

Table A1. Table showing the number of adult *Anatalanta aptera* flies sampled to generate metabolic profiles at different time steps during a week-long daily heating experiment. For each time step, six female (F) and six male (M) flies were exposed to daily heating from 4 °C to 8 °C or 24 °C, however some did not survive. Sampling times were: before heating ( $T_{7_0}$ ), immediately following heating on the first ( $T_{14}$ ) and seventh ( $T_{7_14}$ ) days, during the cool down phase on the first ( $T_{18}$ ) and seventh ( $T_{7_18}$ ) days, and at the end of the week-long exposure period ( $T_{end}$ ). No flies were sampled at  $T_{7_18}$  in the 8 °C treatment.

Time	F - 8 °C	F - 24 °C	M - 8 °C	M - 24 °C
T0	6	6	4	4
T14	6	6	6	6
T18	6	6	4	5
T7-14	6	6	6	6
T7-18	-	6	-	5
Tend	4	6	4	5

Table A2. P-values associated with one-way ANOVAs for individual metabolites measured in *Anatalanta aptera* female and male adults exposed to different daily heating treatments (heating from 4 °C to 8 °C or 4 °C to 24 °C) over one week. Individuals were sampled at different time points (before, immediately following exposure on first or seventh days, during cool down on first day or seventh days, at the end of the seventh day). See Table A1 for the number of individuals of each sex and treatment surviving at each timestep (and therefore used for metabolomic analysis) and Figure A1 for details on heating regimes. An \* indicates a significant result ( $p < 0.05$ ) after application of the Benjamini-Hochberg procedure to account for false discovery rate.

Metabolite	Timing	Sex	Treatment
valine	1.951628e-04*	5.665189e-02	0.16596874
leucine	9.312806e-05*	5.868469e-01	0.55839092
aminobutyric acid	1.841600e-03*	2.927679e-02*	0.16596874
aspartic acid	1.951628e-04*	6.514652e-09*	0.12545975
citric acid	1.438346e-02*	3.580152e-02*	0.08850348
malic acid	1.056602e-02*	3.049393e-02*	0.02073998
phosphoric acid	4.638481e-01	9.122348e-02	0.06250114
citrulline	4.281354e-01	3.952394e-06*	0.16596874
g3p	8.190875e-01	5.979380e-03*	0.55839092
g6p	3.735148e-01	1.637108e-01	0.04428697
glucose1	4.270325e-01	7.133060e-01	0.17424104
glucose2	4.281354e-01	9.893844e-01	0.18905744
glycerol	1.040416e-01	9.835618e-02	0.96983682
glycine	5.365126e-01	7.133060e-01	0.85431791
inositol	3.521296e-01	1.219275e-09*	0.24252268
maltose	1.450731e-05*	1.642416e-04*	0.37384216
methionine	3.224228e-01	5.140269e-02	0.16596874
ornithine	2.644050e-01	1.873685e-10*	0.08850348
proline	3.521296e-01	6.576457e-05*	0.24252268
serine	4.378868e-01	1.642416e-04*	0.06250114
trehalose	9.704964e-02	6.041372e-01	0.94435194
xylitol	1.502428e-01	2.138430e-03*	0.27275320

Table A3. Table showing R<sup>2</sup> and p-values associated with RDA of metabolic profile of adult *Anatalanta aptera* flies exposed to daily heating from 4 °C to 8 °C (Int8) or 4 °C to 24 °C (Int24) and sampled at different time points throughout this exposure period (i.e., before, during, and after on the first or seventh days – see Figure A2 for specifics). P-values are associated with differences in profiles given the timing of sampling.

<b>Treatment</b>	<b>Sex</b>	<b>R<sup>2</sup></b>	<b>p-value</b>
Int8 (8 °C)	Female	0.24	0.086
Int8 (8 °C)	Male	0.15	0.58
Int24 (24 °C)	Female	0.063	0.92
Int24 (24 °C)	Male	0.13	0.63

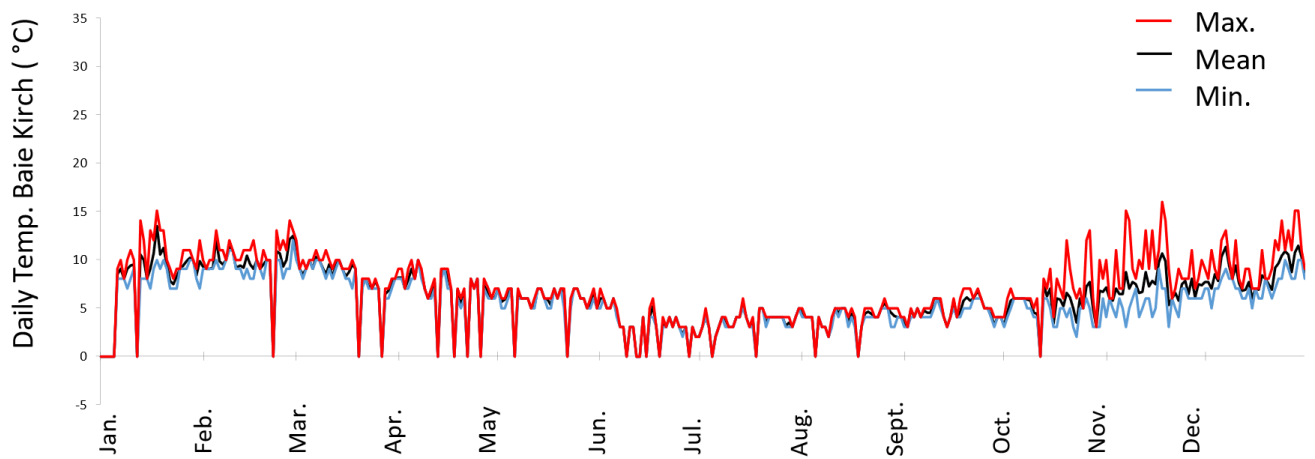
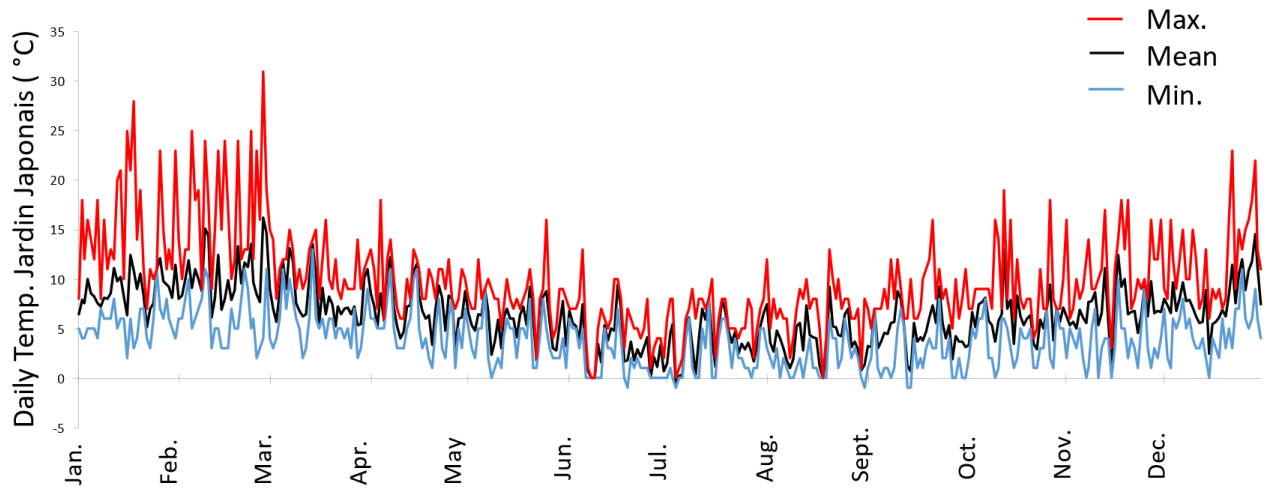


Figure A1. Daily low (blue), mean (black), and high (red) surface temperatures at Jardin Japonais (top) and Baie Kirch (bottom) in the Crozet Archipelago measured by TMS4 TOMST loggers in 2022.

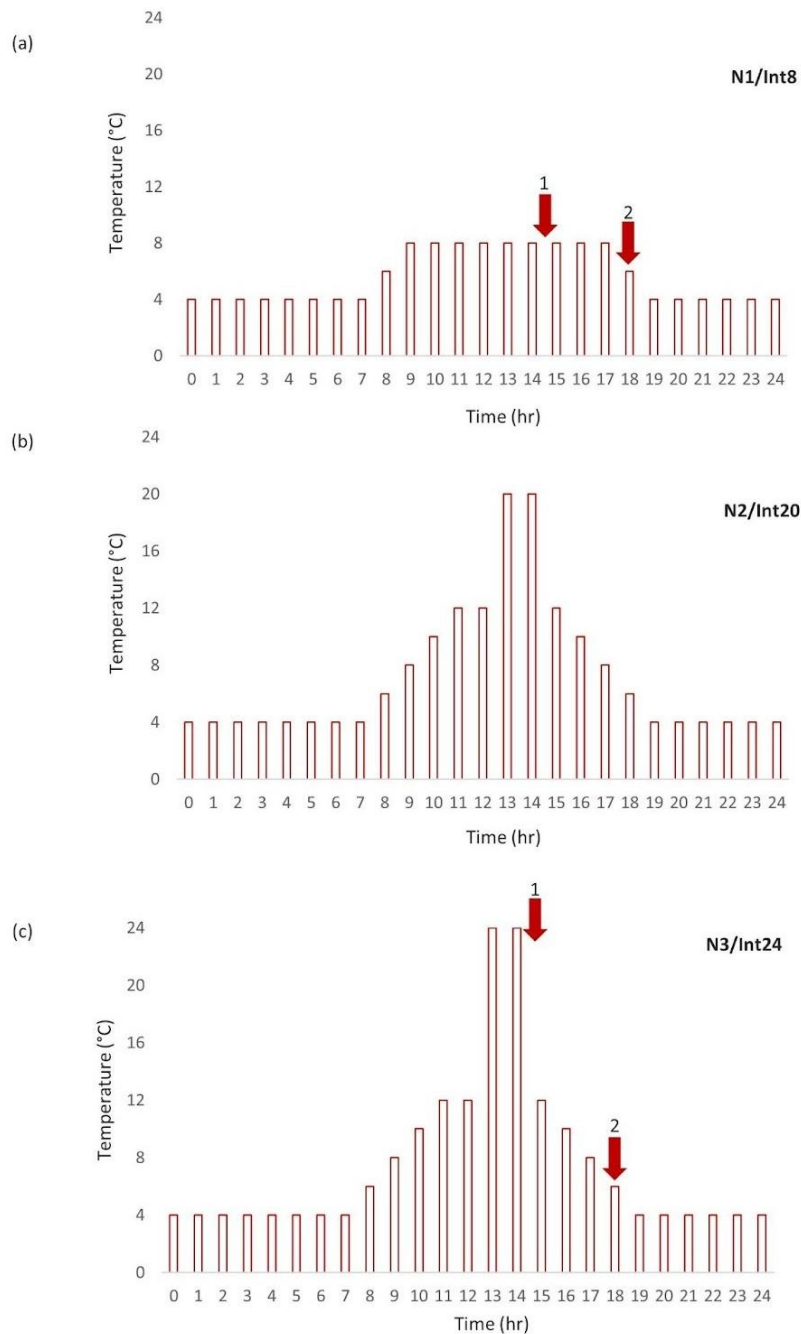


Figure A2. Daily heating regimes applied to *Anatalanta aptera* adults during a 12-day period to test the effects of varying intensities of heat stress on fecundity. Daily regimes involved gradual heating to different temperatures. (a) shows the control treatment Int8, with a heat peak at 8 °C, which *A. aptera* is frequently exposed to in its native sub-Antarctic range. (b) and (c) show experimental heating treatments that exceed average temperatures in the sub-Antarctic, 20 °C (Int20) and 24 °C (Int24) respectively. Arrows represent the timings of removal of individuals for metabolomic analysis. In panel (c), arrow 1 represents when individuals were taken just after the 24 °C heat peak during the first and seventh cycles, while arrow 2 represents when individuals were taken during the 6 °C cool down phases of the same cycles. In panel (a), representing the control treatment, arrows 1 and 2 show that individuals were taken following the same timings from panel (c).

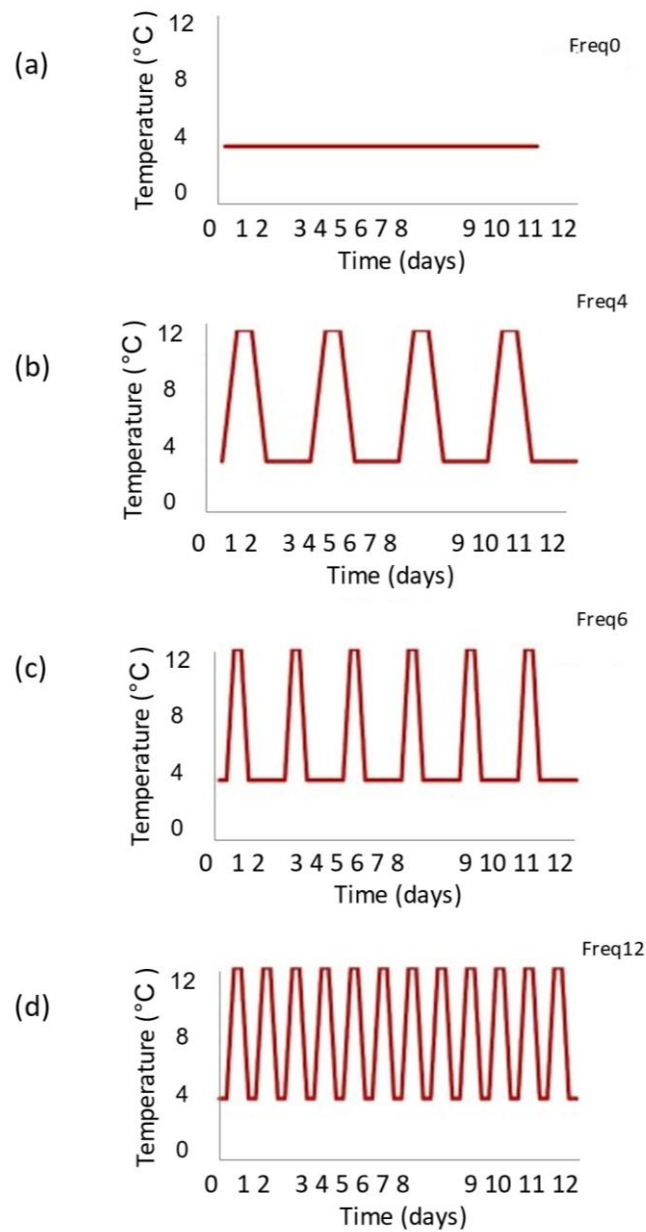


Figure A3. Varying exposures to heat stress (12 °C from baseline 4 °C) for groups of cold-adapted fly *Anatalanta aptera* to test for the effect of thermal stress frequency on fecundity. Panel (a) shows the control condition, Freq0, in which individuals were kept at a constant temperature of 4 C for the duration of the experiment. Panels (b) - (d) show experimental treatments with four (Freq4), six (Freq6), and 12 (Freq12) cycles of heating during the 12-day experimental period.

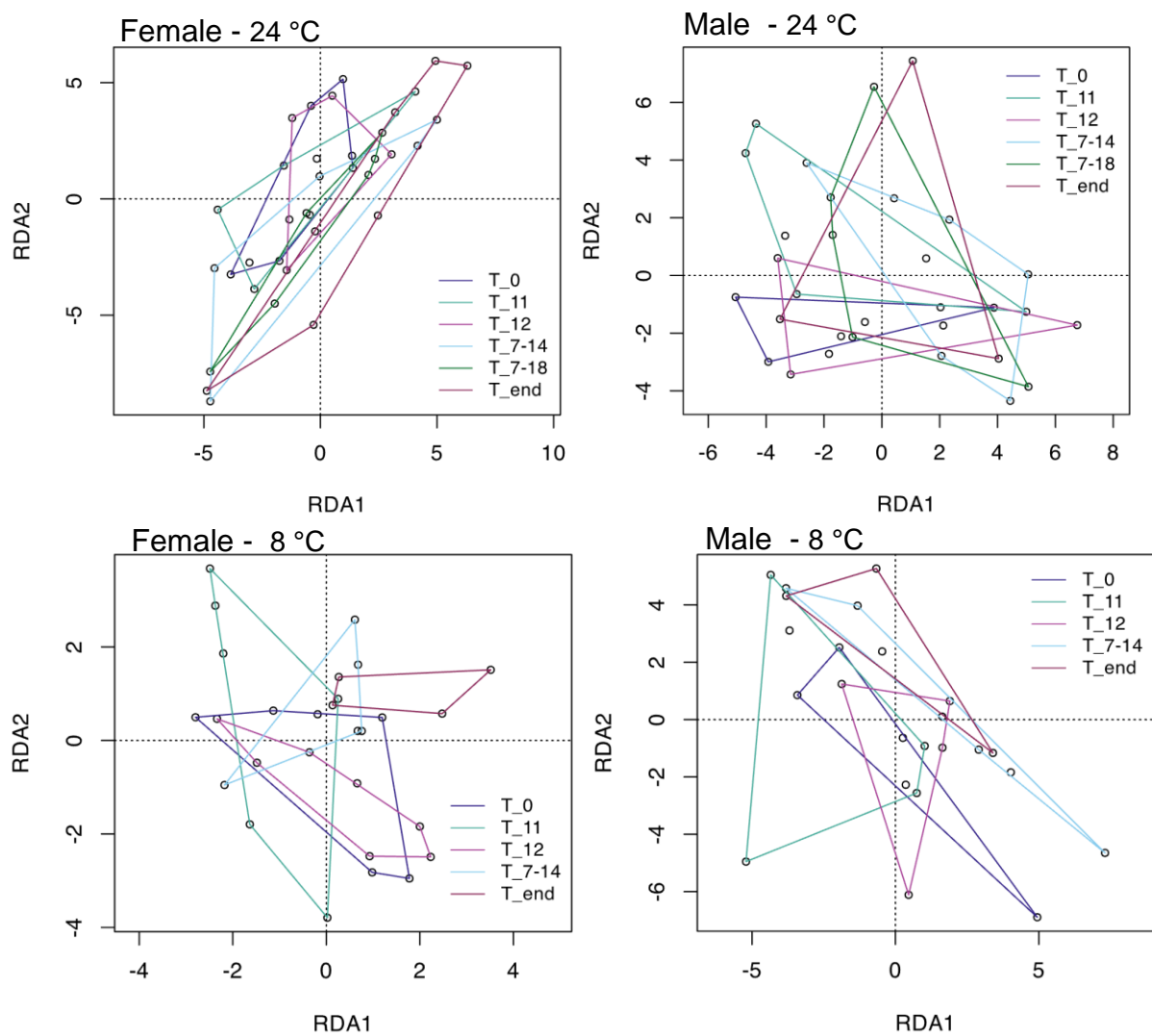


Figure A4. Metabolic profiles for female (left) and male (right) adult *Anatalanta aptera* flies exposed to two experimental heating regimes. Flies were heated daily from 4 °C to 8 °C (top – Int8) or 24 °C (bottom – Int24). Individuals were sampled before heating (T\_0), immediately following the heating on the first (T\_11) and seventh (T\_71) days, during the cool down

phase on the first (T<sub>12</sub>) and seventh (T<sub>72</sub>) days, and at the end of the week-long exposure period (T<sub>end</sub>) for metabolic analysis.