

This file is meant as a descriptive README for the scripts and data files included for Dam, HG et al. *Rapid, but limited, zooplankton adaptation to simultaneous warming and acidification*. 2021.

Notes for scripts and Data

To use these scripts, first download R (<https://www.r-project.org/>) and R Studio (<https://www.rstudio.com/>) for the most effective implementation of the scripts.

The Script titled: "Phenotypic_analysis_all_traits.R" is used to analyze all life-history traits discussed in the manuscript (survival, egg production rate, egg hatching success, development time, sex ratio, and population fitness).

Survival, development time, and sex ratio analyses are all calculated from the available survival data "Survival_data_total.txt" file. Data columns are listed as "Date", "x", "Generation", "Treatment", "Temp", "pH", "Rep", "Beak", "time", "nx", "lx", "Ndev", "Cdev", "F.Ratio", and "M.Ratio". Data in "Date" and "x" are not used in the analysis. Data in "Generation" refers to the corresponding generation when survival was measured. Data in "Treatment" refers to corresponding treatment evaluated. Data in "Temp" and "pH" refers to the target temperature and pH, respectively, for each treatment. Data in "Rep" refers to the treatment replicate. Data in "Beak" refers to the replicate beaker used in survival assays. Data in "time" refers to the number of days after the start of the experiment. Data in "nx" refers to the number of surviving individuals. Data in "lx" proportion of surviving individuals remaining. Data in "Ndev" refers to the number of individuals who mature from naupliar stages to copepodid stages. Data in "Cdev" refers to the number of individuals who mature from copepodid stages to adults. Data in "F.Ratio" and "M.Ratio" refer to the corresponding ratio of females:males, and males:females, respectively.

Egg production and egg hatching success analyses are all performed using the "EPR_HF_data_total.txt" file. Data columns are listed as "Generation", "Rep", "Treatment", "Temp", "pH", "Hatched", "Unhatched", "Total", "EPRtot", and "HFtot". Data in "Generation" refers to the corresponding generation when survival was measured. Data in "Treatment" refers to corresponding treatment evaluated. Data in "Temp" and "pH" refers to the target temperature and pH, respectively, for each treatment. Data in "Rep" refers to the treatment replicate. Data in "Hatched" refers to the number of hatched eggs observed. Data in "Unhatched" refers to the number of unhatched eggs observed. Data in "Total" refers to the sum of "Hatched" and "Unhatched" columns. Data in "EPRtot" refers to the rate of eggs produced per female per day. Data in "HFtot" refers to the frequency of hatching success.

Resulting population fitness data can be accessed directly from the "lambda_results_devtime_surv_epr_hf_sex_standardized_relative.txt" file. The corresponding values of survival, egg production, hatching success, development time and sex ratio used to calculate

fitness are listed according to their correlated fitness value under the following column headings: "surv", "epr", "hf", "dev.time", and "sex". This also includes relative fitness values (rel.fitness) and standardized fitness values (stand.fitness) as well as standardized and relative values for survival, epr, hatching success, development time, and sex ratio.

The Script titled: "A_tonsa_physical_data.R" is used to analyze all temperature, pH, pCO₂, alkalinity, and accompanying carbonate chemistry data collected during the transgenerational experiment. The resulting data is summarized in the MS Excel workbook titled "A_tonsa_physical_data_complete_MS.xls". Included, you will find all the collected temperature and pH measurements, as well as all calculated values of pCO₂, alkalinity, omega CA, omega AR, fCO₂, and DIC. There is also all the statistically evaluated temperature and pH contrast results.

The script used to evaluate genetic (nucleotide) diversity is titled "genetic_diversity.md". It includes sections of bash script for bioinformatic analysis and subsequent R code for analysis of nucleotide diversity. Accompanying sequence data is located at <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA590963>.

Thank you for expressing interest in our research! For an easy-to-understand video that explains more about our research, check out: <https://youtu.be/Yrl2188-ejM>