Supplementary material

Trehalose provisioning in *Daphnia* resting stages reflects local adaptation to the harshness of diapause conditions

**Authors**

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## **Table S1**. List of *Daphnia magna* genotypes, their origin (country, GPS coordinates), and the habitat type (summer-dry (sd) and winter-freeze (wf): Yes (Y) or No (N)). N1 and N2 are the number of replicates (N1 excludes replicates with absorbance below 0.100; N2 contains all replicates). Last two columns show mean percentage of trehalose per dry weight, excluding replicates with absorbance below 0.100 (tre1) and including all replications (tre2).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Genotype** | **Country** | **Latitude** | **Longitude** | **sd** | **wf** | **N1** | **N2** | **tre1** | **tre2** |
| BE-HO-1 | Belgium | 50.1451 | 5.0771 | N | N | 8 | 8 | 8.32 | 8.32 |
| BE-WE-G59 | Belgium | 51.0678 | 3.7736 | N | N | 8 | 8 | 8.11 | 8.11 |
| BY-G-9 | Belorussia | 52.4215 | 31.0138 | N | Y | 7 | 8 | 7.40 | 7.24 |
| CH-H-1 | Switzerland | 47.5578 | 8.8626 | N | Y | 8 | 8 | 7.02 | 7.02 |
| CY-PA2-1 | Cyprus | 35.0328 | 33.9551 | Y | N | 6 | 8 | 8.18 | 7.84 |
| CY-PA3-1 | Cyprus | 35.0341 | 33.9549 | Y | N | 7 | 8 | 10.23 | 9.50 |
| CZ-KO-1 | Czech-Republic | 50.1254 | 14.8687 | N | Y | 8 | 8 | 9.63 | 9.63 |
| DK-RL-3 | Denmark | 55.9642 | 9.5964 | N | Y | 6 | 8 | 10.95 | 9.00 |
| ES-D-BDE1 | Spain | 37.1481 | -6.0366 | Y | N | 8 | 8 | 9.00 | 9.00 |
| ES-HT-1 | Spain | 38.7752 | -1.4102 | Y | N | 8 | 8 | 12.66 | 12.66 |
| FI-FAT-1-3 | Finland | 60.0217 | 19.9021 | Y | Y | 8 | 8 | 11.10 | 11.10 |
| FI-FUT1-2-1 | Finland | 60.3471 | 27.4785 | Y | Y | 8 | 8 | 12.08 | 12.08 |
| FI-OER-3-3 | Finland | 59.7886 | 23.1741 | Y | Y | 8 | 8 | 12.64 | 12.64 |
| FI-SK-58-2 | Finland | 59.833 | 23.2574 | Y | Y | 8 | 8 | 11.76 | 11.76 |
| FI-SKW-2-1 | Finland | 59.833 | 23.2563 | Y | Y | 8 | 8 | 11.13 | 11.13 |
| GB-C1-1 | Great-Britain | 51.7344 | -1.3363 | N | N | 5 | 8 | 4.83 | 4.70 |
| GB-EK2-6 | Great-Britain | 55.6977 | -2.3434 | N | N | 8 | 8 | 10.33 | 10.33 |
| GB-FML-1 | Great-Britain | 52.5311 | -1.9559 | N | N | 8 | 8 | 6.78 | 6.78 |
| HU-AG-03 | Hungary | 47.5146 | 19.0813 | Y | Y | 8 | 8 | 8.88 | 8.88 |
| IE-DUB-1 | Ireland | 53.3267 | -6.2341 | N | N | 7 | 8 | 10.64 | 9.92 |
| IL-BM-1 | Israel | 30.5113 | 34.6121 | Y | N | 8 | 8 | 11.97 | 11.97 |
| IL-M1-8 | Israel | 31.7782 | 35.2206 | Y | N | 8 | 8 | 13.37 | 13.37 |
| IT-MDV-1 | Italy | 37.6855 | 12.6175 | Y | N | 8 | 8 | 11.10 | 11.10 |
| IT-PER-2 | Italy | 37.5192 | 14.3073 | Y | N | 7 | 8 | 10.84 | 10.15 |
| MA-ES-3 | Morocco | 31.4907 | -9.7644 | Y | N | 7 | 8 | 12.27 | 11.80 |
| NO-AA-1 | Norway | 60.051 | 5.0744 | N | Y | 8 | 8 | 10.29 | 10.29 |
| NO-F-1 | Norway | 63.5877 | 10.729 | N | Y | 8 | 8 | 11.60 | 11.60 |
| NO-LADE-1 | Norway | 63.449 | 10.4529 | N | Y | 8 | 8 | 7.52 | 7.52 |
| NO-RO-1 | Norway | 67.5274 | 12.1268 | N | Y | 8 | 8 | 11.59 | 11.59 |
| RU-BOL1-1 | Russia | 66.4497 | 33.8567 | Y | Y | 8 | 8 | 13.08 | 13.08 |
| RU-KA1-205 | Russia | 45.5994 | 45.2975 | Y | Y | 8 | 8 | 12.95 | 12.95 |
| RU-KOR1-1 | Russia | 66.4519 | 33.799 | Y | Y | 7 | 8 | 7.17 | 6.49 |
| RU-R2-1 | Russia | 56.425 | 37.6027 | N | Y | 8 | 8 | 12.04 | 12.04 |
| SE-G1-9 | Sweden | 60.4217 | 18.5102 | Y | Y | 8 | 8 | 12.22 | 12.22 |
| SE-GN2-3A10 | Sweden | 60.4971 | 18.4316 | Y | Y | 8 | 8 | 11.19 | 11.19 |
| SE-H1-1 | Sweden | 58.3423 | 11.218 | Y | Y | 8 | 8 | 11.01 | 11.01 |
| UA-KR-1-7 | Ukraine | 45.0937 | 36.3107 | Y | Y | 8 | 8 | 14.86 | 14.86 |

## **Table S2.** List of individual *Daphnia magna* (replicates) used for each genotype in this study. Detailed data for each replicate (rep), with description of habitat type, specifically summer-dry (sd) and winter-freeze (sd): Yes (Y) or No (N); sample volume in mg (vsample), and trehalose percentage per dry weight (conc\_trehalose). Biological sample replicates with an asterisk (\*) had absorbance below 0.1 and were excluded from the main analysis.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **genotype** | **rep** | **sd** | **wf** | **vsample** | **conc\_trehalose** |
| BE-HO-1 | 1 | N | N | 0.062 | 5.85 |
| BE-HO-1 | 2 | N | N | 0.086 | 10.88 |
| BE-HO-1 | 3 | N | N | 0.065 | 5.92 |
| BE-HO-1 | 4 | N | N | 0.058 | 10.67 |
| BE-HO-1 | 5 | N | N | 0.069 | 6.00 |
| BE-HO-1 | 6 | N | N | 0.063 | 10.88 |
| BE-HO-1 | 7 | N | N | 0.068 | 4.41 |
| BE-HO-1 | 8 | N | N | 0.068 | 11.93 |
| BE-WE-G59 | 1 | N | N | 0.060 | 1.67 |
| BE-WE-G59 | 2 | N | N | 0.058 | 16.82 |
| BE-WE-G59 | 3 | N | N | 0.054 | 5.75 |
| BE-WE-G59 | 4 | N | N | 0.053 | 13.02 |
| BE-WE-G59 | 5 | N | N | 0.048 | 4.44 |
| BE-WE-G59 | 6 | N | N | 0.059 | 6.37 |
| BE-WE-G59 | 7 | N | N | 0.068 | 4.13 |
| BE-WE-G59 | 8 | N | N | 0.062 | 12.66 |
| BY-G-9 | 1\* | N | Y | 0.059 | 6.11 |
| BY-G-9 | 2 | N | Y | 0.069 | 7.52 |
| BY-G-9 | 3 | N | Y | 0.054 | 6.44 |
| BY-G-9 | 4 | N | Y | 0.067 | 6.66 |
| BY-G-9 | 5 | N | Y | 0.058 | 0.73 |
| BY-G-9 | 6 | N | Y | 0.064 | 11.28 |
| BY-G-9 | 7 | N | Y | 0.063 | 3.00 |
| BY-G-9 | 8 | N | Y | 0.068 | 16.19 |
| CH-H-1 | 1 | N | Y | 0.063 | 3.25 |
| CH-H-1 | 2 | N | Y | 0.077 | 18.29 |
| CH-H-1 | 3 | N | Y | 0.067 | 6.36 |
| CH-H-1 | 4 | N | Y | 0.078 | 6.73 |
| CH-H-1 | 5 | N | Y | 0.078 | 1.31 |
| CH-H-1 | 6 | N | Y | 0.070 | 8.91 |
| CH-H-1 | 7 | N | Y | 0.071 | 4.14 |
| CH-H-1 | 8 | N | Y | 0.077 | 7.15 |
| CY-PA2-1 | 1\* | Y | N | 0.061 | 5.89 |
| CY-PA2-1 | 2 | Y | N | 0.053 | 13.34 |
| CY-PA2-1 | 3 | Y | N | 0.047 | 5.17 |
| CY-PA2-1 | 4 | Y | N | 0.059 | 4.15 |
| CY-PA2-1 | 5 | Y | N | 0.047 | 3.95 |
| CY-PA2-1 | 6 | Y | N | 0.044 | 8.95 |
| CY-PA2-1 | 7\* | Y | N | 0.046 | 7.76 |
| CY-PA2-1 | 8 | Y | N | 0.044 | 13.53 |
| CY-PA3-1 | 1 | Y | N | 0.056 | 9.35 |
| CY-PA3-1 | 2 | Y | N | 0.058 | 12.73 |
| CY-PA3-1 | 3 | Y | N | 0.059 | 8.83 |
| CY-PA3-1 | 4 | Y | N | 0.055 | 9.97 |
| CY-PA3-1 | 5\* | Y | N | 0.058 | 4.44 |
| CY-PA3-1 | 6 | Y | N | 0.058 | 9.85 |
| CY-PA3-1 | 7 | Y | N | 0.054 | 7.90 |
| CY-PA3-1 | 8 | Y | N | 0.055 | 12.96 |
| CZ-KO-1 | 1 | N | Y | 0.086 | 8.13 |
| CZ-KO-1 | 2 | N | Y | 0.064 | 20.35 |
| CZ-KO-1 | 3 | N | Y | 0.075 | 7.38 |
| CZ-KO-1 | 4 | N | Y | 0.079 | 9.03 |
| CZ-KO-1 | 5 | N | Y | 0.074 | 0.47 |
| CZ-KO-1 | 6 | N | Y | 0.071 | 8.69 |
| CZ-KO-1 | 7 | N | Y | 0.065 | 11.63 |
| CZ-KO-1 | 8 | N | Y | 0.073 | 11.38 |
| DK-RL-3 | 1 | N | Y | 0.079 | 12.43 |
| DK-RL-3 | 2 | N | Y | 0.075 | 13.55 |
| DK-RL-3 | 3 | N | Y | 0.079 | 9.68 |
| DK-RL-3 | 4 | N | Y | 0.075 | 10.43 |
| DK-RL-3 | 5\* | N | Y | 0.080 | 3.20 |
| DK-RL-3 | 6\* | N | Y | 0.081 | 3.15 |
| DK-RL-3 | 7 | N | Y | 0.085 | 9.46 |
| DK-RL-3 | 8 | N | Y | 0.080 | 10.14 |
| ES-D-BDE-1 | 1 | Y | N | 0.049 | 2.27 |
| ES-D-BDE-1 | 2 | Y | N | 0.054 | 13.57 |
| ES-D-BDE-1 | 3 | Y | N | 0.047 | 8.82 |
| ES-D-BDE-1 | 4 | Y | N | 0.047 | 9.52 |
| ES-D-BDE-1 | 5 | Y | N | 0.054 | 7.25 |
| ES-D-BDE-1 | 6 | Y | N | 0.063 | 7.53 |
| ES-D-BDE-1 | 7 | Y | N | 0.055 | 10.47 |
| ES-D-BDE-1 | 8 | Y | N | 0.047 | 12.55 |
| ES-HT-1 | 1 | Y | N | 0.050 | 9.79 |
| ES-HT-1 | 2 | Y | N | 0.044 | 16.02 |
| ES-HT-1 | 3 | Y | N | 0.061 | 7.72 |
| ES-HT-1 | 4 | Y | N | 0.054 | 11.90 |
| ES-HT-1 | 5 | Y | N | 0.053 | 10.18 |
| ES-HT-1 | 6 | Y | N | 0.047 | 15.79 |
| ES-HT-1 | 7 | Y | N | 0.048 | 16.38 |
| ES-HT-1 | 8 | Y | N | 0.059 | 13.54 |
| FI-FAT-1-3 | 1 | Y | Y | 0.065 | 9.97 |
| FI-FAT-1-3 | 2 | Y | Y | 0.056 | 16.49 |
| FI-FAT-1-3 | 3 | Y | Y | 0.065 | 6.31 |
| FI-FAT-1-3 | 4 | Y | Y | 0.063 | 9.62 |
| FI-FAT-1-3 | 5 | Y | Y | 0.065 | 8.94 |
| FI-FAT-1-3 | 6 | Y | Y | 0.073 | 11.77 |
| FI-FAT-1-3 | 7 | Y | Y | 0.061 | 11.63 |
| FI-FAT-1-3 | 8 | Y | Y | 0.067 | 14.08 |
| FI-FUT1-2-1 | 1 | Y | Y | 0.072 | 11.42 |
| FI-FUT1-2-1 | 2 | Y | Y | 0.063 | 16.92 |
| FI-FUT1-2-1 | 3 | Y | Y | 0.064 | 3.69 |
| FI-FUT1-2-1 | 4 | Y | Y | 0.067 | 12.74 |
| FI-FUT1-2-1 | 5 | Y | Y | 0.061 | 5.87 |
| FI-FUT1-2-1 | 6 | Y | Y | 0.060 | 9.68 |
| FI-FUT1-2-1 | 7 | Y | Y | 0.061 | 19.16 |
| FI-FUT1-2-1 | 8 | Y | Y | 0.065 | 17.16 |
| FI-OER-3-3 | 1 | Y | Y | 0.058 | 13.75 |
| FI-OER-3-3 | 2 | Y | Y | 0.053 | 19.46 |
| FI-OER-3-3 | 3 | Y | Y | 0.050 | 7.36 |
| FI-OER-3-3 | 4 | Y | Y | 0.058 | 14.00 |
| FI-OER-3-3 | 5 | Y | Y | 0.060 | 7.95 |
| FI-OER-3-3 | 6 | Y | Y | 0.055 | 15.98 |
| FI-OER-3-3 | 7 | Y | Y | 0.063 | 8.44 |
| FI-OER-3-3 | 8 | Y | Y | 0.065 | 14.20 |
| FI-SK-58-2 | 1 | Y | Y | 0.062 | 14.38 |
| FI-SK-58-2 | 2 | Y | Y | 0.067 | 11.96 |
| FI-SK-58-2 | 3 | Y | Y | 0.067 | 10.46 |
| FI-SK-58-2 | 4 | Y | Y | 0.066 | 17.01 |
| FI-SK-58-2 | 5 | Y | Y | 0.075 | 5.01 |
| FI-SK-58-2 | 6 | Y | Y | 0.070 | 14.38 |
| FI-SK-58-2 | 7 | Y | Y | 0.069 | 6.71 |
| FI-SK-58-2 | 8 | Y | Y | 0.062 | 14.19 |
| FI-SKW-2-1 | 1 | Y | Y | 0.060 | 14.53 |
| FI-SKW-2-1 | 2 | Y | Y | 0.071 | 16.18 |
| FI-SKW-2-1 | 3 | Y | Y | 0.067 | 4.33 |
| FI-SKW-2-1 | 4 | Y | Y | 0.059 | 12.57 |
| FI-SKW-2-1 | 5 | Y | Y | 0.061 | 4.10 |
| FI-SKW-2-1 | 6 | Y | Y | 0.066 | 8.19 |
| FI-SKW-2-1 | 7 | Y | Y | 0.059 | 14.18 |
| FI-SKW-2-1 | 8 | Y | Y | 0.057 | 14.94 |
| GB-C1-1 | 1 | N | N | 0.059 | 4.36 |
| GB-C1-1 | 2 | N | N | 0.074 | 5.16 |
| GB-C1-1 | 3 | N | N | 0.067 | 1.63 |
| GB-C1-1 | 4 | N | N | 0.061 | 2.42 |
| GB-C1-1 | 5\* | N | N | 0.058 | 4.44 |
| GB-C1-1 | 6\* | N | N | 0.078 | 3.30 |
| GB-C1-1 | 7\* | N | N | 0.062 | 5.69 |
| GB-C1-1 | 8 | N | N | 0.046 | 10.59 |
| GB-EK2-6 | 1 | N | N | 0.059 | 10.63 |
| GB-EK2-6 | 2 | N | N | 0.068 | 19.94 |
| GB-EK2-6 | 3 | N | N | 0.062 | 7.89 |
| GB-EK2-6 | 4 | N | N | 0.064 | 15.13 |
| GB-EK2-6 | 5 | N | N | 0.069 | 1.92 |
| GB-EK2-6 | 6 | N | N | 0.066 | 10.59 |
| GB-EK2-6 | 7 | N | N | 0.071 | 6.01 |
| GB-EK2-6 | 8 | N | N | 0.066 | 10.56 |
| GB-FML-1 | 1 | N | N | 0.079 | 1.99 |
| GB-FML-1 | 2 | N | N | 0.065 | 10.42 |
| GB-FML-1 | 3 | N | N | 0.103 | 1.86 |
| GB-FML-1 | 4 | N | N | 0.066 | 9.48 |
| GB-FML-1 | 5 | N | N | 0.069 | 0.85 |
| GB-FML-1 | 6 | N | N | 0.073 | 8.81 |
| GB-FML-1 | 7 | N | N | 0.067 | 8.89 |
| GB-FML-1 | 8 | N | N | 0.061 | 11.93 |
| HU-AG-03 | 1 | Y | Y | 0.056 | 3.20 |
| HU-AG-03 | 2 | Y | Y | 0.058 | 15.30 |
| HU-AG-03 | 3 | Y | Y | 0.054 | 4.85 |
| HU-AG-03 | 4 | Y | Y | 0.052 | 8.61 |
| HU-AG-03 | 5 | Y | Y | 0.055 | 3.83 |
| HU-AG-03 | 6 | Y | Y | 0.056 | 12.07 |
| HU-AG-03 | 7 | Y | Y | 0.060 | 10.06 |
| HU-AG-03 | 8 | Y | Y | 0.047 | 13.11 |
| IE-DUB-1 | 1 | N | N | 0.050 | 4.19 |
| IE-DUB-1 | 2 | N | N | 0.041 | 11.54 |
| IE-DUB-1 | 3 | N | N | 0.045 | 10.65 |
| IE-DUB-1 | 4 | N | N | 0.057 | 12.72 |
| IE-DUB-1 | 5\* | N | N | 0.052 | 4.90 |
| IE-DUB-1 | 6 | N | N | 0.045 | 15.17 |
| IE-DUB-1 | 7 | N | N | 0.051 | 7.55 |
| IE-DUB-1 | 8 | N | N | 0.044 | 12.67 |
| IL-BM-1 | 1 | Y | N | 0.043 | 12.98 |
| IL-BM-1 | 2 | Y | N | 0.044 | 16.86 |
| IL-BM-1 | 3 | Y | N | 0.048 | 4.55 |
| IL-BM-1 | 4 | Y | N | 0.046 | 11.93 |
| IL-BM-1 | 5 | Y | N | 0.042 | 10.23 |
| IL-BM-1 | 6 | Y | N | 0.047 | 12.09 |
| IL-BM-1 | 7 | Y | N | 0.053 | 10.11 |
| IL-BM-1 | 8 | Y | N | 0.041 | 17.01 |
| IL-M1-8 | 1 | Y | N | 0.051 | 16.50 |
| IL-M1-8 | 2 | Y | N | 0.063 | 14.01 |
| IL-M1-8 | 3 | Y | N | 0.055 | 11.48 |
| IL-M1-8 | 4 | Y | N | 0.055 | 12.39 |
| IL-M1-8 | 5 | Y | N | 0.058 | 9.87 |
| IL-M1-8 | 6 | Y | N | 0.052 | 15.08 |
| IL-M1-8 | 7 | Y | N | 0.060 | 11.15 |
| IL-M1-8 | 8 | Y | N | 0.061 | 16.44 |
| IT-MDV-1 | 1 | Y | N | 0.063 | 11.97 |
| IT-MDV-1 | 2 | Y | N | 0.054 | 19.37 |
| IT-MDV-1 | 3 | Y | N | 0.055 | 8.86 |
| IT-MDV-1 | 4 | Y | N | 0.052 | 19.09 |
| IT-MDV-1 | 5 | Y | N | 0.055 | 7.45 |
| IT-MDV-1 | 6 | Y | N | 0.057 | 10.50 |
| IT-MDV-1 | 7 | Y | N | 0.064 | 3.91 |
| IT-MDV-1 | 8 | Y | N | 0.061 | 7.68 |
| IT-PER-2 | 1 | Y | N | 0.058 | 12.91 |
| IT-PER-2 | 2 | Y | N | 0.051 | 15.55 |
| IT-PER-2 | 3 | Y | N | 0.063 | 6.86 |
| IT-PER-2 | 4 | Y | N | 0.061 | 12.94 |
| IT-PER-2 | 5 | Y | N | 0.051 | 5.91 |
| IT-PER-2 | 6 | Y | N | 0.061 | 9.62 |
| IT-PER-2 | 7\* | Y | N | 0.067 | 5.31 |
| IT-PER-2 | 8 | Y | N | 0.064 | 12.11 |
| MA-ES-3 | 1 | Y | N | 0.038 | 6.91 |
| MA-ES-3 | 2 | Y | N | 0.037 | 16.72 |
| MA-ES-3 | 3 | Y | N | 0.037 | 17.93 |
| MA-ES-3 | 4 | Y | N | 0.036 | 11.45 |
| MA-ES-3 | 5 | Y | N | 0.039 | 10.02 |
| MA-ES-3 | 6 | Y | N | 0.039 | 12.54 |
| MA-ES-3 | 7\* | Y | N | 0.042 | 8.50 |
| MA-ES-3 | 8 | Y | N | 0.042 | 10.34 |
| NO-AA-1 | 1 | N | Y | 0.069 | 15.53 |
| NO-AA-1 | 2 | N | Y | 0.062 | 11.56 |
| NO-AA-1 | 3 | N | Y | 0.073 | 8.11 |
| NO-AA-1 | 4 | N | Y | 0.070 | 11.21 |
| NO-AA-1 | 5 | N | Y | 0.060 | 5.95 |
| NO-AA-1 | 6 | N | Y | 0.058 | 12.31 |
| NO-AA-1 | 7 | N | Y | 0.084 | 8.59 |
| NO-AA-1 | 8 | N | Y | 0.073 | 9.04 |
| NO-F-1 | 1 | N | Y | 0.051 | 10.61 |
| NO-F-1 | 2 | N | Y | 0.052 | 12.12 |
| NO-F-1 | 3 | N | Y | 0.054 | 11.09 |
| NO-F-1 | 4 | N | Y | 0.048 | 10.33 |
| NO-F-1 | 5 | N | Y | 0.055 | 12.72 |
| NO-F-1 | 6 | N | Y | 0.050 | 10.84 |
| NO-F-1 | 7 | N | Y | 0.050 | 10.03 |
| NO-F-1 | 8 | N | Y | 0.054 | 15.01 |
| NO-LADE-1 | 1 | N | Y | 0.074 | 5.02 |
| NO-LADE-1 | 2 | N | Y | 0.054 | 9.10 |
| NO-LADE-1 | 3 | N | Y | 0.064 | 10.01 |
| NO-LADE-1 | 4 | N | Y | 0.062 | 8.18 |
| NO-LADE-1 | 5 | N | Y | 0.068 | 3.35 |
| NO-LADE-1 | 6 | N | Y | 0.077 | 7.66 |
| NO-LADE-1 | 7 | N | Y | 0.061 | 6.57 |
| NO-LADE-1 | 8 | N | Y | 0.071 | 10.25 |
| NO-RO-1 | 1 | N | Y | 0.061 | 13.87 |
| NO-RO-1 | 2 | N | Y | 0.063 | 14.39 |
| NO-RO-1 | 3 | N | Y | 0.060 | 7.49 |
| NO-RO-1 | 4 | N | Y | 0.065 | 10.33 |
| NO-RO-1 | 5 | N | Y | 0.063 | 5.53 |
| NO-RO-1 | 6 | N | Y | 0.061 | 13.12 |
| NO-RO-1 | 7 | N | Y | 0.064 | 12.08 |
| NO-RO-1 | 8 | N | Y | 0.063 | 15.92 |
| RU-BOL1-1 | 1 | Y | Y | 0.061 | 13.34 |
| RU-BOL1-1 | 2 | Y | Y | 0.073 | 12.11 |
| RU-BOL1-1 | 3 | Y | Y | 0.067 | 13.86 |
| RU-BOL1-1 | 4 | Y | Y | 0.067 | 12.87 |
| RU-BOL1-1 | 5 | Y | Y | 0.070 | 11.45 |
| RU-BOL1-1 | 6 | Y | Y | 0.056 | 10.91 |
| RU-BOL1-1 | 7 | Y | Y | 0.069 | 10.82 |
| RU-BOL1-1 | 8 | Y | Y | 0.065 | 19.30 |
| RU-KA1-205 | 1 | Y | Y | 0.070 | 11.30 |
| RU-KA1-205 | 2 | Y | Y | 0.069 | 18.00 |
| RU-KA1-205 | 3 | Y | Y | 0.067 | 14.90 |
| RU-KA1-205 | 4 | Y | Y | 0.075 | 14.25 |
| RU-KA1-205 | 5 | Y | Y | 0.064 | 8.03 |
| RU-KA1-205 | 6 | Y | Y | 0.066 | 10.28 |
| RU-KA1-205 | 7 | Y | Y | 0.064 | 16.66 |
| RU-KA1-205 | 8 | Y | Y | 0.056 | 10.17 |
| RU-KOR1-1 | 1 | Y | Y | 0.058 | 9.31 |
| RU-KOR1-1 | 2 | Y | Y | 0.052 | 7.86 |
| RU-KOR1-1 | 3 | Y | Y | 0.167 | 1.76 |
| RU-KOR1-1 | 4\* | Y | Y | 0.048 | 7.84 |
| RU-KOR1-1 | 5 | Y | Y | 0.043 | 7.70 |
| RU-KOR1-1 | 6 | Y | Y | 0.052 | 2.39 |
| RU-KOR1-1 | 7 | Y | Y | 0.048 | 9.54 |
| RU-KOR1-1 | 8 | Y | Y | 0.040 | 5.56 |
| RU-R2-1 | 1 | N | Y | 0.065 | 10.84 |
| RU-R2-1 | 2 | N | Y | 0.069 | 13.43 |
| RU-R2-1 | 3 | N | Y | 0.061 | 9.10 |
| RU-R2-1 | 4 | N | Y | 0.058 | 10.75 |
| RU-R2-1 | 5 | N | Y | 0.074 | 7.84 |
| RU-R2-1 | 6 | N | Y | 0.063 | 10.61 |
| RU-R2-1 | 7 | N | Y | 0.063 | 19.54 |
| RU-R2-1 | 8 | N | Y | 0.063 | 14.23 |
| SE-G1-9 | 1 | Y | Y | 0.067 | 4.11 |
| SE-G1-9 | 2 | Y | Y | 0.050 | 17.34 |
| SE-G1-9 | 3 | Y | Y | 0.071 | 10.73 |
| SE-G1-9 | 4 | Y | Y | 0.063 | 9.10 |
| SE-G1-9 | 5 | Y | Y | 0.061 | 12.23 |
| SE-G1-9 | 6 | Y | Y | 0.053 | 15.30 |
| SE-G1-9 | 7 | Y | Y | 0.063 | 13.28 |
| SE-G1-9 | 8 | Y | Y | 0.058 | 15.68 |
| SE-GN2-3A10 | 1 | Y | Y | 0.050 | 12.16 |
| SE-GN2-3A10 | 2 | Y | Y | 0.043 | 11.77 |
| SE-GN2-3A10 | 3 | Y | Y | 0.039 | 9.79 |
| SE-GN2-3A10 | 4 | Y | Y | 0.043 | 13.08 |
| SE-GN2-3A10 | 5 | Y | Y | 0.056 | 5.64 |
| SE-GN2-3A10 | 6 | Y | Y | 0.050 | 12.55 |
| SE-GN2-3A10 | 7 | Y | Y | 0.045 | 7.77 |
| SE-GN2-3A10 | 8 | Y | Y | 0.050 | 16.79 |
| SE-H1-1 | 1 | Y | Y | 0.067 | 13.57 |
| SE-H1-1 | 2 | Y | Y | 0.060 | 9.72 |
| SE-H1-1 | 3 | Y | Y | 0.067 | 8.16 |
| SE-H1-1 | 4 | Y | Y | 0.063 | 13.66 |
| SE-H1-1 | 5 | Y | Y | 0.056 | 9.61 |
| SE-H1-1 | 6 | Y | Y | 0.054 | 13.98 |
| SE-H1-1 | 7 | Y | Y | 0.056 | 9.73 |
| SE-H1-1 | 8 | Y | Y | 0.060 | 9.66 |
| UA-KR-1-7 | 1 | Y | Y | 0.052 | 10.41 |
| UA-KR-1-7 | 2 | Y | Y | 0.058 | 21.05 |
| UA-KR-1-7 | 3 | Y | Y | 0.055 | 10.74 |
| UA-KR-1-7 | 4 | Y | Y | 0.055 | 14.28 |
| UA-KR-1-7 | 5 | Y | Y | 0.055 | 13.64 |
| UA-KR-1-7 | 6 | Y | Y | 0.047 | 13.21 |
| UA-KR-1-7 | 7 | Y | Y | 0.058 | 12.68 |
| UA-KR-1-7 | 8 | Y | Y | 0.048 | 22.90 |

## Table S3. Analysis of variance for the effect of habitat type and host genotype on trehalose concentration of *Daphnia magna* resting eggs, including all samples. Significant *p-*values are shown in bold (p ≤ 0.01).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Factor | Df | Mean of squares | | F value | | P value | |
| Summer-dry | 1 | | 370.6 | 11.74 | | **0.0016** | | |
| Winter-freeze | 1 | | 73.3 | 2.32 | | 0.137 | | |
| Summer-dry: Winter-freeze | 1 | | 12.4 | 0.39 | | 0.534 | | |
| Residuals | 33 | | 31.6 | - | - | |
| Error: Genotype | 259 | | 15.76 |  |  | |

## Section S4 – R scripts used for the analysis of this study

**Analysis of variance**

# R packages used in this analysis

>library(lme4)

# Input data for main analysis

>dat1 <-read.table("input\_file.csv", sep = ";", head=TRUE)

# Analysis of variance

> variance\_analysis<-aov(dat1$conc\_trehalose ~ dat1$sd \* dat1$wf + Error(dat1$genotype))

>summary(variance\_analysis)

# Analysis of variances and tests for checking normality of data and homogeneity of variances were repeated by using all data entries including ones with absorbance below 0.1, following the same methods.

#Normality test – Shapiro test – Normality of data residuals

> model<-lm(dat1$conc\_trehalose~ dat1$sd \* dat1$wf + (dat1$genotype), data=dat1)

>res<-resid(model)

>plot(fitted(model), res)

>qqnorm(res)

>hist(res)

>plot(density(res))

>shapiro.test(res)

#Bartlett's test – Homogeneity of variances

>bartlett.test(conc\_trehalose ~ interaction(sd,wf,genotype), data=dat1)

**Analysis of variance of genotypes component**

#R packages used in this analysis

>library(lme4)

# Analysis of variance of genotype component

>genotype\_variance<- lmer(conc\_trehalose ~ (1|genotype), data=dat1) #genotype is the random variable

>summary(genotype\_variance)

# Calculation of genotype variance component

>genotype\_variance\_perc<- “variance of genotype”/( “variance of genotype ”+”variance of residual”) \* 100

#in this case the genotype variance percentage value was obtained by the following

>genotype\_variance\_perc<- 2.635/(2.635+ 15.739) \*100

## Section S5 – Comparison of methods for trehalose quantification in *Daphnia magna* resting eggs.

*This section profited from the contributions of* *Ralph O. Schill and Arnd G. Heyer from the University of Stuttgart, Germany.*

**Overview:** Estimations of trehalose concentration presented in this study largely diverged from the ones estimated in Hengherr *et al.* (2011). Our main study reported an average of 10.55 % of trehalose per *D. magna* resting egg dry weight, whereas Hengherr *et al*. (2011) reported 0.5 % of trehalose per dry weight. The main difference between the two studies is the trehalose quantification method. In our study we used a calorimetric method following the Megazyme trehalose kit (Megazyme Bray, Ireland). Hengherr *et al*. (2011) used a high-performance liquid anion exchange chromatography (HPAEC) using a CarboPac PA-1 collumn on a Dionex DX-500 gradient chromatography system coupled with pulsed amperometric detection by a gold electrode (Dionex, Sunnyvale, CA).

Here we report on a comparison of the estimations of trehalose concentration in *Daphnia magna* resting eggs based on the two different quantification methods (our main study and Hengherr *et al.* 2011). The method applied in our study was run in the University of Basel and the method applied in Hengherr *et al*. (2011) was run in the University of Stuttgart. The samples were produced in duplicate at the University of Basel and the same biological material was split into two, in order to be analysed separately by the two distinct methods in the two research groups.

**Methods:** Four sets of thirty *Daphnia magna* resting eggs were decapsulated from the ephippial shell providing four samples. For each samples the total egg volume was calculated and a samples solution of extracted trehalose was prepared following the same methodology implemented both in our study and Hengherr *et al*. 2011. Furthermore, three blanks samples (ultrapure water) and three samples with trehalose of known concentration (180.9 mg/L) were produced. At this stage, each sample was separated into two portions of 120 µL (to be used in HPAEC method) and 20 µL (to be used in Megazyme trehalose kit method). Samples were frozen until measurements were performed (which took place in the two laboratories within three days). Sample codes were replaced with random numbers (Table S5.1).

Trehalose was quantified in each of the duplicated samples by both High-performance liquid anion exchange chromatography method in the University of Stuttgart, as in Hengherr *et al.* (2011), and trehalose Megazyme kit (Megazyme, Bray, Ireland), in the University of Basel, as described in the main experiment of our study.

|  |  |  |
| --- | --- | --- |
| Code | Sample | Origin of Daphnia eggs |
| B1 | Sample B | SE-H1-4 |
| B2 | Blank 1 | - |
| B3 | Sample A | SE-H1-4 |
| B4 | Trehalose 1 | - |
| B5 | Blank 2 | - |
| B6 | Sample C | FI-FUT1-2-1 |
| B7 | Blank 3 | - |
| B8 | Sample D | FI-FUT1-2-1 |
| B9 | Trehalose 2 | - |
| B10 | Trehalose 3 | - |

**Table S5.1** – Description of samples used in this experiment. Note that egg-samples B and A are from a different genetic *Daphnia* *magna* background than samples C and D, and are expected to differ to some degree.

**Results:** For both quantification methods, blank samples B2, B5 and B7 showed no trehalose, as expected. The quantities obtained by the two methods for the egg samples (trehalose concentration (g/L and mol/L)) were very similar. Specifically, the average trehalose concentration present on egg samples (B1, B3, B6 and B8) were of 4.2x10-4 mol/L for HPAEC method and 3.8x10-4 mol/L Megazyme trehalose kit method, resulting in, on average, about 10 % higher estimates by HPAEC method. In regard to the trehalose standard samples (B4, B9 and B10), average estimation was 5.97x10-4 mol/L and 4.78x10-4 mol/L, for each HPAEC and Megazyme trehalose kit method respectively. The trehalose samples comprised a known trehalose solution of 180.9 mg/L concentration (i.e. 4.8x10-4 mol/L). The HPAEC method overestimated this amount by 24 % and Megazyme trehalose kit method estimations were very close to the expected. However, the difference between the two methods was not significant (paired T-test: t=1.98, df=2, *p*-value=0.19).

Additionally, for each egg samples, trehalose per egg (g), trehalose per wet weight of egg (g/g) and trehalose per dry weight of egg (g/g) was calculated (*see* Table S5.2). Trehalose per wet weight was measured based on the estimation of eggs volume included in each sample, assuming a density of 1 (equal to water). Trehalose per dry weight was measured considering previous estimations in our laboratory of the ration between wet and dry egg for *Daphnia magna* resting eggs (i.e. estimations based on four samples of 100 eggs each). Mean of wet weight and dry weight per egg was estimated as 15.0 µg and 6.375 µg, respectively (thus, ration between egg dry and wet weight is 0.425). For comparison, the dry weight reported in Hengherr *et al.* (2011) is 7.39 µg. On average trehalose per egg dry weight was estimated as 10.7 % and 9.8 %, respectively for HPAEC and Megazyme trehalose kit methods (Table S5.2). This difference is not significant (paired T-test: t=1.85, df=3, *p*-value=0.16).

**Table S5.2** – Estimations of trehalose per egg (g), trehalose per egg wet and dry weight (g/g) for the trehalose quantification methods HPAEC and Megazyme trehalose kit performed in this study.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Samples Egg weight | | | | High-performance liquid anion exchange chromatography (Stuttgart) | | | Megazyme trehalose kit (Basel) | | |
| Code | Sample | eggs wet weight [mg] | eggs dry weight  [mg] | Trehalose per egg  [g] | Trehalose per egg wet weight [g/g] | Trehalose per egg dry weight [g/g] | Trehalose per egg  [g] | Trehalose per egg wet weight [g/g] | Trehalose per egg dry weight [g/g] |
| B1 | Sample B | 0.56 | 0.24 | 6.86E-07 | 0.036 | 0.086 | 6.21E-07 | 0.033 | 0.078 |
| B3 | Sample A | 0.58 | 0.24 | 6.60E-07 | 0.034 | 0.081 | 6.53E-07 | 0.034 | 0.080 |
| B6 | Sample C | 0.50 | 0.21 | 9.00E-07 | 0.054 | 0.128 | 7.31E-07 | 0.044 | 0.103 |
| B8 | Sample D | 0.48 | 0.20 | 9.09E-07 | 0.057 | 0.134 | 8.75E-07 | 0.055 | 0.129 |
| Average | | 0.53 | 0.23 | 7.89E-07 | 0.043 | 0.107 | 7.20E-07 | 0.042 | 0.098 |

**Discussion:** The results of this experiment showed that the two methods used to quantify trehalose resulted in similar estimates and that the amounts of trehalose provisioning in *D. magna* resting eggs are in accordance to the estimates of our main study.

The discrepancy between the reported values in our main study and in Hengherr *et al.* (2011) cannot be explained by these new quantifications, because this discrepancy seems neither be attributable to a difference among the two research groups nor to a difference in the quantification methods applied. Examination of the data presented in Hengherr *et al.* (2011) shows that egg dry weight is roughly similar between them and this study (7.39 µg and 6.375 µg, respectively). *Daphnia magna* resting eggs can vary by factor two in volume, even for eggs from the same female. Also, genotypes differ in the size of egg they produce. In addition, the Hengherr *et al.* (2011) reported hatching rates of previously dried resting eggs of 14 %, which fits the lower end of hatching rates reported otherwise. We conclude that, in regard to these parameters the *D. magna* genotype used by Hengherr *et al.* (2011) is not unusual. Estimations of trehalose content on *D. magna* resting eggs remain distinct between the two studies. An explanation might be due to an extreme case of biological material used by Hengherr *et al.* (2011).

The trehalose level of the resting stages of *Daphnia magna* reported in our study are in the general range observed for other organisms, for example *Polypedilum vanderplanki* (insecta), *Artemia* (lower Crustacea) and nematods, and where trehalose levels were reported to be between 10 % and 20 % of dry weight (Clegg 1965; Madin and Crowe 1975; Watanabe *et al.* 2002).

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