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## Research

### Simulated heatwave conditions associated with global warming affect development and competition between hyperparasitoids

Cong Chen, S. Helena Donner, Arjen Biere, Rieta Gols and Jeffrey A. Harvey

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Anthropogenic global warming and attendant effects like heatwaves affect the biology and ecology of both individuals and species within and across different trophic levels. Here, we examined the effects of a simulated heatwave on development of and competition between two hyperparasitoid wasps, *Lysibia nana* and *Acrolyta nens* when attacking the same host, cocoons of the primary parasitoid, *Cotesia glomerata*. Parasitized hosts were exposed to three different day and night temperature regimes (low, medium and high) that reflect cool, normal and heatwave conditions in the Netherlands. We found that higher temperatures decreased survival to eclosion more strongly in the hyperparasitoids than in their host. Heatwave conditions also shortened development time and led to the production of smaller adult wasps of both hyperparasitoid species in singly parasitized hosts. In multiparasitized hosts, *L. nana* won most of the contests when it oviposited first, irrespective of the time interval between the first and second parasitism, whereas *A. nens* only dominated when it had a 24 h head start or longer. Most importantly, our results show that *L. nana* in particular benefited in competition at higher temperatures, perhaps due to an increase in the metabolic rate and more rapid egg and/or larval development. This may potentially reduce opportunities for coexistence following heat waves. Our results suggest that heatwaves associated with global warming will enhance the rate of development, but negatively affect survival and other fitness-related traits in (hyper)parasitoids. Moreover, the outcome of larval competition may be determined via physiological responses that are species-specific and thus influence phenology.

Keywords: *Acrolyta nens*, climate warming, *Cotesia glomerata*, host–parasitoid interactions intrinsic competition, *Lysibia nana*, temperature

#### Introduction

The frequency of extreme short-term climatic events associated with global warming, such as heatwaves, droughts and torrential downpours, has increased in much of Europe in recent decades (Beniston et al. 2007). During extreme climate events such as heatwaves maximum daily temperatures exceed tropical thresholds of 30 degrees

over several days. These conditions stress ecological communities at all levels of organization (Jentsch et al. 2007), but may have more negative effects on ectotherms, including arthropods and some vertebrates, for instance reptiles and amphibians, than on endotherms such as mammals and birds (Buckley et al. 2012). The body temperature of ectotherms relies critically on ambient environmental temperature, and their metabolic rate increases in response to increasing temperature until a maximum is reached (Angilletta et al. 2010). This translates into more rapid growth and development in plants and animals and perhaps a reduction in their longevity (Paital et al. 2016). Under this scenario, critical resources for consumers may be available for shorter periods during the course of a growing season (Visser and Holleman 2001). When species in different trophic levels respond differently to heatwaves, this can affect the strength of top-down and bottom-up processes that regulate the structure and functioning of food webs (Petchey et al. 1999, Van der Putten et al. 2010, Shurin et al. 2012). The effects of these changes on community structure and ecosystem functioning is difficult to predict but there is ample evidence to suggest that there will be negative consequences if warming at the current rate continues unabated (Walther et al. 2002, Stireman et al. 2005, Zimova et al. 2016, Yee et al. 2017).

In nature, many organisms compete intra- and inter-specifically for resources that may be temporally limiting or scarce (Chesson and Kuang 2008, Harvey et al. 2013). Climate warming may change both the availability and quality of resources for consumers and in some cases intensify competition. Among insect natural enemies, the majority of parasitoid wasps, whose larvae develop on or inside the bodies of other insects, are specialized, and thus attack one or only a few types of closely related hosts in nature (Godfray 1994). Because they depend on limited resources (the host) for their development, parasitoids are under strong selection for the optimal exploitation and allocation of host resources to different and often competing fitness functions such as reproduction and survival (Wajnberg et al. 2012). In almost all host-parasitoid associations, only one parasitoid species can successfully parasitize and develop in an individual host, thus competition for hosts means invariably that there will be only one winner. Importantly, under global warming, competition may favor parasitoid species that are more adapted to warmer conditions, resulting in a shift in the community structure of parasitoids. For example, in the Netherlands, caterpillars of *Plutella xylostella* that used to be parasitized predominantly by *Diadegma semiclausum* (Ichneumonidae) are now also attacked by *Cotesia vestalis* (Braconidae) which is the dominant parasitoid in southern Europe (Kos et al. 2011, Li et al. 2016). At 25°C, *C. vestalis* is the superior competitor when hosts are also parasitized by *D. semiclausum* (Shi et al. 2004). Temperature-related differences in larval competitive interactions between the two species in northern Europe may therefore favor *C. vestalis* if the warming continues unabated. Warming-induced shifts in community structure have also been observed in other host-parasitoid systems (Tougeron et al. 2018).

In larval competition, the time lag between the attack of the host by the first and second parasitoid species usually plays an important role in determining the outcome of interspecific competition. Several studies have shown that the first parasitoid species that parasitizes the host often wins the competition (De Moraes and Mescher 2005, Harvey et al. 2009a). However, there are some other factors that can affect the outcome of larval competition such as duration of embryo development, egg size, larval feeding strategy, larval aggression, host quality and host specificity (Jervis and Kidd 1986, De Moraes et al. 1999, Harvey et al. 2013). Importantly, given that they are ectotherms and their development rate is correlated with temperature, the effects of these factors on competition between parasitoid larvae could be strongly affected under simulated heatwave conditions (Hance et al. 2007, Jeffs and Lewis 2013, Harvey 2015).

Here, we investigate the effects of a simulated heat wave and different temperature regimes on survival, development and larval competition in two hyperparasitoid species *Lysibia nana* (Hymenoptera: Ichneumonidae, Fig. 1a) and *Acrolyta nens* (Hymenoptera: Ichneumonidae, Fig. 1b) that display similarity in several life-history traits. Both species are idiobionts that attack non-growing host stages or prevent further host development and are largely specialized

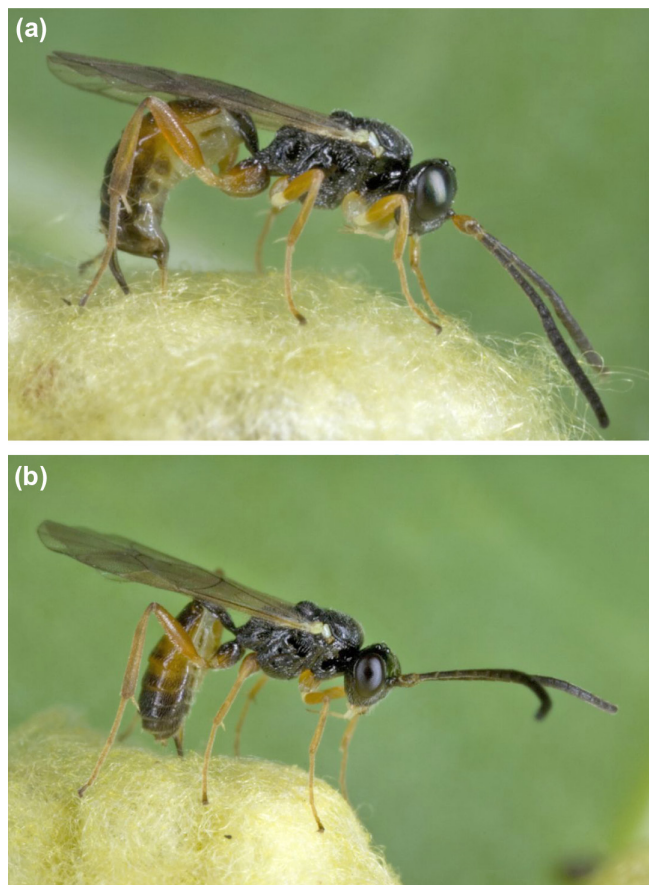


Figure 1. *Lysibia nana* (a) and *Acrolyta nens* (b) females parasitizing *Cotesia glomerata* cocoons.

on cocoons of *Cotesia* species in nature, with *C. glomerata* appearing to be one of their more important hosts (Shwarz and Shaw 2000). The two hyperparasitoids appear to be bi-voltine and have very overlapping niches, both spatially and temporally (Harvey et al. 2014). Several field studies have found that both species frequently emerge from single cocoon clusters of *C. glomerata* in the field, strongly suggesting that they do compete for hosts (Poelman et al. 2013, Harvey et al. 2014, Zhu et al. 2015). To address whether different temperature regimes affect the outcome of larval competition in the two species, we simulated three day–night temperature regimes in climate cabinets representing cool, normal and hot summer conditions in the Netherlands where the two species are native.

We predict that 1) exposure to high temperatures resembling heatwave conditions will reduce the survival of both hyperparasitoid species (and the pupal stage of their host) since they are all adapted to temperate zones with modest temperature regimes; 2) longer time intervals between the first and second parasitism event will benefit the hyperparasitoid that parasitized the host first. This effect will be stronger under warmer conditions because the development rate of both hyperparasitoids is positively correlated with temperature, but will favor the species that can most strongly adjust its development rate at the higher temperature.

## Material and methods

### Insects

All cultures of insects were maintained in a climate room at  $22 \pm 2^\circ\text{C}$ , 50–70% RH and a photophase of 16L:8D at the Netherlands Institute of Ecology (NIOO). Caterpillars of *Pieris brassicae* (Lepidoptera, Pieridae) were used as a host for the primary parasitoid *Cotesia glomerata*. Healthy unparasitized and parasitized hosts were reared on Brussels sprout plants (*Brassica oleracea* var. *gemmifera* cv. Cyrus) which had been grown at the Unifarm greenhouse facilities of Wageningen University. *Acrolyta nens* and *L. nana* were in turn reared on *C. glomerata* cocoons. *Pieris brassicae* and *L. nana* were obtained from the Laboratory of Entomology. All species used in this study were originally collected from cabbage fields near the University.

*Cotesia glomerata* is a gregarious species that attacks L1–L3 caterpillars of *P. brassicae*. To obtain *C. glomerata* cocoons, leaves containing aggregations of L2 host larvae were placed into the rearing cage with adult wasps for several minutes. The parasitized hosts were reared on potted Brussels sprout until larval parasitoid egression and pupation. Parasitoid cocoons were collected from the plants and placed in petri dishes. Only fresh cocoons (<24 h), which are of high quality for hyperparasitoid development (Harvey et al. 2006), were used in this study. Both *L. nana* and *A. nens* were maintained in rearing cages ( $35 \times 35 \times 35$  cm) and were provided with honey and water, which were replaced twice a week. Cocoons of *C. glomerata* were presented to either *L. nana* or

*A. nens* for at least 8 h in cages ( $35 \times 35 \times 35$  cm) after which they were transferred to petri dishes ( $\phi = 10$  cm) until adult eclosion. Newly emerged hyperparasitoids were then used for the experiments or further rearing.

### Experimental design

Different temperature environments were created using Sanyo incubators model MIR-553. The incubator day and night temperatures were  $20^\circ\text{C}/12^\circ\text{C}$ ,  $25^\circ\text{C}/17^\circ\text{C}$  and  $30^\circ\text{C}/22^\circ\text{C}$  (day/night) respectively, with a photoperiod of 16 h. These temperatures reflect the lowest to highest mean average day and night summer temperatures in the Bilt in the Netherlands measured from 1981 to 2010 (<[www.wintergek.nl/data/lijst-gemiddelde-temperatuur-nederland](http://www.wintergek.nl/data/lijst-gemiddelde-temperatuur-nederland)>).

Fresh cocoon broods of *C. glomerata* were separated using forceps. A previous study has shown that there is a strong relationship between host size of *C. glomerata* and adult body size of both hyperparasitoids (Harvey et al. 2009c). Single *C. glomerata* cocoons were weighed on a microbalance (accuracy  $\pm 1 \mu\text{g}$ ) and then hyperparasitized individually by presenting them to a female hyperparasitoid of one of the two species in a vial. The female was observed ovipositing into the cocoon, a process which usually takes between two and six minutes. Following parasitism by the first parasitoid, the cocoons were multiparasitized by a female of the second hyperparasitoid species in the same way as described for the first parasitism event at time intervals of 0, 6, 24, 48 and 72 h. Each temperature regime (3)  $\times$  time interval (5)  $\times$  first parasitizing species (2) combination was replicated 50 times. From the first parasitism event onwards, individual cocoons were placed in petri dishes ( $\phi = 6$  cm) and maintained at the assigned temperature regime until no more wasps eclosed. Two cohorts of 150 *C. glomerata* cocoons (50 per temperature regime) were singly parasitized by *A. nens* and *L. nana*, respectively, and served as control groups.

The fate of the cocoons was scored into one of four categories: 1) *A. nens* emerged, 2) *L. nana* emerged, 3) *C. glomerata* emerged and 4) no emergence. Egg-to-adult development times were recorded in days. Newly emerged wasps (wasp eclosion was monitored several times each day) were sexed and anaesthetized using  $\text{CO}_2$  and the adult parasitoid body mass was measured. The experiment was repeated three times in which temperature regimes were switched among incubators to account for incubator effects.

To study the effect of the different temperature regimes on the survival and development time of host cocoons themselves, we also placed five petri dishes ( $\phi = 10$  cm), each containing 30 unparasitized fresh *C. glomerata* cocoons, in the three incubators with the different temperature regimes. These petri dishes were checked for parasitoid eclosion daily, and adult parasitoids were counted and taken out. These controls were also repeated three times along with the temperature switching of the incubators.

To verify that the hyperparasitoids actually lay an egg in the cocoon of a previously parasitized host, an additional set of multiparasitized cocoons were cut open using a scalpel

and micro-scissors and photographed underneath a stereomicroscope. We checked cocoons that were multiparasitized at two of the five time intervals between the first and second parasitism: 1) at the 0-h interval, and 2) at the 72 h interval under each of the three temperature regimes (30 cocoons were used in total). The cocoons were dissected immediately after the second parasitism event. The photographs confirmed that the hyperparasitoids laid a second egg in previously parasitized cocoons (Supplementary material Appendix 1 Fig. A5c), and that a cocoon that already had a 72 h-old hyperparasitoid larva could still be multiparasitized by the second hyperparasitoid species (Supplementary material Appendix 1 Fig. A5d).

### Statistical analysis

Effects of temperature regime on the development time and survival of host parasitoids (*C. glomerata*) were analyzed using one-way ANOVA and Kruskal–Wallis tests, respectively. The proportion of host cocoons that were successfully parasitized by *A. nens* or *L. nana* in the control treatments (single species parasitism) was analyzed using a generalized linear model (GLM) with a logit link function and binomial error distribution. The temperature regime, species and their interactions were set as fixed factors. For the outcome of intrinsic competition, two parameters were analyzed: 1) the proportion of successfully parasitized cocoons (cocoons that produced either a *L. nana* or an *A. nens*) and 2) the proportion of successfully parasitized cocoons that produced *L. nana* (note that, invariantly, from multiparasitized cocoons only one of the two hyperparasitoid species emerges). These analyses were also performed GLMs with a logit link function and binomial error distribution. Temperature regime, time interval between the two parasitism events, and order of parasitism (which species oviposited first) and their interactions were included as fixed factors. Multiple comparisons were conducted based on Tukey-adjusted test when effects of temperature treatments, time intervals or their interactions were significant. For singly parasitized host cocoons, data on development time and adult body mass of *L. nana* and *A. nens*, respectively, were analyzed using ANCOVA with cocoon weight as a covariate and temperature regime as a fixed factor. When host cocoons were multiparasitized, data on development time and adult body mass of *L. nana* and *A. nens* were separately compared using ANCOVA with cocoon weight as a covariate. Temperature regimes, time intervals between parasitism events, order in which species parasitized host cocoons and their interactions were included as fixed factors. A post hoc test (Tukey HSD) was carried out for multiple comparisons if significant effects were found. As the sex ratios of both hyperparasitoid species were highly male-biased (*L. nana*: 82% and *A. nens*: 72%), we only used data from body mass and development time of males in these analyses. To illustrate covariate effects, we show simple linear regressions of adult hyperparasitoid body mass on cocoon weight for the three different temperature regimes. All data was analyzed using the R statistical computing environment ver. 3.4.0 (<[www.r-project.org](http://www.r-project.org)>).

### Data deposition

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.34g9b66>> (Chen et al. 2019).

### Results

Development time of host cocoons of *Cotesia glomerata* was significantly affected by temperature treatments ( $F_{2,42} = 1403$ ,  $p < 0.001$ ; Fig. 2a). Host cocoons developed almost twice as fast under the high than under the low temperature regime. Moreover, temperature treatments also influenced the survival of *C. glomerata* ( $\chi^2 = 7.59$ ,  $df = 2$ ,  $p = 0.02$ ; Fig. 2b).

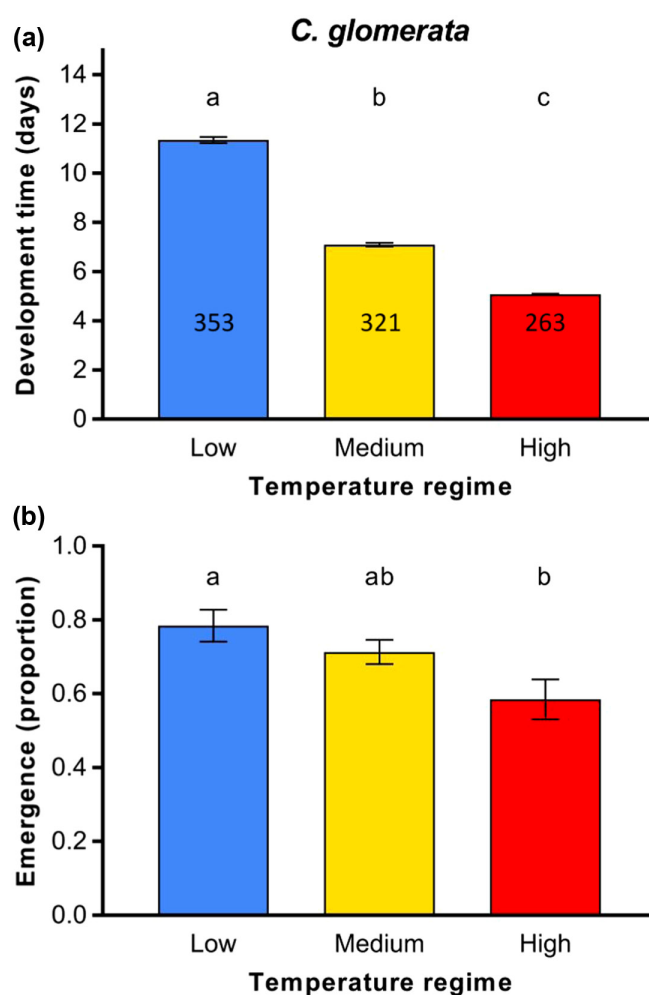


Figure 2. Mean ( $\pm$ SE) development time (a) and adult emergence (b) of *Cotesia glomerata* cocoons when exposed to three different day–night temperature regimes (low, medium and high). Different letters indicate significant differences ( $p < 0.5$ ) between the three temperature treatments, based on pairwise  $t$  tests (development time) and pairwise wilcox test (adult emergence proportions). Numbers in the bars depict the number of individuals that emerged as adults of which development time was determined.

Survival of *C. glomerata* cocoons was around one quarter (60 versus 80%) lower under the highest than under the lowest temperature regime.

The proportion of host cocoons that were successfully parasitized by a single parasitism was significantly higher for *A. nens* than for *L. nana* ( $\chi^2 = 43.9$ ,  $df = 1$ ,  $p < 0.001$ ; Fig. 3). Temperature regime significantly affected the emergence of singly parasitized cocoons ( $\chi^2 = 108$ ;  $df = 2$ ;  $p < 0.001$ ). Emergence of both hyperparasitoids was reduced by more than half when they developed under the high-temperature than under the low and medium-temperature regimes ( $p < 0.05$ ; Fig. 3).

In multiparasitized cocoons, the emergence success of hyperparasitoids was significantly affected by temperature ( $\chi^2 = 436$ ,  $df = 2$ ,  $p < 0.001$ ), time interval between parasitism events ( $\chi^2 = 33.2$ ,  $df = 4$ ,  $p < 0.001$ ) and the order in which species parasitized the cocoons ( $\chi^2 = 9.41$ ,  $df = 1$ ,  $p = 0.002$ ). The proportion of multiparasitized cocoons from which a hyperparasitoid successfully emerged dropped from around 80% under the low and intermediate temperature regime to approximately 50% under the high temperature regime (Fig. 4a–b). Across all time intervals, the proportion of successfully emerging hyperparasitoids was slightly lower when *L. nana* was the first to parasitize the host than when *A. nens* parasitized first. Parasitism success also varied depending on the time intervals between the two parasitism events, but here no consistent pattern could be discerned and it differed depending on which species oviposited first (time  $\times$  order interaction;  $\chi^2 = 37.7$ ,  $df = 4$ ,  $p < 0.001$ ; Fig. 4).

For singly parasitized cocoons, adult body mass of both hyperparasitoid species positively correlated with the initial mass of the host cocoons (*L. nana*:  $F_{1,133} = 300$ ,  $p < 0.001$ ; *A. nens*:  $F_{1,211} = 288$ ,  $p < 0.001$ ; Fig. 5a–b). More importantly, adult body mass of both species was significantly lower under high-compared to low- and medium-temperature

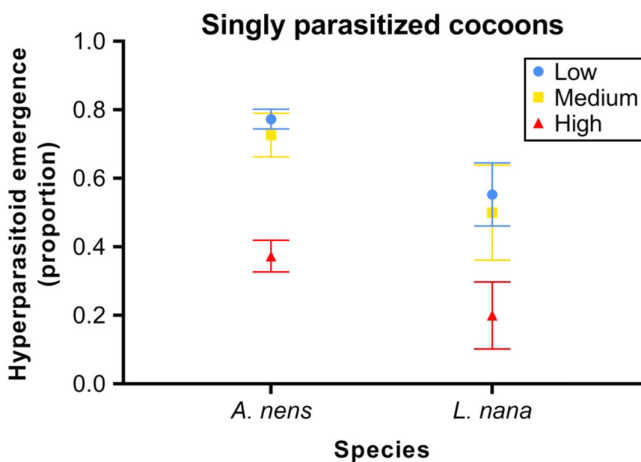


Figure 3. Proportion of adult *Lysibia nana* and *Acrolyta nens* emerging from host cocoons ( $n = 150$ ) when singly parasitized under the three different day–night temperature regimes: low (blue), medium (orange) and high (red).

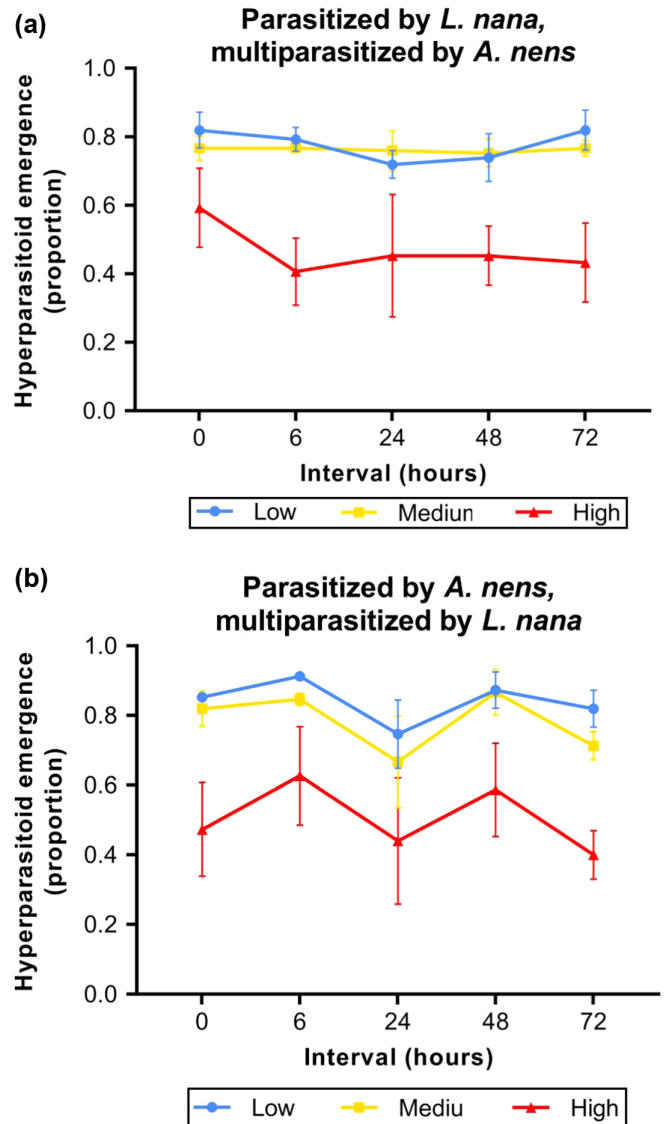


Figure 4. Proportion of successfully parasitized host (*Cotesia glomerata*) cocoons (total hyperparasitoid emergence) when cocoons ( $n = 150$ ) were multiparasitized at each of five time intervals (0, 6, 24, 48 and 72 h) under three different day–night temperature regimes: low (blue), medium (orange) and high (red). (a) *Lysibia nana* parasitized the cocoon first and *Acrolyta nens* second; (b) *A. nens* parasitized the cocoon first and *L. nana* second.

conditions (*L. nana*:  $F_{2,133} = 11.50$ ,  $p < 0.001$ ; *A. nens*:  $F_{2,211} = 19.45$ ,  $p < 0.001$ ; Fig. 5a–b, Supplementary material Appendix 1 Fig. A1). Likewise, in multiparasitized cocoons, temperature significantly affected adult body mass of *L. nana* ( $F_{2,1245} = 19.45$ ,  $p < 0.001$ ) and *A. nens* ( $F_{2,923} = 14.66$ ,  $p < 0.001$ ); hyperparasitoids were lighter with increasing temperature (Supplementary material Appendix 1 Fig. A2a, c). In addition, time interval between parasitism events influenced adult body mass of *A. nens*, but not that of *L. nana* (*A. nens*:  $F_{4,923} = 4.23$ ,  $p = 0.002$ ; *L. nana*:  $F_{4,1245} = 1.01$ ,  $p = 0.40$ ; Supplementary material Appendix 1 Fig. A3, A4). Adult

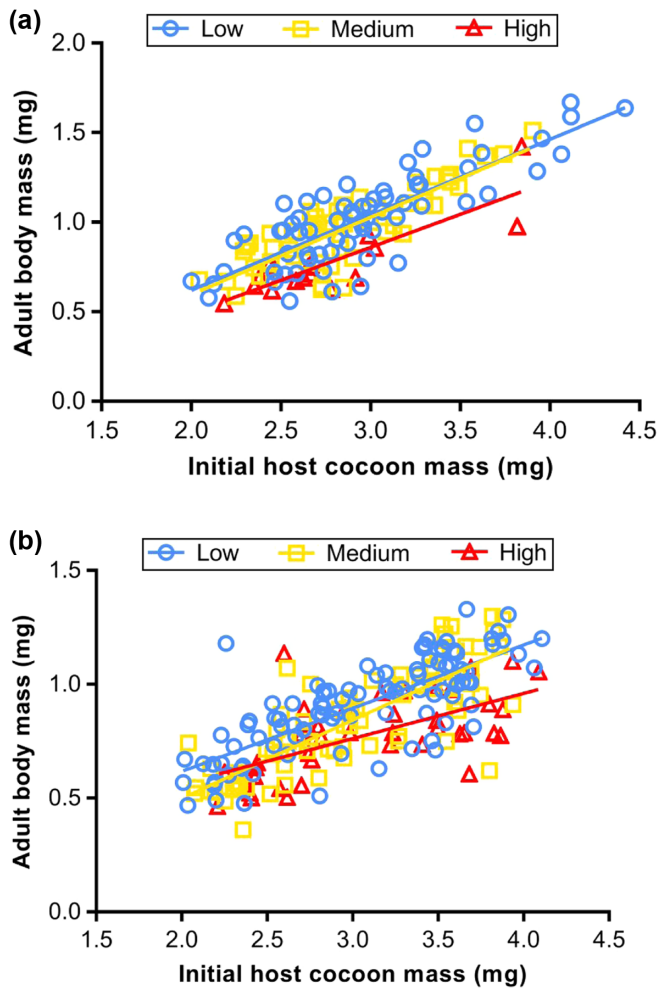


Figure 5. Relationship between initial host cocoon mass and adult body mass of male *Lysibia nana* (a) and *Acrolyta nens* (b) from singly parasitized cocoons under three different day–night temperature regimes: low (blue), medium (orange) and high (red). Regression equations: (a)  $y(\text{low}) = -0.21 + 0.42x$ ,  $r^2 = 0.68$ ;  $y(\text{medium}) = -0.27 + 0.43x$ ,  $r^2 = 0.71$ ;  $y(\text{high}) = -0.25 + 0.37x$ ,  $r^2 = 0.73$ . (b)  $y(\text{low}) = 0.06 + 0.28x$ ,  $r^2 = 0.58$ ;  $y(\text{medium}) = -0.19 + 0.34x$ ,  $r^2 = 0.66$ ;  $y(\text{high}) = 0.17 + 0.20x$ ,  $r^2 = 0.39$ .

body mass of *A. nens* was higher at 0 h than at the other four time intervals ( $p < 0.05$ ).

When cocoons were singly parasitized, development time of both hyperparasitoid species was significantly shorter at the medium- and high-temperature regimes than at the low-temperature regime (*L. nana*:  $F_{2,133} = 386$ ,  $p < 0.001$ ; *A. nens*:  $F_{2,211} = 503$ ,  $p < 0.001$ ; Fig. 6). The effect was similar when the cocoons were multiparasitized both when *L. nana* parasitized the cocoons first ( $F_{2,1245} = 815$ ,  $p < 0.001$ ; Supplementary material Appendix 1 Fig. A2b) and when *A. nens* parasitized the cocoons first ( $F_{2,923} = 255$ ,  $p < 0.001$ ; Supplementary material Appendix 1 Fig. A2d). In addition, time interval between parasitism events affected development time of both species (*L. nana*:  $F_{4,1245} = 2.70$ ,  $p = 0.03$ ; *A. nens*:  $F_{4,923} = 3.68$ ,  $p = 0.006$ ; Supplementary material Appendix 1 Fig. A3, A4). No consistent pattern could be detected here.

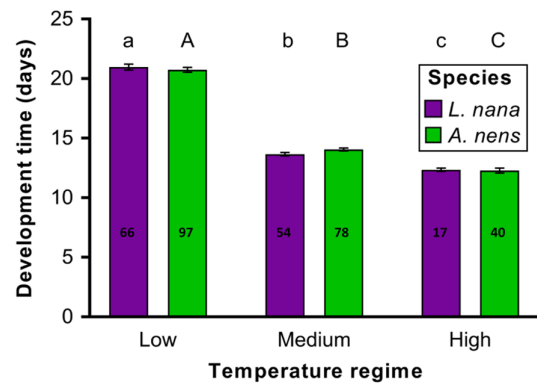


Figure 6. Mean development time of male *Lysibia nana* (purple bars) and *Acrolyta nens* (green bars) emerging from *C. glomerata* cocoons that had been singly parasitized under three different day–night temperature regimes (low, medium and high). Different small (*L. nana*) or capital letters (*A. nens*) indicate significant differences between means ( $p < 0.05$ , Tukey HSD test).

Also the outcome of competition, i.e. which species won the contests was significantly affected by temperature ( $\chi^2 = 12.0$ ,  $df = 2$ ,  $p = 0.003$ ), time interval between the parasitism events ( $\chi^2 = 205$ ,  $df = 4$ ,  $p < 0.001$ ) and the order ( $\chi^2 = 592$ ,  $df = 1$ ,  $p < 0.001$ ) in which the species parasitized the cocoons (Fig. 7). However, the effect of temperature regime and time lapse between parasitism events, depended on which species parasitized the cocoon first (order–temperature interaction:  $\chi^2 = 15.0$ ,  $df = 2$ ,  $p < 0.001$ , order time–lapse interaction: ( $\chi^2 = 168$ ,  $df = 4$ ,  $p < 0.001$ ). When host cocoons were first parasitized by *L. nana* and subsequently parasitized by *A. nens*, *L. nana* invariably was the superior competitor (Fig. 7a), even though in singly parasitized cocoons it was less successful than *A. nens* (Fig. 3). High temperature consistently enhanced the competitive advantage of *L. nana* in multiparasitized cocoons when it was the first one to parasitize the hosts (Fig. 7b). Conversely, when host cocoons of *C. glomerata* were first parasitized by *A. nens*, *L. nana* only outcompeted *A. nens* when both species parasitized the host cocoons simultaneously, and increasingly lost the competition from *A. nens* as the time lag increased (Fig. 7b), whereas the effect of temperature on the outcome of competition when cocoons were first parasitized by *A. nens* (Fig. 7b) was marginal and not consistent across time intervals.

## Discussion

This study shows that pupae of healthy (=unparasitized) *Cotesia glomerata* cocoons completed their development to adult eclosion twice as fast under the heatwave conditions than when exposed to the lower temperatures, but also that their survival decreased from around 80 to 60%. Similarly, the survival to adulthood of both hyperparasitoid species emerging from singly and multiparasitized cocoons dropped even more dramatically (from around 80 to 40% for *A. nens* and from around 60 to 20% for *L. nana*) under the high-temperature regime. High temperature also accelerated the

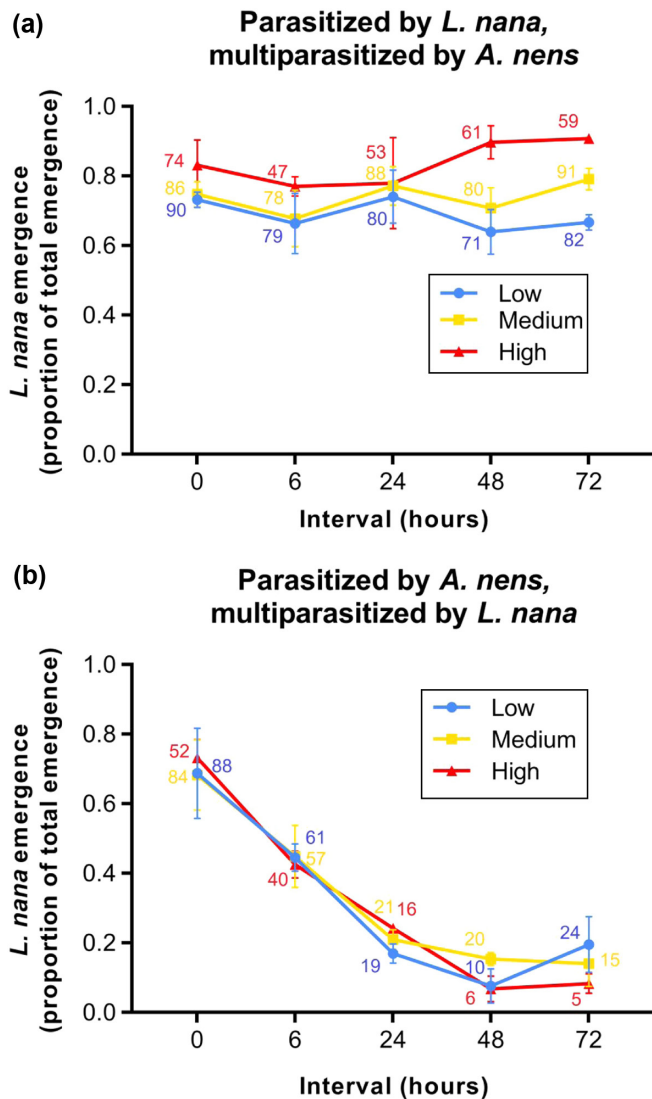


Figure 7. Proportion of adult *Lysibia nana* out of total hyperparasitoid emergence from *Cotesia glomerata* cocoons that were multiparasitized by *Acrolyta nens* and *L. nana* at each of five time intervals (0, 6, 24, 48 and 72h) under three different day–night temperature regimes: low (blue), medium (orange) and high (red) and that produced and adult wasp. Note that only one hyperparasitoid species emerged from multiparasitized cocoons. (a) *L. nana* parasitized the cocoon first and *A. nens* second; (b) *A. nens* parasitized the cocoon first and *L. nana* second. The number of emerging *L. nana* are given for each time point and temperature regime combination.

rate of hyperparasitoid development, but at the same time reduced their adult body mass for a given host cocoon mass. The accelerated development rate and reduced adult body mass is consistent with previous studies on the effects of high temperatures on the performance of ectotherms such as insects (Willott and Hassall 1998, Zuo et al. 2012). High temperatures have been reported to negatively affect survival and/or reproduction of primary parasitoids. For instance, Roux et al. (2010) showed that the survival and reproduction

of the aphid parasitoid *Aphidius avenae* was already negatively affected by a 1-h heat shock. In contrast with our results, Gillespie et al. (2012) found that development time of two aphid parasitoids was longer when exposed to heatwave conditions. Chen et al. (2019) also reported that the reproductive success of two secondary hyperparasitoids were negatively affected under simulated heatwave conditions. Although little studied, the perceived benefits of reduced development time in parasitoids include escaping from predation or success in aggressive competition (Godfray 1994). However, our findings suggest that heatwaves can also have profoundly negative impacts on the fitness of *L. nana* and *A. nens* via reduced survival as a result of physiological stress and lower biomass. In general, benefits of faster development time in ectotherms may be offset or even outweighed by increased mortality when temperatures reach levels beyond the thermal neutral range.

In multiparasitized hosts, *L. nana* was superior to *A. nens* when it oviposited first or just after *A. nens*, whereas *A. nens* only outcompeted *L. nana* when it oviposited 24h or more before *L. nana*. Previous studies examining larval competition in parasitoids have also reported that the outcome of competition (i.e. who is the winner) is strongly influenced by time lags between the first and second oviposition (Tillman and Powell 1992, De Moraes et al. 1999, Harvey et al. 2009a). However, despite their inferiority under conditions of larval competition, *A. nens* was actually more successful in parasitizing cocoons than *L. nana* when cocoons were not multiparasitized by the other species. Thus, although *L. nana* is superior when competing with *A. nens* larvae in the same host, *A. nens* appears to parasitize cocoons of *C. glomerata* more successfully in the absence of *L. nana* eggs or larvae.

Previous work has shown that life-history strategies (e.g. development, reproduction, longevity) of the two species are very similar (Harvey et al. 2009c). However, there are potential differences in traits such as egg maturation rate. The duration of embryogenesis and larval development in parasitoids is shorter at high than at low temperatures, as is generally observed in arthropods and ectotherms. It takes about 36–48 h for eggs of both hyperparasitoids to hatch after oviposition (Harvey et al. 2009b). Newly hatched larvae of cocoon hyperparasitoids, including *L. nana* and *A. nens*, rapidly crawl over the surface of the host pre-pupa or pupa and kill the eggs of other hyperparasitoids by biting them (Harvey et al. 2013). If the larvae encounter other larvae, they presumably engage in physical combat in which there is only one winner. Our results suggest that eggs of *L. nana* develop at a slightly faster rate than eggs of *A. nens*, or else that larvae of *L. nana* are better ‘fighters’ than similarly aged larvae of *A. nens* and this can explain why *L. nana* is the better competitor at higher temperatures. Coexistence at higher trophic levels may be disrupted by global warming when its conditions favor organisms that are better adapted to warmer conditions. Furthermore, when the time lag between parasitism events increases (three days), high temperature appears to enhance the competitive superiority of the first species to



parasitize the host. This is most likely caused by the fact that hyperparasitoid development is accelerated under warmer conditions, giving the first species to oviposit an even faster 'head start' over later competitors. Our study therefore shows that oviposition timing is even more critical under warmer conditions when two species compete for a shared and limited resource.

Several field studies have found that *L. nana* and *A. nens* frequently emerge from single cocoon clusters of *C. glomerata* in the field, strongly suggesting that they do compete for hosts (Poelman et al. 2013, Harvey et al. 2014, Zhu et al. 2015). Furthermore, cocoons of *C. glomerata* remain suitable for parasitism for less than four days at 25°C (Harvey et al. 2006), and this decreases rapidly under warmer conditions (Chen et al. 2019). Parasitism success is not only determined by larval survival to adulthood, but also by the ability of the adult mothers to find and parasitize suitable hosts. Thus, selection pressure for both host finding and host exploitation by hyperparasitoids (indeed, parasitoids in general) may increase under hotter conditions such as those that occur during heatwaves (Jeffs and Lewis 2013, Harvey 2015) reducing the temporal window in which suitable hosts are available.

The effects of exposure to higher temperatures in parasitoids in terms of competition among guilds have thus far been little studied. Co-existence among parasitoids attacking the same host species is often mediated through the specific adaptations of species – for example rate of egg maturation, egg size, ecto- or endo-parasitism, the specificity of host stage attacked, and the expression of many other traits involved in resource allocation and acquisition (Price 1972). However, there is compelling evidence that abiotic conditions, such as temperature, also affect the expression of physiological traits such as lipogenesis that determine how successfully host stages ('resources') are partitioned among parasitoids attacking the same or different host stages (Le Lann et al. 2014). When exposed to heatwaves, parasitoids may differ in their physiological responses that reduce the efficiency of trait divergence or trait diversity and create 'winners' and 'losers' that ultimately reduce the diversity of parasitoids associated with specific hosts (Le Lann et al. 2014). Given that *C. glomerata* cocoons alone harbour as many as 10 hyperparasitoids that both share many overlapping and different traits, it would be interesting to measure the longer-term demographics of hyperparasitoids associated with this primary parasitoid host. This would enable us to determine if there are changes in the relative abundance of them under warming conditions, and the effect of temperature on traits that mediate their success.

Conditions under the simulated heatwave conducted in our experiments were not extreme; in the Netherlands, daily maximum temperatures of 30 degrees or more are required over three of five days with the other two days reaching maximum highs of 25 degrees or more to constitute an official heatwave according to the Royal Netherlands Meteorological Institute (KNMI). However, recent heatwaves have been extended with temperatures reaching as high as 35–37 degrees in parts of the east and south of the country. The summer of

2018 was the warmest in at least 300 years in the Netherlands (<[www.knmi.nl/nederland-nu/klimatologie/maand-en-seizoensoverzichten/2018/zomer](http://www.knmi.nl/nederland-nu/klimatologie/maand-en-seizoensoverzichten/2018/zomer)>) and was accompanied by two extended heatwaves and a protracted drought that lasted over three months. The effects of these extreme events on plant–insect interactions will only be discernible over time. However, if we take the results of our study on body mass and survival under simulated heatwave conditions into account, then it is possible to assume that a wide range of multitrophic interactions have suffered.

Recent rises in surface temperatures across temperate and tropical ecosystems have coincided with significant declines in the abundance of both vertebrate and invertebrate taxa, with insects being particularly vulnerable. For instance, recent dramatic multi-decadal declines in insects have been reported from both temperate (Hallmann et al. 2017, Sánchez-Bayo and Wyckhuys 2019) and tropical (Lister and Garcia 2018) biomes. Although the factors driving these alarming declines are unclear, climate change is almost certainly involved in some of them (Lister and Garcia 2018). Our study has shown that temperature can have a non-linear effect on cocoon survival in the host, *C. glomerata*, with high mortality once a critical temperature is passed that in turn leads to the precocious death of developing hyperparasitoids. Sales et al. (2018) found that male flour beetles became effectively sterile when exposed to temperatures five to seven degrees above optimum for five days, conditions that frequently occur during heatwaves. More studies on the physiological effects of warming on insects will likely yield similar effects. In effect, warming and attendant heatwaves may lead to 'developmental traps' for many ectotherms (Van Dyck et al. 2015) with significant effects on phenology, dispersal and other parameters that ultimately have broader ecological consequences (Parmesan 2006, Travis et al. 2013). Further studies are urgently required to elucidate how a combination of stresses mediated by heatwaves and other extreme climate events like droughts will affect a broader range of insect multitrophic interactions and in turn how this impacts ecological communities.

## Speculation

Most plant–insect food chains consist of at least three trophic levels (plant, herbivore, natural enemy) or even four or five with tertiary predators or hyperparasitoids (Pimm and Lawton 1978, Harvey et al. 2009b). Most predators are generalists and when prey availability changes with temperature they can easily switch to feed on alternate prey. By contrast, most parasitoids are specialists that attack discrete host stages (e.g. eggs, larvae or pupae) that are only suitable for a few days at most. Consequently, in parasitoids we argue that exposure to longer or more severe heat waves, in addition to generating often lethal physiological stresses, will increase selective pressures on males to find mates and females to find suitable hosts that may be available for shorter periods of time. This may act to reduce the intensity of larval competition among different parasitoids within the same guild unless different

females find hosts, but at the same time increase pressure on female parasitoids to find hosts. Furthermore, increased exposure to heatwaves will generate a suite of ecophysiological stresses at different stages in the life cycles of insects at all trophic levels (Abarca et al. 2019). This in turn will affect all kinds of resource–consumer interactions up the food chain that may be amplified from the bottom–up as the abundance or availability of food or hosts decreases (Voigt et al. 2003). Given that parasitoids are important members of most terrestrial food chains, we believe that the broader implications of heatwaves, droughts and other processes associated with climate warming will be to weaken the strength of top–down interactions and thus negatively impact food webs and ecological communities at larger scales (Carnicer et al. 2011).

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Supplementary material (available online as Appendix oik-06538 at <[www.oikosjournal.org/appendix/oik-06538](http://www.oikosjournal.org/appendix/oik-06538)>). Appendix 1.