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Differential effects of climate warming on reproduction and functional responses on insects in the fourth trophic level

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Abstract

1. Understanding the effects of anthropogenic global warming (AGW) on species interactions is essential for predicting community responses to climate change. However, while effects of AGW on resource–consumer interactions at the first and second trophic level have been well studied, little is known about effects on interactions at higher trophic levels at the terminal end of food chains (e.g. in the third and fourth trophic levels).

2. Here, we examined the effects of temperature variability by simulating heatwaves on functional responses of two species at the fourth trophic level (hyperparasitoids) that parasitize host species at the third trophic level (parasitoid cocoons).

3. We found that host cocoons developed faster under simulated heatwave conditions, decreasing the temporal window of susceptibility of the host cocoons to parasitism by the two hyperparasitoids, and consequently parasitism declined with temperature. However, the effects of a simulated heatwave markedly differed among the two hyperparasitoid species; temperature and host quality had a much stronger effect on early reproduction in the less fecund hyperparasitoid *Gelis agilis*, than in the more fecund species *Acrolyta nens*.

4. Our results suggest that exposure to heatwaves, that are expected to increase in frequency, will affect the ability of species at higher trophic levels to exploit transient resources whose suitability is temperature-dependent. In turn, the observed effects of AGW on the functional responses of the hyperparasitoids may disrupt trophic interactions and have profound impact on population dynamics and ecological processes.

KEYWORDS
anthropogenic global warming, functional responses, host, hyperparasitoids, parasitoid interactions, parasitoids, reproduction

1 | INTRODUCTION

Rising atmospheric concentrations of greenhouse gases (primarily CO_2) driven by a suite of anthropogenic processes has resulted in rapid warming of the planet's surface since the Industrial Revolution and particularly over the past three decades (IPCC, 2014). Furthermore, scientists argue that the frequency of extremely warm years will increase with rising greenhouse gas levels, and that the harmful effects of anthropogenic global warming (AGW) on biodiversity and ecosystems across the biosphere will therefore only escalate (IPCC, 2013). Understanding the effects of AGW and attendant short-term heatwaves on species interactions is essential for predicting community responses to climate change (Urban, 2015; Walther et al., 2002). To predict how AGW is going to affect community structure, and then to scale this up to the level of ecosystems and even biomes, it is important to investigate how changes

in temperature affect resource–consumer interactions. Species within and at different trophic levels are likely to respond differently to changes in climate (Both, van Asch, Bijlsma, van den Burg, & Visser, 2009; Davis, Lawton, Shorrocks, & Jenkinson, 1998; Watt & McFarlane, 2002). Differences in these responses are especially apparent in interactions between the first and second trophic levels, that is between plants and herbivores, especially ectotherms such as insects (Visser & Holleman, 2001). However, the outcomes of elevated concentrations of CO_2 and higher temperatures on species and species interactions across variable spatial and temporal scales are complex. For example, AGW may enhance the growth rates of plants (Myneni, Keeling, Tucker, Asrar, & Nemani, 1997), but this effect might be offset by increased drought stress (Barber, Juday, & Finney, 2000). Moreover, a combination of increased CO_2 and warmer conditions may be concomitant with higher primary production by plants but a decrease in foliar concentrations of nitrogen, leading to compensatory feeding by insect herbivores and thus more damage (Coley, 1998; O'Connor, Piehler, Leech, Anton, & Bruno, 2009). Furthermore, exposure to higher temperatures may shorten the development time of insect herbivores, thus reducing their temporal window of exposure to natural enemies such as predators and parasitoids (the "slow-growth-high-mortality hypothesis" (Clancy & Price, 1987; Benrey & Denno, 1997). By escaping from their natural enemies, we might also expect an increase in the number of pest outbreaks (Coley, 1998).

Various studies have also reported that the physiological and temporal synchrony between plants and herbivores and in turn between herbivores and their natural enemies may be disrupted by AGW (Hunter & Elkinton, 2000; Klapwijk, Grobler, Ward, Wheeler, & Lewis, 2010; Visser & Holleman, 2001). Empirical and theoretical evidence has shown that higher trophic levels are affected more by climate change than lower trophic levels (Gilman, Urban, Tewksbury, Gilchrist, & Holt, 2010) because higher trophic levels are less numerous to begin with and also depend on the ability of lower trophic levels to adapt to rapid environmental changes such as AGW. Therefore, AGW is predicted to affect ecosystem functioning by simplifying the structure of food webs and thus potentially reducing primary production (O'Connor et al., 2009).

Parasitoid wasps make model organisms for studying the effects of AGW and short-term heatwaves on resource–consumer interactions (Gillespie, Nasreen, Moffat, Clarke, & Roitberg, 2012; Stireman et al., 2005; Thomson, Macfadyen, & Hoffmann, 2010). Parasitoids are insects whose offspring develop in or on the bodies other arthropods, usually other insects, whereas the adult stage is free-living (Godfray, 1994). For parasitoids, the host represents a finite resource that is often not much larger than the attacking adult female parasitoid (Godfray, 1994). Because of this, parasitoids are under very strong selection to optimize the exploitation and allocation of these limited resources to different fitness functions such as reproduction and survival (Jervis, Ellers, & Harvey, 2008). Parasitoids are also much more specialized than most insect predators and attack not only a limited number of host species in nature but also specific host stages such as eggs, larvae or pupae (Godfray, 1994). This

makes them highly susceptible to changes in the availability of hosts caused by warming that either disrupt the temporal phenology of the host–parasitoid interaction or else reduce the temporal window of accessibility of parasitoids to specific host stages (Harvey, 2015; Jeffs & Lewis, 2013).

Idiobionts are parasitoids that attack non-growing host stages such as eggs or pupae or else which prevent further development of their host at parasitism via envenomation (Askew & Shaw, 1986). The quality of these hosts for the development of parasitoid offspring often decreases rapidly with host age making them vulnerable to parasitism for only a short period of time (Harvey, 2008). For example, studies have shown that the pupal stages of hosts remain optimal for parasitoid development for only a few days, after which host resources are inaccessible because of the differentiation and sclerotization of body tissues (Harvey, Vet, Witjes, & Bezemer, 2006; Otto & Mackauer, 1998). At higher temperatures, the increased developmental rate of hosts reduces their susceptibility to parasitism even further, increasing the pressure exerted on adult parasitoids to find and exploit them. If the temporal window of susceptibility of the host to its parasitoids is reduced beyond some critical threshold, or else the phenology of the host and its parasitoid become separated in space and time, then this may cause local populations of the parasitoids to become extinct.

The ability of parasitoids to respond to temporal changes in the accessibility of their hosts mediated by heatwaves may hinge on certain aspects of their reproductive biology that determine their functional responses (Holling, 1966). For example, species that can mature large numbers of eggs rapidly and/or sustain higher egg loads may have a competitive edge over species that mature eggs slowly and/or sustain lower egg loads because they can more quickly parasitize larger numbers of hosts over a shorter period of time. On the other hand, parasitoid species that are highly specialized may be at higher risk in a warming world than more generalized species, because the latter group has the capacity to switch to alternative hosts.

In this study, we examine the effects of different temperature regimes, including simulated heatwave conditions, on host exploitation and early reproduction (e.g. functional responses) in the idiobiont hyperparasitoids *Acrolyta nens* Hartig (Hymenoptera: Ichneumonidae) and *Gelis agilis* Fabricius (Hymenoptera: Ichneumonidae) in the fourth trophic level. We compared parasitism under conditions where temperature and host cocoon age (and thus quality) were manipulated. Early reproduction was compared in hyperparasitoids of different age and physiological status given continuous access to 25 cocoons over several days under different temperature regimes that represent low, normal and high summer day–night temperature regimes in the Netherlands based on temperatures measured since 1983 (data source from KNMI, The Netherlands). Both hyperparasitoid species attack cocoons of *Cotesia glomerata* in the Netherlands (Harvey, 2008; Harvey, Wagenaar, & Bezemer, 2009). We hypothesize that (a) exposure to high temperatures will negatively affect the efficiency with which the hyperparasitoids exploit host cocoons due to a smaller window of host susceptibility under warmer conditions, and (b) temperature will affect the reproduction of two hyperparasitoids

species differently because of their different reproductive strategies and rates of egg maturation. Furthermore, based on differences in their biology and egg maturation rates, we predict that *A. nens* will be able to parasitize most *C. glomerata* cocoons, even under less optimal conditions, whereas *G. agilis* will only be able to parasitize a small fraction, even when all conditions are very favourable.

2 | MATERIALS AND METHODS

2.1 | Insects

Insects were originally collected in fields in the vicinity of Wageningen and were maintained at 22 ± 2°C (16:8 L:D, 60 RH) at the Netherlands Institute of Ecology (NIOO). The primary endoparasitoid *C. glomerata* served as host for the hyperparasitoids *G. agilis* and *A. nens*. *C. glomerata* is a gregarious parasitoid that attacks young caterpillars of *Pieris brassicae*. *Gelis agilis* is an asexually reproducing solitary generalist species whose females are wingless and host-feed for egg production, whereas the more specialized *A. nens*, which is also solitary but winged, reproduces sexually (Harvey, 2008; Harvey, Wagenaar, & Gols, 2011). In both hyperparasitoids, host quality rapidly decreases after 60 hr in hosts reared in the laboratory at 22°C (Harvey et al., 2009, 2011). Moreover, *A. nens* and *G. agilis* exhibit quite differing reproductive and host utilization strategies. Both species emerge as adults with no mature eggs, but adult female *G. agilis* obligatorily feed on host haemolymph for egg production and mature eggs only in very small numbers (e.g. a maximum egg load of 2–4). However, females can produce these eggs within 2–3 days of host-feeding. By contrast, *A. nens* does not host-feed and matures eggs in larger numbers (e.g. a maximum egg load of 40–50 that is reached 4–6 days after eclosion). *Cotesia glomerata* produces broods of up to 50 cocoons that represent a transient, clustered resource. *Cotesia glomerata* was reared on first- or second-instar larvae of *P. brassicae*, which were obtained from the general insect rearing at Wageningen University, the Netherlands. The larvae of *C. glomerata* develop inside host larvae for about two weeks depending on the temperature, before they emerge from their host and spin cocoons (Harvey et al., 2011). Parasitized *P. brassicae* hosts were maintained in rearing cages (100 × 60 × 60 cm) and were provided with Brussels sprout plants (*Brassica oleracea* var. gemmifera, cv. Cyrus) as food until the parasitoid larvae egressed from their hosts and pupated. At this point, the cocoon clusters were collected and presented to either *G. agilis* or *A. nens* for at least 8 hr in cages (35 × 35 × 35 cm) in which they were provided with honey and water. Following exposure to the hyperparasitoids, the cocoons were collected and transferred to Petri dishes (ø = 10 cm) until eclosion of either *C. glomerata* or the hyperparasitoids. Newly emerged hyperparasitoids were then used for the experiments (see below) or rearing.

2.2 | Experimental design

To investigate the effect of temperature variability on reproduction of the two hyperparasitoids, we exposed host cocoons to the hyperparasitoids under different temperature regimes using incubators

(ECD01, Snijders Labs, The Netherlands). The incubators were set at day/night temperature regimes of either 20/12°C, 25/17°C or 30/22°C, respectively, and a photoperiod of 16:8 hr L:D. 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 (https://www.knmi.nl).

2.2.1 | Experiment 1: Effects of temperature regimes on survival and development of *C. glomerata* cocoons

To determine whether the temperature regimes affected survival and development time of host cocoons, five unparasitized cocoons of *C. glomerata* were placed in a small Petri dish (ø = 5.5 cm) and were maintained in the incubators set at the temperature regimes described above (*n* = 10 dishes per temperature regime). Petri dishes were monitored for wasp emergence daily, and the number and sex of emerged parasitoids were recorded.

2.2.2 | Experiment 2: Effects of temperature regimes on longevity of hyperparasitoids

To investigate whether the different temperature regimes influenced longevity of the two hyperparasitoids, newly emerged females obtained from the culture were placed in Petri dishes (ø = 10 cm, one female per dish) and were maintained in the incubators set at the same temperature regimes as described above. This was replicated five times for each species at each temperature regime. Each group of females was maintained at the same temperature regime when the temperature regimes were switched among three incubators (see Experiment 3). The females were provided with water and honey, which was replaced every 2 days until the wasps died. Longevity of the females since adult eclosion was recorded in days.

2.2.3 | Experiment 3: Effects of temperature regimes on reproduction of hyperparasitoids

The age of the *C. glomerata* cocoons affects their quality as hosts for the hyperparasitoid, that is it declines with cocoon age (Figure 1; Harvey et al., 2011). Therefore, we used two cocoon age classes: 12 hr and 60 hr since cocoon formation. Twenty-five cocoons per age class were gently separated using forceps and placed in a Petri dish (ø = 10 cm). Females of both hyperparasitoid species enclose as adults with no mature eggs yet (Harvey et al., 2009). In addition, *G. agilis* needs to host-feed for egg development (Harvey, 2008). In both species, maximum egg loads are attained after 4–6 days, but maximum egg loads are significantly lower in *G. agilis* (four eggs on average) than in *A. nens* (41 eggs on average) (Harvey, 2008; Harvey et al., 2009). We included the physiological status of the hyperparasitoids as an additional factor. We used newly emerged and 4-day-old females of both hyperparasitoid species, and the four-day-old *G. agilis* females were either provided with cocoons of *C. glomerata* for host-feeding 48 hr prior to initiation of the temperature exposure
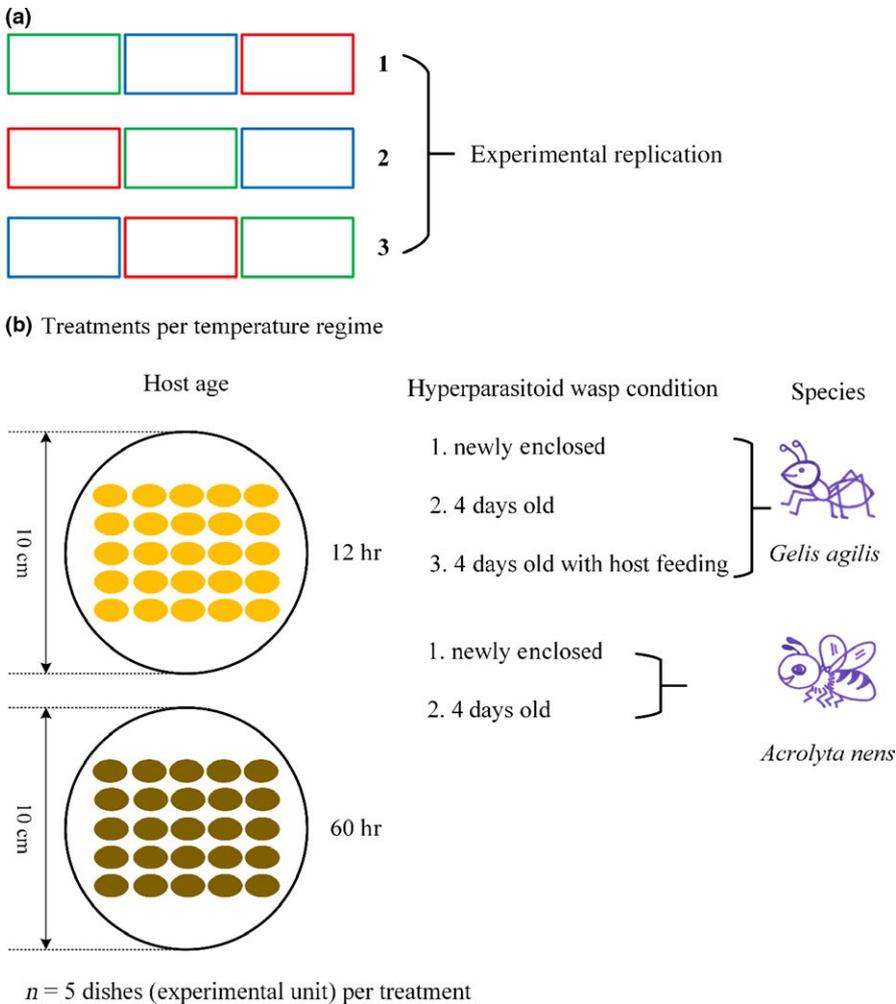
(a)

(b) Treatments per temperature regime

$n = 5$ dishes (experimental unit) per treatment

FIGURE 1 Design of Experiment 3. (a) Three incubators were set at different day–night temperature regimes (green, blue and red represent 20–12, 25–17 and 30–22°C, respectively) that were switched among incubators in the three replicated experiments. (b) Petri dishes each with 25 cocoons and a single hyperparasitoid female (*Acrolyta nens* or *Gelis agilis*) were placed in these incubators. The host cocoons were either 12 or 60 hr old. Hyperparasitoid females were either newly enclosed, 4 days old or host fed (*G. agilis* only). Petri dishes served as experiment unit with five replicates per treatment in each of the three replicated experiments

treatment or not. Single females were placed in Petri dishes with 25 cocoons of either age class. Until this point, all insects were maintained in a climate room under the general rearing conditions: 22 ± 2°C, photoperiod 16:8 hr L:D. Subsequently, the Petri dishes were exposed to one of the three temperature regimes. The hyperparasitoid females were provided with water and honey which was replaced daily. After a week, the hyperparasitoid females were removed from the Petri dishes and from here on the dishes were checked daily for wasp emergence. The fate of the cocoons was recorded as (a) *G. agilis* or *A. nens*, (b) *C. glomerata*, (c) unknown death. Each treatment (two host conditions (12 hr old or 60 hr old) and two or three hyperparasitoid conditions (newly enclosed, 4 days old, or host fed only in *G. agilis*)) was replicated five times at each temperature regime (150 dishes in total). The experiment was repeated three times where temperature regimes were switched among the incubators. A total of 270 females of *G. agilis* and 180 females of *A. nens* were used in the experiments.

2.3 | Statistical analyses

Data on survival of *C. glomerata* cocoons that had not been exposed to hyperparasitoids were analysed using a generalized linear model (GLM) with a binomial distribution and logit link function and temperature

regime as a fixed factor. Data on development time of *C. glomerata* from pupa to adult were analysed using a two-way ANOVA with temperature regime, parasitoid sex and their interaction as explanatory variables. Data on longevity of the hyperparasitoids were analysed using a one-way ANOVA with temperature regime as the explanatory variable. Data were analysed separately for the two hyperparasitoid species. When the effects of temperature regime were significant, means were compared pairwise using Tukey HSD tests.

A generalized linear mixed model (GLMM) with a binomial distribution and logit link function was used to analyse the effect of (a) the temperature regime, (b) the physiological condition of the hyperparasitoids and (c) the age of the *C. glomerata* host cocoons on the emergence of *A. nens* or *G. agilis*. A dish with 25 cocoons was the experimental unit, and the proportion of *C. glomerata* cocoons out of 25 developing into adult hyperparasitoid was the response variable in the analysis. The temperature treatments (three levels), the physiological condition of the hyperparasitoids (newly enclosed, 4 days old or host fed only in *G. agilis*) and the age of the *C. glomerata* host cocoons (12 hr old or 60 hr old), as well as their interactions, were included as explanatory variables. The replicates (dishes within an incubator) and incubator (1–3) were entered as random factors in the GLMM. Multiple comparison tests were conducted based on "lsmeans function" when any of the main effects or their interaction terms were significant. When

FIGURE 2 Mean (± *SE*) survival of cocoons (a) and development time from pupa to adult (b) of *Cotesia glomerata* when exposed to three different day–night temperature regimes (low, medium and high represent 20–12, 25–17 and 30–22°C, respectively). In (b), development time is given for females (black circles) and males (open circles). Different letters indicate significant differences (*p* < 0.5) between the three temperature treatments for development time of *C. glomerata*
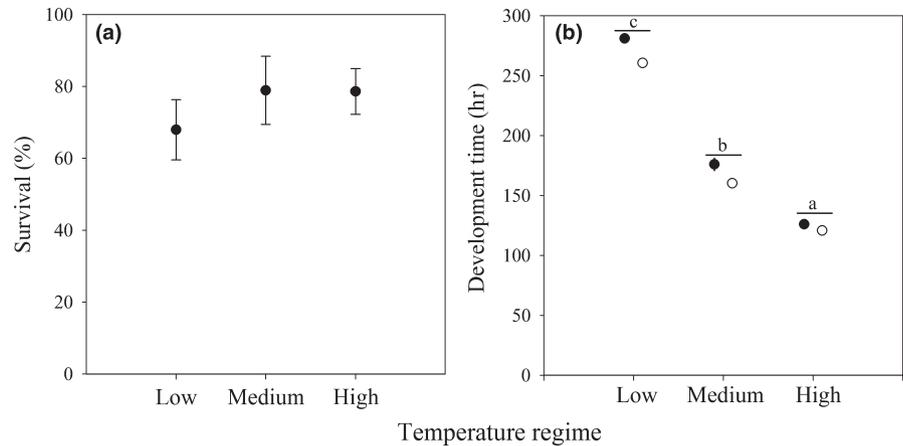
effects of hyperparasitoid condition for *G. agilis* were significant, three contrasts were analysed (newly enclosed vs. 4 days old, 4 days old vs. 4 days old with host-feeding and newly enclosed vs. 4 days old with host-feeding) to test whether the effects were significantly different between the three hyperparasitoid conditions. All analyses were done in R 3.4.0 (R Development Core Team, 2017)

3 | RESULTS

3.1 | Experiment 1: Effect of temperature regime on unparasitized *C. glomerata* cocoons

The survival of unparasitized *C. glomerata* cocoons was not affected by temperature regime (χ^2 = 1.45; *df* = 2, *p* = 0.48, Figure 2a). On average, 75% of the cocoons developed into adult *C. glomerata*. However, development time of *C. glomerata* from pupa to adult was influenced by temperature regime ($F_{2,118}$ = 1851.86, *p* < 0.001). Females developed slower than males (sex: $F_{1,118}$ = 34.48; *p* < 0.001), but the interaction between temperature and sex was also significant ($F_{2,118}$ = 4.31; *p* = 0.016) (Figure 2b). With increasing temperature, development time of *C. glomerata* decreased and this effect was stronger for females than for males. *Cotesia glomerata* exposed to the high-temperature regime took only half the time to develop from pupation to adulthood compared to conspecifics exposed to the low-temperature regime.

3.2 | Experiment 2: Effect of temperature regime on longevity of the hyperparasitoids

There was a significant effect of temperature regime on longevity of *A. nens* ($F_{2,12}$ = 11.32; *p* = 0.002) and *G. agilis* ($F_{2,12}$ = 10.34; *p* = 0.002) (Figure 3). Longevity of both hyperparasitoid species decreased with increasing day/night temperature regimes, although the difference between the high- and medium-temperature treatments was not significant. Longevity of the hyperparasitoids in the low-temperature regime was more than twice as high as in the high-temperature regime.

3.3 | Experiment 3: Effect of temperature regime, host age and hyperparasitoid condition on the reproductive success of the hyperparasitoids

For the hyperparasitoid species *A. nens*, temperature regime, host age and hyperparasitoid physiological state had a significant effect on the number of offspring produced, measured as the proportion of host cocoons out of 25 producing a hyperparasitoid (Table 1). The effects of temperature on *A. nens* reproduction depended on host age and hyperparasitoid physiological condition (see significant interaction terms for temperature × hyperparasitoid condition and temperature × host age × hyperparasitoid condition, Table 1, Figure 4). Compared with the low- and medium-temperature regimes, successful hyperparasitoid development was reduced when females were

FIGURE 3 Mean longevity (± *SE*) of adult female of *Acrolyta nens* (a) and *Gelis agilis* (b) when exposed to three different day–night temperature regimes (low, medium and high represent 20–12, 25–17 and 30–22°C, respectively). Different letters indicate significant differences (*p* < 0.5) between the three temperature treatments for each species
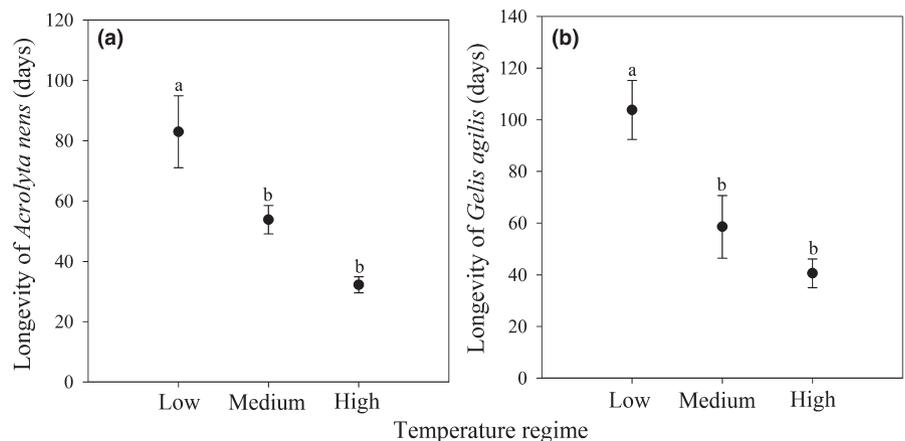
TABLE 1 Results of the statistical analyses (generalized linear mixed model) for the effects of temperature regime, host cocoon age, hyperparasitoid condition and their interactions on the proportion of host cocoons (*Cotesia glomerata*) developing into adult hyperparasitoids, *Acrolyta nens* and *Gelis agilis*

Effects	A. nens	G. agilis
Temperature	$\chi^2 = 396.69$; $df = 2$; $p < 0.001$	$\chi^2 = 66.54$; $df = 2$; $p < 0.001$
Host age	$\chi^2 = 39.17$; $df = 1$; $p < 0.001$	$\chi^2 = 41.62$; $df = 1$; $p < 0.001$
Hyper-condition	$\chi^2 = 107.21$; $df = 1$; $p < 0.001$	$\chi^2 = 39.44$; $df = 2$; $p < 0.001$
Temperature × Host-age	$\chi^2 = 3.21$; $df = 2$; $p = 0.201$	$\chi^2 = 3.68$; $df = 2$; $p = 0.159$
Temperature × Hyper-condition	$\chi^2 = 27.27$; $df = 2$; $p < 0.001$	$\chi^2 = 4.89$; $df = 4$; $p = 0.299$
Host age × Hyper-condition	$\chi^2 = 7.24$; $df = 1$; $p = 0.007$	$\chi^2 = 0.71$; $df = 2$; $p = 0.702$
Temperature × Host-age × Hyper-condition	$\chi^2 = 31.80$; $df = 2$; $p < 0.001$	$\chi^2 = 9.31$; $df = 4$; $p = 0.054$

exposed to the highest temperature regime (Figure 4). The effect of host cocoon age on *A. nens* development depended on temperature and the physiological condition of the hyperparasitoid. For newly emerged *A. nens* (Figure 4a), offspring production was significantly lower on older host cocoons when they were raised at the high-temperature regime. However, for 4-day-old *A. nens* (Figure 4b), the negative effect of host age was only significant at the low-temperature regime (Figure 4b). Overall, offspring production was higher when *A. nens* females were exposed to the different temperature regimes when they were 4 days old than when they were exposed as freshly emerged females (Figure 4).
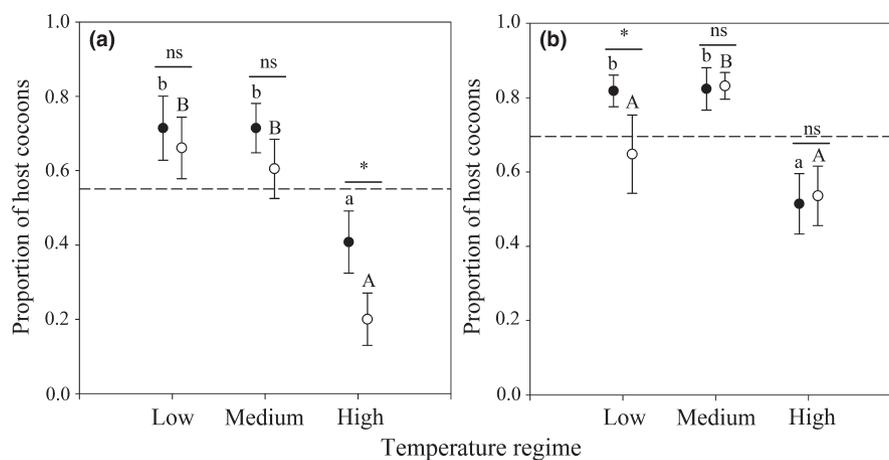
Similar to what was observed for *A. nens*, temperature regime, host age and hyperparasitoid physiological state had a significant effect on the number of offspring produced by *G. agilis*, but none of the interactions with temperature were significant (Table 1, Figure 5). The proportion of host cocoons developing into adult *G. agilis* (8.8%) was much lower than for *A. nens* (69.6%). In general, successful adult *G. agilis* emergence declined from low- to medium- to high-temperature conditions and was higher when they were provided with young than with old cocoons. The proportion of cocoons developing into adult hyperparasitoids was similar for newly emerged females and for females that were already 4 days old (*p* > 0.05, Figure 5a,b). Offspring numbers were the highest for females that were 4 days old and had been provided with hosts for host-feeding prior to the initiation of the temperature treatments (*p* < 0.05, Figure 5c).

4 | DISCUSSION

In insects, metabolic rate increases with increasing ambient temperature (Baffoe, Dalin, Nordlander, & Stenberg, 2012; Mann, Axtell, & Stinner, 1990). Pupal mortality in cocoons of *C. glomerata* was not significantly affected by temperature, whereas the duration of pupal development of *C. glomerata* in cocoons to adult eclosion decreased dramatically with an increase in temperature. *Acrolyta nens* and *G. agilis* exhibited differing functional responses to cocoon clusters under differing temperatures and physiological conditions. Both species were significantly less successful in developing in *C. glomerata* cocoons under simulated heatwave conditions than under summer ambient and below-average conditions representative of Dutch summers. The effect of temperature on parasitism success, however, differed markedly between the two hyperparasitoids and depended on their physiological condition (age, egg load, prior host-feeding experience in

FIGURE 4 Mean proportion (± *SE*) of host cocoons (*Cotesia glomerata*) developing into adult *Acrolyta nens* under three different day–night temperature regimes (low, medium and high represent 20–12, 25–17 and 30–22°C, respectively). Individual hyperparasitoid females had been given access to 25 cocoons for 7 days as newly emerged adults (a) or when they were already 4 days old (b). Host cocoons were either 12 (black circles) or 60 (white circles) hour old when *A. nens* females were introduced. Asterisks and "ns" indicate significant and non-significant differences, respectively, between host age classes within temperature regimes. Different small or capital letters indicate significant differences between temperature regimes for each host age class. Dashed lines represent the grand means of all treatments within a panel

FIGURE 5 Mean proportion (± *SE*) of host cocoons (*Cotesia glomerata*) developing into adult *Gelis agilis* under three different day–night temperature regimes (low, medium and high represent 20–12, 25–17 and 30–22°C, respectively). Individual hyperparasitoid females had been given access to 25 cocoons for 7 days as newly emerged adults (a), 4-day-old adults without host cocoons (b) or 4-day-old adults with host cocoons before onset of the experiment (c). Host cocoons were either 12 (black circles) or 60 (white circles) h old when *G. agilis* females were introduced. Asterisk and "ns" indicate significant (*p* < 0.05) or non-significant differences, respectively, between host-age classes within temperature regime means. Different small or capital letters indicate significant differences between temperature regimes for each host age class. Dashed line represents the grand means of all treatments within a panel
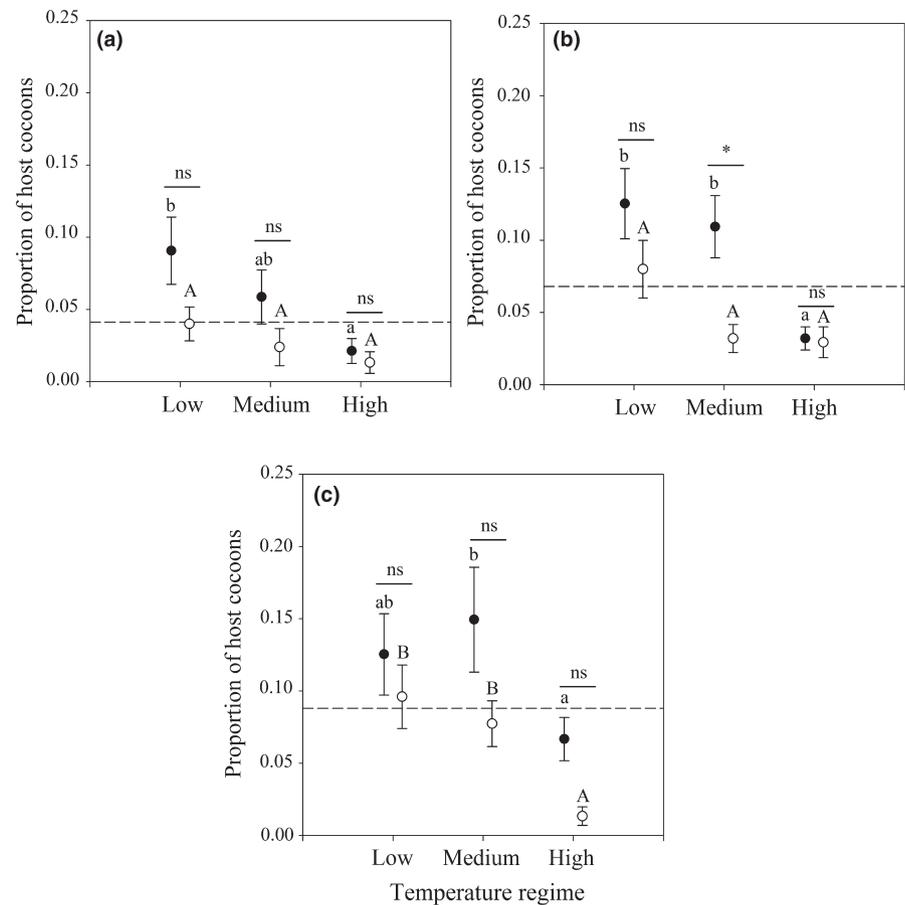
G. agilis). *Gelis agilis*, which matures small numbers of eggs, was clearly more constrained in its functional response at the higher temperature than the more specialized *A. nens*, which matures a larger number of eggs. In pupal parasitoids, including hyperparasitoids, host quality has been shown to decline with host age because host tissues begin to differentiate in pupae and are no longer suitable resources for parasitoid development (Harvey, van Dam, & Gols, 2003; Harvey et al., 2011). The faster development of host cocoons under simulated heatwave conditions therefore decreased the temporal window of susceptibility of the host cocoons to parasitism by the two hyperparasitoids.

Newly emerged females of both species emerge with no ripe eggs and therefore must wait for eggs to mature before they can oviposit (Harvey, 2008; Harvey et al., 2009). *Acrolyta nens* is able to mobilize stored fats and proteins that are carried over from larval development, producing a maximum egg load of 40–50 about 4–6 days after eclosion at the mid-temperature regime (Harvey et al., 2009). *Gelis agilis* females also emerge with no eggs, but, unlike *A. nens*, they can only mobilize proteins for egg production by mutilating (and subsequently killing) some hosts with their ovipositor and drinking haemolymph which seeps from the wound, a process known as "host-feeding" (Jervis & Kidd, 1986). This process limits their egg production. Moreover, *G. agilis* females are only able to produce around 50 or fewer progeny in their lifetimes (Harvey, 2008). *Gelis* species produce very large yolky "anhydropic" eggs (Jervis & Kidd, 1986) in only tiny numbers; *G. agilis* can only carry a maximum of 2–4 eggs at a given time, and new eggs are matured at a rate of

only around the same number per day (Harvey, 2008). This greatly constrains their ability to respond to large clusters of *C. glomerata* cocoons, even under cooler temperatures that prolong the suitability of *C. glomerata* pre-pupae and pupae as hosts. Indeed, even under the most favourable conditions of temperature, host age and physiological state, *G. agilis* only parasitized a small proportion of *C. glomerata* cocoons (e.g. around 15% of those in the cluster of 25).

Acrolyta nens was able to respond to temperature-mediated changes in host quality much more effectively than *G. agilis* and was able to parasitize a much higher proportion of host cocoons irrespective of age, physiological state and temperature than *G. agilis* was under any conditions. Even newly enclosed *A. nens* females with no mature eggs were able to successfully parasitize 20 per cent of older host cocoons at the highest temperature. Under low- and medium-temperature regimes, *A. nens* typically parasitized over 70% of the 25 hosts in a cocoon cluster, and only under the simulated heatwave conditions did this drop appreciably. However, older females with fully mature egg loads responded to both younger and older hosts equally well under the highest temperatures.

Our results therefore reveal that *A. nens* is clearly better able to respond to age- and temperature-related differences in host quality and availability than *G. agilis*. *Acrolyta nens* is a largely specialized hyperparasitoid of *Cotesia* species, whereas *G. agilis* and other gelines possess very broad host ranges. For instance, they are known to parasitize hosts as phylogenetically unrelated as spider egg sacs and moth cocoons in addition to parasitoid cocoons (Cobb & Cobb, 2004; Toth &

Lukáš, 2005). Furthermore, a mature egg load of *A. nens* approximates a typical brood (cocoon cluster) size in *C. glomerata* (Gu, Wang, & Dorn, 2003). In heatwaves, we would expect specialists like *A. nens* to exhibit functional responses that enable them to exploit their hosts more effectively than generalists such as *G. agilis*. However, this may not necessarily be the case, but is simply an example of the latter species "losing the battle but winning the war." A great advantage of generalism in a warming world and under heatwaves is that generalists have the capacity to switch to alternative hosts. Thus, whereas specialized species like *A. nens* are evolutionarily obligated to find a narrow range of host species, generalists like *G. agilis* can exploit a wide range of hosts in their habitat. However, this advantage is countered by the fact that many gelines lack wings and thus are able to forage only over a very limited spatial area compared with winged species like *A. nens*. How differences in these traits play out in a warming world is hard to gauge. There are costs and benefits of specialism and generalism that may be trait-dependent, but this far this area has been little studied.

In insects, AGW may have negative consequences for several life-history traits (e.g. smaller body size, reduced longevity) but positive effects on other traits (e.g. through reduced development time) (Hance, van Baaren, Vernon, & Boivin, 2007) that affect reproduction and hence fitness. How these differing and even opposite effects will affect the demographics and survival of insects in nature is very difficult to predict. It is possible that the costs of smaller body size and reduced longevity on fecundity under warmer conditions, and especially mediated by short-term heatwaves, may be balanced against the benefits of faster development time that in turn might lead to more generations over the course of a year. On the other hand, increasing temperatures may tend to reduce the difference in development time between male and female of *C. glomerata*. This may have consequences for local mating that in *C. glomerata*, whereby males often emerge first and wait for their siblings to emerge and then mate with them (Elias, Dorn, & Mazzi, 2010). If the time differential between the eclosion of males and females is reduced under warmer conditions, then this might have consequences for sib-mating. Parasitoids are haplo-diploid, whereby unfertilized eggs produce males and fertilized eggs produce females (Heimpel & de Boer, 2008). Many species, including *C. glomerata*, exhibit single-locus complementary sex determination, where inbreeding can lead to complementary alleles producing diploid males that are often sterile. A reduction in local mating opportunities because of warming thereby confers both fitness costs and benefits to *C. glomerata*. If non-sibs are hard to find, then mating opportunities may decrease; on the other hand, less sib-mating means more outcrossing and the production of less diploid males.

A major concern of climate warming on tri-trophic interactions involving plants, herbivores and parasitoids is that climatic variability will hinder the ability of parasitoids to track host populations (Davis et al., 1998; Hance et al., 2007; Jeffs & Lewis, 2013; Stireman et al., 2005). These studies, however, generally pay less attention to traits, such as development and reproduction, in the insects that should be considered when exploring phenology-based disruptions caused by warming on multitrophic interactions, and instead focus on broader phenological processes. Phenology involves not only understanding whether warming can lead to asynchrony in the life cycles of hosts and their parasitoids, but also what ecophysiological mechanisms might drive this asynchrony. As we have shown, heatwave conditions can reduce the temporal window of host suitability, putting more pressure on parasitoids to find hosts over a limited time. Furthermore, even when they find aggregated hosts their ability to parasitize them may be constrained by their reproductive biology. Parasitoids that produce large eggs in small numbers and that take a long time to lay individual eggs may be much more vulnerable than parasitoids that produce small eggs in large numbers which they can lay rapidly (Jervis et al., 2008).

Species in the fourth trophic level, such as hyperparasitoids, may be particularly vulnerable to rapid warming, because their survival and persistence depend on the presence of several trophic levels beneath them. However, warmer conditions may also mean more generations per year in multivoltine insects leading to the risk of herbivores emerging when suitable food plants or hosts are scarce or unavailable. This will clearly have a knock-on effect on higher trophic levels up the food chain. Moreover, species which are dependent on resources that are available for only a very short period of time may be seriously affected by warming. For example, species that exploit resources of a very specific age (e.g. newly opening plant shoots, unripe siliques) may be unable to persist whether warming and localized heatwaves lead to desynchronization with their resources. This example of "phenological mismatching" has been shown in a diverse range of plant–herbivore and predator–prey interactions as well as interactions over three trophic levels (e.g. plant–herbivore–predator) leading to rapid declines in the abundance of both the predator and its prey (bottom-up effects) (Both et al., 2009; Visser & Holleman, 2001; Watt & McFarlane, 2002). Thus, climate warming can negatively affect both intrinsic (species level) and extrinsic (trophic and community level) parameters over variable scales.

In summary, our result shows that exposure to variable temperature regimes, including those occurring under simulated heatwave conditions, affects the development rate of a primary parasitoid wasp during the pupal stage. In turn, this alters the temporal "window of suitability" of the host for two of its secondary hyperparasitoids. The functional responses of these two hyperparasitoid species in exploiting hosts depend not only on temperature, but on other factors including egg maturation rate, egg load dynamics, host-feeding behaviour and host age. Previous studies have shown that various trophic levels often respond differently to climate change (MacLennan, Arnott, & Strecker, 2012; Van der Putten, Macel, & Visser, 2010; Thackeray et al., 2010; Voigt et al., 2003). The functioning and stability of ecosystems often depend on the structure of food webs embedded in them (Petchey, McPhearson, Casey, & Morin, 1999; Vinebrooke et al., 2003). We have shown that variable temperatures affect the functional responses of two hyperparasitoid species that may in turn disrupt trophic interactions and have a profound impact on their population dynamics. Exposure to higher temperatures may disrupt the developmental synchrony between parasitoids and hyperparasitoids, potentially leading to changes in the structure of food chains in which they are embedded. Overall, these results emphasize the

importance of incorporating higher trophic levels in future studies projecting the effects of climate change and the ecological consequences of elevated temperatures.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

C.C. and J.A.H. conceived the ideas and designed the study; C.C. conducted the experiment and collected the data; C.C. carried out the statistical analyses with helps of R.G. and A.B.; C.C., R.G. and J.A.H. wrote the manuscript. All authors contributed to the various drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.dg124q0 (Chen, Gols, Biere, & Harvey, 2019).

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