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FINAL REPORT

Summer Microzooplankton in the Bering Sea
NPRB BSIERP Project B55 Final Report

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Abstract

Microzooplankton (20-200 micron size fraction of zooplankton) abundance, biomass, contribution to chlorophyll \( a \) and grazing on phytoplankton were estimated as part of the BEST-BSIERP integrated ecosystem project during the summers of 2008, 2009 and 2010. One celled grazers, primarily ciliates and dinoflagellates, dominated the microzooplankton biomass, and at times microzooplankton biomass was higher than phytoplankton biomass in surface waters on the inner and middle shelf. A confounding factor was the contribution of mixotrophic (combination of feeding and photosynthesis) ciliates to chlorophyll \( a \), which is a proxy for phytoplankton biomass. On average, mixotrophic ciliates comprised 66% of the ciliate biomass, and in the North Middle Domain, sometimes contributed over 50% of the chlorophyll \( a \) in surface waters. Near the shelf break and in the “bloom” waters, microzooplankton biomass was about one-half that on the inner and middle shelf. At the shelf break and in the outer shelf domain, microzooplankton consumed 67-78% of phytoplankton daily growth but in the middle and inner shelf domains, microzooplankton grazing exceeded phytoplankton daily growth. On the northern Middle Shelf, there was usually a prominent deep chlorophyll maximum (DCM); microzooplankton ingestion of chlorophyll was high in the DCM, indicating a role for the DCM in trophic transfer. On the eastern Bering Sea shelf in summer, microzooplankton consumed most of the phytoplankton production and accounted for many of the larger size cells in the plankton; indicating that they are an important trophic link in food webs supporting larger zooplankton, which in turn support fish and larger organisms.

Keywords: planktonic ciliates, dinoflagellates, subarctic seas, subpolar continental shelf, microzooplankton grazing, dilution experiments, mixotrophy, plastidic ciliates, grazing in DCM

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Introduction

The BSIERP program was designed to study ecosystem processes and the effects of inter-annual and decadal variation in weather and climate on food webs that support fisheries and marine bird and mammal populations in the eastern Bering Sea. Summer data on microzooplankton were a gap in the program. Microzooplankton are responsible for most of the grazing on phytoplankton in the sea (Landry and Calbet, 2004; Sherr and Sherr, 2009). They are an important link in the food chain between phytoplankton and zooplankton, such as copepods and krill (Calbet and Saiz, 2005; Campbell et al., 2009), which are food for zooplanktivorous fish such as larval and juvenile walleye pollock and forage fish that support top predators (Hunt et al. 2002, 2011). Microzooplankton grazing is also a key factor regulating phytoplankton blooms (Landry and Calbet, 2004; Sherr and Sherr, 2009). The project “Summer Microzooplankton in the Bering Sea” contributes to the goals of the program by providing data for summers 2008, 2009, and 2010 on standing stocks of microzooplankton and their grazing activities. Abundance, biomass, size distribution and composition of larger microzooplankton were determined. Experiments were conducted at sea to measure grazing by microzooplankton on phytoplankton. Microzooplankton production was estimated. Information on microzooplankton is necessary to construct models linking changes in climate and weather to changes in food webs that support fish, marine birds and mammals in this highly productive, but potentially vulnerable, marine ecosystem. This project specifically addresses the BSIERP hypothesis that “Climate-induced changes in physical forcing will modify the availability and partitioning of food for all trophic levels through bottom-up processes”. A key goal was to determine how the microzooplankton link is influenced by regional differences in physical forcing and climate-induced changes in Bering Sea food webs.

Previous to this study, there were only three summer investigations of microzooplankton in the eastern Bering Sea (Liu et al., 2002; Olson and Strom, 2002; Strom and Fredrickson, 2008, Sherr et al., in press), but this limited data set illustrated the inter-annual variability in microzooplankton and raised interesting questions about coupling between phytoplankton and microzooplankton in this ecosystem. Two of the studies were conducted in summer of 1999 (Liu et al., 2002; Olson and Strom, 2002) and the third study was conducted in summer 2004 (Strom and Fredrickson, 2008). A fourth study was part of the BEST program and conducted during spring sea ice conditions of 2008, 2009 and 2010 (Sherr et al., in press). The first recorded bloom in the Bering Sea of the coccolithophorid alga, *Emiliana huxleyi*, occurred in 1997, and the bloom persisted in patches into 1999. In 1999, reduced microzooplankton grazing on < 10 µm phytoplankton (the size class containing *Emiliana huxleyi*) was observed inside bloom patches (Olson and Strom, 2002). The conventional paradigm is that microzooplankton are primarily the grazers of small phytoflagellates, not large diatoms. Surprisingly, Olson and Strom (2002)
observed a greater impact of microzooplankton grazing on net growth of the >10 µm fraction of phytoplankton than on the <10 µm fraction, and on average, MZ grazing consumed 100% of the phytoplankton growth in the > 10 µm size fraction of phytoplankton. Very high abundance of microzooplankton (22-227 cells ml\(^{-1}\)) and high microzooplankton biomass were observed. These data suggest that in 1999 top-down control of microzooplankton by larger zooplankton was weak and microzooplankton grazing was important in regulating phytoplankton composition. Microzooplankton grazing appears to have promoted the persistence of the coccolithophorid bloom that channeled phytoplankton production into the microbial food web. It is not known if this increased channeling of production into the microbial food web was a consequence of reduced top-down control of large cell-size microzooplankton by larger zooplankton or whether it was due to “bottom-up” control by the environment favoring particular phytoplankton. Probably it was due to an interaction of these factors.

The most recent summer investigation of microzooplankton in the Bering Sea prior to the BEST/BSIERP Program was in 2004, a year with low ice cover, intense summer stratification at some stations, and anomalously high surface water temperatures (Strom and Fredrickson 2008). In 2004 phytoplankton growth rates were similar to 1999, but microzooplankton grazing mortality of phytoplankton was about half that observed in 1999. Interestingly, pennate diatoms in the genus *Pseudo-nitzschia* were the dominant diatoms at some stations (Strom and Fredrickson, 2008). Biomass of large (>20 µm) MZ in 2004 was about half that observed in 1999. Strom and Fredrickson (2008) observed that the intense stratification in the anomalously warm SE Bering Sea in 2004 lead to phytoplankton nutrient limitation and hypothesized that this was the cause of the reduced grazing and lower microzooplankton standing stock that they observed. This reduced trophic coupling at lower trophic levels may have negatively affected zooplankton stocks and thus perhaps also higher trophic levels that depend on summer zooplankton production.

The results from these prior investigations in the SE Bering Sea suggested several hypotheses concerning the influence of summer sea surface temperature and stratification on trophic coupling low in the food web. These were:

1. In “warmer” years, microzooplankton coupling to phytoplankton growth is reduced compared to more average years due to increased stratification and nutrient depletion in the mixed layer (from Strom and Fredrickson, 2008). *Alternate hypothesis*: In “warmer” years, microzooplankton coupling to phytoplankton growth is increased due to a shift of production to smaller size phytoplankton that are more efficiently grazed by microzooplankton.
2. In “warmer” years, large microzooplankton (>20 µm) biomass is low compared to more average years; less microzooplankton prey is available to zooplankton during “warm” summers. **Alternate hypothesis:** Biomass of large microzooplankton in “warm” years is ≥ than the biomass in average years.

3. At stratified stations, if a deep chlorophyll layer is present at the pycnocline, microzooplankton abundance and biomass per m³ is higher in this layer than in the mixed layer above. The pycnocline is a potential site of enhanced trophic transfer of microzooplankton carbon to higher trophic levels under stratified conditions. **Alternate hypothesis:** Microzooplankton abundance and biomass per m³ in the deep chlorophyll layer is ≤ abundance and biomass in the mixed layer.

These hypotheses were formulated and partially tested by previous investigations in the Bering Sea. However, given the inter-annual variability and spatial heterogeneity of the eastern Bering Sea, there was not enough data to conclusively test any of these hypotheses. The project reported herein was designed to yield comparable data, as much as possible, to the existing data base to facilitate comparison of results across years. It was anticipated that the 2008-2010 field years would include “warm” years with reduced sea ice during spring and that comparisons could be made between “warm” and “cold” years within the project data set. However all three years were cold with average stratification indices (Ladd and Stabeno, 2012; Stabeno et al., 2012b). The data on microzooplankton for summers 2008, 2009 and 2010 demonstrate the important domain (regional) differences in microzooplankton. The 2008-2010 data also document year-to-year differences within a “cold” period in microzooplankton abundance and biomass and microzooplankton grazing. Thus these data provide insights into the structure and functioning of lower trophic levels in the eastern Bering Sea and provide input on microzooplankton abundance, biomass and rate processes useful to zooplankton ecologists and modelers interested in predicting the response of the Bering Sea ecosystem to inter-annual and decadal variation in physical forcing and biotic responses.

**Overall Objectives**

The overall objective was to contribute to the goals of BSIERP by providing data on standing stocks of microzooplankton and their grazing activities. The specific objectives were to:

1. **Determine the abundance, biomass and composition of the larger (>20 µm) microzooplankton.**

Describe inter-annual and spatial (domain) variability in microzooplankton.

We achieved this objective by sampling mixed layer microzooplankton on summer cruises in 2008, 2009, and 2010 in the eastern Bering Sea and analyzing the samples microscopically (Chapter 1, sections 2.1
and 2.2). Stations were grouped by physical domain (Chapter 1, Table 1) and two-way analysis of variance was used to test for differences among domains by year (Chapter 1, section 2.4). Results are presented in Chapter 1, Table 2 and Figures 2-4).

2. Determine if microzooplankton biomass in the upper mixed layer is statistically associated with sea surface temperature, indices of stratification, inorganic nutrient availability, chlorophyll $a$ (< 20 and >20 µm size classes and total), specific phytoplankton blooms or domain (inner, middle or outer shelf).

We partially achieved this objective by using Pearson Product Moment Correlation analyses to determine if microzooplankton biomass was significantly associated with chlorophyll $a$ and found that it was not (Chapter 1, section 3.2 and Figure 5). Because size fractionated chlorophyll was not available from the CTD casts from most stations, we only included total chlorophyll. Likewise, information on phytoplankton species composition was not available for most stations so we did not have the data for these analyses. Instead of presenting correlations between biomass and sea surface temperature and inorganic nutrient availability, we decided to examine the association between microzooplankton grazing and these factors because data on these factors was available for most “process station” casts, which we used for the grazing experiments, but not for most routine sampling stations that we used for the biomass estimates. We found no significant correlations between microzooplankton grazing and water temperature or inorganic nutrients (Chapter 2, section 3.2). We microscopically determined the abundance of Phaeocystis pouchetii in samples from the experimental stations and found no statistically significant correlations with microzooplankton grazing (Chapter 2, section 3.2). Indices of stratification were not available for individual stations and thus we did not include this factor in our statistical analyses. Two-way analysis of variance tested for domain differences in microzooplankton biomass (Chapter 1, Table 2 and Figures 2-4) and grazing (Chapter 2 Section 3.2 and Table 3).

3. Estimate phytoplankton growth rates ($\mu$) and mortality ($g$) due to microzooplankton grazing (<200 µm fraction) with the seawater dilution method. Calculate $g:\mu$ to estimate grazing as a fraction of total phytoplankton production.

We achieved this by conducting seawater dilution grazing experiments on all three summer cruises (Chapter 2, section 2.1 and 2.2, Figure 1 and Table 2). Results ($\mu$, $g$, and $\mu/g$) are given Chapter 2, section 3.2 and Table 3 and Figure 2.

4. Based on estimated phytoplankton mortality due to microzooplankton grazing (dilution experiments), carbon:chlorophyll ratios and microzooplankton gross growth efficiencies from the literature, estimate microzooplankton production.
Estimates of microzooplankton production based on chlorophyll ingestion are presented in Chapter 2, section 3.2 and Figure 4.

5. Compare microzooplankton data from this investigation to data from the literature to assess interannual and regional variability.

This was achieved for both microzooplankton biomass (Chapter 1, sections 4.3 and 4.4, Table 3) and grazing (Chapter 2, section 4, Table 6).

6. Provide data to other BEST/BSIERP field investigators and modelers; collaborate in assessing the role of MZ in the SE Bering Sea Ecosystem and in ecosystem responses to inter-annual and decadal changes in weather and climate.

Data from all cruises has been submitted. We are collaborating with other BEST/BSIERP investigators.
Chapter 1.

Microzooplankton: Abundance, Biomass and Contribution to Chlorophyll in the Eastern Bering Sea in Summer

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Abstract

Summer microzooplankton abundance and biomass were determined for three years, 2008, 2009, and 2010, during a four year “cold” period in the eastern Bering Sea. Average microzooplankton densities ranged from $4 \times 10^3$ to $25 \times 10^3$ cells l$^{-1}$ in the mixed layer. Microzooplankton biomass was 21-25 $\mu$g C l$^{-1}$ in the mixed layer on the middle shelf (South and North Middle Domains) which has relatively low chlorophyll during summer stratification. However, microzooplankton biomass was about one-half that in the less stratified waters near the shelf break, greenbelt, and in the “bloom” waters in the Pribilof Island Domain. Although phytoplankton biomass was higher in deep chlorophyll maxima (DCM) than in surface waters on the shelf, microzooplankton biomass was generally not elevated in the DCM. High ratios (>1) of microzooplankton biomass to phytoplankton biomass were observed at chlorophyll $a$ concentrations < 1 $\mu$g l$^{-1}$. At times, average microzooplankton biomass was higher than the calculated phytoplankton biomass in the mixed layer in coastal (Inner Domain) and middle shelf (South and North Middle Domain) waters. A confounding factor in comparing microzooplankton and phytoplankton biomass was the contribution of plastid-retaining, mixotrophic, ciliates to chlorophyll $a$. On average, mixotrophic ciliates comprised 66% of the ciliate biomass, and in the North Middle Domain, on some cruises, contributed over 50% of the chlorophyll $a$ in the mixed layer. The 2008-2009 data suggest that extent of summer stratification, presence of localized blooms, and domain differences all have major influences on coupling of microzooplankton to phytoplankton stocks in summer in the eastern Bering Sea.
1. Introduction

The eastern Bering Sea supports productive commercial and subsistence fisheries as well as major marine mammal and bird populations (Hunt et al., 2008, 2011). It is also highly sensitive to interannual and decadal-scale climate variability, with differences between “warm”, “cold” and “average” years reflected in secondary productivity and fish, bird, and mammal populations, particularly on the southern shelf (Aydin and Mueter, 2007; Hunt et al., 2002, 2011). The spatial extent of winter sea ice and the timing of sea ice retreat have a large impact on the timing and characteristics of the spring bloom and the ecology of the Bering Sea. Winter-spring conditions set up the shelf for the summer, influencing summer phytoplankton and zooplankton populations (Stabeno et al., 2012b). In addition, summer weather, particularly storms and wind mixing, break down stratification (Ladd and Stabeno, 2012) resulting in large but ephemeral blooms (Sambrotto et al., 1986) that result in enhanced net community production (Mordy et al., 2012).

Microzooplankton are an important link between primary production and higher trophic levels in polar and sub-polar as well as temperate and tropical waters (Levinsen and Nielsen, 2002; Calbet and Saiz, 2005; Campbell et al., 2009). In both “warm” and “cold” years and spring as well as summer, microzooplankton are major consumers of primary production in the eastern Bering Sea (Liu et al., 2002; Olson and Strom, 2002; Strom and Fredrickson, 2008; Sherr et al., accepted; Stoecker et al., this issue). Microzooplankton are important prey for mesozooplankton, including large crustacean zooplankton such as euphausiids and copepods (e.g., Calanus., Neocalanus, and Metridia spp.) as well as for small crustacean zooplankton such as Acartia, Pseudocalanus and Oithona spp. (Gifford and Dagg, 1991; Levinsen and Nielsen, 2002; Campbell et al., 2009; Stoecker 2012) and fish larvae (Fukami et al., 1999; Figueiredo et al., 2007; Montagnes et al., 2010). Crustacean zooplankton are food for larvae and juveniles of many important Bering Sea fish species including pollock (Howell-Kübler et al., 1996; Coyle et al., 2008; Hunt et al., 2011). When chlorophyll is low and dominated by small phytoplankton, microzooplankton can be crucial to the survival and fecundity of mesozooplankton. Microzooplankton are particularly important as a food for mesozooplankton after the spring bloom, during late spring and summer stratification (Ohman and Runge, 1994; Fileman et al., 2010).

Studies during both cold (1999) and warm (2004) years have shown that summer microzooplankton abundance and biomass in the eastern Bering Sea shelf is relatively high and dominated by heterotrophic and mixotrophic dinoflagellates and ciliates. Most data are from the southern Bering shelf and the productive area around the Pribilof Islands (Olson and Strom, 2002; Strom and
However, relatively little information is available on variation in microzooplankton abundance and biomass across the shelf or on the shelf north of St. Matthew Island. The Eastern Bering Sea Shelf is extensive and can be divided into regions by its hydrography and bathymetry; coastal (<50m depth, Inner Domain), middle shelf (50-100m; Middle Domain), and outer shelf (100-200m depth; Outer Domain) (Hunt et al., 2002). Each domain varies in physical forcing, susceptibility to climate change and biology, as does the northern and southern portions of the shelf (Stabeno et al., 2012a). In summer, the south middle shelf is a thermally stratified system with a wind mixed surface layer typically 20-30m thick and a tidally mixed bottom layer. The middle shelf is separated from the inshore, shallow well-mixed coastal domain by the Inner Front, and from the offshore, outer domain by the Middle Transition Zone (Kachel et al., 2002; Stabeno et al. 2012a). Thus, Inner, Middle, and Outer domains now are subdivided into southern and northern regions with the transitional area corresponding to ~60°N, the position of the MN line (Fig. 1) (Stabeno et al., 2012a). The area around the Pribilof Islands on the southern shelf is a separate domain. The Pribilof Domain is supplied with nutrient and plankton rich water from the slope and outer shelf as well as being subject to tidal mixing; it can have new production and localized phytoplankton blooms in summer even when the rest of the south middle shelf is highly stratified (Hunt et al., 2008, Sambrotto et al., 2008).

The southern region is the most susceptible part of the eastern Bering Sea to climate change due to inter-annual variation in the marginal ice zone (Stabeno et al., 2012a, 2012b). During 2007-2010 (cold years) the south middle shelf became well mixed after the retreat of sea ice, with thermal stratification starting in May (Stabeno et al., 2012b). The two-layer structure usually persists through October, however, the magnitude and extent of thermal stratification is not correlated with warm or cold years or spring sea ice extent on the southern shelf (Stabeno et al., 2012b). Wind mixing determines nutrient supply to the euphotic zone, and hence net community production (Mordy et al. 2012), during summer on the southern middle shelf.

On the northern shelf, the average amount of sea ice is greater and persists later in the year than in the south. The north middle domain is usually stratified in summer due to combination of salinity and temperature (Stabeno et al., 2012a). Mixed layer water temperature in summer on the northern shelf is several degrees cooler than in the south. In contrast to the southern middle domain, subsurface phytoplankton blooms are common in summer on the northern middle shelf. The euphotic zone (~20-40 m deep) extends at least partially into the pycnocline (Stabeno et al., 2012a); phytoplankton growth may occur in the euphotic portions of the pycnocline. However, deep chlorophyll maxima resulting from sedimentation of surface blooms may also occur in the pycnocline. No data were available on the association of microzooplankton with these subsurface, high chlorophyll layers.
As part of the BEST-BSIERP ecosystem study in the eastern Bering Sea, we sampled microzooplankton in both the southern and northern shelf on approximately month long cruises in summers of 2008, 2009, and 2010. This was during a four year “cold” period with extensive sea ice cover in spring (Stabeno et al., 2012b). Our primary goal was to investigate summer microzooplankton abundance, biomass, and composition across domains. We were also interested in documenting the relative biomass of microzooplankton to phytoplankton in both the mixed layer and in the deep chlorophyll maxima because this ratio is an indicator of the potential importance of microzooplankton as food for mesozooplankton. The biomass of autotrophic and mixotrophic ciliates is often high in polar seas during summer (Putt, 1990; Simé-Ngando et al., 1992; Sorokin et al., 1996; Suzuki and Taniguchi, 1998; Levinsen and Nielsen, 1999; Montagnes et al., 2008). One of our goals was to determine if plastidic ciliates were important biomass components in the Eastern Bering Sea and to estimate their contribution to chlorophyll $a$ in summer.

2. Materials and Methods

2.1 Microzooplankton sampling and onboard observations

Samples for abundance and biomass estimates of microzooplankton were collected on BEST/BSIERP summer cruises in 2008, 2009, and 2010 on the USCG Healy, R/V Knorr and R/V T.G. Thompson, respectively. In 2008, the cruise was in June but in 2009 and 2010, the cruise was later, from mid-June to mid-July (Table 1). Samples were collected from routine sampling stations along established transects including the cross shelf NP, MN and SL lines and south to north mid-shelf 70-m line (Figure 1). In 2009 the XB and XB2 transects were added to sample the greenbelt area. Mixed layer samples were routinely collected at a predetermined depth, usually 5 or 10 m. Microzooplankton samples were also collected from productivity casts from the depth of 55% of surface incident PAR irradiance that varied from 3-15 m depending upon the station (Lomas et al., 2012). In all cases, this depth was within the surface mixed layer. At stations with a pronounced deep chlorophyll $a$ fluorescence maximum (DCM), additional samples were obtained. The fluorescence maximum samples were from depths of 14 to 46 m. When multiple maxima were sampled, data are presented for the depth with the highest measured chlorophyll $a$. 

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Duplicate samples (250 ml) for microzooplankton were collected with 30 liter Niskin bottles attached to a CTD and water samples were immediately preserved with 10% (final concentration) acid Lugol’s solution in amber glass bottles on HY0803 in 2008 and with 5% (final concentration) acid Lugol’s solution in 2009 (KN195-10) and 2010 (TN-250). In 2008, a 10 sample subset of samples was fixed with 2%, 5%, and 10% acid Lugol’s solution. The estimated abundance with the different strengths were compared; the abundance estimate with 10% acid Lugol’s was not higher than with the 5% solution (data not shown). We switched to 5% acid Lugol’s in 2009 and 2010 since this lower concentration reduces cell shrinkage of ciliates and matched the fixative concentration used on the spring cruises (Sherr et al., 2012). Lugol’s fixed samples were stored in the dark until analysis at Horn Point Laboratory.

Onboard, we examined “fresh” samples from some stations, either live or fixed with 1% glutaraldehyde with transmitted light and/or epifluorescence microscopy in Utermöhl chambers with an inverted microscope. Our objective was to examine cells for the presence or absence of plastids and characterize the types of microzooplankton and microphytoplankton present. We determined which morphotypes of ciliates had plastids (i.e., were mixotrophs) and which were strictly heterotrophic.

2.2 Microzooplankton sample analysis

At Horn Point Laboratory we enumerated > 20 µm protistan microzooplankton (> ~15 µm when fixed with acid Lugol’s), using a Nikon Eclipse TE 2000-U inverted microscope, after settling 25 or 50 ml volumes overnight in Utermöhl chambers (Gifford and Caron, 2000). Our detection limit was ~40 cells liter⁻¹. A SPOT v 4.0 RT camera and SPOT diagnostic software were used to record images and to measure cells. Biovolumes were calculated from the measurements using appropriate geometric formulae. In accordance with earlier microzooplankton studies in the Bering Sea (Olson and Strom, 2002; Strom and Fredrickson, 2008), all dinoflagellates within the designated size range were included in abundance and biomass estimates because most if not all photosynthetic dinoflagellates are also phagotrophic (Jeong et al., 2010).

The ciliates were placed in the following functional and taxonomic categories: 1. *Mesodinium rubrum* (sym. *Myrionecta rubra*); this photoautotrophic ciliate consumes cryptophytes and other small prey. 2. Mixotrophic ciliates; these are non-loricate spirotrich ciliates (strombidiids) that graze on phytoplankton (usually nanophytoplankton) but have chlorophyll and are also photosynthetic. Only ciliates which we observed to have chlorophyll at sea and which we could recognize as the same
morphospecies in the Lugol’s fixed samples are included in this category. Thus, it is a minimum estimate of mixotrophic ciliates. 3. Non-loricate strictly heterotrophic spirotrich ciliates (this includes heterotrophic strombidiids and strobiliids). This category may include a few mixotrophs that we were not able to recognize in the Lugol’s fixed samples. The ciliates in this category are mostly grazers on phytoplankton. 4. Tintinnid ciliates. These were all heterotrophs. 5. Other=All other ciliates.

We used the “C=0.19*V” conversion factor for ciliates from Putt and Stoecker (1989) for the samples fixed in 5% acid Lugol’s solution. This conversion factor is commonly used (for example, in the studies of Bering Sea microzooplankton by Olson and Strom, 2002; Strom and Fredrickson, 2008; Sherr et al. 2012). To correct for shrinkage in the 2008 samples fixed with 10% acid Lugol’s solution we used a correction factor of “C= 0.251*V” based on the Putt and Stoecker factor, but corrected for cell shrinkage in 10% acid Lugol’s solution using data in Stoecker et al. (1994).

Dinoflagellates were categorized as thecate or non-thecate. Most of the dinoflagellates were non-thecate and we were not able to separate many of the smaller non-thecate dinoflagellates by morphotype in the Lugol’s fixed samples and thus could not classify them as plastidic or non-plastidic. Therefore, we aggregated all dinoflagellates in one group. The dinoflagellates did not shrink differently in 5% and 10% acid Lugol’s data (data not shown), so we used the dinoflagellate specific algorithm of Menden-Deuer and Lessard (2000) to estimate the biomass of morphotypes in both the 5% and 10% acid Lugol’s samples. This procedure is the same as in other recent Bering Sea investigations of microzooplankton (Olson and Strom, 2002; Strom and Fredrickson, 2008; Sherr et al., 2012).

We also enumerated and sized other microzooplankton sized protist cells that could not be readily classified taxonomically or which were rare. These included unidentified heterotrophic microflagellates, testate amoebae, and the mixotrophic silicoflagellates. We did not include the colonial mixotrophic flagellate, Dinobryon spp., in our estimates because it did not preserve well in the Lugol’s fixed samples. Biomasses of non-ciliate and non-dinoflagellate microzooplankton were estimated from biovolume using the general protist algorithm of Menden Deuer and Lessard (2000). Metazoan microzooplankton (crustacea and rotifers) were rare compared to protists and are not included in our abundance and biomass estimates.

2.3 Chlorophyll a and determination of Phytoplankton Biomass

For samples collected in conjunction with productivity casts, chlorophyll data available from the primary productivity study were used (Lomas et al., 2012). For other stations, we used chlorophyll data
collected as part of core hydrographic measurements. At stations for which our sampling depth did not match the sampling depths for chlorophyll, we interpolated between depths when possible. We estimated phytoplankton biomass from chlorophyll $a$ using an empirically determined Carbon to Chlorophyll $a$ ratio of 50 (Lomas et al., 2012). Only data for stations and depths for which both microzooplankton and chlorophyll data were available were used in comparing microzooplankton and phytoplankton biomasses.

2.4 Data Analyses and Statistics

We grouped stations by physical domain (Figure 1) (Hunt et al., 2008; Lomas et al. 2012; Stabeno et al. 2012a) with the number of stations sampled in each domain each year (N) given in Table 1. Data for the MN line, a transitional area between the northern and southern shelves of the eastern Bering Sea, do not belong in the other domains (Stabeno et al. 2012a) and are separate. Data are presented only for domains for which microzooplankton data are available for 2 or more stations in each year. Data are not available for all stations in all years and the distribution of data among domains varied from year to year. For example, the representation of middle and inner shelf domains was higher in 2010 than 2008 or 2009 (Table 1). Over-all yearly averages of abundance and biomass are influenced by station distribution.

All statistical tests were run using Sigmaplot version 9.0. Two way Analysis of Variance (ANOVA) was used to test for differences in the microzooplankton abundance or biomass among domains by year. We applied standard transformations (log 10, square root or arcsin) when data did not meet assumptions for ANOVA. In cases in which the transformed data still did not meet the assumptions, one way ANOVAs were conducted on data for each domain separately or a one way ANOVA on ranks was run using the combined data. Pair-wise multiple comparisons were made using the Holm-Sidak method. We used the Pearson Product Moment Correlation to examine association between microzooplankton abundance or biomass and chlorophyll $a$.

3. Results

3.1 Abundance and Composition

During summers 2008-2010 the abundance of protistan microzooplankton was high in the eastern Bering Sea (Table 2). Protistan microzooplankton occurred at average densities of $4 \times 10^3$ to $25 \times 10^3$ cells l$^{-1}$ in the mixed layer on the shelf. Year and the “domain X year” interaction were significant
sources of variation in microzooplankton abundance (Table 2). On the north middle shelf and along the
MN transect, the mixed layer MZ abundance was higher in 2010 than in 2009 and higher in 2009 than in
2008 (p<0.05). On the South Outer shelf, abundances were also higher in 2010 than in 2008 (p<0.05).

Ciliates and heterotrophic and mixotrophic dinoflagellates numerically dominated the
microzooplankton, with other taxa contributing <5% to this assemblage. Ciliates ranged in average
abundance in the mixed layer samples from $1.4 \times 10^3$ to $13.0 \times 10^3$ cells l$^{-1}$ (Fig 2a). In the South Outer
domain, South Inner domain and along the MN transect, ciliates were more abundant in 2010 than 2008
or 2009 (p<0.05). In the North Middle domain they were more abundant in 2009 and 2010 than in 2008
(p<0.05). Ciliates accounted for 30-53% of the microzooplankton in surface waters.

Over 95% of the ciliates were non-loricate spirotrich ciliates (mostly strombidiids and
strobilidiids). The most common ciliates were mixotrophic strombidiids (*Strombidium* spp. and *Laboea
strobilia*) with algal plastids. Mixotrophic ciliates were particularly prevalent in surface waters on the
shelf where they numerically contributed 68-75% of the ciliate microzooplankton. The average
abundance of mixotrophic ciliates was $0.4 \times 10^3$ to $7.7 \times 10^3$ cells l$^{-1}$ (Fig. 3). Similar to the total ciliates,
mixotrophic ciliates were more abundant in 2009 and 2010 than in 2008 in the North Middle Domain
(Fig. 3). In the south inner domain mixotrophic ciliates were more abundant in 2010 than 2008 or 2009.
The primarily photosynthetic ciliate, *Mesodinium rubra*, and tintinnid ciliates each made relatively minor
(1-6%) numerical contributions to the ciliate assemblage.

Heterotrophic and mixotrophic dinoflagellates were co-dominant with the ciliates and ranged in
abundance from $1.3 \times 10^3$ to $15.7 \times 10^3$ cells l$^{-1}$ (Fig. 2b). In the South Outer domain, dinoflagellates
were more abundant in surface waters in 2009 and 2010 than in 2008 and along the MN transect and in
the North Middle Domain they tended to be more abundant in 2010 than in 2009 and 2008 (p<0.05).
Heterotrophic and mixotrophic dinoflagellates contributed an average of 45-67% of the microzooplankton
in the mixed layer. The dinoflagellates were dominated by non-thecate heterotrophic dinoflagellates, with
*Gyrodinium* and *Gyrodinium*-like species common. Thecate dinoflagellates such as *Protoperidinium*
were rare.

3.2 Biomass and correlations with Chlorophyll a

Average microzooplankton biomass in the mixed layer was 12-14 µg C l$^{-1}$ in the south outer
domain, at the shelf break station and at the Pribilof Island stations. Average microzooplankton biomass
was 21-25 µg C l$^{-1}$ on the shelf (middle domains, south inner domain) (Figure 4). Biomass along the MN
transect, which crossed from the inner to the middle and outer shelf between the south and north, and thus contained a mixture of domains, averaged 16 µg C l\(^{-1}\). In the South Outer Domain, microzooplankton biomass was significantly higher in 2010 than 2008. Average microzooplankton biomass was similar for the three summer cruises for the South Middle Domain, MN transect, and Shelf Break. In the South Outer Domain, microzooplankton biomass was significantly higher in 2010 than 2008 (Figure 4).

Microzooplankton biomass in the mixed layer was not correlated with chlorophyll \(a\) concentration (Pearson Product Moment Correlation, N=132, p>0.05). High ratios (>1) of microzooplankton biomass to calculated phytoplankton biomass were observed at chlorophyll \(a\) concentrations of < 1 µg Chl l\(^{-1}\) with ratios > 2 observed occasionally observed at chlorophyll concentrations of < 0.5 µg Chl l\(^{-1}\) (Figure 5). Domain had a significant effect on the ratio of microzooplankton to phytoplankton biomass in the mixed layer during summer (Figure 6). In the South Inner and North Middle domains in summer 2008, average microzooplankton biomass in the mixed layer was greater than estimated phytoplankton biomass (Figure 6). In the North and South Middle domains and along the MN transect in summer 2010, microzooplankton biomass was greater than phytoplankton biomass in the mixed layer (Figure 6). In summer 2009, compared to summer 2008 or 2010, the ratio of microzooplankton to phytoplankton biomass was low in north middle domain. In general, ratios were highest on the inner and middle shelf and lowest on the outer shelf and shelf break (Figure 6).

The relative contribution of ciliates and dinoflagellates to microzooplankton biomass was roughly similar to their numerical contribution. Heterotrophic and mixotrophic dinoflagellates averaged 64% and ciliates 35% of microzooplankton biomass. Non-thecate heterotrophic dinoflagellates contributed almost all of the biomass in the dinoflagellate fraction. Among the ciliates, non-loricate choreotrich ciliates contributed 88% of the ciliate biomass and were dominated by mixotrophic species, contributing ~65% of the ciliate biomass. Other protistan taxa contributed < 5% of the microzooplankton biomass at all stations.

3.3 Microzooplankton in the Deep Chlorophyll Maxima (DCM)

On the middle shelf, there was often a pronounced deep chlorophyll maximum layer (DCM). In the South Middle Domain a DCM was observed at 41% of the stations and occurred at average depth of 24-26 m; in the North Middle Domain, a pronounced DCM was observed at 70% of the stations sampled and occurred at average depths of 30-34 m. The DCMs were usually located at or below the base of the thermocline and were rich in chain-forming diatoms. Although phytoplankton were elevated in
abundance in the DCM, microzooplankton were not. Estimated biomass of microzooplankton in the south or north middle domain DCMs was not different from average biomass in the mixed layer for the domain and year (Figure 7). As a result, the ratio of microzooplankton to phytoplankton biomass in the DCM was lower than the ratio in the corresponding mixed layer. In the South Middle Domain, the microzooplankton biomass ranged from an average of 33 to 103% in the mixed layer and 25 to 49% of phytoplankton biomass in the DCM depending on year (Figure 7a). In the North Middle Domain, the microzooplankton biomass ranged from an average of 31 to 281% in the mixed layer and 5 to 22% in the DCM of phytoplankton biomass depending on year (Figure 7b).

3.4 Estimated contribution of ciliates to chlorophyll a.

Using the mean chlorophyll a to carbon conversion factor of 0.0164 pg chl (pg C)^{-1} derived for ciliates, we estimated mixotrophic ciliates (strombidiid species) chlorophyll in mixed layer samples. The mean estimated ciliate chlorophyll a l^{-1} was 0.09 (SD, 0.094), the median 0.07, and the range 0.00 to 0.49 (Fig. 8). Estimated ciliate chlorophyll was not correlated with total chlorophyll (Pearson Product Moment Correlation, N=135; p>0.05). Estimated ciliate contribution to total chlorophyll was highly variable across years and domains (Figure 8). In the North Middle Domain, the contribution was sometimes over 50%, with the estimated contribution greater in 2008 and 2010 than in 2009 when it was ~11%. In 2010, the contribution of ciliates to chlorophyll in the North Middle Domain was significantly higher than in the South Middle Domain or South Outer Domain, all other comparisons were not statistically significant (p>0.05).

4. Discussion

4.1. Confounding factors

Several factors confound the comparison of microzooplankton among seasons and years (Table 3). Timing of cruises is undoubtedly important. The Olson and Strom (2002) and Strom and Fredrickson (2008) data were from cruises in late July through August, about a month later than our cruises. Domains in the eastern Bering Sea differ in their physics and biology as well as the timing of events (Hunt et al., 2008; Stabeno et al., 2012a). The mix of stations sampled varied among studies and years, with some domains being better represented in some studies and years than others. For example, in 1999 and 2004,
sampling was focused around the Pribilof Domain and the southern shelf (Olson and Strom, 2002; Strom and Fredrickson, 2008) whereas our investigation included the northern domains (Figure 1). Even within our investigation, the cruise track and distribution of stations among domains varied from year to year (Table 1). The spring cruises focused on the marginal ice zone, with the distribution of stations depending on year-to-year variation in ice conditions (Sherr et al. accepted). In addition, although the methods were quite similar in all the studies (Table 3), there were differences in sampling schemes, fixation and counting procedures that may have influenced abundance and biomass estimates.

All three of our summer cruises were within a four year “cold” period with extensive sea ice in winter and spring (Stabeno et al., 2012b). However, there were year to year differences in the summer distribution and biomass of microzooplankton among our cruises. In 2009, the average ratio of microzooplankton to phytoplankton biomass in the mixed layer was low, particularly in the North Middle Domain. The contribution of mixotrophic ciliates to biomass was also relatively low in 2009. The average temperature for our mixed layer samples in the North Middle Domain was colder (3.7±0.76°C) in summer 2009 than in summer 2008 or (6.3±0.51°C) or 2010 (5.9±0.22°C). Although 2009 is classified as an average year in terms of the August stratification index, stratification set-up was unusually late (4 June) and breakdown early at M2 (station on south middle shelf) compared to most other years (Ladd and Stabeno, 2012). It appears that the typically summer microzooplankton assemblage, dominated by mixotrophic ciliates and heterotrophic dinoflagellates adapted to low chlorophyll, stratified waters, was not well developed by the time of our cruise in 2009.

Particularly in 2009 we noted small blooms of gelatinous colonial phytoplankton, *Phaeocystis pouchetti* and *Chaetoceros socialis*, on parts of the middle and outer shelf. These colonial phytoplankton are often poor food for microzooplankton (Nejstgaard et al., 2007). The presence of these bloom species may partially explain the relatively low ratio of microzooplankton to phytoplankton biomass in summer 2009. Strom and Fredrickson (2008) also noted that few large ciliates and dinoflagellates were present at high chlorophyll “green belt” stations. Localized mixing events and storms can lead to episodic blooms and relative low ratios of microzooplankton grazing to phytoplankton growth on the shelf (Stoecker et al., this issue).

4.2. Comparison between spring and summer

During spring sea ice conditions, microzooplankton biomass was more variable than during summer, particularly at bloom stations, however, average microzooplankton biomass during spring and
summer were similar (Table 3). The ratio of microzooplankton to phytoplankton is higher in summer than in spring (Figure 6; Sherr et al., accepted). In spring, microzooplankton biomass was positively related to chlorophyll $a$ (Sherr et al., accepted), but in summer we found no such relationship. In early spring, microzooplankton do not increase in abundance until phytoplankton populations are high enough to support their growth and thus there is temporary “uncoupling” of microzooplankton growth from phytoplankton growth at the onset of blooms (Sherr and Sherr, 2009; Sherr et al., accepted). Although integrated rates of Net Primary Production (NPP) are lower in summer than in spring, estimated growth rates of phytoplankton are about twice as high in summer (Lomas et al., 2012); phytoplankton turn-over rapidly in summer and thus can support relatively high populations of microzooplankton for the observed chlorophyll $a$ standing stock. Another factor that leads to persistence of high biomass of microzooplankton relative to phytoplankton after blooms is acquired phototrophy in mixotrophic ciliates (Dolan and Perez, 2000; Stoecker et al., 2009).

### 4.3 Comparisons among Domains

Summer microzooplankton abundance and biomass were surprisingly high in the low chlorophyll water found in the mixed layer over the middle shelf, with average estimates of microzooplankton biomass greater than in higher chlorophyll waters in the Pribilof Domain and at frontal and shelf break stations. In the North Middle Domain there was usually a well-developed DCM, but the biomass of microzooplankton was not usually elevated in the DCM relative to the mixed layer.

Both top down and bottom up factors may be responsible for the high biomass of microzooplankton in these relatively low chlorophyll waters. Top down control of microzooplankton may be lower on the middle shelf compared to the more productive Pribilof Domain and “Green Belt” areas that have higher mesozooplankton populations (Coyle et al., 2008; Hunt et al., 2008). In the Pribilof Domain and in the “Green Belt”, large oceanic crustacean zooplankton are advected onto the shelf along with nutrient rich slope waters (Hunt et al., 2008). In polar waters, copepod grazing can have a strong top-down impact on microzooplankton populations, particularly large cell-size ciliates (Levinsen and Nielsen, 2002; Campbell et al., 2009).

Secondly, although phytoplankton biomass was higher at “bloom” stations in the Pribilof Domain and in the “Green Belt” and frontal areas, the phytoplankton may not have been as suitable a food for microzooplankton as the mixed populations of phytoflagellates and small diatoms on the middle shelf. Particularly in 2009, blooms of *Phaeocystis pouchettii* were present at the high chlorophyll stations,
including some Shelf Break, Pribilof Island, South Outer, and North Middle domain stations. The food
value of *P. pouchettii*, particularly the colonies, is a subject of debate (reviewed in Nejstgaard et al.,
2007). Although *P. pouchettii* is consumed by some microzooplankton, it may, in general, be a poor food
for microzooplankton (Calbet et al., 2011 and references cited therein). Most microzooplankton may not
be able to consume large colonies (Nejstgaard et al., 2007; Calbet et al., 2011), which dominated at some
“bloom” stations. At other “bloom” stations, we observed *Chaetoceros socialis*, a colonial gelatinous
diatom that also may not be readily ingested by most microzooplankton.

Likewise, although phytoplankton were more abundant in DCMs than in the mixed layer above,
microzooplankton usually were not more abundant in the DCM. Mesozooplankton grazers may
congregate in the DCM and/or the *Phaeocystis pouchettii* and *Chaetoceros socialis* colonies that form
many DCMs may be poor foods for microzooplankton. Some DCMs contained “old” phytoplankton,
including sea ice diatoms left-over from the spring, which may be poor quality foods for micro-grazers.

4.4 Comparison between warm and cold years

The average biomass and composition of microzooplankton we found were within the ranges
reported previously for microzooplankton in surface waters of the eastern Bering Sea (Table 3). The
average biomass of microzooplankton we observed appears to be somewhat lower than observed by
Strom and Fredrickson (2008) during a warm year with a high stratification index (2004), however,
differences in sampling locations confound this broad comparison by cruise averages. A higher
proportion of their stations were in slope waters, the “green belt” or Pribilof Domain than in our study in
which more sampling was on the middle shelf. If comparisons are by domain, the interpretation is
different. In summer 2004, at the middle south domain station (M2), microzooplankton biomass was
consistently $<15 \mu g C l^{-1}$ (Strom and Fredrickson 2008); this value is lower than the 21-25 $\mu g C l^{-1}$ that
we observed in the middle and inner domains during 2008-2010 (Table 3). In the summer of 1999, a cold
year with low stratification, microzooplankton biomass estimates were perhaps slightly higher than for
summer 2004, however the biomass ranges overlap (Table 3). There is no good evidence that
microzooplankton biomass is higher in summers of “warm” than “cold” years. Although for the one
warm year for which there are data on microzooplankton, summer stratification was also high (Table 3),
over-all the strength of summer stratification is not correlated with “warm” or “cold” years as defined by
depth averaged water temperature (Ladd and Stabeno, 2012). Domain, stratification, intensity, and timing
of cruises in relationship to mixing events may have a greater effect on observed microzooplankton
biomass and coupling to phytoplankton than temperature.
4.5 Contributions of mixotrophic ciliates to chlorophyll \( a \)

One of the surprising results of our cruises is the high abundance and biomass of mixotrophic strombidiid ciliates (\textit{Laboea strobilia}, \textit{Strombidium} spp.) and their estimated contribution to total chlorophyll. The primarily autotrophic ciliate, \textit{Mesodinium rubrum} (\textit{Myrionecta rubra}) was also present, but not abundant. On average, mixotrophic ciliates were 62\% of ciliate abundance and 66\% of estimated ciliate biomass in the mixed layer. Sorokin et al. (1996) estimated that in late spring-early summer, 30-40\% of the ciliates on the shelf in the western Bering Sea were mixotrophs; Booth et al. (1993) estimated that in the western sub-Arctic, 30-50\% of the ciliates were mixotrophs. In the subarctic Atlantic (Iceland, Greenland and Barents Seas), Putt (1990) observed that 58-65\% of the ciliates in surface waters were mixotrophs. In summer, in stratified sub-arctic seas, mixotroph ciliates are generally very abundant and important components of the microzooplankton.

Calculation of estimated contribution of ciliates to chlorophyll is very sensitive to the factors used to estimate chlorophyll per unit biovolume or biomass. In the North Middle Domain, where chlorophyll levels were very low in the mixed layer in 2008 and 2010, average estimated contribution of mixotrophic ciliates to total chlorophyll was over 50\% using the mean value of 3.12 fg Chl \( a \mu m^3 \) calculated for mixotrophic ciliates (refer to methods). Putt (1990), based on determination of the chlorophyll content of ciliates collected at sea, estimated that ciliates accounted for 4-15\% of total chlorophyll \( a \) in summer in surface waters of the Nordic Seas. This is similar to our estimate of 5-14\% for the south shelf domains, but lower than our estimate of 22-35\% for the more northern shelf (MN transect and North Middle Domain).

About 64\% of the chlorophyll in the Bering Sea in summer is due to cells < 5 \( \mu m \) in size (Lomas et al., 2012). Assuming 36\% of the chlorophyll in the mixed layer in all domains is > 5 \( \mu m \) and using the mean value for chlorophyll content of mixotrophic ciliates, the average contribution of ciliates to chlorophyll in the > 5 \( \mu m \) size class should range from 15\% to 96\%. It is clear that the contribution of ciliates to chlorophyll (and probably photosynthesis), particularly in the size fraction that is readily accessible to crustacean zooplankton, is considerable, particularly in the northern domains. This also implies that bulk chlorophyll measurements overestimate the biomass of phytoplankton and that the ratios of microzooplankton biomass to chlorophyll \( a \) or phytoplankton biomass are underestimates.

4.6. Importance of microzooplankton to crustacean zooplankton
Crustacean zooplankton, particularly large crustacean zooplankton, are important in food webs that support higher trophic levels in the southeastern Bering Sea (Coyle et al., 2008; Hunt et al., 2008; Stabeno et al., 2012b). Crustacean zooplankton consume both phytoplankton and microzooplankton, but often have higher clearance rates for microzooplankton than phytoplankton, particularly when phytoplankton are not abundant and/or are small in size (Gifford and Dagg, 1991; Calbet and Saiz, 2005; Campbell et al., 2009). After the spring bloom, when phytoplankton are scarce, predation on microzooplankton can maintain fecundity of copepods (Ohman and Runge, 1994). Large, mixotrophic ciliates are particularly important as prey for copepods (Dutz and Peters, 2008) and young fish larvae (Figuierdo et al., 2007). Under these conditions, photosynthesis in ciliates may “boost” microbial food web efficiency and hence carbon availability to higher trophic levels (Stoecker et al., 2009). This may be important in stratified Bering Sea shelf waters where chlorophyll levels were relatively low (mean total Chl $a \sim 0.9 \, \mu g \, l^{-1}$) and composed of small cells ($\sim 64\%$ of chl $a < 5 \, \mu m$) in summer (Lomas et al., 2012). Most crustacean zooplankton do not effectively graze on cells $< 5 \, \mu m$ in size. Almost all cells larger than $10 \, \mu m$ in size in the mixed layer on the inner and middle shelf (with the exception of the Pribilof Domain) were microzooplankton (personal obs). Estimated microzooplankton biomass in the mixed layer was 98% (SD 166%) of estimated phytoplankton biomass with a median value of 55% (Maximum 155%, Minimum 2%, N=132). In low chlorophyll surface waters, such as on the middle shelf in summer, about 50% of the diet of copepods is ciliates (Calbet and Saiz, 2005). Although microzooplankton probably are very important in the diet of crustacean zooplankton on Bering Sea Shelf in summer, data on predation rates on microzooplankton for this season are lacking.

4.7. Climate variability and the microzooplankton trophic link

Climate, weather and ocean acidification all have the potential to influence the future ecology of the eastern Bering Sea (Mathis et al 2010; Overland et al., 2012; Stabeno et al., 2012b; Wang et al 2012). It is not clear if the pattern of the last few years of decreased annual variability in temperature will continue or if the Eastern Bering Sea will return to strong inter-annual variability (Overland et al. 2012). During the “warm” years of 1998 and 2001-2005, surface water temperatures on the southern middle shelf rose above 10°C during the summer, although during “average” and “cold” years surface waters temperatures remain below 10°C (Stabeno et al., 2012b). The timing and strength of stratification controls the availability of nutrients for phytoplankton growth and hence has a strong influence on new production, phytoplankton species composition, and perhaps the suitability of phytoplankton as food for microzooplankton (Sambrotto et al., 1986, 2008; Strom and Fredrickson, 2008; Mathis et al., 2010).
During July and August 2004, intense stratification in the southeastern Bering Sea led to phytoplankton nutrient limitation and reduced microzooplankton grazing (Strom and Fredrickson, 2008). Contrary to expectations, stratification index does not correlate with “warm” and “cold” years in the eastern Bering Sea (Ladd and Stabeno, 2012). In the eastern Bering Sea, weather and tides are important in controlling stratification. Advection of water onto the shelf and wind and tidal mixing make nutrients available to phytoplankton and can lead to localized blooms even during summer (Sambrotto et al. 1986, 2008).

However, in the Bering Sea Shelf, where surface water temperatures during “cold” and “normal” years are below 10°C even in summer, there might be a direct, positive effect of increased temperature on coupling between microzooplankton and phytoplankton growth. Based on a compendium of laboratory studies of cultured protists, Rose and Caron (2007) found that the maximum growth rate of herbivorous microzooplankton was lower than the maximum growth rates of phytoplankton below 10°C. If this relationship holds in natural communities, then the coupling of microzooplankton to phytoplankton would be expected to increase with increases in water temperature. However, modeling of the general responses of microzooplankton herbivory to warming suggest that it may increase the ratio of microzooplankton grazing to phytoplankton growth under eutrophic but not oligotrophic conditions (Chen et al., 2012). This indicates that stratification and mixing, by largely controlling nutrient availability, may control the coupling of microzooplankton and phytoplankton. Our knowledge of how climate variability will affect key phytoplankton groups, and their interactions with microzooplankton, is very limited, but it is clear that interactive effects of temperature, stratification/mixing and nutrients are important (Olson and Strom, 2002; Rose et al 2009; Boyd et al., 2010).

Ocean acidification is another climate change phenomenon that has the potential to affect phytoplankton and hence the microzooplankton food web link in the Bering Sea. Ocean acidification induced changes in calcium carbonate mineral saturation states are most evident in high latitude seas, such as the Bering Sea (Mathis et al., 2010). Most of the microzooplankton in the Bering Sea are not calcifying organisms, and thus unlikely to be directly influenced by ocean acidification. However, indirect effects due to changes in net primary production, phytoplankton species composition or biochemical composition of phytoplankton are possible (Suffrian et al., 2008; Hopkinson et al., 2010; Yoshimura et al., 2010). Hare et al. (2007) addressed synergistic effects of “greenhouse ocean” conditions (i.e., increased temperature and pCO₂ in combination) on plankton communities in manipulative, shipboard experiments conducted on the Bering Sea shelf. They found that simulated “greenhouse ocean” conditions decreased phytoplankton biomass but increased biomass-specific photosynthetic rates and cause a shift from diatoms towards nanophytoplankton (Hare et al. 2007), changes which may favor microzooplankton grazers. Somewhat similar experiments conducted in
temperate waters during the North Atlantic spring bloom suggest a transient positive effect of increased temperature and pCO$_2$ on microzooplankton abundance and grazing, but after the initial stimulation, microzooplankton abundance decreased (Rose et al., 2009). The changes in microzooplankton assemblages were attributed to changes in phytoplankton composition rather than the direct effects of temperature or pCO$_2$ on microzooplankton, although no direct measurements on microzooplankton were made (Rose et al., 2009).

5. Conclusions

a. Microzooplankton biomass equaled or exceeded summer phytoplankton biomass in the mixed layer in some domains. Microzooplankton were relatively more important in stratified shelf waters than in the “green belt”, Pribilof Island Domain or at the Shelf Break.

b. Mixotrophic strombidiid ciliates were the dominant component of the ciliate microzooplankton. They made important contributions to both microzooplankton biomass and chlorophyll, especially in the larger size classes.

c. Based on our data and previous investigations, the range of summer microzooplankton biomass was similar in “warm” and “cold” years, with differences among years obscured by differences among domains, derived in part by differences in mixing, nutrient supply and phytoplankton composition.

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References


Table 1. Number of microzooplankton samples by domain, cruise and layer (ML=mixed layer; DCM=chlorophyll max) on summer BEST/BSIERP cruises in SE Bering Sea in 2008 (HLY-08-03, July 3 to July 31), 2009 (KNORR 195-10, June 14 to July 13) and 2010 (TN-250, June 16 to July 14). See Figure 1 for station locations; not all stations sampled on all cruises.

<table>
<thead>
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<th>Domain</th>
<th>Layer</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
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<td>South Outer</td>
<td>ML</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>South Middle</td>
<td>ML</td>
<td>10</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>DCM</td>
<td>7</td>
<td>8</td>
<td>11</td>
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<td>ML</td>
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<td></td>
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<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Pribilof Islands</td>
<td>ML</td>
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<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Shelf Break</td>
<td>ML</td>
<td>4</td>
<td>3</td>
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</tr>
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</table>

Table 2. Summer mixed layer microzooplankton abundance in the eastern Bering Sea (cells X 10^3 l^-1).

<table>
<thead>
<tr>
<th>Domain</th>
<th>2008 Mean</th>
<th>2008 SD</th>
<th>2009 Mean</th>
<th>2009 SD</th>
<th>2010 Mean</th>
<th>2010 SD</th>
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<td>13.21</td>
<td>7.986</td>
<td>24.85</td>
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<td>South Inner</td>
<td>6.82</td>
<td>3.794</td>
<td>7.68</td>
<td>2.574</td>
<td>24.58</td>
<td>16.564</td>
</tr>
<tr>
<td>North Middle</td>
<td>3.96</td>
<td>2.750</td>
<td>15.58</td>
<td>5.317</td>
<td>24.40</td>
<td>8.852</td>
</tr>
</tbody>
</table>
Table 3. Microzooplankton biomass and composition in “warm” and “cold” years and in years with “high” and “low” summer stratification index in the eastern Bering Sea.

<table>
<thead>
<tr>
<th>Year</th>
<th>Season</th>
<th>Biomass (µg C l⁻¹)</th>
<th>Composition of microzooplankton</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992 Spring</td>
<td>1-10 (Slope waters)</td>
<td>Dinoflagellates 48-54% of integrated biomass; remainder mostly ciliates</td>
<td>Howell-Kubler et al. (1996)</td>
<td></td>
</tr>
<tr>
<td>1999 (cold; summer SI low)</td>
<td>Summer (Emiliania huxleyi bloom year)</td>
<td>57 (18-164)</td>
<td>Dinoflagellates ~50%, ciliates ~50%</td>
<td>Olson and Strom (2002)</td>
</tr>
<tr>
<td>2004 (warm; high summer SI)</td>
<td>Summer</td>
<td>38 (11-118)</td>
<td>~70% biomass HD (included dinoflagellates &lt;20µm); remainder mostly ciliates</td>
<td>Strom and Fredrickson (2008)</td>
</tr>
<tr>
<td>Year(s)</td>
<td>Season</td>
<td>Conditions</td>
<td>Profiles:</td>
<td>Biomass and Ciliates</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------</td>
<td>--------------------------</td>
<td>-----------</td>
<td>----------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23 +/- 22 (bloom conditions)</td>
</tr>
<tr>
<td>2008, 2009, 2010</td>
<td>Summer</td>
<td>(cold; average summer SI)</td>
<td>12-14 (outer domain, shelf break and Pribilofs)</td>
<td>Heterotrophic and mixotrophic dinoflagellates 64% biomass; remainder mostly ciliates</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21-25 (middle and inner domains)</td>
</tr>
</tbody>
</table>

1. “Warm” and “cold” years as defined by depth averaged water temperature (Stabeno et al. 2012a).
2. SI=Stratification index at station M2 (middle south domain) in August. SI is compared to average values \( \pm 1 \text{ SD} \) in August (Ladd & Stabeno 2012).
Figure Legends

Fig. 1. Location of transects and stations. The MN transect stations lie between the S and N domains. All stations were not sampled in all years (refer to Table 1).

Fig. 2. Abundance of ciliates (A) and heterotrophic/mixotrophic dinoflagellates (B) in the mixed layer samples by domain/transect (SO=South Outer, SM=South Middle, SI=South Inner, MN=MN transect, NM=North Middle, SB=Shelf Break, Pri=Pribilof Islands) and year. Ciliate abundances were greater in 2010 than 2008 or 2009 (p<0.05) with the year X domain interaction significant. Abundances of dinoflagellates were greater in 2010 than 2008 and in 2009 than 2008 (p<0.05) with the year X domain interaction significant. Across years, domain was not a significant source of variation for abundance of ciliates (p=0.416) or dinoflagellates (p=0.081). Within a domain, different letters indicate significant differences among years (p<0.05). 2-Way ANOVA on square root transformed data; df=144).

Fig. 3. Abundance of mixotrophic ciliates in the mixed layer by domain/transect (SO=South Outer, SM=South Middle, SI=South Inner, MN=MN transect, NM=North Middle, SB=Shelf Break, Pri=Pribilof Islands) and year. Mixotrophic ciliates were more abundant in 2010 than 2008 and more abundant in 2009 than 2008 with domain X year interaction significant (p<0.05). Across years, domain was not a significant source of variation for abundance of mixotrophic ciliates (p=0.326). Within a domain, different letters indicate significant differences among years (p<0.05). 2-Way ANOVA on log 10 transformed data; df=144).

Fig. 4. Biomass of microzooplankton in the mixed layer samples by domain/transect (SO=South Outer, SM=South Middle, SI=South Inner, MN=MN transect, NM=North Middle, SB=Shelf Break, Pri=Pribilof Islands). 1-way ANOVAs tested the effect of year for each domain except SI and NM, for which the data did not meet assumptions for ANOVA. Within a domain, different letters indicate significant differences among years (p<0.05).
Fig. 5. Ratio of estimated microzooplankton biomass to phytoplankton biomass versus Chlorophyll \( \text{a} \) (Chl \( \text{a} \)) in mixed layer, eastern Bering Sea summers 2008, 2009 and 2010. Data are from all samples for which there were both microzooplankton data and estimates of chlorophyll \( \text{a} \) (N=132).

Fig. 6. Ratio of estimated microzooplankton biomass to phytoplankton biomass in the mixed layer samples by domain/transect (SO=South Outer, SM=South Middle, SI=South Inner, MN=MN transect, NM=North Middle, SB=Shelf Break, Pri=Pribilof Islands). The ratio of microzooplankton biomass to phytoplankton biomass was greater in 2010 than in 2008 or 2009. The ratio in the Middle domains and along the MN transect was greater than in the South Outer domain and the ratio in North Middle Domain was greater than at the Shelf Break (2-Way ANOVA). Within a domain, different letters indicate significant differences among years (p<0.05).

Fig. 7. Comparison of estimated biomass of microzooplankton and phytoplankton in the mixed layer and in the deep chlorophyll maximum (DCM) in the: A. South Middle Domain, and B. North Middle Domain. Note difference in y-axes between A and B. There were no significant differences in microzooplankton biomass between the mixed layer and the DCM in the south or north middle domains (2-Way ANOVAs of year and layer; p>0.05). Mean +/- Standard deviation.

Fig. 8. Estimated contribution of mixotrophic ciliates to chlorophyll \( \text{a} \) in the mixed layer samples by domain/transect (SO=South Outer, SM=South Middle, SI=South Inner, MN=MN transect, NM=North Middle, SB=Shelf Break, Pri=Pribilof Islands). Ciliate chlorophyll was estimated using an average of 3.12 fg chl a/um\(^3\) for mixotrophic strombidiids. Mean +/- Standard deviation.

In 2010, estimated contribution of mixotrophic ciliates to chlorophyll \( \text{a} \) was higher in North Middle domain than in South Middle Domain or South Outer Domain (p<0.05). For the North Middle Domain, the percent contribution of ciliates to chlorophyll a was higher in 2008 and 2010 than in 2009 (p<0.05).
Fig. 1.
Fig. 2A
Abundance (Dinoflagellates $l^{-1}$)

- SO
- SM
- SI
- MN
- NM
- SB
- Pri

- 2008
- 2009
- 2010

Fig. 2B
Abundance (Mixotrophic Ciliates \textsuperscript{1})

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Abundance of mixotrophic ciliates in different locations and years.}
\end{figure}
Chlorophyll a (μg l⁻¹)
Biomass ($\mu g$ C l$^{-1}$)

Mixed Layer

Deep Chlorophyll Maximum

Fig. 7a
Biomass (µg C l⁻¹)

<table>
<thead>
<tr>
<th>Year</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microzooplankton</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mixed Layer | Deep Chlorophyll Maximum

Fig. 7b
Fig. 8

Estimated % Total Chl. α

- SO
- SM
- SI
- MN
- NM
- Pri
- SB

Legend:
- 2008
- 2009
- 2010
Microzooplankton Grazing in the Eastern Bering Sea in Summer

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Dilution experiments to estimate microzooplankton grazing on phytoplankton were conducted during the summers of 2008, 2009, and 2010 in the eastern Bering Sea as part of the BEST-BSIERP integrated ecosystem project. All three summers followed cold springs in the Bering Sea. Average microzooplankton grazing coefficients were relatively similar among regions, ranging from 0.16 to 0.34 $d^{-1}$ in simulated *in situ* incubations with mixed layer water collected from the depth of the 55% isolume. In off shelf and outer shelf domains, microzooplankton consumed 67-78% of phytoplankton daily growth but in the middle and inner shelf domains, microzooplankton grazing exceeded phytoplankton daily growth. Regional estimates of microzooplankton ingestion of phytoplankton carbon ranged from 4.4 to 11.0 $\mu g C d^{-1}$, with highest ingestion in the Off Shelf, Outer Shelf, and Alaska Peninsula regions and, lower ingestion in the Middle Shelf and Inner Shelf regions. On the northern Middle Shelf, a deep chlorophyll maximum (DCM) occurred at most stations. Grazing coefficients in the DCM were similar in magnitude to coefficients in the corresponding mixed layer. However, because of the higher phytoplankton biomass in the DCM, estimated microzooplankton ingestion and secondary production $l^{-1}$ were higher in the DCM than in the mixed layer. Measurements of variable fluorescence in whole seawater and diluted treatments indicated that with some plankton assemblages, dilution has a negative effect on phytoplankton physiology and could compromise their growth rates. This could also result in an underestimation of microzooplankton grazing. Nevertheless, it is clear that microzooplankton grazing consumes most of the phytoplankton production in summer, and that microzooplankton must be an important link in food webs supporting larger zooplankton and in carbon flow in the eastern Bering Sea.

**Keywords:** Bering Sea, microzooplankton grazing, dilution method, deep chlorophyll maximum
1. Introduction

Microzooplankton (defined as the <200 µm fraction which includes nanozooplankton) are an important link between primary producers and higher trophic levels in sub-polar and polar waters as well as in temperate and tropical waters (Levinsen and Nielsen, 2002; Calbet and Saiz, 2005; Campbell et al., 2009; Sherr et al., submitted). They are important grazers on pico, nano and microplankton, including large diatoms (Sherr et al., 2009, submitted). Previous studies have shown microzooplankton (defined as the <200 µm fraction, which includes nanozooplankton, for dilution experiments) are the major consumers of primary production in summer in the eastern Bering Sea (Liu et al., 2002; Olson and Strom, 2002; Strom and Fredrickson, 2008). In Arctic and sub-Arctic as well as temperate and tropical seas, microzooplankton are important prey for mesozooplankton, including both small and large crustacean zooplankton (Levinsen and Nielsen, 2002; Campbell et al., 2009) and hence are a significant component of the food web and carbon cycle.

Although microzooplankton can graze on large as well as small phytoplankton, including chain forming dinoflagellates (Strom et al., 2007; Sherr et al., submitted) their grazing rates, particularly microzooplankton biomass specific rates, can be influenced by phytoplankton species composition, physiological state and cell size (Olson and Strom, 2002; Strom and Fredrickson, 2008). In the eastern Bering Sea, phytoplankton < 5 µm comprise ~70% of the chlorophyll a in summer and autotrophic biomass is dominated by phytoflagellates (Lomas et al., 2012; Moran et al., 2012). Sporadic blooms of chain forming diatoms (mostly Chaetoceros and Thalassiosira spp.) and blooms of the solitary or colonial phytoflagellate Phaeocystis pouchetti occur in response to tidal and storm mixing or intrusion of deeper water onto the shelf (Sukhanova et al., 1999; Sambrotto et al., 2008). In late spring and summer, prolonged blooms of diatoms and P. pouchetti are associated with the shelf break and shelf partition fronts (Flint et al., 2002). During the anomalously warm and stratified spring of 1997, a bloom of the coccolithophorid, Emiliania huxleyi, developed on the southeastern shelf and also during summers 1998-
2000 the bloom was present (Stockwell et al., 2001; Merico et al., 2004). In summer, particularly on the northern shelf, a deep chlorophyll maximum (DCM) composed primarily of diatoms has often been encountered. It is not clear if the DCM is a resident low light population or physiologically inactive settled material (Moran et al., 2012; Stabeno et al., 2012a), and to what extent it is grazed by microzooplankton.

Microzooplankton were sampled as part of the BEST-BSIERP ecosystem study in the eastern Bering Sea in summers of 2008, 2009, and 2010 (Stoecker et al., this issue), all part of a four year “cold” period characterized by extensive sea ice in spring (Stabeno et al., 2012b). Average summer microzooplankton (defined as 20-200 µm size range) densities ranged from 4 X 10^3 to 25 X 10^3 cells l^{-1} in the mixed layer in stratified shelf waters but were about half that concentration in less stratified waters near the shelf break. High ratios (>1) of microzooplankton biomass to phytoplankton biomass were observed when chlorophyll concentrations were below 1 µg l^{-1} in the mixed layer (Stoecker et al., this issue). In coastal (inner domain) and middle shelf (middle domain) waters, the average biomass of microzooplankton in the mixed layer was often equal to or higher than that of phytoplankton.

Microzooplankton were also found in the deep chlorophyll maxima (DCM) on the shelf; densities of 20-200 µm microzooplankton in these high chlorophyll layers were usually similar to densities in the lower chlorophyll, mixed layer. Microzooplankton abundance and biomass data from summers 2008-2010, along with results from previous studies during both “warm” and “cold” years in the Eastern Bering Sea (Liu et al., 2002; Olson and Strom, 2002; Strom and Fredrickson, 2008), indicate that summer microzooplankton population differences among domains are far greater than differences due to year-to-year variations in sea ice extent and water temperature.

We report on dilution grazing experiments conducted in conjunction with microzooplankton sampling on the BEST-BSIERP summer cruises in 2008, 2009, 2010. Prior to our study, data on microzooplankton grazing for the eastern Bering Sea were limited to the southern shelf and the productive waters around the Pribilof Islands (Olson and Strom, 2002; Hunt et al., 2008; Strom and Fredrickson,
2008). Our goal was to conduct dilution grazing experiments across a spectrum of environments and to compare microzooplankton grazing among domains. We also wanted to determine if microzooplankton grazing and its impact on phytoplankton were correlated with the biomass of microzooplankton (Stoecker et al., this issue) and/or to dominance of certain phytoplankton taxa. Low microzooplankton grazing can occur during blooms of coccolithophorids (Olson and Strom, 2002) and Phaeocystis pouchetti (reviewed in Nejstgaard et al., 2007). We were also interested in determining if microzooplankton grazing was important in the DCM since this is a characteristic feature of the northern shelf in summer (Stabeno et al., 2012a).

2. Materials and Methods

2.1. Sampling

Microzooplankton grazing experiments were conducted on BEST/BSIERP summer cruises in 2008, 2009 and 2010 on the USCG Healy (HLY-08-03, July 3 to July 31), R/V Knorr (KNORR 195-10, June 14 to July 13) and R/V T.G. Thompson 2010 (TN-250, June 16 to July 14). The stations at which dilution grazing experiments were conducted are shown in Figure 1; the stations and depths of experiments for each year are given in Table 1. In most cases the dilution grazing experiments were undertaken in conjunction with the phytoplankton biomass and primary productivity casts with water collected from the depth of the 55% of surface PAR irradiance level (Lomas et al., 2012). At all stations the 55% irradiance level was in the surface mixed layer, with the sampling depth for mixed layer incubations ranging from 3-10 m (Table 1). In 2010 we also undertook microzooplankton grazing experiments using assemblages from DCM which was located based on chlorophyll fluorescence profiles.
from the core CTD casts. Supporting information including water temperature, salinity, chlorophyll \( a \), inorganic nutrients and irradiance were obtained from core program measurements or from the productivity casts (Lomas et al., 2012).

### 2.2. Dilution Experiments

Dilution grazing experiments are the only method available for estimating microzooplankton community grazing on the whole phytoplankton community. This method is used universally to estimate phytoplankton growth rates (\( \mu \)) and mortality of phytoplankton due to microzooplankton grazing (\( g \)) (Landry, 1993). Dilution grazing experiments include all grazers <200 \( \mu \)m in size, including small heterotrophic and mixotrophic flagellates, as well as the larger ciliates and dinoflagellates. Whole seawater (WSW), containing natural assemblages of phytoplankton and microzooplankton, is diluted with filtered, particle free seawater (FSW) from the same sample. Dilution reduces microzooplankton encounter rates with phytoplankton prey; in highly dilute treatments net growth rate (NGR) of phytoplankton approaches the intrinsic growth rate (\( \mu \)). Phytoplankton mortality due to microzooplankton grazing is calculated as \( \mu \)-NGR. A modified dilution method, the two-point method (Landry et al., 2008) was used because it is more efficient than the original method. The original and the two-point method have been compared in grazing experiments conducted in coastal Gulf of Alaska (Strom et al., 2006) and in the SE Bering Sea (Strom and Fredrickson, 2008) and found to provide similar results.

In nutrient limited waters, which can occur in summer on the Bering Sea Shelf (Strom and Fredrickson, 2008), nutrient regeneration due to micrograzers can be important in supplying inorganic nutrients for phytoplankton growth. This would violate the first assumption of the dilution technique, that phytoplankton growth rate is not influenced by dilution (Landry, 1993). To solve this problem, inorganic nutrients are added to all the bottles. However, then estimated phytoplankton growth rates are no longer
similar to *in situ* rates. In this situation, it is usual to run incubations with and without nutrient addition. In *in situ* phytoplankton growth rate, $\mu$, is estimated from the incubation without added nutrients and, if nutrient limitation is important, $g$ can be determined from NGR and $\mu$ in the incubations with added nutrients. To evaluate this, we undertook some, but not all, incubations with and without the addition of nutrients. In 2008, nutrient additions were 5 $\mu$M N as NaNO$_3$. In 2009, nutrient additions were as 5 $\mu$M N as NH$_4$Cl, and in 2010 nutrient additions were 5 $\mu$M N as NaNO$_3$ combined with 0.3 $\mu$M P as Na$_2$HPO$_4$. These additions are similar to those used by Strom and Fredrickson (2008) in earlier experiments in the Bering Sea, which found no difference between N addition as NH$_4$Cl and NaNO$_3$.

All tubing, carboys, filter cartridges and incubation bottles were cleaned with 10% HCl, and rinsed 3 or more times with de-ionized water, and then rinsed with filtered seawater prior to use and between experiments (Landry, 1993). As mentioned earlier, in “mixed layer” experiments, water was collected with 30 L Niskin bottles on a CTD rosette from the depth corresponding to 55% surface irradiance level and in “DCM” experiments WSW was collected from the depth of chlorophyll fluorescence maximum. Seawater was gently siphoned (using silicone tubing) from Niskin bottles into black plastic covered polycarbonate carboys in the CTD bay. During siphoning, water was prescreened through a 200 $\mu$m Nitex mesh to remove larger zooplankton. This prescreened “whole seawater” contained phytoplankton and microzooplankton, however when phytoplankton >200 $\mu$m (long diatom chains and large colonial phytoplankton) were present, the pre-screening removed them. Thus, the “WSW” contains a variable fraction of the total *in situ* chlorophyll. Filtered seawater (FSW) was prepared using gravity filtration and 0.2 $\mu$m pore size sterile Pall Capsule Filters. Treatments consisted of 100% WSW and diluted whole seawater. In early experiments we used 5% WSW for the diluted treatments (95% FSW), but found that in low chlorophyll waters it was difficult to obtain consistent chlorophyll measurements due to the low chlorophyll in the diluted treatment. We switched to using 20% WSW (80% FSW and 20% WSW) when *in situ* chlorophyll levels were low. Silicon tubing was used to gently transfer, without bubbling, water (WSW or diluted seawater) from 20 liter polycarbonate carboys.
to triplicate 1 liter polycarbonate incubation bottles. If a nutrient addition series was included in an experiment, nutrient stock solution was added with a micropipette directly to triplicate WSW and diluted WSW incubation bottles.

At the beginning of each experiment, triplicate samples for total chlorophyll (in effect, <200 μm chlorophyll because of the prescreening) and size fractionated (<20 μm) chlorophyll were taken from the WSW and diluted seawater carboys. Samples for microzooplankton and Phaeocystis enumeration were siphoned directly from the Niskin bottles into 125 ml sampling bottles and fixed with acid Lugol’s solution as described (Stoecker et al., this volume). In mixed layer experiments (Table 1), the triplicate bottles for each treatment were incubated on deck in flowing sea-water with neutral density screening to approximate 55% surface irradiance. In DCM experiments, the bottles were incubated in the low light either on ice or in a 4°C cold room (Table 1). At the end of the incubations, chlorophyll samples (total chlorophyll a and < 20 μm chlorophyll a) were taken from each bottle. Chlorophyll samples were filtered onto 25 mm GF/F filters using gentle vacuum filtration, extracted in 90% acetone at -20°C for 24 h, and analyzed at sea with a pre-calibrated fluorometer (Turner Designs TD-700).

Chlorophyll a was used as a proxy for phytoplankton biomass. Based on Landry et al. (2008), microzooplankton grazing (g) and phytoplankton growth rates (μ) were calculated from net growth rates of phytoplankton in the WSW (K) and diluted treatment (K_d) and the fraction of WSW in the diluted treatment (x). K or K_d values were calculated for each triplicate using average initial values of chlorophyll for each treatment. A one-way ANOVA was used to determine if the K and K_d values for an experimental series were statistical different (p<0.05), indicating phytoplankton mortality. The instantaneous rate of phytoplankton mortality (d⁻¹), or grazing coefficient, was calculated as g = (K_d - K)/(1 - x). The instantaneous rate of phytoplankton growth (μ, d⁻¹) was calculated as μ = K + g. Calculations were done separately for experiments with and without added nutrients (Landry 1993, Olson & Strom 2002). The fraction of phytoplankton growth grazed per day was estimated as g/μ. Daily ingestion (I) of phytoplankton biomass (μg C l⁻¹ d⁻¹) was estimated from chlorophyll consumption using a C:Chl ratio of
50 for the Bering Sea (Lomas et al., 2012) as $I = \text{(Chl a)}(50)(g)$. Assuming an average gross growth efficiency of 35% for strictly heterotrophic microzooplankton, secondary production based on herbivory was estimated (Landry and Calbet, 2004).

2.3. Variable fluorescence measurements

In several studies, insignificant grazing coefficients and/or statistically significant “negative” microzooplankton grazing rates have been reported from a proportion of the stations in the Bering Sea and also from other sub-Arctic and Arctic seas (Olson and Strom, 2002; Strom and Fredrickson, 2008; Calbet et al., 2011; Sherr et al., submitted). “Negative grazing” is impossible, but calculation of negative “g” results when the $\mu$ of phytoplankton in the diluted treatment is lower than in the WSW. Lower growth of phytoplankton in the diluted treatments than in WSW is usually attributed to lack of regenerated nutrients due to low numbers of micrograzers in the diluted treatments. However, nutrient addition usually did not eliminate the effect in our experiments. To explore the possibility that dilution itself, or chemicals released from plankton into the dilution water during preparation of FSW, have a negative effect on phytoplankton we measured variable fluorescence (Fv/Fm) of phytoplankton in the undiluted WSW and diluted treatments. Fv/Fm was measured after incubation of subsamples in the dark for ~1 h in a Automated Laser Fluorometer (ALF)(Chekalyuk and Hafez, 2008). Fv/Fm was measured in samples from the triplicate incubation bottles in the 100%WSW, 20%WSW, 100%WSW+nuts, 20%WSW+nuts treatments at the end of the 24 h incubations in mixed layer experiments at 14 stations (C-55, W-4b, SL-8, NP-7, NP-14, NP-11, LS-16, P14-4.5, P14-2, MN-6, MN-12, MN-19 and SL-14) in 2008 (Table 1). Samples were collected in 500 ml amber glass bottles, and stored in the dark for about 30 min, to minimize the impacts of non-photochemical quenching before analysis in the ALF.
2.4. Enumeration of *Phaeocystis pouchettii*

The bloom forming phytoplankter *P. pouchettii* has been associated with inhibition of grazing in dilution experiments (Calbet et al., 2011). To determine if low grazing coefficients were associated with abundance of *Phaeocystis* cells, we enumerated this phytoplankter in water samples fixed with acid Lugol’s (refer to Stoecker et al., this volume) using a 1-ml capacity Sedgwick-Rafter counting chamber at 400X magnification.

2.5. Statistical Analyses

Analysis of variance (ANOVA) was used to test statistical significance (p<0.05) of differences between coefficients from incubations with and without addition of nutrients, mixed layer versus DCM incubations, and among domains. T–tests were used to compare variable fluorescence measurements between diluted and undiluted treatments within an experiment. Pearson product-moment correlation tested for statistically significant associations between factors. If the data did not fulfill the assumptions for ANOVA, we applied appropriate transformations. We used ANOVA on ranks if data still did not meet the assumptions. We used Sigmaplot version 9.0 for all statistical analyses.

3. Results

3.1. Effect of nutrient additions
We compared the results of paired dilution experiments conducted with and without the addition of nutrients (Table 2). In calculating means and standard deviations, we used data from all paired incubations, whether or not grazing was statistically significant; we substituted “0” for negative “g” values in calculating means. In 2008, we did 21 comparative experiments, with and without the addition of 5 µM nitrogen as sodium nitrate. Mean phytoplankton growth rates (µ, d\(^{-1}\)) in the control (no nutrient addition) and nutrient addition treatments were similar, however grazing coefficients (g, d\(^{-1}\)) were significantly lower in the nutrient treatments (Table 2). In 2009, we did 5 comparative experiments in which the 5 µM nitrogen nutrient treatment was attained using ammonium chloride. The effects on phytoplankton growth and grazing were both non-significant (Table 2). In 2010, we did 15 experiments in which the nutrient treatment was addition of 5 µM nitrogen as sodium nitrate in combination with 0.3 5 µM phosphate as sodium phosphate. The nutrient treatment significantly increased mean phytoplankton growth but not estimation of microzooplankton grazing (Table 2). Based on these results, we report microzooplankton grazing based upon the no addition dilution series. Olson and Strom (2002) also noted that nutrient enrichment can sometimes result in a decrease in phytoplankton growth rates in dilution experiments in the Bering Sea.

3.2. Phytoplankton growth, microzooplankton grazing, ingestion and production in the mixed layer

The mixed layer dilution experiments were with WSW samples collected at the depth of 55% of surface irradiance (PAR) and incubated under simulated in situ conditions. The sample depth varied from 3 to 10 m (Table 1) and was always within the surface mixed layer. Water was prescreened with a 200 µm Nitex mesh prior to set-up of the dilution series to remove mesozooplankton so large phytoplankton chains and colonies were not included in the incubations. The <200 µm fraction ranged from an average
of 69% at the off shelf, 79-90% at the outer shelf stations, 41-98% at the middle shelf stations, 77% at the inner shelf stations and 74% at the Alaska Peninsula stations of the total chlorophyll (Table 3). Grazing coefficients were greater than “0” in 61% of the 59 dilution experiments conducted with mixed layer assemblages (Table 3). To avoid biasing the data against low growth and grazing rates, we included all coefficients, whether or not they were significant, in our estimates of average rates, however we did replace negative grazing rates with “0” in calculating means. Estimated average phytoplankton growth coefficients for most regions were in the range of 0.24-0.32 d\(^{-1}\) (Table 3). The average phytoplankton growth coefficient for the mid north Middle Shelf was low (Table 3) because of an experiment with a negative growth rate (\(\mu = -0.80\)); without this one experiment, the average was 0.37 d\(^{-1}\). The average phytoplankton growth rate in the Alaska Peninsula region appeared to be higher than rates in the other areas, but differences among regions were not statistically significant (1-WAY ANOVA, \(p>0.05\)).

Likewise, estimates of average microzooplankton grazing were relatively similar across regions, ranging from 0.16 to 0.34 d\(^{-1}\) (Table 3) and differences among regions were not statistically significant (1-WAY ANOVA, \(p>0.05\)). Correlations between \(\mu\) or \(g\) and mixed layer water temperature, inorganic nutrients (phosphate, silicate, nitrate, ammonium) and estimated total PAR for incubations (data not shown) were not statistically significant (Pearson Product Moment Correlation; \(p>0.05\)). Both microzooplankton grazing and phytoplankton growth coefficients correlated negatively with total chlorophyll \(a\) estimated either as total chlorophylls from CTD bottles as part of core measurements or by the primary production study (Lomas et al., 2012). The regressions only accounted for a small amount of the variability (Figure 3 A and B). Microzooplankton grazing (<200 \(\mu\)m fraction) was not correlated with the biomass of 20-200 \(\mu\)m ciliate and dinoflagellate microzooplankton (\(p>0.05\)) (data not shown). Microzooplankton grazing was also not correlated with abundance of Phaeocystis cells (\(p>0.05\)) (data not shown).

We estimated the proportion of phytoplankton growth grazed by phytoplankton as “g/\(\mu\)” from the average “g” and “\(\mu\)” for regions (Table 3). Estimates for individual incubations could not be made because of negative or zero coefficients. Estimated average proportion of phytoplankton growth in the
<200 µm fraction grazed by phytoplankton appeared to vary among regions (Figure 2). In the off shelf and outer shelf regions, microzooplankton grazing consumed 67-78% of phytoplankton growth but on the middle shelf and inner shelf (with the exception of the south middle shelf) microzooplankton grazing coefficients were greater than phytoplankton growth rates in the mixed layer (Figure 2 and Table 3).

Estimated phytoplankton carbon ingestion by microzooplankton was 11.0 µg carbon l⁻¹ d⁻¹ in the Alaska Peninsula region, 8.2 off shelf, 7.7 on the outer shelf, 4.7 on the middle shelf and 4.4 d⁻¹ on the inner shelf, with differences among regions not statistically significant (1 Way ANOVA, p>0.05) (Figure 4). Assuming that all microzooplankton were strictly heterotrophic and a gross growth efficiency of 35%, the estimated secondary production of microzooplankton in the mixed layer was 3.8 µg carbon l⁻¹ d⁻¹, 2.9, 2.7, 1.6, and 1.5 for the Alaska Peninsula, off shelf, outer shelf, middle shelf and inner shelf regions, respectively.

3.3. Microzooplankton grazing, ingestion and production in the DCM

We conducted dilution grazing experiments with deep chlorophyll maximum assemblages at 6 stations in 2010, four of these were paired with mixed layer incubations at the same station (Table 4). Irradiance levels and water temperatures were low in the DCM so we decided to incubate the DCM experiments in the dark either in a cold room (~4°C) or on ice (~0 °C), whichever provided conditions closer to in situ (Table 4). Grazing coefficients in the DCM ranged from ~0 to 0.70 d⁻¹ and in 4 out of 6 DCM incubations, the grazing coefficients were significant (p<0.05) (Table 4). It is interesting that in paired mixed layer and DCM incubations, the DCM grazing coefficients were similar in magnitude to the mixed layer coefficients (Table 4) however, because of the higher phytoplankton biomass in the DCM than the mixed layer estimated ingestion of phytoplankton carbon by microzooplankton in the DCM was higher than in the mixed layer (Figure 5). Assuming similar gross growth efficiencies for
microzooplankton in the DCM and in the mixed layer, microzooplankton secondary production in the DCM on a per liter basis is ~4-fold higher than in the mixed layer.

3.4. Non-significant and “negative” grazing coefficients and Fv/Fm

In 39% of our dilution grazing experiments with mixed layer assemblages the grazing coefficients were not statistically significant (p>0.05) and in 3% we found negative grazing coefficients that were statistically significant. Estimation of microzooplankton grazing in dilution experiments is based on the assumption that phytoplankton growth rate is the same in diluted and undiluted treatments. To determine if dilution was having a negative effect on the physiology of phytoplankton, and potentially on their growth rates, we measured variable fluorescence (Fv/Fm) in both the undiluted (WSW) and diluted (20% WSW) treatments at the end of paired incubations with and without added nitrate (Table 1). Fv/Fm was significantly lower (t-test, p<0.05) in the diluted than undiluted treatments in 11 out of 14 incubations without added nutrients and in 7 out of 14 incubations with added nutrients (Table 5). Fv/Fm was significantly higher (p<0.05) in the diluted treatments with than without added nitrate in 3 of the 14 experiments (data not shown). In only two experiments did this alleviate the negative effect of dilution on variable fluorescence of phytoplankton (Table 5). Low estimates of microzooplankton grazing coefficient were associated with experiments in which dilution had a relatively large negative effect of on Fv/Fm (Figure 6).

4. DISCUSSION
During summers of 2008-2010, all “cold years” in the eastern Bering Sea (Stabeno et al., 2012b), the over-all average mixed layer microzooplankton grazing coefficient was 0.26 d\(^{-1}\), which is quite similar to the average coefficient of 0.29 d\(^{-1}\) observed on the SE shelf by Olson and Strom during summer 1999, another cold year (Table 6). In 2004, a “warm year” (Ladd and Stabeno, 2012), microzooplankton grazing was low on the SE Shelf (Strom and Fredrickson, 2008)(Table 6). The low grazing in 2004 was probably a response of microzooplankton to poor food quality caused by phytoplankton nutrient limitation due to intense stratification (Strom and Fredrickson, 2008) however “warm” years are not always associated with high stratification on the Bering Sea shelf (Ladd and Stabeno, 2012), so it is unlikely that summer microzooplankton grazing is predictable from temperature alone. During 2004, phytoplankton growth rates responded strongly to addition of nutrients (Strom and Fredrickson, 2008) but we only observed increases in phytoplankton growth in response to nutrient additions in one year, 2010 (Table 2). Contrary to expectations, nutrient addition sometimes resulted in lower estimates of phytoplankton growth and sometimes microzooplankton grazing. Possible inhibition of growth and grazing due to nutrient addition has been previously reported (Gifford, 1988; Olson and Strom, 2002).

Average microzooplankton grazing coefficients during spring sea ice conditions are <50% of summer grazing coefficients (Table 6). The lower grazing coefficients in spring are probably due to a combination of factors including lower ratios of microzooplankton biomass to phytoplankton biomass in spring than in summer (Sherr et al., submitted; Stoecker et al., this volume), differences in size distribution and species composition of phytoplankton (Lomas et al., 2012) and lower water temperatures in spring.

It is interesting that both the growth rate of phytoplankton (\(\mu\)) and microzooplankton grazing (\(g\)) were correlated negatively with total chlorophyll \(a\) in the mixed layer. Negative or no correlation of growth and grazing coefficients with chlorophyll have also been noted by Olson and Strom (2002), Strom et al. (2007), and Calbet et al. (2011) in northern seas in summer. This is consistent with domination of the phytoplankton by < 5 \(\mu\)m cells, and a rate, rather than biomass, controlled production system.
dependent on nutrient recycling (Lomas et al., 2012). Based on primary production measurements,
integrated phytoplankton growth rates ($\mu$) averaged 0.42 d$^{-1}$ (SD, 0.17) (Lomas et al., 2012), which is
within the wide range of phytoplankton growth rates we estimated in mixed layer dilution experiments
(Table 6). In the north middle and inner domains, the biomass of microzooplankton to phytoplankton was
high in summer (Stoecker et al., this issue), consistent with the high ratios of microzooplankton grazing to
phytoplankton growth. A confounding factor in estimating phytoplankton growth and microzooplankton
grazing from chlorophyll in dilution experiments on the Bering Sea shelf is the high biomass of plastidic
ciliates in summer (Stoecker et al., this issue). The incorporation of phytoplankton chloroplasts into
ciliates may result in underestimation of microzooplankton grazing and an overestimate of
“phytoplankton” biomass.

For the 2008 summer cruise, the spatial pattern of mixed layer phytoplankton growth and
microzooplankton grazing (Figure 7) can be compared with the spatial distribution of chl $\alpha$, variable
fluorescence and phytoplankton composition from HPLC (Goes et al., this issue). The high phytoplankton
growth ($\mu$, d$^{-1}$) and moderate microzooplankton grazing (g, d$^{-1}$) were measured on the north middle shelf,
where diatom and cryptophytes patches, probably remnants of spring ice associated blooms, were
observed. Elevated phytoplankton growth and microzooplankton grazing were observed on the middle
shelf near 60°N, at the border between the St. Matthews and North Inner Shelf regions. This area was
characterized by relatively low surface chlorophyll $\alpha$ and moderate variable fluorescence. Diatom patches
were to the east and haptophytes to the west of 170°W in this area. Phytoplankton growth and
microzooplankton grazing were also relatively high on parts of the north outer shelf and off shelf north
regions on the inner edge of the greenbelt. The greenbelt was characterized by low to moderate surface
chlorophyll $\alpha$, moderate variable fluorescence and dominance of phytoplankton biomass by cryptophytes
and haptophytes. The Pribilof Island area was a hot spot for phytoplankton growth, with patches of both
diatoms and haptophytes present, but microzooplankton grazing was moderate in this region. In contrast,
the southeastern shelf and Peninsula area tended to have lower phytoplankton growth and
microzooplankton grazing coefficients, although small flagellates, including cryptophytes and haptophytes, dominated (Goes et al., this issue).

In 2004, weak trophic coupling of phytoplankton growth to microzooplankton grazing was observed (Strom and Fredrickson, 2008), but strong coupling was observed in 1999 (Olson and Strom, 2002) and in summers of 2008, 2009, and 2010 (Table 6). On the shelf, microzooplankton grazing coefficients (g) often exceeded phytoplankton growth coefficients (µ) in the mixed layer, but g/µ was <1 in Alaska Peninsula, Off Shelf and Outer Shelf waters in summer (Table 3). On average, grazing is equivalent to phytoplankton growth in the sea (Banse, 1992), with ratios of g to µ exceeding 1 often found during the demise of blooms. For example, in the more southerly Gulf of Alaska, the ratio of microzooplankton grazing to phytoplankton growth on the middle and inner shelf reaches a maximum in summer with g/µ > 1.0 whereas in summer the ratio is lower on the outer shelf (Strom et al., 2007), similar to our observations in the eastern Bering Sea. Another example is the Sea of Okhotsk, Liu et al. (2009) found that microzooplankton grazing (g) was about 3X higher than phytoplankton growth (µ) in late summer in nutrient-depleted shelf waters whereas in the higher nutrient shelf break and strait waters, g/µ estimates were <0.5. Ratios of microzooplankton grazing to phytoplankton growth in excess of 1 may be a general, although transient, feature of highly stratified boreal and arctic shelf ecosystems in summer.

Large copepods, such as Neocalanus spp., Calanus glacialis, and Metridia longa, have a strong prey preference for microzooplankton, but are largely absent from the middle and inner shelf waters in summer (Vidal and Smith, 1986; Gifford, 1993; Campbell et al., 2009; Hunt et al., 2008). A reduction in top down control of microzooplankton on the eastern Bering Sea shelf may be partially responsible for the high biomass of microzooplankton (Stoecker et al., this issue) and their high grazing impact on the Bering Sea shelf in summer. A similar phenomenon occurs on the Gulf of Alaska shelf, where summer populations of large copepods are low and large cell-size microzooplankton are very abundant due to diminished top down control (Strom et al., 2007).
Although the grazing impact of microzooplankton on phytoplankton growth (g/µ) was highest in middle and inner shelf waters, estimated microzooplankton ingestion of phytoplankton carbon and secondary production of microzooplankton was highest in the Alaska Peninsula, off shelf and outer shelf waters. This was due to higher chlorophyll levels than on the middle and inner shelf. However, microzooplankton (20-200 µm) biomass in the off shelf and outer shelf regions was about half that in the inner and middle domains (Stoecker et al., this issue). One reason for this discrepancy may be that grazing by nanozooplankton (which are not included in the microzooplankton biomass estimates) makes a larger contribution to grazing in the higher chlorophyll regions. Nanozooplankton have been shown to be more abundant in frontal areas on the outer shelf and at the shelf break than in lower chlorophyll inner and middle domains in summer (Flint et al., 2002). The relatively low biomass of 20-200 µm microzooplankton (ciliates and large dinoflagellates) and high biomass of nanozooplankton in Peninsula, off shelf and outer shelf waters suggests a trophic cascade in which top down control of large microzooplankton by crustacean zooplankton releases nanozooplankton from grazing control. Conversely, large microzooplankton are probably relatively more abundant on the shelf in summer due to relaxation in top down control by copepods which are mostly absent from the shelf in summer (Vidal and Smith, 1986; Hunt et al., 2008).

During the summer, the DCM in the northern domain can be very well developed with > 10 µg chlorophyll a l⁻¹ at some stations (Lomas et al., 2012). Thus, the processes within the DCM may be very important to carbon flux and trophic transfer. Microzooplankton biomass (Stoecker et al., this issue) and grazing coefficients in the DCM were usually similar to those in the mixed layer above. The DCM samples ranged in depth from 26 to 35 m (Table 4), which was near or below the average depth of the 1% PAR isolume, 30 m (Lomas et al., 2012). If we assume that phytoplankton growth was usually low at these depths, it is clear that the ratio of grazing to phytoplankton growth must have been very high. Microzooplankton grazing is often important in erosion of “plankton patches” (Menden-Deuer and Fredrickson, 2010). Although grazing coefficients were roughly similar in the mixed layer and
corresponding DCM, the ingestion rates in the DCM were 3-30X higher than in the mixed layer (Figure 5). If we assume that microzooplankton growth efficiency was similar in the mixed layer and DCM, the DCM may be an important site of microzooplankton production during summer, particularly on the northern shelf. The lack of accumulation of microzooplankton biomass in the DCM suggests that predation pressure on microzooplankton is high and thus that these layers may be important in trophic transfer to higher trophic levels. The impact of microzooplankton grazing on phytoplankton and export fluxes can be under-estimated if grazing near the base of the euphotic zone is not included (Landry et al., 2011).

The decrease in variable fluorescence during the incubations from some stations suggest that dilution had a negative impact on phytoplankton photosynthetic physiology and hence potentially growth rate. This effect was not simply due to N limitation alone in the diluted treatments; it occurred in control and, in most cases, the paired nitrate amended dilutions. One of the assumptions of the dilution method is that dilution does not change the growth rate of phytoplankton, and this assumption likely was violated in at least some dilution experiments. A decrease in phytoplankton growth rate with dilution would result in an underestimation of the grazing coefficients. This may partially account for the low or non-significant grazing coefficients. Low grazing rates of microzooplankton for the biomass of microzooplankton have previously been reported in the SE Bering Sea during intense summer stratification and nutrient limitation of phytoplankton (Strom and Fredrickson, 2008). Non-significant microzooplankton grazing coefficients have also been reported during the spring at both non-bloom and diatom bloom ice edge stations (Sherr et al., submitted). In addition to non-significant grazing coefficients, statistically significant negative rates occur. Negative dilution grazing results are usually not reported (Dolan and McKeon, 2005), or the negative coefficients treated as “0” (Strom and Fredrickson, 2008). Negative rates can only occur when the growth rate of phytoplankton, $\mu$, is lower in the diluted treatment and than in the whole seawater. In the polar and subpolar ecosystems, non-significant and negative results in dilution experiments are common, particularly during Phaeocystis blooms (Calbet et al., 2011; Caron et al., 2000).
In culturing phytoplankton, a “lag” phase typically occurs after transfer of cells to new media, this has been ascribed to the time it takes cells to “ramp up” to better growth conditions, the “shock” of transfer and to lack of “conditioning factors” in the media. Perhaps something similar happens when phytoplankton are diluted with filtered seawater. Another possibility is that the mechanical stress involved in passage through filters results in release of “toxic” or “inhibitory” compounds into the filtered seawater used to make the dilutions. Strom and Fredrickson (2008) noted that release of diatom extracts from filters used to prepare filtered seawater may have inhibited growth of phytoplankton. During certain growth phases, Phaeocystis pouchetti and many bloom forming diatoms produce cytotoxic aldehydes that are inhibitory to phytoplankton growth, including their own (Hansen and Eilertsen, 2007; Paul et al., 2009). Mechanical stress can trigger the release of these compounds from cells (Hansen & Eilertsen, 2007). We hypothesize that decreases in variable fluorescence and low or negative phytoplankton growth rates in diluted treatments could be due to presence of toxic aldehydes released from phytoplankton during preparation of filtered seawater. Preliminary results indicate that with some diatom and Phaeocystis blooms, treatment of filtered seawater with activated carbon to remove organic material prior to its use in dilution can reverse the negative effects on both “u” and “g” (Stoecker and Nejstgaard, unpubl. data). However, contrary to expectation, grazing coefficients were not negatively correlated with abundance of Phaeocystis pouchetti cells in the Bering Sea. Production of inhibitory compounds by Phaeocystis varies with life form and bloom stage (Nejstgaard et al., 2007), thus not all Phaeocystis cells will have the same impact on water chemistry. Furthermore, diatoms are also a source of cytotoxic aldehydes and are bloom dominants in the Bering Sea (Flint et al., 2002; Lomas et al., 2012). A simple relationship between one of these factors (Phaeocystis cells) and apparent low grazing coefficients is unlikely due to confounding factors.

Although the overestimation of microzooplankton grazing by the dilution technique may occur under some circumstances (Dolan et al., 2000; Dolan and McKeon, 2005), it is possible that dilution experiments underestimate microzooplankton grazing during summer in the Bering Sea. Whether or not
grazing is underestimated, it is clear that microzooplankton grazing consumes most of phytoplankton production and that secondary production by microzooplankton is important. Most phytoplankton production is by < 5 µm phytoplankton and thus passes through the microzooplankton link before it is available to crustacean zooplankton in summer. On the northern shelf, the DCM may often be a site of enhanced trophic transfer. Microzooplankton can comprise 49% or more of the food available to larger zooplankton on the Bering Sea shelf in summer (Stoecker et al., this volume).

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Reference


Goes, J.I. et al., this volume, Distribution of phytoplankton communities in the Bering Sea during the summer of 2008 that followed a cold spring. --


Figure Legends

Fig. 1. Stations where r dilution experiments were performed in the eastern Bering Sea during summers 2008, 2009, and 2010. Refer to Table 1 for list of stations, regions and experimental parameters; experiments were not performed at all stations in all years.

Fig. 2. Estimated fraction of phytoplankton daily growth consumed by microzooplankton grazing (g/u) in the mixed layer, Eastern Bering Sea. Means (SD) of regions within larger domains. AK PEN=Alaska Peninsula region; OFF=Off Shelf; OUTER=Outer Shelf; MIDDLE=Middle Shelf; INNER= Inner Shelf or Coastal. Data for summers 2008, 2009, and 2010.

Fig. 3. Microzooplankton grazing (g, d-1) (A) and phytoplankton growth (u, d-1) (B) vs. chlorophyll a, Eastern Bering Sea. Data for summers 2008-2010.

Fig. 4. Estimated microzooplankton ingestion of phytoplankton carbon in the mixed layer. Means (SD) of stations within a region, Eastern Bering Sea. AK PEN=Alaska Peninsula region; OFF=Off Shelf; OUTER=Outer Shelf; MIDDLE=Middle Shelf; INNER= Inner Shelf or Coastal. Data for summers 2008, 2009 and 2010.

Fig. 5. Comparison of estimated microzooplankton ingestion of phytoplankton carbon, paired mixed layer and DCM experiments, Eastern Bering Sea, summer 2010.
Fig. 6. Estimated microzooplankton grazing coefficients (g, d⁻¹) vs. the difference in variable fluorescence (Fv/Fm) between WSW and diluted treatments in an experiment. Low grazing was associated with decreased Fv/Fm in the diluted compared to the whole seawater treatments.

Fig. 7. Spatial distribution of phytoplankton growth (μ, d⁻¹) (upper panel) and microzooplankton grazing (g, d⁻¹)(lower panel) in the eastern Bering Sea, summer 2008.
Fig. 2

![Bar chart showing the comparison of different groups (AK PEN, OFF, OUTER, MIDDLE, INNER) with their respective mean values and standard deviations (±SD).]
Fig. 3

A. $R^2=0.072, p<0.05$

B. $R^2=0.236, p<0.001$
Fig. 4

![Graph showing µg Carbon / d]
Microzooplankton Ingestion (µg C l⁻¹ d⁻¹)

- Mixed Layer
- DCM

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Fig. 6

$r_{12}=0.579, p=0.030$
Fig. 7

Growth rates

Grazing rates

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<td>2</td>
<td>07/14/08</td>
<td>NP-7</td>
<td>7</td>
<td>5.21 N</td>
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<td>70m-53</td>
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</tr>
<tr>
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<td>NP-7</td>
<td>7</td>
<td>5.25 N*</td>
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<td>70m-58</td>
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<td>3.30 N+P</td>
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<td>70m-25</td>
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<td>07/06/10</td>
<td>ML-3</td>
<td>7</td>
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<td>70m-25</td>
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<td>10</td>
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<td>SL-11</td>
<td>6</td>
<td>5.68 B</td>
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<td>70m-25</td>
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</tr>
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<td>4</td>
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<td>5.69 C</td>
</tr>
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<td>NP-14</td>
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<td>NP-14</td>
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<td>07/09/08</td>
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<td>Value</td>
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<td>-------</td>
<td>------</td>
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<td></td>
</tr>
<tr>
<td>07/24/08</td>
<td>MN-12</td>
<td>7</td>
<td>N</td>
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<td>2</td>
<td>N</td>
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<td></td>
</tr>
<tr>
<td>07/25/08</td>
<td>MN-19</td>
<td>9</td>
<td>N</td>
<td>06/23/09</td>
<td>NP-15</td>
<td>4</td>
<td>N*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>07/03/09</td>
<td>MN-19</td>
<td>10</td>
<td>6.03</td>
<td>06/26/09</td>
<td>P14-7</td>
<td>3</td>
<td>5.86</td>
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<tr>
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<td>XB16</td>
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<td>06/28/10</td>
<td>P14N-10</td>
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<td>06/29/10</td>
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<td>6.22</td>
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</tr>
<tr>
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<td>07/21/08</td>
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<tr>
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<td>5.86</td>
<td>06/25/09</td>
<td>SB-7</td>
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<tr>
<td>06/27/10</td>
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<td>06/19/09</td>
<td>CN-20</td>
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<td>6.31</td>
<td>N</td>
<td></td>
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<tr>
<td>07/03/10</td>
<td>MN-16</td>
<td>7</td>
<td>5.36</td>
<td>07/06/08</td>
<td>PIT-1</td>
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<td>N</td>
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<tr>
<td>07/03/10</td>
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<td>07/07/08</td>
<td>sta21</td>
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</tr>
<tr>
<td>07/04/10</td>
<td>TM4</td>
<td>35</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>07/05/10</td>
<td>ML-13</td>
<td>3</td>
<td>5.97</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
Table 2. Effect of nutrient additions (+nuts) on phytoplankton growth (µ) and microzooplankton grazing (µ) in dilution experiments, mixed layer, summer, Eastern Bering Sea 2008, 2009, 2010. Mean (SD).

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Experiments</th>
<th>Control</th>
<th>+ Nuts&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Control vs. Nuts&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µ</td>
<td>g</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>21</td>
<td>0.31(0.178)</td>
<td>0.31(0.204)</td>
<td>ns</td>
</tr>
<tr>
<td>2009</td>
<td>5</td>
<td>0.17(0.178)</td>
<td>0.18(0.159)</td>
<td>ns</td>
</tr>
<tr>
<td>2010</td>
<td>15</td>
<td>0.28(0.157)</td>
<td>0.36(0.165)</td>
<td>**</td>
</tr>
</tbody>
</table>

<sup>a</sup> 2008: 5 µM N as sodium nitrate; 2009: 5 µM N as NH₄Cl; 2010: 5 µM N as sodium nitrate + 0.3 µM P as sodium phosphate.

<sup>b</sup> Repeated Measures ANOVA: ns=non-significant, *p<0.05, **p<0.01.
Table 3. Growth coefficients of phytoplankton (µ, d⁻¹) and microzooplankton grazing coefficients (g, d⁻¹), and proportion phytoplankton daily growth grazed by microzooplankton (g/µ). Mixed Layer data for 2008, 2009, 2010 combined by region. Mean (SD). For g and u, mean of all incubations without added nutrients, but “0” used for negative g. *Number of experiments in which g was statistically significant (p<0.05). C=without outlier

<table>
<thead>
<tr>
<th>Region</th>
<th>Chl a &lt;200 µm (µg l⁻¹)</th>
<th>% Chl a &lt;200 µm</th>
<th>Incubation PAR (µE s⁻¹ m⁻²)</th>
<th>µ</th>
<th>g</th>
<th>g/u</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Off Shelf</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southeast (n=3, 1*)</td>
<td>0.77(0.44)</td>
<td>nd</td>
<td>742(300.1)</td>
<td>0.24(0.16)</td>
<td>0.16 (0.16)</td>
<td>.67</td>
</tr>
<tr>
<td>North(n=6, 4*)</td>
<td>0.60(0.54)</td>
<td>69(32)</td>
<td>278(119.3)</td>
<td>0.19(0.30)</td>
<td>0.30(0.12)c</td>
<td>.77c</td>
</tr>
<tr>
<td><strong>Outer Shelf</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South (n=3, 2*)</td>
<td>0.79(0.22)</td>
<td>90(14)</td>
<td>210(151.6)</td>
<td>0.32(0.20)</td>
<td>0.25(0.15)</td>
<td>.78</td>
</tr>
<tr>
<td>North(n=14, 9*)</td>
<td>0.89(0.64)</td>
<td>79(14)</td>
<td>328(141.5)</td>
<td>0.25(0.24)</td>
<td>0.19(0.12)</td>
<td>.76</td>
</tr>
<tr>
<td><strong>Middle Shelf</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South (n=3, 2*)</td>
<td>0.38(0.22)</td>
<td>98(2)</td>
<td>517(146)</td>
<td>0.27(0.11)</td>
<td>0.24(0.06)</td>
<td>.89</td>
</tr>
<tr>
<td>Mid-North(n=4, 3*)</td>
<td>0.40(0.35)</td>
<td>41(49)</td>
<td>241(41.0)</td>
<td>0.08(0.62)</td>
<td>0.19(0.20)</td>
<td>1.56</td>
</tr>
<tr>
<td>St. Matthew (n=8, 6*)</td>
<td>0.52(0.52)</td>
<td>62(1)</td>
<td>344(184.5)</td>
<td>0.25(0.15)</td>
<td>0.28(0.22)</td>
<td>1.17</td>
</tr>
<tr>
<td>North(n=8, 4*)</td>
<td>0.33(0.41)</td>
<td>97(25)</td>
<td>440(188.4)</td>
<td>0.24(0.21)</td>
<td>0.34(0.28)</td>
<td>1.42</td>
</tr>
<tr>
<td><strong>Inner Shelf</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(South, Mid-North &amp; North combined)(n=6, 3*)</td>
<td>0.47(0.28)</td>
<td>77(50)</td>
<td>263(116.2)</td>
<td>0.19(0.14)</td>
<td>0.22(0.25)</td>
<td>1.16</td>
</tr>
<tr>
<td><strong>AK Peninsula</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=4, 2*)</td>
<td>0.71(0.47)</td>
<td>74(30)</td>
<td>334</td>
<td>0.45(0.23)</td>
<td>0.27(0.22)</td>
<td>.60</td>
</tr>
</tbody>
</table>
Table 4. Deep chlorophyll maximum (DCM) Dilution Experiments, Middle Shelf, Summer 2010. For experiments with a paired Mixed Layer (ML) experiment, both presented. Means (SD).

<table>
<thead>
<tr>
<th>Domain (region)</th>
<th>Station</th>
<th>Sample Depth</th>
<th>Temp. (°C)</th>
<th>Avg. Incub. Irradiance</th>
<th>Chlorophyll a (t₀) (µg l⁻¹)</th>
<th>µ</th>
<th>g</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(m)</td>
<td>In situ</td>
<td>Incubation</td>
<td>µ E s⁻¹ m⁻²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle (10)</td>
<td>SL-11</td>
<td>6-ML</td>
<td>5.6</td>
<td>~6</td>
<td>347</td>
<td>0.30</td>
<td>0.16</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>33-DCM</td>
<td>-1.2</td>
<td>4</td>
<td>Dark</td>
<td>1.08</td>
<td>-1.03</td>
</tr>
<tr>
<td>Middle (10)</td>
<td>BN-3</td>
<td>5-ML</td>
<td>5.7</td>
<td>~6</td>
<td>524</td>
<td>0.10</td>
<td>0.29</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30-DCM</td>
<td>-1.2</td>
<td>0</td>
<td>Dark</td>
<td>0.85</td>
<td>0.03</td>
</tr>
<tr>
<td>Middle(6)</td>
<td>70m-25</td>
<td>3-ML</td>
<td>6.5</td>
<td>~6</td>
<td>262</td>
<td>0.16</td>
<td>0.39</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28-DCM</td>
<td>-0.3</td>
<td>4</td>
<td>Dark</td>
<td>1.84</td>
<td>-0.03</td>
</tr>
<tr>
<td>Outer (8)</td>
<td>ML-13</td>
<td>3-ML</td>
<td>6.0</td>
<td>~6</td>
<td>330</td>
<td>0.11</td>
<td>0.34</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35-DCM</td>
<td>-1.1</td>
<td>4</td>
<td>Dark</td>
<td>5.36</td>
<td>0.04</td>
</tr>
<tr>
<td>Outer (8)</td>
<td>MN-18</td>
<td>26-DCM</td>
<td>1.6</td>
<td>~4</td>
<td>Dark</td>
<td>1.21</td>
<td>0.03</td>
<td>0.18</td>
</tr>
<tr>
<td>Outer (8)</td>
<td>TM-4</td>
<td>35-DCM</td>
<td>1.7</td>
<td>4</td>
<td>Dark</td>
<td>0.65</td>
<td>0.08</td>
<td>0.15</td>
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</table>
Table 5. Variable Fluorescence (Fv/Fm) at the end of mixed layer dilution experiments in undiluted (WSW) and diluted (20% WSW) treatments without and with the addition of 0.05 µM N as sodium nitrate. All experiments conducted in summer 2008. N=2 or 3.

<table>
<thead>
<tr>
<th>Station</th>
<th>Mean (SD), No nutrient addition</th>
<th>T-test</th>
<th>Mean(SD), Nutrient addition</th>
<th>T-test</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>WSW</td>
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<td>20%WSW</td>
<td></td>
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<tr>
<td>C-55</td>
<td>0.345(0.0071)</td>
<td>p</td>
<td>0.348(0.0318)</td>
<td>0.353(0.4596)</td>
</tr>
<tr>
<td>W-4b</td>
<td>0.237(0.0379)</td>
<td>p&lt;0.05</td>
<td>0.245(0.0283)</td>
<td>0.283(0.0318)</td>
</tr>
<tr>
<td>SL-8</td>
<td>0.350(0.0433)</td>
<td>p&lt;0.05</td>
<td>0.353(0.0404)</td>
<td>0.426(0.0871)</td>
</tr>
<tr>
<td>NP-7</td>
<td>0.330(0.0100)</td>
<td>p&lt;0.05</td>
<td>0.310(0.00635)</td>
<td>0.37(0.0200)</td>
</tr>
<tr>
<td>NP-14</td>
<td>0.183(0.1626)</td>
<td>p&lt;0.05</td>
<td>0.268(0.0104)</td>
<td>0.267(0.0189)</td>
</tr>
<tr>
<td>NP-11</td>
<td>0.341(0.0136)</td>
<td>p&lt;0.05</td>
<td>0.362(0.0076)</td>
<td>0.300(0.0250)</td>
</tr>
<tr>
<td>LS1-6</td>
<td>0.343(0.0152)</td>
<td>p&lt;0.05</td>
<td>0.345(0.0087)</td>
<td>0.265(0.0071)</td>
</tr>
<tr>
<td>P14-4.5</td>
<td>0.253(0.0144)</td>
<td>p&lt;0.05</td>
<td>0.268(0.0293)</td>
<td>0.183(0.0231)</td>
</tr>
<tr>
<td>P14-2</td>
<td>0.298(0.0126)</td>
<td>p&lt;0.05</td>
<td>0.313(0.0058)</td>
<td>0.245(0.0229)</td>
</tr>
<tr>
<td>MN-6</td>
<td>0.280(0.0173)</td>
<td>p&lt;0.05</td>
<td>0.307(0.0115)</td>
<td>0.247(0.0058)</td>
</tr>
<tr>
<td>MN-12</td>
<td>0.245(0.0071)</td>
<td>p&lt;0.05</td>
<td>0.273(0.0289)</td>
<td>0.150(0.0141)</td>
</tr>
<tr>
<td>MN-19</td>
<td>0.250(0.0173)</td>
<td>p&lt;0.05</td>
<td>0.240(0.0265)</td>
<td>0.163(0.0321)</td>
</tr>
<tr>
<td>SL-14</td>
<td>0.263(0.0306)</td>
<td>ns</td>
<td>0.283(0.0643)</td>
<td>0.233(0.0106)</td>
</tr>
</tbody>
</table>
Table 6. Comparison of hydrographic conditions, phytoplankton growth coefficients and microzooplankton grazing coefficients from mixed layer dilution experiments conducted in the Bering Sea. Years classified as warm or cold, with high or low stratification index (Ladd and Stabeno, 2012; Stabeno et al., 2012b). Phytoplankton growth rates (µ) and MZ grazing rates (g) per day. Average with SD in parentheses, or range.

<table>
<thead>
<tr>
<th>Cruise dates and hydrographic conditions in SE Bering Sea</th>
<th>Water, °C</th>
<th>Growth</th>
<th>Grazing</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 99: south Bering Sea (off shelf and outer shelf) (cold year)</td>
<td>5.3-7.7</td>
<td>0.2-0.6 0.41 (n=5)</td>
<td>0.1-0.4 0.27 (n=5)</td>
<td>Liu et al., 2002</td>
</tr>
<tr>
<td>July-August 99; SE Bering Sea (cold year with low stratification index)</td>
<td>5.8-8.4</td>
<td>-0.7-0.6 0.33 (n=13)</td>
<td>0.1-0.5 0.29 (n=13)</td>
<td>Olson and Strom, 2002</td>
</tr>
<tr>
<td>July-August 04: SE Bering Sea (warm year with high stratification index)</td>
<td>9.3-13.4</td>
<td>0.0-1.0 0.35 (n=18)</td>
<td>0.0-0.27 0.13 (n=18)</td>
<td>Strom and Fredrickson, 2008</td>
</tr>
<tr>
<td>April-May, 2008, 2009, 2010-Non bloom, Eastern Bering Sea, ice edge (cold years)</td>
<td>-0.3(1.4) n=17</td>
<td>0.17(0.14)</td>
<td>0.08(0.12) n=17</td>
<td>Sherr et al., submitted</td>
</tr>
<tr>
<td>April-May, 2008, 2009, 2010-Bloom, Eastern Bering Sea, ice edge (cold years)</td>
<td>0.8(1.7) n=21</td>
<td>0.21(0.12)</td>
<td>0.09(0.08) n=21</td>
<td>Sherr et al., submitted</td>
</tr>
<tr>
<td>June-July, 2008, 2009, 2010-Eastern Bering Sea (cold years; low stratification index in 2008, stratification classification for 2009 &amp; 2010 not yet available)</td>
<td>2.3-7.9</td>
<td>-0.8-0.8 0.26 (n=61)</td>
<td>0.0-0.9 0.26 (n=61)</td>
<td>This study</td>
</tr>
</tbody>
</table>
Conclusions

There are regional (domain) differences in microzooplankton coupling to phytoplankton in the eastern Bering Sea in summer (Chapters 1 and 2). In stratified shelf waters, microzooplankton (defined as < 200 µm, includes nanozooplankton) consumed most of primary production (Chapter 2). Microzooplankton biomass (20-200 µm) often exceeded phytoplankton biomass in the mixed layer (Chapter 1). Large and small crustacean zooplankton do not efficiently feed on small (<10 µm) phytoplankton (reviewed in Calbet and Saiz, 2005), and often most of the biomass of > 10 cell was 20-200 µm microzooplankton, not large phytoplankton such as diatoms. These data indicate that if zooplankton are present in stratified shelf waters in summer, they must be largely dependent on microzooplankton as food. In less stratified waters near the shelf break, in the “green belt” and in the Pribilof Island Domain, the ratio of microzooplankton biomass (20-200 µm) to phytoplankton biomass was lower but microzooplankton (<200 µm fraction) still consumed about 67-78% of daily phytoplankton production (Chapters 1 and 2). This indicates an important role for combined nanozooplankton and microzooplankton in carbon cycling and nutrient regeneration in these higher chlorophyll waters.

In the northern middle domain there was a well developed deep chlorophyll maximum (DCM) (Chapter 1). Although microzooplankton grazing coefficients were not elevated in the DCM, microzooplankton ingestion of phytoplankton carbon was higher than in the mixed layer due to the high phytoplankton biomass (Chapter 2). These observations suggest that the DCM may be an important site of trophic transfer of phytoplankton carbon to microzooplankton, and in turn to zooplankton, on the northern shelf in summer.

Mixotrophic ciliates were a dominant component of the 20-200 µm fraction of the plankton in stratified shelf waters, particularly on the northern shelf (Chapter 1). Mixotrophic ciliates are members of the microzooplankton because they ingest phytoplankton, but they are also functionally members of the phytoplankton due to their chlorophyll content and photosynthetic activities. The dominance of the ciliate microzooplankton by mixotrophs may be a common feature of high latitude, stratified seas in summer. The mixotrophic ciliates, because of their relatively large cell sizes, were probably important as food for crustacean zooplankton in the mixed layer (Dutz and Peters, 2008). Due to photosynthesis, mixotrophic ciliates can have higher gross growth efficiency than strict heterotrophs (Stoecker et al., 2009). In the eastern Bering Sea shelf in summer, this may have had a positive impact on food web transfer efficiency to crustacean zooplankton.

Several factors complicate comparison of the role of microzooplankton in summers of “warm” versus “cold” years in eastern Bering Sea. One is that summer stratification and episodic mixing influence
phytoplankton biomass and coupling of microzooplankton grazing to phytoplankton growth. Ladd and Stabeno (2012) show that in the existing data set, stratification was not significantly correlated with “warm” or “cold” years. Thus, it is probably not possible to predict the role of microzooplankton in Bering Sea food webs based on temperature classifications alone. Another important factor in comparing years is differences in stations sampled and cruise timing in different years (Chapter 2). If domain is considered, there are no clear differences in microzooplankton biomass among “warm” and “cold” years (Chapter 1).

**Project Connections**

I liked working on the larger Bering Sea Project and enjoyed learning about and being exposed to the higher “vertebrate” trophic levels; this was new to me because on previous cruises that I have participated in as a plankton ecologist there were no vertebrate ecologists.

I was disappointed that I was not able to link my results more strongly to “bottom up” and “top down” results. It would have helped to have more data available on phytoplankton assemblages, not just chlorophyll, from the larger project. Food species do matter, even to microzooplankton. I think the general species composition of blooms makes a big difference to trophic transfer to microzooplankton and higher trophic levels.

Discussions and collaborations with the phytoplankton investigators, especially M. Lomas and D. Stockwell, were very useful. Collaboration with J. Goes, a guest on the 2008 cruise, helped tremendously in interpretation of the results from the dilution experiments. Our collaboration on dilution experiments resulted in general insight into the application of this technique. I also wish there had been research on the summer cruises on mesozooplankton in general (not just krill) to which to link results on microzooplankton. Specifically it would have been good to have data on zooplankton populations and zooplankton grazing --- I had to resort to the “general” literature, not data specific to the Bering Sea to link my results to higher trophic levels. One question is whether microzooplankton are abundant on the shelf in summer because there are not enough mesozooplankton to exert significant top down control on them.

**Management or policy implications**

The effects of global warming, ocean acidification and climate variability (changes in storm frequency, mixing events, stratification as well as timing of the spring bloom) will impact marine food webs through changes not just in “chlorophyll” but in phytoplankton and microzooplankton composition. This along with photosynthetic biomass and size fraction is the “frontline” for biological, bottom up
effects. Phytoplankton composition, at least “rough” taxonomic composition of biomass, should be included in long-term monitoring. The dominance of certain species (for example *Phaeocystis pouchetti* and *Emiliania huxleyi*) may largely determine what type of planktonic food web develops. Likewise the species and size distribution of microzooplankton matter since they can be an important prey for crustacean zooplankton that support pelagic fisheries. In low chlorophyll waters dominated by small cell size phytoplankton, such as observed on the Bering Sea Shelf in summer, plastidic ciliates appear to be a particularly important link between primary production and crustacean zooplankton. Although plastidic ciliates are common in temperate as well as arctic waters during summer stratification, they appear to occur on the Bering Sea shelf (and in other summer stratified arctic and sub-arctic seas) at higher biomass than in temperate waters. It is unclear if climate change will influence the magnitude and role of this “trophic link” under summer, low chlorophyll conditions.

**Publications**


**Poster and Oral Presentations**

ASLO/AGU, Feb 2010, Portland Oregon, Oral presentation: Summer importance of microzooplankton on Bering Sea Shelf. (co-authors Blattner, Stockwell)

Invited Speaker, 5th International Zooplankton Production Symposium, Population Connections, Community Dynamics and Climate Variability. March 2011, Pucón, Chile. Acquired phototrophy in ciliates: Does it boost trophic transfer to mesozooplankton? (co-authors Blattner, Weigel and Stockwell)

**Outreach**

Diane Stoecker and Kristen Blattner participated in outreach during the summer cruise 2008 by preparing presentations and talking with oceanography summer camp students, teachers and citizens of St. George Island. Diane Stoecker participated in an outreach webinar during the summer cruise. Diane Stoecker will use material from the cruise in teaching a graduate course in Biological Oceanography at University of Maryland. Diane Stoecker has digital micrographs of Bering Sea plankton (obtained in collaboration with John Casey) and digital photographs of research activities from the cruise which were...
available to other researchers on the cruise and which are available to the BSIERP program. Diane Stoecker used material from the 2008 cruise in teaching a graduate course in Biological Oceanography at University of Maryland.

**Acknowledgements**

Refer to acknowledgements in Chapters 1 and 2.

**Literature cited**

Refer to references in Chapters 1 and 2.