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Zooplankton grazing and egestion shifts particle size distribution in natural communities

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ABSTRACT: Marine plankton communities can be viewed in terms of their size structure rather than their taxonomic composition, revealing how allometric relationships affect the functioning of the community. Oceanic particle size spectra can be used to explain and predict variability in carbon export efficiency, because large particles generally sink faster than small particles. Since plankton trophic interactions impact particle size in the surface ocean, this size-structured view is a useful simplification for connecting plankton ecology with biogeochemistry. We conducted a series of grazing experiments to test the hypothesis that mesozooplankton shift particle size spectra toward larger particles, in a predictable manner that reflects their community size structure, through grazing and egestion of fecal pellets. These experiments were carried out over several months, and used natural communities of mesozooplankton and their microplankton prey collected in the coastal Gulf of Maine. After incubation, we analyzed the samples to determine size distribution and taxonomic information. Our results show that mesozooplankton grazing impacts microplankton in proportion to their abundance. Size relationships between plankton predators and prey that have been established at the individual level do not linearly translate to the community level. Further, while grazing itself does not significantly alter the particle size spectrum, repackaging of prey into mesozooplankton fecal pellets shifts particle size spectra toward larger particles.

KEY WORDS: Plankton grazing · Egestion · Particle size distribution · Imaging cytometry · Carbon flux

INTRODUCTION

Plankton communities in the ocean can be viewed in terms of their size structure as well as their species structure. The interchangeability of different species of similar size is central to a size-based view. The finding that zooplankton grazers have an optimal size range in which they feed regardless of the prey type supports this concept (Kiørboe 2008, Wirtz 2012, Boyce et al. 2015). Plankton community size structure is usually characterized by a power-law relationship between body size and biomass within a size class (Sheldon et al. 1972), with exponentially more biomass in the smaller size classes than in the larger size classes. Differences in this relationship among ecosystems can be attributed to bottom-up controls, such as nutrient availability (Sprules & Munawar 1986, Taniguchi et al. 2014), and top-down controls, such as predation (Zhou 2006, Taniguchi et al. 2014). Predation itself is constrained by size, with individual predators or grazers selecting prey within a narrow size range of approximately 10% of their own body size (Kiørboe 2008). Other researchers have found a log-linear relationship between predator and prey size, corroborating narrow prey selection (Wirtz 2012, Boyce et al. 2015).

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Early grazing experiments that used natural assemblages of microplankton prey showed that mesozooplankton select prey based on a variety of factors, including size, abundance and morphology. Hargrave & Geen (1970) found that prey selectivity by some copepods was primarily based on prey size. Other studies found that copepods consume particles within a particular size range first, followed by the most abundant particles (Poulet 1973, Richman et al. 1977). Parsons et al. (1967) found that morphology and size were both important factors. In his seminal study on the feeding behavior of copepod *Calanus pacificus*, Frost (1972) determined that copepods adjust their ingestion rates to achieve the same carbon uptake by feeding on higher concentrations of small cells or lower concentrations of large cells. Due to strong size structuring in the ocean, size is considered a 'master trait' in marine ecology (Litchman & Klausmeier 2008, Barton et al. 2013, Litchman et al. 2013) and food web models based on size spectra (abundance as a function of individual length or mass) are well established in the field of oceanography (Zhou et al. 2010, Banas 2011, Taniguchi et al. 2014).

Size is also an important variable in understanding the efficiency of the biological carbon pump. The biological carbon pump is a set of processes that moves biogenic carbon from the surface to the deep ocean (Ducklow et al. 2001). When biogenic carbon sinks deep enough, often out of the surface mixed layer, it is decoupled from the atmosphere–ocean interface (de la Rocha & Passow 2007). When a higher proportion of particles produced at the ocean's surface sink below this depth, the biological carbon pump is sequestering carbon more efficiently. In the field, the production of larger particles can explain increases in carbon export efficiency (Boyd & Newton 1999), because particle size is directly related to sinking rate (Guidi et al. 2008). Therefore, understanding the processes that give rise to particle size structure is critical to understanding variability in the ocean carbon cycle (de la Rocha & Passow 2007).

Interactions among plankton trophic levels play a central role in shaping particle size structure through grazing, respiration, excretion and egestion (Legendre & Michaud 1998, Ward et al. 2014). These metabolic rates and processes often scale allometrically with body size (Brown et al. 2004). Particles that are not consumed by grazers sink if they are large and dense enough; for example, large, ballasted diatoms often contribute disproportionately to the particle flux measured at depth (Buesseler 1998). Small particles that would otherwise not sink can be aggregated by grazers and egested as larger fecal pellets, which also contribute to the measured particle flux (Wiedmann et al. 2014, Laurenceau-Cornec et al. 2015, Turner 2015). Larger mesozooplankton produce larger fecal pellets, which are more likely to sink below the surface mixed layer (Uye & Kaname 1994, Mauchline 1998, Stamieszkin et al. 2015). Mesozooplankton size structure therefore impacts particle size and flux potential in 2 ways: (1) by consuming particles within a particular size range but leaving those that are too small or too large; and (2) by creating larger particles from those consumed.

In this study, we present results from a series of experiments designed to examine the net effect of mesozooplankton grazing and egestion on particle size structure. The experiments used natural assemblages of mesozooplankton grazers, as well as phytoplankton and microzooplankton prey, collectively termed microplankton. We analyzed the experiments using imaging cytometry, which allowed us to consider both size and species simultaneously. We were interested in how the plankton community size structure changes with and without mesozooplankton grazing. We hypothesized that the impact of mesozooplankton grazing on the microplankton community will reflect the size preferences of the grazers, and that when we increase the mesozooplankton grazing pressure, gaps in the microplankton size structure reflecting the narrow size-selection window will appear. We also hypothesized that as microplankton are consumed and fecal pellets are produced, these larger fecal particles will reflect the size structure of the mesozooplankton community, shifting the particle size spectrum toward larger particles in a predictable manner.

MATERIALS AND METHODS

We conducted 5 experiments in 2014 at different times of year to capture the seasonal variability in plankton community structure and physical conditions. We used natural assemblages of both mesozooplankton grazers and microplankton prey.

Field collections

Plankton were collected at one station (DMC2) approximately 9 km from the mouth of the Damariscotta River in the Gulf of Maine (43° 45.0' N, 069° 20.2' W) (Fig. 1). Sampling and experiments were conducted over 7 mo, from the beginning of April to the end of October 2014 (Table 1). We col-

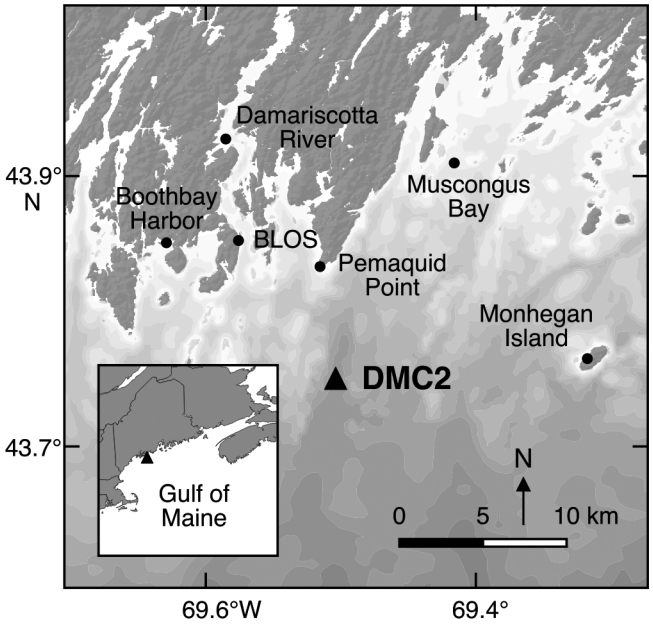

Fig. 1. Map of the study region. Station DMC2 is marked by a triangle; Bigelow Laboratory for Ocean Sciences (BLOS) and other landmarks are indicated by dots

lected the microplankton community (<200 µm) by sampling 24 l of whole seawater from the chlorophyll maximum or near-surface, using 3 l Niskin bottles. We targeted the chlorophyll maximum when a functioning CTD was available. Otherwise, samples from the top 1 m were collected. All samples came from within the top 2 m. We gently passed the water through tubes fitted with 210 µm mesh directly from the Niskin bottles into a large cooler to remove mesozooplankton. The mesozooplankton (≥200 µm) community was sampled with a 200 µm mesh, 0.5 m ring net and a non-filtering cod end. We conducted one vertical tow to approximately 100 m depth, which was 5 to 10 m above the seafloor, thereby sampling the entire mesozooplankton community. Once onboard, the live mesozooplankton were immediately diluted and transferred into containers of whole seawater, and placed into a cooler with ice.

Laboratory incubations

Laboratory work was conducted at Bigelow Laboratory for Ocean Sciences (BLOS) in East Boothbay, Maine, USA (Fig. 1). We started the laboratory experiments within 2 to 3 h of the field collections, ensuring that the plankton were alive and active. Each experiment included treatment and control containers, in triplicate. Each of the 6 incubation containers consisted of 2 nested 1 l tripour beakers. The inner beaker had a 210 µm mesh bottom, so that fecal pellets produced by mesozooplankton would fall through the mesh and be protected from coprophagy (Urban-Rich 2001). The incubation containers were filled with 600 ml of 210-µm-filtered seawater containing the microplankton community. We then added 100 ml live mesozooplankton suspension to the treatment containers and 100 ml of 210-µm-filtered mesozooplankton suspension water (without grazers) to the control containers, so that there was 700 ml of water with natural prey concentrations in all containers. The containers were incubated at or slightly below ambient near-surface temperature for 4 h. A preliminary experiment showed that there was no significant difference when the containers were incubated in the dark versus the light, likely due to

Table 1. Summary of experimental conditions in 2014. Incubation temperature is as close to ambient temperature as possible. Microplankton area-based diameters (ABDs) are from FlowCam analysis. Mesozooplankton prosome lengths (PLs) are measurements made after the experiments were conducted, using ImageJ software and preserved samples. Numbers reported are the mean and median ± 1 SD

Date of experiment	Incubation temperature (°C)	Mean microplankton ABD (µm)	Median microplankton ABD (µm)	Mean mesozooplankton PL (µm)	Median mesozooplankton PL (µm)
03 April	2	26.5 ± 15.0 n = 759	23.7 ± 3.1 n = 759	230 ± 156 n = 557	182 ± 16 n = 557
06 May	5.5	48.2 ± 28.4 n = 1114	39.0 ± 6.3 n = 1114	653 ± 748 n = 93	596 ± 370 n = 93
07 July	14	23.8 ± 14.7 n = 5967	19.9 ± 2.1 n = 5967	1320 ± 962 n = 142	1280 ± 542 n = 142
25 August	15	32.5 ± 17.3 n = 4203	28.9 ± 0.4 n = 4203	874 ± 371 n = 94	807 ± 32 n = 94
31 October	12	32.0 ± 18.6 n = 2516	26.3 ± 1.3 n = 2516	691 ± 224 n = 124	691 ± 52 n = 124

the short incubation time (paired $t(2) = -1.3$, $p = 0.32$); we therefore incubated all containers in the dark. The incubation temperature was determined based on readings from an oceanographic buoy (Buoy E, http://neracoos.org) approximately 5 km from the sampling station (43° 42.6′ N, 069° 21.0′ W) at 1 m depth.

After the 4 h incubation, the inner mesh-bottom beaker was removed and the strained contents (zooplankton ≥210 µm) were preserved in 5–6% buffered formalin for analysis. The beakers containing the remaining 700 ml seawater, microplankton that had not been grazed and fecal pellets were placed into an ice bath to halt grazing and degradation, until they were subsampled (150 ml) for FlowCam® analysis (see 'Laboratory analysis') usually 0 to 1.5 h after incubation. On one occasion, a sample sat on ice for 3.5 h before processing. The rest of the sample (550 ml) was refrigerated at 3–5°C to minimize degradation processes, until it was processed via microscopy for fecal pellet number and size. The fecal pellet analysis was performed no more than 8 h after the incubations took place.

Laboratory analysis

We analyzed 150 ml of the incubated seawater with a Fluid Imaging Technologies FlowCam® (VS IV) in 'trigger mode', which excites particles using a green laser (532 nm) and captures images of the naturally fluorescent particles within the sample as they travel through a flow cell, due to chlorophyll autofluorescence (Fig. 2A). While this method of data collection

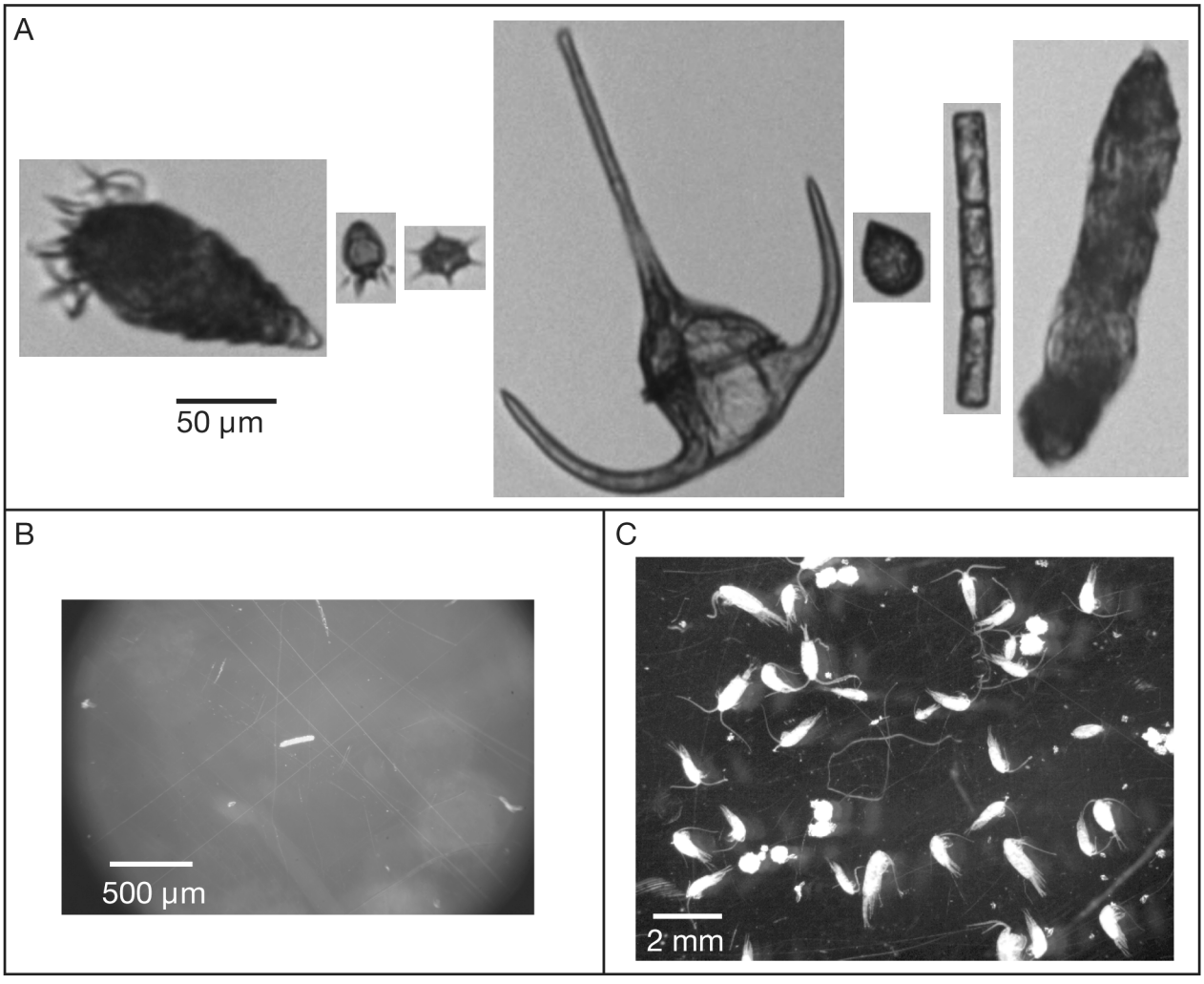

Fig. 2. Example images from the analysis. (A) FlowCam images. From left to right: *Laboea* sp., small ciliate, silicoflagellate, large *Ceratium* sp., small dinoflagellate, small chain diatom, mesozooplankton fecal pellet. (B) Fecal pellets analyzed with ImageJ software. (C) Copepod grazers analyzed with ImageJ software

does capture heterotrophic organisms that have chlorophyll in their guts, it should be noted that this method excludes heterotrophic organisms feeding on non-fluorescent material from the results. This mode of data acquisition has been used previously in grazing experiments (Ide et al. 2008). The FlowCam software, VisualSpreadsheet®, also collects a suite of measurements for each particle image, including area-based diameter (ABD). We used a lower limit of 10 µm ABD because smaller particles were unidentifiable and close to the instrument's lower range of particle detection using a 4× objective (resolution = 1 µm per pixel). The upper limit of microplankton size was 200 µm due to how we defined and separated meso- and microplankton. Fecal pellets collected in the incubation containers from the remaining 550 ml were processed within 8–16 h of the incubation after being stored at 3–5°C. We subsampled using a 5 ml Stempel pipette, and photographed a minimum of 50 representative fecal pellets from a known fraction of the sample collected using a Canon Rebel T3i digital camera attached to an Olympus SZ60 microscope (Fig. 2B). Similar to the fecal pellets, the preserved mesozooplankton from all experiments were subsampled and photographed using a Leica DFC290 with FireCam® software (Fig. 2C). We used open-source ImageJ software to estimate ABD for fecal pellets and prosome length (PL) for mesozooplankton (Abràmoff et al. 2004).

Particle classification and data analysis

We estimated the size and concentration of particles before (initial samples) and after (control and treatment samples) the incubations using the FlowCam. The FlowCam used for this analysis captures images of approximately one-third of the particles that pass through it; we therefore applied a correction factor to estimate particle concentration (no. ml^{-1}). The correction factor was calculated as the width of the FlowCam camera field of view (1.02 mm) divided by the flow cell width (3 mm). When calculating the effect of mesozooplankton grazing on the microplankton community, we also had to account for microzooplankton grazing (Nejstgaard et al. 1997). The difference in microplankton community concentration and size structure between the initial samples (pre-incubation) and the control samples (post-incubation, no mesozooplankton) was due to microzooplankton grazing (hetero- or mixotrophs <200 µm). Therefore, when calculating the impacts of mesozooplankton grazing (>200 µm), treatment samples (post-incubation, mesozooplankton present) were compared with control samples, rather than with initial samples. Incubations times were short (4 h) to limit possible trophic cascade effects inside the incubation containers (Klaas et al. 2008).

We binned the collected images into taxonomic and size categories to explore the results using a histogram function (Matlab v. R2014b). VisualSpreadsheet software was used for automated identification of particles from the FlowCam samples. We binned the particles into general taxonomic categories: microzooplankton, chain diatoms, centric or pennate diatoms, dinoflagellates, colonial phytoplankton, silicoflagellates and unidentified. Microplankton were labeled 'unidentified' if they were too small (mostly <20 µm) to identify by taxon or out of focus. The unidentified microplankton were included in the size analyses. All classifications were confirmed by eye.

Binning data by size inherently adds some subjectivity to an analysis. We therefore chose bin widths based on the sizes of the organisms in our samples. We also tested several bin widths to make sure those that we selected did not conceal any important information about the community size structure. We calculated mean and median particle and mesozooplankton sizes for each of the 5 experiments by analyzing measurements from all 3 triplicates together. Therefore, these values represent variability among the different communities present during each experiment. When calculating mean particle and grazer concentrations, and mean grazing impact, we calculated the means and standard deviations among triplicates for each experiment to demonstrate the reproducibility of our experimental design. When comparing the particle size distributions of the control (no mesozooplankton present) and treatment (mesozooplankton present) containers, we used relative proportions of the particles in each size bin. We did this because each experiment throughout the year inherently had different concentrations of particles and grazers. Since we were interested in the impact of grazing on size structure, we present these changes in terms of relative proportions of particles in each size bin, across all experiments.

We compared size distributions of the particles consumed by the mesozooplankton grazers with the size distributions of the grazers themselves. This was done by calculating the number of microplankton consumed that fell within a range of size bins, as described above. We then parameterized a log-linear relationship between the abundances of grazers and prey using a least-squares linear regression (Matlab v. R2014b). We also compared the abundances using

published relationships (Kiørboe 2008, Wirtz 2012, Boyce et al. 2015). We estimated a relationship between a grazer size metric and the volume of particles produced during the incubations, again using a linear regression function (Matlab v. R2014b). All paired comparisons were completed with paired *t*-tests (Matlab v. R2014b).

Finally, we estimated the change in particle sinking rate with grazing. For this rough approximation, we assumed that particle density, water viscosity and gravitational acceleration were all equal, and that sinking rate was proportional to particle radius squared, as suggested by Stokes Law (McCave 1975). While caveats to using Stokes Law for estimating particle sinking rates in the ocean abound (e.g. those presented in Smayda 1971, Miklasz & Denny 2010, and others), we used it as a rough approximation to demonstrate how the repackaging of microplankton into fecal pellets can change particle size, and therefore estimated sinking rate.

RESULTS

Our goal in conducting multiple experiments over a year was to sample the entire annual seasonal cycle, but an extraordinarily severe winter prevented us from completing work after November 2014. The ambient sea surface temperature (1 m) during the study period (April to November) ranged from 2 to 15°C.

The taxonomic composition of the autofluorescent microplankton community (<200 µm) varied over the duration of the study (Fig. 3). Microzooplankton constituted 42% of the microplankton by abundance in April and 70% in May. Most of the microzooplankton present in April were small (≤60 µm), whereas approximately half of the microzooplankton present in May were large ciliates of the genus *Laboea*. Small chain diatoms (≤40 µm) dominated in July, small dinoflagellates were the most abundant microplankter in August (≤50 µm) and silicoflagellates were most abundant in October (Fig. 3). Unidentified microplankton made up 35% of the total, on average.

Microplankton size structure in the field shifted throughout the experiments (Fig. 4). Mean microplankton size ranged from 23.8 ± 14.7 µm in July to 48.2 ± 28.4 µm in May and median microplankton size ranged from 19.9 ± 2.1 µm in July to 39.0 ± 6.3 µm in May (Table 1). The smallest microplankter included in the analysis was 10.0 µm ABD, due to instrument limitation based on the microscope objective used (4×), and the largest microplankter measured was 164.5 µm ABD. The natural communities of microplankton had log-linear distributions in all experiments except for the one conducted in May (Fig. 4B).

Mesozooplankton community composition and size structure varied throughout the experiments as well. Copepods dominated the mesozooplankton community during all experiments except in April, when barnacle nauplii (*Balanus* spp.) were most abundant (Fig. 5A). Barnacle nauplii were still abundant in May, but mixed with calanoid copepods, including *Calanus finmarchicus* copepodids and adults, and genus *Pseudocalanus* (Fig. 5B). In July, the mesozooplankton grazers were composed mostly of calanoid copepods, including late stage *C. finmarchicus*, and genera *Acartia*, *Pseudocalanus*, *Temora* and *Centropages*. Cladocera were also present in the July mesozooplankton grazer community (Fig. 5C). During the August experiment, the mesozooplankton included the genera *Centropages*, *Temora* and *Pseudocalanus*, as well as the species *C. finmarchicus* (Fig. 5D). One of the 3 treatment containers also contained a chaetognath; however, no consistent pattern in particle abundance indicated that the chaetognath significantly altered the grazing impact of the mesozooplankton community. The chaetognath was not included in the grazer body size analyses. The mesozooplankton grazer community in the final experiment, conducted at the end of October, was composed mostly of the genus *Temora*, and also contained some *Pseudocalanus*

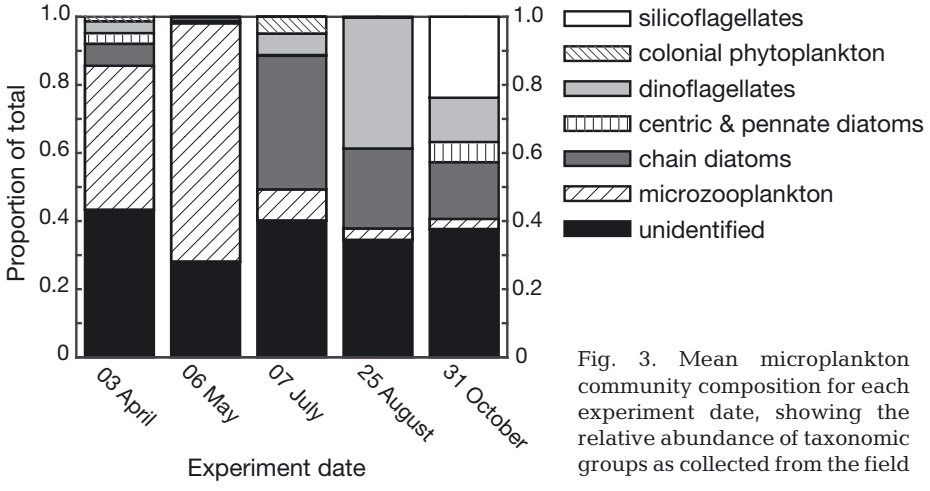

Fig. 3. Mean microplankton community composition for each experiment date, showing the relative abundance of taxonomic groups as collected from the field

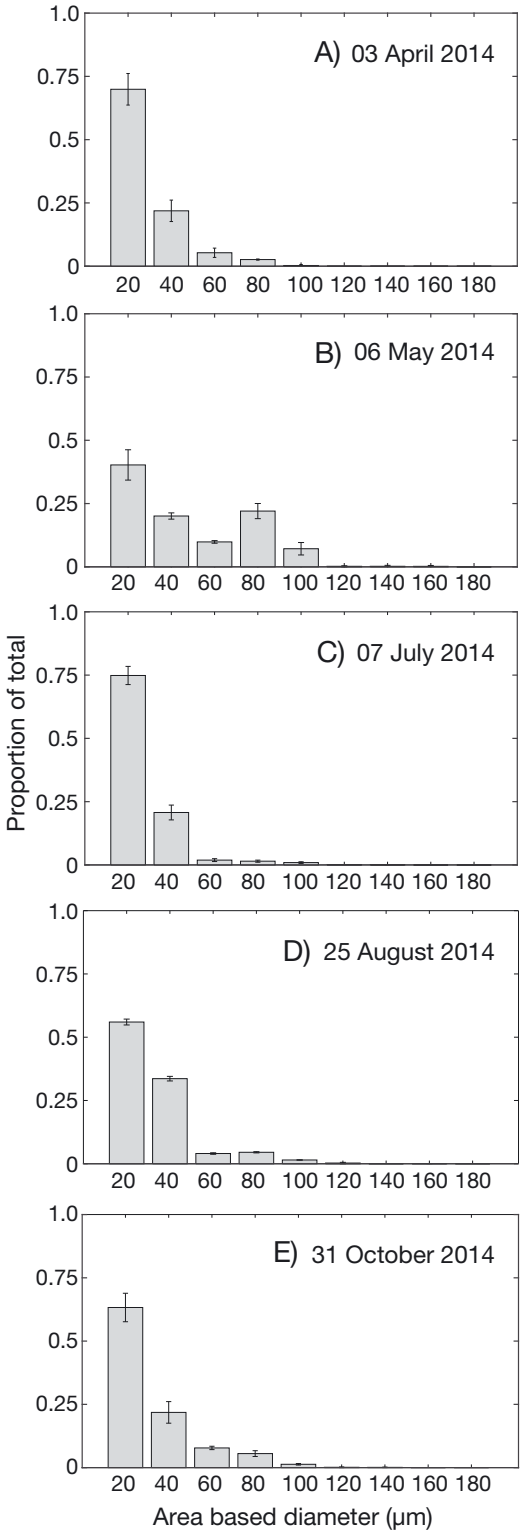

Fig. 4. Microplankton size distribution in the field from the 5 experiments: (A) 03 April 2014, (B) 06 May 2014, (C) 07 July 2014, (D) 25 August 2014 and (E) 31 October 2014. The distributions are represented as the mean proportion of total individuals in each size bin (±SD). Bin width is 20 µm and size is estimated as area-based diameter (µm)

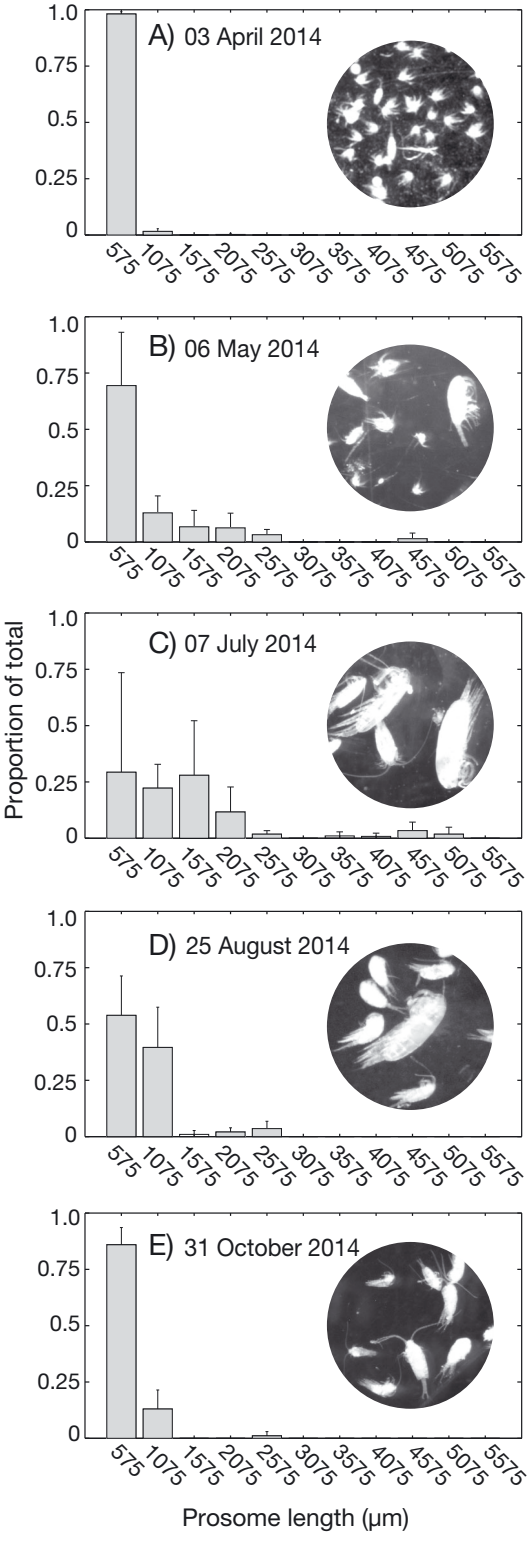

Fig. 5. Mesozooplankton size distribution from the 5 experiments: (A) 03 April 2014, (B) 06 May 2014, (C) 07 July 2014, (D) 25 August 2014 and (E) 31 October 2014. The distributions are represented as the mean proportion of total individuals in each size bin (±SD). Bin width is 500 µm and size is estimated as prosome length (µm). Inset images show shifts in composition

Table 2. Microplankton and mesozooplankton grazer concentrations from each experiment in 2014. Numbers reported are the mean ± SD of replicate measurements (n = 3), except the initial microplankton concentration from 03 April 2014 (n = 2). Note: grazer concentration has been increased from that in the field and does not reflect natural concentrations

Date of experiment	Initial microplankton concentration (no. ml^{-1})	Control microplankton concentration (no. ml^{-1})	Treatment microplankton concentration (no. ml^{-1})	Grazer concentration (no. ml^{-1})	Grazing by mesozooplankton (%)
03 April	7.5 ± 2.1	7.4 ± 0.9	5.8 ± 0.9	1.5 ± 0.2	21.7 ± 3.2
06 May	7.3 ± 0.3	5.7 ± 0.6	5.1 ± 1.0	0.1 ± 0.0	10.4 ± 1.2
07 July	39.5 ± 6.6	26.2 ± 8.4	11.0 ± 3.0	0.9 ± 0.1	56.0 ± 12.5
25 August	27.4 ± 2.6	19.5 ± 1.1	11.0 ± 1.3	0.3 ± 0.0	43.5 ± 3.5
31 October	16.4 ± 2.3	12.4 ± 0.4	9.0 ± 0.3	0.3 ± 0.1	29.9 ± 0.9

and *Centropages*. Late stage *C. finmarchicus* were also present, but sparse (Fig. 5E).

Mean mesozooplankton prosome length (PL) ranged from 230 ± 156 µm in April to 1320 ± 962 µm in July and median mesozooplankton PL ranged from 182 ± 16 µm in April to 1280 ± 542 in July (Table 1). These results were driven by the overwhelming dominance of small barnacle nauplii in April and late stage *C. finmarchicus* in July (Fig. 5A,C, respectively). The smallest mesozooplankter measured 76 µm in PL and was found in the May experiment. The largest mesozooplankter measured 5310 µm in PL and was found in the July experiment.

The goal of these experiments was to study the grazing impact of mesozooplankton on particle size structure. We therefore calculated the percentage of microplankton grazed to show that grazing did indeed occur, rather than quantifying specific grazing rates. We found that mesozooplankton grazing resulted in an average net loss of at least 10.4 ± 1.2% of microplankton in May, and at most 55.7 ± 12.6% in July (Table 2). However, grazing by the mesozooplankton did not result in a distinct change in microplankton size structure, as hypothesized. The size distribution of microplankton in the control containers, which contained no mesozooplankton grazers, was rarely different from the distribution of microplankton in the treatment containers, in which grazers were grazing (Fig. 6A,C,E,G,I). The only significant changes in size distribution related to grazing were observed in July (paired t (2) = −4.8, p = 0.04) and August (paired t (2) = −10.3, p = 0.01), when particle relative abundance decreased in the 10 to 50 µm size bin, and subsequently increased in the 50 to 90 µm size bin (Table 3, Fig. 6A,C). Given the known relationships between individual mesozooplankton grazer size and prey size range (Kiørboe 2008, Wirtz 2012, Boyce et al. 2015), we looked for a community-level relationship between grazer community size structure and the size structure of microplankton prey consumed. No such relationship was apparent in this study and prey were consumed in proportion to their abundance (Fig. 7).

Grazing by mesozooplankton alone did not greatly alter the microplankton size structure. However, a shift in particle size distribution was observed in some of the experiments when the production of fecal pellets that resulted from grazing was included in the analysis. When fecal pellets produced during grazing were included in the particle size spectrum, the average size of particles increased in all experiments, significantly so in August (paired t (2) = −4.9, p = 0.04; Fig. 6H) and October (paired t (2) = −12.6, p = 0.01; Fig. 6J), and nearly significantly in April (paired t (2) = −3.0, p = 0.09; Fig. 6B) and July (paired t (2) = −3.6, p = 0.07; Fig. 6F). In May, average particle size did not significantly change (paired t (2) = −1.5, p = 0.28; Fig. 6D). These results are summarized in Table 3.

While we did not find a relationship between grazer community size structure and the size structure of microplankton consumed, we did find a log-linear relationship between median grazer prosome length (PL) and mean volume of particles produced during the incubation period (V_{mean}, R^2 = 0.65, p ≪ 0.01, Fig. 8):

$$\log_{10}(V_{mean}) = 1.4 \log_{10}(PL) + 6.7 \quad (1)$$

We compared this community-level relationship with individual-based relationships in the literature (Table 4) and found that they were consistently log-linear, and that the model parameters are close and of the same magnitude (Uye & Kaname 1994, Mauchline 1998, Stamieszkin et al. 2015). This indicates that the known body length to fecal pellet volume relationship can be scaled up from individual organisms to a community.

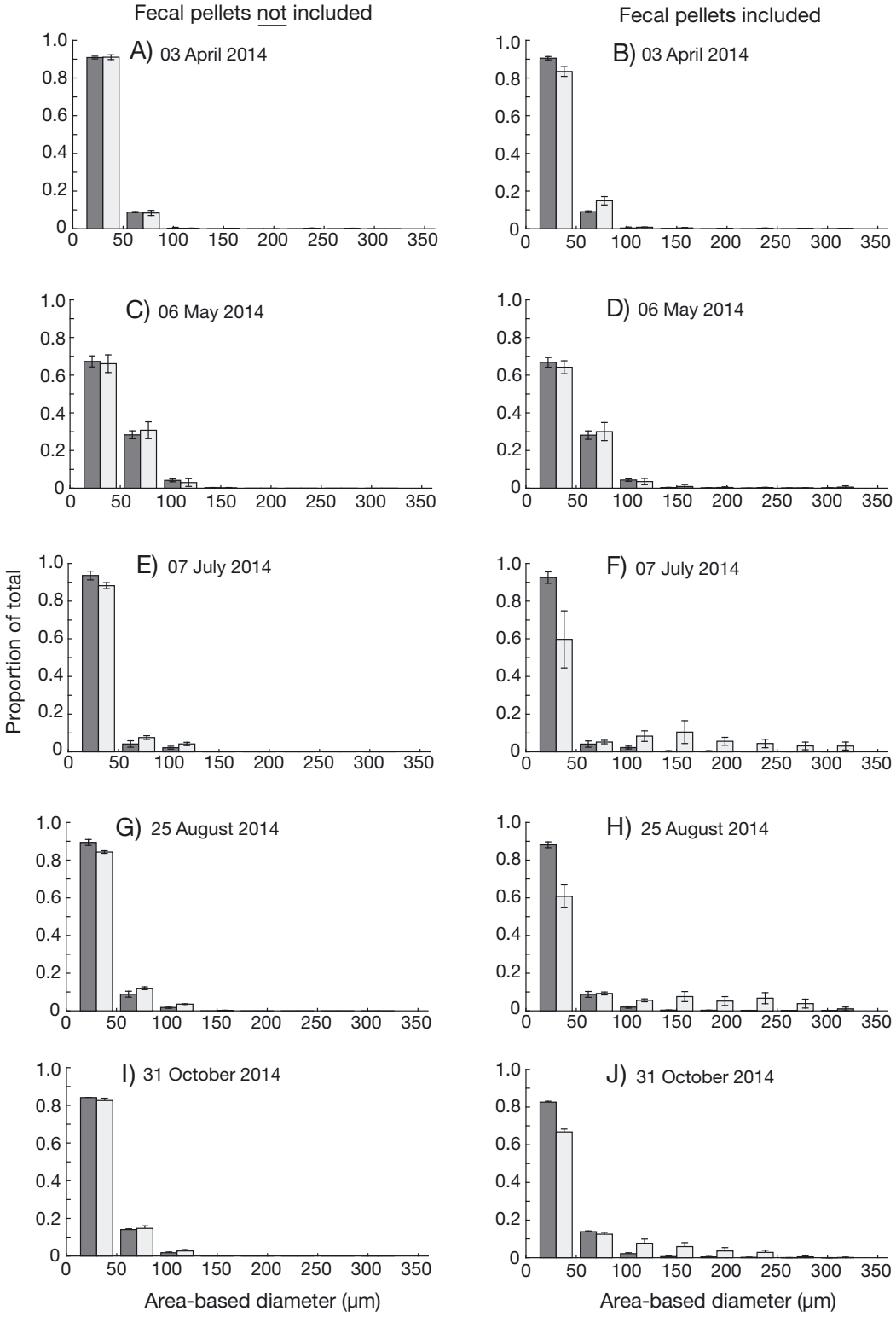

Fig. 6. Grazing impact on microplankton size distributions from the 5 experiments: (A,B) 03 April 2014, (C,D) 06 May 2014, (E,F) 07 July 2014, (G,H) 25 August 2014 and (I,J) 31 October 2014. Dark gray bars represent results from the containers without mesozooplankton grazers ('control') and light gray bars represent results from the containers with mesozooplankton grazers ('treatment'). The left column shows effects of grazing on the particle size distribution and the right column shows the effects of grazing and fecal pellet production on the particle size distribution. The distributions are represented as the mean proportion of total particles in each size bin (±SD). Bin width is 40 μm and size is estimated as area-based diameter (μm)

Table 3. Particle size from each experiment, both excluding and including fecal pellets produced during the incubation periods. Numbers reported are the mean ± SD of replicate measurements (n = 3). Mean particle size in control (no mesozooplankton grazing) and treatment (mesozooplankton grazing occurring) containers were compared using a paired *t*-test (Matlab R2014b)

Date of experiment	Control	Treatment	Results of paired *t*-test comparing particle size in control and treatment containers
Experiment excluding fecal pellets			
03 April 2014	28.4 ± 0.9	28.6 ± 0.1	paired t (2) = –0.5, p = 0.69
06 May 2014	44.1 ± 1.5	43.5 ± 3.2	paired t (2) = 0.3, p = 0.78
07 July 2014	25.4 ± 2.3	30.4 ± 1.2	paired t (2) = –4.8, p = 0.04
25 August 2014	32.8 ± 0.9	36.3 ± 0.5	paired t (2) = –10.3, p = 0.01
31 October 2014	33.2 ± 0.3	34.8 ± 0.9	paired t (2) = –2.6, p = 0.12
Experiment including fecal pellets			
03 April 2014	28.6 ± 1.0	32.7 ± 1.6	paired t (2) = –3.0, p = 0.09
06 May 2014	45.3 ± 0.6	48.1 ± 2.8	paired t (2) = –1.5, p = 0.28
07 July 2014	27.6 ± 3.5	82.9 ± 27.4	paired t (2) = –3.6, p = 0.07
25 August 2014	34.9 ± 0.5	80.4 ± 15.9	paired t (2) = –4.9, p = 0.04
31 October 2014	35.6 ± 0.4	58.2 ± 3.4	paired t (2) = –12.6, p = 0.01

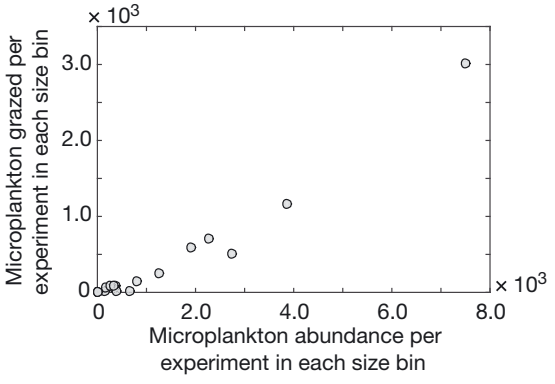

Fig. 7. Number of microplankton in each of 15 size bins (bin width of 20 µm) from the initial samples (the microplankton available for consumption) versus the number of microplankton grazed by mesozooplankton from each size bin. The results of all experiments were combined

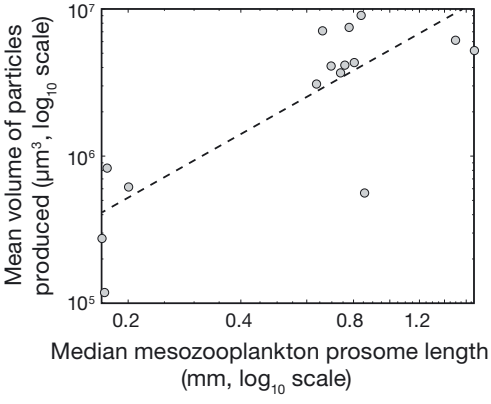

Fig. 8. Median mesozooplankton prosome length (PL, mm) versus mean volume of particles produced during the incubations (V_{mean}, µm^3); most of these particles were mesozooplankton fecal pellets. Both axes are on a $\log_{10}$ scale. The dotted line is a line of best fit (R^2 = 0.65, $p \ll 0.01$): $\log_{10}(V_{mean}) = 1.4 \log_{10}(PL) + 6.7$

The change in particle size distribution that results from mesozooplankton grazing and fecal pellet production increased estimated mean particle sinking rate in all but one experimental triplicate. This increase ranged from 100 ± 9% in April to 1800 ± 1100% in July. In May, the increase was 32 ± 56%, with a decrease of 16% in sinking rate in one of the triplicates. The change in sinking rate was significant in all experiments except the May experiment: April paired t (4) = –3.0, p = 0.04; July paired t (4) = –3.2, p = 0.03; August paired t (4) = –4.1, p = 0.02; October paired t (4) = –9.2, $p < 0.01$; and May paired t (4) = –1.2, p = 0.31.

One advantage of using the FlowCam to analyze the results of these experiments is that we were able to take a closer look at outliers in the size spectrum. A large species of the dinoflagellate genus *Ceratium* (example image in Fig. 2A) was relatively abundant during the July experiment and present in the August and October experiments, and stands out in the particle size spectrum (Fig. 9). However, when its abundances in the treatment (grazing) and control (no grazing) containers were compared, they were not significantly different: July paired t (4) = –0.6, p = 0.56; August paired t (4) = 1.3, p = 0.26; and October paired t (4) = –1.7, p = 0.17. This indicates that these large *Ceratium* spp. are not eaten by mesozooplankton grazers in these experiments.

DISCUSSION

Experimental approaches to explaining plankton grazing patterns have tended to emphasize the taxon-centric view and consider the effects of grazing by only one or a few grazer species. For example, it has been shown that calanoid copepods often prefer ciliate prey (Atkinson 1996, Nejstgaard et al. 1997, Rollwagen Bollens & Penry 2003, Castellani et al. 2008, Fileman et al. 2010). It has also been shown that prey preference can also change with season (Teegarden et al. 2001, Rollwagen Bollens & Penry 2003, Castellani et al. 2008), diel cycles (Wu et al. 2010) and prey toxicity (Teegarden 1999, Ger et al. 2016). Rather than focus on species, in this study we used size as an organizing trait, thus abstracting taxonomic

Table 4. Comparison of the relationship between copepod prosome length (PL) and fecal pellet volume (V_{mean}) from previous studies, and mean mesozooplankton PL and V_{mean} in the present study; the relationship is in the form $\log_{10}(V_{mean}) = \alpha \log_{10}(PL) + \beta$, where V_{mean} is in μm^3 and PL is in mm

α	β	Source
2.6	5.4	Uye & Kaname (1994)
2.5	5.2	Mauchline (1998)
2.6	5.4	Stamieszkin et al. (2015)
1.4	6.7	Present study

denominations. This approach allows us to connect trophic dynamics directly to particle size spectra.

Based on plankton predator–prey size relationships (Kiørboe 2008, Wirtz 2012, Boyce et al. 2015), we hypothesized that grazing by mesozooplankton on microplankton would create gaps in the particle size spectrum, and that these gaps would be predictable based on the size structure of the grazers. However, in our grazing experiments, we found little evidence for preference, either for size or taxa. The shapes of the prey size spectra after grazing were similar to those with no grazing (Fig. 6A,C,E,G,I), indicating that mesozooplankton in our experiments were eating prey in proportion to their abundance (Fig. 7). Non-selective feeding has been documented in older literature and our results align well with these patterns (Parsons et al. 1967, Poulet 1973, Richman et al. 1977). Different from previous studies is our use of FlowCam imaging technology for analyzing grazing experiments. This method enables a more standardized and precise analysis of particle size simultaneous with taxonomic classification (Verity & Paffenhöfer 1996). Further, by processing live samples rather than preserved, the likelihood of losing particles or altering their size through preservation (Stoecker et al. 1994, Menden-Deuer et al. 2001) decreases.

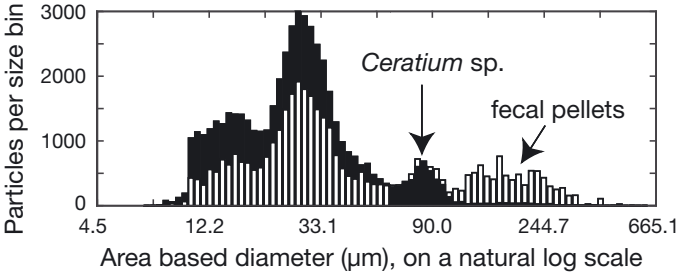

Fig. 9. Particle size distribution for all data combined; bars are approximately 6.5 µm wide. Black bars represent the particles in control containers after incubation (no mesozooplankton grazing) and white bars represent the particles in treatment containers after incubation (mesozooplankton present and grazing). Particle abundance per size bin is the FlowCam count per 700 ml sample container

These experiments are unique because they use the natural assemblage of grazers and prey. Previous work using natural prey communities has focused on a limited number of mesozooplankton grazer types (Parsons et al. 1967, Poulet 1973, Richman et al. 1977, Atkinson 1995, Nejstgaard et al. 1997, Teegarden et al. 2001, Rollwagen Bollens & Penry 2003, Castellani et al. 2008, Fileman et al. 2010, Wu et al. 2010), and published size relationships between grazers and prey are based on individual organisms, rather than whole communities (Kiørboe 2008, Wirtz 2012, Boyce et al. 2015). When the cumulative effects of individual grazers are considered, the emergent community-scale patterns are quite different. Rather than grazing down narrow size ranges of prey, as predicted by individual predator–prey size relationships, the mesozooplankton community has a uniform grazing effect. This effect results in microplankton being consumed in proportion to their abundance.

The uniform grazing effect could be explained in 2 ways. First, the predators may be feeding indiscriminately across the full range of prey sizes and types. This seems unlikely given the large range of predator sizes (Table 1). Instead, we propose that the grazer and microplankton communities are tightly coupled and have developed over time to fit one another in the field; therefore, when we intensify grazing in our treatment containers, the particle abundance changes, but not the size distribution.

Another way to consider this is a reshaping of the Red Queen hypothesis, borrowed from evolutionary biology (VanValen 1973). The Red Queen hypothesis posits an evolutionary arms race between predator and prey in which the 2 are constantly evolving in tandem to maximize consumption by the predator and escape by the prey, allowing both to accumulate biomass at converse times. In a plankton community, microplankton proliferate within the restrictions of nutrients and physical factors, and mesozooplankton life cycles are adapted to time periods of high prey availability (Longhurst 1995, Tommasi et al. 2013, Friedland et al. 2015). Hence, there is usually a tight coupling between plankton trophic levels, and it would be expected that available prey items will be grazed. This scenario gives rise to the even distribution of grazing over a prey size spectrum.

Exceptions to this pattern of even grazing can be observed over short timescales, since it takes time for predator communities to 'adapt' when a prey species diverts resources to protect itself from predation or

outcompetes other microplankton, thereby accumulating biomass. These experiments reveal a 'snapshot' in time of the plankton communities, allowing us to observe the proliferation of a species, before the predator community has shifted to take advantage of the relatively abundant prey or because the necessary nutrients are no longer available. Large individuals of the genus *Ceratium* were a notable example of this in our experiments. Their relatively large size (~50 to 100 µm) and spines likely protect them from significant grazing (Verity & Paffenhöfer 1996). Using size as an organizing trait allows outliers like *Ceratium* to stand out as not being grazed; its abundance remained nearly the same between control and treatment containers (Fig. 9). By investing in spines and size, *Ceratium* spp. are able to reduce their predation mortality, although likely using resources that could be used for reproduction. In addition to morphological investments in self-preservation, phytoplankton defenses against grazing can include toxicity and decreased edibility (induced defenses reviewed in Van Donk et al. 2011). Another explanation for the persistence of *Ceratium* spp. cells during incubation could be that we did not collect grazers large enough to consume these large *Ceratium* spp. cells. Based on the individual predator to prey size ratio of 10:1 (Kiørboe 2008, Boyce et al. 2015), the mesozooplankton grazers in our experiments could have consumed these cells, but did not. This suggests that while grazing in proportion to abundance may be the default, there are opportunities for individual species to break out of the size spectrum through a major investment in adaptations that reduce mortality.

In this study, we considered not only particles that are grazed, but also particles that are produced. The production of fecal pellets clearly adds particles to the larger size bins within the particle size spectrum considered (Fig. 9). By collectively grazing most particles in the 10 to 50 µm size range, and aggregating those into larger fecal pellets in the 100 to 500 µm size range, mesozooplankton grazers in our experiments increase mean particle size. Larger fecal pellets sink faster and are therefore more likely to contribute to particle flux out of the surface ocean (Stamieszkin et al. 2015). In these experiments, mesozooplankton grazing and egestion increased estimated sinking rate, and therefore increased the potential for fecal pellet carbon flux. When mesozooplankton feed, they also create smaller particles by breaking apart prey items (Poulet 1973). It is likely that smaller particles were produced during these experiments. However, the particles were either unidentifiable or smaller than 10 µm and not included in this analysis. Future experiments could use additional technology, such as flow cytometry, to capture a wider size range of particles.

In the analysis of these experiments, we considered particle size as a proxy for carbon content. While this is generally a reasonable assumption (Mullin et al. 1966, Alldredge 1998, Menden-Deuer & Lessard 2000), the carbon content and stoichiometry of microplankton change depending on taxonomy and evolutionary history (Menden-Deuer & Lessard 2000, Quigg et al. 2003), nutrient availability (Geider & la Roche 2002), as well as other factors reviewed by Klausmeier et al. (2008). The stoichiometry of zooplankton fecal pellets also varies depending on the composition of prey being consumed (Urban-Rich 2001) and amount of prey available (Atkinson et al. 2012). In future iterations of these experiments, nutrient analysis of microplankton and fecal pellet stoichiometry would provide useful information for quantifying the relationships between mesozooplankton grazing, nutrient cycling and carbon export.

Predator–prey size relationships in the literature primarily focus on individual-level selection; however, these relationships do not directly scale up to the community level. Furthermore, models of optimal mesozooplankton prey size have limited applicability to natural plankton communities, since a grazer may never cross paths with prey of the optimal size, but must still feed. To understand the trophic dynamics that affect particle size spectra, and therefore the efficiency of the biological carbon pump, it is essential to be able to scale up from the individual organism to the community level. Future experimental and modeling work should take into consideration trophic dynamics at the community level, and consider grazing and particle production along a continuum. The abstraction of taxonomic information using size as a descriptive trait allows us to take that community view and work toward a mechanistic understanding of how trophic interactions affect particle flux potential.

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