Supplementary Material

**C:\Users\nkn\OneDrive - Aalborg Universitet\phD\Articles for Publication\Groenlandicus metabolomics\Results\plots\collection site_Side_3.tif**

**Figure S1:** The fieldwork site (A) is a grass-covered and dry habitat located along the bank of Tunulliarfik Fjord. Adult *N. groenlandicus* was collected from the grasses (B and C) using a sweep net.

**Table S1:** Overview of dates and times for field collection of *N. groenlandicus*, start times of thermal assays (heat knockdown time (HKDT) and chill coma recovery temperature (Trecovery)), and microhabitat temperature summary. The insects were kept in glass containers in the shadow in the field site until assay start. Stars (\*) indicate time points when samples for GC-MS analysis were collected simultaneously with individuals for thermal tolerance tests. Microhabitat temperatures were summarized by average, minimum, maximum, and daily range (max-min) in the 1 hour timespan prior to assay start.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Sampling date | HKDT assay start | Trecovery assay start | Avr | Min | Max | Range |
| Day 1 | 07-08-2018 | 08:30 | 08:28 | 9.4 | 7.0 | 11.5 | 4.5 |
| 07-08-2018 | 12:10 | 12:09 | 25.3 | 22.0 | 28.0 | 6.0 |
| 07-08-2018 | 16:08 | 16:06 | 24.5 | 23.5 | 26.0 | 2.5 |
| 07-08-2018 | 19:58 | 19:58 | 17.1 | 15.0 | 19.5 | 4.5 |
| Day 2 | 10-08-2018 | 08:22 | 08:14 | 10.7 | 10.0 | 12.0 | 2.0 |
| 10-08-2018 | 12:20 | 12:15 | 26.3 | 23.0 | 28.5 | 5.5 |
| 10-08-2018 | 17:08 | 17:09 | 23.6 | 22.0 | 24.5 | 2.5 |
| 10-08-2018 | 20:35 | 20:32 | 15.9 | 14.5 | 17.0 | 2.5 |
| Day 3 | 11-08-2018 | 08:14 | 08:08 | 10.1 | 8.5 | 11.0 | 2.5 |
| 11-08-2018 | 12:15 | 12:09 | 18.0 | 17.5 | 19.0 | 1.5 |
| 11-08-2018 | 16:16 | 16:09 | 21.5 | 20.0 | 24.0 | 4.0 |
| 11-08-2018 | 20:20 | 20:11 | 12.5 | 12.0 | 13.5 | 1.5 |
| Day 4 | 22-08-2018\* | 08:51 | 08:44 | 8.1 | 7.0 | 8.5 | 1.5 |
| 22-08-2018\* | 12:22 | 12:15 | 10.2 | 10.0 | 10.5 | 0.5 |
| 22-08-2018\* | 16:22 | 16:15 | 13.0 | 11.5 | 14.5 | 3.0 |
| 22-08-2018\* | 20:20 | 20:15 | 8.8 | 7.0 | 10.0 | 3.0 |
| Day 5 | 27-08-2018\* | 08:20 | 08:13 | 1.0 | 0.5 | 2.5 | 2.0 |
| 27-08-2018\* | 12:39 | 12:33 | 22.2 | 18.0 | 25.0 | 7.0 |
| 27-08-2018\* | 16:29 | 15:25 | 16.0 | 13.5 | 21.5 | 8.0 |
| 27-08-2018\* | 20:25 | 16:21 | 8.6 | 7.0 | 10.0 | 3.0 |



**Figure S2:** A) Mean heat knockdown time (seconds) and B) chill coma recovery temperature (°C) for female (red) and male (blue) *N. groenlandicus* collected across different times and days. Bars are standard errors of the mean.

**Table S2:** Summary of two-way ANOVAs on rank inverse transformed HKDT and Trecovery of individuals (n=20 pr assay) as dependent variables and ‘day’ and ‘Time of day’ as independent variables.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **ANOVA Heat knockdown time** | | | | | |  |
| sex | variable | Df | Sum sq | mean sq | F value | Pr(>F) |  |
| females | day | 4 | 27.25 | 6.81 | 8.62 | 1.14E-06 | \*\*\* |
| time | 3 | 23.37 | 7.79 | 9.86 | 2.83E-06 | \*\*\* |
| day:time | 12 | 47.61 | 3.97 | 5.02 | 9.15E-08 | \*\*\* |
| Residuals | 379 | 299.47 | 0.79 |  |  |  |
|  |  |  |  |  |  |  |  |
| males | day | 4 | 28.30 | 7.08 | 7.70 | 5.65E-06 | \*\*\* |
| time | 3 | 0.70 | 0.24 | 0.26 | 0.855 |  |
| day:time | 12 | 20.00 | 1.67 | 1.82 | 0.044 | \* |
| Residuals | 379 | 348.60 | 0.92 |  |  |  |
|  |  |  |  |  |  |  |  |
|  | **ANOVA chill coma recovery temperature** | | | | | |  |
| females | day | 4 | 40.80 | 10.21 | 11.77 | 5.10E-09 | \*\*\* |
| time | 3 | 9.20 | 3.06 | 3.52 | 0.015 | \* |
| day:time | 12 | 18.90 | 1.57 | 1.81 | 0.045 | \* |
| Residuals | 378 | 327.80 | 0.87 |  |  |  |
|  |  |  |  |  |  |  |  |
| males | day | 4 | 32.50 | 8.13 | 9.07 | 5.22E-07 | \*\*\* |
| time | 3 | 12.20 | 4.06 | 4.53 | 0.004 | \*\* |
| day:time | 12 | 13.60 | 1.13 | 1.26 | 0.238 |  |
| Residuals | 379 | 339.40 | 0.90 |  |  |  |

**Table S3:** Metabolites detected by GC-MS on whole-body extract on female *N. groenlandicus*. A total of 33 metabolites were detected. Two metabolites (marked by \*) occurred at concentrations below the quantification limit.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Free amino acids*** | ***Sugars*** | ***Polyols*** | ***Metabolic intermediates*** | ***Other metabolites*** |
| Alanine (Ala) | Fructose (Fru) | Adonitol | Citric acid | Citrulline |
| Isoleucine (Ile) | Galactose (Gal) | Arabitol | Fumaric acid | Ethanolamine\* | |
| Leucine (Leu) | Glucose (Glc) | Inositol | Glyceric acid | Phosphoric Acid |
| Lysine (Lys) | Glucose-6-phosphate (G6P) | Glycerol | Lactic acid |  |
| Glutamic acid (Glu) | Trehalose (Tre) | Glycerol-3-Phosphate | Malic acid |  |
| Glycine (Gly) |  | Xylitol\* | Succinic acid |  |
| Ornithine (Orn) |  |  |  |  |
| Phenylalanine (Phe) |  |  |  |  |
| Proline (Pro) |  |  |  |  |
| Serine (Ser) |  |  |  |  |
| Threonine (Thr) |  |  |  |  |
| Tyrosine (Tyr) |  |  |  |  |
| Valine (Val) |  |  |  |  |

**Table S4:** Raw data of detected metabolite concentrations from *N. groenlandicus* whole-body extract by GC-MS. Concentrations (nmol.mg-1) are listed for each metabolite (columns) and each replicate (rows) of the different sampling times and days (Excel).

**Table S5:** Results from one-way Analysis of Variance (ANOVA) performed on log-transformed metabolite data for day 4 and day 5 separately. The dependence of sampling time (morning, midday, afternoon, and evening) on metabolite concentration was examined for each metabolite (Excel).

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**Figure S3:** Between-class Principal Component Analyses (PCA) based on GC-MS on whole-body extracts of female *N. groenlandicus* sampled in the field at four consecutive sampling time points (8:00 am, 12:00 pm, 4:00 pm, 8:00 pm) on **A)** day 4 and **B)** day 5. Scores for PC1 and PC3 are depicted. Lines represents individual sample position respective to centroids (n=8). Correlations of metabolite concentrations (relative proportions) to PCs in the between-class PCA. A and B represent metabolite correlations to PC2 for day 4 and day 5, respectively.