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Microzooplankton grazing responds to simulated ocean acidification indirectly through changes in prey cellular characteristics

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ABSTRACT: Microzooplankton (MZP) grazing is a factor that regulates oceanic primary production and is a controlling mechanism for marine biogeochemical cycling. Despite the prominent ecological role of MZP, few studies have explored their responses to ocean acidification (OA). Studies to date generally indicate that MZP are affected indirectly by OA through changes in phytoplankton prey composition and biomass concentration. Here, we conducted a series of experiments testing whether OA-induced changes in cellular characteristics of individual prey species can cause changes in MZP grazing. Two tintinnid ciliates (*Eutintinnus* sp. and *Schmidingerella* sp.) and a heterotrophic dinoflagellate (*Oxyrrhis marina*) were offered phytoplankton prey (*Emiliania huxleyi*) cultured under 3 pCO_2 concentrations. Using linear mixed effects models, we found that *Eutintinnus* sp. and *O. marina* exhibited a step-wise increase in ingestion rates on *E. huxleyi* cells cultured under elevated pCO_2. *Schmidingerella* sp. ingestion showed a non-linear response, whereby cells cultured under high pCO_2 were ingested at higher rates than cells from moderate pCO_2. The percentages of all 3 MZP populations observed feeding were higher on *E. huxleyi* cells cultured under elevated pCO_2, with *Eutintinnus* sp. showing a step-wise increase. We postulate that this response is caused by the observed increased coccosphere volume in *E. huxleyi* cells cultured under elevated pCO_2. If changes in phytoplankton cell volume are widespread under OA, this could be an important mechanism by which MZP grazing behavior shifts and planktonic food web dynamics are altered in the future ocean.

KEY WORDS: Microzooplankton grazing · Ocean acidification · *Emiliania huxleyi* · Plankton food web

INTRODUCTION

For decades, microzooplankton (MZP) ecologists have been measuring per capita rates of MZP grazing potential across the world's oceans and coastal ecosystems. These measurements have demonstrated that when unencumbered by their own top-down regulation, MZP are capable of consuming >100% primary production (PP) in open-ocean and coastal environments (e.g. McManus & Ederington-Cantrell 1992, Neuer & Cowles 1994, Olson et al. 2008, Sherr et al. 2009). In a synthesis of available data on *in situ* MZP grazing rates, Calbet & Landry (2004) showed that across contrasting marine environments, MZP consume on average 67% of PP, exceeding that of the ocean's other dominant grazer, copepod mesozooplankton (e.g. Dagg 1993). This recognition of the dominant role of MZP in consuming phytoplankton led to further work demonstrating their regulatory role in the microbial loop (Pomeroy 1974, Azam et al. 1983, Pomeroy et al. 2007), including high rates of nutrient remineralization (Caron & Goldman 1990, Ferrier-Pages & Rassoulzadegan 1994) and bacterivory (e.g. Strom 2000).

Despite their governing role in marine microbial dynamics and biogeochemical cycling, it is striking

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that only a limited number of studies have addressed potential effects that ocean acidification (OA) will have on MZP biology and ecology (Suffrian et al. 2008a, Rose et al. 2009, Aberle et al. 2013, Horn et al. 2016). OA is a well-described climate stressor resulting from ocean carbonation (Orr et al. 2005, Doney et al. 2009) and acidifying coastal processes (Borges & Gypens 2010, Feely et al. 2010, Cai et al. 2011, Sunda & Cai 2012). The paucity of OA studies focusing on MZP is in stark contrast to the extensively studied effects of OA on other wide-ranging marine taxa (Doney et al. 2009, Kroeker et al. 2013, Mostofa et al. 2016).

OA has the potential to affect MZP both directly and indirectly. Direct OA effects to MZP may arise from decreased external pH precipitating changes in intracellular pH, cell-surface membrane potential, and enzyme activity (Felle 1994, Nimer et al. 1994, Langer et al. 2006, Nielsen et al. 2010). However, MZP are largely tolerant to low pH, but may show reduced growth rates at pH 8.8 and above (Pedersen & Hansen 2003a,b, Weisse & Stadler 2006). A more likely mechanism for an OA effect on MZP is through indirect pathways. Microzooplankton grazers are acutely sensitive to the physiological, biochemical, and morphological characteristics of their phytoplankton prey (Olson & Strom 2002, Tillmann 2004). This acute sensitivity engenders selective feeding behavior by MZP that, when exhibited by the ocean's dominant grazers, has implications for many biogeochemical processes, including, but not limited to, nutrient turnover, export flux efficiency, dissolved organic carbon (DOC) production, and top-down influence on phytoplankton community and size composition.

OA can affect phytoplankton growth rates (Nimer et al. 1994, Iglesias-Rodriguez et al. 2008, Langer et al. 2009, Lohbeck et al. 2012, Müller et al. 2012), cell and coccosphere volume (Iglesias-Rodriguez et al. 2008, Müller et al. 2012, Olson et al. 2017), photosynthetic rate (e.g. Feng et al. 2008, Borchard & Engel 2012), organic and inorganic carbon production (reviewed by Findlay et al. 2011, Meyer & Riebesell 2015), dimethylsulfoniopropionate (DMSP) concentration and production (e.g. Avgoustidi et al. 2012, Spielmeyer & Pohnert 2012, Arnold et al. 2013, Webb et al. 2016, Olson et al. 2017), and toxicity (Sun et al. 2011). Community photosynthetic and nitrogen fixation rates are also sensitive to OA (e.g. Tortell et al. 2002, 2008, Hare et al. 2007, Hutchins et al. 2007, Hopkinson et al. 2010). Phytoplankton community composition can also change under OA conditions (Tortell et al. 2008, Feng et al. 2009), but this effect is not universally observed (Nielsen et al. 2010). Given that these characteristics are all known to affect MZP feeding ecology, the strongest link between OA and MZP may be through trophic interactions with the more OA-sensitive prey they consume.

Attempts to understand the effects of OA on MZP come exclusively from a few micro- and mesocosm experiments that, while holistic in nature, provide community-based assessments that have inherently low species-level resolution (e.g. Suffrian et al. 2008, Aberle et al. 2013, Rossoll et al. 2013, Calbet et al. 2014, Horn et al. 2016). In all cases, MZP exhibited high tolerance to OA, and any observed indirect effects to MZP were attributed to OA and temperature reshaping the phytoplankton prey field (Rose et al. 2009, Aberle et al. 2013, Schulz et al. 2013, Horn et al. 2016, Lischka et al. 2017). Experimental designs whereby a single consumer is offered only a single prey item do omit the ecological complexity, genetic variability, and community-wide ecophysiological responses to OA that exist in natural systems (Rossoll et al. 2013, Calbet et al. 2014). However, a simplified system allows potential mechanisms for change to be uncovered. Also, detailed interspecies-level interactions may be occurring in mesocosms that are ecologically significant in more stable and tightly coupled ecosystems (e.g. subtropical gyres), but that are obscured by taking a community-wide approach with a diverse plankton assemblage. The results of controlled microcosm studies can be coupled with whole-community experiments or observations of natural systems, and this approach serves to enhance the interpretation of both types of study.

Given the dominant ecological role of MZP in marine carbon cycling, and the observed sensitivity of phytoplankton to OA, the goal of this research was to determine whether MZP modify their feeding rates when exposed to phytoplankton acclimated to OA. We chose to use a microcosm design with the globally-significant phytoplankton *Emiliania huxleyi* and 3 species of common MZP grazers. *E. huxleyi* is a bloom-forming phytoplankton shown to respond morphologically (Iglesias-Rodriguez et al. 2008, Müller et al. 2012, Olson et al. 2017) and physiologically and biochemically to OA (reviewed by Meyer & Riebesell 2015). This prey was chosen because *E. huxleyi* is a well-characterized model phytoplankton, the subject of studies ranging from genetics to ecology, and this expansive knowledge allows experimental results obtained here to be placed within a broad ecological context. Secondly, it is a representative, globally important calcifier, and has been shown to exhibit some physiological response to OA under the conditions and on the time scale of these planned experiments (Olson et al. 2017). The MZP grazers, i.e. 2 tintinnid

ciliates and a heterotrophic dinoflagellate, were selected to represent important classes of MZP with differing feeding strategies, for which *E. huxleyi* is an acceptable prey species (Hansen et al. 1996, Strom & Bright 2009). These experiments were designed to detect whether species-specific changes in phytoplankton resulting from OA do indeed induce changes in MZP feeding rates. Because MZP play such a vital role in planktonic food webs, any shifts in MZP feeding behavior could result in changes to trophic energy transfer, carbon fluxes, and nutrient cycles.

MATERIALS AND METHODS

Experimental organisms and maintenance culture conditions

The calcifying *Emiliania huxleyi* strain 2668, obtained from the National Center for Marine Algae and Microbiota, was maintained at 15°C under 75 µmol photons s^{-1} m^{-2} irradiance on a 12:12 h light:dark diurnal cycle. To ensure *E. huxleyi* remained in exponential growth, stock cultures were diluted every 7 d in autoclaved 0.2 µm filtered seawater (AFSW) amended with f/2 nutrient concentration. The AFSW was collected from the Salish Sea at a depth of 35 m and a salinity of 31 psu. One large-volume collection provided all experimental water needed for experiments. Three MZP grazers were used in these tests: 2 tintinnid ciliates, *Eutintinnus* sp. and *Schmidingerella* sp., and the heterotrophic dinoflagellate *Oxyrrhis marina*. All MZP were isolated from the Salish Sea/Puget Sound, Washington State (USA), and maintained on mixed diets of the haptophytes *Isochrysis galbana* and *E. huxleyi* strain 374, the cryptomonad *Rhodomonas* sp., the autotrophic dinoflagellate *Heterocapsa rotundata*, and the chlorophytes *Dunaliella tertiolecta*, *Micromonas pusilla*, and *Mantoniella squamata*. Microzooplankton grazers were cultured in ciliate media (Gifford 1993) at 15°C and under approximately 5 µmol photons s^{-1} m^{-2} irradiance on a 12:12 h light:dark irradiance cycle. All MZP cultures were diluted twice weekly and supplied with fresh ciliate media and saturating prey concentrations.

CO_2 experimental system

A novel system used here to elevate pCO_2/reduce pH is described in detail in Love et al. (2017). Briefly, pCO_2 treatments were made by first compressing and then stripping CO_2 from ambient air. Research-grade CO_2 was subsequently mixed with the CO_2-free air using mass flow controllers to achieve the treatment CO_2 concentrations. This air was then distributed to 2 channels for each treatment. One channel bubbled air through fine-pore air stones into AFSW amended with f/50 nutrient concentration, producing pre-equilibrated AFSW at treatment pCO_2 concentrations for use in experiments (experimental water). The other channel delivered air to 120 l volume atmospheric simulation chambers (ASCs) made from clear acrylic that were housed in a light- and temperature-controlled room. Experimental cultures were placed inside the ASCs, and transfer of CO_2 gas across the air/water interface helped maintain experimental water at treatment pCO_2 concentrations, and minimized changes to water chemistry driven by organismal metabolic activity. This system was designed to accommodate delicate protists whose behavior and survival are affected by the shear stress resulting from direct bubbling of enriched CO_2 gas into culture vessels. Carbonate chemistry parameters of pre-equilibrated AFSW and experimental water were quantified by spectrophotometric pH (Agilent 2853, m-cresol purple method after Dickson et al. 2007) and total alkalinity (A_T) using a Metrohm Titrando and a modified Gran titration method as described by Millero et al. (1993). Sets of replicate measurements of certified reference material for A_T (Batch 104) had standard deviations between 2 and 7 µeq l^{-1}. Temperature (*in situ* and at time of analysis) and salinity of the AFSW were measured and, along with pH and A_T, were used to calculate remaining carbonate parameters using CO2sys for Microsoft Excel/VBA (Lewis & Wallace 1998).

E. huxleyi acclimation and characterization experiments

E. huxleyi were inoculated at 2.5×10^3 cells ml^{-1} into 1 l experimental flasks containing 500 ml of experimental water pre-equilibrated to 1 of 3 pCO_2 concentrations (400, 850, and 1000 µatm) (Houghton et al. 2001). Each pCO_2 treatment was maintained in triplicate. Cultures were placed into the ASC held at 15°C under 250 µmol photons s^{-1} m^{-2} irradiance on a 14:10 h light:dark irradiance cycle. Cell densities in treatment flasks were measured daily using a BD FACSCaliber flow cytometer. Under these conditions, *E. huxleyi* cells grew exponentially in batch culture until cell densities reached 1.0×10^5 cells ml^{-1}. After this point, experimental cultures were maintained semi-continuously by daily determina-

tion of initial cell densities, followed by removing and replacing culture volume with the appropriate amount of pre-equilibrated experimental treatment water to reduce cell densities to 4.0×10^4 cells ml^{-1}. The volume removed from each treatment replicate was used for daily measurements of pH and every other day measurements of A_T. *E. huxleyi* populations were cultured semi-continuously for 8–10 d, which allowed for steady state acclimation to treatment pCO_2 conditions (Olson et al. 2017). Acclimated *E. huxleyi* cells from each treatment replicate were harvested for analysis of intrinsic growth rate (μ, d^{-1}), coccosphere volume (μm^3), particulate inorganic carbon (PIC; pg $cell^{-1}$), and particulate organic carbon (POC) and nitrogen (PON) (pg $cell^{-1}$) (Table 1).

E. huxleyi population growth rates from each treatment replicate were calculated according to:

$$\mu = \ln(N_2) - \ln(N_1)/t_2 - t_1 \quad (1)$$

where N_2 is *E. huxleyi* cell concentration on the final day of acclimation (t_2), and N_1 is *E. huxleyi* cell concentration after dilution from the previous day (t_1).

Coccosphere volumes (μm^3) of *E. huxleyi* from each treatment replicate were quantified by imaging live cells at 100× magnification using an Olympus CH30 microscope networked to a Photometrics CoolSNAP camera. For each treatment replicate, the first 200 coccospheres encountered were imaged. Volume of the sphere was calculated as:

$$V = 4/3\pi r^3 \quad (2)$$

where r was calculated as:

$$r = \sqrt{A/\pi} \quad (3)$$

where area, A, is determined from 2D images of each coccosphere.

On the last day of each experiment, duplicate 100 ml samples from each treatment replicate were filtered through 13 mm GF/F filters muffled previously at 450°C for 4 h. One of the paired filter samples was placed in a tin capsule and the other in a silver capsule. Both filter sets were dried at 60°C for 24 h. Afterward, PIC was removed from cells held in the silver capsules by acid fumigation for 24 h. After fumigation, silver capsules and filters were dried again at 60°C for 24 h. The cellular masses of POC and PON were determined using a CE Elantech Flash EA 1112 elemental analyzer. PIC was calculated by subtracting POC from total particulate carbon calculated from samples contained in tin capsules.

Multi-generational studies addressing long-term changes may be more properly termed OA experiments, while short-term exposure studies are pCO_2 sensitivity experiments (McElhany 2017). We note that the 8–10 d acclimation period, while not long, encompassed ~15 generations in the *E. huxleyi* populations. We retain the use of the term OA here for brevity and because episodic changes in pCO_2 on this time scale are common in coastal waters (Hofmann et al. 2011). As used in this study, OA effects refer to potential responses to a relatively short-term exposure to elevated pCO_2, allowing for acclimation but probably not adaptation to these conditions.

Microzooplankton grazing experiments

Epifluorescence microscopy was used to determine MZP ingestion rates on *E. huxleyi* cells acclimated to the 3 pCO_2 treatments. This technique renders *E. huxleyi* cells ingested by grazers easily visible inside grazer food vacuoles when illuminated under blue-light excitation. Counting *E. huxleyi* cells within MZP food vacuoles in subsamples of the mixed culture over time provides estimates of grazer ingestion rate. Prior to experiments, MZP were allowed to consume maintenance food to low concentrations. Once maintenance prey was grazed to near completion, MZP were concentrated using reverse filtration through 20 μm mesh sieves and allowed time for voiding of food vacuoles. The duration of time that the respective MZP grazers needed to clear food vacuoles of background prey was determined from preliminary experiments. Care was taken to assure adequate time for each grazer species to void food vacuoles, yet not allow grazers to engage in behavior or physiological impairment caused by prolonged starvation.

Table 1. Microzooplankton (MZP) grazers tested, duration of experiment, and measured *Emiliania huxleyi* metrics for each experiment. In all experiments, the prey was *E. huxleyi* strain 2668. GR: *E. huxleyi* growth rate (μ, d^{-1}); CV: *E. huxleyi* coccosphere volume (μm^3); PIC: *E. huxleyi* particulate inorganic carbon (pg $cell^{-1}$); POC: *E. huxleyi* particulate organic carbon (pg $cell^{-1}$); PON: *E. huxleyi* particulate organic nitrogen (pg $cell^{-1}$); IR: grazer ingestion (prey cells $grazer^{-1}$); PPF: percentage of MZP population observed feeding

Expt	Grazer	Duration (d)	Grazing time points (min)	Metrics
1	*Eutintinnus* sp.	10	15, 30, 45	GR, CV, PIC, POC, PON, IR, PPF
2	*Schmidingerella* sp.	8	15, 30, 45	GR, CV, PIC, POC, PON, IR, PPF
3	*Oxyrrhis marina*	10	30, 60, 90	GR, CV, PIC, POC, PON, IR, PPF

For feeding experiments, grazers of one species were added to 150 ml polycarbonate bottles that contained 100 ml of AFSW pre-equilibrated to ambient pCO_2. The 2 ciliate species were at a density of 20 ciliates ml^{-1}, and *O. marina* was at a density of 150 *O. marina* ml^{-1} in grazing bottles. Grazing experiments started when the MZP were inoculated with *E. huxleyi* from each pCO_2 treatment at saturating prey C concentrations of ~400 µg C l^{-1}, thus assuring that neither food concentration nor encounter rate was limiting for grazers. Diet treatments were carried out in triplicate, for a total of 9 bottles per experiment. For the 2 ciliates, all treatment replicates were sampled immediately after prey addition (time point 0), and again at 15, 30, and 45 min, whereas *O. marina* was sampled at 0, 30, 60, and 90 min. At each time point, 20 ml were withdrawn from each well-mixed treatment replicate and dispensed into an amber glass bottle containing 1 ml of 10% glutaraldehyde fixative (0.5% final concentration) and 0.2 ml of 10 µg ml^{-1} 4,6-diamidino-2-phenylindole (DAPI) nucleic acid stain. Samples were stored at 6°C for 24 h to allow sufficient penetration of DAPI into grazer and *E. huxleyi* nuclei. After 24 h, samples were filtered onto 25 mm Nucleopore polycarbonate filters, mounted on slides with immersion oil, and frozen at −20°C until analysis. For analysis, the first 100 grazers randomly encountered from each treatment replicate were assessed for presence and number of ingested *E. huxleyi* cells using a Leitz epifluorescent microscope under blue-light excitation at 100×. When no *E. huxleyi* cells were present in a grazer, it was recorded as a 0 and used to calculate the percent of the MZP population that was observed feeding. MZP cells ingested $grazer^{-1}$ at each sampling time point were calculated and are expressed here in 2 ways. First, assessments were calculated using all grazers (both those with and without ingested *E. huxleyi* cells) and are denoted as community ingestion (I_c). Second, ingestion rates were calculated based on only the actively feeding MZP, i.e. only those MZP that were observed with ingested cells. Ingestion rates calculated this way are denoted I_a. See Table 1 for experimental overviews.

Data analyses

All data presented here are mean values from triplicate treatment measurements with error estimates of ±1 SD. Differences in treatment response for each *E. huxleyi* metric were assessed using ANOVA and Tukey HSD tests on the last day of the acclimation period. All data displayed normal distributions (Shapiro-Wilk test) and variances were homogenous (Levene's test). A significance threshold of $p < 0.05$ was used for all tests. Normality and homoscedasticity were further verified by inspection of Q-Q plots and residual plots. Time and pCO_2 treatment were modeled as predictors of MZP ingestion rates and percent population feeding using a series of linear mixed effects models and a maximum likelihood method (SPSS 24). Factors in each model included time (main effect, 3 levels), pCO_2 (main effect, 3 levels), the interaction of time and pCO_2, and bottle as a random factor. These factors were used to predict I_c and I_a, and the percent population feeding for each of the 3 MZP species. The correlation structure of each model was varied, and the model that produced the best fit was selected using Akaike's information criterion (AIC), log likelihood comparisons. Models with unstructured variance and heterogeneous compound symmetry failed to converge, and a scaled identity structure was preferred over a diagonal or autoregressive structure (AR1). In all cases, variance assigned to bottle was non-significant and small relative to the residuals. Least Significant Difference (LSD) post hoc tests were used to compare pCO_2 treatments.

RESULTS

Microzooplankton ingestion experiments

The pCO_2 treatment concentrations during *Emiliania huxleyi* acclimation periods varied across experiments. In particular, Expt 1 had lower values across all treatments in comparison to Expts 2 and 3, which were maintained near target concentrations. Despite across-experiment variability, pCO_2 concentrations were significantly different across treatments in all experiments. The full suites of carbonate chemistry values for experimental water are presented in Table 2.

Each MZP species investigated here ingested significantly more *E. huxleyi* cells cultured under elevated pCO_2 when all grazers (I_c) were considered in the analyses (Fig. 1, Table 3). Two MZP (*Eutintinnus* sp. and *Oxyrrhis marina*) exhibited stepwise increases in I_c with pCO_2, while for *Schmidingerella* sp. I_c, only the moderate and high pCO_2 treatment were significantly different from each other. In the 2 ciliates, the interaction of time and pCO_2 was a significant predictor of I_c, indicating that the effect of pCO_2 varied over time. This interaction is evident, particularly in *Eutintinnus* sp., where increasing separation of the treatments over time can be observed,

Table 2. Three treatments (Treat.) were generated: low (L), moderate (M), and high (H) partial pressure of CO_2 (pCO_2) relative to current atmospheric conditions. Measured carbonate chemistry parameters include pH on the total scale and total alkalinity (A_T). Parameters calculated via CO2sys include total carbon (TC), pCO_2, and calcite saturation state (Ω Calcite) and were calculated at *in situ* temperature (15°C) and at measured salinity (mean: 31.2 PSU, range 30–33); n: number of samples analyzed over the course of the experiment. Means ± SD are shown

Exp.	Treat.	pH (total)	A_T (µeq kg^{-1})	TC (µmol kg^{-1})	pCO_2 (µatm)	Ω Calcite	n
1	L	8.21 ± 0.10	2166 ± 28	1898 ± 49	260 ± 78	4.6 ± 0.8	18
	M	7.98 ± 0.09	2172 ± 59	2009 ± 62	479 ± 122	3.0 ± 0.5	18
	H	7.85 ± 0.07	2145 ± 24	2028 ± 22	644 ± 125	2.3 ± 0.3	18
2	L	8.05 ± 0.01	2095 ± 22	1922 ± 19	385 ± 10	3.3 ± 0.1	17
	M	7.84 ± 0.02	2128 ± 31	2018 ± 23	676 ± 31	2.2 ± 0.1	17
	H	7.71 ± 0.01	2143 ± 25	2066 ± 21	926 ± 21	1.7 ± 0.0	17
3	L	8.05 ± 0.05	2088 ± 28	1901 ± 29	379 ± 43	3.3 ± 0.4	18
	M	7.83 ± 0.02	2107 ± 31	2004 ± 32	683 ± 36	2.1 ± 0.1	18
	H	7.73 ± 0.04	2108 ± 32	2033 ± 36	864 ± 86	1.7 ± 0.2	18

and in *Schmidingerella* sp., where I_c for the low pCO_2 treatment varies greatly relative to the other 2 treatments over time.

When quantifying ingestion using only actively feeding grazers (I_a), the dinoflagellate *O. marina* maintained a significant stepwise trend of ingesting more *E. huxleyi* cells cultured with increasing pCO_2 (Fig. 2, Table 3). Greater I_a was observed for the ciliate *Eutintinnus* sp. in the high *E. huxleyi* pCO_2 treatment compared to the low treatment (Fig. 2, Table 3). The pCO_2 factor did not significantly predict I_a for *Schmidingerella* sp.

As is evident by the differences between the active and community metrics, there were differences in the percentage of the MZP populations that were observed feeding (PPF) (Fig. 3, Table 3). A significant effect of prey pCO_2 condition on PPF was found for all grazer species. That is, when offered *E. huxleyi* cells cultured under high pCO_2, significantly more grazers were observed with ingested cells compared to low pCO_2 treatments (Fig. 3, Table 3). These differences were stepwise with pCO_2 for *Eutintinnus* sp. For *O. marina*, the moderate and high pCO_2 treatments grouped together, while low and moderate treatments grouped together in *Schmidingerella* sp. The ciliate *Eutintinnus* sp. showed the greatest deviation in the PPF between treatments, with 16, 25, and 24% more of the population observed feeding on high-pCO_2 cultured *E. huxleyi* compared to low-pCO_2 cultured cells at 15, 30, and 45 min, respectively. The increase in PPF in high pCO_2 diet treatments compared to low pCO_2 diet treatments diminished with time, going from 17 to 8 to 0% over 45 min for the ciliate *Schmidingerella* sp. and from 13 to 4 to 3% for the dinoflagellate *O. marina* over 90 min. This waning of the effect of pCO_2 is reflected in the significant interaction effect between time and pCO_2 factors in the models for PPF in these MZP.

E. huxleyi characterization

E. huxleyi coccosphere volume significantly increased with elevated pCO_2 in all 3 experiments (Fig. 4, Table 4). The coccosphere volumes from the low to moderate treatments increased by 22, 7, and 9% for Expts 1, 2, and 3, respectively. In Expt 1, the increase in *E. huxleyi* coccosphere volume from the low to moderate treatment was higher than in the other 2 experiments, but Expt 1 also had particularly

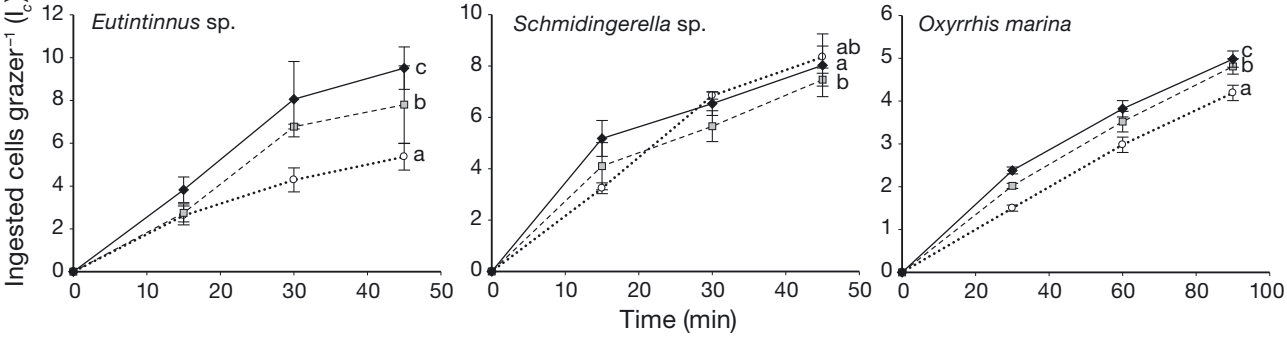

Fig. 1. Mean ± SD number of ingested *Emiliania huxleyi* cells per grazer in community ingestion experiments (I_c) over time (min). *E. huxleyi* prey cells were cultured under either low (○), moderate (▣) or high (◆) pCO_2. Different letters indicate significant LSD post hoc differences between pCO_2 treatments. See Table 3 for significance

Table 3. Summary of fixed effects from linear mixed effects models with scaled identity variance structure. Each model consisted of bottle as a random factor (9 levels) and main effects of time (3 levels), pCO_2 (3 levels), and the interaction of time and pCO_2. Estimates that are significant at $p < 0.05$ are shown in **bold**. Pairwise similarity of pCO_2 treatments (low – moderate – high) by LSD post hoc comparisons are indicated in the LSD column. Additional details in Table A1 in the Appendix

Factor	*Eutintinnus* sp.			*Schmidingerella* sp.			*Oxyrrhis marina*		
	F	p	LSD	*F*	p	LSD	*F*	p	LSD
Cells ingested (whole community; I_c)									
Intercept	$F(1,27) = 1329$	**<0.001**		$F(1,27) = 3742$	**<0.001**		$F(1,27) = 18266$	**<0.001**	
Time	$F(2,27) = 74$	**<0.001**		$F(2,27) = 118$	**<0.001**		$F(2,27) = 980$	**<0.001**	
pCO_2	$F(2,27) = 31$	**<0.001**	a-b-c	$F(2,27) = 5$	**0.008**	ab-a-b	$F(2,27) = 98$	**<0.001**	a-b-c
Time × pCO_2	$F(4,27) = 3$	**0.022**		$F(4,27) = 5$	**0.002**		$F(4,27) = 0.4$	0.788	
Cells ingested (only active feeders; I_a)									
Intercept	$F(1,27) = 1505$	**<0.001**		$F(1,9) = 2845$	**<0.001**		$F(1,27) = 19469$	**<0.001**	
Time	$F(2,27) = 51$	**<0.001**		$F(2,18) = 69$	**<0.001**		$F(2,27) = 627$	**<0.001**	
pCO_2	$F(2,27) = 6$	**0.007**	a-ab-b	$F(2,9) = 0.5$	0.601	–	$F(2,27) = 58$	**<0.001**	a-b-c
Time × pCO_2	$F(4,27) = 1$	0.368		$F(4,18) = 2.4$	0.086		$F(4,27) = 0.2$	0.902	
Percent population feeding (PPF)									
Intercept	$F(1,27) = 3288$	**<0.001**		$F(1,9) = 12345$	**<0.001**		$F(1,9) = 156364$	**<0.001**	
Time	$F(2,27) = 14$	**<0.001**		$F(2,9) = 21$	**<0.001**		$F(1,18) = 1108$	**<0.001**	
pCO_2	$F(2,27) = 26$	**<0.001**	a-b-c	$F(2,18) = 18$	**0.001**	a-a-b	$F(1,9) = 105$	**<0.001**	a-b-b
Time × pCO_2	$F(4,27) = 0.8$	0.511		$F(4,18) = 7$	**0.001**		$F(1,18) = 27$	**<0.001**	

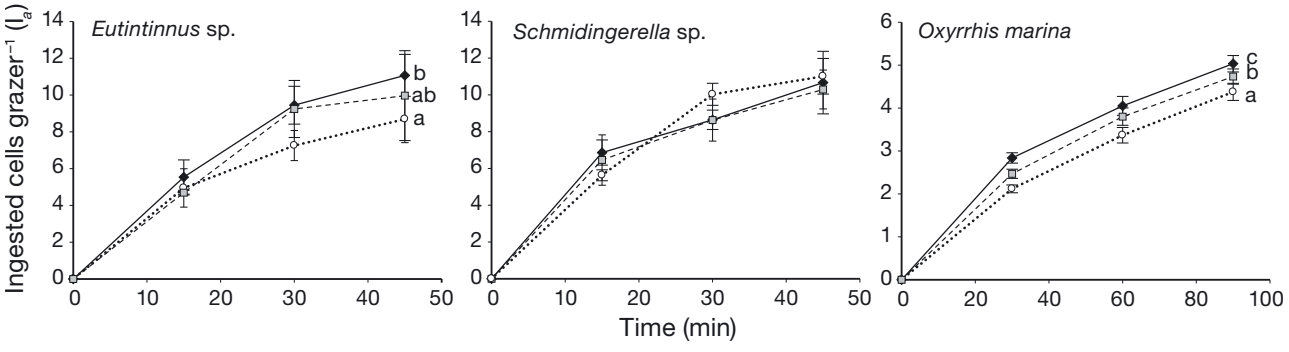

Fig. 2. Mean ± SD number of ingested *Emiliania huxleyi* cells per grazer from actively feeding grazer populations (I_a) over time (min). Other details as in Fig. 1

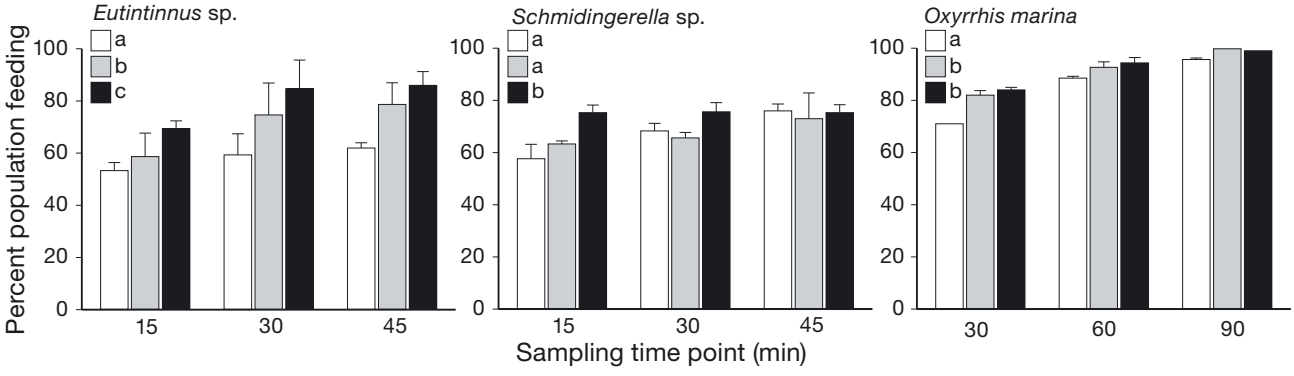

Fig. 3. Percentages of the respective microzooplankton populations that were observed with ingested *Emiliania huxleyi* prey (actively feeding populations) at each sampling interval. *E. huxleyi* prey cells were cultured under either low (open bars), moderate (grey bars), or high (black bars) pCO_2. Boxes and letters represent results from LSD post hoc tests showing significant treatment differences. See Table 3 for significance

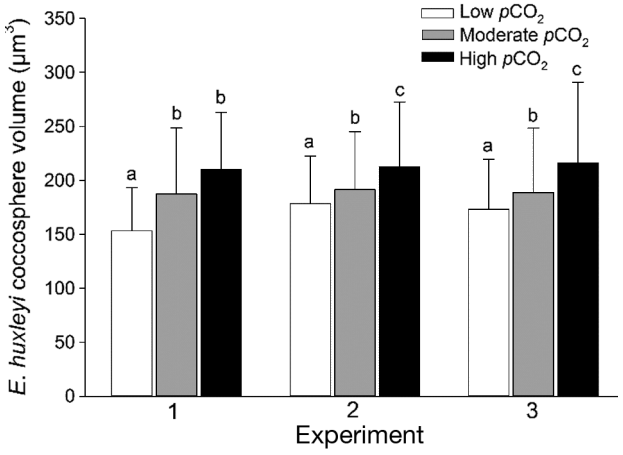

Fig. 4. *Emiliania huxleyi* coccosphere volumes across pCO_2 treatments during experiments. Letters above bars are results from Tukey's HSD post hoc tests with different letters showing significant treatment differences at $p < 0.05$

low pCO_2 in the lowest treatment. The volume increase from moderate to high treatments was similar across experiments, increasing by 12, 11, and 15%, in Expts 1, 2, and 3, respectively, despite the differences in treatment pCO_2 achieved in each experiment.

Per cell POC significantly increased from the low to high pCO_2 in 2 of the 3 experiments, and trended in the same direction in all experiments (Table 4). Differences in POC were 45, 20, and 9% from the low to high treatments in Expts 1, 2, and 3, respectively. In Expt 1, the difference between POC was 27% between the moderate and high treatment, whereas for Expts 2 and 3, differences between these treatments were negligible. However, when normalized to coccosphere volume, differences in POC across low to high pCO_2 treatments increased by only 9 and 2% in Expts 1 and 2, and decreased by 10% in Expt 3. All other *E. huxleyi* metrics characterized here did not show significant differences across pCO_2 treatments (Table 4).

Table 4. Mean ± SD growth rates (GR: µ, d^{-1}), coccosphere volumes (CV, $µm^3$), and cellular mass (pg $cell^{-1}$) of particulate inorganic carbon (PIC), particulate organic carbon (POC), and particulate organic nitrogen (PON) of *Emiliania huxleyi* prey cells determined on Day 10 of respective pCO_2 concentration acclimation. Treatment differences were calculated using 1-way ANOVA (F: $^{**}p < 0.01$; $^{***}p < 0.001$; ns: no significant difference). Paired superscripts represent statistically similar values according to Tukey post hoc tests

Expt	Metric	pCO_2 treatment			
		Low	Moderate	High	F
1	GR	1.58 ± 0.26	1.35 ± 0.25	1.26 ± 0.34	2.4^{ns}
	CV	153.24 ± 39.87^{a}	187.27 ± 61.30^{b}	210.10 ± 52.84^{b}	16.9^{***}
	PIC	9.21 ± 1.95	8.93 ± 0.92	11.04 ± 3.15	0.8^{ns}
	POC	14.04 ± 0.38^{a}	16.91 ± 0.16^{a}	22.22 ± 2.81^{b}	19.2^{**}
	PON	1.38 ± 0.34	1.21 ± 0.49	1.85 ± 0.70	1.2^{ns}
2	GR	1.11 ± 0.05	1.12 ± 0.02	1.13 ± 0.06	0.1^{ns}
	CV	178.37 ± 44.00^{a}	191.41 ± 53.67^{b}	212.54 ± 59.87^{c}	21.3^{***}
	PIC	10.36 ± 0.85	9.38 ± 0.86	10.24 ± 0.20	1.7^{ns}
	POC	15.55 ± 0.29^{a}	19.66 ± 0.43^{b}	19.04 ± 0.61^{b}	68.6^{***}
	PON	1.48 ± 0.16	1.74 ± 0.54	1.65 ± 0.13	0.5^{ns}
3	GR	1.11 ± 0.04	1.12 ± 0.03	1.13 ± 0.05	0.1^{ns}
	CV	173.36 ± 46.22^{a}	188.63 ± 59.68^{b}	216.13 ± 74.70^{c}	24.7^{***}
	PIC	6.57 ± 4.33	8.87 ± 2.38	9.54 ± 1.36	0.8^{ns}
	POC	17.96 ± 2.74	19.28 ± 2.25	19.57 ± 1.53	0.4^{ns}
	PON	1.46 ± 0.34	1.70 ± 0.16	1.77 ± 0.19	1.3^{ns}

DISCUSSION

We used a microcosm design to test whether MZP feeding behavior changes in response to effects of simulated OA on the cellular characteristics of a single species of phytoplankton. This approach allowed the investigation of species-specific interactions resulting from elevated pCO_2 that would likely be obscured in community-based experimental designs in productive environments, yet could be an agent for change in complex systems over time. These interactions could also be important structuring mechanisms in more static environments where predator–prey dynamics are more tightly coupled, i.e. in oligotrophic oceanic environments. We found that ingestion rates of all 3 MZP were affected by the pCO_2 treatment environment of their prey cells, indicating an indirect effect of OA on MZP grazing caused by direct cellular effects in prey species.

Mechanisms for indirect OA effects on MZP grazing

Despite contrasting feeding strategies that constrain prey specificity, all MZP tested here were indirectly affected by elevated pCO_2 through changes in prey state. These effects were particularly evident for *Eutintinnus* sp. and *Oxyrrhis marina*, which showed step-wise increases in I_c on *Emiliania huxleyi* cells grown under increasing pCO_2. For the ciliate *Schmidingerella* sp., this same trend was seen at the first sampling time point, but by the end of the

experiment, ingestion rates between treatments were similar. Short-term responses cannot be used to predict longer-term responses, but the interaction effects over time in these short-term experiments can offer some clues as to potential patterns which could emerge. For the 2 ciliates, there was an interaction between prey pCO_2 treatment and time, but in opposite directions. With time, the disparity in *Eutintinnus* sp. I_c diverged, with ingestion rates becoming increasingly higher on high-pCO_2 cultured *E. huxleyi*, while the low treatment did not increase ingestion of cells at proportional rates (positive interaction effect between low pCO_2 and Time 1, see Table A1 in the Appendix). *Schmidingerella* sp. exhibited the opposite trend, where ingestion rates slowly converged after 15 min of feeding (seen in the negative interaction effect between low pCO_2 and Time 1, Table A1). The effect of pCO_2 on I_c of *O. marina* did not change over time, indicating that the differences between treatments that became apparent in the first time step were maintained. These 3 MZP demonstrate the 3 potential patterns of short-term response: amplification over time as rate differences are maintained, convergence over time as rate differences diminish, and differences which manifest quickly and then are preserved although rates do not differ between treatments over later time intervals. No general patterns relating to MZP characteristics can be made here, but which of these patterns is most common among the diverse assemblage of MZP could determine the scope of indirect effects of OA-induced prey quality on a larger scale.

One mechanistic difference that can be resolved with these data is whether differences in I_c result from a greater proportion of MZP grazing under elevated pCO_2 conditions, or if the actively feeding MZP are grazing at increased rates. All 3 MZP showed higher percentages of the populations feeding on OA-acclimated *E. huxleyi.* This pattern suggests that a common OA-induced trigger exists in *E. huxleyi* that elicits equivalent responses in MZP that have contrasting functional characteristics controlling feeding behavior. In addition, the data indicate that some changes in community grazing rates persisted when only the actively feeding MZP were considered. Both *Eutintinnus* sp. and *O. marina* exhibited increased ingestion on high pCO_2 prey in both the I_c and I_a metrics as well as increased PPF. These 2 MZP had the strongest and most consistent OA response to elevated pCO_2, and the I_c response was likely due to increases in MZP PPF, and increased ingestion rates among those actively feeding MZP. In the case of *Schmidingerella* sp., the treatment-induced effects at the community level are more modest, and appear to be largely due to differences in whether the MZP are feeding or not (PPF) because pCO_2 does not predict rates among the actively feeding population. Therefore, species-specific MZP grazing responses to OA-induced changes in their prey may manifest in both the proportion of the population feeding and potentially also in their ingestion rates.

There are several candidate mechanisms for the cues exhibited by prey cells that could deter and retard grazing. Microzooplankton grazers are acutely sensitive to chemical (Wolfe 2000, Pohnert et al. 2007, Strom et al. 2007), behavioral (Jakobsen et al. 2006), and morphological characteristics (Goldman & Dennett 1990, Hansen et al. 1994, Wirtz 2012) of their phytoplankton prey. This sensitivity can govern the rate at which MZP ingest prey and serve as a mechanism to engender selective feeding behavior. Chemical characteristics can be on the cell surface (Olson & Strom 2002, Strom et al. 2012, 2017) or dissolved in water surrounding prey cells (Fredrickson & Strom 2009). Further, some phytoplankton, including strains of *E. huxleyi*, can retard grazing through chemical protection (Wolfe 2000, Kolb & Strom 2013). We measured several potential triggers for an MZP grazing response, including intrinsic growth rates, POC cell^{-1}, PON cell^{-1}, and PIC cell^{-1}. The observed increase in *E. huxleyi* coccosphere volume was the only parameter which showed consistent differences under simulated OA conditions in the algal cells, potentially triggering the grazing responses in the MZP populations.

Size is known to influence MZP grazing. Kiørboe (2008) stated that zooplankton prey selection is primarily guided by the size of the prey, and that other selective mechanisms may be operational, but they modify rather than drive selectivity. Alternative explanations to the primacy of the cell volume effect seem unlikely. For the MZP grazing responses observed here to be driven by size-independent characteristics, each grazer would need to possess similar cell-surface receptors idealized for the recognition of chemical and behavioral cues specific to *E. huxleyi*. It would also necessitate the expression of a secondary characteristic by *E. huxleyi* acute enough to over-ride size-based grazing responses. Excluding coccosphere size, POC cell^{-1} is the only *E. huxleyi* cellular characteristic that showed a pCO_2 response. However, this cellular response was observed in just 2 out of the 3 experiments. Furthermore, POC normalized to coccosphere volume had no significant response to pCO_2. That is, increased POC cell^{-1} paralleled increased coccosphere volume.

We cannot omit the possibility that some other non-quantified *E. huxleyi* metric contributed significantly to the observed MZP ingestion responses. Some possibilities include cellular and dissolved DMSP, and transparent exopolymeric substances. One functional role of DMSP is as an MZP grazing deterrent, the efficacy of which appears to be MZP grazer-specific (Strom et al. 2003, Fredrickson & Strom 2009). Although it was not measured here, in a companion study using the same *E. huxleyi* strain, Olson et al. (2017) found that DMSP cell^{-1} increased in response to OA. If that response to OA occurred in these experiments, a reduction in grazing rate under OA due to elevated DMSP may be expected. We found the opposite MZP grazing response, suggesting that contrary to the aforementioned studies, DMSP would have had to act as a grazing attractant rather than a deterrent if it contributed to the observed effects. To our knowledge, the effects of OA on the structure of transparent exopolymeric substances remains unknown. Another prey characteristic that could engender an alteration in MZP feeding response is an OA-induced change in prey cell surface protein shape and function. That is, if pH changes on the magnitude expected by the end of this century are enough to slightly denature prey cell surface proteins, signaling efficiency between MZP grazers and prey may change, resulting in modified grazing dynamics. If operational, this potential mechanism is unlikely to have driven the grazing response observed here. Because the plasma membrane and associated surface proteins of the *E. huxleyi* prey cells were obstructed by inert coccoliths, contact between grazer and prey surface receptors was impeded.

Prey size constraints on MZP grazing

Given that size selectivity is a strong candidate explanatory factor in the observed OA effects, a more detailed look at feeding mechanics in these MZP species is warranted. Ciliates are constrained to a relatively narrow window of prey sizes; for spirotrich ciliates, spacing between adjacent membranelles limits the lower end of the available prey sizes, and fixed cytosome diameters and prey handling constraints limits the upper ranges. Optimal prey size for tintinnid ciliates has been found to be ~20% of the lorica oral diameter (Dolan 2010, Dolan et al. 2013). *Eutintinnus* sp. can ingest cells between 5 and 32 µm and ingests those between 8 and 18 µm equivalent spherical diameter (ESD) most efficiently, while *Schmidingerella* sp. can ingest cells between 4.5 and 49 µm ESD, and ingests cells between 15 and 36 µm most efficiently (Fenchel 1987, Dolan et al. 2013). *E. huxleyi* cells grown under low pCO_2 conditions were at the smaller end of cells available to these ciliate MZP grazers, with an average ESD of 6.9 µm. Therefore, the increased cell volume in the prey cells brought them closer to the center of the range in which these ciliate MZP feed most efficiently. Heterotrophic dinoflagellates that use specialized feeding mechanisms (e.g. peduncles or palliums) can prey on cells much larger than themselves, resulting in predator:prey size ratios as low as 0.15 (Tillmann 2004). However, those dinoflagellates that ingest prey via phagocytosis (e.g. *O. marina*) are generally more limited in their prey size range, with predator:prey size ratios supporting optimal growth ranging from 1:1 to 2.4:1 (Naustvoll 2000a,b). The predator:prey ratio in Expt 3 was 2.25:1 in the low and 2.04:1 in the high treatment for *O. marina,* trending away from the outer limits of optimal conditions as pCO_2 increases. Our conclusion that the elevated feeding response in *O. marina* was driven by OA-induced increases in coccosphere volume is supported by Hansen et al. (1996), who showed consistent selection of larger cells by *O. marina* for a variety of algal species and mixed algal diets, including *E. huxleyi*.

Given that ingestion events for any grazer are constrained to specific sizes and morphologies of prey, a change in prey cell size that trends toward an optimal predator:prey size ratio would, in the absence of any interactive secondary selective mechanisms, result in increased ingestion efficiency, and as a result, higher ingestion rates in the MZP feeding on those larger cells (Hansen et al. 1996). Because prey was saturating in all pCO_2 treatments, encounter rates were neither a limiting factor nor a variable factor across treatments. Our observation that the differences in the PPF across treatments diminished over time for 2 grazers shows that these grazers were physically able to ingest cells from all treatments. For this reason, it is possible that the large differences in the PPF in early sampling times resulted from active decisions by the grazers rather than decreased ingestion efficiency. To illustrate, energy expenditure of an ingestion event is the aggregate energy required to locate, possibly pursue, handle and manipulate prey cells, and finally ingest cells through various mechanisms depending on the MZP species. While the *E. huxleyi* cells used here were immotile, because of their smaller size, cells cultured under low pCO_2 may be on the lower end of acceptable predator:prey size ratios, and would thus be more difficult to handle and manipulate, and energetically costly to

ingest. As such, the MZP offered low pCO_2 diets may be actively rejecting encountered small cells up to a point when rejection of cells is no longer energetically favorable. The exception to this pattern is *Eutintinnus* sp., for which differences in PPF were maintained throughout the experiment. Difficulty handling the smaller low pCO_2-conditioned cells may contribute to the particularly marked effect on *Eutintinnus* sp. PPF, and the increase in the size of this effect over time. The size of prey cells offered in the low pCO_2 treatment was closest to the lower size limit for ingestion for this MZP. This result suggests that OA-driven size changes could sometimes shift which species are suitable prey for a given MZP, potentially introducing changes in selective pressures on both phytoplankton and MZP assemblages.

Ecological implications

These microcosm studies have revealed that MZP grazing rates can be altered by OA-induced changes to their prey. To what extent are these results ecologically applicable? These short-term experimental results cannot necessarily predict longer-term responses, or be applied directly to more complex mixed communities. They can, however, indicate prey selection mechanisms which could be used to help interpret changes observed over longer time spans. Currently, our knowledge of indirect effects to MZP, keystone members of the planktonic community, come from mesocosm experiments conducted in productive coastal ecosystems (Suffrian et al. 2008b, Nielsen et al. 2010, Calbet et al. 2014, Lischka et al. 2017) or from a continuous culture design employed in the North Atlantic during the spring bloom (Rose et al. 2009). In many of these community-based experiments, nutrients were added to simulate bloom conditions (Suffrian et al. 2008a, Nielsen et al. 2010, Aberle et al. 2013, Rossoll et al. 2013, Schulz et al. 2013, Calbet et al. 2014). Results from these studies are conflicting. Many show no effect on MZP (Suffrian et al. 2008a, Nielsen et al. 2010, Aberle et al. 2013, Rossoll et al. 2013, Langer et al. 2017), while others show indirect effects on MZP precipitated through changes in prey species composition and biomass concentration (Rose et al. 2009, Calbet et al. 2014, Horn et al. 2016, Lischka et al. 2017). Perhaps the lack of clear and consistent indirect effects to MZP resulting from OA is due to the inherent ecological richness and chemical and physical stochasticity in natural systems (Rossoll et al. 2013). That is, in a natural system with a wide diversity of prey to choose from, each of which may show a different tolerance to highly ephemeral OA conditions, effects on zooplankton may be dampened or modified in comparison to when diet breadth is minimal and consists largely of OA-sensitive prey. Under these ephemeral conditions, it is difficult to attribute any observed biological response or trophic interaction to a projected increase in baseline pCO_2 or pH given the broad temporal fluctuation in carbonate chemistry. While microcosms do not include the species richness and diversity of a natural community, the mechanisms uncovered here, such as size selectivity as a lever operated upon by OA, can then be applied to interpret results from complex mixed communities. They can also guide future studies, such as increased recognition of the importance of quantifying changes in cell volume in response to OA. Sommer et al. (2015) found that several Baltic Sea phytoplankton species increased cell volume within mesocosms exposed to OA. Unfortunately, MZP dynamics were not included in that research, so it remains unknown if the MZP feeding responses observed here, presumably to changes in prey cell size, operate in natural assemblages.

These results may also be directly applicable in understanding how OA affects MZP feeding ecology in more hydrodynamically stable environments where pCO_2 fluctuations are small, and systems are dominated by very low phytoplankton species and size diversity. If the elevated grazing rates observed here are consistent across MZP functional groups and extend over longer time spans when grazing on OA-exposed phytoplankton, rates of phytoplankton mortality could potentially increase and, depending on assimilation efficiencies under elevated grazing, the cycling and remineralization of nutrients in oligotrophic environments could also increase. In addition, *E. huxleyi* is a consistent bloom former over many of the world's oceans and seas and contributes significantly to biogeochemical cycling, including but not limited to sulfur, calcium carbonate, and organic carbon, nitrogen, and phosphorus. Most studies exploring the grazing of *E. huxleyi* by MZP show that it is grazed at low rates (e.g. Fileman et al. 2002, Olson & Strom 2002), and is an inferior food to support high growth rates (Naustvoll 2000a). Any changes in *in situ* rates of *E. huxleyi* grazing mortality has the potential to realign rates of biogeochemical cycles, and export flux of calcium carbonate.

To what degree increased MZP ingestion rates under OA will influence organic matter cycling in the ocean depends, in part, on the biochemical nature of prey. We found here, and in Olson et al. (2017), a per

cell increase in POC under OA. However, when normalized to concomitant increases in coccosphere volumes, the POC per coccosphere volume in these studies was unchanged, or decreased with increasing pCO_2, resulting in a volume-normalized scaling or dilution of an essential cellular constituent. Volume normalization is not common in studies of this kind (Olson et al. 2017), and to our knowledge changes in the density of nutritional components in prey cells have not been demonstrated in OA studies on other strains or species. We have found consistent increases in coccosphere volume in *E. huxleyi* (Olson et al. 2017, this study), and also in the cell volume of the cryptophyte *Rhodomonas* sp. (Still 2016). However, these changes may not always scale consistently with pCO_2 conditions. For example, high pCO_2 conditions in Expt 1 produced cells of similar volume to the other 2 experiments despite relatively lower pCO_2 conditions across all treatments in that experiment. The extent to which changes in coccosphere and cell size are exhibited across wide species and ecological contexts under OA, and the extent to which changes in cellular constituents are decoupled from size changes remain unknown, but these effects could be an important aspect of the influence of OA on marine food webs. For a grazer with limited vacuole space capacity, a vacuole filled with fewer large cells under OA compared to smaller cells under low pCO_2 conditions, would result in comparatively less vacuole nutrition under OA. To offset any nutritional deficit that may come from consuming larger, nutritionally dilute cells, grazers may alter assimilation efficiencies, which would have implications for the quantity and quality of organic matter transferred to adjacent trophic levels. Alternatively, grazers could increase their ingestion rates, which would be a longer-term parallel to the short-term grazing rate response observed in these experiments, which appears to be driven mostly by prey cell size selection. Future studies exploring longer-term responses and combined direct and indirect effects are warranted. For direct effects, this includes simultaneously culturing grazers under elevated pCO_2.

CONCLUSIONS

This study demonstrated consistent increases in short-term grazing rates of several MZP on *E. huxleyi* cells raised under simulated OA conditions, and showed that the primary mechanism driving these changes was likely increased cell volume in the prey cells cultured under high pCO_2. While direct ecological inferences based on these controlled laboratory studies are not appropriate, these results do suggest that indirect effects, through changes in cellular characteristics of their phytoplankton prey, are a primary mechanism through which OA could influence MZP. Furthermore, the cell volume response of phytoplankton cells to OA has the potential to change the rate at which they are grazed and their nutritional value.

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Appendix. Parameter estimates from linear mixed effects models

Table A1. Summary of parameter estimates from linear mixed effects models with scaled identity variance structure. Each model consisted of bottle as a random factor (9 levels) and main effects of time (3 levels; T1 to T3), pCO_2 (3 levels), and the interaction of time and pCO_2. Parameter estimates for Time 1, Time 3, low pCO_2 (L pCO_2), high pCO_2 (H pCO_2), and the interaction of time with pCO_2 are given relative to time point 2 and moderate pCO_2 which have estimates set at 0. Values in parentheses are standard errors of the estimates. Estimates that are significant at $p < 0.05$ are shown in **bold**. AIC: Akaike's information criterion

Factor	Est(SE) Time 1	Est(SE) Time 3	Est(SE) L pCO_2	Est(SE) H pCO_2
Eutintinnus sp. – Cells ingested (whole community) (–2 log likelihood 65.125, AIC 85.128)				
Intercept	**6.78** (0.47)	–		
Main effect	**–4.02** (0.66)	1.03(0.66)	**–2.49** (0.66)	1.28(0.66)
Interaction	T1×L: **2.36** (0.93)	T3×H: 0.42(0.93)	T3×L:0.53(0.93)	T1×H:0.2(0.93)
Eutintinnus sp. – Cells ingested (only active feeders) (–2 log likelihood 79.509, AIC 99.509)				
Intercept	**9.24** (0.61)	–		
Main effect	**–4.55** (0.86)	0.73(0.86)	**–1.99** (0.86)	0.20(0.86)
Interaction	T1×L: 2.44(1.21)	T3×H: 0.89(1.21)	T3×L: 0.70(1.21)	T1×H: 0.63(1.21)
Eutintinnus sp. – Percent population feeding (PPF) (–2 log likelihood 176.01, AIC 196.01)				
Intercept	**74.67** (3.64)	–		
Main effect	**–16.00** (5.15)	4.00(5.15)	**–15.33** (5.15)	10.00(5.15)
Interaction	T1×L: 10.0(7.28)	T3×H: ,–2.66(7.28	T3×L: –1.33 (7.28)	T1×H: 0.67(7.28)
Oxyrrhis marina – Cells ingested (whole community) (–2 log likelihood –33.93, AIC –11.93)				
Intercept	**3.52** (0.07)	**–**		
Main effect	**–1.5** (0.08)	**1.29**(0.09)	**–0.54** (0.01)	**0.30**(0.01)
Interaction	T1×L: 0.02(0.15)	T3×H: –0.13(0.15)	T3×L: –0.08(0.15)	T1×H: 0.06(0.15)
O. marina – Cells ingested (only active feeders) (–2 log likelihood –31.11, AIC –9.11)				
Intercept	**3.80** (0.08)	**–**		
Main effect	**–1.33** (0.11)	**1.01**(0.11)	**–4.35** (0.11)	**0.25**(0.11)
Interaction	T1×L: 0.08(0.16)	T3×H: ,–0.03(0.16	T3×L: –0.001(0.16)	T1×H: 0.12(0.16)
O. marina – PPF (–2 log likelihood 75.05, AIC 97.06)				
Intercept	**92.66** (0.57)	**–**		
Main effect	**–10.67** (0.72)	**7.33**(0.72)	**–4.17** (0.81)	**1.67**(0.81)
Interaction	T1×L: **–6.83**(1.01)	T3×H: **–2.67**(1.01)	T3×L: –0.17(1.01)	T1×H: 0.33(1.01)
Schmidingerella sp.– Cells ingested (whole community) (–2 log likelihood 41.6, AIC 63.61)				
Intercept	**5.66**(0.30)	**–**		
Main effect	**–1.56**(0.43)	**1.80**(0.43)	**1.19**(0.42)	**0.88(0.43)**
Interaction	T1×L: **–2.05**(0.60)	T3×H: –0.32(0.60)	T3×L: –0.30(0.60)	T1×H: 0.203(0.60)
Schmidingerella sp.– Cells ingested (only active feeders) (–2 log likelihood 65.4, AIC 87.4)				
Intercept	**8.69**(0.47)	**–**		
Main effect	**–2.23**(0.65)	**1.61**(0.65)	1.35(0.66)	–0.04(0.65)
Interaction	T1×L: **–2.17**(0.92)	T3×H: 0.41(0.92)	T3×L: –0.64(0.92)	T1×H: 0.45(0.92)
Schmidingerella sp.– PPF (–2 log likelihood 138.0, AIC 160.0)				
Intercept	**65.33**(1.80)	–		
Main effect	–2.00(2.48)	**7.66**(2.48)	3.00(2.54)	**10.33**(2.54)
Interaction	T1×L: **–8.66**(3.05)	T3×H: **–8.00**(3.51)	T3×L: 0.00(3.51)	T1×H: 1.66(3.05)

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