



stazione zoologica anton dohrn

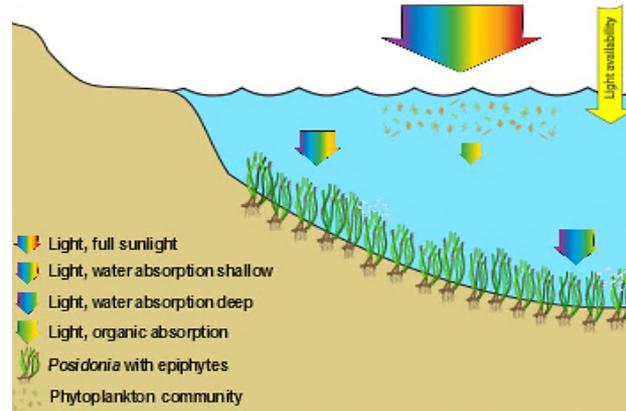
Meccanismi di risposta di *Posidonia oceanica* alle variazioni ambientali indotte dai cambiamenti climatici

Gabriele Procaccini

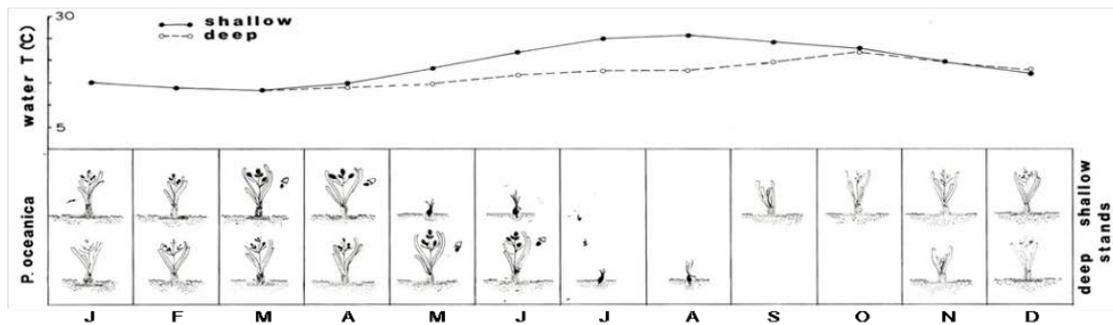
Stazione Zoologica Anton Dohrn, Napoli, Italy



Meadows grow from the surface to 35 m depth



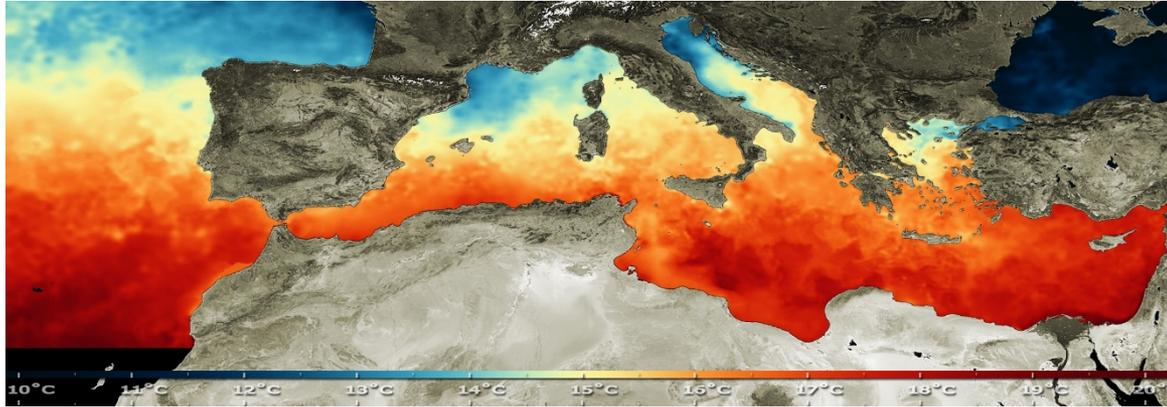
- Meadows experience **different light quantity and quality** along their distribution.
- The **same genotype** can experience differences in light from 50 to 1000 μE .
- Plants also experience **strong differences in temperature** when thermocline is stable



from Buia e Mazzella, 1991

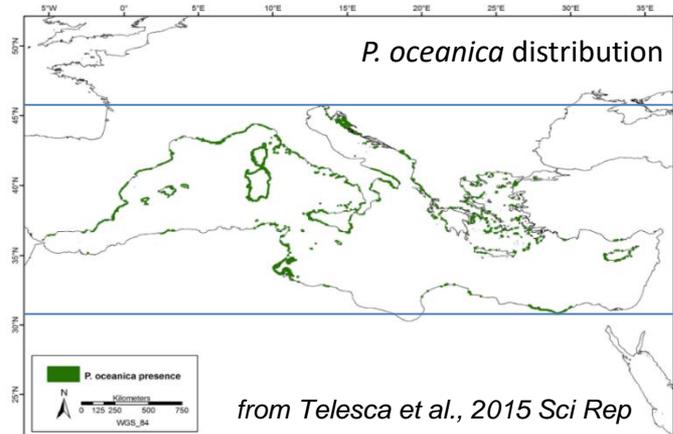
Plants growing at different depth show phenological differences

Meadows grow from from southern to northern Mediterranean coasts



https://www.esa.int/spaceinimages/Images/2008/10/Mediterranean_sea_surface_temperature

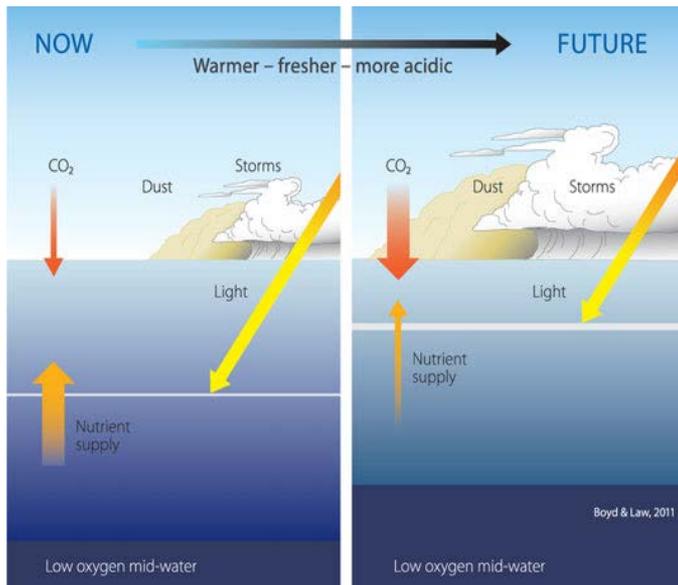
- Meadows experience **different light quantity** along their distribution.
- Plants experience **strong differences in temperature** associated to strong differences in SST



Seagrasses and environmental changes

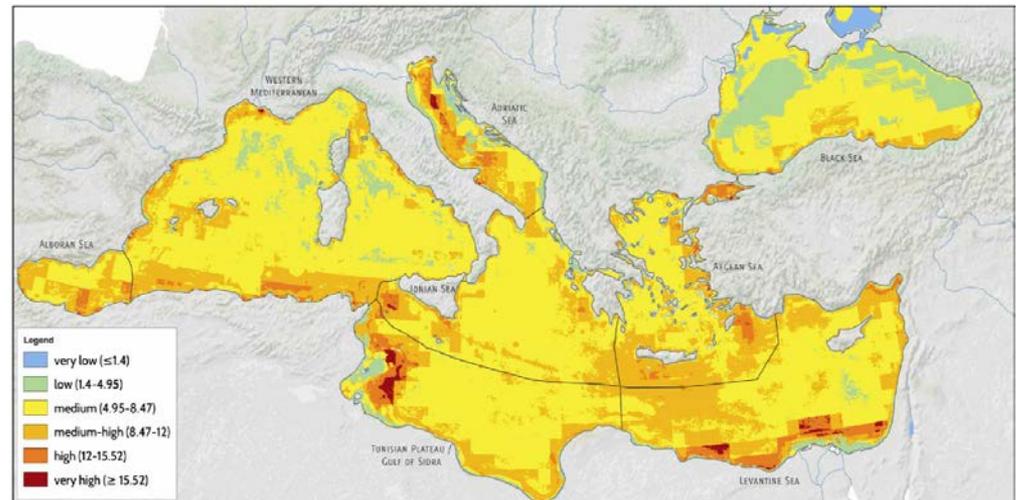
The marine biota is threatened by global climate change and direct human pressures

Consequences of Global Climate Change



from Reusch and Boyd 2012. Evolution

Map of cumulative impact



from Micheli et al 2013. Plos One

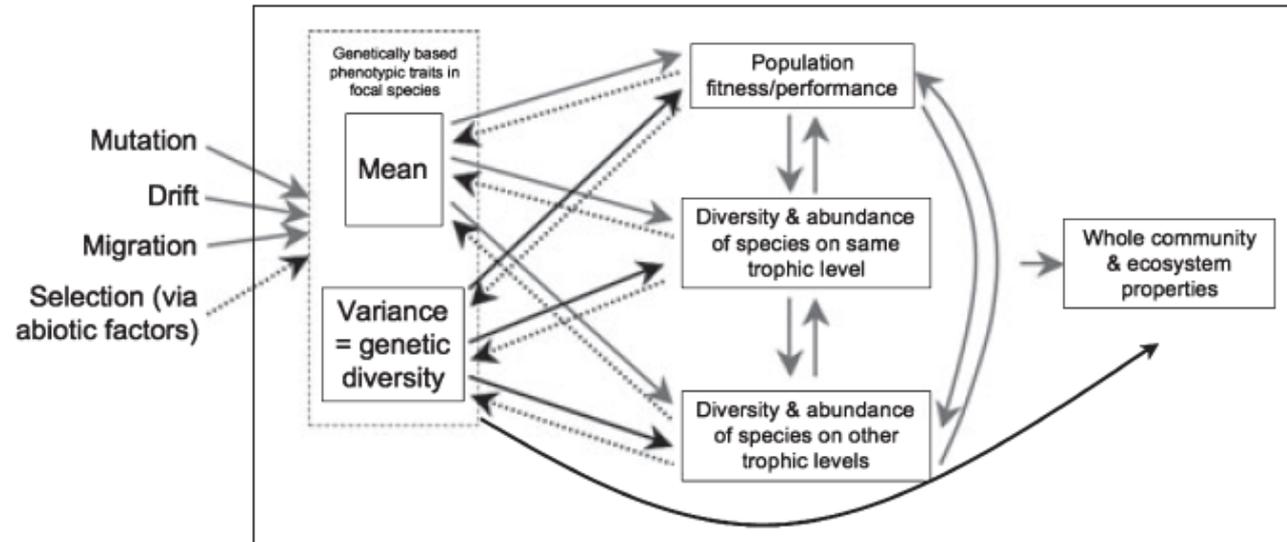
Seagrass ecosystems are declining worldwide due global changes and to direct human pressures (e.g. urbanisation, discharges, harbour development, boating activities, tourism facilities)

Indicators of changes and state

Genetic approaches to seagrass conservation

Ecosystem state indicators

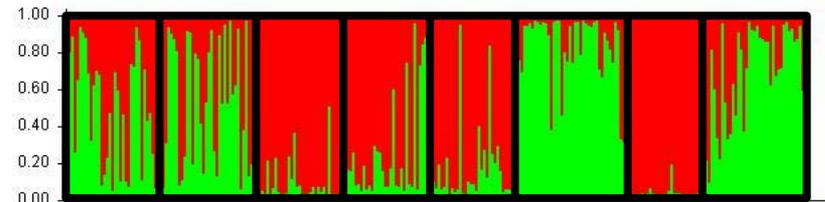
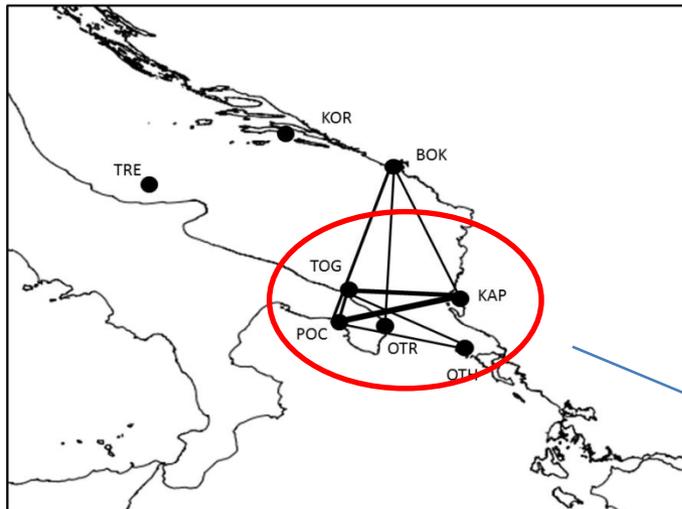
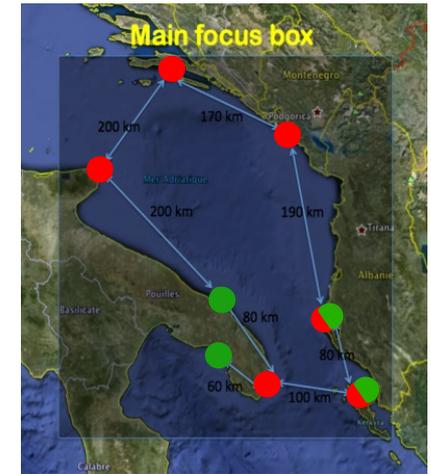
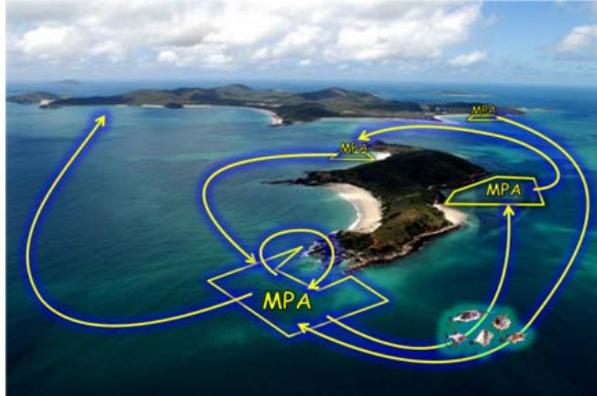
Analysis of genetic diversity to understand ecosystem state (seagrass potential for resistance and resilience)



from Hughes et al 2008 Ecol. Lett.

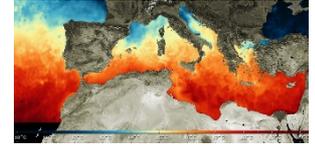
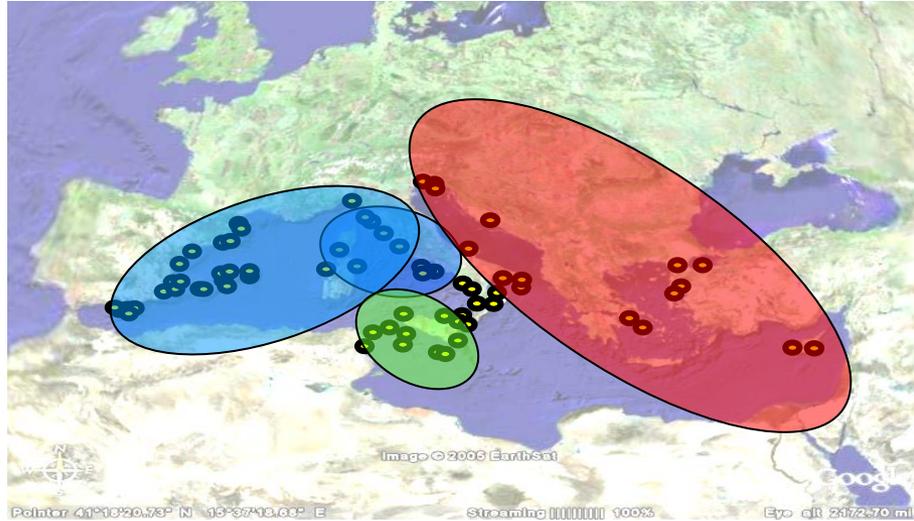
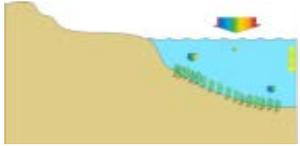
Genetic diversity and connectivity

Assessment of connectivity for establishing network of MPAs

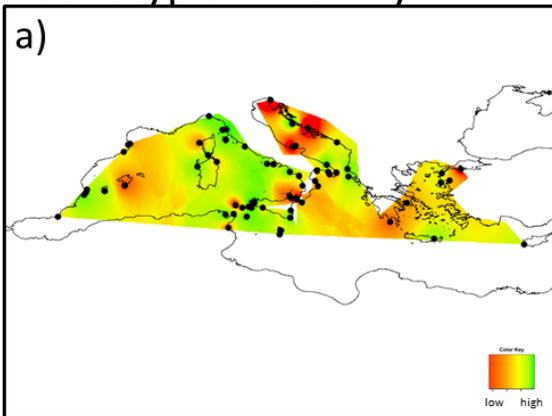


Minimum MPAs network system

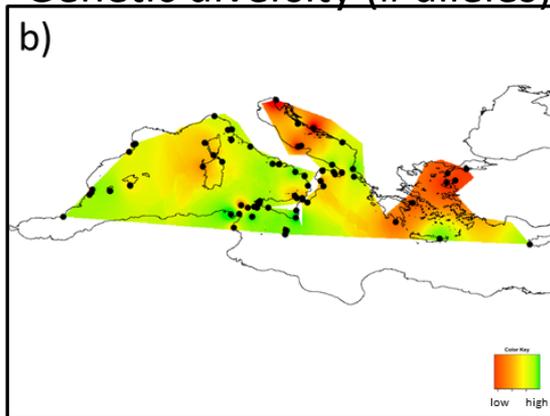
Genetic diversity along geographic and depth gradients



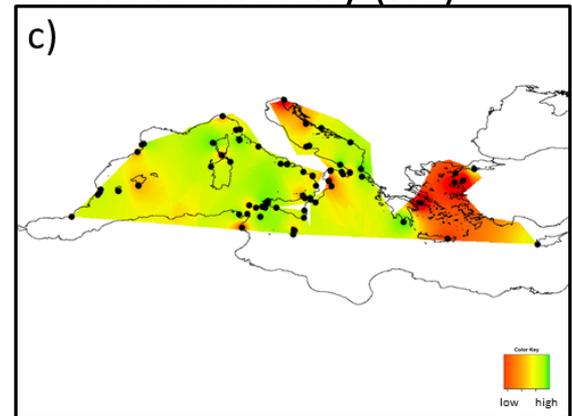
Genotypic diversity



Genetic diversity (# alleles)



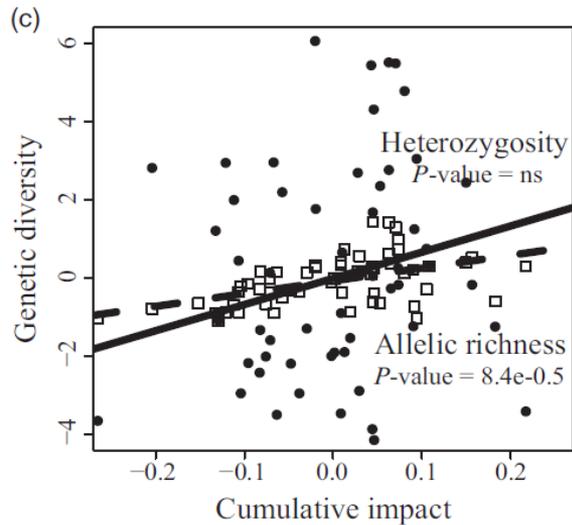
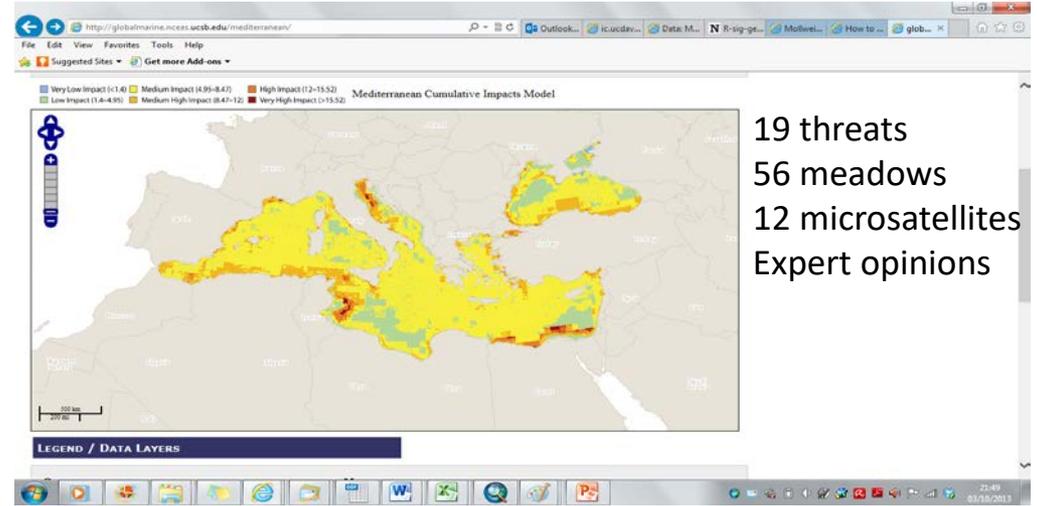
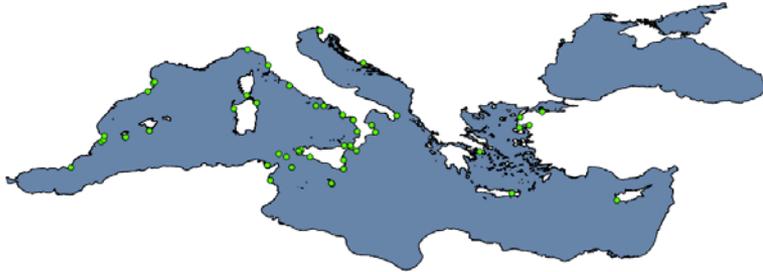
Genetic diversity (Ho)



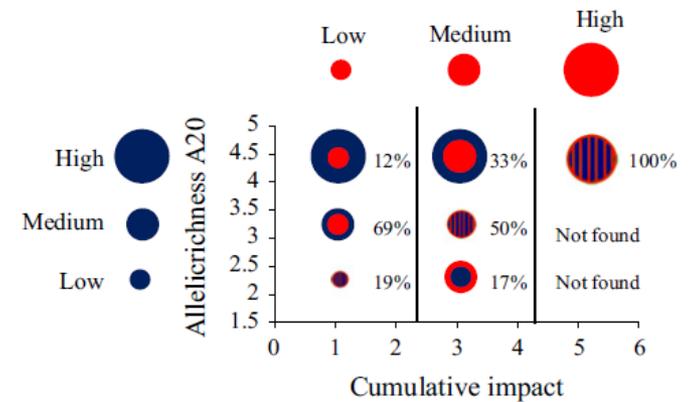
QGIS (Quantum GIS Development Team 2013)

Role of genetic variation

Disturbance and Diversity

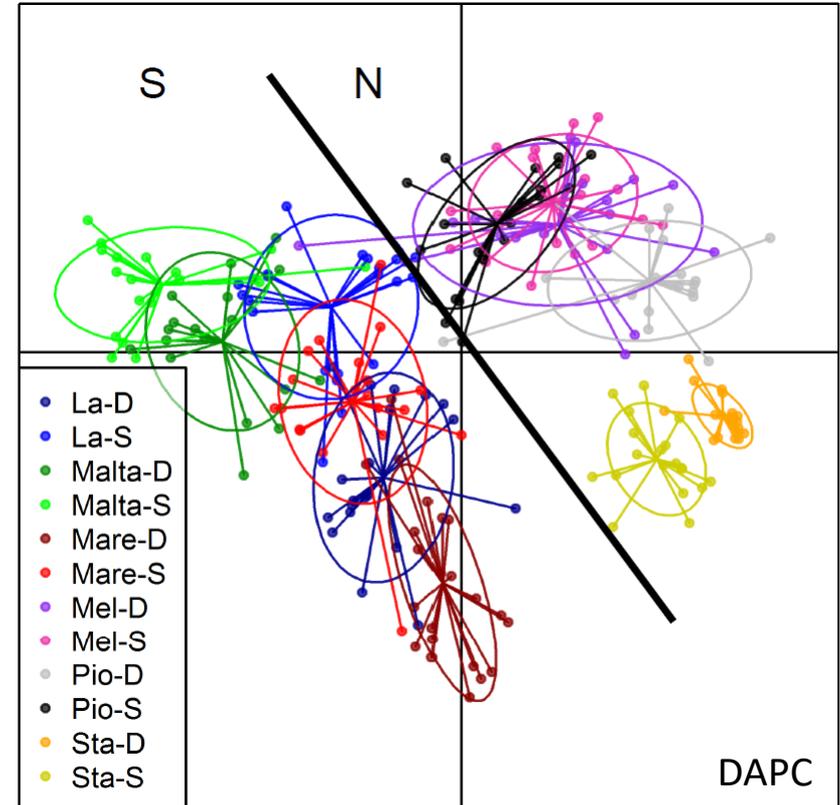
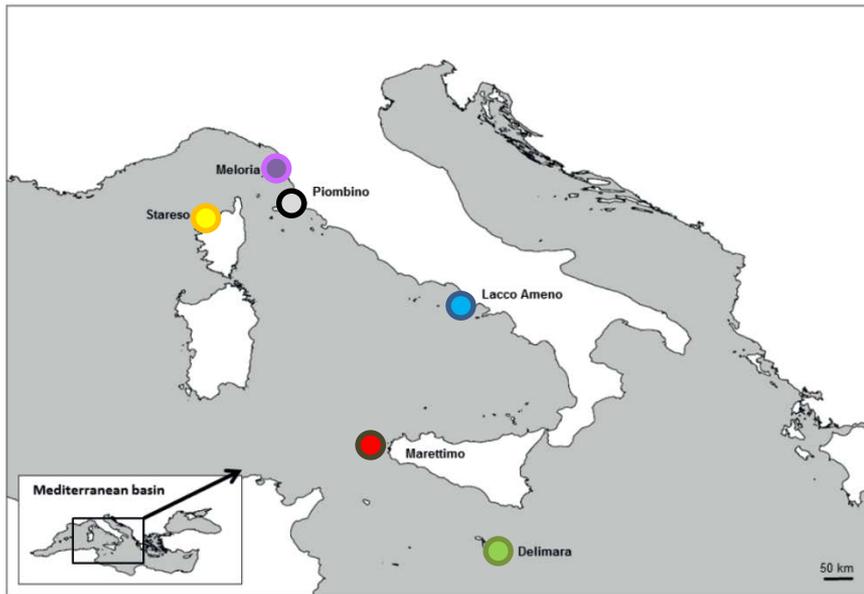


No populations were found with low allelic richness and high cumulative impact, suggesting that these were not able to cope with high impact



Role of genetic variation and structure

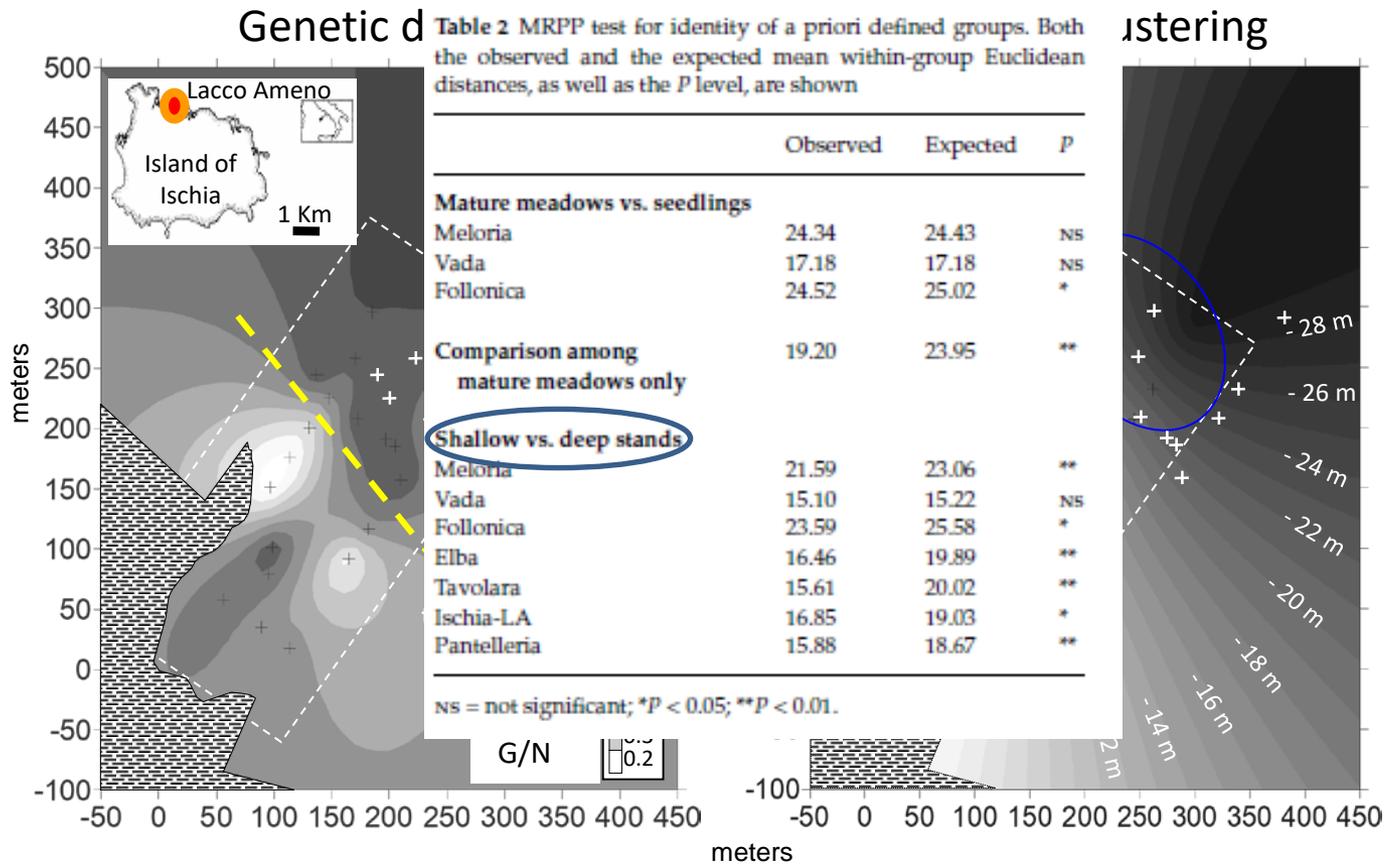
Analysis conducted with 24 microsatellite loci. Four outlier loci related to depth and two outlier loci related to latitude were identified.



Northern and southern and shallow and deep meadow stands appear to be genetically distinct

Genetic structure at a meadow scale

Microsatellites based analysis identified clear genetic distinction between plants growing above and below the summer thermocline (~ -15m)



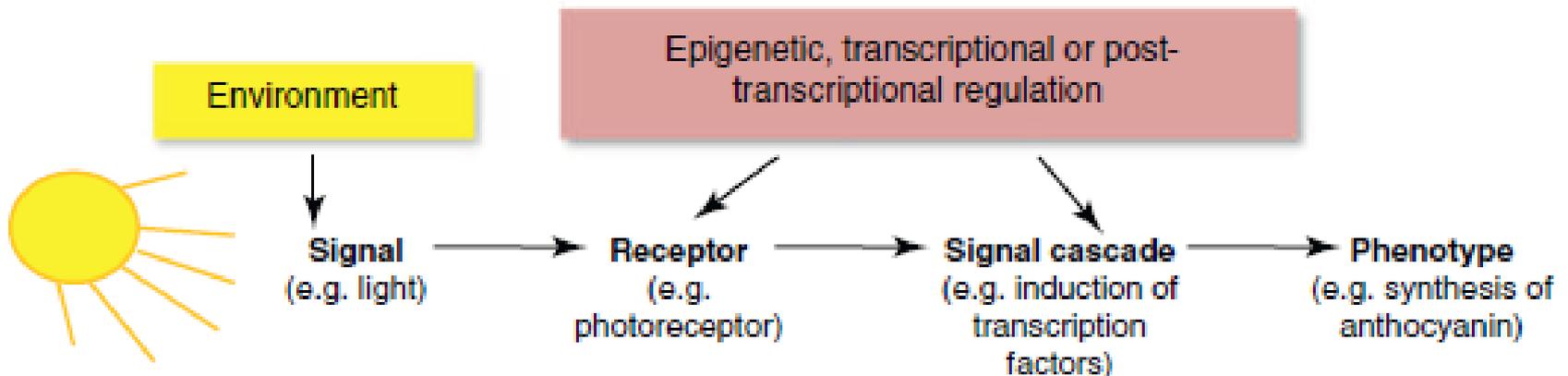
Indicators of changes and state

Genetic approaches to seagrass conservation



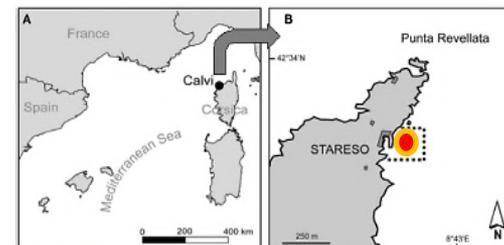
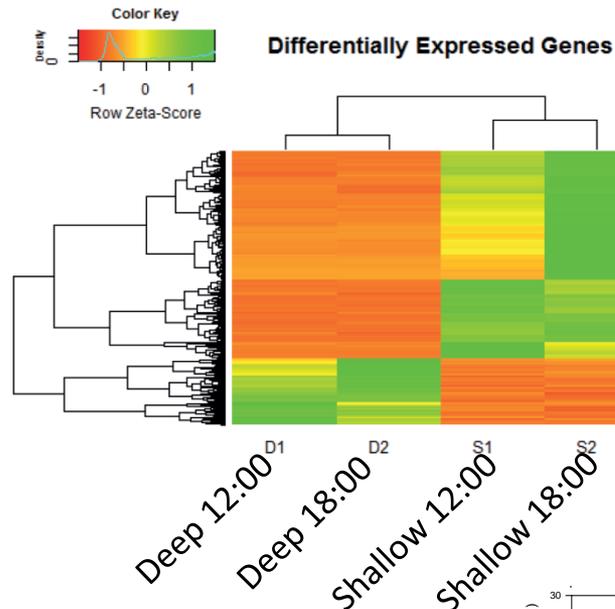
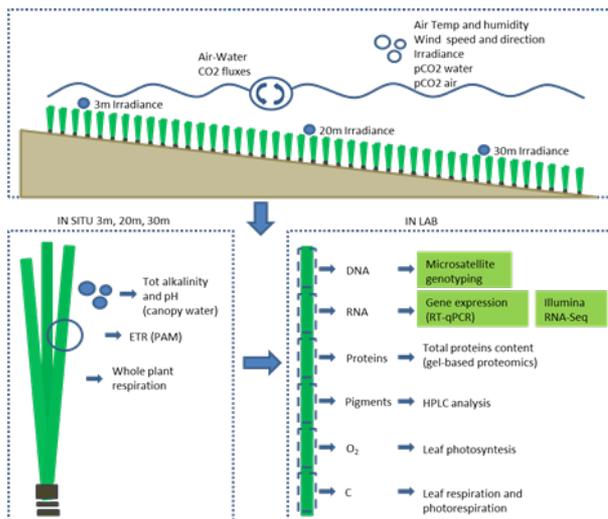
Indicators for detecting
**response to changes in
drivers**

Analysis of gene
expression for selecting
early warning indicators



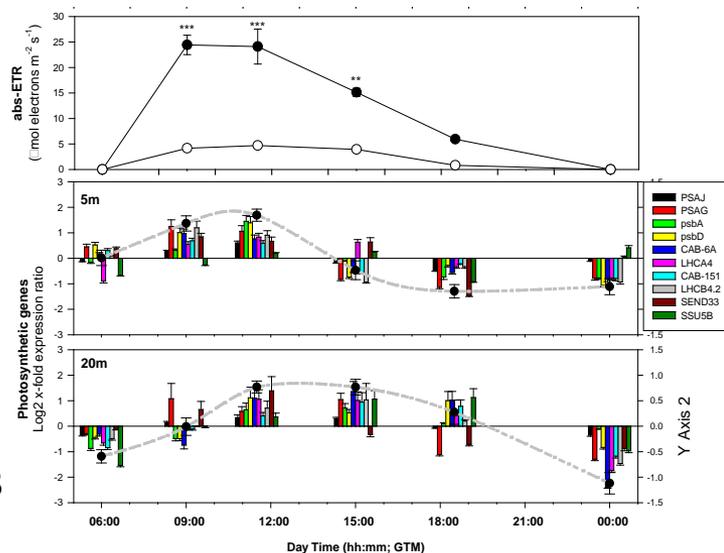
P. oceanica differential response along gradients

Difference in gene expression along depth



Shallow and deep plants are **locally adapted** to the existing environmental conditions and segregate according to depth

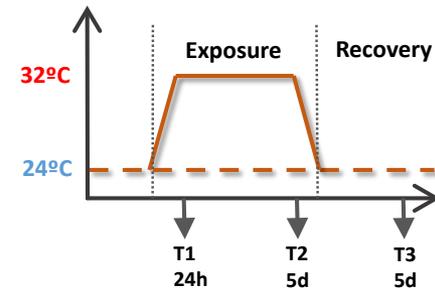
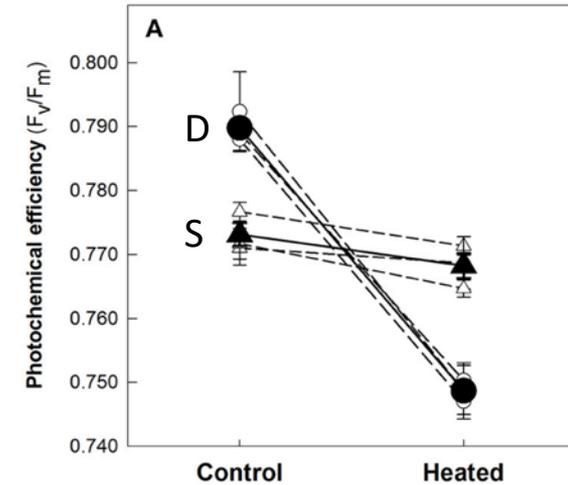
Photosynthesis



P. oceanica differential response along gradients

Difference in gene expression along depth

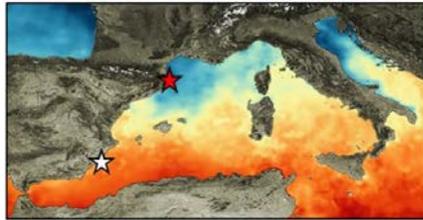
Shallow and deep plants have a different capability to respond to a simulated temperature increase (heat wave)



Depth	PHOTOSYNTHESIS	HSPs & CHAPERONES	RESPIRATION	HSFs
Shallow plants	psbB (5.2) atpF (6.8) psbC (13.6) atpA (5.5) psbD (13.2) atpB (5.1) psbE (7.7) rbcL (2.8) psaA (7.6) psbR (58.7) psaB (7.9) psbK (65.3) petA (9.5) petB (7.4) ndhB (17.8) ndhF (7.0) ccsA (18.5)	CPN60II (3.5) HSP1 (3.2) FES1 (3.3) HSP90-6 (2.4) CPN10 (2.3) AHSA1 (3.9) HSP70 (2.4) HSP90 (2.2) HSC70-1 (39.2) HSP70 (3.9) HSP90-7 (6.4) ATJ6-like (2.8) FKBP62-like (3.0)	COA6 (2.3) Protein midA-like (2.1) Ubiquinol-Cytochrome C reductase (2.2) H ⁺ -translocating NAHD-quinone oxidoreductase (34.8)	HSF A6b (2.7) HSF B2b (2.2)
Deep plants	psbR (23.6) psbK (12.5)	BAG6 (13.9) Chaperone-domain superfamily (6.8) DNAJA1 (5.2) HSP82 (9.1) HSP83 (10.1) Class I HSP (12.0) sHSP (9.2)	APX1 (2.2) APX2 (2.0) CCS (3.3) FER3 (4.5)	RECQL5 (2.4) MSH1 (2.4) MSH7 (3.2) BRCA1 (3.6) B3GALT5 (4.0) Ash2I (91.3) RPL7D (6.5) rsmB (7.1) TOP3 (4.6)
			HRPA2 (101.0)	TOP3 (4.3)

P. oceanica differential response along gradients

Long term exposure to heat stress of plants from a latitudinal gradient (24 libraries – Ion Torrent) *P. oceanica* – *C. nodosa*



SAMPLING:

- PAM: weekly
- Mortality: weekly
- Antioxidant activity: T2 & T3
- Carbohydrates: T2 & T3
- Photosynthesis & Respiration: T2 & T3
- Pigments: T2 & T3
- Growth: T2 & T3
- Morphology: T2 & T3

Seagrass sampling sites

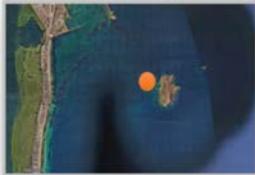


Medas



P. oceanica – Cold-plants

Isla Grosa



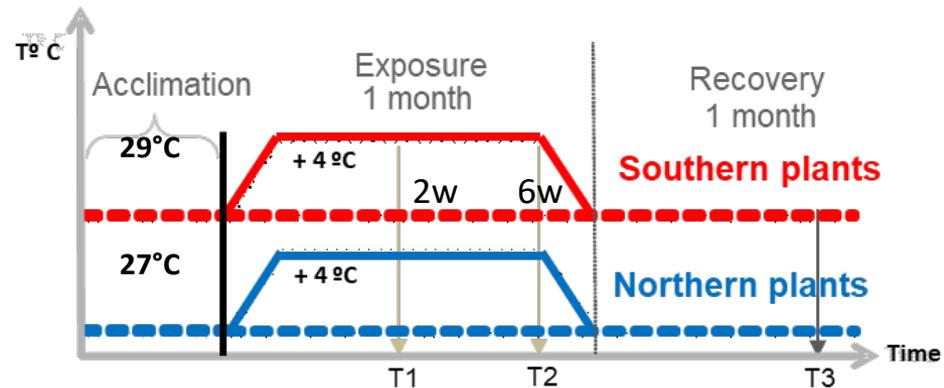
P. oceanica – Warm-plants

Ebro Delta



C. nodosa – Warm-plants

C. nodosa – Cold-plants

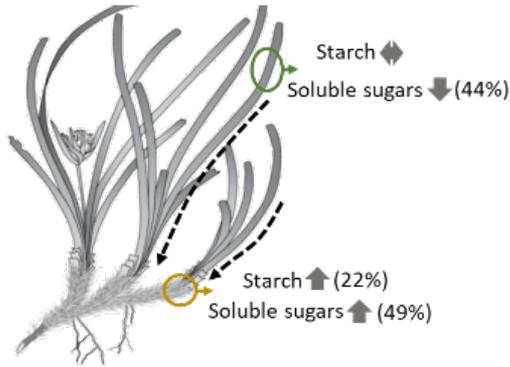


P. oceanica differential response along gradients

Warmer environment

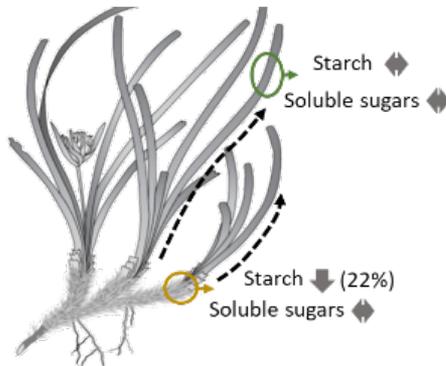
- P:R ratio ↕
- Growth ↓
- Storage ↑

Posidonia oceanica



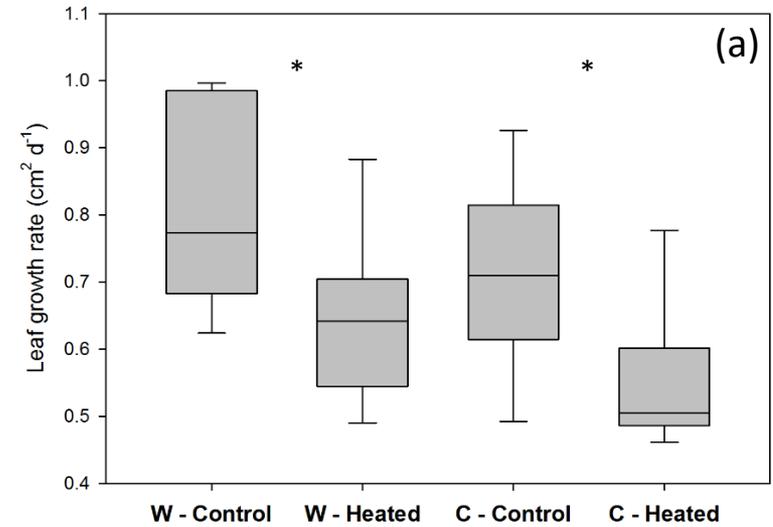
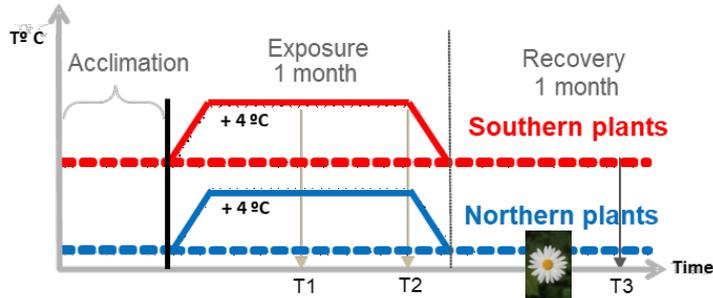
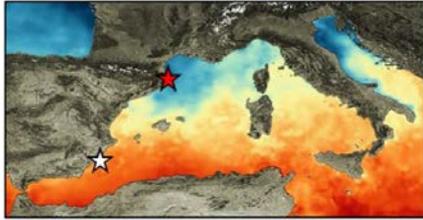
Colder environment

- P:R ratio ↓
- Growth ↓
- Storage ↓

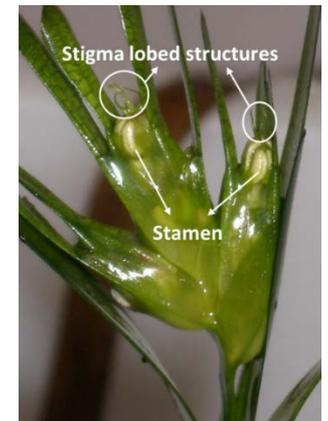
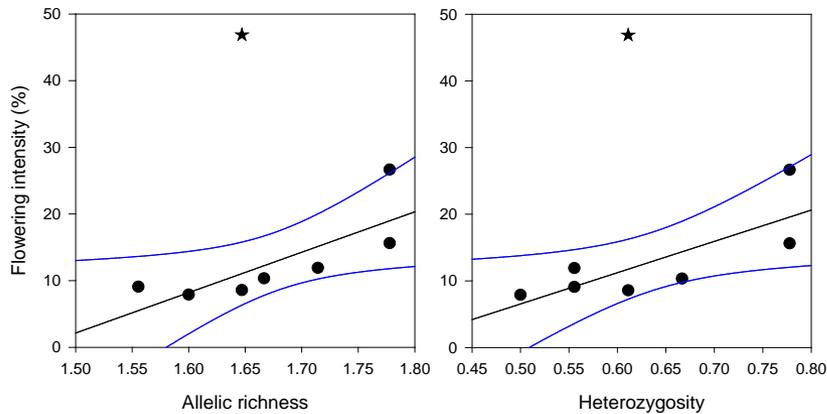


Plants from colder environments are more affected by warming.

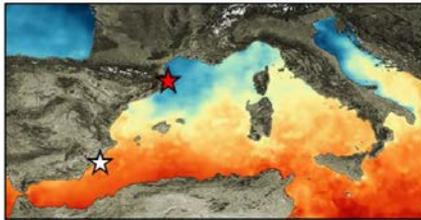
Stress response to warming



Genetic diversity of flowering plants correlate with flowering intensity

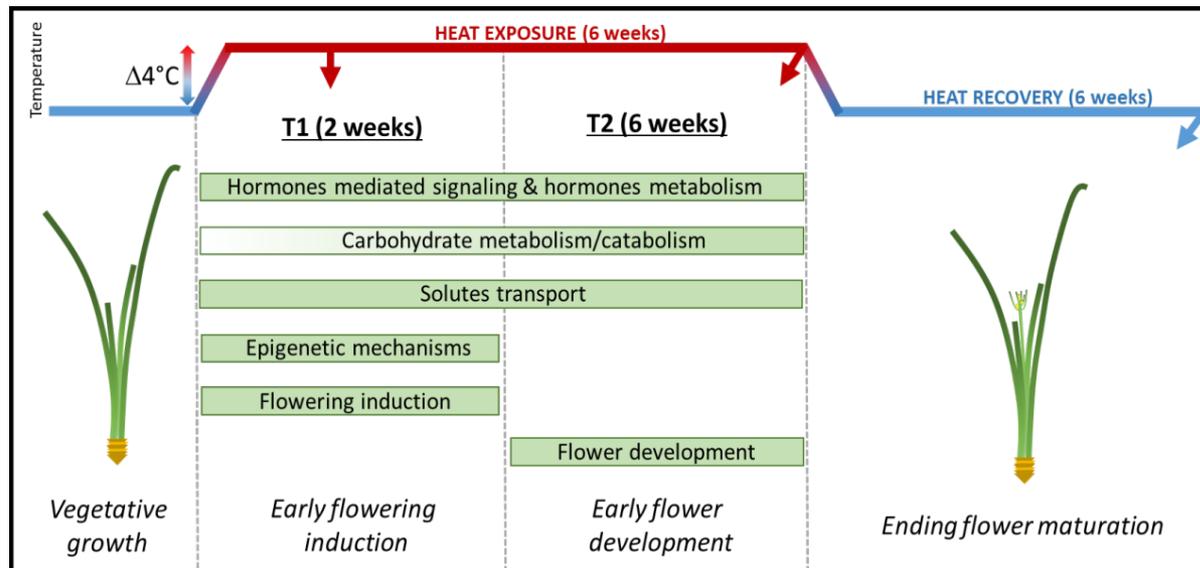
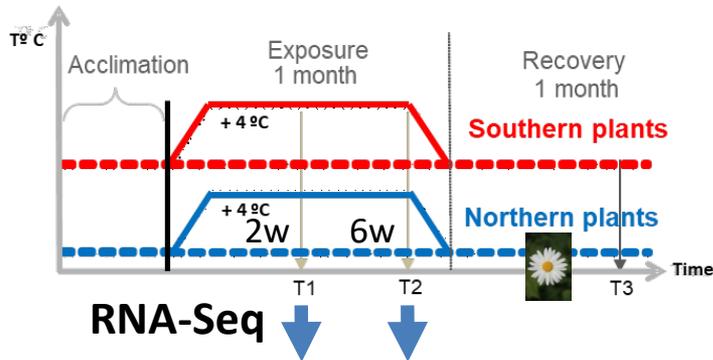


Stress response to warming



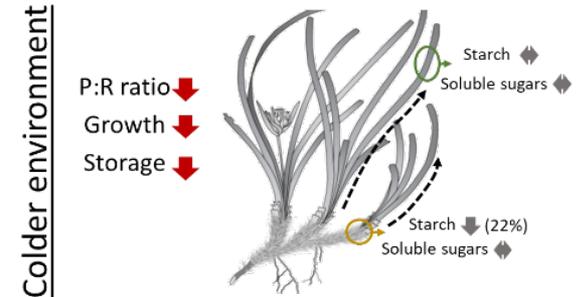
Flowering induction starts already after two weeks of warming

Flower development genes are expressed when flowers are still not visible

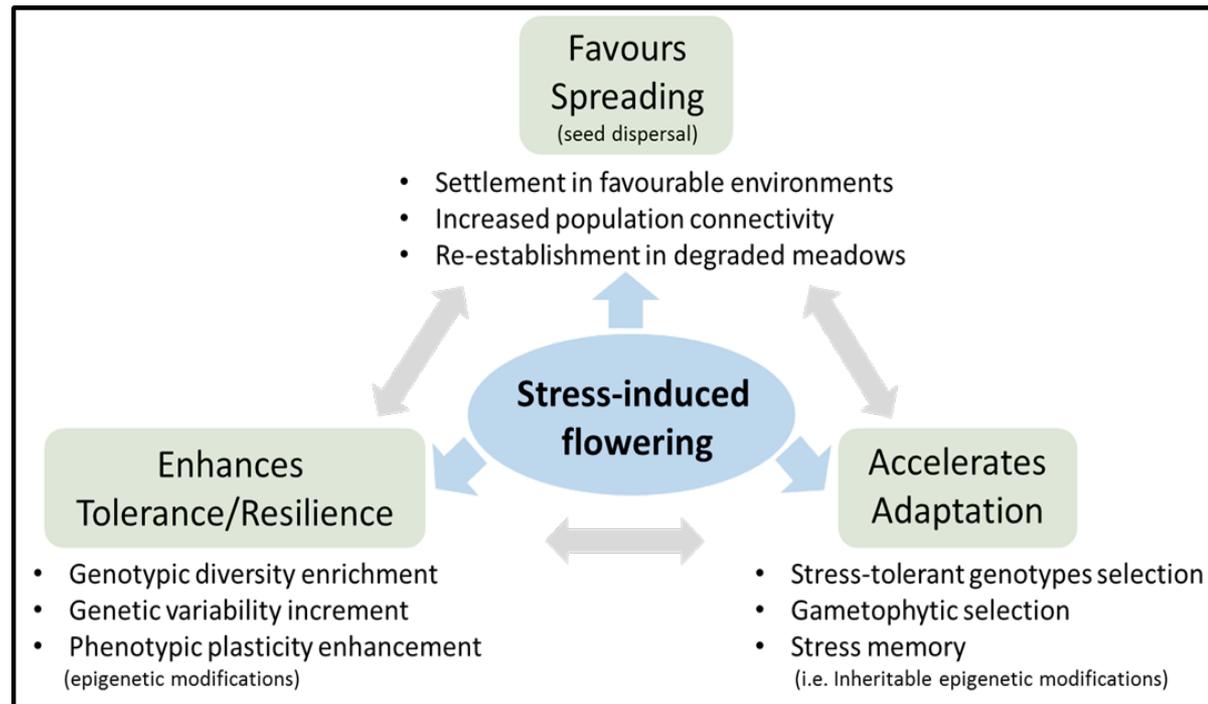


Stress response to warming

Altered expression levels of several transcriptional factors, photoperiodic-genes and gene families, which **regulates flowering time by modulating the photoperiod pathway**



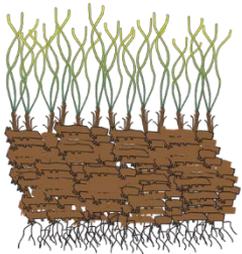
Plants invest energy in the response to stress



Multiple stressors and early warning indicators

SPECIES

Posidonia oceanica



FACTORS

CO₂ and NUTRIENTS

NUTRIENTS and BURIAL

NUTRIENTS and GRAZING

EXPERIMENTAL DESIGN

Two levels of CO₂ and three levels of nutrients. At volcanic vents - Ischia.

Two levels (medium and high) of nutrients and burial. In the field

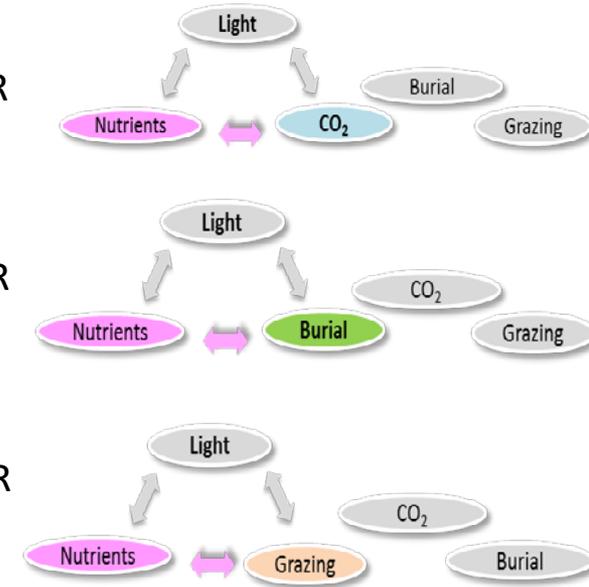
Two levels of intensity (high and control) and temporal variance (pulse and press) of nutrients and grazing activities (natural and overgrazing). In the field

APPROACH

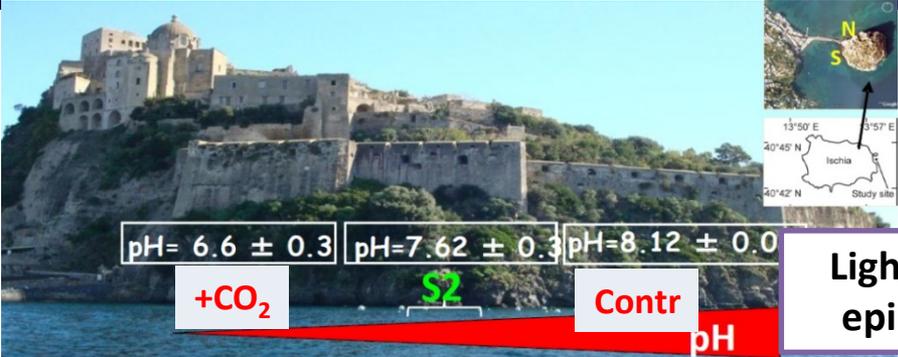
RT-qPCR

RT-qPCR

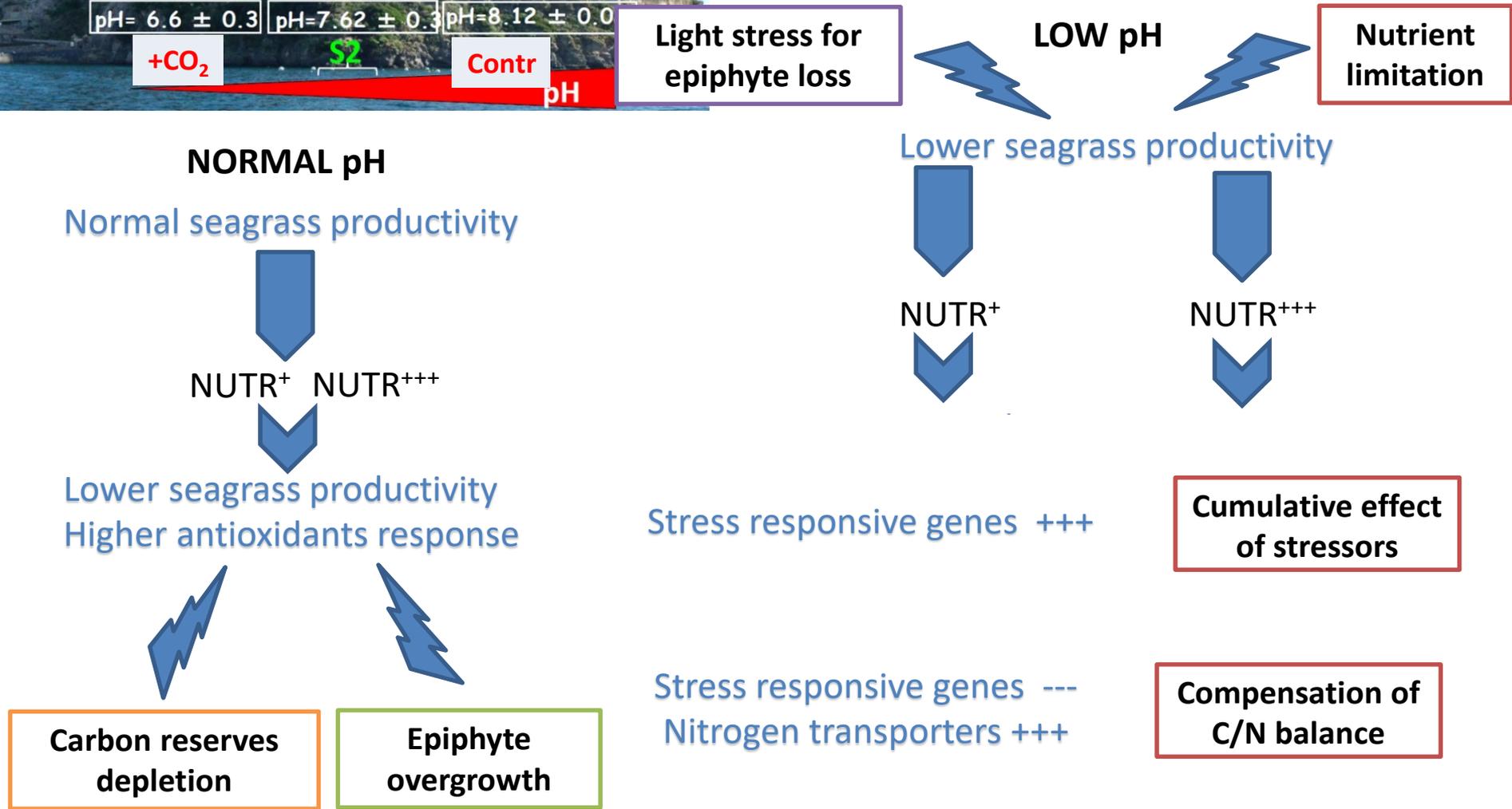
RT-qPCR



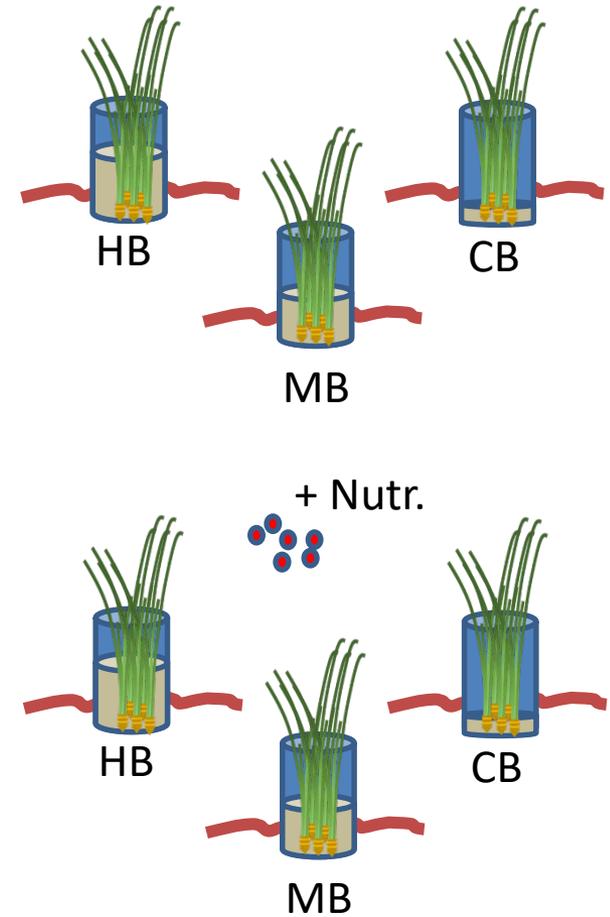
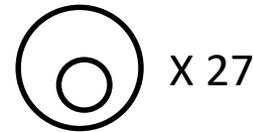
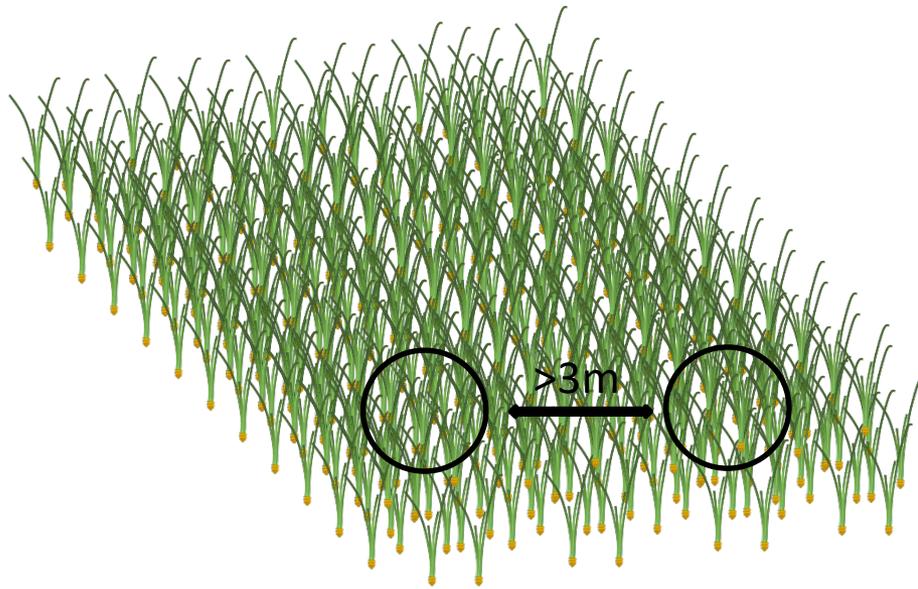
Effects of CO₂ and nutrient supply on plant performance



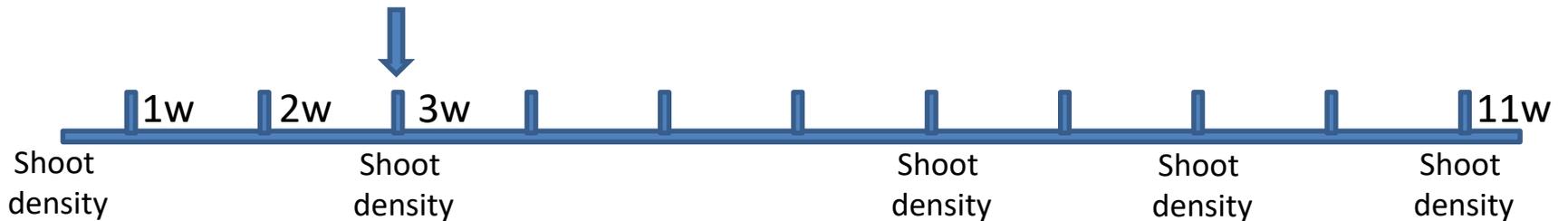
Plants were treated with different levels of nutrients High N, Mod N
 The experiment lasted 15 months



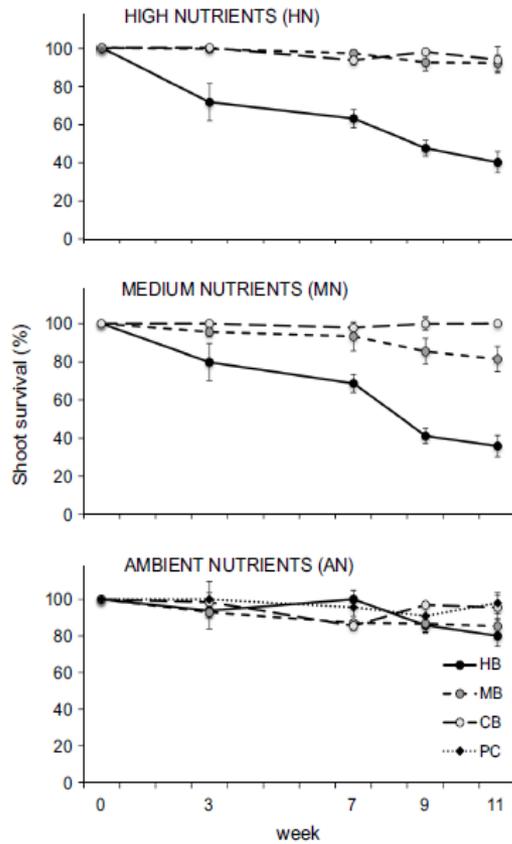
Multiple stressors and early warning indicators



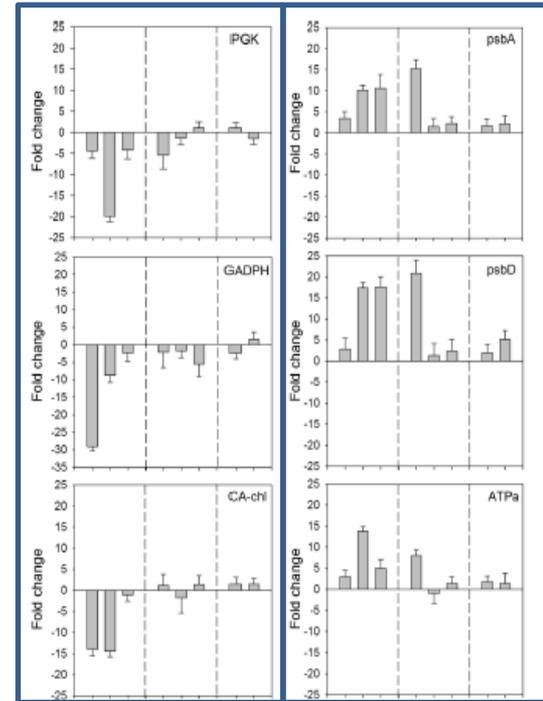
6 - morphological variables
 11- physiological biochemical variables
 10 – stress related genes



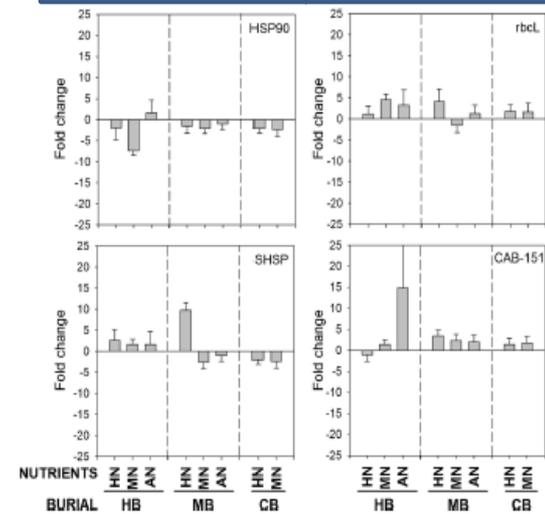
Early warning indicators



Carbon metabolism genes



Photosynthetic genes



Early warning indicators

The multiple regressions conducted with morphological, antioxidant, isotope, and genetic covariates explained the 27.8%, 36.4, 33.2 and 60.6 (Adjusted R^2 values) of the variance in shoot survival, respectively

Effect	Estimate	SE	<i>t</i> value	<i>p</i> value
Morphological/growth				
Epiphyte load	- 0.00365	0.00104	- 3.50	0.00193
Shoot biomass	- 0.17576	0.10531	- 1.66	0.10869
Leaf growth	0.06651	0.03766	1.766	0.09066
Adjusted $R^2 = 0.2779$	$F_{3,23} = 4.33$ <i>p</i> value 0.0146			
Physiological and biochemical (antioxidant)				
ORAC	- 0.0004	0.00013	- 3.045	0.00557
Phenolics	0.0007	0.00021	3.697	0.00113
Adjusted $R^2 = 0.3644$	$F_{2,24} = 8.45$ <i>p</i> value 0.0016			
Physiological and biochemical (isotopes)				
Leaf N	- 0.4591	0.2735	- 1.678	0.1075
Leaf $\delta^{15}\text{N}$	- 0.1782	0.1195	- 1.491	0.1501
Leaf S	- 0.5854	0.4482	- 1.306	0.2050
Leaf $\delta^{34}\text{S}$	0.0627	0.0266	2.356	0.0278
Adjusted $R^2 = 0.3321$	$F_{2,22} = 4.23$ <i>p</i> value 0.0108			
Gene expression				
CA-chl	0.0546	0.00972	5.623	0.00000
ATPa	- 0.0216	0.01205	- 1.796	0.08510
Adjusted $R^2 = 0.6056$	$F_{2,24} = 20.96$ <i>p</i> value 0.0000			

Variable	Stressor effect	Association to survival
Epiphyte load	↑N ↑B	Very high
Necrosis	↑N ↑B	-
Leaf growth rate	↑N	Low
ORAC	No Ha	Medium
TEAC	↑N	-
Phenolics	↓N ↓B	High
Leaf $\delta^{34}\text{S}$	No effect	Medium
GADPH	No Ha	-
HSP90	No Ha	-
PGK	↓B	-
psbA	No Ha	-
CA-chl	↓ B	Very high
ATPa	↑ B	Low

The positive estimate of Ca-chl suggests that overexpression of this gene at week 3 is positively correlated with shoot survival at week 11

The chloroplast carbonic anhydrase (CAchl) catalyzes the conversion of HCO_3 into CO_2

84% of the variation in shoot mortality explained by the model

Overall conclusions

- **Genetic diversity matters in responding to adverse conditions**
- **Seagrass meadows are locally adapted to existing environmental conditions**
- **Reaction norm and response plasticity depend from the environmental conditions plants experience in their distribution areal**
- **Flowering is a stress response allowing plants to escape from adverse conditions**
- **Target genes can be used as early warning indicators**



Thank you for your attention



Hans von Marées, 1873 (particular) - Fresco Room
Stazione Zoologica Anton Dohrn

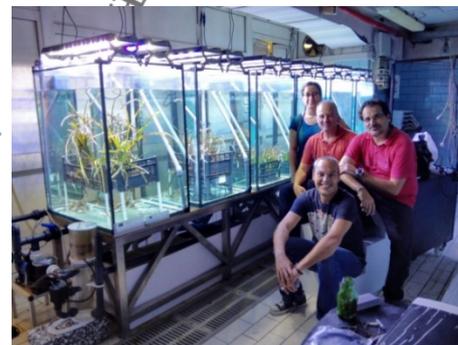
- Laura Pereda – IMEDEA, CSIC
- Juan Manuel Ruiz Fernandez – IEO, Murcia
- Fernando Tuya - University of Las Palmas de G.C.
- Jeanine L. Olsen – University of Groningen
- Giulia Ceccherelli – Università di Sassari
- Fabio Bulleri – Università di Pisa



- Miriam Ruocco
- Lazaro Marin Guirao
- Emanuela Dattolo
- Daniela D'Esposito
- Marlene Jahnke
- Laura Entrambasaguas
- Claudia Traboni



- Hung Nguyen
- Jessica Pazzaglia
- Alex Santillan Sarmiento
- Ludovica Pedicini



<https://www.researchgate.net/lab/Gabriele-Procaccinis-Group-Molecular-Ecology-of-Seagrasses-Gabriele-Procaccini>

<https://gpgroupsxn.wixsite.com/website>