

Supplementary Material

Magnitude and predictability of pH fluctuations shape plastic responses to ocean acidification

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Supplementary Methods

Autonomous oceanographic monitoring of coastal and lagoon habitats

Autonomous oceanographic monitoring of the coastal site was conducted between September 2016 and August 2017 using SeaFET pH sensors (Martz, Connery, and Jonson 2010) and Seabird Electronic (SBE25) profilers with 60-minute sampling frequencies. Sensors were deployed on a buoy submerged to 2 m in a total water depth of 80 m. Detailed sensor deployment and quality control methodology are reported previously, with an estimated error in pH_T of ± 0.01 units (Kapsenberg et al. 2017b). Monitoring of the lagoon site was conducted between October 2016 and June 2017 using SeaFET pH and SBE37 sensors with twenty-minute sampling frequencies. For pH, SeaFET data were calibrated using SeaFET temperature data (± 0.004 units pH_T error; Kapsenberg and Hofmann 2016) and multiple field collected pH

reference samples per deployment (Bresnahan et al. 2014). Open cell titration was used to determine total alkalinity (A_T) necessary to calculate *in situ* pH (Dickson, Sabine, and Christian 2007). Spectrophotometric measurements of pH samples were performed on discrete samples using *m*-cresol indicator (Dickson, Sabine, and Christian 2007) with an accuracy of ± 0.006 units pH_T (determined by Kapsenberg *et al.* 2017b). Based on 13 reference samples, spatio-temporal mismatch error of the reference sampling was ± 0.014 units pH_T (following calculations in Kapsenberg and Hofmann 2016). The final estimated standard uncertainty of pH data from the lagoon is ± 0.016 units pH_T .

pH manipulation system

Following common garden conditioning, a subset of mussels from each population were randomly selected and transferred to a pH manipulation system to assess population-specific responses to the benign and stressful pH treatments. The experimental system was a flow-through seawater pH-manipulation system described by Kapsenberg et al. 2017c. Filtered seawater within the system was maintained at a constant temperature of 15.5°C and seawater pH was controlled in four header tanks using a glass pH electrode feedback system (IKS aquastar) and pure CO₂ gas addition and constant CO₂-free air aeration. Two header tanks were used per treatment to account for potential header tank effects. Each header tank distributed water at a rate of 8 L hr⁻¹ into two, replicate 8 L treatment buckets ($n = 4$ replicates per treatment), resulting in equal pH exposure across replicates (table S1). Honeywell Durafet pH sensors were deployed in each header tank and one downstream replicate bucket to continually monitor temperature and pH throughout the course of the experiment (see Kapsenberg *et al.* 2017c for calibration methods). pH accuracy of Durafet calibration was 0.01 units pH_T . Samples for A_T and salinity were obtained from the header tanks daily (table S1).

Supplementary Tables and Figures

Supplementary Table 1. Environment conditions within the common garden and pH manipulation system. Values are reported as mean across all sampling points (+/- S.D.) Aragonite saturation and $p\text{CO}_2$ were computed using parameter mean values.

Treatment	pH _r	Ω	$p\text{CO}_2$ (μatm)	AT (μmol/kg)	T (°C)	SAL (ppt)
Common Garden	7.84 (+/- 0.) (n = 12)	2.0	770	2585 (n = 1)	16.3 (+/-0.4) (n = 12)	38.4 (n = 1)
Stressful pH_1	7.69 (+/- 0.05) (n = 1,754)	1.4	1116	2554 (n = 5)	15.5 (+/-0.3) (n = 1,754)	38.2 (+/-0.1) (n = 5)
Stressful pH_2	7.69 (+/- 0.05) (n = 1,754)	1.4	1116	2558 (n = 5)	15.5 (+/-0.3) (n = 1,754)	38.2 (+/-0.1) (n = 5)
Benign pH_1	8.19 (+/- 0.01) (n = 1,754)	3.7	292	2556 (n = 5)	15.5 (+/-0.4) (n = 1,754)	38.2 (+/-0.1) (n = 5)
Benign pH_2	8.19 (+/- 0.01) (n = 1,754)	3.6	301	2558 (n = 5)	15.4 (+/-0.4) (n = 1,754)	38.2 (+/-0.1) (n = 5)

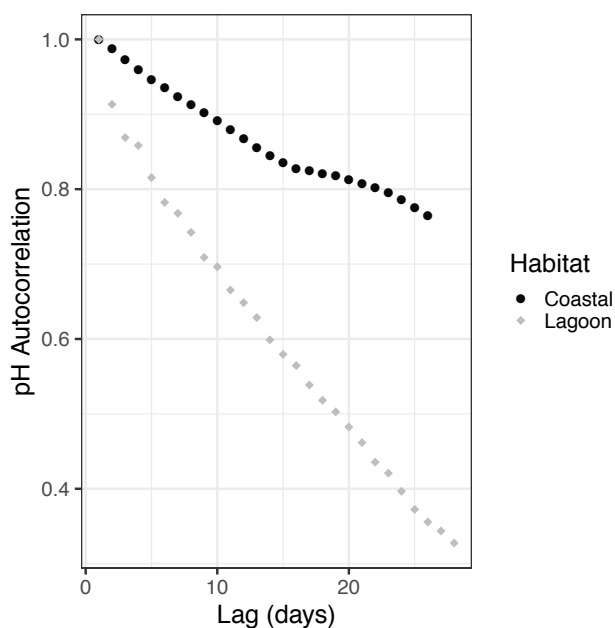


Figure S1. pH autocorrelation across daily lag intervals in the coastal (black circles) and lagoon (grey diamond) habitats.

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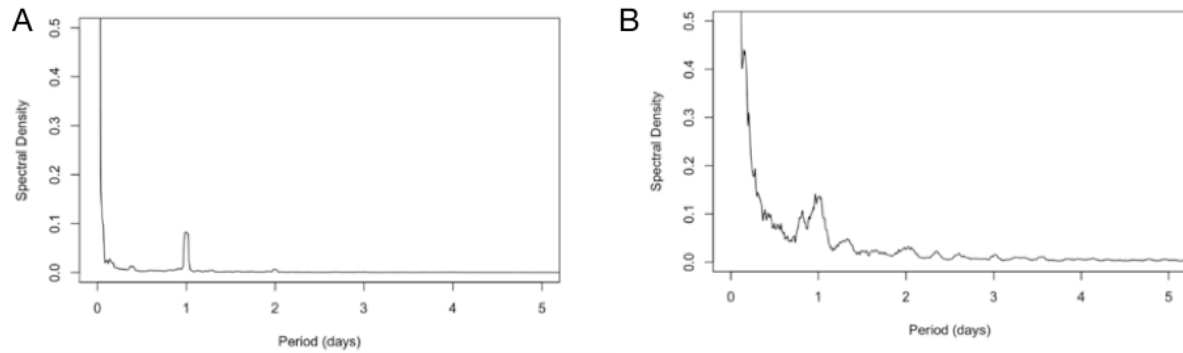


Figure S2. Spectral analysis of pH time-series from the coastal (A) and lagoon (B) sites.

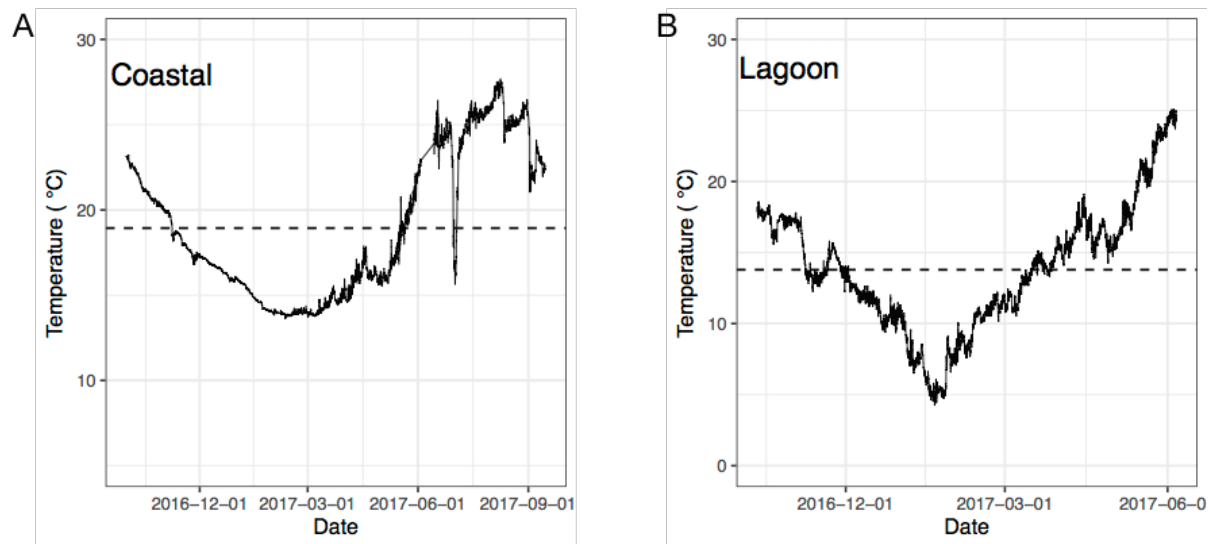


Figure S3. Temperature (oC) profiles of the coastal (A) and lagoon (B) habitats throughout the observation windows.

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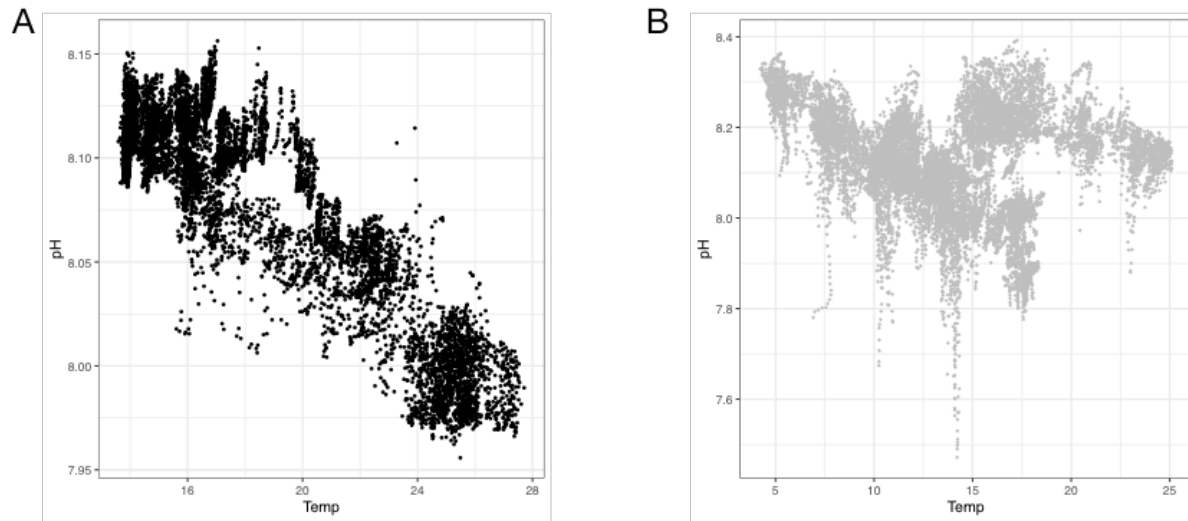


Figure S4. Correlation between temperature and pH at the (A) coastal ($R^2 = -0.9$, $t = -180.86$, $p < 0.001$) and (B) lagoon ($R^2 = -0.13$, $t = -22.63$, $p < 0.001$) habitats.

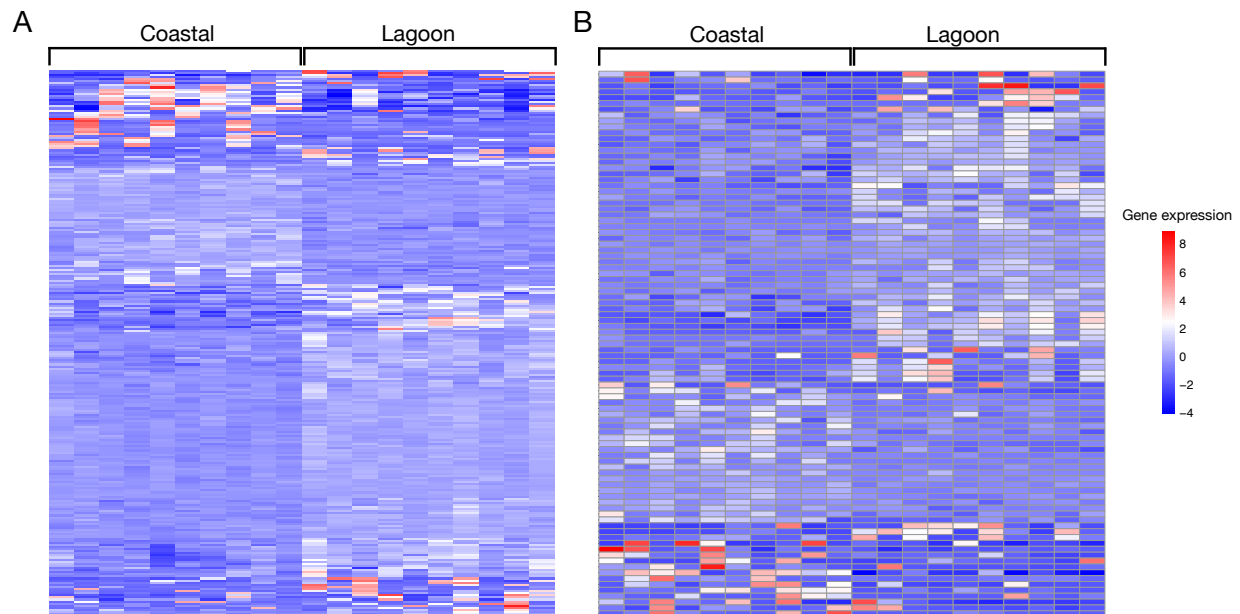


Figure S5. Heatmap depicting patterns of expression at genes differentially expressed between populations in the (A) benign and (B) stressful pH treatments. Each row corresponds to a differentially expressed gene, while columns correspond to individual mussels. Cell color corresponds gene expression, here computed as a regularized log-transformation of the transcript abundance data.

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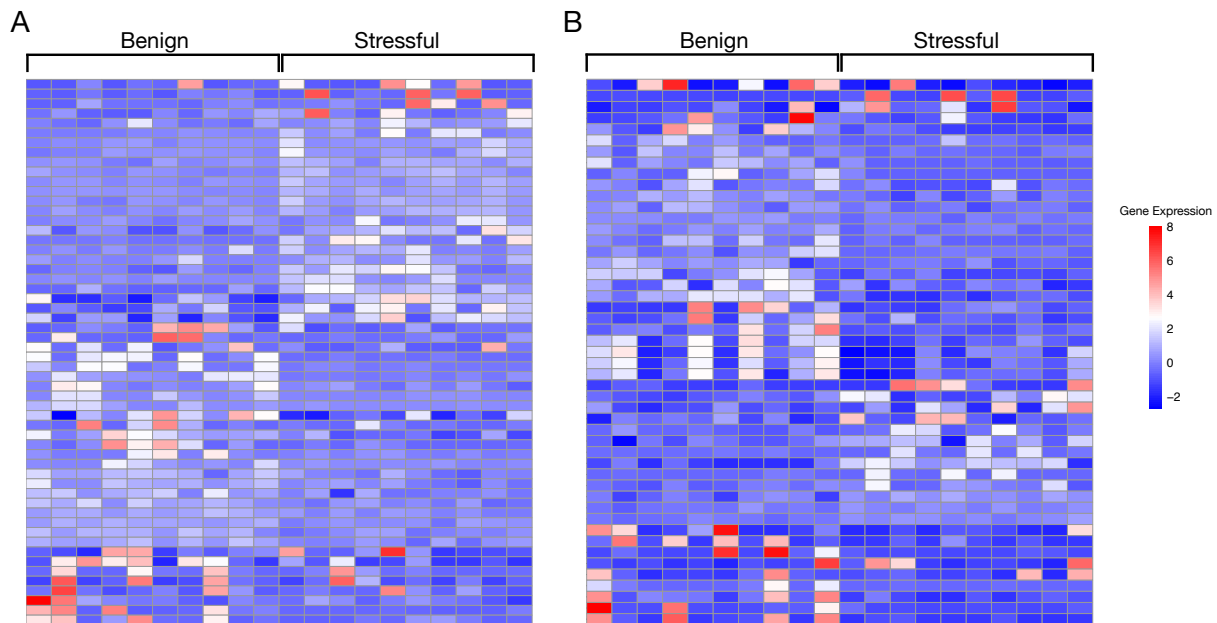


Figure S6. Heatmap depicting patterns of expression at genes differentially expressed in response to stressful pH exposure in the coastal (A) and lagoon (B) populations. Each row corresponds to a differentially expressed gene, while columns correspond to individual mussels. Cell color corresponds to gene expression, here computed as the regularized log-transformation of the transcript abundance for each individual/gene.