Basin-wide transcriptomic response of the eelgrass *Zostera marina* to a simulated heatwave

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Heat waves are a major threat for *Zostera marina*, the predominant seagrass in the northern hemisphere. In previous studies, *Z. marina* was found to be more heat tolerant at the southern edge of its European distribution (Mediterranean, [1]). Here, **we asked whether** inherent heat resistance is a unique characteristic of Mediterranean **seagrass** or if *Z. marina* **adapted to warm temperatures in parallel** along the American and European thermal clines.



METHODS

Sampling sites

Weekly average sea surface temperature (SST) at four sampling sites with contrasting thermal regimes.



Common-garden heat-stress

Following 50 days of acclimation, we created a realistic heatwave and recovery period.

Bioinformatics data analysis

Workflow of data analysis with color codes representing grouping of samples/libraries

Location	G (Gabicce Mare)	D (Doverodde)	W (Waquoit)	GB (Great Bay)	
Atlantic/Mediterranean	М	А			



Samples for RNAseq were taken at two time points under heatstress (T2 and T3: 0 and 5 days at 25.5°C) and at three time points under recovery (T5. T7. and T9: 1, 20, and 30 days at 19°C) along with control samples at all five time points.



RESULTS AND DISCUSSION

Heat response

Heat-responsive genes (ca. 3300) were more differently expressed between Mediterranean and Atlantic samples than between Northern and Southern samples, both under heatstress and under control/recovery conditions.

Hierarchical cluster on the first five principal components of heat-responsive gene expression in all samples.

Adaptive differentiation

At 21 genes, Mediterranean samples showed expression differences from Atlantic samples that were putatively **adaptive to contrasting temperatures**, as these genes were also adaptively differentiated between Northern and Southern samples.

Venn diagram of adaptively differentiated genes

A vs M

Frontloaded genes

In 498 genes the Mediterranean samples showed **higher con**stitutive expression than the Atlantic samples.

Enriched molecular functions in the frontloaded frontloaded genes were dominated by'methyltransferase activity' (epigenetic regulation of gene expression).

transferase	amylase	alpha-amylase activity	Weight carte and divide Will part
activity	activity		a an an frenkri



D: Doverodde, G: Gabicce Mare, GB: Great Bay, W: Waquoit; c: control samples, w: stressed samples. Numbers indicate sampling time points.

Faster recovery of gene expression in both southern populations suggests **reduced sensitivity to global warming at the species' southern edge** of distribution N vs S 3327 149 21 3

Differentially expressed (gray numbers) or adaptively differentiated (black numbers) genes between Atlantic (A) and Mediterranean (M) samples and between Northern (N) and Southern (S) samples.

methyltransferase transferase transferring amylase activity Anternation (1967) activity activity one-carbon hosphoserir groups fucosidase phosphatase activity activity carbohyurate activity acetolactate structural RNA binding molecule kinase activity Iomoserin activity transferase kinase peroxiredoxin activity, activity activity transferring DNA structural **DNA-directed** glycosyl groups polymerase quinone constituent primary amine DNA processivity prenyltransferase binding oxidase of ribosome polymerase factor activity activity

The size of the rectangles reflect the p-value for enrichment.

Much of the previously observed North-South differentiation along the European coast [e.g. 1] might be explained by the adaptive transcriptomic differentiation between Mediterranean and Atlantic seagrass.

Frontloading of heat-responsive genes may convey heathardening in Mediterranean seagrass. Differential methylation could explain the rapid transcriptomic differentiation of Mediterranean from Atlantic seagrass.

CONCLUSIONS

Faster recovery and parallel transcriptomic differentiation of the two Southern seagrass populations suggest that *Z. marina* **adapted to warm temperatures at least partly (at 24 genes) in parallel** along the European and Atlantic thermal clines. Most of the transcriptomic North-South differentiation along the European coast, however, can be explained by strong adaptive differentiation (at 170 genes) between Atlantic and Mediterranean seagrass. Transcriptomic adaptation to the thermally unique Mediterranean must have been rapid and might have been driven by epigenetic regulation of gene expression.

Constitutive overexpression of heat responsive genes might be energetically costly and, instead of reducing sensitivity to climate change, could **undermine** the **potential of Mediterranean seagrass to respond to additional anthropogenic stress**.



References

[1] Franssen SU, Gu J, Bergmann N, Winters G, Klostermeier UC, Rosenstiel P, Bornberg-Bauer E & Reusch, TBH (2011): Transcriptomic resilience to global warming in the seagrass *Zostera marina*, a marine foundation species. *Proceedings of the National Academy of Sciences* 108(48):19276–19281

