Dual benefit of ocean acidification for the laminarialean kelp *Saccharina latissima*: enhanced growth and reduced herbivory

Craig S. Young, Michael H. Doall, Christopher J. Gobler*

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

ABSTRACT: The laminarialean kelp *Saccharina latissima* is a common macroalga along rocky shorelines that is also frequently used in aquaculture. This study examined how ocean acidification may alter the growth of *S. latissima* as well as grazing on *S. latissima* by the gastropod *Lacuna vincta*. Under elevated nutrients, *S. latissima* experienced significantly enhanced growth at pCO₂ levels ≥1200 μatm compared to ambient pCO₂ (~400 μatm). Elevated pCO₂ (≥830 μatm) also significantly reduced herbivory of *L. vincta* grazing on *S. latissima* relative to ambient pCO₂. There was no difference in grazing of *S. latissima* previously grown under elevated or ambient pCO₂, suggesting lowered herbivory was due to harm to the gastropods rather than alteration of the biochemical composition of the kelp. Decreased herbivory was specifically elicited when *L. vincta* were exposed to elevated pCO₂ in the absence of food for ≥18 h prior to grazing, with reduced grazing persisting 72 h. Elevated growth of *S. latissima* and reduced grazing by *L. vincta* at 1200 μatm pCO₂ combined to increase net growth rates of *S. latissima* more than 4-fold relative to ambient pCO₂. *L. vincta* consumed 70% of daily production by *S. latissima* under ambient pCO₂ but only 38 and 9% at 800 and 1200 μatm, respectively. Collectively, decreased grazing by *L. vincta* coupled with enhanced growth of *S. latissima* under elevated pCO₂ demonstrates that increased CO₂ associated with climate change and/or coastal processes will dually benefit commercially and ecologically important kelps by both promoting growth and reducing grazing pressure.

KEY WORDS: Ocean acidification · Kelp · Gastropods · Grazing

1. INTRODUCTION

Ocean acidification is changing marine ecosystems. As anthropogenic CO₂ accumulates in the atmosphere and surface oceans, levels of pH, CO₃²⁻, and the saturation states of calcium carbonate are declining (Doney et al. 2009, Feely et al. 2009). In addition, many coastal ecosystems can experience partial pressure of CO₂ (pCO₂) levels not projected to occur in open ocean systems until the year 2100 (>1000 μatm) due to a multitude of processes, including upwelling (Feely et al. 2008), riverine discharge (Vargas et al. 2016), macrophyte respiration (Wahl et al. 2018), and eutrophication-enhanced microbial respiration (Wallace et al. 2014). These changes in ocean chemistry stand to reorganize the function of coastal ecosystems, as acidification can be inhibitory to some marine animals (Poloczanska et al. 2016), especially calcifiers (Talmage & Gobler 2010, Young et al. 2019), but may benefit other ocean organisms (Koch et al. 2013, Young & Gobler 2016).

*Saccharina latissima* (also known as sugar kelp) is a bladed, cold-water brown macroalga that is widely distributed across the North Atlantic, Pacific, and Arctic Oceans (Brinkhuis et al. 1983, Sivertsen & Bjørgen 2015). *S. latissima* can form dense, highly productive and biologically diverse beds that provide numerous ecosystem services, including nursery

*Corresponding author: christopher.gobler@stonybrook.edu
habitat, refuge from predators, coastal defense, carbon and nitrogen sequestration, and food sources for other organisms (Norderhaug et al. 2005, Chung et al. 2013, Smale et al. 2013). More recently, the commercial importance and aquaculture of S. latissima and other species of kelp has grown (Marinho et al. 2013). Excessive grazing, however, can significantly lower kelp abundance and ecosystem function (Scheibling et al. 1999, Norderhaug & Christie 2009). Moreover, global distributions of S. latissima are declining due to increasing temperatures (Filbee-Dexter et al. 2016), eutrophication (Moy & Christie 2012), and overfishing of the predators of kelp grazers (Steckley et al. 2002). However, recent evidence suggests that elevated pCO2 associated with ocean acidification could benefit some species of kelp, potentially countering the processes contributing to their decline (Hepburn et al. 2011).

Beyond climate change, eutrophication may also act to negatively impact kelp communities. In some temperate regions, excessive nutrient loading can initiate a succession whereby kelp forests become overgrown by turf algae (Eriksson et al. 2002, Connell et al. 2008) and this succession may be accelerated by acidification (Connell & Russell 2010). Still, kelp can also exist in ecosystems where turf algae are rare, and kelp can be purposely grown in more eutrophic locales for aquaculture purposes (Kim et al. 2015, Jiang et al. 2020). While climate change and eutrophication are strong environmental drivers in coastal ecosystems, the manner in which nutrients and acidification act, and potentially interact, to directly alter kelp growth is not fully clear.

The northern Lacuna snail Lacuna vincta (Gastropoda) is a common grazer in coastal North Atlantic and North Pacific ecosystems (Chavanich & Harris 2002, Janiak & Whitlatch 2012) and is a well-known grazer of macroscopic kelp sporophytes (Brady-Camperell et al. 1984, Johnson & Mann 1986). Grazing by large populations of L. vincta can cause extensive damage to kelp blades in kelp beds (Chenelot & Konar 2007, Krumhansl & Scheibling 2011), consuming up to 10% of the surface area of S. latissima (Molis et al. 2010, Krumhansl & Scheibling 2011). The resulting damage done by the grazer on the kelp blade, meristem, and stipe can significantly lower the tensile strength of the kelp, making it more susceptible to breakage (Chenelot & Konar 2007, Molis et al. 2010). High grazing intensity by L. vincta on the reproductive sorus tissue of S. latissima may exacerbate recruitment limitation and further hinder the recovery of degraded kelp beds (O’Brien & Scheibling 2016). Compared to other macroalgae native to the northern Atlantic Ocean, laminarialean kelps are preferred by L. vincta due to overall lower phlorotannins (anti-grazing defense) and relatively high palatability (Wakefield & Murray 1998). While ocean acidification can disrupt the grazing by L. vincta on some macroalgae (Young et al. 2019), the manner in which L. vincta herbivory on kelps might be altered by this process is unknown.

The overarching objective of this study was to assess how coastal acidification may affect Northwest Atlantic populations of S. latissima. Experiments quantified the growth rates as well as elemental and isotopic content of S. latissima under treatment levels of pCO2 with and without nutrients. Experiments were also performed to quantify herbivory rates of L. vincta on S. latissima under normal and elevated levels of pCO2. Given that prior research has determined that the effects of acidification on grazing by L. vincta are dose-dependent with regard to both pCO2 levels and exposure duration (Young et al. 2019), experiments exploring differing levels of pCO2 and differing durations of exposure were performed. Given that the effects of acidification on invertebrates can depend on food supply (Melzner et al. 2011, Thomsen et al. 2013, Pansch et al. 2014), exposures to acidification were made following periods of ad libitum and restricted feeding. Finally, the net growth rates (growth minus grazing) of S. latissima were determined under treatment pCO2 conditions, providing a novel examination of the net effects of ocean acidification on a keystone macrophyte. While studies exploring how ocean acidification affects herbivory have been limited, to our knowledge this is the first study to assess how ocean acidification concurrently alters growth and grazing for a macrophyte.

2. MATERIALS AND METHODS

2.1. Collection and preparation of Lacuna vincta and Saccharina latissima

Lacuna vincta used for this study were collected by hand during low tide from Shinnecock Bay, NY, USA (40.85°N, 72.50°W), part of New York’s South Shore Estuary Reserve (NYSSER) (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m664p087_supp.pdf). L. vincta are an abundant macroalgae grazer in estuaries of the northeastern USA (Chavanich & Harris 2002, Janiak & Whitlatch 2012) and were identified based on morphology. Saccharina latissima used in the study was cultured from blades collected from
Long Island Sound, spawned on line, and grown along horizontal longlines at a commercial oyster farm (i.e. Great Gun Shellfish) in Moriches Bay, NY (40.78° N, 72.78° W), a lagoon contiguous with Shinnecock Bay to the east. Large, well-pigmented blades of *S. latissima* were selected from samples collected by hand at low tide (Fig. S1). *S. latissima* were cut from their holdfasts as close to the longlines as possible. Following collection, *S. latissima* and *L. vincta* were immediately placed in seawater-filled containers and transported to the Stony Brook Southampton Marine Science Center of Stony Brook University within 30 min of collection. Upon arrival to the facility, *L. vincta* were placed in a 20 l polycarbonate vessel filled with filtered (0.2 μm) seawater taken from the collection site. The vessel was supplied with air and recently collected *S. latissima* as a food source until experiments were initiated (Duffy et al. 2014). *S. latissima* used in experiments were placed in a large, round ~2000 l tank filled with flowing, 1 μm filtered seawater and assigned, without 1 μm filtered seawater from Shinnecock Bay.

For experiments performed to quantify algal growth rates in response to elevated pCO$_2$ and/or nutrients, individual *S. latissima* rectangular sporophytes were prepared by cutting the stipe 2.5 cm below the blade–stipe interface and cutting the blade 5 cm above the blade–stipe interface. This was done to standardize initial kelp blade tissue type and size (Boderskov et al. 2016). *S. latissima* samples were weighed on a Scientech ZSA 120 digital micro-balance (±0.0001 g) to obtain initial fresh weight in grams. For experiments performed to quantify grazing rates on *S. latissima*, rectangular sections (2 × 4 cm, length × width) of the algae were cut from large blades with care taken to ensure uniformity of size, shape, and tissue type. Sections from the upper blades of *S. latissima* were used due to *L. vincta*’s preference for this section of the organism over the lower blade, meristem, or stipe (Molis et al. 2010). All samples were spun in a salad spinner to remove debris and epiphytes, extensively rinsed with filtered (0.2 μm) seawater before being spun again to further remove any remaining debris, epiphytes, and excess seawater (Young & Gobler 2016), and weighed as described above.

### 2.2. Preparation of experiments

Two experiments were performed to assess the effects of pCO$_2$ and nutrients on the growth rates of *S. latissima* and 5 experiments were performed to assess the effects of elevated pCO$_2$ on the herbivory rates of *L. vincta* on *S. latissima*. Each experiment was performed in 1 l polycarbonate vessels that were acid-washed (10% HCl) and liberally rinsed with deionized water. All experimental containers were placed in an environmental control chamber set to a temperature (~10–18°C), light intensity (~250 μmol photons m$^{-2}$ s$^{-1}$), and duration (12 h light:12 h dark cycle) that matched ambient conditions at the collection site to allow for optimal conditions for *L. vincta* and *S. latissima*. As temperatures rose through the spring, so did the temperatures used for experiments to minimize thermal shock to the kelp and snails. All containers were filled with filtered seawater, randomly placed within the environmental control chamber, and randomly assigned in quadruplicate to each treatment, which varied based on the experiment performed. For grazing experiments (see below), 2 additional containers were filled with filtered seawater and assigned, without *L. vincta*, to assess *S. latissima* residual growth (i.e. additional growth the *S. latissima* samples experienced during the grazing period). During those experiments, the lights of the environmental control chamber were turned off 24 h prior to the introduction of *Lacuna* in order to minimize residual *S. latissima* growth (Nelson et al. 2008, Young et al. 2019). For each grazing experiment, 8 *L. vincta* (~3 mm) were added to each separate container, mimicking densities found on macroalgae at the collection site (0.5–1 grazer cm$^{-2}$) and reported in the literature (Chenelot & Konar 2007, Dubois & Iken 2012, Young et al. 2019).

Dissolved gases were delivered into each experimental container via air diffusers (Pentair) connected to 1 ml polystyrene serological pipettes inserted into the bottom of each vessel and connected via Tygon tubing to an air source. The containers that were subjected to treatment CO$_2$ conditions utilized multtube gas proportioner systems (Cole Parmer® Flowmeter) to mix ambient air with 5% CO$_2$ (Talmage & Gobler 2010). The containers subjected to ambient conditions utilized single tube proportioner systems to introduce only ambient air into the containers. The gases were mixed through gang valves and were delivered at a flow rate of 2500 ± 5 ml min$^{-1}$ to experimental containers through serological pipettes inserted through plexiglass covers on the containers. The bubbling rates in the containers turned over the volume >1000 times d$^{-1}$, and bubbling was initiated at least 2–3 d prior to the initiation of experiments to allow CO$_2$ levels and carbonate chemistry to reach a state of equilibrium. A Honeywell DuraFET III ion-sensitive field effect transistor-based (ISFET) solid-state pH sensor (±0.01 pH unit, total scale) was used to measure pH.
To quantify the effects of elevated pCO2, experiments (designated as ‘Co-effects of pCO2 and nutrients’; Table 1) were performed to assess how high and low pCO2 would influence the growth rates of S. latissima. Estuaries are dynamic environments where levels of nutrients vary in time and space (Wallace et al. 2014; R. B. Wallace et al. unpubl. data). Given prior studies (Olshager et al. 2012, Zhang et al. 2020), we hypothesized that elevated pCO2, and or nutrients on the growth of S. latissima, 2.3. Assessing the effects of elevated nutrients and pCO2 on S. latissima

Table 1. Mean (±SD) pH (total scale), temperature (°C), salinity (g kg−1), pCO2 (μatm), total dissolved inorganic carbon (DIC; μmol kg−1 seawater (SW)), total alkalinity (TA; μmol kg−1 SW), and HCO3− (μmol kg−1 SW), and the saturation states of aragonite and calcite (Ωarag and Ωcalc, respectively) for all experiments.

| Experiment                                      | pCO2 level | pH      | Temp. | Salinity | pCO2 | Total DIC | TA | HCO3− | Ωarag | Ωcalc |
|------------------------------------------------|------------|---------|-------|----------|------|-----------|----|--------|-------|-------|
| Co-effects of pCO2 and nutrients                | Ambient    | 7.98 ± 0.04 | 14.9 ± 0.7 | 28.4 ± 0.4 | 377 ± 11 | 1710 ± 66 | 1857 ± 74 | 1591 ± 59 | 1.65 ± 0.11 | 2.61 ± 0.17 |
|                                              | Elevated   | 7.31 ± 0.03 | 15.1 ± 0.5 | 28.4 ± 0.4 | 2053 ± 132 | 1877 ± 12 | 1836 ± 15 | 1775 ± 12 | 0.36 ± 0.02 | 0.60 ± 0.04 |
| pCO2 dose response                              | Ambient    | 7.89 ± 0.01 | 20.3 ± 0.4 | 28.7 ± 0.9 | 479 ± 9 | 1961 ± 5 | 2084 ± 3 | 1844 ± 6 | 1.48 ± 0.02 | 2.34 ± 0.03 |
|                                              | Low        | 7.69 ± 0.02 | 20.3 ± 0.4 | 28.0 ± 0.4 | 802 ± 2 | 2057 ± 41 | 2122 ± 45 | 1957 ± 38 | 1.01 ± 0.04 | 1.60 ± 0.06 |
|                                              | Medium     | 7.50 ± 0.04 | 20.2 ± 0.4 | 27.8 ± 0.1 | 1232 ± 6 | 2061 ± 7 | 2073 ± 6 | 1965 ± 7 | 0.66 ± 0.00 | 1.05 ± 0.01 |
|                                              | High       | 7.27 ± 0.05 | 20.2 ± 0.4 | 30.5 ± 1.5 | 2388 ± 3 | 2118 ± 3 | 2051 ± 3 | 1994 ± 3 | 0.35 ± 0.00 | 0.56 ± 0.00 |
| Effects of high and low pCO2 on herbivory       | Ambient    | 7.90 ± 0.03 | 14.4 ± 1.0 | 29.0 ± 0.0 | 425 ± 11 | 1729 ± 16 | 1866 ± 14 | 1614 ± 16 | 1.57 ± 0.01 | 2.47 ± 0.02 |
|                                              | Elevated   | 7.30 ± 0.04 | 14.4 ± 0.9 | 29.0 ± 0.0 | 1804 ± 191 | 1958 ± 51 | 1933 ± 65 | 1857 ± 53 | 0.47 ± 0.07 | 0.75 ± 0.12 |
| Direct vs. indirect effects of elevated pCO2 on herbivory | Ambient    | 7.95 ± 0.05 | 15.5 ± 1.7 | 29.3 ± 0.4 | 453 ± 9 | 1831 ± 64 | 1985 ± 68 | 1702 ± 59 | 1.80 ± 0.08 | 2.82 ± 0.12 |
|                                              | Elevated   | 7.41 ± 0.05 | 15.5 ± 1.7 | 29.3 ± 0.4 | 1808 ± 28 | 1921 ± 3 | 1908 ± 6 | 1824 ± 3 | 0.52 ± 0.01 | 0.82 ± 0.02 |
| Co-effects of elevated pCO2 and food restriction on herbivory | Ambient    | 8.00 ± 0.04 | 16.0 ± 0.7 | 30.0 ± 0.3 | 459 ± 25 | 1939 ± 71 | 2088 ± 70 | 1811 ± 68 | 1.72 ± 0.03 | 2.72 ± 0.05 |
|                                              | Elevated   | 7.28 ± 0.03 | 15.8 ± 0.7 | 29.9 ± 0.1 | 2035 ± 33 | 1856 ± 0 | 1814 ± 2 | 1753 ± 1 | 0.37 ± 0.01 | 0.58 ± 0.01 |
| Effective minimum exposure duration             | Ambient    | 7.87 ± 0.05 | 19.7 ± 0.3 | 29.1 ± 0.1 | 453 ± 8 | 1906 ± 2 | 2084 ± 6 | 1759 ± 0 | 2.10 ± 0.04 | 3.28 ± 0.06 |
|                                              | Elevated   | 7.24 ± 0.03 | 19.3 ± 0.3 | 29.9 ± 0.1 | 2843 ± 5 | 1934 ± 4 | 1874 ± 4 | 1816 ± 4 | 0.36 ± 0.00 | 0.56 ± 0.00 |
| Effective minimum dose                          | Ambient    | 7.92 ± 0.01 | 20.3 ± 0.1 | 27.8 ± 0.2 | 451 ± 6 | 1701 ± 3 | 1848 ± 5 | 1579 ± 2 | 1.73 ± 0.02 | 2.70 ± 0.04 |
|                                              | Low        | 7.70 ± 0.02 | 20.4 ± 0.1 | 27.7 ± 0.0 | 830 ± 1 | 1745 ± 2 | 1814 ± 2 | 1653 ± 2 | 1.03 ± 0.01 | 1.61 ± 0.00 |
|                                              | Medium     | 7.46 ± 0.03 | 20.4 ± 0.1 | 27.4 ± 0.3 | 1418 ± 74 | 1807 ± 9 | 1820 ± 5 | 1719 ± 8 | 0.65 ± 0.02 | 1.02 ± 0.04 |
|                                              | High       | 7.21 ± 0.05 | 20.4 ± 0.1 | 28.8 ± 1.0 | 2453 ± 79 | 2044 ± 42 | 2007 ± 41 | 1933 ± 39 | 0.48 ± 0.01 | 0.75 ± 0.01 |
vated nutrients might be inhibitory (Connell et al. 2008). *S. latissima* was placed in 1 of 4 treatments established in quadruplicate (for this and all experiments): a control with ambient $p$-$CO_2$ levels (350–450 μatm) and no nutrient additions, a treatment with ambient $p$-$CO_2$ and nutrient additions (50 μM nitrate, 3 μM phosphate), a treatment with elevated $p$-$CO_2$ levels (~1800–2100 μatm) and no nutrient additions, and a treatment with elevated $p$-$CO_2$ and nutrient additions. For this experiment, *S. latissima* samples were incubated under these conditions for 7 d. The elevated nutrients and $p$-$CO_2$ concentrations were higher than levels present at the collection site (0–10 μM nitrate, 0–1 μM phosphate, 400–800 μM $p$-$CO_2$), but consistent with concentrations present in some US East Coast estuaries, including those in the nearby New York region during winter and spring (Gobler et al. 2006, Wallace et al. 2014). For example, total dissolved nitrogen concentrations exceeding 50 μM have been reported in Quantuck Bay (Gobler et al. 2011), the estuary contiguous with and 10 km from the *S. latissima* collection site, and $p$-$CO_2$ levels in the range of 1000–3000 μatm have recently been reported for Shinnecock Bay and the Peconic Estuary (Wallace et al. unpubl. data), systems contiguous with the *S. latissima* collection site. Given the ability of future ocean acidification to synergistically depress pH values when coupled with eutrophication-driven acidification (Sunda & Cai 2012), we expect future climate change scenarios to increase regional $p$-$CO_2$ concentrations to levels higher than presently observed.

For the second experiment (‘$p$-$CO_2$ dose response’; Table 2), the goal was to identify the minimum level of $p$-$CO_2$ needed to yield enhanced growth rates considering the levels currently present in regional estuaries (Wallace et al. 2014, Wallace et al. unpubl. data) and levels that may be present in the coming centuries due to climate change (IPCC 2014). We hypothesized that, like other carbon-limited autotrophs (Raven et al. 2020), growth rates would increase linearly with increasing levels of $p$-$CO_2$. *S. latissima* samples were placed in 1 of 4 treatments: a control with ambient $p$-$CO_2$ (350–450 μatm), a treatment with 750–850 μatm $p$-$CO_2$, a treatment with 1200–1500 μatm $p$-$CO_2$, and a treatment with 1800–2100 μatm $p$-$CO_2$. While the higher $p$-$CO_2$ levels used in this and the prior experiment exceed 21st century climate change projections, they capture the range of levels observed in coastal ecosystems influenced by eutrophication (Wallace et

| Experiment | Incubation period (d) | Figure no. | Experimental conditions |
|------------|------------------------|------------|------------------------|
| Co-effects of $p$-$CO_2$ and nutrients | 7 | 1 | *Saccharina latissima* incubated under ambient or elevated $p$-$CO_2$ with and without nutrient additions |
| $p$-$CO_2$ dose response | 14 | 2 | *S. latissima* incubated under treatment $p$-$CO_2$ levels with elevated nutrients |
| Effects of high and low $p$-$CO_2$ on herbivory | 24 | 4 | *Lacuna vincta* starved and grazed under ambient or elevated $p$-$CO_2$ on *S. latissima* that was grown at ambient $p$-$CO_2$ levels |
| Direct vs. indirect effects of elevated $p$-$CO_2$ on herbivory | 24 | 5 | *L. vincta* starved under ambient or elevated $p$-$CO_2$; half of snails from each group grazed under ambient and elevated $p$-$CO_2$ on *S. latissima* that was pre-incubated for ~1 wk under ambient or elevated $p$-$CO_2$ |
| Co-effects of elevated $p$-$CO_2$ and food restriction on herbivory | 24 | 6 | *L. vincta* starved or fed under ambient or elevated $p$-$CO_2$ for 24 h and then grazed on *S. latissima* that was grown at ambient $p$-$CO_2$ levels |
| Effective minimum exposure duration | 24 | 7 | *L. vincta* starved under elevated $p$-$CO_2$ for 0, 6, 12, 18, or 24 h before grazing on *S. latissima* that was grown at ambient $p$-$CO_2$ levels |
| Effective minimum dose | 24 | 8 | *L. vincta* starved and grazed on *S. latissima* under a range of $p$-$CO_2$ levels. *S. latissima* was grown at ambient $p$-$CO_2$ levels prior to the experiment |
al. 2014), upwelling (Feely et al. 2008), macrophyte respiration (Wahl et al. 2018), or riverine discharge (Vargas et al. 2016). Each treatment was supplied with nutrients (50 μM nitrate, 3 μM phosphate), and \( S. \) \( \text{latissima} \) samples were incubated for ~2 wk. At the end of the incubation periods for this and the first experiment, samples were removed from their respective treatments, weighed as described above, and final length and width measurements were made. Weight-based growth rates were determined by the following formula:

\[
\text{mg d}^{-1} = \frac{(W_{\text{final}} + W_{\text{initial}}) \times 1000}{\Delta t}
\]

where \( W_{\text{final}} \) and \( W_{\text{initial}} \) are the final and initial fresh weights, in grams, and \( \Delta t \) is the duration of the experiment in days.

### 2.4. Herbivory by \( L. \) \( \text{vincta} \) on \( S. \) \( \text{latissima} \)

Five herbivory experiments were performed, the first of which (designated as ‘Effects of high and low \( p\text{CO}_2 \) on herbivory’) gauged the herbivory rates of \( L. \) \( \text{vincta} \) feeding on \( S. \) \( \text{latissima} \) under ambient (350–400 μatm) and elevated (1800–2100 μatm) \( p\text{CO}_2 \) levels (Table 2). We hypothesized that \( L. \) \( \text{vincta} \) herbivory rates would be lower under higher \( p\text{CO}_2 \) levels (Young et al. 2019). \( L. \) \( \text{vincta} \) were placed in ambient or elevated \( p\text{CO}_2 \) conditions without \( S. \) \( \text{latissima} \) and starved for 24 h and then placed into containers with \( S. \) \( \text{latissima} \) (never exposed to elevated \( p\text{CO}_2 \)) under ambient or elevated \( p\text{CO}_2 \) for 48 h. At the end of the grazing period, \( L. \) \( \text{vincta} \) and \( S. \) \( \text{latissima} \) were removed from the containers and \( S. \) \( \text{latissima} \) samples were weighed as described above. Herbivory rates were calculated by obtaining the difference in the initial and final corrected weights divided by the number of grazers and the elapsed time of the grazing period and multiplied by 1000 to convert weights to mg (mg grazer\(^{-1}\) d\(^{-1}\)). Final weights were corrected using residual growth of the additional \( S. \) \( \text{latissima} \) samples grown with \( L. \) \( \text{vincta} \) (see above).

The next experiment (‘Direct vs indirect effects of elevated \( p\text{CO}_2 \) on herbivory’; Table 2) was performed to determine if lowered herbivory rates of \( L. \) \( \text{vincta} \) under high \( p\text{CO}_2 \) were caused by direct effects on \( L. \) \( \text{vincta} \) or indirectly by altering the palatability of \( S. \) \( \text{latissima} \). Given prior studies of this snail, we hypothesized that \( L. \) \( \text{vincta} \) herbivory rates would be lowered due to direct exposure to high \( p\text{CO}_2 \) levels and not due to exposure of \( S. \) \( \text{latissima} \) to high \( p\text{CO}_2 \) (Young et al. 2019). \( L. \) \( \text{vincta} \) were starved under ambient (350–450 μatm) or elevated (1800–2100 μatm) \( p\text{CO}_2 \) levels and feed \( S. \) \( \text{latissima} \) incubated under either ambient or elevated \( p\text{CO}_2 \), with the intent of placing one-half of the \( L. \) \( \text{vincta} \) from each \( p\text{CO}_2 \) group in either the \( p\text{CO}_2 \) level they were starved in or the opposite \( p\text{CO}_2 \) group. For this experiment, \( S. \) \( \text{latissima} \) was incubated for ~1 wk under ambient or elevated \( p\text{CO}_2 \); on the final day of the incubation period, \( L. \) \( \text{vincta} \) were starved in separate vessels under ambient or elevated \( p\text{CO}_2 \) without \( S. \) \( \text{latissima} \) for 24 h. At the end of the 1 wk \( S. \) \( \text{latissima} \) incubation period and concurrent 24 h \( L. \) \( \text{vincta} \) starvation period, the lights of the environmental chamber were turned off, and \( L. \) \( \text{vincta} \) were introduced into the vessels containing \( S. \) \( \text{latissima} \) for a total of 4 treatments: a control with ambient \( p\text{CO}_2 \) containing \( L. \) \( \text{vincta} \) starved under ambient \( p\text{CO}_2 \) and allowed to graze on \( S. \) \( \text{latissima} \) incubated under ambient \( p\text{CO}_2 \), a treatment with elevated \( p\text{CO}_2 \) containing \( L. \) \( \text{vincta} \) starved under ambient \( p\text{CO}_2 \) and allowed to graze on \( S. \) \( \text{latissima} \) incubated under elevated \( p\text{CO}_2 \), and a treatment with elevated \( p\text{CO}_2 \) containing \( L. \) \( \text{vincta} \) starved under elevated \( p\text{CO}_2 \) and allowed to graze on \( S. \) \( \text{latissima} \) incubated under elevated \( p\text{CO}_2 \). Once in their respective containers, \( L. \) \( \text{vincta} \) were allowed to graze for 96 h, with \( S. \) \( \text{latissima} \) samples being replaced every 24 h with the same source of \( S. \) \( \text{latissima} \) (high or ambient \( p\text{CO}_2 \) exposure). At the end of each 24 h grazing period, \( S. \) \( \text{latissima} \) samples were removed from the containers and were weighed as described above and herbivory was calculated.

Given that the effects of acidification on invertebrates can depend on food supply (Melzner et al. 2011, Thomsen et al. 2013, Pansch et al. 2014), for the next experiment (‘Co-effects of elevated \( p\text{CO}_2 \) and food restriction on herbivory’; Table 2), herbivory rates of \( L. \) \( \text{vincta} \) were quantified on individuals that were either starved or fed under ambient (350–450 μatm) or elevated \( p\text{CO}_2 \) (1800–2100 μatm) for 24 h. Given prior studies, we hypothesized that \( L. \) \( \text{vincta} \) exposure to high \( p\text{CO}_2 \) during the starvation period would yield lowered herbivory rates while exposure during the grazing period would not (Young et al. 2019). There were 4 treatments: starved under ambient \( p\text{CO}_2 \), fed at ambient \( p\text{CO}_2 \), starved under high \( p\text{CO}_2 \), or fed at high \( p\text{CO}_2 \). After 24 h, fresh \( S. \) \( \text{latissima} \) blade portions were placed in the containers and \( L. \) \( \text{vincta} \) were allowed to graze for 24 h. At the end of the grazing periods, \( L. \) \( \text{vincta} \) and \( S. \) \( \text{latissima} \) were removed from the containers and
S. latissima samples were weighed as described above and herbivory was calculated.

The final experiments were performed to determine how the intensity and duration of CO2 exposure altered herbivory of L. vincta grazing on S. latissima. These experiments specifically assessed the effective minimum duration of elevated pCO2 required to alter herbivory rates ('Effective minimum exposure duration'; Table 2) and the effective minimum concentration of pCO2 to alter herbivory rates of L. vincta ('Effective minimum dose'; Table 2). Given that pCO2 concentrations can vary diurnally within shallow estuaries (Baumann et al. 2015) and that even short-term exposure to acidification can disrupt herbivory (Young et al. 2019), the 'effective minimum exposure duration' experiment was designed to identify the minimum duration exposure needed to disrupt herbivory in L. vincta. We hypothesized that exposure to high pCO2 for less than 24 h would still lower grazing rates (Young et al. 2019). There were 5 treatments in this experiment: a control with ambient pCO2 (350–450 μatm) during the 24 h starvation period (no dose), a treatment with elevated pCO2 (1800–2100 μatm) during the entire 24 h starvation period, and treatments with 6, 12, and 18 h of exposure to elevated pCO2 during the starvation period with 18, 12, and 6 h, respectively, of ambient pCO2 exposure prior to exposure to elevated pCO2. At the end of the starvation period, L. vincta were placed in containers with ambient pCO2 levels, S. latissima samples were introduced, and L. vincta were allowed to graze for 24 h, after which herbivory rates were calculated. Given that pCO2 levels can be dynamic in estuaries (Wallace et al. 2014) and that levels of pCO2 are expected to more than double this century, the 'effective minimum dose' experiment was conducted to assess how levels of pCO2 found in estuaries today (Wallace et al. 2014) as well as those projected for the future in less impacted regions (IPCC 2014) affect herbivory by L. vincta. Given prior studies with L. vincta, we hypothesized that moderately elevated (>1500 μatm) concentrations of pCO2 would significantly lower herbivory rates (Young et al. 2019). This experiment established 4 treatments: a control with ambient pCO2 (350–450 μatm), a treatment with slightly elevated pCO2 (750–850 μatm), a treatment with moderately elevated pCO2 (1200–1500 μatm), and a treatment with high pCO2 (1800–2100 μatm). During this experiment, L. vincta were placed in 1 of the 4 treatments and starved for 24 h. At the end of the starvation period, S. latissima samples were introduced, and L. vincta were allowed to graze for 24 h at their respective pCO2 levels. At the end of the grazing period, L. vincta and S. latissima were removed from the containers and S. latissima samples were weighed as described above and herbivory was calculated.

2.5. Post-experimental analyses

For carbon (C) and nitrogen (N) analyses of S. latissima, frozen samples were dried at 60°C for 24 h, and then homogenized into a fine powder with a mortar and pestle. Tissue C, N, and δ13C were analyzed using an elemental analyzer interfaced to a Europa 20-20 isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility. The measured δ13C levels of the CO2 gas (−80%) used in experiments and isotopic mixing models (Young & Gobler 2016, 2017) were used to identify the relative use of CO2 and HCO3− by S. latissima.

Net algal growth rates were calculated using growth rates and total herbivory rates from the ‘pCO2 dose response’ and ‘effective minimum dose’ experiments, respectively. The herbivory rates for each replicate in each pCO2 treatment in the ‘effective minimum dose’ experiment were calculated by subtracting the final S. latissima fresh weight from the initial fresh weight. We note that the S. latissima in this herbivory experiment were all raised at ambient pCO2. The mean total herbivory rate for each pCO2 treatment was subtracted from the S. latissima growth rates in the corresponding pCO2 treatment in the ‘pCO2 dose response’ experiment to obtain the net growth rate (growth minus herbivory) for 4 pCO2 levels.

One-way ANOVAs were performed within SigmaPlot 11.0 to assess significant differences in herbivory rates in the ‘pCO2 dose response’, ‘effects of high and low pCO2 on herbivory’, ‘effective minimum exposure duration’, and ‘effective minimum dose’ experiments (n = 4 treatment−1 for each experiment). Two-way ANOVAs were performed with SigmaPlot to assess herbivory rates in the ‘co-effects of pCO2 and nutrients’, ‘direct vs. indirect effects of elevated pCO2 on herbivory’, and ‘co-effects of elevated pCO2 and food restriction on herbivory’ experiments (n = 4 treatment−1 for each experiment), where the main treatment effects were pCO2 and nutrient levels (ambient or elevated for both) for the ‘co-effects of pCO2 and nutrients’ experiment, pCO2 level for the S. latissima incubation and L. vincta starvation periods (ambient and elevated for both) for the ‘direct vs. indirect effects of elevated pCO2 on herbivory’ experiment, and pCO2 level (ambient or elevated) and starvation (starved or not starved) for the ‘co-effects of elevated
pCO₂ and food restriction on herbivory’ experiment. Normality and equal variance were tested via the use of Shapiro-Wilk and Leven tests within SigmaPlot 11.0; assumptions of equal variance and normality were met for all data. For all experiments, if significant differences were detected, a Tukey’s HSD test using R v.3.4.0 within RStudio v.1.0.143 was performed (R Core Team 2020, RStudio Team 2020).

3. RESULTS

3.1. Effects of elevated pCO₂ and nutrients on *Saccharina latissima* growth rates

In the ‘co-effects of pCO₂ and nutrients’ experiment, growth rates of *Saccharina latissima* were significantly higher in elevated than in ambient pCO₂ by ~70% (2-way ANOVA and Tukey’s HSD, p < 0.05; Fig. 1, Tables S1 & S2 in the Supplement). Furthermore, growth rates were significantly higher by ~50% under elevated nutrient conditions relative to treatments that did not receive nutrient additions (2-way ANOVA and Tukey’s HSD, p < 0.05; Fig. 1, Tables S1 & S2), and there was no interaction between nutrient additions and pCO₂ levels (Fig. 1).

For the ‘pCO₂ dose response’ experiment, growth rates in the ~1200 and ~2300 μatm pCO₂ treatments were double the ambient (~500 μatm) pCO₂ treatment (1-way ANOVA and Tukey’s HSD, p < 0.05 for both; Fig. 2, Tables S3 & S4) but were not different from each other. While growth rates were 45% higher in the 800 μatm pCO₂ treatment relative to the ambient treatment, the difference was not statistically different (1-way ANOVA and Tukey’s HSD, p > 0.05; Fig. 2, Tables S3 & S4). Further, there was no difference in growth between the 800, ~1200, and ~2300 μatm pCO₂ treatments (1-way ANOVA and Tukey’s HSD, p > 0.05 for both; Fig. 2, Tables S3 & S4).

3.2. *S. latissima* tissue analyses

During the ‘co-effects of pCO₂ and nutrients’ experiment, tissue δ¹³C was significantly lower under elevated pCO₂ (−29‰) relative to ambient conditions (−16‰) (2-way ANOVA and Tukey’s HSD, p < 0.05; Fig. 3, Tables S5 & S6), while nutrients did not significantly alter δ¹³C (2-way ANOVA, p > 0.05; Fig. 3, Tables S5 & S6). The mixing model performed for this experiment showed the actual δ¹³C signatures following exposure to elevated pCO₂ (−27.9‰) to be similar to predicted δ¹³C for exclusive uptake of CO₂ (−26.8‰) as opposed to exclusive HCO₃⁻ uptake (−16.3‰). In the ‘pCO₂ dose response’ experiment, the ~2300 μatm pCO₂ treatment had significantly lower tissue δ¹³C (−22‰) than in the ~500 and ~800 μatm pCO₂ treatments (~18 and ~19‰, respectively) (2-way ANOVA and Tukey’s HSD, p < 0.05 for both; Fig. 3, Tables S5 & S6). While tissue δ¹³C in the ~1200 μatm pCO₂ treatment (~21‰) was significantly lower than in the ~500 μatm pCO₂ treatment (2-way ANOVA and Tukey’s HSD, p < 0.05; Fig. 3, Tables S5 & S6), there was no significant difference in δ¹³C between the ~1200 and ~800 μatm pCO₂ treatments (2-way ANOVA and Tukey’s HSD; p > 0.05; Fig. 3, Tables S5 & S6). There was no significant
difference in δ13C between the ~500 and ~800 μatm pCO2 treatments (2-way ANOVA and Tukey’s HSD, p > 0.05; Fig. 3, Tables S5 & S6). Mixing models performed for the ~800, ~1200, and ~2300 μatm pCO2 treatments showed the actual δ13C signatures (~18.7, −21.6, and −22.2‰, respectively) of *Saccharina latissima* shifted from being similar to predicted δ13C signatures from exclusive HCO3− use (~15.8‰ for all) to predicted signatures from exclusive CO2 use (~28.1, −27.6, and −25.2‰, respectively) as pCO2 levels increased across treatments. There were no significant differences in tissue C, N, and C:N between any of the pCO2 treatments (2-way ANOVA and Tukey’s HSD, p > 0.05 for all; Table 3, Tables S7–S9).

### 3.3. Herbivory rates of *Lacuna vincta* grazing on *Saccharina latissima*

During the ‘effects of high and low pCO2 on herbivory’ experiment, which examined how high and low pCO2 affected herbivory of *Lacuna vincta* that were fed *Saccharina latissima*, grazing rates in the elevated pCO2 treatment were significantly reduced by 60% relative to the ambient pCO2 after 48 h (1-way ANOVA, p < 0.05; Fig. 4, Table S10). For the ‘direct vs. indirect effects of elevated pCO2 on herbivory’ experiment, *L. vincta* herbivory rates were sensitive to pCO2 levels during the starvation period, but not the pCO2 conditions that *S. latissima* were incubated under (Fig. 5). Specifically, for the 24, 48, and 72 h timepoints, herbivory was significantly decreased by ~78, ~86, and ~94% when *Lacuna* were starved under elevated pCO2 relative to ambient conditions (2-way ANOVA, p < 0.05 for all; Fig. 5, Table S11). In contrast, herbivory was not significantly altered by the pCO2 conditions in which *S. latissima* were grown under throughout the experiment (2-way ANOVA, p > 0.05 for all; Fig. 5, Table S11). After 96 h, herbivory rates of *L. vincta* exposed to elevated pCO2 recovered and were no longer affected by the pCO2 levels it had been exposed to during the starvation period (2-way ANOVA, p > 0.05 for both; Fig. 5, Table S11).

In the ‘co-effects of elevated pCO2 and food restriction on herbivory’ experiment, pCO2 levels and being starved or fed prior to grazing was examined in a 2 × 2 experimental design. While herbivory rates of *L. vincta* on *S. latissima* during this experiment were not significantly affected by whether snails were starved under ambient pCO2 (2-way ANOVA, p > 0.05; Fig. 6, Tables S12 & S13), exposure to elevated pCO2 during the starvation period significantly depressed herbivory rates of starved snails (2-way ANOVA and Tukey’s HSD, p < 0.05; Fig. 6, Tables S12 & S13). Within the elevated pCO2 treatments, *L. vincta* that were starved had herbivory rates that were ~85% lower than individuals that were fed throughout experimentation (Tukey’s HSD, p < 0.05; Fig. 6, Tables S12 & S13). During the ‘effective minimum exposure duration’ experiment, exposure to elevated pCO2 levels for 6

| Experiment                  | Treatment          | Tissue C    | Tissue N    | C:N        |
|-----------------------------|--------------------|-------------|-------------|------------|
| Co-effects of pCO2          | Control            | 0.32 ± 0.01 | 0.0104 ± 0.0004 | 30.5 ± 2.4 |
|                             | Nutrients          | 0.33 ± 0.01 | 0.0109 ± 0.0007 | 29.6 ± 2.4 |
|                             | CO2                | 0.33 ± 0.00 | 0.0098 ± 0.0005 | 33.7 ± 1.7 |
|                             | CO2/Nutrients      | 0.33 ± 0.02 | 0.0095 ± 0.0011 | 35.2 ± 4.8 |
| pCO2 dose response          | Ambient pCO2       | 0.33 ± 0.01 | 0.0160 ± 0.0032 | 21.4 ± 4.4 |
|                             | Low pCO2           | 0.33 ± 0.02 | 0.0160 ± 0.0036 | 21.6 ± 6.7 |
|                             | Medium pCO2        | 0.33 ± 0.02 | 0.0169 ± 0.0022 | 19.8 ± 4.0 |
|                             | High pCO2          | 0.34 ± 0.01 | 0.0148 ± 0.0018 | 22.9 ± 3.3 |
or 12 h did not alter herbivory rates compared to the control (1-way ANOVA and Tukey’s HSD, p > 0.05 for all; Fig. 7, Tables S14 & S15) but 18 and 24 h exposure did, yielding rates significantly lower than the 0, 6, and 12 h exposure treatments (1-way ANOVA and Tukey’s HSD, p < 0.05 for all; Fig. 7, Tables S14 & S15). There was no significant difference in herbivory between the 18 and 24 h exposure treatments (1-way ANOVA and Tukey’s HSD, p < 0.05; Fig. 7, Tables S14 & S15).

Finally, during the ‘effective minimum dose’ experiment, pCO2 concentrations reduced herbivory rates of L. vincta feeding on S. latissima in a dose-dependent manner. Herbivory rates within the ~830, ~1420, and ~2450 μatm pCO2 treatments were all significantly lower than in the ambient (~450 μatm) pCO2 treatment (1-way ANOVA and Tukey’s HSD, p < 0.05 for all; Fig. 8, Tables S16 & S17). While herbivory rates in the ~830 μatm pCO2 treatment were significantly higher than in the ~1420 and ~2450 μatm pCO2 treatments (1-way ANOVA and Tukey’s HSD, p < 0.05 for both; Fig. 8, Tables S16 & S17), there was no significant difference in herbivory between the 1420 and 2450 μatm pCO2 treatments (1-way ANOVA and Tukey’s HSD, p > 0.05; Fig. 8, Tables S16 & S17).
3.4. Net growth rates

Net growth rates of *S. latissima* (growth − grazing) were determined by considering growth under the range of pCO$_2$ levels in the ‘pCO$_2$ dose response’ experiment and herbivory rates of *L. vincta* grazing on *S. latissima* under approximately the same pCO$_2$ conditions in the ‘effective minimum dose’ experiment. Net growth rates increased as pCO$_2$ levels increased above 1200 μatm. At 1230−1420 and 2340−2450 μatm pCO$_2$ levels, net growth (~14 mg d$^{-1}$) was significantly higher than the ambient (3 mg d$^{-1}$ at 430−450 μatm) and 800−830 μatm (5 mg d$^{-1}$) pCO$_2$ levels by more than 4- and 2-fold, respectively (1-way ANOVA and Tukey’s HSD, p < 0.05 for all; Fig. 9, Tables S18 & S19). There were no significant differences in net growth between the 430−450 and 800−830 μatm pCO$_2$ treatments or between the 1230−1420 and 2340−2450 μatm pCO$_2$ treatments (1-way ANOVA and Tukey’s HSD, p > 0.05 for all; Fig. 9, Tables S18 & S19).
CO₂ (Maberly et al. 1992, Raven et al. 2002, Hepburn et al. 2011). In the present study, *S. latissima* had δ¹³C signatures that ranged from −16 to −18‰ when exposed to normal conditions, suggesting it primarily, but not exclusively, uses HCO₃⁻ as a C source under ambient pCO₂ conditions (Maberly et al. 1992, Raven et al. 2002, Hepburn et al. 2011). Exposure to elevated pCO₂ significantly lowered δ¹³C signatures (−18 to −29‰) of *S. latissima*, indicating that when exposed to higher pCO₂ levels, this alga obtained a larger fraction of its C from CO₂, an observation consistent with previous studies of stable carbon isotope discrimination of *S. latissima* and other laminarian kelp species (Maberly et al. 1992, Fernández et al. 2015). Furthermore, in the present study, growth rates increased under increasing pCO₂ levels up to ~1200 μatm, suggesting that *S. latissima* may become substrate-saturated at this concentration. While this level of pCO₂ is not predicted to occur in open ocean regions for more than a century, it can occur regularly in some coastal systems (Feely et al. 2008, Melzner et al. 2013, Wallace et al. 2014). Be it in the future or due to present day coastal processes, elevated pCO₂ levels may directly benefit the growth of laminarian kelp species such as *S. latissima*.

Nutrients also increased *S. latissima* growth rates, although there was no interaction with pCO₂ levels. Eutrophication can initiate phase shifts in coastal ecosystems whereby turf algae benefit from the overloading of nutrients and cover substrate onto which kelp may grow and recruit, causing declines in kelp forests (Eriksson et al. 2002, Gorgula & Connell 2004, Connell et al. 2008). However, kelps including *S. latissima* can grow robustly using aquaculture approaches in eutrophic ecosystems (Kim et al. 2015, Jiang et al. 2020), and the results shown here demonstrate that, without competition, elevated nutrients can benefit *S. latissima* via enhanced growth rates.

Beyond growth, herbivory on *S. latissima* also changed under elevated pCO₂. The effects of elevated pCO₂ and the absence of food on the herbivory rates of *L. vincta* are consistent with prior observations regarding the manner in which acidification alters herbivory by this gastropod grazing on the green alga, *Ulva* (Young et al. 2019). In the present study, herbivory by *L. vincta* on *S. latissima* was even more sensitive to acidification, with food limitation decreasing herbivory rates at 830 μatm, slightly lower than the level at which *L. vincta* grazing on *Ulva* was reduced (850 μatm; Young et al. 2019). Exposure to elevated pCO₂ levels may cause a state of acidosis that can disrupt metabolism and homeostatic function, thus diverting energy from critical functions such shell and somatic growth, shell repair, and gametogenesis (Lindinger et al. 1984, Pörtner et al. 1998, Hendriks et al. 2010). *L. vincta* exposed to elevated pCO₂ and starved displayed lower respiration rates (Young et al. 2019), a finding interpreted as metabolic depression within other marine gastropods during periods of food limitation (Maas et al. 2011). The ability of acidification alone to slow the metabolism of gastropods has also been previously reported (Hendriks et al. 2010, Melatunan et al. 2011), which may serve as a survival strategy to match lowered energy supply (Bishop & Brand 2000) and may result in an increased reliance on anaerobic respiration (Pörtner et al. 1998, Melatunan et al. 2011). Reductions in herbivory under elevated pCO₂ were not observed when *L. vincta* was fed ad libitum, however. Similarly, when provided with an adequate food source, some other calcifying organisms (Melzner et al. 2011, Thomsen et al. 2013, Pansch et al. 2014) and early life stage fish (Gobler et al. 2018) are resistant to acidification.

In an ecosystem setting, kelp beds may provide a refuge to grazers by simultaneously buffering carbonate chemistry and providing ample quantities of food. Previous studies have demonstrated the ability of macroalgae to buffer carbonate chemistry and promote the growth and survival of calcifying organisms (Wahl et al. 2018, Young & Gobler 2018). However, such a buffering effect can be species- and/or site-specific (Rivest et al. 2017). Daytime primary productivity within kelp beds has been shown to significantly reduce pCO₂ compared to outside the bed on diel and even seasonal timescales (Delille et al. 2000, 2009). Furthermore, vertical gradients in pCO₂ can also form in kelp canopies due to enhanced primary productivity in surface waters (Hofmann et al. 2011). The lower quantities of food and relatively higher levels of pCO₂ on the vertical or horizontal margins of the kelp beds may be the regions more likely to facilitate disruption of gastropod herbivory. Similarly, acidified regions with low-density kelp blades could similarly be disruptive to snail grazing. All these conditions would be exacerbated by nocturnal acidification (Wallace et al. 2014, Tomasetti & Gobler 2020).

While the pCO₂ levels in the present study are not expected for the open ocean for decades or centuries to come, eutrophication, upwelling, riverine discharge, and other coastal processes may result in the diurnal and/or seasonal accumulation of CO₂ in coastal zones that can produce the pCO₂ and nutrient levels shown here to accelerate the growth of, and reduce grazing on, *S. latissima* (Feely et al. 2008,
Melzner et al. 2013, Wallace et al. 2014). In the present study, 18 h of exposure to elevated pCO₂ in the absence of food suppressed L. vincta herbivory, with this effect persisting for 72 h. In an ecosystem context, L. vincta and other gastropods and grazers sensitive to elevated pCO₂ may be vulnerable to diurnal and seasonal shifts in pH and pCO₂. During nighttime and/or low tides, eutrophic estuaries may become strongly net heterotrophic and produce acidified conditions that can persist >18 h (Wallace et al. 2014, Baumann et al. 2015). These conditions are mostly likely to occur in temperate estuaries during the late summer, when microbial respiration rates accelerate acidification, and may persist into the fall (Wallace et al. 2014), which is the beginning of the growing season for numerous kelp species (Kim et al. 2015, Augyte et al. 2017). Aside from the diurnal and seasonal shifts in pCO₂, acidified conditions often co-occur with low oxygen conditions in estuaries (Tomasetti & Gobler 2020), and the combination of hypoxia and acidification has been shown to additively and synergistically disrupt grazing by L. vincta (Young & Gobler 2020). Finally, the elevated nutrients associated with eutrophication may also affect herbivory rates by L. vincta and growth by S. latissima. Nutrient enrichment can reduce the abundance of common estuarine grazers, including Lacuna, due to potentially toxic concentrations of ammonia (Atalah & Crowe 2012). While eutrophication indirectly harms S. latissima via reduced light availability and overgrowth by epiphytic and filamentous algae (Moy & Christie 2012), the alga can directly benefit from the increased nutrient concentrations (Boderskov et al. 2016, this study) and the acidification wrought from eutrophication (this study).

Despite the potential benefits that ocean acidification may provide to some primary producers (Koch et al. 2013, Hattenrath-Lehmann et al. 2015, Young & Gobler 2016), grazing pressure by common herbivores, such as gastropods, may limit the extent of these benefits through top-down controls on growth (Baggini et al. 2015). Among the factors that limit kelp abundance, overgrazing is a major biotic driver of kelp loss via consumption of kelp blades, inhibition of new recruitment, and/or grazing damage causing blade breakage (Steneck et al. 2002, Molis et al. 2010, Filbee-Dexter & Wernberg 2018). As L. vincta is the primary mesograzer of S. latissima macroscopic kelp sporophytes in the Northwest Atlantic (Brady-Campbell et al. 1984, Johnson & Mann 1986), large population increases in the gastropod can consume significant quantities of kelp biomass, which can result in significant losses of kelp canopy biomass due to wave action (Krumhansl & Scheibling 2011). Studies have shown that L. vincta grazing of kelp can remove up to 10% of the total surface area of blades in natural settings (Molis et al. 2010, Krumhansl & Scheibling 2011) and larger amounts (>40%) in experimental settings (Chenelot & Konar 2007). In the present study, L. vincta displayed significantly reduced herbivory when exposed to elevated pCO₂ and prior food restriction, while S. latissima growth was significantly increased under elevated pCO₂. When considering the responses of S. latissima across a pCO₂ gradient, net growth rates increased more than 4-fold from under ambient pCO₂ to ~1200 μatm (Fig. 9). L. vincta had the potential to consume 70% S. latissima productivity per day under ambient pCO₂ but only 38 and 9% at 800 and 1300 μatm, respectively, meaning that as pCO₂ levels rise, L. vincta, and other gastropods that experience reduced herbivory rates under elevated pCO₂, may become incapable of controlling kelp proliferation.

Beyond growth and grazing, competition with other algae will also influence the fate of S. latissima populations in high-CO₂ environments (Young & Gobler 2017). During the last decade, turf-forming algae have begun to replace kelp beds in many ecosystems, primarily due to warming and eutrophication (Moy & Christie 2012, Filbee-Dexter & Wernberg 2018). In regions with naturally occurring high CO₂ conditions from volcanic vents, however, turf algal communities decline in diversity and abundance with decreasing pH and increasing pCO₂ (Porzio et al. 2011). Hence, beyond the ability of high pCO₂ to foster higher net growth rates in S. latissima, these conditions may also allow it to maintain dominance in regions where it may be competing with turf-forming algae, possibly offsetting losses associated with other anthropogenic processes.

Within the northwestern Atlantic, L. vincta can reproduce year-round on kelp in cooler climates (Maney & Ebersole 1990), with a distinctive peak in spawning during January and February (Johnson & Mann 1986), larval recruitment and hatching occurring thereafter, followed by a summer maxima (Southgate 1982). Within the same regions, kelp species such as S. latissima begin their growing season during the fall, grow rapidly in spring, and experience rapid decay and mortality during the summer as thermal thresholds are surpassed (Krumhansl & Scheibling 2011, Kim et al. 2015, Augyte et al. 2017). The emergence of marine gastropod grazers with the onset of the warmer summer weather accelerates the seasonal demise of kelp (Johnson & Mann 1986, Krumhansl & Scheibling 2011). As coastal waters sea-
sonally warm, accelerating rates of ecosystem metabolism can cause a supersaturation of pCO$_2$ (Wallace et al. 2014, Baumann et al. 2015) that may enhance the net growth rates of *S. latissima* and prolong the growing season of the kelp that might otherwise slow due to warmer temperatures and grazing (Wernberg et al. 2010, Filbee-Dexter et al. 2016). In the future, as climate change intensifies acidification, shortened growing seasons due to warming may be offset by increased net growth due to higher pCO$_2$. Future experiments should consider the interactive effects of temperature and pCO$_2$ on the growth of *S. latissima*.

5. CONCLUSIONS

Elevated levels of pCO$_2$ have a dual benefit for the kelp *Saccharina latissima*. The net growth rates of *S. latissima* at higher pCO$_2$ (>1200 μatm) exceeded those at ambient pCO$_2$ by 4-fold due to both accelerated growth by *S. latissima* and suppressed herbivory by the gastropod *Lacuna vincta*. The pCO$_2$ conditions that elicited reduced herbivory (~830 μatm) and enhanced kelp growth (~1200 μatm pCO$_2$) are seasonally found in many estuaries today and will become more common in the future as climate change accelerates. Eutrophicication associated with coastal acidification may benefit *S. latissima* directly through nutrient- and CO$_2$-enhanced growth rates and may further reduce herbivore grazing due to hypoxia and/or high ammonia levels. Since only 18 h of acidification suppressed herbivory by *L. vincta*, nocturnal acidification associated with eutrophicication (Wallace et al. 2014) may further suppress herbivore grazing. While *S. latissima* may buffer carbonate chemistry to the benefit of calcifying organisms such as *L. vincta*, these benefits may only be realized in the center of dense kelp beds as opposed to horizontal and vertical margins where acidification and gastropod starvation may become more common. Lastly, given that turf-forming algae may not directly benefit from increased pCO$_2$, the combination of increased growth and suppressed herbivory experienced by *S. latissima* may allow the alga to maintain dominance facilitated by high-CO$_2$ conditions.

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