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SEASONAL PROTISTAN GRAZING IN NARRAGANSETT BAY

Caitlyn Lawrence
University of Rhode Island, clawrence0718@gmail.com

SEASONAL PROTISTAN GRAZING IN NARRAGANSETT BAY

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CAITLYN LAWRENCE

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CAITLYN LAWRENCE

APPROVED:

Thesis Committee:

Major Professor: Susanne Menden-Deuer
Tatiana Rynearson
Christopher Lane

Nasser H. Zawia
DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND
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ABSTRACT

The impact of heterotrophic protist grazing on phytoplankton abundance was measured in Narragansett Bay, RI, USA, a coastal estuary, from January 2010 to February 2011. Plankton samples were collected within the long-term phytoplankton monitoring project in Narragansett Bay, initiated in the 1950s. Concurrent with weekly dilution experiments, samples were assessed for phytoplankton species composition and environmental conditions at the sampling site were recorded. Over the year, grazing removed an average of 94% (range 20 - 200%) of daily primary production, with peaks in both phytoplankton growth and heterotrophic grazing rates occurring during the summer. Phytoplankton growth rates averaged 0.69 ± 0.58 day^{-1} for the year, while protistan grazing rates averaged 0.66 ± 0.61 day^{-1}. Phytoplankton growth rates were negative in both winter and spring. Negative growth rates in the winter did not result from nutrient limitation, although nutrient limitation was evident during the summer. There was no relationship between protistan grazing rates and ambient chl *a* concentration. Grazing rates were related to temperature as well as changing phytoplankton community composition. Seasonal patterns of protistan grazing and phytoplankton community composition and abundance may be better understood when examined in relation to species composition and environmental conditions rather than bulk measures of biomass, including chl *a*. Overall, results suggest that grazing by heterotrophic protists accounts for a large proportion of phytoplankton mortality in Narragansett Bay.

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PREFACE

This thesis is written in Manuscript Format for submission to Botanica Marina.

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MANUSCRIPT

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The Effect of Protistan Grazing in a Coastal Estuary: Seasonal Signal of Plankton Community Composition and Environmental Conditions

Caitlyn M. Lawrence and Susanne Menden-Deuer

Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, USA

Corresponding Author: Caitlyn Lawrence
Graduate School of Oceanography
University of Rhode Island
225A, Coastal Institute, 210 S Ferry Rd
Narragansett, RI 02882, USA
Phone: +1-401-874-6264
Email address: caitlynl@gso.uri.eud

ABSTRACT

The impact of heterotrophic protist grazing on phytoplankton abundance was measured in Narragansett Bay, RI, USA, a coastal estuary, from January 2010 to February 2011. Plankton samples were collected within the long-term phytoplankton monitoring project in Narragansett Bay, initiated in the 1950s. Concurrent with weekly dilution experiments, samples were assessed for phytoplankton species composition and environmental conditions at the sampling site were recorded. Over the year, grazing removed an average of 94% (range 20 - 200%) of daily primary production, with peaks in both phytoplankton growth and heterotrophic grazing rates occurring during the summer. Phytoplankton growth rates averaged 0.69 ± 0.58 day^{-1} for the year, while protistan grazing rates averaged 0.66 ± 0.61 day^{-1}. Phytoplankton growth rates were negative in both winter and spring. Negative growth rates in the winter did not result from nutrient limitation, although nutrient limitation was evident during the summer. There was no relationship between protistan grazing rates and ambient chl *a* concentration. Grazing rates were related to temperature as well as changing phytoplankton community composition. Seasonal patterns of protistan grazing and phytoplankton community composition and abundance may be better understood when examined in relation to species composition and environmental conditions rather than bulk measures of biomass, including chl *a*. Overall, results suggest that grazing by heterotrophic protists accounts for a large proportion of phytoplankton mortality in Narragansett Bay.

CHAPTER 1

INTRODUCTION

Primary production in the ocean accounts for approximately 50% of global oxygen production (Field et al., 1998). Of this, heterotrophic microzooplankton, such as ciliates and dinoflagellates, can consume on average 67% of daily global primary production (Calbet and Landry, 2004). It has been suggested that even a quantitatively small disruption of predation pressure relative to phytoplankton growth can lead to large scale phenomena such as the North Atlantic Spring Bloom (Behrenfeld, 2010). It is therefore important to understand the role and magnitude of protistan grazing, as well as to understand the factors that may govern variation of protistan grazing in order to better understand plankton dynamics in the global oceans.

Near shore waters, such as coastal estuaries, appear to have different phytoplankton patterns than the open ocean (Longhurst, 1995). Estuarine systems are often more productive than the open ocean as a result of near-land associations such as nutrient enrichment (Cloern and Jassby, 2008). Narragansett Bay is a well-mixed, relatively shallow (mean depth 9m), highly productive estuary located on the Northeast coast of the United States (Martin, 1965; Borkman and Smayda, 2009a). It is the site of the longest phytoplankton monitoring project in the US, which characterizing weekly plankton community composition and environmental variables (e.g. Pratt, 1959; Borkman and Smayda, 2009a). Lower Narragansett Bay, the site of these experiments, is dominated by diatoms (Pratt, 1959; Karentz and Smayda, 1984; Borkman and Smayda, 2009a), often large or chain forming species. For several

decades, a weekly sample has been analyzed for various physical, chemical and biological components; however, grazing has not been systematically quantified as a part of this sampling. Given the significant impact grazers have on phytoplankton biomass, it is an important component to consider when assessing phytoplankton abundance, distribution and potential for primary production.

Little is known about annual changes in the extent of protistan grazing. Few studies have measured seasonal variation in natural assemblages of protistan grazers and their impact upon phytoplankton growth and mortality. In Narragansett Bay, the seasonal magnitude of protistan grazing on nanoplankton was previously assessed using a modified dilution experiment (Verity, 1986). On average 62% of daily primary production was grazed by protists in Narragansett Bay over the course of one year (Verity, 1986). Given that Narragansett Bay is the site of long-term phytoplankton monitoring, it is an optimal location for assessing variations in planktonic communities and environmental parameters with relation to protistan grazing. Seasonal changes in planktonic biodiversity and environmental conditions give rise to seasonal variability in protistan grazing.

In this study, the magnitude of protistan grazing was assessed for one year in Narragansett Bay. Our study measured the impact of seasonal variation on phytoplankton community composition, environmental conditions and heterotrophic protist grazing. Seasonal variation is likely to precipitate changes in all other factors whether directly or indirectly. To provide quantitative estimates of heterotrophic protistan grazing within the environmental context in which they occurred, we measured weekly phytoplankton growth and heterotrophic grazing rates along with

phytoplankton community composition and abundance, as well as measures of environmental parameters.

CHAPTER 2

MATERIALS AND METHODS

Study site and sampling program

Water samples were collected weekly at a mid-bay station (41° 31.25'N, 71° 24.31'W, Fig. 1) to determine the effect of grazing by heterotrophic protists on the phytoplankton community in Narragansett Bay. Net phytoplankton growth and grazer induced mortality rates were measured using the dilution method (Landry and Hassett, 1982) in a two-point modification (Landry et al., 2008; Strom and Fredrickson, 2008). A total of 45 dilution experiments were conducted from 26 January 2010 through 21 February 2011. Whole surface seawater samples (WSW) were collected and gently filtered through a 200-µm mesh to remove mesozooplankton predators. Sample water was kept in the dark while in transit to the laboratory. A portion of the water was then gravity filtered through a 0.2-µm filter (Pall) to yield filtered seawater (FSW). Whole seawater was diluted with FSW to 10% WSW. Triplicates for each dilution level (10% and 100%) were incubated in clear, 1L polycarbonate bottles in ambient seawater and light and temperature for 24 hours, rotating at 2 to 3 rpm in a flow-through seawater incubator. Chlorophyll *a* (Chl *a*) was extracted in triplicate at initial time (T_0) and in triplicate from each of the triplicate bottles after 24 hours (T_F) for total chl *a* concentration as measured following Graff and Rynearson (2011). The volume filtered ranged from 50 to 200 mL depending on phytoplankton abundance. Acid washed polycarbonate bottles and silicon tubing were used throughout to eliminate toxicity effects on heterotrophic microzooplankonton (Price et al., 1986).

Dilution experiments were performed weekly from January 2010 to February 2011. In summer 2010, 3 nutrient amended experiments were conducted, in which nutrients were added to parallel samples. Evidence of significant nutrient limitation during the summer led to further nutrient amended experiments to determine the seasonal extent of nutrient limitation. Biweekly nutrient amended experiments were conducted from October 2010 through February 2011. In each of these 12 experiments, triplicates of 100% and 10% WSW were prepared as before with the addition of non-limiting concentration of nitrate and phosphate to a final concentration of 10 μM and 2 μM respectively. Nutrient concentrations for amendments were based on the average monthly nutrient concentration between spring 2003 and January 2010 from the long-term phytoplankton monitoring dataset.

In order to determine the impact of copepod grazers on phytoplankton growth and protistan grazing *Acartia tonsa* were added to 2 dilution experiments, representing separate weekly samples (19 July and 6 August). *Acartia tonsa* is a copepod that is considered to be one of the dominant zooplankton grazers in the summer in Narragansett Bay (Deason, 1980; Thompson et al., 1994). Copepod amended experiments were conducted, in which the copepod amended dilution experiment was set up as above, with an additional 100% treatment with 5 female *Acartia tonsa* per liter, approximating average concentrations of *A. tonsa* (Durbin, personal communication).

Grazing rate (g, day^{-1}) and net phytoplankton growth rate (k, day^{-1}) can be calculated by measuring the change in chl *a* concentration. Net phytoplankton growth was calculated using $k = (1/t)(\ln(P_t/P_0)$, where P_t = final concentration of chl *a*, P_0 =

initial concentration of chl *a* and t = length of incubation period in days. Previous studies have shown that the net phytoplankton growth rate (k, day^{-1}) was not significantly different from the instantaneous growth rate (μ, day^{-1}) when comparing the two-point method with a multi-point dilution experiment (Strom et al., 2007; Strom and Fredrickson, 2008). As such, net phytoplankton growth was used as an approximation of the instantaneous growth rate. Grazing rate was calculated as the difference in growth rates between the two dilution factors. Samples with negative values of grazing and net phytoplankton growth were modified as in Calbet and Landry (2004); negative phytoplankton growth rates were set to 0.01 day^{-1} while negative grazing rates were transformed to 0 day^{-1}. The use of the exponential growth equation ($P_n = P_0 e^{rt}$) assumes that nutrients were not limiting during the incubation. Samples with negative growth were included in the analysis if growth rates were not limited by nutrient availability; however, samples with negative net growth where no nutrient added control was available were removed.

Historical Data Set

The dilution experiments were done with samples from the same site as those from the long-term phytoplankton monitoring program, initiated in 1952 (Pratt, 1959; Smayda, 1998). Samples starting in 1999 were taken to establish baseline measurements of water quality and phytoplankton community composition and all data is freely available (http://gso.uri.edu/phytoplankton). Sample collection for the monitoring program includes weekly analysis of plankton community composition, size fractionated chl *a*, macronutrients (NH^+_4, DIP, NO_3^{2+}, NO^-_3, NO_2, DIN, and Si), turbidity and temperature, salinity and diissolved measured using an *in situ* profiler

(Yellow Springs instrument YSI 6920 V2). Weekly samples were collected for 636 weeks over the 12 year period (98% of weeks).

In addition to water quality analysis, local meteorological variables, such as wind and precipitation (monitored at T.F. Green Airport by the National Oceanic and Atmospheric Administration, http://www.ncdc.noaa.gov/oa/ncdc.html), as well as irradiance (monitored by Woods Hole Oceanographic Institution, http://cis.whoi.edu/science/PO/climate) were compiled. These meteorological variables, as well as cell counts, temperature, salinity and percent dissolved oxygen (%DO) gathered for the long-term phytoplankton monitoring program, were used in the analysis of the dilution experiment.

For every grazing experiment, plankton community composition and numerical abundance from field samples was determined in accordance with methods for the long-term data set. A Sedgwick-Rafter (1 mL volume) chamber was used to enumerate live plankton samples to the lowest taxonomic level possible (genus or species) using an Eclipse E800 light microscope equipped with phase contrast (Nikon). In order to determine initial abundance of less frequent heterotrophic protists, 10 to 50 mL of 3% Lugol's preserved sample were counted for all weeks (Utermöhl, 1931). Samples were counted to genus where possible and grouped into the following three classification types: loricate and aloricate ciliates, and heterotrophic dinoflagellates. 'Dominance' was assigned to those groups that were numerically dominant on a specific date.

Carbon content was estimated for the top 10 most abundant taxa (genus or species) during the dilution experiment. 100 to 1000 cells were photographed with a

microscope mounted camera (Allied Vision Technology, Stingray F-146) and the length and width for each cell were measured using ImageJ software (National Institute of Health). Cell volume was calculated assuming a sphere, cylinder or prolate spheroid depending on cell shape. Cell volumes were converted to carbon content using regression equations from Menden-Deuer and Lessard (2000).

Statistical Analysis

A paired t-test was used to determine if growth rates differed significantly between dilution experiments and parallel incubations with either nutrient or copepod addition. Linear regression analysis (Model 1) was used to describe the association between chl *a* concentration ($\mu g\ L^{-1}$) on grazing rate (day^{-1}). When relating temperature (°C) to grazing rate (day^{-1}), different regression models were applied and the one with maximal R^2 and p-value was chosen. To determine the relationship of phytoplankton community composition and season, multivariate analysis in PRIMER-E v6 (Plymouth Routines in Multivariate Ecological Research) was used. Multidimensional scaling (MDS) analysis was used to reduce multivariate data of the 58 different taxa that were present over the course of the year. Genus/species groups were fourth-root transformed to reduce bias of taxa with high cell densities. Phytoplankton abundances were compared to season and grazing as well as environmental data. Seasons were delineated as follows: winter=December, January, and February; spring=March, April, and May; summer= June, July, and August; fall=September, October, and November. Variations in environmental conditions were compared to season using analysis of similarity (ANOSIM, PRIMER-E). The association between environmental data and season is described by the global R

statistic, which ranges from -1 to 1, where 1 and -1 indicate strong similarity and dissimilarity respectively and 0 indicates no relationship.

The ratio between grazing rate (g, day^{-1}) and phytoplankton growth rate (μ, day^{-1}) was used to determine percent primary production consumed (%PP consumed, g/μ). Only samples with phytoplankton growth rates > 0.1 day^{-1} were used to eliminate skew as a result of a small denominator. Statistical significance was assigned at p-values ≤ 0.05.

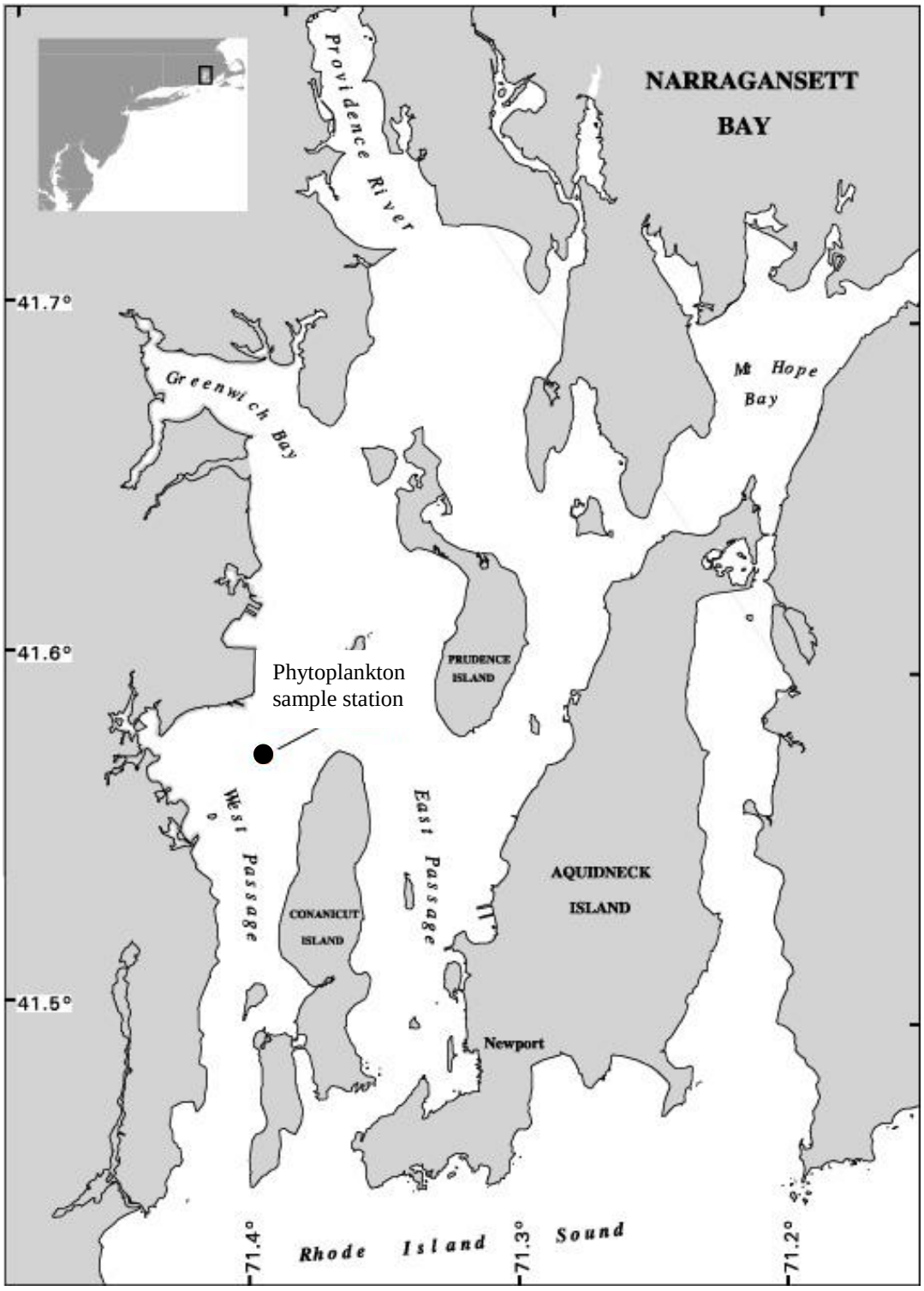

Figure 1. Map of Narragansett Bay, Rhode Island, USA with the location of the long-term phytoplankton monitoring site indicated (41° 34.2'N, 71° 23.4'W, modified from http://gso.uri.edu/phytoplankton).

CHAPTER 3

RESULTS

From January 2010 through February 2011, phytoplankton growth ranged from 0.01 day^{-1} to 2.4 day^{-1} (average 0.69 day^{-1}, Fig 2) in Narragansett Bay. Non-nutrient amended phytoplankton growth rates were fastest during the summer, which was the only season in which no negative growth rates were recorded. During the fall, there was only one week with negative growth, while winter and spring both experienced substantial periods of negative phytoplankton growth (8 of 11 weeks and 8 of 13 weeks respectively, negative points in spring not graphed due to possible nutrient limitation). On average, phytoplankton growth rates were positive for 66% of all weeks sampled. Nutrients did appear to significantly limit phytoplankton growth during the summer ($p=0.007$), when growth increased by 3 to 4 fold after nutrient addition, significantly altering protistan grazing rates (Table 1). In fall and winter, nutrient addition did not significantly increase phytoplankton growth rates ($p=0.48$), and growth rates remained negative even with nutrients added. Addition of the copepod *A. tonsa* did not significantly alter growth or grazing rates. Average growth on 19 July was 1.3 day^{-1} and 1.5 day^{-1} on 6 August, with or without copepods added.

Heterotrophic protist grazing rates were similar in magnitude and seasonal pattern to phytoplankton growth rates (Fig 2). Heterotrophic protist grazing ranged from 0 to 3.7 day^{-1} (average 0.79 day^{-1}). Of the weeks sampled, 18% had negative grazing rates and all instances of negative grazing rates occurred in the winter, generally when phytoplankton growth was also negative. Above average grazing was

observed during the summer and one week several weeks after the 2010 winter-spring bloom, which was observed on 26 January 2010.

The ratio of heterotrophic grazing rates and phytoplankton growth (g/μ) provides a measure of the percent primary production consumed by heterotrophic protists (Fig 3). Between 20 and 200% (average 94%) of primary production was grazed throughout the course of the year. When nutrient limitation was ameliorated by nutrient addition, %PP consumed did not exceed 130%. Percent primary production consumed was greatest in the summer when temperatures were warmest.

Heterotrophic protist grazing rates did not appear to be related to initial chl *a* concentrations (Fig 4). Initial chl *a* ranged from 0.79 to 29.8 μg L^{-1} (%CV= 8.1%) for all weeks, including those with negative growth rates. The entirety of the range of measured grazing rates could be observed at low to intermediate chl *a* concentration. Grazing rates did relate to initial grazer community present (Fig 5). Loricate ciliates tended to dominate during the spring and fall, while heterotrophic dinoflagellates were more abundant during the summer. When heterotrophic dinoflagellates were numerically dominant, the average grazing rate was 1.02 day^{-1}, a factor of 1.2 higher than the overall average grazing rate (0.79 day^{-1}). There was no association between aloricate ciliate concentration and grazing rate. When aloricate ciliates were dominant, above average grazing rates were observed 50% of the time (average grazing rate = 0.70 day^{-1}). However, numerical dominance of loricate ciliates was associated with below average grazing rates. Loricate ciliate dominance was only associated with above average grazing 17% of the time, with an average grazing rate of 0.35 day^{-1}.

Phytoplankton community composition was strongly correlated with season based on a comparison of carbon content of the 10 most abundant phytoplankton using ANOSIM (Table 2). The composition of the phytoplankton community was most similar in winter and spring, while spring and summer were most different from one another ($p = 0.001$). The summer phytoplankton community was most different from all other seasons. These seasonal phytoplankton associations were found irrespective of biomass or numerical abundance of phytoplankton. The only difference between the two analysis approaches is that the difference between spring and summer communities was less pronounced when numerical abundance of all 58 taxa was included rather than carbon content of the 10 most abundant species.

Weekly counts of phytoplankton showed that diatoms were the most numerically abundant. *Skeletonema* spp. was present year round, with maximum abundance in the winter and summer. Flagellates too were abundant year round, though numerical abundance was greatest during the summer and fall. *Thalassiosira nordenskioeldii* and *Heterocapsa cf triquetra* were abundant during the winter and early spring, when temperatures were low (below 12°C). *Leptocylindrus minimus* and *Cylindrotheca closterium* were only abundant during the summer. *Chaetoceros debilis* dominated biomass during the late fall.

Seasonal shifts in environmental conditions in Narragansett Bay appear to be related to changes in temperature, irradiance, wind, salinity, precipitation and surface %DO (Fig 6). Surface temperature varied broadly from 0 to 24°C. Irradiance ranged from 250 to 8600 Wh m^{-2}, averaging 4650 Wh m^{-2}. In the surface, %DO ranged from 78 to 136%; at depth %DO ranged from 47 to 98.5%. Irradiance and temperature

were maximized from late spring to early fall, while %DO at depth was minimized during the summer. Surface %DO appeared more strongly associated with phytoplankton abundance than season. When relating environmental conditions to phytoplankton community composition, temperature appeared to be most strongly correlated with changes in species composition (Spearmen correlation coefficient $\rho=0.289$, $p=0.001$). Temperature had a significant ($p<0.001$) exponential association with grazing (Fig 7). The highest grazing rates occurred when temperatures were warmest, with the exception of one week in February 2010, following the winter-spring bloom (when temp=1.38°C and grazing=1.13 day^{-1}).

Table 1. Comparison of phytoplankton growth and heterotrophic protist grazing rates (day^{-1}) from parallel incubations with and without added nutrients for a subset of all experiments. During summer 2010 phytoplankton growth was nutrient limited ($p=0.007$, * delineate dates with significantly faster growth with nutrients added). During fall and winter 2010, negative growth rates were observed even in nutrient amended incubations and were not significantly different from non-amended incubations ($p=0.48$).Protistan grazing rates increased as phytoplankton growth rates increased.

Date	Growth without nutrients	Growth with nutrients	Grazing without nutrients	Grazing with nutrients
28-Jun-10*	0.54	2.5	0.89	2.45
12-Jul-10*	0.71	2.2	1.36	2.17
26-Jul-10*	1.0	2.9	1.56	3.68
18-Oct-10	0.60	0.65	0.11	0.08
16-Nov-10	0.53	0.61	0.20	0.35
29-Nov-10	0.05	0.01	0.15	0.20
14-Dec-10	0.02	-0.04	-0.04	-0.02
30-Dec-10	-0.01	-0.06	-0.35	-0.32
11-Jan-11	0.05	0.06	0.11	0.09
1-Feb-11	-0.10	-0.17	-0.47	-0.55
21-Feb-11	-0.07	-0.10	0.00	-0.01

Table 2. ANOSIM using the carbon content ($\mu g\ L^{-1}$) of the top ten most abundant plankton species. Plankton community composition in summer and spring were most different from one another and communities in winter and spring were most similar to one another. All values were significant $p < 0.05$ (* indicate $p<0.05$, **indicate $p \leq 0.001$).

Groups	Global R
Summer, Spring	0.57**
Summer, Winter	0.53**
Summer, Fall	0.48**
Winter, Fall	0.43**
Spring, Fall	0.29*
Winter, Spring	0.14*

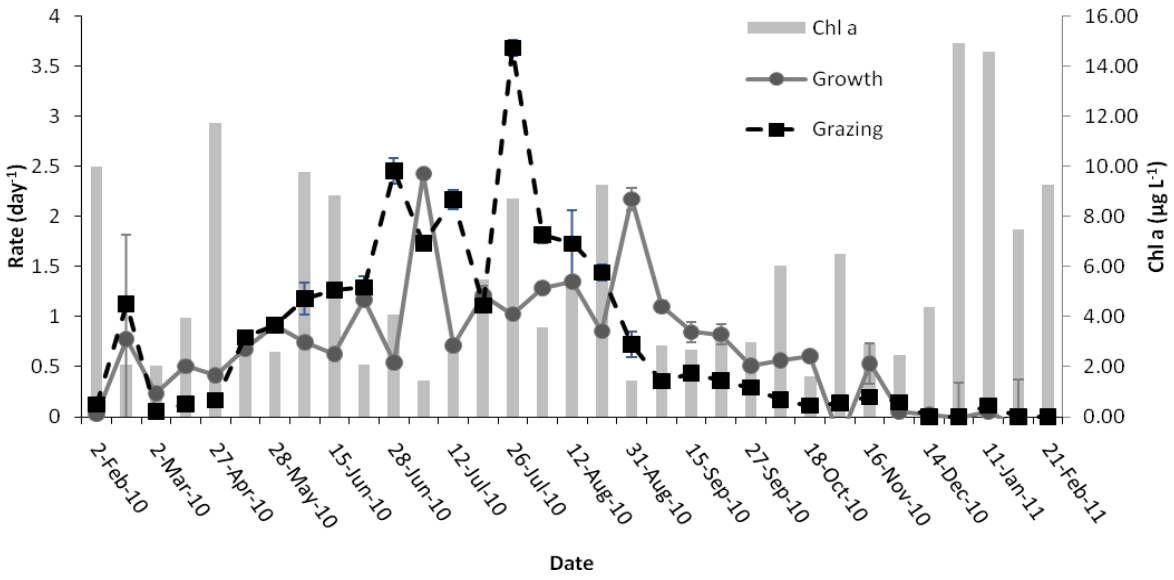

Figure 2. Weekly measured rates of phytoplankton growth (day^{-1}, solid gray line), heterotrophic grazing rates (day^{-1}, dashed line) and chl *a* (µg L^{-1}, grey solid bars) for all dates with positive, or non-nutrient limited growth. Error bars are one standard deviation of triplicate measurements. Experiments with significantly higher phytoplankton growth rates with nutrients added (28 June, 12 July, and 26 July 2010) are represented by the nutrient-amended grazing rates. Phytoplankton growth rates ranged from -0.22 and 2.4 day^{-1} (average 0.68 day^{-1}). Heterotrophic grazing rates ranged from -0.47 to 3.7 day^{-1} (average 0.79 day^{-1}). For the weeks shown, chl *a* ranged from 1.44 to 14.9 ug L^{-1} (average 5.49 ug L^{-1}). Both phytoplankton growth rates and grazing rates were greatest in the summer, while chl *a* ranged broadly throughout the year.

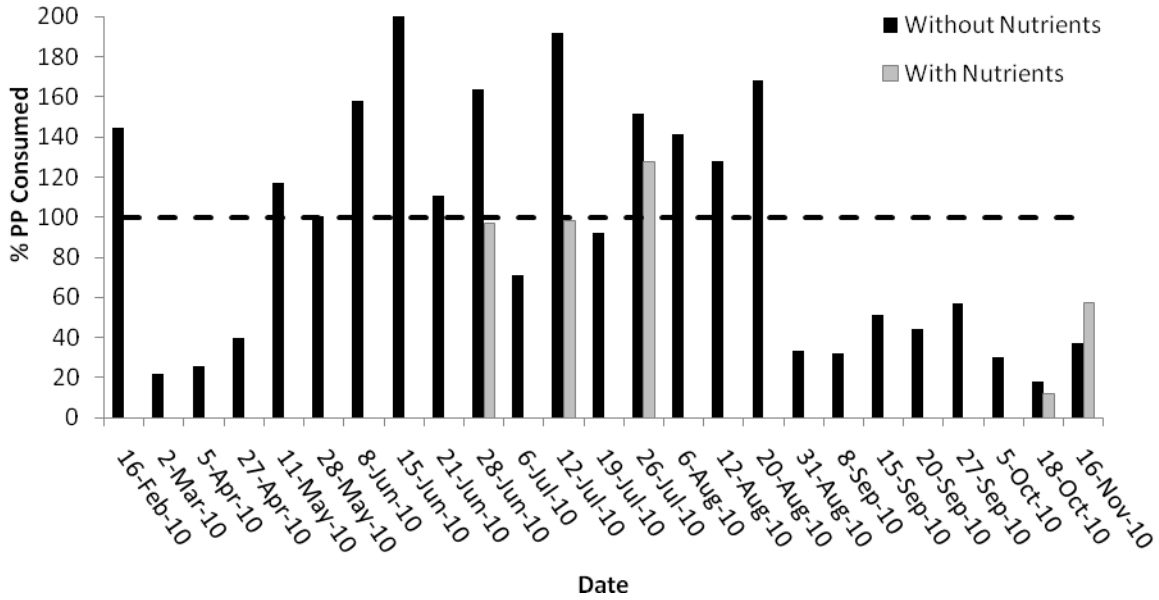

Figure 3. Percent of primary production consumed by heterotrophic protists. Percent primary production consumed ranged from 20 to 200% (average 94%). Dark bars represent %PP consumed for non-nutrient amended treatments, while the light bars indicate %PP consumed when nutrients were added. If percent primary production consumed was greater than 100%, there is a standing stock decrease, if it is less than 100% there is a standing stock increase and grazing conditions are such that a bloom may occur. The horizontal dashed line represents consumption of 100% PP. During the summer grazing rates exceeded phytoplankton growth, depleting phytoplankton stocks. When nutrients were added in the summer, grazing pressure was eased as phytoplankton growth was approximately equal to heterotrophic protist induced mortality.

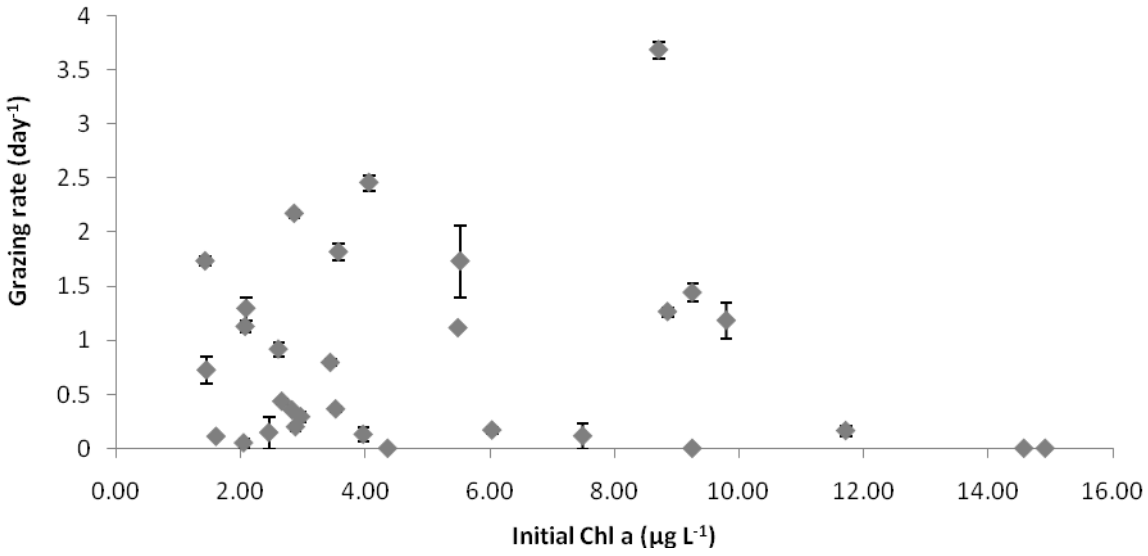

Figure 4. Heterotrophic protist grazing rates (day^{-1}) versus total initial chl *a* concentration ($\mu g\ L^{-1}$). No significant relationship was found between chl *a* concentration and measured grazing rates ($p=0.68$). Error bars indicate one standard deviation of triplicate measures. This indicates that bulk biomass, as measured by chl *a*, is a poor indicator of heterotrophic protist grazing pressure.

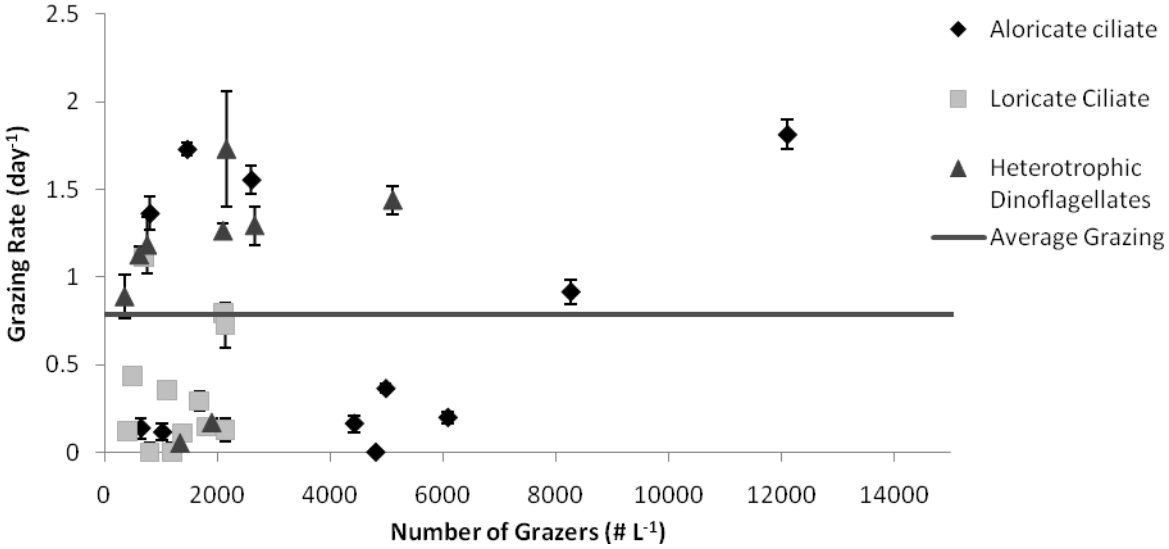

Figure 5. Heterotrophic grazing rate (day^{-1}) versus number of grazers present. Error bars represent one standard deviation of triplicate measures. Broad grazer groupings considered were aloricate ciliates (◆), loricate ciliate (▩) and heterotrophic dinoflagellates (▲). The solid line represents average grazing rates for all dates sampled. One point with > $3x10^4$ aloricate ciliates L^{-1} and a grazing rate 0 day^{-1} was omitted from the graph. Heterotrophic dinoflagellates dominance was associated with above average grazing rates 78% of the dates. There was no association between loricate ciliates and grazing rates (50%) and loricate ciliates were associated with below average grazing (17% above average).

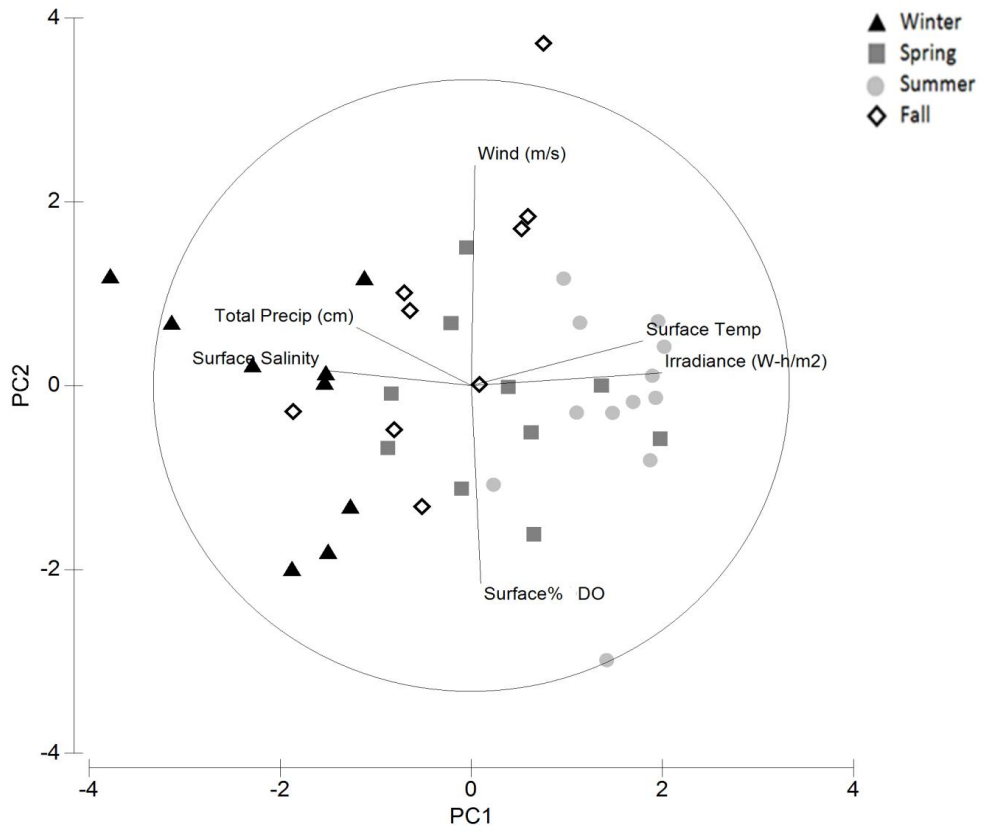

Figure 6. Principal component analysis of environmental conditions categorized by the season during which the sample was taken. Axes 1 and 2 explain 62% the total variance observed. Data points for 3 weeks representing extreme flooding in March 2010 were removed as they obscured all other relationships. Winter dates were closely related and were generally associated with increased precipitation and reduced salinity, temperature and irradiance, while summer, whose dates were also closely related, was more strongly associated with increased temperature, irradiance and salinity but reduced precipitation. Fall and spring do not appear to group as strongly as summer and winter, indicating they were broadly associated with all variables. Seasonal shifts in the environment appeared to be most strongly related to changes in temperature, irradiance, wind and %DO.

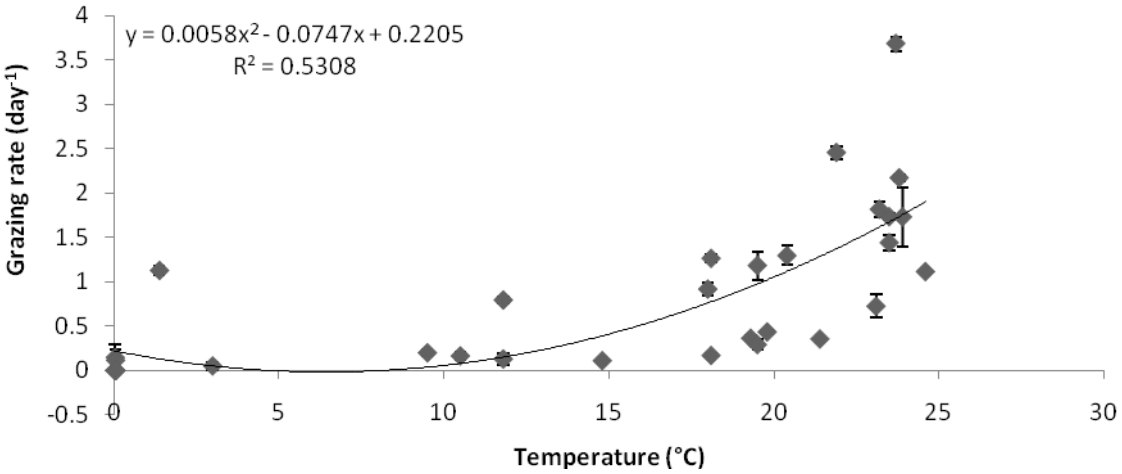

Figure 7. Heterotrophic grazing rate (day^{-1}) versus surface temperature for all weeks with positive or non-nutrient limited growth rates. Error bars represent standard deviation of triplicate measures. There was a significant, exponential relationship between temperature and grazing rate ($p<0.001$).

CHAPTER 4

DISCUSSION

Protistan Grazing

Previous studies have found that on average heterotrophic protists graze over half of daily global primary production (Calbet and Landry, 2004). In Narragansett Bay heterotrophic protists consume up to 200% of primary production during the summer and nearly 100% on average. In this study and in general, protistan grazing rates often exceed phytoplankton growth, which demonstrates a mechanism for the majority of phytoplankton mortality. In highly productive estuaries, it is especially important to understand the magnitude of protistan grazing in order to understand the plankton community dynamics.

Though grazing was substantial, especially in the summer, seasonal changes in chl *a* concentration were not a predictor of grazing in Narragansett Bay. A lack of relationship between chl *a* as an indicator of prey abundance and grazing rate has previously been observed in the North Pacific (Strom et al, 2001; Sherr et al., 2009; Menden-Deuer and Fredrickson, 2010). Chl *a* as a measure of apparent prey availability may be a poor indicator because chl *a* does not access the palatability of the prey item to predators. Though grazing by protists in Narragansett Bay was not related to the bulk biomass available, as measured by chl *a*, protistan grazing was related to the abundance of specific organisms in the Bay. An increase in the numerical abundance of phytoplankton in the Bay was related to grazing rates greater than 1 day^{-1}. When grazing rates were high (>1 day^{-1}), *Skeletonema* spp., *Chaetoceros*

spp., and *Letocylindrus miniums* cell concentrations were 5 to 60 times greater than when grazing rates were low (<0.5 day^{-1}). Grazing rates were lower when organisms such as *Chaetoceros socialis, Thalassiosira nordenskioeldii* and *Heterocapsa cf triquetra* were numerically abundant. *Heterocapsa triquetra* is a common dinoflagellate in estuarine systems and often blooms when heterotrophic grazing pressure is low (Litaker et al., 2002). *Chaetoceros socialis* forms large colonies and *T. nordenskioeldii* is a large chain forming organism with chitinous threads extending outward, perhaps making these species difficult for protists to ingest, though it was not within the scope of this study to determine prey palatability to protists.

There was a strong relation between grazing and *Skeletonema* spp. abundance. High grazing rates were associated with increased abundance of *Skeletonema* spp. A historical study of protistan grazing in Narragansett Bay conducted by Verity (1986) found that protistan grazers consumed on average 62% of daily primary production, while in our study protists consumed 94% of daily primary production. Verity's study was conducted in 1982, shortly after a shift to lower abundance of *Skeletonema* spp. occurred; however, during our study, *Skeletonema* spp. concentrations were comparable to those before the 1980 shift (Borkman and Smayda, 2009a). The 1980 decrease in *Skeletonema* spp. abundance appeared to be associated with transition from a negative NAO to a positive NAO regime (Borkman and Smayda, 2009b). Shifts to increased concentrations of *Skeletonema* spp. abundance may again be a result of a return to a negative NAO regime (NOAA Climate Prediction Center). Grazing rates in Narragansett Bay appear to have increased as levels of *Skeletonema* spp. abundance have increased. The magnitude of grazing may be greater in the

coming decades if the NAO remains negative and if a long-term association between protistan grazing rates and *Skeletonema* spp. does exist. Grazing by heterotrophic protists may be better parameterized by plankton community composition and abundance than by bulk biomass as measured by chl *a*.

Seasonal Patterns

Seasonal patterns were observed in multiple measures characterized by changes in temperature and phytoplankton community composition. Phytoplankton growth in Narragansett Bay was greatest during the summer in spite of apparent nutrient limitation. Grazing by heterotrophic protists was also greatest during the summer, grazing up to 3.7 day^{-1} or 130% of the non-nutrient limited standing stock. This begs the question: how are high rates of phytoplankton growth and biomass able to persist in spite of nutrient limitation and substantial grazing pressure? We suggest that the rate of nutrient recycling by protistan grazers was great enough to continually stimulate phytoplankton growth. Heterotrophic protists are efficient nutrient recyclers (Sherr and Sherr, 2002; Sherr and Sherr, 2009; Glibert, 1997), especially as temperatures increase (Glibert et al., 1992). Sustained grazing by heterotrophic protists on diatoms may have recycled nutrients, allowing phytoplankton growth to persist, rather than loss of nutrient as a result of export to the benthos via copepod fecal pellets or diatoms sinking (Legendre and Rassoulzadegan, 1996; Turner, 2002). Excretion of nutrients by heterotrophic protists may lead to persistence of the bloom, especially when nutrients are limiting during the summer.

Environmental conditions often impact phytoplankton growth and protistan grazing, but grazing may impact environmental conditions as well. During the

summer, temperatures and irradiance increased, providing suitable environment for phytoplankton growth. Though growth was quite substantial throughout the summer, sinking of organic matter never led to oxygen depleted conditions near the benthos typical of hypoxic conditions (hypoxia defined as $\leq$3 mg L^{-1}). Hypoxia is uncommon at this location (Bergondo, 2006; Deacutis et al., 2006; Deacutis, 2008); however, on one occasion bottom dissolved oxygen was as low as 3.7 mg L^{-1}. This low bottom oxygen concentration (6 July 2010) occurred one week after the only date during the summer with below average heterotrophic grazing rates (28 June 2011). Perhaps reduction of grazing pressure by heterotrophic protists in the water column increased export of organic matter and degradation in the benthos, reducing benthic oxygen concentrations. While there are many factors that influence hypoxia, we speculate that grazing by heterotrophic protists can reduce the likelihood of hypoxic events during summer periods with high phytoplankton biomass. While changes in environmental conditions are well described to induce changes in the biological factors, feedbacks of biology on environment have been documented less frequently. Protistan grazing may reduce benthic export, thus reducing benthic oxygen limitation.

Environmental conditions during the winter were suitable for bloom formation during both winters sampled. Borkman and Smayda (2009a) found that winters with bright, cold and windy conditions as well as low abundance of the copepod *Acartia hudsonica* are suitable for *Skeletenema* spp. bloom formation. In both 2010 and 2011, these conditions were present and blooms did occur, but blooms differed each year. In 2010, there was a large, rapid spike in *Skeletonema* spp. abundance but growth appeared to be negative, suggesting that the experiment may have been conducted

after the initiation and development of the bloom and when phytoplankton growth was no longer positive. In 2011, peak chl *a* concentrations were observed concomitant with the only positive winter phytoplankton growth rates. It is noteworthy that phytoplankton growth was only slight (0.05 day^{-1}), suggesting growth need not be very large in order to reach bloom concentrations (Behrenfeld, 2010). There was no peak in protistan grazing seen after the *Skeletonema* spp. bloom; however, there appeared to be an increase in copepod abundance, though copepod abundance was not enumerated systematically in this study. This suggests that both years had suitable environmental conditions for bloom formation, but the mechanism for termination may have been different. It is possible that weekly sampling frequency was not great enough to resolve protistan grazing activity immediately after the bloom in either case. Copepod additions with the dominant winter species *Acartia hudsonica* could shed light on bloom termination mechanisms. This could be valuable knowledge if *Skeletonema* spp. abundance continues to remain high as *Skeletonema* spp. has been attributed great importance as an environmental indicator in Narragansett Bay (Oviatt et al., 2002) and to compare historical observations (Borkman and Smayda 2009a) of environmental controls on phytoplankton abundance and bloom formation to present conditions.

Acartia tonsa did not appear to have a significant impact on phytoplankton growth and phytoplankton grazing during the summer. Copepod grazing has clearly been shown to be important in Narragansett Bay (Gifford and Dagg, 1988; Thompson et al., 1994). It is possible that *A. tonsa* did have a significant impact on phytoplankton growth and protistan grazing, but the true impact may not have been

discernable because nutrients were limiting. Copepod additions were conducted during the summer, when nutrients significantly limited phytoplankton growth; however, no nutrients were added to incubations with *A. tonsa*. It is therefore possible that the impact of *A. tonsa* was not apparent because nutrient limitation masked the influence of the predator on both phytoplankton and protists. *Acartia tonsa* should be added to nutrient-amended dilution experiments in order to determine if grazing by *A. tonsa* significantly alters plankton dynamics when nutrients are non-limiting.

Dominant Grazers

Heterotrophic dinoflagellates and aloricate ciliates were associated with higher grazing rates than loricate ciliates. Dinoflagellates have high metabolic costs (Geider and Osborne, 1989; Langdon 1993; Hitchcock et al., 2010), and may graze at higher rates to meet energy demands. Heterotrophic dinoflagellates may be especially successful grazers in Narragansett Bay as they are known to be dominant grazers of larger phytoplankton such as diatoms (Sherr and Sherr, 2009) and have long starvation capacity (Menden-Deuer et al, 2005). Loricate ciliates do not graze large diatoms effectively (Verity and Villareal, 1986). Verity (1986) found that loricate ciliates were dominant grazers at this site; however, heterotrophic dinoflagellates were not included in his analysis. Aloricate ciliates did not dominate during any particular season and were thus abundant when there were variable phytoplankton communities. The average number of total grazers was greatest when aloricate ciliates were dominant. When aloricate ciliates were dominant and grazing rates were above average, all 3 grazer groups were present, perhaps indicating that aloricate ciliate grazing success was attributable to diversified grazing communities which could graze on diverse

phytoplankton communities. Diverse grazer communities can exploit many prey types, which may lead to apparent increase in grazing as a result of increasingly varied phytoplankton and grazer community. Grazing rates may depend upon grazer group present and the ability of that grazer to successfully process the phytoplankton community that is present.

CHAPTER 5

CONCLUSION

In Narragansett Bay, grazing by heterotrophic protists was important as protists consumed a vast fraction of primary production. It is impossible to say whether biotic or abiotic factors contributed most strongly to the grazing rates observed. Both temperature and species composition were related to changes in grazing rates and changed concomitantly with season. Rose et al. (2009) were also unable to determine whether bottom-up or top-down factors controlled grazing rate variability, finding that temperature increase resulted in a changed protistan community composition and physiology but also influenced phytoplankton community composition. Factors such as temperature and plankton species composition may be better related to grazing than chl *a*, which is commonly used to parameterize grazing.

APPENDICES

The following tables and figures are those from the grazing experiment that were not included in the manuscript.

Table A1. Sizes of the 10 most abundant species. Over 100 cells of each species or genus was measured using a Stingray camera on an epifluorescent microscope and analyzed using ImageJ. *Chaetoceros* spp., and to a lesser degree *Thalassiosira* spp., have large %CV which is probably as a result of the broad morphological diversity of across genera. *Skeletonema* spp. may have a large %CV as a result of seasonal differences in volume.

Organism	***volume type***	***Average Volume (cubic microns)***	***% CV***
Chaetoceros debilis	prolate spheroid	20768	62.0
Chaetoceros socialis	prolate spheroid	4822	69.8
***Chaetoceros* spp**	prolate spheroid	1772	277
Cylindrotheca closterium	prolate spheroid	631	92.9
Flagellate unknown	sphere	141	81.4
Heterocapsa/Scripsiella spp	prolate spheroid	37423	32.3
Leptocylindrus minimus	cylinder	409	66.0
***Skeletonema* spp.**	prolate spheroid	333	86.6
Thalassiosira nordenskoeldii	cylinder	26655	68.3
***Thalassiosira* spp.**	cylinder	23343	79.1

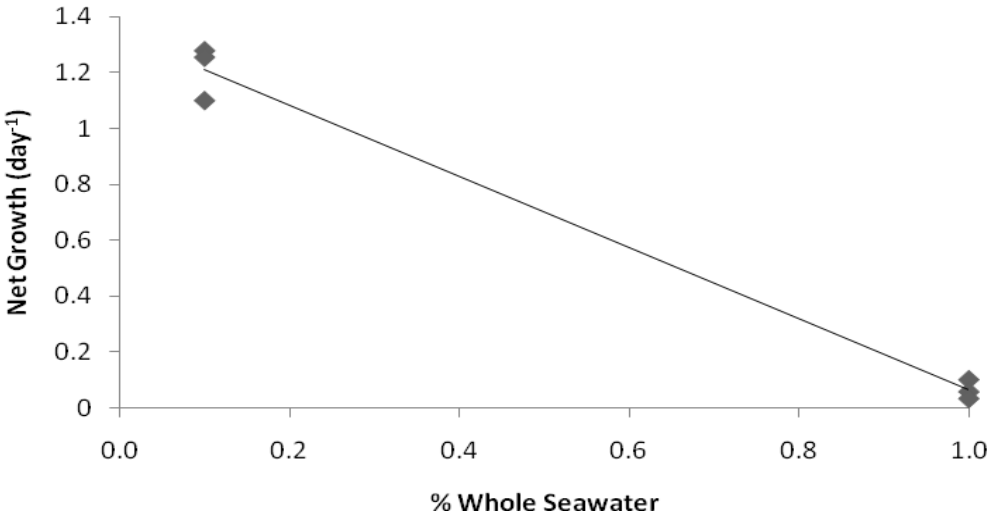

Figure A1. Example of a two-point dilution experiment outcome. Each point represents the growth rate (*k*, day^{-1}) determined using the exponential growth equation $k=(1/t)(\ln(P_t/P_0)$. The differential growth between the full versus reduced grazing pressure is used to estimate the grazing rate, which can also be determined by taking the slope of line of regression. The y-intercept of this line provides an estimate of the intrinsic growth rate (μ).

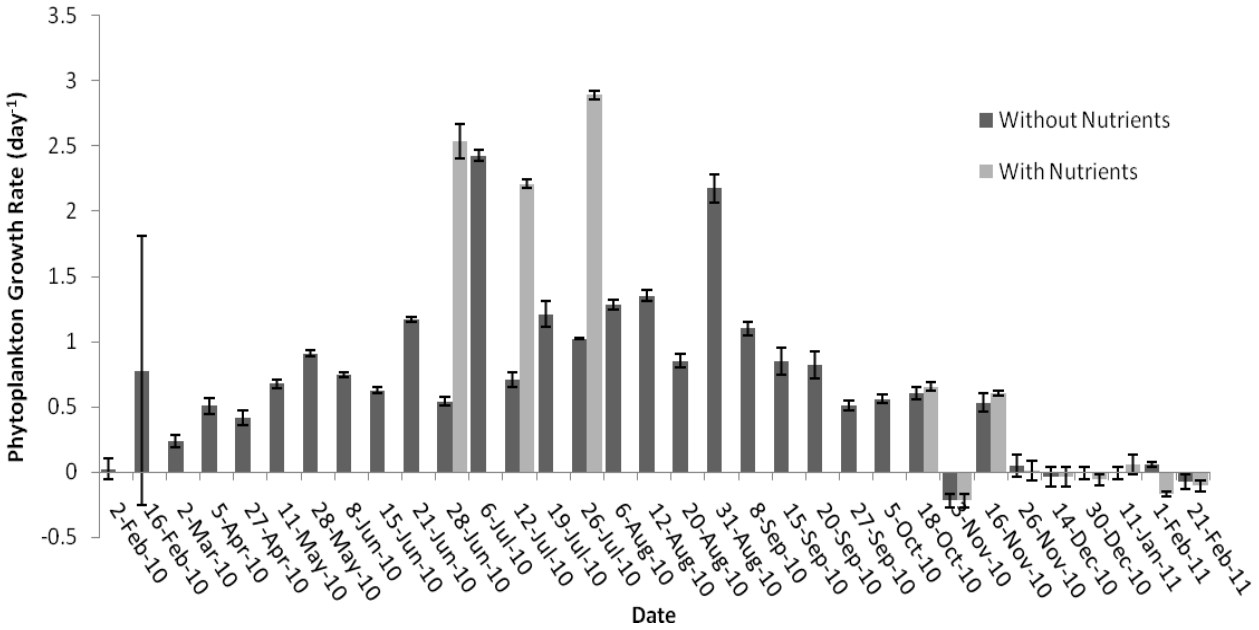

Figure A2. Phytoplankton growth rates for all dates with positive or non-nutrient limiting growth. Throughout the sampling period, 66% of the weeks had positive growth (29 of 44). During the winter 30% (3 of 10 weeks) had positive growth, spring had 46% (6 of 13) positive, summer 100% (12 of 12) positive, and fall 89% (8 of 9 weeks). Of these, winter had 4 weeks that were not nutrient limited. Interestingly, the date of the winter-spring bloom (26 Jan 2010) experienced negative net growth. Perhaps this negative growth was, in fact, as a result of nutrient (Si) limitation. One cannot say whether the 7 weeks with negative net phytoplankton growth was due to nutrient limitation or not as nutrient amended experiments were not conducted at this time. The weeks with negative growth during the fall and winter 2010/2011 were not nutrient limited.

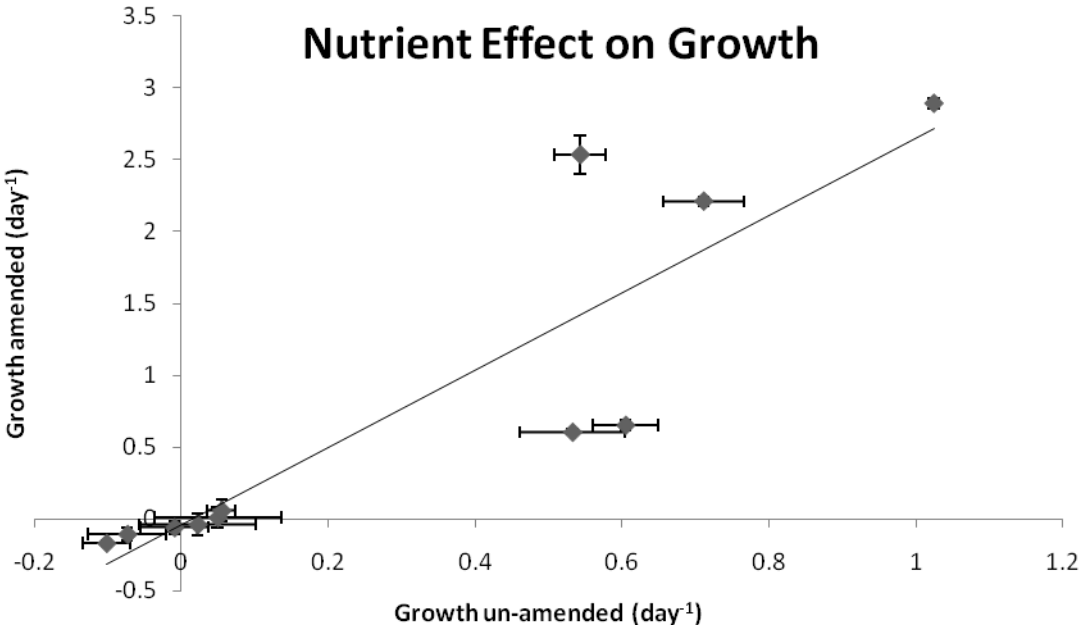

Figure A3. Phytoplankton growth rates for nutrient amended dilution experiments versus phytoplankton growth rates in parallel dilutions without nutrients added. The equation for the line is y=2.6925x-0.0396, indicating that phytoplankton growth rates were underestimated in experiments that were not nutrient amended, especially as the growth rate increased.

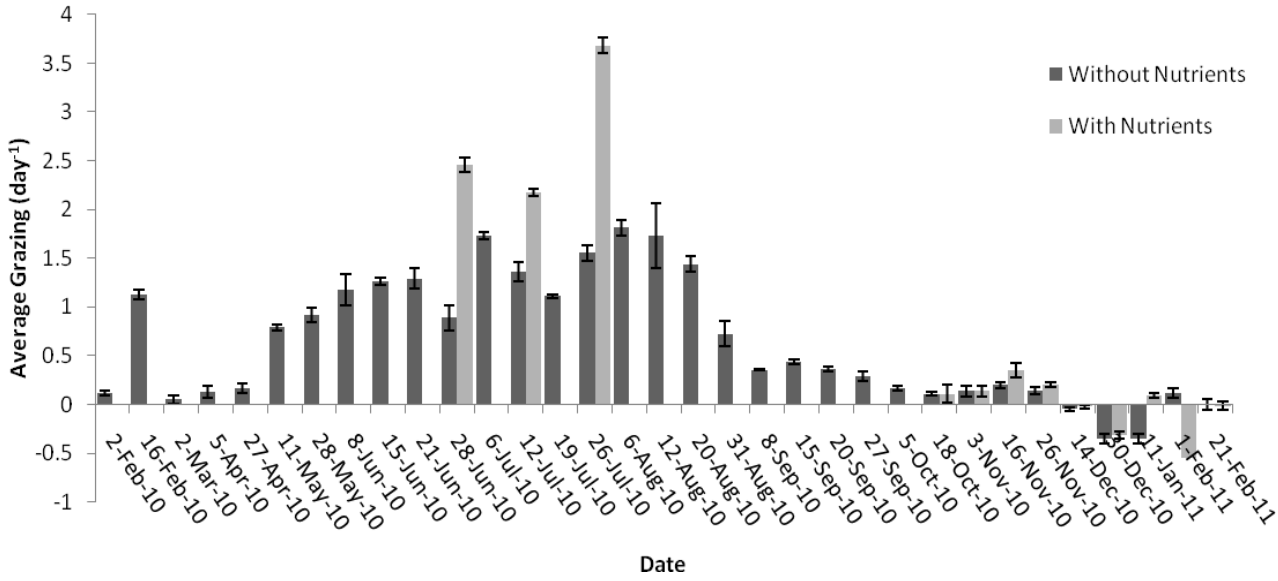

Figure A4. Mortality due to grazing for all dates with positive or non-nutrient limited growth. Grazing appeared to be highest during the summer. Samples appeared to be nutrient limited during the summer but not in the fall or winter for which nutrient experiments were conducted. Heterotrophic grazing rates ranged from 0 to 3.7 day^{-1} (average 0.79 day^{-1}, negative grazing values were set to 0 in calculations of range and average) and were greatest in the summer. During the summer 83% of weeks exhibited above average grazing rates, while for the whole data set, only 33% of weeks had above average grazing.

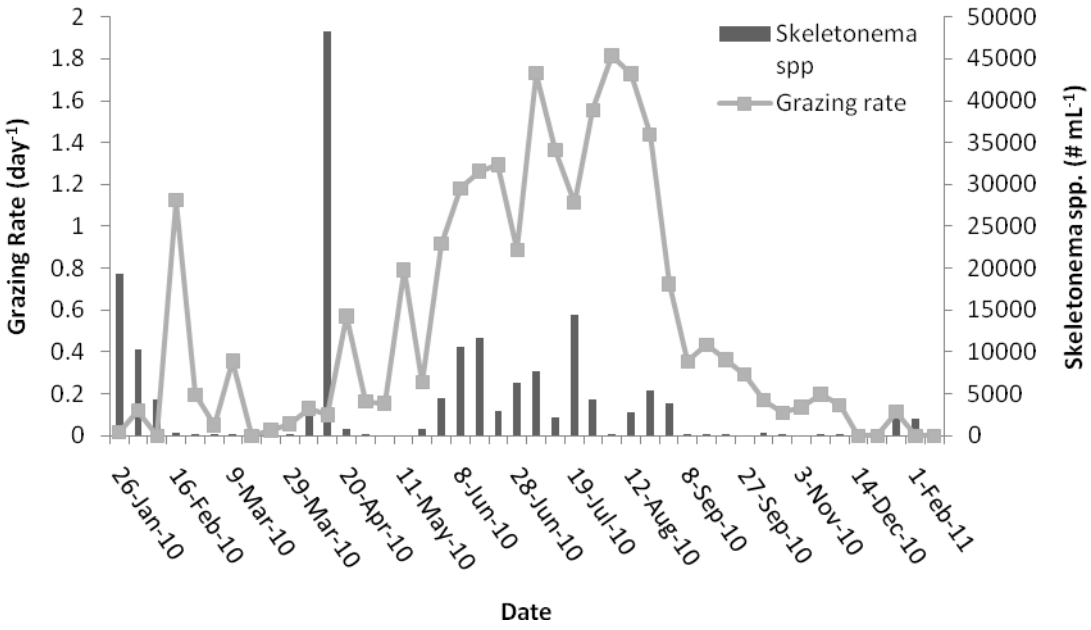

Figure A5. Abundance of *Skeletonema* spp. over the course of the dilution experiments compared to heterotrophic grazing rates. Grazing appears to increase a week to a few weeks after *Skeletonema* spp. increases. *Skeletonema* spp. bloom formation in April was as a result of extreme flooding in late March.

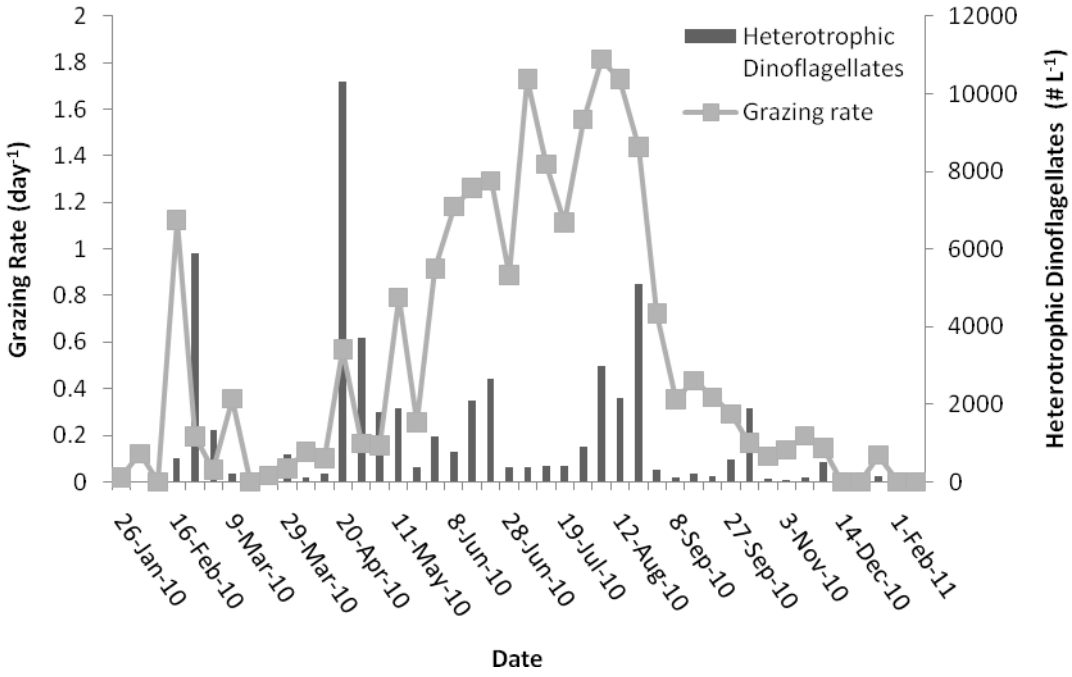

Figure A6. Heterotrophic grazing rates and known heterotrophic dinoflagellates throughout the year for which dilution experiments were conducted. Abundance of heterotrophic dinoflagellates often seem to be related to grazing rates, though the two are not significantly related (linear correlation, p=0.12).

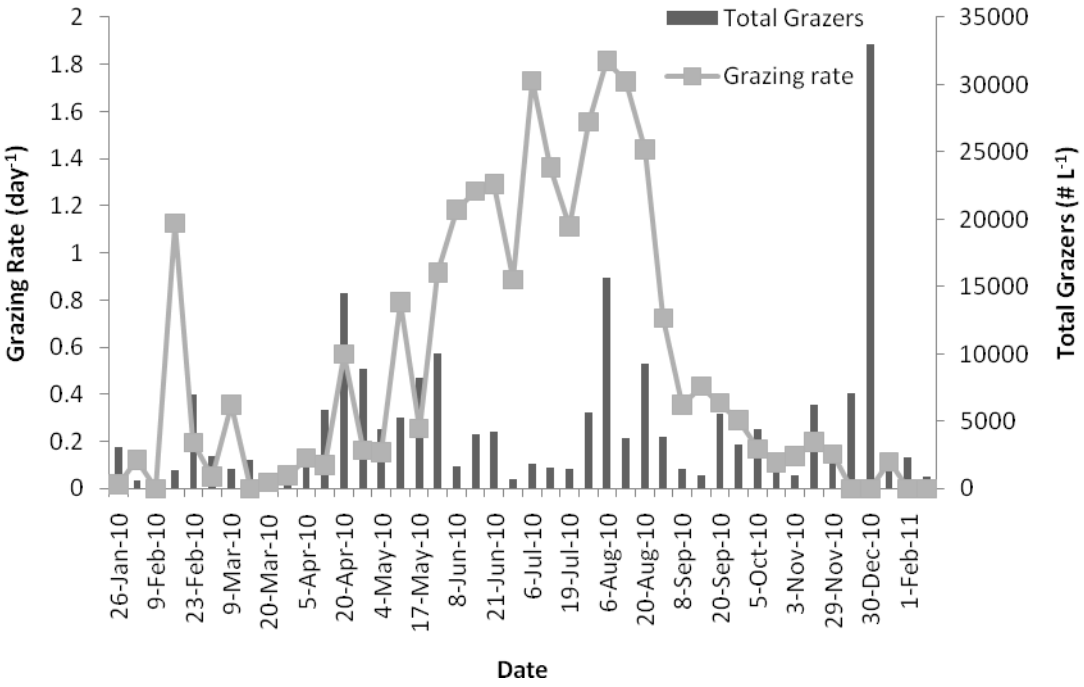

Figure A7. Heterotrophic grazing rate and total grazer abundance throughout the year for which dilution experiments were conducted. There appears to be no relationship between heterotrophic protist abundance and grazing rates (linear correlation p=0.81).

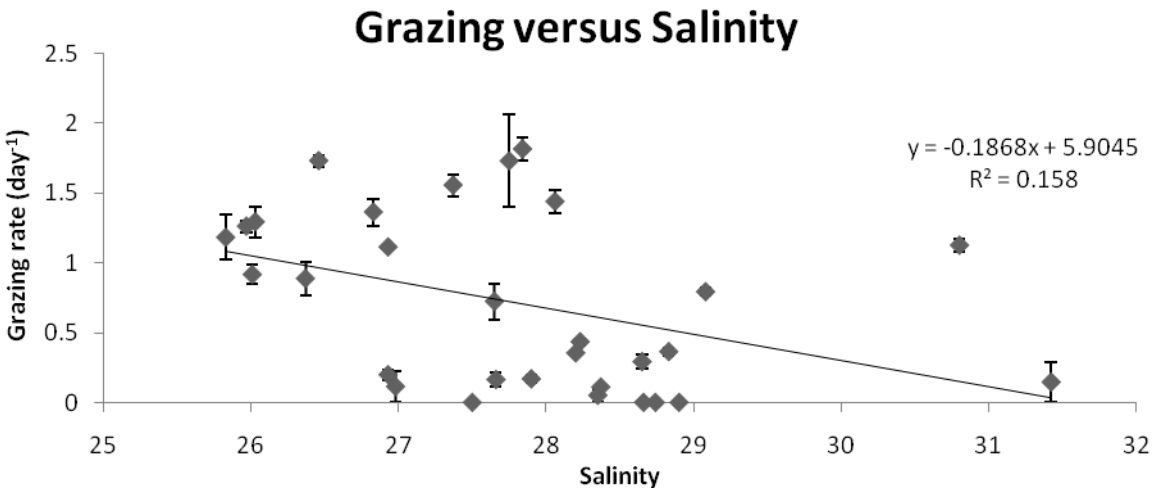

Figure A8. Grazing rate versus salinity for all weeks with positive growth. There is a significantly negative relationship between salinity and grazing rate (p=0.03). Error bars represent standard deviation of triplicate measures. An extreme flooding event was removed as it represented one week with an extreme freshwater bias.

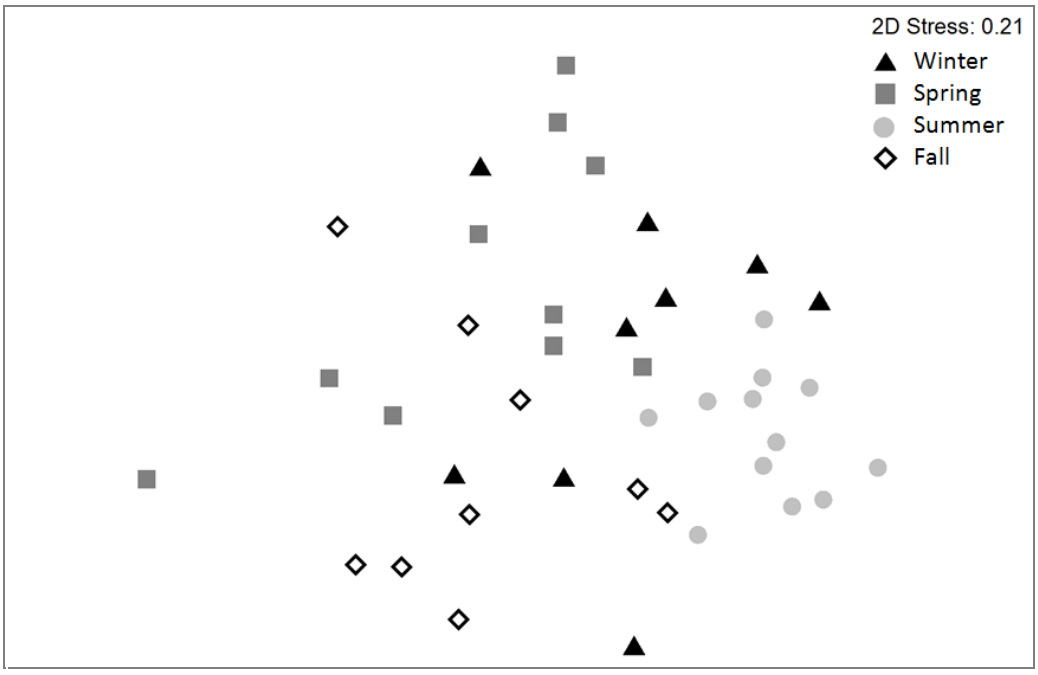

Figure A9. MDS representing the association of plankton community with season, generated by PRIMER-E. Each point represents the plankton community composition for a specific date during a specified season (Winter=December, January, February; Spring= March, April, May; Summer= June, July, August; Fall= September, October, November.) Values have been fourth root transformed. Communities in winter and spring are most similar to one another, while the communities of fall and summer were most different (ANOSIM, p=0.001). Data points for 3 weeks, representing extreme flooding in March 2010 were removed as they obscured all other relationships.

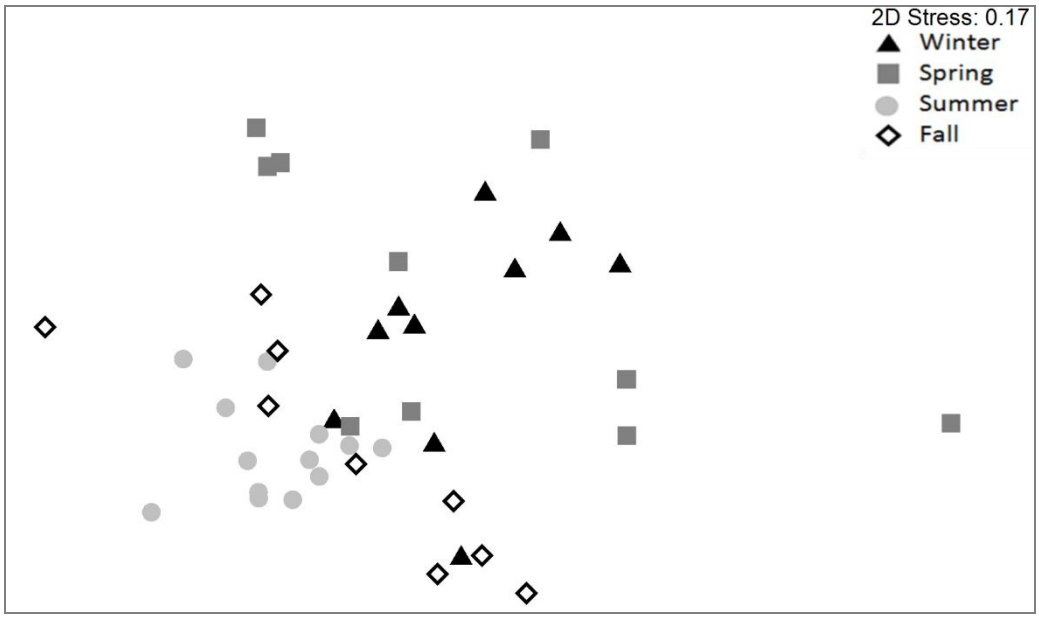

Figure A10. MDS representing the association of carbon content of top 10 most abundant plankton species with season, generated by PRIMER-E. Each point represents the plankton community composition for a specific date during a specified season (Winter=December, January, February; Spring= March, April, May; Summer= June, July, August; Fall= September, October, November.) Values have been fourth root transformed. Communities in winter and spring are most similar to one another, while the communities of fall and summer were most different (ANOSIM, p=0.001). Data points for 3 weeks, representing extreme flooding in March 2010 were removed as they obscured all other relationships.

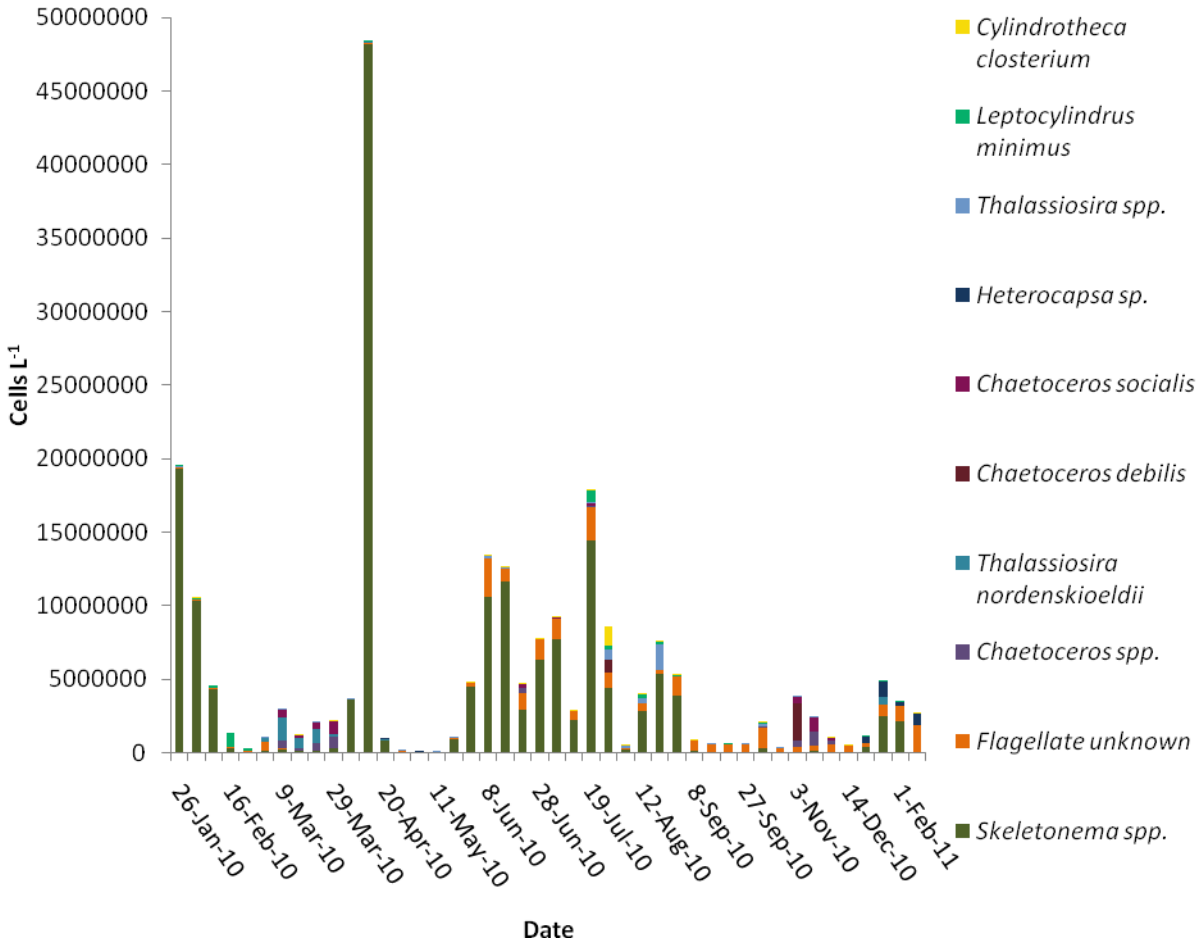

Figure A11. Numerical abundance of the top ten most abundant species for the year during which the dilution experiments were conducted. The large peak in early April is as a result of a *Skeletonema* spp. bloom after an extreme flooding event.

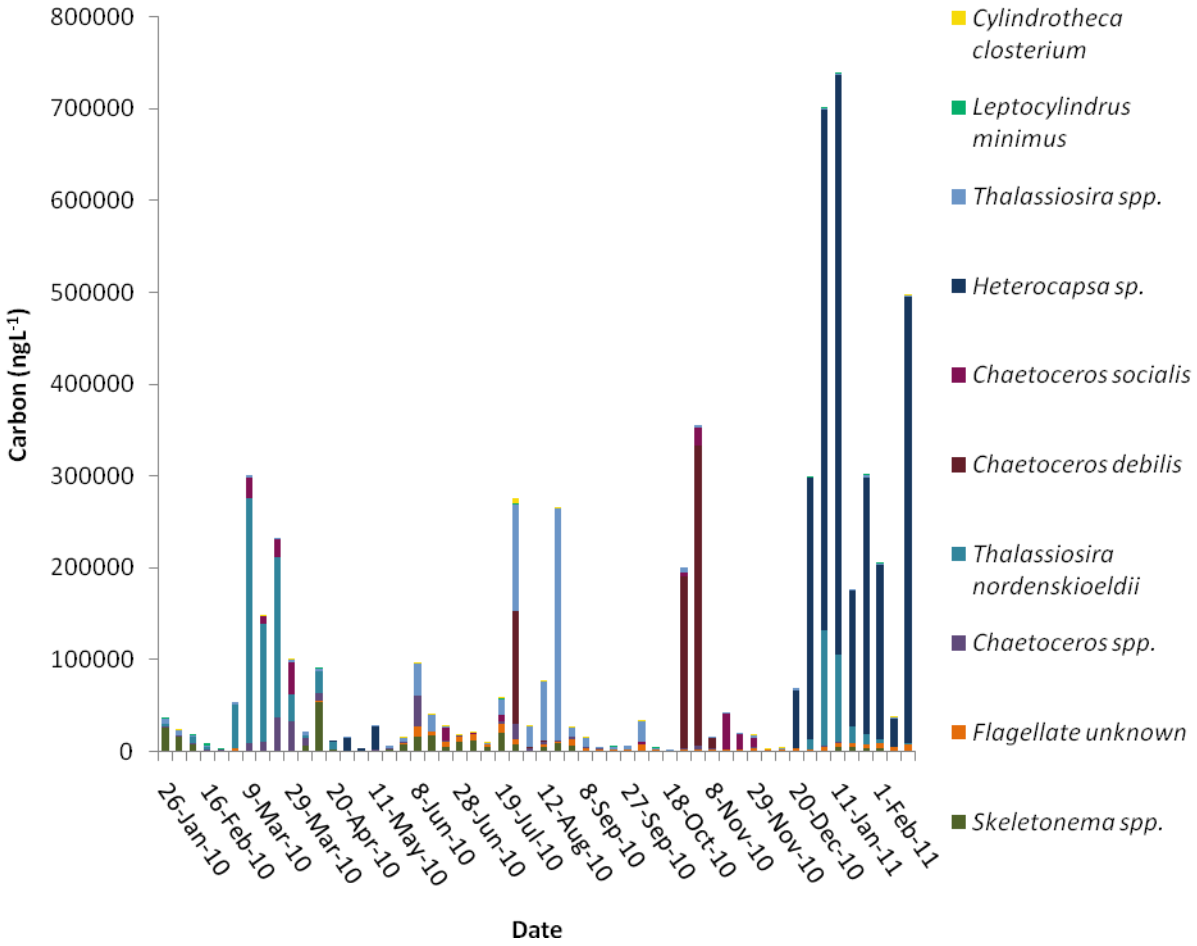

Figure A12. Carbon content (ng L^{-1}) of cells present for each date during which the dilution experiment was conducted. The apparent numerical dominance of *Skeletonema* spp. is overshadowed by those cells, that while less abundant, are larger, and thus contain more carbon. Carbon content is determined using the conversion from Menden-Deuer and Lessard (2000).

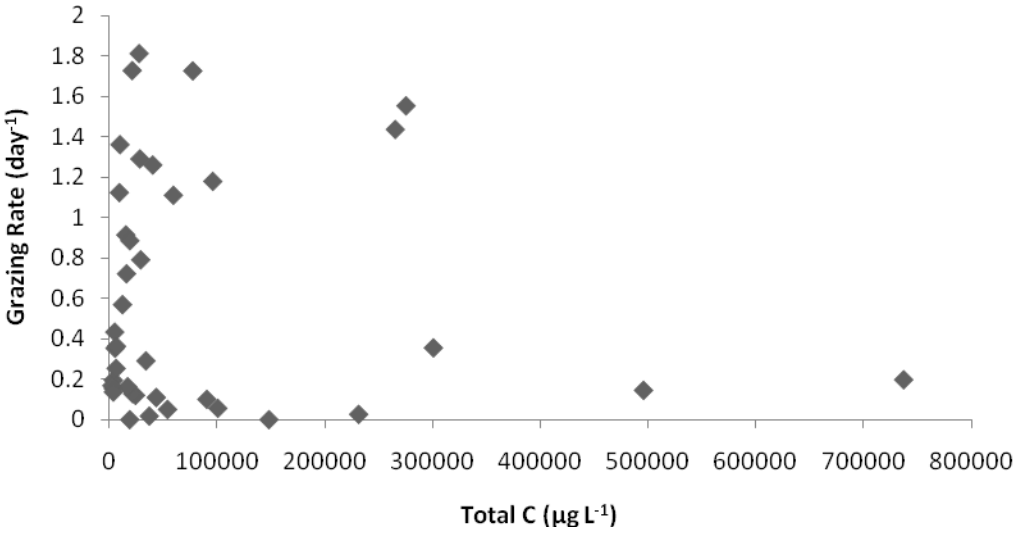

Figure A13. Heterotrophic grazing rate versus total carbon content (µg L^{-1}). Grazing rate does not appear to be related to the total carbon content (the top 10 most abundant plankton groups) of the initial phytoplankton composition (linear correlation, p= 0.65).

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