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Ocean acidification and food limitation combine to suppress herbivory by the gastropod *Lacuna vincta*

Craig S. Young, Alyson Lowell, Bradley Peterson, Christopher J. Gobler*

School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, NY 11968, USA

ABSTRACT: While ocean acidification has different effects on herbivores and autotrophs, how acidification may influence herbivory is poorly understood. This study examined how grazing by the gastropod *Lacuna vincta* (hereafter *Lacuna*) on the macroalgae *Ulva* spp. (hereafter *Ulva*) is influenced by ocean acidification. Herbivory by *Lacuna* was significantly reduced under elevated partial pressure of carbon dioxide (pCO_2; 1500–2000 µatm) relative to ambient pCO_2 (~400 µatm). This significant decrease in herbivory was unrelated to the physiological status of *Ulva* but rather was specifically elicited when *Lacuna* was exposed to elevated pCO_2 in the absence of food for 18 to 24 h prior to grazing *Ulva*. The negative effects of elevated pCO_2 on *Lacuna* were absent at 400 to 800 µatm pCO_2 or when fed but persisted for up to 72 h following a 24 h exposure to elevated pCO_2 without food. Depressed respiration rates in *Lacuna* following exposure to high pCO_2 without food indicated these conditions produced metabolic suppression potentially associated with acidosis. Collectively, the lasting (72 h) nature of grazing inhibition of *Lacuna* following brief exposure (18 h) to moderate pCO_2 levels (>~1500 µatm) when food was not available suggests this process could have broad effects on the dynamics of macroalgae in estuaries where *Lacuna* is a dominant grazer; these effects will be amplified as climate change progresses.

KEY WORDS: Ocean acidification · Gastropods · Macroalgae · Grazing

1. INTRODUCTION

The progressive delivery of CO_2 into surface oceans is depressing levels of pH, CO_3^{2-}, and the saturation states of calcite ($\Omega_{calcite}$) and aragonite ($\Omega_{aragonite}$) (Doney et al. 2009, Feely et al. 2009). Beyond this process of ocean acidification, coastal zones can experience further acidification via upwelling, riverine discharge, and eutrophication-accelerated microbial respiration (Feely et al. 2008, Cai et al. 2011, Melzner et al. 2013, Wallace et al. 2014). In many cases, the seasonal accumulation of CO_2 in some modern coastal zones can create levels that exceed those projected by the year 2100 (>1000 µatm) (Feely et al. 2008, Melzner et al. 2013, Wallace et al. 2014).

Ocean acidification is deleterious to a variety of calcifying organisms, including corals (Kleypas et al. 1999), coralline algae (Gao & Zheng 2010), echinoderms (Wood et al. 2008), bivalves (Gazeau et al. 2007, Talmage & Gobler 2011), and other molluscs, including gastropods (Shirayama & Thorton 2005, Coleman et al. 2014). For gastropods, reduced pH and/or elevated CO_2 levels have been shown to reduce fertility (Hendriks et al. 2010), shell growth (Shirayama & Thorton 2005), and metabolism (Melatunan et al. 2011), with depressed metabolic rates potentially related to an increased reliance on anaerobic metabolism (Bishop & Brand 2000, Seibel & Walsh 2003). Furthermore, elevated partial pressure of carbon dioxide (pCO_2) can create conditions chemically unfavorable for shell

*Corresponding author: christopher.gobler@stonybrook.edu

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synthesis (Manno et al. 2012). Such adverse reactions to ocean acidification juxtapose those of some marine autotrophs, such as seagrasses (Palacios & Zimmerman 2007, Young et al. 2018), phytoplankton (Fu et al. 2012, Hattenrath-Lehmann et al. 2015), and macroalgae (Hepburn et al. 2011, Young & Gobler 2016), that may benefit from increased pCO_2.

While the effects of ocean acidification on the growth and/or survival of various marine herbivores or autotrophs in isolation have been well studied during the past decade, little is known regarding the effects of elevated CO_2 on autotroph–herbivore interactions. Previous studies have found that for numerous autotrophs, elevated pCO_2 can alter tissue C:N ratios by increasing non-structural carbohydrates (Fonseca et al. 1997, Zimmerman et al. 1997) or by enhancing C or N assimilation (Xu et al. 2010), which can affect palatability for grazers (Wakefield & Murray 1998, Lapointe et al. 2004). Recent studies indicate that macroalgae such as *Ulva* can undergo rapid growth and assimilation of C and N under eutrophic and acidified conditions (Pedersen & Borum 1997, Wallace & Gobler 2015, Young & Gobler 2016). As such, gastropods could ultimately benefit from enhanced macroalgal growth under eutrophic and acidified conditions.

The northern *Lacuna* snail *Lacuna vincta* (Gastropoda) is a common grazer in Northwest Atlantic coastal ecosystems that consumes various genera of macroalgae including *Laminaria* and *Ulva* (Brady-Campbell et al. 1984, Chavanich & Harris 2002, Nelson et al. 2008). In ecosystems where *L. vincta* is abundant, it can consume up to 25% of macroalgal productivity (Nelson et al. 2008, Molis et al. 2010). Few, if any, studies have assessed how grazing by *L. vincta* is affected by elevated pCO_2. Given that some genera of macroalgae, such as *Ulva*, benefit from ocean acidification (Olischläger et al. 2013, Young & Gobler 2016), and given *L. vincta*'s preference for this alga (Nelson et al. 2008), it is important to understand how acidification may affect grazer–autotroph interactions (Falkenberg et al. 2013).

The objective of this study was to assess how elevated pCO_2 affects the grazing behavior of *L. vincta* feeding on Northwest Atlantic populations of the macroalgae *Ulva*. A series of experiments was performed to quantify changes in grazing following exposure to elevated pCO_2 and to determine if altered herbivory was associated with changes within the macroalgae or the grazers. Multiple levels and durations of exposure to elevated pCO_2 were assessed in parallel with co-stressors for the snail (e.g. starvation) to obtain a refined understanding of how acidification may alter grazing by *L. vincta*.

2. MATERIALS AND METHODS

2.1. Collection and preparation of *Lacuna vincta* and *Ulva*

Ulva and *L. vincta* used for this study were collected from Shinnecock Bay, NY, USA (40.85° N, 72.50° W; Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m627p083_supp.pdf) during low tide. Large, well-pigmented fronds of *Ulva* and *L. vincta* were collected by hand, placed in seawater-filled containers, and transported to the Stony Brook Southampton Marine Science Center within 15 min of collection. Upon arrival at the facility, *L. vincta* were placed in a 20 l polycarbonate vessel filled with filtered (0.2 µm polysulfone filter capsule, Pall©) seawater taken from the collection site. Aeration was supplied, and recently collected *Ulva* was introduced as a food source until experiments were initiated. Consistent with our previous sequencing efforts and microscopy (Young & Gobler 2016), *Ulva* samples were confirmed as *Ulva rigida*. *L. vincta* are known to be an abundant macroalgae grazer in estuaries of the northeastern US (Chavanich & Harris 2002, Janiak & Whitlatch 2012) and were identified based on morphology. For this study, we refer to the algae as *Ulva* due to the inconsistent macroalgal taxonomic nomenclature as well as sequencing similarities within the internal transcribed spacer region of the ribosome of *Ulva* species (Kirkendale et al. 2013). We refer to *L. vincta* as *Lacuna* for the sake of consistency.

For all experiments, circular sections (~3.5 cm in diameter) of *Ulva* were cut from large thalli with care taken to avoid the potentially reproductive outer region of the organism (Wallace & Gobler 2015). All samples were placed in a salad spinner to remove debris and epiphytes, extensively rinsed with filtered seawater, and spun again to further remove any debris and epiphytes (Young & Gobler 2016). Samples were placed on a flat white board with a scale and a transparency film placed over the samples. A PVC cylinder was used to flatten the samples, taking care to eliminate creases in the samples but without causing any tears. A digital camera was used to image capture the samples, and the images were analyzed using ImageJ software, with the scale of each image individually calibrated. This procedure was performed 15 min prior to the introduction of *Ulva* in experiments (described in Section 2.3).

2.2. Preparation of experiments

Thirteen experiments were performed, in series, to assess the effects of elevated pCO_2 on the grazing behavior of *Lacuna* on *Ulva*. All experiments were performed in 1 l polycarbonate vessels that were acid washed (10% HCl) and liberally rinsed with deionized water prior to use. Experimental vessels were placed in an environmental control chamber set to a temperature (20–23°C), light intensity (300–500 µE m^{-2} s^{-1}), and duration (14 h light:10 h dark cycle) that matched ambient conditions observed at the collection site and optimal conditions for the animals and algae. Vessels were filled with filtered seawater and randomly assigned, in quadruplicate, to each treatment, which varied based on the experiment performed. Two additional containers were filled with filtered seawater and assigned to each treatment, without *Lacuna*, to assess residual *Ulva* growth. To minimize *Ulva* growth during the grazing period of the experiments, the lights in the environmental control chamber were turned off 24 h prior to the introduction of *Lacuna* (Nelson et al. 2008). For all grazing experiments, 8 *Lacuna* (~2 mm) were added to each vessel. The number of *Lacuna* used mimicked densities found on *Ulva* assemblages at the collection site (0.5–1 grazer cm^{-2}) and reported in the literature (Chenelot & Konar 2007, Dubois & Iken 2012).

Dissolved gases were delivered into each experimental container through aeration via air diffusers (Pentair) connected to 1 ml polystyrene serological pipettes inserted into the bottom of each container and connected via Tygon tubing to an air source. Containers were subjected to varying CO_2 levels via multitube gas proportionator systems (Cole Parmer® Flowmeter) that mixed ambient air with 5% CO_2 gas (Talmage & Gobler 2011). The gases were mixed and delivered at a flow rate of 2500 ± 5 ml min^{-1} through gang valves into the serological pipettes that were fit through an opening in the plexiglass used to cover the experimental containers, which resulted in bubbling rates that turned over the volume of the experimental containers >1000 times daily. Bubbling was initiated 3 d prior to the start of each experiment to allow CO_2 levels and carbonate chemistry to reach a state of equilibrium. A Honeywell DuraFET III ion-sensitive field-effect transistor-based solid-state pH sensor (±0.01 pH unit, total scale) was used to measure pH within containers daily. Water samples were taken at the beginning and conclusion of experiments to directly measure dissolved inorganic carbon (DIC) within experimental containers. The samples were preserved using a saturated mercuric chloride solution and stored at ~4°C until analysis on a VINDTA 3D delivery system coupled with a UIC Inc. coulometer (model CM5017O) as reported in Young et al. (2018). CO_2 levels (Table 1) were calculated from measured levels of DIC, pH, temperature, and salinity as well as the first and second dissociation constants of carbonic acid in seawater (Millero 2010) using the program CO2SYS (http://cdiac.ornl.gov/ftop/co2sys/). Certified reference material (CRM; provided by A. Dickson, Scripps Institution of Oceanography, University of California, San Diego; batch 159 = 2027.14 µmol DIC kg^{-1} seawater) was used as a quality assurance of measure, and analyses only proceeded when recovery of CRM was 99.9 to 100%.

Table 1. Values of pH_{total}, temperature (°C), salinity (g kg^{-1}), partial pressure of carbon dioxide (pCO_2; µatm), total dissolved inorganic carbon (DIC; µmol kg^{-1} seawater [SW]), HCO_3^- (µmol kg^{-1} SW), total alkalinity (µmol kg^{-1} SW), saturation state of calcite ($\Omega_{calcite}$), and saturation state of aragonite ($\Omega_{aragonite}$) for Expts 1 to 13. Data values represent means ± SD. Data for individual experiments appear in Table S1 in the Supplement

Parameter	Ambient CO_2	Elevated CO_2
pH_{total}	8.04 ± 0.10	7.42 ± 0.08
Temperature	22.4 ± 1.0	22.4 ± 1.1
Salinity	29.5 ± 0.8	29.6 ± 0.8
pCO_2	443 ± 119	1911 ± 371
Total DIC	1833 ± 91	2036 ± 140
HCO_3^-	1676 ± 94	1932 ± 132
Alkalinity	2031 ± 89	2040 ± 136
$\Omega_{calcite}$	3.61 ± 0.54	1.08 ± 0.20
$\Omega_{aragonite}$	2.33 ± 0.35	0.70 ± 0.13

2.3. Experimental designs

The first 2 experiments were performed to gauge the herbivory rates of *Lacuna* feeding on *Ulva* incubated under ambient (~400 µatm) and elevated (~2000 µatm) pCO_2 (Table 2). For Expts 1 and 2, *Ulva* was incubated for ~1 wk under ambient and elevated CO_2 concentrations. At the conclusion of the incubation period, *Lacuna* were moved to separate vessels without *Ulva* (i.e. starved) under ambient or elevated pCO_2 (24 h). *Lacuna* were introduced into *Ulva*-containing vessels with ambient or elevated pCO_2 and allowed to graze for 24 h. Two additional experiments (Expts 3 and 4; Table 2) were performed to quantify herbivory of *Lacuna* when starved and allowed to graze on *Ulva* that was not being pre-exposed to elevated CO_2 conditions. *Lacuna* were starved for 24 h in ambient or elevated pCO_2 and

Table 2. Experiments with their respective starvation and grazing periods and conditions. Figs. S2, S3, & S4 available in the Supplement. pCO_2: partial pressure of carbon dioxide

Series no.	Expt. no.	Starvation period (h)	Grazing period (h)	Fig. no.	Experimental condition
1	1	24	24	1A	*Lacuna* grazed on *Ulva* incubated for ~1 wk under ambient or elevated pCO_2
	2	24	24	S2	
	3	24	24	S3	*Lacuna* grazed on *Ulva* that was not pre-incubated
	4	24	24	1B	
	5	0	24–72	1C	
2	6	24	24	S4	*Lacuna* starved under ambient or elevated pCO_2; half of snails from each group grazed under ambient and elevated pCO_2 on *Ulva* that was not pre-incubated
	7	24	24	2A	
	8	24	24–72	2B	
	9	24	24	2C	*Lacuna* acclimated to ambient or elevated pCO_2 for 1 wk; starved and grazed under ambient or elevated pCO_2
3	10	24	24	3A	*Lacuna* starved under elevated pCO_2 for 0, 6, 12, 18, or 24 h before grazing on *Ulva* that was not pre-incubated
	11	24	24	3B	*Lacuna* starved and grazed on *Ulva* that was not pre-incubated under a range of pCO_2 levels
	12	24	24–120	3C	*Lacuna* starved under elevated pCO_2 for 24 h and grazed on *Ulva* that was not pre-incubated
	13	24	24	4	*Lacuna* respiration measured pre-starvation, post starvation, and post grazing under ambient or elevated pCO_2

then placed into vessels with *Ulva* under ambient or elevated pCO_2 for 24 h. A fifth experiment (Expt 5; Table 2) followed the same procedure as Expts 3 and 4, where *Lacuna* grazed on *Ulva* not exposed to elevated CO_2, but the *Lacuna* were not starved for 24 h and were allowed to graze for up to 72 h, with *Ulva* samples replaced, and herbivory was quantified every 24 h. At the end of the grazing periods for all experiments, *Lacuna* and *Ulva* were removed from the vessels, and a digital camera was used to capture images of the *Ulva* with analysis using ImageJ as described in Section 2.1. Surface areas of *Ulva* samples grazed on by *Lacuna* were corrected for residual growth using the difference in surface area of *Ulva* samples without *Lacuna*. Herbivory rates were calculated by obtaining the difference in the initial and the final corrected surface areas divided by the number of grazers and the time elapsed of the grazing period (mm^2 $grazer^{-1}$ d^{-1}).

A second set of experiments was performed to quantify herbivory rates of *Lacuna* when starved under ambient or elevated pCO_2 and then allowed to graze on *Ulva* under ambient or elevated pCO_2. During the 2 such reciprocal transplant experiments (Expts 6 and 7; Table 2), *Lacuna* were placed in ambient or elevated pCO_2 and starved for 24 h as described above. At the end of the starvation period, the lights of the environmental chamber were turned off, and half of the *Lacuna* from both CO_2 treatments were introduced into *Ulva*-containing vessels with the same CO_2 level that they were starved in, while the other half were placed in containers with the opposite CO_2 level, for a total of 4 treatments: a control with *Lacuna* that were starved and grazed in ambient pCO_2, a treatment with *Lacuna* starved in ambient pCO_2 that grazed in elevated pCO_2, a treatment with *Lacuna* starved in elevated pCO_2 that grazed in ambient pCO_2, and a treatment with *Lacuna* that were starved and grazed in elevated pCO_2. *Ulva* was exposed to only ambient CO_2 conditions prior to the start of the experiments. Once in their respective containers, *Lacuna* could graze for 24 h. A similar procedure was used for Expt 8, with the only modification being that *Lacuna* were starved for 24 h and then allowed to graze for 72 h, with *Ulva* pre-conditioned at ambient pCO_2, replaced every 24 h, and processed to quantify herbivory (Table 2). Expt 9 was similar to Expts 6 to 8 except that *Lacuna* were placed in ambient or elevated pCO_2 with *Ulva* and allowed to acclimate for 1 wk prior to the 24 h starvation period (Table 2). At the end of the starvation period, the lights of the environmental chamber were turned off, and *Lacuna* were introduced into *Ulva*-containing vessels with elevated or ambient pCO_2 and allowed to graze for 24 h on *Ulva* that was previously exposed to only ambient CO_2 conditions prior to the start of the experiment. At the end of the grazing periods, *Lacuna* and *Ulva* were removed, and grazing rates were calculated.

The final set of experiments (Expts 10–13; Table 2) were performed to produce a refined sense of how CO_2 exposure altered herbivory in *Lacuna*. These experiments specifically quantified the effective minimum dose of CO_2 to alter herbivory rates of *Lacuna*, assessed the effective minimum duration of elevated CO_2 exposure required to alter rates of herbivory, and assessed the duration of CO_2-altered grazing post exposure. For Expt 10, which altered the duration of high CO_2 exposure, 5 treatments were established: a control with ambient CO_2 during the 24 h starvation period (no dose); a treatment with elevated pCO_2 during the entire 24 h starvation period; and treatments with 6, 12, and 18 h of elevated pCO_2 exposure during the starvation period with 18, 12, and 6 h, respectively, of ambient pCO_2 exposure prior to the elevated pCO_2 exposure. At the end of the starvation period, *Lacuna* were transferred to containers with ambient pCO_2, *Ulva* samples were introduced, and *Lacuna* could graze for 24 h, after which rates of herbivory were quantified. For Expt 11, 4 treatments were established: a control with ambient pCO_2 (~400 µatm), a moderate pCO_2 treatment (~850 µatm), an elevated pCO_2 treatment (~1500 µatm), and a high pCO_2 treatment (~2050 µatm). For this experiment, *Lacuna* were placed in one of the 4 CO_2 treatments and starved for 24 h. At the end of the starvation period, *Ulva* samples were introduced, and *Lacuna* could graze for 24 h at their respective CO_2 levels. At the end of the 24 h grazing period, *Ulva* samples were removed, a new set of *Ulva* samples were introduced, and *Lacuna* could graze for another 24 h (48 h total), and after that 24 h period, the process was repeated (72 h total). Herbivory was quantified in each vessel from each time point at each level of CO_2. For Expt 12, the starvation period lasted 24 h, during which *Lacuna* were starved under ambient and elevated pCO_2 but could graze only under ambient pCO_2 during the grazing period, which persisted for 120 h, with new *Ulva* samples introduced every 24 h (Table 2). At the end of the grazing periods for all experiments, *Lacuna* and *Ulva* were removed, and rates of herbivory were quantified.

A single experiment (Expt 13; Table 2) was performed to measure respiration rates of 2 *Lacuna* groups that were exposed to 3 successive conditions: when incubated under ambient CO_2 levels with food, when starved for 24 h under elevated or ambient CO_2 levels, and 24 h after grazing on *Ulva* under elevated or ambient CO_2 levels. A PyroScience® FirestingO_2 optical oxygen sensor was used to quantify changes in dissolved oxygen (DO) concentrations within 2 ml respiration vials. Each vial was slowly filled with seawater from their respective treatments without bubbling, and *Lacuna* from their respective treatments were carefully transferred to each vial. For each of the 3 experimental conditions, 5 *Lacuna* of similar size were selected and analyzed for each group. DO levels within each vial were recorded continuously for 3 h, during which there were near-linear declines in DO with concentrations never declining by more than 50% or <4 mg l^{-1}. At the conclusion of the experiment, *Lacuna* dry tissue weights were obtained by weighing individuals after drying at 60°C for 72 h, combusting them at 450°C for 4 h, and weighing them again. Respiration rates were calculated using the changes in DO and dry tissue weight and were expressed as mg O_2 h^{-1} g^{-1} dry tissue.

2.4. Post-experimental analyses

One-way ANOVA was performed within SigmaPlot 11.0 to assess significant differences in herbivory rates or, in the case of Expt 13, respiration rates under ambient and elevated pCO_2 in Expts 1 to 5, 10, 11, and 13 (Table 2). Two-way ANOVA was performed within SigmaPlot to assess herbivory rates in Expts 6 to 9 and 12, where the main treatment effects were pCO_2 during the starvation and grazing period (ambient or elevated for both; Expts 6–8), pCO_2 during acclimation and the starvation and/or grazing period (ambient or elevated for both; Expt 8), and pCO_2 during the starvation and/or grazing period (ambient, low, medium, and high) and time point (24, 48, and 72 h; Expt 12). Normality was tested via the use of Shapiro-Wilks tests within SigmaPlot 11.0. In the case of Expts 10, 12, and 13, if significant differences were detected, a Tukey's HSD test using R 3.4.0 within RStudio 1.0.143 was performed.

3. RESULTS

3.1. First experimental series: assessing the impact of high CO_2

Lacuna herbivory rates on *Ulva* were highly sensitive to changes in pCO_2. For Expt 1, herbivory rates were significantly lower in elevated pCO_2 relative to ambient conditions, declining from 23 mm^2 $grazer^{-1}$ d^{-1} to zero (1-way ANOVA; $p < 0.05$; Fig. 1A; Table S2). For Expt 2, herbivory rates under elevated pCO_2 were 86% lower than within ambient treatments (1-way ANOVA; $p < 0.05$; Fig. S2; Table S2). During Expts 3 and 4, the sensitivity of *Lacuna* herbivory rates to high CO_2 persisted, even when *Ulva* was

not incubated under high CO_2. In Expt 3, herbivory rates under elevated pCO_2 decreased by ~50% relative to ambient treatments (1-way ANOVA; $p < 0.05$; Fig. S3; Table S2). For Expt 4, herbivory rates within elevated CO_2 treatments were close to zero and ~12-fold lower than herbivory rates in ambient CO_2 treatments (1-way ANOVA; $p < 0.05$; Fig. 1B; Table S2). For Expt 5, when *Lacuna* was not starved before being allowed to graze on *Ulva*, elevated pCO_2 did not significantly affect herbivory rates at the 24, 48, or 72 h time points (1-way ANOVA; $p > 0.05$ for all; Fig. 1C; Table S2).

3.2. Second experimental series: identifying the critical time of exposure

For Expts 6, 7, and 8, *Lacuna* herbivory rates were found to be dependent on the CO_2 concentrations in which they were starved in. In Expt 6, when *Lacuna* were starved under elevated pCO_2, herbivory decreased by nearly 4-fold relative to ambient treatments (2-way ANOVA; $p < 0.05$; Fig. S4; Table S3). Herbivory was not significantly changed by the pCO_2 *Lacuna* were exposed to during the grazing period ($p > 0.05$). In Expt 7, herbivory was reduced by 6-fold when *Lacuna* were starved under elevated pCO_2 (2-way ANOVA; $p < 0.05$; Fig. 2A; Table S3). Herbivory was not significantly affected by the pCO_2 *Lacuna* were exposed to during the grazing period ($p > 0.05$). For Expt 8, herbivory was significantly lower for *Lacuna* after being exposed to elevated pCO_2 during the starvation period at all time points (2-way ANOVA; $p < 0.05$ for all; Fig. 2B; Table S3) but was not significantly affected by the pCO_2 present when *Lacuna* grazed ($p > 0.05$ for all). During Expt 9, *Lacuna* herbivory rates were significantly lower by 5-fold when *Lacuna* were exposed to elevated pCO_2 during the starvation and/or grazing period when pCO_2 was kept constant for both periods

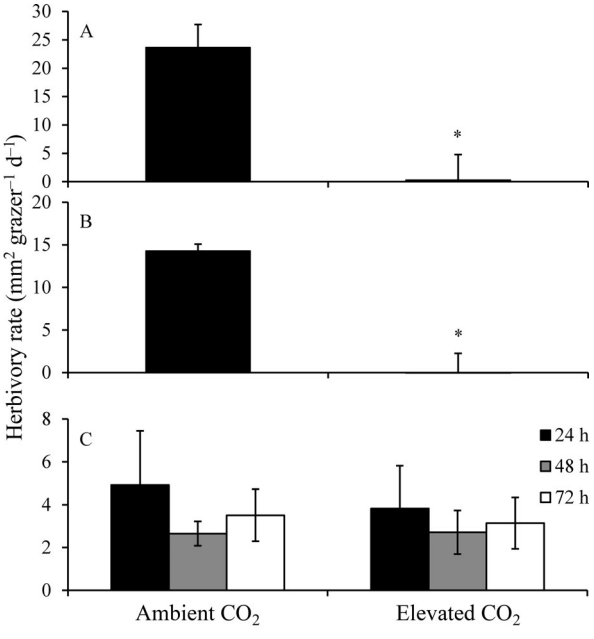

Fig. 1. Herbivory rates of *Lacuna* grazing on *Ulva* under ambient and elevated partial pressure of carbon dioxide (pCO_2) for (A) *Ulva* pre-incubated for 1 wk under ambient and elevated pCO_2 (Expt 1; Table 2), (B) *Ulva* not pre-incubated (Expt 4; Table 2), and (C) *Ulva* not pre-incubated and with-out a starvation period for *Lacuna* (Expt 5; Table 2). Asterisks represent significant differences between treatments ($p < 0.05$). Bars and error bars represent means ± SD

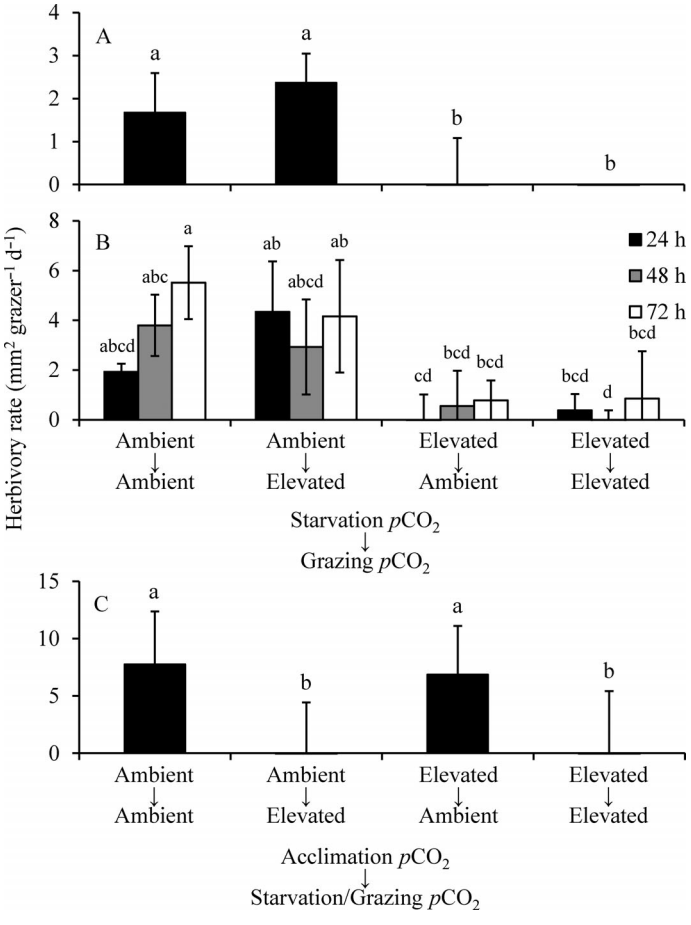

Fig. 2. Herbivory rates of *Lacuna* starved under ambient and elevated partial pressure of carbon dioxide (pCO_2) and allowed to graze on *Ulva* under both CO_2 concentrations for (A) 24 h (Expt 7; Table 2), (B) 24 to 72 h (Expt 8; Table 2), or (C) 24 h following an initial 1 wk to acclimate under ambient and elevated pCO_2 (Expt 9; Table 2). Letters on bars represent significant differences among groups as determined by Tukey Honest Significant Difference tests performed for each 24 h grazing period. Bars and error bars represent means ± SD

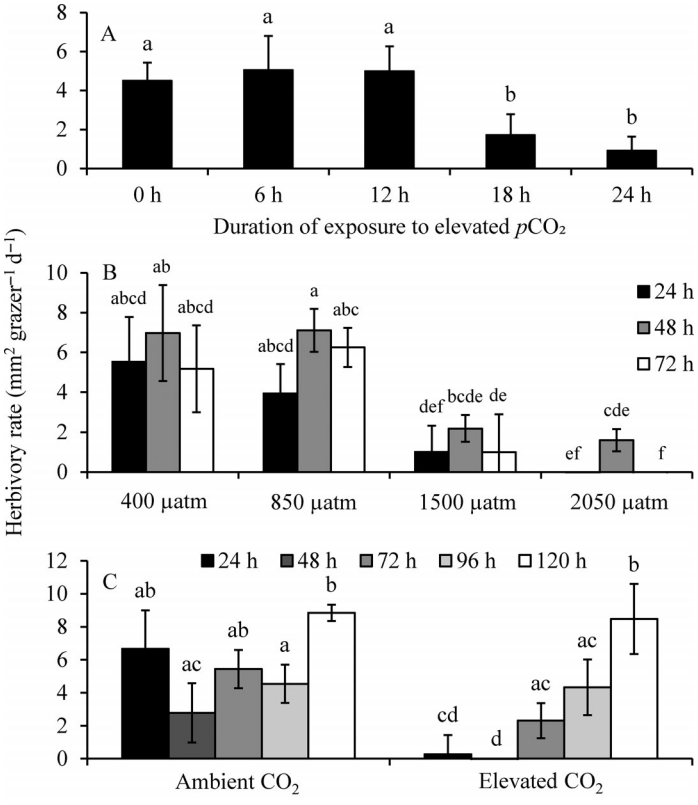

Fig. 3. Herbivory rates of *Lacuna* (A) starved for various lengths of time under ambient and elevated partial pressure of carbon dioxide (pCO_2; Expt 10; Table 2), (B) starved for 24 h and allowed to graze on *Ulva* under varying pCO_2 for 24 to 72 h (Expt 11; Table 2), and (C) starved for 24 h under ambient or elevated pCO_2 and allowed to graze on *Ulva* under only ambient pCO_2 for 24 to 120 h (Expt 12; Table 2). Letters on bars represent significant differences among groups as determined by Tukey Honest Significant Difference tests

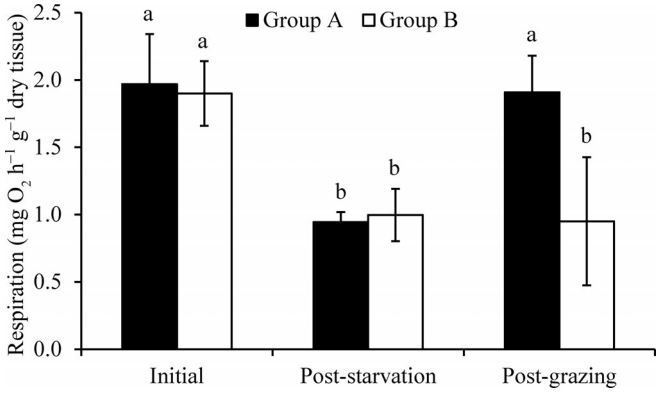

Fig. 4. Respiration rates of *Lacuna* following incubation under ambient partial pressure of carbon dioxide (pCO_2), post starvation, and post grazing for individuals starved under ambient or elevated pCO_2 for 24 h (Groups A and B, respectively; Expt 13; Table 2). Significant differences between Group A and B represent p-values less than 0.05. Columns and error bars represent means ± SD

(2-way ANOVA; $p < 0.05$; Fig. 2C; Table S4). However, herbivory was not significantly altered by the pCO_2 when exposed 1 wk prior to the experiment and supplied food ad libitum ($p > 0.05$).

3.3. Third experimental series: assessing the minimal effective dose and respiration

For Expt 10, herbivory rates differed significantly as a function of the duration of exposure to elevated pCO_2 during the starvation period (1-way ANOVA; $p < 0.05$; Fig. 3A; Table S5). Herbivory rates of individuals exposed to elevated pCO_2 for 18 and 24 h during the starvation period were significantly lower than rates for individuals exposed for 0, 6, and 12 h (Tukey's HSD; $p < 0.05$ for all; Fig. 3A; Table S6). For Expt 11, herbivory rates were found to be inhibited by pCO_2 in a dose-dependent manner, with rates under 1500 and 2050 µatm pCO_2 being significantly and over 4-fold lower than rates of individuals exposed to 400 and 850 µatm (Tukey's HSD; $p < 0.05$ for both; Fig. 3B; Table S7). This trend persisted throughout the 72 h experiment ($p < 0.05$ for both). During Expt 12, *Lacuna* starved under elevated pCO_2 had significantly lower herbivory rates after 24, 48, and 72 h (1-way ANOVA; $p < 0.05$ for all; Fig. 3C; Tables S8 & S9), but the inhibitory effect dissipated by 96 h ($p > 0.05$).

Finally, in Expt 13, respiration rates of *Lacuna* were sensitive to elevated pCO_2 and feeding. Prior to experimental exposure, respiration rates of 2 groups of *Lacuna* incubated under ambient pCO_2 were not significantly different (1-way ANOVA; $p > 0.05$; Fig. 4; Tables S10 & S11). Following the 24 h starvation period, the respiration rates of *Lacuna* starved under both ambient and elevated pCO_2 were both 50% lower than before starvation but not significantly different from each other ($p > 0.05$). Following the 24 h grazing period, the respiration rates of *Lacuna* that were starved and grazed under ambient pCO_2 were significantly higher than individuals under elevated pCO_2 ($p < 0.05$).

4. DISCUSSION

During this study, herbivory rates of *Lacuna* were significantly reduced when snails were exposed to elevated CO_2 concentrations and deprived of food. This occurred whether *Ulva* had been exposed to high or ambient pCO_2 prior to the experiment. Allowing *Lacuna* to acclimate to elevated pCO_2 for 1 wk did not mitigate the suppression of herbivory.

The response of *Lacuna* to the negative effects of elevated pCO_2 and starvation did not occur linearly with increasing CO_2 but rather declined at a threshold of 1500 µatm pCO_2 or higher with an exposure of at least 18 h needed to significantly depress herbivory. Reduced herbivory persisted for 72 h following a 24 h exposure period to elevated pCO_2, and starving and reductions in grazing were accompanied by lowered respiration rates. Collectively, this study revealed a novel mechanism by which acidification can disrupt a fundamental ecological process in coastal zones: gastropod herbivory on macroalgae.

Exposure to elevated pCO_2 in the absence of food led to a metabolic depression in *Lacuna* as evidenced by lowered respiration rates. Consistent with our measurements, marine gastropods have been shown to experience significantly reduced respiration during prolonged periods of food limitation (Maas et al. 2011). Furthermore, lowered respiration and metabolic rates in response to elevated CO_2 levels have been documented in other gastropods (Bibby et al. 2007, Hendriks et al. 2010, Melatunan et al. 2011) and other common estuarine organisms (Lindinger et al. 1984, Pörtner et al. 1998, Todgham & Hofmann 2009). The gross growth efficiency for marine gastropods can be as low as ~6% (Odum & Smalley 1959) and is dependent on food supply (Carefoot 1967). Exposure to elevated pCO_2 can cause acidosis, which can disrupt metabolism, homeostatic functions, and the function of energy transduction and divert energy from shell and somatic growth (Lindinger et al. 1984, Pörtner et al. 1998, Marchant et al. 2010). Depressed metabolism may also serve as a survival strategy to match the lowered energy supply (Bishop & Brand 2000, Seibel & Walsh 2003) available due to starvation and, potentially, an increased reliance on anaerobic respiration (Pörtner et al. 1998, Melatunan et al. 2011). In the present study, the suppression of grazing rates under elevated pCO_2 and in the absence of food likely reflects a broad-scale suppression of *Lacuna* metabolism associated with concurrently experiencing acidosis and starvation.

Beyond the direct effects on herbivory in *Lacuna,* elevated pCO_2 may have a multitude of other effects on this and other marine gastropods. The continued delivery of CO_2 into coastal ecosystems is expected to negatively affect the growth and survival of gastropods and other calcifying organisms through reductions in the availability of carbonate. Exposure to elevated pCO_2 and/or low pH conditions for prolonged periods of time has been shown to reduce shell growth, repair rates, and physiological integrity in numerous marine gastropods (Shirayama & Thorton 2005, Bibby et al. 2007, Manno et al. 2012, Coleman et al. 2014). *Lacuna* are particularly vulnerable to reductions in $\Omega_{calcite}$ (Taylor & Reid 1990) and may need to reallocate extra energy to counteract shell dissolution (Manno et al. 2012). As mentioned previously, diversion of energy toward maintenance of shell integrity may have contributed to metabolic depression in *Lacuna* in the present study.

While tissue C:N ratios and anti-grazing compounds in macroalgae can be altered by elevated pCO_2 (Gordillo et al. 2001, Young & Gobler 2016) and may affect grazing behavior (Wakefield & Murray 1998, Falkenberg et al. 2013), the exposure of *Ulva* to high CO_2 prior to grazing did not alter herbivory rates of *Lacuna* during this study. Herbivory rates of *Lacuna* were significantly reduced when exposed to elevated pCO_2 prior to feeding on *Ulva* that was grown under high or low CO_2. Furthermore, grazing rates of *Lacuna* on *Ulva* under high CO_2 were not reduced when the snails had not been starved for 24 h prior to grazing.

Beyond the delivery of CO_2 via the combustion of fossil fuels, eutrophication-enhanced microbial respiration is a strong source of CO_2 into coastal zones that can result in pCO_2 that exceeds levels projected for the year 2100 (>2000 µatm) (Feely et al. 2008, Cai et al. 2011, Melzner et al. 2013, Wallace et al. 2014). While *Ulva* experienced enhanced growth under elevated pCO_2 in this study (Fig. S5) and has been shown to experience enhanced growth under elevated CO_2 and nutrient concentrations (Olischläger et al. 2013, Young & Gobler 2016, Ober & Thornber 2017), nutrient enrichment can reduce the abundances of numerous common estuarine grazers, including gastropods such as *Lacuna*, due to potentially toxic levels of ammonia (Atalah & Crowe 2012). Furthermore, the proliferation, death, and decomposition of macroalgal mats can result in the accumulation of sulfides, significantly reducing DO concentrations (Hauxwell et al. 2001) and increasing levels of pCO_2 (Wallace et al. 2014). During short-term hypoxic events, tolerance to hypoxia by marine gastropods is reduced by elevated hydrogen sulfide concentrations (Hiroki 1978), and as this study revealed, their respiration rates may decline in response to acidification. Given that *Ulva* is capable of undergoing rapid growth under eutrophic conditions (Pedersen & Borum 1997, Wallace & Gobler 2015) and the depression of *Lacuna* herbivory rates after exposure to excess CO_2 when not actively grazing, it would seem that eutrophication and eutrophication-promoted acidification that inhibit grazing on *Ulva* could synergistically facilitate the proliferation of algal bio-

mass and events known as green tides (Zhao et al. 2013) via both top-down and bottom-up processes. Moreover, elevated CO_2, hypoxia, and high nutrient concentrations symptomatic of eutrophication may establish a positive feedback loop with regard to *Ulva* blooms in coastal zones in that the reduction of herbivory rates of *Lacuna* would facilitate the overgrowth of *Ulva* and, through the eventual decomposition of the algae, lead to the accumulation of CO_2, sulfides, ammonia, and hypoxia zones that could collectively contribute to further declines in grazer abundance and herbivory rates, promoting continued macroalgal overgrowth.

In the present study, *Lacuna* herbivory rates did not decrease linearly with increasing pCO_2 but rather declined after exposure to ~1500 µatm, with the potential for negative effects to take effect above 850 µatm, which is within the range of projected pCO_2 for world oceans as early as the end of the 21st century (Doney et al. 2009, Feely et al. 2009). In the present study, 18 h of exposure to elevated pCO_2 in the absence of food suppressed *Lacuna* herbivory rates, with the grazing suppression persisting for 72 h. In an ecosystem context, this suggests that gastropods sensitive to elevated pCO_2 may be vulnerable to both diurnal and seasonal shifts in pH and pCO_2. Shallow eutrophic estuaries can become strongly net heterotrophic at night and during low tides, with acidified conditions sometimes persisting 18 h (O'Boyle et al. 2013, Wallace et al. 2014, Baumann et al. 2015). Such conditions are most common in temperate estuaries during late summer when high microbial respiration rates intensify benthic acidification, with declines in pH and Ω and increases in pCO_2 above levels that negatively affected *Lacuna* in the present study (>1500 µatm) (Wallace et al. 2014). Such acidification is often coupled with hypoxia (Melzner et al. 2013, Wallace & Gobler 2015), which may further exacerbate deleterious effects on herbivory rates (Melatunan et al. 2011, Russell et al. 2013, Gobler & Baumann 2016).

In the present study, *Lacuna* was tolerant of elevated pCO_2 when fed ad libitum. Previous studies have found that growth and calcification of blue mussels *Mytilus edulis* (Melzner et al. 2011, Thomsen et al. 2013) and other calcifying organisms (Pansch et al. 2014) were resistant to acidification when there was an abundance of food. Similarly, forage fish *Menidia beryllina* and *Cyprinodon variegatus* were found to be more vulnerable to the harmful effects of elevated pCO_2 when their food supply was restricted (Gobler et al. 2018). Adequate food availability yielding optimal energy supplies may increase the likelihood that individuals will be able to upregulate the biochemical pathways needed to resist the negative effects of elevated pCO_2 (Thomsen et al. 2013, Pansch et al. 2014). Given that *Lacuna* utilize macroalgae as a habitat and a food source (Martel & Chia 1991, Chavanich & Harris 2002), moderate levels of algae have the potential to minimize the harmful effects of exposure to acidification.

Macroalgae such as *Ulva* could partially mitigate the harmful effects of elevated pCO_2 on gastropods. Beyond its role as a food source, *Ulva* may buffer carbonate chemistry through photosynthetic activity, serving as a refuge for organisms negatively affected by elevated pCO_2 levels (Krause-Jensen et al. 2015, Wahl et al. 2018, Young & Gobler 2018). Given that *Lacuna* were not significantly impacted by elevated pCO_2 when able to graze on *Ulva* and that photosynthesis by *Ulva* can partly counteract acidification via photosynthesis (Young & Gobler 2018), it seems likely that if *Lacuna* were exposed to elevated pCO_2, a robust population of *Ulva* could create a refuge to protect *Lacuna.* The extent to which *Ulva* may mitigate the harmful effects of acidification on *Lacuna* is likely dependent on several factors including water residence time, water column depth, growth stage and abundance of *Ulva*, and extent of eutrophication. Deeper water bodies with rapid flushing times, poorly growing or scarce *Ulva*, and/or lower nutrient loading rates would be less likely to experience the carbonate buffering of macroalgae. Furthermore, the beneficial nature of *Ulva* could seasonally transform into a harmful effect for *Lacuna* when *Ulva* dies off and creates high CO_2 and low food conditions potentially coupled with poor water quality (high ammonia and sulfides, low oxygen).

Reduced herbivory by grazers under elevated pCO_2 could have consequences for other macroalgae. While *Ulva* is an important food source for *Lacuna* (Nelson et al. 2008), *Lacuna* and other marine gastropods also often consume laminarialean kelps due to their lower anti-grazing defenses (phlorotannins) and high palatability (Wakefield & Murray 1998). As a common grazer in coastal Northwest Atlantic ecosystems (Chavanich & Harris 2002, Janiak & Whitlatch 2012) and the only mesograzer in the region known to consume macroscopic kelp sporophytes (Brady-Campbell et al. 1984, Johnson & Mann 1986), large population increases in *Lacuna* can result in significant consumption of kelp blades and the loss of canopies of kelp beds in the Northwest Atlantic region (Fralick et al. 1974, Krumhansl & Scheibling 2011). Elevated pCO_2 is known to significantly enhance the growth of various kelp species

(Swanson & Fox 2007, Hepburn et al. 2011). If increased CO_2 suppresses *Lacuna* herbivory on kelp, future CO_2 increases associated with climate change may indirectly benefit kelp beds by both promoting growth and potentially reducing *Lacuna* herbivory rates.

In conclusion, exposure of *Lacuna* to elevated pCO_2 in the absence of food reduced herbivory rates on *Ulva*. Reduced respiration rates of *Lacuna* following exposure to elevated pCO_2 in the absence of food evidenced a metabolic depression as a response to these dual stressors. These impacts may be common in estuaries given that the threshold dose that elicited the effects (18 h, 1500 µatm pCO_2) are found in many eutrophic estuaries during summer (Feely et al. 2008, Melzner et al. 2013, Wallace et al. 2014) and will be more common in the future (Doney et al. 2009). The lasting effect on grazing suppression (72 h) demonstrates that even ephemeral transitions into low food, high CO_2 conditions could have lasting ecosystem ramifications. Reduced herbivory facilitated by acidification may limit the consumption of macroalgal biomass, allowing green tides to proliferate unchecked, ultimately promoting the accumulation of sulfides, hypoxia, and CO_2 and perhaps further reducing top-down control of macroalgal biomass. The extent to which gastropods may be released from the negative effects of acidification by macroalgae buffering carbonate chemistry and providing food is unknown and warrants future investigation.

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