***Oviposited eggs are sensitive to experimental heatwaves***

*Summary:*

This study investigates the consequences of short- and long-term heatwave exposures on oviposited insect eggs (*Tribolium castaneum*) and subsequence embryonic survival as well as developmental stage failures were quantified. Overall, from an outbred stock population (non-overlapping generations) we set up these assays. This work constitutes data files (.csv) and an Rscript file.

Everyone involved (with the exception of MJGG) in the study contributed to data collection. Miss. Natasha Brown (research technician) helped with some of the pilot study counts, helped curate the material and run the assays.

*R Session Info:*

R version 4.5.0 (2025-04-11)

Platform: aarch64-apple-darwin20

Running under: macOS Sequoia 15.5

Matrix products: default

BLAS: /System/Library/Frameworks/Accelerate.framework/Versions/A/Frameworks/vecLib.framework/Versions/A/libBLAS.dylib

LAPACK: /Library/Frameworks/R.framework/Versions/4.5-arm64/Resources/lib/libRlapack.dylib; LAPACK version 3.12.1

locale:

[1] en\_US.UTF-8/en\_US.UTF-8/en\_US.UTF-8/C/en\_US.UTF-8/en\_US.UTF-8

time zone: Europe/London

tzcode source: internal

attached base packages:

[1] stats graphics grDevices utils datasets methods base

other attached packages:

[1] ggpubr\_0.6.0 lubridate\_1.9.4 forcats\_1.0.0 stringr\_1.5.1 dplyr\_1.1.4 purrr\_1.0.4 readr\_2.1.5 tidyr\_1.3.1

[9] tibble\_3.2.1 tidyverse\_2.0.0 Rmisc\_1.5.1 plyr\_1.8.9 lattice\_0.22-6 ggplot2\_3.5.2

loaded via a namespace (and not attached):

[1] gtable\_0.3.6 crayon\_1.5.3 compiler\_4.5.0 ggsignif\_0.6.4 tidyselect\_1.2.1 Rcpp\_1.0.14

[7] scales\_1.4.0 R6\_2.6.1 labeling\_0.4.3 generics\_0.1.4 Formula\_1.2-5 backports\_1.5.0

[13] car\_3.1-3 pillar\_1.10.2 RColorBrewer\_1.1-3 tzdb\_0.5.0 rlang\_1.1.6 broom\_1.0.8

[19] stringi\_1.8.7 timechange\_0.3.0 cli\_3.6.5 withr\_3.0.2 magrittr\_2.0.3 grid\_4.5.0

[25] rstudioapi\_0.17.1 hms\_1.1.3 lifecycle\_1.0.4 vctrs\_0.6.5 rstatix\_0.7.2 glue\_1.8.0

[31] farver\_2.1.2 abind\_1.4-8 carData\_3.0-5 tools\_4.5.0 pkgconfig\_2.0.3

>

**1) Filename:**  **UEA\_Egg HW Results\_2025.csv**

Dataset describing adult counts (from lab A) when eggs were exposed to short-term experimental heatwaves across temperatures (°C) and durations (hours – h).

Fodder quantity (7.5 grams – g)

Column A: Location

Lab A where the assays were conducted

Column B: Day

Day when the experiments were run, levels : 5 (as short-term exposures were run across five independent days)

Column C: temp

Experimental heatwave exposure (°C)

Column D: duration (hours- h)

Duration the eggs were exposed, levels : 2 (2 h and 5 h)

Column E: count

Adult counts after eggs were exposed

Column F: Date Collected

Date when the heatwaves were run and the offspring counted

**2) Leeds\_Egg HW Results\_2025\_sHW.csv**

Dataset describing adult counts (from lab B) when eggs were exposed to short-term experimental heatwaves across temperatures (°C) and durations (hours – h).

Column A: temp

Experimental heatwave exposure (°C)

Column B: fodder

Fodder quantity (7.5 grams – g)

Column C: duration (hours- h)

Duration the eggs were exposed, levels : 2 (2 h and 5 h)

Column D: Rep

Individual replicates within each treatment and a random ID given to the samples

Column E: Rand.Code

A random code to blind all the information, this allowed to control/avoid any biases when running the assays

Column F: Off.Counts

Offspring counts

Column G: Pupae

Dead pupae counts within the batches

Column H: Larvae

Dead larval counts

Column I: Treat

Short-term heatwave treatment (description only)

Column J: Day

The day that the assays were run (random blocks) in Lab B

**3) Leeds\_Egg HW Results\_2025.csv**

Dataset describing adult counts (from lab B) when eggs were exposed to **long-term experimental heatwaves** across temperatures (°C) and durations (hours – h).

Fodder: 7.5 g

Column A: ID

Replicate sample ID

Column B: Temp

Experimental heatwave exposure (°C)

Column C: Days.Exposed

Heatwave exposure (in days)

Column D: Counts

Offspring counts (adults that eclosed after eggs were exposed)

Column E: Life.Stage

Life stages that failed during development after the fertilised, unhatched oviposited eggs were exposed to long-term heatwaves

Column F: Notes

Nominal data describing the stage (dead or alive)

Column G: Heatwave

Long-term heatwave (descriptive)

**4) Leeds\_Egg.Counts.Fodder.csv**

Dataset describing egg counts (from labs A and B) from a subset of samples (in 7.5 g of fodder) from the short-term heatwaves, frozen at the time of setting up the assays. These counts were done on anonymised batches that were then, de-anonymised the sample IDs, compared, once the egg counts were completed for plotting.

Column A: ID

Replicate sample ID

Column B: Loc

Location the samples were collected from

Column C: Fodder.mass (grams – g), without oats, just flour + yeast.

Mass of the container with the fodder was measured using an electronic AND balance to the second decimal point (XX.XX g)

Column D: Egg.Counts

Eggs counted

Column E: Larvae

Whether at the point of eggs being frozen, whether any of the eggs hatched or not. So the presence of larvae was checked at the time of the egg counts

Column F: Assayed

When the egg count assays were carried out (day)

Column G: Sample.Block

Block the samples came from (e.g., A-E or Sat and Sun)

Column A: Scoop

Not applicable

**5) Life Stage Deaths Long HW\_2025.csv**

Dataset describing life stage deaths (from lab B, in 7.5 g of fodder) from the short-term heatwaves.

Column A: Rep

Replicate sample ID

Column B: Temp (°C)

Heatwave temperature (°C)

Column C: Fodder (grams – g),

Fodder mass used

Column D: Duration (hours - h)

Duration in hours

Column E: Offspring

Total number of offspring counted across replicate pots

Column F: Deaths

Life stages that had died during their development

Column G: Prop.Dead (number)

Proportion dead, life stage – Deaths/Offspring

Column H: Stage (nominal)

Life stage that died

Column I: Day (nominal)

Day the assays were carried out

**6) Lifestage failures\_LHW\_210525.csv**

Dataset describing life stage deaths (from lab B, in 7.5 g of fodder) from the long-term heatwaves.

Column A: Temp (°C)

Heatwave temperature (°C)

Column B: Duration (days - d)

Duration in hours

Column C: Off.Counts

Total number of offspring counted across replicate pots

Column D: Deaths

Life stages that had died during their development

Column E: Percent (number)

Percentage of the life stage that died, calculated as – Deaths/Off.Counts

Column F: Life.Stage (nominal)

Life stage that died

Column G: Replicates

Replicate sample number

Column H: Fodder (grams – g),

Fodder mass used

Column I: Type (nominal)

Heatwave type

**7) Lifestage failures\_sHW\_210525.csv**

Summarised dataset describing life stage deaths (from lab B, in 7.5 g of fodder) from the short-term heatwaves.

Column A: Temp (°C)

Heatwave temperature (°C)

Column B: Off.Counts

Total number of offspring counted across replicate pots

Column C: Deaths

Life stages that had died during their development

Column D: Percent (number)

Percentage of the life stage that died, calculated as – Deaths/Off.Counts

Column E: Life.Stage (nominal)

Life stage that died

Column F: Day (nominal)

Day the assays were run

Column G: Duration (hours - h)

Duration the heatwaves were run for

Column H: Replicates

Replicate sample number

Column I: Fodder (grams – g),

Fodder mass used

Column I: Type (nominal)

Heatwave type

**8) Supporting Information\_Fig 1.3\_2025.csv**

Summarised dataset describing impacts of short-term heatwaves on oviposited eggs when this was initially run as a pilot study.

Column A: Blinded\_code

Temperature treatments (°C) that were blinded using a code

Column B: ID

Replicate ID for each sample

Column C: Counts

Offspring counts

Column D: Duration (hours - h)

Duration the heatwaves were run for

Column E: ForT (grams – g),

Fodder mass used

Column F: Rep

Replicate sample number

Column G: Counted by

User who counted the offspring

Column H: Species

Species ID

Column I: Set up

User for setting up

Column J: Expt

Experiment ID

Column K: Date

Not applicable