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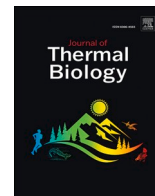
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The consequences of heatwaves for the reproductive success and physiology of the wingless sub-Antarctic fly *Anatalanta aptera*

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ABSTRACT

Sub-lethal effects of warming temperatures are an important, yet sometimes overlooked impact of climate change that may threaten the long-term survival of numerous species. This, like many other effects of climate change, is especially concerning for cold-adapted ectotherms living in rapidly warming polar regions. This study examines the effects of warmer temperatures on cold-adapted Diptera, using the long-lived sub-Antarctic sphaeroцерid fly, *Anatalanta aptera*, as a focal species. We conducted two experiments to assess heat stress in adult flies, one varying the intensity of the heat stress (daily heating from 4 °C to 8 °C, 20 °C, or 24 °C) and one varying the frequency of heat stress exposure (heating from 4 °C to 12 °C every one, two, or three days) and examined consequences for reproductive success and metabolic responses. We found that more heat stress reduced reproductive output, but not timing of reproduction. Surprisingly, individuals sampled at different times during heat stress exposure were undifferentiable when all metabolite concentrations were analysed with redundancy analysis, however some individual metabolites did exhibit significant differences. Overall, our findings suggest that warmer temperatures in the sub-Antarctic may put this species at greater risk, especially when combined with other concurrent threats from biological invasions.

1. Introduction

Climate change poses a significant threat to biodiversity at all organisational levels through countless impacts on environmental, biological and ecological processes (Bellard et al., 2012). Ectothermic organisms, such as insects, are particularly affected by climate because their body temperatures, and therefore biological cycles, are largely controlled by the temperature of the environment (Everatt et al., 2015). Thermal variations, and the increased occurrence of extreme meteorological events, increase the risks and frequency of short exposure to extreme temperatures, which can constitute significant stress for these animals (Colinet et al., 2015; Renault et al., 2022). In some instances, the duration and/or intensity of the thermal stress can have biological and physiological impacts causing moderate to serious injuries necessitating for the organism to mount compensatory responses (Bodlah et al., 2023; Neven, 2000). The repair of temperature-induced physiological damage may occur at the cost of other biological functions, such as reproductive ability or longevity (Bahrndorff et al., 2021; Walsh et al., 2021). There is increasing evidence that the effects of novel temperature regimes, and extreme thermal events, alter the reproductive success of

individuals (Walsh et al., 2021), with the reduction of fertility due to heat stress being of particular concern (Jørgensen et al., 2006; Zizzari and Ellers, 2011).

In cold environments, such as polar, subpolar, and mountainous regions, insects may suffer more intensely from the effects of warming temperatures and extreme thermal events such as daily heat spikes (Bahrndorff et al., 2021). Despite this, data on the heat tolerance of polar arthropods is lacking, inhibiting the application of many predictive modelling approaches (Bahrndorff et al., 2021). Moreover, polar species often exist primarily in range-edge populations and have limited options for range shifting or expansion to escape stressful conditions (Rehm et al., 2015). This is especially true for species on small, geographically isolated sub-Antarctic islands where refuge options in the face of warming temperatures (Daly et al., 2023) are scarce, which could drive some species to extirpation or extinction as they are unable to withstand novel regimes (Harvey et al., 2023).

One at risk species is the sphaeroцерid fly *Anatalanta aptera* Eaton, 1875; Renault et al. (2022). This flightless endemic is active year-round and is notable for its exceptional longevity (up to 2 years in mostly the adult stage in captivity), cold tolerance (Vernon and Vannier, 1996),

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large altitudinal range (living from sea level to 600 m above sea level, higher than any other insects in the region (Hullé and Vernon, 2021)), and improved locomotor ability in response to cold stress in old age (Lalouette et al., 2010). *A. aptera* is an important decomposer (Vernon et al., 1998), meaning threats to its long-term survival could have significant ecological impacts, but we are just beginning to understand its capacity to cope with heat stress. Previous experiments have shown that individuals can survive temperature spikes mimicking projected local warming scenarios for up to 10–15 days before survival is hampered (Renault et al., 2022). Here, we were thus interested in examining if such exposures could also affect reproduction. Sub-lethal effects, such as physiological responses and delayed or reduced reproduction in response to heat stress, could pose demographic threats and need to be better understood to predict long-term persistence (Bahrndorff et al., 2021).

In particular, we lack understanding of the whole set of physiological mechanisms involved in enhanced heat tolerance and plasticity, resulting in shorter recovery times and enhanced survival after heat stress (Bahrndorff et al., 2021; Buchon et al., 2014; Black and Subject, 1990). However, metabolomics is a powerful tool that can detect system-wide changes in physiological homeostasis and phenotype, in an unbiased fashion, and in a wide range of ecological contexts (Colinet and Renault, 2018; Dahl and Renault, 2022; Noer et al., 2022). Such approaches have been used to show how metabolite concentrations in the Antarctic midge *Belgica antarctica* Jacobs, 1900 (Diptera, Chironomidae) respond to heat stress, allowing for the formation of hypotheses about the physiological cycles and pathways affected (Michaud et al., 2008). Specifically, researchers have found increases in both α -ketoglutarate and putrescine and decreases in glycerol, glucose, and serine in response to stress exposure indicating possible effects on the Krebs cycle and glycolysis (Michaud et al., 2008). Comparison of metabolite profiles has also been useful in understanding how Arctic insects cope with large daily temperature fluctuations between microhabitats and provided evidence that concentrations are actively altered on a daily basis (Noer et al., 2022). Additionally, examination of metabolite changes can contribute to a more mechanistic understanding of heat hardening (Kalra et al., 2017), which is crucial to predict long-term sub-lethal impacts like reduced fecundity due to global change.

In order to better understand the future impacts of warming on cold-adapted insects, we examined the effects of heat spikes on the reproductive success and physiology of the fly *A. aptera*. We exposed flies to environmental conditions representing current and future climatic conditions in their native range with different intensities and frequencies. We measured the effects of these conditions on their reproductive timing and output (number of hatching larvae, developmental rates). We hypothesise that flies exposed to hotter or more frequent heat spikes will have decreased reproductive success as they should incur more damage, and therefore more energetic cost, than flies in cooler non-stressful conditions. We also expect that reproduction will be delayed in flies exposed to warmer conditions, as they should require time to repair metabolic damage prior to breeding. To look for physiological signatures of heat stress in this species, we also explored metabolic consequences of experimental heating on a smaller sample of these flies. Concerning physiological responses to heat, we hypothesise that heating will induce a distinguishable physiological response between individuals exposed to heat stress and those kept at constantly low temperatures (the controls). We expect bigger differences between controls and individuals exposed to high heat than more moderate heat. Additionally, due to heat hardening, we expect greater differences between control individuals and individuals sampled after one heat exposure as compared to multiple exposures.

2. Material and methods

2.1. Sampling and rearing of flies

A. aptera individuals were sampled in two locations in north-western part of Ile de la Possession (Crozet Archipelago, 46°24'27"S 51°45'27"E): Jardin Japonais and Baie Kirch (Fig. 1) in January 2018. Baie Kirch and Jardin Japonais have similar average temperatures, though Jardin Japonais has higher maximum temperatures possibly due to the presence of a penguin colony (Fig. A1). For the subsequent rearing, individuals were bred at the laboratory of the Alfred Faure research station (Ile de la Possession). Adult *A. aptera* were randomly selected from these two subpopulations, and bred together to create a lab population which was further used for the experiments. Although we lack genetic data on these populations, and that could cause important differences relating to heat stress, other studies have found no differences in the survival of individuals from these populations (Renault et al., 2022).

The flies were reared for two generations in groups of 40–50 under controlled conditions: 4 ± 1 °C and natural L:D cycle in plastic enclosures (12 × 9 × 5 cm) containing a layer of 3 cm of dark sand (making it easier to see the whitish eggs and larvae) from Baie du Marin (Ile de La Possession, Crozet Archipelago). Prior to being used for experiments, the sand was sieved at 2 mm, washed with freshwater and dried at 60 °C overnight. Every 10 days, the sand of the enclosures was humidified; the flies were fed *ad libitum* with chicken egg yolks as described by Vernon and Vannier (1996) and were provided water with a 2 mL microtube closed with a wet cotton mesh. New pupae were regularly removed and subsequently stored in new enclosures to control the age of the individuals. All flies were mature adults (at least 70 days old) when exposed to experimental trials. Flies were kept at a baseline temperature of 4 ± 1 °C and exposed to different temperature conditions depending on experimental groups.

2.2. Effects of daily exposure to heat spikes on reproduction

To investigate the effects of heat stress on the reproduction of adult *A. aptera*, individuals were exposed to one of three experimental treatments:

- (i) A control group was exposed to thermal conditions typically found in the species' native range during the summer period which corresponds to the species' breeding season: 34 females and 49 males were exposed to temperatures oscillating from 4 to 8 °C on a daily basis (Fig. A2a).
- (ii) the second group (20 °C) (i.e., 34 females and 26 males) was exposed to moderate warming fluctuating from 4 to 20 °C and held at the maximum temperature of 20 °C for 2 h (Fig. A2b).
- (iii) The third group (24 °C), which included 54 females and 59 males, was experimentally exposed to strong warming, with temperatures oscillating from 4 to 24 °C on a daily basis, and the flies were held at the maximum temperature of 24 °C for 2h (Fig. A2c).

Temperature conditions experienced by flies in from group 2 (20 °C) and 3 (24 °C) are atypical, but can and do occasionally occur in the region (e.g., Fig. A1). Each of the groups was placed in separate incubators (Panasonic, MIR154) that allowed an accurate control of the exposure duration at each desired experimental temperature. Following a 12-day exposure period at each condition, groups of 10 males and 10 females per treatment (8, 20, and 24 °C) were placed in separate enclosures in the control condition of 4 °C. Observations began after a week in the control condition to give the flies time to reproduce. Afterwards, the effects of the thermal treatment on reproduction were examined by counting the number of eggs and the number of larvae over a 70-day observation period during which all eggs laid within the first 30 days should have hatched.

The small size and white-to-transparent colour of eggs prevented

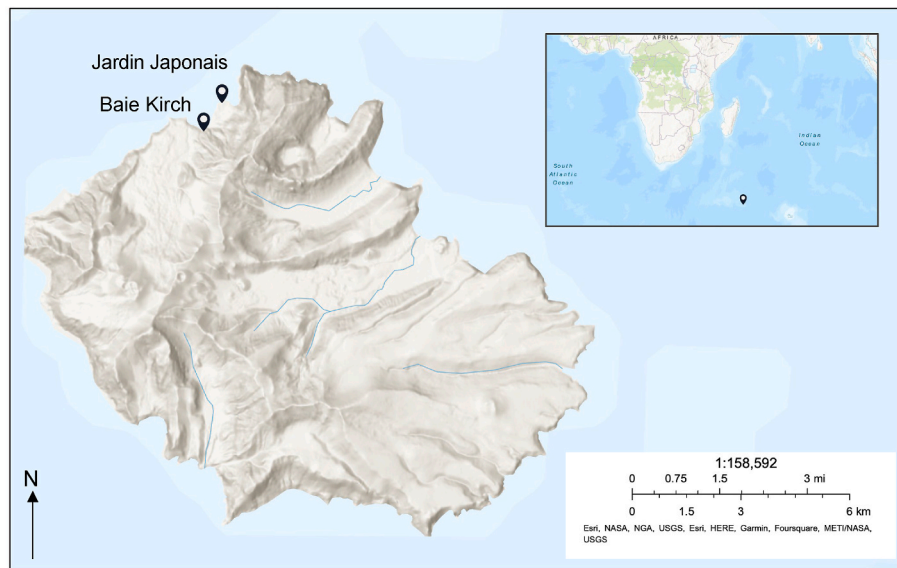


Fig. 1. Map showing sampling locations of *Anatalanta aptera* individuals for thermal stress experiments in the Crozet Archipelago. Adults were selected randomly among a generation of flies from Baie Kirch and from Jardin Japonais sites on Crozet's Possession Island (46° 24' 41" S, 51° 45' 22" E) and bred together to form one experimental population.

highly precise counting and so categorical values were defined for reporting egg presence (0 = 'Absence', 1–10 = 'Few', >10 = 'Many' eggs). The absolute number of larvae was recorded. Any eggs or larvae produced before the observation period began were removed and not counted. During the first seven days of the experiment, observations were taken every day. Thereafter, observations were taken every two to three days for 33 days (days 8–40) when copulations were expected to decline, then every five days for 10 days (days 41–50), and finally every 10 days for 20 days (days 51–70) to check if individuals kept reproducing.

We used an ordinal logistic regression with the `polr` function from the R package `mass` (Venables and Ripley, 2002) to test for association between treatment category and egg category. We modelled the number of larvae present on observation days using a generalized additive model (GAM) with the `bam` function from the R package `mcgv` (Wood, 2011), and tested the different treatments for significant post-hoc comparisons using the Wald test using the `wald_gam` function from the R package `itsadug` (van Rij et al., 2022). All statistical analyses were done using R version 4.1.1 (R Core Team, 2021).

2.3. Effects of daily exposure to heat spikes on metabolites and metabolic profiles

To study the metabolic consequences of daily heat spikes on the flies, 60 female and 60 male flies were exposed to conditions 8 °C and 24 °C (Figs. A2a and c). In this part of the experiment, the duration of the exposure was reduced to seven days to limit the risks of flies entering heat coma which makes the assessment of their status (dead or alive) more difficult at the end of the exposure period. During the course of the treatment, 12 individuals (six females and six males) from each group were taken at different time points throughout the exposure (Fig. A2): (a) on the first day, just after the 24 °C heat peak (14 h into the experiment (T₁₄)), and in the 6 °C cool down phase following the heat peak (18 h into the treatment (T₁₈)), (b) at the seventh day, at the same periods of the daily cycle (T₇₋₁₄ and T₇₋₁₈, respectively), and (c) the last 12 flies from each group were sampled at the end of the cycle (T_{end}) (Fig. A2). Several individuals did not survive the duration of the experiment and thus were not included in the biochemical assays (Table A1). Six additional flies of each sex were also taken from the laboratory rearing and served as controls (T₀), i.e. they were not

subjected to heating and were kept at a constant 4 °C.

To conduct the metabolomic analysis, each individual was weighed (mg) before being snap-frozen with liquid nitrogen. Samples were then ground with two tungsten beads at 25 hz for 1 min 30 s with a bead beater (MM400 Retsch GmbH, Haan, Germany) in a mixture of 600 µL of methanol and 300 µL of chloroform. Once the ground material was obtained, 600 µL of ultra-pure water was added (methanol/chloroform/water ratio: 2/1/2, v/v/v) and samples were vortexed, and then centrifuged for 10 min at 4000 g. A volume of 850 µL of the supernatant liquid, which contained the extracted metabolites for the subsequent metabolomics assays, was collected for each sample. Then, an aliquot of 120 µL of the supernatant was pipetted and transferred to a clean glass vial. All samples were vacuum-dried (Speedvac MiVac; Genevac Ltd., Ipswich, England) for 40 min at room temperature.

Metabolic composition of the samples were described as in Thiébaud et al. (2021). The dry residue of each sample was re-suspended in 30 µL of 25 mg mL⁻¹ methoxyamine hydrochloride in pyridine. After incubation at 40 °C for 60 min under orbital shaking, a 30 µL volume of BSTFA (N,O-Bis(trimethylsilyl)trifluoroacetamide; CAS number: 25561-30-2) was added to each sample. Derivatization was conducted at 40 °C for 60 min under orbital shaking. The timing of sample preparation was standardised by using a CTC CombiPal autosampler (PAL System, CTC Analytics AG, Zwingen, Switzerland). A gas chromatography/mass spectrometry (GC/MS) platform, consisting of a 5977B GC/7890 MS platform (Agilent Technologies, CA, USA) was used to analyse the samples. One µL of the sample was injected (split ratio: 25:1; Injector temperature: 250 °C) and the oven temperature was raised from 70 to 170 °C at 7 °C min⁻¹, from 170 to 280 °C at 7 °C min⁻¹, and from 280 to 320 °C at 15 °C min⁻¹. At the end of the temperature ramps, the oven remained at 320 °C for 4 min. The samples were run under the selected ion monitoring (SIM) mode rather than the full-scan mode; thus, we only screened for the 60 pure reference compounds included in our custom spectral database. Calibration curves for 60 pure reference compounds at concentrations of 5, 10, 20, 50, 100, 200, 500, 750, 1000, and 1500 µM were established.

Chromatograms were deconvoluted using MasHunter, and metabolite concentrations were quantified based on the calibration curves of each reference compound. We divided all samples by the body mass of each individual and considered the different steps of the preparation of the samples that resulted in dilutions of metabolite quantities prior to

analysis. We conducted one-way ANOVAs for each metabolite using sampling time, sex, and treatment as factors with a correction for false discovery rate (Benjamini and Hochberg 1995) to look for changes in individual metabolites using the R package *MetabolomicsBasics* (Jaeger and Liseč 2018). We expected differences in certain metabolites observed to change in other flies with heat stress and for those differences to be larger between flies in the treatment group than the control. We analysed variations in metabolite profiles between individuals sampled at different points during the heat stress with redundancy analysis (RDA) with the R package *vegan* version 2.6–4 (Oksanen et al., 2012) to see if differences could be observed in individuals before, during, and after exposure. We performed this separately for males and females exposed of each treatment and tested for significance with the ANOVA function in the R package *vegan* (Oksanen et al., 2012).

2.4. Effects of varying frequency of thermal fluctuations on fecundity

In natural environments, average temperatures may vary in a relatively uniform manner, or through larger sporadic increases in daytime or night-time temperatures. For this reason, it is important that investigations not only consider the effects of varying thermal stress intensities on the biology and ecology of insects, but also examine the effects of different frequency and, to a certain extent, unpredictability of thermal fluctuations. To compare the effects of different warming scenarios, and more particularly improve our understanding of different time period occurrence of warmer periods, flies were assigned to one of four following conditions (a) control condition (Freq0) held flies at a constant temperature of 4 °C, (b) three treatment groups experienced four (Freq4), six (Freq6) or twelve (Freq12) cycles of light warming from the baseline temperature of 4 °C–12 °C (Fig. A3). In this experiment, 12 °C was used as the heat stress temperature, as little fly mortality was reported over 12d in this condition (Renault et al., 2022). Each experimental condition had three replicates, each replicate consisting in 34 adult flies (17 females and 17 males). Flies were provided water - but no food - during the 12-day incubation period. Following incubation, individuals were transferred to optimal rearing conditions and left to breed at a temperature of 4 °C.

The plastic enclosures containing the flies were moved every day for one week into a cold room (7–8 °C max.) for initial observations. Following this observation time, observations of the number of larvae, pupae, and active copulations were recorded once per week. After three months of observations, adults were removed and the spacing of observations moved to once per 10 days. Adult offspring were removed following emergence, starting around 6 months after the first larvae hatched, to prevent production of another generation. The experiment was carried out until all enclosures were completely empty during a period of approximately one year (July 2019 to July 2020). As with the stress intensity experiment, we employed GAM using the *bam* function from *mcgv* (Wood, 2011) and a post-hoc Wald test using R package *itsadug* (van Rij et al., 2022) function *wald.gam* to analyse the number of larvae produced and the number of pupae produced.

3. Results

3.1. Effects of daily exposure to heat stress on reproduction

To understand the effects of varying intensities of daily heat stress, we subjected three groups of *A. aptera* to daily stresses with different maximum temperatures and measured effects on the fecundity of these flies. Using ordinal logistic regression, we found that egg production was significantly decreased in groups exposed to 20 °C (OR = 12.00, 95% CI = 5.39, 28.22, $p < 0.05$) or 24 °C (OR = 7.30, 95% CI = 3.76, 14.72, $p < 0.05$) relative to control groups exposed to 8 °C warming. As larvae were more reliably counted than eggs, were able to analyse the number of individual offspring. We modelled the number of individuals using a GAM smoothed by day of experiment, which explained 74.8% of the

deviance. Post-hoc analysis of the GAM (Fig. 2) with a Wald test to compare treatment levels showed that the moderate heat stress 20 °C group produced significantly more larvae than the control (Wald's test statistic = 6.279, $p = 0.012$) and high heat stress 24 °C group flies (Wald's test statistic = 19.519, $p = 9.96e-06$), but that the 24 °C group was not statistically different from the control (Wald's test statistic = 3.7, $p = 0.054$). The number of larvae began to increase from around day 40 in all groups (Fig. 2).

3.2. Effects of daily exposure to heat spikes on metabolites and metabolic profiles

To better understand the relationship between heat exposure and metabolic responses, we examined differences in individual metabolites as well as metabolic profiles of individuals at several time steps during one week of daily heat stress exposures. Individuals were exposed to 8 °C or 24 °C from a baseline of 4 °C, as with the thermal intensity experiment, and were sampled for their metabolic profiles at select time points before, during, and following heat exposure (see Fig. A2 for specific regimes). We performed this procedure for 12 adult flies, 6 female and 6 male. Several individuals did not survive and so were excluded from the analysis (see Table A1 for counts). No individuals were sampled at the 7–18 timepoint in the group exposed to 8 °C (control flies). We expected differences in certain metabolites (glycerine, glucose, serine, and trehalose) between individuals sampled at different time points throughout the heating treatments in heating regimes. However, examination of individual metabolites using ANOVAs with treatment, timing, and sex as factors (Table A2) showed that there were no significant differences in concentrations of any of these metabolites for individuals sampled at different time points, treatments, or sexes ($p > 0.05$). The one exception was serine, whose concentrations were significantly different between sexes ($p = 0.00016$), however not for treatment or timing ($p > 0.05$). We did observe significant differences between concentrations of some metabolites ($p < 0.05$) sampled at different time points, most of which were acids (aminobutyric acid, aspartic acid, citric acid, malic acid, leucine, and valine) with the exception of maltose (Table A2).

We also tested for differences in metabolic profiles between individuals sampled at different times (i.e., before, during, and after heating) for each sex and treatment. Sex-partitioned RDA ordinations of individuals from both treatments revealed that the timing during the exposure when individuals were sampled explained a small amount of the variance (maximum 24%, Fig. A4). However, significance testing showed that our model was not significant for females or males in either treatment group (Table A3) indicating that there was no strong separation between groups based on the metabolites covered. As flies sampled during or following heat exposure demonstrated no statistical difference in profile to flies of either sex sampled prior to heating, we did not test for differences between treatment groups.

3.3. Effects of varying frequency of thermal fluctuations on fecundity

We varied the frequency of exposure to heat stress to examine the effect it had on the fecundity of *A. aptera* by exposing different groups of flies to heat stress daily (Freq12), every two (Freq6) or three days (Freq4). We also kept one control group at a constant temperature of 4 °C, which was the baseline temperature for the experimental groups. We used a maximum temperature known to be sub-lethal in this species (12 °C) and the majority of flies survived the experimental warming in all groups, with only one or no flies dying in each group. The one exception was one of three replicates for the intermediate intensity stress that exposed flies every two days (Freq6), which lost three flies during the exposure period.

As this data was non-linear, we used a GAM smoothed by day to model the number of larvae and pupae produced by individuals subjected to the different heating regimes. The model for larvae explained

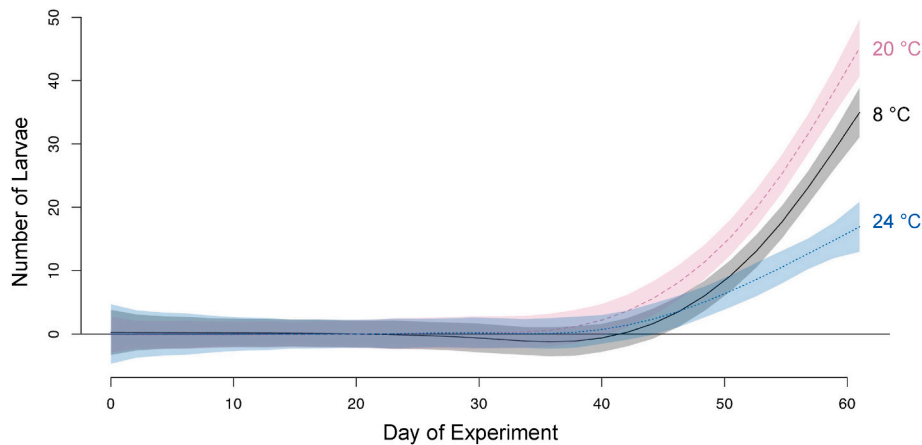


Fig. 2. Smoothed generalized additive mixed model curve of number of larvae produced by *Anatalanta aptera* adults exposed to daily heat peaks of different intensities for 12-days before being allowed to reproduce. The 8 °C control condition represents the average high temperature in their native sub-Antarctic range, while the 20 °C and 24 °C heat peaks represent extreme temperatures.

79.5% of the deviance. Post-hoc comparisons of the treatment groups with a Wald test showed that the only group which significantly differed from the control (Freq0) was Freq6 (Wald test statistic = 9.097, $p = 0.003$), which produced more larvae. There were, however, significant differences between treatment groups. Specifically, the group exposed to four heating cycles (Freq4) had significantly fewer larvae than those exposed to six (Freq6, Wald test statistic = 14.318, $p = 1.56e-04$) or 12 heating cycles (Freq12, Wald test statistic = 4.851, $p = 0.028$) (Fig. 3a). The pupae data was also non-linear and so we modelled it with a GAM

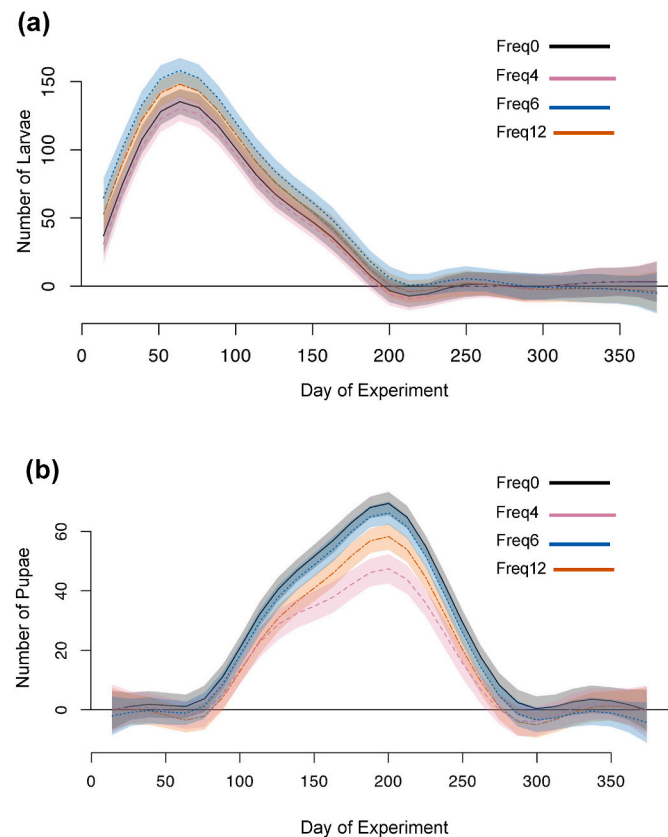


Fig. 3. Smoothed generalized additive mixed model curves of number of (a) larvae and (b) pupae produced by *Anatalanta aptera* adults exposed to heat stress (12 °C from baseline 4 °C) at different frequencies (Freq0 - no exposure, Freq4 - exposure every three days, Freq6 - exposure every two days, and Freq12 - daily exposures) for 12-days before being allowed to reproduce.

smoothed for day, which explained 80.4% of the deviance. We found more significant differences in pupae than larvae produced by the different groups in this experiment. A post-hoc Wald test showed that all treatment groups produced significantly fewer pupae than the Freq0 control (Fig. 3b): Freq4 (Wald test statistic = 34.815, $p = 3.63e-09$), Freq6 (Wald test statistic = 4.266, $p = 0.039$), and Freq12 (Wald test statistic = 18.875, $p = 1.40e-05$). This also revealed that the treatment with heat exposures every other day, Freq6, produced significantly more pupae than the more (Freq12, Wald test statistic = 5.194, $p = 0.023$) and less frequent treatments (Freq4, Wald test statistic = 14.707, $p = 1.26e-04$). The only non-significant treatment-treatment comparison was in between Freq4 and Freq12 (Wald test statistic = 2.421, $p = 0.120$), which were also the two groups with the lowest pupal production (Fig. 3b).

The count trends for larvae and pupae were similar across all groups. For larvae, the number of individuals peaked around day 60 followed by a rapid decline until no individuals in that life stage were left by day 200 (Fig. 3a). The number of pupae began to rise rapidly around day 75 (following the larval peak), peaking around day 200, followed by a rapid decline leaving no individuals in this life stage left at day 300 (Fig. 3b).

4. Discussion

4.1. Thermal stress and reproductive performance of *Anatalanta aptera*

Climate change, and consequent thermal stress, is a growing concern for sub-Antarctic insects, and in particular for native dipterans (Renault et al., 2022). Thermal stress has a strong impact on the survival and the fecundity of ectotherms like insects (Ratte, 1985; Sales et al., 2021), and this impact varies with the severity (e.g., frequency and intensity) of the stress and the conditions to which the organism is adapted. Warmer environments can lead to earlier reproduction in ectothermic species (Chick et al., 2019) and increase the number of generations produced per year (Forrest, 2016). The acceleration of reproduction in response to temperature change may be especially important for species that do not employ reproductive diapause, including *A. aptera* (Chevrier, 1996) which lays eggs throughout its life. However, although we found that the reproduction of *A. aptera* was impacted by thermal stress in both the varying intensity and frequency experiments, there were no differences when larvae were first observed between conditions in either case (Figs. 2 and 3). Prior work by Chevrier (1996) demonstrated that a 4 °C increase within a non-stressful range (4 °C–8 °C) could accelerate the appearance of first *A. aptera* clutches, from 16 weeks to 11 weeks on average. We used this same temperature difference for our control group (from 4 °C to 8 °C), but included daily basis variation. It may be the case

that any developmental speed gained after this heat peak is marginal, and this could be why we did not observe any large difference in the timing of the life cycle between experimental groups.

In addition to leading to earlier reproduction, warmer environments can also increase heat stress in ectothermic species incurring energetic costs for the maintenance of physiological homeostasis, at the expense of reproductive performance (Colinet et al., 2015). This has been demonstrated in many species including aphids (Zhao et al., 2014) and flies from Drosophilidae (Evans et al., 2018; Krebs and Feder, 1997) and Agromyzidae (Huang et al., 2007). Physiological flexibility favouring survival at the cost of reproduction is likely especially important in insects with extended adult lifespans like *A. aptera*. We expected an overall decline in reproductive output in response to higher thermal stress in cold-adapted *A. aptera*. However, we found that the number of larvae produced by flies exposed to the control condition (heating from 4 to 8 °C) and the higher stress treatment (heating from 4 to 24 °C) were statistically indistinguishable (Fig. 2), while significantly more were produced by the lower stress treatment (heating from 4 to 20 °C) than the control. The 20 °C heat spike group also produced approximately double the larvae than the 24 °C group (Fig. 2), which may indicate the existence of a threshold within this 4 °C range where heat switches from a boon to a hindrance.

The larvae results contrast somewhat with our egg production results, as the 20 °C treatment group had higher odds of producing no or few eggs relative to the control. *A. aptera* can lay eggs on the surface or inside substrate and in dead organic matter, and so some eggs may have been difficult to observe if they were more buried in the substrate. Though temperature can modulate behaviours associated with insect reproduction, including copulation, responses to pheromones, and oviposition (e.g., Huey and Pascual, 2009; Abram et al., 2017), which could explain the fewer eggs observed, it is unlikely that this phenomenon would uniquely affect observations of one treatment group (exposed to 20 °C) and not the other (exposed to 24 °C). Another explanation could be that some carrying capacity exists in the rearing enclosures such that groups with more eggs also experience higher mortality of eggs and early instar larvae. Although food was not limited during the experiment, we did not test the carrying capacity of the enclosures and so this remains a possible explanation for the difference in egg and larvae results. Finally, this could be explained by heat stress more severely impacting male fertility, or the fertilization process, than female fertility as unfertilized females are able to lay eggs (Chevrier, 1996). Reductions in male fertility (David et al., 2005; Iossa et al., 2019; Walsh et al., 2019; Domenach and Fricke 2023) including reduced ability to pass sperm from male to female following heating (Rinehart et al., 2000) have been demonstrated in other dipterans and such declines are considered good predictors of vulnerability to climate change (van Heerwaarden and Sgrò 2021).

Variation in heating frequency also affected fecundity, with differential effects depending on life stage and exposure frequency. Individuals exposed to more frequent stresses sometimes produced significantly more larvae (Fig. 3a), but always produced fewer pupae than the control group (Fig. 3b). In the larval count model, exposure to 'intermediate' stress frequency, i.e. warming (4 °C–12 °C) every second day (Freq6), produced significantly more offspring than those held at a constant cool temperature (Freq0). However, larvae produced by adults exposed every day (Freq12) and those exposed every 3 days to thermal fluctuations (Freq4), were not significantly different from the control (Freq0). In contrast, significantly fewer pupae were produced by all exposure groups (Freq4, Freq6, and Freq12) compared to the control, representing a life stage dependent decline in offspring viability. Transgenerational effects of heat stress are known in insects, including decreased reproductive fitness (Sales et al., 2018), improved heat response (Green et al., 2019), and increased mortality (Rukke et al., 2018). The effects of heat stress on *A. aptera* individuals may therefore be dependent on heat stress experienced by its parents and may be life stage dependent (Fig. 3). This represents a potential viability threat as

deleterious effects can slowly deteriorate populations as fitness is reduced through generations (Rukke et al., 2018).

Reductions in fecundity with increasing intensity or frequency of thermal stresses may be due to induction of necessary metabolic repairs incurring a cost to the reproductive fitness of the individuals, which has been observed in other insects exposed to thermal (Hoffmann et al., 2003) and other stresses (Chippindale et al., 1996). As *A. aptera* adults are particularly long-lived, the existence of a trade-off between reproduction and survival, aimed at maximising adult survival cannot be excluded. This type of compromise between reproduction and survival is widespread in nature. For example, increased temperatures can accelerate insect development at the expense of their size, with individuals growing faster but becoming smaller (Colinet et al., 2015; Forrest, 2016) or reduce production of gametes in favour of the synthesis of other protective or reparative compounds (Evans et al., 2018; Silbermann and Tatar, 2000).

We examined the effects of daily heat spikes on the metabolic profiles of adult *A. aptera* sampled before, immediately after, and long after daily exposures on the first and seventh days of the week-long experiment (Fig. A2). We expected differences in the metabolic profiles sampled at different points as changes in individual metabolite concentrations can be produced when heat stress induces the synthesis of protective compounds (Malmendal et al., 2006). Polyols like glycerol are protective against thermal stress and desiccation in many species (Everatt et al., 2015; Colinet et al., 2012; Storey, 1983), including cold-adapted *B. antarctica* (Michaud et al., 2008), while sugars like trehalose and glucose, as well as serine may be important in other flies like *D. melanogaster* (Benoit et al., 2009; Michaud et al., 2008; Malmendal et al., 2006). However, we found no significant difference in the metabolic profiles (Table A3, Fig. A4) or in the expected metabolites (Table A2), though there were differences in some individual metabolites: maltose, a disaccharide, and several acids (Table A2).

Maltose has been shown to respond to heat stress in Hymenoptera (Hildago et al., 2019) possibly hydrolysing to release protective glucose (Michaud et al., 2008; Malmendal et al., 2006). However, without corresponding significant changes in glucose observed in our samples, it is difficult to draw any conclusions. *A. aptera* develops at colder temperatures than *D. melanogaster*, and typically experiences a much narrower range of temperatures than *B. antarctica*. As a consequence, the non-response of the metabolite profiles and unexpected responses in individual metabolites could indicate that it does not have the same capacity to respond as other species more accustomed to heat stress. Alternatively, it could indicate that the thermal stress is insufficiently stressful to elicit a response, however the reproductive impacts would indicate otherwise. In either case, a more targeted study is necessary to better understand the metabolic response of *A. aptera* to thermal stress.

4.2. Future outlook and implications

In the present study, we have confirmed the existence of deleterious sub-lethal and transgenerational impacts of high temperatures, which have significant implications for the future population dynamics of this polar insect. Although we cannot isolate the exact mechanisms by which heat stress influences reproduction *A. aptera*, we have identified fertilization, male fertility, and transgenerational survival as interesting avenues for future study. Our findings, along with others showing that cold stress responses are age and life stage dependent in *A. aptera* (Lalouette et al., 2010), emphasize the importance of life history in the study of thermal stress in this species. As sub-lethal effects of thermal stress may reduce long-term survival of this species, it also threatens the fragile ecosystems that depend on it as a prey species and important decomposer (Tréhen et al., 1985). Any additional threats to this species could prove disastrous as it already grapples with pressure from introduced predators (Ouisse et al., 2017) and potential competitors (Chevrier et al., 1997) that benefit from warming temperatures (Daly et al., 2023). Further studies could help to define the precise mechanisms and

thresholds concerning reproductive effects to improve our ability to forecast the future survival of this species in the context of climate change and prioritise refugia for management activities.

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CRediT authorship contribution statement

Ella Daly: Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Mathilde Defourneaux:** Writing – review & editing, Methodology, Investigation. **Camille Legrand:** Methodology, Investigation. **David Renault:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

The data presented in this study are openly available in GitHub at https://github.com/davidrenault/Anatalanta_Heat_stress_and_reproduction. R scripts from the analyses in this article were developed by ED and are openly available in GitHub at https://github.com/davidrenault/Anatalanta_Heat_stress_and_reproduction.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2024.103910>.

References

- Abram, P.K., Boivin, G., Moiroux, J., Brodeur, J., 2017. Behavioural effects of temperature on ectothermic animals: unifying thermal physiology and behavioural plasticity. *Biol. Rev.* 92 (4), 1859–1876. <https://doi.org/10.1111/brv.12312>.
- Bahrndorff, S., Lauritzen, J.M.S., Sørensen, M.H., Noer, N.K., Kristensen, T.N., 2021. Responses of terrestrial polar arthropods to high and increasing temperatures. *J. Exp. Biol.* 224 (7), jeb230797 <https://doi.org/10.1242/jeb.230797>.
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., Courchamp, F., 2012. Impacts of climate change on the future of biodiversity. *Ecol. Lett.* 15 (4), 365–377. <https://doi.org/10.1111/j.1461-0248.2011.01736.x>.
- Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. Roy. Stat. Soc. B* 57 (1), 289–300.
- Benoit, J.B., Lopez-Martinez, G., Elnitsky, M.A., Lee, R.E., Denlinger, D.L., 2009. Dehydration-induced cross tolerance of *Belgica antarctica* larvae to cold and heat is facilitated by trehalose accumulation. *Comp. Biochem. Physiol. A, Mol. Integr. Physiol.* 152, 518–523.
- Black, A.R., Subjeck, J.R., 1990. In: Schlesinger, M.J., Santoro, M.G., Garaci, E. (Eds.), *Stress Proteins: Induction and Function*. Springer Verlag, Berlin, pp. 101–107.
- Bodlah, M.A., Iqbal, J., Ashiq, A., Bodlah, I., Jiang, S., Mudassar, M.A., Rasheed, M.T., Fareen, A.G.E., 2023. Insect behavioral restraint and adaptation strategies under heat stress: an inclusive review. *J. Saudi Soc. Agric. Sci.* 22 (6), 327–350. <https://doi.org/10.1016/j.jssas.2023.02.004>.
- Buchon, N., Silverman, N., Cherry, S., 2014. Immunity in *Drosophila melanogaster* – from microbial recognition to whole-organism physiology. *Nat. Rev. Immunol.* 14 (12), 796–810. <https://doi.org/10.1146/annurev-ento-010814-021017>.
- Chevrier, M., 1996. Introduction de deux espèces d'insectes aux Îles Kerguelen: Processus de colonisation et exemples d'interactions. Université de Rennes 1, Thesis.
- Chevrier, M., Vernon, P., Frenot, Y., 1997. Potential effects of two alien insects on a sub-Antarctic wingless fly in the Kerguelen Islands. In: Battaglia, B., Valencia, J., Walton, D.W.H. (Eds.), *Antarctic Communities: Species, Structure, and Survival*. Cambridge University Press, Cambridge, pp. 424–431.
- Chick, L.D., Strickler, S.A., Perez, A., Martin, R.A., Diamond, S.E., 2019. Urban heat islands advance the timing of reproduction in a social insect. *J. Therm. Biol.* 80, 119–125. <https://doi.org/10.1016/j.jtherbio.2019.01.004>.
- Chippindale, A.K., Chu, T.J.F., Rose, M.R., 1996. Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution* 50 (2), 753–766. <https://doi.org/10.2307/2410848>.
- Colinet, H., Renault, D., Charoy-Guével, B., Com, E., 2012. Metabolic and proteomic profiling of diapause in the aphid parasitoid *Praon volucre*. *PLoS One* 7 (2), e32606. <https://doi.org/10.1371/journal.pone.0032606>.
- Colinet, H., Sinclair, B.J., Vernon, P., Renault, D., 2015. Insects in fluctuating thermal environments. *Annu. Rev. Entomol.* 60 (1), 123–140. <https://doi.org/10.1146/annurev-ento-010814-021017>.
- Colinet, H., Renault, D., 2018. Similar post-stress metabolic trajectories in young and old flies. *Exp. Gerontol.* 102, 43–50.
- Dahl, J.E., Renault, D., 2022. Ecophysiological responses of the lesser mealworm *Alphitobius diaperinus* exposed to desiccating conditions. *Front. Physiol.* 13.
- Daly, E.Z., Gerlich, H.S., Frenot, Y., Høye, T.T., Holmstrup, M., Renault, D., 2023. Climate change helps polar invasives establish and flourish: evidence from long-term monitoring of the blowfly *Calliphora vicina*. *Biology* 12 (1). <https://doi.org/10.3390/biology12010111>. Article 1.
- David, J.R., Araripe, L.O., Chakir, M., Legout, H., Lemos, B., Pétavy, G., Rohmer, C., Joly, D., Moreteau, B., 2005. Male sterility at extreme temperatures: a significant but neglected phenomenon for understanding *Drosophila* climatic adaptations. *J. Evol. Biol.* 18 (4), 838–846. <https://doi.org/10.1111/j.1420-9101.2005.00914.x>.
- Domenach, B.C., Fricke, C., 2023. Developmental heat stress interrupts spermatogenesis inducing early male sterility in *Drosophila melanogaster*. *J. Therm. Biol.* 114, 103589 <https://doi.org/10.1016/j.jtherbio.2023.103589>.
- Evans, R.K., Toews, M.D., Sial, A.A., 2018. Impact of short- and long-term heat stress on reproductive potential of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). *J. Therm. Biol.* 78, 92–99. <https://doi.org/10.1016/j.jtherbio.2018.09.011>.
- Everatt, M.J., Convey, P., Bale, J.S., Worland, M.R., Hayward, S.A.L., 2015. Responses of invertebrates to temperature and water stress: a polar perspective. *J. Therm. Biol.* 54, 118–132. <https://doi.org/10.1016/j.jtherbio.2014.05.004>.
- Forrest, J.R., 2016. Complex responses of insect phenology to climate change. *Curr. Opin. Insect Sci.* 17, 49–54. <https://doi.org/10.1016/j.cois.2016.07.002>.
- Green, C.K., Moore, P.J., Sial, A.A., 2019. Impact of heat stress on development and fertility of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). *J. Insect Physiol.* 114, 45–52. <https://doi.org/10.1016/j.jinsphys.2019.02.008>.
- Harvey, J.A., Tougeron, K., Gols, R., Heinen, R., Abarca, M., Abram, P.K., Basset, Y., Berg, M., Boggs, C., Brodeur, J., Cardoso, P., De Boer, J.G., De Snoo, G.R., Deacon, C., Dell, J.E., Desneux, N., Dillon, M.E., Duffy, G.A., Dyer, L.A., et al., 2023. Scientists' warning on climate change and insects. *Ecol. Monogr.* 93 (1), e1553. <https://doi.org/10.1002/ecm.1553>.
- Hildago, K., Beaugéard, E., Renault, D., Dedeine, F., Lécureuil, C., 2019. Physiological and biochemical responses to thermal stress vary among genotypes in the parasitic wasp *Nasonia vitripennis*. *J. Insect Physiol.* 117, 103909 <https://doi.org/10.1016/j.jinsphys.2019.103909>.
- Hoffmann, A.A., Sørensen, J.G., Loeschcke, V., 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Therm. Biol.* 28 (3), 175–216. [https://doi.org/10.1016/S0306-4565\(02\)00057-8](https://doi.org/10.1016/S0306-4565(02)00057-8).
- Huang, L.-H., Chen, B., Kang, L., 2007. Impact of mild temperature hardening on thermotolerance, fecundity, and Hsp gene expression in *Liriomyza huidobrensis*. *J. Insect Physiol.* 53 (12), 1199–1205. <https://doi.org/10.1016/j.jinsphys.2007.06.011>.
- Huey, R.B., Pascual, M., 2009. Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline. *Ecology* 90 (7), 1715–1720.
- Hullé, M., Vernon, P., 2021. Terrestrial macro-arthropods of the sub-Antarctic islands of Possession (Crozet Archipelago) and Kerguelen: inventory of native and non-native species. *Zoosystema* 43 (22), 549–561. <https://doi.org/10.5252/zoosystema2021v43a22>.
- Iossa, G., Maury, C., Fletcher, R.M., Eady, P.E., 2019. Temperature-induced developmental plasticity in *Plodia interpunctella*: reproductive behaviour and sperm length. *J. Evol. Biol.* 32 (7), 675–682. <https://doi.org/10.1111/jeb.13447>.
- Jaeger, C., Lisek, J., 2018. Statistical and multivariate analysis of MS-based plant metabolomics data. In: António, C. (Ed.), *Plant Metabolomics, Methods and Protocols*. Springer, New York.
- Jørgensen, K.T., Sørensen, J.G., Bundgaard, J., 2006. Heat tolerance and the effect of mild heat stress on reproductive characters in *Drosophila buzzatii* males. *J. Therm. Biol.* 31 (3), 280–286. <https://doi.org/10.1016/j.jtherbio.2005.11.026>.
- Kalra, B., Tamang, A.M., Parkash, R., 2017. Cross-tolerance effects due to adult heat hardening, desiccation and starvation acclimation of tropical drosophilid *Zaprionus indianus*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 209, 65–73. <https://doi.org/10.1016/j.cbpa.2017.04.014>.
- Krebs, R.A., Feder, M.E., 1997. Deleterious consequences of Hsp70 overexpression in *Drosophila melanogaster* larvae. *Cell Stress & Chaperones* 2 (1), 60–71.
- Lalouette, L., Vernon, P., Amat, H., Renault, D., 2010. Ageing and thermal performance in the sub-Antarctic wingless fly *Anatalanta aptera* (Diptera: sphaeroceridae): older is better. *Biol. Lett.* 6 (3), 346–349. <https://doi.org/10.1098/rsbl.2009.0873>.
- Malmendal, A., Overgaard, J., Bundy, J.G., Sørensen, J.G., Nielsen, N.C., Loeschcke, V., Holmstrup, M., 2006. Metabolic profiling of heat stress: hardening and recovery of homeostasis in *Drosophila*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 291 (1), R205–R212. <https://doi.org/10.1152/ajpregu.00867.2005>.
- Michaud, M.R., Benoît, J.B., Lopez-Martinez, G., Elnitsky, M.A., Lee, R.E., Denlinger, D.L., 2008. Metabolomics reveals unique and shared metabolic changes in response to heat shock, freezing and desiccation in the Antarctic midge, *Belgica antarctica*. *J. Insect Physiol.* 54 (4), 645–655. <https://doi.org/10.1016/j.jinsphys.2008.01.003>.
- Neven, L.G., 2000. Physiological responses of insects to heat. *Postharvest Biol. Technol.* 21 (1), 103–111. [https://doi.org/10.1016/S0925-5214\(00\)00169-1](https://doi.org/10.1016/S0925-5214(00)00169-1).

- Noer, N.K., Sørensen, M.H., Colinet, H., Renault, D., Bahrndorff, S., Kristensen, T.N., 2022. Rapid adjustments in thermal tolerance and the metabolome to daily temperature changes – a field study on the Arctic seed bugs *Nysius groenlandicus*. *Front. Physiol.* 13 <https://doi.org/10.3389/fphys.2022.818485>.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Sólymos, P., Stevens, M.H.H., Wagner, H., 2012. *Vegan: Community Ecology Package*.
- Ouisse, T., Laparie, M., Lebouvier, M., Renault, D., 2017. New insights into the ecology of *Merizodus soledadinus*, a predatory carabid beetle invading the sub-Antarctic Kerguelen Islands. *Polar Biol.* 40 (11), 2201–2209. <https://doi.org/10.1007/s00300-017-2134-z>.
- R Core Team, 2021. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Ratte, H.T., 1985. Temperature and insect development. In: Hoffmann, K.H. (Ed.), *Environmental Physiology and Biochemistry of Insects*. Springer, pp. 33–66. https://doi.org/10.1007/978-3-642-70020-0_2.
- Rehm, E.M., Olivas, P., Stroud, J., Feeley, K.J., 2015. Losing your edge: climate change and the conservation value of range-edge populations. *Ecol. Evol.* 5 (19), 4315–4326. <https://doi.org/10.1002/ece3.1645>.
- Renault, D., Leclerc, C., Collet, M.-A., Boutet, A., Hotte, H., Colinet, H., Chown, S.L., Convey, P., 2022. The rising threat of climate change for arthropods from Earth's cold regions: taxonomic rather than native status drives species sensitivity. *Global Change Biol.* 28 (20), 5914–5927. <https://doi.org/10.1111/gcb.16338>.
- Rinehart, J.P., Yocum, G.D., Denlinger, D.L., 2000. Thermotolerance and rapid cold hardening ameliorate the negative effects of brief exposures to high or low temperatures on fecundity in the flesh fly, *Sarcophaga crassipalpis*. *Physiol. Entomol.* 25 (4), 330–336. <https://doi.org/10.1111/j.1365-3032.2000.00201.x>.
- Rukke, B.A., Sivasubramaniam, R., Birkemoe, T., Aak, A., 2018. Temperature stress deteriorates bed bug (*Cimex lectularius*) populations through decreased survival, fecundity and offspring success. *PLoS One* 13 (3), e0193788. <https://doi.org/10.1371/journal.pone.0193788>.
- Sales, K., Vasudeva, R., Dickinson, M.E., Godwin, J.L., Lumley, A.J., Michalczyk, Ł., Hebberecht, L., Thomas, P., Franco, A., Gage, M.J.G., 2018. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nat. Commun.* 9 (1) <https://doi.org/10.1038/s41467-018-07273-z>. Article 1.
- Sales, K., Vasudeva, R., Gage, M.J.G., 2021. Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. *R. Soc. Open Sci.* 8 (3) <https://doi.org/10.1098/rsos.201717>, 201717.
- Silbermann, R., Tatar, M., 2000. Reproductive costs of heat shock protein in transgenic *Drosophila melanogaster*. *Evolution* 54 (6), 2038–2045.
- Storey, K.B., 1983. Metabolism and bound water in overwintering insects. *Cryobiology* 20 (3), 365–379. [https://doi.org/10.1016/0011-2240\(83\)90025-1](https://doi.org/10.1016/0011-2240(83)90025-1).
- Thiébaud, G., Tarayre, M., Jambon, O., Le Bris, N., Colinet, H., Renault, D., 2021. Variation of thermal plasticity for functional traits between populations of an invasive aquatic plant from two climatic regions. *Hydrobiologia* 848 (9), 2077–2091. <https://doi.org/10.1007/s10750-020-04452-2>.
- Tréhen, P., Bouché, M., Vernon, Ph, Frenot, Y., 1985. Organization and dynamics of Oligochaeta and Diptera on possession island. In: Siegfried, W.R., Condy, P.R., Laws, R.M. (Eds.), *Antarctic Nutrient Cycles and Food Webs*. Springer, pp. 606–613. https://doi.org/10.1007/978-3-642-82275-9_82.
- van Heerwaarden, B., Sgrò, C.M., 2021. Male fertility thermal limits predict vulnerability to climate warming. *Nat. Commun.* 12 (1), 2214. <https://doi.org/10.1038/s41467-021-22546-w>.
- van Rij, J., Wieling, M., Baayen, R.H., van Rijn, H., Itsadug: interpreting time series and autocorrelated data using GAMMs. R package. version 2.4.1. <https://CRAN.R-project.org/package=itsadug/>.
- Venables, W.N., Ripley, B.D., 2002. *Modern Applied Statistics with S, fourth ed.* Springer, New York.
- Vernon, P., Vannier, G., 1996. Developmental patterns of supercooling capacity in a subantarctic wingless fly. *Experientia* 52 (2), 155–158. <https://doi.org/10.1007/BF01923362>.
- Vernon, P., Vannier, G., Trehen, P., 1998. A comparative approach to the entomological diversity of polar regions. *Acta Oecol.* 19 (3), 303–308. [https://doi.org/10.1016/S1146-609X\(98\)80034-9](https://doi.org/10.1016/S1146-609X(98)80034-9).
- Walsh, B.S., Parratt, S.R., Hoffmann, A.A., Atkinson, D., Snook, R.R., Bretman, A., Price, T.A.R., 2019. The impact of climate change on fertility. *Trends Ecol. Evol.* 34 (3), 249–259. <https://doi.org/10.1016/j.tree.2018.12.002>.
- Walsh, B.S., Parratt, S.R., Mannion, N.L.M., Snook, R.R., Bretman, A., Price, T.A.R., 2021. Plastic responses of survival and fertility following heat stress in pupal and adult *Drosophila virilis*. *Ecol. Evol.* 11 (24), 18238–18247. <https://doi.org/10.1002/ece3.8418>.
- Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J. Roy. Stat. Soc. B Stat. Methodol.* 73 (1), 3–36. <https://doi.org/10.1111/j.1467-9868.2010.00749.x>.
- Zhao, F., Zhang, W., Hoffmann, A.A., Ma, C.-S., 2014. Night warming on hot days produces novel impacts on development, survival and reproduction in a small arthropod. *J. Anim. Ecol.* 83 (4), 769–778. <https://doi.org/10.1111/1365-2656.12196>.
- Zizzari, Z.V., Ellers, J., 2011. Effects of exposure to short-term heat stress on male reproductive fitness in a soil arthropod. *J. Insect Physiol.* 57 (3), 421–426. <https://doi.org/10.1016/j.jinsphys.2011.01.002>.