

Fig. S1: Two examples of NMR-spectra from blank samples (black lines) compared to a biological sample (red line). The blank samples were obtained by sampling the buffer droplet that had been placed for up to 45 min. The biological sample is from a cold exposed *D. melanogaster*. On the basis of such comparisons we identified regions of the NMR spectra that were contaminated and these were omitted from the analysis (See Fig. 1 in the main manuscript).

The individual metabolites found in the spectra are listed in table S1. The variation in metabolite content can also be seen from a more simple correlation analysis between the relative intensities of the individual metabolites and the cold tolerance measure LT_{e50} (Fig. S1A-D). The metabolite intensities have first been normalized to the total intensity in each spectrum which allows us to compare the intensities regardless of how many flies were used in each sample. However it is important to remember that the spectra allow us to “see” H-atoms on different molecules. This means that if there are more of “the same” H-atoms on a given molecules the intensity of its signal will increase accordingly.

Therefore the metabolite intensities shown in Fig. S1 are also normalized to the number of hydrogen atoms that were present in the signals used for each metabolite (given in table S1). This allows the y-axes in figure S1 to be directly compared even though the intensities themselves are still only relative (to the total signal intensity). This is important since it gives us the ability to see that trehalose is by far the most abundant metabolite of the four (appr. 40 times more abundant than sarcosine) which means that it is also far more important as an osmolyte.

Table S1: Identified metabolites in the NMR spectra. No. corresponds to the numbers given in the spectrum in Fig. 2.

No	Name	Chemical shift	No. of hydrogen atoms
1	Guanine/Xanthine	7.67 ppm	1
2	Formate	6.50 ppm	1
3	O-phosphotyrosine	7.22, 7.17 ppm	2
4	Tyrosine	7.18, 7.89 ppm	2
5	Maltose	5.39, 5.22 ppm	1
6	Glucose	5.21, 3.97 ppm	1
7	Trehalose	5.18, 3.44 ppm	2
8	O-phosphocholine	4.15, 3.21 ppm	9
9	Methanol	3.38 ppm	3
10	unidentified metabolite	3.01, 1.71 ppm	?
11	methionine sulfoxide or sarcosine	2.74 ppm	3
12	β -alanine	2.54 ppm	2
13	Glutamine	2.44 ppm	2
14	Sarcosine	2.39 ppm	2
15	Proline	2.34, 2.06, 1.99 ppm	2
16	Unidentified metabolite	1.09 ppm	?
17	Unidentified fatty acid	0.85 ppm	3

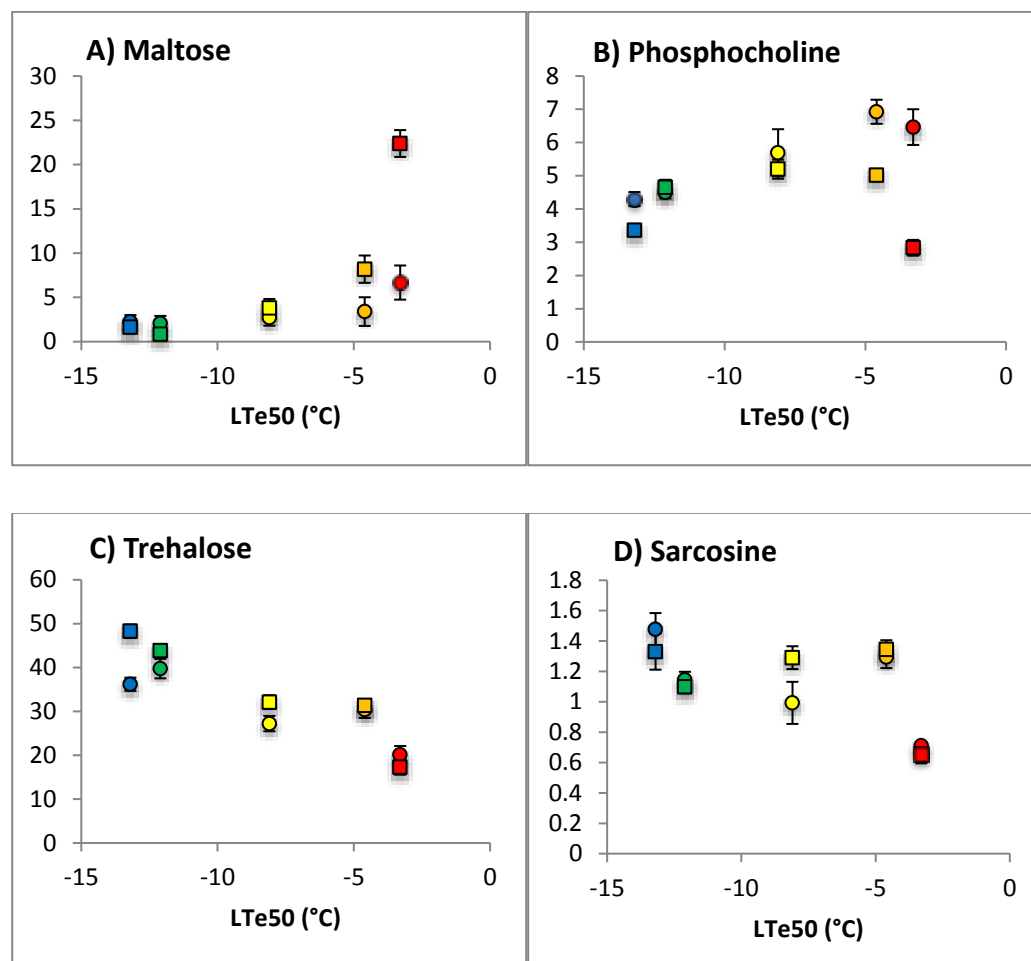


Fig. S2: The relative intensities of four specific metabolites identified in the five species before (circle) and after cold treatment (square). The metabolites are A) Maltose B) Phosphocholine C) Trehalose and D) Sarcosine