


Macroalgae may mitigate ocean acidification effects on mussel calcification by increasing pH and its fluctuations

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Abstract

Ocean acidification (OA) is generally assumed to negatively impact calcification rates of marine organisms. At a local scale however, biological activity of macrophytes may generate pH fluctuations with rates of change that are orders of magnitude larger than the long-term trend predicted for the open ocean. These fluctuations may in turn impact benthic calcifiers in the vicinity. Combining laboratory, mesocosm and field studies, such interactions between OA, the brown alga *Fucus vesiculosus*, the sea grass *Zostera marina* and the blue mussel *Mytilus edulis* were investigated at spatial scales from decimetres to 100s of meters in the western Baltic. Macrophytes increased the overall mean pH of the habitat by up to 0.3 units relative to macrophyte-free, but otherwise similar, habitats and imposed diurnal pH fluctuations with amplitudes ranging from 0.3 to more than 1 pH unit. These amplitudes and their impact on mussel calcification tended to increase with increasing macrophyte biomass to bulk water ratio. At the laboratory and mesocosm scales, biogenic pH fluctuations allowed mussels to maintain calcification even under acidified conditions by shifting most of their calcification activity into the daytime when biogenic fluctuations caused by macrophyte activity offered temporal refuge from OA stress. In natural habitats with a low biomass to water body ratio, the impact of biogenic pH fluctuations on mean calcification rates of *M. edulis* was less pronounced. Thus, in dense algae or seagrass habitats, macrophytes may mitigate OA impact on mussel calcification by raising mean pH and providing temporal refuge from acidification stress.

The uptake of rising anthropogenic CO₂ by the ocean is leading to substantial shifts in seawater carbonate chemistry: concentrations of CO₂, H⁺ and HCO₃⁻ are increasing, whereas CO₃²⁻ concentration and the saturation states of calcite (Ω_{Ca}) and aragonite (Ω_{Ar}) are decreasing (e.g., Feely et al. 2004). This on-going process called ocean acidification (OA) is expected to reduce surface open ocean pH by 0.3–0.4 units until the end of the century (Orr et al. 2005; Doney et al. 2009; IPCC 2013). However, these modeling projections for the open ocean are not always applicable to near-shore environments (Duarte et al. 2013; Müller et al. 2016). Coastal

ecosystems, especially when shallow and sheltered, are often subject to large fluctuations of the carbonate system caused by hydrodynamics and/or biological activity (Duarte et al. 2013; Waldbusser and Salisbury 2014). Continuous time series at various sites revealed that pH fluctuations in coastal waters can exceed those in the open ocean by an order of magnitude or more (e.g., Hofmann et al. 2011). In addition to seasonal pH fluctuations (e.g., Thomsen et al. 2013), a number of studies have reported substantial diurnal oscillations of pH and pCO₂ in productive coastal communities driven by biological photosynthesis/respiration cycles in, e.g., tide or rock pools (Truchot and Duhamel-Jouve 1980; Bjork et al. 2004; Beer et al. 2006; Wootton et al. 2008), coral reefs (Shamberger et al. 2011; Gray et al. 2012; Price et al. 2012; Smith et al. 2013), seagrass meadows (Invers et al. 1997; Semesi et al. 2009; Unsworth et al. 2012; Buapet et al. 2013; Hendriks et al. 2014) and macroalgal habitats (e.g., Middelboe and Hansen 2007; Krause-Jensen et al. 2015; Krause-Jensen and Duarte 2016). For shallow stands of *Fucus*

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vesiculosus, the most important macroalgal species in the Baltic Sea, diurnal amplitudes of about one pH unit can typically be observed during summer months (Middelboe and Hansen 2007; Saderne and Wahl 2013). Generally, the rate and amplitude of pH changes are inversely proportional to the spatial scale considered, i.e., small scale pH fluctuations can be fast (sec-min) and dramatic (1–3 units) in the diffusive boundary layer of macroalgal thalli whereas large-scale seasonal pH fluctuations in coastal waters are much slower (weeks-months) and less strong (< 1 unit) (Wahl et al. 2016). In addition, macroalgal photosynthesis may raise the mean pH in their vicinity, an effect which increases with increasing daily photoperiod (Krause-Jensen et al. 2016).

OA may represent a threat, in particular to calcifying taxa (e.g., Orr et al. 2005; Fabry et al. 2008; Hofmann et al. 2010; Clark and Gobler 2016; Ramajo et al. 2016), and numerous studies revealed negative impacts of OA on their growth and survival (reviewed by Kroeker et al. 2010; Harvey et al. 2013). The dominance of negative effects reported in numerous OA studies (e.g., Kroeker et al. 2010; Harvey et al. 2013; Nagelkerken et al. 2016), may in part be attributable to the fact that the great majority of these investigations were conducted under non-fluctuating (and mono-factorial) treatments and in the absence of species interactions. Both, fluctuations and biotic interactions, have a substantial potential to modulate OA impacts (e.g., Boyd et al. 2016; Gunderson et al. 2016; Wahl et al. 2016). If not buffered by physiological plasticity (e.g., Hofmann and Todgham 2010), natural fluctuations of the carbonate system may either enhance OA impact such as reported for reef calcification (Shaw et al. 2012) or mitigate it as found for various calcifiers living in algal boundary layers (Hurd et al. 2011; Cornwall et al. 2013, 2014, 2015; Hurd 2015). Such apparently contradictory effects of pH fluctuations on calcification were found even within the group of bivalves where fluctuations were reported to mitigate (Frieder et al. 2014) or not to mitigate the impact of OA on larval calcification (Clark and Gobler 2016). In consequence, understanding whether and when pH fluctuations enhance or mitigate the impact of progressing acidification on calcifying biota is crucial. Apart from the immediate effect of fluctuations, sparse available evidence suggests that strong spatial and temporal variability in near-shore coastal habitats (such as large portions of the Baltic Sea) may indeed select for populations more tolerant to extreme pH situations (Melzner et al. 2009; Johannesson et al. 2011; Thomsen et al. 2013; Frieder et al. 2014; Pansch et al. 2014).

In the Baltic Sea, “anthropogenic” eutrophication in combination with warming-enhanced stratification and intense benthic remineralization promotes the occurrence of oxygen-poor, CO₂-enriched bottom waters in late summer, which regularly reaches the surface by wind-driven upwelling (Myrberg and Andrejev 2003; Melzner et al. 2013; Thomsen et al. 2013). During such upwelling events, peak *p*CO₂-values of up to 2300 μatm are common in this region,

corresponding to pH values as low as 7.4 (Thomsen et al. 2010; Saderne et al. 2013). With respect to mussels (*Mytilus edulis*), the resulting acidification stress may, however, be compensated to some degree by elevated food (phytoplankton) supply in nutrient-rich waters (Thomsen et al. 2013; Ramajo et al. 2016). Whether biogenic fluctuations of O₂ and the carbonate system driven by macrophytes also helps compensating acidification stress impacts on calcifiers, remains to be verified. Biogenic fluctuations around a potentially stressful mean will only be beneficial for calcifying organisms if the benefit of low-stress phases outweighs the harm suffered during enhanced-stress phases. Calcifiers could benefit from pH fluctuations if they were capable of maintaining overall rates by shifting acidification-sensitive processes toward time windows of high pH (Thomsen et al. 2015). Since macrophytes, when photosynthesizing, can temporarily raise Ω_{Ca} and Ω_{Ar} (saturation state of calcite and aragonite, respectively) considerably, they may create a refuge for calcifiers during daylight hours as observed in various habitats (e.g., Manzello et al. 2012; Duarte et al. 2013; Hendriks et al. 2014). The strength of the modification of local seawater carbonate chemistry by primary production depends on light regime, salinity, macrophyte abundance, and density (Binzer and Sand-Jensen 2002; Buapet et al. 2013), as well as on the residence time of the water within the macrophyte habitat (e.g., Truchot and Duhamel-Jouve 1980; Perez-Llorens et al. 2004). The potential of such fluctuating conditions to reduce acidification stress and, thus, enhance local calcification rates has been claimed repeatedly (Semesi et al. 2009; Price et al. 2012; Unsworth et al. 2012; Findlay et al. 2013; Saderne and Wahl 2013), experimental evidence, however, is scarce.

Here, we present a comprehensive study on calcification and growth of the blue mussel *M. edulis*, an ecologically and economically important calcifier in the Baltic Sea, under various pH settings (fluctuating or constant, high and low) - with or without the putatively protective neighborhood of macrophytes. In a laboratory experiment, we examined the extent of changes in seawater carbonate chemistry by photosynthetic activity of a macrophyte (the bladder wrack *F. vesiculosus*), and how this modulates OA impacts on calcification rates in *M. edulis*, during net photosynthesis (day) and net respiration (night) of the macrophyte. In a second approach, we investigated interactive effects of acidification and warming on mussel calcification in simulated mussel-macrophyte communities in the Kiel Outdoor Benthocosms (Wahl et al. 2015a). These experiments featuring controlled conditions were complemented by two field studies on a meso-scale level (10–100 m), where we (1) compared in situ growth rates of mussels in small (1–2 m diameter) patches of *Fucus*, seagrass or sand, and (2) assessed calcification rates of *M. edulis* incubated in seawater taken at different spots along a transect into a large *Fucus* bed.

We hypothesized that (1) the activity of macrophytes imposes substantial fluctuations on oxygen and carbonate system conditions in their vicinity, that (2) such macrophyte-

driven fluctuations of the carbonate system may facilitate the calcification of *M. edulis* under acidified conditions and that (3) this effect increases with macrophyte biomass to water ratio, e.g., from periphery to the center of a macrophyte habitat.

Materials and methods

An overview of the experiments, the experimental parameters modulated, their replication and all measurements taken is given in Supporting Information Table 1.

Study organisms

In the Baltic Sea, the bladder wrack *F. vesiculosus* is the most widespread canopy forming macroalga (Kautsky and Kautsky 2000; Torn et al. 2006). It provides habitat, substratum and food for a large number of invertebrates (e.g., Kautsky et al. 1992). Another important habitat-forming primary producer in the Baltic ecosystems is the eelgrass *Zostera marina*, which—like *Fucus* sp.—contributes significantly to high local biodiversity, especially due to the associated epifaunal assemblages (Bostrom and Bonsdorff 1997, 2000). The blue mussel *M. edulis* tends to dominate benthic fouling communities in the Baltic Sea (Durr and Wahl 2004; Enderlein and Wahl 2004) and is undoubtedly one of the most common calcifiers in the Baltic (Hiebenthal et al. 2013; Thomsen et al. 2013). Blue mussels often occur in close proximity to the macrophyte stands formed by *Fucus* sp. or *Z. marina* (Reusch et al. 1994; Bostrom and Bonsdorff 1997; Wikstrom and Kautsky 2007; Vinther et al. 2008). More than 95% of the shell's wet weight consist of bimineralic calcium carbonate (Yin et al. 2005) deposited as aragonite in the inner layer and calcite in the outer layer (Dalbeck et al. 2006).

Total alkalinity and pH measurements and estimation of calcification rates

Total alkalinity (TA) and pH measurements were used to estimate mussel calcification rates and derive other CO₂ system parameters, like *p*CO₂ and Ω_{Ca}. Water samples for TA were kept in polypropylene tubes (Falcon) at 4°C for 1–2 d until analysis. When longer storage was anticipated, seawater samples (50 mL) for TA measurements were poisoned with 50 μL saturated HgCl₂-solution to inhibit biological activity (following Dickson et al. 2007), and samples were stored at room temperature until analysis. TA was measured in duplicates via potentiometric titration with an automated open cell titrator (Titroline 7000, SI analytics, Germany) using 25 mL of the sample and 0.05 M HCl (precision ± 8 μmol kg⁻¹), followed by TA calculation using the Gran approximation by determining the second endpoint of the titration curve (Grasshoff 1983). The gained TA data were corrected based on repeated measurements of Dickson's certified reference material (CRM batch 103; Dickson et al. 2003). Net calcification rate (*G*) was then calculated as the difference in TA between the initial and final sample. Standardization of calcification was achieved by dividing *G* by soft tissue dry

weight (DW) of the mussel used in the experiment and by the duration (*h*) of the respective incubation. Calcification rates were calculated after the following equation, with *V* as the volume of the incubation chamber and with a water density ρ of 1.012 kg L⁻¹ (at a temperature of 15°C and a salinity of 17 PSU):

$$G \left[\frac{\mu\text{mol CaCO}_3}{\text{g DW} \times h} \right] = \frac{\frac{\Delta\text{TA}}{2} \left[\frac{\mu\text{mol}}{\text{kg}} \right] \times V [L] \times \rho_{\text{seawater}} \left[\frac{\text{kg}}{L} \right]}{\text{DW} [\text{g}] \times t_{\text{incubation}} [h]}$$

For all (potentiometric) pH measurements performed within this study, glass electrodes were calibrated with NBS-buffers (3-point calibration: pH 4, 7, 10) and results are given at the NBS scale, if not stated differently (pH values are expressed on the total scale for the macrophyte patches field survey.). Exact types of the glass electrodes used in this study are specified in the respective chapters below.

Further carbonate system parameters (*p*CO₂, Ω_{Ca}) for the laboratory experiment were calculated from TA, pH, salinity, and temperature using the CO2SYS macro (Pierrot et al. 2006 with *K*₁ and *K*₂ according to Millero et al. 2006 and KHSO₄ after Dickson 1990).

Laboratory experiment

We tested the interactive effects of the factors OA (2 levels), daytime (2 levels, i.e., light and dark) and amount of simulated density of *F. vesiculosus* (5 levels) on calcification rates of *M. edulis*.

Specimens of *F. vesiculosus* growing on cobbles and small boulders at depths of 0.3–1 m were collected in Kiel Fjord (Strande: N54°26'48" E10°11'2") on 17 October 2012. Only vegetative thalli of healthy individuals were chosen. Algae were transferred to GEOMAR, Kiel, and allowed to acclimatize to laboratory conditions for 48 h in large (39 L) tanks filled with filtered seawater from Kiel Fjord at a constant temperature of 15°C (corresponding to in situ temperature). The tanks received constant aeration (ambient *p*CO₂) and seawater flow-through (two tank volumes per 24 h). Artificial light (per aquarium: four neon tubes, Hobar T5HO 39W/10,000K) was provided with a 12-h day/night-cycle. Specimens of *M. edulis* were collected from a subtidal population in Kiel Fjord at GEOMAR pier (N54°19'48" E10°9'1") on 19 October 2012 and kept in aerated (ambient *p*CO₂) natural seawater until the start of the experiment.

Seawater from Kiel Fjord was filtered by a sand filter and pumped into two header tanks in which two different *p*CO₂ levels were established by direct aeration of the water body with compressed ambient *p*CO₂-air (ca 400 μatm, present conditions) and CO₂-enriched air (1120 μatm, prediction for 2100 under more severe scenarios in IPCC 2013), respectively, using an automatic CO₂ mixing facility (Linde Gas & HTK Hamburg, Germany; Bleich et al. 2008). Each header tank was connected in parallel to three replicate series of aquaria. In each series, five 5 L plastic aquaria were connected in-line with a height

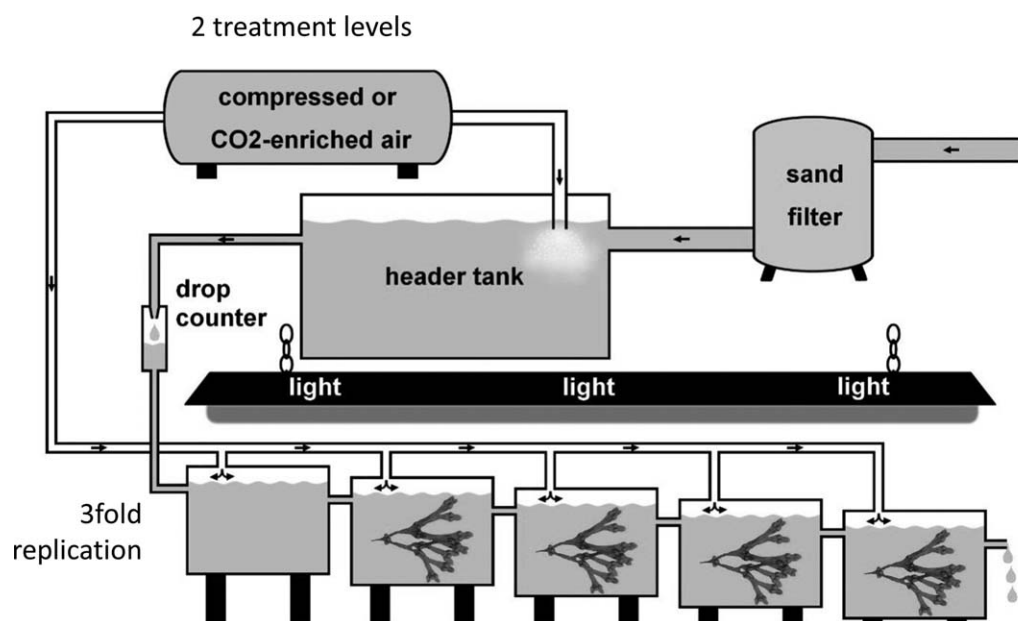


Fig. 1. Schematic illustration of the laboratory set-up. The graphic shows one header tank connected to one series of aquaria as an example for an acidification treatment (not to scale, replicates are not shown). Non-acidified units were bubbled with non-CO₂-enriched air. The pictured system was replicated thrice for each treatment level. Aquaria to the right (down-flow) represent a position closer to the center of an algal stand, aquaria to the left represent a position in the periphery.

difference of 1.2 cm between adjacent aquaria (Fig. 1) to ensure a steady directional flow (2.4 L h^{-1}) from the header tank downstream through aquaria #1 through #5. The aquaria were sealed with transparent plastic foil (permeable for photosynthetic active radiation) to minimize gas exchange with the atmosphere. The headspace between plastic covering and water surface in the aquaria was additionally aerated with ambient or CO₂-enriched air (see above). The experiment was started when the $p\text{CO}_2$ of the water in the header tanks reached equilibrium with the $p\text{CO}_2$ of the applied gas mixture and the pH had stabilized (Supporting Information Table 2).

F. vesiculosus thalli ($20 \pm 1 \text{ g}$ wet weight) were cut from the substratum and cleaned of all macroscopic epibionts the day before the experiment started. One thallus was placed into each aquarium of a series except the first. Thus, the conditioned water of the header tank was modulated by 0, 1, 2, 3, and 4 *Fucus* individuals (simulating an increasingly dense macroalgal stand or a position increasingly deep into a *F. vesiculosus* patch), from aquarium #1 to aquarium #5, respectively.

Temperature, salinity, oxygen concentration, total alkalinity (TA), and pH_{NBS} were measured before and immediately after each 12 h “daytime” treatment using a WTW Oxi 3315 analyser equipped with a WTW FDO925 optical sensor for oxygen measurements and a WTW Cond 315i salinometer with a WTW TETRACON 325 probe for temperature and salinity. Measurements of pH_{NBS} were performed using a WTW 3310 pH-meter with a WTW SenTix 81-electrode. The algae were incubated in two runs (“day”/“night”) for 12 h

each: under continuous illumination (four neon tubes, Hobar T5HO 39W/10,000K) and in the dark, respectively. At the end of each run, two 1 L plastic beakers were filled with water from each aquarium. Eight small mussels (shell length $24 \pm 3.8 \text{ mm}$ (mean \pm SD)) were placed in the first beaker containing the water from the various treatment combinations (daytime \times $p\text{CO}_2$ level \times *Fucus* density) and incubated in the pre-treated seawater for 12 h in the dark. The second plastic beaker containing the same water but no mussels served as a reference to identify changes in seawater chemistry which were not caused by the mussels. Immediately after the end of the incubation, the second seawater sample for TA was taken (see above) from the plastic beaker. During the 24 h of incubation, no food was provided. Dry weight and calcification rates of the mussels as well as further carbonate system parameters were determined as described above. Replication in this experiment was threefold.

Benthocosm experiment

Calcification of mussels from Kiel Fjord was assessed under naturally fluctuating pH and temperature conditions of different means in the Kiel Outdoor Benthocosm infrastructure (details in Wahl et al. 2015a). The benthocosm facility consists of 12 experimental units of 1500 L each. The tanks are thermally insulated and were covered by a transparent hood, which allows for control of the headspace atmosphere composition. Each sub-unit is equipped with a water pump driving an internal circulation via a wave-generator. All sub-units are monitored by a set of four

sensors (temperature, salinity, oxygen und pH_{NBS}), the data of which are continuously logged. The crossed 2-factorial approach in this experiment consisted of warming and acidification: warming by 5°C relative to the temperature control tanks (ambient temperature in Kiel Fjord) was produced by three heaters (600 watt, Schego Titan, Schemel & Goetz, Offenbach am Main, Germany) per sub-unit. Acidification was obtained by increasing the pCO_2 of the headspace atmosphere to $1100 \mu\text{atm}$. The intense mixing created by the wave-generators accelerates the air-sea gas exchange between headspace atmospheres (CO_2 treatment) and the water of the sub-units, enabling a rapid equilibration between atmospheric CO_2 and water carbonate chemistry. Both treatments caused add-ons to the natural fluctuations in temperature and pH which were driven by the season, weather, fjord hydrography, and biology with regard to the inflowing fjord water and, in addition to that, by the photosynthetic and respiratory activities of the organisms in the benthocosms.

During the experiment presented here (total duration 3 months), the community in the benthocosms consisted of bladder wrack (*F. vesiculosus*, wet weight biomass: 672 ± 103 (SE) g per 1500 L tank volume), mussels (*M. edulis*), sea stars (*Asterias rubens*), snails (*Littorina littorea*), isopods (*Idotea baltica*), amphipods (*Gammarus* spp.) and fishes (*Gasterosteus aculeatus*) in proportions typical for a bladder wrack community of the Kiel Fjord region (see Graiff et al. 2015a). Nutrients, plankton and microbes were provided by unfiltered through-flowing fjord water (ca 1.5 tank volumes per day).

In this near-natural setting of the benthocosms, calcification rates were assessed of “naïve” *M. edulis* specimen (2–3 cm long), collected at the GEOMAR pier in late June 2013. They were cleaned and placed in triplicates into each of twelve 300-mL glass jars, filled with water from one of the 12 benthocosm units, and left inside for 2–3 h for recovery and acclimatization. Subsequently, incubations were run for 3 h with 300 mL fresh water taken from the respective benthocosm unit. Before and after incubation, pH_{NBS} (Seven Multi + InLab Expert Pro, Mettler Toledo GmbH, Giessen, Germany) was measured and a 50 mL water sample was taken for TA measurements. Incubations with the same mussel individuals were conducted twice, at 15:00 h and at 09:00 h. Thus, the afternoon incubations took place in water conditioned by daytime activity (photosynthesis-dominated) and the morning incubations in water conditioned mainly by night activity (respiration-dominated) of the benthocosm community. Replication in this experiment was threefold.

Small macrophyte habitats - patches

A survey of O_2 concentration ($[\text{O}_2]$ TA, pH_T (total scale), seston and mussel growth was performed in three different components of a mosaic benthic habitat in Kiel Fjord (Kiel: $\text{N}54^\circ22'30''$, $\text{E}10^\circ9'34''$): *F. vesiculosus* (40% of mosaic patches), *Z. marina* (15%) and sand patches (45%). In each of the patches the cover by *Fucus*, *Zostera* and sand,

was $>90\%$. Five randomly arranged patches of each type with a size of $1\text{--}2 \text{ m}^2$ at a depth of $1\text{--}2 \text{ m}$ were selected for the in situ exposure of mussels. Thus, replication in this experiment was fivefold.

At the center of each of the 15 patches, 5 mussels (pseudoreplicates) in a mesh bag were attached to a float at $\sim 20 \text{ cm}$ from the bottom. Prior to deployment, a mark was set on the edge of the mussel shells with a rotary tool grinder (Dremel Multi-pro, U.S.A.). After 5 weeks of deployment (19 August 2013 to 23 September 2013), the distance between the mark and the new shell edge was measured with a Vernier calliper. We refrained from assessing a change in wet weight here, since this metric is prone to changes unrelated to growth (gut content, mantle water content, gonad maturation, etc.).

During the deployment phase, seawater sampling in the 15 patches was performed by snorkelling twice a week, at dusk (11 events, between 19:00 and 20:30 h, depending on seasonal shift of sunset) and the following dawn (10 events, between 6:00 and 7:00 h, depending on seasonal shift of sunrise). Immediately after sampling, $[\text{O}_2]$ was measured with a WTW Oximeter ProfiLineOxi 3315 equipped with a FDO 925 sensor (WTW, Germany), salinity and voltage for pH_T assessment were measured using a Mettler-Toledo (USA) SG 7/8 equipped with the InLab 731 conductivity sensor and the Expert Pro pH sensor, and temperature was recorded from the thermistor integrated in the O_2 probe. Additionally, seston (food for mussels) was determined by taking a seawater sample of 200 mL at each patch and time point, filtering it through a pre-weighed GF/F filter and drying it at 60°C until constant weight. The filtered seawater was used for TA determination.

The voltage recorded by the pH sensor in the field was converted into pH_T after Dickson et al. (2007) by comparing the voltage of the sample at the field temperature with the voltage of a homemade TRIS pH_T buffer at salinity 15 measured in the same week at GEOMAR at the same temperature, and assuming Nernstian behavior (-59 mV/pH unit) of the electrode (Saderne and Wahl 2013).

Macrophyte habitat - transects

Water samples were collected along two 10 m transects from the outer rim to the center of a *Fucus* meadow in Kiel Fjord just north of Mönkeberg marina ($\text{N}54^\circ21'22''$ $\text{E}10^\circ10'43''$) on 30 May 2013 and 31 May 2013. The weather was calm and sunny with a gentle breeze from NNE (wind speed $4\text{--}5 \text{ m s}^{-1}$) and wavelets. Both transects started 12 m (ca. 0.7 m deep) and ended 2 m (ca. 0.3 m deep) from the shore. The distance between the two replicate transects was 20 m. On both days water samples were taken at 15:00 h and at 5:00 h (i.e., around the expected maximum and minimum peaks of pH, respectively, as driven by photosynthesis and respiration in the *Fucus* dominated community). Water samples were taken with glass jars 20–30 cm above the sea

floor at each meter along the transects. Jars were opened and closed underwater at the spot of sampling. Temperature and pH_{NBS} (Mettler Toledo Seven Multi + InLab Expert Pro) were measured in the jars within 15 min after sampling.

On 07 June 2013, calcification and respiration rates of *M. edulis* (in addition to temperature and pH_{NBS}) were measured in waters from five spots along the same two transects at 15:00 h. The day was sunny, however with substantially more water movement (along-shore wind NNE, 5–10 m s^{-1}) than on the day of the previous samplings (offshore wind SE, 2–7 m s^{-1}) and water exchange in the *Fucus* stand was presumably more intense. Water samples were collected as described above and five small mussels (ca 2 cm shell length) were placed in each jar and incubated for 1 h in 450 mL water. Before and after the incubation, in each jar oxygen and pH were measured and a 50 mL water sample was taken for TA measurements just before and after the calcification period.

Data analysis and statistics

In order to compare the effect of the alga-modulated seawater on mussel calcification, the differences between day and night calcification rates were transformed to Log Effect Ratios, i.e., Log (day calcification divided by night calcification). All data were checked for normal distribution and homogeneity of variance prior to testing.

The effects of acidification, its modulation by light (day, night) and of density of *Fucus* (position) in the laboratory experiment were analyzed in a 3-way-ANOVA followed by Tukeys HSD posthoc tests. The influence of increasing simulated *Fucus* density on the proportional partitioning of calcification between day and night calcification was analyzed by linear regression, separately for acidified and non-acidified conditions.

Calcification rates in the benthocosm experiment were analyzed in a 3-way-ANOVA including the factors headspace $p\text{CO}_2$ (ambient/high), temperature (ambient, high) and daytime (day, night). Growth of mussels in the field patch experiment were analyzed by an ANOVA with one factor (“habitat”) and three levels (Sand, *Zostera*, *Fucus*).

The calcification rate of mussels at different positions in the *Fucus* transects were not analyzed since these were not sufficiently replicated (2 transects only) for an ANOVA. We plotted the trend only.

Results

Across all laboratory experiments, mussel calcification, on average, increased by 40% ($1 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ h}^{-1}$) when pH increases by half of a unit (e.g., from 7.8 to 8.3, Fig. 2, calcification rate = $2.12 * \text{pH} - 14.96$, $r^2 = 0.49$, $p < 0.0001$). In all experiments (laboratory, benthocosms, field) presented here, mean pH ranged from 7.73 (laboratory experiment, high CO_2 , without *Fucus*) to 9.03 (benthocosms, low $\text{CO}_2 \times$ low temperature, with *Fucus*), with day-night amplitudes ranging

from 0.1 (without *Fucus*) to more than 1 pH unit (inner zone of the in situ *Fucus* bed; Fig. 3). As a general trend across all experiments, mean pH and the amplitude of diurnal pH fluctuations increased with increasing *Fucus* biomass to water volume ratio (Figs. 2, 3). Additionally, in the benthocosms the diurnal amplitude of pH_{NBS} fluctuations driven by the biology of the macroalgae was larger by 24% under acidified conditions as compared to non-acidified conditions (Wilcoxon paired *t*-test, $n = 7$, $t = 3.72$, $p < 0.01$).

Laboratory experiment

The replicate sequence of the five aquaria connected in a flow through system (Fig. 1) in the laboratory experiment is henceforth referred to as aquarium #1 to #5 downstream beginning with the aquarium that contained no thallus (“aquarium #1”) and followed by four aquaria with one *Fucus* thallus each (“aquarium #2 - #5”). Both, pH and calcification varied between day and night, between low and high $p\text{CO}_2$ treatment and with upstream *Fucus* biomass (Fig. 2, Supporting Information Table 2a). After 12 h of light, pH_{NBS} increased from 7.74 ± 0.05 (mean \pm SD, aquarium #1) along the series of *Fucus* to 8.50 ± 0.05 (aquarium #5) in the high CO_2 treatment ($1120 \mu\text{atm } p\text{CO}_2$). In the ambient CO_2 treatment ($400 \mu\text{atm } p\text{CO}_2$) pH increased from 8.08 ± 0.04 (aquarium #1) to 8.54 ± 0.06 (aquarium #5) (Fig. 4). The pH_{NBS} values in the high and ambient CO_2 treatments were almost indistinguishable in aquaria #3 to #5 (Fig. 4). After 12 h of incubation in the dark, only a weak (not statistically significant) decrease in pH_{NBS} was observed along the series of *Fucus* (i.e., with increasing *Fucus* “patch size”) in both CO_2 treatments. Changes in $p\text{CO}_2$ and Ω_{Ca} (Fig. 4) mirrored, as usual, changes in pH across treatments, and undersaturation (< 1) with regard to Ω_{Ca} was only reached under high CO_2 treatments in the absence of *Fucus* (aquaria #1) and during night incubations in all high CO_2 aquaria. Remarkably, under high CO_2 in the light treatment, $p\text{CO}_2$ dropped below the aquarium #1 level of the ambient treatment already in aquarium #2 ($456 \pm 116 \mu\text{atm}$). After 12 h of “day” incubation, Ω_{Ca} increased from a slightly undersaturated level (0.97 ± 0.09) in aquarium #1 to a highly saturated level (4.71 ± 0.39) in aquarium #5, in the high CO_2 treatment. Oxygen concentration of the seawater increased under “day” conditions with increasing number of *Fucus* thalli conditioning the water, whereas measured values of the “night” incubation remained close to the start level.

Increasing *Fucus* biomass in the series of aquaria could have affected mussel calcification by two impacts on the CO_2 system: (1) under light conditions, it raised the mean pH_{NBS} (and Ω_{Ca}) from 8.1 (2.1) to 8.25 (5.5) in the ambient CO_2 treatment and from 7.7 (1.0) to 8.1 (5.2) in the high CO_2 treatment (Figs. 2, 4; aquarium #5), (2) it imposed circadian fluctuations of pH_{NBS} with amplitudes increasing from 0.14 (aquarium #1) to 0.63 (aquarium #5) in the series of non-acidified aquaria and from 0.1 (aquarium #1) to 0.9

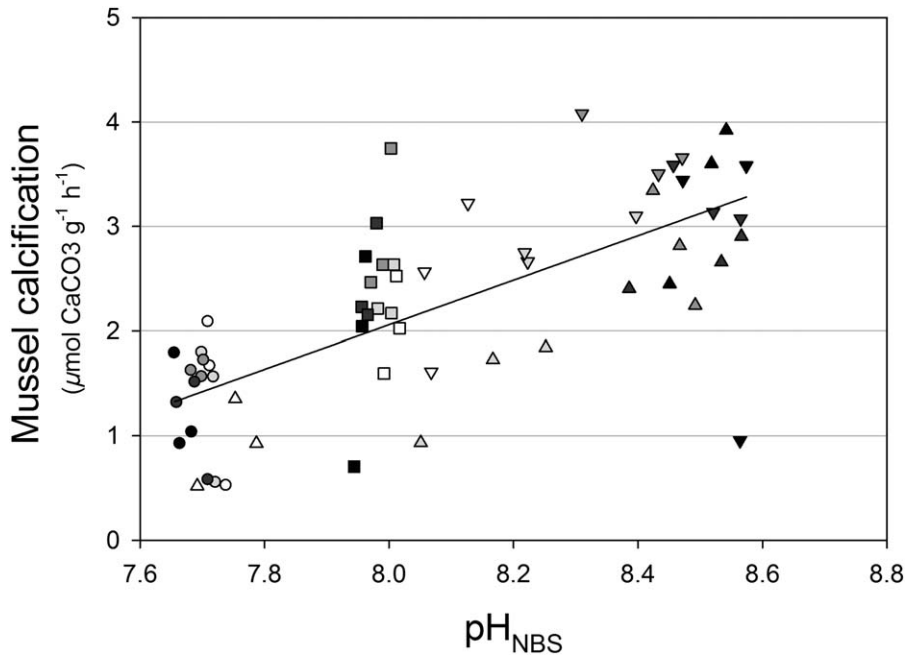


Fig. 2. Mussel calcification in response to pH_{NBS} . Represented are the calcification rates measured in the 30 laboratory aquaria subjected to the various treatment combinations (acidification \times daytime \times number of upstream *Fucus*). Triangle pointing upwards: hi pCO_2 at day time, triangles pointing downwards: lo pCO_2 at day time, dots: hi pCO_2 at night time, squares: lo pCO_2 at night time, filling: shading darkens with increasing algal biomass. Regression: $y = -15 + 2.13 \cdot x$, $R^2 = 0.49$, $p < 0.0001$).

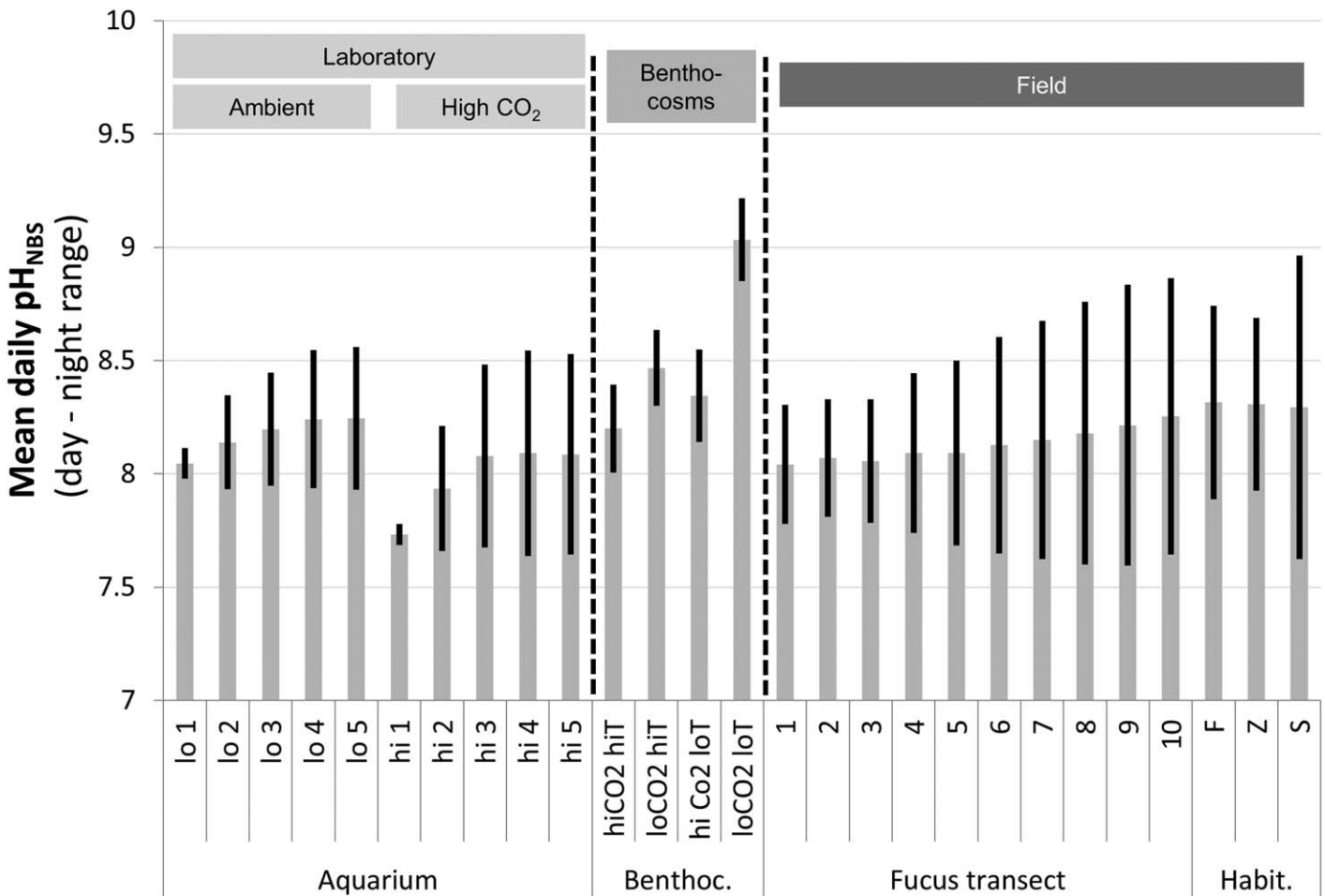


Fig. 3. pH_{NBS} conditions in the laboratory, benthocosm and field experiments. Depicted are the overall mean and the average day-night amplitude over the duration of the respective experiments (pH_{T} values measured for the habitats *Fucus* (F), *Zostera* (Z) and Sand (S) were converted to the NBS scale by adding 0.14 pH units).

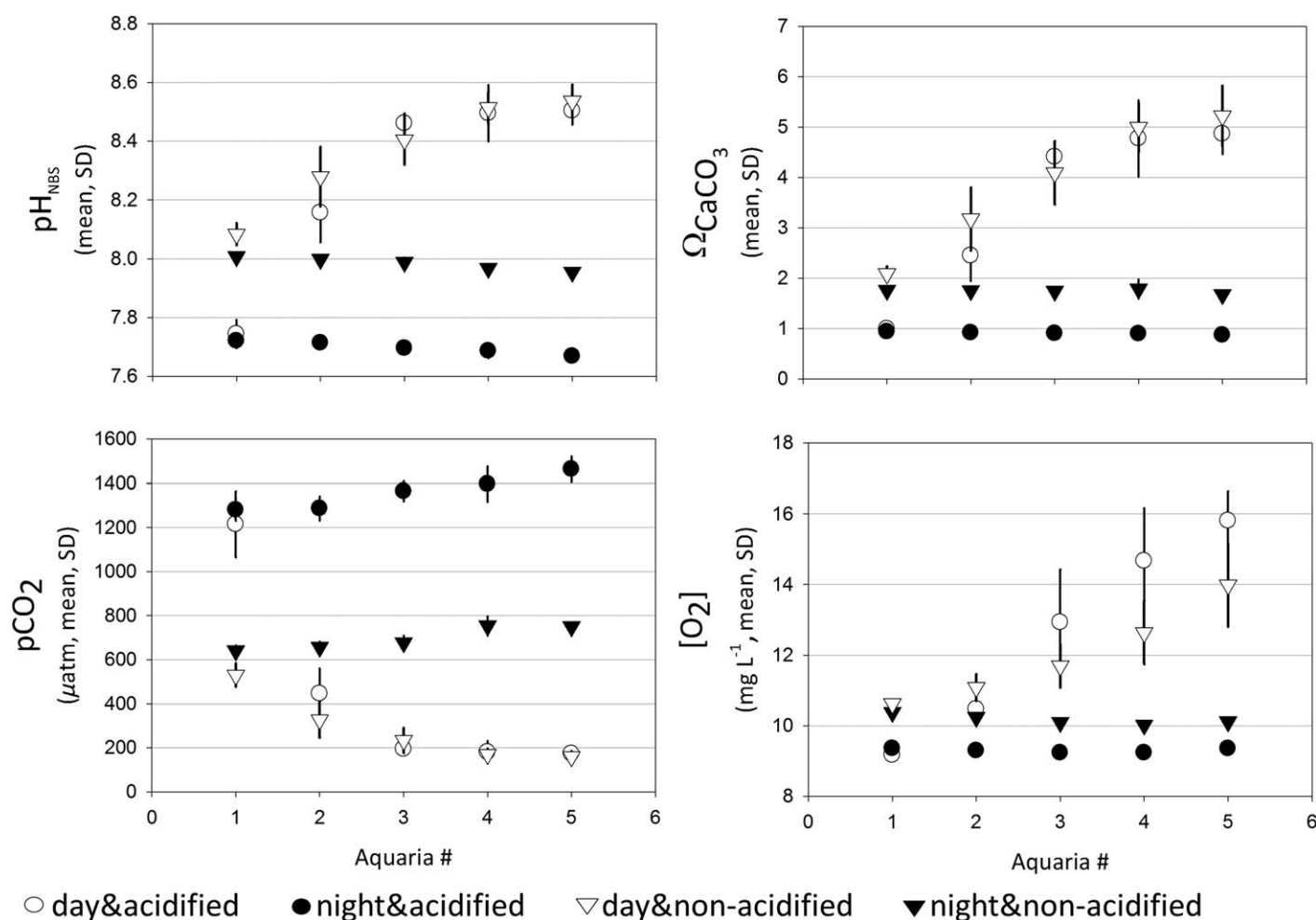


Fig. 4. Abiotic conditions regarding pH_{NBS} , pCO_2 , O_2 concentration and Ω_{Ca} in the series of aquaria subjected to acidified ($1120 \mu\text{atm pCO}_2$, dots) and non-acidified ($400 \mu\text{atm pCO}_2$, triangles) conditions during day time (light, white symbols) and night time (dark, black symbols). The serial aquaria at positions 2 through 5 contained one *Fucus* each so that the water in these aquaria were conditioned by 1, 2, 3, and 4 bladder wrack individuals, respectively, simulating a transect from outside into a *Fucus* stand.

(aquarium #5) in the acidified aquaria (Fig. 3). Mussel calcification was significantly lower under night conditions and under acidified conditions (Fig. 5; Table 1, Supporting Information Tables 2b, 3). At ambient conditions, calcification tended to peak at intermediate *Fucus* density (Fig. 5A), but this relation was not significant (polynomial regression; day: adj $R^2 = 0.64$, $p > 0.05$; night: adj. $R^2 = 0.73$, $p > 0.05$). At acidified conditions, in contrast, calcification increased linearly and significantly (linear regression, $R^2 = 0.91$, $p < 0.05$) with increasing *Fucus* density at daytime but not at nighttime conditions (Fig. 5B). Likewise, the cumulated 24 h-calcification increased significantly with *Fucus* density (Fig. 5B). The presence of *Fucus* entirely compensated the impact of acidification from aquarium #3 onwards (overlapping CIs in Fig. 5, Supporting Information Table 3). Furthermore, time of day and the number of upstream *Fucus* interacted

significantly as compensation of the acidification was mainly visible during day time: In the high CO_2 treatment of the “day” incubation, net calcification increased significantly ca 3.5-fold from $0.93 \pm 0.42 \mu\text{mol CaCO}_3 \text{ gDW}^{-1} \text{ h}^{-1}$ in aquarium #1 to $3.32 \pm 0.77 \mu\text{mol CaCO}_3 \text{ gDW}^{-1} \text{ h}^{-1}$ in aquarium #5. During “night” incubation, net calcification was generally low, independent of the downstream position. Again, from aquarium #3 onwards, day calcification was significantly larger than night calcification under acidified conditions (non-overlap of CIs in Fig. 5B) whereas under non-acidified conditions only in aquarium #4 this difference was significant. Differences in calcification rates between day and night (displayed as Log Effect Ratios in Fig. 6) were larger in the high CO_2 treatment than under ambient CO_2 conditions in all but the first two positions and this difference increased along the series of increasing *Fucus* biomass substantially

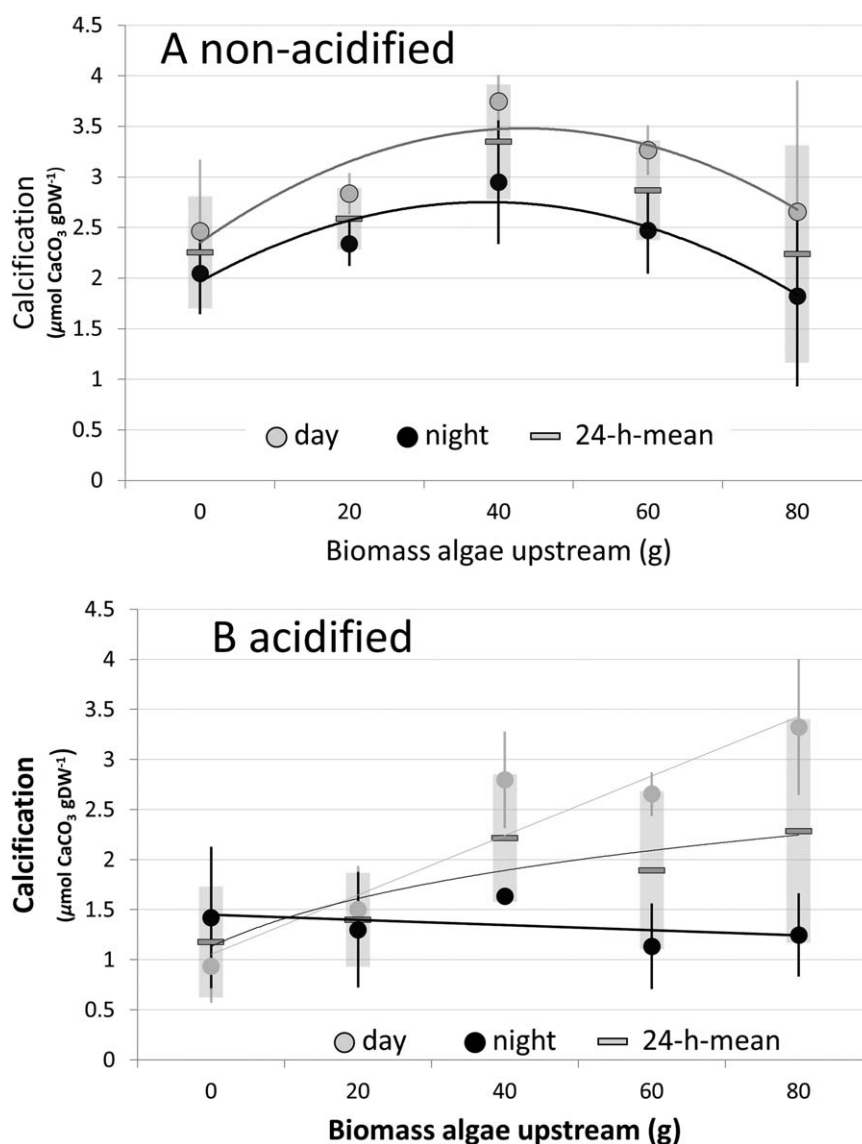


Fig. 5. Mussel calcification (means \pm CI) under non-acidified (**A**) and acidified (**B**) conditions during daytime (light gray dots and CIs), night time (black dots and CIs) and as daily mean (dark gray bars and transparent gray CIs). The serial aquaria at positions 2 through 5 contained one *Fucus* each so that the water in these aquaria were conditioned by 1, 2, 3, and 4 bladder wrack individuals, respectively, simulating a transect from outside into a *Fucus* stand. Regressions: Non-acidified day (panel **A**, gray line, $y = 2.34 + 0.052x - 0.0006x^2$, $R^2 = 0.76$, $p = 0.1$), non-acidified night (panel **A**, black line, $y = 1.965 + 0.41x - 0.0005x^2$, $R^2 = 0.86$, $p = 0.07$), acidified day (panel **B**, gray line, $y = 1.055 + 0.03x$, $R^2 = 0.9$, $p < 0.01$), acidified night (panel **B**, black line, $y = 1.45 - 0.003x$, $R^2 = 0.186$, $p = 0.47$), acidified 24-h-mean (panel **B**, dashed dark gray line, $y = -0.0135x + 1.253$, $R^2 = 0.76$, $p = 0.054$).

faster under acidified (slope of 0.15) than under non-acidified conditions (slope of 0.022) ($p < 0.05$, Fig. 6, Supporting Information Table 4).

Benthocosm experiment

During the Benthocosm experiment in June 2013 (details in Wahl et al. 2015a,b, monitored system data in Pangaea doi: 10.1594/PANGAEA.842739), temperature in the warm treatments ranged between 17°C and 23°C with typical day-night amplitudes of approximately 2°C (Supporting Information Fig. 1a). In the cold treatment temperature ranged between 12°C

and 17°C, again with day-night amplitudes of about 2°C. Under the CO₂ enriched headspace, water pH_{NBS} was consistently lower by 0.1 to 0.5 pH_{NBS} units compared to the ambient CO₂ treatment (Supporting Information Fig. 1b). The day-night amplitude of the pH_{NBS} fluctuations driven by photosynthesis-respiration cycles was larger under acidified (~ 0.6 pH_{NBS} units) than under non-acidified conditions (~ 0.4 pH_{NBS} units). Calcification (Fig. 7; Table 2) was significantly reduced by about 40% in the acidification treatment. In addition, calcification was significantly reduced by about 60% during the night as compared to daytime calcification rates.

Table 1. Three-factorial ANOVA on mussel calcification in the lab experiment in response to three factors, i.e., acidification level (“ $p\text{CO}_2$ ”, acidified, non-acidified), daytime (day, night) and position in the simulated transect into a *Fucus* belt (“Aqu#”1 through 5). All three factors affect calcification significantly with a significant interaction between daytime and position. Effects with p -values in bold are significant.

Effect	SS	Df	MS	F	p
Intercept	297.5272	1	297.5272	739.9119	0.000000
Day,night	9.1650	1	9.1650	22.7923	0.000024
$p\text{CO}_2$	11.2407	1	11.2407	27.9541	0.000005
Aqu #	7.7763	4	1.9441	4.8347	0.002822
Day,night* $p\text{CO}_2$	0.1915	1	0.1915	0.4763	0.494078
Day,night*Aqu #	4.4747	4	1.1187	2.7820	0.039503
$p\text{CO}_2$ *Aqu #	3.1959	4	0.7990	1.9869	0.115036
Day,night* $p\text{CO}_2$ *Aqu #	2.1329	4	0.5332	1.3261	0.276929
Error	16.0845	40	0.4021		

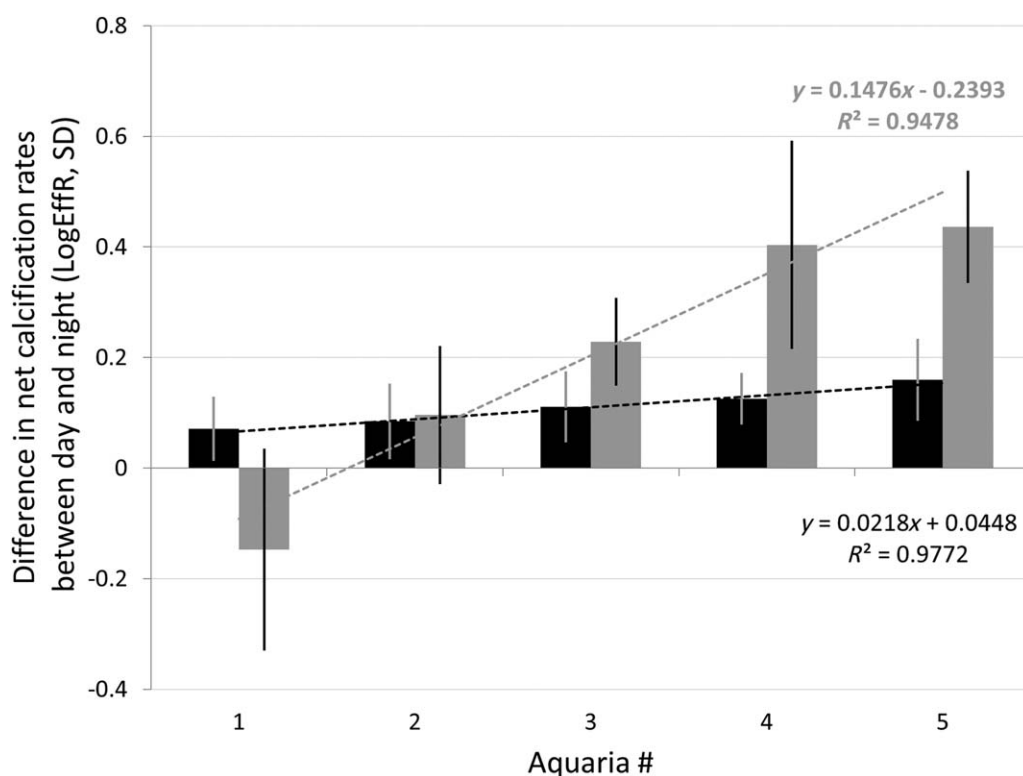


Fig. 6. Differences between calcification rates in day vs. night time under acidified (gray bars) and non-acidified (black bars) conditions. The ratio of day-to-night calcification is expressed as log response ration where a value of 1 means that calcification is 10 times stronger during daytime than during nighttime, a value of -1 means that the former is 10 times weaker. Under non-acidified conditions the increasing *Fucus* biomass does not affect the day-to-night ratio substantially. In contrast, under acidified conditions daytime calcification becomes increasingly important with increasing *Fucus* biomass.

Warming did not affect calcification and the three factors ($p\text{CO}_2$, temperature, day vs. night) did not significantly interact.

Field study

Macrophyte patches

The three habitat types (*Fucus* patches, *Zostera* patches, sand patches) turned out to be very similar considering seston, temperature, salinity, as well as with regard to daily mean and

day-night fluctuations of oxygen and pH (Fig. 8, Supporting Information Fig. 2). While temperature, salinity, and seston did not exhibit substantial day-night fluctuations, oxygen and pH were consistently higher during the day as compared to the night in all three habitat types. During the experimental run of 35 d, mussel length (mean, SD) increased by 2.2 ± 0.4 mm ($6.6\% \pm 0.7\%$) in the *Zostera* patches, by 2.8 ± 0.9 mm ($8.3\% \pm 1.0\%$) in the sand patches and by 2.6 ± 0.7 mm

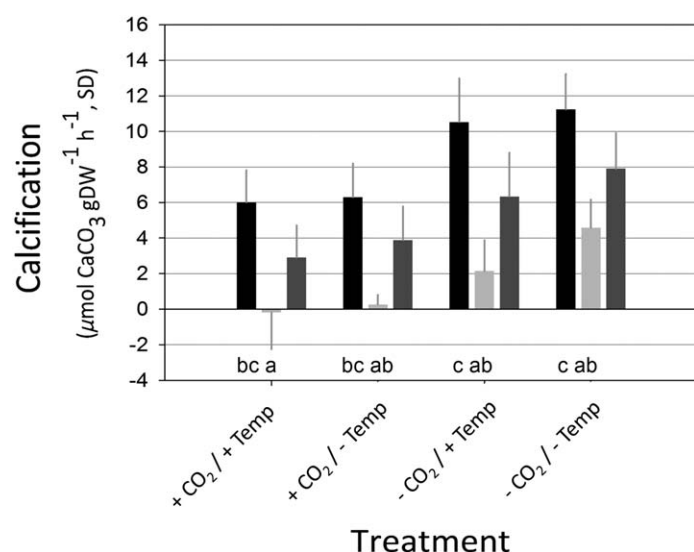


Fig. 7. Calcification rates in the benthocosms during daytime (black), night time (light gray) and averaged over 24 h (dark gray). Day and night calcification not sharing the same letters are significantly different. Error bars represent SD.

(7.7% ± 0.9%) in the *Fucus* patches (Fig. 8B). There was no significant effect of habitat type on mussel growth (ANOVA, $df = 2$, $F = 0.80$, $p = 0.475$).

Macrophyte transect

On the calm and sunny day along the transect from the outer rim (position #1) to the inner zone (position #9) of the *Fucus* belt, strong and opposite pH gradients were found during the day and night samplings, respectively. During day, pH_{NBS} increased from 8.30 outside to 8.83 10 m inside the *Fucus* belt (Fig. 9). During night, pH_{NBS} decreased from pH_{NBS} 7.78 outside to pH_{NBS} 7.59 10 m inside the belt. The amplitude of day-night pH_{NBS} fluctuations was 0.5 pH_{NBS} units outside the *Fucus* habitat, while in the inner part of the belt the pH_{NBS} reached an amplitude of 1.2 units. At the end of a sunny day, $[O_2]$ increased to 12 mg L⁻¹ at the outer edge and to 15 mg L⁻¹ in the inner part of the belt. After a calm night, $[O_2]$ had decreased to about 9 mg L⁻¹ (outside the belt) and to 6.5 mg L⁻¹ (at the inner part of the belt).

On the windy day of calcification measurements, the gradients in daylight pH_{NBS} along the transect were substantially less steep (Fig. 9). Nonetheless, changes in pH_{NBS} along the transect explained 52% of the variation in calcification rates but, due to large variation, this trend is not significant (Fig. 10; $df = 5$, $F = 3.27$, $p > 0.05$).

Discussion

Fluctuations in environmental variables have recently been suggested to alter the magnitude and/or direction of the effects caused by superimposed long-term changes (e.g., Cornwall et al. 2014; Gunderson et al. 2016; Wahl et al.

Table 2. Three-factorial ANOVA on mussel calcification in the benthocosm experiment with the factors acidification (“ pCO_2 ,” acidified, non-acidified), warming (“ T° ,” ambient, warmed by 5°C) and daytime (“night-day,” day, night). Acidification and daytime affect calcification significantly, without any interaction among the three factors. Effects with p -values in bold are significant.

Effect	SS	df	MS	F	p
Intercept	230517.7	1	230517.7	159.4726	<0.001
pCO_2	35803.1	1	35803.1	24.7687	0.000166
T°	2083.5	1	2083.5	1.4414	0.248531
Night-day	102346.7	1	102346.7	70.8037	<0.001
$pCO_2 * T^\circ$	785.2	1	785.2	0.5432	0.472484
$pCO_2 * \text{night-day}$	1113.4	1	1113.4	0.7703	0.393975
$T^\circ * \text{night-day}$	514.1	1	514.1	0.3557	0.559812
$pCO_2 * T^\circ * \text{night-day}$	342.8	1	342.8	0.2372	0.633295
Error	21682.5	15	1445.5		

2016). It is important to distinguish in this context between extreme events and fluctuations. The former are sporadic disturbances (e.g., Wernberg et al. 2013) which may have ecosystem-wide effects because they are unlikely to allow for acclimation or adaptation. The latter rather represent recurrent excursions from the mean with alternating phases of high stress and recovery which may alter global change effects at the organismic scale (e.g., Gunderson et al. 2016) and in the long run facilitate a “hardening” of populations (e.g., Pansch et al. 2014). The main goal of the investigations presented here was to investigate macrophyte-driven biogenic fluctuations of the seawater carbonate system at different scales, and to determine their influence on calcification rates and growth of the blue mussel *M. edulis*. We show that the diurnal photosynthesis/respiration cycles of macrophytes produce fluctuations of the carbonate system that, at least at small scales (dm to m), facilitated calcification even under acidified conditions, particularly so during daytime. The concurrently fluctuating $[O_2]$ may have contributed to beneficial daytime conditions since anti-stress mechanisms are supposed to be energy- and, thus, O_2 -demanding (e.g., Ramajo et al. 2016) but this effect was not in focus of this study.

Macrophytes modulate seawater carbonate chemistry

The metabolism (photosynthesis and respiration) of macrophytes cause diurnal DIC fluctuations together with fluctuations of other carbonate system parameters (e.g., pCO_2 , pH) in the surrounding seawater. Day-night amplitudes of such fluctuations may exceed one pH unit in dense macrophyte stands (Middelboe and Hansen 2007; Pajusalu et al. 2013; Saderne and Wahl 2013; Hurd 2015; all experiments here as shown in Fig. 3) but are strongly scale-dependent (Wahl et al. 2016 and references therein). The amplitude of biogenic diurnal pH fluctuations increases with algal biomass as evidenced along the aquaria series and along the in situ

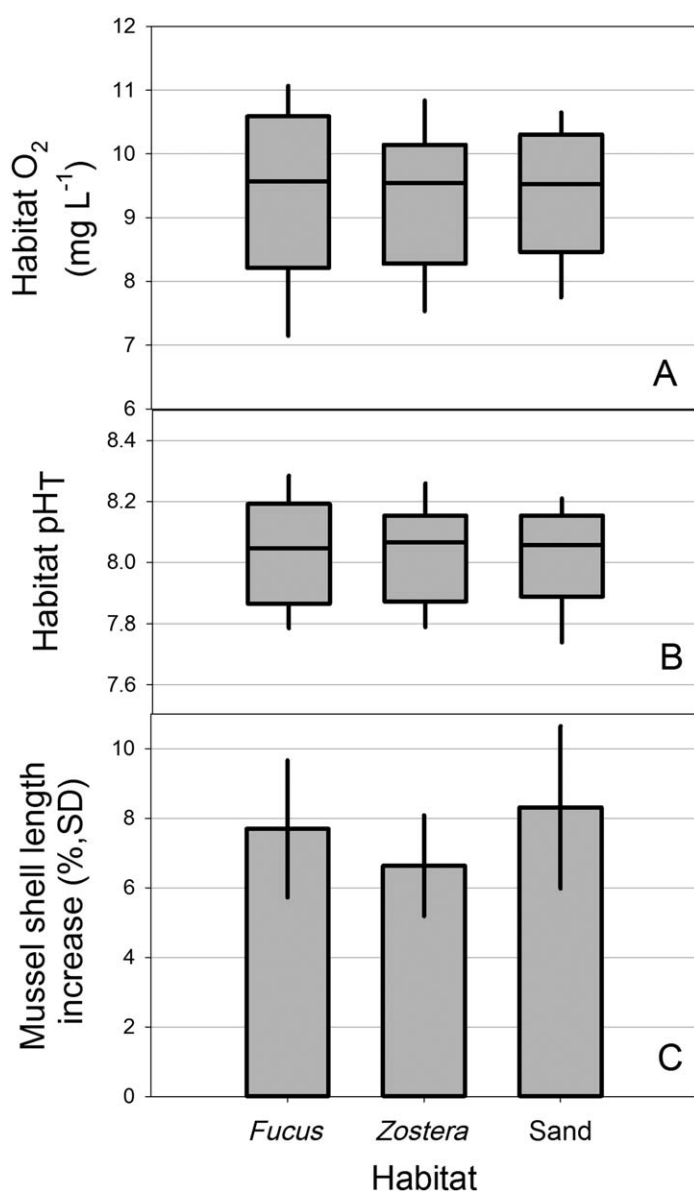


Fig. 8. Oxygen (A) and pH_T (B) conditions in the three microhabitats (as median, 25–75 percentiles, and 10–90 percentiles). The *Fucus*, *Zostera* and sand patches did not differ significantly in mean values but the *Fucus* habitats tends to vary most. Mussel growth (C) expressed as the increase in shell length (+SD) over the duration of the in situ experiment. Growth of mussel did not differ among the microhabitats.

transect into a *Fucus* belt (Fig. 3). We here demonstrate that biogenic activity may impact the carbonate system stronger than predicted future increase in pCO_2 means: Both in the laboratory tanks and in the benthocosm experiment, the amplitude of biogenic day-night fluctuations exceeded the (simulated) shift in seawater pH predicted for the end of the century (Fig. 3, Supporting Information Fig. 1b). The laboratory experiment illustrated how the pH drop caused by elevated pCO_2 levels was completely masked by only two *Fucus* individuals upstream of the mussel (i.e., in aquarium #3,

Fig. 4). Photosynthesis/respiration cycles of the macroalgae also incurred diurnal fluctuations in $[O_2]$ (e.g., by 7 mg L^{-1} in the large *Fucus* belt, Fig. 9) and an overall slight shift in mean pH (e.g., by 0.2 units along the aquaria series, Fig. 3). At this point, our data do not permit to clearly disentangle the impact of these aspects of macroalgal activity on mussel calcification.

Macrophytes may offer daytime refuge from OA

High levels of seawater pCO_2 associated with decreased pH can reduce mussel calcification (Gazeau et al. 2013; Thomsen et al. 2015 and Fig. 2). Reduced calcification may entail slower growth and/or higher predation risk jeopardizing mussel survival (Enderlein et al. 2003; Thomsen et al. 2013) if they do not adapt in time to the predicted acidification. When considering the results it should be noted that the experiments were too short to allow for any adaptation on the side of the mussels. Other recent transgenerational experiments suggest, however, that such an adaptation to OA could require numerous generations in *M. edulis* (Thomsen et al. 2017). In the present study, a decrease of the mean pH by half a unit (caused by a pCO_2 increase to $1120 \mu\text{atm}$, as predicted for the end of the century in some scenarios [IPCC 2013]), reduced mussel calcification by about 40% (Fig. 2). This effect was, however, partly mitigated by biogenic pH modulation in the system. In all of our experiments, from the laboratory to the field, the presence and activity of macrophytes increased the overall *mean* pH by 0.01 to 0.2 units (Figs. 3,4,9,10), which per se, should be beneficial to mean calcification of mussels (Gazeau et al. 2013; Thomsen et al. 2015) and other organisms (Kroeker et al. 2010; Cyronak et al. 2016). Similar beneficial effects by primary producers were reported by some tropical studies showing that photosynthesizing macrophytes could mitigate the negative impact of OA on marine calcifiers such as corals at the habitat scale (Kleypas et al. 2011; Manzello et al. 2012; Unsworth et al. 2012; Jokiel et al. 2014). In addition to the increase of the mean pH, the substantial diurnal fluctuations in pH, pCO_2 , and Ω_{Ca} represented phases favorable (daytime) or adverse (nighttime) to calcification. The mussels proved capable of benefitting from these temporal refuges from acidification stress and maintained high calcification by shifting a major portion of this process to the photosynthesis dominated periods (i.e., daytime: Fig. 6). Thus, the presence of *Fucus* enhanced mussel calcification, especially so under OA conditions, twofold to threefold (laboratory experiment, Fig. 5). Based on the numerical relation between pH and calcification rates (Fig. 2) the rise of *mean* pH_{NBS} along the series of aquaria by 0.35 pH_{NBS} units should increase calcification by $0.74 \mu\text{mol CaCO}_3 \text{ g DW}^{-1} \text{ h}^{-1}$. Instead it increased by $1.1 \mu\text{mol CaCO}_3 \text{ g DW}^{-1} \text{ h}^{-1}$ from aquarium 1 to aquarium 5. These additional 49% of calcification should be attributable to the biogenic fluctuations and the conspicuous increase of calcification under favorable

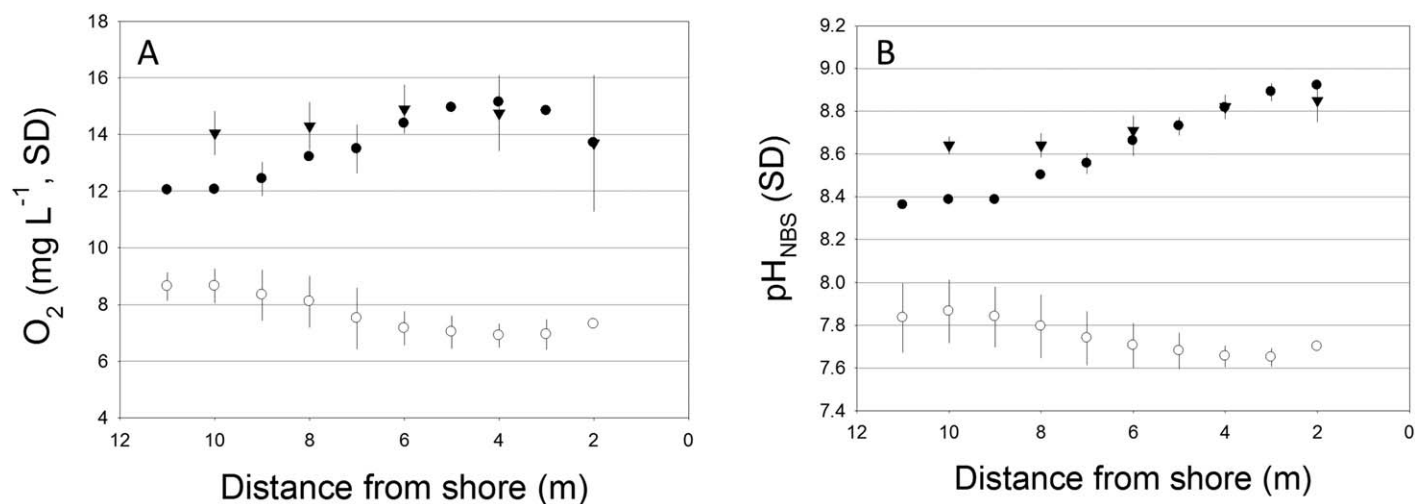


Fig. 9. Oxygen (A) and pH_{NBS} (B) profile through a *Fucus* belt from outside (12 m offshore) to deep into the belt (2 m offshore) under calm conditions daytime = black dots, night-time = white dots) and on a windy day (black triangles).

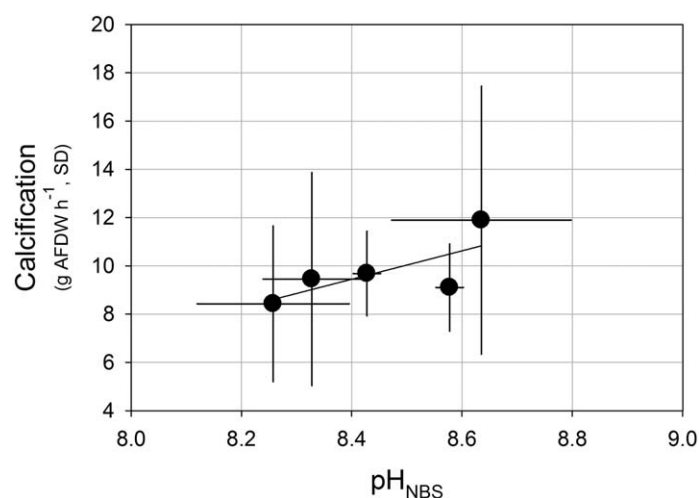


Fig. 10. Calcification of mussels under different pH_{NBS} conditions along the transect into a *Fucus* belt.

conditions produced by photosynthesis during daytime – especially so under acidification (Fig. 6). Under ambient $p\text{CO}_2$ conditions and in the absence of macrophyte-induced pH fluctuations, mussels calcified at similar rates during day- and night-time (laboratory experiment, Fig. 5), indicating that *M. edulis* does not feature an internal circadian rhythm in calcification. Why mussel calcification under non-acidified conditions peaked at intermediate algal density cannot be explained at present.

In a review article, Hofmann and Todgham (2010) emphasize the importance of physiological plasticity as a strategy of marine organisms to cope with changing environmental conditions such as acidification and heat stress. Regarding calcification, “physiological plasticity” means a reallocation of resources needed for biomineralization. This

should be facilitated if the available resources, e.g., food, are abundant as described for mussels in a eutrophied habitat (Thomsen et al. 2013). The already prevalent and in some regions still progressing nutrient enrichment in many coastal areas may increase phytoplankton density, thus mitigate the impact of ongoing acidification for plankton-feeding calcifiers (Ramajo et al. 2016). However, intensified plankton blooms may, in the course of sedimentation and remineralization, also lead to hypoxia of deeper water, which, when upwelled, may stress organisms by low [O₂], high $p\text{CO}_2$ and H₂S and low pH conditions (Melzner et al. 2013). Here, we did not quantify day-time vs. night-time concentrations of plankton since fluctuations at this high frequency are likely to be buffered by the mussel’s energy storage capacity. In the field, seston concentrations were similar in all microhabitats (Supporting Information Fig. 1).

Beneficial effects of macrophytes on calcifiers were described before. Semesi et al. (2009) demonstrated for the calcifying algae *Hydrolithon* sp., *Mesophyllum* sp. and *Halimeda* sp. that calcification was favored by the vicinity of seagrass driving substantial diurnal pH fluctuations with amplitudes of up to 1 pH unit. Also, the coralline alga *Arthrocardia corymbosa* calcified more and grew better at OA conditions when a thick diffusive boundary layer protected the alga’s surface at low-flow conditions, likely by enhancing daytime pH (Cornwall et al. 2014). In an acidification experiment, Saderne and Wahl (2013) showed that calcifying epibionts located in the thin (< 1 mm) diffusive boundary layer sheeting *Fucus serratus* thalli which displays strong diurnal pH and [O₂] fluctuations (e.g., Hurd 2015; Wahl et al. 2016), maintain growth and calcification even under high levels of $p\text{CO}_2$ (1200 μatm). In contrast to our observation, Cornwall et al. (2013) describe how pH fluctuations reduce growth and recruitment of a coralline alga both under acidified and

non-acidified conditions. In the Cornwall study, however, the pH changes were chemically (not biologically) induced and instantaneous (instead of gradual) and may not be comparable to the more sinusoidal fluctuations of pH (and $p\text{CO}_2$, $[\text{O}_2]$, Ω) imposed by biological processes in the field.

Macrophyte density and hydrodynamics mediate the biogenic refuge

In the relatively large *Fucus* belt (ca 20 m wide in an onshore-offshore direction and 200 m along shore), on calm and sunny days (field study, Fig. 9), biogenic fluctuations in pH were similar to or slightly larger than those found in the laboratory experiment (aquarium #5; Fig. 4) and in the benthocosm system (Supporting Information Fig. 1b). This is a result of the high biomass to seawater ratio, typical of shallow macroalgal habitats (Bjork et al. 2004; Middelboe and Hansen 2007; Pajusalu et al. 2013) in the absence of strong water movement. Fluctuations just outside the *Fucus* belt offshore were presumably caused by plankton activity mainly, whereas inside the *Fucus* habitat, both plankton and macrophytes contributed to the diurnal fluctuations. Overall, differences in pH between day and night ranged from 0.3 to 1.2 pH units (Fig. 3). This amplitude is similar to findings in field studies of Middelboe and Hansen (2007) and Pajusalu et al. (2013) who described diurnal amplitudes of about 1 pH unit for *Fucus* dominated macroalgal fields of the Baltic Sea, but higher than those of Saderne and Wahl (2013) who reported a mean diurnal amplitude in pH of 0.3 in a Baltic *Fucus* belt. Vertical gradients in pH already occur within the upper centimetres in a shallow macroalgae-dominated habitat (Middelboe and Hansen 2007; Krause-Jensen et al. 2015). Our study complements these observations by adding the factor of a horizontal pH gradient at the scale of meters (field study, Fig. 9). Comparing the $[\text{O}_2]$ and pH gradient into the belt on a calm (water movement estimated at 1 cm/s) and on a more windy day (water movement estimated at 3–5 cm/s, Fig. 9), illustrates how much the amplitude of the biogenic fluctuations depend on the biomass to water volume ratio. Such flow velocity dependency of a biogenic pH gradient was shown at a smaller scale, i.e., in the diffusive boundary layer of macrophytes, by Cornwall et al. (2014) and Wahl et al. (2016). In the small habitat patches of *Zostera* and *Fucus* ($\sim 1\text{--}2\text{ m}^2$), we did not detect a significant impact of the macrophytes on the carbonate chemistry (field study; Fig. 8A) and, consequently, with regard to mussel growth (field study; Fig. 8B). It is likely that the selected habitats were too small and the biomass to water volume ratio too little to substantially alter the carbonate system of the surrounding seawater. The high load of seston (i.e., mussel food; $\sim 8\text{ mg L}^{-1}$) during this field experiment could have contributed to the uniformly good growth of mussels in all habitat patches (Thomsen et al. 2013; Ramajo et al. 2016). Similarly to our findings, Middelboe and Hansen (2007) and Pajusalu et al. (2013) reported that fluctuations in CO_2 and

pH are less pronounced in a patchy habitat than in densely covered macrophyte meadows. Thus, both laboratory and field experiment illustrate how biogenic mitigation of acidification can be important at the m-scale while depending strongly on the macrophyte biomass to water volume ratio, i.e., water exchange in the macrophyte habitat.

Interacting effects of OA and warming

The human-induced shift in $p\text{CO}_2$ and oceanic mean pH is often superimposed by warming effects – the other (first known) side of the CO_2 problem (IPCC 2013). While the CO_2 treatment of the benthocosm experiment impacted calcification rates of *M. edulis*, temperature had no effect, neither alone nor in combination with the CO_2 treatment. Long-term experiments on *M. edulis* revealed that growth was not significantly affected by temperatures below 25°C and below seawater $p\text{CO}_2$ of $1600\ \mu\text{atm}$ in combined acidification and warming treatments (Hiebenthal et al. 2013; Keppel et al. 2015). In other studies, however, exposure to warming substantially reduced shell strength of the mussels (Mackenzie et al. 2014).

Macrophytes can increase their metabolic activity under elevated levels of CO_2 (Fig. 4; Falkenberg et al. 2013; Graiff et al. 2015a) but may suffer severely from ocean warming when passing a certain threshold temperature (Graiff et al. 2016). The performance of macrophytes under the combination of acidification, warming and eutrophication—probable future scenarios for many coastal regions—may be severely jeopardized by filamentous epiphytes thriving under these conditions (Werner et al. 2016a,b) and depriving their *Fucus* host of light and nutrients (Wahl 2008). Macrophyte decline would certainly reduce the calcification window of mussels. It seems likely, that the favorable effect of *Fucus* for mussel calcification and future acidification may be important in all seasons except summer when macroalgae tend to be increasingly stressed by warm temperatures and proliferating epiphytes (Werner and Matthiessen 2013; Graiff et al. 2015b).

Conclusions

The common macroalga *F. vesiculosus* has the capacity to raise mean pH in its vicinity and to provoke strong diurnal fluctuation of $[\text{O}_2]$, $p\text{CO}_2$, pH and Ω_{Ca} driven by its photosynthesis/respiration cycle. The calcification of the mussel *M. edulis* decreases with pH (and associated shifts in the carbonate system). Acidification impacts on mussel calcification may be neutralized by the alga-driven shifts and fluctuations of the carbonate system. Both traits of the carbonate system shifts, increased mean pH and enhanced amplitude of diurnal pH fluctuations contribute to the enhancement of calcification. The strength of this beneficial effect, however, relates to the ratio of algal biomass to water volume and in our series of experiments decreases from the laboratory over the benthocosms to the field.

Thus, *Mytilus*—and likely other calcifiers—can temporarily find refuge from future OA by increased mean pH and

pH variability already a few meters into a macrophyte belt. This ecological refuge offered by macrophyte habitats may, however, change in the course of future multifactorial change in two contrasting scenarios (see Graiff et al. 2015a; Werner et al. 2016a,b): (1) On-going CO₂ increase and progressing eutrophication in many coastal areas (but see Andersen et al. 2017 for recent signs of trend reversal in the Baltic Sea) fertilize macroalgae (Gutow et al. 2014; Graiff et al. 2015a,b), increase the biomass to water ratio at a local scale and, thus, benefit calcifiers (but see Britton et al. 2016). At the same time, eutrophication may lead to denser plankton. This may have two contrasting effects on the OA-macrophyte-calcifier interactions. The shading by plankton may reduce the beneficial effect on calcifiers of the macrophyte-driven diel fluctuations of pH and the slight rise of its mean. At the same time plankton blooms may indirectly enhance the prevalence of stressful hypoxic conditions in upwelling waters. On the other hand, the increased supply of suspended food renders calcifying filter feeders more robust to OA (e.g., Thomsen et al. 2013; Ramajo et al. 2016). (2) If *Fucus* benefits less from pCO₂ and nutrient increase than its epiphytes and/or consumption by mesograzers is strongly enhanced by ocean warming (e.g., Werner et al. 2016b) macroalgal biomass may decrease and progressive OA would continuously narrow the time window in which calcification by mussels is still possible. This capacity of macroalgae to mitigate stress such as hypoxia and low pH associated with summer upwelling events (Melzner et al. 2013), should be a further motivation for the protection of coastal macroalgal habitats which are increasingly endangered by various anthropogenic factors such as coastal construction, dredging, harvesting or eutrophication (Wahl et al. 2015b).

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Conflict of Interest

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