IMPACTS OF OCEAN ACIDIFICATION ON INTERTIDAL MACROALGAE AND ALGIVORE PREFERENCE

By

Andrea M. Fieber

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Committee Membership

Dr. Paul Bourdeau, Committee Chair

Dr. Frank Shaughnessy, Committee Member

Dr. Joe Tyburczy, Committee Member

Dr. David S. Baston, Committee Member

Dr. Erik Jules, Committee Member

Dr. Paul Bourdeau, Graduate Program Coordinator

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ABSTRACT

IMPACTS OF OCEAN ACIDIFICATION ON INTERTIDAL MACROALGAE AND ALGIVORE PREFERENCE

Andrea M. Fieber

Ocean acidification, a facet of global climate change, has the potential to induce changes in marine macroalgae that modify their existing interactions with algivorous invertebrates. In this study, I examined the effects of elevated carbon dioxide (pCO_2) on several species of intertidal macroalgae (Phaeophyta, Rhodophyta) and evaluated the present-day and predicted future preferences of algivores (*Pugettia producta* and *Tegula funebralis*) by assessing grazing rates on untreated algal tissue and on algae exposed to high-pCO_2 seawater. Both red and brown algae grew faster in elevated pCO_2 than in ambient seawater, and algae in intermediate pCO_2 generated more new growth overall than those in highly elevated *p*CO2. The effect of pCO_2 on the carbon and nitrogen contents of algae depended on species identity, and C:N ratios decreased slightly with increasing pCO_2 for four of the five species studied. Total phenolic content in each alga was unaffected by pCO_2 treatment, although similar (distinct) levels between untreated species became distinct (similar) when those same species were compared after high-pCO_2 treatment. Algivores demonstrated contrasting responses to changes in their food sources; *P. producta*, a specialist crab grazer, did not modify its preference for the brown alga *Egregia menziesii* when offered high-pCO_2 treated individuals, but the generalist

snail *T. funebralis* adjusted its feeding behavior to choose algae with low phenolic contents, which created different patterns of preference for untreated and pCO_2-treated algae. C:N ratios of algae did not appear to be a strong driver of preference for either grazer in feeding experiments. These results indicate that algae may be well-equipped to benefit from moderate increases in seawater pCO_2, but they exhibit species-specific rates of growth and phenolic production, which in turn affect their appeal to a generalist algivore. Intertidal algal communities will therefore face altered patterns of predation under future ocean acidification conditions as generalist algivores adjust to new variation in algal palatability.

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INTRODUCTION

As atmospheric carbon dioxide levels rise, the world's oceans absorb a large portion of these anthropogenic emissions (Sabine et al., 2004; Takahashi et al., 2006), and increasing inorganic carbon inputs prompt changes in ocean carbonate chemistry. Dissolution of CO_2 in seawater forms carbonic acid (H_2CO_3), which dissociates into bicarbonate (HCO_3^-) and hydrogen (H^+) and further into carbonate (CO_3^{2-}) and hydrogen (H^+), increasing overall dissolved inorganic carbon (DIC) content and reducing pH in a process termed ocean acidification (OA). Although fluxes in carbonate chemistry have occurred throughout earth's history, current and future climatic changes are expected to progress at unprecedented rates (IPCC, 2015), creating rapidly developing ecological stressors that may outpace the physiological adaptability of marine life. As a result, there is concern for the fitness and survival of these organisms as OA introduces increasingly novel conditions.

A wide variety of taxa spanning multiple trophic levels are susceptible to OA-induced stress. Adverse effects have been most notably observed in calcifying organisms such as corals and shelled mollusks, though a growing body of evidence demonstrates negative effects in more cosmopolitan groups such as phytoplankton, diatoms, fleshy macroalgae, crustaceans, echinoderms, and fish (Hurd et al., 2019; Kroeker et al., 2010). Moreover, acidification does not occur evenly across all oceanic zones, and many intertidal ecosystems face more extreme conditions than pelagic zones as upwelling

systems draw deep, low-pH water into shallower habitats (Feely et al., 2008). As the climate changes, the northeast Pacific is expected to experience more frequent (Bakun et al., 2015), prolonged (Wang et al., 2015), and intense (Bakun, 1990; Snyder et al., 2003) periods of upwelling, inundating nearshore habitats with seawater that has a lower pH and higher DIC than surface water. California's species-rich intertidal communities, which are exposed to these deep waters for increasingly extended upwelling seasons (García-Reyes & Largier, 2010), host a variety of OA-sensitive taxa such as macroalgae, mussels, snails, barnacles, and crabs. Although these ecosystems are highly valued for their economic and recreational resources (Wilson et al., 2005), the impacts of OA on rocky-shore species and their trophic interactions remain understudied.

Marine macroalgae, which are primary producers in intertidal ecosystems and directly utilize carbon dioxide and DIC for photosynthesis, exhibit a variety of growth responses to ocean acidification (Hurd et al., 2009). Though elevated inorganic carbon availability often increases productivity in terrestrial and aquatic autotrophs (Koch et al., 2013), marine algal responses are difficult to generalize (Connell et al., 2018; Kroeker et al., 2010). Much of this metabolic diversity is the product of different photosynthetic pathways among macroalgae, which allow many species to take advantage of OA through enhanced passive carbon acquisition (Falkenberg et al., 2013; Hepburn et al., 2011). Other macroalgae with carbon-concentrating mechanisms (CCMs), which uptake bicarbonate to provide ample carbon for photosynthesis, may be unaffected by small changes in ambient pCO_2 concentrations if their CCMs remain more beneficial than

passive diffusion (Koch et al., 2013). Several CCM-operating chlorophytes and phaeophytes (Graham et al., 2016; Moroney, 2001) can thus exhibit neutral responses to elevated pCO_2 (Hepburn et al., 2011; Israel & Hophy, 2002). However, large increases in CO_2 abundance prompt the downregulation of CCMs (Hepburn et al., 2011), reducing the energetic cost of carbon acquisition for these algae. For example, the brown alga *Cystoseira compressa* has been shown to increase photosynthesis and carbon uptake under pCO_2 enrichment (Celis-Plá et al., 2015), and several species of green algae are reported to grow more rapidly in high-pCO_2 conditions (Gordillo et al., 2001; Israel & Hophy, 2002; J. Xu & Gao, 2012). Species lacking CCMs can also deviate from predicted responses to OA, such as the red alga *Hypnea musciformis*, which can experience greatly reduced growth and even tissue decay in low-pH conditions (Israel & Hophy, 2002). Carbon use strategy is thus not always an adequate predictor of an alga's growth response to OA (van der Loos et al., 2019), and secondary physiological responses to elevated pCO_2 further complicate the predicted effects of OA on marine macrophytes.

Focusing on OA-induced changes in algal biomass alone fails to account for species' ecological roles, such as their palatability to grazers, which are largely influenced by nutrient acquisition and secondary metabolism (Mattson, 1980; Targett & Arnold, 1998; Van Alstyne et al., 2001). When elevated pCO_2 allows for increased photosynthetic uptake of inorganic carbon but ambient nitrogen availability remains unchanged, carbon-nitrogen ratios (C:N) increase (Mercado et al., 1999), reducing the

nutritional value of algal tissue. Such changes may prompt preference shifts or compensatory feeding behavior in algivores, who must then consume different or more algae to obtain sufficient nutrition (Cruz-Rivera & Hay, 2000; Duarte et al., 2016). Additionally, high seawater pCO_2 has also been shown to induce algal production of phenolic compounds (Celis-Plá et al., 2015; Swanson & Fox, 2007), and these secondary metabolites are widely hypothesized to deter generalist grazers in both terrestrial and marine systems (Bennett & Wallsgrove, 1994; Erickson et al., 2006; Steinberg, 1985; Wright et al., 2004). Thus, while increasing pCO_2 may manifest as changes in algal growth, the altered quality of that growth carries implications for shifting alga-algivore interactions.

Intertidal invertebrates, many of which are primarily algivorous grazers (Ricketts et al., 1985), are likely to exhibit divergent responses to changing food quality as a result of differing feeding strategies. Generalist herbivores show low fidelity to any particular host plant or alga, instead selecting tissues with both high nitrogen and low phenolic contents for optimal nutrition and digestion (Cruz-Rivera & Hay, 2000; van der Meijden, 1996). Specialist grazers are often adapted to, or co-evolved with, their host plants and therefore are not easily deterred by high levels of phenolic compounds in their preferred food source (Leimu et al., 2005; Wei et al., 2015). However, both feeding strategies can be affected by the availability of high-quality food, as specialists have also been shown to selectively graze particular host plant tissues that are high in nitrogen (Berner et al., 2005; Bracken & Stachowicz, 2007). Thus, implications for future alga-algivore interactions

depend upon algal nitrogen and phenolic contents and their influence on the community's generalist and specialist grazers. Because much of our knowledge about herbivore preference is derived from terrestrial systems, the responses of marine algivores to altered food quality are still not well-understood, particularly in the context of OA.

My study examined the potential changes that may take place in upwelling-exposed intertidal ecosystems as OA progresses to near- and far-future conditions. I conducted experiments using macroalgae and algivores from the northern coast of California, an eastern Pacific boundary experiencing strong seasonal upwelling and therefore amplified present-day exposure to acidified seawater (Feely et al., 2008; Wang et al., 2015). These intertidal ecosystems are also highly species-rich algal assemblages and numerous algivores representing a variety of phyla, feeding strategies, and microhabitat preferences (Augyte & Shaughnessy, 2014; Light, 2007). To investigate predicted future feeding preferences and explore the potential driving factors in algivore food choice, I selected five locally abundant macroalgae (phaeophytes *Egregia menziesii* and *Laminaria setchellii*; rhodophytes *Mazzaella splendens*, *Neorhodomela larix*, and *Odonthalia floccosa*) and two algivores, the specialist northern kelp crab *Pugettia producta* Randall, 1840 and the generalist black turban snail *Tegula funebralis* A. Adams, 1855. In laboratory mesocosms, I exposed algae to elevated levels of pCO_2, examined subsequent changes in growth and tissue composition, and assessed feeding preferences for present- and future-condition algae in order better understand the effects that OA may have on trophic interactions in the intertidal upwelling ecosystem.

METHODS

Selection of Study System

I based laboratory experiments on macroalga-algivore interactions naturally occurring on northeast Pacific rocky shores. For macroalgae, I selected five species representing the phaeophytes (kelps *Egregia menziesii* (Turner) Areschoug and *Laminaria setchellii* P.C. Silva) and rhodophytes (*Mazzaella splendens* (Setchell & N.L. Gardner) Fredericq, *Neorhodomela larix* (Turner) Masuda, and *Odonthalia floccosa* (Esper) Falkenberg). These epilithic algae are abundant throughout the mid- and low-intertidal zones of California, Oregon, and Washington, USA (Abbott & Hollenberg, 1976; Augyte & Shaughnessy, 2014), and previous research has indicated their palatability to grazers (Dobkowski, 2017; Hultgren & Stachowicz, 2010; Leighton, 1966; Ruesink, 2000; Steinberg, 1985; Thornber et al., 2008). For algivores, I chose to study the northern kelp crab (*Pugettia producta*) and black turban snail (*Tegula funebralis*) because of their local abundance and complementary habitat selection. *P. producta* is generally found in lower intertidal and subtidal zones (Hines, 1982; Hultgren & Stachowicz, 2008), whereas *T. funebralis* primarily inhabits mid- and high-intertidal areas (Byers & Mitton, 1981; Paine, 1969), and in visual surveys, both grazers encountered the selected algal assemblage within their respective ranges (personal observation). These grazers also represent distinct phyla (Arthropoda and Mollusca, respectively) and feeding habits; kelp crabs are typically regarded as specialists (Dobkowski et al., 2017; Hines, 1982;

Leighton, 1966) whereas black turban snails are well-documented generalists (Ricketts et al., 1985; Steinberg, 1985), and this allowed me to examine how dissimilar grazers responded to similarly treated algae.

Algal Growth Experiments

I collected all algae from Baker Beach (41°02’57.1” N, 124°07’36.7” W), an intertidal boulder field in Trinidad, Humboldt County, California. In May 2019, I collected 50 individuals of each species by detaching discrete thalli, including their holdfasts, from mid- and low-intertidal rocks. I collected the smallest available individuals to improve the likelihood of growth in the laboratory and minimize the presence of tissue greater than six months old, and I sourced all algae from this single site to standardize site-specific characteristics that might otherwise confound treatment-induced differences. I then transported algae to the Humboldt State University Telonicher Marine Laboratory (TML) in Trinidad, where I checked each thallus for evidence of grazing and retained only undamaged individuals for experiments.

To assess algal growth responses to different carbonate chemistry treatments, I situated algae in experimental mesocosms at TML. After rinsing each individual in fresh water to remove epiphytes and small invertebrates, I measured initial mass by first drying the individual in a manual centrifuge for 10 s before measuring its weight. This process non-destructively removes excess water and yields an accurate fresh weight of the individual (Ross et al., 2017). I then attached four individuals of each alga to a perforated

plastic sheet using small cable ties, randomly assigning their position to control for unintentional shading or facilitation among individuals. I assembled 12 of these sheets for a total of 240 individuals and placed them into 147-liter (39-gallon) aquaria under an array of 54-watt full-spectrum (12,000k) grow lights (Sun Wave™ HO T-5, Wave-Point, Sturgeon Bay, WI) set to a 12-hour daytime photoperiod. All aquaria received cold (12.4 ± 0.7°C) inflowing seawater at an average rate of 35.1 mL/s (±5.2 mL/s) from the laboratory flow-through system. I blocked the aquaria into three pCO_2 treatments, with a total of 80 algal individuals subjected to each treatment type. In a second experiment, to produce additional treated algae for feeding-preference trials, I collected an additional 16 individuals of each species and arranged them in four aquaria subjected only to the highest pCO_2 treatment.

I established pCO_2 treatments using a system of header tanks, monitoring probes, and CO_2 gas delivery. I assigned two header tanks to each of three pCO_2 treatments. "Ambient" (hereafter AM) pCO_2 tanks transferred unaltered seawater (pH 7.83 ± 0.06) from the laboratory's recirculating system to aquaria, "moderate" (hereafter MOD) pCO_2 tanks with seawater adjusted to a target pH of 7.70 (mean pH = 7.73 ± 0.07) before outflowing to aquaria, and "high" (hereafter HI) pCO_2 tanks adjusted to a target of 7.60 (mean pH = 7.65 ± 0.12) before outflowing. I outfitted MOD and HI tanks with pH probes (APEX Double Junction pH Probe, Neptune Systems, Morgan Hill, CA) connected to a Reefkeeper Elite V2 computer (Digital Aquatics, Woodinville, WA), which released flow from CO_2 gas cylinders when the pH of the header tanks rose above

programmed limits. I calibrated probes at least once per week using the computer's standard two-point calibration system, which conditioned probes in pH 7.02 (±0.01) and 10.04 (±0.02) buffer solutions (BioPharm, San Mateo, CA), and took additional instantaneous pH readings with a handheld probe (Hach IntelliCAL™ PHC101 and HQ40d, Hach, Loveland, CO). I also collected weekly discrete water samples, which I poisoned with mercuric chloride to halt all biological activity and sealed to prevent interaction with atmospheric gases. I then analyzed these samples with a highly accurate (± 0.1%) seawater carbonate chemistry analyzer ("Burke-o-lator"), at Hog Island Oyster Company in Arcata, CA. This system uses a strip gas to extract CO_2 from water samples and transport it to a nondispersive infrared (NDIR) gas analyzer (Bandstra et al., 2006; Hales et al., 2004), and I converted NDIR outputs (pCO_2 and tCO_2) to pH using the "seacarb" package in R (Gattuso et al., 2019).

To evaluate treatment-specific growth, I first allowed algae to acclimate to laboratory conditions for two weeks to minimize artefacts and assure the survival of the individuals during experiments. I re-weighed algae after this acclimation period to confirm that algae were growing and to obtain a new starting weight for each individual prior to the treatment period. Algae remained in treatment for four weeks during Experiment 1 (8 June to 13 July) and two weeks during Experiment 2 (29 July to 13 August). I weighed each individual every two weeks to assess growth. At the end of each experiment, I removed all individuals from laboratory treatments to use their tissue in feeding-preference trials and compositional analyses.

Algal Composition

To explore biochemical changes occurring in experimentally treated algae, I collected all algal tissue not used in feeding trials (*E. menziesii* = 1588 g, *L. setchellii* = 690.93 g, *M. splendens* = 402.38 g, *N. larix* = 174.89 g, *O. floccosa* = 568.59 g), separated them by species and treatment into airtight bags, and prepared them for use in laboratory assays. I first placed all 15 bags of treated algae and an additional five bags of untreated algae collected from Baker Beach (*E. menziesii* = 331.2 g, *L. setchellii* = 73.92 g, *M. splendens* = 94.48 g, *N. larix* = 90.96 g, *O. floccosa* = 142.34 g) into a freeze dryer (Preservator 220 & Freezemobile 42, VirTis Company, Inc., Gardiner, NY) for five days. Each sample bag was sufficiently dry once the change in algal mass decreased by less than 1% in a 24-hour period. I then removed the bags from the freeze-dryer and homogenized each dried sample (Bistro Electric Coffee Grinder, Bodum, Triengen, Switzerland), removing residual ground algae between samples by cleaning the grinder with a brush and grinding 0.25 g of silica gel sorbent (Mesh 28-200, Grade 12, CAS 63231-67-4, Fisher Scientific, Hampton, NH) for 10 s. After grinding, I stored algae samples in glass jars (Qorpak, Bridgeville, PA) placed within a desiccator to maintain consistently dry tissue for massing.

I first analyzed algal samples for their total carbon and nitrogen contents to better understand their nutritional value to grazers. To determine total carbon in each alga, I used a loss-on-ignition (LOI) combustion method (Ince et al., 2007). In the Humboldt State University Biology Core Research Facility (Arcata, CA), I weighed 0.500 g of each

sample into a 15 mL high-form porcelain crucible (CoorsTek, Golden, CO), noted the total initial weight of each filled container, and placed crucibles into a muffle furnace for 18 hours at 500°C. After allowing samples to cool for three hours, I recorded the total mass after combustion, noting that the change in sample weight represented the amount of all organic and inorganic carbon lost from the sample.

I estimated total nitrogen in each sample using the Kjeldahl digestion method and post-digestion analysis (Kalra, 1997). I combined 0.200 g of dried algae with a catalyst mixture [selenium (CAS 7782-49-2, Fisher Scientific), cupric sulfate pentahydrate (CAS 7758-99-8, Fisher Scientific), and potassium sulfate (CAS 7778-80-5, Fisher Scientific)] in a Lachat digester tube, added 5 mL sulfuric acid (CAS 7664-93-9, Fisher Scientific), and digested the reactants at 370°C for 30 minutes. I then diluted each sample by adding Nanopure water to produce a final volume of 20 mL for analysis by a Shimadzu TOC-L/TNM-L unit (Shimadzu Corporation, Kyoto, Japan), which utilized an infrared detector to measure the amount of NH_3 in each volatilized sample.

To verify these analyses, I shipped a subset of samples to Washington State University's Stable Isotope Core Laboratory in Pullman, WA, USA. This facility employed an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA) to process 1.50-2.00 mg of each algal species from either the MOD or HI treatment and determine total carbon and nitrogen proportions of each sample.

Finally, I analyzed all samples for total phenolic content, as the presence of these compounds in algae is likely to influence their palatability to grazers (Kathryn L. Van

Alstyne & Paul, 1990; Wright et al., 2004). Using a modified Folin-Ciocalteu method adapted from Zhang et al. (2006), I weighed 0.250 g of each sample into 15-mL centrifuge tubes (Falcon, Corning, NY) and extracted soluble contents with a methanol-water solution (1:1) followed by an acetone-water solution (7:3). After preparing serial standards of phloroglucinol dihydrate solution (CAS 6099-90-7, FW 162.14 g/mol, Alfa Aesar, Ward Hill, MA), I reacted the extracts and standards with sodium carbonate anhydrous solution (CAS 497-19-8, FW 105.99, Fisher Scientific, Hampton, NH) and Folin-Ciocalteu reagent (Spectrum Chemical, New Brunswick, NJ) and pipetted 250 µL of each solution into a Costar 96-well black clear-bottomed microplate. I then analyzed each plate using a SpectraMax i3 spectrophotometer (Molecular Devices, San Jose, CA) at 750 nm and converted sample and control values into units of phloroglucinol equivalent per gram (PGE g^{-1}).

Feeding Preference Trials

For feeding preference trials, I collected *P. producta* and *T. funebralis* individuals from three intertidal sites along the northern California coast: Point St. George in Crescent City (Del Norte Co.; 41°47’08.0” N, 124°22’42.47” W), Baker Beach in Trinidad (Humboldt Co.; 41°02’57.1” N, 124°07’36.7” W), and Devil’s Gate near Cape Mendocino (Humboldt Co.; 41°23’45.4” N, 124°15’23.1” W). Each site contains an abundance of the five algal species used in the study; however, I collected equal numbers of invertebrates from each location to control for any site-specific foraging preferences.

To minimize the organisms' holding time in the lab, I revisited each site before beginning a new set of feeding trials and collected approximately 10 new crabs and 70 new snails for each phase of the experiments.

I conducted a series of feeding trials to determine algivore preferences for both untreated and HI-treated algae. After transporting grazers from the field to the laboratory, I starved them for 48 h to standardize hunger levels and improve the likelihood of measurable feeding behavior. I then blotted, weighed, and assigned the grazers to small 8.5-L aquaria either individually, for *P. producta*, or in groups of four, for *T. funebralis*. I covered aquaria containing *P. producta* with a sheet of black plastic to mimic nocturnal conditions and maximize feeding activity, as overhead lighting in the laboratory prevents natural dark hours. Each individual crab or group of four snails participated in only one feeding trial to eliminate any acclimation bias across replicates.

For untreated preference trials, I collected fresh individuals of the study algae from Baker Beach, cleaned them of epiphytes in fresh water, and placed a 4-g piece of each species in an 8.5-L aquarium with the grazer(s) for 24 h. Control replicates excluded grazers to establish a known rate of autogenic loss of algae during trial periods. At the end of each trial, I re-weighed each piece of algae from experimental replicates, subtracted the average amount of autogenic loss, scaled consumed mass to grazer mass. For high-pCO_2 preference trials, I replicated this setup using algae from the HI treatment to determine whether elevated pCO_2 caused feeding preferences to deviate from those observed with untreated algae.

In both untreated- and HI-preference trials, I also included no-choice replicates, which evaluated grazing activity on a single 20-g piece of algae. Either multiple-choice or no-choice trials, used alone, tell an incomplete story; whereas choice trials fail to indicate the preference's strength by lacking statistical independence within each replicate, no-choice trials restrict a grazer's ability to adequately demonstrate preference by removing the "context" of feeding behavior and may prompt compensatory consumption, which confounds results (Peterson & Renaud, 1989; Roa, 1992). A combination of multiple-choice and no-choice trials provided a robust and effective way to evaluate preference, elucidating whether observed behaviors represented true preference or were due to random effects, such as the order in which grazers encountered different algae.

Statistical Analyses

I performed all statistical analyses in R version 3.5.1 (R Core Team, 2018), produced visualizations with the "ggpubr" package (Kassambra, 2020), and used the "FSA" and "nortest" packages to check datasets for normality and homoscedasticity (Gross & Ligges, 2015; Ogle et al., 2019). First, to assess the validity of experimental conditions, I compared the pH and pCO_2 levels of seawater within each treatment type, combining measurements for both header tanks and aquaria for each condition. I used one-way analyses of variance (ANOVAs) to compare treatment groups, but because some of these datasets were non-normal, I employed Kruskal-Wallis rank sum tests where

appropriate. To compare pH values between algae-lacking header tanks and algae-containing aquaria, whose differences indicate photosynthetic activity in the experimental algae, I used a Wilcoxon rank sum test for each treatment group.

I evaluated algal growth and carbon, nitrogen, and phenolic contents with two-way ANOVAs, treating algal species and pCO_2 treatment as fixed factors in each analysis. I checked data for normality and homogeneity of variances and used a square root transformation to improve the fit of the model for phenolic content of algae. I used Tukey's Honest Significant Difference tests to determine which pairwise differences contributed to the overall significance of each model.

I assessed grazer preference using several ANOVAs. Untreated preference data was non-normal and heteroscedastic, so I used Kruskal-Wallis rank sum tests and Dunn's tests for multiple comparisons to evaluate differences in each trial type (choice or no-choice) for both grazers. Because high-pCO_2 preference data were normally distributed with equal variances, I used a 1-way ANOVA for each combination of grazer and trial type. I also assessed the effects of algal species and treatment on each algivore's order of preference by separating data into individual replicates (one choice aquarium or five no-choice aquaria), ranking each alga by its amount consumed in that replicate, and analyzing each grazer-trial combination with a two-way ANOVA.

RESULTS

Experimental Conditions

Discrete water samples confirmed that carbonate chemistry was consistent with the intended experimental treatments (Fig. 1). Seawater pCO_2 values were higher in HI treatments (1235.69 ± 78.02 ppm, $n = 36$) than in AM treatments (889.37 ± 72.93, $n = 31$) for both the first ($\chi^2 = 4.96$, $df = 2$, $P = 0.040$) and second ($F_{2,15} = 5.20$, $P = 0.015$) growth experiments. AM and HI treatments were also distinct when both experiments were considered together ($\chi^2 = 10.63$, $df = 2$, $P = 0.005$). Handheld pH sensor readings indicated significant differences in pH among all treatments ($F_{2,69} = 23.83$, $P < 0.001$); AM was higher in pH than both MOD (Tukey's HSD, $P = 0.004$) and HI (Tukey's HSD, $P < 0.001$), and MOD had a higher pH than HI (Tukey's HSD, $P = 0.002$). These combined results indicate that experimental conditions matched proposed treatments during both growth periods; conservatively, HI seawater was at least higher in pCO_2 than unaltered seawater as represented in the AM treatment, thus carbonate chemistry is a likely factor in observed algal and grazer responses to these two seawater conditions.

Water chemistry also provided evidence for photosynthetic activity by experimental algae during laboratory treatment. For all treatments in Experiment 1, daytime pH values in aquaria were significantly higher than in the header tanks that supplied them, likely indicating an uptake of inorganic carbon by growing algae (Fig. 2).

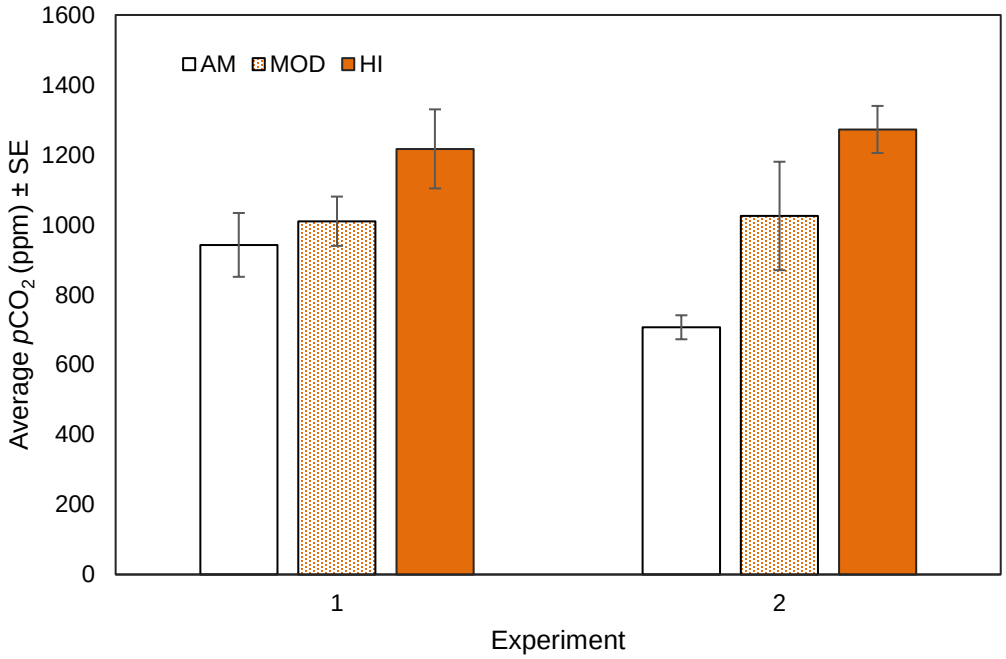


Figure 1. AM treatments were significantly lower in pCO_2 than HI treatments for both Experiment 1 (AM = 942.56 ± 91.25 ppm; HI = 1217.12 ± 67.24 ppm) and Experiment 2 (AM = 707.02 ± 34.32 ppm; HI = 1272.76 ± 67.24 ppm). MOD treatments (1013.65 ± 73.03, n = 31) were marginally higher in pCO_2 than AM (Dunn's test, P_{adj} = 0.070) and marginally lower than HI treatments throughout both experiments (Dunn's test, P_{adj} = 0.087).

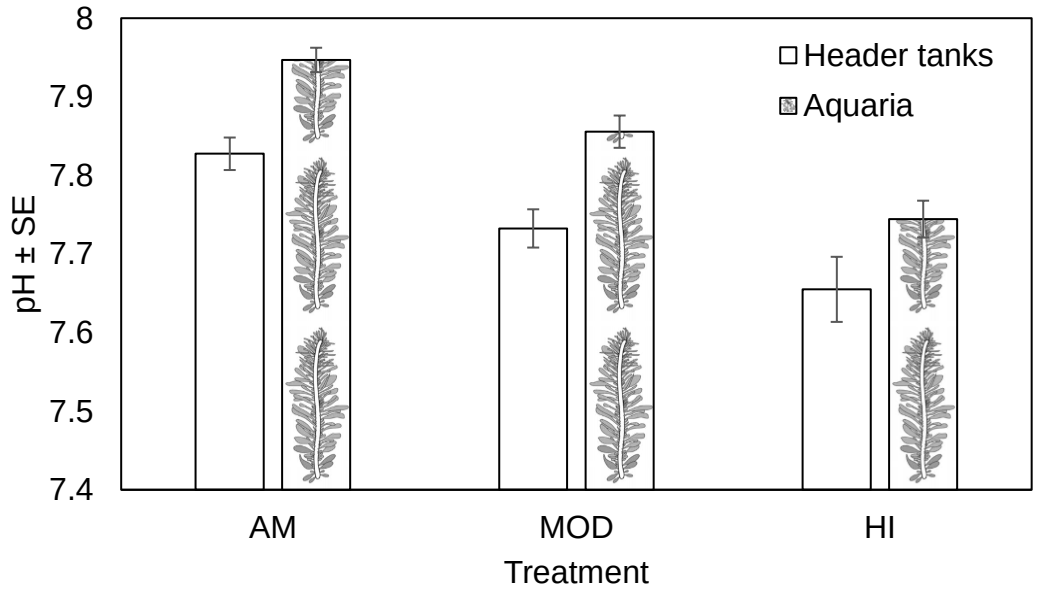


Figure 2. pH readings from a handheld sensor, averaged across four weeks during Experiment 1, indicated that algae-containing aquaria maintained a higher pH than algae-lacking header tanks within the same treatment. AM ($W = 15$, $P = 0.003$), MOD ($W = 19.5$, $P = 0.007$), and HI ($W = 30.5$, $P = 0.043$) treatments all demonstrated this effect.

Algal Growth

All algal species generated new growth during at least half of each experiment, though most occurred in the first two weeks of treatment (Table 1). Red algae (*M. splendens*, *N. larix*, and *O. floccosa*) responded rapidly to elevated pCO_2, growing quickly during the first two weeks in the HI treatment (Fig. 3*C*-*E*), but these rates declined during the remainder of treatment. Brown algae (*E. menziesii* and *L. setchellii*) responded more slowly than red algae to increased pCO_2, but they maintained steadier growth throughout the experimental period (Fig. 3*A*-*B*). During the entire treatment period, all species but *M. splendens* exhibited a net increase in biomass.

Species identity significantly affected algal growth ($F_{4,222} = 2.42$, $P = 0.049$), with *L. setchellii* generating the highest rate of daily growth across all treatments (Fig. 4). MOD-treated *L. setchellii* grew more rapidly ($1.39 \pm 1.01\%$ day^{-1}) than any other species growing in the AM or HI treatments and more than MOD-treated *M. splendens.* Levels of pCO_2 also influenced growth, with the majority of new growth occurring in the MOD treatment ($F_{2,222} = 4.65$, $P = 0.011$). MOD individuals grew, on average, $1.230 \pm 2.20\%$ per day, whereas AM and HI treatments only saw increases of $0.236 \pm 2.08\%$ and $0.264 \pm 1.72\%$ per day, respectively. AM and HI treatments yielded similar growth rates (Tukey's HSD, $P = 0.992$) when averaged across all species.

Table 1. Growth rates (proportional change per day ± SE) varied throughout Experiment 1, with the greatest change occurring in the first two weeks of the study (n = 237). Kelps *Egregia menziesii* and *Laminaria setchellii* demonstrated similar rates of growth regardless of time period, whereas rhodophytes demonstrated initial rapid growth with later declines in growth rates.

	Growth period		
Species	**0-2 weeks**	**2-4 weeks**	**0-4 weeks**
Egregia menziesii	0.20 ± 0.06	0.18 ± 0.17	0.11 ± 0.06
Laminaria setchellii	0.28 ± 0.06	0.30 ± 0.23	0.30 ± 0.04
Mazzaella splendens	0.37 ± 0.14	-0.18 ± 0.04	-0.06 ± 0.06
Neorhodomela larix	0.31 ± 0.14	-0.07 ± 0.04	0.05 ± 0.04
Odonthalia floccosa	0.19 ± 0.08	-0.02 ± 0.03	0.09 ± 0.03
Combined avg.	0.27 ± 0.05	0.04 ± 0.06	0.10 ± 0.02

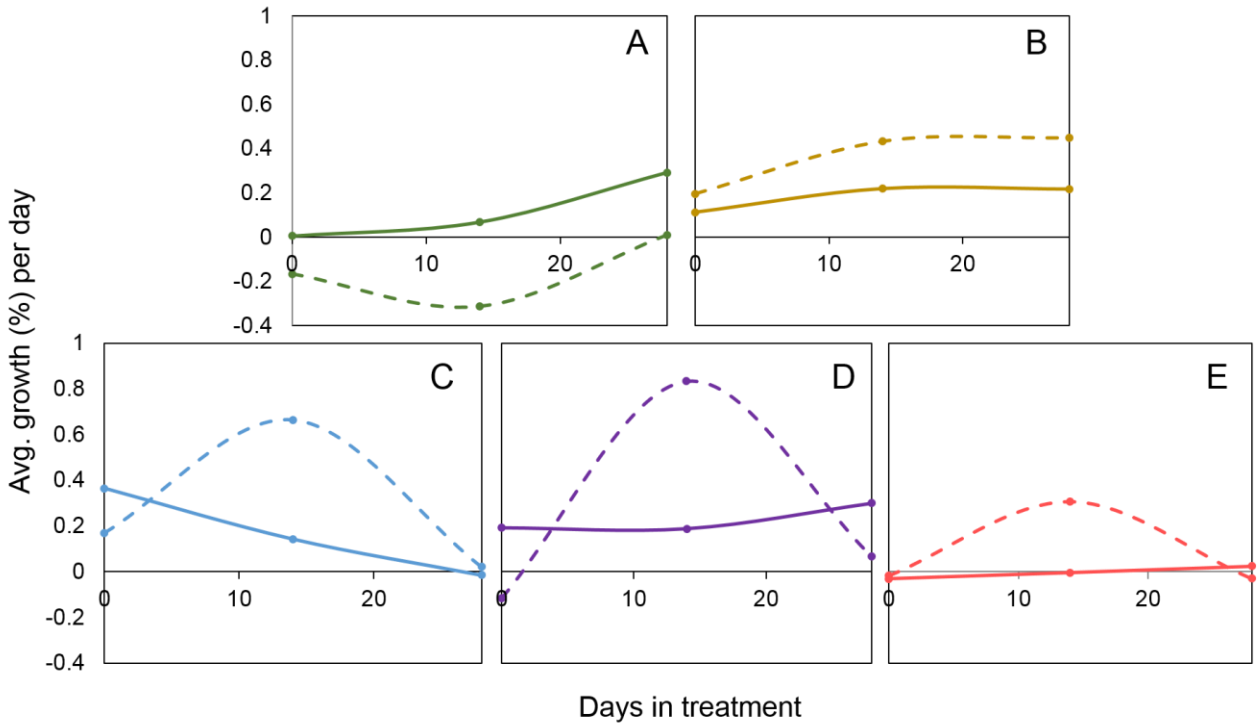


Figure 3. Algal growth relative to starting biomass for (*C*) *M. splendens*, (*D*) *N. larix*, and (*E*) *O. floccosa*, peaked during the second week for all HI treatments (*dashed line*) while MOD treatments (*solid line*) were much less variable. Brown algae (A) *E. menziesii* and (B) *L. setchellii* demonstrated steadier, less dramatic fluctuations in biomass than red algae. Biomass values are relative to conspecifics in the AM treatment at each time point, which is represented along the x-axis.

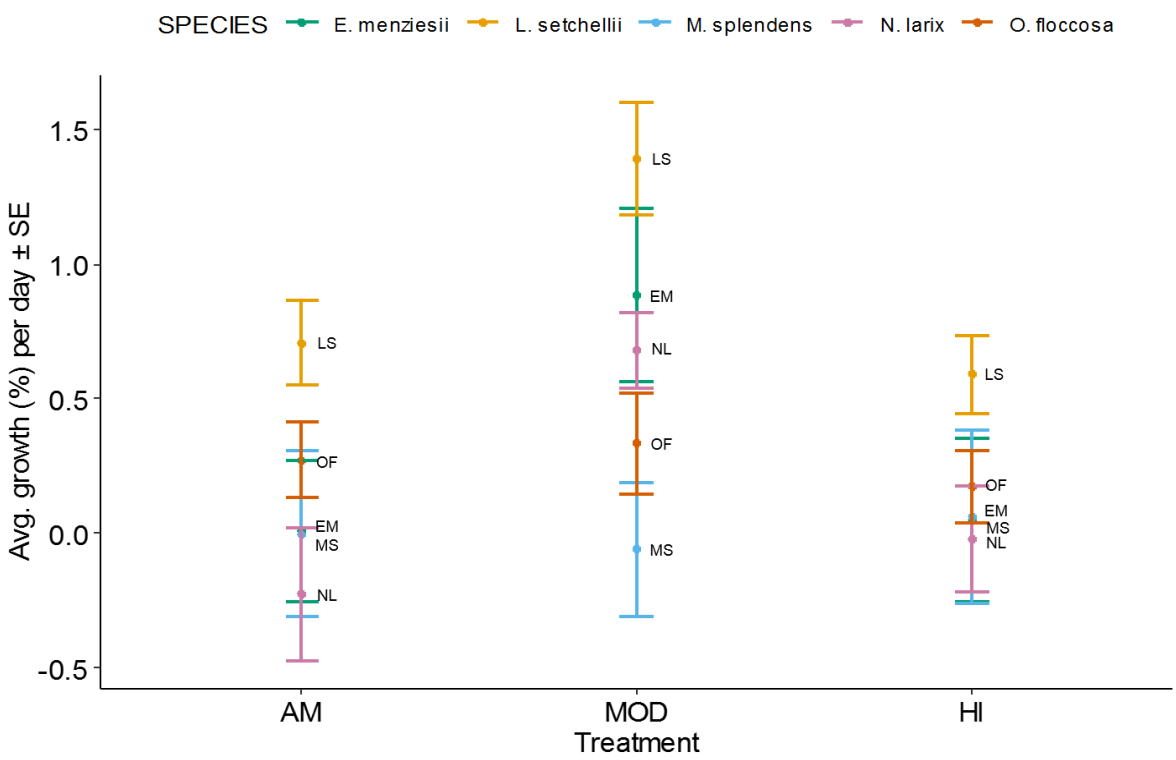


Figure 4. Across all treatments, the kelp *L. setchellii* grew more quickly (0.896 ± 0.76% day^{-1}) by weight than any other species in the test assemblage. Relatively high productivity in brown algae (*E. menziesii* and *L. setchellii*), particularly in the MOD treatment, produced significant effects of both species and treatment on algal growth, though interactive effects were not statistically significant ($F_{8,222} = 1.28$, $P = 0.257$).

Algal Composition

Total carbon content of algae varied by species ($F_{4,19} = 647.02$, $P < 0.001$) and treatment ($F_{3,19} = 33.76$, $P < 0.001$). The interaction between species and treatment also influenced carbon proportions ($F_{12,19} = 179.56$, $P < 0.001$), with most comparisons showing significant differences but some similarities occurring between *L. setchellii* and *O. floccosa* and between *E. menziesii* and *N. larix* (Fig. 5*A*).

Nitrogen content also varied by species ($F_{4,40} = 424.76$, $P < 0.001$) and treatment ($F_{3,40} = 9.71$, $P < 0.001$); all species but the kelps *E. menziesii* and *L. setchellii* differed, and individuals in the AM and HI treatments were comparable whereas all other treatment comparisons yielded significant differences. I also observed strong interactive effects between species and treatment ($F_{12,40} = 156.27$, $P < 0.001$), with 166 of 190 comparisons indicating differences in nitrogen contents (Fig. 5*B*). Interestingly, all but one comparison between a brown and a red alga showed differences in nitrogen. These results indicate that while nitrogen content was influenced by both species and treatment across the full assemblage, brown algae in particular had similarly low N contents regardless of treatment.

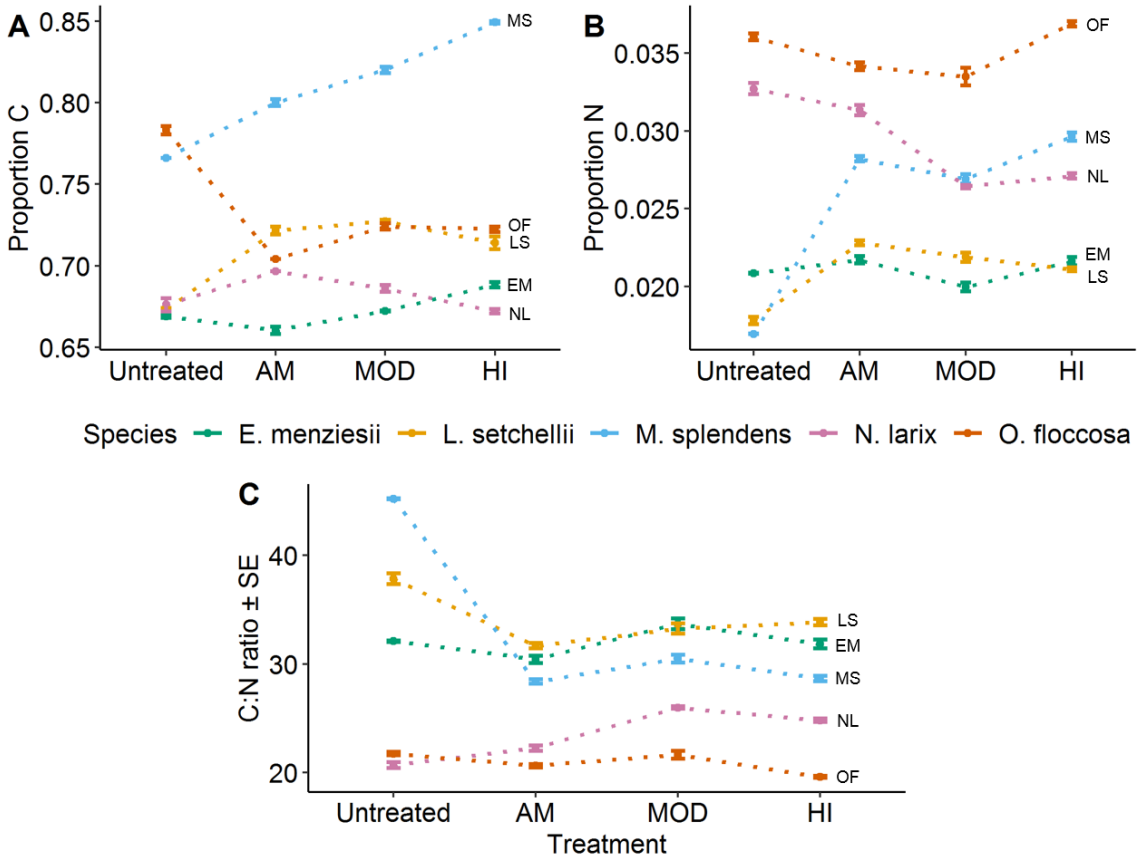


Figure 5. Interactive effects between species and treatment influenced both (*A*) carbon and (*B*) nitrogen contents of experimental algae, with lower levels of nitrogen found in brown algae. (*C*) Carbon-nitrogen ratios were lowest in red algae, which had higher proportions of nitrogen in their tissues than brown algae, regardless of treatment.

Phenolic content of laboratory algae strongly varied among species ($F_{4,161}$ = 49.05, $P < 0.001$) and was affected by the interaction between species and treatment ($F_{8,161} = 3.44$, $P = 0.001$; Fig. 6). Generally, each species contained a distinct level of phenolic compounds, which remained similar across laboratory treatments. Untreated algae showed few interspecific differences ($F_{4,55} = 11.72$, $P < 0.001$), with *M. splendens*' very low levels responsible for four of the five significant species comparisons. HI-treated algae presented greater variation ($F_{4,55} = 27.58$, $P < 0.001$) than other treatments, with the brown alga *L. setchellii* differing from both *E. menziesii* (Tukey's HSD, $P < 0.001$) and *N. larix* (Tukey's HSD, $P < 0.001$) after HI pCO_2 exposure. The only species to vary on the basis of laboratory treatment was *L. setchellii*, which contained different levels of phenolic compounds between AM and HI treatments (Tukey's HSD, $P = 0.014$).

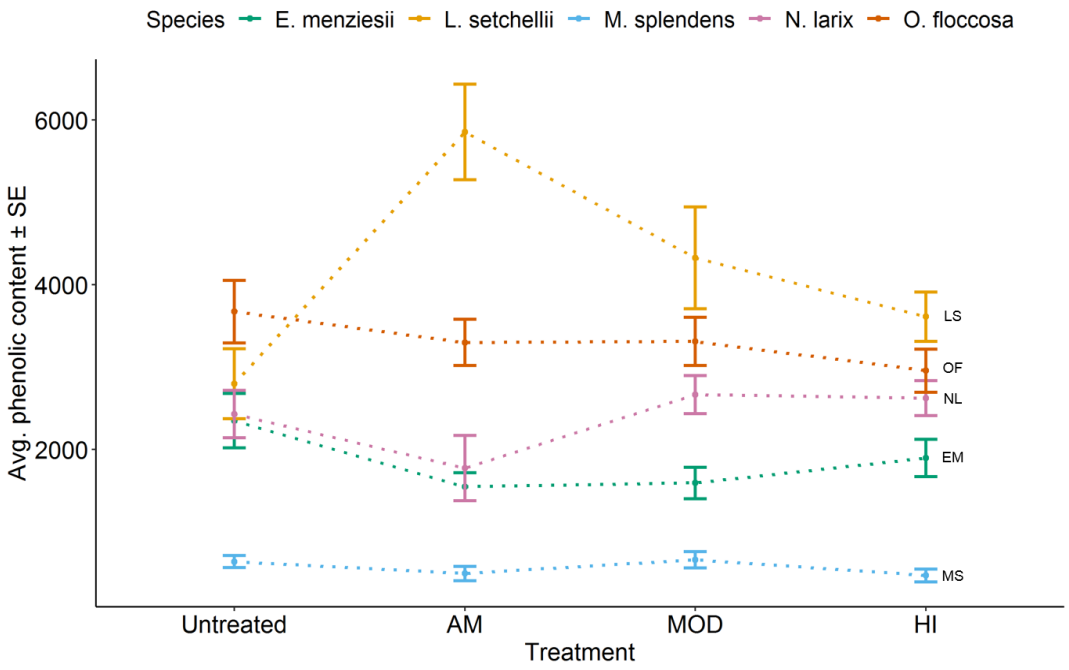


Figure 6. There was no significant effect of treatment on levels of phenolic compounds (represented as phloroglucinol equivalent, PGE) in laboratory algae ($F_{2,161}$ = 0.56, P = 0.573). However, variation in phenol concentration was significant among species and in the interaction between species and treatment, excluding untreated algae.

Algivore Preference

In both multiple-choice and no-choice feeding trials, *P. producta* favored brown algae over red algae. Crabs preferred the kelp *E. menziesii* in multiple-choice trials, consuming more untreated *E. menziesii* (0.121 ± 0.108 g) than two other untreated species (χ^2 = 18.13, *df* = 4, *P* = 0.001) and more treated *E. menziesii* (0.119 ± 0.023 g) than all four other treated species ($F_{4,15}$ = 70.54, *P* < 0.001; Fig. 7, top panels). In no-choice trials, *P. producta* consumed all five untreated species at similar rates but demonstrated a preference for treated kelps over treated red algae (Fig. 8, top panels). These results indicate a weak preference among untreated algae but a strong preference among algae growing in high-pCO_2 conditions. Treatment did not affect algal rankings for *P. producta* ($F_{1,40}$ = 0.67, *P* = 0.417), but treated *E. menziesii* ranked higher than untreated conspecifics in no-choice trials (*t* = 3.80, *df* = 5, *P* = 0.013; Table 2), thus preference for the kelp was strengthened by the HI treatment.

T. funebralis also preferred *E. menziesii*, though their preference was weaker than that of crabs. In choice trials, the snail consumed an average of 0.033 ± 0.026 g of untreated and 0.020 ± 0.005 g of treated *E. menziesii*, the latter of which was greater than the consumption of any treated red alga (Fig. 7, bottom panels). Multiple-choice and no-choice trials yielded similar patterns of preference for untreated algae, indicating that *T. funebralis* consistently favored *E. menziesii* over one to two other untreated species. However, no-choice trials with treated algae showed no preference in snails and therefore failed to substantiate the preference demonstrated in multiple-choice trials; preference for

treated algae was weaker than that for untreated algae. Treatment had no influence on algal ranks in either choice or no-choice trials ($F_{1,40} = 0.11$, $P = 0.740$; $F_{1,40} = 1.49$, $P = 0.229$), indicating that the ranking of algae by snails did not depend on whether the algae was treated. Thus, while treatment did not affect the order of snails' preference, it likely influenced the strength of this preference relative to other algal species, particularly in multiple-choice trials.

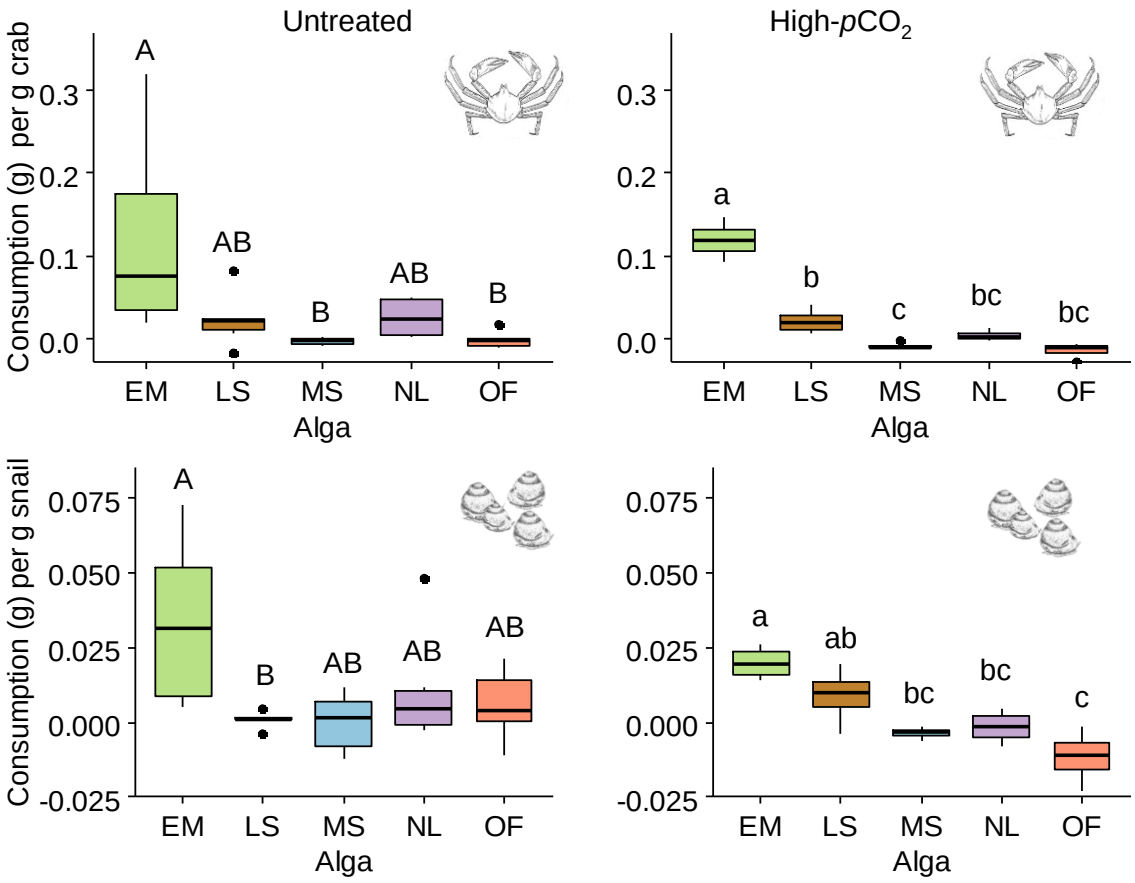


Figure 7. In multiple-choice trials, grazers demonstrated stronger preferences among HI-treated algae (right) than among the untreated assemblage (left). *P. producta* preferred *E. menziesii* (EM) over two species of red algae ($\chi^2 = 18.13$, $df = 4$, $P = 0.001$) in natural preference trials, but in high-pCO_2 (HI) trials, the crab consumed more *E. menziesii* than any other species ($F_{4,15} = 63.77$, $P < 0.001$). *T. funebralis* weakly preferred *E. menziesii* in untreated trials ($\chi^2 = 9.97$, $df = 4$, $P = 0.041$) but demonstrated a moderate preference for the kelp after high-pCO_2 treatment, choosing it over all red algal species ($F_{4,15} = 12.09$, $P < 0.001$).

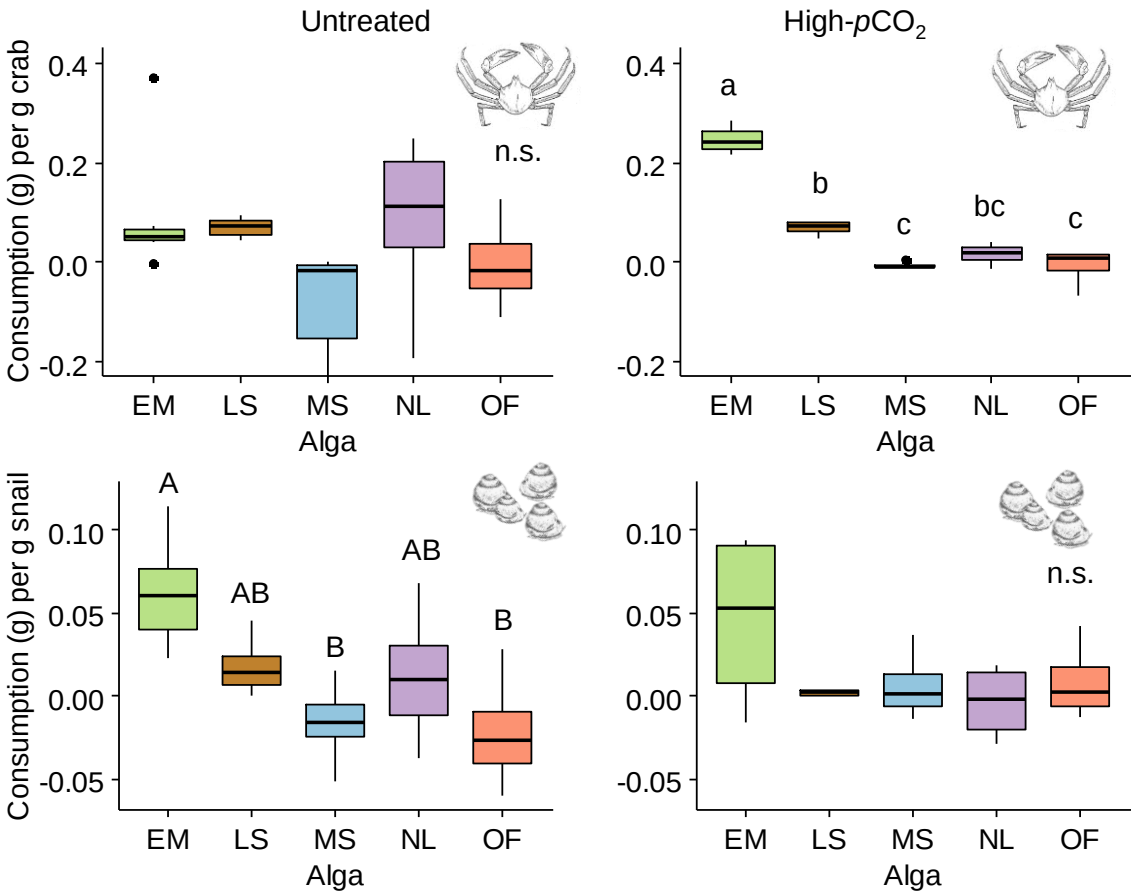


Figure 8. No-choice trials indicated that *P. producta* would eat all untreated algae in relatively equal quantities (χ^2 = 12.46, *df* = 4, *P* = 0.014); however, the crab consumed markedly more *E. menziesii* (EM) than any other HI species, ($F_{4,15}$ = 70.54, *P* < 0.001) indicating strong preference. Snails, somewhat preferring untreated *E. menziesii* (χ^2 = 15.88, *df* = 4, *P* = 0.003), demonstrated no preference in HI trials ($F_{4,15}$ = 1.69, *P* < 0.206), thus indicating weak preference.

Table 2. Mean rank (± 1 SD) of algal species in feeding trials. The rank of *E. menziesii* was significantly lower than that of all other species, which indicates that the kelp is the first choice of both *P. producta* ($F_{4,40}$ = 24.32, P < 0.001) and *T. funebralis* ($F_{4,40}$ = 5.22, P = 0.002). Lower rank values indicate higher degrees of preference.

	Alga	Choice, Untreated	Choice, Treated	No-choice, Untreated	No-choice, Treated
P. producta 	*E. menziesii*	1.33 ± 0.52	1.00 ± 0.00	2.17 ± 0.75	1.00 ± 0.0
	L. setchellii	2.17 ± 0.98	2.00 ± 0.00	2.33 ± 0.82	2.00 ± 0.00
	M. splendens	4.50 ± 0.55	4.25 ± 0.50	4.50 ± 0.84	4.50 ± 0.58
	N. larix	2.67 ± 0.82	3.00 ± 0.00	2.33 ± 1.75	3.50 ± 1.00
	O. floccosa	4.33 ± 0.82	4.75 ± 0.50	3.67 ± 1.37	4.00 ± 0.82
T. funebralis 	*E. menziesii*	1.50 ± 0.84	1.25 ± 0.50	1.33 ± 0.82	2.25 ± 1.50
	L. setchellii	3.67 ± 1.21	2.25 ± 1.26	2.33 ± 0.52	3.25 ± 2.06
	M. splendens	3.33 ± 1.63	3.25 ± 0.50	4.33 ± 0.52	3.00 ± 1.15
	N. larix	3.00 ± 1.26	3.50 ± 1.29	2.83 ± 1.33	4.00 ± 1.41
	O. floccosa	3.50 ± 1.38	4.75 ± 0.50	4.17 ± 1.17	2.50 ± 1.00

DISCUSSION

My results indicate that ocean acidification has the potential to induce changes in marine macroalgal growth rates and secondary metabolism, which in turn may modify existing interactions with algivores. Specifically, preferences for particular algae may either strengthen or weaken as acidification progresses, and this effect is likely modulated by the feeding strategies (i.e., degree of specialization) of consumers. These data also suggest that the relative phenolic content of algae, which has previously been shown to be altered by high-pCO_2 conditions (Celis-Plá et al., 2015; Swanson & Fox, 2007) and influential to palatability and grazing behavior (Hay & Fenical, 2016), acts as a driver of these changes in algivore preference. Both algae and the grazers which rely on them are thus expected to be affected by ocean acidification, although individual responses appear dependent upon a variety of factors.

Observed variation in algal responses to increased pCO_2 during growth experiments may have been the result of a variety of carbon-use strategies within the assemblage. Red algae initially benefitted from elevated carbon availability, which likely allowed for increased passive acquisition. Although these species' growth rates in the highest-pCO_2 treatment declined in the latter stage of the experiment, this effect may be the result of prolonged exposure to low-pH seawater, which can induce tissue decay in red and brown algae (Israel & Hophy, 2002). Both kelps, on the other hand, utilized higher levels of inorganic carbon throughout the experiments, allocating this extra

resource to enhanced photosynthesis and the increased production of carbon-based phenolics, particularly in the case of *L. setchellii*. Brown algal growth was, however, slower and less extreme than the initial pulse of red algal growth. The addition of DIC to presently under-saturated seawater (Koch et al., 2013) has the capacity to enhance algal metabolism for both red and brown algae (Swanson & Fox, 2007), either by boosting direct diffusion or by allowing for the downregulation of energetically costly CCMs (Hepburn et al., 2011; Hurd et al., 2009; Swanson & Fox, 2007), and differential responses in experimental algae may be explained by the presence or absence of CCMs in these species. While these red algae likely rely on passive CO_2 diffusion and are thus more sensitive to elevated pCO_2, kelps and other brown algae often utilize CCMs to enhance internal CO_2 concentration regardless of ambient levels (Hepburn et al., 2011), which makes them less reactive to small pCO_2 changes than red algae. Regardless of their carbon-use strategies, many algae demonstrated overall growth rates that, contrary to expectation, did not vary linearly or even monotonically with pCO_2 concentration and length of treatment. This may be attributed to artefact or the degree to which laboratory lighting and temperature conditions differed from natural ones, but the effect may also be partially explained by long-term exposure of these algal populations to local intertidal conditions.

Experimental algae were most capable of taking advantage of elevated pCO_2 when it mimicked the timing or intensity of the natural variability experienced by seaweeds on the northern California coast. Situated in a seasonal upwelling system, these

populations regularly encounter short-term (multi-day) periods of increased pCO_2 concentrations (Huyer, 1983; Iles et al., 2012) within a range (Feely et al., 2008; Hales et al., 2005). Experimental algae exhibited the most growth at moderate pCO_2 levels and in the first two weeks of treatment; each of these factors is consistent with those found during summer in upwelling zones of the northeast Pacific, thus local populations may be acclimatized to respond positively to short-term or moderate increases in pCO_2. Acclimatization to growth stressors has been shown with exposure to variable temperatures, after which algae were better equipped to adjust to future fluctuations (Padilla-Gamiño & Carpenter, 2007), and with exposure to naturally variable pCO_2, after which algae were less sensitive to subsequent changes (Padilla-Gamiño et al., 2016). While the degree of plasticity exhibited by algal populations is likely to be species-specific, an idea supported by the variable responses in my test assemblage, algae that are locally acclimatized demonstrate unique tolerances for the stressors frequently experienced by that population (Harley et al., 2012). Here, peaking growth rates at moderate pCO_2 and rapid growth rates of red algae under short-term pCO_2 enrichment may indicate population-level adjustments to some of the conditions established by natural upwelling events.

Although algal growth patterns indicate how this trophic level might respond in isolation to future ocean carbon conditions, they tell us little about the effects of ocean acidification on algal palatability to grazers. Herbivores are generally expected to select plant tissues with the greatest relative proportion of nitrogen, a limiting nutrient in most

systems (Mattson, 1980). Nitrogen contents of algae underwent subtle changes with increasing pCO_2 exposure, but effects were highly dependent on species identity. Although the slightly lower C:N ratio of *E. menziesii* with respect to *L. setchellii* may help explain crabs' consistent preference between these two kelps, red algal species had markedly higher N content than either kelp regardless of treatment, with the exception of untreated *M. splendens*. Therefore, nutritional value alone does not explain algivore preference, but its role is likely influenced by phenolic contents. Greater consumption of apparently N-deficient brown algal tissue by the specialist *P. producta* may be explained by the elevated phenolic contents of those algae, as phenolics can reduce herbivores' ability to digest nitrogen from tissues, resulting in compensatory consumption of these algae (Arnold et al., 1995; Cruz-Rivera & Hay, 2000; Mattson, 1980). Additionally, phenolic compounds have been shown to deter generalist herbivores even when the nutritional value of the food source is high (Newman et al., 1996; Speiser & Rowell-Rahier, 1991). Here, *E. menziesii* and *N. larix* slightly increased phenolic production with increasing pCO_2 while *L. setchellii*, *M. splendens*, and *O. floccosa* showed decreased or unchanged levels, and grazers sensitive to phenolics responded by decreasing (increasing) feeding on algae that contained higher (lower) levels of phenolics. Other factors, such as structural defense in *M. splendens* (Gaines, 1985), are almost certainly influencing palatability, as evidenced by grazers' preference for kelps regardless of C:N ratios or phenolic content. Regardless, divergence in the composition of untreated and

high-pCO_2 treated algae in these experiments indicates that algal palatability is likely to change, at least for some grazers, as OA progresses.

Differing levels of anti-herbivory compounds did not produce identical preference responses in the two algivores studied here, which should be expected for grazers with dissimilar feeding strategies. Though high-pCO_2 treatment appeared to induce stronger preferences in kelp crabs, the same conditions weakened the preference of turban snails. Kelp crabs, a specialist grazer, chose untreated *E. menziesii* over others in choice trials but showed no preference in no-choice trials, indicating that this is a weak preference. In high-pCO_2 trials, however, crabs chose *E. menziesii* over all others in both choice and no-choice trials, signifying strong preference for the kelp following pCO_2 treatment. Snails demonstrated the opposite pattern. *T. funebralis* consistently chose *E. menziesii* in untreated trials, both choice and no-choice, and therefore had a strong preference for the alga under present-day conditions. In high-pCO_2 trials, snails only weakly favored *E. menziesii* as evidenced by their consumption of all algal species in relatively equal proportions during no-choice trials. Interestingly, it was preference for the same alga (*E. menziesii*) that generated such contrasting responses, implicating differential feeding strategies as a cause for this pattern.

Kelp crabs, known to favor low-intertidal and subtidal kelps including *E. menziesii* (Bracken & Stachowicz, 2007; Dobkowski, 2017; Leighton, 1966), showed little variation in their untreated and high-pCO_2 preferences. *P. producta* selected nearly identical rankings of algae in multiple-choice trials and appeared to reestablish its

preference for high-pCO_2 *E. menziesii* in no-choice trials, indicating that it is likely well-adapted to this particular alga, regardless of the levels of anti-herbivory phenolics found in the algal tissue. In both marine and terrestrial systems, specialist grazers like kelp crabs are adapted to the secondary metabolites of their host plants (Hay & Fenical, 2016; Wei et al., 2015), undeterred by these compounds and even rely on them as chemical cues for detection and recognition of their host plants (van der Meijden, 1996). Perhaps, having developed an inherent preference for *E. menziesii*, kelp crabs are unaffected by small deviations in nitrogen and phenolic content relative to other palatable species, consistently choosing this alga regardless of pCO_2-induced changes.

As would be expected of a generalist grazer, turban snails demonstrated a much more plastic feeding behavior than kelp crabs, responding to algal changes with adjustments to feeding preferences. Snails selected a low-phenol, high-nutrient alga in untreated trials as it was likely the most palatable option among the assemblage. However, as phenolic contents of treated algae diverged (Appendix 1), comparisons between species appeared to dictate snails' feeding behavior. For example, untreated *N. larix* and *E. menziesii* contained similar levels of phenolics (2427.7 ± 993.6 and 2346.7 ± 1141.5 PGE g^{-1}, respectively) and were grazed at similar rates by *T. funebralis*, but when treated, *N. larix* contained somewhat greater phenolic compounds (2664.3 ± 802.5 PGE g^{-1}) than *E. menziesii* (1894.9 ± 785.7 PGE g^{-1}) and was grazed significantly less. Other species comparisons, such as that of *L. setchellii* and *O. floccosa*, further support this pattern of contrasting preferences relative to phenolic content. The generalist feeding

strategy allows preference to shift based on palatability, which itself shifted in this assemblage. Elevated levels of secondary metabolites, such as phenols, in host plant tissue have been shown to deter generalist herbivores (Wei et al., 2015), which can then select foods without such compounds (Speiser & Rowell-Rahier, 1991). Unlike the specialist kelp crab, which appeared unaffected by shifting palatability, *T. funebralis* behavior indicates that generalist responses to OA-affected algae are likely to be more dynamic and dependent upon changes in algal secondary metabolism.

Despite that all algae in this experiment demonstrated enhanced growth with increasing pCO_2, differential nutrient contents and phenolic production indicate that overall macroalgal responses to OA may be species-specific, at least in terms of their appeal to intertidal grazers. Further, algivores exhibit contrasting strategies to manage these changes in algal palatability. Although specialists may be expected to consume relatively greater amounts of their preferred alga in future ocean carbon conditions, generalists may begin to diversify their choices, placing new grazing pressures on algae that are currently uninfluenced by their herbivory. These results suggest that intertidal community dynamics can be expected to change as OA progresses, though continued examination of algal and algivore responses is necessary to elucidate the effects of multiple stressors (elevated temperature, increased ultraviolet light exposure, hypoxia) on these interactions (Celis-Plá et al., 2015; Xu et al., 2010) in upwelling systems. Further study should also seek to identify the underlying mechanisms of preference, which may help explain the extent to which algivory and competition between algivores may be

modified. Understanding the nature and strength of these relationships in future climate conditions is a key step towards preserving biodiverse and resilient ecosystems in the face of climate change.

REFERENCES

Abbott, I. A., & Hollenberg, G. J. (1976). *Marine algae of California*. Stanford University Press.

Arnold, T., Tanner, C., & Hatch, W. (1995). Phenotypic variation in polyphenolic content of the tropical brown alga Lobophora variegata as a function of nitrogen availability. *Marine Ecology Progress Series*, *123*(1–3), 177–183. https://doi.org/10.3354/meps123177

Augyte, S., & Shaughnessy, F. J. (2014). A floristic analysis of the marine algae and seagrasses between Cape Mendocino, California and Cape Blanco, Oregon, USA. *Botanica Marina*, *57*(4), 251–263. https://doi.org/10.1515/bot-2013-0032

Bakun, A., Black, B. A., Bograd, S. J., García-Reyes, M., Miller, A. J., Rykaczewski, R. R., & Sydeman, W. J. (2015). Anticipated Effects of Climate Change on Coastal Upwelling Ecosystems. *Current Climate Change Reports*, *1*(2), 85–93. https://doi.org/10.1007/s40641-015-0008-4

Bakun, Andrew. (1990). Global Climate Change and Intensification of Coastal Ocean Upwelling. *Science*, *247*(4939), 198–201. https://doi.org/10.1126/science.247.4939.198

Bandstra, L., Hales, B., & Takahashi, T. (2006). High-frequency measurements of total CO2: Method development and first oceanographic observations. *Marine Chemistry*, *100*(1–2), 24–38. https://doi.org/10.1016/j.marchem.2005.10.009

Bennett, R. N., & Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New Phytologist*, *127*(4), 617–633. https://doi.org/10.1111/j.1469-8137.1994.tb02968.x

Berner, D., Blanckenhorn, W. U., & Körner, C. (2005). Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged. *Oikos*, *111*(3), 525–533. https://doi.org/10.1111/j.1600-0706.2005.14144.x

Bracken, M. E. S., & Stachowicz, J. J. (2007). Top-down modification of bottom-up processes: Selective grazing reduces macroalgal nitrogen uptake. *Marine Ecology Progress Series*, *330*, 75–82. https://doi.org/10.3354/meps330075

Byers, B. A., & Mitton, J. B. (1981). Habitat choice in the intertidal snail Tegula funebralis. *Marine Biology*, *65*(2), 149–154. https://doi.org/10.1007/BF00397079

Celis-Plá, P. S. M., Hall-Spencer, J. M., Horta, P. A., Milazzo, M., Korbee, N., Cornwall, C. E., & Figueroa, F. L. (2015). Macroalgal responses to ocean acidification depend on nutrient and light levels. *Frontiers in Marine Science*, *2*(MAY), 26. https://doi.org/10.3389/fmars.2015.00026

Connell, S. D., Doubleday, Z. A., Foster, N. R., Hamlyn, S. B., Harley, C. D. G., Helmuth, B., Kelaher, B. P., Nagelkerken, I., Rodgers, K. L., Sarà, G., & Russell, B. D. (2018). The duality of ocean acidification as a resource and a stressor. *Ecology*, *99*(5), 1005–1010. https://doi.org/10.1002/ecy.2209

Core Writing Team. (2015). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (R. K. Pachauri & L. Meyer (eds.)). Intergovernmental Panel on Climate Change. http://www.ipcc.ch.

Cruz-Rivera, E., & Hay, M. E. (2000). Can Quantity Replace Quality? Food Choice, Compensatory Feeding, and Fitness of Marine Mesograzers. *Ecology*, *81*(1), 201. https://doi.org/10.2307/177144

Dobkowski, K. (2017). The role of kelp crabs as consumers in bull kelp forests-evidence from laboratory feeding trials and field enclosures. *PeerJ*, *2017*(5), e3372. https://doi.org/10.7717/peerj.3372

Dobkowski, K. A., Kobelt, J., Brentin, S., Van Alstyne, K. L., & Dethier, M. N. (2017). Picky Pugettia: a tale of two kelps. *Marine Biology*, *164*(11), 210. https://doi.org/10.1007/s00227-017-3244-4

Duarte, C., López, J., Benítez, S., Manríquez, P. H., Navarro, J. M., Bonta, C. C., Torres, R., & Quijón, P. (2016). Ocean acidification induces changes in algal palatability and herbivore feeding behavior and performance. *Oecologia*, *180*(2), 453–462. https://doi.org/10.1007/s00442-015-3459-3

Erickson, A. A., Paul, V. J., Van Alstyne, K. L., & Kwiatkowski, L. M. (2006). Palatability of macroalgae that use different types of chemical defenses. *Journal of Chemical Ecology*, *32*(9), 1883–1895. https://doi.org/10.1007/s10886-006-9116-x

Falkenberg, L. J., Russell, B. D., & Connell, S. D. (2013). Contrasting resource limitations of marine primary producers: Implications for competitive interactions under enriched CO2 and nutrient regimes. *Oecologia*, *172*(2), 575–583. https://doi.org/10.1007/s00442-012-2507-5

Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., & Hales, B. (2008). Evidence for upwelling of corrosive “acidified” water onto the continental shelf.

Science, *320*(5882), 1490–1492. https://doi.org/10.1126/science.1155676

Gaines, S. D. (1985). Herbivory and Between-Habitat Diversity: The Differential Effectiveness of Defenses in a Marine Plant. *Ecology*, *66*(2), 473–485.

García-Reyes, M., & Largier, J. (2010). Observations of increased wind-driven coastal upwelling off Central California. *Journal of Geophysical Research: Oceans*, *115*(4). https://doi.org/10.1029/2009JC005576

Gattuso, J.-P., Epitalon, J.-M., Lavigne, H., & Orr, J. (2019). *seacarb: Seawater Carbonate Chemistry.* (R package version 3.2.12). https://cran.r-project.org/package=seacarb

Gordillo, F. J. L., Niell, F. X., & Figueroa, F. L. (2001). Non-photosynthetic enhancement of growth by high CO 2 level in the nitrophilic seaweed Ulva rigida C. Agardh (Chlorophyta). *Planta*, *213*(1), 64–70. https://doi.org/10.1007/s004250000468

Graham, L. E., Graham, J. M., Wilcox, L. W., & Cook, M. E. (2016). *Algae* (Third). LJLM Press, LLC.

Gross, J., & Ligges, U. (2015). *nortest: Tests for Normality.* (R package version 1.0-4.). https://cran.r-project.org/package=nortest

Hales, B., Chipman, D., & Takahashi, T. (2004). High-frequency measurement of partial pressure and total concentration of carbon dioxide in seawater using microporous hydrophobic membrane contactors. *Limnology and Oceanography: Methods*, *2*(11), 356–364. https://doi.org/10.4319/lom.2004.2.356

Hales, B., Takahashi, T., & Bandstra, L. (2005). Atmospheric CO2 uptake by a coastal upwelling system. *Global Biogeochemical Cycles*, *19*(1), 1–11. https://doi.org/10.1029/2004GB002295

Harley, C. D. G., Anderson, K. M., Demes, K. W., Jorve, J. P., Kordas, R. L., Coyle, T. A., & Graham, M. H. (2012). EFfects Of Climate Change On Global Seaweed Communities. *Journal of Phycology*, *48*(5), 1064–1078. https://doi.org/10.1111/j.1529-8817.2012.01224.x

Hay, M. E., & Fenical, W. (2016). Marine Plant-Herbivore Interactions: The Ecology of Chemical Defense. *Annual Review of Ecology and Systematics*, *19*(May), 111–145.

Hepburn, C. D., Pritchard, D. W., Cornwall, C. E., Mcleod, R. J., Beardall, J., Raven, J. A., & Hurd, C. L. (2011). Diversity of carbon use strategies in a kelp forest

community: Implications for a high CO2 ocean. *Global Change Biology*, *17*(7), 2488–2497. https://doi.org/10.1111/j.1365-2486.2011.02411.x

Hines, A. H. (1982). Coexistence in a Kelp Forest: Size, Population Dynamics, and Resource Partitioning in a Guild of Spider Crabs (Brachyura, Majidae). *Ecological Monographs*, *52*(2), 179–198. https://doi.org/10.2307/1942610

Hultgren, K. M., & Stachowicz, J. J. (2008). Alternative camouflage strategies mediate predation risk among closely related co-occurring kelp crabs. *Oecologia*, *155*(3), 519–528. https://doi.org/10.1007/s00442-007-0926-5

Hultgren, K. M., & Stachowicz, J. J. (2010). Size-related habitat shifts facilitated by positive preference induction in a marine kelp crab. *Behavioral Ecology*, *21*(2), 329–336. https://doi.org/10.1093/beheco/arp192

Hurd, C. L., Beardall, J., Comeau, S., Cornwall, C. E., Havenhand, J. N., Munday, P. L., Parker, L. M., Raven, J. A., & McGraw, C. M. (2019). Ocean acidification as a multiple driver: How interactions between changing seawater carbonate parameters affect marine life. *Marine and Freshwater Research*, *71*(3), 263–275. https://doi.org/10.1071/MF19267

Hurd, C. L., Hepburn, C. D., Currie, K. I., Raven, J. A., & Hunter, K. A. (2009). Testing the effects of ocean acidification on algal metabolism: Considerations for experimental designs. *Journal of Phycology*, *45*(6), 1236–1251. https://doi.org/10.1111/j.1529-8817.2009.00768.x

Huyer, A. (1983). Coastal upwelling in the California current system. *Progress in Oceanography*, *12*(3), 259–284. https://doi.org/10.1016/0079-6611(83)90010-1

Iles, A. C., Gouhier, T. C., Menge, B. A., Stewart, J. S., Haupt, A. J., & Lynch, M. C. (2012). Climate-driven trends and ecological implications of event-scale upwelling in the California Current System. *Global Change Biology*, *18*(2), 783–796. https://doi.org/10.1111/j.1365-2486.2011.02567.x

Ince, R., Hyndes, G. A., Lavery, P. S., & Vanderklift, M. A. (2007). Marine macrophytes directly enhance abundances of sandy beach fauna through provision of food and habitat. *Estuarine, Coastal and Shelf Science*, *74*(1–2), 77–86. https://doi.org/10.1016/j.ecss.2007.03.029

Israel, A., & Hophy, M. (2002). Growth, photosynthetic properties and Rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO2 concentrations. *Global Change Biology*, *8*(9), 831–840. https://doi.org/10.1046/j.1365-2486.2002.00518.x

Kalra, Y. P. (1997). *Handbook of Reference Methods for Plant Analysis*. CRC Press. https://doi.org/10.2135/cropsci1998.0011183x003800060050x

Kassambra, A. (2020). *ggpubr: "ggplot2" Based Publication Ready Plots.* (R package version 0.2.5.). https://cran.r-project.org/package=ggpubr

Koch, M., Bowes, G., Ross, C., & Zhang, X. H. (2013). Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology*, *19*(1), 103–132. https://doi.org/10.1111/j.1365-2486.2012.02791.x

Kroeker, K. J., Kordas, R. L., Crim, R. N., & Singh, G. G. (2010). Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecology Letters*, *13*(11), 1419–1434. https://doi.org/10.1111/j.1461-0248.2010.01518.x

Leighton, D. L. (1966). Studies of Food Preference in Algivorous Invertebrates of Southern California Kelp Beds. *Pacific Science*, *20*(1), 104–113. https://scholarspace.manoa.hawaii.edu/bitstream/10125/7726/1/v20n1-104-113.pdf

Leimu, R., Riipi, M., & Stærk, D. (2005). Food preference and performance of the larvae of a specialist herbivore: Variation among and within host-plant populations. *Acta Oecologica*, *28*(3), 325–330. https://doi.org/10.1016/j.actao.2005.06.004

Light, S. F. (2007). *The Light and Smith manual: intertidal invertebrates from central California to Oregon* (Fourth). University of California Press.

Mattson, W. J. (1980). Herbivory in Relation to Plant Nitrogen Content. *Annual Review of Ecology and Systematics*, *11*(1), 119–161. https://doi.org/10.1146/annurev.es.11.110180.001003

Mercado, J. M., Javier, F., Gordillo, L., Xavier Niell, F., & Figueroa, F. L. (1999). Effects of different levels of CO2 on photosynthesis and cell components of the red alga Porphyra leucosticta. *Journal of Applied Phycology*, *11*(5), 455–461. https://doi.org/10.1023/A:1008194223558

Moroney, J. V. (2001). Carbon concentrating mechanisms in aquatic photosynthetic organisms: A report on CCM 2001. *Journal of Phycology*, *37*(6), 928–931. https://doi.org/10.1046/j.1529-8817.1999.37601-2.x

Newman, R. M., Kerfoot, W. C., & Hanscom, Z. (1996). Watercress allelochemical defends high-nitrogen foliage against consumption: Effects on freshwater invertebrate herbivores. *Ecology*, *77*(8), 2312–2323. https://doi.org/10.2307/2265733

Ogle, D. H., Wheeler, P., & Dinno, A. (2019). *FSA: Fisheries Stock Analysis.* (R package version 0.8.25). https://github.com/droglenc/FSA.

Padilla-Gamiño, J. L., & Carpenter, R. C. (2007). Seasonal acclimatization of Asparagopsis taxiformis (Rhodophyta) from different biogeographic regions. *Limnology and Oceanography*, *52*(2), 833–842. https://doi.org/10.4319/lo.2007.52.2.0833

Padilla-Gamiño, J. L., Gaitán-Espitia, J. D., Kelly, M. W., & Hofmann, G. E. (2016). Physiological plasticity and local adaptation to elevated pCO2 in calcareous algae: an ontogenetic and geographic approach. *Evolutionary Applications*, *9*(9), 1043–1053. https://doi.org/10.1111/eva.12411

Paine, R. T. (1969). The Pisaster-Tegula Interaction: Prey Patches, Predator Food Preference, and Intertidal Community Structure. *Ecology*, *50*(6), 950–961. https://doi.org/10.2307/1936888

Peterson, C. H., & Renaud, P. E. (1989). Analysis of feeding preference experiments. *Oecologia*, *80*(1), 82–86. https://doi.org/10.1007/BF00789935

Ricketts, E. F., Calvin, J., Hedgpeth, J. W., & Phillips, D. W. (1985). *Between Pacific Tides.* Stanford University Press. https://doi.org/10.2307/2420532

Roa, R. (1992). Design and analysis of multiple-choice feeding-preference experiments. *Oecologia*, *89*, 509–515.

Ross, M. E., Stanley, M. S., Day, J. G., & Semião, A. J. C. (2017). A comparison of methods for the non-destructive fresh weight determination of filamentous algae for growth rate analysis and dry weight estimation. *Journal of Applied Phycology*, *29*(6), 2925–2936. https://doi.org/10.1007/s10811-017-1157-8

Ruesink, J. L. (2000). Intertidal mesograzers in field microcosms: Linking laboratory feeding rates to community dynamics. *Journal of Experimental Marine Biology and Ecology*, *248*(2), 163–176. https://doi.org/10.1016/S0022-0981(00)00170-2

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., Wong, C. S., Wallace, D. W. R., Tilbrook, B., Millero, F. J., Peng, T. H., Kozyr, A., Ono, T., & Rios, A. F. (2004). The oceanic sink for anthropogenic CO2. *Science*, *305*(5682), 367–371. https://doi.org/10.1126/science.1097403

Snyder, M. A., Sloan, L. C., Diffenbaugh, N. S., & Bell, J. L. (2003). Future climate change and upwelling in the California Current. *Geophysical Research Letters*, *30*(15). https://doi.org/10.1029/2003GL017647

Speiser, B., & Rowell-Rahier, M. (1991). Effects of Food Availability, Nutritional Value, and Alkaloids on Food Choice in the Generalist Herbivore Arianta arbustorum (Gastropoda: Helicidae). *Oikos*, *62*(3), 306. https://doi.org/10.2307/3545495

Steinberg, P. D. (1985). Feeding Preferences of Tegula Funebralis and Chemical Defenses of Marine Brown Algae. *Ecological Monographs*, *55*(3), 333–349. https://doi.org/10.2307/1942581

Swanson, A. K., & Fox, C. H. (2007). Altered kelp (Laminariales) phlorotannins and growth under elevated carbon dioxide and ultraviolet-B treatments can influence associated intertidal food webs. *Global Change Biology*, *13*(8), 1696–1709. https://doi.org/10.1111/j.1365-2486.2007.01384.x

Takahashi, T., Sutherland, S. C., Feely, R. A., & Wanninkhof, R. (2006). Decadal change of the surface water pCO2 in the North Pacific: A synthesis of 35 years of observations. *Journal of Geophysical Research: Oceans*, *111*(7), C07S05. https://doi.org/10.1029/2005JC003074

Targett, N. M., & Arnold, T. M. (1998). Predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. *Journal of Phycology*, *34*(2), 195–205. https://doi.org/10.1046/j.1529-8817.1998.340195.x

Thornber, C., Jones, E., & Stachowicz, J. (2008). Differences in herbivore feeding preferences across a vertical rocky intertidal gradient. *Marine Ecology Progress Series*, *363*, 51–62. https://doi.org/10.3354/meps07406

Van Alstyne, K. L., Whitman, S. L., & Ehlig, J. M. (2001). Differences in herbivore preferences, phlorotannin production, and nutritional quality between juvenile and adult tissues from marine brown algae. *Marine Biology*, *139*(1), 201–210. https://doi.org/10.1007/s002270000507

Van Alstyne, Kathryn L., & Paul, V. J. (1990). The biogeography of polyphenolic compounds in marine macroalgae: temperate brown algal defenses deter feeding by tropical herbivorous fishes. *Oecologia*, *84*(2), 158–163. https://doi.org/10.1007/BF00318266

van der Loos, L. M., Schmid, M., Leal, P. P., McGraw, C. M., Britton, D., Revill, A. T., Virtue, P., Nichols, P. D., & Hurd, C. L. (2019). Responses of macroalgae to CO2 enrichment cannot be inferred solely from their inorganic carbon uptake strategy. *Ecology and Evolution*, *9*(1), 125–140. https://doi.org/10.1002/ece3.4679

van der Meijden, E. (1996). Plant defence, an evolutionary dilemma: contrasting effects of (specialist and generalist) herbivores and natural enemies. In *Proceedings of the*

9th International Symposium on Insect-Plant Relationships (pp. 307–310). Springer Netherlands. https://doi.org/10.1007/978-94-009-1720-0_69

Wang, D., Gouhier, T. C., Menge, B. A., & Ganguly, A. R. (2015). Intensification and spatial homogenization of coastal upwelling under climate change. *Nature*, *518*(7539), 390–394. https://doi.org/10.1038/nature14235

Wei, X., Vrieling, K., Mulder, P. P. J., & Klinkhamer, P. G. L. (2015). Testing the Generalist-Specialist Dilemma: The Role of Pyrrolizidine Alkaloids in Resistance to Invertebrate Herbivores in Jacobaea Species. *Journal of Chemical Ecology*, *41*(2), 159–167. https://doi.org/10.1007/s10886-015-0551-4

Wilson, M. A., Costanza, R., Boumans, R., Liu, S., & others. (2005). Integrated assessment and valuation of ecosystem goods and services provided by coastal systems. *The Intertidal Ecosystem: The Value of Ireland's Shores*, 1–24. www.planbleu.org/indexa.htm

Wright, J. T., De Nys, R., Poore, A. G. B., & Steinberg, P. D. (2004). Chemical defense in a marine alga: Heritability and the potential for selection by herbivores. *Ecology*, *85*(11), 2946–2959. https://doi.org/10.1890/03-4041

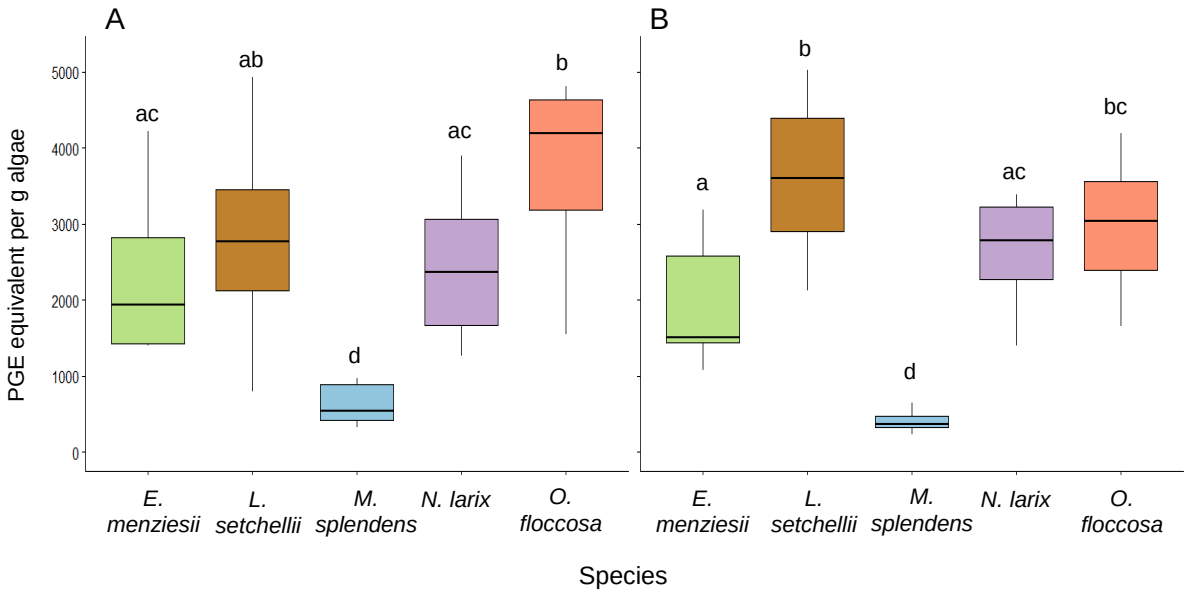
Xu, J., & Gao, K. (2012). Future CO2-induced ocean acidification mediates the physiological performance of a green tide alga. *Plant Physiology*, *160*(4), 1762–1769. https://doi.org/10.1104/pp.112.206961

Xu, Z., Zou, D., & Gao, K. (2010). Effects of elevated CO2 and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalga Gracilaria lemaneiformis (Rhodophyta). *Botanica Marina*, *53*(2), 123–129. https://doi.org/10.1515/BOT.2010.012

Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D. A., & Barrow, C. J. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology*, *18*, 445–450. https://doi.org/10.1007/s10811-006-9048-4

APPENDICES

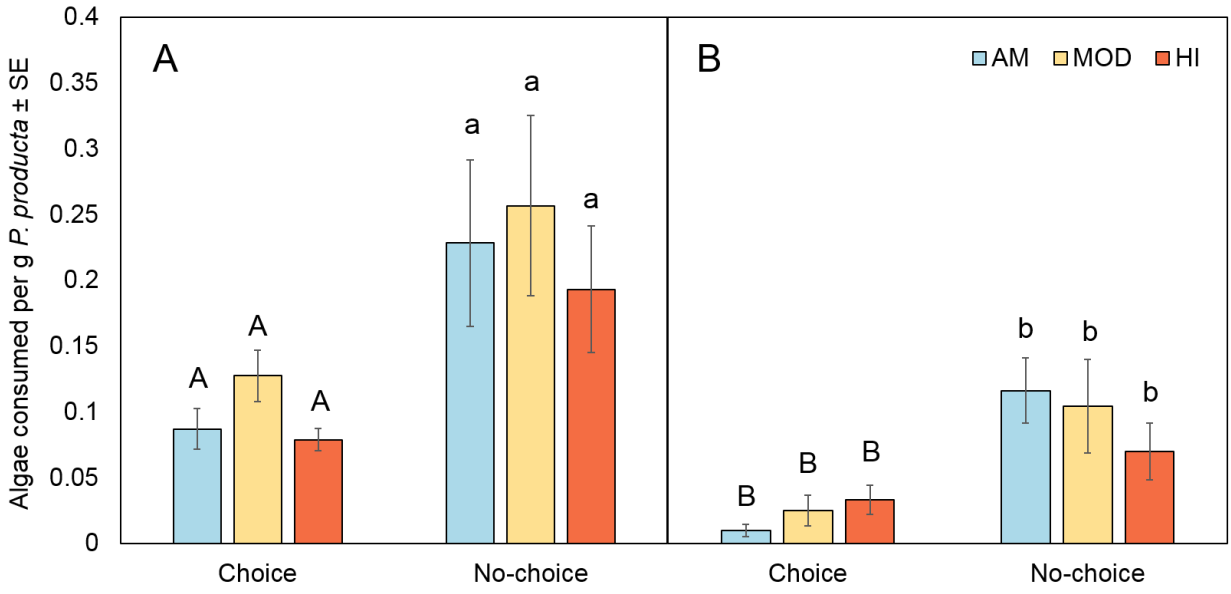
Appendix 1**.** Differences in phenolic contents between (*A*) untreated and (*B*) HI-treated algae. Letters indicate significance across both panels.



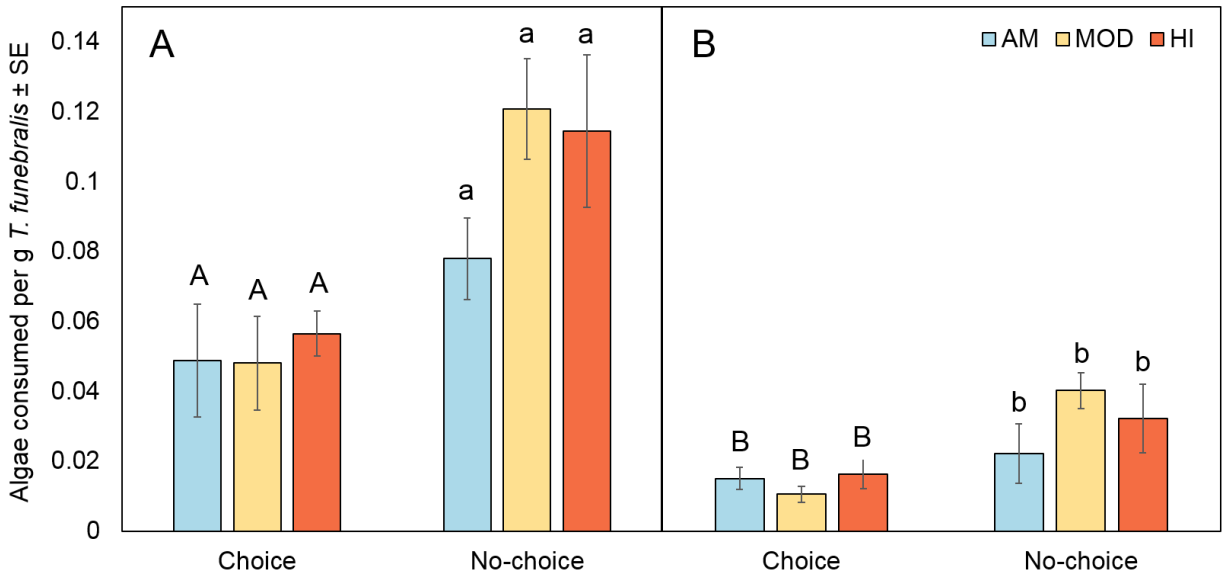
Appendix 2. Methods and results for pCO_2 preference trials.

To evaluate algivore affinity for algae growing in a particular pCO_2 condition, I conducted an additional set of feeding trials similar to those for untreated and HI-treated algae. I offered grazers a choice of three 4-g algal pieces, each sourced from a different experimental treatment (AM, MOD, or HI). Because these trials took place after the untreated-preference experiment, I selected each grazer's two most naturally preferred algal species for study and allowed them to choose from the three lab-treated pieces. I re-weighed algal pieces at the end of each 24-h experimental period, scaling consumed mass to grazer body mass. I also included control (no-choice) replicates, which offered grazers a single 12-g piece of algae from only one treatment, to evaluate grazing behavior when choice was not a factor. I analyzed these data with four 1-way ANOVAs to test each grazer's preference for each experimental alga.

In *these* preference trials, neither grazer demonstrated a preference for algae grown in a particular treatment. When each algivore was fed its respective most-preferred species from each pCO_2 condition, consumption did not differ by treatment in either choice or no-choice trials (Appendix 2a, 2b).



Appendix 2a. When offered conspecific algae grown under all laboratory treatments, crabs consumed similar amounts of algae from all pCO_2 conditions for (**A**) *E. menziesii* choice ($F_{2,15}$ = 2.429, P = 0.122) and no-choice trials ($F_{2,15}$ = 0.339, P = 0.718) and for (**B**) *L. setchellii* choice ($F_{2,15}$ = 1.209, P = 0.326) and no-choice trials ($F_{2,15}$ = 0.62, P = 0.551).



Appendix 2b**.** Snails showed no preference for the growing conditions of (**A**) *E. menziesii* in choice ($F_{2,15}$ = 0.115, P = 0.892) and no-choice ($F_{2,15}$ = 1.632, P = 0.228) trials. They responded similarly to all treated (**B**) *N. larix* options in both choice ($F_{2,15}$ = 0.703, P = 0.511) and no-choice ($F_{2,15}$ = 1.044, P = 0.376) experiments.

Appendix 3. Average %C, %N, and C:N ratios of algal samples as obtained by various methods of analysis show agreeability for nitrogen data, a key component of algal palatability. Dry analysis has been shown to capture slightly more nitrogen than Kjeldahl reactions, as represented here. Carbon discrepancies can be explained by the presence of inorganic carbon in algal tissues, which was not combusted by the loss-on-ignition (LOI) method but reduced the %C value of each sample in the elemental analyzer. C:N ratios are similarly affected.

Species	**Treatment**	**%N** Kjeldahl	**%N** Elemental analyzer	**%C** LOI	**%C** Elemental analyzer	**C:N** Kjeldahl + LOI	**C:N** Elemental Analyzer
E. menziesii	**HI**	2.161	2.444	68.831	27.795	31.856	11.373
L. setchellii	**HI**	2.109	2.265	71.400	30.429	33.848	13.432
M. splendens	**MOD**	2.690	3.049	82.000	31.284	30.483	10.262
N. larix	**MOD**	2.641	3.159	68.600	29.644	25.977	9.382
O. floccosa	**MOD**	3.349	4.127	72.400	30.482	21.618	7.385