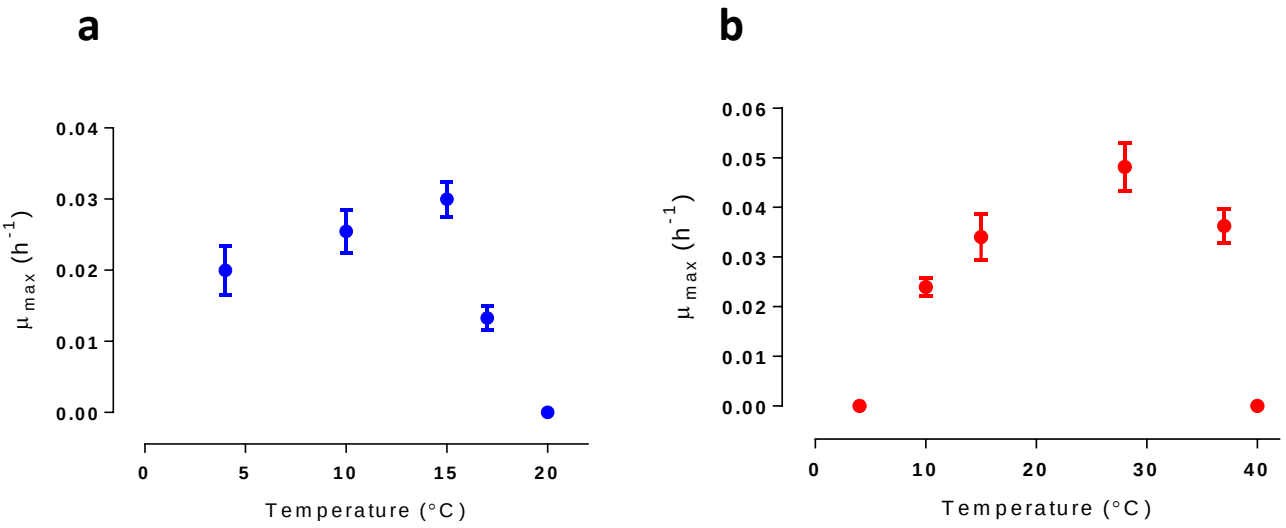

Figure 1. Maximum growth rate (μ_{MAX}) of exponentially growing algal cultures at various temperatures**. (a)** *Chlamydomonas* sp. UWO241 **(b)** *Chlamydomonas reinhardtii*. Data are the means ± SD of at least six biological replicates

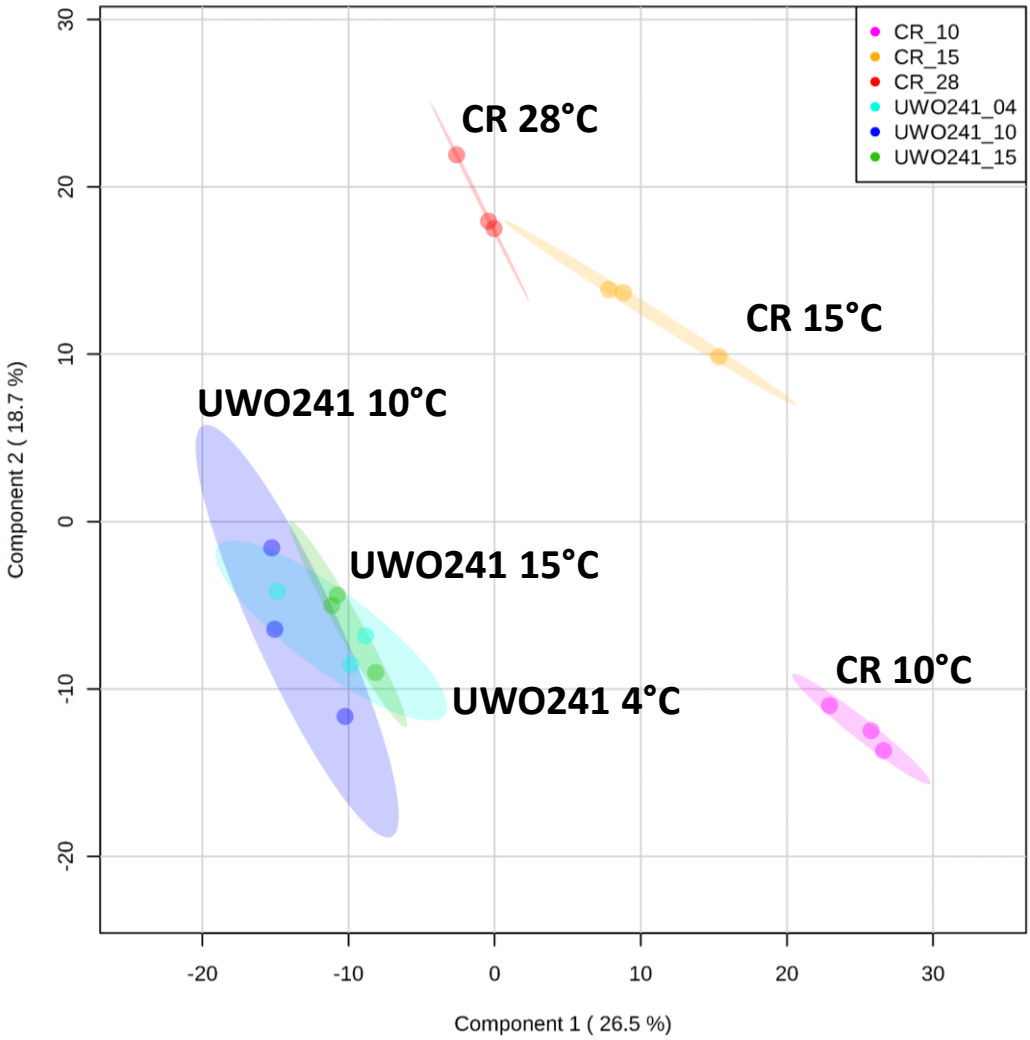

Figure 2: Principal component analysis (PCA) of the primary metabolome of the two *Chlamydomonas* species acclimated to different steady-state temperatures. *C. reinhardtii* was grown at 10°C (magenta; CR_10), 15°C (orange; CR_15), and 28°C (red; CR_28). UWO241 was grown at 4°C (cyan; UWO241_04), 10°C (blue; UWO241_10) and 15°C (green; UWO241_15). The analysis includes all 771 quantified metabolites separated along the first two principal components that explained the largest degree of variation in the datasets, and the 95% confidence interval for each treatment.

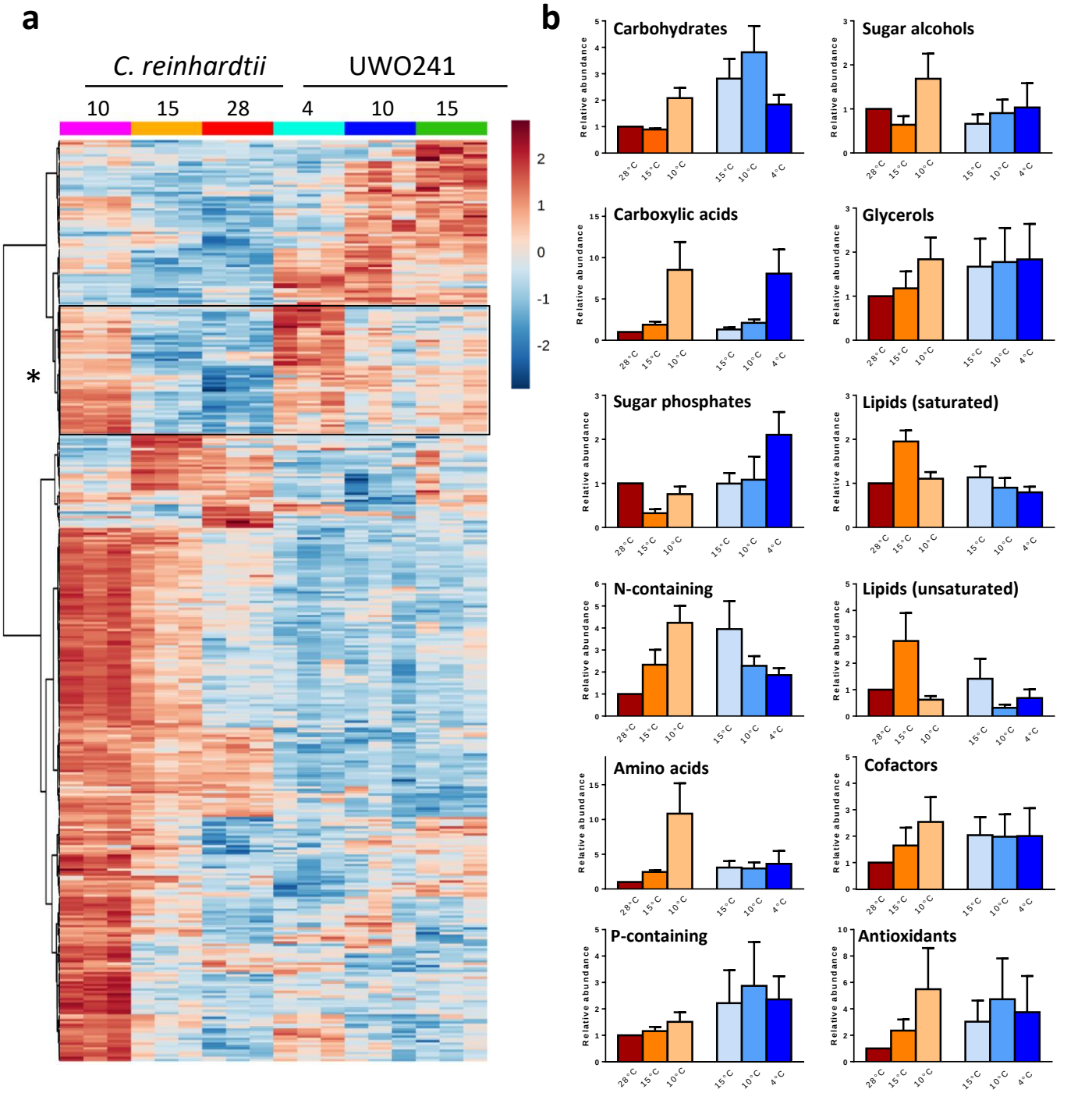

Figure 3. Differences in the primary metabolome of *C. reinhardtii* and UWO241, acclimated at different steady-state temperatures. (**a**) Heat map showing the relative changes in metabolite abundances between growth temperatures in the two algal species. Only metabolites which are significantly different are shown (392 metabolites, ANOVA, P<0.01). In each treatment, three biological replicates are represented using a color based metabolite profile as indicated (red – increase in abundance; blue – decrease in abundance). Hierarchical clustering is based on Euclidean distances and Ward's linkage. A cluster of metabolites present in both species at the lowest temperature is highlighted by a *. (**b**) Relative abundance of metabolites classified based on their chemical nature. Only metabolites which were positively identified based on their GC-MS spectra and retention times were taken into consideration. In this analysis, the metabolite abundance corresponding to *C. reinhardtii* grown at 28°C was arbitrarily set to 1 and all other treatments were compared to this sample.

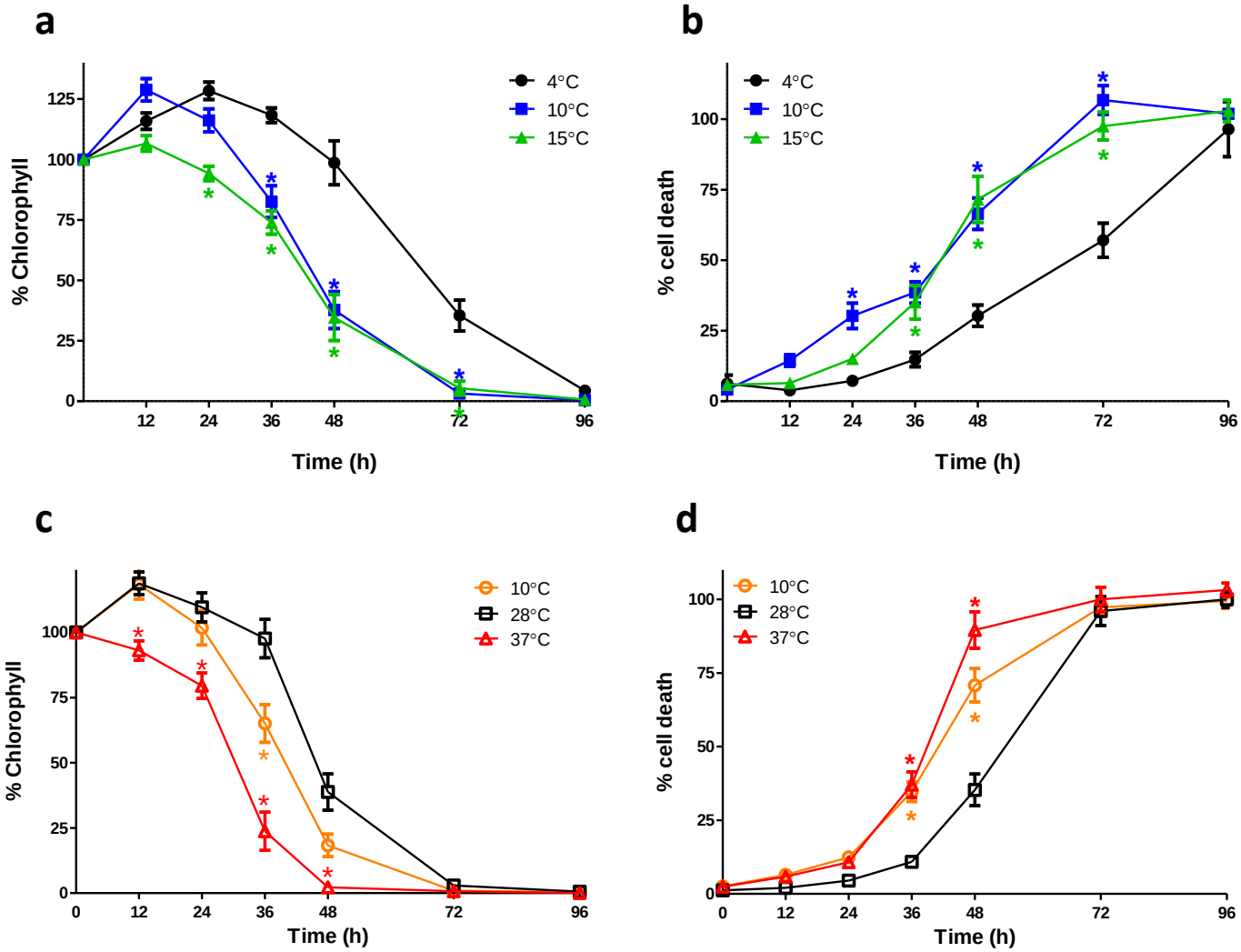

Figure 4. Kinetics of cell death in UWO241 **(a,b)** and *C. reinhardtii* **(c,d)** acclimated to different growth temperatures and exposed to non-permissive conditions (24°C and 42°C, respectively). Cell death was estimated as the loss of chlorophyll in cells exposed to heat **(a,c)** or as a percentage of algal cells stained with 0.5% Evans Blue that accumulates in cells with damaged membranes **(b,d)**. Algal cells treated with 1% v/v chloroform were taken as a positive control and used to calculate 100% cell death. Data are means ± SD of at least three independent experiments and analyzed by two-way ANOVA followed by Bonferroni post-test comparing each treatment with 4°C (UWO241) and 28°C (*C. reinhardtii*). Statistical significance ($P<0.01$) is indicated as *

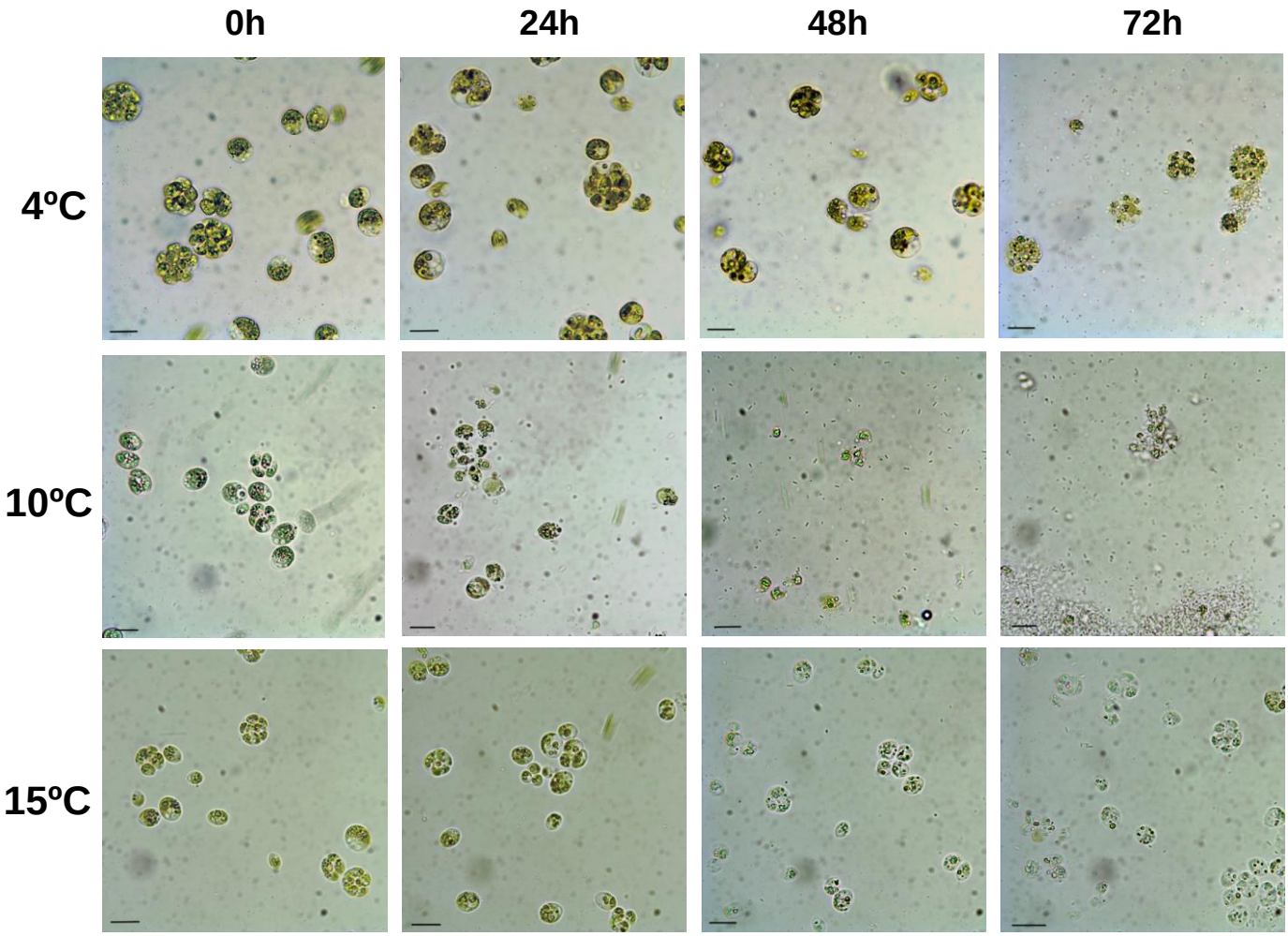

Figure 5. Light microscope images of UWO241 acclimated to different steady state temperatures (4°C, 10°C, 15°C) and exposed to non-permissive temperature (24°C) for 24h, 48h and 72h. Algae are present as single cells or palmelloid colonies. Scale bar = 15 μm (400x total magnification)

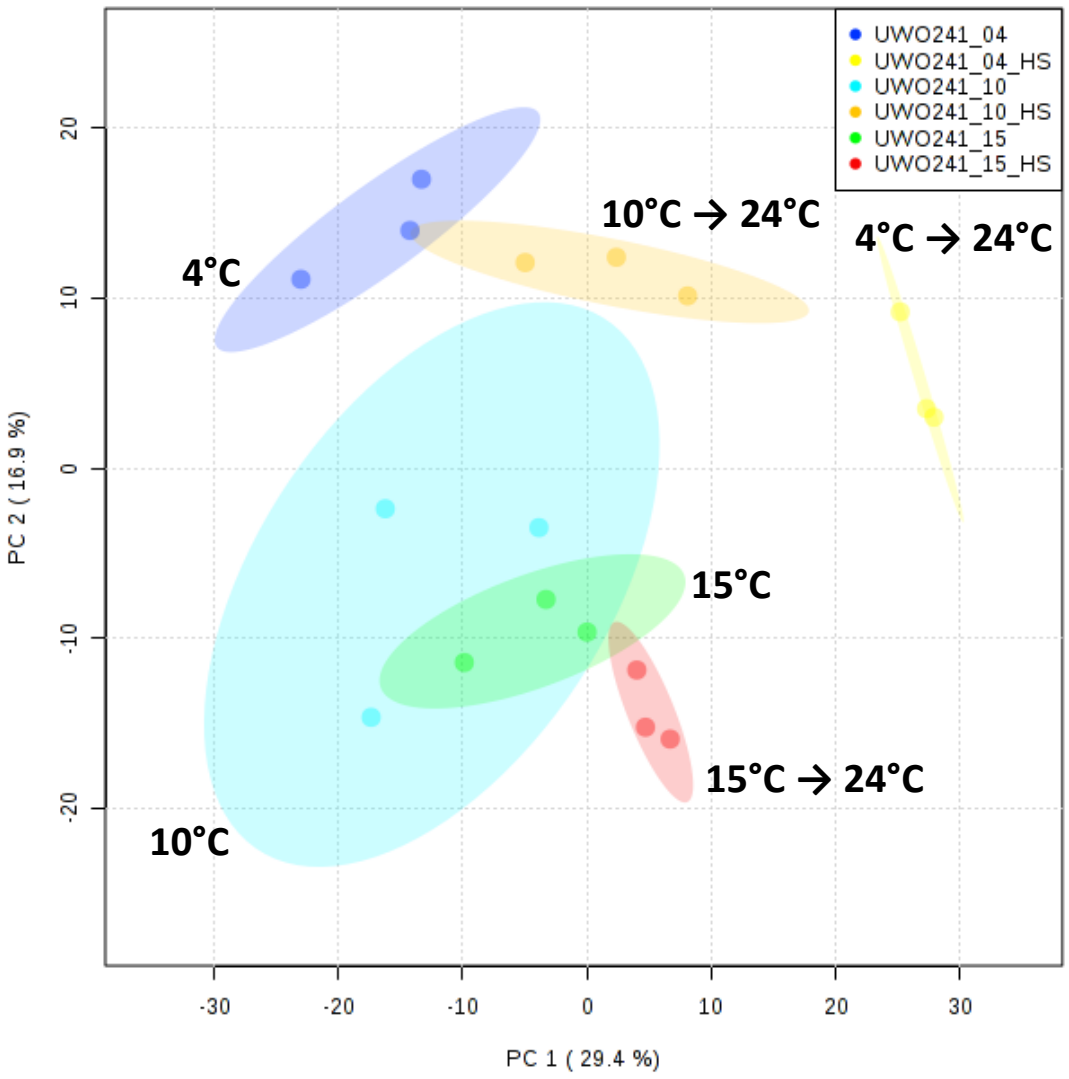

Figure 6. Principal component analysis (PCA) of the primary metabolome of UWO241 acclimated to different steady-state temperatures and subsequently exposed to non-permissive temperature for 6 hours. UWO241 was grown at 4°C (blue, UWO241_4) and exposed to 24°C (yellow, UWO241_4_HS); grown at 10°C (cyan; UWO241_10) and exposed to 24°C (orange, UWO241_10_HS); and grown at 15°C (green; UWO241_15) and exposed to 24°C (red, UWO241_15_HS). The analysis includes all quantified metabolites separated along the first two principal components and the 95% confidence interval for each treatment.

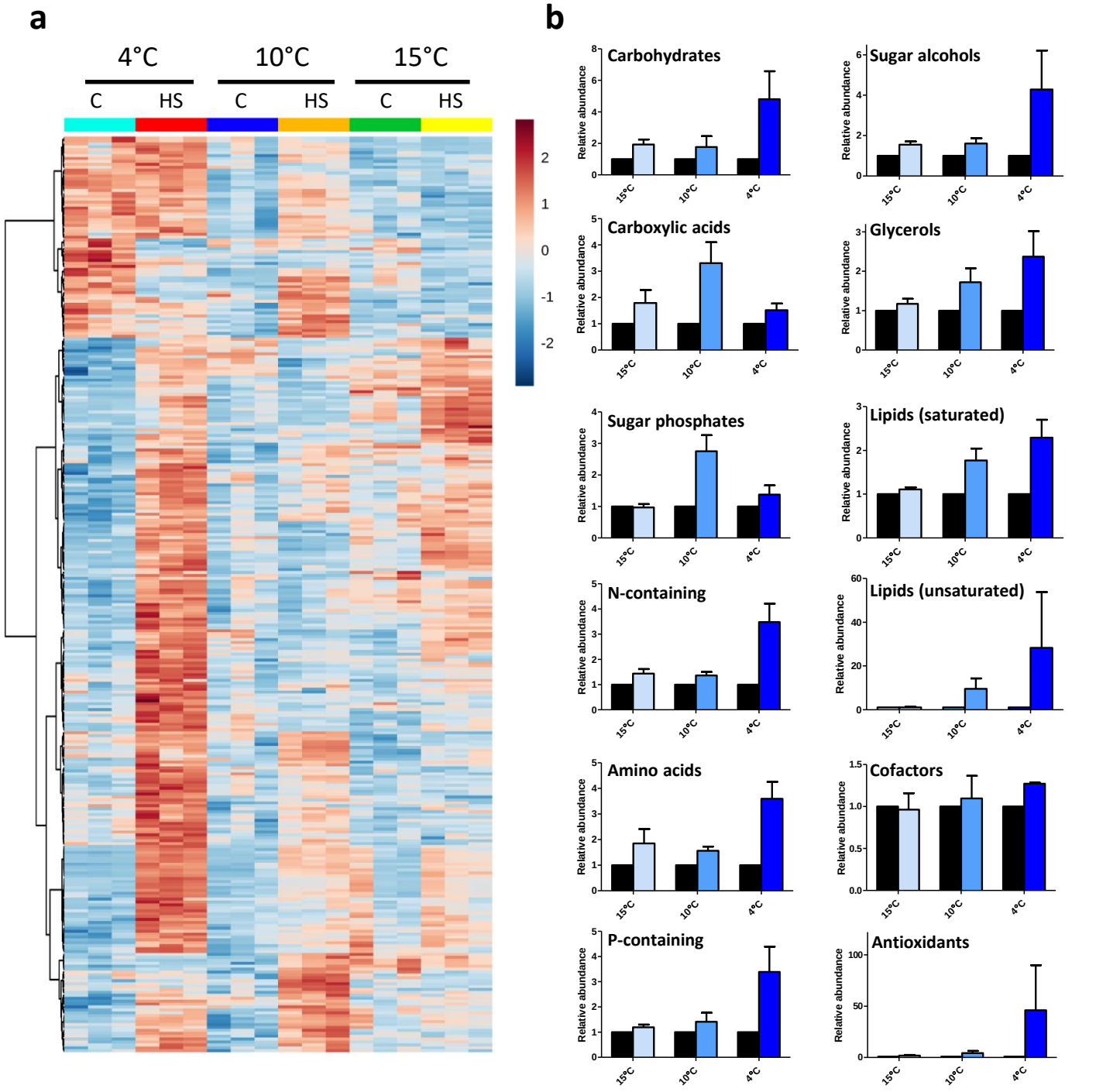

Figure 7. Differences in the primary metabolome in UWO241 acclimated at different temperatures (4°C, 10°C, 15°C) and subsequently exposed to heat stress (24°C) for 6 hours. (**a**) Heat map showing the relative changes in metabolite abundances between control samples at each steady-state growth temperature (C) and heat-treated samples (HS). Only metabolites that are significantly different are shown (314 metabolites, ANOVA, $P<0.01$). Three biological replicates are represented using a color-based metabolite profile as indicated (red – high abundance; blue – low abundance). Hierarchical clustering is based on Euclidean distances and Ward's linkage (**b**) Relative abundance of metabolites classified based on their chemical nature. Only metabolites which were positively identified based on their GC-MS spectra and retention times were taken into consideration. In this analysis, the metabolite abundance in algae grown at the three different steady state temperatures were arbitrarily set to 1 (black bars) and all the heat stress treatments were compared to the corresponding control sample (blue bars).

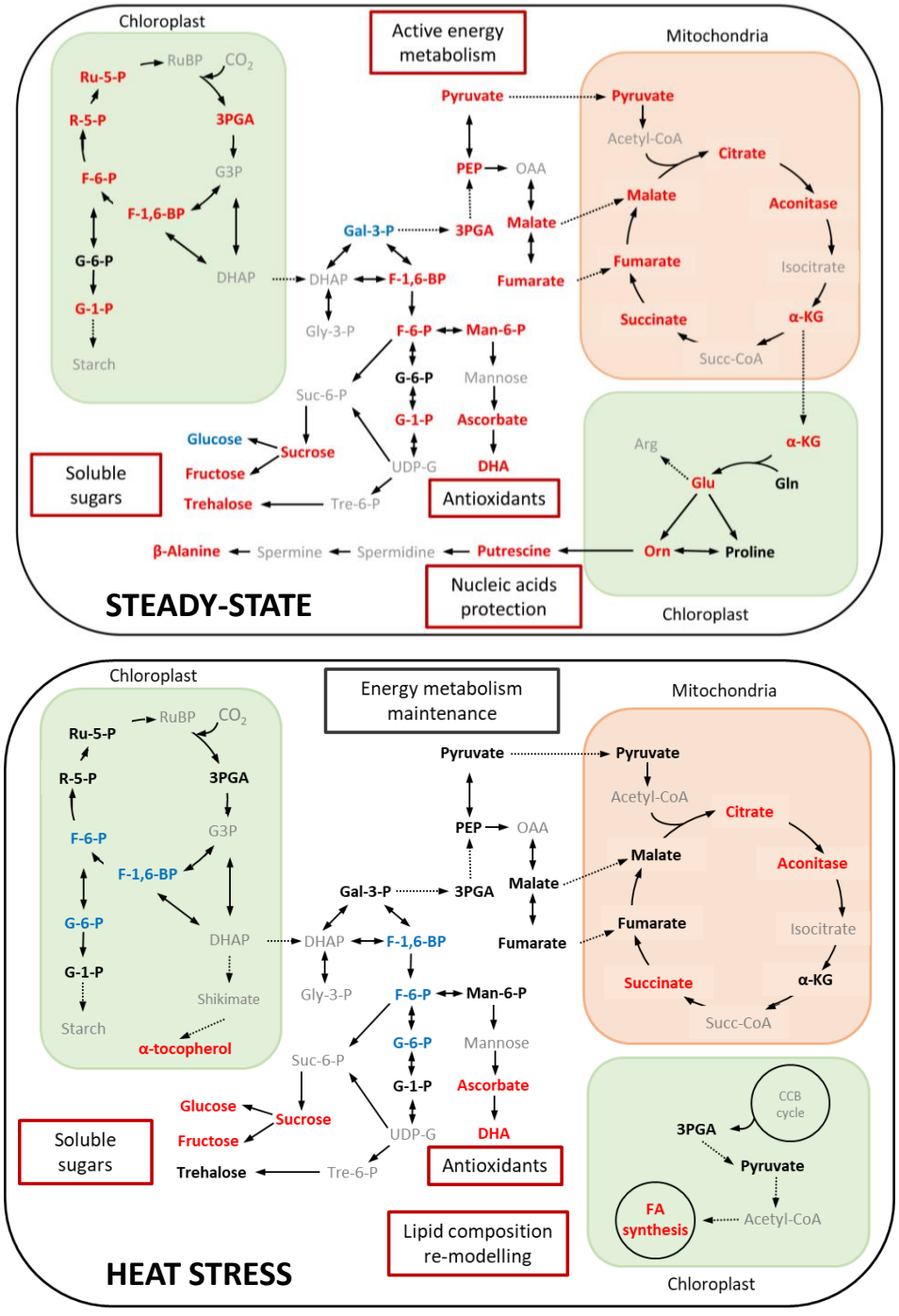

Figure 8: (a) UWO241 grown at temperatures closest to its natural environment in Lake Bonney has an active central metabolism and constitutively accumulates metabolites important for life at low temperatures, including soluble sugars, antioxidants and compounds involved in nucleic acid protection. This simplified pathway map shows key metabolites that are increased (red) or decreased (blue) in UWO241 at 4°C, when compared to *C. reinhardtii* at 28°C. Metabolites shows in black did not change significantly, and those in gray were not detected in this study. We propose that this metabolic state provides UWO241 with the ability to cope with environmental stress**. (b)** When UWO241 is exposed to short-term heat stress at 24°C, many metabolites characteristic for cold adaptation, including soluble sugars and antioxidants, are maintained or even increased. The maintenance of energy metabolism could provide the energy to drive the production of protective compounds during heat stress. We show key metabolites that are increased (red) or decreased (blue) in, when compared to UWO241 at 4°C. Metabolites shows in black did not change significantly, and those in gray were not detected in this study.