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Elevated nest temperature has opposing effects on host species infested with parasitic nest flies

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ABSTRACT

1. Hosts have developed or evolved defense strategies, including tolerance and resistance, to reduce damage caused by parasites. Environmental factors, such as elevated temperature, can influence the effectiveness of these different host defenses but also can directly affect parasite fitness. Therefore, the net effect of elevated temperature on host-parasite relationships are determined by its direct effects on the host and the parasite. Furthermore, because host species can defend themselves differently against their parasites, the net effect of temperature might differ across each host's interaction with the same parasite.

2. Few studies have determined the net effects of temperature on both host defenses and parasites in a multi-host system. To address this gap, we experimentally manipulated temperature and parasite presence in the nests of two host species who defend themselves differently to the same parasitic nest fly (*Protocalliphora sialia*). Specifically, we conducted a factorial experiment by increasing temperature (or not) and removing all parasitic nest flies (or not) in the nests of tolerant eastern bluebirds (*Sialia sialis*) and resistant tree swallows (*Tachycineta bicolor*). We then quantified parasite load in nests and measured nestling body size metrics, blood loss, and survival.

3. If temperature predominately affected parasite fitness, then elevated temperature would cause similar directional effects on parasite abundance across species. If temperature has different effects on hosts, then parasite abundance would differ in response to elevated temperature across host species.

4. In contrast to previous years, we found that bluebird nests had half as many parasites as compared to swallow nests. Elevated temperature affected parasite abundance differently in each host species. Swallows from heated nests had fewer parasites compared to non-heated nests,

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suggesting that they were more resistant to the parasites. Interestingly, swallows from heated nests were also more tolerant to the effects of parasites than controls. In contrast, bluebirds from heated nests had more parasites and lower body mass compared to controls, suggesting that they lost tolerance, and resistance, to the parasites.

5. Our results suggest that a changing climate could have complex net effects on host-parasite interactions, including on host defenses, with implications for host health and parasite survival.

Keywords: eastern bluebird, ectoparasites, host defenses, *Protocalliphora sialia*, temperature effects, tolerance, tree swallow, resistance.

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1 | INTRODUCTION

Parasites and pathogens can cause significant damage to their hosts. In turn, hosts have developed or evolved two types of defense strategies to reduce damage. First, hosts can invest in resistance mechanisms, such as immune responses, to reduce parasite damage by reducing parasite fitness (Medzhitov et al., 2012, Råberg et al., 2007). Second, hosts can invest in tolerance mechanisms, such as energy compensation, to reduce parasite damage without reducing parasite fitness (Miller et al., 2006, Medzhitov et al., 2012, Råberg et al., 2007). Although effective host defense mechanisms can increase host fitness when they are parasitized, these defenses can be costly to produce (Read et al., 2008, Roy & Kirchner, 2000). Therefore, host defense strategies can have trade-offs with other factors, such as physiological and behavioral processes, and the outcome of these trade-offs can affect host fitness (Medzhitov et al., 2012). Because the life history of each host is different, the balance between energy invested in defense strategies and challenges to their fitness will likely vary among different host species (Sears et al., 2015, Sorci, 2013). However, few studies have determined the environmental conditions that influence variation in defenses against the same parasitic taxa across host species.

Environmental conditions, such as elevated temperatures, can influence the effectiveness of host defenses, including resistance and tolerance (Martin et al., 2010, Beard and Mitchell, 1987). For example, previous studies shows that sticklebacks (*Gasterosteus aculeatus*) have a weakened immunological resistance to tapeworm infection as temperatures increase, which leads to an increase in infection risk (Franke et al., 2017, Scharsack et al., 2016). Elevated temperatures could also positively affect host immune response, as in a study on sea fan corals (*Gorgonia ventalina*) for which elevated temperatures increased anti-fungal activity, thereby increasing fungal inhibition (Ward et al., 2007). For hosts that predominately rely on tolerance

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mechanisms, tolerance to parasitism can become compromised when hosts have to spend energy maintaining homeostasis under temperatures outside of their own optimal thermal range (Greenspan et al., 2017, Cohen et al., 2017). For example, heat stress can cause a loss of tolerance (via decreasing body mass and survival) under higher parasite abundances (Franke et al., 2017).

Parasite fitness can also be directly negatively affected by elevated temperatures if temperatures fall outside of a parasite's optimal thermal performance range (Cohen et al., 2017, Scharsack et al., 2016, Shim et al., 2012). Because parasites and hosts can have different optimal thermal ranges in which they best perform, elevated temperatures could concurrently affect the host and parasite differently, thereby shifting the dynamics of the interaction (i.e., "thermal mismatch hypothesis", Cohen et al., 2017). A hosts' balance between defense strategies and a parasites' response to these host defenses creates an intersection in how elevated temperatures will have influence throughout the host-parasite relationship. Because of the complexity of temperature effects on host-parasite interactions, experimental studies are needed to further understand the net consequences of changing temperatures on multi-host-parasite interactions.

Box-nesting birds, such as tree swallows (*Tachycineta bicolor*) and eastern bluebirds (*Sialia sialis*), and their parasitic nest flies (*Protocalliphora sialia)* are an ideal system to study the effect of elevated temperature on multi-host-parasite interactions. Adult *P. siala* are non-parasitic but lay their eggs in the nests of birds soon after nestlings hatch and the larvae then feed non-subcutaneously on the blood of nestlings (Boyd, 1951). The host species differ in their defenses against *P. sialia* (Grab et al., 2019). Tree swallows are resistant to *P. sialia* because they mount an effective immune response that decreases parasite abundance in nests. In contrast,

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bluebirds are more tolerant to *P. sialia* because they do not mount an effective immune response and, on average, have twice as many parasites compared to swallows (Grab et al., 2019).

The goal of our study was to determine the effect of elevated temperature on parasitic nest fly abundance and whether interactions between parasite and nest temperature might affect the health of tree swallow and eastern bluebird nestlings. We experimentally elevated nest temperature (hereon, heated) or not (hereon, non-heated) and manipulated parasite presence by removing all parasites (hereon, non-parasitized treatment) or allowing for natural parasitism (hereon, parasitized treatment). We then quantified parasite abundance and parasite density (number of parasites per gram of nestling) to account for difference in host body size. We also measured nestling body size metrics (body mass, tarsus length, first primary feather length) and hemoglobin levels (proxy of blood loss to parasitism) when nestlings were 9-10 days old, and nestling survival (fledging success).

We predicted that if nest temperature impacted parasite fitness directly, then parasite abundance would be similar in response to elevated nest temperatures for both species. For example, since heat can negatively affect parasite fitness (Dawson, Hillen, et al., 2005, Castaño-Vázquez et al., 2018), we expected that the heat treatment would decrease parasite abundance in all nests, regardless of host species and host defense. Nest temperature could also influence the effectiveness of host defenses, which could then indirectly affect parasite abundance. If nest temperature influences host defenses, we predicted the effect of heat treatment on parasite abundance would differ between resistant swallows and tolerant bluebirds. Elevated temperatures can cause heat stress in developing nestlings (Salaberria et al., 2014, Rodriguez & Barba, 2016, Andreasson et al., 2017), which could affect the defense against parasites. Heat-stressed swallow nestlings might lose their ability to immunologically resist parasites, and

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therefore might have higher parasite abundances than non-heat stressed swallows. Consequently, heated, parasitized swallow nestlings might have poor body condition (body mass, size and survival) compared to swallows from non-heated nests. Because bluebirds are generally tolerant to parasites and therefore do not affect parasite fitness with an immune defense, we predict that bluebird nestlings likely will have similar parasite abundance across temperature treatments independent of the possible direct effects of heat treatment on parasites. However, heat stress might reduce tolerance to the parasite and therefore heated, parasitized bluebird nestlings are predicted to be in worse condition compared to bluebirds from all other treatments. Overall, understanding the causal effects of elevated temperatures on host-parasite interactions is important because temperature is proposed to be one of the most important environmental factors affecting disease (Studer et al., 2010).

2 | MATERIAL AND METHODS

2.1 | Study system

Our study was conducted from May to August 2018 in northern Minnesota near the University of Minnesota Itasca Biological Station and Laboratories (47°13′33″N, −95°11′42″W) in Clearwater and Hubbard Counties. In 2018, approximately 170 nest boxes were established haphazardly on the properties of landowners and within Itasca State Park. Tree swallows and eastern bluebirds are abundant in the area and nest readily in artificial cavities. *Protocalliphora sialia* is the primary nest ectoparasite that infests the nests of tree swallows and bluebirds at this site (Grab et al., 2019). Tree swallow clutch size ranges from one to nine eggs, which are incubated for 13-14 days, and nestlings spend an average of 20 days in the nest (Grab et al.,

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2019). Bluebird clutch size ranges from one to seven eggs, which are incubated for 13-14 days and nestlings spend an average of 18.8 days in the nest (Gowaty & Plissner, 2015).

2.2 | Experimental manipulation of parasites and temperature

Nest boxes were checked once a week for evidence of nesting activity (i.e. nest construction). Once eggs were found, lay date was determined, and then nests were checked every other day until the eggs hatched. During the nestling stage, we conducted a two-by-two factorial experiment by manipulating parasite presence (parasites or no parasites) and nest temperature (heat or no heat). At hatching, the nestlings and the top liner of the nests were removed for the parasite treatment. For the control treatment, nests were treated with sterile water to allow for natural parasitism (parasitized). For the experimental treatment, nests were treated with a 1% permethrin solution to remove all parasites (non-parasitized; Permectrin II) (Knutie et al., 2016, DeSimone et al., 2018). Parasite treatment for each species was initially determined by a coin flip, and all subsequent nests were alternately assigned to a treatment.

For the heat treatment, we used a metal spatula to lift nest material from the bottom of the box and placed a fresh UniHeat 72+ Hour heat pack (heated) or an exhausted heat pack (non-heated) in the open space (Fig. S1). The packs contained a mixture of charcoal, iron powder, vermiculite, salt, sawdust, and moisture, and produced elevated temperatures between 35-40°C for two days when exposed to the air (Dawson, Hillen, et al., 2005). Nest boxes were revisited every two days to replace active heat packs so that nest boxes continually had elevated temperatures while nestlings were 0 to10 days old, or to lift nest material with a metal spatula to cause similar disturbance in control nests. Heat packs were always checked for parasites before they were removed; any parasites that were on the heat pack were returned to the nest. To record

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internal nest temperature a data logger (Thermochron iButton DS1921G, Dallas Semiconductor, USA) was placed under the nest liner in 17 bluebird nests. Data loggers were programmed to record internal nest temperature once every hour from day of treatment until nests were collected. Heat treatment for each species was initially determined by a coin flip and all subsequent nests were alternately assigned to a treatment.

2.3 | Nestling growth metrics and survival

At hatching, nestlings were weighed to the nearest 0.1g using a portable digital scale balance (Ohaus CL2000). Nests were revisited when nestlings were between 9-10 days old to take new weight measurements and to measure tarsus length (to the 0.01mm), bill length (0.01mm), and first primary feather length (0.01mm) using analog dial calipers from Avinet. Nestlings were also banded at approximately 10 days old with a uniquely numbered Fisheries and Wildlife metal band (Master's banding #23623). When nestlings were approximately 15 days old, the boxes were checked every other day from a distance (to prevent pre-mature fledging) and the age of fledging or death was recorded.

2.4 | Blood collection and hemoglobin levels

When nestlings were 9-10 days old, a small blood sample (~20µL) was collected from the brachial vein of a subset of nestlings using a 30-gauge sterile needle. Hemoglobin levels (g/dl) were then quantified from the whole blood using a HemoCue® HB +201 portable analyzer.

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2.5 | Quantifying parasite abundance

When nests were empty, they were carefully removed, along with the heat packs and iButton, from the nest box and stored in a gallon-sized, labeled plastic bag. Nest material was then dissected over trays lined with a white piece of paper. All *P. sialia* larvae (1st, 2nd, and 3rd instars), pupae, and pupal cases were counted to determine total parasite abundance for each nest. The length and width (0.01 mm) of up to ten pupae were haphazardly selected and measured with digital calipers. These measurements were used to calculate pupal volume ($V = \pi*[0.5*width]^2*length$). Larvae and pupae were reared to adulthood to confirm that they were *P. sialia*.

2.6 | Statistical Analyses

To confirm that heat treatment effectively increased temperatures in bluebird nests, we modeled nest temperature over 24 hours in heated and non-heated nests using a general additive mixed model (GAMM) with the gamm function in the mgcv package in R. In this model, the response was nest temperature measured every hour. The predictors included heat treatment and smoothed term of time in hours. The effect of time on nest temperature was allowed to vary according to heat treatment. We accounted for the non-independence of temperature observations within nests and dates by including random intercept terms for nest and date. Temperature data collected from tree swallow nests were excluded because only three nests had sufficient data.

To determine the effect of temperature treatment on parasite load within nests, we examined parasite abundance and parasite density. Parasite abundance was the total number of *P. sialia* larvae, pupae, and pupal cases counted within each nest. Parasite density was the number of parasites per gram of nestling mass, calculated as parasite abundance divided by the total mass

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of nestlings within a nest. To evaluate how the effect of heat treatment on parasite abundance differed across bird species, we used a generalized linear model with a Poisson error distribution. The response was parasite abundance, and the predictors were heat treatment, bird species, and the interaction of heat treatment and bird species. To evaluate how the effect of heat treatment on parasite load differed across bird species, regardless of the differences in body size of the two species, we used a linear model with parasite density as the response, and heat treatment, bird species, and the interaction of heat treatment and bird species as the predictors. Both of these models examining parasite load also included two covariates, log-transformed nest mass and day the first egg within a nest hatched. In addition, these models excluded observations from the non-parasitized treatment since no parasites were found in the nests. To determine if heat treatment influenced the size of pupae within nests across bird species, we used a linear mixed effect model with pupal size, calculated as cylindrical volume, as the response and heat treatment, bird species, and the interaction of heat treatment and bird species as predictors. The model also included a covariate of parasite abundance, to account for the intraspecific competition among parasites which might also affect size.

To examine the effects of parasite and heat treatments on the fledging success of bluebird and swallow nestlings, we used two logistic regressions, one model for each species. In each model, the response was proportion of nestlings that successfully fledged (number of nestlings that survived to the end of the observation period, number of nestlings that died), and the predictors were heat treatment, parasite treatment, and the interaction of heat and parasite treatments. Both of these models examining fledging success also included two covariates, log-transformed nest mass and day first egg within a nest hatched.

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To test for the effects of heat and parasite treatments on growth of bluebirds and swallows, we completed two permutational analysis of variance (PERMANOVA) models, one model for each species. For each model, the response was a resemblance matrix constructed with Euclidean distances of normalized values of average nestling mass, average bill length, average tarsus length, and average first primary feather length of birds within nests, and the predictors were heat treatment, parasite treatment, and the interaction of heat and parasite treatments. Both of these models also included two covariates, log-transformed nest mass and day first egg within a nest hatched. We evaluated 9999 permutations using residuals under a reduced model and examined test statistics associated with Type I sums of squares to determine if there were any effect of treatments after accounting for the effects of the two covariates. To visualize the results of the PERMANOVA, we used distance-based redundancy analysis (dbRDA). The dbRDA was based on an appropriate resemblance matrix as previously described. The underlying predictors were parasite and heat treatments (as categorical variables) and log-transformed nest mass and day the first egg within a nest hatched (as continuous variables). In the dbRDA plot, we show the centroid values for the four experimental treatments. Ellipses surrounding points represent 95% confidence intervals of groups based on standard errors and were made using the ordiellipse function in the '*vegan*' package in R. PERMANOVA models and the dbRDA were completed using PERMANOVA+ for PRIMER version 7 (PRIMER-E Ltd, Plymouth, UK). For ease of visualization of the dbRDA point and vector plots, data from PERMANOVA+ for PRIMER were exported, and plots were made using '*ggplot2*' package in R.

We followed significant results from PERMANOVA models with univariate tests to evaluate which endpoints drove the multivariate effect. Univariate tests were linear mixed effects models with individual birds' body mass, bill length, tarsus length, or first primary length as the

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response, and heat treatment, parasite treatment, and the interaction of heat and parasite treatments as the predictors. All univariate models also included two covariates, log-transformed nest mass and day the first egg within a nest hatched, and a random intercept term of nest ID.

To test for the effects of heat and parasite treatments on blood loss for bluebirds and swallows, we used two linear models, one for each species. For each model the response was hemoglobin level of a single bird within a nest, and the predictors were heat treatment, parasite treatment, and the interaction of heat and parasite treatments. In addition, the models included a single covariate, mass of individual birds. To examine how parasite abundance contributed to these patterns, we used two additional linear models, one for each species. The form of the models was identical to those previously described, except the model included parasite abundance as a predictor instead of parasite treatment. For all univariate models, test statistics associated with Type III sums of squares were evaluated. All univariate models and figures were conducted in R version 3.6.1.

3 | RESULTS

3.1 | Effect of temperature treatment on parasite load

In bluebird nests, heated nests had increased nest temperature compared to nests that were non-heated (t = -2.863, P = 0.004, Fig. 1). Treating nests with permethrin was effective at eliminating parasites within nests for both species; no parasites were found in any nests treated with permethrin. Parasite abundance varied according to heat treatment, bird species, and the interaction of heat treatment and bird species (Table S1). For non-heated nests, parasite abundance was greater in swallow nests compared to bluebird nests, but for heated nests, parasite abundance was greater in bluebird nests compared to swallow nests (Fig. 2A). Accounting for

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the difference in the mass of the birds according to species, parasite density was driven by bird species and the interaction between heat treatment and bird species, but not the main effect of heat treatment (Table S1). Similar to the effects on parasite abundance, in non-heated nests parasite density was still greater in nests with tree swallows compared to nests with bluebirds (Fig. 2B). However, in heated nests, parasite densities were similar between bluebird and tree swallow nests (Fig. 2B). Size of parasite pupae was not influenced by heat treatment, bird species, or the interaction of heat treatment and bird species (Table S1).

3.2 | Fledging success and nestling growth

Survival of bluebird nestlings was not affected by heat treatment, parasite treatment, or their interaction (Fig. 3, Table S2). Survival of swallow nestlings was influenced by the interaction of heat and parasite treatments, but not the main effects of heat or parasite treatments (Fig. 3, Table S2). Overall, swallow survival was greater with heated nests compared to non-heated nests across both parasite treatments. The multivariate model for tree swallows showed no effects of heat treatment, parasite treatment, or their interaction on size and mass (Table S3).

The multivariate model showed that bluebird size and mass were driven by a marginal effect of heat ($P = 0.057$) and the interaction of parasite and heat treatments, but not the main effect of parasite treatment (Table 1, Table S3). The dbRDA point plot demonstrates the largest difference in size and mass of eastern bluebirds was between heat and no heat (Fig. S2A). Bluebird nestlings from non-heated nests had larger body mass compared to nestlings from heated nests (Fig. S2A, B). Bluebird size and mass were more similar between parasitized and non-parasitized nests in heated nests compared to non-heated nests (Fig. S2A). This effect was likely driven by a combination of variation in bill length, first primary feather length, and tarsus

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length (Fig. S2A, B). Univariate tests of bluebird size and mass showed no effect of heat treatment, parasite treatment, or their interaction on bill length or tarsus length (Table 1, Table S4). Bluebird first primary feather length was influenced by the interaction of parasite treatment and heat treatment but not their main effects (Table 1, Table S4). Within non-parasitized nests, nestlings from non-heated nests had longer first primary feathers compared to nestlings from heated nests, and this effect was reversed when nests were parasitized. Bluebird mass was affected by heat treatment but not parasite treatment or the interaction of heat and parasite treatments (Table 1, Table S4). In agreement with the dbRDA plot, nestling bluebirds from heated nests weighed less than nestlings from non-heated nests.

3.3 | Nestling hemoglobin

Bluebird hemoglobin levels were not affected by heat treatment, parasite treatment, or their interaction (Table 1, Table S5). Similarly, bluebird demonstrated tolerance to parasitism because hemoglobin was not influenced by heat treatment, parasite abundance, or their interaction (Table S6, Fig. 4A).

For swallows, there was a marginal effect of parasite treatment on hemoglobin levels ($P = 0.053$), but no effect of heat treatment or their interaction (Table S5). Hemoglobin levels on average were lower for swallows in parasitized nests compared to non-parasitized nests. Parasite abundance and the interaction of parasite abundance and heat treatment affected swallow hemoglobin levels (Table S6). The main effect of heat treatment on hemoglobin levels was not significant (Table S6). For swallow nestlings from non-heated nests, hemoglobin levels declined as parasite abundance increased. However, for nestlings from heated nests, hemoglobin levels remained fairly stable as parasite abundance increased (Fig. 3B).

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4 | DISCUSSION

Our study demonstrates elevated temperature can have varying effects on the host, parasite, and their interaction. We found a contrasting effect of elevated temperature on the parasite abundance between two host species; bluebird nests had more parasites and swallow nests had fewer parasites in heated nests compared to non-heated nests. Each host species responded to the interaction of heat and parasitism differently; tree swallows were in better condition than bluebirds in heated and parasitized nests. Interestingly, neither elevated heat nor parasitism affected blood loss in bluebirds, while swallows from heated nests were more tolerant to blood loss, compared to non-heated nests, by maintaining a high level of hemoglobin despite an increase in parasite abundance. Surprisingly, non-heated swallow nests had more parasites than non-heated bluebird nests, which contrasts past results on these populations (Grab et al., 2019) and suggests that host defenses might vary annually. Our results suggest that there are direct effects of elevated temperature on hosts in response to parasitism, but these effects vary across species.

We found that the effect of heat on parasite abundance was likely mediated by the host. Swallow nestlings from heated nests are more resistant to parasites compared to nestlings from non-heated nestlings. One mechanism by which swallows are more resistant to parasitism is through the IgY antibody response, which has been documented in swallows previously (DeSimone et al., 2018, Grab et al., 2019). Tree swallow nestlings also maintained high hemoglobin (lower blood loss) in heated-treated nests despite increasing parasite abundance, which suggests that swallows are tolerant of the sublethal effects of parasitism when exposed to heat. Previous studies have found a negative relationship between nestling hemoglobin levels and parasite load, since ectoparasites remove blood from their hosts (Grab et al., 2019, Sun et al.,

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2019, DeSimone et al., 2018). Ardia (2013) found higher nest microclimate resulted in higher hematocrit levels (more red blood cells) in nestling tree swallows. Elevated nest temperatures might result in faster red blood cell recovery in nestlings via changing oxygen demands, which could explain our results (Niedojadlo et al., 2018, Fair et al., 2007, Bradley et al., 2020). These results could provide support for how some hosts are able to tolerate the effects of parasitism under warmer temperatures.

Overall, tree swallow nestling survival was greater in heated nests, even when nestlings were parasitized. Other studies have similarly found that tree swallow nestlings can have higher survival in response to elevated temperatures, which supports our findings (Dawson, Lawrie, et al., 2005, McCarthy & Winkler, 1999). One potential mechanism for greater survival is that elevated temperatures allowed nestlings to devote less energy toward maintaining an optimal temperature and more energy to growth and immunity to parasitism (Ganeshan et al., 2019).

The number of parasites were generally low in non-heated bluebird nests compared to non-heated swallow nests. This pattern was reversed compared to other years, which suggests that non-heated bluebirds might have invested more heavily into a resistance mechanism in 2018. In other years, bluebirds from this population generally did not produce a robust IgY immune response to the parasites (Grab et al., 2019), unless under high resource availability (Knutie, 2020). Resource availability might have been higher in 2018 facilitating higher resistance in non-heated nests, but then our heat treatment reduced this resistance resulting in higher parasite abundances. Similarly, other studies have found that heat can have a negative effect on the immune response (Calefi et al., 2016). In our study, we did not quantify IgY production of nestlings, which could have explained our results. Future studies are still needed to understand the mechanisms to how heat specifically could be benefiting host resistance.

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Parasitism nor heat affected bluebird nestling survival. However, we found an interaction between the effect of parasitism and heat treatment on bluebird growth. Within non-parasitized nests, nestlings from non-heated nests had longer first primary feathers compared to nestlings from heated nests, and this effect was reversed when nests were parasitized. Similar to our study, Murphy (1985) found eastern kingbirds (*Tyrannus tyrannus*) had longer first primary feathers with higher ambient temperatures, but lower mass gain. Other studies have proposed first primary growth to be prioritized in nestling development as a potential driver for successful or earlier fledging (Saino et al., 1998, Andreasson et al., 2017). When faced with parasitism, bluebird nestling first primary feathers may be prioritized to help fledge sooner and therefore escape parasitism, and this growth could be accommodated by elevated temperatures.

Bluebird nestlings from heated nests had lower body mass and size than non-heated nests. Nestling thermoregulation under heat stress requires energy that could otherwise be invested toward growth (Cunningham et al., 2013). Adverse environmental conditions, such as the heat treatment in our study, may have had a negative effect on the energy bluebird nestlings allocated toward growth. In great tits (*Parsus major)*, heated nestlings had lower body mass when temperatures reached a point to cause thermal stress (Rodriguez & Barba, 2016). Similar to these results, bluebird nestlings in our study likely experienced temperatures outside of their thermal optimal range and diverted energy away from mass gain to regulate body temperature.

To explain the difference in parasite abundance in the nests across hosts, we still must consider that elevated temperature could have had a direct effect on *P. sialia*. As an insect ectoparasite, *P. sialia* can be affected by environmental temperature apart from their hosts (Martinez & Merino, 2011, Bennett & Whitworth, 1991). Previous studies have found that elevated nest temperatures can have a negative or a positive effect on parasite fitness (Castano-

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Vazquez et al., 2021, Dube et al., 2018). Variation in parasite intensity across years and in response to environmental factors has also been recorded in other studies (Musgrave et al., 2019, Merino & Potti, 1996, Schultz et al., 2018). In our study, heat treatment increased nest temperatures by about 10°C, creating an environment of 35-45°C in heated nests. This elevated temperature was at the higher end of a curvilinear relationship found between *Protocalliphora* sp. and temperature in swallow nests from another study (Dawson, Hillen, et al., 2005). For this reason, we should expect parasite survival to be lower in the heated nests of both swallows and bluebirds. However, parasite abundance was greater in heated bluebird nests, suggesting that elevated heat is likely having a larger indirect effect on the parasite (via the host) than a direct effect.

Climate change poses many threats to the dynamics of host-parasite interactions, with elevated temperatures a particular concern (Martinez & Merino, 2011, Studer et al., 2010). Changing temperatures could have a direct influence on both parasite and host, and therefore it is important to understand how temperature will influence the net effects for each interaction (Studer et al., 2010, Scharsack et al., 2016). In this study, we found important implications for the effects of elevated temperature interacting with an effect of parasitism. Specifically, infestation with the same parasite, *P. sialia*, decreased in tree swallow nests and increased in eastern bluebird nests in response to elevated nest temperature. In natural host-parasite interactions, elevated temperatures could have consequences for the health of different host species and potentially alter the balance between defense strategies that the hosts use against infection, thereby changing the dynamics of the relationship with the parasite. Elevated temperatures may also directly affect the parasite apart from, or at the same time as, directly affecting the host. The relationship between temperature and parasitism throughout the

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interaction presented in this study provide new questions into the role of a changing climate on the interconnectedness of host-parasite interactions.

ACKNOWLEDGMENTS

We thank Steve Knutie and Doug Thompson for building nest boxes and the University of Minnesota Itasca Biological Station and Laboratories for logistical support. We also thank the following people for the interest in our work, along with access to nest boxes located on their property: Lesley Knoll and Aaron Hebbeler, Helen Perry, Doug and Dawn Thompson, Pioneer Farms, and Rock Creek General Store. The work was funded by Summer Undergraduate Research Fellowship award and Katie Bu award from the University of Connecticut and Savaloja Research Grant from the Minnesota Ornithologists' Union to LA, and start-up funds from the University of Connecticut to SAK. All applicable institutional guidelines for the care and use of animals were followed (University of Connecticut IACUC protocol #A18-005). The authors and collaborators also wish to acknowledge the Ojibwe people who have cared for and occupy the land in which our research was conducted. The University of Minnesota Itasca Biological Station and Laboratories is located on the land ceded by the Mississippi and Pillager Bands of Ojibwe in the Treaty of Washington, commonly known as the 1855 Treaty. This treaty affirms the reserved rights doctrine and the inalienable rights of Ojibwe people to uphold their interminable relationship to the land. With affiliation the forementioned academic institutions, it is our responsibility to acknowledge Native rights and the institutions' history with them. We are committed to continue building relationships with the Ojibwe People through recognition, support, and to advocate for all Native American Nations. We strive to be good stewards of our

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place and privilege. *This land acknowledgement was revised and written with support from Rebecca Dallinger and Joe Allen. It is a living document open to changes.*

AUTHOR CONTRIBUTIONS

LA and SAK conceived the ideas and designed methodology; LA, AP, SAK collected data; SR and GV analyzed the data; LA, SAK, GV, SR contributed to the writing of the manuscript. All authors gave final approval of the manuscript.

DATA AVAILABILITY STATEMENT

Data available will be made available via FigShare upon acceptance.

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explain higher resting metabolic rates in nestling birds with hematophagous ectoparasites? *Journal of Avian Biology, 51(2),* 1-8. https://doi.org/10.1111/jav.02264

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Figure Legends

Figure 1. The effect heat treatment on nest temperature. In eastern bluebird nests, heat increased nest temperatures throughout the course of a day. Lines are predictions from generalized additive mixed models, which included random intercept terms for nest and date to account for the non-independence of individual observations within nests and dates. Error ribbons are standard errors of model predictions.

Figure 2. The effect of heat treatment and bird species on parasite abundance. A) When no heat was applied to nests, parasite abundance was greater in nests with tree swallows compared to nests with eastern bluebirds, but when heat was applied this effect was reversed. Means and Poisson standard errors are shown. B) Accounting for the difference in the mass of the birds according to species, when no heat was applied parasite density was still greater in nests with tree swallows compared to nests with eastern bluebirds, but when heat was applied, parasite densities were similar between eastern bluebird nests and tree swallow nests. Means and standard errors are shown.

Figure 3. The influence of heat and parasite treatment on fledging success and growth. A) There was no significant effect of heat and parasite treatments or their interaction on eastern bluebird survival. B) For non-parasitized nests, tree swallow nestling survival was greater with heat compared to no heat, and the magnitude of this difference between heat and no heat increased when nests were parasitized. In both panels, means and binomial standard errors are shown.

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Figure 4. The effect of heat treatment and parasites on hemoglobin levels in tree swallows. A) Eastern bluebird hemoglobin levels were not influenced by heat treatment, parasite abundance, or their interaction. B) When no heat was applied to tree swallow nests, hemoglobin levels declined as parasite abundance increased. When heat was applied to nests, hemoglobin levels remained fairly stable as parasite abundance increased.

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Table 1. Effect of parasite and heat treatment on bluebird and tree swallow nestling growth and physiology. Numbers are mean ± SE and numbers in parentheses are number of nests.

	Parasitized		Non-parasitized	
Measurement	No Heat	Heat	No Heat	Heat
Eastern Bluebird				
Tarsus (mm)	18.62 ± 0.30 (12)	18.70 ± 0.17 (13)	19.11 ± 0.19 (10)	18.56 ± 0.29 (10)
1st Primary (mm)	13.94 ± 1.09 (12)	17.12 ± 0.88 (13)	16.58 ± 0.78 (10)	14.51 ± 0.99 (10)
Bill length (mm)	5.17 ± 0.11 (12)	5.39 ± 0.08 (13)	5.34 ± 0.07 (10)	5.24 ± 0.09 (10)
Mass (g)	26.11 ± 0.75 (12)	24.21 ± 0.66 (13)	26.65 ± 0.72 (10)	23.97 ± 0.56 (10)
Hemoglobin (g/dl)	10.54 ± 0.59 (11)	10.46 ± 0.73 (13)	11.78 ± 0.49 (10)	10.86 ± 0.22 (9)
Tree Swallow				
Tarsus (mm)	11.59 ± 0.17 (7)	11.57 ± 0.14 (8)	11.60 ± 0.08 (8)	11.55 ± 0.19 (7)
1st Primary (mm)	12.34 ± 1.02 (7)	13.66 ± 1.30 (8)	12.88 ± 1.41 (8)	15.40 ± 1.05 (7)
Bill length (mm)	4.29 ± 0.12 (7)	4.53 ± 0.07 (8)	4.45 ± 0.14 (8)	4.70 ± 0.17 (7)
Mass (g)	19.45 ± 0.69 (7)	19.73 ± 0.74 (8)	20.76 ± 0.85 (8)	20.89 ± 0.55 (7)
Hemoglobin (g/dl)	10.25 ± 1.09 (6)	12.30 ± 0.29 (7)	11.74 ± 0.37 (8)	12.32 ± 0.18 (6)

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Figure 1.

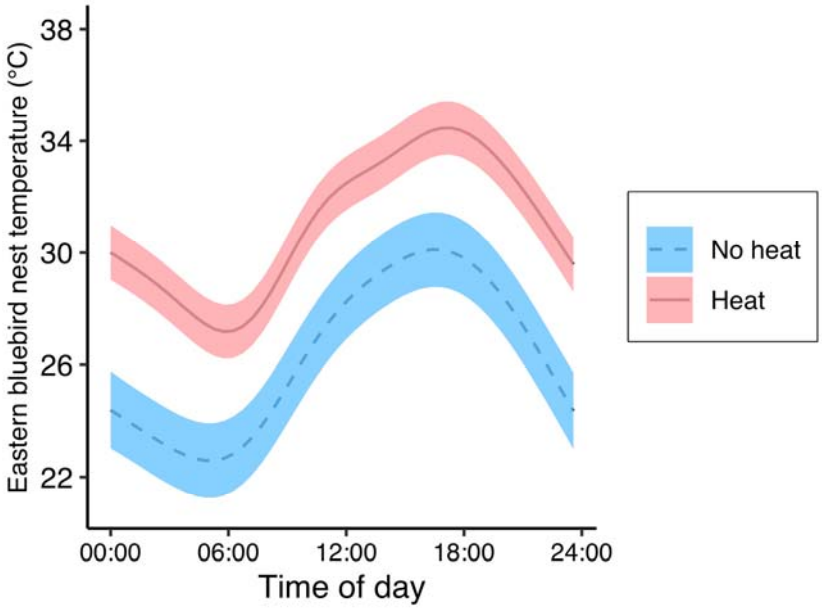

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Figure 2.

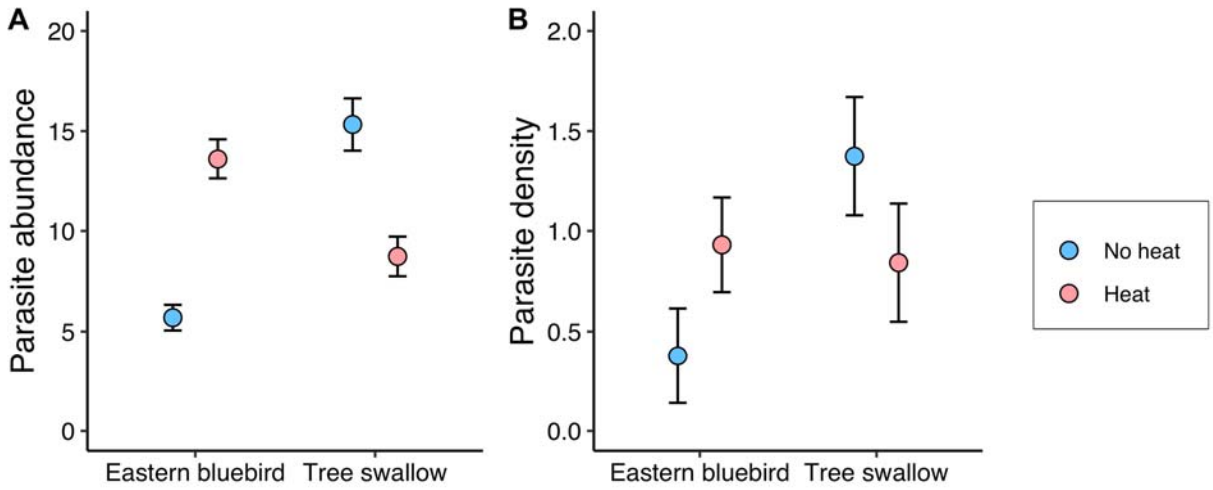

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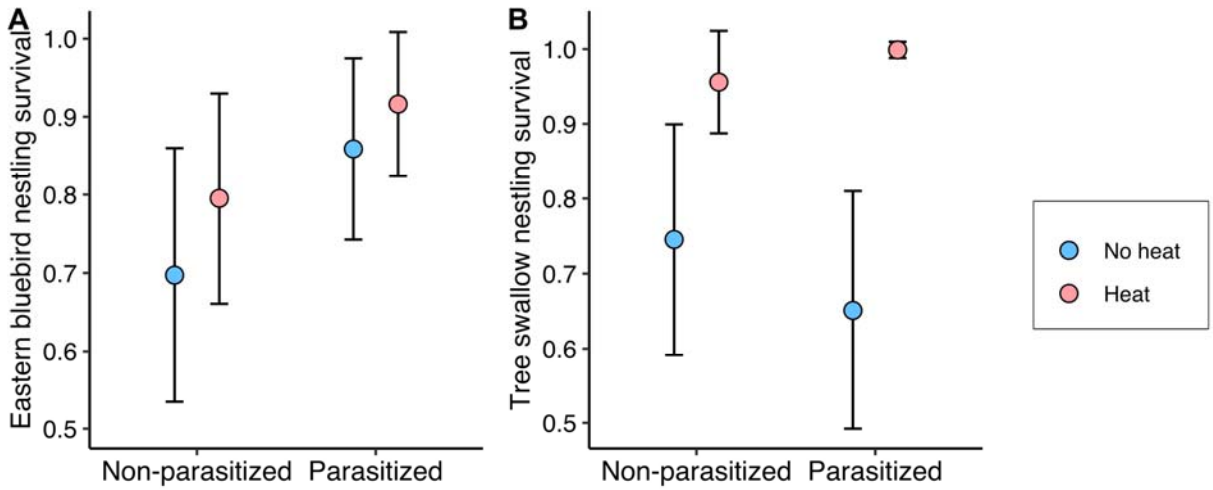
Figure 3.



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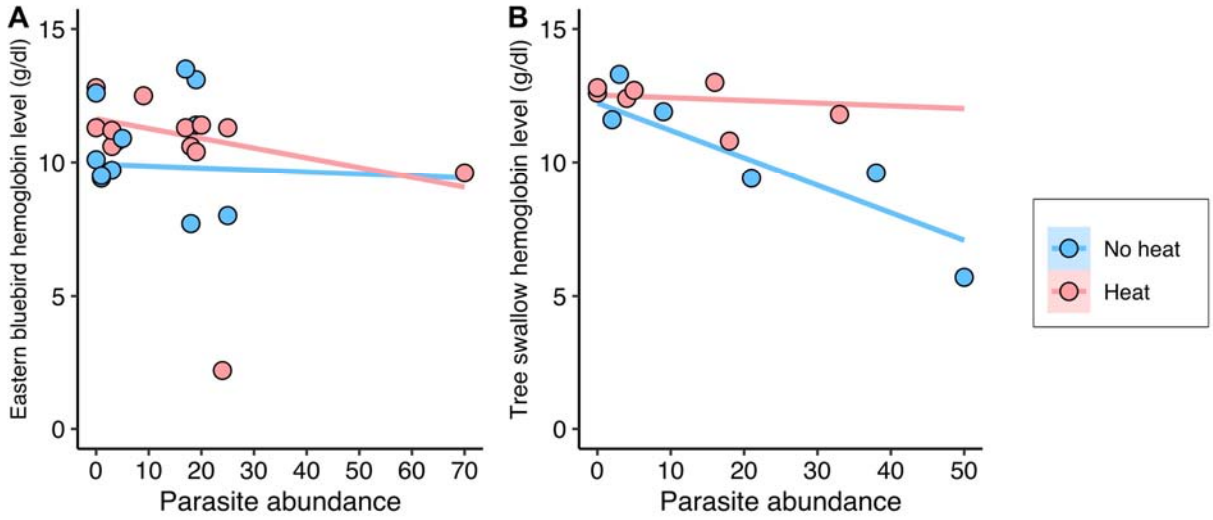
Figure 4.