

High temperatures cause reduced growth, plant death and metabolic changes in eelgrass *Zostera marina*

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Text S1.

Materials and methods

Metabolomics

Ten mg of lyophilized and homogenized plant material (leaf, rhizome and root) were extracted in 1 mL methanol/acetonitrile/water (4:4:1 [v/v/v]) and dried in a speed-vac. The dried samples were methoximated and silylated as outlined in Weckwerth *et al.* (2004). The metabolites were separated on Agilent 7890B gas chromatograph equipped with a DB5-MS Ultra-inert column (30 m, 0.25 mm, 0.25 μ m) (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 7200 GC-QTOF-MS (Agilent Technologies, Santa Clara, CA, USA) after injecting 1 μ l in split 1:20 mode. The GC temperature gradient was at 60 °C for 1 min, followed by a ramp of 10 °C per min to 325 °C held for 10 min. The TOF acquisition rate was set to 10 spectra s⁻¹ in the extended dynamic range mode (2GHz). Data were collected, examined, deconvoluted, aligned and annotated in Masshunter (Agilent Technologies, Santa Clara, CA, USA). Analytes were considered as putatively annotated (MSI level 2, after Sumner *et al.*, 2007) by matching the deconvoluted and aligned mass spectra against an in-house library as well as the Fiehn-lib (Kind *et al.*, 2009) (match factor >80) and annotation was further supported by manual comparison of retention indices. Peak areas were standardized for sample weight and to the internal standard and relatively quantified. Later baselined by unit scaling (mean-centered and divided by standard deviation of each variable) and log₂ transformed. The effect of temperature increase was analyzed in MetaboAnalyst (Xia *et al.*, 2015) by univariate and multivariate methods. A cut-off value of P < 0.05 was considered as significant in the one-way ANOVA (Tukey's multiple comparison test) applying a Benjamini Hochberg false discovery rate of 5 % for multiple testing corrections.

Results

Chlorophyll a

Leaf chlorophyll *a* content (Figure S1) did not vary significantly, but tended to decrease with temperature ($P = 0.085$, One-way ANOVA). The content varied from $18.0 \pm 0.5 \mu\text{g cm}^{-2}$ (mean \pm SE, $N = 3$) in the 22 °C treatment over $16.0 \pm 1.8 \mu\text{g cm}^{-2}$ in the 26 °C treatment to $12.8 \pm 1.4 \mu\text{g cm}^{-2}$ in shoots grown at 30 °C.

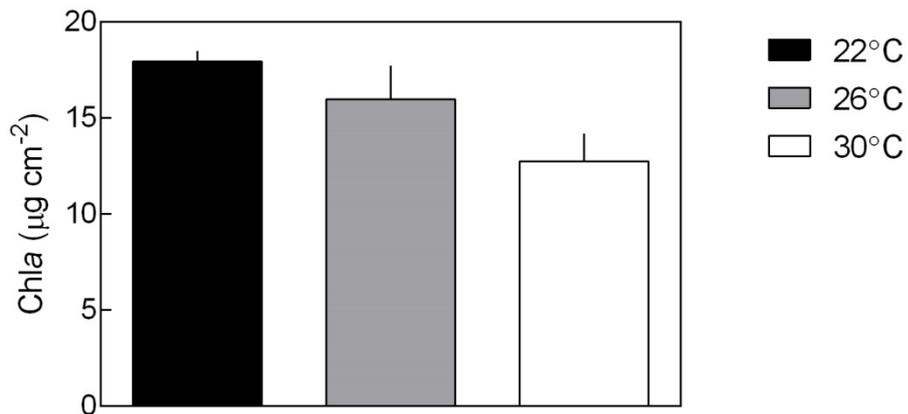


Fig. S1. Chlorophyll *a* content ($\mu\text{g cm}^{-2}$) in the second youngest leaf after 26 days of incubation at 22, 26 and 30 °C (Not significant, One-way ANOVA, $P = 0.085$, $N = 3$). Mean \pm SE.

Fig. S2. A

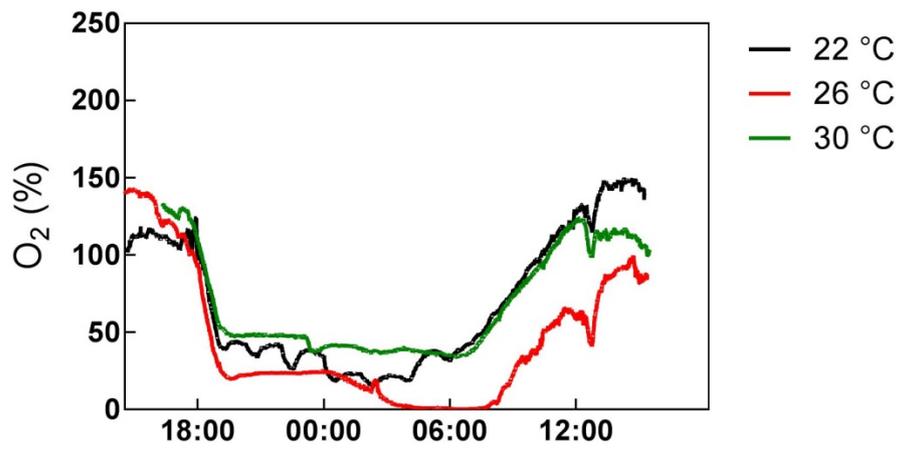
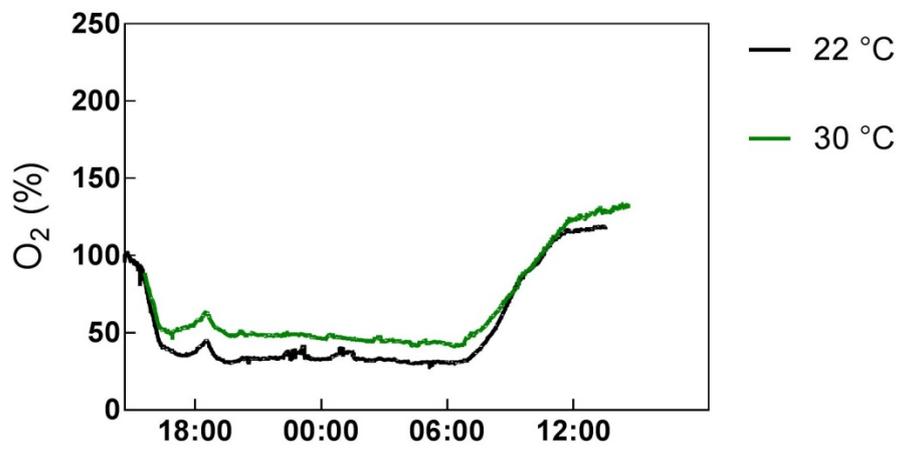
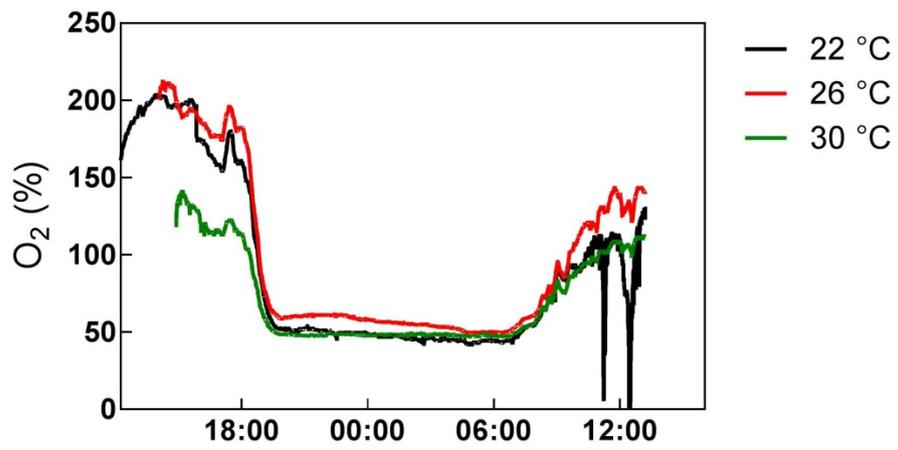


Fig. S2. B

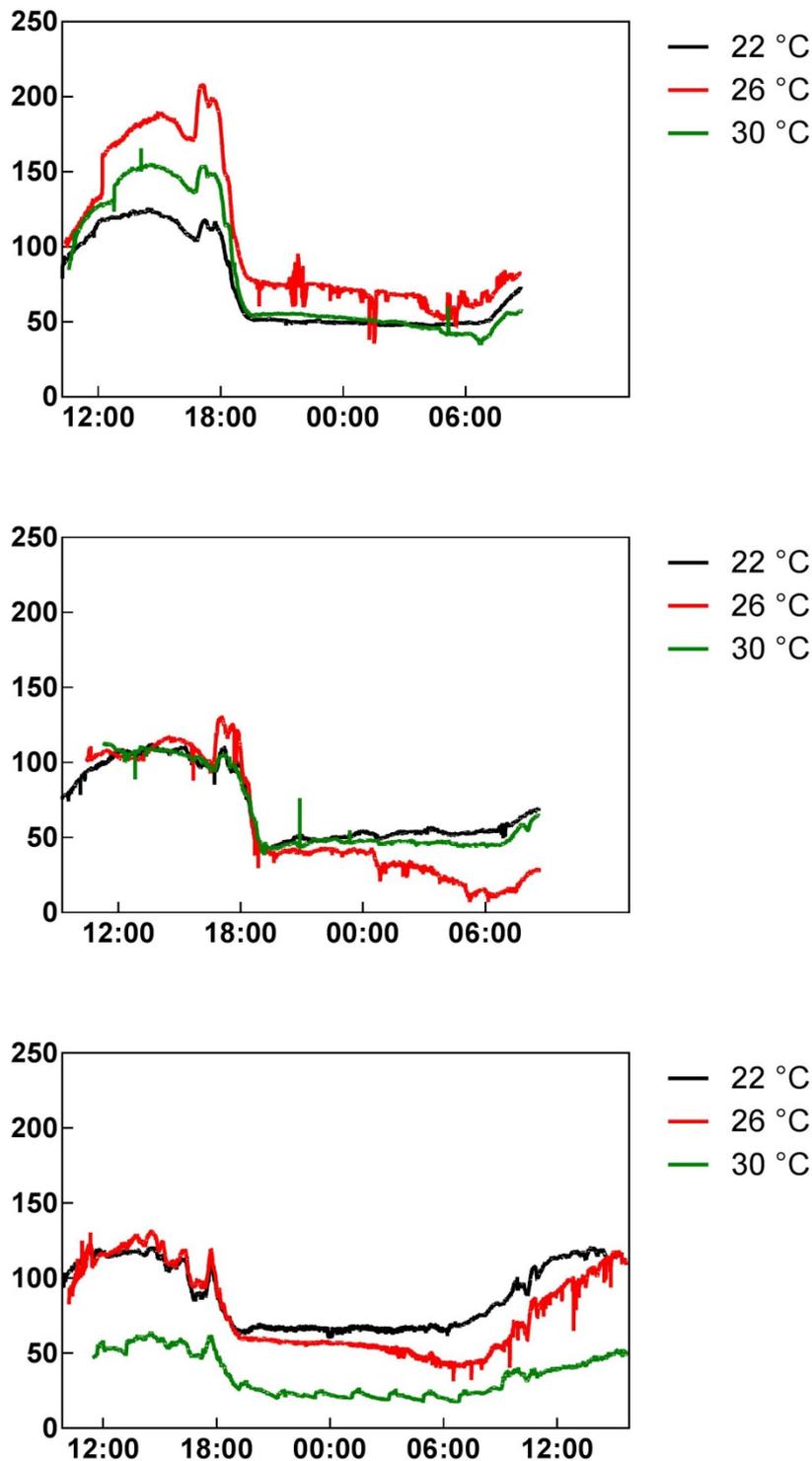


Fig. S2. A and B Diel changes in oxygen content (% of air saturation) in meristematic tissue of shoots grown at 22, 26 and 30 °C for three consecutive days halfway (S1 A: day 6, 7 and 8) and at the end of the experimental period (S1 B: day 20, 21 and 22). Traces are variable among the individual shoots, but in general meristematic oxygen concentrations were above saturation during the day and below during night. With few exceptions night time oxygen concentrations were not critically low suggesting that meristematic tissues remained oxic

throughout the diel cycle. The consistent rise in internal oxygen concentrations in the morning indicates that the photosynthetic capacity was maintained in all shoots independent on temperature treatment and experimental period.

Literature cited

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