ABSTRACT

BURSEY, JOSEPH RYAN. Protistan-Copepod Trophic Interactions in Bogue Sound, NC. (Under the direction of Dr. Astrid Schnetzer).

Protists are the main primary producers (i.e. phytoplankton) in marine and coastal waters and also major consumers (e.g. ciliates) of phytoplankton. These organisms, in turn are consumed by larger zooplankton (i.e. copepods) that play an important ecological role as regulators of energy flux to higher trophic levels. In this study, the copepod-protist trophic link was examined in Bogue Sound, an estuarine embayment along the NC coast. Grazing experiments were conducted in June and October of 2014 for which changes in chlorophyll *a* and cell abundance (carbon biomass) were measured in the absence and presence of copepod grazers (>100 µm). Copepod assemblage structure varied significantly among June and October with different prevalent species, life stages, and feeding modes which was reflected in prey selections during the feeding experiments. Adult copepods and copepodites consumed mostly larger phytoplankton (especially diatoms within the 20 – 100 µm size class) in June, while some microzooplankton (i.e. dinoflagellates), and in some incidences also copepod nauplii, contributed to adult copepod diet in October. In agreement with microscopy data, results from pigment analyses (20 – 100 µm size class) supported the removal of larger phytoplankton (i.e., diatoms) by copepods in June while no feeding was detected for microphytoplankton in October. Pigment analyses were also conducted for nanophytoplankton within the 5 – 20 µm size range. While no apparent grazing on these cells was detected in June, nanophytoplankton were removed at significant rates in the October incubations, likely by both adult copepods and a large number of copepod nauplii that were abundant in October but not June. Experimental feeding rates based on microscopy counts indicated that the natural copepod assemblage only removed 4.3% and 4.4% of the microplankton carbon stock in June and in October, respectively. The findings of this study emphasize the role of temporal variation in protistan and grazer assemblages on protistan-copepod trophic interactions and carbon flow through planktonic assemblages.

Protistan-copepod Trophic Interactions in Bogue Sound, NC

by
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DEDICATION

I dedicate this work to the memory of both my grandfather Edward Krasnowski and my aunt, Marlene “Boobsie” Ranucci who always listened and encouraged me regardless of what scientific facts I was telling them or if they ever even understood them. This thesis wouldn’t exist without them.

BIOGRAPHY

As a child, I was always taken by nature. Some kids asked for toys, I asked for books about sharks. I collected and identified insects and reptiles as a child and eventually begin studying biology at Winthrop University. Here, I was able to further study the marine ecosystem as I met Dr. Julian Smith who introduced me to marine meiofauna ecology. I finished up here and accepted a job at NC State where I helped set up the lab I would eventually switch into graduate school and produce this body of work.

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My committee members; Astrid Schnetzer, Dan Kamykowski, and Dave Eggleston, have been amazing in their guidance on this research project. I would also like to thank my outstanding lab group for their contributions and support.

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INTRODUCTION

Autotrophic protists (phytoplankton) are the main primary producers in marine coastal waters where they fix carbon and provide energy for upper trophic levels within the food web. Heterotrophic and mixotrophic protists (e.g., ciliates and dinoflagellates) are major grazers of algae and, in turn are consumed by larger zooplankton such as copepods. In coastal waters, copepods are typically the most abundant mesozooplankton and play an important ecological role as regulators of energy flux. They provide a link between the lower autotrophic and heterotrophic levels they consume and the higher trophic levels that prey upon them (Fessenden & Cowles 1994, Murrel 2004, Schnetzer 2005). Top-down control by copepods can directly impact biomass and diversity within protistan assemblages (Pace et al. 1998, Schnetzer 2005, Caron et al. 2012). Copepods may remove an average ~30% of the primary production in coastal systems (Calbet & Saiz 2005).

Copepods are known to feed on a variety of prey types using different feeding modes including passive suspension feeding or raptorial feeding (Leising et al. 2005). Passive suspension feeding is generally used to filter and concentrate particles that are too small to be efficiently collected individually, whereas copepods that prefer larger prey tend to actively consume them using a raptorial feeding mode (Paffenhöfer & Knowles 1978, Price & Paffenhöfer 1986). These feeding modes may vary by copepod species, life stage, prey availability and prey motility (Fessenden & Cowles 1994, Turner et al. 2001, Calbet & Saiz 2005). Understanding the extent to which copepods drive changes in natural protistan communities over varying spatiotemporal scales is important as their grazing activities govern energy flow to higher trophic levels (Pavés et al. 2015).

Most studies of copepod-prey dynamics have examined copepod grazing impact by measuring bulk changes in total chlorophyll (chl *a*), or by examining the effects of copepod grazing on single prey organisms (cultured species) in laboratory experiments (Gismervik & Andersen 1997, Menden-Deuer & Lessard 2000). Chl *a* is a proxy for phytoplankton biomass and does not allow for consideration of non-pigmented prey. Relatively few studies have investigated copepod feeding using natural protistan assemblages and/or derived carbon ingestion rates (IRs) for specific prey types (i.e. major groups such as diatoms, ciliates, and dinoflagellates) (López & Anadón 2008, Saiz & Calbet 2011). Overall, these studies indicate that natural copepod diets can be complex and that copepod fitness during varying life stages is dependent upon the consumption of mixed prey (Kleppel 1993). Feeding studies using natural prey assemblages and microscopy may be labor intensive compared to most culture studies, but they can resolve selectivity in copepod grazing and shifts in prey assemblage structure due to top down control. Copepod selective feeding may be guided by mechanical and chemical cues for prey detection. For instance, motile-prey can generate disturbances which copepods detect leading to ambush predation (Kiørboe et al. 2009) or copepods may distinguish prey items based on their chemical characteristics (Steinke et al. 2006, Gonçalves & Kiørboe 2015).

This study investigated changes in natural protistan prey abundances and taxa composition in response to copepod grazing pressure in Bogue Sound, North Carolina, USA, a tidally flushed, oligotrophic, 23-mile embayment located in the southwestern region of the Albemarle-Pamlico Sound.

MATERIALS AND METHODS

Study Site and Grazing Experiments

Natural phytoplankton assemblages and copepod grazers for feeding experiments were obtained in June and October of 2014 from Bogue Sound, North Carolina, USA (34°43'18" N and 76°45'34" W, Fig. 1). Whole seawater (WSW) was collected in the morning (~0930) using a 50 L carboy, and copepods were collected using tow-yo net tows (net diameter 100 cm, 150 µm mesh) in the upper water column (0 - 2 m) in both months (water column depths ~ 5 m). A flow meter (Sea Gear Inc.) was used to measure the approximate total volume that was filtered for each tow and to calculate ambient copepod densities following the manufacturer's instructions. All samples were transported back to the laboratory and grazing experiments were started within 3 hrs of collection. First, to remove metazoan grazers, WSW was pre-filtered through a 100 µm Nitex mesh to fill twelve 2 L polycarbonate bottles. The mesh screen size allowed for the removal of most adult copepods and copepodites while the majority of protists passed through. Three control bottles (without grazer additions) were processed to obtain measurements at the beginning of the experiments (T_0). Each experiment was comprised of three replicates for each of three treatments 1) control bottles with no copepod additions, 2) bottles to which copepods were added at ~10 ind. L^{-1}, and 3) bottles to which copepods were added at ~20 ind. L^{-1}. The chosen grazer densities represented ranges typical for copepod abundances within Bogue Sound. For the grazer treatments, adult copepods were handpicked randomly from the net tows and rinsed in 0.2 µm filtered seawater to reduce transfer of prey organisms with the copepod additions. Immediately after copepods were transferred into their respective bottles, the six bottles (three for each of the two grazer addition treatments) and three control bottles were transferred into a culture incubator (Percival), where they were incubated for 24 hours at ambient water temperatures determined during sample collection with 26.6ºC for June and 22°C for October. Bottle placement inside the incubator was random at a light:dark cycle of 12:12 hours (~290 µEinsteins m^{-2} s^{-1}) both months. Sampling at T_0 and at the final time point, after 24 hours (T_{24}), followed the same protocol. The water from each bottle was gently prescreened using a 100 µm filter to remove copepod grazers and subsampled (see details next). All retained zooplankton were rinsed off the 100 µm mesh, visually inspected to confirm they were still alive and then they were preserved using formalin (5% final concentration) for later counting, sizing and identification via microscopy (Cushing 2000)**.**

Chlorophyll *a* Measurements

Size-fractionated chlorophyll (chl *a*) samples were collected onto GF/F filters (0.45µm) running the seawater (200 ml) through a series of nitex screens to distinguish between size fractions from 5 to <20 µm and 20 to 100 µm, respectively. The filters were kept at -20°C until analysis following a non-acidification protocol as detailed in (Welschmeyer 1994) and using a Trilogy fluorometer (Turner Designs). Briefly, 7 ml of 100% acetone was added and the samples extracted overnight at -20°C. Analyses took place within one week of collection to minimize pigment loss from extended freezing (Graff & Rynearson 2011).

Plankton Enumeration and Biomass Calculations

Changes in microplankton abundance and community structure (20–100 µm) in response to copepod predation were examined by comparing the abundance and biomass of protists at T_0 in the control bottles to the grazer addition bottles over the time course of the experiments (see below). Protists were enumerated by light microscopy (Olympus BX53) using standard settling techniques for samples (50-mL) preserved with either acid-Lugol's solution (10% final concentration) or formalin (1% final concentration) with Lugol's added later (Utermöhl 1958, Stoecker et al. 1994). Protists were classified into broad taxonomic groups (i.e., diatoms, ciliates, and dinoflagellates) using reference guides (Tomas 1997). Cell biovolumes (BV) were calculated using approximated geometric shapes for 20-40 cells for each of the major taxonomic groups and average BVs converted into carbon (C) biomass by applying published conversion factors for ciliates (0.19 pgC mm^{-3}), diatoms (pgC mm^{-3} = 0.288BV$^{0.811}$), and dinoflagellates (pgC mm^{-3} = 0.76BV$^{0.819}$) (Putt & Stoecker 1989, Menden-Deuer & Lessard 2000).

Copepod Ingestion Rates

Copepod ingestion rates (IRs) (µg chl *a* cop^{-1} day^{-1}, cells cop^{-1} day^{-1}, and µg C cop^{-1} day^{-1}) were calculated by comparing changes in control bottles with those in grazer treatments and normalizing individual rates for the number of adult copepods and copepodites within each bottle (Frost 1972). Changes in chl *a*, cell abundance and C biomass were assumed to be the result of copepod predation but were more accurately the result of both micro- and metazooplankton predation.

Statistical analyses

Multivariate analyses were conducted (PRIMER v7 statistics package, Anderson et al. 2008) to examine whether shifts in prey abundances and/or taxa composition occurred in control compared to grazer bottles. IRs based on chl *a*, cell abundance and C biomass were square-root-transformed and Euclidean distances were computed to creative a resemblance matrix comparing replicate bottles within a treatment and among the varying treatments. Negative IRs were replaced by zero values and the individual effects of copepod abundance (treatment) and month on IRs were tested via ANOSIM permutation analyses (Clarke & Warwick 2001). Comparison of overall final community structure were conducted using relative abundances from microscopy counts after square-root transformation and computing Bray-Curtis similarities for Cluster analyses (Clarke & Warwick 2001). Finally, initial chl *a* levels, C biomass and cell abundances among June and October were also compared (t-test).

RESULTS

Bogue Sound Conditions

Environmental conditions at the start of each experiment were recorded. In June the conditions were cloudless, water temperature was 26.6°C, and high/low tides occurred at 1021/1612. In October, the weather was mostly sunny, water temperature was 22°C, and high/low tides occurred at 0906/1536.

Natural Copepod Abundance and Taxonomic Composition

Mean concentrations of adult copepods in Bogue Sound were similar during June and October 2014 with 5.7 (±0.7 SD) and 5.2 copepods L^{-1} (±0.6 SD), respectively. The copepod assemblage in June consisted mainly of adult copepods, some copepodites and a few nauplii whereas in October, a high number of nauplii and some polychaete larvae were present. In both grazing experiments, copepods were added to the grazer treatments to increase abundances by x2 and x3 in June (10 and 17 copepods L^{-1}, respectively) and by x2 and x3.5 in October (10 and 18 copepods L^{-1}, respectively) compared to ambient abundances (Fig. 2). During the experiment in October, a few smaller copepods and a considerable number of nauplii passed through the 100 µm screen (see control treatments in Fig. 2). All zooplankton were assigned to major phyla or copepod order at the end of the incubations. In addition, three randomly selected copepods were identified to genus, if possible to species, for each triplicate bottle. In June the most common copepods belonged to the orders of calanoids, harpacticoids and cyclopoids, whereas in October calanoids and cyclopoids dominated (Table 1). Copepodites were only assigned to major order (i.e., calanoids, cyclopoids or harpacticoids). The most abundant adult copepod species in June were *Euterpina acutifrons, Oithona* spp., and *Paracalanus indictus* whereas in October *Paracalanus aculeatus, Parvocalanus crassirostris, Acartia tonsa*, and *Oithona oculata* were most abundant (Table 1).

Initial Microplankton Abundances and Biomass

The initial microplankton assemblage in surface waters of Bogue Sound was analyzed by measuring phytoplankton pigment concentration, quantifying cell concentrations and estimating carbon biomass (Table 2). Initial total chl *a* concentrations (5 – 100 µm size fraction) averaged lower in June (4.1 ±0.1 SE µg Chl *a* L^{-1}) than October (6.9 ±0.5 SE µg Chl *a* L^{-1}) (t-test; $p < 0.05$, n =3). Microplankton C biomass in June exceeded that in October 2014 (1,639 and 976 µg C L^{-1}, respectively), though without significant statistical difference (t-test; $p = 0.16$, n = 3). At the level of major taxa a significant difference in initial biomass of diatoms (1,372 and 390 µg C L^{-1} for June and October, respectively; $p = 0.0002$) and ciliates (86 and 410 µg C L^{-1} for June and October, respectively; p = 0 .038) was observed, but not for dinoflagellates (182 and 177 µg C L^{-1}, for June and October, respectively; (p = 0.47). Diatoms were the most abundant microplankton in June (96% of all cells), with an average of 1,433 cells mL^{-1} and *Guinardia*, *Leptocylindrus*, and *Asterionellopsis* spp., making up ~76% of all diatoms (Table 3). Dinoflagellates contributed ~3% to the entire microplankton count and ciliates only ~1% (Table 3). In October, diatoms were again the most abundant microplankton (80% of all cells) with an average of 412 cells mL^{-1} and with the genera *Skeletonema*, *Leptocylindrus*, and *Nitzschia* making up ~67% of all diatoms.

Dinoflagellates contributed ~12% and ciliates ~8% to the microplankton assemblage. In both months, *Prorocentrum* spp. dominated the dinoflagellate assemblage and aloricate ciliates were the most common ciliates (Table 3). Comparison of overall microplankton community structure indicated significant differences between June and October, with no further distinction between final assemblage structures in response to copepod presence or absence (SIMPROF, $p < 0.05$).

Copepod Ingestion Rates

Copepod IRs were derived using differences between control and copepod addition bottles for chl *a* (µg chl *a* cop^{-1} day^{-1}, Fig. 4), cell abundances for the three major taxonomic groups (cells cop^{-1} day^{-1}, Fig. 5, Table. 4), and C biomass (µg C cop^{-1} day^{-1}, Fig. 6). Comparing total pigment concentrations in either month revealed no effect of copepod abundance (treatment) on chl *a* IR (i.e., x2 versus x3 treatment in June; ANOSIM, $p < 0.05$). However, significant differences were seen between months in regard to what phytoplankton cell sizes were consumed, with ingestion of cells 20–100 µm in size in June and cells 5-20 µm in size in October (ANOSIM, $p < 0.05$) (Fig. 4). IRs based on cell abundances in June showed grazing on diatoms, ciliates and dinoflagellates at x2 copepod concentrations but no grazing at x3 copepod concentrations (Fig. 5a and b). In October, grazing on dinoflagellates within the 20-100 µm size range was seen based on cell counts in only one of 3 replicate bottles which resulted in high standard error values (Fig. 5). With high variability among replicate bottles, no statistically significant differences could be detected among IRs based on cell abundance or C biomass among the treatments or months (ANOSIM, $p <0.05$). IRs based on C biomass reflected results based on cell counts (Fig. 6) and showed how ciliates and dinoflagellates contributed more strongly to C ingestion due to their higher C cell content compared to diatoms (x2 treatment in Fig. 6a). Inconsistencies were observed among grazer treatments in June as diatoms and ciliates showed reduced growth in x2 copepod addition treatments and fair growth in x3 copepod additions (Fig. 7). In October, diatoms generally flourished in all copepod addition bottles (x2 and x3.5) while dinoflagellates exhibited reduced growth (Fig. 7).

Genera-specific abundance changes were observed for the 6 most common microphytoplankton taxa which contributed on average 90% and 84% of the total cell counts in June and October, respectively. Cell abundances indicated differing growth/grazer responses (Figs. 8a and 8b) and were used to calculate genera-specific IRs (Figs. 9a and 9b). For example in June, *Guinardia* spp. and *Asterionellopsis* spp. were the most grazed, followed by *Leptocylindrus* spp. and *Pseudo-nitzschia*, spp. while *Chaetoceros* spp. was seemingly avoided in the x2 grazer treatment (Fig. 9a). At x3 copepod concentrations, ingestion of these same taxa could not be observed (Fig. 9a) despite the fact that these genera were available for consumption (Fig. 8a). Compared to June, and consistent with the trends seen for chl *a*, microphytoplankton ingestion in October was extremely low or undetectable (Fig. 9b).

DISCUSSION

Copepod Grazing on Phototrophic and Heterotrophic Prey

Chl *a* has traditionally been used to approximate phytoplankton ingestion by copepods, however, chl *a* values cannot account for non-pigmented prey, such as ciliates or heterotrophic dinoflagellates. Moreover, this approach cannot resolve selective feeding within phytoplankton genera. Thus, the present study also incorporated microscopy analyses that facilitated calculations of IRs for both autotrophic and heterotrophic prey. Chl *a* IRs indicated grazing on the larger phytoplankton (microphytoplankton within the 20-100 µm size range) in June and was in agreement with the IRs of diatoms and dinoflagellates detected in the x2 grazer treatment. Conversely, in October, consumption only of nanophytoplankton (5-20 µm size range) was detected. In both experiments the increase in top-down grazing pressure correlated with an increase in cell growth for diatoms at the highest copepod additions (i.e. x3 and x3.5-fold numbers of copepods) (Fig 7). A possible explanation for these increases could have been an increase in remineralized nutrients in bottles that contained the highest grazer densities (i.e., via sloppy feeding or excretion), conditions that have been seen to lead to accelerated phytoplankton growth (Sunda & D 2007, Saba et al. 2011).

A closer look at IRs for specific genera/groups of prey in June indicated that the most common types, *Guinardia* and *Asterionellopsis* spp., were readily consumed while *Chaetoceros* spp., a spine-forming chain-former was avoided. Avoidance of *Chaetoceros* spp. by copepods had been reported in other studies (Koski et al. 2008, Nishibe et al. 2010).

In October, higher variability among replicate bottles for both IRs and growth rates made it difficult to discern trends in genus/group-specific grazing rates. All identified copepods were known omnivores except for the herbivore *E. acutifrons* which made up 41% of the grazer assemblage in June (Table 1). As such, *E. acutifrons* likely played a key role in the disappearance of diatoms in June; the genus had also shown to prefer diatoms over ciliates (Broglio et al. 2004).

A major difference between the June and October experiment was seen in the contribution of nauplii to the incubation bottles. A copepod/copepodite to nauplii ratio of 1:3 in the x2 grazer treatments in October seemed in good agreement with the trends that were seen for chl *a* IRs within the nanophytoplankton range (5 – 20 µm). Depending on life stage, nauplii are capable of grazing on particles within this size range with a preference for ciliates and diatoms, however nauplii sizes and life stages were not determined in this experiment (Jefferson et al. 2001, Saiz et al. 2014). In October the most abundant adult copepods included *Oithona* which can also graze preferentially on small phototrophic (as well as heterotrophic) flagellates (2-8 µm) (Uye & Kasahara 1983). A dramatic drop in nauplii concentrations between the control or x2 grazer treatments compared to the x3.5 grazer bottles (48.7 and 59.3 compared to 26.3 nauplii L^{-1}, respectively) indicated that adult copepods/copepodites may have preyed on these early life stages instead of phytoplankton prey. While the identified copepods for October were general omnivores, at least *Oithona* is a known grazer on *A. tonsa* nauplii (Saiz et al. 2014).

Copepod Influence on Microplankton Biomass in Bogue Sound

The impact of copepod grazing on microplankton biomass was calculated as % biomass removed by the copepod community per day using mean C IRs at x2 grazing pressure for June and October. In June the total biomass removed per day for diatoms was ~1.7%, dinoflagellates ~10.0% and ciliates ~1.3% day^{-1} whereas in October only one treatment bottle allowed for the calculation of dinoflagellate removal with 14.0% day^{-1}. The average copepod diet in June consisted of 87% diatoms, 11% ciliates, and 2% dinoflagellates and, as mentioned, only one value was derived for October that resulted in dinoflagellates making up 100%. Overall, copepods grazed readily on microzooplankton (Table 4) despite their low numbers compared to diatoms. Other studies conducted in oligotrophic systems like Bogue Sound showed C IR for ciliates and dinoflagellates within a similar range (Fessenden & Cowles 1994, Schnetzer 2005) (Table 5). Interestingly, during the October experiment, the highest copepod addition treatments correlated with a strong decrease in nauplii, and nauplii can be readily grazed on by omnivorous adult copepods (Landry 1981, Durbin et al. 2008). Assuming the nauplii in this experiment were preyed upon by the carnivorous copepods, this resulted in a removal rate of up to 0.62 nauplii cop^{-1} day^{-1} (Fig. 2). Given limited published information on nauplii predation rates by copepods, this potentially important trophic link warrants further investigations. It should further be noted, that the timing of the experiments in this study did not coincide with copepod peak abundances in Bogue Sound and are therefore not representative of maximum grazing impacts within this coastal system. If repeated, grazing experiments should also further account for the trophic importance of nanoflagellates which may contribute significantly to carbon flow to higher trophic levels (directly or via a trophic cascade) (Lampitt & Gamble 1982, Zöllner et al. 2009, Böttjer et al. 2010).

The findings of this study emphasize the importance of considering temporal variability in both the protistan and grazer assemblage when examining protistan-copepod trophic interactions. While trophic dynamics strongly differed among June and October, ambient copepod grazing pressure seemed to have a minor impact on microplankton standing stocks in Bogue Sound during either month.

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Table 1. Identified copepod grazers listed by studies. Feeding modes H; herbivory, O; omnivory, and C; carnivory.

Copepods	**June**	**October**	**Feeding Mode**	**Source**
Calanoida	9	23		
Acartia tonsa	0	5	O	Turner 1984, Lonsdale et al., 1979,
Paracalanus aculeatus	0	6	O	Turner 1984b, Barquero et al., 1998, Cheng et al., 2014
Paracalanus indictus	1	0	O	Turner 1984b, Barquero et al., 1998, Cheng et al., 2014
Parvocalanus crassirostris	0	6	O	Turner 1984b, Barquero et al., 1998, Cheng et al., 2014
Paracalanus parvus	0	4	O	Turner 1984b, Barquero et al., 1998, Cheng et al., 2014
Copepodites	8	2	-	
Cyclopoida	6	4		
Oithona oswaldocruzi	1	0	O	Nakamura and Turner 1997
Oithona nana	4	0	O	Nakamura and Turner 1997
Oithona oculata	0	4	O	Nakamura and Turner 1997
Copepodites	1	0		
Harpacticoida	14	0		
Euterpina acutifrons	12	0	H	Camus and Zeng (2012)
Copepodites	2	0		

Table 2. Cell abundances (cells mL^{-1}) and carbon biomass (μg C L^{-1}) in initial seawater samples in June 2014 and October 2014. Taxa-specific cell carbon (pg C $cell^{-1}$) and carbon biomass (μg C L^{-1}) in initial seawater samples. ± Standard Error.

		Cells mL-1	n	μg C L-1
Diatoms	June	1433.3(212)	20	1371.7 (154.8)
	October	411.7(68)	20	389.6 (64.7)
Dinoflagellates	June	48.9(16)	10	181.7 (60.6)
	October	63.3(28)	10	177.0 (79.0)
Ciliates	June	16.3(0)	10	86.3 (0.0)
	October	40.7(24)	10	409.7 (236.6)

Table 3. Average abundance of varying prey groups at the beginning of each experiment (cells ml^{-1}). nd; no detectable grazing.

Major Taxa	Genus / Group	June	October
Pennates	*Leptocylindrus*	233.5	86.0
	Pseudo-nitzschia	43.4	18.1
	Nitzschia	nd	22.6
	Thalassionema	21.7	9.0
	Asterionellopsis	352.9	22.6
	Chaetoceros	65.1	4.5
	Rhizosolenia	32.6	nd
	Pleurosigma	nd	0.3
	Unidentified Diatoms	nd	4.5
Centrics	*Skeletonema*	nd	230.7
	Guinardia	618.9	nd
	Eucampia	nd	0.3
	Navicula	nd	0.3
	Cylindrotheca	5.4	nd
	Corethon	5.4	nd
	Pleurosigma	5.4	nd
	Odontella	nd	9.0
	Bacteriastrium	5.4	4.5
	Coscinodiscus	0.1	nd
	Hemialus	38.0	nd
	Other Centric Diatoms	5.4	nd
	Total	1,433.4	412.6
Dinoflagellates	*Gymnodinium*	0.0	nd
	Prorocentrum gracile	5.4	27.1
	Prorocentrum	10.9	36.2
	Other Dinoflagellates	32.6	0.0
	Total	48.9	63.3
Ciliates	*Aloricate Ciliates*	16.3	36.2
	Loricate Ciliates	nd	4.5
	Total	2,231.6	829.7

Table 4. IRs based on shifts in chl *a*, cell abundance, and C biomass. ± SE, *none determined.

		June		October	
Method	**Class**	**x2**	**x3**	**x2**	**x3.5**
Chl *a* (ug chl *a* cop-1 d-1)	20-100 µm	0.48(0.43)	0.34(0.32)	*	*
	5-20 µm	-0.01(0.00)	0.01(0.02)	0.09±0.03	0.31±0.25
Cell abundance (cells cop-1 d-1)	Diatoms	2.70(1.65)	-1.14(0.25)	-0.39(0.19)	-1.09(0.17)
	Ciliates	0.34(0.18)	-0.01(0.01)	*	*
	Dinoflagellates	1.32(2.06)	-0.83(0.26)	2.04±2.03	0.09±0.09
C biomass (ug C cop-1 d-1)	Diatoms	2.28(1.40)	-1.14(0.23)	-0.37(0.19)	-1.04(0.16)
	Ciliates	1.82(0.97)	-0.04(0.04)	*	*
	Dinoflagellates	0.11(0.22)	-0.27(0.13)	5.71(5.68)	0.25(0.25)

Table 5. Comparison of C IRs with other field experiments.

	Location	Dates	Temp °C	Species	Prey	µg C copepod^{-1} day^{-1}(SE)
This Study	Bogue Sound North Carolina, USA	June 2014	22.0	x2 Mixed Copepods	Diatoms	2.28(1.40)
					Ciliates	1.82(0.97)
					Dinos	0.11(0.22)
		October 2014	18.0	x2 Mixed Copepods	Diatoms	*
					Ciliates	*
					Dinos	5.71(5.68)
Schnetzer and Caron, 2005	San Pedro Channel, California, USA	August 2002	20.5	Mixed Copepods	Ciliates	2.06(0.03)
		March 2003	17.0		Hetero. Dinos	0.78(0.04)
Calbet 2005	Polar Regions to Tropics			Mixed copepods	Hetero. Dinos	0.4 to 18.0
Broglio et al., 2004	Mediterranean Sea	May 1999 - Jul 2000	12.5 to 24.0	Oithona spp	Ciliates	0.25
				Average	>5 µm Phyto	0.10
				Paracalanus parvus	Ciliates	0.42
				Average	>5 µm Phyto	0.51
				Euterpina acutifrons	Ciliates	0.08
				Average	>5 µm Phyto	0.21
Fessenden and Cowles, 1994	Oregon, USA	Jan-August 1991	10.0	*Centropages abdominalis*	Ciliates	0.7(0.4)
				Pseudocalanus sp.		4.6(1.1)
				Calanus Pacificus		6.5(2.4)
Barquero et al., 1998	Spain Shelf and coastal	May 1994 (bloom)	12.0	Small copepods	Gen. Phyto.	0.30
				Medium copepods	Gen. Phyto.	1.30

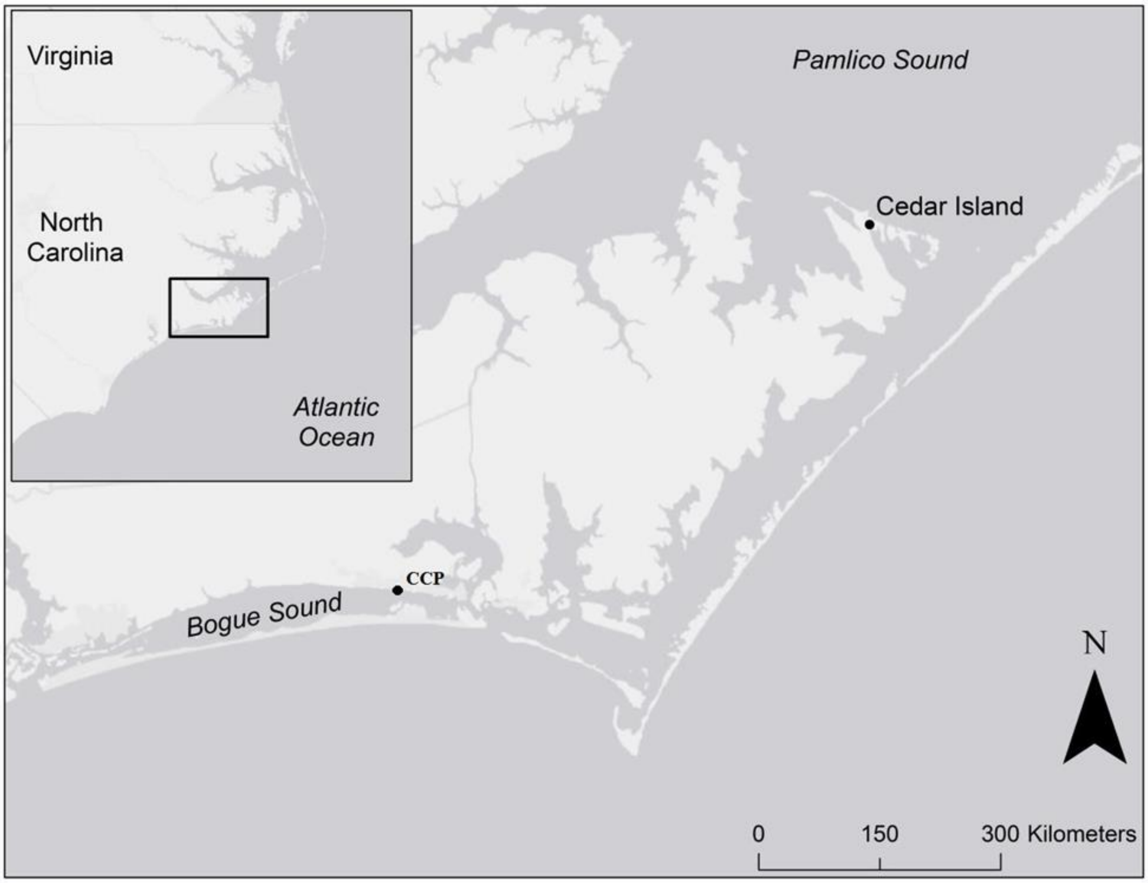

Figure 1: Bogue Sound is located within the southwestern part of the Albemarle-Pamlico Sound estuary. Sampling location at Carteret Community College Pier (CCP)

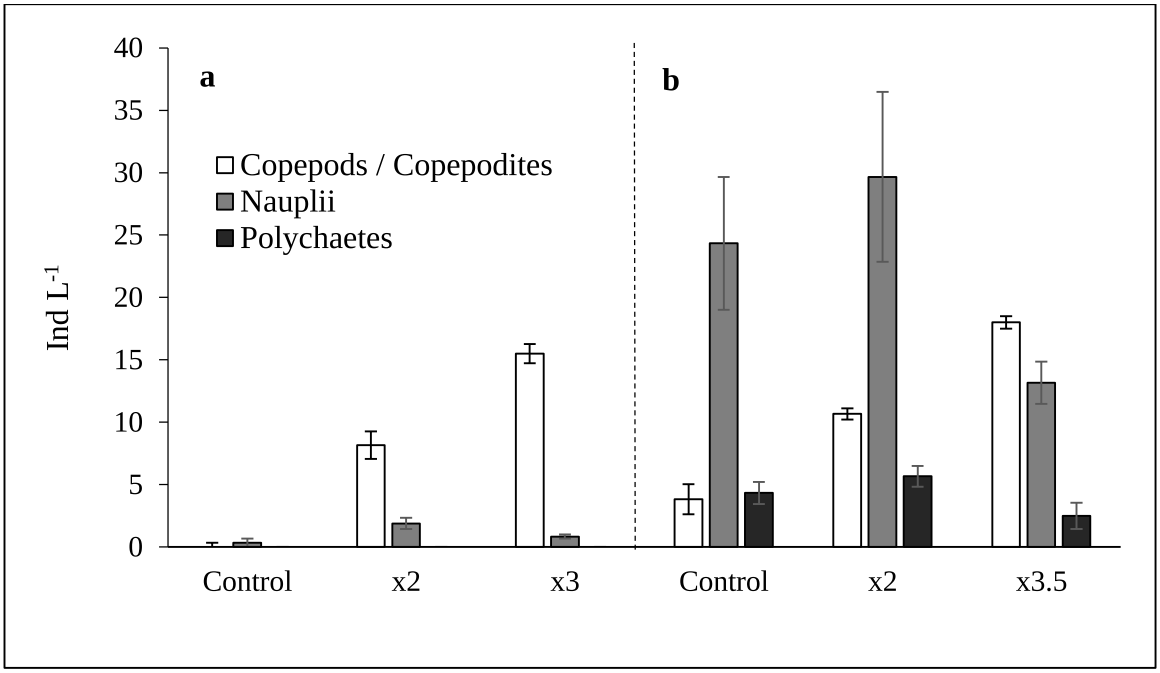

Figure 2. Number of copepods plus copepodites, nauplii, and polychaete larvae (Ind. L^{-1}) during June 2014 (a) and October 2014 (b). Means derived from three bottles for each treatment and are shown with standard error (±SE).

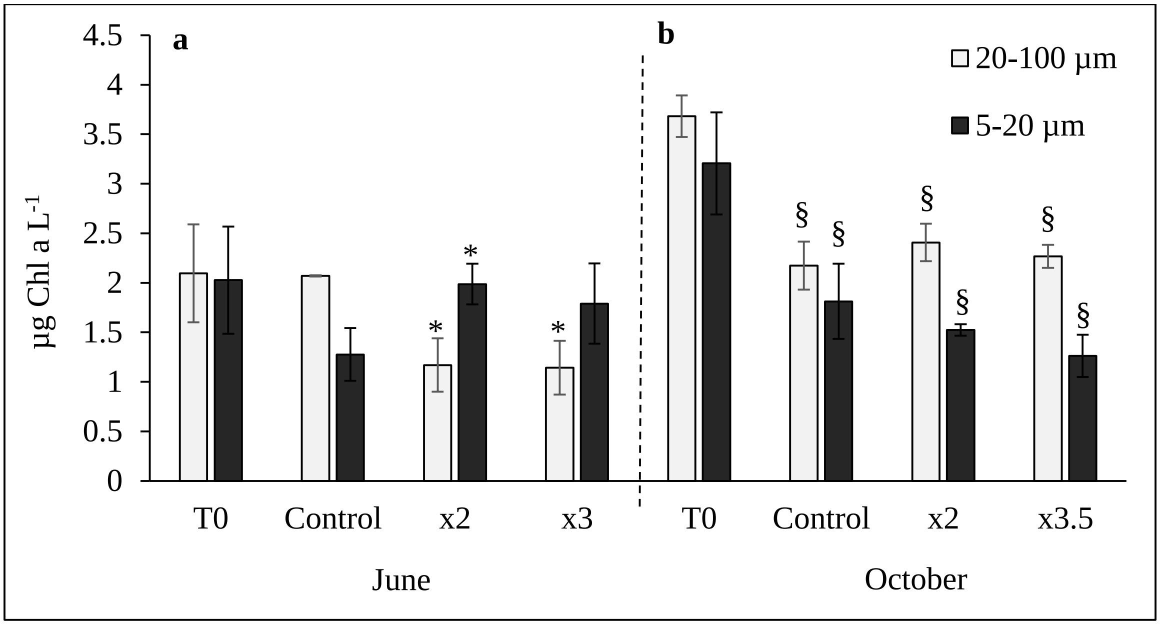

Figure 3. Initial and final Chl *a* concentrations distinguished by size fractions (20-100 and 5-20 µm) in June and October. * indicates that the final concentrations in grazer treatments were significantly different from the control, § indicates that final concentrations were significantly different from T0. Shown with standard error (±SE).

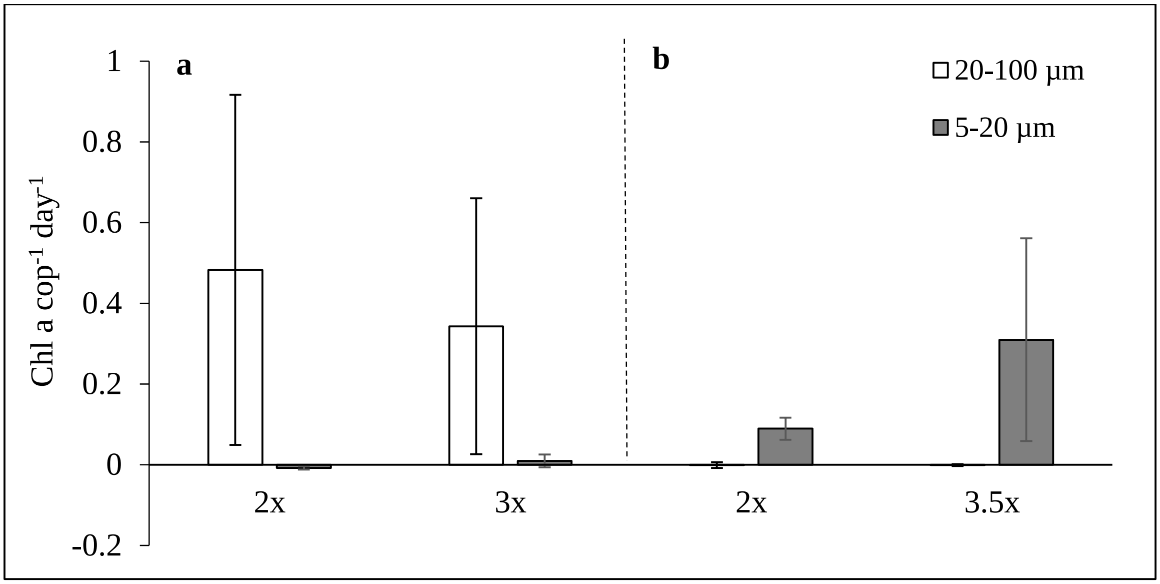

Figure 4. Chl *a* IRs for the 5-20 and 20-100 phytoplankton size ranges in June (a) and October (b) in grazer addition treatments. Values are shown as means from triplicate bottles with their standard error (±SE).

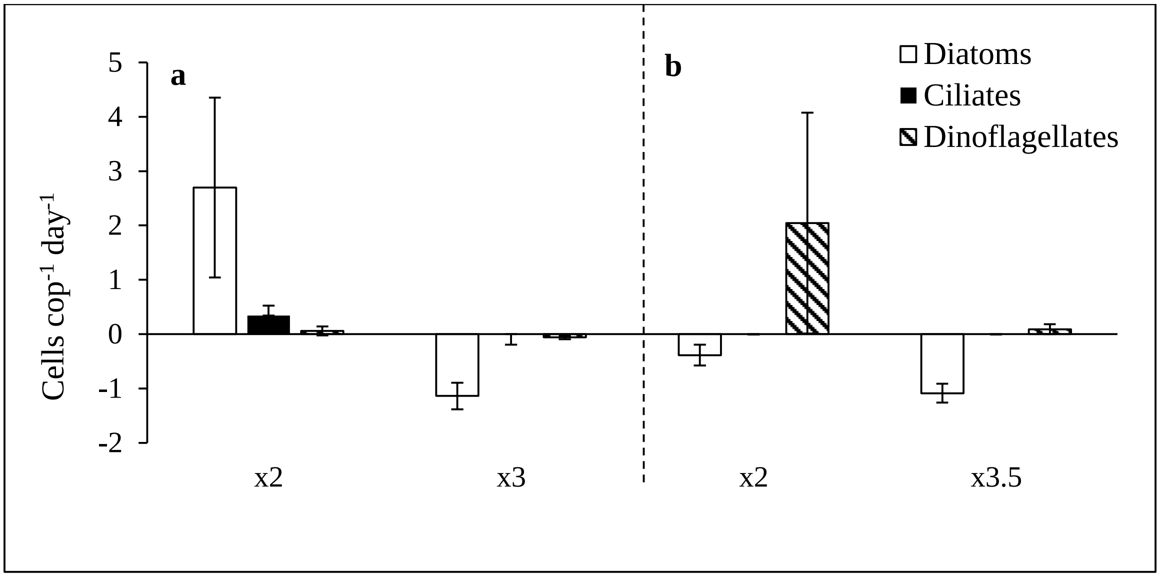

Figure 5. Cell IRs for diatoms, ciliates and dinoflagellates in grazer addition treatment for June (a) and October (b). Values are shown as means from triplicate bottles with their standard error (±SE).

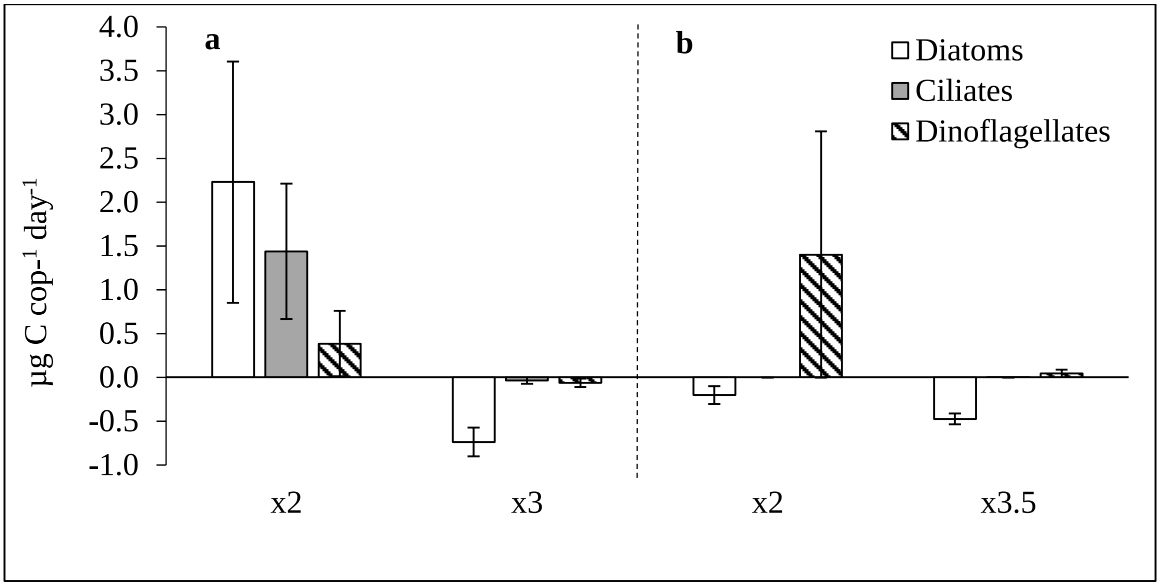

Figure 6. Copepod C IRs for diatoms, ciliates and dinoflagellates in grazer addition treatment for June (a) and October (b). Values are shown as means from triplicate bottles with their standard error (±SE).

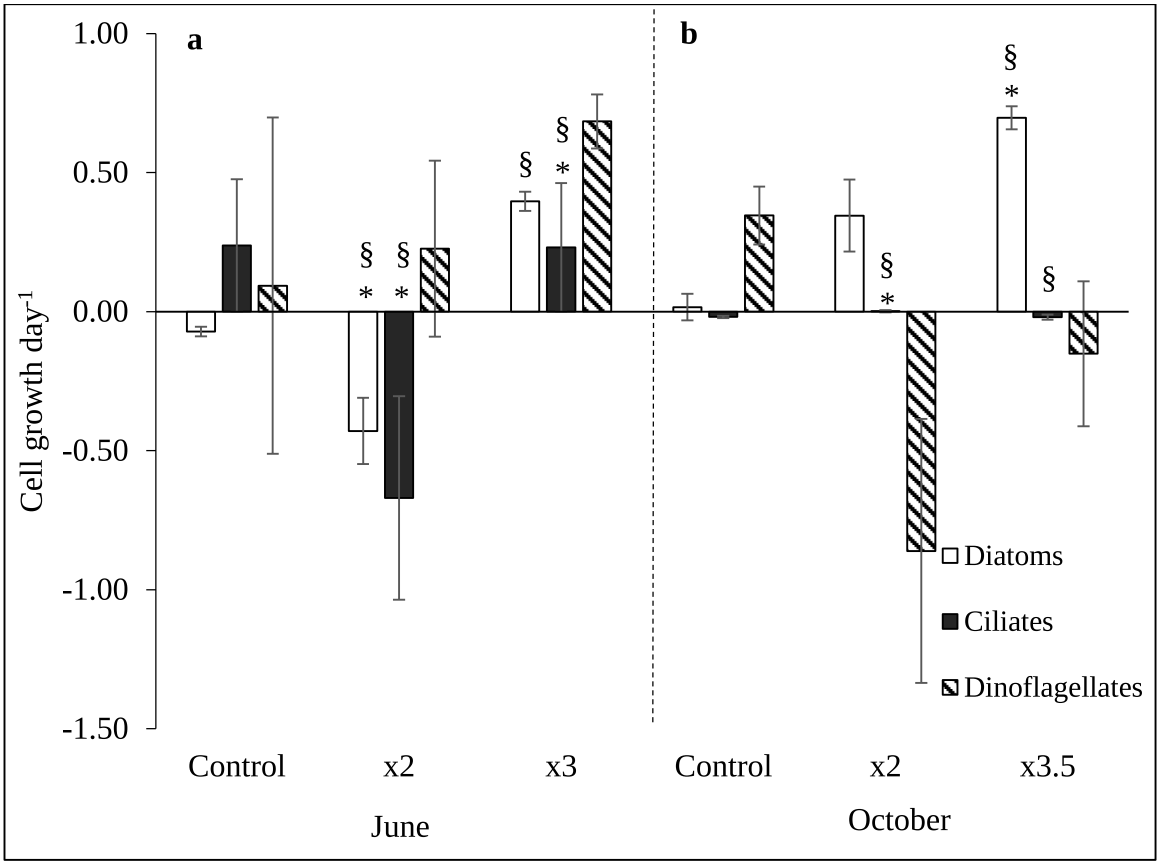

Figure 7. Cell growth in the varying treatments in June (a) and October (b). Values are shown as means from triplicate bottles with their standard error (±SE). * indicates significantly different from control, § indicates significantly different from other grazer treatment.

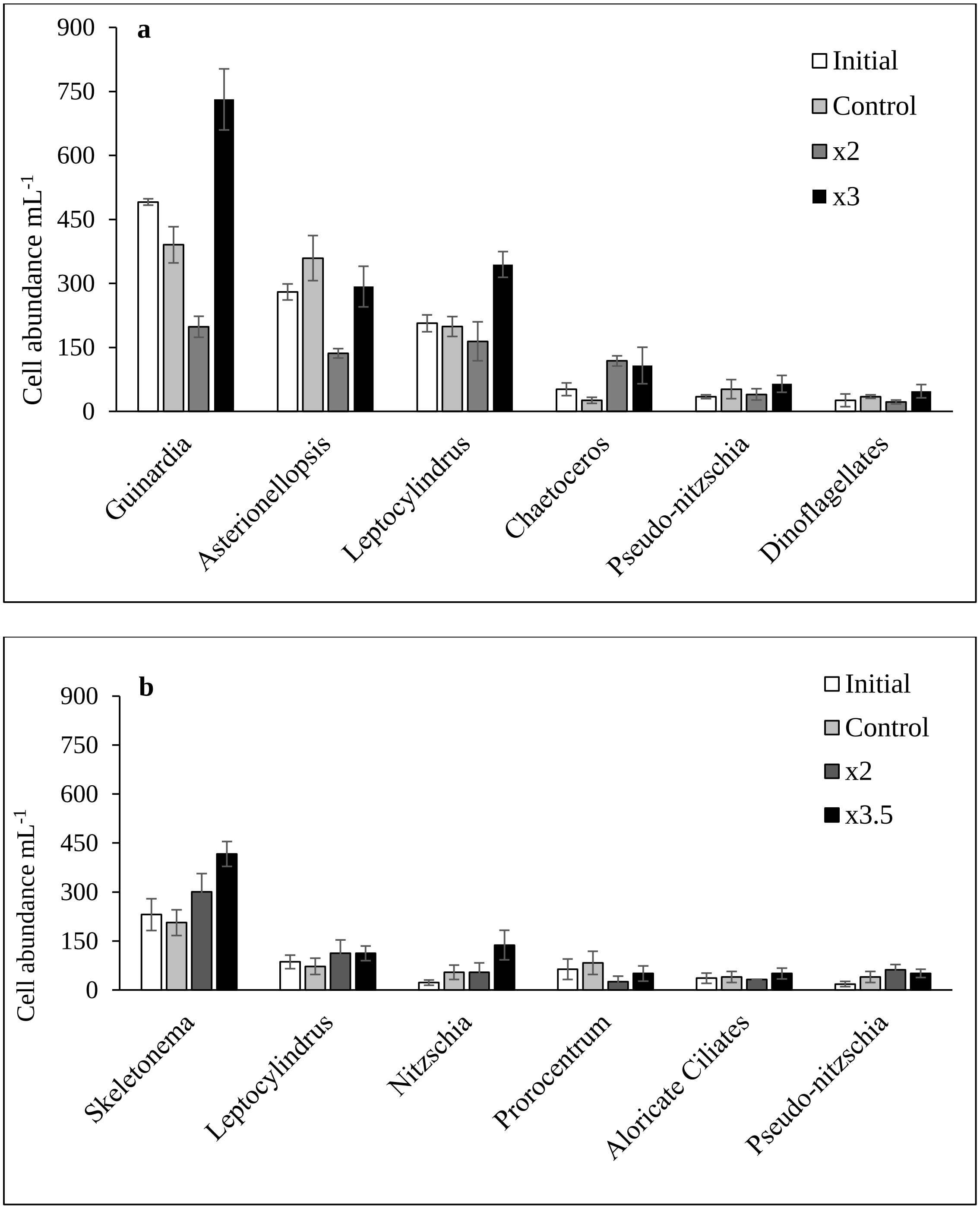

Figure 8. Cell abundances at the beginning and the final time points of each experiment shown for the most abundant 6 genera/groups in June (a) and October (b).

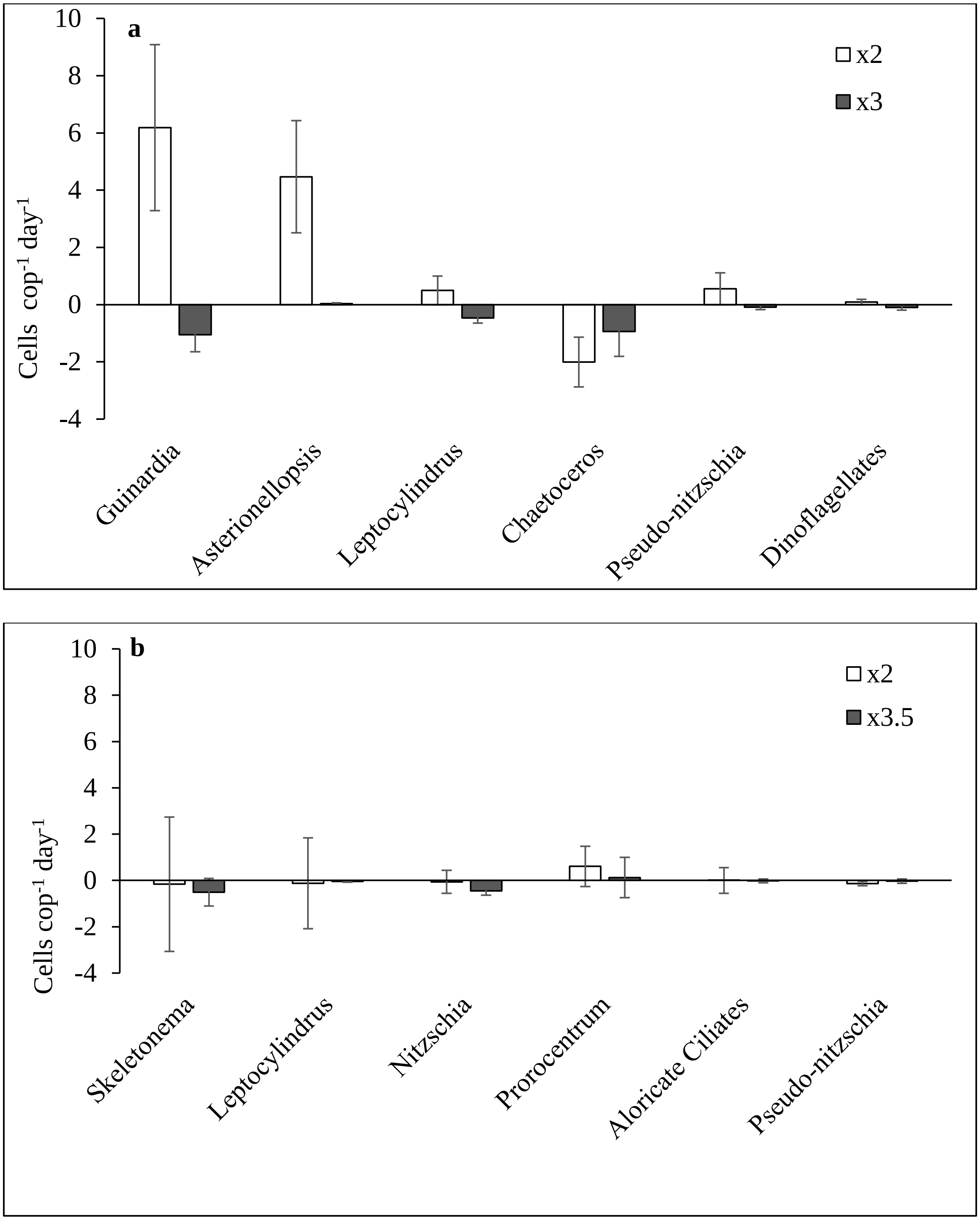

Fig. 9. IRs for the most abundant 6 genera/groups in June (a) and October (b).