Journal of Experimental Marine Biology and Ecology 547 (2022) 151662

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Effects of ocean acidification on the performance and interaction of fleshy macroalgae and a grazing sea urchin

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ARTICLE INFO

Keywords:
Climate change
Elevated pCO_2
Direct effects
Physiology
Indirect effects
Herbivory

ABSTRACT

When predicting the response of marine ecosystems to climate change, it is increasingly recognized that understanding the indirect effects of ocean acidification on trophic interactions is as important as studying direct effects on organism physiology. Furthermore, comprehensive studies that examine these effects simultaneously are needed to identify and link the underlying mechanisms driving changes in species interactions. Using an onshore ocean acidification simulator system, we investigated the direct and indirect effects of elevated seawater pCO_2 on the physiology and trophic interaction of fleshy macroalgae and the grazing sea urchin *Lytechinus variegatus*. Macroalgal (*Dictyota* spp.) biomass increased despite decreased photosynthetic rates after two-week exposure to elevated pCO_2. Algal tissue carbon content remained constant, suggesting the use of alternative carbon acquisition pathways beneficial to growth under acidification. Higher C:N ratios driven by a slight reduction in N content in algae exposed to elevated pCO_2 suggest a decrease in nutritional content under acidification. Urchin (*L. variegatus*) respiration, biomass, and righting time did not change significantly after six-week exposure to elevated pCO_2, indicating that physiological stress and changes in metabolism are not mechanisms through which the trophic interaction was impacted. Correspondingly, urchin consumption rates of untreated macroalgae (*Caulerpa racemosa*) were not significantly affected by pCO_2. In contrast, exposure of urchins to elevated pCO_2 significantly reduced the number of correct foraging choices for ambient macroalgae (*Dictyota* spp.), indicating impairment of urchin chemical sensing under acidification. However, exposure of algae to elevated pCO_2 returned the number of correct foraging choices in similarly exposed urchins to ambient levels, suggesting alongside higher C:N ratios that algal nutritional content was altered in a way detectable by the urchins under acidification. These results highlight the importance of studying the indirect effects of acidification on trophic interactions simultaneously with direct effects on physiology. Together, these results suggest that changes to urchin chemical sensing and algal nutritional quality are the driving mechanisms behind surprisingly unaltered urchin foraging behavior for fleshy macroalgae under joint exposure to ocean acidification. Consistent foraging behavior and consumption rates suggest that the trophic interaction between L. *variegatus* and fleshy macroalgae may be sustained under future acidification. However, increases in fleshy macroalgal biomass driven by opportunistic carbon acquisition strategies have the potential to cause ecological change, depending on how grazer populations respond. Additional field research is needed to determine the outcome of these results over time and under a wider range of environmental conditions.

1. Introduction

Changes in seawater carbonate chemistry and temperature associated with climate change have demonstrated impacts to the physiology, abundance, and distribution of marine organisms, resulting in alterations to community structure and biodiversity in a wide variety of marine ecosystems (Doney et al., 2012). One of the main climate change stressors facing marine environments is ocean acidification, or the decline in ocean pH and the shift in seawater carbonate chemistry equilibrium associated with rising atmospheric CO_2 (carbon dioxide) levels (Doney et al., 2009). Predicting the impacts of climate stressors like ocean acidification on marine ecosystems requires an understanding

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https://doi.org/10.1016/j.jembe.2021.151662
Received 10 November 2020; Received in revised form 4 July 2021; Accepted 28 October 2021
Available online 24 November 2021

of the direct effects on species as well as the indirect effects on complex species interactions (Kroeker et al., 2012; Rosenblatt and Schmitz, 2016). It has become increasingly recognized that modified species interactions under ocean acidification have the potential to produce changes in an ecosystem on a much larger scale than those generated by altered species physiology alone (Kroeker et al., 2012; Gaylord et al., 2015).

The trophic interaction between primary producers and grazers is of particular importance in marine ecosystems because changes in herbivory have been shown to result in community phase shifts in which the dominant, habitat-forming species are substantially reduced or replaced, such as shifts from coral to macroalgal dominance on coral reefs under reduced herbivory rates (Hughes et al., 2007; Vergés et al., 2014). Ocean acidification has been shown to impact algae-herbivore interactions (Duarte et al., 2016; Kamya et al., 2017; Rich et al., 2018; Young et al., 2019), alter the relative abundance of herbivore (Kroeker et al., 2011; Baggini et al., 2015) and algae taxa (Connell et al., 2013; Edmunds et al., 2016; Poore et al., 2016), and produce changes in community structure through these impacts (Enochs et al., 2015; Connell et al., 2018). Predicting impacts to coral reef health induced by changes in algae-herbivore interactions is crucial because coral reefs are responsible for a host of essential goods and services, including habitat provision that supports high biodiversity, shoreline protection, and contribution to healthy economies through fisheries and tourism (Moberg and Folke, 1999). On reefs in the Caribbean, changes in coral health, cover, and community composition have been directly linked to fluctuations in the populations of grazing sea urchins and non-calcifying, fleshy macroalgae, where lower rates of urchin herbivory and higher macroalgal abundance negatively impact the success of corals (Mumby et al., 2006; Idjadi et al., 2010; Sangil and Guzman, 2016). Therefore, understanding the direct effects of ocean acidification on the performance of urchins and fleshy macroalgae and the resulting indirect effects on their trophic interaction is critical for predicting changes in coral reef community structure under future climate change.

In response to ocean acidification, some fleshy macroalgae species demonstrate a positive effect on production, possibly because elevated CO_2 levels have the potential to fertilize photosynthesis (Johnson et al., 2014). Studies conducted along volcanic CO_2 seeps, where the pH naturally simulates end-of-century ocean acidification conditions, reveal that elevated seawater pCO_2 (partial pressure of carbon dioxide) can indeed support increased growth and abundance of fleshy macroalgae on coral reefs (Porzio et al., 2011; Connell et al., 2013; Cornwall et al., 2017; Agostini et al., 2018). In contrast, many sea urchin species, as calcifying organisms, demonstrate negatively impacted physiology under acidification. Some urchins show increased metabolic rates (Catarino et al., 2012; Carey et al., 2016; Rich et al., 2018) and/or reduced somatic and reproductive growth in adults (Siikavuopio et al., 2007; Stumpp et al., 2012; Challener et al., 2014; Dupont and Thorndyke, 2014) under acidification, likely in response to a higher overall energy demand generated by energy reallocation to acid-base regulation of intra- and extracellular pH (Stumpp et al., 2012; Dupont and Thorndyke, 2014).

Alterations to the consumption rates of urchins under ocean acidification have been reported as the direct result of changes in urchin physiology (Siikavuopio et al., 2007; Stumpp et al., 2012; Kurihara et al., 2013; Burnell et al., 2013; Taylor et al., 2014; Rich et al., 2018), such as increased consumption driven by higher metabolic demand (Rich et al., 2018). Additionally, grazing marine invertebrates have been shown to adjust their consumption rates relative to changes in the nutritional quality of their food under ocean acidification (Falkenberg et al., 2013; Burnell et al., 2013; Duarte et al., 2016; Boada et al., 2017; Kamya et al., 2017; Leung et al., 2018). Indeed, the indirect impacts of modified nutrient content and palatability in primary producers may play a more substantial role in altering herbivory rates under acidification than the direct impacts on grazers (Burnell et al., 2013; Falkenberg et al., 2013; Duarte et al., 2016; Kamya et al., 2017). Some species of fleshy macroalgae experience reduced protein and organic content when exposed to ocean acidification (Borell et al., 2013; Duarte et al., 2016), and reduced algal nutritional content has been shown to increase consumption as a form of energy compensation in no-choice feeding assays on grazing invertebrates (Cruz-Rivera and Hay, 2003; Duarte et al., 2016), including sea urchins (Boada et al., 2017). However, reduced preference for algae with lower nutritional content results in contrastingly decreased consumption in multiple choice feeding assays (Borell et al., 2013; Duarte et al., 2016), highlighting the role that foraging behavior plays in mediating rates of herbivory. Foraging behavior may also be negatively impacted by ocean acidification through the impaired ability of grazers to orient to the chemical cues produced by their food, which has been documented in urchins (Barry et al., 2014), molluscs (Queirós et al., 2015; Horwitz et al., 2020), and crustaceans (de la Haye et al., 2012). Therefore, predicting the effects of ocean acidification on urchin herbivory is complicated by the interaction of complex urchin and macroalgae physiology and the resulting impacts to multiple facets of their trophic interaction. Understanding these changes requires simultaneous measurements of direct and indirect effects in order to disentangle and identify the mechanisms driving changes in urchin herbivory of fleshy macroalgae under future climate change.

In this study, we aim to understand the impacts of ocean acidification on the performance and interaction of the grazing sea urchin *Lytechinus variegatus* and two fleshy macroalgae species (*Dictyota* spp. and *Caulerpa racemosa*) found within the Florida Reef Tract. For this, we investigate the direct effects of elevated seawater pCO_2 on macroalgae and urchin physiology as well as indirect effects on urchin foraging behavior and consumption rate. We test these effects simultaneously to isolate the key underlying mechanisms influencing the trophic interaction (i.e., which direct effects interact to produce the indirect effects we observe) under elevated pCO_2. We hypothesize: (1) ocean acidification will positively impact the production of fleshy macroalgae, increasing biomass and photosynthesis while reducing nutritional content (i.e., increasing tissue C:N ratios); (2) ocean acidification will negatively impact urchin physiology, increasing metabolism (i.e., respiration) and stress (i.e., righting time) while reducing biomass; (3) ocean acidification will alter the trophic interaction, negatively impacting foraging behavior (i.e., reducing the proportion of correct foraging choices) while increasing consumption rates of algae in urchins (Table 1).

2. Materials and methods

To test our hypotheses, we conducted two experiments within the

Table 1
A breakdown of the experimental variables, their hypothesized results, and their actual results in this two-part study, with a superscript of 1 or 2 indicating the experiment in which these variables were measured and = indicating no significant effect.

Effects of Ocean Acidification	Ecological Role (Species Type)	Variables Measured	Hypotheses	Results
Direct Effects	Primary Producer (Fleshy Macroalgae)	Biomass[1]	↑	↑
		Photosynthesis[1]	↑	↓
		C:N Ratio (Nutritional Content)[1]	↑(↓)	↑(↓)
	Grazer (Sea Urchin)	Respiration (Metabolism)[1]	↑	=
		Change in Biomass[2]	↓	=
		Righting Time (Stress Response)[2]	↑	=
Indirect Effects	Trophic Interaction	Correct Foraging Choices (Foraging Behavior)[1]	↓	=
		Consumption Rates[2]	↑	=

Climate and Acidification Ocean Simulator (CAOS) outdoor experimental system at Mote Marine Laboratory's Elizabeth Moore International Center for Coral Reef Research and Restoration in Summerland Key, Florida, USA. The study species for these experiments, *L. variegatus* (sea urchin), *Dictyota* spp. and *C. racemosa* (fleshy macroalgae), were chosen because of their co-occurrence on hardbottom, nearshore habitats, including coral reefs, in the Florida Keys. *L. variegatus* was chosen because it is a calcifier with well-studied physiology (Watts et al., 2013), especially under elevated pCO_2 (Challener and McClintock, 2013; Challener et al., 2014; Emerson et al., 2017), and well-recognized ecological importance as a key grazer on coastal habitats in Florida and the Caribbean (Valentine and Heck, 1991; Klumpp et al., 1993; Heck and Valentine, 1995; Rose et al., 1999). *Dictyota* spp. was chosen for its ecological relevance as an overly abundant, bloom-forming macroalgae on coral reefs in the Florida Keys (Beach and Walters, 2000) and as a known food for L. *variegatus* (Cobb and Lawrence, 2005; Souza et al., 2008). *C. racemosa* was chosen for its role as a preferred food of L. *variegatus* (Souza et al., 2008) and for its tendency to grow in abundance when *Dictyota* spp. experiences seasonal die-offs in the Florida Keys (R. Nowicki, personal communication). In Experiment 1, conducted from May to July 2019, we investigated the response of *Dictyota* spp. biomass, photosynthesis, and C, N, and P content as well as L. *variegatus* respiration and foraging behavior to ocean acidification (Table 1). In Experiment 2, conducted from September to October 2019, we investigated the response of L. *variegatus* consumption rates of *C. racemosa*, biomass, and righting time to ocean acidification using different individuals (Table 1).

2.1. Organism collection and maintenance

For each experiment, a total of 60 adult L. *variegatus* urchins were collected from Horseshoe Hole in Spanish Harbor Key, Florida (Lat. 24.655642°, Long. −81.302088°) and divided evenly between two 140 L holding tanks, each containing a similar size distribution of urchins. Experiment 1 had size distributions (mean ± SD urchin wet weight) of 16.6 ± 10.7 g (range 4–50 g) and 16.6 ± 9.5 g (range 6–41 g) in ambient and elevated pCO_2 tanks, respectively (Fig. S1). Experiment 2 had size distributions (mean ± SD urchin wet weight) of 27.2 ± 10.5 g (range 6–56 g) and 27.2 ± 6.7 g (range 16–45 g) in ambient and elevated pCO_2 tanks, respectively (Fig. S1). Urchins were fed 30 g of *Laurencia* spp. macroalgae per tank every three days. *Laurencia* spp. was hand collected from the seagrass flats (Lat. 24.660323°, Long. -81.454718°) located near Mote Marine Laboratory's Summerland Key facility.

In June 2019 (Experiment 1), approximately 120 g of *Dictyota* spp. was collected from the fore reef near Looe Key off Summerland Key, Florida and divided evenly between two additional 140 L holding tanks with the same pCO_2 treatments as the urchin holding tanks. Algae were separated into 20 replicates per tank, with 2 g of algae per replicate. Algae replicates were placed into sterile 118 mL sample collection cups drilled with 30 evenly spaced 3 mm-diameter holes to allow water to flow through the cups. The plastic cups were acid-washed prior to the beginning of the exposure period, weighed down with three 0.5 g lead fishing weights each, and scrubbed daily to remove epiphytes (i.e., turf algae and cyanobacteria). All holding tanks were siphoned and scrubbed of epiphytes on a biweekly basis. Unlike in Experiment 1, algae for Experiment 2 was not collected for incubation in the pCO_2 treatments but rather for use as food in the consumption rate experiment. In October 2019 (Experiment 2), approximately 160 g of *C. racemosa* was collected from Horseshoe Hole the morning of each consumption trial to minimize autogenic mass loss. Prior to use in the experiment, the algae were briefly held in a 140 L holding tank with ambient seawater pH and flow.

2.2. Exposure to ocean acidification

In both experiments, the organisms were exposed to ambient or elevated pCO_2 treatments within the CAOS system. The 140 L flow-through holding tanks were housed in a 1000 L water bath supplied by a dual heat exchange system to assist in temperature control. The temperature of the water bath was maintained at 28.5 °C in accordance with average in situ conditions on reefs in the Florida Keys (Platz et al., 2020; Kuffner, 2013), and tanks were kept under a shade tarp (mean ± SE light: 215 ± 20 μE m^{-2} s^{-1}) to prevent UV overexposure. Two powerhead pumps enhanced water circulation and homogenization in each tank. Seawater was supplied to the tanks from a manifold at 38 mL s^{-1}. The treatment water was sourced from two of four 5000 L header tanks, which were rotated every two weeks to manage any possible biofouling and limit pseudoreplication (Hurlbert, 2009; Cornwall and Hurd, 2015). The seawater for each header tank is sourced from the Atlantic side of the Florida Keys. The elevated pCO_2 treatment was created by injecting CO_2 into filtered seawater (filtered using a sand and 20 μm particle filter) using a Venturi pump system. CO_2-dosing was controlled using Walchem W900 controllers (Iwaki America Inc., Holliston, MA) and monitored using pH values obtained from both daily pH measurements and biweekly water sampling of the header tanks.

A target pH offset of −0.3 compared to ambient seawater was set for the elevated pCO_2 treatment. This pH offset was chosen based on the IPCC (2019) end-of-century projection for acidification under a business-as-usual CO_2 emission scenario (RCP 8.5). Prior to the exposure period, seawater pH in the elevated pCO_2 treatment was gradually decreased over the course of 72 h to allow for acclimation of the organisms (Ho and Carpenter, 2017; Rich et al., 2018; Rodríguez et al., 2018). In Experiment 1, urchins were exposed to the treatments for six weeks, while algae were exposed for two weeks. In Experiment 2, only urchins were exposed to the treatments for six weeks.

Throughout the exposure period, seawater temperature, salinity, dissolved oxygen, and pH_{NBS} were monitored daily in each tank using a YSI Professional Plus handheld multiparameter instrument (Xylem Inc., Rye Brook, NY). Dissolved oxygen and pH_{NBS} were calibrated daily while salinity was calibrated weekly. Water samples were collected weekly in glass borosilicate bottles and immediately poisoned with a saturated solution of mercuric chloride ($HgCl_2$) for laboratory analysis of dissolved inorganic carbon (DIC) and total alkalinity (TA) following best practices (Dickson et al., 2007). DIC was measured using an Apollo AS-C3 DIC Analyzer (Apollo SciTech, Newark, DE) equipped with a LI-COR LI-7000 gas-analyzer (LI-COR Biosciences, Lincoln, NE), while TA was analyzed using open-cell acid titration with a Metrohm 905 Titrando titrator (Metrohm, Herisau, Switzerland). Accuracy and precision of the instruments were checked using Certified Reference Materials for Seawater CO_2 Measurements (Dickson Laboratory, Scripps Institution of Oceanography, San Diego, CA) every five samples for DIC measurements and every 20 samples for measurements of TA. Data were corrected when measurements deviated more than 1% from the certified value; this only occurred for TA data in Experiment 2.

DIC and TA values measured in weekly water samples, along with associated temperature and salinity measurements, were used to calculate pH_T, pCO_2, CO_2, HCO_3^-, CO_3^{2-}, and Ω_{arag} using the *seacarb* package (Lavigne et al., 2011) in R version 3.5.2 (R Core Team, 2019). K1 and K2 constants from Lueker et al. (2000) were used in these calculations. The mean (± SE) seawater parameters maintained in the holding tanks throughout the exposure period for Experiment 1 and 2 are presented in Table 2.

2.3. Direct effects on algal physiology

2.3.1. Algal photosynthesis and biomass

Immediately following the exposure period in Experiment 1, we measured photosynthesis and biomass of *Dictyota* spp. to reveal changes to algal production under ocean acidification. Algal photosynthesis was measured by incubating algae replicates in 300 mL respirometry chambers fitted with FireSting O_2 optical oxygen sensors (PyroScience GmbH, Aachen, Germany) for 30 mins under 158 μE m^{-2} s^{-1} mean light

Table 2
Mean ± SE seawater parameters maintained in the holding tanks throughout the exposure periods in Experiment 1 and 2. Carbonate chemistry was calculated using measured values of temperature, salinity, DIC, and TA using dissociation constants K1 and K2 from Lueker et al. (2000).

pCO_2 Treatment	T (°C)	Salinity (psu)	DO (mg/L)	DIC (μmol/kg)	TA (μmol/kg)	pH_T	pCO_2 (μatm)	CO_2 (μmol/kg)	HCO_3^- (μmol/kg)	CO_3^{2-} (μmol/kg)	Ω_{arag}
Experiment 1											
Lytechinus											
Tanks											
Ambient	28.3 ± 0.1	37.79 ± 0.24	6.02 ± 0.03	2051 ± 9	2297 ± 7	7.87 ± 0.01	633 ± 20	16.3 ± 0.5	1856 ± 11	178 ± 3	2.82 ± 0.05
Elevated	28.4 ± 0.0	37.74 ± 0.29	5.77 ± 0.11	2181 ± 8	2291 ± 5	7.57 ± 0.02	1391 ± 76	35.8 ± 2.0	2045 ± 10	100 ± 5	1.58 ± 0.07
Dictyota											
Tanks											
Ambient	28.3 ± 0.0	37.63 ± 0.33	6.03 ± 0.02	2024 ± 7	2284 ± 4	7.90 ± 0.02	581 ± 31	15.0 ± 0.8	1822 ± 13	187 ± 7	2.95 ± 0.11
Elevated	28.4 ± 0.1	37.64 ± 0.35	5.67 ± 0.07	2181 ± 13	2287 ± 6	7.56 ± 0.02	1420 ± 68	36.5 ± 1.8	2048 ± 15	97 ± 4	1.53 ± 0.06
Experiment 2											
Lytechinus											
Tanks											
Ambient	28.6 ± 0.0	36.91 ± 0.23	5.42 ± 0.67	1970 ± 20	2266 ± 24	7.97 ± 0.01	478 ± 8	12.3 ± 0.0	1749 ± 0	209 ± 0	3.32 ± 16.85
Elevated	28.7 ± 0.0	36.93 ± 0.21	5.20 ± 0.74	2143 ± 19	2264 ± 23	7.60 ± 0.01	1270 ± 37	32.5 ± 0.0	2007 ± 1	104 ± 0	1.66 ± 17.55

saturation provided by two overhanging fluorescent lights. Algae were held in place using fishing line weighed down by three 0.5 g lead fishing weights. Respirometry chambers were filled with new seawater of the same pCO_2 treatment as the algae's holding tank. The temperature of the chambers was maintained at 28.5 °C by an external water bath. The volume of water needed to fill the chamber with the algae in place was recorded for later calculations. Mixing was provided by a magnetic stir bar isolated at the bottom of the chamber. Oxygen was measured as μmol l^{-1} and multiplied by the volume of water in the chamber to produce units of μmol O_2. Photosynthesis was calculated as the slope of the line created by oxygen produced over time to generate units of μmol O_2 h^{-1}. The slope of the oxygen measurements from a neighboring control chamber lacking algae were subtracted from the slopes of the other three chambers in each round to control for oxygen produced or consumed by microbial and picoplanktonic activity. Photosynthesis was then standardized per gram of algal biomass to produce units of μmol O_2 h^{-1} g^{-1}. Final algal biomass was measured after patting each replicate dry with paper towels. Because of autogenic mass loss of *Dictyota* spp. throughout the exposure period, we present changes to algal biomass between treatments as final biomass values, rather than as values of growth.

2.3.2. Algal nutritional content

Following measurements of algal photosynthesis and biomass in Experiment 1, we prepared *Dictyota* spp. for tissue analysis of carbon (C), nitrogen (N), and phosphorus (P) content to reveal changes to algal nutritional content under ocean acidification. A small, healthy-looking portion of each algae replicate was selected, patted dry, and placed in a VWR 51014992 air jacketed CO_2 incubator (Thermo Fisher Scientific, Marietta, OH) to dry for 48 h at 70 °C. The dried algae were then ground to a fine powder using a mortar and pestle and frozen in separate vials at −80 °C. Samples analyzed for C and N were homogenized and ran through a CE Flash 1112 elemental analyzer (Thermo Fisher Scientific, Marietta, OH) using standard procedures. Samples analyzed for P underwent a dry-oxidation acid hydrolysis extraction followed by a colorimetric analysis of phosphate (Solórzano and Sharp, 1980; Fourqurean et al., 1992) with a Shimadzu UV-2450 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). All nutrient analyses were performed by the Blue Carbon Analysis Laboratory at Florida International University (Miami, FL).

2.4. Direct effects on urchin physiology

2.4.1. Urchin respiration

Immediately following the exposure period in Experiment 1, we measured mass-specific respiration rates of L. *variegatus* to reveal changes to urchin metabolism under ocean acidification. Urchins were not fed for 12 h leading up to respiration measurements to eliminate the influence of digestion on respiration (Stumpp et al., 2012). Respiration rates were measured by placing the urchins in 300 mL or 1300 mL (for the larger urchins) respirometry chambers fitted with FireSting O_2 optical oxygen sensors (PyroScience GmbH, Aachen, Germany) for one hour under ambient light conditions. A plastic grate was wedged in place halfway down the chamber to block the urchin's access to the probe without inhibiting the urchin's movements. The volume of water needed to fill the chamber with the urchin and plastic grate in place was recorded for later calculations. Immediately following measurements of respiration, we obtained the wet weight of the urchins and standardized respiration rates per gram of urchin biomass to produce units of μmol O_2 consumed h^{-1} g^{-1}. The first five minutes of measurements were discarded to account for each urchin's initial stress response when placed into the chambers. The longest linear decrease in oxygen concentration between the start and the end of the measurement period was chosen for the calculations (Stumpp et al., 2012; Fig. S2). The same calculations as those used to measure algal photosynthesis were used to quantify urchin respiration rates.

2.4.2. Urchin biomass

In Experiment 2, we measured L. *variegatus* wet weight prior to the exposure period and after consumption trials to reveal individual changes in urchin biomass over the course of the exposure period and to gain insight into urchin physiological fitness under ocean acidification. The urchins were individually identified by comparing before and after photos of their aboral surfaces, which had unique patterns and coloration, and by cross-referencing these with biomass (Fig. S4). The change in urchin biomass was calculated as the total change in wet weight to the nearest 0.01 g of each urchin over the course of the six-week exposure period. The urchins were not starved before weighing, so individual weight to the nearest 0.01 g may be influenced by the fullness of their guts.

2.4.3. Urchin righting time

Following measurements of biomass in Experiment 2, we measured

L. *variegatus* righting time to reveal changes to urchin stress response under ocean acidification. Righting behavior is well-known in echinoderms (Hyman, 1955; Reese, 1966), and righting response, or the time required by an individual to right itself following inversion, has been used alongside changes to environmental parameters as an indicator of physiological stress in many echinoderm species (Lawrence, 1973; Forcucci and Lawrence, 1986; Watts and Lawrence, 1986; Lawrence and Cowell, 1996), including L. *variegatus* (Lawrence, 1975; Böttger et al., 2001; Santos et al., 2013). Immediately following the consumption experiment and removal of the algae, each urchin was kept in the same 9.5 L tank and placed flat on their aboral side. Righting time was then measured as the time, in seconds, it took each individual to reach an angle of 90° relative to the surface.

2.5. Indirect effects on the trophic interaction

2.5.1. Urchin foraging behavior

In Experiment 1, we measured L. *variegatus* foraging behavior for *Dictyota* spp. to gain insight into changes to urchin chemical sensing and algal nutritional quality under ocean acidification. In previous studies, "choice chambers" have frequently been used to study the ability of marine organisms, including sea urchins, to detect and to select for chemical cues, such as those produced by food (e.g., Vadas, 1968; Vadas, 1977; Solandt and Campbell, 2001; de la Haye et al., 2012; Horwitz et al., 2020), settling sites (Ross and Behringer, 2019; Gravinese et al., 2020), and predators (Manríquez et al., 2014). We employed a similar method in which an urchin was placed in the middle of a 18 cm-diameter x 122 cm-long (31,000 mL) cylindrical chamber with a choice on each end of either the algal chemical cue or no chemical cue (the control) (Fig. S3). In this case, we considered a foraging choice to be made once an urchin traveled all the way to one end of the chamber and made direct contact with the mesh screen dividing the urchin from the algae (or absence of algae) on the other side. We considered this foraging choice to be correct if the urchin chose the side with the algal cue. Using a fully factorial design (Table S1), we paired each urchin and algae treatment (i.e. algae treatment x urchin treatment, 4 groups) to reveal whether elevated pCO_2 resulted in changes to the urchin's chemical sensing abilities or to the chemical cue produced by the algae or both.

To minimize effects on urchin physiology, the water supplied to the chamber always matched the treatment of the urchin being trialed. Water entered the chamber from a tube at each end, flowing through the algae where it was present and carrying the chemical cue to the center of the chamber (Fig. S3). Water was pulled out of the chamber at the top by two tubes located at the center, each spaced approximately 30 cm apart, to allow for continued division of flow on each side and minimal mixing at the center. Proper flow was confirmed by a dye test. A mesh window screen on either end of the chamber separated the urchin from the inflow tube and the source of the cue (i.e., the algae). In each trial, 6 g of *Dictyota* spp. algae was placed in a mesh bag of the same material behind the window screen divider. Algae was used for no more than three trials in a row before being returned to its holding tank for another 24 h. The side of the chamber containing the algae was switched between each trial to limit potential artifacts created by the position of the sun and shadows in this outdoor setup. For the same reason, procedural control trials containing no chemical cue on either side were run to confirm that there were no external factors causing urchin preference for one side of the chamber over the other. Over the course of the experiment, each urchin was subjected to trials three times, with three days in between each use, but never solely for the same treatment combination as before to limit the potential of learned behavior biasing our results (e.g., Ross and Behringer, 2019; Fig. S5; Table S2).

Prior to the start of the experiment, the urchins were starved for five days. During the trial, the urchin was given 20 min to make a choice (Solandt and Campbell, 2001), and only the urchin's initial choice, which was made in an average of eight minutes, was analyzed as the urchins would often continue to search the chamber, presumably for physical contact with the source of the cue when the mesh prevented it. We filmed the urchin's movements with a GoPro Hero 5 camera (GoPro, San Mateo, CA) and later analyzed the footage (Video S1). We converted the results to binomial data (0 = did not choose algae, 1 = chose algae) and considered the urchins that did not make a choice (did not travel to either end of the chamber in the allotted 20 mins) to be statistically the same as the urchins that "incorrectly" chose the side with no chemical cue.

2.5.2. Urchin consumption rates

In Experiment 2, we measured L. *variegatus* mass-specific consumption rates of freshly-collected *C. racemosa* to reveal changes to the trophic interaction and underlying species physiology under ocean acidification. The urchins were starved for one week prior to the consumption trials to overcome any possible period of ingestive conditioning (e.g., Solandt and Campbell, 2001). Consumption trials occurred within 9.5 L experimental tanks, for which the temperature was maintained at 28.5 °C using a 490 L water bath. Treatment water was supplied to each of the tanks through a manifold at 38 mL s^{-1}. Prior to each trial, *C. racemosa* were separated into bundles of approximately 4 g that were patted dry, weighed to the nearest 0.01 g, and placed in each tank in contact with the urchin. A subset of algae replicates were placed in control tanks not containing urchins to allow for the calculation of rates of autogenic mass loss caused by the experiment in units of mg algae lost h^{-1}. Seawater temperature, salinity, dissolved oxygen, and pH_{NBS} were then measured in each tank using a YSI Professional Plus handheld multiparameter instrument (Xylem Inc.).

To begin the trials, each urchin was placed in a 9.5 L tank of the same treatment water as their holding tank (i.e., one urchin per tank). To limit the effect of feeding preference (i.e., for different species of algae) on consumption, no food choices other than *C. racemosa* were offered. Furthermore, the algae bundle was placed in direct contact with the urchin to remove any 'search' component of the experiment. Two 5 cm-diameter x 2 mm-thick PVC disks were then placed on top of each urchin to satisfy the covering response upon disturbance that is characteristic of this species (Millott, 1956; Sharp and Gray, 1962; Amato et al., 2008). Consumption trials were run for six hours between approximately 10:00 AM and 6:00 PM local time to eliminate biases associated with day/night feeding cycles. Urchins were then weighed to obtain a feeding rate standardized by biomass. Each algae bundle was then patted dry and weighed to the nearest 0.01 g to determine the amount consumed. This value was divided by the time each urchin was allotted to feed to calculate a rate of feeding with units of mg of algae consumed g^{-1} h^{-1}.

2.6. Statistical analyses

Algal biomass, photosynthesis, and C:N:P data and urchin respiration, biomass, righting time, and consumption rate data were analyzed using linear regressions in R version 3.5.2 (R Core Team, 2019). Outliers were identified and removed (Table S3) prior to analysis using a visual examination of boxplots of the data followed by the boxplot.stats function in the ggplot2 package in R, which removes data points that exceed 150% of the interquartile range away from the median. All data were tested for normality using a Shapiro-Wilks test and for homogeneity of variances using a Levene's Test. Righting time data were log transformed based on their distribution to meet the assumptions of normality. Because the algal C:N:P data could not be transformed to meet the assumptions of normality, they were analyzed using a Kruskal-Wallis rank sum test. The effect of treatment on algal biomass and photosynthesis was assessed using two-sample *t*-tests. Urchin respiration, changes in biomass, righting time, and consumption rate data were analyzed using a multiple linear regression with treatment, urchin biomass, and their interaction as factors. For these models, if the interaction of treatment and urchin biomass was insignificant, the interaction was removed and the model was re-run to assess the significance of the main effects alone. Urchin foraging behavior data were converted to binomial data and

analyzed using a logistic regression with algae treatment, urchin treatment, and their interaction as factors. Foraging behavior data were further analyzed using a post hoc comparison of treatment combinations via the lsmeans function in the lsmeans package in R. All data are reported as mean ± standard error unless otherwise stated.

3. Results

3.1. Exposure to ocean acidification

In both experiments, the elevated pCO_2 treatment effectively simulated ocean acidification conditions projected for the end of the century under a business-as-usual CO_2 emission scenario (IPCC, 2019). Throughout the exposure period in Experiment 1, we maintained a minimum 0.30-differential in pH_T. In the ambient pCO_2 holding tanks, we maintained an average pH_T of 7.88 ± 0.01 and pCO_2 of 617 ± 18 µatm (Table 2). In the elevated pCO_2 holding tanks, we maintained an average pH_T of 7.57 ± 0.02 and pCO_2 of 1400 ± 55 µatm (Table 2). Throughout the exposure period in Experiment 2, we maintained a minimum 0.30-differential in pH_T. In the ambient pCO_2 holding tank, we maintained an average ± pH_T of 7.97 ± 0.01 and pCO_2 of 478 ± 8 µatm (Table 2). In the elevated pCO_2 holding tank, we maintained an average ± pH_T of 7.60 ± 0.01 and pCO_2 of 1270 ± 37 µatm (Table 2). The ambient conditions maintained in our system mimic pH and pCO_2 values observed in the Florida Keys (Manzello et al., 2012; Meléndez et al., 2020; Platz et al., 2020), and in Florida's coastal inlets (Millero et al., 2001; Enochs et al., 2019); each of these coastal habitats in southern Florida experience seawater pCO_2 values exceeding 600 µatm. Urchin mortality over the course of the exposure period in Experiment 1 was very low. We experienced 0% urchin mortality in the ambient pCO_2 treatment and 10% urchin mortality in the elevated pCO_2 treatment, which we do not attribute to pCO_2 because it occurred in the first few days of exposure. During the exposure period in Experiment 2, there was no urchin mortality in either treatment.

3.2. Direct effects on algal physiology

Exposure to elevated pCO_2 had a significant effect on the final biomass of *Dictyota* spp. (df = 37, $P = 0.0136$; Table S3). On average, final algal biomass was 21% greater in the elevated treatment at 1.35 ± 0.06 g, compared to 1.06 ± 0.09 g in the ambient treatment (Fig. 1a). Elevated pCO_2 had a significant negative effect on photosynthesis in *Dictyota* spp. (df = 37, $P = 0.0202$; Table S3). Mean photosynthesis was 14% lower in the elevated treatment at 18.20 ± 0.63 µmol O_2 h^{-1} g^{-1}, compared to 21.24 ± 1.06 µmol O_2 h^{-1} g^{-1} in the ambient treatment (Fig. 1b). Total tissue C content did not differ significantly between treatments ($P = 0.9352$; Table S3) and remained extremely consistent at 24.51 ± 0.31% dry weight in the elevated treatment and 24.57 ± 0.46% dry weight in the ambient treatment on average (Fig. 2a). Similarly, differences in total N content between treatments were not significant ($P = 0.0717$; Table S3). However, mean total N content was slightly reduced in the elevated treatment at 1.79 ± 0.05% dry weight, compared to 1.90 ± 0.05% dry weight in the ambient treatment (Fig. 2b). Driven by the difference in N content, differences in C:N ratios were marginally significant between treatments ($P = 0.0496$; Table S3). On average, C:N ratios were 7% higher in *Dictyota* spp. exposed to elevated pCO_2 at 13.88 ± 0.36, compared to 12.96 ± 0.19 in the ambient treatment (Fig. 2c). Total P content did not differ significantly between treatments ($P = 0.8605$; Table S3) and as a result, neither did differences in C:P ratios between treatments ($P = 0.7925$; Table S3).

3.3. Direct effects on urchin physiology

Mass-specific respiration did not differ significantly between L. *variegatus* exposed to ambient and elevated pCO_2 treatments ($F_{1,49} = 1.315$, $P = 0.2570$; Table S3). On average, urchin respiration was 0.83 ± 0.06 µmol O_2 consumed h^{-1} g^{-1} in the elevated treatment and 0.74 ± 0.06 µmol O_2 consumed h^{-1} g^{-1} in the ambient treatment (Fig. 3a). A multiple linear regression revealed that the interaction effect of treatment and urchin biomass on respiration was not significant ($P = 0.2805$), so it was removed and the model was re-run to interpret main effects only. This revealed a significant effect of biomass on respiration ($P = 0.0183$; Fig. S6a). The relationship between urchin biomass and respiration scaled allometrically, in which increases in urchin biomass correlated to decreases in respiration rate (i.e., larger urchins demonstrated lower respiration rates).

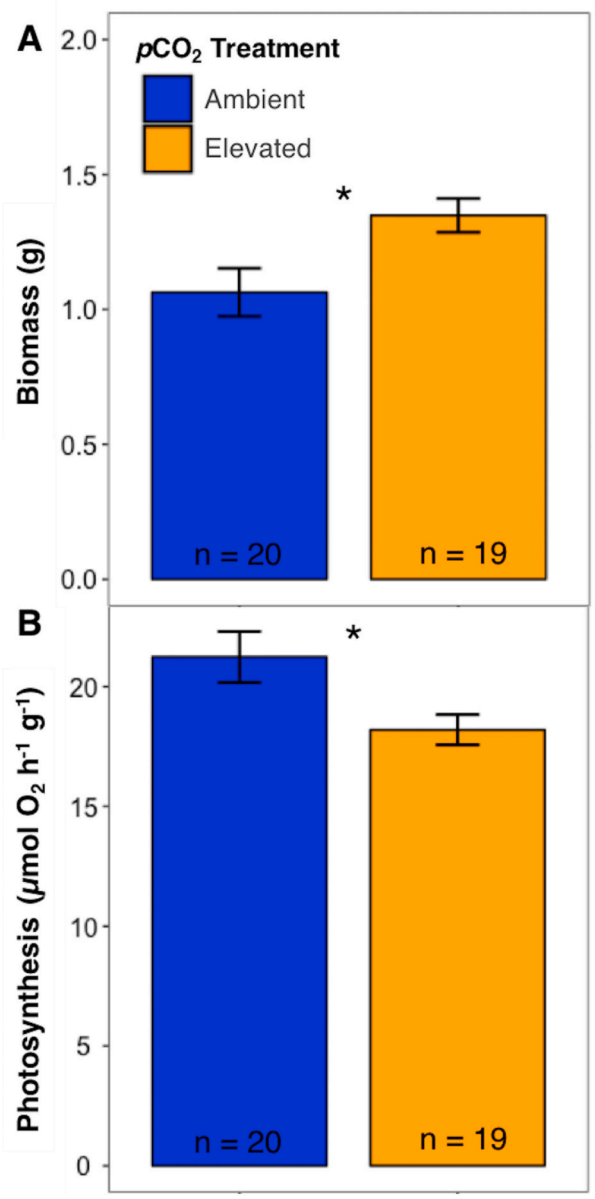

Fig. 1. Mean final biomasses (g) (**A**) and photosynthetic rates (µmol O_2 h^{-1} g^{-1}) (**B**) of *Dictyota* spp. exposed to ambient and elevated pCO_2 treatments, with standard error bars shown. *Asterisks* indicate a statistically significant difference ($\alpha = 0.05$) between treatments.

Eighty percent of urchins demonstrated net loss of biomass by the end of the exposure period (2.6% and 2.3% mean biomass loss in ambient and elevated pCO_2, respectively), potentially as a result of the one-week starvation period before the consumption trial and/or failing to satisfy the urchins' dietary needs. While leftover food observed in the urchin tanks after 24 h indicates that the amount of food we provided was ample, feeding the urchins exclusively algae may not have been

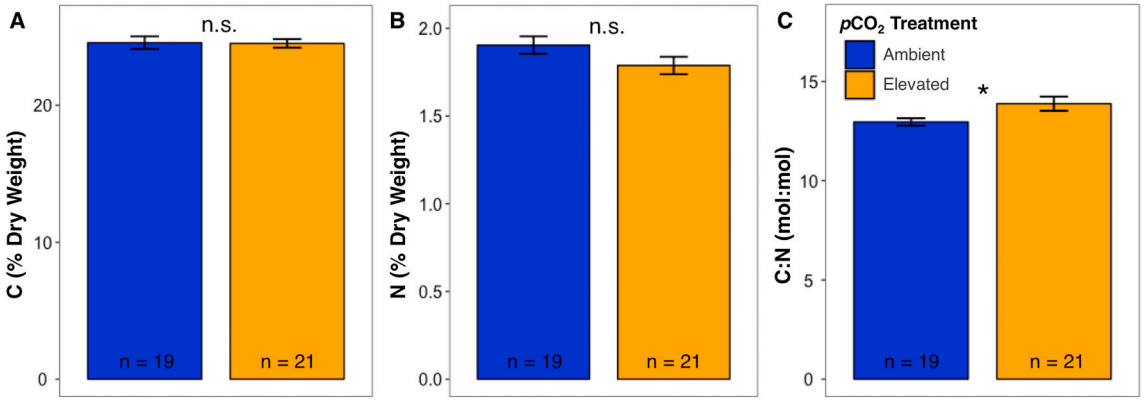

Fig. 2. Mean total tissue C (**A**) and N (**B**) and C:N ratios (**C**) of *Dictyota* spp. exposed to ambient and elevated pCO_2 treatments, with standard error bars shown. *Asterisks* indicate a statistically significant difference ($\alpha = 0.05$) between treatments.

enough to maintain normal growth considering that this species has a facultatively omnivorous diet in the field (Beddingfield and McClintock, 2000). However, changes in urchin biomass over the course of the exposure period did not differ significantly between L. *variegatus* exposed to ambient and elevated pCO_2 treatments ($F_{1,53} = 0.295$, $P = 0.5892$; Table S3). On average, the change in urchin biomass was −0.67 ± 0.13 g in the elevated treatment and −0.76 ± 0.16 g in the ambient treatment (Fig. 3c). A multiple linear regression revealed that the interactive effect of the pCO_2 treatment and initial urchin biomass on the change in urchin biomass during the exposure period was not significant ($P = 0.3747$), so it was removed and the model was re-run to interpret main effects only. This revealed a significant effect of initial biomass on

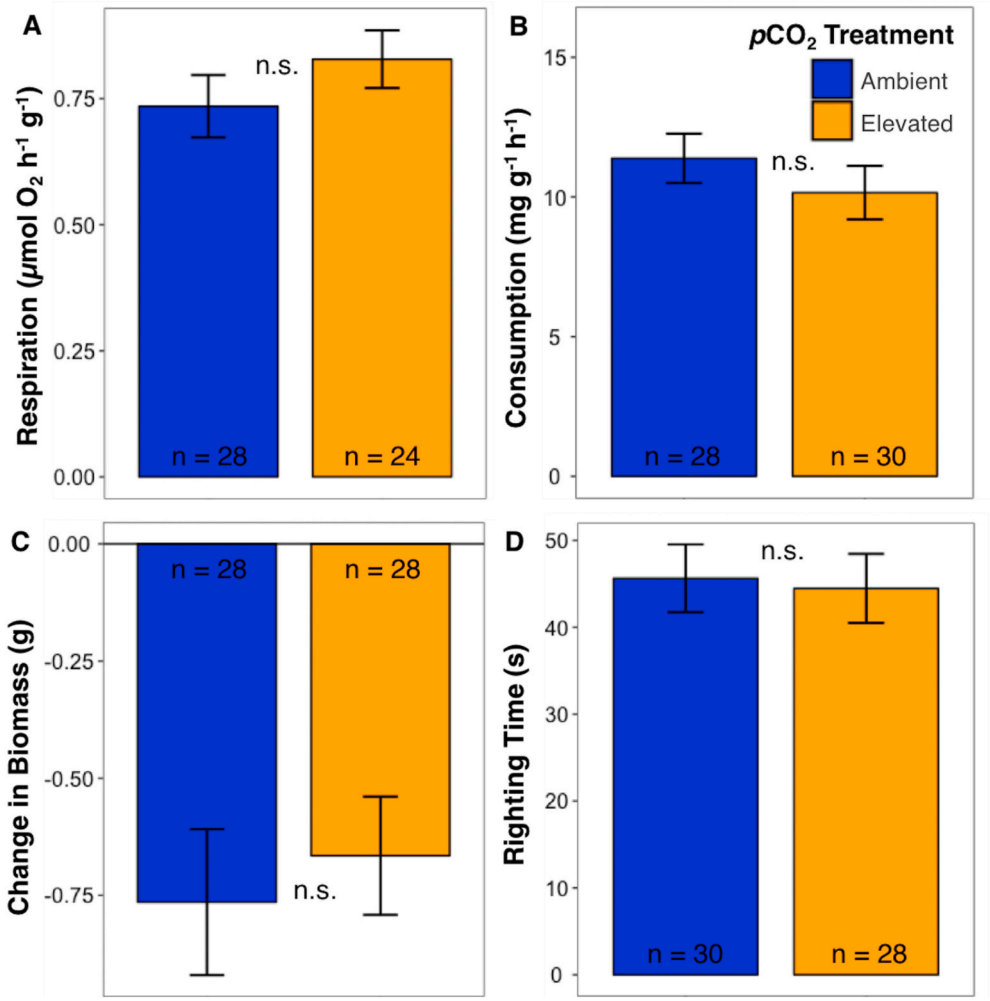

Fig. 3. Mean respiration rates (μmol O_2 consumed h^{-1} g^{-1}) (**A**), consumption rates (mg *Caulerpa racemosa* consumed g^{-1} h^{-1}) (**B**), changes in biomass (g) over six weeks (**C**), and righting times (s) (**D**) of *Lytechinus variegatus* exposed to ambient and elevated pCO_2 treatments, with standard error bars shown. n.s. indicates no significant difference between treatments ($\alpha = 0.05$).

the change in urchin biomass ($P = 0.0011$; Fig. S6c). This relationship scaled allometrically, in which increases in initial urchin biomass correlated to decreases in the change in urchin biomass (i.e., larger urchins demonstrated smaller changes in biomass over the same period of time).

Righting time did not differ significantly among L. *variegatus* exposed to ambient and elevated pCO_2 treatments ($F_{1,55} = 0.071$, $P = 0.7909$; Table S3). On average, righting time was 44.48 ± 3.97 s in the elevated treatment and 45.65 ± 3.90 s in the ambient treatment (Fig. 3d). A multiple linear regression revealed that the interactive effect of the pCO_2 treatment and urchin biomass on righting time was not significant ($P = 0.7031$), so it was removed and the model was re-run to interpret main effects only. This revealed a significant effect of biomass on righting time ($P = 0.0126$; Fig. S6d). The relationship between urchin biomass and righting time scaled allometrically, in which increases in urchin biomass correlated to increases in righting time (i.e., larger urchins demonstrated longer righting times).

3.4. Indirect effects on the trophic interaction

The effect of the urchin pCO_2 treatment on L. *variegatus* foraging behavior for *Dictyota* spp. was significantly influenced by the algae pCO_2 treatment ($P = 0.0103$; Fig. 4). A post hoc comparison of treatment combinations revealed that exposure of the urchins alone to elevated pCO_2 resulted in a marginally significant decrease in the number of correct foraging choices for algae from the ambient treatment ($P = 0.0634$). While 56% of urchins from the ambient treatment made the correct foraging choice for ambient algae, only 20% of urchins from the elevated treatment made the correct foraging choice. Post hoc comparisons revealed that exposure of the algae alone to elevated pCO_2 resulted in no significant difference in the number of correct foraging choices in urchins from the ambient treatment ($P = 0.9423$). However, exposure of both the urchins and the algae to elevated pCO_2 resulted in a return to ambient foraging success ($P = 0.7681$). Urchins exposed to elevated pCO_2 made a significantly higher number of correct foraging choices for algae that was also exposed to elevated pCO_2 (62% correct choices) than for algae exposed to ambient conditions (20% correct choices; $P = 0.0257$).

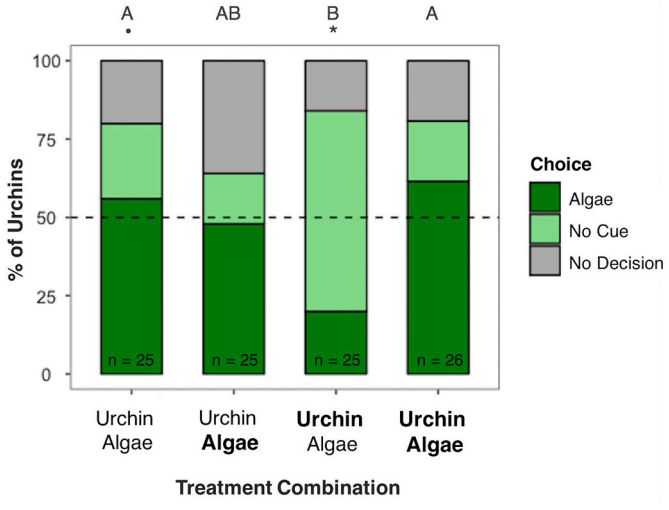

Fig. 4. The percent of *Lytechinus variegatus* that chose the algal (*Dictyota* spp.) cue, no chemical cue, or did not make a choice for each treatment combination. The dotted line denotes equal likelihood of choice. Bolded text within the treatment combination labels indicates exposure of that organism to the elevated pCO_2 treatment, while plain text indicates exposure of that organism to the ambient pCO_2 treatment. *Asterisks* indicate a statistically significant difference ($\alpha = 0.05$) between treatment combinations, while letters designate which of those treatment combinations are statistically similar.

During consumption trials, rates of autogenic mass loss in *C. racemosa* controls were highly variable (range 10–333 mg h^{-1}) but did not vary significantly between treatments ($F_{1,25} = 0.826$, $P = 0.3720$); as such, we assumed equal rates of autogenic loss across algae replicates and did not apply these values to rates of consumption (Poore et al., 2016). These consumption rates, therefore, likely represent high estimates of actual consumption. Mass-specific consumption rates of *C. racemosa* were observed to be highly variable among individuals, ranging from 1.6 to 22.4 mg g^{-1} h^{-1}. Consumption rates did not differ significantly among L. *variegatus* exposed to ambient and elevated pCO_2 treatments ($F_{1,55} = 0.992$, $P = 0.3237$; Table S3). On average, consumption rates were 10.2 ± 1.0 mg g^{-1} h^{-1} in the elevated treatment and 11.4 ± 0.9 mg g^{-1} h^{-1} in the ambient treatment (Fig. 3b). A multiple linear regression revealed that the interaction effect of treatment and urchin biomass on consumption rate was not -significant ($P = 0.2421$), so it was removed and the model was re-run to interpret main effects only. This revealed a significant effect of biomass on consumption rate ($P = 0.00678$; Fig. S6b). The relationship between urchin biomass and consumption rate scaled allometrically, in which increases in urchin biomass correlated to decreases in consumption rate (i.e., larger urchins demonstrated lower consumption rates).

4. Discussion

4.1. Direct effects on algal physiology

Consistent with our hypothesis, our results show that two-week exposure to ocean acidification has a positive effect on macroalgal production in *Dictyota* spp. at a seawater pCO_2 of 1420 μatm and pH_T of 7.56. However, increased macroalgal biomass under elevated pCO_2 was not a result of increased photosynthetic rates (Table 1). Enhanced macroalgal growth despite decreased photosynthesis has also been observed in *Ulva rigida* exposed to elevated pCO_2 and associated pH_{NBS} of 7.7 (Gordillo et al., 2001). Significant increases in macroalgal growth can be due to the downregulation of carbon concentrating mechanisms (CCM) used by the algae in response to the increased abundance of CO_2 in seawater (Gordillo et al., 2001; Cornwall et al., 2017; van der Loos et al., 2018). Species that demonstrate the ability to preferentially alter their carbon acquisition strategy in response to environmental conditions, such as *Dictyota dichotoma* and *C. racemosa*, are found in greater abundance closer to volcanic CO_2 seeps (7.69 pH_T) in the Mediterranean (Cornwall et al., 2017). CCMs are an energetically costly form of inorganic carbon acquisition, compared to diffusive CO_2 uptake, so the inactivation of these mechanisms leaves more energy for the production of biomass (Gordillo et al., 2003). Downregulation of CCM activity in *U. rigida* exposed to elevated pCO_2 was coupled with greater retention of carbon fixed by photosynthesis, evident in consistent C content across pCO_2 treatments (Gordillo et al., 2001). *Dictyota* spp. exposed to elevated pCO_2 in the current study similarly demonstrated consistent C content despite reduced photosynthesis. These findings strongly suggest that *Dictyota* spp. can alter its carbon acquisition strategy to compensate for reduced photosynthesis, resulting in greater energy availability and larger carbon reserves that allow for higher production of biomass under acidification. In the Florida Keys, *Dictyota* has historically overwhelmed reefs with seasonal blooms, occupying as much as 70% of the benthos at a time (Beach and Walters, 2000) and competing with coral for resources, so understanding the resiliency of the trophic interaction between fleshy macroalgae like *Dictyota* spp. and grazers that help mediate its growth under future acidification is critical.

4.2. Direct effects on urchin physiology

Contrary to our hypothesis, our results show that adult L. *variegatus* physiological fitness is not altered by six-week exposure to ocean acidification at a seawater pCO_2 of 1294–1391 μatm and pH_T of 7.57–7.60 (Table 1). Previous studies on adult urchin respiration, biomass, and

righting time show mixed responses to elevated pCO_2 and low pH. However, given an experimental design that employs similar magnitudes of acidification (ΔpH: −0.3) and at least one month of exposure time, the physiology results for multiple tropical and temperate urchin species align with those found in this study. No change in respiration rates, an indicator of metabolism, was observed in *Strongylocentrotus droebachiensis* (Stumpp et al., 2012) and *Echinometra mathaei* (Moulin et al., 2015) urchins exposed to elevated pCO_2 for six weeks and thirteen months, respectively. Similarly, righting time, an indicator of physiological stress, was unchanged in L. *variegatus* (Challener and McClintock, 2013; Emerson et al., 2017) and *Strongylocentrotus fragilis* (Taylor et al., 2014) urchins exposed to elevated pCO_2 for two to three months and one to four months, respectively. Final wet weight was also unchanged in L. *variegatus* exposed to elevated pCO_2 for just over two months (Emerson et al., 2017), but when exposed for a longer period of fourteen weeks, final urchin wet weight, diameter, and dry matter production in L. *variegatus* was reduced (Challener et al., 2014). Other studies on tropical sea urchins employing much longer timespans, however, observed no significant change in the final wet weight of *Hemicentrotus pulcherrimus* (Kurihara et al., 2013) and *E. mathaei* (Moulin et al., 2015) exposed to elevated pCO_2 for nine and thirteen months, respectively. In comparison, the results of the present study indicate that six weeks of exposure to ocean acidification does not result in significant changes to biomass, respiration, or righting time in adult L. *variegatus* urchins, indicating that acidification did not induce a stress response or alter metabolism. Therefore, any changes observed in the trophic interaction between L. *variegatus* and fleshy macroalgae in this study are not likely the result of physiological stress or metabolic mechanisms.

There are three main possible reasons for measuring no significant effects of pCO_2 on urchin physiology. Elevated pCO_2 may have simply had no significant effect on urchin respiration, righting time, and biomass, as indicated. The ability of the individuals used in this study to tolerate ocean acidification may be inherent to this population as a result of the highly variable carbonate chemistry that comprises its habitat. Though we did not sample the carbonate chemistry of the site from which these individuals were collected, inshore sites in the lower Florida Keys demonstrate seasonal fluctuations in seawater carbonate chemistry (Muehllehner et al., 2016), including pCO_2 values ranging from 301 to 395 μatm on the Florida Reef Tract (Manzello et al., 2012). At sites like Florida Bay, seasonal fluctuations in pCO_2 are as large as 325–725 μatm (Millero et al., 2001). Our results, therefore, may reflect preadaption to variability in seawater pCO_2 in this species of urchin (Rivest et al., 2017; Asnicar et al., 2021). Alternatively, elevated pCO_2 may have impacted the urchins' physiology in the short-term, but the urchins may have employed compensatory mechanisms, such as significant gains in internal HCO_3^- concentrations (Stumpp et al., 2012; Moulin et al., 2015), to achieve an acclimation state in which no effect on physiology was measured. Without having measured the acid-base balance of the urchins' coelomic fluids in this study, it is difficult to determine if full extracellular pH compensation occurred as it did in *S. droebachiensis* (Stumpp et al., 2012), *S. fragilis* (Taylor et al., 2014), and *E. mathaei* (Moulin et al., 2015) urchins exposed to elevated pCO_2 for at least one month. During six-month exposure to elevated pCO_2, *Paracentrotus lividus* urchins preadapted to either a high or a low variability environment both experienced significant changes in their physiological and behavioral responses, but both demonstrated the ability to acclimate to elevated pCO_2 as the exposure continued, with individuals preadapted to high variability achieving acclimation faster than those adapted to low variability (Asnicar et al., 2021). Therefore, if the individuals collected for this study are preadapted to high variability in seawater pCO_2, measuring physiological responses at different timepoints during the exposure period may have revealed that acclimation occurred over time. Finally, it is possible that the exposure period in this study was simply not long enough for elevated pCO_2 to produce measurable effects on the physiological responses we tested.

4.3. Indirect effects on the trophic interaction

The foraging behavior results indicate that elevated pCO_2 had a significant effect on the interaction between L. *variegatus* and *Dictyota* spp. Consistent with our hypothesis, exposure of the urchins to elevated pCO_2 reduced the number of correct foraging choices for ambient algae, which suggests that chemical cue sensing was impaired in L. *variegatus* under acidification. In contrast to our hypothesis, however, exposure of the algae to elevated pCO_2 returned the number of correct foraging choices in urchins exposed to elevated pCO_2 to ambient levels (Table 1), which suggests that algal nutritional content was altered in a way detectable by the urchins under acidification. Significantly higher C:N ratios driven by a slight reduction in N content in *Dictyota* spp. exposed to elevated pCO_2 in this study (Table 1) suggest a decrease in nutrition, specifically protein content, under acidification. Correlations between algal nutritional quality and grazer feeding preference, in which lower nutritional quality (i.e., lower protein and organic carbon content and higher C:N ratios) is associated with reduced palatability and preference, have been observed in a number of invertebrates (Falkenberg et al., 2013; Duarte et al., 2016; Kamya et al., 2017; Leung et al., 2018), including sea urchins (Borell et al., 2013). For example, exposure to elevated pCO_2 reduced the protein content of fleshy macroalgae, and this was associated with reduced feeding preference in *Orchestoidea tuberculata* amphipods (Duarte et al., 2016) and *Tripneustes gratilla* urchins (Borell et al., 2013) in multiple choice feeding assays. Another possible reason for altered urchin foraging behavior that was not measured in this study is changes in the concentrations of secondary metabolites, which are shown to be higher in fleshy macroalgae under elevated pCO_2 (Borell et al., 2013) and effectively deter grazing by L. *variegatus* in *Dictyota* species (Barbosa et al., 2004; Cobb and Lawrence, 2005; Souza et al., 2008; Pereira et al., 2010).

Reduced nutritional quality in *Dictyota* spp. under elevated pCO_2 had the potential to negatively impact the number of correct urchin foraging choices through decreased palatability, but it had the opposite effect on L. *variegatus* exposed to elevated pCO_2. Altered algal nutritional quality may have interacted with the chemical sensing ability of L. *variegatus* under ocean acidification to produce the novel results that we observed. Barry et al. (2014) observed impaired chemosensory behavior in urchins exposed to ocean acidification indicated by increased foraging time 4.7 times longer on average than that of control urchins in *S. fragilis* exposed to elevated pCO_2. Impaired chemosensory behavior would explain why less palatable changes to *Dictyota* spp. under elevated pCO_2 positively impacted preference in urchins exposed to elevated pCO_2. This finding suggests that the urchins may be interpreting an unfavorable chemical cue as a favorable one under ocean acidification. These CO_2-induced, maladaptive behavioral choices in chemosensory response have been documented in other marine invertebrates (de la Haye et al., 2012; Manríquez et al., 2014; Ashur et al., 2017; Ross and Behringer, 2019; Gravinese et al., 2020), and impairment of the ability to process information and make decisions has been implicated (de la Haye et al., 2011; Briffa et al., 2012; Horwitz et al., 2020). For example, exposure to elevated pCO_2 impaired the decision-making process of hermit crabs, causing them to be less likely to trade up from a suboptimal to an optimal shell (de la Haye et al., 2011). In post-larval lobsters, exposure to elevated pCO_2 resulted in 75% fewer lobsters making the correct choice for the algal settlement cue compared to individuals reared in ambient conditions (Gravinese et al., 2020). While impaired chemical sensing and maladaptive behavioral choices under ocean acidification have been sparsely documented in crustaceans and molluscs, even fewer studies have been conducted on these responses in echinoderms and urchins, in particular. Thus, we aim to contribute new insights through this study. Additionally, these results highlight the importance of studying the indirect effects of acidification on species interactions simultaneously with direct effects on species physiology. Together, these results suggest that changes to urchin chemical sensing and algal nutritional quality are the driving mechanisms behind surprisingly

unaltered urchin foraging behavior for fleshy macroalgae under joint exposure to acidification.

The trophic interaction once again held consistent under acidification when L. *variegatus* consumption rates of *C. racemosa* experienced no significant changes under elevated pCO_2 (Table 1). In previous studies that exposed urchins to similar magnitudes of acidification (ΔpH: −0.3) for one to four months, consumption rates were similarly unaffected by elevated pCO_2 (Stumpp et al., 2012; Borell et al., 2013; Challener et al., 2014; Collard et al., 2014; Taylor et al., 2014; Carey et al., 2016). While increases in consumption rate have been observed in urchins exposed to elevated pCO_2 for shorter time periods of one to three weeks, this result was attributed to higher metabolic demand (Rich et al., 2018) and decreased nutritional quality of the food (Burnell et al., 2013). In the present study, respiration, indicative of metabolic demand, and food nutritional quality during consumption trials were consistent across both pCO_2 treatments. Therefore, consistent consumption rates of ambient algae in adult L. *variegatus* exposed to ocean acidification are not surprising or novel, and they reflect the stability of L. *variegatus*'s physiological results. Due to logistical limitations encountered during this study, we did not measure urchin consumption rates of fleshy macroalgae exposed to elevated pCO_2. It is important to recognize that altered algal nutritional content under acidification has been implicated as the driving mechanism behind grazer consumption rates, rivaling even direct effects on grazer physiology (Falkenberg et al., 2013). Therefore, further research is needed to determine how altered algal nutrition and palatability under elevated pCO_2 may affect consumption rates in L. *variegatus*.

5. Conclusions

Consistent with our hypotheses, *Dictyota* spp. biomass increased under elevated pCO_2, but increased photosynthesis was not the mechanism through which this occurred. Enhanced biomass production and consistent tissue C content despite reduced photosynthetic rates indicate the use of alternative carbon acquisition pathways beneficial to growth under ocean acidification. As a result, acidification appears to have positive effects on the production of fleshy macroalgae as predicted. In contrast with our hypotheses, elevated pCO_2 did not result in significant changes to adult L. *variegatus* physiological fitness (respiration, biomass, and righting time) or consumption rates of *C. racemosa*, indicating that physiological stress and changes in metabolism are not mechanisms through which the trophic interaction was impacted. As we hypothesized, urchin exposure to elevated pCO_2 altered L. *variegatus* foraging choice for ambient *Dictyota* spp. through putatively impaired urchin chemical sensing, but subsequent exposure of the algae to elevated pCO_2 unexpectedly returned foraging choice to ambient levels through altered algal nutritional quality. These counterintuitive results suggest that less palatable, reduced protein content in acidified *Dictyota* spp. interacted with compromised information processing and maladaptive behavioral choices in acidified urchins, essentially cancelling each other out to produce no net changes to L. *variegatus* foraging behavior under acidification. Our findings reveal that the indirect effects of elevated pCO_2 on the trophic interaction produced entirely different results than the direct effects of elevated pCO_2 on organism physiology, highlighting the importance of studying the role that ocean acidification plays in mediating species interactions as a whole. Additionally, by studying direct and indirect effects simultaneously, we are able to identify urchin chemical sensing and algal nutritional quality as the mechanisms most likely driving this result.

In contrast with our hypothesis, consistent foraging behavior and consumption rates suggest that the trophic interaction between fleshy macroalgae and L. *variegatus* individuals may be sustained under ocean acidification. However, increases in fleshy macroalgal biomass driven by opportunistic carbon acquisition strategies under acidification pose a potential threat to the resiliency implied by this stable trophic interaction, depending on how grazer populations respond. Further field research is needed to determine the ecological outcome of these findings over successive generations and under a wider range of environmental conditions, including simultaneous ocean warming. However, for large marine ecosystems not amenable to experimentation, studies like ours reveal key mechanisms responsible for ecosystem change, allowing them to be considered as contributing factors of future field results. It is research like this, aimed at quantifying both the direct and indirect effects of climate stressors on macroalgae and their grazers, that when applied to a wide variety of species, will help us to better predict the persistence of macroalgae under future climate change.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2021.151662.

Funding

This work was supported by Mote Marine Laboratory Postdoctoral Fellowships (RJN and HNP), Becker Internship Funding, and philanthropic funds to ERH.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Mote Marine Laboratory and their donors for funding our experiments. We would also like to thank all of the Mote staff and interns who helped with experimental setup, daily water quality monitoring, carbonate chemistry analysis, and data entry, most notably Alexandra Fine and Curtie Leipold (staff), Bailey Wallace, Jill Ashley, Arielle Martinka, Emily Ng, Gabi Greenberg, and Sara Casareto (interns).

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