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RESEARCH ARTICLE

Patterns of microzooplankton growth in dilution experiments across a trophic gradient: Implications for herbivory studies

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Abstract To investigate the growth and grazing patterns of microzooplankton (MZP) in environments of differing productivity, dilution experiments measuring phytoplankton growth (μ) and grazing mortality (m) rates were performed using samples from contrasting locations along the Texas coast. Samples were collected from estuaries, coastal lagoons and offshore Gulf of Mexico locations in the spring and summer of 2001. MZP growth rates were determined in each dilution treatment. Although MZP biomass changed over time in most dilution treatments, adjusting μ and m for the actual grazer gradient (represented by geometric mean MZP biomass) did not cause a significant deviation from the nominal dilution gradient. Likewise, these adjustments did not yield significant regressions where none existed before adjustment. The dynamics of MZP taxonomic groups (ciliates, dinoflagellates) and size categories differed suggesting that in some cases internal predation may lead to trophic cascades. MZP biomass was higher in productive coastal waters and included a larger proportion of dinoflagellates than in the oligotrophic, ciliate-dominated waters of the Gulf of Mexico. The MZP biomass-to-chlorophyll *a* ratio was lowest in the hypereutrophic Nueces River, where MZP biomass significantly increased in all dilution treatments (net growth rates up to 2 day^{-1}) suggesting a strong top–down control. In the brown-tide dominated Upper Laguna Madre and the oligotrophic seagrass-dominated Lower Laguna Madre MZP growth was decoupled from that of phytoplankton. At these sites, MZP were likely fueled by bacterial carbon and mixotrophy, respectively. Observing the growth response of MZP in dilution experiments can provide insight into trophic structure and efficiency of the microbial food web.

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Introduction

In aquatic systems, phagotrophic protists consume a significant proportion of primary production (e.g. Calbet and Landry 2004, and references therein) and supply the bulk of regenerated nutrients in the ocean (Legendre and Rassoulzadegan 1995). Microzooplankton (MZP, mainly ciliates and dinoflagellates 20–200 μm) act as an important link between small primary producers and larger consumers (Burkill et al. 1993). MZP respond rapidly to increases in phytoplankton abundance (Strom et al. 2001) and through selective herbivory can restructure phytoplankton communities and influence the function of the microbial food web (Burkill et al. 1987; Gaul and Antia 2001). Conversely, the avoidance or deterrence of grazing by MZP has been implicated as a factor in the formation of algal blooms (Buskey and Hyatt 1995;

Stoecker et al. 2000; Irigoien et al. 2005). As top-predators in the microbial food web, MZP form trophic cascades that can indirectly influence picoplankton (Samuelsson and Andersson 2003), nitrifying bacteria (Lavrentyev et al. 1997), and nutrient regeneration rates (Sherr and Sherr 2002). In turn, MZP serve as a significant energy source for mesozooplankton in the open ocean (Calbet and Landry 1999) as well as in coastal waters, where ciliates are selectively preyed upon by copepods (Calbet and Saiz 2005). MZP are also important as a food source for larval fish (Buskey et al. 1993) and benthic invertebrates (Lavrentyev et al. 1995).

Information about MZP herbivory is commonly generated from serial dilution experiments (Landry and Hassett 1982), which provide simultaneous estimates of both rates of phytoplankton growth (μ) and the grazing impact (m) of MZP. In this method, natural seawater is progressively diluted with particle-free seawater (typically < 0.2 μm). In theory, phytoplankton growth is independent of the dilution and it follows that decreasing the herbivore-phytoplankton encounter rate will relieve grazing pressure in proportion to the dilution gradient. Thus, the central assumption of the dilution method is that the grazer impact is proportional to the dilution factor (Landry et al. 1995).

It is well established that MZP, particularly ciliates, can match and even exceed the growth rates of their prey (Banse 1982; Montagnes and Lessard 1999; Lavrentyev et al. 2004). While accounting for herbivore growth in undiluted samples has been suggested (Landry 1993; Gallegos et al. 1996), few studies have considered the variation in MZP growth along the full dilution gradient. In one study, tintinnid and oligotrich ciliates grew at low dilutions but declined precipitously in highly diluted samples (Dolan et al. 2000). The extent to which uneven grazer growth along the dilution gradient may bias MZP grazing impact estimates has since been debated (Dolan and McKeon 2005; Landry and Calbet 2005).

Comparison of the microbial food web structure and dynamics suggests that the dominant groups of MZP and their activities vary with trophic status and ambient conditions (Boissonneault-Cellineri et al. 2001; Fileman and Burkill 2001). MZP display a variety of trophic behaviors including omnivory, bacterivory, and mixotrophy (i.e. the ability to switch between or combine autotrophic and phagotrophic modes of nutrition), which allows their communities to adapt to changing conditions (Dolan and Perez 2000). Phytoplankton community structure can be an important factor determining the composition of MZP communities (Lavrentyev et al. 2004) and the outcome of herbivory experiments (Fahnenstiel et al. 1995; Lewitus et al. 1998). Furthermore, the impact of mesozooplankton predation and trophic interactions among MZP can vary over a trophic gradient (Calbet 2001; Samuelsson and Andersson 2003; Liu et al. 2005).

These considerations lead to the following questions: (1) How do the growth patterns of MZP in dilution experiments translate into experimental estimates of herbivory? (2) How does the growth of major MZP groups and their grazing impacts vary across natural productivity gradients? To address these questions, serial dilution experiments were conducted at several contrasting coastal and offshore sites representing a trophic gradient from a highly productive river (Nueces River, TX, USA) to the oligotrophic Gulf of Mexico. The response of different MZP assemblages to the dilution gradient was observed to provide insight into the function of microbial food webs. To assess potential consequences of MZP growth on herbivory estimates the dilution gradient was adjusted to account for changes in the grazer community.

Methods

Dilution experiments

Sampling and experiments were conducted at several locations along the Texas coast and the open Gulf of Mexico during spring and summer 2001 (Table 1; Fig. 1). Estuarine stations varied from hypereutrophic conditions (Nueces River) to an oligotrophic, seagrass-dominated system (Lower Laguna Madre). High salinity in Laguna Madre results from high evaporation and low freshwater input. In Upper Laguna Madre, the brown-tide forming pelagophyte, *Aureoumbra lagunensis*, has persisted for over a decade (Buskey and Hyatt 1995). Water from the estuarine Corpus Christi Bay and the oligotrophic open Gulf of Mexico was collected at several times over the spring and summer. The average depth of Corpus Christi Bay is 3-m and water was collected directly below the surface near the center of the Bay. Open Gulf of Mexico water was collected in low turbidity water, usually >50-km from the coast. At estuarine sites, surface water was collected into 20-l Nalgene polyethylene carboys. Open Gulf of Mexico and Laguna Madre samples were collected with 10-l Niskin bottles from 5-m below and at the surface, respectively. All materials used in handling samples were first acid-washed with 10% HCl and triple rinsed with deionized water. Laguna Madre experimental incubations were started immediately after sampling aboard the R/V *Longhorn*. All other

Table 1 Station identification, sample date, temperature and salinity of experimental stations

ID	Location	Date (2001)	Temperature (°C)	Salinity	μ (±SE) (day^{-1})	m (±SE) (day^{-1})	μ_0 (±SE) (day^{-1})	r^2 (±SE)	N
C1	Corpus Christi Bay	20-March	16.3	25.0	0.30 (±0.08)*	0.56 (±0.14)*	–0.36 (±0.07)	0.62 (±0.11)	10
C2	Corpus Christi Bay	29-May	28.0	27.3	1.10 (±0.08)**	1.34 (±0.14)**	0.32 (±0.03)	0.90 (±0.14)	11
G1	Gulf of Mexico	22-March	19.6	29.9	–0.5 (±0.26)	–0.01 (±0.37)	–	–0.10 (±0.36)	12
G2	Gulf of Mexico	1-June	28.0	32.0	0.94 (±0.23)*	0.68 (±0.38)	–	0.16 (±0.43)	12
G3	Gulf of Mexico	11-June	28.0	32.0	1.64 (±0.26)**	1.40 (±0.43)*	0.44 (±0.10)	0.46 (±0.49)	12
NB	Nueces Bay	5-June	27.9	29.6	1.55 (±0.12)**	1.76 (±0.19)**	0.88 (±0.08)	0.88 (±0.22)	12
NR	Nueces River	11-June	31.1	0.5	–0.07 (±0.29)*	0.09 (±0.46)	–	–0.11 (±0.49)	11
UL	Upper Laguna Madre	27-June	30.0	49.0	–0.12 (±0.08)	0.08 (±0.13)	–	–0.06 (±0.15)	12
LL	Lower Laguna Madre	28-June	33.0	28.2	–0.28 (±0.13)	0.12 (±0.21)	–	–0.07 (±0.23)	12

Experimental results of dilution experiments are shown. The growth rate (μ; day^{-1}), grazing mortality (m; day^{-1}), non-nutrient amended phytoplankton growth (μ_0; day^{-1}), and r^2 values are shown (±standard error)

*$P < 0.05$; **$P < 0.001$

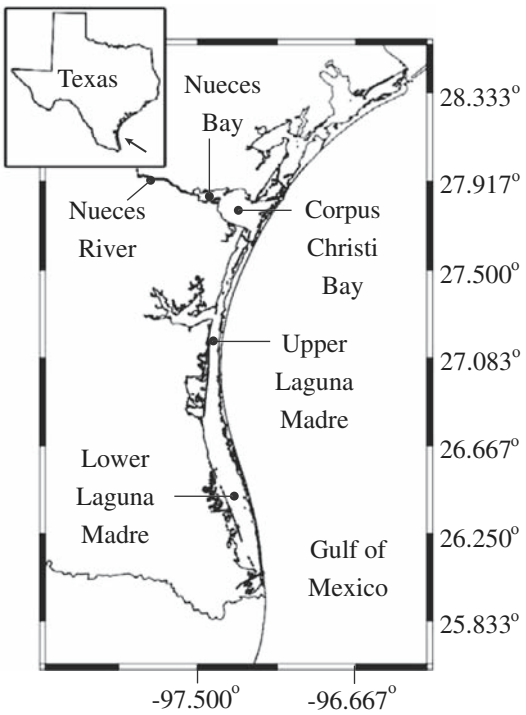

Fig. 1 Map displaying sampling locations of coastal station (generated from National Geophysical Data Center—http://www.rimmer.ngdc.noaa.gov/coast). See Table 1 for sampling identifications and descriptions

experimental incubations started within 5-h of sampling.

In order to remove mesozooplankton, water was screened through a 153-μm Nitex mesh that was rinsed with Nanopure water to remove any collected debris. Roughly between 30 and 50-l of seawater were gravity-filtered sequentially through two Gelman capsules (3.0 and 0.22-μm) connected by silicon tubing and collected into 20-l Nalgene carboys. A series of dilutions were prepared by mixing the 153-μm-screened water (from here on called whole Seawater, WSW) with the 0.22-μm-filtered water (100, 60, 30, 15% WSW; 100, 75, 50, and 25% for stations C1 and G1). The reason for the change in the dilution treatments after the C1 and G1 trials was to incorporate a higher dilution point (i.e. 15% WSW). Treatments were enriched with N (4-μM NH_4Cl for open Gulf and Lower Laguna Madre, 8-μM for all others) while non-amended WSW was incubated as a control (Landry et al. 1995). Treatments additionally amended with P (0.6 and 0.3-μM $NaHPO_4$ to C1 and G1, respectively) did not exhibit phytoplankton growth significantly different from N-amended samples. Therefore, P was not added in further experiments. Triplicates for each dilution and control were placed into clear polycarbonate 1-l Nalgene bottles filled to the mouth to eliminate headspace and tightly sealed.

Prior to incubation, three samples for chlorophyll *a* (CHL) were collected from each treatment. Between 25 – 300-mL of water was filtered through 47-mm diameter 0.2-μm Nuclepore membrane filters. Filters were placed in 14-ml Falcon tubes, filled with 10-ml of 90% acetone, and CHL was extracted at –20 ±2°C in the dark for at least 24-h. CHL was measured with a Turner Designs fluorometer (TD-700) by the non-acidic method (Welschmeyer 1994). Equal aliquots from each dilution replicate were pooled and preserved in Lugol's iodine (5% final concentration) for analysis of MZP. All preserved samples were kept refrigerated (5 ±2°C) and dark until the time of analysis.

All experimental bottles were incubated for 24 h in an incubator tank shaded with blue Plexiglas to reduce ambient light. The incubator was kept within 1°C of the station temperature by a microprocessor controlled, Neslab RTE circulator bath (Jochem et al. 2004). At the end of the incubation, samples for CHL

and organism counts were collected and processed as described above. Net growth rates (r; d^{-1}) of phytoplankton were calculated from the exponential growth equation:

$$r = \frac{1}{t} \ln \frac{P_t}{P_0} \quad (1)$$

where P_t and P_0 are the final and initial CHL concentrations, respectively. A linear regression model was used to plot the best-fit relationship between r and dilution level. Phytoplankton growth (μ) and grazing mortality rates (m) are defined as the *y*-intercept and the negative slope of this relationship, respectively. For comparison, phytoplankton growth (μ_0) was calculated as the sum of net growth rate in control samples without nutrient amendments and the grazing impact from the linear relationship (Landry 1993; Landry et al. 1995). This calculation accounts for the potentially higher phytoplankton growth in nutrient amended treatments.

Microzooplankton counts

In order to quantify the MZP community, between 20 and 100-ml (depending on the plankton concentration, water turbidity, and dilution) of Lugol's preserved water samples were settled in Utermöhl chambers for at least 18-h. After settling, the chamber was scanned on an Olympus phase contrast inverted microscope with transects at 200–400X, for small size and abundant cells. The entire chamber was scanned to quantify larger size and less abundant MZP. Length and width (taken as the longest dimensions) of 3–50 cells per group were estimated using an eyepiece micrometer calibrated against a stage micrometer at 400 – 600X magnification and averaged for each station.

Cell volume was calculated from these parameters by the approximate geometric shape, usually either ellipsoid or conical (Wetzel and Likens 1991). Cell volume was converted to carbon biomass using published relationships for ciliates (Putt and Stoecker 1989) and dinoflagellates (Menden-Deuer and Lessard 2000). Dinoflagellate cell volumes were corrected for shrinkage due to preservatives (Menden-Deuer et al. 2001). Ciliates can also shrink in preservatives (Stoecker et al. 1994) but their volume is already corrected in the regression (Putt and Stoecker 1989). MZP were identified to the lowest possible taxonomic level using literature for ciliates (Small and Lynn 1985; Carey 1992; Lynn and Gilron 1993; Petz 1995) and dinoflagellates (Steidinger and Tangen 1993). Net MZP growth rates (r_z) were calculated from initial and final biomass using Eq. 1.

Analysis

In order to gauge the effect of predator net growth or loss rate (r_z) on measurements of μ and m, we used the geometric mean predator biomass (GMPB) to reassess the experimental outputs (Gallegos et al. 1996):

$$\text{GMPB} = Z_0 \, e^{r_z \, \Delta t/2} \quad (2)$$

where Z_0 is the initial predator biomass concentration. We measured MZP net growth rates in all dilution treatments in this study and defined the "predator" (i.e. the grazer) as either ciliates or total MZP (dinoflagellates and ciliates). These large groupings are necessary for the inclusion of cells present in WSW but absent or depleted in high dilutions. Also, many populations present at low concentrations (i.e. < 100 cells-l^{-1}) can potentially introduce artificially high net rates of growth or mortality and these large categories dampened this effect. Phytoplankton growth is plotted against the GMPB at each dilution normalized to GMPB in 100% water.

MZP were also sorted into size categories based on equivalent spherical diameter (ESD) derived from cell volume. Considering the distribution of cell volumes, we defined three size classes: < 20, 20–40, and >40-µm ESD, each with a similar contribution to total biomass. Typically, cells smaller than 20-µm are classified as nanoplankton. In this study, we included small ciliates (e.g. the choreotrich, *Lohmaniella* sp.) and dinoflagellates (e.g. *Gymnodinium* sp. typically >15-µm) below this arbitrary size cutoff. Rates of initial net phytoplankton productivity were calculated as the product of initial phytoplankton biomass and net growth in each dilution treatment.

Results

Initial CHL in WSW ranged from 0.2 to 46 µg l^{-1} (Fig. 2a) and total initial MZP biomass ranged from 3.6 to 75 µg C l^{-1} (Fig. 2b). The MZP:CHL ratio was lowest at the hypereutrophic NR (0.9) and reached a high of 49 at G2 and LL (Fig. 2c). In four of the nine dilution experiments, the relationship between net phytoplankton growth rate and the dilution gradient was linear (Table 1). Of these four experiments, the grazing rate ranged from 0.56-day^{-1} (C1) to 1.76-day^{-1} (NB). Estimates of μ ranged from 0.3-day^{-1} (C1) to 1.64-day^{-1} (G3). In all cases, $\mu_0 < \mu$ suggesting that all stations were nutrient limited. High concentration of suspended detritus in NB prevented accurate MZP counts; therefore these data are not reported.

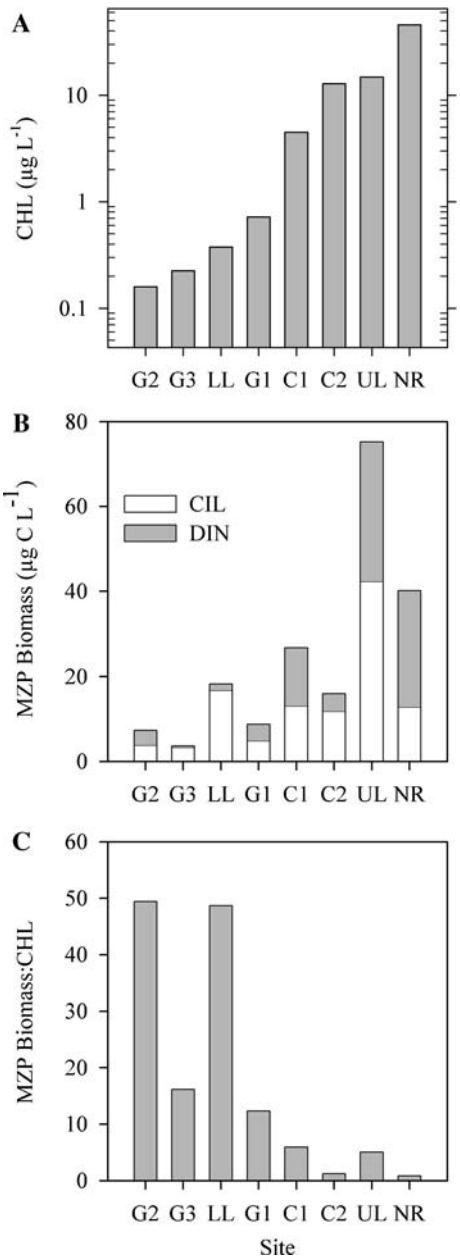

Fig. 2 **a** Initial CHL (abbreviations explained the text) in WSW at sampling locations, ranked from low to high (note the log scales). **b** Initial MZP biomass and contribution from both ciliates (*CIL*) and dinoflagellates (*DIN*). **c** Ratio of initial MZP biomass to phytoplankton biomass at sampling locations

The GMPB-adjusted dilution gradient did not yield μ and m values outside the range of error of nominal dilution estimates (Fig. 3). Likewise, adjusting the nominal dilution values to MZP biomass and growth did not establish a significant linear relationship where none existed without adjustment. This same analysis was performed defining each MZP size class as the dominant herbivore. Adjusting the growth curve to the mean biomass of each of these groups yielded similar results to ciliate and total MZP adjustments (data not shown). The ratios of m/μ were roughly balanced, ranging from 0.9 to 1.9. However, the ratios of m/μ_0 were much greater than balanced, ranging from 2.0 to 3.9 (Fig. 3c), indicating the grazing impact is several times the non-nutrient amended phytoplankton growth rate. The value of m/μ_0 for C1 (although not calculated because $\mu_0 < 0$) could potentially exceed this range.

Changes in MZP abundance occurred in all dilution treatments. However, the net growth rate patterns varied among the sites and different MZP groups. At C1 dinoflagellate abundance increased over time in all dilution treatments, while total ciliates declined (Fig. 4a). The opposite pattern was observed at C2, where total ciliates grew at net rates of ≥1-day^{-1} in some treatments (Fig. 4b). The overall decline in ciliates at C1 was driven mainly by the disappearance of *Strombidinopsis* sp., which composed 78% of the initial ciliate biomass (Fig. 5a). Other subgroups of ciliates such as oligotrichs (20–40-µm ESD, e.g. *Tontonia* sp., and *Strombidium* sp, comprising 14% of initial ciliate biomass) had small but positive net rates of growth and productivity (Fig. 5b). At G1, phytoplankton declined at roughly the same rate in all dilution treatments. Although MZP declined in all dilution treatments, the rate of decline was greatest in the two highest dilutions (Fig. 6a). The growth rate of ciliates at G2 was >1-day^{-1} in 100% WSW but the MZP growth rate in other dilution treatments was either low or negative (Fig. 6b). MZP displayed uneven growth across the dilution treatment in G3, the only Gulf of Mexico experiment with significant linear measurements of μ and m. Here, MZP grew in 100% WSW and declined precipitously in highly diluted water (Fig. 6c)

Phytoplankton did not show a clear response to dilution and declined in all treatments at both Laguna Madre locations (Fig. 7). In UL, high initial ciliate biomass was mainly due to the hypotrich ciliate *Euplotes gracilis*. However, ciliates declined in 100% WSW and did not grow in any dilution treatment (Fig. 7a). In contrast, dinoflagellates increased significantly in 100% WSW, decreasing with the dilution gradient. Water column CHL at LL was low and comparable to open Gulf of Mexico locations. The MZP community at this site was composed of the mixotrophic ciliates *Myrionecta rubra*, *Tontonia* sp., and *Laboea strobila*, which increased slightly in 100% WSW, declined at intermediate dilutions, and showed no changes in high dilutions (Fig. 7b).

In NR, high MZP net growth rates (e.g. >2-day^{-1} for ciliates in 60% WSW) and rates of productivity were observed in all dilution treatments (Fig. 8). After removing an apparent outlier in 15% WSW, there was

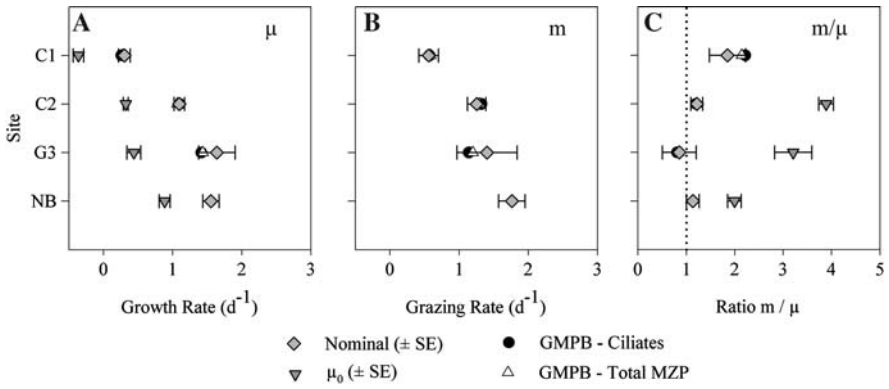

Fig. 3 **a** Phytoplankton growth rate (μ; day^{-1}), **b** grazing mortality rate (m; day^{-1}), and **c** the fraction of primary production grazed (m/μ). Values of μ and m adjusted to the Geometric Mean Predator Biomass (GMPB) of both ciliates and total MZP. Also shown are phytoplankton growth rates from incubations without nutrient amendments (μ_0). Stations are identified in Table 1

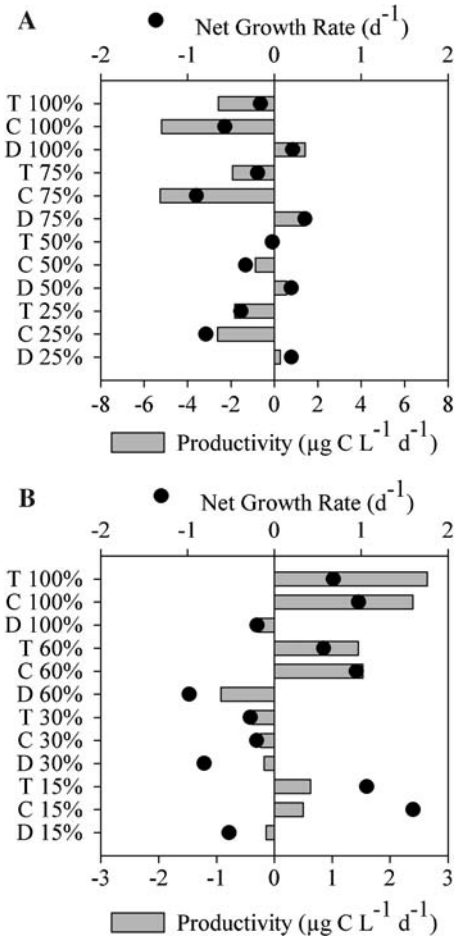

Fig. 4 Corpus Christi Bay net rates of MZP growth (day^{-1}) and initial productivity (μg C L^{-1} day^{-1}) for **a** C1 and **b** C2. MZP rates are displayed as Total MZP (*T*), Ciliates (*C*), and Dinoflagellates (*D*)

a significant difference between phytoplankton net growth rates in WSW and highly diluted treatments in NR (–0.34 and 0.10-day^{-1}, respectively, ANOVA, $p < 0.01$), indicating phytoplankton were impacted by grazers.

In some experiments, net rates of MZP growth and productivity for varied among the three size classes in 100% WSW (Fig. 9). For example, at G2 both large MZP (>40-μm) and small MZP (< 20-μm) increased while the intermediate size class (20–40-μm) declined. At UL, the opposite trend occurred; large and small MZP declined while the intermediate size class increased. Both the small and intermediate size classes increased concurrently with a decrease in the large MZP size class at C1. All size classes increased in NR and decreased in G1 (Fig. 9).

Discussion

Phytoplankton concentrations spanned two orders of magnitude at the stations examined. In this study, phytoplankton in situ growth rate was estimated as both the y-intercept of the nutrient amended phytoplankton growth curve and the sum of the grazing rate (i.e. the slope) and net phytoplankton growth in undiluted control incubations. The latter method, in theory, provides a more accurate estimate of phytoplankton in situ growth rate (Landry 1993). MZP grazing rates exceeded the in situ phytoplankton growth rates. However, this high grazing impact may have been partially caused by a decrease in top-down control of MZP by crustacean mesozooplankton (e.g. Calbet 2001), which were reduced or eliminated by screening the water. Higher values of phytoplankton net growth in the nutrient-amended samples at both coastal and offshore stations may be indicative of phytoplankton N-limitation. In most experiments in this study, MZP biomass increased during the incuba-

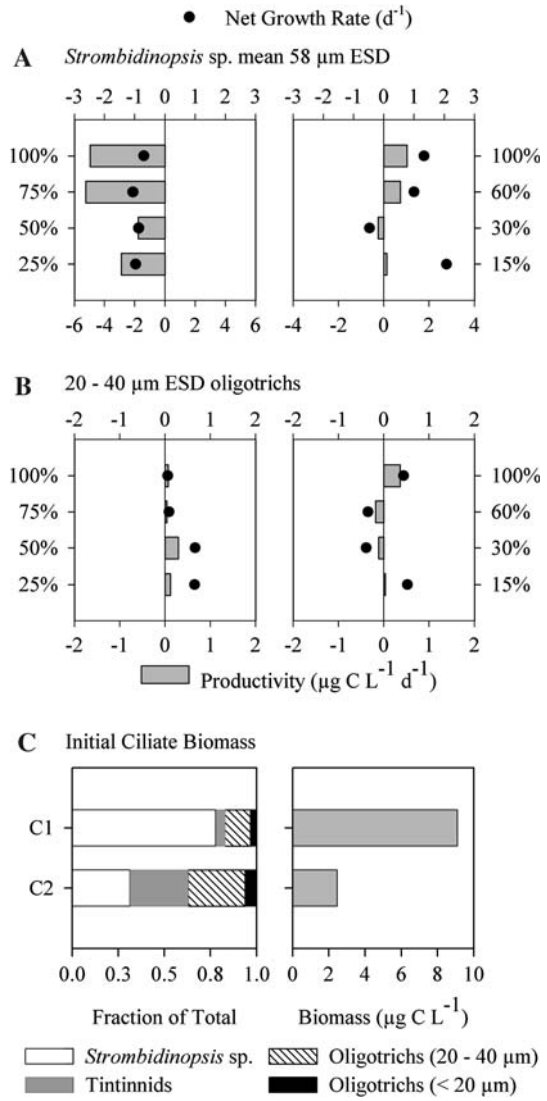

Fig. 5 Net rates of growth and productivity for **a** *Strombidinopsis* sp. and **b** 20–40 µm ESD oligotrichs at both Corpus Christi Bay experiments; C1 (*left panels*) and C2 (*right panels*)

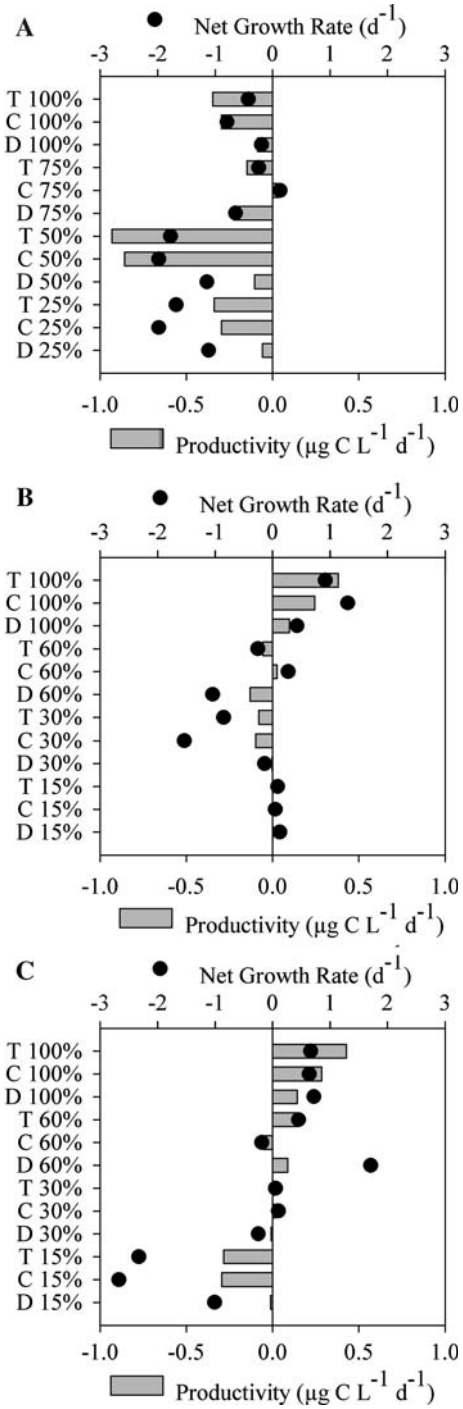

Fig. 6 Gulf of Mexico net rates of MZP growth (day^{-1}) and initial productivity (µg C L^{-1} day^{-1}) for **a** G1, **b** G2 and **c** G3. MZP rates are displayed as Total MZP (*T*), Ciliates (*C*), and Dinoflagellates (*D*)

tions indicating that grazer dynamics are an inherent component of dilution experiments. In many cases, the net rates of MZP growth exceeded those of phytoplankton. However, there was no clear trend found between the trophic status of the system and the patterns of MZP net growth rates in the dilution treatments. For example, the MZP response to the dilution gradient varied among experiments from Corpus Christi Bay (C1 and C2) and the open Gulf of Mexico (G1, G2, and G3). This suggests that the dominant trophic pathways in the microbial food web can vary both spatially and temporally.

Although MZP biomass changes over the incubation were large in many cases, these changes were not large enough to offset the initial dilution gradient. Overestimation of MZP grazing can occur when grazer abundance increases in undiluted treatments and decreases in high dilutions (Dolan et al. 2000). We observed this MZP response at G3 and found that the GMPB-adjusted μ and m were slightly lower but not

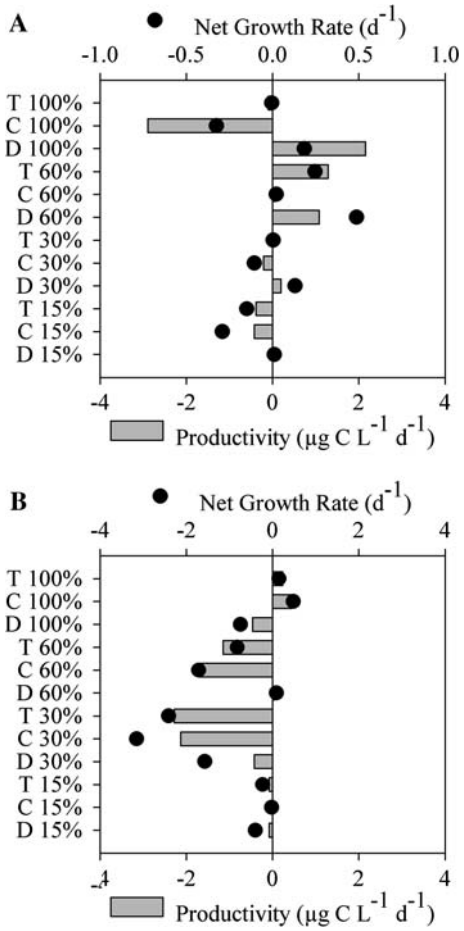

Fig. 7 Laguna Madre net rates of MZP growth (day^{-1}) and initial productivity (µg C L^{-1} day^{-1}) for **a** UL and **b** LL. MZP rates are displayed as Total MZP (*T*), Ciliates (*C*), and Dinoflagellates (*D*)

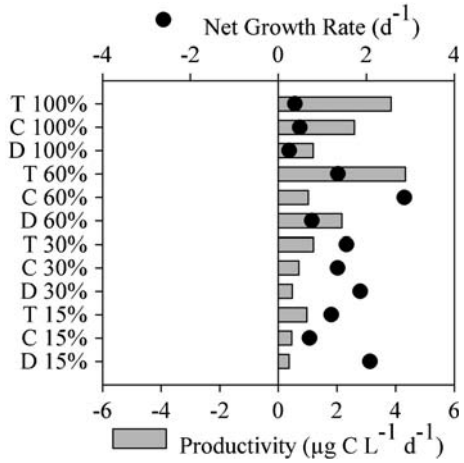

Fig. 8 Nueces River net rates of MZP growth (day^{-1}) and initial productivity (µg C L^{-1} day^{-1}). MZP rates are displayed as Total MZP (*T*), Ciliates (*C*), and Dinoflagellates (*D*)

significantly different from unadjusted μ and m. Adjusting phytoplankton growth to GMPB assumes that the MZP category defined as predator is the major herbivore. MZP communities consist of multiple populations each characterized by specific trophic habits and adaptations. For example, oligotrich ciliates are omnivores with flexibility in prey selection (Samuelsson and Andersson 2003). They can switch between raptorial and suspension feeding depending on the availability of nanoplankton or picoplankton prey, respectively (Fenchel 1987). Summing the biomass and calculating growth rates of major MZP categories (e.g. ciliates or MZP < 20-µm) can conceal fluctuations in smaller taxonomic groups or trophic guilds. For instance, the decline in the abundant *Strombidinopsis* sp. masked the increase of other ciliates at C1. Also, broad categories based on cell size obscure more relevant groupings based on prey preference (Turner and Roff 1993).

Many MZP components (particularly dinoflagellates) may be mixotrophic and it is difficult to ascertain what nutrition mode they use at any given time. It also should be noted that ''microzooplankton'' herbivory is in reality a microbial community process and includes nanozooplankton, picoeukaryotes, and viruses (Nagasaki et al. 2005). Ciliates alone could not account for the overall grazing rate estimates in some dilution experiments without assigning per-capita clearance rates outside of realistic ranges (Dolan and McKeon 2005). In this study we did not consider heterotrophic nanoflagellates, which can be important herbivores (Archer et al. 1996). Other groups of pigmented flagellates also can resort to phagotrophy when nutrient-limited (e.g. Arenovski et al. 1995). The nitrogen limited conditions in Texas coastal waters (McCarthy et al. submitted) should be conducive to mixotrophic herbivory.

In general, the relative abundance of MZP was greatest in the oligotrophic stations (G2, G3, LL). An exception to this occurred a C1, where the relatively high MZP abundance may explain the high rates of grazing relative to phytoplankton growth. Additionally, low μ at C1 may be explained by the lower phytoplankton gross growth rates in the colder spring water compared with the higher temperatures occurring in summer (Suzuki and Takahashi 1995). The general trend of increasing μ and m with increasing temperature has been documented previously, even in systems where these rates were unbalanced (Murrell et al. 2002). Low temperatures also limit ciliate growth (Müller and Geller 1993) and the lower concentration of grazers can decrease grazing rate by limiting the encounter rate with phytoplankton. At G1, phyto-

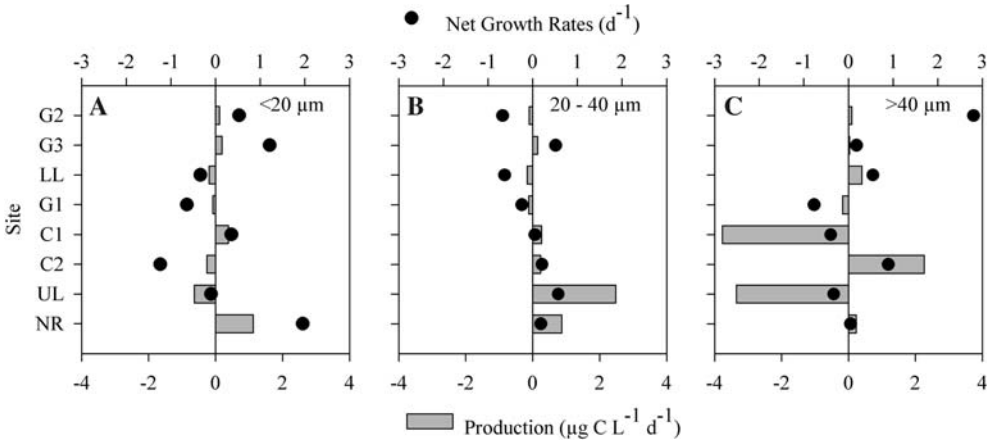

Fig. 9 Net rates of MZP growth (day^{-1}) and initial productivity ($\mu g\ C\ L^{-1}\ day^{-1}$) in 100% WSW in all dilution experiments. Total MZP are subdivided according to the average equivalent spherical diameter (*ESD*) grouped into cells **a** <20 µm ESD, **b** 20–40 ESD and **c** > 40 µm ESD

plankton declined in all treatments. Approximately 25% of the phytoplankton biomass was in the diatom size range (i.e. >5-µm) at this station. The lack of herbivory could be attributed to MZP selective predation on heterotrophic nanoflagellates (Fonda Umani and Beran 2003).

Nonlinear or non-significant linear phytoplankton responses to dilution gradients are not uncommon (Dolan et al. 2000) nor are outcomes such as positive growth slopes (e.g. Juhl and Murrell 2005). As with any experimental manipulations, bottle effects are certainly introduced. Problems such as the shading effect of turbid waters (Murrell and Hollibaugh 1998) and contamination (Landry 1993) have been suggested as possible sources of errors in dilution experiments. Although we cannot completely rule out these sources of error in our experiments, considering the MZP dynamics in each nonlinear or non-significant dilution experiment in detail may reveal some interesting patterns.

Upper Laguna Madre has been a site of a brown tide bloom since 1990 (Buskey and Hyatt 1995) and high concentrations of the brown tide alga were observed during this experiment. Exopolymeric secretions (EPS) produced by this alga are known to deter MZP grazing (Liu and Buskey 2000). In addition, EPS likely promotes bacterial production. Hypotrich ciliates (like the abundant *E. gracilis*) are capable of grazing bacteria (Wilks and Sleigh 1998) and at the same time can be predators on heterotrophic nanoplankton. Therefore, the microbial loop (where dissolved organic carbon is transferred to higher trophic levels via a number of trophic links involving heterotrophic protests—Azam et al. 1983) may be the dominant energy flow at this station.

Although LL had a comparatively low phytoplankton biomass, like UL, phytoplankton did not grow in any treatment. At LL, seagrass production contributes substantially to overall ecosystem net primary production (Kaldy et al. 2002). Low primary production in the water column potentially limits the biomass of larger grazers, resulting in a food web dominated by bacteria and nanoflagellates (Samuelsson et al. 2002). Alternatively, MZP at this site may be adapted to the lack of edible phytoplankton and therefore employ mixotrophy as indicated by the significant relative abundance of *M. rubra*. These examples illustrate that a temporary mismatch between grazers and phytoplankton composition may determine the outcome of herbivory experiments.

The lowest MZP:CHL ratio was observed in NR, where the absolute biomass of MZP was lower than two other estuarine locations with substantially lower phytoplankton abundance (UL and C1). The low initial biomass of MZP at this location may be due to top-down control from zooplankton preying on large MZP, which can lead to a reduction in grazing rates and the initiation of algal blooms (Christaki and Van Wambeke 1995; Froneman 2002; Irigoien et al. 2005). Additionally, the high biomass of filamentous cyanobacteria in NR may have deterred MZP grazing (Sellner et al. 1993). MZP exhibited high net positive rates of growth and production in all dilution experiments. Removing mesozooplankton is generally part of dilution experiment methodology, and removal of these grazers may have translated into differential responses in contrasting food webs. It is possible that the initial 153-µm screening had a stronger impact on MZP-phytoplankton interactions in these productive systems than in offshore waters. While mesozooplankton predation on MZP may be disproportionately high in oligotrophic waters (Calbet and Saiz 2005), the removal or reduction of mesozooplankton may not cascade down to picoplanktonic producers in

these open ocean environments (Calbet and Landry 1999).

Changes in MZP abundance or grazing activity have been cited as a potential explanation for nonlinear or nonsignificant dilution experiments (Gallegos 1989; Dolan et al. 2000). Modified experimental techniques have incorporated both changes in MZP abundance (Landry 1993; Gallegos et al. 1996) and the relative uptake of tracer cells (Landry et al. 1995) as verification that the nominal dilution gradient represents the grazing gradient. Changes in grazer abundance can also provide clues as to trophic interactions within MZP. Factors such as the number of trophic links and the degree of omnivory can affect overall phytoplankton community structure and microbial food web efficiency (Reckermann and Veldhuis 1997; Samuelsson and Andersson 2003).

The results of this study suggest that microzooplankton growth is an inherent part of dilution experiments and must be taken into account. Although this study confirms that dilution is a valid tool for assessing MZP herbivory impacts, it also stresses the need to examine the composition and dynamics of both prey and grazer populations. This is particularly important in coastal waters, where phytoplankton can include various taxonomic and size groups. Under such conditions, changes in total chlorophyll may not reveal intense trophic interactions between MZP and subset of the phytoplankton community. Internal trophic interactions among MZP can also impact the dominant herbivores, causing a non-linear grazing response as trophic links are severed with increasing dilution. The complexity of the coastal microbial food web can make interpreting the results of grazing experiments less than straightforward. It is important not to understate this complexity as the simplifications commonly employed (e.g. size class, taxa) do not approach the actual food web complexity (Pomeroy 2001). At the same time, dilution can help gain valuable insights into this intertwined system by gradually unraveling trophic links between protists and their prey.

Increased information about the feeding and growth patterns of predominant MZP taxa can provide insight in the function and efficiency of the microbial food web. Examining the response of an entire microbial system from viruses to MZP to dilution may expose interesting patterns. The application of microscopy and flow-cytometry can enhance dilution experiments (Fahnenstiel et al. 1995; Landry et al. 1995; Jochem et al. 2004). Further work in plotting interactions and constraining the regulatory forces in the microbial food web promises a deeper understanding of energy flow in aquatic systems.

Declaration

The authors declare all work presented here was conducted in compliance of the laws of the United States of America.

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