

Non-additive effects of simulated heat waves and predators on prey phenotype and transgenerational phenotypic plasticity

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Abstract

Understanding the effects of extreme climatic events on species and their interactions is of paramount importance for predicting and mitigating the impacts of climate change on communities and ecosystems. However, the joint effects of extreme climatic events and species interactions on the behaviour and phenotype of organisms remain poorly understood, leaving a substantial gap in our knowledge on the impacts of climatic change on ecological communities. Using an aphid–ladybeetle system, we experimentally investigated the effects of predators and heat shocks on prey body size, microhabitat use, and transgenerational phenotypic plasticity (i.e., the asexual production of winged offspring by unwinged mothers). We found that (i) aphids were smaller in the presence of predators but larger when exposed to frequent heat shocks; (ii) frequent heat shocks shifted aphid distribution towards the plant's apex, but the presence of predators had the opposite effect and dampened the heat-shock effects; and (iii) aphids responded to predators by producing winged offspring, but heat shocks strongly inhibited this transgenerational response to predation. Overall, our experimental results show that heat shocks inhibit phenotypic and behavioural responses to predation (and vice versa) and that such changes may alter trophic interactions, and have important consequences on the dynamics and stability of ecological communities. We conclude that the effects of extreme climatic events on the phenotype and behaviour of interacting species should be considered to understand the effects of climate change on species interactions and communities.

KEYWORDS

aphid, body size, climate change, extreme temperatures, heat stress, ladybeetle, phenotypic plasticity, spatial distribution, trophic interactions, wing polyphenism

1 | INTRODUCTION

Increases in climate variability and extreme climatic events are currently emerging as two of the most important facets of climate change (Easterling et al., 2000; IPCC, 2013; Thompson, Beardall, Beringer, Grace, & Sardina, 2013). Yet, previous ecological studies have largely focused on how changes in mean temperature affect

populations and community structure while neglecting variability and extreme events (reviewed in Easterling et al., 2000; Parmesan, Root, & Willig, 2000; Thompson et al., 2013). Extreme hot temperatures are fundamentally different from mild warming because they place organisms outside their thermal threshold for growth and reproduction, thereby drastically reducing their development rate, fecundity, and survival (Colinet, Sinclair, Vernon, & Renault, 2015; Dowd, King,

& Denny, 2015; Gillespie, Nasreen, Moffat, Clarke, & Roitberg, 2012; Huey et al., 2012; Magozzi & Calosi, 2015; Sentis, Hempinnee, & Brodeur, 2013). As a consequence, the detrimental effects of extreme temperatures on individual fitness and population dynamics are, in many cases, more important than what would be expected from shifts in average temperatures (Benedetti-Cecchi, Bertocci, Vaselli, & Maggi, 2006; Dowd et al., 2015; Easterling et al., 2000; Ma, Rudolf, & Ma, 2015; Thompson et al., 2013; Vasseur et al., 2014). Thus, investigations on how changes in the frequency and amplitude of extreme climate events influence organisms and their interactions are much needed to improve our understanding of climate change impacts on ecological communities.

Phenotypic plasticity constitutes a common response to environmental stress and can modulate an individual's physiology, morphology, and behaviour to cope with the change (Chevin, Lande, & Mace, 2010; Donelson, Munday, McCormick, & Pitcher, 2011; Huey et al., 2012; Sentis, Morisson, & Boukal, 2015). These phenotypic responses influence species interactions (Sentis et al., 2015) as well as the speed and direction of evolutionary trajectories (Chevin et al., 2010; Donelson et al., 2011; Ghalambor et al., 2015; Munday, Warner, Monro, Pandolfi, & Marshall, 2013); they are thus of paramount importance for the dynamics and persistence of communities facing rapid environmental changes. However, very few studies have investigated phenotypic responses to extreme temperatures (Colinet et al., 2015). Recent studies have proposed that average warming and extreme temperatures should induce divergent and sometimes opposite phenotypic responses depending on the degree of warming relative to the shape and position of the organism's thermal performance curve (Dowd et al., 2015; Gardner, Peters, Kearney, Joseph, & Heinsohn, 2011). For instance, many ectotherm species are predicted to become smaller with mild warming (Daufresne, Lengfellner, & Sommer, 2009; Sheridan & Bickford, 2011) but would become larger with heat waves as larger organisms are more tolerant to heat and desiccation than smaller ones (Baudier, Mudd, Erickson, & O'Donnell, 2015; Gardner et al., 2011; Kaspari, Clay, Lucas, Yanoviak, & Kay, 2015). In addition to adaptive responses, extreme temperatures can disrupt homeostasis and cellular functioning, which may cause plastic responses to be amplified or inhibited. We thus predict that phenotypic and behavioural responses to extreme temperatures will depend on the frequency and amplitude of heat waves that determine both an organism's physiological state (Dowd et al., 2015; Sentis et al., 2013) and its ability to display adaptive responses to environmental factors (Chevin et al., 2010).

Previous studies have highlighted the importance of including biotic interactions in models and experiments to predict the effects of increasing mean temperatures on a species' abundance and distribution (Petchey, McPhearson, Casey, & Morin, 1999; Sentis, Hempinnee, & Brodeur, 2012; Stenseth et al., 2002; Suttle, Thomsen, & Power, 2007; Tylianakis, Didham, Bascompte, & Wardle, 2008; Van der Putten, Macel, & Visser, 2010). Indeed, biotic interactions influence the phenotype, behaviour, and spatio-temporal distribution of interacting species to such an extent that it can modulate the effects of temperature on organisms. These responses, generally referred to

as trait-mediated or non-consumptive effects (Abrams, 2007; Werner & Peacor, 2003), can involve modifications of behaviour (e.g., habitat shift, reduction in activity, altered feeding rate), life history traits (e.g., achieving maturity at a different time and/or size), morphology (e.g., defensive structure, colour, shape), or a combination of any of these traits (Boersma, Spaak, & De Meester, 1998; Dixon & Agarwala, 1999; Hammill, Rogers, & Beckerman, 2008; Pigliucci, 2003; Spitze, 1992; Tollrian, Duggen, Weiss, Laforsch, & Kopp, 2015; Tollrian & Harvell, 1999; Touchon & Warkentin, 2008). In some cases, phenotypic responses to predation can be transgenerational, whereby offspring have an altered trait or a distinct alternate phenotype that reduces predation risk (Spitze, 1992). Such transgenerational responses have been well described for *Daphnia* and aphids that, when exposed to predator or parasite cues, produce offspring developing defensive crests and spines in the former or wings and larger embryos in the latter (Dixon & Agarwala, 1999; Kaiser & Heimpel, 2016; Spitze, 1992). These non-consumptive effects can have strong impacts on the dynamics of interacting species and communities only by influencing the behaviour and phenotype of the prey (Agrawal, 2001; Suraci, Clinchy, Dill, Roberts, & Zanette, 2016). However, very little information exists on how the frequency and intensity of heat waves may affect prey phenotypic and behavioural responses to natural enemies.

While there is a growing interest in studying the impacts of extreme climatic events on organisms and their interactions, the combined effects of extreme temperature and species interactions on prey phenotypic and behaviour remain largely unexplored. In a previous experimental study on trophic interactions and population dynamics in a simple plant–aphid–ladybeetle food chain (Sentis et al., 2013), we found that (i) heat shocks did not affect plant biomass and (ii) ladybeetle and heat shocks significantly decreased aphid density, but (iii) food chains including predatory ladybeetles were more resistant to heat shocks than a simple plant–aphid association, with aphid abundance being less influenced by heat shocks in the presence of ladybeetle (Sentis et al., 2013). In this study, we used non-published data from the same experiment to investigate the effects of predatory ladybeetles and heat shock frequency and amplitude on aphid body size, microhabitat shift (i.e., changes in its spatial location on the host plant), and transgenerational phenotypic plasticity (i.e., the asexual production of winged offspring by unwinged mothers). Our study reveals the complexity of animal responses to multiple stressors and emphasizes the importance of considering these responses to better understand the effects of rapid environmental changes on the dynamics and evolution of ecological communities.

2 | MATERIALS AND METHODS

2.1 | Biological system

The experimental food chain consisted of the predatory ladybeetle *Coleomegilla maculata lengi* Timberlake (Coleoptera: Coccinellidae), the green peach aphid *Myzus persicae* Sulzer (Homoptera: Aphididae), and the sweet pepper *Capsicum annuum* L. cv. Bell Boy.

Approximately 4,000 adult *C. maculata* were collected in October 2009 in a field at Saint-Mathieu-de-Beloeil (45°35'N, 74°45'W, Québec, Canada), brought to the laboratory, reared in mesh cages (60 × 40 × 40 cm), and fed pollen and *M. persicae*. A colony of *M. persicae*, established from an individual collected in greenhouses from Agriculture and Agri-Food Canada (St-Jean-sur-Richelieu, Québec, Canada) was maintained on sweet pepper grown from seed. All insects and plants were maintained in growth chambers (Conviron® E15) at 23 ± 1°C, 50%–60% relative humidity, and under a 16L:8D photoperiod at a light intensity of 150 μmol m⁻² s⁻¹.

2.2 | Experimental design

A full factorial experiment was set up to measure the effects of heat shocks (five temperature regimes described below) and predators (presence or absence) on the distribution of aphids on their host plant, aphid phenotype (winged/unwinged), and body mass. Aphids were submitted to five temperature regimes for 10 days: (i) a constant temperature of 23°C, (ii) one heat wave of 30°C twice a week (with 2 days at constant 23°C between the heat shocks), (iii) one heat wave of 30°C per day, (iv) one heat wave of 40°C twice a week (with 2 days at constant 23°C between the heat shocks), and (v) one heat wave of 40°C per day (see Table S1 for more details). Thirteen replicates per temperature regime—predation treatment—were performed. Temperature was kept constant at 23°C on days with no heat wave, and each temperature regime was designed so that the daily average temperature over the duration of the experiment was 23°C.

Four-week-old pepper plants with four unfurled leaves were placed individually in plastic cylinders (ø: 20 cm; h: 45 cm). The top of the cylinder and the two lateral openings were covered with mesh muslin for ventilation. During the experiment, pepper plants were fertilized every 3 days with Nitrophoska (12-4-14) at a nitrogen concentration of 100 ppm (Plant-Prod®, Montréal, Canada). At the onset of the experiment, four parthenogenetic adult female *M. persicae* obtained from synchronous cohorts were transferred to the unfurling young leaves at the apex of each plant using a fine paintbrush and allowed to acclimatize and reproduce for 24 hr. For the predation treatment, one newly hatched first instar *C. maculata* larva was introduced at the bottom of each plant stem with its head oriented upwards. After 10 days, the number of aphids per plant, aphid phenotype (winged/unwinged), and aphid distribution on the plant (number of aphids per leaf) were recorded. We then randomly sampled 15 unwinged adult aphids from each experimental cylinder and weighed them together to the nearest microgram (Mettler Toledo MT5 microbalance; Mettler-Toledo, Inc., Mississauga, Ontario, Canada). In *M. persicae*, nymphs, winged and apterous phenotypes can be distinguished by their colour: winged nymphs are pink, whereas unwinged nymphs are green (Blackman & Eastop, 2000). Moreover, we conducted a preliminary experiment to confirm that nymph colour is an accurate predictor of winged/unwinged phenotypes: we isolated ca. 20 nymphs (from all instars) of each colour (green or pink) and reared them until they became adults and then

recorded their phenotype. Pink and green nymphs always produced winged and unwinged adults, respectively. During the experiments, the temperature and humidity were recorded continuously using Hobo U12 (Hobo®) units.

Because of logistical constraints, only two controlled-temperature cabinets were available to house the experiment. We therefore set up an experimental design that minimized potential pseudoreplication. At the beginning of the experiment, two temperature regimes were randomly selected and each was assigned to a growth chamber. Six or seven replicates of the treatments were assigned to each cabinet, where they were arranged in three blocks to control for potential environmental heterogeneity within the chambers. At the end of this first batch, two new temperature regimes were again randomly selected and the whole process was repeated until the 13 replicates were repeated for each treatment. We avoided running the two repetitions involving a particular temperature regime at the same time or in the same growth chamber to ensure that differences between temperature regimes would not confound an effect of experimental date and/or growth chamber.

2.3 | Statistical analyses

First, we analysed the effects of predators, temperature regimes, and their interactions on the fresh mass of aphids with an LMM (linear mixed model), the proportion of winged aphids (adults + nymphs) with a binomial GLMM (generalized linear mixed model), and the proportion of aphids located on the plant's apical region (the apex + the first two leaves) with a binomial GLMM. We did not take into account possible correlations among the three response variables as we are not aware of a statistical analysis that would allow testing the effects of multiple explanatory variables (and their interactions) on multiple response variables (with different distribution families) and that would also take into account random effects. A Brown–Forsythe test was used to compare the variances of aphid mass between treatments. In a previous study focusing on the effects of heat shocks on the abundance and biomass of the plant, the aphid, and the ladybeetle (Sentis et al., 2013), we found that heat shocks did not affect plant biomass, whereas aphid density was influenced by both heat shocks and predation (Appendix S1 and Fig. S1). Therefore, we did not include plant biomass in our analyses but included aphid density as an explanatory variable in the models of this study to account for the density-mediated effects of heat shocks and predation on the three dependent variables. Blocks nested within growth chambers were included in the models as random factors nested within temperature regimes (Sentis et al., 2013).

Secondly, when the temperature regimes or the interactions with the temperature regimes had a significant effect on the dependent variable, the regime with constant temperature was not taken into consideration and mixed models (LMM for aphid weight and binomial GLMMs for proportions) were used to investigate the effects of predators, heat-wave amplitude, heat-wave frequency, and their interactions. Likelihood ratio tests were used to assess the significance of the fixed model terms, and post-hoc Tukey tests were used

to determine significant differences among means. For the proportion of aphids at the plant's apex, we detected model overdispersion that was corrected by including the experimental units (i.e., plastic cylinders) nested within blocks as individual-level random effects (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). For the proportion of winged aphids, the GLMM failed to converge, probably because of the very low number of winged aphids at the 40°C daily temperature regime (see below). We thus performed a transformation (arcsine square root) on the proportion of winged aphids and analysed the effects of predators, temperature regimes, and their interactions using an LMM. LMM and GLMMs were fitted using the LME4 package (Bates, Mächler, Bolker, & Walker, 2015) in R 3.1.1 (R Development Core Team, 2013).

3 | RESULTS

3.1 | Aphid body mass

Aphid body mass was significantly affected by the temperature regime ($\chi^2 = 14.08$; $df = 4$; $p = .007$; Figure 1). The presence of predators led to a decrease in aphid body mass ($\chi^2 = 42.18$; $df = 1$; $p < .0001$), and this effect was independent of the temperature regime (interaction temperature regime \times predation: $\chi^2 = 2.48$; $df = 4$; $p = .648$). Aphid body mass was not significantly influenced by aphid density ($\chi^2 = 0.53$; $df = 1$; $p = .466$).

When investigating the effects of predators, heat-wave amplitude, heat-wave frequency, and their interactions on aphid body mass, we found that aphid body mass was not significantly influenced by the three-way interaction or any of the three-two-way interactions ($p > .05$). Moreover, we found no effect of heat-wave amplitude ($\chi^2 = 0.09$; $df = 1$; $p = .765$) but a significant effect of

heat-wave frequency ($\chi^2 = 8.28$; $df = 1$; $p = .001$), with larger aphids being produced in the two treatments with daily heat shocks compared to the two treatments with weekly heat shocks. Finally, the presence of predators significantly influenced aphid body mass ($\chi^2 = 25.37$; $df = 1$; $p < .0001$), with smaller aphids being observed in the presence of predators. However, variance in aphid mass was not affected by the presence of predators (Brown–Forsythe test: $F_{1,125} = 0.1732$; $p = .678$).

3.2 | Proportion of winged aphids

The proportion of winged aphids was affected by the temperature regimes ($\chi^2 = 11.97$; $df = 4$; $p = .0176$; Figure 2), the presence of predators ($\chi^2 = 19.78$; $df = 1$; $p < .0001$), and the interaction between temperature regimes and predators ($\chi^2 = 26.50$; $df = 4$; $p < .0001$). In the absence of predators, the proportion of winged aphids was not affected by the temperature regimes. Predator presence significantly increased the proportion of winged aphids, but this effect was weaker with heat shocks and became non-significant at the most severe temperature regime (40°C daily; Figure 2). The proportion of winged aphids was not significantly influenced by aphid density ($\chi^2 = 3.65$; $df = 1$; $p = .0578$).

The effects of heat-wave amplitude and heat-wave frequency on the proportion of winged aphids depended on the presence/absence of predators (significant interactions for predator \times amplitude and for predator \times frequency; Table 1). In the absence of predators, the proportion of winged aphids was not influenced by heat-wave frequency, heat-wave amplitude, or their interaction. However, in the presence of predators, the proportion of winged aphids significantly decreased with heat-wave frequency and heat-wave amplitude, but it was not affected by their interaction (Table 1, Figure 2).

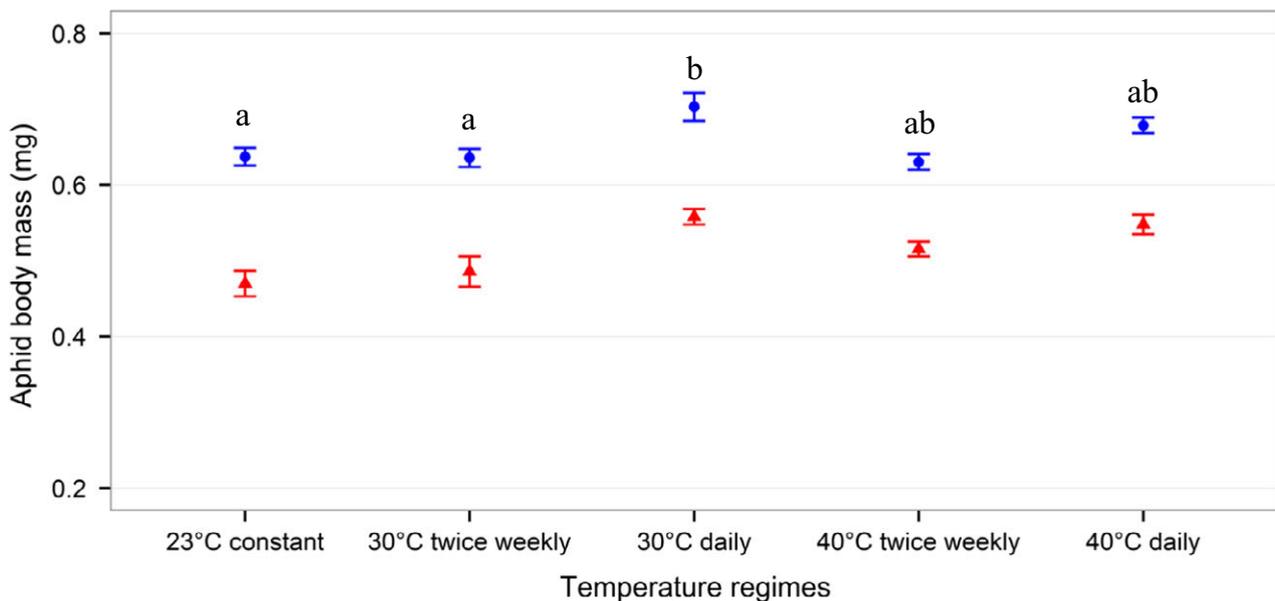


FIGURE 1 Mean aphid body mass (\pm SE) in the five temperature regimes with (red triangles) or without (blue dots) predators. Different letters denote significant differences ($p < .05$) among temperature regimes. These differences are not affected by the presence of predators

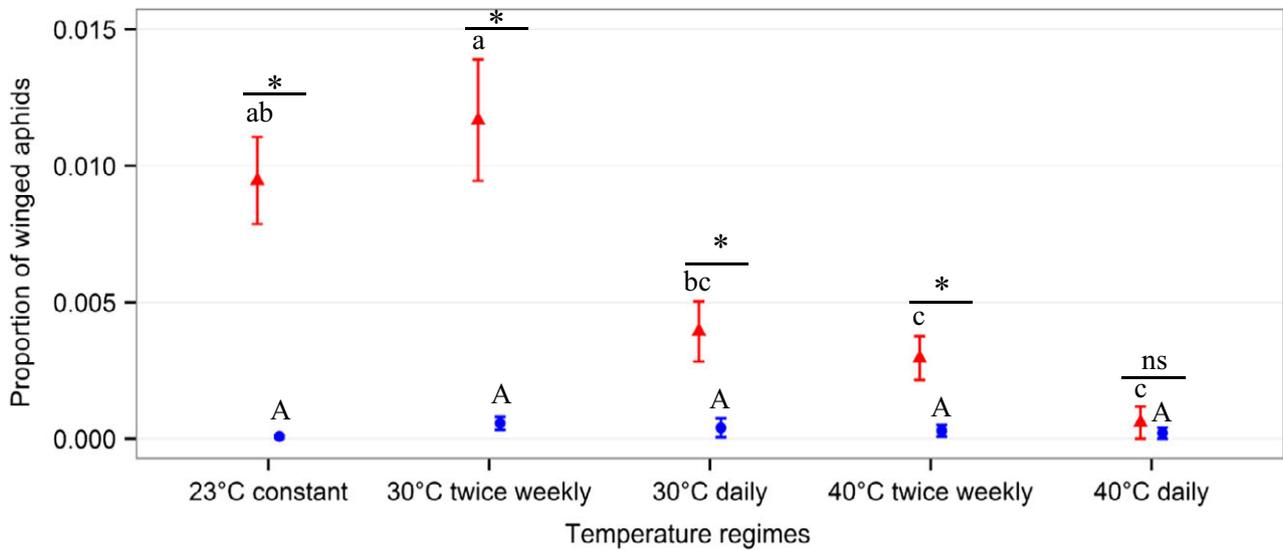


FIGURE 2 Mean proportion of winged aphids on pepper plants (\pm SE) in the five temperature regimes with (red triangles) or without (blue dots) predators. Different small or capital letters denote significant differences ($p < .05$) among temperature regimes in the presence or absence of predators, respectively. Within each temperature treatment, an asterisk or “ns” denotes, respectively, a significant ($p < .05$) or non-significant ($p > .05$) effect of predators

TABLE 1 χ^2 and p values of the LMM for the effects of predator, heat-wave amplitude, heat-wave frequency, and their interactions on the proportion of winged aphids (first column); χ^2 and p values of the LMM for the effects of heat-wave amplitude, heat-wave frequency, and their interactions on the proportion of winged aphids without predators (second column) and with predators (third column)

Effect	Full model (with and without predators)	Without predators	With predators
Frequency	$\chi^2 = 5.49$; $df = 1$; $p = .019$	$\chi^2 = 1.04$; $df = 1$; $p = .308$	$\chi^2 = 9.45$; $df = 1$; $p = .002$
Amplitude	$\chi^2 = 6.15$; $df = 1$; $p = .013$	$\chi^2 = 1.33$; $df = 1$; $p = .249$	$\chi^2 = 10.06$; $df = 1$; $p = .002$
Frequency \times amplitude	$\chi^2 = 0.90$; $df = 1$; $p = .342$	$\chi^2 = 0.21$; $df = 1$; $p = .643$	$\chi^2 = 1.01$; $df = 1$; $p = .315$
Predator	$\chi^2 = 12.57$; $df = 1$; $p = .001$	—	—
Frequency \times predator	$\chi^2 = 10.68$; $df = 1$; $p = .001$	—	—
Amplitude \times predator	$\chi^2 = 11.96$; $df = 1$; $p = .001$	—	—
Amplitude \times frequency \times predator	$\chi^2 = 0.77$; $df = 1$; $p = .381$	—	—

Significant effects are shown in bold.

3.3 | Aphid distribution on plants

The proportion of aphids located at the plant's apex greatly decreased in the presence of predators ($\chi^2 = 65.98$; $df = 1$; $p < .0001$), and the temperature regimes did not affect this pattern ($\chi^2 = 6.43$; $df = 4$; $p = .169$). However, we found a significant temperature regime \times predation interaction ($\chi^2 = 15.90$; $df = 4$; $p = .003$), indicating that the effect of temperature depends on the presence/absence of predators. The temperature regimes influenced aphid distribution only in the absence of predators (Figure 3). In addition, the proportion of aphids at the plant's apex was influenced by aphid density on the plant ($\chi^2 = 8.06$; $df = 1$; $p = .004$). However, this effect depended on the temperature regime and the presence of predators (significant temperature regime \times aphid density \times predator interaction: $\chi^2 = 17.40$; $df = 4$; $p = .002$). In the absence of predators, the proportion of aphids on the apex decreased with aphid density, whereas we observed the opposite relationship in the presence of predators (Figure 4). In both cases, heat shocks

weakened this density-mediated effect, which was not significant in the most severe temperature regimes (Figure 4).

We found a significant interaction between predation and heat-wave amplitude/frequency (Table 2) and thus examined the data by predator treatment. In the presence of predators, there were no interaction between heat-wave frequency and heat-wave amplitude, no effect of heat-wave amplitude, and no effect of heat-wave frequency (Table 2). With no predator, significantly more aphids were located at the apex of plants when heat shocks occurred daily compared to treatments with weekly heat shocks (Figure 3). However, aphid distribution was influenced neither by heat-wave amplitude nor by the interaction between heat-wave frequency and amplitude.

4 | DISCUSSION

Heat waves already cause a number of severe social, economic, and ecological disturbances (Easterling et al., 2000; Ma et al., 2015;

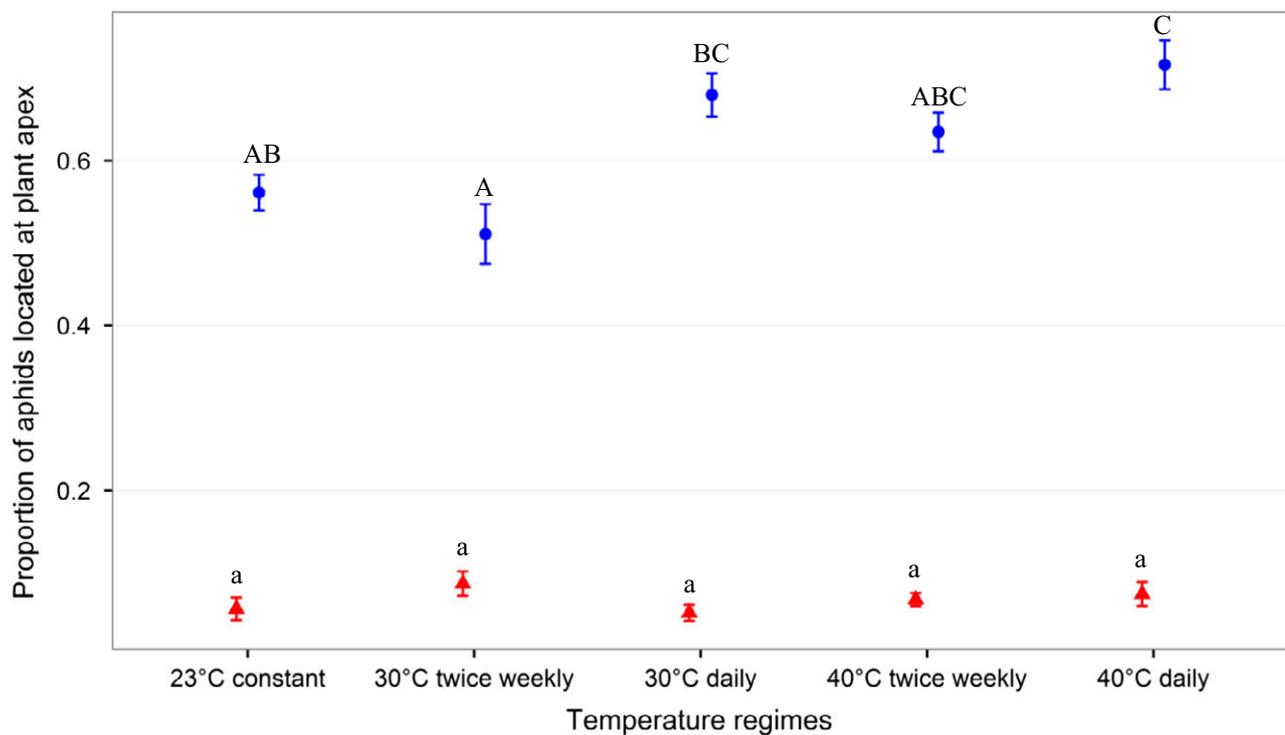


FIGURE 3 Mean proportion of aphids (\pm SE) located at the apex of pepper plants (apex + two-first leaves) in the five temperature regimes with (red triangles) or without (blue dots) predators. Different small or capital letters denote significant differences ($p < .05$) among temperature regimes in the presence or absence of predators, respectively

Parmesan et al., 2000) and are expected to increase in intensity and frequency along with climate warming (Easterling et al., 2000; IPCC, 2013). However, the interacting effects of extreme climatic events and other stresses on the behaviour and phenotype of organisms remain largely unexplored. In this study, we found that changes in the frequency and amplitude of simulated heat waves altered body size, transgenerational phenotypic plasticity, and microhabitat use in aphids (Figure 5). Importantly, these thermal effects were both independent (for body size) and dependent (for transgenerational wing polyphenism and microhabitat use) on the presence of predators, revealing the complexity of the links between biotic and abiotic factors in shaping the behavioural and phenotypic responses of species. Together, these results emphasize the importance of accounting for extreme temperature events, trophic interactions, and phenotypic responses to natural enemies and temperature when predicting how climate change will affect communities.

4.1 | Effects of heat shocks and predators on aphid body size

To the best of our knowledge, this is the first experimental evidence of aphids being significantly smaller in the presence of predators. While aphid density was influenced by the presence of predators, we did not find an effect of aphid density on aphid body mass, suggesting that the observed reduction in aphid body size is not mediated by a density-dependent effect. However, a control treatment for the mere presence of the predator (e.g., ladybeetle with jaws glued) would have been useful to exclude any potential density-mediated effects.

At least two non-mutually exclusive mechanisms could explain the body size reduction of aphids in response to predation: phenotypic plasticity (i.e., ladybeetles modify the aphid reaction norm of size and age at maturity) and size-selective predation (i.e., ladybeetles more often consume large rather than small aphids, which reduces the average aphid body size). However, directional selection typically reduces trait variance, and aphid weight variance was not affected by the presence of predators. Moreover, ladybeetles are generally more efficient at capturing small than large aphids (Sloggett, 2008), which would lead to an increase in average body size that would be the opposite of our observations. The observed reduction in aphid body size is thus likely driven by a shift in size and age at maturity in response to predation risk (i.e., rapid juvenile development at the expense of size), as reported in previous studies of predator-prey interactions in aquatic systems (Beckerman, Rodgers, & Dennis, 2010; Lima, 1998).

Interestingly, heat shocks influenced aphid body size, with larger aphids being more common in thermal regimes with frequent heat shocks; this effect was independent of the presence/absence of predators. This finding is consistent with previous studies that showed that larger individuals have lower transpiration loss and higher thermal tolerance compared to small individuals (Baudier et al., 2015; Gardner et al., 2011). However, it contrasts with previous experimental results that showed that the body size of many ectotherms (Forster, Hirst, & Atkinson, 2012; Horne, Hirst, & Atkinson, 2015), including aphids (Kindlmann & Dixon, 1992) shrinks with mild warming. This implies that the selective advantages of smaller size under a gradual rise in mean temperature might become

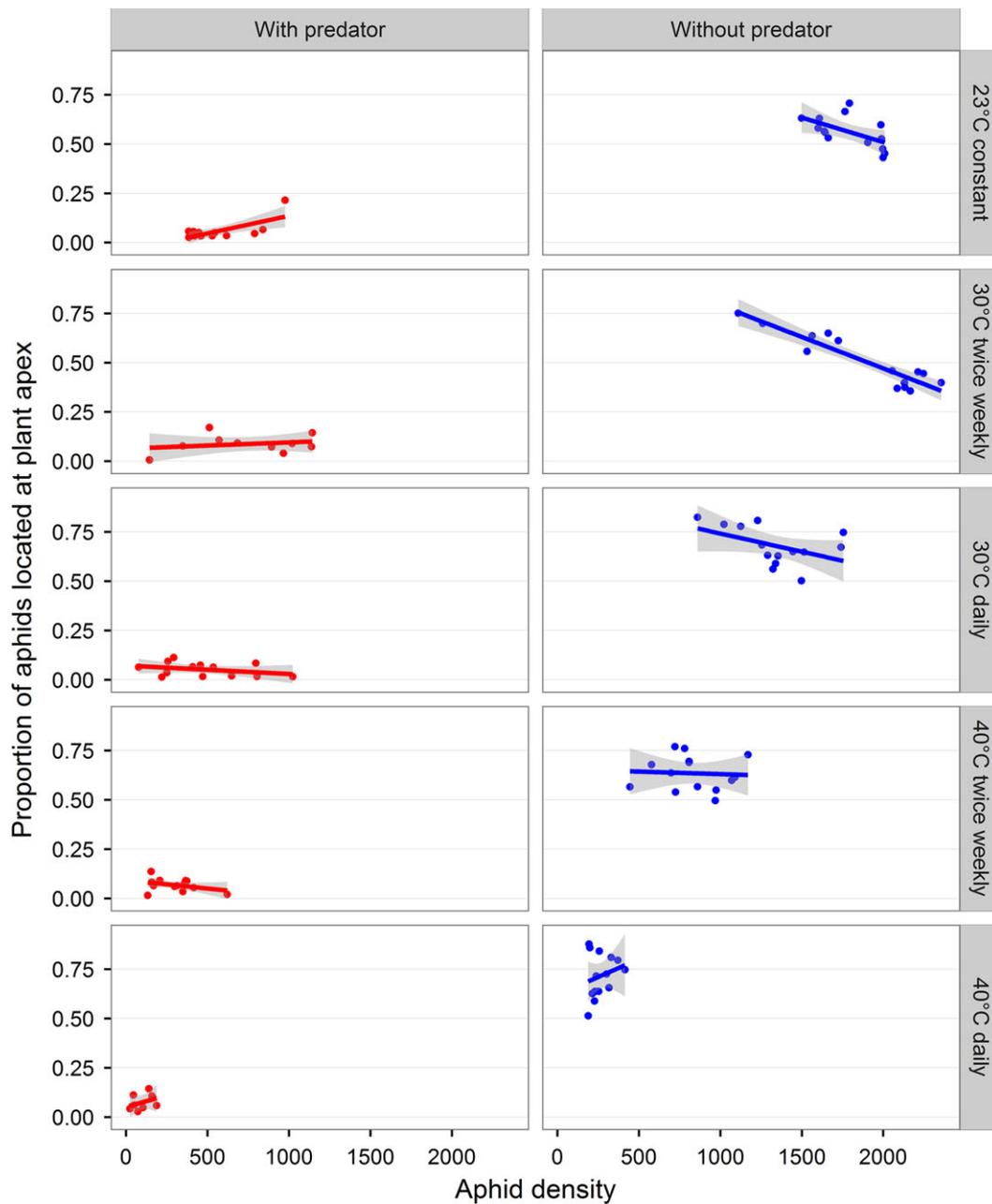


FIGURE 4 The relationship between aphid density and the proportion of aphids located at the plant's apex in the five temperature regimes with (red) or without (blue) predators. Red and blue lines represent the slopes of linear models between aphid density and the proportion of aphids located at the plant's apex. Grey bands represent the 95% confidence intervals of the regression slopes

disadvantageous under short-term exposure to temperature extremes (Gardner et al., 2011). Overall, our results indicate that the direction of the selection on body size is predicted to shift depending on the nature and extent of the temperature change and the presence or absence of predators. Because any change in a species' body mass can have important consequences on fecundity, population growth rate, species interactions, and community stability (Brose, Williams, & Martinez, 2006; Brown, Gillooly, Allen, Savage, & West, 2004; Gardner et al., 2011; Peters, 1983; White, Ernest, Kerkhoff, & Enquist, 2007), a more detailed investigation of the

mechanisms of size selection in response to biotic and abiotic factors is crucial for better understanding and predicting the eco-evolutionary consequences of global change.

4.2 | Effects of heat shocks and predators on transgenerational wing polyphenism

Consistent with previous studies (Dixon & Agarwala, 1999; Weisser, Braendle, & Minoretti, 1999), we found a higher proportion of winged aphids in the presence of predators. Because there were

TABLE 2 χ^2 and p values of the GLMM for the effects of predator, heat-wave amplitude, heat-wave frequency, and their interactions on the proportion of aphids located at the plant's apex (first column); χ^2 and p values of the GLMM for the effects of heat-wave amplitude, heat-wave frequency, and their interactions on the proportion of aphids located at the plant's apex without predators (second column) and with predators (third column)

Effect	Full model (with and without predators)	Without predators	With predators
Frequency	$\chi^2 = 1.07$; $df = 1$; $p = .300$	$\chi^2 = 5.51$; $df = 1$; $p = .019$	$\chi^2 = 2.26$; $df = 1$; $p = .133$
Amplitude	$\chi^2 = 2.12$; $df = 1$; $p = .146$	$\chi^2 = 2.60$; $df = 1$; $p = .107$	$\chi^2 = 0.07$; $df = 1$; $p = .794$
Frequency \times amplitude	$\chi^2 = 0.39$; $df = 1$; $p = .534$	$\chi^2 = 0.57$; $df = 1$; $p = .449$	$\chi^2 = 3.81$; $df = 1$; $p = .051$
Predator	$\chi^2 = 49.44$; $df = 1$; $p < .0001$	—	—
Frequency \times predator	$\chi^2 = 8.14$; $df = 1$; $p = .004$	—	—
Amplitude \times predator	$\chi^2 = 1.03$; $df = 1$; $p = .309$	—	—
Amplitude \times frequency \times predator	$\chi^2 = 4.83$; $df = 1$; $p = .028$	—	—

Significant effects are shown in bold.

very few winged aphids in the absence of predators (Fig. S2), we attribute this result to a transgenerational phenotypic response to predators and not to a phenotype-selective predation that could also have led to an increase in the proportion of the less-consumed phenotype. Interestingly, the proportion of winged aphids was not affected by heat shocks in the absence of predators. In contrast, this proportion decreased with the amplitude and frequency of heat shocks in the presence of a ladybeetle larva. In the most severe thermal regime (40°C daily), the presence of predators did not modify the proportion of winged aphids even though they were being actively preyed upon (A. Sentis, personal observation). Our results corroborate those of previous studies showing that warm temperatures inhibit the production of winged aphids in response to crowding or poor nutritional conditions (Braendle, Davis, Brisson, & Stern, 2006; Müller, Williams, & Hardie, 2001). One proximal explanation for this result is that extreme temperatures interfere with or inhibit the hormonal and epigenetic pathways that control wing polyphenism in aphids (Brisson, 2010). Alternatively, under thermal stress, the costs of developing wings (i.e., longer development time and lower fecundity) outweigh the benefit of escaping predators. Indeed, one of the key assumptions concerning the evolution of predator-induced defences is that the benefits of induction are balanced by its costs, which leads to reduced fitness in the absence of predation (Agrawal, 2001; Hammill et al., 2008; Tollrian & Harvell, 1999). Extreme temperatures may influence the costs and benefits of wing induction by, for instance, affecting the aphid's energy budget or decreasing survival during flight dispersion. Moreover, the benefit of producing winged offspring for escaping extreme weather needs to be further explored because (i) the full generation time between exposure and response to extreme temperatures might be too long to respond to heat waves, which typically last for just a few days, and (ii) heat waves generally occur over large areas so that aphids would have to fly long distances to escape. These factors may contribute to inhibit the production of winged offspring by parents when exposed to extreme temperatures.

Induced defences are a key example of trait-mediated or sublethal effects that underpin direct and indirect interactions and significantly influence the structure and dynamics of food webs

(Agrawal, 2001; Beckerman, Wieski, & Baird, 2007; Bolker, Holyoak, Křivan, Rowe, & Schmitz, 2003; Mougi & Kishida, 2009; Peacor & Werner, 2001). Although the causes and mechanisms remain to be investigated in more detail, the patterns we observed clearly indicate that heat shocks can disrupt the aphid's transgenerational phenotypic response to predation. Based on our results, extreme temperature events should reduce winged aphid abundance and thus decrease the probability of dispersal to new plants with reduced predation risk and/or higher nutritional quality. We thus predict that this sublethal effect of heat shocks should hasten aphid population declines and restrict their spatial distribution, which could have feedback effects on predator populations, causing community destabilization.

4.3 | Effects of predators and heat shocks on aphid distribution on host plants

Many prey respond to predators by escaping to safe microhabitats. This reduces the immediate predation risk at a cost of garnering resources in suboptimal environments (Downes, 2001; Lima, 1998; Sih, Englund, & Wooster, 1998). Here, we found that, in the absence of ladybeetles, aphids typically stay at the plant's apex, which is their preferred feeding site due to the high nutritional quality of vigorously growing apical tissues (Cibils-Stewart, Sandercock, & McCornack, 2015; Cornelissen, Wilson Fernandes, & Vasconcellos-Neto, 2008; Costamagna, McCornack, & Ragsdale, 2013; Dixon, 1998). In the presence of predators, the aphid's spatial distribution significantly shifted towards the lower leaves of the plants. Because coccinellids tend to forage more often and longer on the plant's upper parts (Costamagna & Landis, 2007; Hodek & Honěk, 1996), we attribute this spatial distribution shift to a predator avoidance behaviour that, as reported in previous studies, reduces predation risk at the cost of feeding on lower-quality plant tissues (Costamagna & Landis, 2011; Costamagna et al., 2013).

Little is known about extreme thermal effects on the distribution of animals within microhabitats (but see Baudier et al., 2015; Cailion, Suppo, Casas, Arthur Woods, & Pincebourde, 2014), and to what extent this temperature-driven shift may affect predation risk–

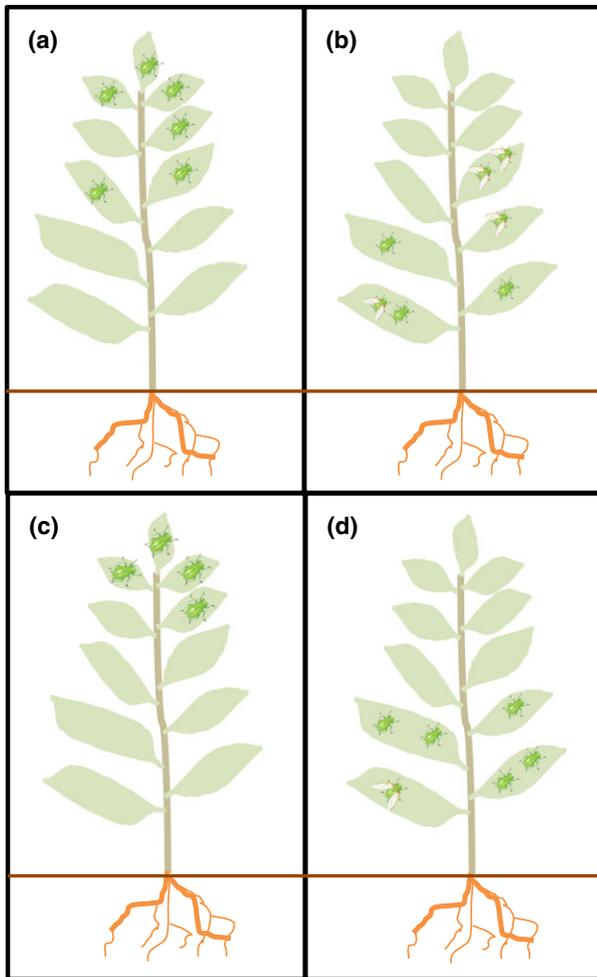


FIGURE 5 Schematic representation of the body size, transgenerational wing polyphenism, and within-plant distribution of aphids in the (a) absence of heat shocks and predators, (b) presence of predators, (c) occurrence of heat shocks, and (d) presence of predators and occurrence of heat shocks. In all panels, aphid size scales with body mass. In the absence of heat shocks and predators (a), aphids are medium sized, unwinged, and distributed towards the plant's apex. In the presence of predators (b), aphids are small, mainly winged, and distributed towards the lower leaves. In the occurrence of heat shocks (c), aphids are large, unwinged, and clustered at the plant's apex. In the presence of predators and when heat shocks occur (d), aphids are larger than in the presence of predators only but smaller than in the treatment without predators and heat shocks. They are mainly unwinged and distributed towards the lower leaves

resource acquisition trade-offs. We found that, in the absence of predators, the increasing frequency of heat shocks led to a higher proportion of aphids being located at the plant's apex, probably because of its high nutritional quality. In contrast, heat shocks did not influence aphid distribution in the presence of predators, suggesting that the aphid's behavioural response to predation (i.e., microhabitat shift towards lower plant parts) is not affected by heat shocks. This result indicates that the presence of predators can buffer the impact of heat shocks on aphid microhabitat distribution.

Understanding and predicting the impacts of climate change on the phenotype, distribution, and interactions of species is a major challenge for ecologists (Fussmann, Schwarzmüller, Brose, Jousset, & Rall, 2014; Seebacher, White, & Franklin, 2015). A common approach in global change biology is to relate the average change of a climatic variable to biological processes (Jentsch, Kreyling, & Beierkuhnlein, 2007). However, focusing on mean effects can be unrealistic because the variance—in time or space—can also be ecologically meaningful. Here, we show that simulated heat waves can inhibit phenotypic and behavioural responses to predation (and vice versa) that may alter trophic interactions and have important consequences on the dynamics and stability of ecological communities. Moreover, extreme temperatures induced considerable changes in our tritrophic system that could not be predicted from observations of a bitrophic system (e.g., inhibition of transgenerational wing induction in the presence of predators). We conclude that considering the links between thermal tolerance, behaviour, phenotypic plasticity, and species interactions is crucial to better understanding the consequences of thermal extremes on ecological communities.

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