Humidity modifies age-dependent heat wave effects in an insect host-parasitoid interaction

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Abstract

1. Climate change is projected to increase the likelihood of extreme heat events, but it may also alter humidity levels, leading to the potential for coupled thermal and hydric stress. While increasing frequency and intensity of extreme heat events have been well-documented for their negative effects on species and their interactions, how humidity modulates the impacts of heat waves is currently unknown. 2. We investigated how humidity interacted with heat waves of different timings and durations to affect the life histories in an insect host-parasitoid interaction, comprising the Indian meal moth, Plodia interpunctella, and its endoparasitoid wasp, Venturia canescens. Hosts parasitised as 4th instar larvae and unparasitized hosts were maintained in a high humid (60.8% RH) or low-humid (32.5% RH) environment at a constant temperature of 28. They were then exposed to 38 heatwaves with a duration of 6 or 72 hours in either the 4th or 5th instar. 3. Humidity and heat waves did not affect the adult emergence of unparasitized hosts, but longer heat waves and lower humidity increased the probability that host adults emerged from parasitized hosts, indicating the negative effect of these conditions on the survival of parasitoids. Furthermore, juvenile development time and body size of hosts and parasitoids responded differently to timing and duration of heat waves, and high humidity decreased larval development time of unparasitized hosts and increased the size of both hosts and parasitoids. In addition, humidity negatively interacted with larval stage and duration of heat waves to affect the size of unparasitized hosts, but this effect was not found in parasitoids. 4. Our results show that humidity modulates the life history of hosts and parasitoids, highlighting the importance of humidity in maintaining host-parasitoid interactions. Humidity should be considered when predicting the impact of temperature extremes on species’ population dynamics and their interactions.

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4. Our results show that humidity modulates the life history of hosts and parasitoids, highlighting the importance of humidity in maintaining host-parasitoid interactions. Humidity should be considered when predicting the impact of temperature extremes on species’ population dynamics and their interactions.

Key words
Environmental stress, species interactions, insect decline, extinction, top-down effect, phenotypic traits

1 Introduction
Climate warming, manifested primarily through increasing temperature and the greater frequency and intensity of heat stress events, such as short-lived extremes or heat waves (Rahmstorf and Coumou, 2011, Sun et al., 2019), has prominent impacts across ecological scales. How extreme heat waves affect biodiversity and ecosystems has yielded mixed findings (Ruthrof et al., 2018, Li et al., 2017, Cope et al., 2023) as extreme high temperatures have been shown to have positive, negative, or neutral effects on the individual fitness
of living organisms, such as insects (Skendžić et al., 2021, Ma et al., 2021). However, as insects’ responses to thermal stress may not be consistent throughout ontogeny, the timing and duration of heat events experienced at different life stages may lead to contrasting fitness outcomes (Moore et al., 2022, Valls et al., 2020). Furthermore, differences in thermal tolerance and sensitivity between interacting species may result in differential responses to high temperatures, with trophic interactions in particular likely being disrupted (Moore et al., 2021, Bannerman and Roitberg, 2014). Hence, understanding how extreme high temperature impact on insect species and their trophic interactions is important to understand how they might respond to future climate change.

In addition to extreme temperatures, climate warming may increase the rate of surface evaporation, resulting in a combined stress of high heat and high humidity (Schär, 2016). Such ‘humid heat waves’ have been observed in many parts of the world and are projected to escalate under future climate change scenarios (Gershunov and Guirguis, 2012, Russo et al., 2017, Wang et al., 2021). However, how humidity modulates the effect of extreme temperatures has not been examined widely, leading to considerable uncertainty about the abundance and distribution of species (Chown et al., 2011, Simmons et al., 2023). Within this context, efforts to forecast the persistence of trophic interactions and their dynamics may be limited (Brown et al., 2023, Rozen-Rechels et al., 2019).

Despite its pronounced influence on regulating insects’ life cycles, evidence for how humidity modulates thermal responses is rather underdeveloped (Brown et al., 2023). Humidity levels are not only related to the availability of water vapour that insects acquire from the environment, but they also affect the ability to regulate water loss through spiracular respiration (Shipp et al., 1988). For example, when exposed to low humidity environments, adult cockroaches (Naupheota cinera) exhibited a slower rate of water loss due to longer periods of spiracular closure (Schimpf et al., 2009). However, some insects were able to gain moisture when air humidity increased, subsequently lowering the risk of desiccation (e.g., Salin et al., 1999, Johnson, 2010). Importantly, temperature is of fundamental importance in driving many physiological processes including spiracular control, thus the responses of insects to humidity may also be temperature-dependent (Heinrich and Bradley, 2014). As such, if the control of spiracles is an adaptive mechanism to balance the trade-off between water loss and gas exchange against changing environments (Oladiupo et al., 2022), humidity can either positively or negatively affect insect survival when temperatures increase.

Parasitoids are top-down regulators of their host’s population dynamics, they are a key component of terrestrial food webs, and they may be important in biological control (Jeffis and Lewis, 2013, Furlong and Zalucki, 2017). Koinobiont endoparasitoids lay their eggs inside their hosts, and their larvae continue to develop and feed inside the hosts as it grows and reaches the optimal size for adult parasitoid eclosion. There is a growing body of evidence that temperature can have both a direct impact on koinobiont endoparasitoid life history and an indirect impact via the response of their hosts to temperature (e.g., Cavigliasso et al., 2021, Meisner et al., 2014, Abarca and Spahn, 2021). In general, parasitoids are less tolerant of high temperatures than their hosts (Abarca and Spahn, 2021, Furlong and Zalucki, 2017), and hosts may suppress parasitism if parasitoids experience extreme high temperatures during early development stages (Moore et al., 2022). On the other hand, endoparasitoids may adapt to sublethal temperatures, via multiple ontogenetic responses as the result of the host development (Harvey and Strand, 2002, Harvey et al., 1994). For example, Additionally, as hosts are the only source of moisture for endoparasitoid larvae, humidity may indirectly affect parasitoids through the direct impact of humidity on their host. Previous work has shown that interactions between environmental factors, such as fluctuating temperatures and resource quality, influence the life history traits of both hosts and parasitoids in ways that could not be predicted from each factor alone (Mugabo et al., 2019), emphasising why it is important to consider combinations of environmental factors and their impacts on interacting species.

We investigated the effect of humidity on the responses of an insect host and its parasitoid when they were exposed to heat waves of different durations and experienced at different host ages, using the Indian meal moth Plodia interpunctella and its koinobiont endoparasitoid wasp Venturia canescens. We carried out a single generation life-history experiment where hosts were kept individually either in a humid or non-humid
environment at a constant temperature of 28°C, with or without being parasitized early in the fourth instar. All parasitised and unparasitized hosts subsequently experienced either no heat wave (0 control), a 6-hour, or 72-hour heat wave of 38°C, applied either in the host fourth or fifth instars. We measured key life history parameters (larval development time, and adult emergence and body size) of both hosts and parasitoids to evaluate the combined effects of humidity and heat waves on this trophic interaction (see Figure 1 for experimental design).

We first assessed how the life history of hosts and parasitoids responded to the heat waves individually, and then assessed whether these responses were modified by a high humid environment. Further, we examined the direct effects of heat waves on hosts and parasitoids and the indirect effect through their trophic interaction, and investigated if these direct and indirect pathways were different between humidity levels. We predicted that 1) increasing heat wave duration would negatively affect hosts and parasitoids; 2) parasitoids will be more affected by heat waves than their hosts when they are earlier in their development; 3) humidity will modify heatwave effects on the hosts directly, and modify heatwave effects on parasitoids indirectly.

2 Materials and methods

2.1 Study organisms

The laboratory culture of the host *P. interpunctella* and larval hosts parasitised by *V. canescens* larvae were kept in temperature controlled incubators at University of Leeds, UK, under a constant temperature of 28°C and 16:8 light: dark cycles (Jones et al., 2015, Mugabo et al., 2019). Adult *V. canescens* were kept at ambient temperature, with a sugar water solution for maintenance. Larval hosts were provided with a wheat bran diet (same with Mugabo et al., 2019). No artificial source of moisture was provided inside the incubator for the laboratory culture to date (Figure S1).

2.2 Experimental procedure

We conducted a life history experiment where hosts were kept individually for their entire life cycles. Eggs were collected for 24 hours from approximately 50 randomly-selected newly emerged host adults from the laboratory culture (Jones et al., 2015). The eggs were then transferred into 25 well (1cm x 1cm) clear plastic plates (Sterilin Limited, Thermo Fisher Scientific, UK), with a single egg plus 0.3g food added into each cell, which is sufficient for complete host development (see Mugabo et al., 2019). A piece of 2-ply tissue paper and a nylon mesh were placed between the well-plate and lid to provide ventilation, while also preventing host larvae from moving between cells or escaping.

We prepared 1360 eggs for the experiment, and assigned these eggs in different treatment combinations. We first placed half of the eggs (i.e., 680 eggs), in an incubator with artificially manipulated humidity, with the other half placed in an incubator with no humidity manipulation, both under a constant 28°C with 16:8 light: dark cycle. Artificial humidity was created by placing ~1000 ml of a saturated sodium chloride solution in the humidity treatment incubators (Solomon, 1951). Pilot data showed that at a constant temperature of 28°C or 38°C the solution can increase the relative air humidity (RH) from 30% ± 5% in the incubators with no solution to 60% ± 5% (Fig S1 - S4).

Larval development was checked daily to identify when they were at either the 4th instar or 5th instar. To avoid changes in the microclimate during monitoring of host development inside the 25-well plates, which may affect their development rates, 200 extra eggs were set up in the same way and monitored to estimate the overall developmental status of the experimental cohort. When host larvae had reached their early fourth instar, half of them were parasitised in each humidity treatment (i.e., 340 eggs per humidity treatment; hereafter ‘H-P’ treatment) and half were unparasitized in each humidity treatment (hereafter ‘H’ treatment). To parasitize hosts in the ‘H-P’ treatment, we took each host out of its cell, and placed it in a petri dish under normal laboratory conditions. A newly emerged parasitoid wasp from the stock
culture was placed with a host until it was parasitised, which was confirmed when \textit{V. canescens} performed a unique cocking motion (Rogers, 1972). Each parasitized host was put back into its original cell and original treatment, to continue development.

170 parasitized hosts and 170 unparasitized hosts that were kept in a humid or non-humid environment, were then exposed to heat waves in their fourth instar (immediately after parasitizing), and the rest were kept in their original incubators until they were exposed to heat waves in their fifth instar (~5\textsuperscript{th} day after parasitizing). Heat waves were generated by transferring parasitised and unparasitized larvae into incubators at a constant 38 incubators (either with or without artificially increased humidity) for different durations of 6 hours or 72 hours. Control parasitised and unparasitized larvae were kept at 28\textdegree C (i.e. no heatwave). Larvae exposed to the heatwaves were returned to 28\textdegree C and corresponding humidity conditions and monitored daily until host or parasitoid adults emerged and died naturally. Collectively, the treatments comprised unparasitized hosts and hosts parasitised in the 4\textsuperscript{th} instar, which were kept in a humid or non-humid environment, and exposed to heatwaves of durations of 0, 6, or 72 hours in their fourth or fifth instar. There were 50 individual larvae for the 0-hour treatment, 60 larvae for the 6-hour heat waves, and 60 larvae for 72-hour heat waves, in each combination of humidity x parasitism x larval stage.

We measured the following traits in unparasitized hosts: 1) host emergence 2) juvenile development time (i.e., from egg to adult emergence) 3) mid femur length (which is correlated with body size, Jones et al., 2015, Mugabo et al., 2019). For parasitized hosts, we measured 1) relative emergence success —whether a host (i.e. parasitoids were killed by heat stress; where encapsulation was not considered), wasp (i.e. parasitoids survived and killed the host), or neither emerged (i.e., both were killed) 2) wasp juvenile development time (i.e., from parasitism to wasp emergence) 3) hind tibia length (as a measure of adult body size, Harvey et al., 2001).

### 2.3 Data analysis

Data consisted of multiple trait measurements in unparasitized and parasitized hosts as responses, and three experimental treatments i.e., humidity, larval stage, duration of heatwaves were predictors. Humidity and larval stage were categorical variables, each with two levels, and duration of heat waves was treated as a continuous variable on a log (x+1)-transformed scale. The number of wasps that emerged from host larvae (i.e., without heat waves) as controls for those exposed during the 5\textsuperscript{th} instar were less than the minimum requirements of statistical tests (i.e. < 3), which may bias overall estimates if treated as an independent group. Therefore, we increased the number of observations in this group by shuffling all no-heatwave controls within each humidity treatment and split equally in numbers into two larval stages, using ‘sample_n()’ function in the ‘tidyverse’ package (Wickham et al., 2019).

Statistical analyses were conducted using R (v 4.3.2, RCoreTeam, 2022). We used a generalised linear model (GLM) with a binomial error distribution to investigate if humidity, larval stage, and duration of heat waves affected the adult emergence of unparasitized hosts. In parasitized hosts, we performed a multinomial logit regression model to investigate if the relative success of hosts and parasitoids was affected by the experimental manipulations, using ‘nnet’ package (Ripley et al., 2016). Probability of success was measured by three possible outcomes of each parasitized host: 1) parasitoid emergence – where the host was killed by the successful emergence of a parasitoid; 2) host adult emergence – where a parasitoid failed to kill the host; 3) none – where both the host and the parasitoid failed to emerge (i.e., both were killed). Humidity, larval stage, duration of heat waves, and their interactions were included as predictors. To compare how the survival of parasitoids was changed due to experimental manipulations, we set up the outcome of ‘parasitoid emergence’ as the reference group, indicating that any changes in the outcomes in parasitized larvae (i.e., from parasitoid emergence to host emergence; or from parasitoid emergence to no emergence) were the results of experimental treatments (where encapsulation was not considered). The multinomial logit coefficients of ‘host adult emergence’ and ‘none’ relative to ‘parasitoid emergence’ were estimated and compared using two-tailed Wald z-tests (Kwak and Clayton-Matthews, 2002).
Host and parasitoid larval development were assumed to be gamma-distributed under the constant temperature (Li et al., 2022), so the GLMs were fitted with a log-link Gamma error distribution for hosts and parasitoids that emerged to the adult stage. Body size of unparasitized adult hosts and parasitoids were analysed using GLMs with a log-link gaussian error distribution. Humidity, larval stage, duration of heat waves, and their interactions were included as independent variables in these models. Model residuals were checked using a simulation-based approach by running n=250 simulations in ‘DHARMa’ package (Hartig and Hartig, 2017) and tested for normality using Kolmogorov-Smirnov test.

Finally, we used a piecewise structural equational model (‘piecewiseSEM’ package, Lefcheck, 2016) to investigate the direct and indirect effect of humidity, larval stage, and duration of heat waves on hosts, and how they subsequently affected the phenotype of parasitoids (body size). The advantage of this method is that piecewise SEMs can switch from global to local estimation, allowing for the fitting of equations with a range of distributions (Shipley, 2000). As the emergence of parasitoids resulted in the death of the host, we quantified the host’s contribution to the parasitoid using host larval survival time (i.e., the date of parasitoid emergence minus the date of host egg laying). The hind tibia length of parasitoids was used as a measure of parasitoid performance to avoid the correlation between two endogenous variables (i.e., host survived time and parasitoids’ sizes). As such, our piecewise SEM included two underlying structured equations which specified a) the effect of larval stage and duration of heat waves on host survival time, and b) the effect of host larval survival time, duration of heat waves, and host larval stage on the hind tibia of parasitoids. Both equations were fitted by a GLM with a log-link gaussian distribution (Gamma GLMs were not supported), with model assumptions checked by the ‘DHARMa’ package (Hartig and Hartig, 2017). To examine if the effect of heat waves on hosts and parasitoids were modified by humidity, we performed a multigroup analysis incorporating humidity as a grouping variable. Whilst constraining both paths may result in a saturated model (i.e., having 0 degrees of freedom to evaluate model fit), the process of automatically adjusting coefficients in piecewise SEMs allows us to examine if any of the paths varied among groups (Lefcheck, 2016). Thus, any significant interactions indicated whether heat waves interacted with humidity, and any constrained effects implied the path to the hosts or parasitoids did not differ in humidity. Standardized coefficients were calculated for non-categorical variables.

3 Results

Humidity, duration of heat waves, larval stage, and their interactions did not affect adult emergence in unparasitized hosts (binomial GLM, all $p > 0.05$). However, in parasitized hosts, the relative success of host and parasitoid adults was significantly affected by experimental treatments (Fig. 2). Longer heat waves increased the probability of host adult emergence in parasitized hosts (Table 1), and experiencing 72 hours of heatwave in the early stage resulted in complete mortality of parasitoids, regardless humidity levels. Increased humidity decreased the probability of host adult emergence (Table 1). Larval stage when the heatwaves were applied did not affect the relative success of hosts and parasitoids, but it positively interacted with humidity, and negatively interacted with duration of heat waves to affect the probability of host adult emergence (Table 1). The effect of the interaction between duration of heat waves and larval stage on host adult emergence depended on humidity levels (three-way interaction, Table 1). Humidity, duration of heat waves, and larval stage did not affect the probability of no adult emergence (Table 1, Fig 2), indicating that the mortality of hosts was not affected by the treatments, but depended on the survival of parasitoids.

The juvenile development time of unparasitized hosts increased when experiencing longer heat waves (log-link Gamma GLM, estimates = 0.036, t = 4.110, $p < 0.001$, Fig. 3a), and decreased in high humid conditions (log-link gamma GLM, estimate = -0.290, t = -7.429, $p < 0.001$, Fig. 3a). Humidity positively interacted with duration of heat waves to affect host juvenile development time (log-link gamma GLM, estimate = 0.038, t = 2.976, $p = 0.003$). For parasitoids, experiencing heat waves at a later stage decreased juvenile development time (log-link Gamma GLM, estimate = -0.068, t = -2.123, $p = 0.036$, Fig. 3b), if they survived
from heat stress. Neither humidity nor duration of heat waves affected the juvenile development time of parasitoids individually, however, increasing heat wave durations in the 5th instar stage decreased juvenile development time (log-link Gamma GLM, estimate = 0.035, t = 2.177, \( p = 0.031 \), Fig. 3b). The mid femur length of unparasitized hosts increased if hosts developed in high humidity (log-link gaussian, estimate = 0.189, t = 5.100, \( p < 0.001 \), Fig. 4a) or experiencing heat waves at later stage (log-link gaussian, estimate = 0.081, t = 2.206, \( p = 0.029 \), Fig. 4a). Moreover, humidity negatively interacted with duration of heat waves (log-link gaussian, estimate = -0.142, t = -2.871, \( p < 0.001 \), Fig. 4a) to affect the mid femur length of unparasitized hosts. The hind tibia length of parasitoids increased if hosts developed in high humidity (log-link gaussian, estimate = 0.144, t = 3.032, \( p = 0.003 \), Fig. 4b), or experienced heat waves during the 5th instar stage (log-link gaussian, estimate = 0.082, t = 2.246, \( p = 0.026 \), Fig. 4b). Larval stage negatively interacted with duration of heat waves to affect the hind tibia length of parasitoids (log-link gaussian, estimate = -0.048, t = -2.577, \( p = 0.011 \)).

Larval stage and duration of heat waves directly affected the survival time of hosts, but not the size of parasitoids (Fig. 5). Instead, the hind tibia length of parasitoids was negatively correlated with host survival time (Fig. 5), suggesting an indirect effect of larval stage and duration of heat waves on the size of parasitoids. All the paths to parasitoids were constrained to a global estimation (\( p > 0.05 \), more in Appendix Table S1), indicating that the effect of heat waves and hosts did not depend on humidity. However, the unconstrained paths to hosts indicated that the effect of heat waves on host survival time depended on humidity levels (\( p < 0.05 \), Appendix Table S1 and S2). In low humidity conditions, host survival time was affected by larval stage and duration of heat waves, whilst it was only affected by duration of heat waves in high humidity conditions.

4 Discussion

To our knowledge, empirical evidence of how humidity regulates the responses of hosts and parasitoids to extreme temperatures is limited. We investigated how the life history traits of hosts and parasitoids were affected by different timing and duration of heat waves, and how the effect of heat waves was modified by different humidity levels. We showed that humidity interacting with heatwaves can strongly affect the life histories of hosts and parasitoids, with a direct effect on hosts and an indirect effect on parasitoids.

Whilst duration of heat waves did not affect host emergence directly, exposing the parasitised hosts to longer heat waves increased the mortality of parasitoids, including complete mortality after 72 hours of heat waves exposed in the early life stage, leading to greater host emergence from parasitized hosts. This supports the general idea finding that parasitoids are less tolerant to heat stress than their hosts (Wenda et al., 2023, Hance et al., 2007), but emphasises that heat wave duration rather than the level of heat wave may be more important. Thus, the detrimental effects on parasitoids experiencing high temperatures for a long period of time can disrupt the trophic interactions, and suggests that pest outbreaks might be more likely if such conditions are experienced in nature. In addition, the juvenile development of survived hosts and parasitoids was longer when exposed to longer heat waves, indicating a risen energy expenditure for hosts and parasitoids to survive from sublethal heat environments. Hosts and parasitoids may able to cope with heat stress by accumulating heat shock proteins and biogenic amines (Gonzalez-Tokman et al., 2020), allowing them to acclimate to the new environment, but with an extra energetic cost. This energetic cost may result in prolonged developmental time (Gillespie et al., 2012, Zhang et al., 2019), and reduced fitness in the adult stage (Yu et al., 2022, Nguyen et al., 2013) as a trade-off.

The timing of heat waves is known to be important for host-parasitoid interactions, as some development stages of parasitoids are more sensitive to heat stress than other stages (Zhang et al., 2019, Valls et al., 2020, Simaz and Szűcs, 2021). In accordance with our predictions, our results showed that experiencing longer heat waves at an early stage increased the mortality of parasitoids, but had no effect on unparasitized hosts, suggesting that experiencing heat waves at early stage of ontogeny may be critical to the survival of parasitoids. Moore et al. (2021) found that compared with other larval stages, experiencing heat waves at
the embryonic stage resulted in complete mortality of the parasitoid *Cotesia congregata*. In our system, *V. canescens* starts to build body mass after 5 days following parasitism of *P. interpunctella* (Harvey et al., 1994), suggesting that the parasitoids may be in the embryonic stage when they experienced heat waves during the 4th instar. Furthermore, the timing of heat waves could modify the life history of surviving parasitoids (Simaz and Szűcs, 2021, Zhang et al., 2019), as our results also showed that parasitoids experiencing heat waves at a later stage decreased their juvenile development time and increased their hind tibia length. Although the physiological mechanisms underpinning these life history changes of surviving parasitoids were not clear, our results highlighted that the effect of heat stress on the success of parasitism was stage specific.

Of potential critical importance for the maintenance of host-parasitoid interactions experiencing heat stress was our finding that higher humidity increased the survival of parasitoids against heat stress. Furthermore, modification of key life history traits of both hosts and parasitoids demonstrated a key role of humidity in modulating the effect of heat stress that has largely been unreported. One possible explanation is that humidity affects host suitability for parasitism through regulating its water budget (Johnson, 2010), indirectly causing fitness-related responses in parasitoids. For example, Mainali and Lim (2013) found high (90-95%) humidity increased the adult emergence of an egg parasitoid *Ooencyrtus nezarae*, due to reduced water loss in host eggs. In our system, *V. canescens* is a koinobiont endoparasitoid, which feeds on host larvae after parasitisation, and it is critical for the host to reach the final (5th) instar for it to complete its own development and eclose as an adult wasp. The responses of hosts to humidity may further affect their nutritional quality for the development of parasitoids. However, disentangling this effect requires further investigation on how the suitability of hosts for parasitism could be driven by different humidity regimes.

The path analysis showed that heat waves affected hosts directly, but indirectly affected parasitoids through host survived time, at least in terms of adult size. This suggested that heat waves were having an indirect effect on parasitoids via their direct effects on the host. Previous studies found that *V. canescens* exhibited flexible growth patterns to accommodate the growth of their hosts (Harvey, 1996, Harvey and Vet, 1997), therefore the responses of hosts to heat stress could result in indirect phenotypic responses of parasitoids. To our knowledge, there are few studies examining these direct and indirect effects across two trophic levels. Simaz and Szűcs (2021) found a direct effect of heat waves on both the host *Halyomorpha halys* and the parasitoid *Trissolcus japonicus*, with an indirect effect found beyond the first generation. However, we demonstrated that the direct and indirect effect of heat waves could be found within a single generation, as the responses of host may affect the ontogenetic development of parasitoids. This highlights that humidity may also play an important role in moderating direct and indirect effects of heat wave effects on hosts and parasitoids, which have important implications for their co-existence, extinction risk, and potential for more pest outbreaks under similar conditions in nature.

We attributed the humidity effect to the differences in the average moisture levels between treatments, but variation in humidity, which was not measured in this study, might also affect some critical life history stages such as diapause (Wetherington et al., 2017, Seymour and Jones, 2000). Higher variation in humidity may trigger more humidity-induced diapause in parasitoids (Wetherington et al., 2017), but in the present study this effect was unconsidered. Furthermore, the optimum humidity levels, which may depend on the temperature, in our system is unclear. Gross (1988) found a significant decline in parasitoid emergence *Trichogramma pretiosum* over 80% or below 40% RH. Duale (2005) found that the optimum humidity for the development of a parasitoid *Pediobius furvus* was between 60-80% RH. It is possible for a nonlinear effect of humidity on the life history performance of hosts and parasitoids, and so a greater range of humidity x temperature combinations should be explored in future.

The present study has implications for pest management and biological control. Humid environments may increase the development of hosts and possibly increase the fitness of their adult stage, resulting in more frequent and severer pest outbreaks (Stireman et al., 2005, Wetherington et al., 2017). Experiencing long heat waves especially at certain larval stage is detrimental to parasitoids, suggesting that the success of biological control under continuous heat events is stage specific. From this point of view, more evaluations of stage specific effect of heat events may provide practical insights into the successful applications of biological
control in the field.

5 Conclusion

Our findings showed how humidity can play an important role in modulating the life history response of hosts and parasitoids to heat waves, through a direct effect on the hosts and an indirect effect on parasitoids. Our results highlight the importance of accounting for humidity in predicting the population dynamics and distribution of species in the context of climate change. Furthermore, we emphasised how interactions between environmental stressors can affect trophic interactions through different pathways, ultimately modulating the interactions between species and their responses to the changing climate. Disentangling the direct and indirect effect of humidity and heat waves will bring us one step closer to the ability of predicting community-level responses to climate change.

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Data availability statement

Data is free to access in GitHub repository (https://github.com/Dongboli/experimental-data.git) and will also make it available in Dryad once published.

Author contributions

D.L., S.M.S, C.C.C, M.S.F, and Y.C designed the experiment, D.L., B.B., and J.R. conducted the experiment and collected the data. D.L. led the writing of the manuscript. All authors gave their approval before submission for publication.

Conflict of interest

The authors declare no conflict of interests.

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Table 1  The parameter estimates of the multinomial logit regression model on the relative success of parasitoids, hosts, and none in parasitized hosts. Reference level = parasitoid emergence. Significant differences ($p < 0.05$) were denoted with bold.

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<td>Duration</td>
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<td>Larval stage (LS)</td>
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<td>Duration: LS</td>
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<td>0.344</td>
<td>-2.354</td>
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<td>Humidity: duration: LS</td>
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<tr>
<td>None</td>
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<td>-0.595</td>
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<td>-0.883</td>
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Figure 1 Schematic diagram of the experiment. Host eggs were placed in either a humid or non-humid incubator at a constant temperature of 28 until they reached the early 4th instar. Half of the hosts were parasitized whilst the other half unparasitized. Both parasitized and unparasitized hosts experienced a 38 heat wave either as early 4th instar or 5th instar larvae. After exposure to the heat wave, all the hosts were kept at constant 28 until adult emergence. Credit with Biorender.com.
Figure 2 Multinomial logit regression model predicted the probabilities of relative emergence success of (left panel) the parasitoid, (middle panel) the host, and (right panel) neither species. Parasitized hosts developed either in high humidity (blue) or low humidity (yellow), with different duration of heat waves (on a log(x+1) transformed scale) exposed during either 4\textsuperscript{th} (solid lines) or 5\textsuperscript{th} (dashed lines) instar larvae. Probabilities were predicted by the full model with 95% confidence intervals.

Figure 3 The juvenile development time of hosts (a) and parasitoids (b). Hosts developed in either high humidity (blue) or low humidity (yellow), experiencing heat waves as 4\textsuperscript{th} instar (left) or 5\textsuperscript{th} instar (right) for different durations. Data were jittered as points for clarity. The maximum and minimum limits are shown by the end of the whiskers, and the first and third quartiles of responses are shown by the end of boxplots. The lines inside the boxplots represent the medians. Note that in (b) parasitoid mortality was 100% when the heatwave was applied in 4\textsuperscript{th} instar larvae for 72 hours so there are no data on juvenile parasitoid development.
Figure 4 The mid femur length of hosts (a) and hind tibia length of parasitoids (b). For annotations, see legend for figure 3. Note that in (b) parasitoid mortality was 100% when the heatwave was applied in 4th instar larvae for 72 hours so there are no data on adult parasitoid hind tibia length.

Figure 5 Multigroup SEM results showed both larval stage and duration of heat waves directly affected host survival time, and indirectly affected the size of parasitoids through response of host. The direct and indirect effect of larval stage and duration of heat waves on host-parasitoids depended on whether they developed in high humidity (blue) or in low humidity (yellow) levels. Significant paths were denoted with solid arrows whereas nonsignificant paths were denoted as dashed arrows. Standardized coefficients (/ when standard coefficients were unavailable) and significant levels were given on the top or bottom of the arrows. Significant levels: '*' .001 ** .01 *** .05 'ns.' This figure showed that both larval stage and duration of heat waves directly affected host survival time, and indirectly affected the size of parasitoids through response of hosts.