in perspective of the sites spread across Québec.

boreal forest in southeastern Canada (Fig. 1). In total, we collected 1237 individuals from 63 species broadly distributed across the odonate phylogenetic tree. See the Supporting information for detailed information on sample sizes and species compositions.

Immune response

Melanin, particularly eumelanin, is the encapsulating defense molecule and is dark brown in color (González-Santoyo and Córdoba-Aguilar 2012), so by quantifying the darkness of a proxy that has been inserted and melanized within a live odonate (to simulate a mite feeding tube), we can determine the relative strength of its immune response (Rantala and Roff 2007, Córdoba-Aguilar et al. 2009, Ilvonen and Suhonen 2016).

For full details of the immune assays see the Supporting information. Briefly, we inserted a sterilized 3 mm piece of nylon monofilament (hereafter implant) into the venter of the odonate thorax. This region of the body was chosen because it is where mites tend to latch onto their host, and the implant is meant to imitate the feeding tube of the parasite (Hasik et al. 2023a). After 12 h we removed and photographed the implant directly above (~30 cm between implant and camera lens, centering the lens over the implant) using the flash from the camera (Canon EOS Rebel T6s, lens extension MP-E 65 mm). We then photographed the complete odonate animal, sacrificed them with acetone, and then stored them in glassine envelopes for subsequent identification and mite counting.

Quantifying immune response

We used ImageJ (Schneider et al. 2012) to quantify the degree of melanization of each implant. We converted images of the nylon implant to 8-bit greyscale, which allowed us to measure average grey value of the pixels. Converting images to greyscale also allows us to extract darkness values ranging from 0 (completely black) to 255 (completely white), which we used to obtain the value representing immune response strength.

We selected six squares on each implant that had dimensions equal to the width of the nylon monofilament, ensuring that the areas were all proportional as every filament has the same width (0.4 mm) across images. The six squares that we measured were: 1) the deepest point of insertion, 2) the area nearest the point of insertion (just under the cuticle), 3) the area directly in between point 1 and 2, 4) the end of the side of the nylon not inserted into the specimen, 5) the region just outside of the cuticle that was not inserted, and 6) the region directly in the middle of 4 and 5 (Supporting information). See the Supporting information for further information on our use of six measurement points. We compared the average grey value of the three measurements spanning the inserted portion (the inserted average, denoted SA) to the average grey value of the three measurements spanning the portion that was not inserted (the inserted control, denoted CA). The immune response for a given individual i was calculated by

taking the difference between the control average for that individual (CA_i) and the inserted average (SA_i):

$$IR_i = CA_i - SA_i$$

Note that higher values of IR_i indicate a stronger melanization response, since CA_i and SA_i are grey-scale values where 0 represents a completely black value and 255 represents a white value. As such, the higher the melanization in the sample, the closer to zero the value for SA_i will be.

Host characteristics

Parasitism and immune function are known to vary with body size (Hasik and Siepielski 2022b). To accurately quantify body mass, we weighed each of the dried odonate host individuals once using an electronic analytical scale. To mitigate specimen damage and prevent limb or fragment loss, specimens were removed from their glassine envelope with metal pliers and placed in small plastic square weighing boats. Furthermore, weighing boats were replaced and the scale was tared between each measurement to minimize static interference and ensure consistency in recorded mass.

Parasitism

To quantify parasitism for each odonate host individual, we examined dry specimens under a dissecting microscope (LoScerbo et al. 2020). We counted the ectoparasitic mites that were attached to the host and noted the points of attachment on the host body in accordance with previous studies (Forbes et al. 1999). We included detached, engorged mites found within the individual odonate collection envelope in the total mite count for the host individual.

Abiotic environment

We quantified the ambient climate and water pH at each collection site. We extracted mean annual temperature (°C; BIO1) and mean annual precipitation (mm; BIO12) associated with each lake from WorldClim ver. 2.1 (Hijmans et al. 2005) as climatic variables representing physiological constraints on hosts and parasites in our models. These data are calculated using climate data from 1970 to 2000. We recorded lake pH using an Oakton WD-35634-35 Series 50 Waterproof Pocket Ph/Cond/TDS/Salinity Tester. pH was quantified as the mean of five measurements taken at least 5 m apart where possible, and between 1 and 3 m away from the shoreline.

Statistical packages

We conducted all statistical analyses in R ver. 4.1.2 (www.r-project.org). Moran's I was calculated using the 'morantest' R package ver. 1 (www.r-project.org, Cooper 2020). Generalized additive models (GAMs) were estimated using the *gam* function from the 'mgcv' package ver. 1.9.1 (Wood

2017), using restricted maximum likelihood to estimate variance parameters (Wood 2010). We used the 'ape' package ver. 5.8.1 (Paradis et al. 2004) and the 'MRFtools' package ver. 0.0.2 (Pedersen and Simpson 2023) to calculate phylogenetic precision matrices.

Testing for evidence of spatial and phylogenetic dependence in immune response

Our core questions centered around whether average odonate immune response varied predictably as a function of local environmental variables. However, our sampling scheme involved sampling sites in spatially clustered locations (Fig. 1). Further, not all species were found in all sites, and it is well-known that species traits (e.g. body size) can be strongly phylogenetically structured (LoScerbo et al. 2020). As such, spatial or phylogenetic autocorrelation in both predictors and outcomes could result in spurious environment-immune response relationships due to spatial or phylogenetic confounding (Warton 2021). As such, we tested for the presence of both spatial and phylogenetic signal in immune response.

We used Moran's I test (Moran 1950), using inverse distances between sample locations as weights, to test for the presence of spatial autocorrelation in melanization ability and in our key environmental and biotic predictors. We also calculated local Moran's I values using the 'pgirmess' package (Giraudoux et al. 2024) to understand the spatial scale at which any potential effects of the local environment are manifesting.

To test for phylogenetic signal in our covariates and outcome, we used the time-calibrated odonate phylogeny of Waller and Svensson (2017), as it is the most comprehensive and well-resolved phylogeny for this group. We pruned this tree to include only those species which we collected during the field surveys using the 'ape' package (Paradis et al. 2004). Though our analyses included 63 total species, only 53 of those species have been mapped to the odonate phylogeny, thus we were not able to include all species in our phylogenetic analyses.

We used a model comparison approach to test for both variation in species-level average trait values, as well as phylogenetic information in the patterns of trait similarities. We transformed the phylogenetic tree into a variance-covariance matrix assuming a Brownian motion model of trait evolution, which we used as part of a phylogenetic hierarchical linear regression model for each trait as a function of species. Here we use the mixed effect model notation suggested by Zuur and Leno (2016) throughout when describing estimated models, where coefficients for fixed effect terms are implicit. Each trait model took the form of:

$$\text{Trait}_{ijs} = \text{Intercept} + \text{HostSpecies}_s + \epsilon_{ijs}$$

$$\text{HostSpecies}_s \sim \text{Normal}(0, \sigma_{\text{species}} \Sigma_{\text{phy}})$$

$$\epsilon_{ijs} \sim \text{Normal}(\sigma_{\text{individual}})$$

where i , j , and s are indices that refer to the specific individual, site, and species associated with a given observation, HostSpecies_s is the estimated species-specific mean of the trait, $\sigma_{\text{species}} \Sigma_{\text{phy}}$ is the Brownian motion phylogenetic variance-covariance matrix scaled by an estimated variance parameter σ_{species} , and ϵ_{ijs} is the assumed normally distributed residual error. This model includes the potential for phylogenetic signal in trait means through the Σ_{phy} matrix, as it acts to penalize estimated mean trait values of related species towards one another, with more closely related species being more penalized toward a common mean. We compared this model with a simple species-level random effect model that still allowed for species-specific means for each trait without requiring phylogenetic signal (as above except assuming $\text{HostSpecies}_s \sim \text{Normal}(0, \sigma_{\text{species}})$). For each trait, we calculated an adjusted R^2 value for species (to estimate the fraction of variation in that variable predicted by species identity) from the random effects model. A high R^2 means that most of the variation in that variable occurs among species (rather than within individuals of the same species). We then calculated ΔAIC differences between the random effect and phylogenetic models for each trait, using AIC calculated via 'mgcv' (which corrects for smoothing when calculating model degrees of freedom; Wood 2017). Here, there would be little evidence of phylogenetic signal if the random effects model has a lower AIC than the phylogenetic model.

To illustrate the species-level patterns of variation in host traits, we calculated species-level averages for biotic variables (weight, melanization, and mite abundance) then visualized these alongside the phylogenetic tree.

Modeling immune response as a function of local environment

Our first question was concerned with understanding if the local environment and parasitism were associated with immune response over large spatial scales. To test this, we used our entire dataset (i.e. records from every species and population) to relate melanization response to environmental factors and parasitism. This dataset included observations of immune response for 1237 individuals from 63 species. Using these data, we estimated the following GAM:

$$\begin{aligned} \text{IR}_{ijs} = & \text{Intercept} + \text{pH}_j + \text{Precip}_j + s(\text{Temperature}_j) \\ & + \text{MiteAbundance}_{js} + \text{IndividualDeviation}_{ijs} \\ & + \text{HostSpecies}_s + \text{HostFamily}_s + \text{Site}_j \\ & + \text{Watershed6}_j + \text{Watershed5}_j + \epsilon_{ijs} \end{aligned}$$

$$\text{HostSpecies}_s \sim \text{Normal}(0, \sigma_{\text{species}})$$

$$\text{HostFamily}_s \sim \text{Normal}(0, \sigma_{\text{family}})$$

$$\text{Site}_j \sim \text{Normal}(0, \sigma_{\text{site}})$$

WaterShed6_j ~ Normal(0, σ_{WS6})

WaterShed5_j ~ Normal(0, σ_{WS5})

ϵ_{ijs} ~ Normal(0, σ_{invidual})

Here IR_{ijs} is the immune response index for individual i of species s in site j . The terms pH_j and $Precip_j$ were environmental fixed effects (all variables were Z-score transformed in the full dataset before analysis). As visual inspection indicated that the *Temperature*–*IR* relationship was not well-approximated as a linear relationship, we modeled the effect of temperature as a nonlinear function ($s(\text{Temperature}_j)$) using a thin-plate regression smoother (Wood 2017) with five basis functions. All continuous predictor covariates were Z-score scaled to have a mean of zero and a standard deviation of 1, so slopes were interpretable as standardized coefficients.

MiteAbundance_j and IndividualDeviation_{ijs} refer to the population-level mean mite abundance for species s at site j and individual-level deviation from this mean for individual i of species s at site j , respectively. Using this modeling approach allows us to avoid confounding the effect of mite density on the individual with effects of average mite abundance on individuals in the population. An individual might have a higher immune response because it is living in a population that has a higher than typical average level of mites (i.e. population effect of parasite load on IR_{ijs}) or it might have a higher immune response because it has higher than average levels of parasites itself (or both effects could be present). It could also be that these effects could occur in opposite directions: adaptive evolution could result in IR_{ijs} being stronger on average in areas with high mite abundances, but an individual with a large number of mites might have a reduced immune response if mite infection depleted its energy stores before our melanization test.

HostFamily_s, HostSpecies_s, Site_j, Watershed6_j, and Watershed5_j are random effect terms included to account for potential spatial and species-scale dependence in *IR*. The error term ϵ_{ijs} is residual individual-specific variation in immune response. We did not include phylogenetic covariance as part of the random effect structure for species (HostSpecies_s), as our prior analysis did not demonstrate any strong signal of phylogenetic conservation in any of the model covariates. However, we did nest species within family to account for phylogenetic clustering of our data. To account for the high degree of detected spatial autocorrelation, and the clustered sampling design, we used a hierarchically nested random effect design: we used watershed data from the HydroBASINS database (www.hydrosheds.org/products/hydrobasins, Lehner and Grill 2013) to assign each site to a level-5 and level-6 watershed (with level-6 watersheds nested inside level-5 watersheds; Supporting information), and then included nested random effects for Site_j, Watershed6_j, and Watershed5_j. This approach allows for modeling variation in immune response at different spatial scales. There was no

multicollinearity between environmental predictors (i.e. $r < 0.80$ in all cases).

Structural equation modeling

Our second question was concerned with disentangling the complex and interconnected network of relationships between the local environment, parasitism and immunity. To do so we utilized structural equation models (SEMs, Shipley 2009). The Supporting information contains a graphical representation of the initial SEM we used to test our hypothesis of water mite-mediated indirect relationships between the local aquatic environment and immune defenses. For this analysis we used a subset of our data, limiting it to those species for which we had roughly equivalent representation of infected and uninfected individuals with a minimum of $n=3$ individuals from $n=3$ populations, including observations of immune response for 556 individuals from seven species. We did this because we were interested in understanding potential drivers of parasitism and immunity within populations, thus it was important to include infected and uninfected individuals and remove those species that were rarely infected and had limited sampling. For all models we compared goodness-of-fit using the comparative fit index (CFI, a measure of the amount of variance accounted for in the covariance matrix, Fan et al. 2016) and root mean square error of approximation (a measure of how far a hypothesized model is from a model with a better fit, Xia and Yang 2018). Models with CFI > 0.95, and RMSE < 0.06 are considered to fit the data well and were compared to one another using AIC values (as in Hasik and Siepielski 2022b, Hasik et al. 2024).

Our initial SEM tested the hypothesis that the local abiotic environment (pH) was affected by the local climate (precipitation and temperature), that host size was related to the local climate (precipitation and temperature), that parasitism population-level mean mite abundance was affected by the local environment (pH) and climate (precipitation), that individual-level deviance in infection was affected by the local abiotic environment (pH) and climate (precipitation) and host size, and finally that melanization response was affected by parasitism (population-level and individual-level), the local abiotic environment (pH), and climate (precipitation and temperature). There is, however, a possibility that there are connections between these various predictors that we did not model (i.e. temperature affects parasitism). To test for the presence of other relationships, we identified additional terms to fit to the model by analyzing modification indices using the ‘modindices’ argument in ‘lavaan’ (Rosseel 2012). Modification indices with $\chi^2 > 3.84$ identify what terms, if added to a candidate model, improve model fit (Whittaker 2012). This method allowed us to build a statistically informed network of additional relationships between the local abiotic environment, climate, parasitism, and melanization response. We iteratively added direct predictors of host size and mite abundance (population- and individual-level) with $\chi^2 > 3.84$ until no further modification indices were identified and the model had a CFI > 0.95 and RMSE < 0.06 (as in Hasik et al. 2024). We centered all environmental

Table 1. Summary statistics of tests for spatial signal (Moran's I), species signal (R^2), and phylogenetic signal (ΔAIC) for variables used in the regression model.

Variable	Observed Moran's I (SD)	Moran's I p-value	R^2 explained by species identity	ΔAIC	Top model
Melanization	0.60 (0.01)	< 0.001	44%	15	Random effects
Population mean mite abundance	-0.06 (0.01)	< 0.001	22%	3	Random effects
Temperature	0.81 (0.01)	< 0.001	38%	24	Random effects
Precipitation	0.64(0.01)	< 0.001	54%	11	Random effects
pH	0.28 (0.01)	< 0.001	29%	23	Random effects
Individual mite load	-0.03 (0.01)	< 0.001	7%	18	Random effects
Weight	0.19 (0.01)	< 0.001	95%	5	Phylogenetic

variables to have a mean of 0 and SD of 1, and included host species as a random effect.

Results

Evidence for spatial or phylogenetic signal in covariates

We found strong evidence of spatial autocorrelation in all covariates, with p-values for observed Moran's I statistics < 0.001 for all tested variables. Melanization in particular was moderately and positively spatially autocorrelated at distances of < 2 decimal degrees, but we found that this spatial autocorrelation decayed after ~ 2.2 decimal degrees (~ 270 km, Supporting information). We found that all traits showed a large degree of species-level conservation (R^2 of 30% or higher) except for individual mite load (Table 1), but only body size (Weight; Table 1) showed any evidence of strong phylogenetic conservation; the random effects model was better supported for all other traits, indicating the absence of detectable phylogenetic signal (Table 1). This is consistent with the large degree of variation in average immune response both among closely related host species (Fig. 2) and within host species (Fig. 3). Given the lack of phylogenetic signal, and the lack of qualitative difference between models that did (Supporting information) and did not control for phylogenetic-relatedness among species, we focus our results on models of our full dataset containing all species while still controlling for spatial autocorrelation; though note we still account for the phylogenetic clustering by nesting Species within Family. Partitioning of the variance of our random effects revealed that Site explained a moderate proportion of the variance (partial $R^2=0.13$), while Species, Family, Watershed 6, and Watershed 5 explained very little variance (all partial $R^2 < 0.01$).

The local climate predicts immune defenses

When considering our entire dataset, we found that melanization response strongly declined as mean annual precipitation of each site increased (parametric regression coefficient (SE) = -0.21 (0.02), $F_1 = 133.58$, $p < 0.0001$) yet did not vary with mean annual temperature of each site (EDF = 1.00, $F_1 = 1.21$, $p = 0.27$, Fig. 4). Surprisingly, we found that the relationship between temperature and melanization was best approximated as a linear relationship (i.e. EDF = 1), despite

the seemingly non-linear relationship of the raw data in Fig. 4b. We did not find significant relationships between melanization response and site pH ($F_1 = 0.28$, $p = 0.60$), population-level mite abundance ($F_1 = 0.13$, $p = 0.72$), or individual-level deviance in mite abundance ($F_1 = 0.98$, $p = 0.32$). Our GAM explained 80.1% of the deviance in the data, with an $R^2_{\text{adjusted}} = 0.79$.

Environmental drivers of immunity

Our SEMs revealed that there were no detectable indirect relationships among climate, pH, mite parasitism, and immune response. Instead, we found that melanization weakly increased with body weight and population mean mite abundance while declining moderately, strongly, and weakly with temperature, precipitation, and individual deviance, respectively (Fig. 5). Additionally, we found that pH moderately declined with precipitation (Fig. 5).

Discussion

Understanding geographic and among-population variation in immune defenses is key to predicting the impact of parasites on host populations, but variation in immune defenses themselves relies on myriad factors (Becker et al. 2019). In this study we used data from a widely distributed and well-studied host-parasite system to test the hypothesis that immune defenses respond to geographical variation in abiotic and biotic factors. Contrary to our expectations, the most prominent result is that the abiotic environment, rather than parasitism or host characteristics, best predicted geographic variation in melanization response. Structural equation models applied to a subset of co-distributed species further supported these findings, with additional insight into the influence of parasitism. Overall, our results are consistent with the strength of melanization being a by-product of adaptation to desiccation and to some degree an evolutionary response to the abundance of parasites in the environment. These findings add to a growing body of literature showing spatial variation in immunity in a broad array of taxa such as bank voles (Tschirren 2015), prairie chickens (Bollmer et al. 2011), purple sea fans (Couch et al. 2008), honey bees (Appler et al. 2015), and vampire bats (Becker et al. 2018).

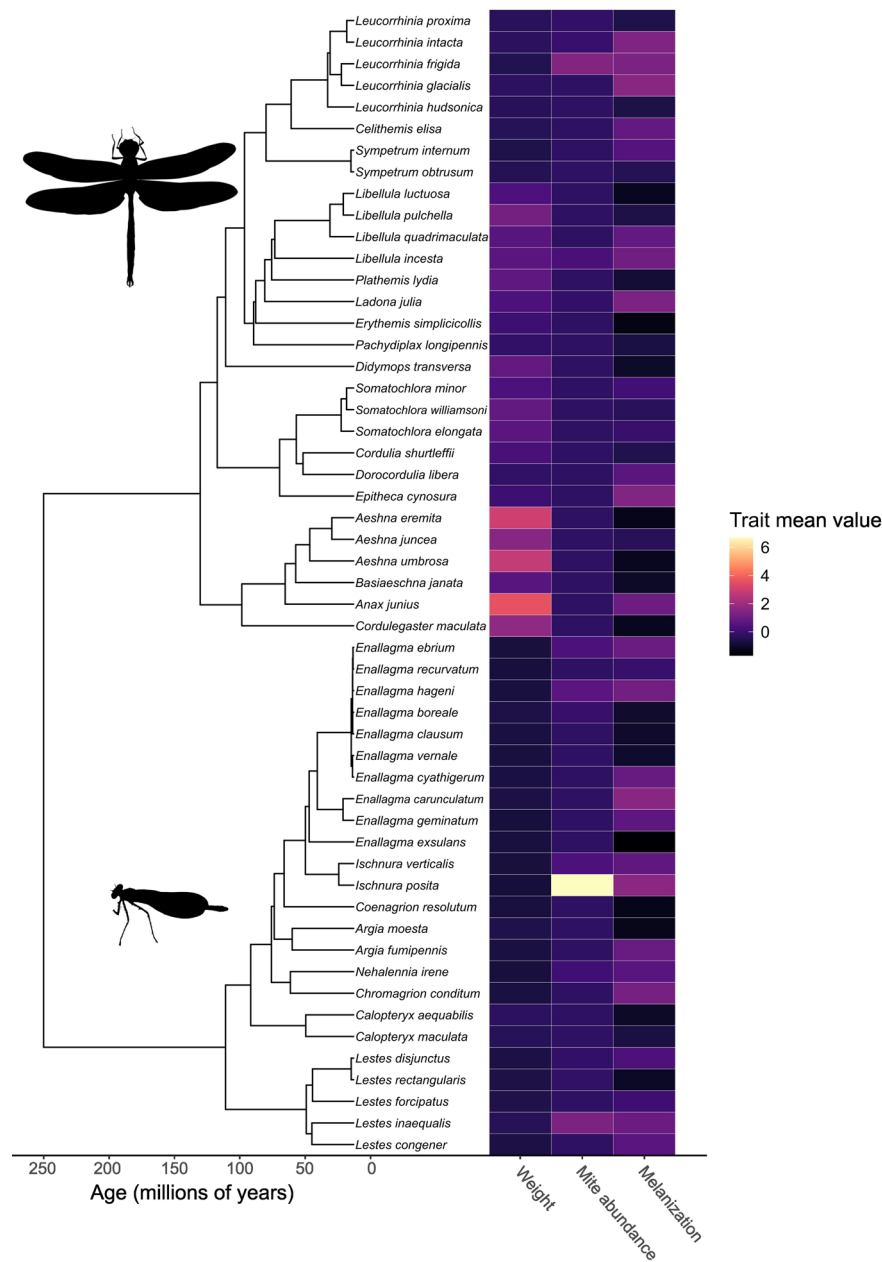


Figure 2. Phylogenetic tree of the species used in our analyses, with trait data on body weight, mite abundance, and melanization mapped onto the tree. All three traits are mean-centered and scaled, such that they have a mean of 0 and SD of 1, with darker colors denoting lower values and lighter colors denoting higher values. Inset images of the dragonfly and damselfly represent the two suborders within the clade.

Our results add to a growing body of literature showing how environmental conditions set the stage for parasitism, in this case via the relationship between environmental factors and immune defenses. Evidence from mammals (Hasik et al. 2025a), insects (LoScerbo et al. 2020, Hasik and Siepielski 2022b, Hasik et al. 2024), fish (Bolnick et al. 2020), and plants (Penczykowski et al. 2014) has revealed that abiotic and biotic factors predict parasitism due to the varied connections between these factors and their effects on the host and/or parasites. In the case of odonates and their water mite parasites, water pH is a key driver of variation in odonate

host community composition (Arrowsmith et al. 2018) and water mite infection levels (LoScerbo et al. 2020, Hasik and Siepielski 2022b, Hasik et al. 2024). The suggested mechanism by which water pH (acidic conditions) affects parasitism is a reduction in water mite larval survival and egg viability at low pH (Edwards 2004). However, the results from our SEM revealed that, at least in the populations and species that we sampled, pH is neither a predictor of melanization nor mite parasitism. Instead, precipitation and to some degree temperature were the primary drivers of melanization. The reasons for this are unclear, and mean annual temperature may not

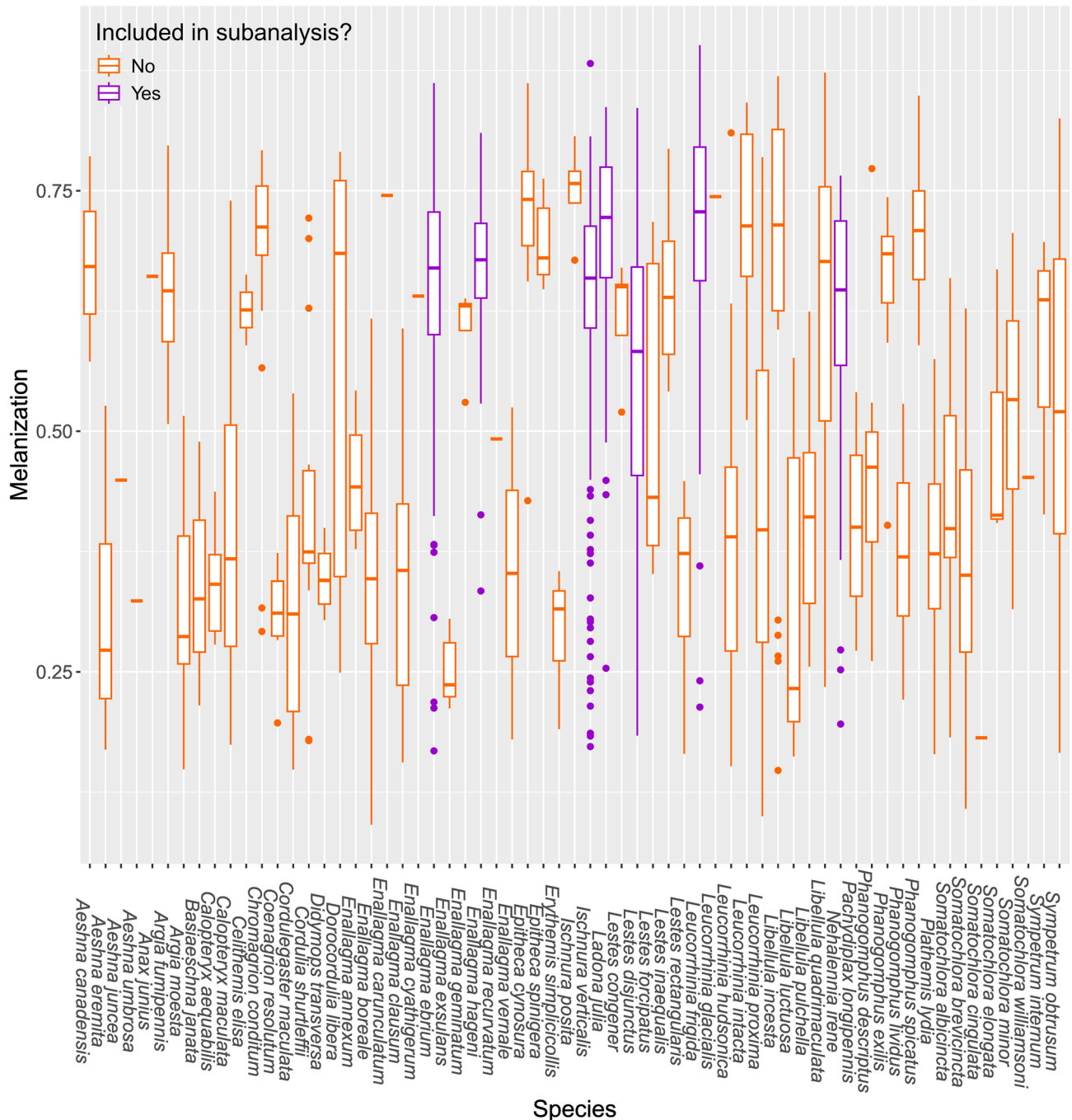


Figure 3. Boxplots representing the variation in melanization response (y-axis) of the various species (x-axis) included in our analyses. Color denotes those species included in the sub-analysis using SEMs.

necessarily be a good indicator of what the temperature was at the time of sampling, but it could relate to how the local environment is expected to affect melanization in insects.

Precipitation best explained variation in the strength of immune response among host individuals and populations. One possible reason behind this pattern is that abiotic factors directly contribute to immunity in ectotherms (Ferguson et al. 2018). In general, high temperatures drive greater melanization responses (Fedorka et al. 2013), though

we did not find evidence for this relationship in our analysis of all individuals. Melanin production is primarily used for three functions: parasite defense (as is the case against mites, Hasik et al. 2023a), thermoregulation via cuticle darkening (Nakhleh et al. 2017), and water retention via reduction in cuticular permeability (King and Sinclair 2015). These functions may covary inversely due to a resource allocation tradeoff and depending on how much they are being selected for (Cotter et al. 2008).

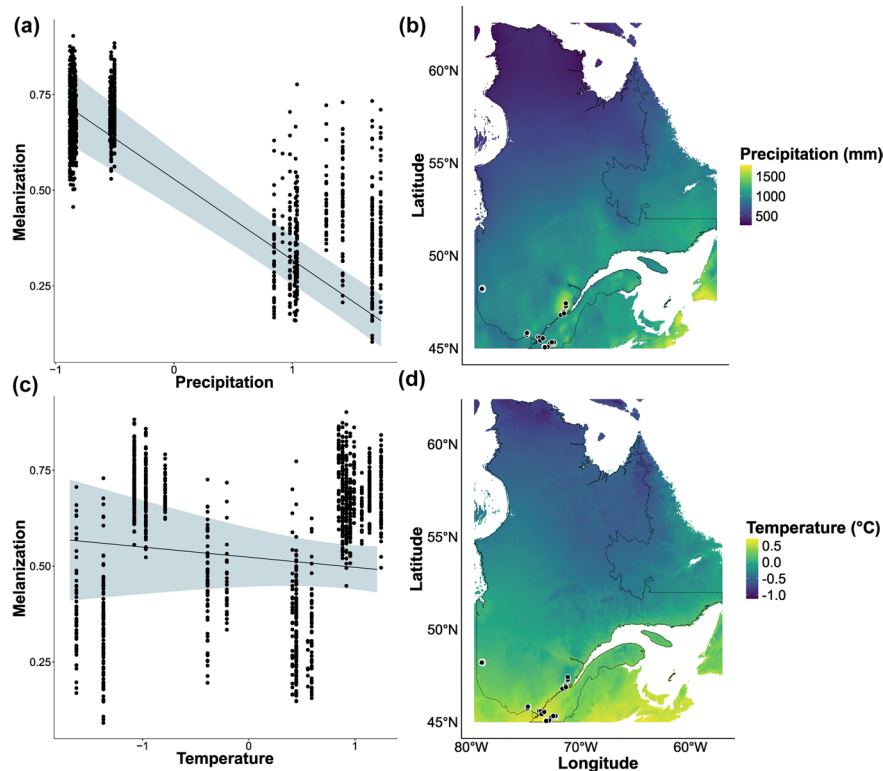


Figure 4. Relationships between melanization and environmental factors. Shown are model-derived predicted regressions (bands denote 95% CI) of melanization response on precipitation (a) and temperature (c), as well as climate maps of Québec denoting mean annual precipitation (b) and temperature (d) with points denoting sampling sites. Note the different x-axes between panels (a) and (c). Points in (a) and (c) denote melanization values for individual odonates.

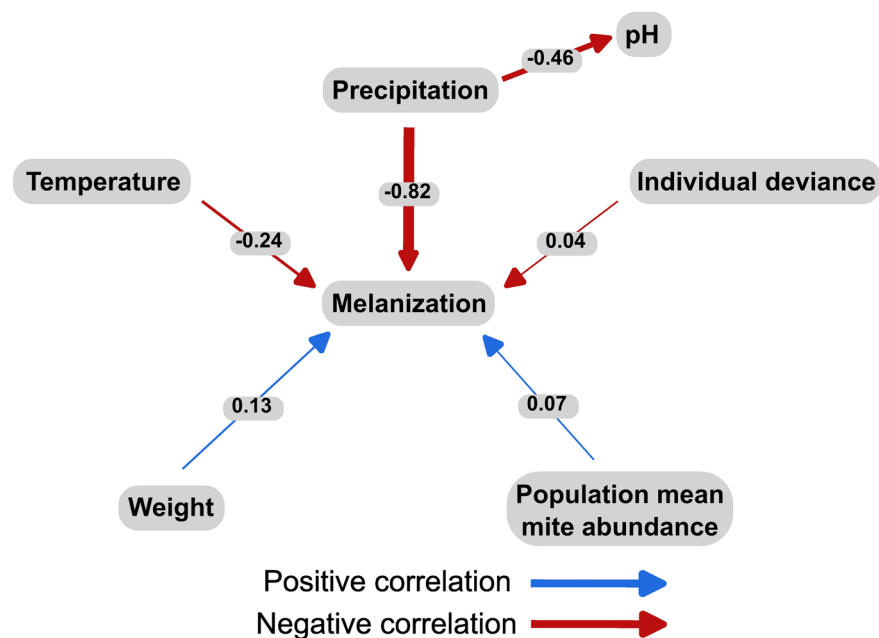


Figure 5. SEM plot of the relationships between climatic variables, the local environment, parasitism, and melanization. Only significant relationships are shown, with numbers representing standardized effect sizes. Width of the arrows denotes effect size magnitude, and color denotes direction of the relationship.

Our results emphasize the strong influence of annual precipitation on melanization response, with the greatest responses recorded in the driest regions. This works supports prior evidence in birds (Fair and Whitaker 2008, Nwaogu et al. 2019) linking precipitation to immune defenses. Nevertheless, some studies have shown that cuticle darkening driven by high cuticular melanin content is affected by how humid an environment is, yet the evidence is equivocal (Clusella-Trullas and Nielsen 2020, Klunk et al. 2022). Similarly, the degree of cuticle darkening varies geographically among wild populations of *D. melanogaster* from Africa (Pool and Aquadro 2006), India (Parkash et al. 2008), and Australia (Telonis-Scott et al. 2011), which indicates that cuticle darkness is an adaptation to regional climatic conditions. Laboratory experiments with *D. melanogaster* further indicate that individuals with darker cuticles are more resistant to desiccation (Ramniwas et al. 2013), which supports the melanization–desiccation hypothesis. The observed increase in desiccation resistance is likely due to a decrease in cuticular permeability. Taken together, these results suggest that the strong melanin encapsulation response observed in the driest part of our study region could be a by-product of the adaptive value of melanin for resistance to desiccation. These results contrast with the common expectation that because wet habitats contain more parasites and predators, host cuticles should exhibit higher cuticle melanization (Delhey 2019, Clusella-Trullas and Nielsen 2020). In such a case, wet environments could select for cuticles that are more heavily melanized at the expense of the immune defenses. An additional possibility is that ultraviolet radiation, which is inversely related to precipitation (i.e. cloud cover), is driving the observed patterns. Ultraviolet B (UVB) radiation is known to affect immune defenses in other invertebrates (Sabockyté et al. 2023, Picard et al. 2025), thus our finding that strong immune response is related to low levels of precipitation could in fact reveal the influence of UVB radiation.

Across all individuals, we found that there was no overall relationship between degree of parasitism and melanization response. The reasons for this in our overall analysis are unclear; however, our interpopulation comparisons using SEMs and a subset of our overall data suggested that local adaptation of the hosts to their water mite parasites may be occurring. That is, the melanization-based immune defense could increase with the degree of local adaptation of odonate hosts to their parasites (Gómez-Llano et al. 2020). Indeed, hosts from populations with greater parasite abundance had a stronger melanization response, independent of the negative relationship we found between individual deviance and melanization, supporting previous work showing that the melanin-based immune defense used in this study is greater in populations experiencing heavy parasitism (Nagel et al. 2010, Kaunisto and Suhonen 2013, Kaunisto et al. 2017). This suggests that hosts from high-risk populations have greater melanization response, which could be an adaptive response, but heavy infections could be depleting or suppressing melanin stores and thereby reducing the melanization response. The latter hypothesis is highly parsimonious given the mechanics

of the melanization response. That is, odonates produce melanin to seal mite feeding tubes and defend themselves from attack (Hasik et al. 2023a). Heavy infections would stimulate the phenoloxidase cascade to produce more melanin than would be seen in a lighter infection, hence the negative relationship with individual deviance. An alternative hypothesis is that mite-mediated pressure is instead a proxy of aquatic bacteria. pH is an important predictor of the distribution of freshwater bacteria (Lindström et al. 2005), and while pH is known to predict variation in water mite infection in this system (LoScerbo et al. 2020, Hasik and Siepielski 2022b, Hasik et al. 2024) it could instead be that the mites themselves are associated with a specific bacterial community in these freshwater lakes that are driving variation in immunity. Future studies should investigate how bacterial communities relate to immune defenses in aquatic invertebrates.

Our analysis testing for a phylogenetic signal of various traits of interest revealed very few significant patterns. Specifically, we only found a phylogenetic signal in body weight, which is consistent with previous work (LoScerbo et al. 2020). The lack of a signal for both mite abundance and immune defenses bolsters our other findings that both of these traits of interest are mediated by the environment and are not constrained by phylogeny, suggesting that accounting for species effects is more important than accounting for phylogeny in analyses of odonate immune defenses. While we did not study evolutionary mechanisms per se, such species-specific variation highlights how environmental differences among host–parasite populations may result in evolutionary hot- and cold-spots (Thompson 2005). That is, our finding that environmental variation best explains variation in immunity, previous findings relating the local environment to water mite parasitism (LoScerbo et al. 2020, Hasik and Siepielski 2022b, Hasik et al. 2024), and the lack of signal in our phylogenetic analyses, suggest that neither the host nor parasite are the primary drivers of the (co)evolutionary relationships. Instead, the local environment shapes these interactions, after which the host and parasite respond. Indeed, the Stockholm Paradigm posits that ecological disruptions generate novel combinations of host and parasite assemblages (Brooks et al. 2015), and it is these novel associations that then drive host–parasite (co)evolution.

Our study is based on a wealth of field data, including data gained from over one thousand immune assays, but we must mention a number of important caveats. First, all our analyses are based on correlational data. Though our modeling framework utilizing SEMs allows for tests of hypothesized ‘casual’ pathways, they cannot infer causality. Second, we used a general measure of parasitism and did not identify water mites to the species level. *Arrenurus* is a remarkably diverse subgenus (Więcek et al. 2023), and while we did not find evidence for parasites being a primary driver of immune defenses, there remains the possibility that such relationships exist on a more species-specific basis. Finally, though our overall dataset contained data collected from 63 species of odonate hosts, some species had better representation than others. Collecting more data from the larger, less-represented

species such as *Aeshna* dragonflies is necessary to understand if the patterns revealed in our study are representative of what was found for the smaller, well-represented species such as *Ischnura* and *Enallagma* damselflies. Having better representation from both more species and more even sampling across sites would also improve the precision of the estimated variance from our nested mixed-effect models.

In conclusion, our results emphasize the role of the local environment in mediating host–parasite interactions. Because the factors driving variation in immunity among individuals and populations are poorly studied and rarely factored into epidemiologic models (Becker et al. 2019), the findings of such models may be biased. Our study demonstrates that analyzing how the climate and environmental factors affect immune defenses reveals nuance inherent to host–parasite interactions. Host characteristics, parasites, and the abiotic environment are known to affect immunity in complex ways (Sweeny and Albery 2022). Understanding and disentangling these relationships can be difficult (Johnson et al. 2019), but future studies using approaches similar to those employed here can improve our knowledge of the emergent differences in parasitism among host individuals and species. Finally, our findings may also be used to understand host–parasite relationships under climate change scenarios, specifically how temperature and precipitation may contribute to patterns of immune defense. Given the synergistic effects of these two factors, increases in temperature or reduction in precipitation at northern latitudes will also influence parasitism rate. Future experimental studies should clarify the nature of this effect.

Acknowledgements – Concordia University is located on unceded Indigenous lands. The Kanien'kehá:ka Nation is recognized as the custodians of Tiohtià:ke/Montreal. We thank Serena Sinno, Gabriel Muñoz, and Javier Ibarra-Isassi for help and advice throughout the project development. AZH benefitted from the musical inspiration of Spiritbox.

Funding – This research project was funded by a NSERC Discovery Grant (RGPIN-2015-06081) to J-PL.

Conflict of interest – The authors declare no conflict of interest.

Author contributions

Adam Z. Hasik: Conceptualization (equal); Formal analysis (equal); Methodology (equal); Writing – original draft (equal); Writing – review and editing (equal). **Maggie Blondeau:** Conceptualization (equal); Data curation (equal); Writing – review and editing (equal). **Jacob Harvey:** Data curation (equal); Writing – review and editing (equal). **Tania Groleau:** Data curation (equal); Writing – review and editing (equal). **Tonia de Bellis:** Data curation (equal); Writing – review and editing (equal). **Eric J. Pedersen:** Conceptualization (equal); Formal analysis (equal); Writing – review and editing (equal). **Alex Córdoba-Aguilar:** Writing – review and editing (equal). **Katie E. Marshall:** Writing – review and editing (equal). **Laura Ferguson:** Writing – review and editing

(equal). **Jean-Philippe Lessard:** Conceptualization (equal); Data curation (equal); Methodology (equal); Project administration (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.m63xsj4fb> (Hasik et al. 2026).

Supporting information

The Supporting information associated with this article is available with the online version.

References

- Albery, G. F., Becker, D. J., Kenyon, F., Nussey, D. H. and Pemberton, J. M. 2019. The fine-scale landscape of immunity and parasitism in a wild ungulate population. – *Integr. Comp. Biol.* 59: 1165–1175.
- Albery, G. F., Morris, A., Morris, S., Kenyon, F., Nussey, D. H. and Pemberton, J. M. 2021. Fitness costs of parasites explain multiple life-history tradeoffs in a wild mammal. – *Am. Nat.* 197: 324–335.
- Appler, R. H., Frank, S. D. and Tarpy, D. R. 2015. Within-colony variation in the immunocompetency of managed and feral honey bees (*Apis mellifera* L.) in different urban landscapes. – *Insects* 6: 912–925.
- Arrowsmith, J., Shivaprakash, K. N., Larrivée, M., Turgeon, J. and Lessard, J.-P. 2018. Environmental filtering along a broad-scale acidity gradient shapes the structure of odonate communities. – *Ecosphere* 9: e02473.
- Becker, D. J., Cziráj, G. Á., Volokhov, D. V., Bentz, A. B., Carrera, J. E., Camus, M. S., Navara, K. J., Chizhikov, V. E., Fenton, M. B., Simmons, N. B., Recuenco, S. E., Gilbert, A. T., Altizer, S. and Streicker, D. G. 2018. Livestock abundance predicts vampire bat demography, immune profiles and bacterial infection risk. – *Philos. Trans. R. Soc. B* 373: 20170089.
- Becker, D. J., Downs, C. J. and Martin, L. B. 2019. Multi-scale drivers of immunological variation and consequences for infectious disease dynamics. – *Integr. Comp. Biol.* 59: 1129–1137.
- Becker, D. J., Albery, G. F., Kessler, M. K., Lunn, T. J., Falvo, C. A., Cziráj, G. Á., Martin, L. B. and Plowright, R. K. 2020. Macroimmunology: the drivers and consequences of spatial patterns in wildlife immune defence. – *J. Anim. Ecol.* 89: 972–995.
- Bollmer, J. L., Ruder, E. A., Johnson, J. A., Eimes, J. A. and Dunn, P. O. 2011. Drift and selection influence geographic variation at immune loci of prairie-chickens. – *Mol. Ecol.* 20: 4695–4706.
- Bolnick, D. I., Reserits, E. J., Ballare, K., Stuart, Y. E. and Stutz, W. E. 2020. Scale-dependent effects of host patch traits on species composition in a stickleback parasite metacommunity. – *Ecology* 101: e03181.
- Brooks, D. R., Hoberg, E. P. and Boeger, W. A. 2015. In the eye of the cyclops: the classic case of cospeciation and why paradigms are important. – *Comp. Parasitol.* 82: 1–8.
- Butler, M. W., Stahlschmidt, Z. R., Ardía, D. R., Davies, S., Davis, J., Guille Jr, L. J., Johnson, N., McCormick, S. D., McGraw, K. J. and DeNardo, D. F. 2013. Thermal sensitivity of immune function: evidence against a generalist–specialist tradeoff among endothermic and ectothermic vertebrates. – *Am. Nat.* 181: 761–774.

- Catalán, T. P., Wozniak, A., Niemeyer, H. M., Kalergis, A. M. and Bozinovic, F. 2012. Interplay between thermal and immune ecology: effect of environmental temperature on insect immune response and energetic costs after an immune challenge. – *J. Insect Physiol.* 58: 310–317.
- Clusella-Trullas, S. and Nielsen, M. 2020. The evolution of insect body coloration under changing climates. – *Curr. Opin. Insect Sci.* 41: 25–32.
- Cooper, M. 2020. Conduct a quick and memory-efficient Moran's I test. – <https://github.com/mcooper/moranfast>.
- Córdoba-Aguilar, A., Jiménez-Cortés, J. G. and Lanz-Mendoza, H. 2009. Seasonal variation in ornament expression, body size, energetic reserves, immune response, and survival in males of a territorial insect. – *Ecol. Entomol.* 34: 228–239.
- Cotter, S. C., Myatt, J. P., Benskin, C. M. H. and Wilson, K. 2008. Selection for cuticular melanism reveals immune function and life-history tradeoffs in *Spodoptera littoralis*. – *J. Evol. Biol.* 21: 1744–1754.
- Couch, C. S., Mydlarz, L. D., Harvell, C. D. and Douglas, N. L. 2008. Variation in measures of immunocompetence of sea fan coral, *Gorgonia ventalina*, in the Florida Keys. – *Mar. Biol.* 155: 281–292.
- Delhey, K. 2019. A review of Gloger's rule, an ecogeographical rule of colour: definitions, interpretations and evidence. – *Biol. Rev.* 94: 1294–1316.
- Duong, T. M. and McCauley, S. J. 2016. Predation risk increases immune response in a larval dragonfly (*Leucorrhinia intacta*). – *Ecology* 97: 1605–1610.
- Edwards, D. D. 2004. Effects of low pH and high temperature on hatching and survival of the water mite *Unionicola foili* (Acari: Unionicolidae). – *Proc. Indiana Acad. Sci.* 113: 26–32.
- Fair, J. M. and Whitaker, S. J. 2008. Avian cell-mediated immune response to drought. – *Wilson J. Ornithol.* 120: 813–819.
- Fan, Y. et al. 2016. Applications of structural equation modeling (SEM) in ecological studies: an updated review. – *Ecol. Processes* 5: 1–12.
- Fedorka, K. M., Lee, V. and Winterhalter, W. E. 2013. Thermal environment shapes cuticle melanism and melanin-based immunity in the ground cricket *Allonemobius socius*. – *Evol. Ecol.* 27: 521–531.
- Ferguson, L. V., Dhakal, P., Lebenzon, J. E., Heinrichs, D. E., Bucking, C. and Sinclair, B. J. 2018. Seasonal shifts in the insect gut microbiome are concurrent with changes in cold tolerance and immunity. – *Funct. Ecol.* 32: 2357–2368.
- Forbes, M. R., Muma, K. E. and Smith, B. P. 1999. Parasitism of *Sympetrum* dragonflies by *Arrenurus planus* mites: maintenance of resistance particular to one species. – *Int. J. Parasitol.* 29: 991–999.
- Forbes, M. R. L. and Baker, R. L. 1991. Condition and fecundity of the damselfly, *Enallagma ebrium* (Hagen): the importance of ectoparasites. – *Oecologia* 86: 335–341.
- Giraudoux, P., Antonietti, J.-P., Beale, C., Pleydell, D. and Treglia, M. 2024. pgirmess: spatial analysis and data mining for field ecologists. – <http://perso.orange.fr/giraudoux>.
- Gómez-Llano, M., Narasimhan, A. and Svensson, E. I. 2020. Male–male competition causes parasite-mediated sexual selection for local adaptation. – *Am. Nat.* 196: 344–354.
- González-Santoyo, I. and Córdoba-Aguilar, A. 2012. Phenoloxidase: a key component of the insect immune system. – *Entomol. Exp. Appl.* 142: 1–16.
- Hasik, A. Z. and Siepielski, A. M. 2022a. Parasitism shapes selection by drastically reducing host fitness and increasing host fitness variation. – *Biol. Lett.* 18: 20220323.
- Hasik, A. Z. and Siepielski, A. M. 2022b. A role for the local environment in driving species-specific parasitism in a multi-host parasite system. – *Freshw. Biol.* 67: 1571–1583.
- Hasik, A. Z., Ilvonen, J. J., Siepielski, A. M. and Murray, R. L. 2023a. Odonata immunity, pathogens and parasites. – In: Córdoba-Aguilar, A., Beatty, C. D. and Bried, J. T. (eds), *Dragonflies and damselflies*. Oxford Univ. Press, pp. 73–84.
- Hasik, A. Z., Tye, S. P., Ping, T. and Siepielski, A. M. 2023b. A common measure of prey immune function is not constrained by the cascading effects of predators. – *Evol. Ecol.* 37: 13–30.
- Hasik, A. Z., Bried, J. T., Bolnick, D. I. and Siepielski, A. M. 2024. Is the local environment more important than within-host interactions in determining coinfection? – *J. Anim. Ecol.* 93: 1541–1555.
- Hasik, A. Z., Butt, S., Maris, K., Morris, S., Morris, A., Turner, R. S., Pemberton, J. M. and Albery, G. F. 2025a. Population density drives increased parasitism via greater exposure and reduced resource availability in wild red deer. – *Parasitology* 152: 724–734.
- Hasik, A. Z., Ilvonen, J. J., Gobbin, T. P., Suhonen, J., Beaulieu, J. M., Poulin, R. and Siepielski, A. M. 2025b. Parasitism as a driver of host diversification. – *Nat. Rev. Biodivers.* 1: 401–410.
- Hasik, A. Z., Blondeau, M., Harvey, J., Groleau, T., de Bellis, T., Pedersen, E. J., Córdoba-Aguilar, A., Marshall, K., Ferguson, L. and Lessard, J. P. 2026. Data from: The macroecology of immunity: predominant influence of climate on invertebrate immune response. – Dryad Digital Repository, <https://doi.org/10.5061/dryad.m63xsj4fb>.
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. and Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. – *Int. J. Climatol.* 25: 1965–1978.
- Ilvonen, J. J. and Suhonen, J. 2016. Phylogeny affects host's weight, immune response and parasitism in damselflies and dragonflies. – *R Soc. Open Sci.* 3: 160421.
- Ilvonen, J. J., Kaunisto, K. M. and Suhonen, J. 2016. Are sexes equally parasitized in damselflies and dragonflies? – *Oikos* 125: 315–325.
- Ilvonen, J. J., Kaunisto, K. M. and Suhonen, J. 2018. Odonates, gregarines and water mites: why are the same host species infected by both parasites? – *Ecol. Entomol.* 43: 591–600.
- Johnson, E. E., Escobar, L. E. and Zambrana-Torrel, C. 2019. An ecological framework for modeling the geography of disease transmission. – *Trends Ecol. Evol.* 34: 655–668.
- Kaunisto, K. M. and Suhonen, J. 2013. Parasite burden and the insect immune response: interpopulation comparison. – *Parasitology* 140: 87–94.
- Kaunisto, K. M., Kaunisto, P., Ilvonen, J. J. and Suhonen, J. 2017. Parasitism, immune response and egg production of the spearhead bluet (*Coenagrion hastulatum*) damselfly. – *Can. J. Zool.* 95: 367–372.
- King, K. J. and Sinclair, B. J. 2015. Water loss in tree weta (*Hemideina*): adaptation to the montane environment and a test of the melanisation–desiccation resistance hypothesis. – *J. Exp. Biol.* 218: 1995–2004.
- Klunk, C. L., Fratoni, R. O., Rivadeneira, C. D., Schaedler, L. M. and Perez, D. M. 2022. Climate and body size have differential roles on melanism evolution across workers in a worldwide ant genus. – *Oecologia* 199: 579–587.

- Lajeunesse, M. J., Forbes, M. R. and Smith, B. P. S. 2004. Species and sex biases in ectoparasitism of dragonflies by mites. – *Oikos* 106: 501–508.
- Lazzaro, B. P. and Little, T. J. 2008. Immunity in a variable world. – *Proc. R. Soc. B* 364: 20080141.
- Lehner, B. and Grill, G. 2013. Global river hydrography and network routing: baseline data and new approaches to study the world's large river systems. – *Hydrol. Processes* 27: 2171–2186.
- Li, D., Brough, B., Rees, J. W., Coste, C. F. D., Yuan, C., Fowler, M. S. and Sait, S. M. 2024. Humidity modifies species-specific and age-dependent heat stress effects in an insect host–parasitoid interaction. – *Ecol. Evol.* 14: e70047.
- Lindström, E. S., Kamst-Van Agterveld, M. P. and Zwart, G. 2005. Distribution of typical freshwater bacterial groups is associated with pH, temperature and lake water retention time. – *Appl. Environ. Microbiol.* 71: 8201–8206.
- LoScerbo, D., Farrell, M. J., Arrowsmith, J., Mlynarek, J. and Lesard, J.-P. 2020. Phylogenetically conserved host traits and local abiotic conditions jointly drive the geography of parasite intensity. – *Funct. Ecol.* 34: 2477–2487.
- Mastore, M., Quadroni, S., Toscano, A., Mottadelli, N. and Brivio, M. F. 2019. Susceptibility to entomopathogens and modulation of basal immunity in two insect models at different temperatures. – *J. Therm. Biol.* 79: 15–23.
- Moran, P. A. P. 1950. Notes on continuous stochastic phenomena. – *Biometrika* 37: 17–23.
- Nagel, L., Robb, T. and Forbes, M. R. 2010. Inter-annual variation in prevalence and intensity of mite parasitism relates to appearance and expression of damselfly resistance. – *BMC Ecol.* 10: 5.
- Nakhleh, J., El Moussawi, L. and Osta, M. A. 2017. Chapter Three - the melanization response in insect immunity. – *Adv. Insect Physiol.* 52: 83–109.
- Nwaogu, C. J., Cresswell, W., Versteegh, M. A. and Tieleman, B. I. 2019. Seasonal differences in baseline innate immune function are better explained by environment than annual cycle stage in a year-round breeding tropical songbird. – *J. Anim. Ecol.* 88: 537–553.
- Paradis, E., Claude, J. and Strimmer, K. 2004. APE: analyses of phylogenetics and evolution in R language. – *Bioinformatics* 20: 289–290.
- Parkash, R., Sharma, V. and Kalra, B. 2008. Climatic adaptations of body melanisation in *Drosophila melanogaster* from Western Himalayas. – *Fly* 2: 111–117.
- Pedersen, E. and Simpson, G. 2023. MRFtools: tools for constructing and plotting Markov random fields in R for graphical data. – <https://github.com/eric-pedersen/MRFtools>.
- Penczykowski, R. M., Walker, E., Soubeyrand, S. and Laine, A. L. 2014. Linking winter conditions to regional disease dynamics in a wild plant–pathogen metapopulation. – *New Phytol.* 205: 1142–1152.
- Picard, C. J., Smith, M. B. and Oonincx, D. G. A. B. 2025. Low intensity UVB irradiation increases expression of the toll pathway in the yellow mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae). – *J. Insects Food Feed* 1: 1–16.
- Pool, J. E. and Aquadro, C. F. 2006. History and structure of sub-Saharan populations of *Drosophila melanogaster*. – *Genetics* 174: 915–929.
- Price, P. V. 1980. Evolutionary biology of parasites. – Princeton Univ. Press.
- Ramniwas, S., Kajla, B., Dev, K. and Parkash, R. 2013. Direct and correlated responses to laboratory selection for body melanisation in *Drosophila melanogaster*: support for the melanisation–desiccation resistance hypothesis. – *J. Exp. Biol.* 216: 1244–1254.
- Rantala, M. J. and Roff, D. A. 2007. Inbreeding and extreme outbreeding cause sex differences in immune defence and life history traits in *Epirrita autumnata*. – *Heredity* 98: 329–336.
- Rolf, J. and Siva-Jothy, M. T. 2003. Invertebrate ecological immunology. – *Science* 301: 472–475.
- Rosseel, Y. 2012. lavaan: an R package for structural equation modeling. – *J. Stat. Softw.* 48: 1–36.
- Sabockytė, A., McAllister, S., Coates, C. J. and Lim, J. 2023. Effect of acute ultraviolet radiation on *Galleria mellonella* health and immunity. – *J. Invertebr. Pathol.* 198: 107899.
- Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. – *Nat. Methods* 9: 671–675.
- Shipley, B. 2009. Confirmatory path analysis in a generalized multilevel context. – *Ecology* 90: 363–368.
- Siva-Jothy, M. T. and Plaistow, S. J. 1999. A fitness cost of eugregarine parasitism in a damselfly. – *Ecol. Entomol.* 24: 465–470.
- Smith, I. M. and Oliver, D. R. 1986. Review of parasitic associations of larval water mites (Acari: Parasitengona: Hydrachnida) with insect hosts. – *Can. Entomol.* 118: 407–472.
- Smith, I. M., Cook, D. R. and Smith, B. P. 2010. Chapter 15 - Water mites (Hydrachnidae) and other arachnids. – In: Thorp, J. H. and Covich, A. P. (eds), Ecology and classification of North American freshwater invertebrates, 3rd edn. Academic Press, pp. 485–586.
- Stephens, P. R. et al. 2016. The macroecology of infectious diseases: a new perspective on global-scale drivers of pathogen distributions and impacts. – *Ecol. Lett.* 19: 1159–1171.
- Sweeny, A. R. and Albery, G. F. 2022. Exposure and susceptibility: the twin pillars of infection. – *Funct. Ecol.* 36: 1713–1726.
- Telonis-Scott, M., Hoffmann, A. A. and Sgrò, C. M. 2011. The molecular genetics of clinal variation: a case study of ebony and thoracic trident pigmentation in *Drosophila melanogaster* from eastern Australia. – *Mol. Ecol.* 20: 2100–2110.
- Thompson, J. N. 2005. The geographic mosaic of coevolution. – Univ. of Chicago Press.
- Tschirren, B. 2015. *Borrelia burgdorferi* sensu lato infection pressure shapes innate immune gene evolution in natural rodent populations across Europe. – *Biol. Lett.* 11: 20150263.
- Tsiotas, D. and Tselios, V. 2022. Understanding the uneven spread of COVID-19 in the context of the global interconnected economy. – *Sci. Rep.* 12: 666.
- Waller, J. T. and Svensson, E. I. 2017. Body size evolution in an old insect order: no evidence for Cope's rule in spite of fitness benefits of large size. – *Evolution* 71: 2178–2193.
- Warton, D. I. 2021. Correlated samples in time, space, phylogeny.... – In: Warton, D. I. (ed.), Eco-Stats: data analysis in ecology. SpringerLink, pp. 151–179.
- Whittaker, T. A. 2012. Using the modification index and standardized expected parameter change for model modification. – *J. Exp. Educ.* 80: 26–44.
- Więcek, M., Broda, Ł., Proctor, H., Dabert, M., Smith, B. P. and Dabert, J. 2023. Species boundaries among extremely diverse and sexually dimorphic *Arrenurus* water mites (Acariformes: Hydrachnida: Arrenuridae). – *Syst. Appl. Acarol.* 28: 322–342.
- Wood, S. N. 2010. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. – *J. R. Stat. Soc. B* 73: 3–36.

- Wood, S. N. 2017. Generalized additive models: an introduction with R, 2nd edn. – Chapman and Hall/CRC.
- Xia, Y. and Yang, Y. 2018. RMSEA, CFI, and TLI in structural equation modeling with ordered categorical data: the story they tell depends on the estimation methods. – Behav. Res. Methods 51: 409–428.
- Zawal, A. and Buczyński, P. 2013. Parasitism of Odonata by *Arrenurus* (Acari: Hydrachnidia) larvae in the Lake Świdwie, nature reserve (NW Poland). – Acta Parasitol. 58: 486–495.
- Zuur, A. F. and Ieno, E. N. 2016. A protocol for conducting and presenting results of regression-type analyses. – Methods Ecol. Evol. 7: 636–645.