

University of Rhode Island

DigitalCommons@URI

Open Access Master's Theses

2013

SEPARATING THE EFFECTS OF TEMPERATURE AND COMMUNITY COMPOSITION ON THE MAGNITUDE OF HETEROTROPHIC PROTIST GRAZING RATES IN NARRAGANSETT BAY

Samantha C. DeCuollo

University of Rhode Island, samantha.decuollo@gmail.com

Follow this and additional works at: <https://digitalcommons.uri.edu/theses>

Terms of Use

All rights reserved under copyright.

Recommended Citation

DeCuollo, Samantha C., "SEPARATING THE EFFECTS OF TEMPERATURE AND COMMUNITY COMPOSITION ON THE MAGNITUDE OF HETEROTROPHIC PROTIST GRAZING RATES IN NARRAGANSETT BAY" (2013). *Open Access Master's Theses*. Paper 168.
<https://digitalcommons.uri.edu/theses/168>

This Thesis is brought to you by the University of Rhode Island. It has been accepted for inclusion in Open Access Master's Theses by an authorized administrator of DigitalCommons@URI. For more information, please contact digitalcommons-group@uri.edu. For permission to reuse copyrighted content, contact the author directly.

SEPARATING THE EFFECTS OF TEMPERATURE AND COMMUNITY
COMPOSITION ON THE MAGNITUDE OF HETEROTROPHIC PROTIST
GRAZING RATES IN NARRAGANSETT BAY

BY

SAMANTHA C. DECUOLLO

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
OCEANOGRAPHY

UNIVERSITY OF RHODE ISLAND

2013

MASTER OF SCIENCE THESIS

OF

SAMANTHA C. DECUOLLO

APPROVED:

Thesis Committee:

Major Professor Susanne Menden-Deuer

David Smith

Tatiana Rynearson

Gavino Puggioni

Nasser H. Zawia
DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND
2013

ABSTRACT

Understanding the relative effects of phytoplankton assemblage and temperature on heterotrophic protist grazing rates remains underdeveloped due to seasonal constraints that result in concurrent changes in both variables. In order to separate effects of temperature and community composition on microherbivory, we used the dilution method to measure grazing rates at *in-situ* and cooled incubation temperatures conducted in parallel during summer/autumn 2012, in Narragansett Bay, Rhode Island, USA. Chain-forming diatoms dominated the microphytoplankton, whereas aloricate ciliates dominated the microzooplankton. Weekly environmental variability –not primarily characterized by temperature– had a significant effect on phytoplankton-species composition. Initial autotrophic biomass averaged $127 \mu\text{g C L}^{-1} \pm 149$ and heterotrophic biomass averaged $459 \mu\text{g C L}^{-1} \pm 281$. Temporal change was the principal factor associated with assemblage structure differences, having a greater effect than temperature and incubation. Total autotrophic biomass increased significantly, 600% at ambient and >200% at cooled temperatures, resulting in a significant change in the phytoplankton assemblage structure over the incubation period. Ambient phytoplankton growth and grazing rates averaged $1.77 \text{ d}^{-1} \pm 0.53$ and $0.63 \text{ d}^{-1} \pm 0.41$, respectively. Temporal changes in phytoplankton species composition did not have a significant effect on grazing rates. An average 6.4°C decrease in temperature significantly lowered rates by an average of 1.9-fold for growth and 3.3-fold for grazing. The percent primary production consumed was on average 1/3 lower

in the cooled treatment. These results suggest that temperature plays a larger role in regulating grazing magnitude than phytoplankton prey species composition.

ACKNOWLEDGMENTS

I would like to thank my major professor Susanne Menden-Deuer for her tremendous mentorship and attentiveness that she has given me in support of my work. This manuscript was greatly improved by the critical review of Drs. S. Menden-Deuer, M. Gomez-Chiarri, T. Ryneerson, D. Smith, G. Puggioni, and E. Harvey. Thank you to: Ed Baker with assistance regarding the experimental set-up, lab mates Amanda Montalbano and Françoise Morrison, as well as phytoplankton identification experts Malcolm McFarland, Dr. Jan Rines, and Dr. Lucie Maranda. In addition, thank you to Narragansett Bay Long-Term Monitoring Program director Dr. T. Ryneerson, Graduate School of Oceanography, University of Rhode Island (USA). Funding for this project was provided by the University of Rhode Island (URI) in support of the Narragansett Bay Long-Term Monitoring Program. Additional funding was provided to S.M.D. by the Office of Naval Research grant no. 0002453 and the National Science Foundation grant no. 0001916. A portion of this work was conducted at the Rhode Island EPSCoR supported Center for Marine Life Science under EPSCoR Grant #1004057 to URI for equipment funding.

I also give great thanks to the many scientists whose dedication, knowledge, and expertise helped to make this project a success. Thank you to friends and family for their love and words of encouragement they have provided over the past two years.

PREFACE

This thesis is written in manuscript style rather than using the traditional segregation of the thesis into chapters. The manuscript text is written in the formatting style appropriate for submission to *Marine Ecology Progress Series*, and is followed by appendices containing detailed, ancillary information regarding analysis techniques and additional findings that will likely stand alone in an additional submission, but that may also be included in the published paper.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGMENTS	iv
PREFACE	v
TABLE OF CONTENTS.....	vi
LIST OF TABLES	vii
LIST OF FIGURES	viii
MANUSCRIPT I	1
CHAPTER 1	2
INTRODUCTION	2
CHAPTER 2	8
MATERIALS AND METHODS	8
CHAPTER 3	16
RESULTS	16
CHAPTER 4	28
DISCUSSION	28
APPENDICES	43
LITERATURE CITED.....	49
FIGURE LEGENDS	57

LIST OF TABLES

TABLE	PAGE
Table 1. Monthly average <i>in situ</i> surface temperature from Narragansett Bay	53
Table 2. <i>In situ</i> temperature, chl <i>a</i> , biomass & rates from experiments	54
Table 3. Comparison of nutrient-amended and unamended phytoplankton apparent growth rates	55
Table 4. Average temperatures over incubation period	56
Table A1. Autotrophic biovolume conversions	43
Table A2. Species-specific growth rates	44

LIST OF FIGURES

FIGURE	PAGE
Figure 1. PCA of Narragansett Bay environmental variables.....	60
Figure 2. Biomass estimates of autotrophic and heterotrophic protist community	61
Figure 3. Autotrophic assemblage MDS	62
Figure 4. <i>In situ</i> chlorophyll <i>a</i> concentration and ambient rates	63
Figure 5. Average rates of phytoplankton specific growth rates and heterotrophic protist grazing for ambient and cooled treatments	64
Figure 6. Regression of phytoplankton specific growth rates and heterotrophic protist grazing rates in ambient vs. cooled treatments	65
Figure 7. %PP consumed for ambient and cooled treatments.....	66
Figure A1. Comparison between the apparent growth rates at the lowest dilution and the phytoplankton specific growth rates	48

MANUSCRIPT – I

**Separating the Effects of Temperature and Community Composition on the
Magnitude of Heterotrophic Protist Grazing Rates in Narragansett Bay**

Samantha C. DeCuollo and Susanne Menden-Deuer*

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

USA

*smenden@gso.uri.edu

This manuscript is formatted for the anticipated publication in the scientific journal

Marine Ecology Progress Series (2014).

Key Words: Heterotrophic Protist, Phytoplankton, Grazing, Estuary, Temperature,

Assemblage structure, Dilution

Running head: Temperature's and Assemblage's Effects on Grazing

INTRODUCTION

Single-celled eukaryotic herbivores within the microzooplankton, commonly termed heterotrophic protists, play a prominent role in the marine food web by grazing on average 67% of the daily global phytoplankton production (Landry & Calbet 2004). The magnitude of protistan herbivory determines the amount of photosynthetically derived carbon that becomes available to higher trophic levels as a result of grazing (Deason & Smayda 1982, Stoecker & Capuzzo 1990, Sherr & Sherr 1994) and has been observed to vary both globally and temporally. Though insight regarding the range of grazing magnitude is well established from a plethora of *in situ* heterotrophic protist grazing rates collected around the globe (Landry & Calbet 2004), reliable predictors of grazing magnitude remain un-developed (Li et al. 2011).

Drivers of Protistan Herbivory

Plankton community structure and temperature have both gained considerable attention as potential variables that mediate heterotrophic protist grazing on phytoplankton. Microzooplankton grazing rates have been positively correlated with temperature in the North Pacific (Strom et al. 2001), the Antarctic (Caron et al. 2000), and the Mediterranean (Modigh & Franzè 2009). The findings that temperature is rate limiting may be regarded as a natural progression of the original research conducted by Eppley (1972), which documented that rates of phytoplankton specific growth increase exponentially with temperature up to ~40°C. More recent developments suggest that metabolic rates, including growth, vary according to a specific, quantitative relationship between body size and temperature (Brown et al. 2004).

However, temperature alone cannot predict rate magnitude. For instance, microzooplankton grazing magnitude also varies the available prey type due to predators' prey preferences. Compiled data from dilution experiments show that grazing magnitude has occasionally exceed 0.4 d^{-1} at low temperatures ($<10^{\circ}\text{C}$) and exhibits a wide range ($0 - >2 \text{ d}^{-1}$) at temperatures $>10^{\circ}\text{C}$, thereby suggesting that species composition likely plays a role in altering grazing magnitude (Caron et al. 2000). Additional evidence points towards the ability of heterotrophic protists to detect and/or ingest desirable over less-desirable prey items (Buskey et al. 1997). At least as much importance is attributed to species composition as to temperature by Lawrence & Menden-Deuer (2012), who observed that grazing in Narragansett Bay was most substantial when *Skeletonema* sp. was abundant or when surface temperatures were highest. However, due to the seasonal constraints that resulted in concurrent changes in both temperature and species composition in their study, the relative effect of temperature and prey species composition on grazing rate could not be differentiated quantitatively. Moreover, several studies attempting to identify driving factors in mediating predator prey interactions are specific to certain species and locations, as well as limited by the scope of species and variables considered (reviewed in Caron & Hutchins 2012). Therefore, tremendous value exists behind evaluating grazing rates within complex phytoplankton assemblages and *in situ* to better predict which factors mediate protistan herbivory. Conducting grazing experiments under controlled and manipulated temperatures but constant species composition may help reveal whether species composition or temperature, or both, mediate the magnitude of heterotrophic protist grazing.

Long-Term Plankton Time Series Station in Narragansett Bay

Narragansett Bay is home to the long-term phytoplankton-monitoring project, possibly one of the world's longest running plankton time series. Initiated in 1957, the sampling and processing methods have since expanded; at present, data collection includes a weekly analysis of the biological (i.e. community composition and abundance of phytoplankton, zooplankton, and ctenophores) and physical (i.e. temperature, salinity, turbidity, size-fractionated chlorophyll *a*, and nutrients) parameters.

The historical context of a well-known time series, such as the Long-Term Monitoring Program (Borkman & Smayda 2009), offers a unique opportunity to concurrently analyze the variation in environmental conditions and phytoplankton assemblages. Predictability in sea surface temperature measurements exists for temperate Narragansett Bay. Based on sea-surface temperatures made weekly from 2007 – 2011 spanning a four-month period between June and September at the Long-Term Time Series Station, summertime water temperatures have been predictably warm (~20.5°C); on average, the variation in temperature across the period was small (i.e. 7.9°C), whereas seasonal variation over an annual scale during the same period was tremendous, averaging 23.0°C, with seasonal variations reaching 15.4°C and 15.8°C during the winter and spring seasons, respectively. Additionally at the station, Karentz & Smayda (1984) observed that *Skeletonema* numerically dominated the diatom genera across a twenty-two year period (1959 - 1980) and exhibited a bimodal maximal occurrence during winter-early spring and again in mid-summer (i.e. August); though Borkman & Smayda (2009) observed a rapid ca. 50% decline in

Skeletonema abundance in 1980, nonetheless *Skeletonema* was observed at relatively stable reduced abundances post-1990. A seasonal study of protistan grazing has identified that these conditions (i.e. peak *Skeletonema* concentrations and warm temperatures) coincide with the annually highest grazing pressure on microphytoplankton (Lawrence & Menden-Deuer 2012). Therefore, the warm temperatures and the presence of the genus *Skeletonema* indicate that summer in Narragansett Bay is an appropriate location to detect optima grazing events and to address the question regarding the relative importance of the effects of temperature and species composition on grazing magnitude.

Use of the Dilution Method to Quantify Heterotrophic Protist Grazing

When predator and prey are similar in size, typical grazing experiments that rely on separation by filtration are not appropriate. The dilution method is a widely used method to quantify grazing by heterotrophic protists on phytoplankton in the same size range (Landry & Calbet 2004, Dolan & McKeon 2005, Weinbauer et al. 2011). The dilution method aims to manipulate the number of predator-prey encounters to measure the grazing rate by comparing the rates of disappearance of phytoplankton pigment across a dilution gradient (Landry & Hassett 1982). The dilution method assumes that (1) phytoplankton growth is unaffected by phytoplankton concentration (i.e. growth is equal at all dilution levels); (2) predator-prey encounter rates are proportional to clearance rates (i.e. grazers will always feed at a constant and maximal rate) and (3) the change in population density of phytoplankton is exponential. A series of dilutions is applied to a homogeneous community of whole seawater and distributed into bottles, where each bottle

represents one dilution. This distributes a well-mixed phytoplankton community across a series of dilutions to create lower prey abundances in more diluted bottles and higher prey abundances in less diluted bottles. If no predators exist, one would expect to observe no significant difference in growth rates across all bottles (i.e. a constant specific growth rate). With grazers, increasing dilution levels decrease the potential for microzooplankton-phytoplankton encounters, decreasing the potential for consumption. Thus, as predator-prey encounter rates increase, the net growth rate decreases. The method is used to determine the rates of phytoplankton growth and heterotrophic protist grazing.

Motivation

The goal of this study was to test the hypothesis that temperature alters the grazing magnitude of heterotrophic protists while controlling for a changing species community composition in order to evaluate the relative importance of each factor. We investigated this by measuring grazing rates in parallel incubations at two temperatures with the same species composition to determine to what degree the magnitude of grazing rates were affected by either incubation temperature or phytoplankton community composition. We conducted experiments during the summer, when temperatures were relatively consistent and when the temperature variation was minimal compared to the annual temperature range as well as inferior relative to other environmental variables, thereby ascertaining an advantage by allowing us to assume trivial temporal temperature variations from week to week. Constraining the study period to the summer minimized weekly temperature

differences and limited additional confounding and indirect effects of temperature, such as those previously associated with the seasonal progression of the plankton assemblage structure (Pratt 1959, Durbin et al. 1975). Fifteen dilution experiments were conducted weekly to bi-weekly from the early summer (June 2012) through mid-autumn (September 2012) on an unfiltered plankton assemblage to separate the effects of temperature and phytoplankton assemblage structure on the grazing rate of heterotrophic protists. Our findings show that temperature significantly altered grazing rates during short-term incubations and that the temporal (i.e. weekly) change was the most significant driver of assemblage structure.

MATERIALS AND METHODS

The individual effects of temperature and community composition on heterotrophic protist grazing rates were assessed on a plankton assemblage collected on fifteen dates from the site of the Narragansett Bay long-term plankton time series from 8 June through 25 September 2012 using the dilution method—a well-established technique used to quantify protistan herbivory rates (Landry & Hassett 1982).

Sampling Methods

Surface seawater was collected from the Long-Term Time Series Station (41° 34.5'N, 71° 24.3'W) located in Narragansett Bay, Rhode Island, USA. For a map of the sampling site location, see Lawrence & Menden-Deuer (2012; Fig. 1). Water was sampled using bucket grabs, gently poured through a 200 µm mesh—hereafter termed *whole seawater*—to remove macrozooplankton grazers, and stored in dark, 10 L carboys during transit to the laboratory. A portion of whole seawater was 0.2 µm gravity-filtered (Pall capsule) to create filtered seawater. Appropriate volumes of whole seawater were added to filtered seawater to create a dilution series, of five dilutions at 10, 25, 50, 75, and 100% whole seawater. Each dilution was partitioned into duplicate clear, 1 L bottles using polycarbonate tubing. The bottles were secured to rotating plankton wheels (rotation rates approximated 3 - 4 rpm) for a 24 h duration under ambient light conditions. Two identical, parallel sets were prepared and incubated on separate plankton wheels - each assigned a temperature treatment (detailed below).

Nutrient limitation of phytoplankton growth has been detected during the summer months in Narragansett Bay (Lawrence & Menden-Deuer 2012). Therefore, to prevent nutrient limitation, bottles were enriched with inorganic nitrogen and phosphorus to a concentration of 10 and 2 $\mu\text{M L}^{-1}$, respectively; these values represent averages of the maximum nutrient concentration observed *in situ* at the Long Term Time Series Station between Spring 2003 and December 2009. To account for the effects of nutrient limitation on phytoplankton growth, two additional bottles of non-nutrient amended whole seawater were included in the ambient temperature treatment. A paired t-test was used to determine if nutrient addition significantly enhanced the apparent net growth rates in nutrient amended and un-amended treatments and a 2-way ANOVA was used to detect the significance of the interaction between nutrient addition and temperature.

Physical data, including salinity, surface seawater temperature ($^{\circ}\text{C}$), and dissolved oxygen percent variation (%), were collected on station using an *in situ* profiler (Yellow Springs Instrument YSI 6920 V2). These data, in addition to photosynthetically available radiation (PAR) ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$), were used for subsequent analysis to identify associations between environmental conditions, growth, grazing, and community composition.

Temperature Treatments

To separate the effects of temperature and community composition on heterotrophic protist grazing magnitude, dilution experiments were completed in pairs, where each dilution series set was assigned to a temperature treatment. An ambient

treatment used flow-through bay water to mimic *in situ* temperature conditions whereas a cooled treatment continuously circulated freshwater through a chiller to a target temperature that was 5°C below the monthly surface seawater temperature average from the last five years observed at the Long-Term Time Series Station (Table 1). Our experimental set-up relied on relatively steady *in situ* temperatures from week to week to control for the effects of temperature. The temperature in each incubator was monitored at 15-minute intervals using HOBOWare equipment (Hobo Inc.).

A cool treatment was selected over a warm treatment, as it guaranteed that experimental temperature did not exceed the maximum temperature tolerable to any one species within the natural assemblage and ensured that the temperature difference between treatments was within the range of ambient temperature variability that could occur in Narragansett Bay over a 24 h period and seasonally.

Rate Analyses

To quantify autotrophic growth within each bottle, chlorophyll *a* and phaeophytin concentrations were measured at the start and endpoint of each 24 h incubation period by filtering triplicate subsamples from each bottle of each dilution level following the method described by Graff & Rynearson (2011). Volumes filtered ranged from 30 to 60 mL. Variation in chl *a* concentration measurements was low; throughout the study period, the coefficient of variation (CV) of all triplicate chl *a* measurements taken from all dilution levels averaged 3.8%, with a range from 0.1% to 11.9%.

Phytoplankton apparent growth rates (k , d^{-1}) were calculated for each bottle as $k = (1/t) * (\ln[P_t/P_0])$, where t represents time and P_t and P_0 represent final and initial chlorophyll a concentrations. A model 1 linear regression analysis of k versus dilution level ($n=10$) was used to yield rates of phytoplankton specific growth (μ , d^{-1}) and heterotrophic protist grazing (g , d^{-1}) from the y-intercept and the negative slope of the regression, respectively (Landry & Hassett 1982). Lack of statistical significance in the regression slope (p -value > 0.05) was interpreted as an event with no measurable grazing (i.e. $g = 0 d^{-1}$). In order to more accurately represent the effects of nutrient limitation on *in situ* growth, phytoplankton specific growth rates were further calculated as $\mu = k + g$, where k represents the apparent phytoplankton growth rate from the undiluted bottle (Landry et al. 2005), and were reported for *in situ* Narragansett Bay conditions.

To compare the relative grazing pressure across sample dates, the percentage of primary production consumed (%PP) by heterotrophic protists was calculated for each treatment on all dates using the equation $\%PP = g/\mu * 100$; %PP was only calculated when phytoplankton growth was significant (i.e. $\mu > 0 d^{-1}$) and when grazing was detected (i.e. $g > 0.01 d^{-1}$) (Landry & Calbet 2004).

Autotrophic & Heterotrophic Protist Biomass Estimates

To assess the taxonomic diversity and to quantify changing community composition over time, subsamples of 100 mL whole seawater were taken at the start of the experiment and again after incubations at each of the two temperature treatments. Samples were preserved by adding acid Lugol's to the subsample to a final

concentration of < 1% Lugol's solution. Phytoplankton cells within the size range 10 - >200 μm were identified at 200x magnification and counted in 1 mL Sedgewick rafter chambers under a light microscope equipped with phase contrast at 100x magnification. Heterotrophic protists (i.e. microzooplankton cells within the size range 10 - 200 μm) were enumerated at 100x magnification in settled 10 mL aliquots, by quantifying each slide in its entirety using inverted microscopy (Utermöhl 1958). Species identification was based on Hoppenrath et al. (2009) and Tomas (1997) and categorized as either autotrophic or hetero/mixotrophic according to the literature. Phytoplankton cells were identified to at least the genus level and heterotrophic protists were designated to belong to a coarse taxonomic or functional group (i.e. aloricate ciliates, ebridian flagellates, heterotrophic dinoflagellates, tintinnids, and radiolaria).

Phytoplankton and heterotrophic protist numerical abundances were converted to biomass estimates ($\mu\text{g C L}^{-1}$) for each sample. A rank order of cell abundance was applied to identify the top ten most abundant phytoplankton species. The top ten most common species comprised over 99% of the total cells observed throughout the study period. The average carbon content ($\mu\text{g C}$) for the top ten most abundant phytoplankton species and for the complete heterotrophic protist community was calculated based on empirical length width measurements taken on 50 - 100 cells of each type using ImageJ software. Biomass for aloricate ciliates, diatoms, ebridian flagellates, heterotrophic dinoflagellates, tintinnids, and radiolaria were calculated using the carbon conversion provided in Menden-Deuer & Lessard (2000) (Appendix

1). In addition, genus- and taxon-specific growth rates were calculated for phytoplankton and heterotrophic protists, respectively.

Statistical Analyses

The experimental design analyzed the response of measured variables to several treatment factors. The first factor addressed the weekly separation between each sample period and is referred to as the *temporal factor*; the second factor addressed the 24 h duration period from each dilution experiment and is referred to as the *incubation factor*. An additional, non-temporal factor arose from the two temperature treatments (i.e. ambient vs. cooled) and is referred to as the *temperature factor*.

To evaluate the effects of temperature and assemblage structure on heterotrophic protist grazing rates, multivariate analyses in PRIMER-E (Plymouth Routines in Multivariate Ecological Research) v6 and PERMANOVA+ were performed separately on the environmental data as well as the autotrophic and heterotrophic protist biomass data. To characterize the variables characteristic of temporal changes in environmental conditions, a principal components analysis (PCA) was used to assess the temporal variation as explained by changes in the normalized variables PAR, temperature, salinity, and dissolved oxygen. PERMANOVA+ was used to detect the presence of significant interaction terms between species composition and sampling date, changes during the incubation, and changes due to temperature (see description below).

All biomass data were fourth-root transformed to down weigh the contribution of the dominant genus/species groups and had a Bray-Curtis resemblance measure applied. A single-linkage CLUSTER and SIMPROF analysis, based on weekly biomass values, were used to segregate sample dates into three statistically significant assemblages. A one-way SIMPER further defined the average similarity within each assemblage and identified those species which contributed most to assemblage discrimination (i.e. that were most influential in typifying an assemblage). A series of ordination plots were used to visualize similarities in assemblage structure and environmental variables across sample dates where distance is proportional to similarity: points that are close together represent similarity in assemblage structure and points that are further apart represent dissimilarity in assemblage structure. In the figures, sample dates are represented in numerical order and increase with increasing sample date (e.g. 1 corresponds to 8-June-12, the first sampling date, and 15 corresponds to the last sampling date).

To determine if temperature altered the phytoplankton specific growth and heterotrophic protist grazing rates, a paired t-test was used to determine statistically significant differences between the ambient and cooled rates. A linear relationship was used to describe the relationship between ambient and cooled rates and to compare the outcome to a 1:1 relationship. In addition, the reliability of a 2-point dilution was assessed using a paired t-test, which compared phytoplankton specific growth rates calculated based on a 2-point and 5-point dilution (Worden & Binder 2003).

For further statistical analysis grazing rates were categorized as low, average, and above average grazing, g (d^{-1}) rates; averages were calculated separately for the

ambient and cooled treatment. The categories were applied as a-priori groups in the form of factors in subsequent multivariate analyses to determine the effect of grazing magnitude on assemblage structure. In addition, the sensitivity of the conclusions to the chosen category was tested and indicated robustness across several divisions of grazing. Unless otherwise stated, all errors presented represent one standard deviation of the mean. Statistical significant was assigned at $p < 0.05$.

Data Sources

Photosynthetically active radiation data were provided by the Narragansett Bay National Estuarine Research Reserve System-wide Monitoring Program, which is supported by a grant under the Federal Coastal Zone Management Act, administered by the Office of Ocean and Coastal Resource Management, National Oceanic and Atmospheric Administration, Silver Spring, MD. Data can be access at the NERRS Centralized Data Management Office, Baruch Marine Field Lab, University of South Carolina at <http://cdmo.baruch.sc.edu>). NOAA/OCRM support research was conducted under an award from the Estuarine Reserves Division, Office of Ocean and Coastal Resource Management, National Ocean Service, National Oceanic and Atmospheric Administration.

RESULTS

In Situ Biological & Environmental Conditions of Narragansett Bay

Throughout the observation period, environmental and biological variations exhibited temporal patterns, which represented the progression of the 2012 summer season. *In situ* temperatures averaged $22.3 \pm 2.2^\circ\text{C}$ and ranged from a low of 17.5°C (8 June 2012) early in the season to a high of 25.0°C (17 August 2012) mid-season. This was on average 1.8°C warmer than average temperature from the same time period between 2007 to 2011 (Table 2).

In situ chl *a* concentrations varied by more than 6-fold and averaged $5.96 \pm 2.52 \mu\text{g L}^{-1}$. Chl *a* values peaked at $10.72 \mu\text{g L}^{-1}$ (17 August 2012) and were lowest at $1.70 \mu\text{g L}^{-1}$ (17 September 2012) towards the end of the study period. No significant relationship between chl *a* concentration and *in situ* temperature was detected ($p = 0.09$).

Heterotrophic protist biomass exceeded autotrophic biomass for all fifteen sampling dates. Autotrophic biomass averaged $127 \pm 149 \mu\text{g C L}^{-1}$ and heterotrophic protist biomass averaged $459 \pm 281 \mu\text{g C L}^{-1}$. The temporal variation in autotrophic biomass was 300-fold and exceeded the temporal variation in heterotrophic protist biomass, which varied by 7-fold. Biomass maxima for both autotrophs and heterotrophic protists coincided with cool *in situ* temperatures. On the earliest and coolest (17.5°C) date, 8 June 2012, biomass values peaked at $574 \mu\text{g C L}^{-1}$ and $1037 \mu\text{g C L}^{-1}$, for the autotrophic and heterotrophic protist community, respectively. Heterotrophic protist biomass reached minima of $150 \mu\text{g C L}^{-1}$ on two occasions; the first occurred on 25 June 2012 and the second on 17 September 2012. The minimum

autotrophic biomass, $2 \mu\text{g C L}^{-1}$, was observed on 23 July 2012 though similar values were observed later in the season.

Temperature contributed minimally to the temporal environmental variation observed in Narragansett Bay. A principal components analysis (PCA) characterized temporal variations in PAR, temperature, salinity, and dissolved oxygen percent variation (Fig. 1). The greatest changes in environmental conditions were due to dissolved oxygen percent saturation, salinity, and PAR; the PC1 axis had a roughly equal weighted combination of contributions from these three variables. The first two axes of the PCA accounted for a combined 72.7% of the explained variation, with the first and second axes explaining 44.4% and 28.3%, respectively, indicating its appropriateness as an indicator of the observed variability among sample dates. Dissolved oxygen percent saturation values ranged from 81 to 113.5%, exhibiting dominance in PC1 and trending with PAR. Salinity values averaged 30.6 and ranged from 29.1 (18 June 2012) to 31.2 (9 July 2012). PAR values averaged $768 \pm 342 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and ranged from a minimum of $45 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (25 June 2012) to a maximum of $1148 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (16 July 2012). Temperature and PAR primarily characterized the PC2 axis. A seasonal trajectory regarding temperature was evident on PC2, in which June was relatively cool ($<20^\circ\text{C}$), July to mid-August became relatively warm ($>22.5^\circ\text{C}$), and a subsequent decrease in temperature continued for the remaining mid-autumn dates. Samples from July through mid-August clustered tightly, indicating similar environmental conditions during this period.

Composition of In Situ Assemblages

Chain-forming diatoms numerically dominated the 10-200 μm observed size fraction throughout the study period. Three species of diatoms dominated the majority of the autotrophic biomass, and are listed with their average relative percentage across the study period: *Ceratulina pelagica* (30%), *Chaetoceros* sp. (28%), and *Skeletonema* sp. (26%). However, over 98% of *C. pelagica*'s biomass was observed on only one date, 8 June 2012, which was characterized by a unique bloom event of this species. The heterotrophic protist species community composition was variable and was dominated by aloricate ciliates, which accounted for 54% of the total observed heterotrophic protist biomass across all sampling dates. The most numerically abundant taxa included aloricate ciliates ($< 30 \mu\text{m}$) followed by heterotrophic dinoflagellates consisting of thecate dinoflagellates ($< 20 \mu\text{m}$) and *Gyrodinium* sp. Tintinnid genera included *Stensomella* sp. and *Favella* sp. We observed only one species of ebridian flagellate, *Ebria tripartita*. Heterotrophic dinoflagellates dominated the heterotrophic protist biomass on 18 June 2012 through 16 July 2012 and again on 7 August 2012 (Fig. 2).

Observed Variability & Temporal Patterns Associated with In Situ Biomass and Assemblage Structure

Temporal change was the principal factor associated with assemblage structure differences, having a greater effect than incubation temperature or the incubation. The passage of time from week to week resulted in significant alterations of the autotrophic assemblage structure ($p < 0.001$) such that sample dates were temporally

segregated into three statistically distinct species assemblages over the study period (Fig. 3). The three assemblages were defined as the late-spring assemblage, the summer assemblage, and the late-summer/autumn assemblage. Biomass of the late-spring assemblage was overwhelmingly dominated by *Cerataulina pelagica*, which composed 98% of the assemblage's biomass. The late-spring assemblage only consisted of a single date, 8 June 2012, which was the day that autotrophic biomass values peaked at $574 \mu\text{g C L}^{-1}$. Due to the lone sample date for this assemblage, an average Bray-Curtis similarity was not calculated. The summer assemblage had an average biomass of $107 \pm 82 \mu\text{g C L}^{-1}$ and exhibited a high degree of similarity, i.e. 74%, made up mainly of contributions from the chain-forming diatom species *Skeletonema* sp., *Chaetoceros* sp., and *Leptocylindrus* sp. With the onset of autumn, Narragansett Bay's *in situ* autotrophic assemblage shifted away from diatoms towards dinoflagellates and relatively smaller ($<10 \mu\text{m}$), un-enumerated flagellates. Exceptions to this temporal pattern included two sample dates, July 23rd and July 30th, which grouped with the autumn samples. The abundance of un-enumerated flagellates likely explains the two orders of magnitude variation in the autotrophic biomass observed. Biomass of the late-summer/autumn assemblage averaged $73 \pm 99 \mu\text{g C L}^{-1}$ and was dominated by *Thalassiosira* sp., followed by *Skeletonema* sp. and *Chaetoceros* sp. The late-summer/autumn assemblage was in general characterized by the inclusion of more dinoflagellates, a greater diversity of autotrophs, and a larger range in biomass values, thereby resulting in a lower average Bray-Curtis similarity, i.e. 58%, compared to the summer assemblage. Similarity of the late-summer/autumn assemblage was made up of contributions from three chain-forming diatom species: *Thalassiosira* sp.,

Skeletonema sp., and *Chaetoceros* sp. It is important to note that although *Thalassiosira* sp. characterized the late-summer/autumn assemblage, the average biomass of the species was still higher in the summer assemblage. The between-assemblage dissimilarity of the summer and late-summer/autumn assemblage was 54.2% and was largely due to contributions by *Skeletonema* sp. and *Chaetoceros* sp., which contributed 10.9% and 10.4% to the total dissimilarity, respectively.

Temporal change also resulted in significant changes in the heterotrophic protist assemblage ($p < 0.001$). We found no evidence of group structure among the biomass of the five heterotrophic protist taxa. Neither the single linkage, complete linkage or group averaged dendrograms yielded significant clustering across all sample dates. Incubation temperature also co-varied with assemblage structure to a significant degree for both the heterotrophic protist ($p = 0.01$) and autotrophic ($p = 0.04$) biomass assemblage.

In Situ Rate Measurements in Narragansett Bay

Phytoplankton growth and heterotrophic protist grazing rates were similar in magnitude throughout the study period (Fig. 4). Phytoplankton specific growth rates averaged $1.14 \text{ d}^{-1} \pm 0.45$ and were positive throughout the study period. Specific growth rates ranged from a minimum of 0.39 d^{-1} (10 September 2012) to a maximum of 2.01 d^{-1} (17 September 2012). Heterotrophic protist grazing rates averaged $0.63 \pm 0.41 \text{ d}^{-1}$. The highest grazing rate, 1.25 d^{-1} , occurred on 16 July 2012. No grazing was measured on 18 June and 25 September 2012. Heterotrophic protist growth rates were positive for all taxa except for ebridian flagellates; average growth rates varied for

each group of heterotrophic protist, with the ebridian flagellates group having the lowest average growth rate of 0.0 d^{-1} and the tintinnid group averaging the highest growth rate of 1.2 d^{-1} .

Grazing rates were unrelated to initial autotrophic and heterotrophic protist biomass as well as unrelated to the biomass of individual phytoplankton genera (maximum $R^2 = 0.23$; minimum $p = 0.05$). The relative percentage of primary production consumed (i.e. $g:\mu$) averaged $68\% \pm 51$ and ranged from a minimum of <1 (18 June & 25 September 2012) to a maximum of 139 (10 September 2012). The percentage consumed was also not significantly related to autotrophic ($p = 0.94$) or heterotrophic protist ($p = 0.66$) biomass. The addition of nutrients significantly increased phytoplankton growth rates at both temperatures ($p < 0.001$); on average, nutrient additions increased phytoplankton growth rates by a factor of 1.6 and 1.3 for the ambient and cooled treatment, respectively, though no significant interaction was detected between nutrient addition and temperature ($p = 0.11$) (Table 3).

Temperature Treatments

The manipulated incubation temperature in the cooled treatment differed significantly from the ambient treatment ($p < 0.001$) by an average of $6.4 \text{ }^\circ\text{C}$ (Table 4). The temperature difference between treatments ranged from a maximum of 7.6°C (13 August 2012) to a minimum of 4.4°C (25 September 2012). The targeted temperature difference of 5.0°C between treatments was exceeded for all but two dates, in which the average difference between treatments reached $4.8 \text{ }^\circ\text{C}$ (18 June 2012) and $4.4 \text{ }^\circ\text{C}$ (25 September 2012). In general, the temperature difference between ambient and

cooled incubators increased with increasing *in situ* temperatures such that the greatest differences (>6 °C) between treatments were observed when it was warmest.

Within the flow-through incubators, ambient and cooled water temperatures averaged $23.0 \pm 2.0^{\circ}\text{C}$ and $16.6 \pm 1.1^{\circ}\text{C}$, respectively. The average daily water temperature for both the ambient and cooled treatments reached minima at similar times resulting in values of $20.0 \pm 1.3^{\circ}\text{C}$ (18 June 2012) and $15.2 \pm 0.6^{\circ}\text{C}$ (8 June and 18 June 2012) respectively. However, we observed maximum water temperatures on different dates for each treatment, neither of which corresponded to the *in situ* maximum. The ambient incubator reached a maximum temperature on 13 August 2012 (average = $25.2 \pm 1.4^{\circ}\text{C}$), whereas the cooled incubator reached a maximum on 16 July 2012 (average = $17.7 \pm 0.9^{\circ}\text{C}$). Temperature within each incubation also varied. Daily variability averaged $4.6^{\circ}\text{C} \pm 1.2$ and $2.3^{\circ}\text{C} \pm 0.8^{\circ}\text{C}$ for the ambient and cooled incubators, respectively (daily variability data not shown). The daily ambient variability exceeded cooled variability and reflects the daily variation in solar irradiance and temperature of the source water for the ambient treatment; because the cooled treatment was hooked up to a chiller, the over variation in temperature was, on average, 2.3°C less in the cooled treatment.

Altered Rates In Response to Temperature Treatments

Temperature had a significant effect on phytoplankton growth and heterotrophic protist grazing rates (Fig. 5). An on average 6.4°C decrease in temperature resulted in a significant decrease in phytoplankton specific growth rate (μ , d^{-1}) by a factor of 1.9 ($y = 0.66011*x - 0.19923$; $R^2 = 0.81$; $p < 0.001$) and a decreased

grazing rate (g, d^{-1}) by a factor of 3.3 ($y = 0.29136*x + 0.010067$; $R^2 = 0.40$; $p = 0.01$) (Fig. 6).

Similar ranges in %PP consumed were observed for both treatments (Fig. 7). Cooling the temperature decreased the %PP consumed by 31% on average, but the difference was not significant between the two treatments ($p = 0.07$), as both growth and grazing rates were lowered by cooled temperature. Protistan herbivory exceeded phytoplankton growth rates in both temperature treatments. On 10 September 2012, the maximum ambient %PP, i.e. 139%, was observed. However, a %PP similar in magnitude, i.e. 138%, was observed on 23 July 2012, when the maximum cooled %PP, 137%, occurred. %PP consumed in the cooled treatment exceeded the ambient treatment for the following two dates: 25 June & 30 July.

Species-specific growth rates were also altered in response to temperature. Contrary to temperature's significant effects on phytoplankton growth, temperature significantly decreased the growth rate of only one heterotrophic protist taxa, (i.e. radiolaria; $p=0.04$). All other heterotrophic protist growth rates were not significantly different across temperature treatments (max $p=0.9$).

Effects of Incubation on Biomass and Assemblage Structure

Over the incubation period, increases in total biomass and shifts in assemblage structure varied in degree relative to trophic level and to temperature. Large, consistent increases in phytoplankton biomass existed in all fifteen incubations for both temperatures. Autotrophic biomass increased significantly ($p = 0.01$), averaging a 600% and 200% increase, across both the ambient and cooled incubations,

respectively; the change in biomass over the incubation period was on the same order of magnitude as the temporal range in autotrophic biomass measured over the entire sampling period, i.e. 300%. The autotrophic assemblage was significantly altered by ambient ($p = 0.003$) and cooled ($p = 0.01$) incubation temperatures; assemblage changes due to an incubation were smaller relative to that variation which resulted from a temporal shift. Discrimination between the initial and final autotrophic assemblages was primarily attributed to increases in the biomass of four species: *Skeletonema* sp., *Chaetoceros* sp., *Eucampia zodiacus*, and *Cerataulina pelagica*. These species were not only the greatest contributors to the observed changes in assemblage structure over the 24 h period, but also made up greater than 90% of the total biomass observed, across all observations *in situ* and after 24 h. Average dissimilarities between the initial and final autotrophic assemblage were 46% and 44% for the ambient and cooled incubations, respectively.

By contrast, incubation generally resulted in smaller biomass changes for heterotrophic protists compared to the large changes in autotrophic biomass. Heterotrophic protist biomass increased by an average of 80% and 60%, which represented a significant increase ($p = 0.003$) for the ambient, but an insignificant increase ($p = 0.05$) for the cooled treatment. The change in biomass was an order of magnitude higher than the temporal variation in heterotrophic protist biomass, i.e. 7%. In seven experiments spanning six experimental dates, i.e. in three ambient treatments and four cooled treatments, total heterotrophic protist biomass declined over the incubation period, with an average decline of 17% and 21%, respectively. However, reduction in biomass did not appear to be associated with temperature. A maximum

decline in heterotrophic biomass, 38%, was observed in a cooled treatment on 17 August. The heterotrophic protist assemblage was also significantly altered by the incubation ($p = 0.01$), but the alteration was not greater than the variation in assemblage structure due to temporal shifts. Changes in tintinnid biomass contributed most to the observed difference over the 24 h period. Interestingly, although tintinnid biomass was the best discriminating group to explain the effect of a 24 h treatment, it did not account for the majority of the heterotrophic protist biomass. The average dissimilarity between the initial and final heterotrophic protist assemblage was 28% for both temperature treatments. For both assemblages, there were no significant interactions between the temporal factor and the 24 h treatment effect (min $p = 0.09$).

Effects of Temperature on Final Biomass and Assemblage Structure

Temperature differences resulted in observed biomass differences, which were amplified in the autotrophic assemblage relative to the heterotrophic protist assemblage. On average, final autotrophic biomass was 1.1-fold higher in the ambient treatment compared to the cooled treatment and this difference was significant ($p = 0.02$). Exceptions to biomass in the ambient treatment exceeding that of the cooled treatment existed on two dates (i.e. 8 June and 18 June), in which cooled autotrophic biomass exceeded ambient biomass by 20% and 28%, respectively. In comparison, heterotrophic protist biomass was, on average, only 0.2-fold higher in the ambient treatment and this difference was not significant ($p = 0.9$). On several dates, heterotrophic biomass in the cooled treatment exceeded ambient heterotrophic biomass (i.e. 8 June, 16 July, 23 July, 13 August, 28 August, and 25 September). It is

important to note that, on average, the biomass increased for all phytoplankton species and for all heterotrophic protist taxa except for radiolarian, implying that the incubation treatments, including temperature manipulations, were reasonably well-tolerated by diverse taxa of both functional groups.

Although the total biomass differed in the two temperature treatments, the assemblage structure was conserved. Temperature has an insignificant effect on the final autotrophic ($p = 0.3$) and the heterotrophic protist ($p = 0.07$) assemblages. Dissimilarities between the ambient and cooled assemblages averaged 44% and 25% for the autotrophic and heterotrophic protist assemblages, respectively. Nonetheless, biomass changes within a few main genera helped to explain the more subtle effects of temperature on each assemblage. Regarding the autotrophic assemblage, the observed differences were attributed to contributions by *Chaetoceros* sp., *Skeletonema* sp., *Cerataulina pelagica*, and *Eucampia zodiacus*, which made up the majority of the observed biomass and, together, accounted for 55% of the average dissimilarity between the ambient and cooled treatments. The species-specific growth rates for these four phytoplankton groups were lowered to the greatest degree in response to lower temperatures, though these differences were insignificant (max $p=0.10$). *Eucampia zodiacus* had its growth rate most altered by temperature. Regarding the heterotrophic protist assemblage, differences were due primarily to changes in tintinnid, ebridian flagellate, and aloricate ciliate biomass, which together accounted for 66% of the average dissimilarity between treatments. Though all heterotrophic protist taxa grew more slowly in the cooled treatment, radiolaria was the only

heterotrophic protist taxa to experience a significant decrease in growth in response to temperature ($p=0.04$).

DISCUSSION

The aim of this study was to elucidate the effects that temperature and community composition have on the magnitude of heterotrophic protist grazing rates within a microplankton assemblage from Narragansett Bay. We observed that a $\sim 6^{\circ}\text{C}$ reduction in temperature for short-term (i.e. 24 h) incubations significantly decreased both phytoplankton growth and heterotrophic protist grazing rates, and that protistan herbivory rates were reduced to a greater extent than autotrophic growth rates. Cooled temperatures decreased phytoplankton growth rates by ~ 2 -fold and decreased microzooplankton grazing rates by ~ 3 -fold. Dual experiments at two temperature treatments successfully maintained consistent phytoplankton and heterotrophic protist species assemblages, making it possible to separate the effects of temperature and community composition on the magnitude of heterotrophic protist grazing rates.

Patterns Regarding In Situ Assemblage Structure

The phytoplankton assemblage was typical of the Narragansett Bay community, as it included the chain-forming diatoms *Chaetoceros* and *Skeletonema*, two well-known genera that constitute part of the principal phytoplankton species in Narragansett Bay (Smayda 1957, Karentz & Smayda 1984, Karentz & Smayda 1998). Phytoplankton abundance in this study varied by ~ 150 -fold and was within the range of cell concentrations previously observed during a summertime study spanning a 22 yr period (Karentz & Smayda 1998).

Over the observation period, temporal changes were the most significant driver of the autotrophic and heterotrophic protist assemblage. Occasional drastic changes

existed in each assemblage from week to week – a variation that has been observed in periods as short as one day (Strom et al. 2001).

A persistent heterotrophic protist assemblage existed throughout the summer. Heterotrophic protists are known to be abundant in estuarine ecosystems and the taxa present in this study were similar to those found previously in Narragansett Bay (Lawrence & Menden-Deuer 2012). Numerically, the abundance of heterotrophic protists exceeded that observed in the Arctic summer (Sherr et al. 2003) and at similar latitudes in a European coastal ecosystem (Modigh & Franzè 2009). At times, heterotrophic dinoflagellates dominated the biomass, which consisted of genera such as *Gyrodinium* and *Protoberidinium*, which are known to feed preferentially on diatoms (Buskey et al. 1997). Heterotrophic protist grazing rates were lower than previously reported from this location; the magnitude of average heterotrophic protist grazing rates measured (i.e. 0.63 d^{-1}) was more similar to the annual average measured at the same location (i.e. 0.66 d^{-1}) than to the average that Lawrence & Menden-Deuer reported for the late spring to summer months (i.e. 1.15 d^{-1}) (2012). However, it is important to note that Lawrence & Menden-Deuer's average grazing for the same period was based on non-nutrient amended experiments whereas our experiments were in nutrient-replete conditions and corrected for nutrient-amendment. The initial biomass of heterotrophic protists was significantly correlated to autotrophic biomass, which supports previous research that suggests that autotrophic biomass is a good indicator of the biomass of heterotrophic species (Burkhill et al. 1995). Strom et al. (2001) further investigated this idea and ultimately detected a strong positive relationship between larger ($> 8 \mu\text{m}$) phytoplankton (based on chl *a* measurements)

and both ciliate and dinoflagellate biomass, which did not extend to the lower-size spectrum. The relationship highlights the important relationship predator-prey interactions between phytoplankton and microzooplankton and is evidence that the microzooplankton assemblage is structurally dependent on the phytoplankton community.

Observed variations in the *in situ* autotrophic and heterotrophic protist assemblage over the four-month period helped to distinguish the inherent temporal characteristics and unique patterns associated in each assemblage. The *in situ* heterotrophic protist assemblage was unstructured, whereas the *in situ* autotrophic assemblage varied by month. Monthly shifts in the autotrophic assemblage structure are well-known phenomena, in which a shift from phytoplankton to smaller flagellates occurs as the summer progresses (Pratt 1959, Durbin et al. 1975). The highly variable heterotrophic protist community composition observed in this study has also been observed previously. Vigil et al. (2009) found evidence of a significant dominant taxa change within one to two weeks with a similarly diverse species assemblage. For both assemblages, temperature and species community composition co-varied, suggesting that both factors likely are influential in mediating grazing on phytoplankton. Our results suggest that the *in situ* heterotrophic protist community structure was extremely variable from week to week and very resilient. The heterotrophic protist community was numerically persistent throughout the season regardless of changes in autotrophic composition; the observation could be indicative of the predators' quick capability to respond to temporal changes, such as autotrophic assemblage shifts, allowing the predators to utilize the niches that become available with a changing autotrophic

community.

Heterotrophic Protist Grazing During Summer Months in Narragansett Bay

Protist grazing accounts for the majority of phytoplankton mortality in the ocean. In this study, on average, heterotrophic protists grazed 79% of the daily phytoplankton primary production and exceeded 100% consumption on six occasions, which supports grazing as a large phytoplankton loss factor. Our values exceeded the overall average for the world's oceans (67%; Landry & Calbet 2004) and were only slightly less than previous observations in Narragansett Bay during the same time period (Lawrence & Menden-Deuer 2012). Variation in heterotrophic protist grazing rates occurred over a wide range of chl *a* concentrations, which varied by approximately 6-fold throughout the study, and were similar to values previously reported for estuarine ecosystems (Durbin et al. 1975, Oviatt 2004). High primary production consumption has been linked to warmer temperatures or peak *Skeletonema* sp. concentrations (Lawrence & Menden-Deuer 2012), two conditions which typically characterize the environmental and biological conditions of summer in Narragansett Bay.

Changes in environmental conditions were not driven by changes in temperature over the four-month study period. The lack of association between temperature and seasonal shifts provided the opportunity to separate the effects of temperature from shifts in phytoplankton assemble structure and the resulting effects on heterotrophic protist grazing rates. The relative consistency in temperature differed from previous research, which found that temperature was the main contributor,

relative to other environmental variables, of the observed environmental variation in Narragansett Bay (Lawrence & Menden-Deuer 2012). We attributed this difference to the length of the observation period. Lawrence and Menden-Deuer's research was conducted over the course of one year whereas our study took place over four months. In temperate regions, such as Narragansett Bay, a short study confined to the summer months holds an advantage over longer studies when testing temperature's effects, as longer study would yield a considerably greater range in *in situ* temperatures compared to a shorter study where temperatures tend to be consistently warm.

Temperature Treatments

Our experimental set-up successfully maintained a significant temperature difference across treatments, thereby allowing us to investigate the individual effects on temperature on a unique natural plankton assemblage multiples times over the four-month study period. The unusually warm summer of 2012 resulted in the unintended consequence of exaggerating temperature differences between treatments. Overall, water temperatures in Narragansett Bay in summer 2012 were $2.5 \pm 1.1^{\circ}\text{C}$ warmer than average. Consequently, our average ambient temperature treatment was consistently higher than the calculated seasonal average, resulting in an achieved average difference of 6.4°C and an overshoot of the targeted 5°C difference between treatments. Previous research has observed an increase in the mean annual water temperature at Woods Hole by $0.04^{\circ}\text{C yr}^{-1}$ since 1960 (Nixon et al. 2004), but the difference is not enough to account for the higher-than-expected water temperatures that we observed. Grazers are thought to graze at higher rates in warmer temperatures

(Burkhill et al. 1995, Caron et al. 2000, Strom et al. 2001), thereby altering the grazing magnitude. As a result, our grazing rates could have been inflated, but on average, our grazing rates were lower than previously observed.

The ambient treatment had a higher daily temperature variability compared to the cooled incubator, because incubators were un-shaded from the Sun's radiation. Exposure to radiation likely heated the ambient tank to a greater degree than the cooled tank, as the chiller buffered temperatures of the latter tank. Un-even heating could have put additional stress on the phytoplankton in the ambient treatment. However, we did not observe any evidence of phytoplankton stress (i.e. mortality) in the incubators and there was no significant difference in taxa-specific mortality rates due to temperature.

Temperature-Induced Shifts of Growth and Grazing Rates

Temperature is a key driver of metabolic rates (Eppley 1872, Gillooly 2001, Gillooly 2002, Brown 2004). We anticipated that a decrease in temperature would result in decreased growth based on a relationship originally described by Eppley (1972). Significant decreases in phytoplankton specific growth rates were observed after exposure to lower temperatures by a factor of 1.9. The outcome of these experiments agrees well with prior documentation that the specific growth rate of phytoplankton is directly related to temperature (Goldman & Carpenter 1974, Suzuki & Takahashi 1995, Montagnes & Franklin 2001, Strom et al. 2001, Montagnes et al. 2003). Heterotrophic protist grazing rates were also significantly lowered in response to a decrease in temperature by a factor of 3.3. The decrease was also expected, as

microzooplankton grazing rates have been previously correlated to ambient seawater temperatures in Narragansett Bay (Lawrence & Menden-Deuer 2012), as well as in the Antarctic (Burkhill et al. 1995), the North Pacific (Strom et al. 2000), and the Mediterranean (Modigh and Franzè 2009). The subsequent decrease in grazing rates due to temperature has also been observed to decrease grazing rates to an exaggerated degree at temperatures $\leq 0^{\circ}\text{C}$ (Caron et al. 2000). Additionally, Rose et al. (2000) completed dilution experiments over the course of three days until reliable microzooplankton grazing rates were obtained because 24 h was too short to detect grazing at low temperatures, but we did not have this issue. It is important to note, however, that a large portion of the studies investigate rate responses using a temperature increase.

Interestingly, on average, the decrease in the magnitude of the rates due to temperature was specific to the metabolic process measured. An average 6.4°C decrease in temperature lowered the grazing response of heterotrophic protists to a greater degree relative to the growth response of phytoplankton (i.e. $3.3 > 1.9$). The observation supports increasing evidence that low temperatures constrain grazing rates relative to phytoplankton growth rates. Investigation of this idea has already been considered for cold environments; Rose & Caron (2007) proposed that low temperatures ($<5^{\circ}\text{C}$) put a relatively larger constraint on microzooplankton growth compared to phytoplankton growth, although the available data $<5^{\circ}\text{C}$ were limited for that study. Additional work completed by Rose et al. (2009) suggested that microzooplankton grazers are more sensitive to temperature-induced shifts compared to phytoplankton. Our results show that similar findings also apply to temperate

environments and have important implications regarding the trophic transfer of energy by providing evidence that the trophic dynamics between microzooplankton predators and their phytoplankton prey are largely controlled by temperature changes.

Percent primary production consumed differed by ~30% across temperature treatments, but the difference was insignificant. If grazing was lowered significantly, and to a greater degree than phytoplankton, then we would have expected to see a significant difference in the percent of primary production consumed, but this was not observed. This lack of significance is likely an expression of tight coupling between predation and growth rates in microplankton. Microzooplankton growth rates can equal or exceed phytoplankton growth, and so the increases in the growth rates of phytoplankton should be quickly matched by microzooplankton (Banse 1992). Though temperature altered the absolute rates, it did not significantly alter the rate of trophic transfer from prey to predator, meaning that the grazers behaved similarly in both treatments by removing relatively equal amounts of phytoplankton. The latter piece of information is highly interesting, in that it provides evidence not only that the heterotrophic protists were able to acclimate to their temperature-manipulated environment, but that at higher temperatures, the microplankton system runs at a higher rate output.

An unequal rate response of predators and prey in response to temperature would have far reaching consequences for phytoplankton ecology. Based on short-term, un-acclimated temperature manipulated experiments, we know that lowering the temperature exaggerates the decrease in grazing rates compared to phytoplankton growth rates, resulting in an increase in phytoplankton biomass. If the same

mechanism applies to warming ocean temperatures, than we can expect microherbivory rates to intensify and to be faster than temperature-induced shifts in phytoplankton growth rates, which is a projection that has already been recognized (López-Urrutia et al. 2006, Chen et al. 2012). The result would be a lower phytoplankton biomass due to the increased metabolic activity of grazers (Keller 1999). Similar observations have already been made for higher trophic-level interactions between predator and prey. For example, zooplankton abundances were observed to decrease in the summer due to ctenophore predation (Oviatt 2004). If projected onto future global temperature projections, which predicts increases between 1.8 and 5.8°C in the next 100 years (IPCC 2001), the change in grazing magnitude would strongly impact future atmospheric CO₂ levels, climate, and export to the deep ocean. Coastal New England ecosystems have already been impacted (Nixon et al. 2004) due to an increase in surface oceanic temperature during the most recent warming period (Levitus et al. 2000). When combined with current global respiration and production values, phytoplankton are projected to consume four gigatons of C yr⁻¹ less by the end of this century - equivalent to about one-third of our current worldwide CO₂ industrial emissions (López-Urrutia et al. 2006). Though it is easy to assume that warmer temperatures might result in grazing rates that increase to a greater degree relative to phytoplankton growth rates, resulting in a decrease in phytoplankton biomass, we have not tested this and therefore cannot assume the effects of warming on a natural assemblage. Sherr & Sherr (2009) point out that blooms occur all over the world over a range of latitudes in which temperature varies and therefore other factors aside from water temperature likely constrain phytoplankton bloom development.

Temperature-Induced Shifts in Assemblage Structure

The autotrophic and heterotrophic protist assemblage structure at the end of the incubations were identical for both temperatures, indicating that the species composition, though not biomass, remained robust in response to an average 6.4°C decrease in temperature. Analyzing the growth rates of those contributors which accounted for assemblage structure differences can help to further investigate a natural assemblage's sensitivity to temperature and to confirm that the assemblage structure was not compromised by the temperature change. First, insignificant changes in the species-specific growth rates of the top autotrophic (i.e. *Chaetoceros* sp., *Skeletonema* sp., *Cerataulina pelagica*, and *Eucampia zodiacus*) and the heterotrophic protist taxa (i.e. tintinnids, ebridian flagellates, and aloricate ciliates) suggest that these organisms thrived within the range of temperatures in the incubators. Second, the average growth rates of all of these groups were positive, which confirms the survival of all groups across treatments. If consistent negative growth rates had been observed, that would suggest that a temperature change amplified the struggle for survival, but this was not observed.

It is important to recognize that some organisms are more sensitive to a temperature change than others, and that this sensitivity can be reflected in subtle, yet amplified, alteration in rates. For example, *Chaetoceros* sp. was the only phytoplankton group that had its growth significantly altered by temperature. Significant differences in growth would suggest that it is either more sensitive to cool temperature (i.e. a direct result of the organism not growing as well in the cool water),

more sensitive to general shifts in temperature (i.e. a direct result of the organism experiencing a new environmental conditions), or grazed on preferentially in cold treatments (i.e. an indirect result of increased grazing in the cooled treatment). The latter is unlikely, as their siliceous spines support evidence that this species is unappetizing to predators. Regarding increased sensitivity to a general shift in temperature, we are limited by only growth in response to cool temperature, and thus do not have ample data to appropriately answer the question. However, separating out these effects requires additional experimentation with and without the presence of grazers, an option that could be incorporated into future experiments had the lowest dilution bottle been inspected. Tintinnids were the most sensitive to the temperature treatment (i.e. highlighted as the main contributor responsible for the differences in the heterotrophic protist assemblage across treatments) but was not the largest in biomass, implying that tintinnids are more temperature-sensitive than other heterotrophic protist taxa. There is likely a limit to the degree of cooling, which heterotrophic protists and/or phytoplankton can withstand, but we found no evidence that the threshold-temperature for the natural assemblages was reached. It is like that some species are more sensitive to temperature than others, but additional experiments observing the performance of phytoplankton and microzooplankton at a range of temperatures would have to be conducted to further address the tolerance of the community to a larger temperature differential.

Effect of Incubation on Biomass and Assemblage Structure

A common outcome of 24 h, nutrient-amended dilution experiments is a

notable increase in the autotrophic biomass, which was observed in our experiment; increases in the diatom assemblage under these conditions have been observed previously (Landry et al, 1995, Juhl and Murrell, 2005, Modigh and Franzè 2009). Increases in the biomass of several phytoplankton species included *Eucampia zodiacus*, *Skeletonema* sp., *Cerataulina pelatica*, and *Chaetoceros* sp. The large increases in these four species yielded high growth rates (i.e. $\geq 1.5 \text{ d}^{-1}$) and explained the significant shift in the autotrophic assemblage over the incubation period. Modigh and Franzè (2009) minimized nutrient additions in order to avoid creating bloom conditions that have the potential to significantly alter assemblage structure. However evidence from previous dilution experiments indicate that summertime nutrient-limitation exists and if left untreated, results in lower phytoplankton specific growth rates (Lawrence & Menden-Deuer 2012). One major assumption of the dilution method is that nutrients must be unlimited across all dilutions so that phytoplankton growth is unlimited. In order to follow in accordance with the assumption and to limit confounding variables, decreasing the amount of nutrients added was not an option for our experiment.

The significant increase in heterotrophic protist biomass over the incubation period has also been observed previously. Modigh & Franzè (2009) found significant changes resulting in a more than 2-fold increase in biomass of the grazer populations, as well as a significant shift in the composition of the heterotrophic protist assemblage and significant changes in ciliate and heterotrophic dinoflagellate abundance over the same length of incubation time. Other research has observed no change in heterotrophic protist biomass (Paterson et al. 2007). Further, others have observed

changes in grazer communities when comparing the initial and final assemblages, especially across various dilution levels (Dolan et al. 2000). For example, tintinnid ciliates have been shown to vary apparent growth rates in proportion to available nanoplanktonic prey, and do not grow well or change their lorica size at low dilutions (Dolan et al. 2000). This suggests that the dilution series can create artifacts, which can lead to a favored survival of different species across the dilution series or the over or underestimation of grazing. When comparing heterotrophic protist growth rates in the ambient and cooled treatment in our study, negative growth was not associated with any one taxa or treatment. Although we do not have data from different dilutions, we propose that our findings suggest that complex interactions unrelated to the level of dilution, such as a lack of a prey item or the presence of a predator was a more likely culprit attributed to these random mortality events of heterotrophic protists rather than a treatment effect. In future experiments, investigating the protistan population at low dilutions will ensure these artifacts do not occur. In summary, evidence exists that grazer communities can be dynamic during dilution experiments, and should be assessed in the future when measuring grazing magnitude, so as not to over or underestimate grazing mortality.

Microzooplankton growth can be used as a relative measure of the efficiency between predator and prey energy transfer, a point stressed by Verity et al. (1986, 1993), and applied to this study to further explain the biomass changes within the heterotrophic protist community. Cooler temperatures constrained heterotrophic protist growth to a greater degree than in the ambient treatment. As a result, heterotrophic protist biomass increased significantly in the ambient treatment due to

ample supply of prey. However, this increase was limited in the cooled treatment – likely due to the decrease in the metabolic rates of heterotrophic protists in response to cooler temperatures, as the environment was likely prey-saturated. Therefore, in both treatments, grazer biomass increased in response to the fast-growing autotrophs, but this increase in grazer population was curtailed in the cooled treatment, thereby limiting the amount of primary production consumed.

One option to minimize the additional dynamics within grazer communities that are introduced due to dilution experiment incubations may be to consider acclimating the plankton assemblages. The dilution experiment and our experiment assumed that the organisms phenotypically acclimate such that a plankton assemblage is forced to survive in an altered environment. Previous research has shown that when environmental conditions change, the most sensitive organisms become excluded and the most resistant individuals become favored, thereby increasing community tolerance but altering the natural assemblage structure (Fogg 2001). For this study, seasonal changes in the *in situ* temperature over the four-month period altered the autotrophic and heterotrophic protist assemblages significantly and to a much greater degree than did a sudden decrease in temperature with the use of a short-term incubation. The incubation period had an insignificant effect on the community composition of the natural phytoplankton assemblage whereas incubation temperature appeared to have a significant effect. Though both of these tests were significant, it does imply that the assemblage structure may not be sensitive to changes in short-term incubation experiments, as the p-value was near the significance cut-off. A incubation period > 24 hr may reveal possible temperature treatment effects, but assumptions of

the dilution method, including the assumption that ample nutrients exist to sustain unlimited phytoplankton growth, could introduce artifacts. In addition, the results provide evidence that temperature acclimation of the phytoplankton may not be necessary when running 24 h dilution experiments. Our results indicate that phytoplankton genera- and heterotrophic protist taxa-specific growth rates in the cooled treatment were comparable to the ambient treatment. Had a catastrophic shock been implemented via the treatments, a higher rate of mortality would have been observed in the cooled treatment. The fact that the species that dominated *in situ* also dominated within our dilution bottles containing 100% seawater, also supports the argument which deems acclimation unnecessary.

Concluding Remarks

Quantitative observations for this study conclude that a 6.4°C temperature shift within a short-term incubation period altered heterotrophic grazing rates to a greater degree than autotrophic rates. Temporal changes, which were not solely characterized by temperature, were the most significant driver of both autotrophic and heterotrophic protist plankton assemblages. Overall, the findings confirm that temperature is a significant modulator of metabolic rates and that it plays a larger role in mediating protistan herbivory than summertime phytoplankton community composition. The study highlights evidence of a differing sensitivity of photosynthetic and respiration rates to temperature that could be applied to future models of microplankton food webs to determine events in which microzooplankton grazing pressure is constrained relative to phytoplankton growth within marine environments.

APPENDICES

Appendix A. Autotrophic Biovolume Conversions

Table A1. The top ten most abundant phytoplankton species observed *in situ* at the long-term monitoring station are listed with the shape used to calculate the biovolume. The average volume (\pm SD), comprised of length-width measurements for 50 - 100 cells imaged, and the total biomass observed *in situ* for every phytoplankton species is listed. Relative percentage refers to the percentage of total *in situ* biomass observed. The specific growth rate, μ , (\pm SD) is based on the change in biomass from the initial to final ambient temperature treatment, which was calculated using the exponential growth equation. These growth rate data are calculated from unreplicated abundance counts that were converted to biomass estimates using the average volume and carbon-equations.

<u>Species</u>	<u>Shape</u>	<u>Average Volume</u> (μm^3)	<u>Total Biomass</u> ($\mu\text{C L}^{-1}$)	<u>Relative Percentage of Biomass</u> (%)	<u>Average Specific Growth Rate, μ</u> (d^{-1})
<i>Skeletonema</i> sp.	cylinder	175.55 \pm 298.91	487	26	1.6 \pm 1.0
<i>Chaetoceros</i> sp.	cylinder	2510.58 \pm 3676.56	531	28	1.5 \pm 1.5
<i>Ceratulina pelagica</i>	cylinder	2089.27 \pm 4308.67	575	30	1.6 \pm 0.9
<i>Leptocylindrus</i> sp.	cylinder	398.09 \pm 183.46	61	3	1.1 \pm 1.3
<i>Cylindrotheca closterium</i>	prolate spheriod	205.33 \pm 200.49	11	1	0.6 \pm 2.4
<i>Prorocentrum gracile</i>	prolate spheriod	2005.65 \pm 697.33	8	0	1.1 \pm 1.6
<i>Thalassionema nitzschoides</i>	rectangular prism	122.90 \pm 69.51 13957.02 \pm	149	8	1.8 \pm 2.1
<i>Eucampia zodiaus</i>	cylinder	18794.05	36	2	1.3 \pm 0.7
<i>Thalassiosira</i> sp.	cylinder	7737.77 \pm 2436.29	11	1	1.2 \pm 1.5
<i>Pseudo-nitzschia</i> sp.	prolate spheriod	846.01 \pm 460.77	35	2	-0.1 \pm 1.0

Appendix B. Species-Specific Growth Rates of Individual Genera/Taxa

Table A2. Growth rates (d^{-1}) of the top ten most abundant autotrophic organisms and the heterotrophic protist taxa observed within a natural phytoplankton assemblage after incubations at ambient (A & C) and cooled (B & D) temperatures from 8 June to 25 September 2012 in Narragansett Bay. Growth rates are calculated as $(1/t) * (\ln[P_t/P_0])$, where t represents time and P_t and P_0 represent final and initial biomass values. On each date, – indicates that the species was not observed, T_00 represents a growth rate beyond detection limits due an initial abundance equal to zero, and T_f0 represents a growth rate beyond detection limits due to a final abundance equal to zero.

	<i>Skeltonema</i> sp.	<i>Chaetoceros</i> sp.	<i>Cerataulina pelagica</i>	<i>Leptocylindrus</i> sp.	<i>Cylindrotheca closterium</i>	<i>Thalassionema</i> sp.	<i>Eucampia zodiacus</i>	<i>Thalassiosira</i> sp.	<i>Pseudo-nitzschia</i> sp.	<i>Prorocentrum gracile</i>	
Ambient	8 Jun	0.9	-0.1	0.3	-0.4	T_00	-	T_f0	-	T_f0	
	18 Jun	1.0	0.4	1.6	0.2	T_00	0.1	0.6	1.6	-1.1	
	25 Jun	1.4	2.4	T_f0	1.3	-	1.8	0.1	0.3	2.5	
	9 Jul	1.6	2.1	-	1.4	-	-	-	T_f0	2.1	T_00
	16 Jul	1.4	0.7	-	1.6	1.1	T_f0	T_00	0.7	1.2	-
	23 Jul	3.0	5.9	-	2.4	1.8	0.0	-	T_f0	1.9	-
	30 Jul	2.0	T_00	-	2.5	1.8	4.2	T_00	1.5	-	-
	7 Aug	0.2	1.2	2.2	0.1	-4.6	-2.0	6.3	2.4	2.2	-
	13 Aug	2.3	0.5	2.6	1.9	1.4	1.4	0.3	0.9	1.1	-1.3
	17 Aug	2.0	2.3	2.2	2.1	2.3	1.1	2.1	1.9	T_f0	0.3
	28 Aug	1.6	0.8	0.6	1.2	1.6	0.9	2.1	1.3	2.6	0.6
	4 Sep	1.3	1.5	T_00	1.8	2.0	1.5	1.3	T_00	-1.6	-
	10 Sep	1.3	1.1	-	-2.3	-2.3	T_00	T_00	T_00	T_f0	T_f0
	17 Sep	0.0	1.9	-	-	T_f0	1.9	-	T_f0	-	T_f0
	25 Sep	3.7	-0.1	-	-	T_00	-	T_00	T_f0	-	-
	average	1.6	1.5	1.6	1.1	0.6	1.1	1.8	1.3	1.2	-0.1
	stdev	1.0	1.5	0.9	1.3	2.4	1.6	2.1	0.7	1.5	1.0

A.

Cooled	8 Jun	-0.1	T _f 0	0.4	0.8	-	-	0.3	-	1.1	-
	18 Jun	0.6	0.9	1.3	-0.6	T ₀ 0	0.3	0.9	2.4	0.7	-
	25 Jun	0.5	0.7	0.7	0.5	-	0.3	-0.1	T _f 0	1.3	-
	9 Jul	1.4	1.0	-	0.3	-	-	-	-0.4	1.2	T ₀ 0
	16 Jul	1.0	0.3	-	1.4	0.7	-0.4	T ₀ 0	T _f 0	0.7	-
	23 Jul	0.5	3.1	-	1.5	0.4	T _f 0	-	T _f 0	0.7	-
	30 Jul	1.8	T ₀ 0	-	2.0	1.0	3.2	T ₀ 0	0.4	-	T ₀ 0
	7 Aug	0.9	1.7	-0.1	0.8	1.8	0.8	1.4	2.4	0.9	T ₀ 0
	13 Aug	0.6	1.1	2.3	0.7	1.0	1.1	-0.2	1.3	-0.8	0.2
	17 Aug	1.5	2.0	1.0	1.5	1.9	0.6	0.7	2.3	T _f 0	1.1
	28 Aug	1.0	0.1	0.1	0.5	0.8	0.7	0.2	1.7	1.4	0.2
	4 Sep	0.8	-0.2	T ₀ 0	1.0	0.9	0.5	0.0	T ₀ 0	T _f 0	-
	10 Sep	0.9	-1.1	-	-1.9	0.9	-	-	T ₀ 0	T _f 0	T _f 0
	17 Sep	1.3	0.2	-	-	T _f 0	0.0	-	-0.3	-	T _f 0
	25 Sep	3.7	-0.6	T ₀ 0	-	T ₀ 0	T ₀ 0	-	-1.4	-	-
	average	1.1	0.7	0.8	0.6	1.0	0.7	0.4	0.9	0.8	0.5
	stdev	0.9	1.1	0.8	1.0	0.5	1.0	0.6	1.4	0.7	0.5

B.

	Radiolaria	Aloricate Ciliates	Dinoflagellates	Tintinnids	Ebridian Flagellates
8 Jun	-	-0.3	1.9	T ₀ 0	-
18 Jun	-	1.6	0.6	T ₀ 0	-
25 Jun	-	1.9	0.5	-	-
9 Jul	-	1.3	0.4	T ₀ 0	-
16 Jul	1.6	-0.4	0.0	1.4	-
23 Jul	-	1.2	1.9	2.2	-
30 Jul	1.0	0.8	1.4	1.7	-
7 Aug	T _f 0	0.4	0.1	0.3	T ₀ 0
13 Aug	1.1	0.2	-1.1	0.3	-0.5
17 Aug	0.3	-0.1	-1.2	1.6	0.8
28 Aug	-	-0.7	-0.7	1.1	T ₀ 0
4 Sep	-	-0.6	-0.5	0.7	-0.8
10 Sep	-	0.5	0.3	0.3	0.5
17 Sep	-	0.2	1.6	4.2	-
25 Sep	-	0.4	1.5	-0.3	-
average	1.0	0.4	0.4	1.2	0.0
stdev	0.5	0.8	1.1	1.2	0.8

C.

	Radiolaria	Aloricate Ciliates	Dinoflagellates	Tintinnids	Ebridian Flagellates
8 Jun	-	0.6	1.1	-	-
18 Jun	-	0.9	0.3	-	-
25 Jun	-	1.1	0.2	-	-
9 Jul	-	0.6	0.2	-	-
16 Jul	0.3	1.7	0.2	0.0	-
23 Jul	-	1.5	2.9	1.3	-
30 Jul	0.6	0.4	1.0	1.1	-
7 Aug	-2.3	-0.5	0.0	1.0	-
13 Aug	0.7	0.3	-0.4	-0.5	0.4
17 Aug	-0.9	-0.6	-1.9	0.1	1.3
28 Aug	-	-0.9	-	2.0	-
4 Sep	-	-0.2	-0.4	0.9	-1.9
10 Sep	-	-0.1	-0.3	-0.7	T _f 0
17 Sep	-	-0.1	2.1	3.9	-
25 Sep	-	0.4	0.7	-0.1	-
average	-0.3	0.3	0.4	0.8	-0.1
stdev	1.3	0.8	1.2	1.3	1.7

D.

Appendix C. Assessing the 2 vs. 5-Point Dilution Method

Questions persist regarding the appropriate number of dilution levels needed to confirm linearity in heterotrophic protist clearance rates across dilution levels (Lawrence & Menden-Deuer 2012). Some scientists have substituted the phytoplankton specific growth rate with the apparent growth rate from the lowest dilution level to work around this caveat. Worden & Binder (2003) developed a 2-point modification of the dilution method to increase the dilution method's spatial and temporal resolution while subsequently reducing the effort required to conduct a dilution experiment. The approach has been shown to be a viable alternative (Strom & Fredrickson 2008), but continues to be widely questioned. To address this question, phytoplankton apparent growth rate values from the lowest dilution level (i.e. 10%

whole seawater) were compared to nutrient-amended phytoplankton specific growth rates using a paired t-test.

A major assumption of the dilution method is that microzooplankton clearance rates are related to the number of predator-prey encounters (i.e. grazers will always feed at a constant and maximal rate). A multi-level dilution method therefore has the advantage of being able to identify non-linear feeding responses, which has been previously observed in productive estuaries (Gallegos 1989, Lessard & Murrell 1998; Worden & Binder 2003). Using a paired t-test, we compared phytoplankton growth and grazing rate estimates based on the empirically determined 5-point dilution to a hypothetical 2-point dilution (i.e. calculating growth and grazing rates based only on the apparent growth rates from 10% and 100% whole seawater). Our results indicate that there was no significant difference between the apparent growth rate (k) obtained from the 10% whole seawater and the specific growth rate (μ) obtained from our 5-point regression for both the ambient ($p = 0.13$) and the cooled ($p = 0.18$) treatments. We observed no difference in rates, irrespective of a 2 or 5-point dilution, over a range of chlorophyll values (i.e. 6-fold variation) and throughout a study period in which the autotrophic and heterotrophic assemblages changed significantly. Though previous authors have suggested a 2-point dilution as providing possibly only a conservative estimate (Lawrence & Menden-Deuer 2012) there was no support for this suggestion. Therefore, we concluded that the rate measurements derived using the 2 and 5-point modification is indistinguishable and confirmed prior research that made the same observations (Strom & Fredrickson 2008; Worden & Binder 2003).

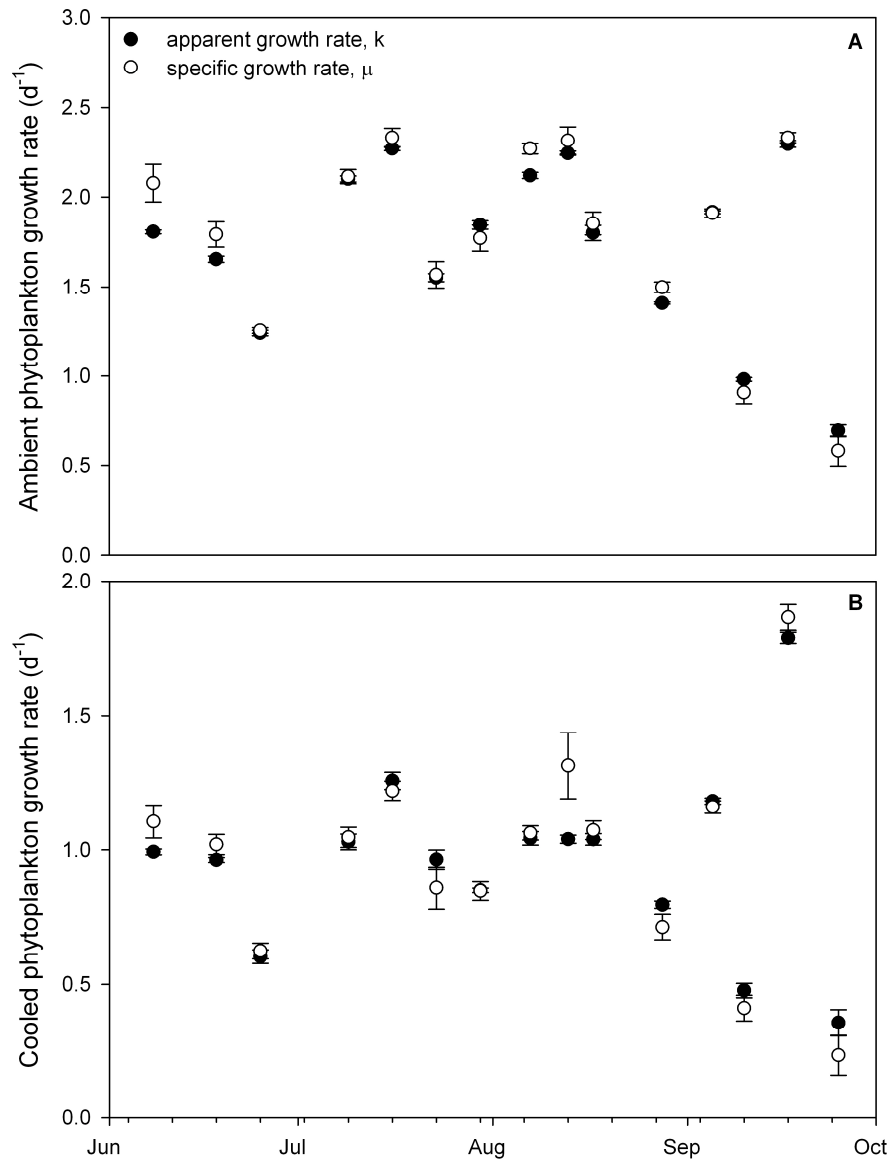


Figure A1. Comparison of the average phytoplankton apparent growth rate from the lowest dilution level (closed circles) to the average phytoplankton specific growth rate (μ , d^{-1}) (open circles) obtained from a set of fifteen ambient (A) and cooled (B) 24 h dilution experiments from each 5-point regression from 8 June through 25 September 2012. Error bars represent the standard deviation from duplicate measurements. There was no significant difference between rates of apparent growth and specific growth despite a wide range (i.e. 6-fold) of chlorophyll *a* values.

LITERATURE CITED

- Banase, K. (1992) Grazing, temporal changes of phytoplankton concentrations, and the microbial loop in the open sea. In Falkowski, P.G. and Woodhead, A.D. (eds), *Primary Productivity and Biogeochemical Cycles in the Sea*. Plenum Press, New York, pp. 409-440.
- Borkman, D. G., & Smayda, T. (2009) Multidecadal (1959–1997) changes in *Skeletonema* abundance and seasonal bloom patterns in Narragansett Bay, Rhode Island, USA. *J Sea Res*, **61**:84–94.
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, B. (2004) Toward a Metabolic Theory of Ecology. *Ecology*, **85**:1771–1789.
- Burkill, P. H., Edwards, E. S. & Sleight, M.A. (1995) Microzooplankton and their role in controlling phytoplankton growth in the marginal ice zone of the Bellingshausen Sea. *Deep-Sea Res Pt II*, **42**:1277–1290.
- Caron, D.A. & Hutchins, D.A. (2012) The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps. *J Plankton Res*, **0**:1-18.
- Caron, D. A., Dennett, M. R., Lonsdale, D. J., Moran, D. M., & Shalapyonok, L. (2000) Microzooplankton herbivory in the Ross Sea, Antarctica. *Deep-Sea Res Pt II*, **47**:3249–3272.
- Chen, B., Landry, M. R., Huang, B., & Liu, H. (2012) Does warming enhance the effect of microzooplankton grazing on marine phytoplankton in the ocean? *Limnol Oceanogr*, **57**:519–526.
- Deason, E. E., & Smayda, T.J. (1982) Ctenophore-zooplankton-phytoplankton interactions in Narragansett Bay, Rhode Island, USA, during 1972-1977. *J Plankton Res*, **4**:203-217.
- Dolan, J.R., Gallegos, C.L & Moigis, A. (2000) Dilution effects on microzooplankton in dilution grazing experiments. *Mar Ecol Prog Ser*, **200**:127-139.
- Dolan, J.R. & McKeon, K. (2005) The reliability of grazing rate estimates from dilution experiments: Have we over-estimated rates of organic carbon consumption by microzooplankton? *Ocean Sci* **1**:1-7.
- Durbin, E. G., Krawiec, R. W. & Smayda, T.J. (1975) Seasonal studies on the relative importance of different size fractions of phytoplankton in Narragansett Bay (USA). *Mar Biol*, **32**:271–287.
- Eppley, R. W. (1972) Temperature and phytoplankton growth in the sea. *Fish B-NOAA*, **70**:1063–1085.
- Falkowski, P. G. (1998). Biogeochemical Controls and Feedbacks on Ocean Primary Production. *Science*, **281**:200–206.
- Field, C., Behrenfeld, M., Randerson, J., & Falkowski, P. (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. *Science*, **281**:237–40.
- Fogg, G.E. 2001. Algal adaptation to stress – some general remarks. In Rai LC, Gaur JP, eds. *Algal adaptation to environmental stresses. Physiological, biochemical and molecular mechanisms*. Berlin, Germany: Springer, 1-20.
- Gallegos, C.L. (1989) Microplankton grazing on phytoplankton in the Rhode River, Maryland: non-linear feeding kinetics. *Mar Ecol Prog Ser*, **57**:23-33.

- Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. & Charnov, E.L. (2001) Effects of Size and Temperature on Metabolic Rate. *Science*, **293**:2248-2251.
- Gillooly, J.F., Charnov, E.L., West, G.B., Savage, V.M & Brown, J.H. (2002) Effects of size and temperature on developmental time. *Nature*, **417**:70-73.
- Goldman JC (1984) Conceptual role for microaggregates in pelagic waters. *B Mar Sci*, **35**:462-476.
- Goldman, J.C & Carpenter, E.J. (1974) A kinetic approach to the effect of temperature in algal growth. *Limnol Oceanogr*, **19**:756-766.
- Graff, J. R., & Rynearson, T. A. (2011) Extraction method influences the recovery of phytoplankton pigments from natural assemblages. *Limnol Oceanogr-Meth*, **9**:129–139.
- Intergovernmental Panel on Climate Change (IPCC). (2011). Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. *Climate Change 2001: The Scientific Basis*, J. Houghton et al., eds. Cambridge University Press, Cambridge, UK, 1-88.
- Juhl, A.R. & Murrell, M.C. (2005) Interactions between nutrients, phytoplankton growth, and microzooplankton grazing in a Gulf of Mexico estuary, *Aquat Microb Ecol*, **38**:147-156.
- Karentz, D. & Smayda, T.J. (1984) Temperature and seasonal occurrence patterns of 30 dominant phytoplankton species in Narragansett Bay over a 22-year period (1959-1980) *Mar Ecol Prog Ser*, **18**:277-293.
- Karentz D. & Smayda, T.J. (1998) Temporal patterns and variations in phytoplankton community organization and abundance in Narragansett Bay during 1959-1980. *J Plankton Res*, **20**:145-168.
- Keller, A. A., Oviatt, C. A., Walker, H. A., & Hawk, J. D. (1999) Predicted impacts of elevated temperature on the magnitude of the winter-spring phytoplankton bloom in temperate coastal waters: A mesocosm study. *Limnol Oceanogr*, **44**:344–356.
- Landry, M. Constantinou, R., J., & Kirshtein, J. (1995) Microzooplankton grazing in the central equatorial Pacific during February and August, 1992. *Deep-Sea Res Pt II*, **42**:657–671.
- Landry, M., & Calbet, A. (2004) Microzooplankton production in the oceans. *ICES J Mar Sci*, **61**:501–507.
- Landry, M.R., Constantinou, J. & Kirshtein, J. (1995) Microzooplankton grazing in the central equatorial Pacific during February and August, 1992. *Deep-Sea Res Pt II*, **42**:657-671.
- Landry, M. R., & Hassett, R. P. (1982) Estimating the grazing impact of marine micro-zooplankton. *Mar Biol*, **67**:283–288.
- Lawrence, C. & Menden-Deuer, S. (2012) Drivers of protistan grazing pressure: seasonal signals of plankton community composition and environmental conditions. *Mar Ecol Prog Ser*, **459**:39-52.
- Lessard, E.J. & Murrell, M.C. (1998) Microzooplankton herbivory and phytoplankton growth in the northwestern Sargasso Sea. *Aquat Microb Ecol*, **16**:173-188.
- Levitus, S. (2000). Warming of the World Ocean. *Science*, **287**:2225–2229.
- Li, Q.P., Franks, P.J.S., & Landry M.R. (2011) Microzooplankton grazing dynamics: parameterizing grazing models with dilution experiment data from the California Current Ecosystem. *Mar Ecol Prog Ser*, **438**:59-69.

- López-Urrutia, A., San Martín, E., Harris, R. P., & Irigoien, X. (2006) Scaling the metabolic balance of the oceans. *P Natl Acad Sci USA*, **103**:8739–44.
- Martin, J.H. (1965) Phytoplankton-zooplankton relationships in Narragansett Bay. *Limnol Oceanogr*, **10**:185-191.
- Menden-Deuer, S., & Lessard, E.J. (2000) Carbon to Volume Relationships for Dinoflagellates, Diatoms, and other protist plankton. *Limnol Oceanogr*, **45**:569–579.
- Modigh, M. & Franzè, G. (2009) Changes in phytoplankton and microzooplankton populations during grazing experiments at a Mediterranean coastal site. *J Plankton Res*, **31**:853–864.
- Montagnes, D. J. S., Kimmance, S. A., & Atkinson, D. (2003) Using Q₁₀ : Can growth rates increase linearly with temperature? *Aquat Microb Ecol*, **32**:307–313.
- Montagnes, D. J. S., & Franklin, D. J. (2001) Effect of Temperature on diatom volume, growth rate, and carbon and nitrogen content: Reconsidering some paradigms. *Limnol Oceanogr*, **46**:2008–2018.
- Nixon, S., Granger, S., Buckley, B., Lamont, M., Rowell, B. (2004) A One Hundred and Seventeen Year Coastal Water Temperature Record from Woods Hole , Massachusetts. *Estuaries*, **27**:397–404.
- Oviatt, C. (2004) The Changing Ecology of Temperate Coastal Waters During a Warming Trend. *Estuaries*, **27**:895–904.
- Pratt, D.M., (1959) The phytoplankton of Narragansett Bay. *Limnol Oceanogr*, **4**:425-440.
- Rhee, G. & Gotham, I. (1981) The Effect of Environmental Factors on Phytoplankton Growth : Temperature and the Interactions of Temperature with Nutrient Limitation. *Limnol Oceanogr*, **26**:635–648.
- Riding, R. (1992). The algal breath of life. *Palaeoclimatology*, **359**:13-14.
- Rose, J. M., & Caron, D. A. (2007) Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. *Limnol Oceanogr*, **52**:886–895.
- Rose, J.M, Feng, Y. DiTullio, G.R., Dunbar, R.B., Hare, C.E., Lee, P.A., Lohan, M., Long, M. Smith Jr., W.O., Shost, B., Tozzi, S., Zhang, Y. & Hutchins, D.A. Synergistic effects of iron and temperature on Antarctic phytoplankton and microzooplankton assemblages. *Biogeosciences*, **6**:3131-3147.
- Schofield, O., Grzymski, J., Moline, M., & Mjovine, R. (1998) Impact of temperature acclimation on photosynthesis in the toxic red-tide dinoflagellate *Alexandrium fundyense* (Ca28). *J Plankton Res*, **20**:1241–1258.
- Sherr, B. F. & Sherr E. B. (1994) Bacterivory and Herbivory: Key Roles of Phagotrophic Protists in Pelagic Food Webs. *Microb Ecol*, **28**:223–235.
- Sherr, E. B., Sherr, B. F., Wheeler, P.A. & Thompson K. (2003) Temporal and spatial variation in stocks of autotrophic and heterotrophic microbes in the upper water column of the central Arctic Ocean. *Deep-Sea Res Pt 1*, **50**:557–571.
- Sherr, E., & Sherr, B. (2009) Capacity of herbivorous protists to control initiation and development of mass phytoplankton blooms. *Aquat Microb Ecol*, **57**:253–262.
- Smayda, T. J. (1957) Phytoplankton studies in lower Narragansett Bay. *Limnol Oceanogr*, **2**:342-359.

- Staehr, P.A. & Birkeland, M.J. (2006) Temperature acclimation of growth, photosynthesis and respiration in two mesophylic phytoplankton species. *Phycologia*, **45**:648-656.
- Staehr, P. & Sand-Jensen, K. (2006) Seasonal changes in temperature and nutrient control of photosynthesis, respiration and growth of natural phytoplankton communities. *Freshwater Biol*, **51**:249–262.
- Stoecker, D. K., & Capuzzo, J. M. (1990) Predation on Protozoa: its importance to zooplankton. *J Plankton Res*, **12**:891-908.
- Strom, S.L. & Fredrickson, K.A. (2008) Intense stratification leads to phytoplankton nutrient limitation and reduced microzooplankton grazing in the southeastern Bering Sea. *Deep-Sea Res II*, **55**:1761-1774.
- Strom, S. L., Macri, E. L. & Olson, M. B. (2007) Microzooplankton grazing in the coastal Gulf of Alaska: Variations in top-down control of phytoplankton. *Limnol Oceanogr*, **52**:1480–1494.
- Strom, S. L., M. A. Brainard, J. L. Holmes, & Olson M. B. (2001) Phytoplankton blooms are strongly impacted by microzooplankton grazing in coastal North Pacific waters. *Mar Biol*, **138**:355–368.
- Suikkanen, S., Pulina, S., Engström-Öst, J., Lehtiniemi, M., Lehtinen, S., & Brutemark, A. (2013) Climate Change and Eutrophication Induced Shifts in Northern Summer Plankton Communities. PLoS ONE, **8**:1–10. E66475.
Doi:10.1371/journal.pone.0066475.
- Suzuki, Y. & Takahashi, M. (1995) Growth responses of several diatom species isolated from various environments to temperature. *J Phycol*, **31**:880-888.
- Tsuda, A. & Kawaguchi, S. (1997) Microzooplankton grazing in the surface water of the Southern Ocean during an austral summer. *Polar Biol*, **18**:240-245.
- Weinbauer, M.G., Bonilla-Findji, O., Chan, A.M., Dolan, J.R., Short, S.M., Šimek, K., Wilhelm, S.W. & Suttle, C.A. (2011) *Synechococcus* growth in the ocean may depend on the lysis of heterotrophic bacteria. *J Plankton Res*, **33**:1465-1476.
- Worden, A.Z. & Binder, B.J. (2003) Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synechococcus* populations in oligotrophic environments. *Aquat Microb Ecol*, **30**:159-174.
- Utermöhl, J. (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt Int Ver Theor Angew Limnol*, **9**:1-38.
- Verity, P.G. (1986) The relative food value of diatoms, dinoflagellates, flagellates, and cyanobacteria for tintinnid ciliates. *Arch Protistenkd* **131**:71-84.
- Verity, P.G., Stoecker, D.K., Sieracki, M.E., Burkhill, P.H., Edwards, E.S. & Tronzo C.R. (1993) Abundance, biomass, and distribution of heterotrophic dinoflagellates during the North Atlantic spring bloom. *Deep-Sea Res II*, **40**: 227-244.
- Vigil, P., Countway, P., Rose, J., Lonsdale, D., Gobler, C. & Caron, D. (2009) Rapid shifts in dominant taxa among microbial eukaryotes in estuarine ecosystems. *Aquat Microb Ecol*, **54**:83–100.

TABLES

Table 1. Monthly average *in situ* surface water temperatures from the long-term monitoring station in Narragansett Bay for the past five years.

Month	Average Temperature (°C ± stdev)
June	18.8 ± 1.5
July	22.3 ± 1.6
August	22.5 ± 1.0
September	20.1 ± 1.1

Table 2. *In situ* temperature, chl a, and biomass measurements as well as growth and grazing rates and percent primary production (%PP) consumed in incubations at ambient temperatures from June - September 2012 at the long-term time series station in Narragansett Bay, R.I.

Date	<i>in situ</i> temperature (°C)	<i>in situ</i> chl <i>a</i> (µg L ⁻¹)	autotrophic biomass (µg C L ⁻¹)	heterotrophic biomass (µg C L ⁻¹)	growth rate, µ (d ⁻¹)	grazing rate, g (d ⁻¹)	%PP consumed g:µ*100
8 Jun	17.48	6.65	574	1037	2.08	0.54	35
18 Jun	19.64	3.45	179	216	1.79	0.00	0
25 Jun	21.50	5.36	20	150	1.26	0.26	26
9 Jul	22.89	3.80	74	235	2.11	0.62	42
16 Jul	23.86	6.14	156	619	2.33	1.23	112
23 Jul	23.02	4.38	2	209	1.57	0.91	138
30 Jul	23.19	4.61	6	348	1.77	0.86	95
7 Aug	24.52	6.46	158	374	2.27	1.20	112
13 Aug	25.03	7.27	162	817	2.31	1.23	113
17 Aug	25.24	10.72	209	693	1.85	0.55	43
28 Aug	23.19	10.67	225	308	1.50	0.84	128
5 Sep	22.89	5.57	125	462	1.91	0.35	22
10 Sep	22.56	8.20	5	850	0.91	0.53	139
17 Sep	20.54	1.70	3	150	2.33	0.32	16
25 Sep	19.26	4.37	7	421	0.58	0.00	0

Table 3. Comparison of phytoplankton apparent growth rates (k , d^{-1}) from nutrient amended (+) and un-amended (-) incubations. A significant nutrient enhancement effect was observed for all but the last sampling date (*italicized*).

Date	<u>ambient</u>		<u>cooled</u>	
	k + nutrients	k - nutrients	k + nutrients	k - nutrients
8 Jun	1.499	-0.022	1.252	-0.027
18 Jun	1.691	-0.602	0.016	0.128
25 Jun	1.021	-0.227	0.409	-0.124
9 Jul	1.571	-0.835	0.797	-0.569
16 Jul	1.224	-0.202	1.113	-0.503
23 Jul	0.745	-0.916	0.435	-0.404
30 Jul	1.008	-0.129	0.388	0.134
7 Aug	1.0802	-0.700	0.8688	-0.373
13 Aug	1.108	-0.454	0.685	-0.163
17 Aug	1.365	0.348	0.843	-0.140
28 Aug	0.645	-0.419	0.582	-0.222
5 Sep	1.59	0.367	0.993	0.460
10 Sep	0.465	-0.439	0.325	-0.047
17 Sep	2.04	1.130	1.8969	1.526
<i>25 Sep</i>	0.43	0.350	0.2592	0.176

Table 4. The average temperature of the ambient and cooled treatment during each incubation from June through September 2012. Averages represent temperature recordings at 15-minute intervals throughout the incubation. Error represents one standard deviation from the mean.

Date	Ambient (°C)	Cooled (°C)	Difference (°C)
8 Jun 12	20.3 ± 1.6	15.2 ± 0.6	5.1 ± 1.2
18 Jun 12	20.0 ± 1.3	15.2 ± 0.6	4.8 ± 1.0
25 Jun 12	21.1 ± 0.6	15.3 ± 0.6	5.7 ± 0.9
9 Jul 12	24.6 ± 1.9	17.5 ± 0.5	7.1 ± 1.6
16 Jul 12	25.2 ± 1.5	17.7 ± 0.9	7.5 ± 1.5
23 Jul 12	23.8 ± 1.3	17.5 ± 0.7	6.3 ± 1.1
30 Jul 12	23.6 ± 1.6	17.4 ± 0.5	6.2 ± 1.3
7 Aug 12	24.9 ± 1.5	17.5 ± 0.6	7.4 ± 1.1
13 Aug 12	25.2 ± 1.4	17.6 ± 0.9	7.6 ± 1.2
17 Aug 12	24.9 ± 1.6	17.3 ± 0.4	7.6 ± 1.4
28 Aug 12	24.2 ± 1.5	17.6 ± 0.9	6.6 ± 1.1
5 Sep 12	23.1 ± 0.2	15.6 ± 0.3	7.5 ± 0.2
10 Sep 12	22.5 ± 1.8	15.7 ± 0.9	6.8 ± 1.2
17 Sep 12	21.0 ± 1.1	15.6 ± 0.4	5.4 ± 0.9
25 Sep 12	20.2 ± 1.3	15.8 ± 0.6	4.4 ± 0.9

FIGURE LEGENDS

Figure 1. Principal component analysis (PCA) characterizing salinity (S), sea-surface temperature (SST), photosynthetically available radiation (PAR), and dissolved oxygen (DO) variation from weekly samples taken between 8 June to 25 September 2012 in Narragansett Bay. The first and second axes of the PCA accounted for 44.4% and 28.3% of the explained variation among sample dates, respectively. Temperature was not a main driver of the seasonal variation observed.

Figure 2. Biomass estimates ($\mu\text{g C L}^{-1}$) of the weekly Narragansett Bay autotrophic and heterotrophic protist assemblages from 8 June through 25 September 2012 *in situ* (A, D) at the Long-Term Monitoring Station, and after a 24 h dilution experiment incubated at ambient (B, E) and cooled (C, F) temperatures.

Figure 3. Non-metric multi-dimensional scaling (MDS) ordination of the three significantly ($p < 0.05$) distinct autotrophic species assemblages, represented by the biomass of the ten most abundant diatom species, observed weekly *in situ* from 8 June to 25 September 2012 in Narragansett Bay. The discriminating species are indicated for each assemblage (e.g. triangle: *Thalassiosira* sp.; circle: *C. pelagica* = *Cerataulina pelagica*; cross: Chae & Skel = *Chaetoceros* sp. & *Skeletonema* sp.). CLUSTER's percent similarity overlay (similarity circles) indicates the degree similarity in assemblage structure.

Figure 4. Initial chlorophyll *a* concentrations (grey bars) as well as ambient rates of phytoplankton specific growth (closed circles) and heterotrophic protist grazing rates (open circles) obtained from dilution experiments using a phytoplankton assemblage from Narragansett Bay. Error bars represent the standard error from triplicate chl *a*

measurements from duplicate bottles. Growth and grazing rates were similar in magnitude and phytoplankton growth exceeded heterotrophic protist grazing for the majority of the sample dates.

Figure 5. Rates of phytoplankton specific growth (A) and heterotrophic protist grazing (B) from 8 June to 25 September 2012 that resulted after an incubation period at ambient (closed circles) and cooled (open circles) temperatures. The error bars represent the standard error from duplicate measurements. Note the change in y-axis range. Ambient rates exceeded cooled rates for all instances when non-zero measurements occurred. An on average 6.4°C decrease in temperature significantly reduced rates of phytoplankton growth and heterotrophic protist grazing.

Figure 6. Linear regressions (solid lines) of cooled versus ambient rate data of phytoplankton specific growth (black circles) and heterotrophic protist grazing rates (grey triangles) from two temperatures across fifteen samples dates from 8 June through 25 September 2012. Relationships are plotted alongside the one-to-one line (dashed line). An on average 6.4°C decrease in temperature resulted in a decreased heterotrophic protist grazing response that was greater in magnitude than the corresponding decrease in the phytoplankton specific growth response.

Figure 7. Percent primary production (%PP) consumed by heterotrophic protists by sample date (A) and *in situ* temperature of Narragansett Bay (B) from incubations at two temperatures -ambient (closed symbols) and cooled (open symbols)- from 8 June through 25 September 2012. In the top graph, the horizontal dashed line indicates where phytoplankton growth is equal to heterotrophic protist grazing. Values of

ambient %PP were on average 1/3 greater than cooled values, but the difference was insignificant and unrelated to *in situ* temperatures.

FIGURES

Fig. 1 DeCuollo & Menden-Deuer

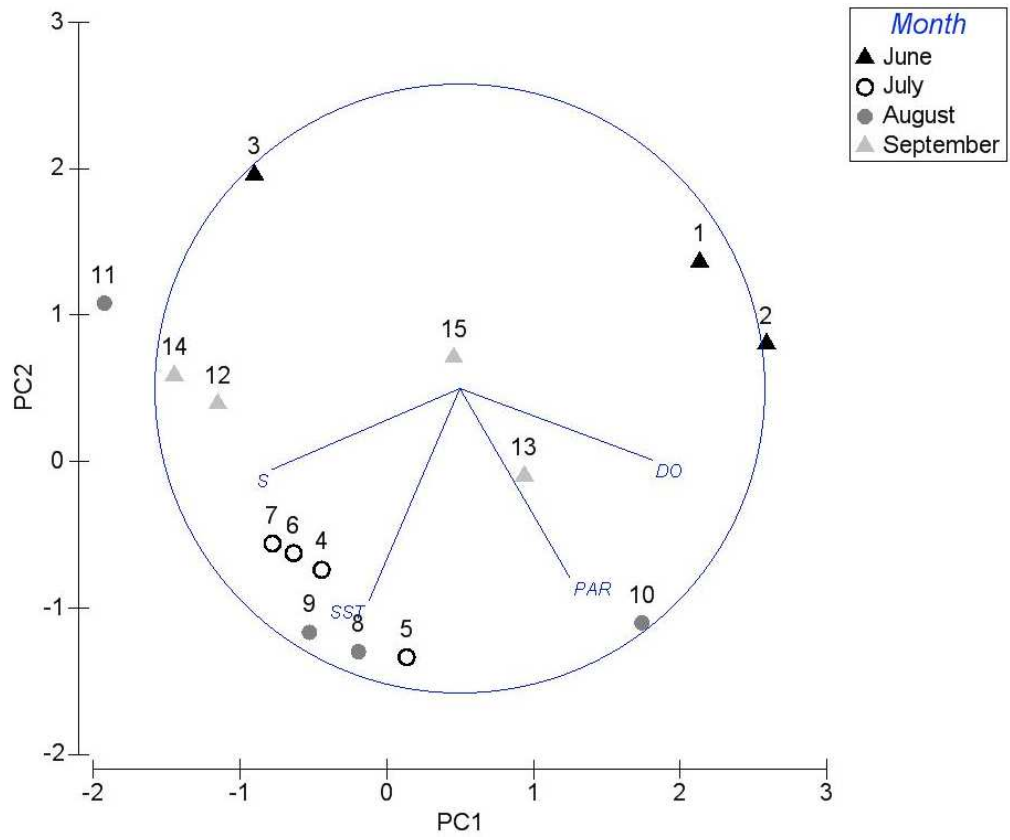


Fig. 2 DeCuollo & Menden-Deuer

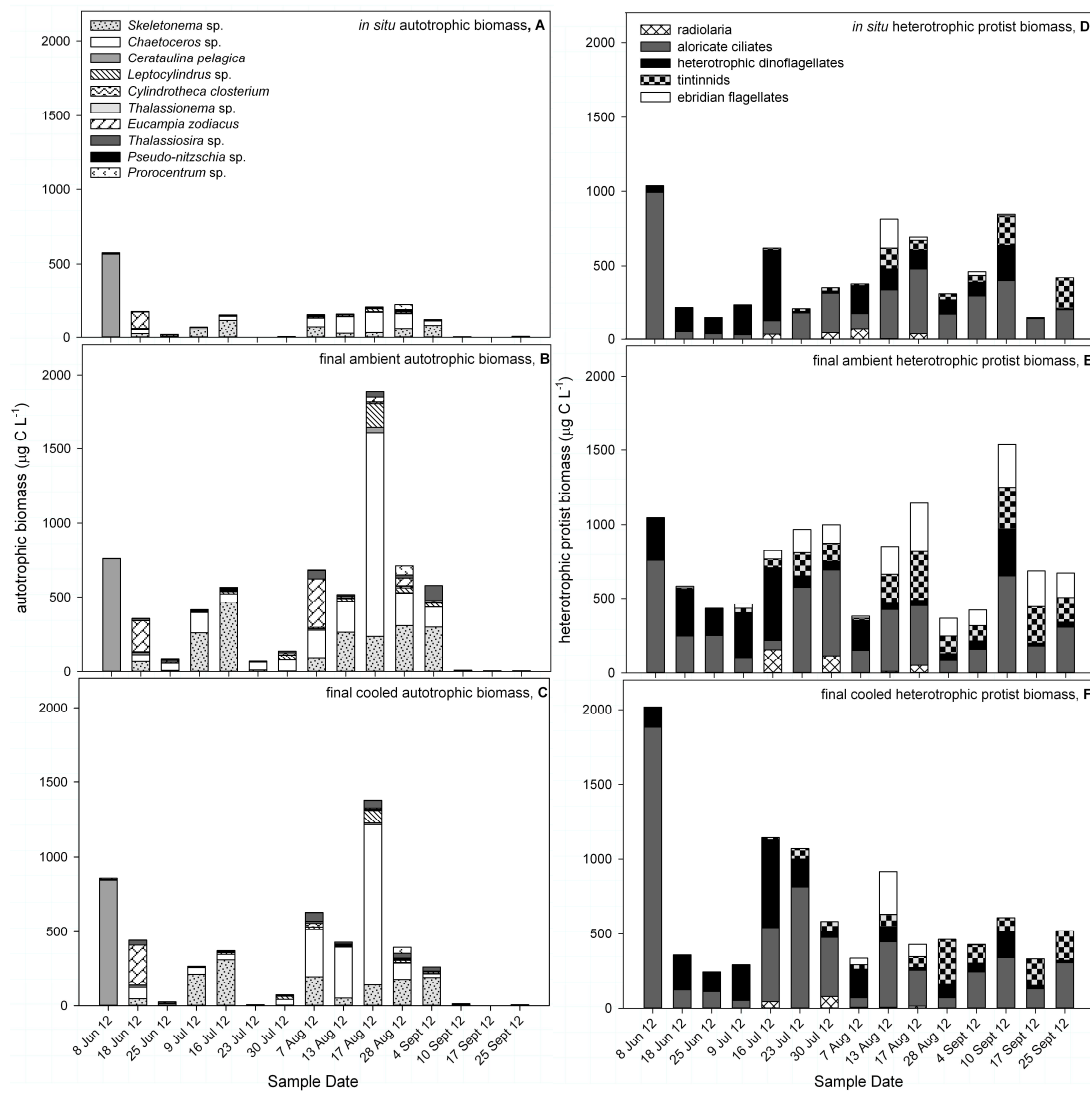


Fig. 3 DeCuollo & Menden-Deuer

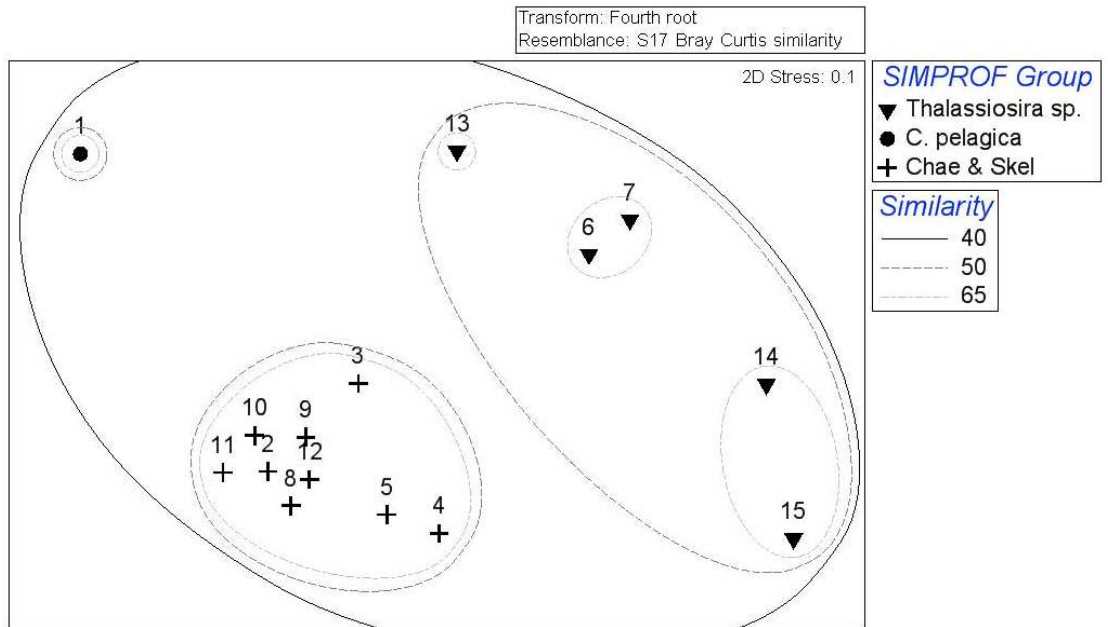


Fig. 4 DeCuollo & Menden-Deuer

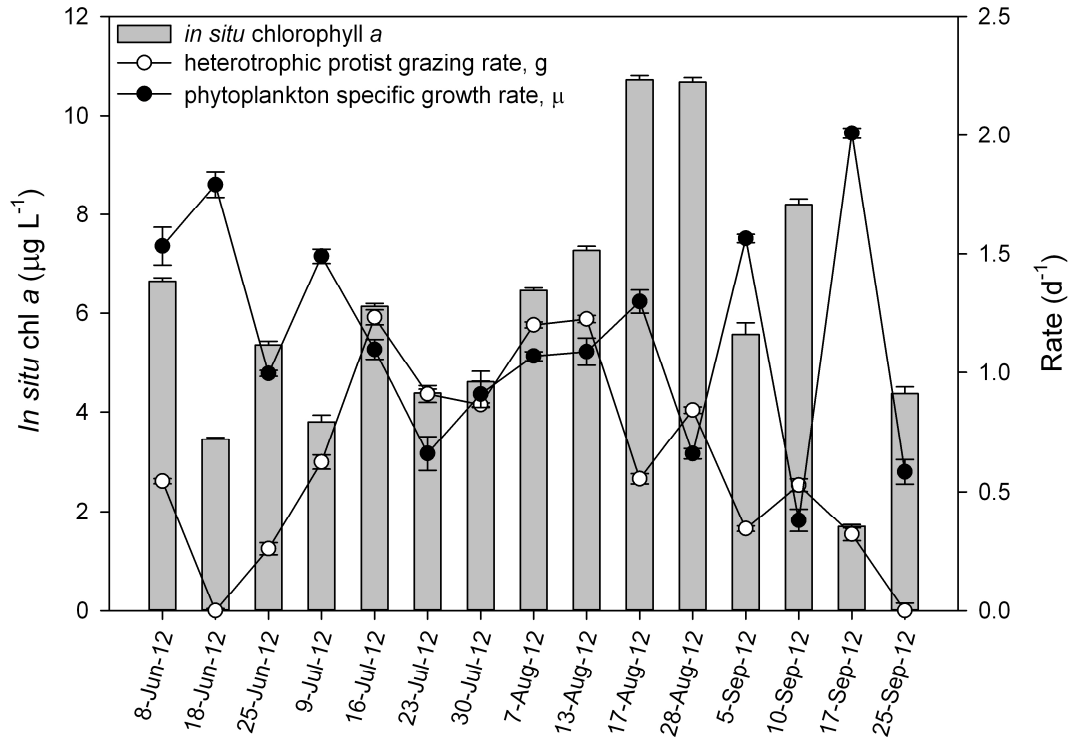


Fig. 5 DeCuollo & Menden-Deuer

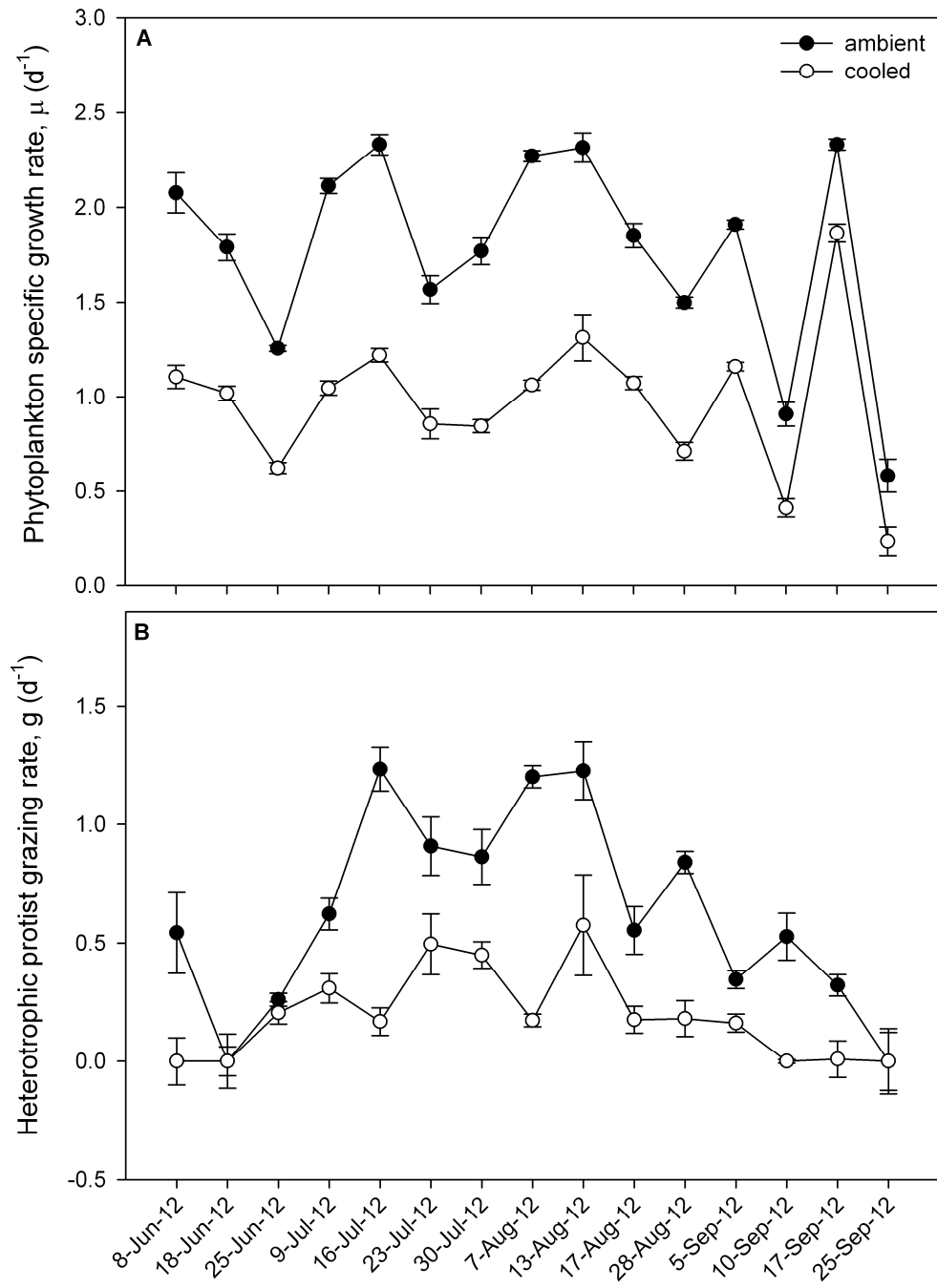


Fig. 6 DeCuollo & Menden-Deuer

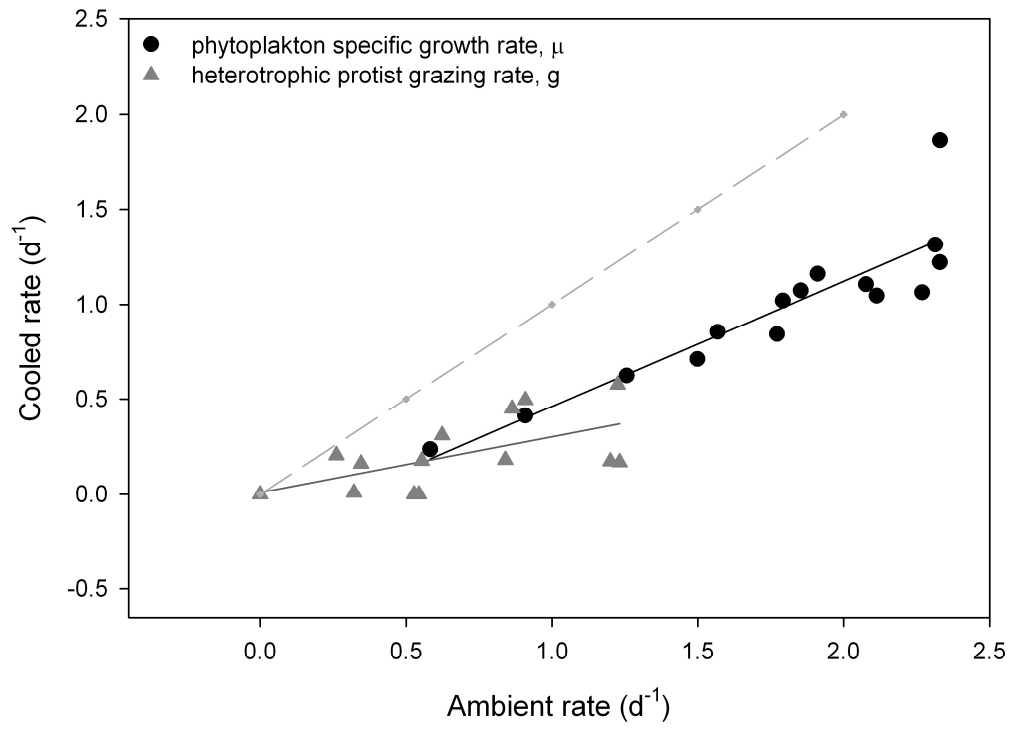


Fig. 7 DeCuollo & Menden-Deuer

