

RESEARCH ARTICLE

Experimental heatwaves facilitate invasion and alter species interactions and composition in a tropical host-parasitoid community

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Abstract

As mean temperatures increase and heatwaves become more frequent, species are expanding their distributions to colonise new habitats. The resulting novel species interactions will simultaneously shape the temperature-driven reorganization of resident communities. The interactive effects of climate change and climate change-facilitated invasion have rarely been studied in multi-trophic communities, and are likely to differ depending on the nature of the climatic driver (i.e., climate extremes or constant warming). We re-created under laboratory conditions a host-parasitoid community typical of high-elevation rainforest sites in Queensland, Australia, comprising four *Drosophila* species and two associated parasitoid species. We subjected these communities to an equivalent increase in average temperature in the form of periodic heatwaves or constant warming, in combination with an invasion treatment involving a novel host species from lower-elevation habitats. The two parasitoid species were sensitive to both warming and heatwaves, while the demographic responses of *Drosophila* species were highly idiosyncratic, reflecting the combined effects of thermal tolerance, parasitism, competition, and facilitation. After multiple generations, our heatwave treatment promoted the establishment of low-elevation species in upland communities. Invasion of the low-elevation species correlated negatively with the abundance of one of the parasitoid species, leading to cascading effects on its hosts and their competitors. Our study, therefore, reveals differing, sometimes contrasting, impacts of extreme temperatures and constant warming on community composition. It also highlights how the scale and direction of climate impacts could be further modified by invading species within a bi-trophic community network.

KEYWORDS

community composition, *Drosophila*, facilitation, global warming, heatwave, interaction modification, invasion, parasitism

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1 | INTRODUCTION

Climate change is leading to large-scale shifts in species' distributions (Chen, Hill, Ohlemüller, et al., 2011; Chen, Hill, Shiu, et al., 2011; Parmesan et al., 1999; Parmesan & Yohe, 2003). Differences in the speed (Mamantov et al., 2021), magnitude (Chen, Hill, Ohlemüller, et al., 2011; Chen, Hill, Shiu, et al., 2011; Sunday et al., 2012) and even direction (Lenoir et al., 2010) of range shifts are causing existing interactions among species to unravel, and new interactions to form.

Despite the widespread occurrence of such novel interactions and evidence that they can influence community responses to climate change (Alexander et al., 2015; Gilman et al., 2010; Nomoto & Alexander, 2021), the ecological ramifications of climate-driven novel species interactions in multi-trophic communities are poorly explored. The impacts of climate change and novel species interactions on a biological community are mutually dependent (Figure 1). Climate change not only affects organisms' performance but also alters the biotic environment (Blois et al., 2013; Davis et al., 2022; Descombes et al., 2020; Nowicki et al., 2021; Romero et al., 2018). Climate-driven changes in the demography or behaviour of competitors, predators, and mutualists could modify the direct effects of climate on individual species. Such influential species can either be pre-existing components of the community (Barton & Ives, 2014; Harmon et al., 2009) or novel species whose arrival is facilitated by climate change (Richman et al., 2020; Schweiger et al., 2010). Furthermore, species interactions, including those between novel species and residents, are themselves climate-dependent (Alexander et al., 2015; LaForgia et al., 2020; Olsen et al., 2016), as two of the dashed lines in Figure 1 represent. As a result, predicting biodiversity response to climate change is challenging because climate change leads to new species interactions as a result of range shifts and new climatic conditions to which species interactions are sensitive (Gilman et al., 2010; HilleRisLambers et al., 2013; Tylianakis et al., 2008; Wallingford et al., 2020).

Experimental approaches have been useful for quantifying the impact of potential novel species interactions outside the current climatic conditions experienced by communities (Alexander

et al., 2016; Descombes et al., 2020; Ravi et al., 2022; Richman et al., 2020). Nevertheless, previous studies have usually focused on a single type of species interaction (e.g., competition, herbivory). Most biological communities, on the other hand, comprise at least a few trophic levels and several species within each level, resulting in motifs with a variety of trophic and non-trophic interaction pathways, both antagonistic and facilitative (Barton & Ives, 2014; Losapio et al., 2021; Thierry et al., 2019). Understanding how the impacts of external drivers such as climate and invading species propagate through species interactions and emerge in multi-species, multi-trophic communities remain a key challenge.

While most empirical research on range shifts has focused on the impact of a constant increase of mean temperature (referred to here as "warming"), climate change also manifests as extreme high-temperature events and higher temperature variability. Tropical regions have experienced the greatest increase in temperature anomalies, such as heatwave frequency and duration (IPCC, 2022; Perkins-Kirkpatrick & Lewis, 2020). Extreme events such as heatwaves also contribute to range dynamics (Diez et al., 2012; Terry et al., 2022; Vasseur et al., 2014), prompting interest in the relative importance of warming versus extreme temperature events (Jentsch et al., 2007; Lynch et al., 2014; Ma et al., 2015). A moderate level of warming generally increases interaction intensity (Klanderud et al., 2015; Roslin et al., 2017) or releases physiological constraints (Crozier, 2004). In contrast, heatwaves often induce mortality or infertility (van Heerwaarden & Sgrò, 2021), temporarily releasing the surviving species from antagonistic biotic interactions (Diez et al., 2012; Wallingford et al., 2020). Different mitigation strategies are required for warming and heatwaves as they operate at different timescales. However, these strategies have seldom been put into practice because the ecological impacts of heatwaves versus warming have rarely been compared (Ma et al., 2021).

Here, we investigate the interacting effects of invading species and different modes of increasing temperatures (i.e., warming and heatwaves) on an experimental host-parasitoid system. Parasitoids are insects whose larvae develop on or in the bodies of other arthropods and eventually kill them (Hardy et al., 1994). Host-parasitoid networks represent relatively tractable, strongly-interacting units containing a range of specialist and generalist interactions (Hrček & Godfray, 2015). Parasitoids play a major role in regulating the abundance and coexistence of arthropods in natural ecosystems and agriculture (Bonsall & Hassell, 1997; Hardy et al., 1994) and as such are often selected as bio-control agents. However, these ecological functions are especially vulnerable to climate change (Furlong & Zalucki, 2017; Hance et al., 2007).

Our focal community comprises *Drosophila* species and their associated parasitoids from Australian rainforests (Jefferies et al., 2021). We focus on high-elevation communities as they are bounded geographically: as the climate warms, these communities face combined threats from changing abiotic and biotic conditions (Shah et al., 2020) without the presence of spatial refuges to buffer these threats. Our previous study has shown that the upland

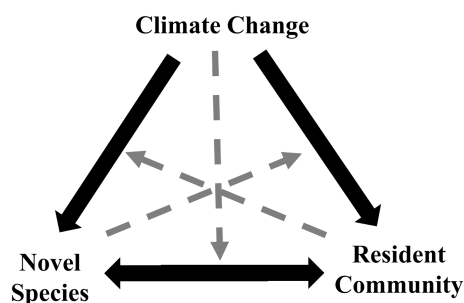


FIGURE 1 The relationships among climate change, invading species, and the resident community. The solid arrows represent direct effects. Dashed arrows indicate that a third factor influences the direct effect between the two factors.

community contains heat-sensitive species, and that interspecific competition prevents lowland species from occupying cooler, higher-elevation locations (Chen & Lewis, 2022). Based on this, we predict that the release of competitive pressure by heatwaves will facilitate the invasion of lowland species into upland communities. Second, we predict that the temporal distribution of excessive heat (manifested in extreme heat events or constant warming) will differ in their impacts on community composition. Third, we predict that the impact of invasion will depend on climate scenarios as the low-elevation invader is only expected to successfully establish in the heatwave treatment. Finally, we expect parasitoids to play a significant role in mediating the impact of temperature and/or the invading species.

2 | MATERIALS AND METHODS

2.1 | Study system

We conducted an experiment using tropical forest *Drosophila* flies and parasitoids in a controlled laboratory environment. Cultures of all species were collected from the Wet Tropics bioregion in Queensland, Australia, which has a high level of species endemism associated with cool, moist upland refugia. Information on the natural distribution patterns of *Drosophila* and their natural enemies, parasitoids, is based on previous trapping using banana baits (Jefferies et al., 2021). Most *Drosophila* species and some of the parasitoids from these communities are now in laboratory culture, allowing multi-species manipulative experiments in the laboratory.

We assembled an experimental community to reflect species compositions at contemporary high-elevation sites (elevation 730 m–880 m), comprising *D. birchii*, *D. rubida*, *D. pallidifrons*, *D. pseudotakahashii*, a generalist pupal parasitoid from the genus *Trichopria* (lab strain 66LD, species identifier drop-Dia127-sp9), and a less generalised larval parasitoid from the genus *Asobara* (lab strain KHB, species identifier drop-Aso-sp8) (Lue et al., 2021). The four *Drosophila* species account for 77%–95% of *Drosophila* abundance sampled at high-elevation sites. Compared to the other *Drosophila* species, *D. pallidifrons* and *D. pseudotakahashii* are high-elevation specialists and are more sensitive to high temperatures (Chen & Lewis, 2022). The distribution of the two parasitoid species is unclear because of their small sample sizes, but both occur at high-elevation sites (Jefferies et al., 2021). To study potential upslope range expansion by a lowland species under climate change we used *D. pandora*. This species is found predominantly at low-elevation (i.e., 70 m) sites (Jefferies et al., 2021), and based on thermal performance trials is better adapted to higher temperatures than the upland species (Chen & Lewis, 2022). As shown in Figure 2a, the two focal parasitoid species differ in their specificity on *D. pseudotakahashii*. Additionally, while the *Trichopria* parasitoid can develop in all 10 *Drosophila* species we have cultured from our study sites, the *Asobara*

parasitoid could only develop in seven of them (Chia-Hua Lue, personal communication).

2.2 | Community initiation

All experimental communities were initiated with the same species on a day/night cycle (24°C, light for 12 h; 18°C, dark for 12 h), mimicking high-elevation conditions (also referred to as upland or control conditions). Each community was kept in a sealed 20 cm × 5 cm × 10 cm plastic cage. Card dividers on the floor of the cage could hold up to 24 standard *Drosophila* vials. In each of the first 3 weeks, 15 females and 15 males of *D. pseudotakahashii*, *D. pallidifrons*, *D. birchii*, *D. rubida*, and 10 females and 10 males of both parasitoid species were added to each cage. The adult flies were of mixed ages, randomly sourced from mass-bred population cages. The parasitoids were one-week-old adults, reared on *D. melanogaster*, a species that does not occur in our study site. After these first 3 weeks, we maintained the community cages without adding insects for two more weeks before starting experimental treatments. During the 5 weeks after the first colonization, each species could expand to a number reflecting their reproduction, competition and resistance to parasitism. The schedule is shown in Figure 2b.

2.3 | Maintenance and treatments

After the fifth week of community initiation, a factorial design of three temperature treatments and two invasion treatments was applied to 30 cages (18 cages in block A and 12 cages in block B, initiated 4 weeks apart). Five cages were randomly assigned to each treatment and maintained for another 20 weeks. Three vials of 6 mL fresh *Drosophila* food (weight/volume concentration: 8% corn flour, 4% yeast, 5% sugar, 1% agar, and 1.67% methyl-4-hydroxybenzoate) were added to the cage twice a week (3–4 days apart). Vials added in the middle of each week were “maintenance vials”. “Maintenance vials” were removed after 40 days so that most flies and parasitoids would have completed one generation within them. Vials added at the start of the week were “sampling vials”, which were removed after 10 days. *Drosophila* flies and parasitoid wasps could freely colonize these vials for 10 days (shorter than the generation time of the fastest species). After removal, they were kept in the same incubator as the communities they were taken from, and the numbers of emerged *Drosophila* and parasitoid adults from these vials were recorded.

2.3.1 | Temperature regimes

As described above, the upland temperature regime alternated between 24°C, light (12 h), and 18°C, dark (12 h). For warming

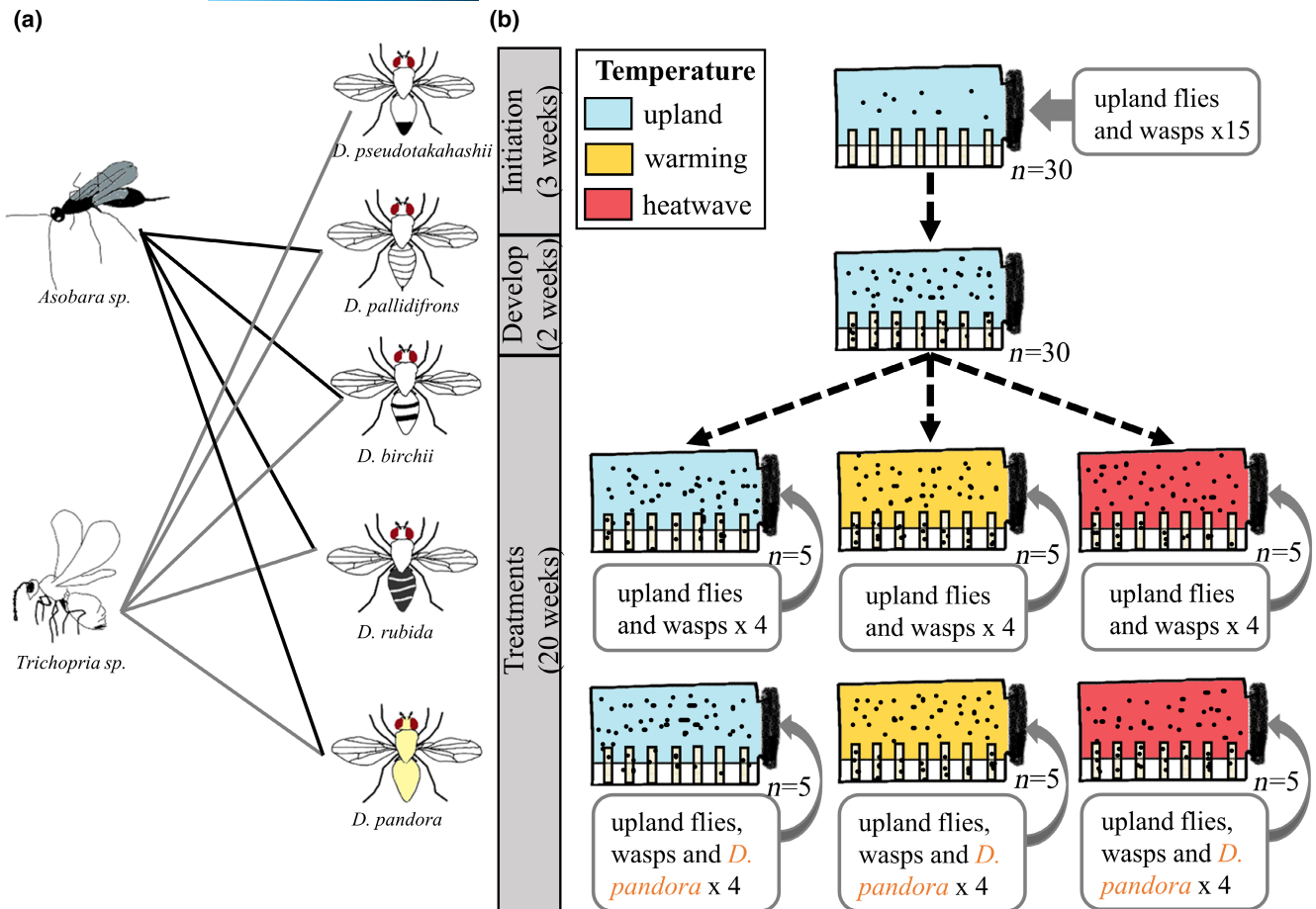


FIGURE 2 The study system and experimental procedures. (a) The known trophic interactions between parasitoids and *Drosophila* flies. These interactions are based on observation of the successful development of the parasitoids. *Drosophila pandora* is a low-elevation specialist, used as a species that is novel to the upland community. Different *Drosophila* species are illustrated by differing abdomen patterns, and these drawings are used in subsequent figures to represent the corresponding species. (b) Experimental communities were maintained in sealed cages with regular food provisions. Fifteen pairs of each upland species were added to every cage for the first 3 weeks. Communities then developed for two additional weeks. Following this, a total of 30 cages (five replicates per treatment combination) underwent combinations of invasion and temperature treatments (blue: upland temperature; yellow: warming; red: heatwave). The boxed text under each cage indicates the species, and the number of pairs of each species, added to the cage each week.

and heatwave treatments, we allocated the same degree-day increase in cumulative temperature (+1°C per day), either in the form of an even increase of temperature or multiple heatwave events. The warming regime increased both the day-time and night-time temperatures by 1°C (25°C, light for 12h; 19°C, dark for 12h), which is anticipated in the study area (0.5–1.4°C) by 2030 (Suppiah et al., 2007). The heatwave regime had the same baseline temperature as the upland regime, but with periodic heatwaves when daytime and night-time temperatures rose by 6°C for five days. For comparison, in February 2017, a heatwave raised the maximum air temperature by 6°C and the average air temperature by 4°C for two consecutive days at our study site (Figure S1a). Heatwaves were applied to all corresponding communities at the same time following a predetermined timing. Heatwaves occurred once every 4 weeks on average, with a two-week

minimum interval between consecutive heatwaves (sequences of heatwave events are shown in Figure S1b). We used three incubators of two similar models (Sanyo MIR-154 & Sanyo MIR-153). To avoid any confounding incubator effects, temperature regimes were rotated among incubators weekly.

2.3.2 | Invasion treatment

Every week, four pairs of the low-elevation specialist, *D. pandora*, were added to cages assigned for the invasion treatment. To avoid the stochastic extinction of resident species, and to mimic natural immigration, four pairs of each *Drosophila* and parasitoid species from the starting community were added to all community cages at the same frequency as *D. pandora*. In the final 2 weeks of the experiment, no animals were added.

2.4 | Data collection

2.4.1 | Regular sampling

The reproductive success of every species was monitored by examining the adults emerging from the "sampling vials". After allowing 10 days of colonization, the three sampling vials were removed from cages and kept for 24 days in the same environmental regime as the communities from which they originated. Emerged fly and parasitoid adults were frozen at -18°C every 3–4 days before being able to lay eggs. Later, adults were identified to species and sexes. Counts of emerged flies and wasps from the three sampling vials were summed. Thus, the number of emerging adults reflects the combined impact of adult population sizes at sampling, adult fecundity, intra- and inter-specific competition, and parasitism. Although we deployed "sampling vials" every week, due to logistic constraints, we only process "sampling vials" of the 7th, 10th, 13th, 16th, 19th, and 22nd week.

2.4.2 | Heatwave-specific sampling

To measure how a single heatwave event altered the reproductive success of flies, fresh food vials were placed into each cage at three time points relative to the final heatwave event: one day before the final heatwave, on the fifth day of the heatwave, and 5 days after the heatwave ended. At each time point, three vials (each containing 6 mL of food) were added to each relevant cage for 24 h, a sufficiently short exposure to avoid larval crowding within vials. These vials were then kept under the upland temperature regime and emerging adult flies were identified and counted. There were no parasitoid offspring as these parasitoids cannot parasitise eggs or first-instar larvae.

2.4.3 | Termination and census

After 25 weeks, all living adult insects in community cages were frozen, identified and counted. Throughout the maintenance of the community cages, the numbers of *Drosophila* or parasitoids increased but did not appear to fluctuate greatly. Therefore, although the final census is only a snapshot, we regard it as a useful representation of community composition. Females of *D. pandora*, *D. pseudo-takahashii*, and *D. birchii* are marginally different in pigmentation and body size but become difficult to separate in a crowded environment which affects their body sizes. Analyses of these three species were therefore based on counts of males if not otherwise specified.

2.5 | Data analysis

The data that support the findings of this study are openly available in Zenodo at <https://doi.org/10.5281/zenodo.7643868> (Chen & Lewis, 2023). Code for statistical models and figures is openly available in GitHub at <https://github.com/Jinlinc/ExperimentalInvasion>.

git. Code was performed with R version 4.0.3 (R Core Team, 2020). We performed diagnostic checks of residuals after fitting (generalised) linear models. Residual plots highlighted no issues.

2.5.1 | Effect of temperature treatment on the lowland species

The final adult abundance of *D. pandora* was modelled as a function of *temperature treatment*, *block* and their interaction term in a generalized linear model with Poisson error distribution. We used the default log link function for Poisson family in all generalized linear model in this study. For tri-weekly sampling data, *D. pandora* numbers were modelled as a function of *temperature treatment* and *block* as fixed effects, *week of sampling* and *cage ID* as random effects in a generalized linear mixed-effect model with Poisson errors.

2.5.2 | Immediate effects of a heatwave event on the reproduction of *Drosophila* species

Counts of offspring of the three most abundant *Drosophila* species were log-transformed ($\log(y + 1)$) and then modelled as a function of *sampling time* (relative to the heatwave event), *species*, *invasion treatment*, *block*, and the interaction between *sampling time* and *species* in a linear model. This strategy was used because residuals were not homogeneous when counts were modelled directly, assuming Poisson errors, in a generalized linear model.

2.5.3 | Community composition

Community compositions of the living adults at the end of the maintenance period were analysed separately for hosts and parasitoids. Compositions of four resident *Drosophila* were visualized using non-metric multidimensional scaling (NMDS) with a dimension of two. Abundances of parasitoids were standardized as z-scores ($\frac{x - \bar{x}}{\sigma}$). Community distance matrices were calculated using the "bray" method (9999 permutations) and assessed through non-parametric MANOVA (Anderson, 2001). Upon detecting overall significant differences among all treatments, post-hoc analyses were performed to test the differences between pairs of treatments. NMDS and non-parametric MANOVA analyses were conducted using the *vegan* package (Oksanen et al., 2020). The *multcompView* package was used to summarise significant pairwise differences based on post-hoc comparisons (Graves, Piepho & Selzer, 2019).

Species abundance in response to treatments was then analysed individually using generalized linear models. The census abundance of each resident species was modelled as a function of *temperature treatment*, *invasion treatment*, their interaction term, and *block*, assuming Poisson error distributions. *p*-values of these predictors of all upland species were adjusted by Bonferroni correction. Post-hoc pairwise comparisons were conducted using the *emmeans* package (Lenth, 2022).

2.5.4 | Inferring indirect effects

To examine whether the impact of a treatment on a species was direct or mediated via other species, we tested whether the effect coefficient of a treatment was reduced by the inclusion in the regression model of the putative mediating species. Directed acyclic graphs (DAGs) were built to describe the direct and potential indirect pathways by which temperature and invasion treatments affect the reproductive outputs of *D. pandora*, *D. pallidifrons*, and *D. pseudotakahashii*. In the DAGs, the two parasitoids were the potential mediators between all treatments and all focal species. Additionally, we added *D. pallidifrons* as a mediator for *D. pandora* and *D. pseudotakahashii*, as we have previously shown that *D. pallidifrons* is a strong competitor that can exclude lowland species from the upland environment (Chen & Lewis, 2022). We first built regression models to identify the effects of treatments on the reproductive success of parasitoids. In these generalized linear mixed-effect models (with Poisson error distributions), we used *temperature treatment*, *invasion treatment*, their interaction, and *block* as fixed effects and *sampling week* and *cage ID* as random effects. The linear mixed-effect model used the *lme4* package (Bates et al., 2015). *p*-values of all tests on parasitoids were then adjusted by Bonferroni correction. For each

focal *Drosophila* species, we first build a regression model following the same modelling strategy as for parasitoids, to test for the overall effects of the experimental treatments. If treatments had significant effects, a further regression model was constructed based on the DAG (Figure 6, left column), adding standardized numbers (*z*-scores) of parasitoids and/or other relevant *Drosophila* as additional predictors. *p*-values from the above two regressions of each *Drosophila* species were grouped and adjusted for multiple comparisons. Model outputs were then compared. If the fitted coefficient of the treatment was reduced after a significant controlling factor was added, we inferred that the overall effect of that treatment was (partially) mediated by the mediating species. The coefficients of treatments in the second model were direct effects which were not mediated by other species.

3 | RESULTS

3.1 | Establishment of the low-elevation species

At the end of the experiment, the censused abundances of the lowland invader *D. pandora* in the upland temperature (control) and warming treatments were both low (Figure 3a; coefficient of

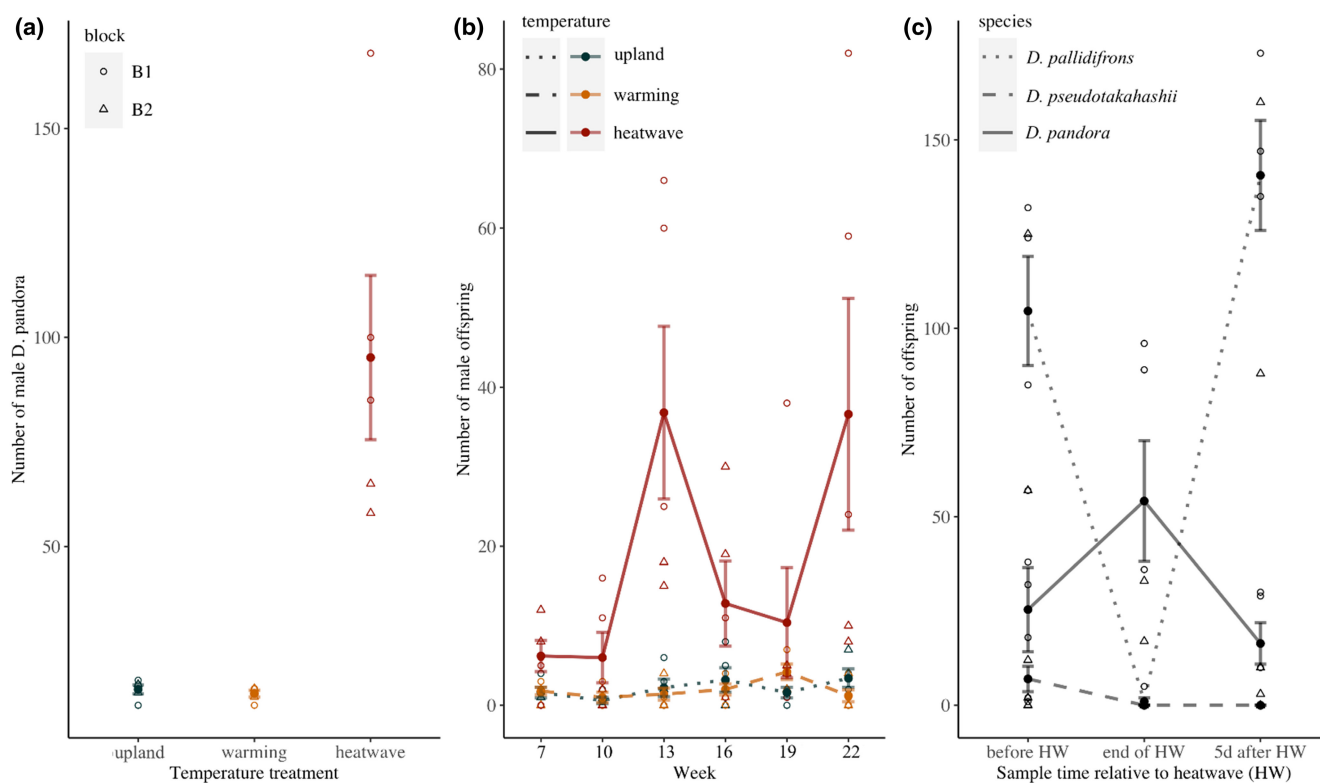


FIGURE 3 Heatwaves promote the establishment of the lowland specialist, *D. pandora*, in upland communities. (a) Numbers of male *D. pandora* adults in community cages upon termination under different temperature treatments. (b) Reproduction of *D. pandora* over time in the upland temperature regime (blue, dotted line), warming treatment (yellow, dashed line) and heatwave treatment (red, solid line). (c) Reproductive success of *D. pallidifrons* (high-elevation specialist; dotted line), *D. pseudotakahashii* (high-elevation specialist; dashed line), and *D. pandora* (solid line) on a single day at different timings relative to the last heatwave event. For all graphs, each open symbol represents the number of individuals censused or sampled from a community replicate ($n=5$). The solid circles represent the means. Shapes of symbols represent experimental block 1 (circle) or block 2 (triangle). Error bars show ± 1 SD.

warming = -0.06 , $p = .75$; degree of freedom = 9). From the same model, the abundance of *D. pandora* in heatwave treatments was significantly higher in both experimental blocks (Figure 3a; coefficient of heatwaves in the first block = 2.02 , $p < .001$; coefficient of heatwave \times block = -0.67 , $p = .008$). Consistent with the census, *D. pandora* produced significantly higher numbers of adult offspring in the heatwave treatment (Figure 3b; coefficient of heatwave treatment = 2.12 , $p < .0001$). The effect of warming was not significant compared with the upland control ($p = .73$). Very few offspring were produced in either the upland temperature or warming treatments. The second block had fewer offspring than the first (coefficient of block = -0.44 , $p = .04$).

To investigate how heatwaves favoured *D. pandora*, we compared one-day snapshots of reproductive success at different timings relative to the final heatwave event (Figure 3c). The heatwaves treatment significantly decreased the reproductive success of both upland specialists ($p_{D. pallidifrons} < .001$; $p_{D. pseudotakahashii} < .001$; degree of freedom = 64). The reproduction of *D. pallidifrons* was able to recover to a similar level as before the heatwave (ratio_{before/5 days after} = 0.63 , $p = .14$), while *D. pseudotakahashii* remained infertile for at least 5 days. In contrast to the upland species, the heatwave temporarily increased the reproductive success of the invader *D. pandora* (ratio_{before/end of heatwave} = 0.21 , $p = .002$).

3.2 | Interacting effects of heatwaves and invaders on the resident community

The temperature treatments and the introduction of the lowland species jointly caused compositional changes in upland communities. Non-parametric MANOVA identified significant differences among treatments in the community composition of upland *Drosophila*

and parasitoid species (*Drosophila*: $F_{5,24} = 7.8$, $p = .0001$, $r^2 = .62$; parasitoids: $F_{5,24} = 6.5$, $p = .0003$, $r^2 = .58$). Post-hoc pairwise analysis revealed significant differences (denoted by different letters on polygons in Figure 4) between groups of communities. Details on individual species are shown in Figure S2. Treatment effects on individual species were analysed using regression models, shown in Table S1.

Our heatwave treatment, but not the warming treatment, significantly changed the community composition of resident *Drosophila* (Figure 4a). This change was driven by a large decrease in the most heat-sensitive species *D. pseudotakahashii* and a small increase in *D. pallidifrons* (Table S1, Figure S2). Invasion only had a significant effect on the local *Drosophila* community when concurrent with the heatwave treatment. Heatwaves and invasion jointly resulted in communities dominated by *D. pallidifrons*. The abundances of *D. birchii* and *D. rubida* remained low across treatments (Figure S2e,f).

Warming and heatwaves reduced the relative abundances of both parasitoid species by a similar degree in the absence of the invading species (Figure 4b). Invasion significantly changed the parasitoid composition by reducing the abundance of *Asobara* parasitoids under both the upland temperature and heatwave treatment but had no detectable effect under warming conditions.

3.3 | Parasitoid-mediated effects of treatments

We used reproductive success over time (raw data are shown in Figure S3) to explore whether the effects of treatments on *D. pandora*, *D. pallidifrons* and *D. pseudotakahashii* were mediated by parasitoids' top-down control. As shown in Table 1, the reproductive performance of *Asobara* was significantly reduced by invasion. Warming and heatwave treatments tend to have opposing effects on both

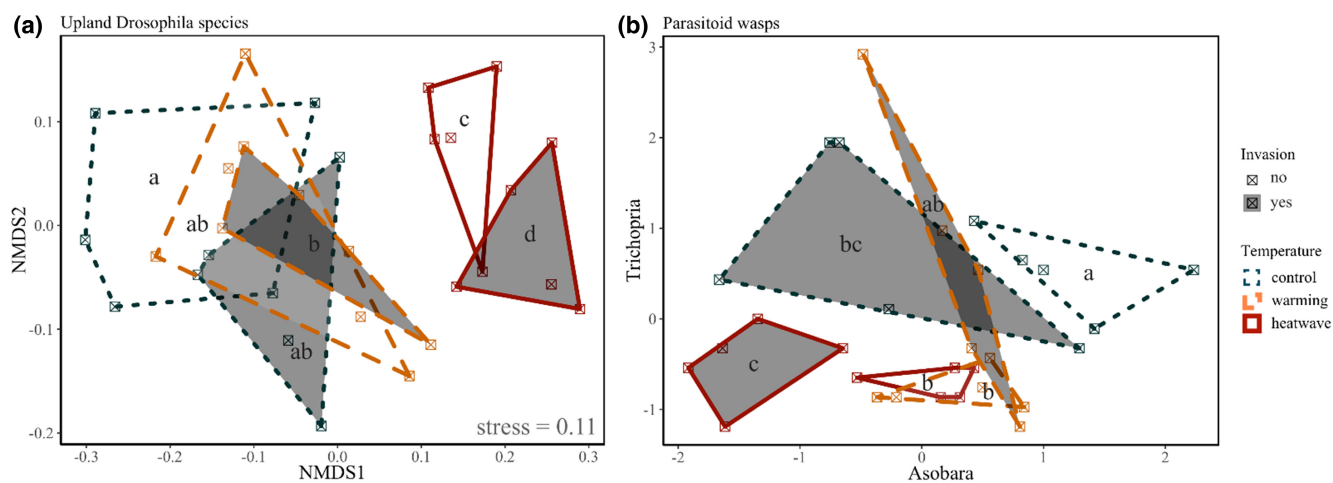


FIGURE 4 Temperature and invasion treatments induced compositional changes of (a) hosts and (b) parasitoids. Hosts composition (four *Drosophila* species) is visualized in two dimensions using non-metric multidimensional scaling. For parasitoids, axes show their standardized abundances. Each point represents a community. Communities of the same treatment are grouped by a minimal convex polygon (replicate = 5). The colours and line types of polygons correspond to temperature treatments, and the shading corresponds to the invasion treatment, as described in the panel. Letters inside the polygons indicate statistically different groups tested by post-hoc non-parametric MANOVA.

Focal species	Predictor	Coefficient	p-value	p_{adjust}
<i>Asobara</i> sp.	Intercept	4.5	<.001	<.001
	Warming	0.13	.04	.59
	Heatwave	-0.09	.17	1
	Invasion	-0.81	<.001	<.001
	Warming × Invasion	0.24	.005	.07
	Heatwave × Invasion	-0.40	<.001	<.001
<i>Trichopria</i> sp.	Intercept	1.22	<.001	<.001
	Warming	0.46	.005	.07
	Heatwave	-1.43	<.001	<.001
	Invasion	0.05	.74	1
	Warming × Invasion	-0.22	.34	1
	Heatwave × Invasion	1.34	<.001	<.001
	Block	-0.01	.96	1

Note: Numbers of individual species are analysed on a log scale. p_{adjusted} is the adjusted p-value following Bonferroni correction. Significant effects after correction for multiple comparisons are in bold. Symbol “x” denotes an interactive effect between two factors.

TABLE 1 The effects of treatments and their interactions on the reproductive success of parasitoids.

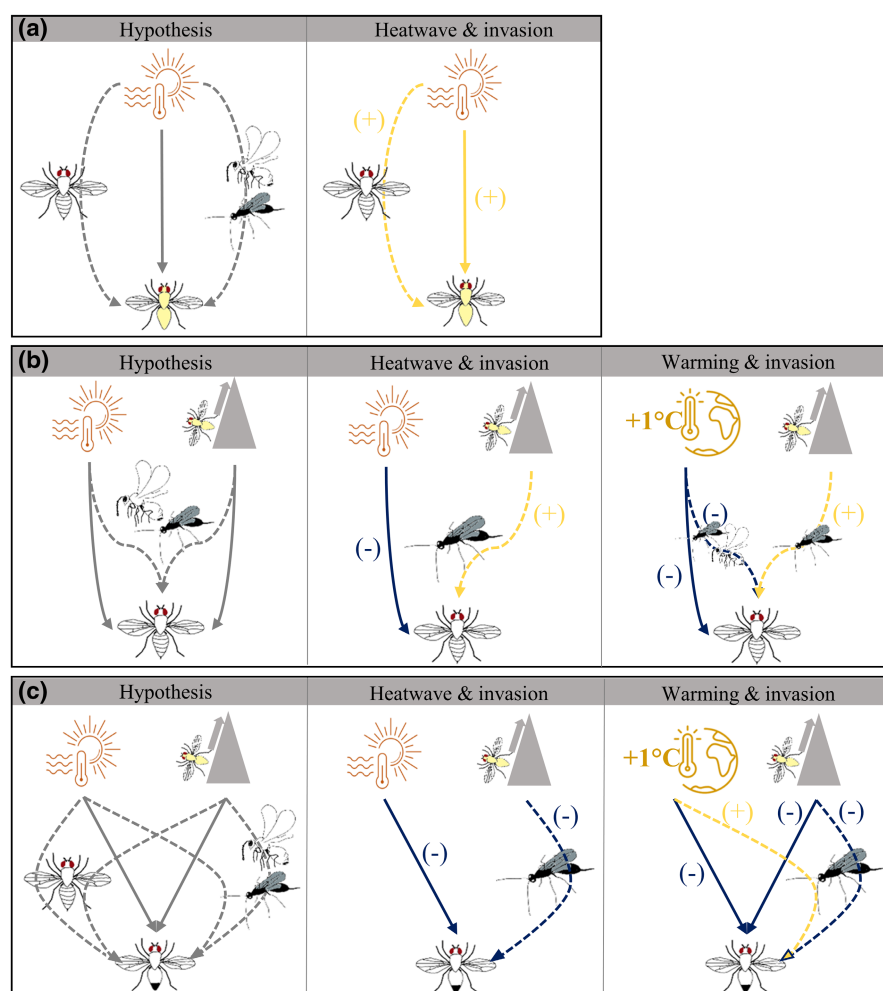


FIGURE 5 The direct and indirect effects of experimental treatments on the reproductive success of (a) *D. pandora* (lowland invader), (b) *D. pallidifrons* (high-elevation specialist) and (c) *D. pseudotakahashii* (high-elevation specialist). The left panel describes the hypothesized direct (solid line) and indirect (dashed lines) effects of experimental treatments on the focal species. The species symbols (defined in Figure 2) on top of the dashed lines represent the species' mediating indirect effects. The middle column shows the effects of heatwave and invasion for each *Drosophila* species. The right column shows the effects of warming and invasion. Blue arrows indicate negative (–) effects and yellow arrows indicate positive (+) effects. Bonferroni correction removed statistical significance only for the effect of invasion on *D. pseudotakahashii* in the “heatwave & invasion” panel.

Asobara and *Trichopria*. For the three focal *Drosophila* species, paths of treatment effects are visualized in Figure 5, and Table 2 shows the corresponding statistical analyses.

For *D. pandora* (Figure 5a), the heatwave treatment directly promoted its reproductive success as the coefficients of the heatwave treatment were similar before and after controlling for putative

TABLE 2 Regression testing the effects of experimental treatments on reproduction of the focal species, with and without controlling for mediating species.

Without mediating species					With mediating species			
Focal species	Predictor	Coefficient	<i>p</i> -value	<i>p</i> _{adjusted}	Predictor	Coefficient	<i>p</i> -value	<i>p</i> _{adjusted}
<i>Drosophila pandora</i>	Intercept	0.67	.05	.51	Intercept	0.67	.04	.41
	Warming	−0.10	.73	1	Warming	−0.31	.28	1
	Heatwave	2.11	<.001	<.001	Heatwave	1.93	<.001	<.001
	Block	−0.44	.04	.40	Block	−0.10	.69	1
					<i>D. pallidifrons</i>	−0.33	<.001	<.001
					<i>Asobara</i>	−0.07	.44	1
					<i>Trichopria</i>	0.06	.12	1
<i>Drosophila pallidifrons</i>	Intercept	3.69	<.001	<.001	Intercept	3.75	<.001	<.001
	Warming	−0.47	<.001	<.001	Warming	−0.17	.003	.056
	Heatwave	−0.19	<.001	.01	Heatwave	−0.19	<.001	.008
	Invasion	0.35	<.001	<.001	Invasion	0.08	.14	1
	Warming × Invasion	0.18	.03	.56	Warming × Invasion	0.01	.90	1
	Heatwave × Invasion	0.09	.26	1	Heatwave × Invasion	−0.09	.26	1
	Block	0.36	<.001	<.001	Block	0.28	<.001	<.001
					<i>Asobara</i>	−0.45	<.001	<.001
<i>Drosophila pseudotakahashii</i>					<i>Trichopria</i>	−0.05	<.001	<.001
	Intercept	2.50	<.001	<.001	Intercept	2.37	<.001	<.001
	Warming	−0.37	.003	.046	Warming	−0.57	<.001	<.001
	Heatwave	−1.98	<.001	<.001	Heatwave	−1.96	<.001	<.001
	Invasion	−0.26	.03	.49	Invasion	0.02	.85	1
	Warming × Invasion	−0.72	<.001	.002	Warming × Invasion	−0.68	<.001	.002
	Heatwave × Invasion	−0.22	.37	1	Heatwave × Invasion	−0.10	.70	1
	Block	0.07	.55	1	Block	0.16	.10	1
					<i>D. pallidifrons</i>	−0.06	.08	1
					<i>Asobara</i>	0.32	<.001	<.001
					<i>Trichopria</i>	0.06	.04	.67

Note: Numbers of individual species are analysed on a log scale. p_{adjusted} is the adjusted *p*-value following Bonferroni correction. Statistically significant effects after correction for multiple comparisons are printed in bold. Symbol “x” denotes an interactive effect between two factors.

mediating species (coefficient_{before} = 2.12 [$p_{\text{adjusted}} < .001$] vs. coefficient_{after} = 1.93 [$p_{\text{adjusted}} < .001$]). The number of *D. pallidifrons* negatively correlated with the number of *D. pandora* (coefficient = −0.33, $p_{\text{adjusted}} < .0001$), reflecting competition. The effects of both parasitoid species were non-significant.

Without controlling for other species, warming and heatwave both significantly reduced the reproduction of *D. pallidifrons*. Invasion significantly increased the reproduction of *D. pallidifrons*. When scaled numbers of parasitoids were added to the model, the offspring numbers of both parasitoid species were negatively associated with the number of *D. pallidifrons* offspring, reflecting parasitism. The effect of the heatwave treatment was direct (Figure 5b) as its effect was not changed (Figure 5b; coefficients before vs. after controlling for other species: −0.19 [$p_{\text{adjusted}} = .01$] vs. −0.19 [$p_{\text{adjusted}} = .008$]). The negative impact of warming treatment was partly mediated by the positive effect of warming on parasitoids (Figure 5b), as the coefficient of warming remained negative despite

a reduction in its magnitude after controlling for the numbers of parasitoids (coefficients before vs. after: −0.47 [$p_{\text{adjusted}} < .001$] vs. −0.17 [$p_{\text{adjusted}} = .056$]). The positive effect of the invader on *D. pallidifrons* appeared to be completely mediated by parasitoids (Figure 5b), as there was no direct effect of the invader after controlling for parasitoid numbers (coefficient_{before} = 0.35, [$p_{\text{adjusted}} < .001$] vs. coefficient_{after} = 0.08, [$p_{\text{adjusted}} = 1$]).

For *D. pseudotakahashii* (Figure 5c), the overall effects of warming and heatwave treatments were significantly negative. Invasion significantly reduced *D. pseudotakahashii* reproduction under the warming treatment, but the effects of invasion were more marginal under other temperature treatments, becoming non-significant following Bonferroni correction (Table 2). When mediating species were added as explanatory variables, the number of *Asobara* parasitoids was positively associated with *D. pseudotakahashii*. The direct effect of warming was more negative than its overall effect (coefficients before vs. after controlling for other species: −0.37 [$p_{\text{adjusted}} = .046$] vs.

-0.57 [$p_{\text{adjust}} < .001$]). The negative effect of heatwaves was mostly direct as it remained the same after including mediating species in the models (coefficient_{before} = -1.98 [$p_{\text{adjust}} < .001$] vs. coefficient_{after} = -1.96 [$p_{\text{adjust}} < .001$]). The negative effect of the invader on *D. pseudotakahashii* appeared to be mediated by the *Asobara* parasitoids as coefficients of invasion treatment were reduced to zero (in control temperature and heatwaves treatments) or partially reduced (in warming treatment) after controlling for its number.

4 | DISCUSSION

Our experimental community corresponds closely to the natural community of closely interacting *Drosophila* and parasitoids in the high-elevation rainforest of the Wet Tropics, Australia. We found that this community of up to seven interacting species in two trophic levels can respond in complex and unpredictable ways to different ways of rising temperatures and climate change-facilitated invasion, mediated by direct and indirect effects within the interaction network. Heatwaves reduced competitive pressure between the local and the lowland *Drosophila* species, enabling the establishment of invaders in the upland community. Periodic extreme heat events and a constant modest increase in temperature (+1°C) influenced component species in different, and sometimes contrasting, ways, leading to distinctive changes in community structure. The invasion of lowland species additionally altered the composition of the wider community. Surprisingly, the invasion treatment significantly reduced the single-generation reproductive success and abundance of a larval parasitoid, *Asobara*, even when the invading species did not establish. Lastly, our data allow us to unravel potential mechanisms underlying these shifts in community composition and highlight the key role of cascading effects mediated by parasitoids within this relatively simple bi-trophic network.

4.1 | Extreme heat events release competitive exclusion

Our results support the hypothesis that extreme heat events facilitate invasion in our study system. Based on the asymmetrical shape of thermal performance curves, theories predict that the temporal distribution of rising temperatures is critical for organismal performance (Dell et al., 2014; Vasseur et al., 2014). At the community level, Polazzo et al. (2023) showed empirically that repeated, short but intense thermal stresses can cause more profound structural changes in communities than longer but less intense stresses. Similarly, the short-term and periodic heatwave treatment in our study had a stronger direct negative impact than constant, modest warming on the single-generation reproductive success of the resident flies. This large decrease in the reproductive performance of the resident species reduced the biotic resistance of the dominant competitors, creating a temporal window when the low-elevation species, *D. pandora*, was able to build up its numbers.

Similar demographic changes induced by extremes have been observed in studies of unmanipulated natural communities, facilitating the population growth of colonising species (Battisti et al., 2006; Jiménez et al., 2011; Sorte et al., 2010; Thibault & Brown, 2008; Wernberg et al., 2013). In comparison, species ranges and community compositions are sometimes stable despite substantial warming in mean temperature (Stuart-Smith et al., 2010). It is a widespread phenomenon that species are living below their thermal optima and that performance drops quickly once the body temperature exceeds optima (Deutsch et al., 2008). This general property of thermal performance curves distinguishes heatwaves from a homogeneous increase of accumulative heat (as in our warming treatment). Nevertheless, the magnitude, duration and timing of warming make a difference to its biological impact, which was not fully explored in our design of the warming treatment. For example, overwintering warming is associated with the range expansion of species whose survival is constrained by the coolest temperature in winter (Crozier, 2004; Figueira & Booth, 2010). Therefore, understanding the mechanisms (e.g., winter temperature, interspecific competition) preventing the establishment of species beyond their current range boundaries is key to predicting which climate factors will drive range shifts. High-resolution observational studies (Harley & Paine, 2009; Thibault & Brown, 2008) and experimental studies (Jentsch et al., 2007) like ours can be complementary to reveal the qualitative distinction between “press” (warming) and “pulse” (heatwave) climatic stresses in structuring biological communities (Easterling et al., 2000; Harris et al., 2018).

4.2 | The impact of invading species is sensitive to climate conditions

Novel interactions involving or driven by range-shifting species could become a crucial driver of community change (Descombes et al., 2020; Wallingford et al., 2020). Compared with typical biological invaders from other bioregions, climate-facilitated invaders from neighbouring communities are more likely to be sensitive to deviations from local temperatures, as they are already at the edge of their realized thermal niche. In our study, the impact of the low-elevation specialist *D. pandora* on resident *Drosophila* community composition was only significant when the community also experienced heatwaves, consistent with our third hypothesis. Alexander et al. (2015) similarly found that novel competitive interactions were more influential when the abiotic conditions matched more closely those typical of the novel species' source populations.

Notably, the effects of invasion in our experiment were sometimes significant on individual species even when the introduction had not resulted in the successful establishment of *D. pandora*. For example, invasion treatment significantly decreased the reproductive success of *Asobara* parasitoids and increased the abundance of *D. pallidifrons* under all thermal regimes. While the successful establishment of the invading species in the heatwave treatment indeed generated the largest shift in both the *Drosophila* and parasitoid

communities, our results on individual species suggest that the initial dispersal and subsequent interactions between neighbouring species and residents could also have a substantial influence on host-parasitoid dynamics.

4.3 | Top-down regulation of coexistence

Parasitoid performance is sensitive to both environmental temperatures and host species composition (Hance et al., 2007; Thierry, Pardikes, Rosenbaum, et al., 2022; Thierry, Pardikes, Ximénez-Embún, et al., 2022). Both heatwave treatment and invasion treatment in our study negatively affected the total number of parasitoids, leading to a top-down effect on host species composition. Introducing *D. pandora* reduced the reproductive success of *D. pseudotakahashii*. Our analysis revealed that this was not a result of direct resource competition but was mediated by parasitoids. The reproductive successes of *D. pseudotakahashii* and *Asobara* parasitoids were associated positively. *Asobara* parasitoids cannot successfully develop on *D. pseudotakahashii* (Chia-Hua Lue, personal communication). However, parasitoids could alter patterns of competitive dominance among hosts (Cornell & Pimentel, 1978). A likely explanation for our observation is that parasitism pressure reduced competition between *D. pseudotakahashii* and other hosts that are susceptible to *Asobara*, such as *D. pallidifrons*, promoting coexistence between the dominant and inferior competitors (Figure 6a).

4.4 | Enemy-mediated facilitation

We observed unexpected facilitation of *D. pallidifrons* by the invader, *D. pandora*, both in the short term (single-generation reproductive success) and long term (abundance in the census). *Asobara* parasitoids can

develop successfully on monocultures of *D. pandora* (Nancie Bowley and Jinlin Chen, unpublished data). Therefore, the addition of *D. pandora* was expected to decrease the population size of *D. pallidifrons* in the long term through direct resource competition and apparent competition (Holt, 1977). Instead, our regressions imply that introducing *D. pandora* lowers the efficiency of *Asobara* parasitizing *D. pallidifrons*. This effect was significant even when most offspring of the introduced *D. pandora* did not successfully develop into adults (in upland temperature or warming regimes). We suspect that the presence of *D. pandora* larvae spared *D. pallidifrons* larvae from attack by parasitoids (Figure 6b), a form of apparent facilitation or apparent mutualism (Holt & Lawton, 1994; Long et al., 2012; Wilbur & Fauth, 1990). Then a large portion of parasitized *D. pandora* did not develop to pupation (and thus failed to generate parasitoid offspring) as a result of competition with *D. pallidifrons*, which is a superior competitor (Chen & Lewis, 2022). An additional possibility is that developing in *D. pandora* hosts leads to low-quality *Asobara* adults, perhaps because of their small body size or unsuitability of *D. pandora*; its close relatives (i.e. *Drosophila bipectinata* and *D. pseudoananassae*) cannot be parasitized successfully by *Asobara* (Chia-Hua Lue, personal communications). If so, low-quality parasitoids would then have lower efficiency in parasitizing *D. pallidifrons*. Our example highlights that the consequences of novel host species integrating into host-parasitoid networks may not be correctly inferred on the basis of competition and parasitism studied in pairwise fashions. Experiments of how host competition alters parasitism success (in quantity and quality) will provide key insights.

4.5 | Limitations

Our manipulation of temperature and invasion treatments can reveal their overall causal effects. However, the underlying pathways and mechanisms are more speculative, based on analyses of associations

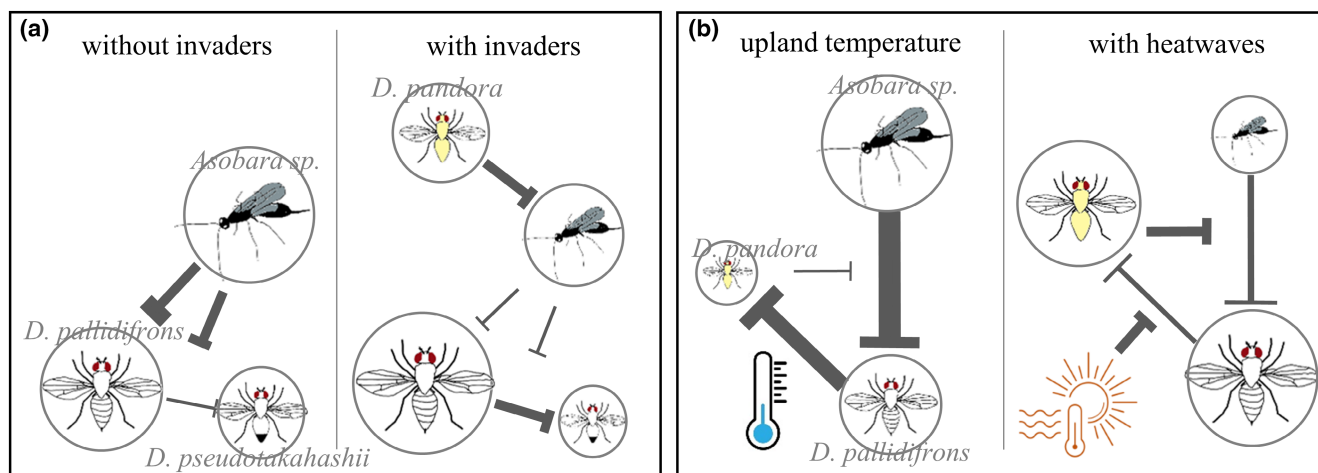


FIGURE 6 Key species interaction motifs that contribute to the community responses to treatments. (a) Summary of how the invading species, *D. pandora*, changes the parasitoid-mediated coexistence between the dominant and inferior *Drosophila* species. (b) Summary of how the heatwave treatment changes the outcomes of species interactions between the invader (*D. pandora*), the high-elevation specialist (*D. pallidifrons*) and their shared parasitoids. Symbols of species were accompanied by their species names when first appeared. Sizes of circles qualitatively represent the abundance of the adult populations. The thickness of the arrow represents the strength of the negative impact.

and prior knowledge of species interactions. The invading species' modification of the *Asobara*-*D. pallidifrons* interaction, and the modification by *Asobara* of *D. pseudotakahashii*-*D. pallidifrons* competition need validation by direct experimental tests. Similar experiments that use communities with or without parasitoids will allow confirmation of how treatment effects are mediated by parasitoids.

While *Drosophila*-parasitoid communities provide tractable units for study at small spatial scales in the laboratory, our experimental setup inevitably differs in several respects from field conditions. One obvious difference is that parasitoids in our experiment are confined to a small space where it is easy for them to locate their hosts. This may explain why the larval parasitoid, *Asobara*, reached high abundance in our experimental communities. If we were to reduce the parasitism rate by increasing the difficulty of foraging for parasitoids, our key results would not be expected to change: (1) the heatwave-facilitated establishment of the lowland invader was not mediated by parasitoids, and it would only become more likely as *D. pandora* is susceptible to both parasitoids; (2) the apparent facilitation effect, resulting from the sparing effect of additional hosts, would become stronger if parasitism was not saturated. Altered ease of foraging might change competitive outcomes between our larval parasitoid and pupal parasitoid, and over-emphasize the mediating effects of *Asobara* parasitoids on the coexistence between hosts. Nevertheless, our experiment reveals the potential for parasitoid-mediated effects. Besides, the situation of high parasitism rate has been seen in agricultural systems where parasitoids are constantly added as biocontrol agents or even in some natural systems (Carton et al., 1986).

4.6 | Practical implications for research, conservation and resource management

Our experiment shows that extreme events such as heatwaves can temporarily and strongly reduce the biotic resistance of local communities to invading species. This suggests that during and shortly after extreme climatic events may be the most crucial and effective time to facilitate the full recovery of the resident community (e.g., if endemic species within it are threatened by invasion), for example by manually removing invading species that are considered harmful (Wallingford et al., 2020). Our study also illustrates that parasitoids are sensitive to both heatwaves and invasion. Parasitoids are widely introduced to regions outside their natural ranges as biocontrol agents (Hardy et al., 1994). When selecting such biocontrol agents, applied entomologists may need to consider the interactions between changing climatic conditions and possible invading species, since climate-facilitated novel interactions could unexpectedly inhibit the integration and effectiveness of biocontrol agents.

4.7 | Conclusion

The interdependence of abiotic and biotic drivers poses a major challenge to efforts to predict biological responses to climate change. Our

work validates the role of heatwaves in facilitating invasion success, a process that is likely to occur more frequently in future alongside extreme climatic events. Constant warming and heatwave treatments had distinctive direct effects and associated biotic risks, emphasizing the need for more informed predictive research and targeting management practices against different components of climate change. Finally, top-down control by parasitoids in our relatively simple bi-trophic community played a significant role in mediating the impacts of climatic and biotic changes. In our experiment, parasitoids enhance the impact of the invading species, mediated by interaction modifications that were not expected a priori; in particular, we found evidence for long-term facilitation between *Drosophila* hosts mediated by a larval parasitoid, a dynamic that has rarely been documented. Our work highlights the uncertainty of the ecological consequences of interactions between parasitoids and invading hosts under changing climates. It also underlines the importance of manipulative research in revealing the key species or interaction motifs that influence communities' responses to climate change.

AUTHOR CONTRIBUTIONS

Jinlin Chen initiated the project. Jinlin Chen and Owen T. Lewis both contributed to the development of ideas. Jinlin Chen designed and conducted the experimental work. Jinlin Chen analysed the results and led the writing of the manuscript. Owen T. Lewis contributed to the writing. Both authors contributed to the revision.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at <https://doi.org/10.5281/zenodo.7643868>.

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SUPPORTING INFORMATION

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