LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 9999, 2020, 1–14

doi: 10.1002/lno.11669

Growth response of calcifying marine epibionts to biogenic pH fluctuations and global ocean acidification scenarios

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Abstract

In coastal marine environments, physical and biological forces can cause dynamic pH fluctuations from microscale (diffusive boundary layer [DBL]) up to ecosystem-scale (benthic boundary layer [BBL]). In the face of ocean acidification (OA), such natural pH variations may modulate an organism's response to OA by providing temporal refugia. We investigated the effect of pH fluctuations, generated by the brown alga *Fucus serratus*' biological activity, on the calcifying epibionts *Balanus improvisus* and *Electra pilosa* under OA. For this, both epibionts were grown on inactive and biologically active surfaces and exposed to (1) constant pH scenarios under ambient (pH 8.1) or OA conditions (pH 7.7), or (2) oscillating pH scenarios mimicking BBL conditions at ambient (pH 7.7–8.6) or OA scenarios (pH 7.4–8.2). Furthermore, all treatment combinations were tested at 10°C and 15°C. Against our expectations, OA treatments did not affect epibiont growth under constant or fluctuating (BBL) pH conditions, indicating rather high robustness against predicted OA scenarios. Furthermore, epibiont growth was hampered and not fostered on active surfaces (fluctuating DBL conditions), indicating that fluctuating pH conditions of the DBL with elevated daytime pH do not necessarily provide temporal refugia from OA. In contrast, results indicate that factors other than pH may play larger roles for epibiont growth on macrophytes (e.g., surface characteristics, macrophyte antifouling defense, or dynamics of oxygen and nutrient concentrations). Warming enhanced epibiont growth rates significantly, independently of OA, indicating no synergistic effects of pH treatments and temperature within their natural temperature range.

Coastal and shallow subtidal zones are naturally characterized by large diurnal environmental fluctuations in factors such as temperature, light intensity, pH conditions, and oxygen concentrations (Feely et al. 2010; Waldbusser and Salisbury 2014). In these habitats, fluctuations in carbonate chemistry may therefore largely exceed acidification levels projected for the open ocean by the end of this century (Duarte et al. 2013). Extensive research on the impacts of ocean acidification (OA) on marine organisms yielded valuable insights into physiological responses of species, and how these responses may translate into its ecosystem-wide impacts (Doney et al. 2009; Havenhand 2012). Recently, studies started to appreciate natural environmental pH variability and its role in modulating organism responses to OA (Wahl et al. 2015; Hoshijima and Hofmann 2019).

Naturally, environmental variations are a result of physical, chemical, and biological processes that often vary from minutes to seasons. Physical processes include currents, upwelling, tidal action, and sunlight (Doney et al. 2009; Sproson and Sahlée 2014), all of which represent important drivers that potentially modify the chemical properties of seawater, as well as biological processes. Chemical processes include reactions of the inorganic carbon cycle. Biological processes include photosynthesis (Graiff et al. 2015; Wahl et al. 2018), respiration, and the uptake and/or release of various inorganic (Hurd 2000) and dissolved organic compounds to and/or from the surrounding seawater (Wetzel 1969; Van Engeland et al. 2011).

There is strong coupling between biological and chemical processes, for example, photosynthetic activity during daytime reduces CO_2 and increases pH of the surrounding seawater. In particular, hydrodynamic conditions increase or dampen the effect of metabolic processes (e.g., photosynthesis and

Additional Supporting Information may be found in the online version of this article.

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respiration) and lead to the enrichment or depletion of metabolic compounds (e.g., O_2, CO_2, CO_3^{2-}, H^+) in the surrounding seawater (Waldbusser and Salisbury 2014; Kapsenberg and Cyronak 2019). This can result in strong diurnal variations of, for example, pH in macrophyte beds, especially when water flow is low (Cornwall et al. 2013*a*; Wahl et al. 2018). Under high-flow conditions, in contrast, seawater pH in macrophyte beds varies less and is closer to open ocean conditions (Hurd et al. 2011; Cornwall et al. 2014). Coupled with other physical drivers like warming, within an organisms' thermal tolerance limits, these processes can modify organism responses (Havenhand 2012; Graiff et al. 2015).

Coastal environments are characterized by high variations of abiotic conditions such as temperature, pH, and light intensity, at various temporal and spatial scales. In temperate regions, macrophytes dominate rocky shores and are recognized as important ecosystem engineers. They can alter the physical state of the surrounding seawater and play an important role in the resource availability to organisms within proximity (Hurd 2015; Teagle et al. 2017). Their metabolic activity coupled with seawater flow intensity can create several boundary layers of varying thickness and chemical concentration gradients (Hermansen et al. 2001; Noisette and Hurd 2018). Depending on hydrodynamic conditions, these processes act at the larger scale (the benthic boundary layer [BBL]), often changing environmental conditions within the entire macrophyte bed (Hurd 2015). At a smaller scale, the macrophyte-seawater interface generates unique microenvironments within the diffusive boundary layer (DBL; Noisette and Hurd 2018). Within boundary layers, diurnal pH fluctuations can be substantial, depending on scale, from few meters (Saderne 2012; Silbiger and Sorte 2018) to sub-mm (Spilling et al. 2010; Noisette and Hurd 2018). The most drastic changes are found in DBLs where diurnal changes in hydrogen ion concentration of up to 30-fold occur, resulting in pH variations of 0.3–1.2 pH units between day and night (Wahl et al. 2018).

These temporal and spatial variations in pH may create both, beneficial (Hurd 2015; Wahl et al. 2018), as well as challenging conditions for organisms, when compared to constant pH (Cornwall et al. 2013*b*). Research proposes that photosynthesis can counterbalance negative OA effects, at least during daytime (Saderne and Wahl 2013) and pH-sensitive processes may therefore follow diurnal pH dynamics (e.g., higher calcification during daytime; Wahl et al. 2018). Under OA the higher availability of CO_2 could enhance macrophyte photosynthesis (Saderne 2012; Cornwall et al. 2017), although such beneficial effects were not observed for all macrophytes (Britton et al. 2016; Cornwall et al. 2017). Yet a "boost" of photosynthesis by OA further increase pH fluctuations in macrophyte boundary layers and could provide increased temporal refugia from acidification stress during daytime for organisms that live inside these boundary layers (Teagle et al. 2017; Bergstrom et al. 2019). The magnitude of pH fluctuations and hence the OA buffering capacity of macrophytes, however, also depends on the structural composition of macrophyte communities and the hydrodynamic conditions that prevail (Hurd 2000; Wahl et al. 2015). Environmental pH fluctuations may potentially render organisms more resistant to environmental changes with important consequences (Saderne and Wahl 2013; Eriander et al. 2016) and applications (Fernández et al. 2019; Kapsenberg and Cyronak 2019).

The vast majority of studies available to date tested the effect of pH reduction on organism performance by applying anticipated constant treatment conditions, which provided important insight into the mechanisms affected by pH. However, recent studies are increasingly incorporating biogenic fluctuations to further understand the ecological significance of OA in coastal and highly variable habitats (Britton et al. 2019; Hoshijima and Hofmann 2019). Furthermore, the studies available testing the effects of constant vs. fluctuating pH requires further development (Havenhand 2012; Wahl et al. 2015), to incorporate complex natural pH conditions and interactions, in the water column, experienced by epibiotic calcifiers. Therefore, the aim of this study was to investigate the interaction of macrophyte metabolic activity and growth response of calcifying epibionts under predicted OA scenarios. Specifically, the experiment tested the response of two important marine calcifiers—growing naturally on biologically active (macrophytes) and on inactive surfaces (e.g., rocks)—to different OA scenarios under consideration of naturally occurring levels of pH fluctuations (from small to large scale; DBL to BBL; Fig. 1). In order to understand potential interacting effects of temperature, all scenarios were tested under two different temperature regimes; 10°C and 15°C as the long-term minimum and maximum temperature mean for the study month May.

We hypothesized that (1) reduced pH (OA conditions) would overall reduce epibiont growth, but (2) that OA-exposed organisms living within DBL and BBL conditions (pH variability) would have higher growth rates than organisms exposed to constant OA treatments (inert substrate – constant pH). To better understand the refugia potential of DBLs of a key macrophyte species, we tested active vs. inactive substratum. Furthermore, we hypothesized (3) that warming (+5°C) would enhance growth rates but with similar growth responses to the two additional drivers (OA and natural pH variability) as hypothesized above.

This study provides a rare but important insight into the effect of OA in an ecologically relevant context that is imperative for numerous macrophyte dominated shallow water ecosystems in temperate habitats.

Materials and methods

Study organisms

Two common calcifiers in the Western Baltic Sea, a barnacle (*Balanus improvisus*) and a bryozoan (*Electra pilosa*), were chosen as study species. The barnacle *Balanus improvisus* is a filter feeder that often dominates shallow water benthic hardbottom

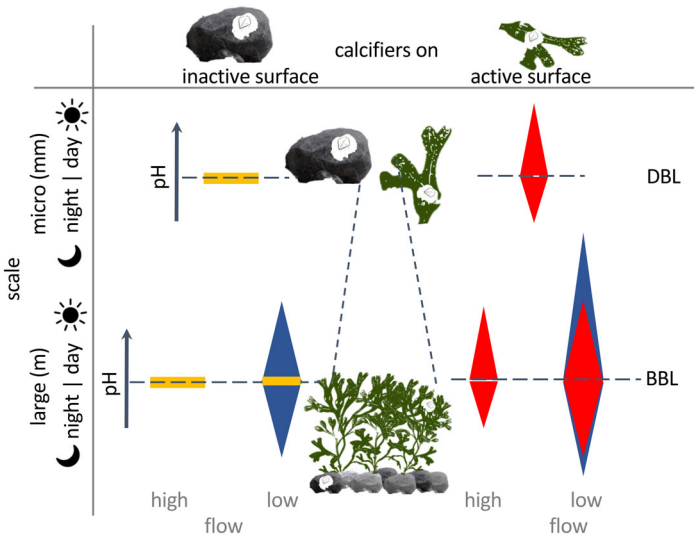

Fig. 1. Magnitude of environmental pH fluctuations experienced by epibionts depending on abiotic and biotic conditions. In nature, organisms can occupy different habitats and thus, the pH that they experience can vary on both temporal (e.g., day and night but also on longer-time scales) as well as at differing spatial scales (from micrometer within the DBL to meter scale within the BBL of foundation species). At the micrometer scale, calcifiers growing on inactive surfaces such as rocks experience rather stable pH conditions (yellow square) compared to those living on biologically active macrophyte surfaces that actively change the pH in their surrounding due to photosynthesis and respiration (red diamond representing diurnal range in pH) within the DBL. Such small-scale variations within the DBL work in conjunction with large-scale BBL seawater pH conditions that itself can vary for instance within dense macrophyte beds. Under stagnant to low-flow conditions, pH can fluctuate considerably between day and night (blue diamond) compared to high-flow conditions with rather constant bulk seawater pH. These natural environmental settings can expose organisms to different levels of pH fluctuations that potentially modulate their response to changes in mean bulk seawater pH as a consequence of global ocean acidification.

communities (Thomsen et al. 2010; Pansch et al. 2012). It is the only marine barnacle species living in the Baltic Sea proper due to its wide salinity tolerance (Jonsson et al. 2018). The bryozoan *Electra pilosa*—also a filter feeder—is a colonial encrusting cheilostome bryozoan consisting of genetically identical individuals called zooids. Both epibionts are formed by free-swimming sexually produced larvae which undergo metamorphosis after settlement onto a firm surface (Ryland 1974; Jonsson et al. 2018). Both calcifiers settle on living (active) and nonliving (inactive) surfaces (Nikulina and Schäfer 2006; Rickert et al. 2015); however, *B. improvisus* settles opportunistically on algal surfaces and *E. pilosa*, occurs nearly obligate on *Fucus* in the Baltic Sea. *Fucus serratus* represents one of the dominating benthic primary producers in the Western Baltic Sea and was therefore chosen as our active surface.

Organism preparation and settlement

The two calcifiers *B. improvisus* and *E. pilosa* were propagated on *F. serratus* blades as active surface and on Plexiglas. Specimen of the macroalgae *F. serratus* were collected with and without colonies of *E. pilosa* from the intertidal zone at Weisennhauser Strand, off the Western Baltic coast (54°18′25″N, 10°45′08″E) in Germany on the 19th of March 2017. Propagation followed established methods for *B. improvisus* (Pansch et al. 2017) and *E. pilosa* (Hermansen et al. 2001). For *B. improvisus*, larvae were derived from an indoor barnacle culture and combined with algal and Plexiglas pieces. After settlement of larvae, the number of barnacle recruits (aged 2–5 d) were standardized to 6–7 individuals per algal or Plexiglas piece by gently removing excess specimen under a stereomicroscope. Pictures were taken to determine the initial size of barnacle recruits.

For *E. pilosa* propagation, *F. serratus* encrusted by *E. pilosa* was cloned onto microscope coverslips following Hermansen et al. (2001). Coverslips containing newly grown zooids were glued onto drilled Plexiglas slides where they continued to expand during the course of the experiment. Prior to the start of the experiment, *E. pilosa* colonies (growing on *F. serratus*) were stained with 60 mg L^{-1} Calcein, for 48 h (modified after Saderne and Wahl 2013). The dye attaches to calcium, which is incorporated into the skeletal wall as the bryozoan grows, thus distinguishing between the start colony from newly formed zooids under fluorescent light. Detailed descriptions of propagation, clonation and settlement onto active and inactive surfaces for both species are available in the "Materials and methods" section in Supporting Information.

Experimental design and setup

The experiments were conducted in the Kiel Indoor Benthocosms (Pansch and Hiebenthal 2019) and lasted for 5 weeks from 10th of May until 15th of June 2017. This study encompassed four different factors in a fully crossed experimental design: (1) seawater temperature (two levels: current and future warming scenario), (2) mean pH (two levels: ambient and acidified), (3) pH variability (two levels: constant and fluctuating), and (4) substrate type (two levels: active and inactive). In detail, this led to four different pH treatments per temperature and substrate: constant-ambient-present-day (pH ~ 8.1, 400 μatm pCO_2), constant-acidified-future (pH ~ 7.8, 1000 μatm pCO_2), fluctuating-ambient-present-day (pH 7.7–8.6), and fluctuating-acidified-future (pH 7.4–8.2). Constant pH conditions represent high-flow and fluctuating pH represents low-flow conditions within macroalgal beds (Fig. 1; Wahl et al. 2015). The resulting four pH treatments were fully crossed with the two different surface types. Active algal surfaces add additional microscale pH fluctuations within the DBL, while inactive Plexiglas surfaces do not, hence resulting in different pH variations experienced by the epibionts in our experimental setup (Fig. 2). Furthermore, all described pH treatments where repeated under two temperature levels, namely 10°C and 15°C.

The experiment was set up in eight mesocosm tanks with each mesocosm corresponding to one of the four different pH

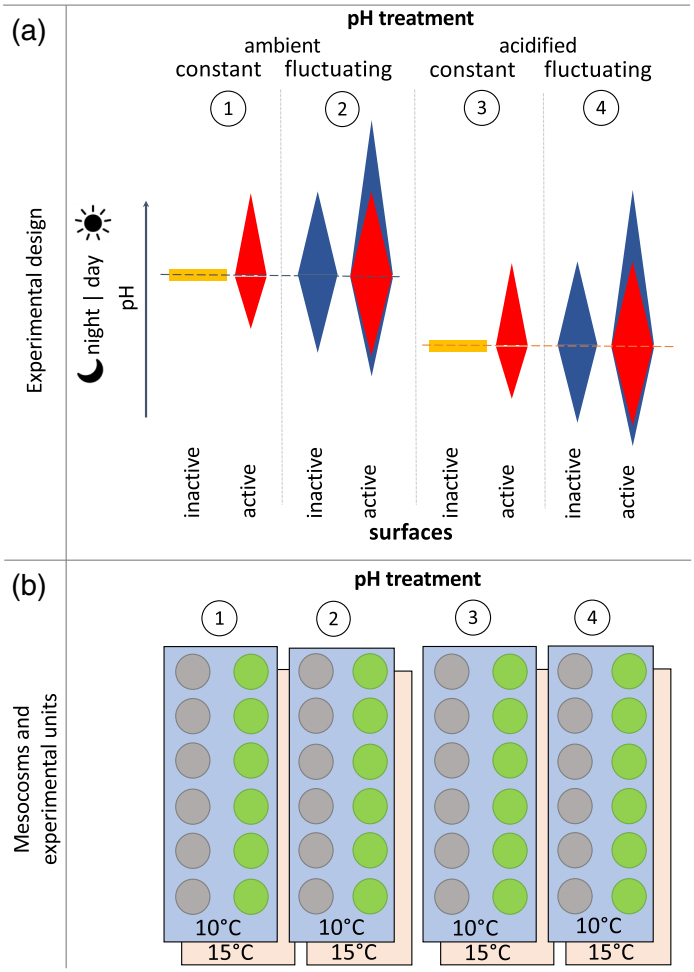

Fig. 2. Experimental design and set-up. (**A**) Treatment conditions were designed to shed light on how different natural pH fluctuations experienced by epibionts (*see* Fig. 1) modulate their growth rates. Two different pH levels and two levels of pH variability representing BBL pH conditions, namely constant (-, yellow square) and fluctuating (∼, blue diamond), were established under both present-day ambient as well future acidified pH conditions. This gives a total of four different pH treatments (1–4). These pH treatments were fully crossed with epibionts growing on inactive and active surfaces to understand the role of small-scale DBL pH variations (inactive surfaces with none—yellow square, and active surfaces with strong pH variations—red diamond). (**B**) A total of eight mesocosms was used with half set up to cold 10°C (blue tanks) and the other half to warm 15°C (orange tanks). All four pH treatments were simulated within each temperature regime. Each mesocosm contained a total of 12 experimental units with separate units for calcifiers on inactive (gray circles; n = 6 units) and active surfaces (green circles, n = 6 units).

treatments (Fig. 2A) and one of the two temperature conditions (Fig. 2B). One mesocosm contained 12 experimental units, resulting in a total of ninety-six 4.5-L experimental units for all treatments. Out of the 12 experimental units per mesocosm, 6 contained active and 6 inactive surfaces representing the true replicates per treatment (n = 6; Fig. 2B). An experimental unit hosted both epibionts with three pseudoreplicates per experimental unit and epibiont (Supporting Information Fig. S1). The entire experiment, thus contained 144 macroalgal and 144 Plexiglas surfaces, per epibiont. Seawater was derived from the Kiel Fjord and passed through a sandfilter and a sequence of finer filters (20, 5, and 1 μm pore size) before it was distributed into 60-liter header tanks located above each mesocosm (Pansch and Hiebenthal 2019). Water was constantly supplied to each experimental unit via a flow-through system from the header tanks through individual silicone tubes (Supporting Information Fig. S1), renewing the seawater within each experimental unit every 3–4 h.

Seawater pH was adjusted by aeration with pCO_2 enriched or reduced air supplied to the experimental units, using a central automated CO_2 mixing facility (Linde Gas & HTK Hamburg, Germany), which provided compressed and dried air with approximately 400, 1000, and 1800 μatm pCO_2. Air with lower pCO_2 than *ambient* (100 μatm pCO_2) was prepared by streaming ambient incoming air through a three-phase system stripping CO_2 and particles out of the air (2x soda lime/chalk filter and 1x AquafilterFCPS5 Polypropylene Melt Blown Filter). The Kiel Indoor Benthocosm system mimicked pH fluctuations between day and night in a 14:10 h cycle by controlling the supply of different pCO_2 concentrations. The carbonate system equilibrated within 2–4 h for day conditions and within less than 2 h for night-conditions (Supporting Information Fig. S2).

Light conditions were synchronized to the pCO_2 day-night rhythm, gradually turning on and off within 2 h after the switch in pCO_2. Light either dimmed prior to the switch or reached full intensity (84 ± 49 [mean ± SD] μmol photons m^{-2} s^{-1}), simulating dusk and dawn conditions, respectively. Epibionts were fed with cultured *Rhodomonas* sp. (cell size ∼ 6 μm) at a mean final concentration of 8000 cells mL^{-1} d^{-1}, supplied in two fractions: 4000 cells mL^{-1} between 08:00 h and 10:00 h, and 4000 cells mL^{-1} between 22:00 h and 00:00 h, after routine pH and temperature measurements were conducted. This *Rhodomonas* concentration has previously been found optimal for *E. pilosa* growth based on preliminary feeding experiments and roughly corresponds to the natural phytoplankton abundance at 4.58 μg L^{-1} for the month of May in the Kiel Fjord (12-yr data set of the Kiel pier; data courtesy, C. Clemmesen-Bockelmann unpubl.). *Rhodomonas* concentration of 4000 cells mL^{-1} is equivalent to a chlorophyll *a* concentration ∼ 5 μg L^{-1} (*see* Clausen and Riisgard 1996 for conversion factor).

Monitoring of abiotic parameters in the experimental units

Temperature and pH_{NBS} (NBS scale) were automatically logged every 10 min by the Kiel Indoor Benthocosm system in one representative experimental unit for each treatment. Manual temperature and pH_T measurements were done twice a day between 08:00 and 10:00 h and 22:00 and 00:00 h. Water samples were taken in parallel to the pH measurements (twice per day) for determination of total dissolved inorganic carbon (100 mL in borosilicate bottles with glass lids) and total

alkalinity (50 mL SARSTEDT falcon tubes) from the different pH treatments (from a random subset, specifically 50% of experimental units from each mesocosm tank) and fixed with 50 μL HgCl (0.02%). Dissolved inorganic carbon was determined with an AIRICA Marianda system (Germany) and total alkalinity was measured by potentiometric titration with an automated open cell titrator (Titroline 7000, SI analytics, Germany). Both were calibrated with Certified Reference Material (Andrew Dickson, Scripps Institution of Oceanography) following best practice procedures (Dickson et al. 2007). Concentrations of CO_2, HCO_3^-, and CO_3^{2-} were calculated based on pH measured on the total scale and dissolved inorganic carbon using the R package seacarb (Lavigne and Gattuso 2010).

Light intensities were measured weekly with an Apogee Light meter (MQ-200 Quantum meter) within a random subset of the experimental units. At all depths within the experimental unit (Supporting Information Fig. S1), organisms were arranged to obtain mean light conditions (84 ± 49 μmol photons m^{-2} s^{-1}) experienced by the active surface affecting DBL conditions. Light values ranged from 38 ± 13 to 147 ± 18 [means ± SD] μmol photons m^{-2} s^{-1} from the bottom to the top of the experimental unit, respectively. These light intensities are within the range of natural light conditions within *Fucus* stands in May (M. Wall unpubl.). Further details on the light regime in the applied mesocosm structure is also given in Pansch and Hiebenthal (2019).

Characterization of DBL at the algal-seawater interface

To determine the pH and oxygen conditions within the DBL of active and inactive surfaces, microsensor measurements were conducted at the end of the 5-week experiment, following established methods and approaches (Hofmann et al. 2016; Noisette and Hurd 2018). The microsensor setup (Unisense, Denmark) consisted of a 1.5-liter flow-through chamber (with a constant laminar flow of ~ 2 cm s^{-1}) containing the sample, a motorized micromanipulator holding two microsensors (a glass pH microsensor and a Clark-type oxygen sensor, both with a tip size of 50 μm) and a four-channel multimeter connecting the microsensors to a computer with the software SensorTrace Suite. The software controlled the motorized stage as well as the microsensor measurements. The pH microsensor was connected to a reference electrode immersed in the same medium (REF401, Radiometer Analytic, Hach Company) and was calibrated using pH 7 and 9 NBS buffer (covering the range of measured pH values in the DBL). The pH sensors exhibited near Nernst behavior with a mean response of approximately 55 mV (± 2 mV) per pH unit (90% response time < 10 s from pH 7 to pH 9). The oxygen sensor was calibrated in oxygen saturated (air bubbled) seawater and in 0% oxygen seawater prepared with sodium nitrite (< 2% stirring sensitivity, 90% response time < 5 s). The flow-through chamber was illuminated (Schott lamp, 3000 K) and light intensity at the substrate surface was measured and adjusted to correspond to mean treatment conditions (60–90 μmol photons m^{-2} s^{-1}). Three to six surfaces per treatment with epibionts were successively mounted in the center of the chamber onto a custom-made sample holder (*see* Wahl et al. 2015 for DBL experienced by epibionts of different heights on *Fucus vesiculosus*). Measurements were done under light and dark conditions using pH (pH_T) levels that resemble those applied in the different experimental treatments during light and dark conditions (e.g., acidified fluctuating pH was 8.17 in light and 7.45 in the dark). We simultaneously measured both pH and oxygen concentration to characterize the two main parameters that change concurrently within the DBL due to photosynthesis and respiration on the active surface *F. serratus*. Measurements were conducted directly at the surface of the algal blade next to the epibiont but neither up- nor downstream of it to measure the algal activity only.

Determination of epibiont growth

The initial size of barnacles (diameter) was determined for ~ 50 randomly chosen individuals on Plexiglas and algal surfaces by analyzing pictures taken under the microscope 1 d prior to the start of the experiment. The resulting sizes were 0.72 ± 0.09 mm on Plexiglass and 0.70 ± 0.09 mm on algal surfaces. Final sizes of *B. improvisus* were determined from pictures taken at the end of the experiment following the same procedure as for initial size measurements.

For *E. pilosa*, colony growth determinations differed slightly between the two surface types. For colonies on inactive surfaces, growth (addition of new zooids) was determined by comparing the number of zooids per colony in the beginning and at the end of the experiment using photographs. For colonies on active surfaces, the number of new zooids were counted directly by counting the zooids established after calcein staining on pictures taken at the end of the experiment, under a fluorescence microscope (*see* "Materials and methods" section in Supporting Information for more details on zooid census). The different procedures were employed to minimize handling of the active surface samples. The growth efficiency (GE) of colonies on both surfaces were calculated based on the number of zooids before the experiment (N_s) and the number of new zooids (N_n) following the equation GE = log10 (N_n/N_s).

Data processing and statistical analysis

The mean growth responses were calculated for each experimental unit by averaging final sizes (*B. improvisus*) and GE (*E. pilosa*) derived from the pseudo-replicates per experimental unit (for *B. improvisus*, 12–21 individuals contributed to the mean of the experimental unit; for *E. pilosa*, three colonies contributed to the mean of the experimental unit). Thus, for our statistical analysis, we considered only one derived value (mean growth per experimental unit) per substrate and treatment (replicates: n = 6; Fig. 2B). All data visualizations and analyses were done with the software R (R-Version 3.4.2; R Core Team 2017). For each pH treatment (multifactorial—with two mean pH

levels and two levels of pH variability), surface type and temperature treatment the mean log response ratios (LnRR) of the response variables final size (*B. improvisus*) and GE (*E. pilosa*) were calculated. LnRR is the ratio of the mean effect in a treatment (X_T) to the mean effect in a control group [X_C; LnRR = ln (X_T/X_C); Hedges et al. 1999]. Our control group was the treatment with the lowest pH variability factor (constant and ambient pH, inactive substrate) at low temperature (10°C). The responses of all other groups were related to this control group. This allowed us to visualize and compare the different epibiont responses in terms of growth and identify whether the effect size differed between treatments. Positive and negative values indicate the response to the treatment. The effect is smaller, the closer the value is to the zero line. The magnitude of variability is indicated by the length of the confidence interval (CI ± 95%). Overlap of CIs indicates that mean effects between compared values are not significant. The mean effect size is considered significant if the CI does not cross the zero line.

A multifactorial ANOVA test was performed to determine the dominating factors that influenced the growth responses of each epibiont (Table 2). The factors tested in a fully crossed design included temperature (10°C vs. 15°C), mean pH (ambient vs. acidified), pH variability (constant vs. fluctuating), and surface type (inactive vs. active surface). Normality of all data was tested using Shapiro–Wilk's test, and the homogeneity of variances determined with Levene's test. When homoscedasticity or normality was not achieved (one subset of barnacle data) even after transformation, the normality of residuals was graphically verified using histograms and normal Q-Q plots. Homogeneity of variances was checked by plotting residuals over fitted values of the model. The ANOVA test and LnRR were used to consolidate statistical and ecological interpretation. For post hoc comparisons, Tukey's Honestly Significant Difference (HSD) was performed.

Results

Experimental conditions representing BBL and DBL habitats

Epibionts were exposed to an average seawater pH_T of 8.06 for ambient and 7.78 for acidified conditions in constant and fluctuating as well as in 10°C and 15°C treatments. Under fluctuating/BBL conditions, pH varied up to 0.66 pH around the mean between day and night. Calcite saturation state ($\Omega_{calcite}$) was > 1 in all ambient pH treatments except at night for the fluctuating treatment at 10°C. In contrast, $\Omega_{calcite} < 1$ occurred at several occasions in all acidified treatments (Table 1).

Within the DBL of the active *F. serratus* surface, pH_T exceeded the pH conditions of the bulk seawater as measured at the surface of the thali. On average, pH was elevated by 0.2–0.45 units during the day (reaching pH values of up to 8.9 in the ambient fluctuating treatment) and by –0.1 units at night (as low as 7.3 in the acidified fluctuating treatment) in ambient and acidified treatments and at 10°C and 15°C (Fig. 3). The pH varied by ≥ 0.5 units at the algal surface within the constant treatments (Fig. 3) and hence, was close to the pH range of the simulated diurnal BBL fluctuations (0.66 pH units; Table 1). Strongest diel pH fluctuations occurred in an active DBL under fluctuating BBL conditions

Table 1. Carbonate chemistry parameters. Data were determined for the different treatment combinations (with two levels of pH variability, namely constant [–] and fluctuating [∼] at ambient and acidified mean pH levels and temperatures of 10°C and 15°C). GHL represents means of pH_T values logged with the Kiel indoor Benthocosm setup. Salinity, pH_T (total scale), total dissolved inorganic carbon (DIC), and total alkalinity (TA) were measured from discrete water samples (*N*) taken throughout the entire experiment. pCO_2 and $\Omega_{calcite}$ were derived from pH_T and DIC using R seacarb package (Lavigne and Gattuso 2010).

				Water samples											
	Treatment	GHL pH_T		*N*	Salinity (ppm)		pH_T		DIC (μmol L^{-1})		TA (μmol L^{-1})		pCO_2 (ppm)		$\Omega_{calcite}$	
10°C	Ambient –	8.10	±0.08	18	15.36	±0.74	8.11	±0.04	1907	±45	2019	±25	359	±37	2.28	±0.24
	Ambient ∼	8.03	±0.39	18	15.36	±0.74	7.70	±0.03	1999	±32	2018	±24	978	±60	0.94	±0.05
				10	15.39	±0.78	8.63	±0.21	1726	±93	2023	±26	100	±54	6.56	±2.30
	Acidified –	7.72	±0.07	12	15.05	±0.69	7.70	±0.03	1996	±23	2011	±17	988	±63	0.92	±0.05
	Acidified ∼	7.83	±0.32	16	15.19	±0.71	7.45	±0.04	2053	±29	2020	±29	1758	±159	0.54	±0.05
				10	15.44	±0.77	8.17	±0.07	1909	±35	2023	±27	313	±54	2.62	±0.37
15°C	Ambient –	8.05	±0.09	18	15.36	±0.74	8.08	±0.05	1892	±37	2018	±31	392	±47	2.60	±0.29
	Ambient ∼	8.04	±0.32	18	15.36	±0.74	7.71	±0.04	1982	±27	2022	±24	992	±97	1.17	±0.14
				10	15.44	±0.77	8.41	±0.11	1782	±66	2027	±26	169	±49	4.95	±1.18
	Acidified –	7.74	±0.09	14	15.04	±0.64	7.67	±0.03	1976	±30	2007	±28	1077	±73	1.06	±0.05
	Acidified ∼	7.82	±0.33	16	15.19	±0.71	7.42	±0.02	2042	±21	2016	±26	1980	±103	0.60	±0.03
				10	15.44	±0.77	8.14	±0.06	1897	±37	2020	±28	342	±46	2.97	±0.39

Table 2. Four-factorial ANOVA for response variables of *Balanus improvisus* and *Electra pilosa*. Temperature and surface types significantly affected the growth of both epibionts, but pH variability (constant vs. fluctuating) and mean pH (pCO_2) had no significant impact. Significance of effects and interactions are indicated by $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and highlighted in bold.

Species	Response variable	Factor	df	Sum sq.	Mean sq.	*F* value	*p* value
B. improvisus	Final size	Temperature (T)	1	62.04	62.04	167.5	**<0.001*** **
		Mean pH (pH)	1	0.010	0.010	0.017	0.897
		pH variability (F)	1	0.080	0.080	0.221	0.640
		Surface type (S)	1	4.670	4.670	12.60	**<0.001*** **
		T:F	1	0.41	0.41	1.108	0.296
		T:S	1	0.36	0.36	0.962	0.330
		F:S	1	0.44	0.44	1.185	0.280
		T:pH	1	0.04	0.04	0.120	0.730
		F:pH	1	0.43	0.43	1.156	0.286
		S:pH	1	1.24	1.24	3.343	0.071
		T:F:S	1	1.480	1.480	4.002	**0.049***
		T:F:pH	1	2.060	2.060	5.560	**0.021***
		T:S:pH	1	0.96	0.96	2.598	0.111
		F:S:pH	1	0.40	0.40	1.075	0.303
		T:pH:F:S	1	4.380	4.380	11.82	**<0.001*** **
E. pilosa	Mean growth efficiency	T	1	2.782	2.782	50.99	**<0.001*** **
		pH	1	0.023	0.023	0.430	0.514
		F	1	0.025	0.025	0.463	0.498
		S	1	2.916	2.916	50.46	**<0.001*** **
		T:F	1	0.030	0.030	0.552	0.460
		T:S	1	0.052	0.053	0.962	0.330
		F:S	1	0.130	0.130	2.380	0.127
		T:pH	1	0.013	0.013	0.233	0.631
		F:pH	1	0.002	0.002	0.043	0.837
		S:pH	1	0.108	0.108	1.972	0.164
		T:F:S	1	0.002	0.002	0.034	0.855
		T:F:pH	1	0.106	0.106	1.935	0.168
		T:S:pH	1	0.038	0.038	0.693	0.408
		F:S:pH	1	0.088	0.089	1.622	0.207
		T:pH:F:S	1	0.333	0.333	6.111	**0.016***

with +0.7 pH units (daytime) and −0.5 pH units (nighttime; Fig. 3) around the mean. In contrast, diel maximum DBL fluctuations were minor at inactive surfaces with ~ 0.03 pH units. On active surfaces, pH fluctuations were accompanied by variations in oxygen, where the bulk seawater had an oxygen concentration of $\sim 300\ \mu mol\ O_2\ L^{-1}$, and the DBL up to $900\ \mu mol\ O_2\ L^{-1}$ during the day and down to $240\ \mu mol\ O_2\ L^{-1}$ in the night (Supporting Information Fig. S3).

Direct effects of temperature on growth of epibionts

Warming had a highly significant and overall enhancing effect on the growth of *B. improvisus* (df = 1, $F = 167.5$, $p < 0.001$) and of *E. pilosa* (df = 1, $F = 50.99$, $p < 0.001$; Figs. 4, 5; Table 2). The effect size of *B. improvisus* was 0.59 ± 0.22 (error terms are specified as 95% CI) across treatments and between 10°C and 15°C, and 0.29 ± 0.12 for *E. pilosa* (Figs. 4, 5). Average increase in growth with temperature was similar for both surfaces. For instance, final size of *B. improvisus* increased from 2.13 ± 0.28 (10°C) to 3.86 ± 0.68 mm (15°C) on inactive surface and from 1.81 ± 0.08 mm (10°C) to 3.30 ± 0.31 mm (15°C) on active surfaces (Supporting Information Fig. S4). Growth efficiency in *E. pilosa* increased from 1.16 ± 0.07 (10°C) to 1.54 ± 0.15 (15°C) and from 0.85 ± 0.08 (10°C) to 1.15 ± 0.12 (15°C) for inactive and active surfaces, respectively (Supporting Information Fig. S5).

Effects of seawater pH_T on epibionts, and changes associated with BBL or DBL pH fluctuations

Constant ambient vs. acidified conditions did not have a significant effect on growth of either epibiont species (Table 2). Similarly, constant vs. fluctuating conditions (mimicking BBL conditions) did not affect the growth of either epibiont, the final size of *B. improvisus* (df = 1, $F = 0.221$, $p = 0.640$) and GE of *E. pilosa* (df = 1, $F = 0.463$, $p = 0.498$,

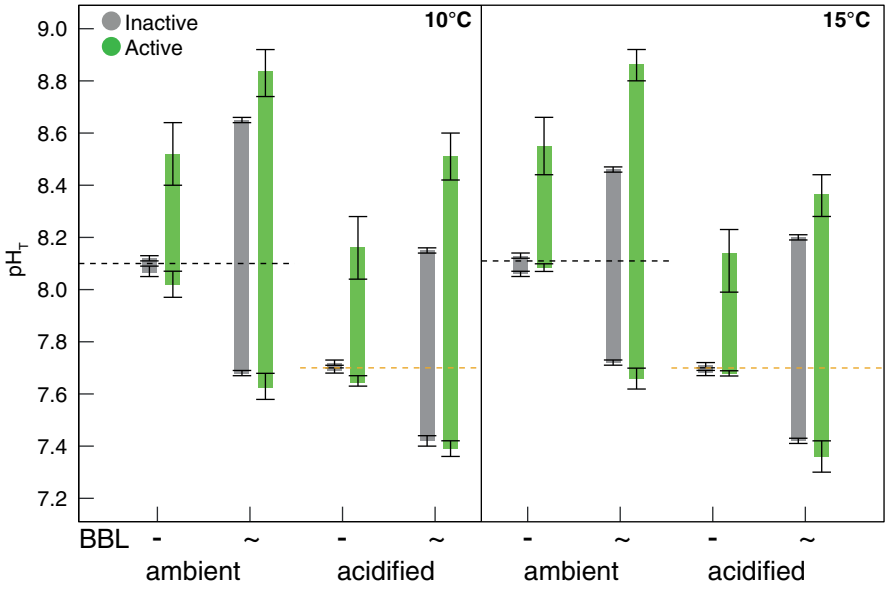

Fig. 3. Diffusive boundary layer conditions on inactive and active surfaces. DBL pH_T (pH on total scale) ranges displayed for both inactive (gray bars) and active (green bars) surfaces. pH_T values were determined under the different treatment combinations (ambient and acidified pH_T levels with both constant (-) and diurnally fluctuating (~) pH conditions within the BBL, and additionally under light and dark conditions (representing daytime and nighttime, respectively), for both, 10°C (right panel) and 15°C (left panel) temperature conditions. The dashed lines represent mean pH_T values of the ambient (black) and the acidified (orange) pH_T treatments. "Ambient" refers to a mean pH of approximately 8.1 and "acidified" a mean pH of approximately 7.7. Measurements were conducted directly at the surface of the algal blade next to the organism but neither up- nor downstream of it to measure only the algal activity. Three to six surfaces per treatment were measured. Data are presented as means ± 95% confidence intervals (CIs).

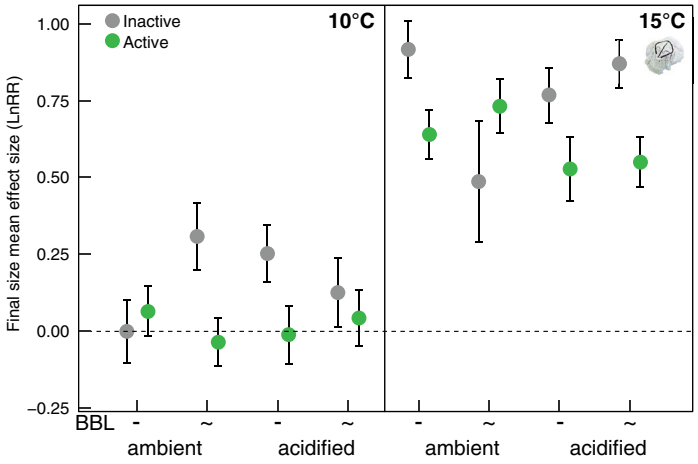

Fig. 4. Final size changes of *Balanus improvisus*. Variation in effect sizes among surface types (inactive vs. active) and pH conditions (ambient vs. acidified, with both constant [-] and fluctuating [~] conditions within the BBL) for the two different temperature treatments cold, 10°C (right panel) and warm, 15°C (left panel). Final size changes were calculated as log response ratios (LnRRs) using final sizes in ambient constant pH at 10°C on inactive surface as reference for both cold and warm treatments. Gray represents inactive and green active surfaces. "Ambient" refers to a mean pH of approximately 8.1 and "acidified" a mean pH of approximately 7.7. Data are presented as means ± 95% CIs. The mean effect size is significant if the CI does not cross the zero line.

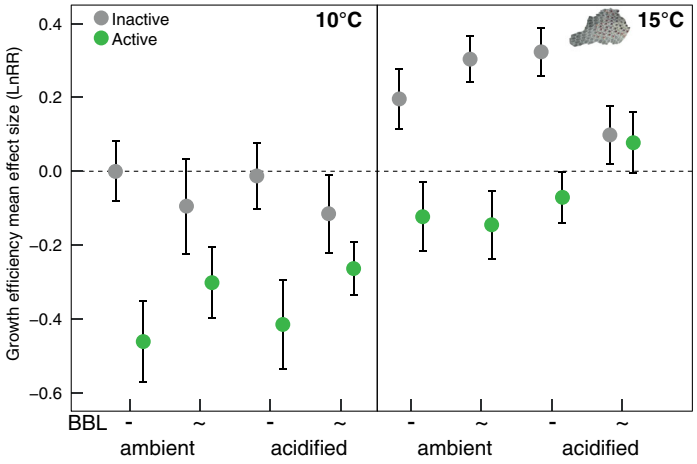

Fig. 5. Growth efficiency of *Electra pilosa*. Variation in growth efficiencies (GE) among surface types (inactive vs. active) and pH conditions (ambient vs. acidified, with both constant [-] and fluctuating [~] conditions within the BBL) for the two different temperature treatments cold, 10°C (right panel) and warm, 15°C (left panel). Changes in growth efficiencies were calculated as log response ratios (LnRRs) using growth efficiency in ambient constant pH at 10°C on inactive surface as reference, for both cold and warm treatments. Gray represents inactive and green active surfaces. "Ambient" refers to a mean pH of approximately 8.1 and "acidified" a mean pH of approximately 7.7. Data are presented as means ± 95% CIs. The mean effect size is significant if the CI does not cross the zero line.

Table 2). Surface type, however, had a significant effect on growth evident in a lower final size of *B. improvisus* (df = 1, F = 12.16, $p < 0.001$, Fig. 4, Supporting Information Fig. S4) and a lower GE of *E. pilosa* (df = 1, F = 50.46, $p < 0.001$, Fig. 5, Supporting Information Fig. S5) on active than on inactive surfaces. This effect was stronger in *E. pilosa* compared to *B. improvisus*. On average the effect was only 0.16 (± 0.18) and 0.15 (± 0.26) for *B. improvisus* compared to 0.30 (± 0.11) and 0.29 (± 0.19) for *E. pilosa* at 10°C and 15°C, respectively. There were no interactive effects between mean pH and pH variability (df = 1, F = 1.156, p = 0.286 for *B. improvisus* and df = 1, F = 0.043, p = 0.837 for *E. pilosa*). In fact, post hoc analysis revealed that mean pH and pH variability interactions were only significant when either or both factors where interacting with one or both of the strongly significant factors, namely temperature and surface type (Supporting Information Tables S1, S2). For example, the temperature × pH variability × surface type, interaction was significant for *B. improvisus* (df = 1, F = 4.002, p = 0.049) but not for *E. pilosa* (df = 1, F = 0.034, p = 0.885). Similarly, the temperature × pH variability × mean pH interactions where significant for *B. improvisus* (df = 1, F = 5.560, p = 0.021) but not for *E. pilosa* (df = 1, F = 1.935, p = 0.168). For both species, interactions were significant when all four factors where combined.

Discussion

The experimental design and setup of this study allowed us to investigate the role of microscale to ecosystem-scale biogenic fluctuations of pH and carbonate chemistry under present-day and future projected pH conditions on calcifying epibiont growth, and how these fluctuations and responses may change with temperature. We measured pH variability above the substrate surface under these scenarios and thus, showed how the algal activity further modulates the pH in direct proximity of the epibionts. At OA conditions, macrophyte photosynthesis was found to raise seawater pH by 0.3 up to almost 0.6 pH units, hence buffering OA conditions at daytime. In contrast to our expectation, however, *B. improvisus* and *E. pilosa* did not seem to have benefitted from these diurnal pH peaks in terms of growth. Instead, epibiont growth was higher on inactive surfaces compared to active surfaces, evidently for *E. pilosa*, which suggests that factors other than the carbonate chemistry drive epibiont growth on *F. serratus*. In summary, neither OA predicted in global change scenarios for the end of 2100 nor large-scale pH fluctuations mimicking BBL fluctuations affected growth significantly. In contrast, warming by 5°C had an overall positive effect on epibiont growth, particularly in *B. improvisus*.

Recent reviews emphasized the importance of naturally pH-variable macrophyte environments such as seagrass meadows, dense algal beds, algal boundary layers, and mangroves to serve as potential natural temporal refugia from OA (Cornwall et al. 2014; Wahl et al. 2018). Furthermore, natural pH fluctuations were suggested to serve as a tool for adapting calcifying organisms in aquaculture (e.g., mussels) to future OA conditions (Fernández et al. 2019). These macrophyte ecosystems have therefore been suggested as potential target areas for future management actions and prioritized restoration (Kapsenberg and Cyronak 2019). For example, in the mussel *Mytilus edulis*, reduced pH was found to reduce mussel growth, which, however, could be mitigated by macroalgal activity (Wahl et al. 2018). Furthermore, Frieder et al. (2014) observed a delay in larval development of two mytilid mussels under reduced pH conditions, which was prevented when exposed to semidiurnal pH fluctuations.

The results of our study, however, do not support the idea that macrophyte communities can serve as temporal refugia from OA. None of the acidified treatments showed that diurnal fluctuations of pH support a higher growth of either *B. improvisus* or *E. pilosa*, when compared to constant pH environments. There are two potential explanations for this: (1) the short-term high pH conditions were too short to cause any measurable benefit (s), or (2) the study species feature a particularly high OA tolerance (e.g., high phenotypic plasticity). The latter explanation is supported by previous studies where calcifiers from the western Baltic Sea revealed strong resistance to changes in seawater pH (Thomsen et al. 2010; Saderne and Wahl 2013), including changes caused by upwelling events, which introduce low-pH and hypoxic water into shallow habitats (Melzner et al. 2013). We acknowledge that pH alone may not be the main driver for the change in an organisms' physiology (e.g., calcification). Similar to the results of the present study, Eriander et al. (2016) found no significant effect of pH variability (constant vs. fluctuating) on barnacle growth (*B. improvisus*) under OA conditions. However, they found a 20-fold higher variability in the trait under fluctuating compared to constant treatment conditions. In the present study, increased variation due to fluctuating pH was less pronounced but evident under some factor combinations (e.g., variation of barnacle final size was higher under fluctuating than under constant pH conditions when growing on inactive surface under ambient pH at 15°C; Supporting Information Table S1). Populations of barnacles from high- and low-variance habitats similarly responded differently to OA, underscoring the important role of environmental history (Pansch et al. 2014).

The results of our study adds to a growing body of literature underscoring that direct exposure to natural variations neither buffers nor intensifies OA effects for non-photosynthesizing calcifying organisms (reviewed in Kapsenberg and Cyronak 2019). Macroalgae still have the potential to offer refuge from OA stress, but this may depend on species, populations, and the studied traits. Epibionts living in the boundary layer of macrophytes may experience a specific, or multiple pH conditions depending on their size, distance from the blade, and topography of the macroalgal surface (Hurd et al. 2011; Wahl et al. 2015). However, even though parts of

the growing epibiont may eventually reach beyond DBL thickness (Wahl et al. 2015), baseplate expansions (calcification) of the barnacles and bryozoans will always occur directly on the algal surface where diurnal fluctuations of the carbonate chemistry are strongest. Although, the results of this study revealed no effect of reduced and fluctuating pH on growth of the two calcifying epibionts, we cannot exclude the possibility that other growth-related features may have been affected as a consequence of shifts in resource allocations (Lombardi et al. 2017). For example, it has been shown that shell strength of *B. improvisus* was strongly and negatively affected by a low pH of 7.7 (Eriander et al. 2016) and that bryozoans shift resources from the maintenance of older zooids to the production of new zooids (Lombardi et al. 2017; Swezey et al. 2017) under OA conditions. Similar strategies have also been demonstrated in a temperate coral species (Fantazzini et al. 2015) and in sea urchins (Hoshijima and Hofmann 2019). In addition, it is possible that other physiological processes than those measured herein were affected in a beneficial or detrimental way by our pH treatments, despite the fact that growth, a critical trait to persist in a competitive environment, was not affected significantly by the predicted OA scenarios.

Surprisingly, while large-scale BBL fluctuations did not have a significant effect on epibiont growth, at the microscale, the DBL conditions of active surfaces have reduced the organism's performance (Figs. 4, 5). This may have the following reasons: (1) The extreme short-term diurnal pH fluctuations on active surfaces (pH of 7.3–8.9) and increased average seawater pH experienced by the epibionts (0.3 pH units), may constitute a rather stressful condition for epibionts, where the organism has to constantly adjust to changing conditions, which may reduce energy investment into growth. (2) Another driver, other than chemical changes in pH, may play a more important role on the algal surface (e.g., algal chemical defense, oxygen fluctuations; Spilling et al. 2010; Lichtenberg and Kühl 2015). (3) Different attributes of active and inactive surfaces (roughness, tension, chemical composition, and biofilm composition) may have added to the observed differences in epibiont growth between surface types. Although a combination of the given reasons is likely driving epibiont growth on algal surfaces, we are still able to address our research aim, which is to determine whether DBLs of macrophytes have the potential to provide refugia from OA. Our study indicates that DBLs of macrophytes and the diurnal maxima of pH within the DBL do not provide a refugia from OA, and that this current conceptual idea may be much more complex. Indeed, several other parameters in the DBL are regulated by the macrophyte, which can affect organism performance. For instance, in the present study, oxygen varied concomitantly with pH, reaching extreme hyperoxic conditions during the day and diminished concentrations during the night (Supporting Information Fig. S3). Both, super- and undersaturation may affect calcification rates as shown in corals with optimum values of 110% oxygen saturation and a decrease in rates beyond and below (Wijgerde et al. 2012). Furthermore, algae excrete various organic compounds and take up nutrients and minerals that shape microbial biofilms on algal surfaces, which in turn modify the chemical microenvironment in DBLs. Macroalgae also excrete chemical defense compounds, which can alter epibiont performance and hamper growth (Brock et al. 2007; Saha et al. 2014). In particular *F. vesiculosus*, closely related to our study species *F. serratus*, has been found to excrete a range of different antifouling compounds (Saha et al. 2018), which successfully hampered barnacle settlement (Brock et al. 2007; Rickert et al. 2015). Furthermore, the biofilms on the surface of *F. vesiculosus* and *F. serratus* fronds were found to hinder the growth of *B. improvisus* larvae (Nasrolahi et al. 2012). Similarly, Manríquez and Cancino (1996) and Hermansen et al. (2001) demonstrated that colonies of bryozoans did not grow faster on macroalgae than those on glass slides, neither in the field nor in laboratory tests, attributing this finding to the chemical defenses of the macrophytes. Even though bryozoans do not seem to benefit from algae in terms of growth, other factors have been suggested that support preferred settlement on algae. Those include low competition for space (Manríquez and Cancino 1996), minimized risk of predation and thus, increased longevity of colony survival (Nikulina and Schäfer 2006; Denley et al. 2014), lower risk of smothering by sediment (Denley et al. 2014), supply of organic matter (food) by macroalgae (Manríquez and Cancino 1996), and higher food availability as the algae sways through the water column (Okamura 1988; Pratt 2008).

The temperature experienced in the Western Baltic Sea typically ranges from 3°C (in February) to 18°C (in August), with long-term average temperatures for May between 10°C and 15°C. In general, metabolic activity and growth of invertebrates increases with temperature (Pistevos et al. 2011; Smith 2014) until they reach an upper thermal limit (usually above local average summer temperatures), where thermal stress occurs. Therefore, it is not surprising that *E. pilosa* and *B. improvisus* grew better under 15°C compared to 10°C, which is also confirmed by previous studies of these species (barnacles: Pansch et al. 2012; Nasrolahi et al. 2013; bryozoans: Menon 1972). While elevated temperatures in spring seem beneficial for epibiont survival and growth in the Kiel Fjord and did not show any interacting effects with pH, elevated temperatures in summer may exceed the organism's thermal optima leading the thermal stress in barnacles (Findlay et al. 2009), and bryozoans (Menon 1972; Pistevos et al. 2011) which may also increase their susceptibility to OA (Rodolfo-Metalpa et al. 2010).

Conclusions

Our study highlights that biologically active surfaces further add complexity to conditions experienced within the DBL that may not be beneficial for calcifiers tested herein. Thus, future studies should consider the following aspects;

(1) the possibility that chemical defenses or other surface characteristics might overwrite the mitigation potential offered by macrophytes under OA scenarios, as shown for the BBL in some studies (Wahl et al. 2018; Bergstrom et al. 2019), and (2) determining how energy resources are exploited when organisms are exposed to different OA conditions (stable or fluctuating). It is imperative that studies explore the concept of refugia and how this is defined (Kapsenberg and Cyronak 2019), but also characterize their biological benefits and drawbacks. Though Kapsenberg and Cyronak (2019) excluded microrefugia from their definition of "refugia in variable environments," these may represent an important training ground for species. In the Baltic Sea, environmental history already renders calcifiers more robust to future changes in mean pH. A similar effect may be derived through fluctuations, and conditions within the DBL. Although DBL conditions in this study did not foster growth, they may select for higher phenotypic plasticity, and therefore increase the adaptive capacity of species, a field that warrants further investigation. Our findings clearly underline that we need to characterize variations not only of the most common drivers such as pH and oxygen, but also direct focus toward the myriad of changes occurring through macroalgal activity, and their role in modulating epibiont performance.

Data availability statement

Data from this article are available at the PANGAEA database: https://doi.pangaea.de/10.1594/PANGAEA.917864 (Johnson et al. 2020).

References

Bergstrom, E., J. Silva, C. Martins, and P. Horta. 2019. Seagrass can mitigate negative ocean acidification effects on calcifying algae. Sci. Rep. **9**: 1–11. doi:10.1038/s41598-018-35670-3

Britton, D., C. E. Cornwall, A. T. Revill, C. L. Hurd, and C. R. Johnson. 2016. Ocean acidification reverses the positive effects of seawater pH fluctuations on growth and photosynthesis of the habitat-forming kelp, *Ecklonia radiata*. Sci. Rep. **6**: 1–10. doi:10.1038/srep26036

Britton, D., C. N. Mundy, C. M. McGraw, A. T. Revill, C. L. Hurd, and J. Norkko. 2019. Responses of seaweeds that use CO_2 as their sole inorganic carbon source to ocean acidification: Differential effects of fluctuating pH but little benefit of CO_2 enrichment. ICES J. Mar. Sci. **76**: 1860–1870. doi:10.1093/icesjms/fsz070

Brock, E., G. M. Nylund, and H. Pavia. 2007. Chemical inhibition of barnacle larval settlement by the brown alga *Fucus vesiculosus*. Mar. Ecol. Prog. Ser. **337**: 165–174. doi:10.3354/meps337165

Clausen, I, and Riisgard, H. U. 1996. Growth, filtration and respiration in the mussel *Mytilus edulis*: no evidence for physiological regulation of the filter-pump to nutritional needs. Mar. Ecol. Prog. Ser. **141**: 37–45. doi:10.3354/meps141037

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

Cornwall, C. E., C. D. Hepburn, C. A. Pilditch, and C. L. Hurd. 2013*b*. Concentration boundary layers around complex assemblages of macroalgae: Implications for the effects of ocean acidification on understory coralline algae. Limnol. Oceanogr. **58**: 121–130. doi:10.4319/lo.2013.58.1.0121

Cornwall, C. E., P. W. Boyd, C. M. McGraw, C. D. Hepburn, C. A. Pilditch, J. N. Morris, A. M. Smith, and C. L. Hurd. 2014. Diffusion boundary layers ameliorate the negative effects of ocean acidification on the temperate coralline macroalga *Arthrocardia corymbosa*. PLoS One **9**: 1–9. doi:10.1371/journal.pone.0097235

Cornwall, C. E., A. T. Revill, J. M. Hall-Spencer, M. Milazzo, J. A. Raven, and C. L. Hurd. 2017. Inorganic carbon physiology underpins macroalgal responses to elevated CO_2. Sci. Rep. **7**: 1–12. doi:10.1038/srep46297

Denley, D., A. Metaxas, and J. Short. 2014. Selective settlement by larvae of *Membranipora membranacea* and *Electra pilosa* (Ectoprocta) along kelp blades in Nova Scotia, Canada. Aquat. Biol. **21**: 47–56. doi:10.3354/ab00569

Dickson, A. G., C. L. Sabine, and J. R. Christian [eds]. 2007. Guide to best practices for ocean CO_2 measurements, p. 191. PICES Special Publication 3. North Pacific Marine Science Organization.

Doney, S. C., V. J. Fabry, R. A. Feely, and J. A. Kleypas. 2009. Ocean acidification: The other CO_2 problem. Ann. Rev. Mar. Sci. **1**: 169–192. doi:10.1146/annurev.marine.010908.163834

Duarte, C. M., and others. 2013. Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. Estuaries Coast. **36**: 221–236. doi:10.1007/s12237-013-9594-3

Eriander, L., A. L. Wrange, and J. N. Havenhand. 2016. Simulated diurnal pH fluctuations radically increase variance in—but not the mean of—growth in the barnacle *Balanus improvisus*. ICES J. Mar. Sci. **73**: 596–603. doi:10.1093/icesjms/fsv214

Fantazzini, P., and others. 2015. Gains and losses of coral skeletal porosity changes with ocean acidification acclimation. Nat. Commun. **6**: 1–7. doi:10.1038/ncomms8785.

Feely, R. A., S. R. Alin, J. Newton, C. L. Sabine, M. Warner, A. Devol, C. Krembs, and C. Maloy. 2010. The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. Estuar. Coast. Shelf Sci. **88**: 442–449. doi:10.1016/j.ecss.2010.05.004

Fernández, P. A., P. P. Leal, and L. A. Henríquez. 2019. Co-culture in marine farms: Macroalgae can act as chemical refuge for shell-forming molluscs under an ocean

acidification scenario. Phycologia **58**: 542–551. doi:10.1080/00318884.2019.1628576

Findlay, H., M. Kendall, and S. Widdicombe. 2009. Post-larval development of two intertidal barnacles at elevated CO_2 and temperature. Mar. Biol. **157**: 725–735. doi:10.1007/s00227-009-1356-1

Frieder, C. A., J. P. Gonzalez, E. E. Bockmon, M. O. Navarro, and L. A. Levin. 2014. Can variable pH and low oxygen moderate ocean acidification outcomes for mussel larvae? Glob. Chang. Biol. **20**: 754–764. doi:10.1111/gcb.12485

Graiff, A., I. Bartsch, W. Ruth, M. Wahl, and U. Karsten. 2015. Season exerts differential effects of ocean acidification and warming on growth and carbon metabolism of the seaweed *Fucus vesiculosus* in the Western Baltic Sea. Front. Mar. Sci. **2**: 112. doi:10.3389/fmars.2015.00112

Havenhand, J. N. 2012. How will ocean acidification affect Baltic Sea ecosystems? An assessment of plausible impacts on key functional groups. Ambio **41**: 637–644. doi:10.1007/s13280-012-0326-x

Hedges, L. V., J. Gurevitch, and P. S. Curtis. 1999. The meta-analysis of response ratios in experimental ecology. Ecology **80**: 1150–1156. doi:10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2

Hermansen, P., P. S. Larsen, and H. U. Riisgård. 2001. Colony growth rate of encrusting marine bryozoans (*Electra pilosa* and *Celleporella hyalina*). J. Exp. Mar. Biol. Ecol. **263**: 1–23. doi:10.1016/S0022-0981(01)00243-X

Hofmann, L. C., M. Koch, and D. de Beer. 2016. Biotic control of surface pH and evidence of light-induced H+ pumping and Ca_2+-H+ exchange in a tropical crustose coralline alga. PLoS One **11**: e0159057.

Hoshijima, U., and G. E. Hofmann. 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**: 1–18. doi:10.3389/fmars.2019.00062

Hurd, C. L. 2000. Water motion, marine macroalgal physiology, and production. J. Phycol. **36**: 453–472. doi:10.1046/j.1529-8817.2000.99139.x

Hurd, C. L. 2015. Slow-flow habitats as refugia for coastal calcifiers from ocean acidification. J. Phycol. **51**: 599–605. doi:10.1111/jpy.12307

Hurd, C. L., C. E. Cornwall, K. Currie, C. D. Hepburn, C. M. McGraw, K. A. Hunter, and P. W. Boyd. 2011. Metabolically induced pH fluctuations by some coastal calcifiers exceed projected 22nd century ocean acidification: A mechanism for differential susceptibility? Glob. Chang. Biol. **17**: 3254–3262. doi:10.1111/j.1365-2486.2011.02473.x

Johnson, M. J., Hennigs, L. M., Sawall, Y., Pansch, C, and Wall, M. 2020. Growth response of the barnacle Balanus improvisus and the bryozoan Electra pilosa from Kiel Fjord to biogenic pH fluctuations and global ocean acidification scenarios. PANGAEA. doi:10.1594/PANGAEA.917864

Jonsson, P. R., A. Wrange, U. Lind, A. Abramova, M. Ogemark, and A. Blomberg. 2018. The barnacle *Balanus improvisus* as a marine model - culturing and gene expression. J. Vis. Exp. (138): 1–11. doi:10.3791/57825

Kapsenberg, L., and T. Cyronak. 2019. Ocean acidification refugia in variable environments. Glob. Chang. Biol. **25**: 1–14. doi:10.1111/gcb.14730

Lavigne, H., and J. Gattuso. 2010. Seacarb: Seawater carbonates chemistry with R package version 3.1.3. [accessed 2017 August 3]. Available from http://cran-project.org/package=seacarb

Lichtenberg, M., and M. Kühl. 2015. Pronounced gradients of light, photosynthesis and O_2 consumption in the tissue of the brown alga *Fucus serratus*. New Phytol. **207**: 559–569. doi:10.1111/nph.13396

Lombardi, C., P. D. Taylor, S. Cocito, C. Bertolini, and P. Calosi. 2017. Low pH conditions impair module capacity to regenerate in a calcified colonial invertebrate, the bryozoan *Cryptosula pallasiana*. Mar. Environ. Res. **125**: 110–117. doi:10.1016/j.marenvres.2017.02.002

Manríquez, P. H., and J. M. Cancino. 1996. Bryozoan-macroalgal interactions: Do epibionts benefit? Mar. Ecol. Prog. Ser. **138**: 189–197. doi:10.3354/meps138189

Melzner, F., J. Thomsen, W. Koeve, A. Oschlies, M. A. Gutowska, H. W. Bange, H. P. Hansen, and A. Körtzinger. 2013. Future ocean acidification will be amplified by hypoxia in coastal habitats. Mar. Biol. **160**: 1875–1888. doi:10.1007/s00227-012-1954-1

Menon, N. R. 1972. Heat tolerance, growth and regeneration in three North Sea bryozoans exposed to different constant temperatures. Mar. Biol. **15**: 1–11. doi:10.1007/BF00347433

Nasrolahi, A., S. B. Stratil, K. J. Jacob, and M. Wahl. 2012. A protective coat of microorganisms on macroalgae: Inhibitory effects of bacterial biofilms and epibiotic microbial assemblages on barnacle attachment. FEMS Microbiol. Ecol. **81**: 583–595. doi:10.1111/j.1574-6941.2012.01384.x

Nasrolahi, A., C. Pansch, M. Lenz, and M. Wahl. 2013. Temperature and salinity interactively impact early juvenile development: A bottleneck in barnacle ontogeny. Mar. Biol. **160**: 1109–1117. doi:10.1007/s00227-012-2162-8

Nikulina, E. A., and P. Schäfer. 2006. Bryozoans of the Baltic Sea. Meyniana **58**: 75–95.

Noisette, F., and C. Hurd. 2018. Abiotic and biotic interactions in the diffusive boundary layer of kelp blades create a potential refuge from ocean acidification. Funct. Ecol. **32**: 1329–1342. doi:10.1111/1365-2435.13067

Okamura, B. 1988. The influence of neighbors on the feeding of an epifaunal bryozoan. J. Exp. Mar. Biol. Ecol. **120**: 105–123. doi:10.1016/0022-0981(88)90083-4

Pansch, C., A. Nasrolahi, Y. S. Appelhans, and M. Wahl. 2012. Impacts of ocean warming and acidification on the larval development of the barnacle *Amphibalanus improvisus*.

J. Exp. Mar. Biol. Ecol. **420–421**: 48–55. doi:10.1016/J.JEMBE.2012.03.023

Pansch, C., I. Schaub, J. Havenhand, and M. Wahl. 2014. Habitat traits and food availability determine the response of marine invertebrates to ocean acidification. Glob. Chang. Biol. **20**: 765–777. doi:10.1111/gcb.12478

Pansch, C., P. R. Jonsson, M. Berglin, E. Pinori, and A.-L. Wrange. 2017. A new flow-through bioassay for testing low-emission antifouling coatings. Biofouling **33**: 613–623. doi:10.1080/08927014.2017.1349897

Pansch, C., and C. Hiebenthal. 2019. A new mesocosm system to study the effects of environmental variability on marine species and communities. Limnol. Oceanogr.: Methods **17**: 145–162. doi:10.1002/lom3.10306

Pistevos, J. C. A., P. Calosi, S. Widdicombe, and J. D. D. Bishop. 2011. Will variation among genetic individuals influence species responses to global climate change? Oikos **120**: 675–689. doi:10.1111/j.1600-0706.2010.19470.x

Pratt, M. C. 2008. Living where the flow is right: How flow affects feeding in bryozoans. Integr. Comp. Biol. **48**: 808–822. doi:10.1093/icb/icn052

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing. [accessed 2017 July 30]. Available from http://www.R-project.org/

Rickert, E., U. Karsten, G. Pohnert, and M. Wahl. 2015. Seasonal fluctuations in chemical defenses against macrofouling in *Fucus vesiculosus* and *Fucus serratus* from the Baltic Sea. Biofouling **31**: 363–377. doi:10.1080/08927014.2015.1041020

Rodolfo-Metalpa, R., C. Lombardi, S. Cocito, J. M. Hall-Spencer, and M. C. Gambi. 2010. Effects of ocean acidification and high temperatures on the bryozoan *Myriapora truncata* at natural CO_2 vents. Mar. Ecol. **31**: 447–456. doi:10.1111/j.1439-0485.2009.00354.x

Ryland, J. S. 1974. Behaviour, settlement and metamorphosis of bryozoan larvae: A review. Thalass. Jugoslavica **10**: 239–262.

Saderne, V. 2012. The ecological effect of CO_2 on the brown algae *Fucus serratus* and its epibionts: From the habitat to the organismic scale. Ph.D. thesis. Christian-Albrechts Universität zu Kiel.

Saderne, V., and M. Wahl. 2013. Differential responses of calcifying and non-calcifying epibionts of a brown macroalga to present-day and future upwelling pCO_2. PLoS One **8**: e70455. doi:10.1371/journal.pone.0070455

Saha, M., M. Rempt, S. B. Stratil, M. Wahl, G. Pohnert, and F. Weinberger. 2014. Defence chemistry modulation by light and temperature shifts and the resulting effects on associated epibacteria of *Fucus vesiculosus*. PLoS One **9**: e105333. doi:10.1371/journal.pone.0105333

Saha, M., F. Goecke, and P. Bhadury. 2018. Minireview: Algal natural compounds and extracts as antifoulants. J. Appl. Phycol. **30**: 1859–1874. doi:10.1007/s10811-017-1322-0

Silbiger, N. J., and C. J. B. Sorte. 2018. Biophysical feedbacks mediate carbonate chemistry in coastal ecosystems across spatiotemporal gradients. Sci. Rep. **8**: 796. doi:10.1038/s41598-017-18736-6

Smith, A. M. 2014. Growth and calcification of marine bryozoans in a changing ocean. Biol. Bull. **226**(3): 203–210. doi:10.1086/BBLv226n3p203.

Spilling, K., J. Titelman, T. M. Greve, and M. Kühl. 2010. Microsensor measurements of the external and internal microenvironment of *Fucus vesiculosus* (phaeophyceae). J. Phycol. **46**: 1350–1355. doi:10.1111/j.1529-8817.2010.00894.x

Sproson, D., and E. Sahlée. 2014. Modelling the impact of Baltic Sea upwelling on the atmospheric boundary layer. Tellus A Dyn. Meteorol. Oceanogr. **66**: 24041. doi:10.3402/tellusa.v66.24041

Swezey, D. S., J. R. Bean, A. T. Ninokawa, T. M. Hill, B. Gaylord, and E. Sanford. 2017. Interactive effects of temperature, food and skeletal mineralogy mediate biological responses to ocean acidification in a widely distributed bryozoan. Proc. Biol. Sci. **284**: 20162349. doi:10.1098/rspb.2016.2349

Teagle, H., S. J. Hawkins, P. J. Moore, and D. A. Smale. 2017. The role of kelp species as biogenic habitat formers in coastal marine ecosystems. J. Exp. Mar. Biol. Ecol. **492**: 81–98. doi:10.1016/j.jembe.2017.01.017

Thomsen, J., and others. 2010. Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. Biogeosciences **7**: 3879–3891. doi:10.5194/bg-7-3879-2010

Van Engeland, T., and others. 2011. Potential uptake of dissolved organic matter by seagrasses and macroalgae. Mar. Ecol. Prog. Ser. **427**: 71–81. doi:10.3354/meps09054

Wahl, M., V. Saderne, and Y. Sawall. 2015. How good are we at assessing the impact of ocean acidification in coastal systems? Limitations, omissions and strengths of commonly used experimental approaches with special emphasis on the neglected role of fluctuations. Mar. Freshw. Res. **67**: 25–36. doi:10.1071/MF14154

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

Waldbusser, G. G., and J. E. Salisbury. 2014. Ocean acidification in the coastal zone from an organism's perspective: Multiple system parameters, frequency domains, and habitats. Ann. Rev. Mar. Sci. **6**: 221–247. doi:10.1146/annurev-marine-121211-172238

Wetzel, R. G. 1969. Factors influencing photosynthesis and excretion of dissolved organic matter by aquatic macrophytes in hard-water lakes. SIL Proc. 1922–2010 **17**: 72–85. doi:10.1080/03680770.1968.11895828

Wijgerde, T., S. Jurriaans, M. Hoofd, J. A. J. Verreth, and R. Osinga. 2012. Oxygen and heterotrophy affect calcification of the scleractinian coral *Galaxea fascicularis*. PLoS One **7**: e52702. doi:10.1371/journal.pone.0052702

Acknowledgments

The authors would like to acknowledge Ulrike Panknin for providing *Rhodomonas* cultures and technical assistance and Mandy Kierspel for analyzing the water samples. We also thank Fabian Wolf, Moritz Baumeister, and Jahangir Vajedsamiei for aiding in the laboratory. This work and M.W. were funded through the German Research Foundation (DFG) project awarded to Y.S.: Exploring functional interfaces: extreme biogenic fluctuations may amplify or buffer environmental stress on organisms associated with marine macrophytes (Grant SA 2791/3-1). Open Access funding enabled and organized by ProjektDEAL.

Conflict of Interest

None declared.

Submitted 03 January 2020
Revised 19 May 2020
Accepted 01 October 2020

Associate editor: Steeve Comeau