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Hidden cost of pH variability in seagrass beds on marine calcifiers under ocean acidification

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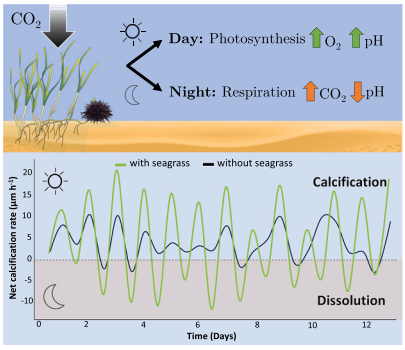
HIGHLIGHTS

- The presence of seagrass creates variability in pH/pCO_2.
- High pCO_2/low pH negatively impacts growth and calcification of sea urchin larvae.
- The variability associated with the presence of seagrass negatively impacts growth under ocean acidification.
- Two different calcification strategies are observed in presence and absence of seagrass.

GRAPHICAL ABSTRACT



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ABSTRACT

Coastal ecosystems experience large environmental variability leading to local adaptation. The key role of variability and adaptation in modulating the biological sensitivity to ocean acidification is increasingly acknowledged. Monitoring and understanding the ecological niche at the right spatio-temporal scale is key to understand the sensitivity of any organism and ecosystems. However, the role of the variability in relevant carbonate chemistry parameters as a driver is often overlooked. For example, the balance between photosynthesis and respiration over the day/night cycle is leading to high pH/pCO_2 variability in seagrass beds. We hypothesized that (i) the calcifying larvae of the sea urchin *Echinus esculentus* exposed to seagrass-driven variability would have some physiological mechanisms to respond to such variability; and (ii) these mechanisms would reach their limit under ocean acidification. We compared the presence and absence of the seagrass *Zostera marina* in flow through mesocosms fed with seawater with 4 pHs. The carbonate chemistry was monitored and biological response of a sea urchin larvae was documented over 3 weeks. Growth and net calcification rates were measured twice a day to encompass diurnal variability. Our results show that larvae growth rate significantly decreased with decreasing average pH_T in both absence and presence of seagrass. Moreover, sea urchin larvae showed a slower growth rate in presence of seagrass, only visible in the lowest pH conditions. In addition, larvae raised in presence of seagrass, maximized calcification during the day, and lower their calcification during the

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night. In contrast, no significant difference was observed between day and night for the net calcification rate in larvae raised in absence of seagrass. Our results demonstrate the limit of local adaptation to the present range of variability under ocean acidification conditions. It also demonstrates that photosynthetic ecosystems such as seagrass may not play a role of refuge against future ocean acidification.

1. Introduction

Heterogeneity in space and time for key physico-chemical parameters can lead to local adaptation and variability in the ability of marine organisms to cope with environmental changes (Boyd et al., 2016; Hofmann et al., 2014; Hofmann et al., 2011). An organism living in a fluctuating environment can be exposed at different times to conditions that are more or less favorable for their fitness. To survive in such environment it can develop strategies that minimize their exposure to these conditions or be phenotypically prepared for expected changes before they occur (Hillebrand et al., 2020). Organisms living in highly variable environments often develop a range of adaptations (e.g. changes in development, behavior and allocation of resources) that allow them to maintain positive fitness (Reed et al., 2010).

Carbonate chemistry differs significantly between regions and is particularly variable in coastal environments. Recent work showed that variability in pCO_2/pH modulates marine species tolerance to ocean acidification leading to thresholds related to the extreme of the present range of natural variability (Vargas et al., 2022).

The potential impact of ocean acidification on marine species that nowadays inhabiting areas of high pCO_2/pH variability have gain attention over the last decade (Vargas et al., 2017), particularly in upwelling zones (Kelly et al., 2013), and rocky shores environments (Cornwall et al., 2018; Dufault et al., 2012; Noisette et al., 2013). Species living in habitat with high fluctuations in pCO_2/pH has been shown to be either sensitive (Cornwall et al., 2013; Johnson et al., 2019), or tolerant to low pH (Dufault et al., 2012; Ramajo et al., 2019). On one hand, highly variable environment can put a given organism at the edge of their physiological tolerance making them highly sensitive to additional environmental challenges; on the other hand, local adaptation to environmental variability can lead to high level of phenotypic plasticity and the physiological ability to cope with more environmental changes (Strader et al., 2022; Wang et al., 2018).

The majority of experiments testing the effects of ocean acidification on marine organisms have been conducted using constant pH conditions and scenarios for the stable open ocean (Riebesell and Gattuso, 2015). Although some recent studies have included the present natural variability in their experimental design (Dufault et al., 2012; Johnson et al., 2019; Mangan et al., 2017), there is still little information on the modulating role of the pCO_2/pH natural variability on biological responses of the species, particularly in coastal habitats.

Seagrass meadows are considered as possible refugia habitat in the context of ocean acidification. This ecosystem is characterized by a high variability in pCO_2/pH due to the balance between the photosynthetic activity and respiration rate over the day/night cycle (Duarte et al., 2013; Hendriks et al., 2014), where the diel fluctuation of pH can be >1.0 unit in tropical seagrass meadows (Semesi et al., 2009). The idea that seagrass meadows can be a refugia against ocean acidification is based on the photosynthetic activity raising the pH during the day, but the increased variability and low pH during the night is often overlooked (Kapsenberg and Cyronak, 2019). This is of critical as recent work highlight the role of extreme low pH of the natural variability as a key factor predicting species sensitivity (Vargas et al., 2017, 2022).

Here we evaluate the biological response (mortality, growth and net calcification rates) of a marine calcifier, the larvae of the sea urchin *Echinus esculentus*, to different levels of variability of pCO_2/pH imposed in the absence or presence of seagrass and under 4 different pHs, relevant in the context of present natural variability and future ocean acidification. The tested hypotheses were that (i) marine calcifiers exposed to seagrass-driven variability would possess some physiological mechanisms to respond to such variability; and (ii) these mechanisms would reach their limit under ocean acidification.

2. Materials and methods

2.1. Biological material

The sea urchin, *Echinus esculentus,* was chosen as a model to test the effect of pCO_2/pH variability. Specimens were collected three months prior experiment (June 2019) by dredging on rocky bottoms at ~30 m in the vicinity of Grundsund, along the Swedish west coast, and transferred to the Kristineberg Marine Research Station, Sweden. They were maintained in natural flowing seawater at 15 °C and pH 8.1 with a diet of *Saccharina latissima*.

Seagrass shoots of *Zostera marina* (eelgrass) were collected in August 2019 from a shallow meadow in the bay of Bokevik in the Gullmars Fjord, next to the Kristineberg Marine Research Station. Plants were collected by snorkeling at 1–2 m water depth and transported to the laboratory within 2 h after collection and maintained in tanks with natural flowing seawater (15 °C).

2.2. Experimental design and larval culture

2.2.1. Aquarium system

The experiment was performed between August and September 2019. We tested the impact of 4 pH treatments (nominal pHs = 7.4, 7.6, 7.9 and 8.2) and the absence/presence of seagrass using a fully crossed design (4 nominal pHs x seagrass absence/presence = 8 treatments). Each treatment was replicated 3 times for a total of 24 experimental 22.5 L tanks. Each experimental tank was fed by aerated surface seawater from a 30 L header tank (15 °C, salinity 32.5 ± 0.5 psu) with a flow of 22 L h^{-1}. pH was maintained in the header tank using a computerized feedback system (AquaMedic) that regulates pH by addition of pure gaseous CO_2 directly into the seawater (±0.03 pH units). Fifteen seagrass plants with 2–4 shoots were planted in 12 experimental tanks (shoot length of 19.3 ± 8.1 cm and shoot width of 0.3 ± 0.09 cm). The sediment used was medium sand collected in the Gullmars fjord at 1 m depth and had a grain size of 236 μm and 0.5 % of organic content, similar to field conditions (Infantes et al., 2016). Light was provided by fluorescent tubes, providing 120–150 PAR and a photoperiod of 12:12 (light: dark, h).

2.2.2. Spawning and culturing

Larval spawning was induced by intracoelomic injection of 0.5 M KCl in filtered seawater (FSW). The sperm was collected using pipette and preserved dry on ice until fertilization. The eggs were collected in 1 L bottles filled with FSW. The gametes were visually examined for viability under the microscope (swimming sperm and healthy eggs). For fertilization, gametes from two females and two males were pooled in FSW to a final concentration of ~1000 sperm egg^{-1}, with a fertilization success close to 100 % (indicated by formation of fertilization membrane). After fertilization, cleaving embryo (two-cell stage) were adjusted to a final concentration of 10 embryos mL^{-1} placed in 4 culture flasks filled with 5 L of aerated FSW at a temperature of 15 °C, and pH_T 8.0 for 5 days. Larvae in culture flasks were then merged and quickly transferred to 1 L glass bottles with final concentration of ~10 larvae per mL.

2.2.3. Experimental exposure of sea urchin larvae

To test the impact of the 8 treatments, sea urchin larvae were transferred at day 5 to 1 L glass bottles at a final concentration of ~10 larvae per mL. Larvae were fed daily with red algae *Rhodomonas sp* at a concentration of ~5000 cells mL^{-1}. Twenty-four 1 L bottles were used and filled with seawater from the corresponding aquarium (one bottle per replicate). Twice a day (6:00 h and 18:00 h before the switch between light and darkness), sea urchin larvae were filtered out of their bottles using a 100 μm mesh, the bottle was filled up with seawater from their respective aquarium with addition of food, and the larvae were transferred back to their bottle. This protocol allowed to expose the larvae to condition mimicking the diurnal fluctuations in the carbonate chemistry of 12:12 (light: dark, h) cycle.

2.3. Seawater parameters

Seawater parameters were monitored twice a day at each water change (at 6:00 h and 18:00 h) throughout the experiment. Discrete samples were taken in each aquarium before each water change, and bottles were immediately filled and closed. This ensured stable carbonate chemistry in the experimental bottles mimicking the conditions in the aquariums. pH_T and temperature were recorded in each aquarium using a Metrohm 827 pH meter (Metrohm, Switzerland) calibrated using TRIS buffer. Dissolved oxygen (mg/L) and salinity (PSU) were also recorded using Pyro Science- Piccolo 2 sensor and YSI PRO-DSS sensor, respectively. Total alkanility (AT) was measured using an automatic titrator (Titroline alpha plus, SI Analytics) at beginning and at end of experiment. The other parameters of the carbonate chemistry, i.e. CO_2 partial pressure (pCO_2) and saturation state of aragonite ΩAr and calcite ΩCa, were calculated for each aquarium using CO_2SYS software (Lewis and Wallace, 1998) (Table 1). In addition to discrete samples, a continuous measurement of pH_T, dissolved oxygen (mg/L) turbidity and conductivity were monitored in each aquarium per time during 24 h using a YSI PRO-DSS multi-parameter water quality meter.

2.4. Mortality

Larvae cultures were maintained for 23 days. At each water change (6:00 h and 18:00 h), 2 sub samples of 10 mL were taken from each bottle and fixed using buffered 4 % paraformaldehyde (PFA) in FSW. The number of larvae in each subsample was counted under a dissecting microscope and was used to estimate the density at each sampling time t (Nt in larvae L^{-1}). For each culture, the relative density was calculated at each sampling time as the proportion of counted live larvae divided by the maximum number of larvae during the experiment. A relative mortality rate (RMR in day^{-1}) was calculated as the coefficient of the significant linear relationship between relative density and time (table S1).

2.5. Growth and net calcification rates

Ten larvae per bottle and sampling time were photographed under a Leica microscope. The body length- (BL, in μm) was measured as the length at midline (Stumpp et al., 2011) as well as the total length of all the skeletal rods (in μm) using the software Image J. The larvae growth rate (μm log $(day)^{-1}$) was calculated using the coefficient of the significant logarithmic regression between BL and time (Stumpp et al., 2011). Net calcification rate (μm h^{-1}) was estimated as the difference in total skeletal rod lengths between 2 consecutive sampling times (12 h) divided by 12.

2.6. Data analysis

All statistical analyses were conducted using R version 4.2.0. All data were checked for normality using a Shapiro-Wilk test ($\alpha = 0.05$), and homoscedasticity with Levene's test. For pH_T, dissolved oxygen and temperature, Kruskall-Wallis (KW) or Wilcoxon (W) were used to test differences between pH treatments and within replicates.

Relative mortality rate (day^{-1}) was plotted against the mean pH_T (average pH_T over the whole experiment in absence/presence of seagrass) using significant linear regression. The effect of absence/presence of seagrass on larvae mortality was tested using *t*-test.

Data of larvae growth rate were transformed using square root to ensure normality and homogeneity of variances. Growth rate (μm log $(day)^{-1}$) was plotted against the mean pH_T (average pH_T over the whole experiment in absence/presence of seagrass) using significant linear regression. The effect of pH on growth rate was tested using analysis of co-variance (ANCOVA) with absence/presence of seagrass as factor and pH_T as covariate.

Net calcification (μm h^{-1}) was plotted against the mean pH_T (average pH_T during whole experiment, and average pH measurements during night and day). ANCOVA were used to test the effect of pH on net

Table 1
Carbonate chemistry parameters (± SEM) measured in aquariums during the sea urchin larvae exposure. Temperature (°C), pH_T (Total scale), salinity of 32 PSU and total alkalinity (AT, ranged from 2374 ± 21.03 μmol Kg^{-1} to 2399 ± 22.92 μmol Kg^{-1}) were used to calculate the partial pressure of CO_2 (μatm) and saturation of carbonate species; calcite (Ω ca) and aragonite (Ω ar).

Treatments	Parameters measured (mean ± SEM)				Parameters estimated (mean ± SEM)		
	T(°C)	pH_T	Dissolved Oxygen (mg/L)	AT (μ mol Kg^{-1})	pCO_2 (μ atm)	Ωca	Ωar
7.4AD	16.8 ± 0.06	7.462 ± 0.01	8.53 ± 0.10	2395 ± 9.39	1898.5 ± 55.22	1.20 ± 0.04	0.77 ± 0.02
7.4AN	15.5 ± 0.06	7.417 ± 0.01	7.29 ± 0.04		2105.7 ± 58.82	1.03 ± 0.03	0.66 ± 0.02
7.4SD	16.8 ± 0.05	7.639 ± 0.02	9.55 ± 0.12	2399 ± 22.92	1240.4 ± 48.32	1.77 ± 0.07	1.14 ± 0.04
7.4SN	15.5 ± 0.05	7.424 ± 0.01	7.21 ± 0.06		2071.2 ± 57.12	1.05 ± 0.02	0.67 ± 0.02
7.6AD	16.8 ± 0.11	7.652 ± 0.01	8.20 ± 0.04	2382 ± 16.69	1179.8 ± 29.78	1.78 ± 0.04	1.14 ± 0.02
7.6AN	15.9 ± 0.08	7.591 ± 0.01	7.47 ± 0.05		1366.2 ± 36.12	1.52 ± 0.04	0.97 ± 0.03
7.6SD	16.6 ± 0.06	7.966 ± 0.05	10.09 ± 0.23	2387 ± 19.59	585.2 ± 73.16	3.54 ± 0.30	2.27 ± 0.19
7.6SN	15.8 ± 0.08	7.581 ± 0.01	7.21 ± 0.06		1399.1 ± 43.24	1.47 ± 0.04	0.94 ± 0.02
7.9AD	16.5 ± 0.03	7.930 ± 0.01	8.25 ± 0.08	2374 ± 21.03	593.1 ± 13.70	3.14 ± 0.06	2.01 ± 0.04
7.9AN	15.5 ± 0.06	7.854 ± 0.01	7.55 ± 0.05		706.6 ± 14.07	2.60 ± 0.04	1.66 ± 0.03
7.9SD	16.7 ± 0.05	8.325 ± 0.03	10.54 ± 0.22	2395 ± 20.51	212.1 ± 18.34	6.72 ± 0.33	4.31 ± 0.21
7.9SN	15.5 ± 0.06	7.840 ± 0.01	7.41 ± 0.04		741.9 ± 20.19	2.55 ± 0.06	1.63 ± 0.04
8.2AD	16.9 ± 0.07	8.375 ± 0.01	8.90 ± 0.11	2384 ± 12.13	170.7 ± 5.63	7.18 ± 0.12	4.60 ± 0.08
8.2AN	15.4 ± 0.04	8.253 ± 0.01	7.67 ± 0.07		243.6 ± 6.09	5.59 ± 0.08	3.57 ± 0.05
8.2SD	16.4 ± 0.43	8.665 ± 0.01	11.17 ± 0.18	2406 ± 3.25	69.7 ± 3.05	10.86 ± 0.19	6.96 ± 0.13
8.2SN	15.4 ± 0.05	8.186 ± 0.01	7.31 ± 0.08		297.2 ± 7.10	5.00 ± 0.07	3.20 ± 0.05

AD- absence of seagrass at day measurements, AN- absence of seagrass at night measurements; SD- presence of seagrass at day measurements, SN- presence of seagrass at night measurements.

calcification (average net calcification during whole experiment), and diel cycle calcification (day/night measurements within each absence/presence of seagrass treatments), using absence/presence of seagrass and cycle (day/night) as factor, respectively.

3. Results

3.1. Seawater chemistry

The carbonate chemistry (mean ± SEM) in all experimental treatments is summarized in Table 1. The mean pH_T for each of the pH treatments ranged from 7.417 ± 0.01 in absence of seagrass at night (pCO_2 = 2105.7 ± 58.82, Ω ca = 1.03 ± 0.03, Ω ar = 0.66 ± 0.02) to 8.665 ± 0.01 in presence of seagrass at day (pCO_2 = 69.7 ± 3.05, Ω ca = 10.86 ± 0.19, Ω ar = 6.96 ± 0.13). pH was significantly different between treatments (KW: $\chi^2(3) = 35.35$, $p < 0.0001$), but pH_T did not vary between replicates within each pH treatments with the exception of the presence of seagrass treatment during the day (see detailed results in table S2). Dissolved oxygen (KW: $\chi^2(3) = 1.62$, $p = 0.65$) and temperature (KW: $\chi^2(3) = 0.81$, $p = 0.85$) did not vary significantly between pH treatments. The diel carbonate chemistry variability (day vs. night) was higher in presence of seagrass as compared to absence of seagrass. The pH_T, dissolved oxygen, Ω ca and Ω ar decreased during the night and increased during the day particularly in presence of seagrass. For example, at the lowest nominal pH 7.4 in presence of seagrass, the average pH was 7.424 ± 0.01 (dissolved oxygen = 7.21 ± 0.06, Ω ca = 1.05 ± 0.02 and Ω ar = 0.67 ± 0.02) at night and 7.639 ± 0.02 (dissolved oxygen = 9.55 ± 0.12, Ω ca = 1.77 ± 0.07 and Ω ar = 1.14 ± 0.04) during the day. This trend was similar in the highest nominal pH 8.2, with an average pH of 8.186 ± 0.01 (dissolved oxygen = 7.31 ± 0.08, Ω ca = 5.00 ± 0.07 and Ω ar = 3.20 ± 0.05) at night and an average pH of 8.665 ± 0.01 (dissolved oxygen = 11.17 ± 0.18, Ω ca = 10.86 ± 0.19 and Ω ar = 6.96 ± 0.13) during the day.

3.2. Biological measurements

3.2.1. Effect of pH on larval mortality and growth rates

Relative mortality rates (RMR) were calculated as the coefficient of the significant regression between relative density and time (see table S1). No significant regression was observed when mortality was analyzed against average pH_T in both absence (RMR = pH x 0.0003 day^{-1}, $p = 0.959$, $R^2 < 0.01$) and presence of seagrass (RMR = pH x 0.0040 day^{-1}, $p = 0.734$, $R^2 = 0.01$, Fig. 1). RMR was slightly higher in presence of seagrass (0.060 ± 0.011 day^{-1} for pH 7.4 and 0.060 ± 0.015 day^{-1} for pH 8.2) than in absence (0.050 ± 0.002 day^{-1} for pH 7.4 and 0.054 ± 0.006 day^{-1} for pH 8.2, Fig. S1). However, there was no significant difference in RMR between absence and presence of seagrass (*t*-test, $p = 0.18$).

The larval growth rate (μm log $(day)^{-1}$) was obtained from the significant logarithmic regression between total body length (μm) and larval development time (day) in each replicate (see table S3, $p < 0.05$). Larvae in three cultures (two 7.6 replicates and one 7.9 replicate) were excluded from analysis due to unstable seawater carbonate chemistry and low larvae density, respectively. Larvae growth rate significantly and linearly decreased with decreasing average pH_T in both absence (GR = pH x 20.4 μm_{BL} log day^{-1}, $p < 0.003$, $R^2 = 0.68$) and presence of seagrass (GR = pH x 26.4 μm_{BL} log day^{-1}, $p < 0.001$, $R^2 = 0.71$, Fig. 2). An analysis of co-variance confirmed that pH had a significant effect on larvae growth rate (model: $F_{1,17} = 32.58$, $p < 0.0001$). The larval growth rate was slightly lower in presence of seagrass ($F_{1,17} = 4.71$, $p < 0.01$) with no significant interaction between absence/presence of seagrass and pH ($F_{1,17} = 0.73$, $p = 0.40$).

3.2.2. Effect of pH on net calcification rate

The net calcification rate was also influenced by pH. Larvae calcification significantly and linearly decreased with decreasing average pH_T in both absence (net calcification = pH x 5.27 μm h^{-1}, $p < 0.001$, R^2 = 0.86) and presence of seagrass (net calcification = pH x 5.38 μm h^{-1}, p < 0.001, R^2 = 0.73, Fig. 3). Net calcification rate decreased with decreasing pH (model: $F_{1,17}$ = 50.62, p < 0.0001), but there was no significant difference between absence and presence of seagrass ($F_{1,17}$ = 0.71, p = 0.40), and no significant interaction between absence/presence of seagrass and pH treatments ($F_{1,17}$ = 0.50, p = 0.48).

During the day, net calcification rates of larvae in absence of seagrass

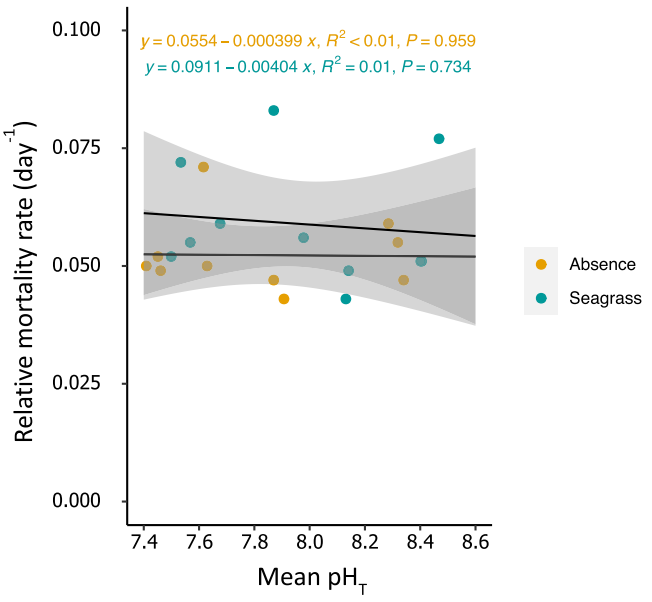

Fig. 1. Relationship between larval mortality rates (day^{-1}) and mean pH_T during all experiment. Each dot represents the regression coefficient extracted from the significant linear relationship between larvae density over time, in aquariums with absence of seagrass and presence of seagrass (see regressions in table S1). Shaded bands represent the 95 % confidence interval.

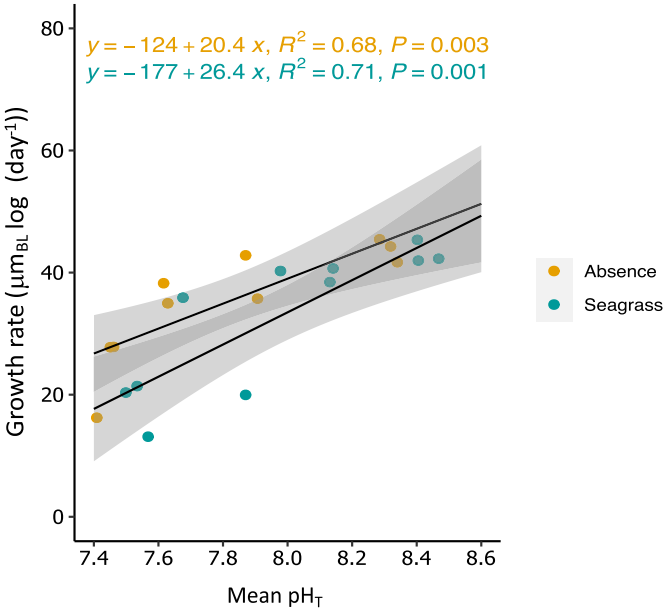

Fig. 2. Relationship between growth rate per developmental time (μm log $(day)^{-1}$) and mean pH_T during all experiment. Each dot represents the regression coefficient extracted from the significant linear relationship between total body length (TL in μm) and time, in aquariums with absence of seagrass and presence of seagrass (see regressions in table S3). Shaded bands represent the 95 % confidence interval.

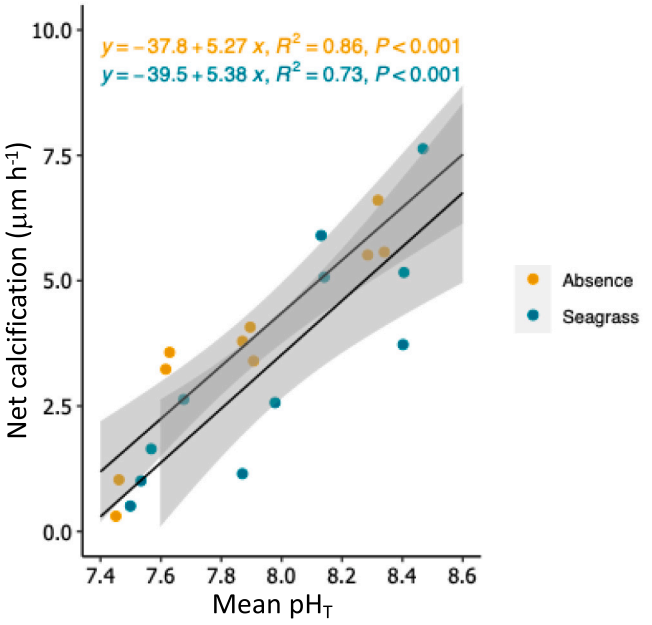

Fig. 3. Relationship between net calcification per developmental time (µm h^{-1}) and mean pH_T. Each dot represents replicates with absence and presence of seagrass. Shaded bands represent the 95 % confidence interval.

varied from min - max: 0.51–6.35 µm h^{-1} in the lowest tested pH_T conditions ($pH_T = 7.462 \pm 0.01$) to 1.83–15.37 µm h^{-1} ($pH_T = 8.375 \pm 0.01$). During the night, it varied between −11.76-0.11 µm h^{-1} ($pH_T = 7.417 \pm 0.01$) and - 4.35-9.32 µm h^{-1} ($pH_T = 8.253 \pm 0.01$) (Fig. 4a). In absence of seagrass, there were no significant effect of pH on larval net calcification (model: $F_{1,18} = 3.34$, $p = 0.08$), no differences between day and night ($F_{1,18} = 3.05$, $p = 0.09$), and no significant interaction between day/night and pH ($F_{1,18} = 0.10$, $p = 0.75$). In presence of seagrass, net calcification rates were different between day and night, with larvae maintaining a high calcification rate during the day (varying only between 9.75 and 13.35 µm h^{-1} in the lowest tested pH ($pH_T = 7.639 \pm 0.02$) and 8.39–18.07 µm h^{-1} in the highest test pH ($pH_T = 8.665 \pm 0.01$). Negative net calcification rates (dissolution) were observed during the night with values ranging between −10.06 - (−7.72) µm h^{-1} ($pH_T = 7.424 \pm 0.01$) and - 7.74 - 6.54 ($pH_T = 8.186 \pm 0.01$) (Fig. 4b). Significant difference in net calcification rates were observed between day and night ($F_{1,18} = 72.63$, $p < 0.0001$) with larvae increasing their calcification during the day while lower their calcification during the night. Significant and linear decrease of larvae calcification rate, leading to dissolution at the lowest pH_T during the night was also observed in seagrass treatments ($F_{1,18} = 5.93$, $p = 0.02$).

4. Discussion

This study shows significant negative impact of low pH/high pCO_2 on different fitness-related biological traits (growth and net calcification rates) of the larval stage of an echinoderm. As a consequence of a balance between respiration and photosynthesis, higher variability in the carbonate chemistry was observed in presence of seagrass, with high pH during the day and low pH during the night. Average pH was a good predictor of the calcification rate in *Echinus esculentus*, independently of the absence or presence of seagrass. However, larvae raised in constant (absence of seagrass) or fluctuating (presence of seagrass) environment used two different strategies for calcification, suggesting a quick selection or acclimation to the environment within the first days of development: (i) in constant environment, the calcification rate did not significantly differ between day and night with a strong negative effect of low pH while (ii) in fluctuating environment, the calcification rate was maximum during the day when the pH is the highest with no influence of decreasing pH, and the calcification rate was low during the night, even leading to dissolution at the lowest pH. These two contrasting mechanisms had consequence for the larval growth. While no significant difference was observed for the mortality rate, a slower growth rate under the near-future ocean acidification conditions was observed in presence of seagrass as compared to constant conditions. It suggests that the physiological mechanisms used by larvae in fluctuating conditions to anticipate the fluctuating pH regimes under present conditions reached their limit under ocean acidification as the associated energy costs for compensatory calcification may be higher than the

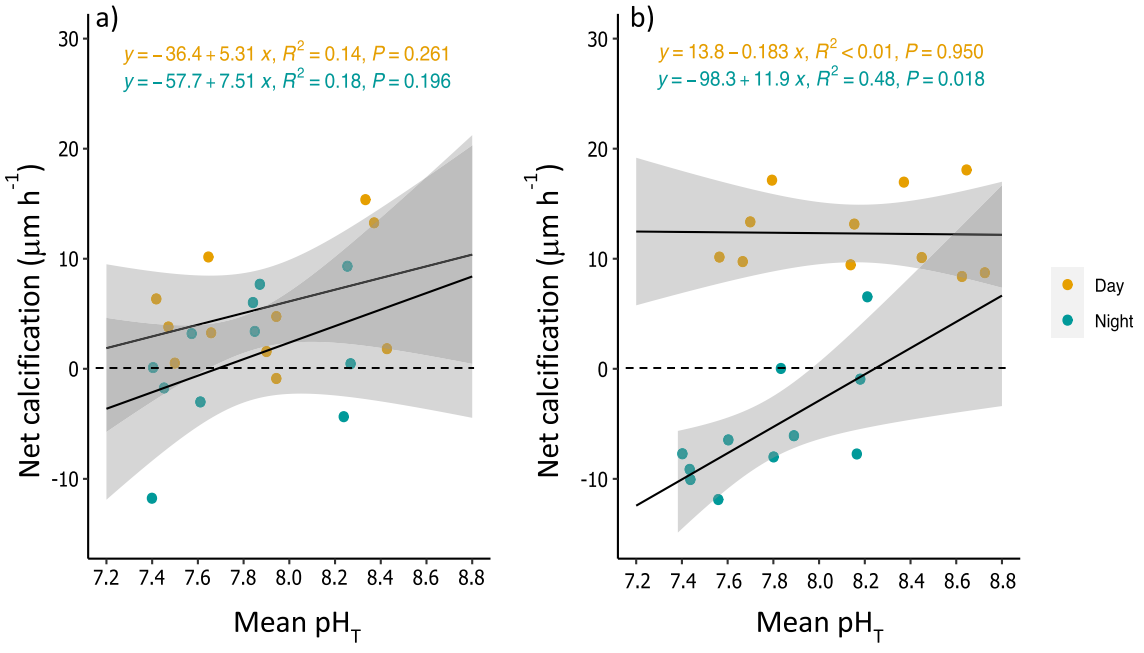

Fig. 4. Relationship between net calcification (µm h^{-1}) and mean pH_T during the day and during the night. Each dot represents replicates where a) absence of seagrass and b) presence of seagrass, during day and night measurements. Shaded bands represent the 95 % confidence interval.

benefits (Bradassi et al., 2013; Ventura et al., 2016). These results have significant implications for the design of experiments involving organisms living in the coastal zone and the understanding of their sensitivity to ocean acidification in variable coastal ecosystems, such as seagrass.

4.1. Impact of low pH/high pCO_2 on mortality and growth rate

Larvae mortality was not significantly impacted by low pH/high pCO_2 in presence and absence of seagrass. However, mortality was slighter higher in presence of seagrass (Fig. 1). This is in accordance with previous studies, where higher pCO_2 did not significantly alter mortality (Chan and Tong, 2020; Stumpp et al., 2011).

The growth rate decreased when larvae were raised under low pH/high pCO_2 in both absence and presence of seagrass. This finding is consistent with previous studies on echinoderm larvae, which showed that low pH/elevated pCO_2 result in smaller growth rate and increased level of abnormalities (Chan et al., 2016; Dorey et al., 2022; Dorey et al., 2013; Stumpp et al., 2011). In addition, our results show that exposure to fluctuating pH condition driven by seagrass resulted in slower larvae growth rate as compared to exposure to constant condition (absence of seagrass). This suggest that fluctuating conditions in seagrass induce an extra energetic cost of larvae and thus change in energy allocation, reducing growth rate. This is consistent with the recent re-analysis of the literature by (Vargas et al., 2022; Vargas et al., 2017) showing that species sensitivity to pH is dependent of local adaptation driven by extreme low pHs. In a given environment, the biological response is then more likely to be driven by extremes than averages and the presence of seagrass leads to an increased variability and then lower extreme pH. For example, it was shown in a previous study that both spicule length and larval body size were smaller in sea urchin, *Strongylocentrotus purpuratus* when reared in upwelling conditions (Strader et al., 2022). Negative physiological effects such as a greater oxidative stress and changes in metabolic rates were reported in mussel, *Mytilus edulis* collected in estuarine habitat (Mangan et al., 2017), and reduced growth rate of coralline macroalga *Arthrocardia corymbosa* (Cornwall et al., 2013) were also reported in fluctuating conditions. In other studies, no negative effects in physiological traits were observed in marine calcifiers exposed to fluctuating conditions (Dufault et al., 2012; Navarro et al., 2020). This can be explained by a physiological plasticity to a wide range of carbonate chemistry conditions as an important mechanism to cope with environmental variability (Hoshijima and Hofmann, 2019; Strader et al., 2022; Wang et al., 2018). However, our results demonstrate that these mechanisms may reach their limit under near-future ocean acidification.

Sensitivity of marine calcifiers living in fluctuating pH/pCO_2 conditions as experienced in coastal ecosystems are highly depending on the present natural variability (Vargas et al., 2022; Vargas et al., 2017). Here, we use as model a sea urchin, *Echinus esculentus* which has a planktonic larvae stage and can experience a strong pH fluctuation of up to 1.0 unit (7.57 to 8.68) in their natural habitat (Dorey et al., 2013). Therefore, the species is likely equipped with the ability to cope with natural variability as in our seagrass treatments. Interestingly, our results also show that when raised in constant condition, larvae used a different mechanism for calcification, maintaining a constant calcification rate at day and night. Long-term and intergenerational studies are needed to explore this concept further and evaluate the cost of maintaining plasticity to persist in these variable conditions under future climate change scenarios.

4.2. Impact of low pH/high pCO_2 on net calcification

Larval net calcification rate was negatively impacted by low pH in both absence and presence of seagrass. This is in accordance with previous studies showing that low pH/high pCO_2 decrease calcification rate of marine calcifiers (Cyronak et al., 2016; Figuerola et al., 2021; Fitzer et al., 2019). Sea urchins have the ability to regulate their intracellular pH (Calosi et al., 2013; Stumpp et al., 2012) and maintain their calcification in low pH conditions (e.g. Dorey et al., 2013). However, acid-base regulation is energetically costly and exposure to a more challenging low pH environment can lead to a shift in energy budget (Pan et al., 2015; Stumpp et al., 2012). The maintenance of calcification under extreme low pH associated with fluctuating conditions can lead to extra energy costs and explain the slower larvae growth rate observed in presence of seagrass as compared to constant (absence of seagrass).

This change in energy budget can also be amplified by the two different strategies observed in larvae raised under constant and fluctuating conditions. While no significant difference was observed for calcification rate between day and night in larvae raised in constant conditions, the larvae raised in fluctuating conditions maximized calcification during the day, while calcification was lower during the night. Under two lower tested pH, it led to dissolution during the night (Fig. 4). Similar findings were observed in previous study in bivalves, in which pH fluctuations fostering by macroalgae, *Fucus vesiculosus* allowed mussels, *Mytilus edulis* to shifting most of their calcification activity into the daytime, while calcification was lower under night conditions and under acidified condition (Wahl et al., 2018). Other studies showed that the presence of marine autotrophs was more suitable for growth and survival of bivalves, even in acidified conditions (see Young et al., 2022; Young and Gobler, 2018).

Here, sea urchin larvae experience some mortality over the first days of development (Fig. 1, table S1) and the two different strategies under fluctuating and constant regime could be an evidence of a strong selection process. For instance, genetic variations were observed in the sea urchins, *Strongylocentrotus purpuratus*. For this species, extreme variable low pH conditions led to a trade-off between survival and growth, with high survival in low pH but with cost of reduced size in surviving larvae, as compared to static pH conditions (Garrett et al., 2020).

Our results confirm previous observations that seagrass meadows is altering the carbonate chemistry by increasing the pH of surrounded seawater during the day (Unsworth et al., 2012). Our experimental design (volume of aquarium, seagrass density, photoperiod, seawater renewal time) to drive a significant impact on the seawater carbonate chemistry. Our main goal was to investigate the physiological mechanisms used by a marine calcifier to cope with such environmental variability. In the field, hydrodynamic conditions and other environmental parameters lead to more complex and dynamic variability in the seawater physico-chemical conditions. Indeed, in situ experiment have shown that in high water residence time, the presence of seagrass increased magnitude of the range in pH (see James et al., 2020). Other experimental designs would be required to fully resolve the impact of more realistic fluctuations in pH on marine species and ecosystems.

The current hypothesis is that photosynthetic activity in seagrass may favor calcification across different taxa (Ramajo et al., 2019; Semesi et al., 2009). However, the buffering effect of photosynthesis during the day is counterbalanced by respiration during the night leading to extremes of low pH (Pacella et al., 2018). Other studies also showed that this diel pH fluctuations can have unfavorable consequences for calcification of marine calcifiers (see Johnson et al., 2019). The idea that high pH experienced during the day in ecosystems dominated by photosynthetic organisms could be used as a refuge under ocean acidification may be over-simplistic and neglect the consequences of being exposed to more extreme pH during the night as well as the inadequacies of physiological adaptation under future environmental conditions (Kwiatkowski et al., 2016). While seagrass bed may not be refuge for ocean acidification, their conservation and restoration is of the highest importance to mitigating effects of climate change (e.g. through coastal protection and sediment stabilization) (see Infantes et al., 2022), as well as to improving biodiversity, benefit local coastal economies and enhance food security.

5. Conclusions

This study evaluates the biological response (mortality, growth and net calcification rates) of a marine calcifier in absence and presence of seagrass in the context of ocean acidification. We found that larvae exposed to seagrass and ocean acidification showed slower growth rate as compared to those exposed to ocean acidification in absence of seagrass. This reinforce that living in variable environments such as seagrass requires more energy than living in a more stable one.

We also showed a clear difference in calcification mechanisms in sea urchin larvae raised in constant or fluctuating conditions (presence of seagrass). In conclusion, seagrass may not act as refuge under ocean acidification for some marine calcifiers as extreme low pH during the night may be more detrimental than the relief of high pH during the day.

CRediT authorship contribution statement

Damboia Cossa: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Funding acquisition. **Eduardo Infantes:** Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. **Sam Dupont:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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Appendix A. Supplementary data

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References

Boyd, P.W., Cornwall, C.E., Davison, A., Doney, S.C., Fourquez, M., Hurd, C.L., Lima, I. D., McMinn, A., 2016. Biological responses to environmental heterogeneity under future ocean conditions. Glob. Chang. Biol. 22, 2633–2650. https://doi.org/10.1111/gcb.13287.

Bradassi, F., Cumani, F., Bressan, G., Dupont, S., 2013. Early reproductive stages in the crustose coralline alga Phymatolithon lenormandii are strongly affected by mild ocean acidification. Mar. Biol. 160, 2261–2269. https://doi.org/10.1007/s00227-013-2260-2.

Calosi, P., Rastrick, S.P.S., Graziano, M., Thomas, S.C., Baggini, C., Carter, H.A., Hall-Spencer, J.M., Milazzo, M., Spicer, J.I., 2013. Distribution of sea urchins living near shallow water CO2 vents is dependent upon species acid–base and ion-regulatory abilities. Mar. Pollut. Bull. 73, 470–484. https://doi.org/10.1016/j.marpolbul.2012.11.040.

Chan, K.Y.K., Tong, C.S.D., 2020. Temporal variability modulates pH impact on larval sea urchin development. Conservation. Physiology 8. https://doi.org/10.1093/conphys/coaa008 coaa008.

Chan, K.Y.K., Grünbaum, D., Arnberg, M., Dupont, S., 2016. Impacts of ocean acidification on survival, growth, and swimming behaviours differ between larval urchins and brittlestars. ICES J. Mar. Sci. 73, 951–961. https://doi.org/10.1093/icesjms/fsv073.

Cornwall, C.E., Hepburn, C.D., McGraw, C.M., Currie, K.I., Pilditch, C.A., Hunter, K.A., Boyd, P.W., Hurd, C.L., 2013. Diurnal fluctuations in seawater pH influence the response of a calcifying macroalga to ocean acidification. Proc. R. Soc. B 280. https://doi.org/10.1098/rspb.2013.2201, 20132201.

Cornwall, C.E., Comeau, S., DeCarlo, T.M., Moore, B., D'Alexis, Q., McCulloch, M.T., 2018. Resistance of corals and coralline algae to ocean acidification: physiological control of calcification under natural pH variability. Proc. R. Soc. B 285. https://doi.org/10.1098/rspb.2018.1168, 20181168.

Cyronak, T., Schulz, K.G., Jokiel, P.L., 2016. The Omega myth: what really drives lower calcification rates in an acidifying ocean. ICES J. Mar. Sci. 73, 558–562. https://doi.org/10.1093/icesjms/fsv075.

Dorey, N., Lançon, P., Thorndyke, M., Dupont, S., 2013. Assessing physiological tipping point of sea urchin larvae exposed to a broad range of pH. Glob. Chang. Biol. https://doi.org/10.1111/gcb.12276 n/a-n/a.

Dorey, N., Butera, E., Espinel-Velasco, N., Dupont, S., 2022. Direct and latent effects of ocean acidification on the transition of a sea urchin from planktonic larva to benthic juvenile. Sci. Rep. 12, 5557. https://doi.org/10.1038/s41598-022-09537-7.

Duarte, C.M., Hendriks, I.E., Moore, T.S., Olsen, Y.S., Steckbauer, A., Ramajo, L., Carstensen, J., Trotter, J.A., McCulloch, M., 2013. Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. Estuar. Coasts 36, 221–236. https://doi.org/10.1007/s12237-013-9594-3.

Dufault, A.M., Cumbo, V.R., Fan, T.-Y., Edmunds, P.J., 2012. Effects of diurnally oscillating p CO $_2$ on the calcification and survival of coral recruits. Proc. R. Soc. B 279, 2951–2958. https://doi.org/10.1098/rspb.2011.2545.

Figuerola, B., Hancock, A.M., Bax, N., Cummings, V.J., Downey, R., Griffiths, H.J., Smith, J., Stark, J.S., 2021. A review and meta-analysis of potential impacts of ocean acidification on marine calcifiers from the southern ocean. Front. Mar. Sci. 8 https://doi.org/10.3389/fmars.2021.584445, 584445.

Fitzer, S.C., Chan, V.B.S., Meng, Y., Rajan, K.C., Suzuki, M., Not, C., Toyofuku, T., Falkenberg, L., Byrne, M., Harvey, B.P., De Wit, P., Cusack, M., Gao, K.S., Taylor, P., Dupont, S., Hall-Spencer, J.M., Thiyagarajan, V., 2019. Established and emerging techniques for characterising the formation, structure and performance of calcified structures under ocean acidification. In: Hawkins, S.J., Allcock, A.L., Bates, A.E., Firth, L.B., Smith, I.P., Swearer, S.E., Todd, P.A. (Eds.), Oceanography and Marine Biology. CRC Press, pp. 89–125. https://doi.org/10.1201/9780429026379-2.

Garrett, A.D., Brennan, R.S., Steinhart, A.L., Pelletier, A.M., Pespeni, M.H., 2020. Unique genomic and phenotypic responses to extreme and variable pH conditions in purple urchin larvae. Integr. Comp. Biol. 60, 318–331. https://doi.org/10.1093/icb/icaa072.

Hendriks, I.E., Olsen, Y.S., Ramajo, L., Basso, L., Steckbauer, A., Moore, T.S., Howard, J., Duarte, C.M., 2014. Photosynthetic activity buffers ocean acidification in seagrass meadows. Biogeosciences 11, 333–346. https://doi.org/10.5194/bg-11-333-2014.

Hillebrand, H., Jacob, U., Leslie, H.M., 2020. Integrative research perspectives on marine conservation. Philos. Trans. R. Soc. B 375. https://doi.org/10.1098/rstb.2019.0444, 20190444.

Hofmann, G.E., Smith, J.E., Johnson, K.S., Send, U., Levin, L.A., Micheli, F., Paytan, A., Price, N.N., Peterson, B., Takeshita, Y., Matson, P.G., Crook, E.D., Kroeker, K.J., Gambi, M.C., Rivest, E.B., Frieder, C.A., Yu, P.C., Martz, T.R., 2011. High-frequency dynamics of ocean pH: a multi-ecosystem comparison. PLoS One 6. https://doi.org/10.1371/journal.pone.0028983 e28983.

Hofmann, G.E., Evans, T.G., Kelly, M.W., Padilla-Gamiño, J.L., Blanchette, C.A., Washburn, L., Chan, F., McManus, M.A., Menge, B.A., Gaylord, B., Hill, T.M., Sanford, E., LaVigne, M., Rose, J.M., Kapsenberg, L., Dutton, J.M., 2014. Exploring local adaptation and the ocean acidification seascape – studies in the California Current Large Marine Ecosystem. Biogeosciences 11, 1053–1064. https://doi.org/10.5194/bg-11-1053-2014.

Hoshijima, U., Hofmann, G.E., 2019. Variability of seawater chemistry in a kelp Forest environment is linked to in situ transgenerational effects in the Purple Sea urchin, *Strongylocentrotus purpuratus*. Front. Mar. Sci. 6, 62. https://doi.org/10.3389/fmars.2019.00062.

Infantes, E., Eriander, L., Moksnes, P.-O., 2016. Eelgrass (*Zostera marina*) restoration in the west coast of Sweden using seeds. Mar. Ecol. Prog. Ser. 545, 31–45.

Infantes, E., Hoeks, S., Adams, M., Van Der Heide, T., Van Katwijk, M., Bouma, T., 2022. Seagrass roots strongly reduce cliff erosion rates in sandy sediments. Mar. Ecol. Prog. Ser. 700, 1–12. https://doi.org/10.3354/meps14196.

James, R.K., van Katwijk, M.M., van Tussenbroek, B.I., van der Heide, T., Dijkstra, H.A., van Westen, R.M., Pietrzak, J.D., Candy, A.S., Klees, R., Riva, R.E.M., Slobbe, C.D., Katsman, C.A., Herman, P.M.J., Bouma, T.J., 2020. Water motion and vegetation control the pH dynamics in seagrass-dominated bays. Limnol. Oceanogr. 65, 349–362. https://doi.org/10.1002/lno.11303.

Johnson, M.D., Rodriguez Bravo, L.M., O'Connor, S.E., Varley, N.F., Altieri, A.H., 2019. pH variability exacerbates effects of ocean acidification on a Caribbean crustose coralline alga. Front. Mar. Sci. 6, 150. https://doi.org/10.3389/fmars.2019.00150.

Kapsenberg, L., Cyronak, T., 2019. Ocean acidification refugia in variable environments. Glob. Chang. Biol. 25, 3201–3214. https://doi.org/10.1111/gcb.14730.

Kelly, M.W., Padilla-Gamiño, J.L., Hofmann, G.E., 2013. Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus*. Glob. Chang. Biol. 19, 2536–2546. https://doi.org/10.1111/gcb.12251.

Kwiatkowski, L., Gaylord, B., Hill, T., Hosfelt, J., Kroeker, K.J., Nebuchina, Y., Ninokawa, A., Russell, A.D., Rivest, E.B., Sesboüé, M., Caldeira, K., 2016. Nighttime dissolution in a temperate coastal ocean ecosystem increases under acidification. Sci. Rep. 6, 22984. https://doi.org/10.1038/srep22984.

Lewis, E., Wallace, D.W.R., 1998. Program Developed for CO2 System Calculations. Department, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S.

Mangan, S., Urbina, M.A., Findlay, H.S., Wilson, R.W., Lewis, C., 2017. Fluctuating seawater pH/ p CO $_2$ regimes are more energetically expensive than static pH/ p CO $_2$ levels in the mussel *Mytilus edulis*. Proc. R. Soc. B 284. https://doi.org/10.1098/rspb.2017.1642, 20171642.

Navarro, J.M., Villanueva, P., Rocha, N., Torres, R., Chaparro, O.R., Benítez, S., Andrade-Villagrán, P.V., Alarcón, E., 2020. Plastic response of the oyster Ostrea chilensis to temperature and pCO2 within the present natural range of variability. PLoS One 15. https://doi.org/10.1371/journal.pone.0234994 e0234994.

Noisette, F., Egilsdottir, H., Davoult, D., Martin, S., 2013. Physiological responses of three temperate coralline algae from contrasting habitats to near-future ocean acidification. J. Exp. Mar. Biol. Ecol. 448, 179–187. https://doi.org/10.1016/j.jembe.2013.07.006.

Pacella, S.R., Brown, C.A., Waldbusser, G.G., Labiosa, R.G., Hales, B., 2018. Seagrass habitat metabolism increases short-term extremes and long-term offset of CO $_2$ under future ocean acidification. Proc. Natl. Acad. Sci. U. S. A. 115, 3870–3875. https://doi.org/10.1073/pnas.1703445115.

Pan, T.-C.F., Applebaum, S.L., Manahan, D.T., 2015. Experimental ocean acidification alters the allocation of metabolic energy. Proc. Natl. Acad. Sci. U. S. A. 112, 4696–4701. https://doi.org/10.1073/pnas.1416967112.

Ramajo, L., Lagos, N.A., Duarte, C.M., 2019. Seagrass Posidonia oceanica diel pH fluctuations reduce the mortality of epiphytic forams under experimental ocean acidification. Mar. Pollut. Bull. 146, 247–254. https://doi.org/10.1016/j.marpolbul.2019.06.011.

Reed, T.E., Waples, R.S., Schindler, D.E., Hard, J.J., Kinnison, M.T., 2010. Phenotypic plasticity and population viability: the importance of environmental predictability. Proc. R. Soc. B 277, 3391–3400. https://doi.org/10.1098/rspb.2010.0771.

Riebesell, U., Gattuso, J.-P., 2015. Lessons learned from ocean acidification research. Nat. Clim. Chang. 5, 12–14. https://doi.org/10.1038/nclimate2456.

Semesi, I., Beer, S., Björk, M., 2009. Seagrass photosynthesis controls rates of calcification and photosynthesis of calcareous macroalgae in a tropical seagrass meadow. Mar. Ecol. Prog. Ser. 382, 41–47. https://doi.org/10.3354/meps07973.

Strader, M.E., Wolak, M.E., Simon, O.M., Hofmann, G.E., 2022. Genetic variation underlies plastic responses to global change drivers in the purple sea urchin, *Strongylocentrotus purpuratus*. Proc. R. Soc. B 289. https://doi.org/10.1098/rspb.2022.1249, 20221249.

Stumpp, M., Wren, J., Melzner, F., Thorndyke, M.C., Dupont, S.T., 2011. CO2 induced seawater acidification impacts sea urchin larval development I: elevated metabolic rates decrease scope for growth and induce developmental delay. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 160, 331–340. https://doi.org/10.1016/j.cbpa.2011.06.022.

Stumpp, M., Hu, M.Y., Melzner, F., Gutowska, M.A., Dorey, N., Himmerkus, N., Holtmann, W.C., Dupont, S.T., Thorndyke, M.C., Bleich, M., 2012. Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification. Proc. Natl. Acad. Sci. U. S. A. 109, 18192–18197. https://doi.org/10.1073/pnas.1209174109.

Unsworth, R.K.F., Collier, C.J., Henderson, G.M., McKenzie, L.J., 2012. Tropical seagrass meadows modify seawater carbon chemistry: implications for coral reefs impacted by ocean acidification. Environ. Res. Lett. 7 https://doi.org/10.1088/1748-9326/7/2/024026, 024026.

Vargas, C.A., Lagos, N.A., Lardies, M.A., Duarte, C., Manríquez, P.H., Aguilera, V.M., Broitman, B., Widdicombe, S., Dupont, S., 2017. Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. Nat. Ecol. Evol. 1, 0084. https://doi.org/10.1038/s41559-017-0084.

Vargas, C.A., Cuevas, L.A., Broitman, B.R., San Martin, V.A., Lagos, N.A., Gaitán-Espitia, J.D., Dupont, S., 2022. Upper environmental pCO2 drives sensitivity to ocean acidification in marine invertebrates. Nat. Clim. Chang. 12, 200–207. https://doi.org/10.1038/s41558-021-01269-2.

Ventura, A., Schulz, S., Dupont, S., 2016. Maintained larval growth in mussel larvae exposed to acidified under-saturated seawater. Sci. Rep. 6, 23728. https://doi.org/10.1038/srep23728.

Wahl, M., Schneider Covachã, S., Saderne, V., Hiebenthal, C., Müller, J.D., Pansch, C., Sawall, Y., 2018. Macroalgae may mitigate ocean acidification effects on mussel calcification by increasing pH and its fluctuations: biogenic fluctuations mitigate OA effects. Limnol. Oceanogr. 63, 3–21. https://doi.org/10.1002/lno.10608.

Wang, J., Russell, B.D., Ding, M.-W., Dong, Y.-W., 2018. Ocean acidification increases the sensitivity of and variability in physiological responses of an intertidal limpet to thermal stress. Biogeosciences 15, 2803–2817. https://doi.org/10.5194/bg-15-2803-2018.

Young, C.S., Gobler, C.J., 2018. The ability of macroalgae to mitigate the negative effects of ocean acidification on four species of North Atlantic bivalve. Biogeosciences 15, 6167–6183. https://doi.org/10.5194/bg-15-6167-2018.

Young, C.S., Sylvers, L.H., Tomasetti, S.J., Lundstrom, A., Schenone, C., Doall, M.H., Gobler, C.J., 2022. Kelp (Saccharina latissima) mitigates coastal ocean acidification and increases the growth of North Atlantic bivalves in lab experiments and on an oyster farm. Front. Mar. Sci. 9 https://doi.org/10.3389/fmars.2022.881254, 881254.