Estuaries and Coasts
DOI 10.1007/s12237-010-9336-8

Microzooplankton Grazing in Green Water—Results from Two Contrasting Estuaries

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Received: 14 October 2009 / Revised: 12 August 2010 / Accepted: 15 August 2010

Abstract We measured seasonal variations in microzooplankton grazing in Long Island Sound (LIS) and San Francisco Bay (SFB). There was consistent evidence of nutrient limitation in LIS, but not SFB. We found higher chlorophyll *a* concentrations in LIS compared with SFB. In spite of differences in phytoplankton, there were no differences in microzooplankton abundance (summer: LIS, $12.4\pm1.8\times10^3$ indiv.L^{-1}; SFB, $14.1\pm3.0\times10^3$ indiv. L^{-1}), biomass (summer: LIS, 30.4 ± 5.0 $\mu g C L^{-1}$; SFB, 26.3 ± 5.9 $\mu g C L^{-1}$), or grazing rates (summer: LIS, 0.66 ± 0.19 day^{-1}; SFB, 0.65 ± 0.18 day^{-1}) between the two estuaries. In common with many other investigators, we found many instances of saturated as well as insignificant grazing. We suggest that saturation in some cases may result from high particle loads in turbid estuarine systems and that insignificant grazing may result from extreme saturation of the grazing response due to the need to process non-food particles.

Keywords Dilution · Zooplankton · Phytoplankton growth · San Francisco Bay · Long Island Sound

Introduction

Microzooplankton (20–200-μm hetero- or mixotrophs) provide one of the major controls on phytoplankton productivity in diverse aquatic environments, from open ocean to estuarine systems (Calbet and Landry 2004; Juhl and Murrell 2005; McManus et al. 2007). They typically graze between 60% and 75% of primary productivity and provide an important link between microbial plankton and higher trophic levels (Calbet and Landry 2004; Calbet and Saiz 2005).

There is only limited information available on the trophic role of microzooplankton in estuarine systems. Calbet and Landry (2004) reviewed microzooplankton grazing rates from 38 oceanic studies, 19 coastal studies, and only 14 estuarine studies. Only two of the estuarine studies were from Pacific estuaries. Eutrophic systems such as estuaries present special problems in measuring microzooplankton grazing. For example, some studies of microzooplankton grazing indicate that phytoplankton abundance may saturate the microzooplankton grazing response more often in estuaries than in oceanic systems (e.g., Gallegos 1989; McManus and Ederington-Cantrell 1992; Gallegos and Jordan 1997; Redden et al. 2002). It is also common for experiments in estuaries to result in low or insignificant estimates of grazing, even when phytoplankton biomass is high (Kamiyama 1994; Murrell and Hollibaugh 1998).

We used the dilution method (Landry and Hassett 1982) to determine the rate of microzooplankton grazing on phytoplankton in two estuaries: Long Island Sound (LIS) and San Francisco Bay (SFB; Fig. 1). The LIS experiments were part of a larger project, the Long Island Sound Integrated Coastal Observing System (LISICOS), whose goal was to study organic matter cycling and processes causing seasonal hypoxia in the western Sound. The experiments in SFB were part of a larger project aimed at understanding food web linkages in the low salinity zone of the SFB leading to a decline of the indigenous delta smelt (*Hypomesus transpacificus*).

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Published online: 09 September 2010

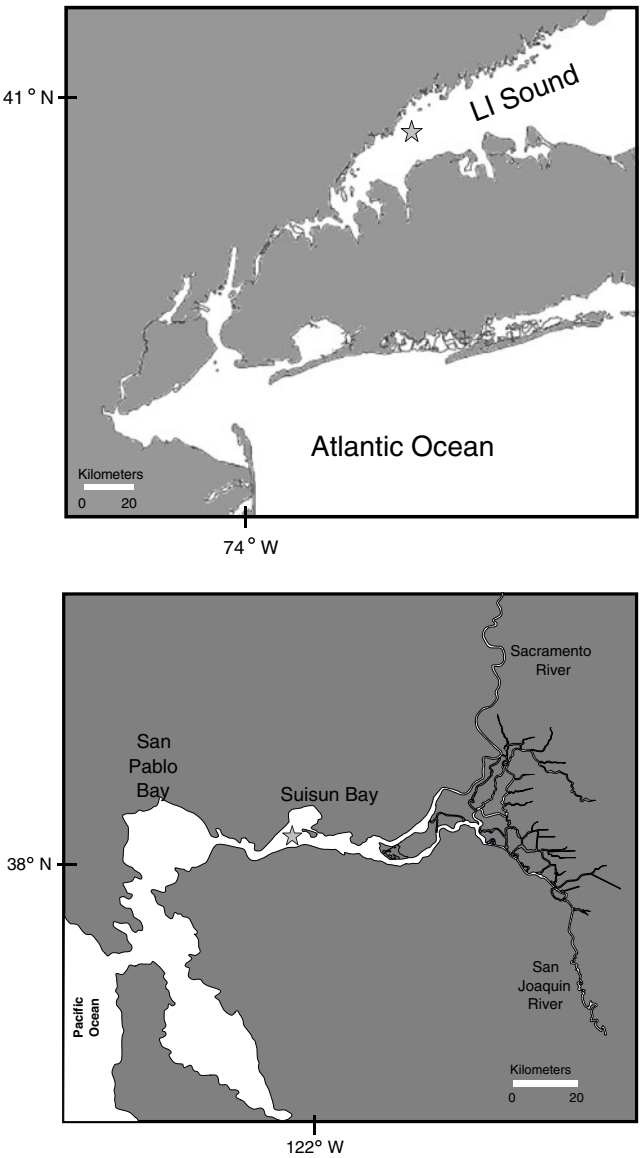

Fig. 1 Experiments in LIS (*upper*) were performed at a cluster of stations in the middle of the western Sound, as indicated by the *star*. In SFB (*lower*), we sampled where the salinity was 2, usually in Suisun Bay

The Long Island Sound region has a temperate climate, with cold winters and hot summers. Precipitation is spread evenly throughout the year, but freshwater input to LIS is increased during the spring snowmelt period. Our work focused on western Long Island Sound, an area surrounded by urban development and numerous sources of pollution. Exchange of water with the open coast is limited to the East River and New York Harbor (Gay et al. 2004; Gay and O'Donnell 2009), minimizing the dispersal of high nutrient loads. Eutrophication in western LIS results in high phytoplankton biomass, which promotes seasonal hypoxia, a phenomenon that is regularly observed in bottom waters (Welsh and Eller 1991; DeJonge et al. 1994; Anderson and Taylor 2001; Goebel et al. 2006). Copepods in the genus *Acartia* dominate the mesozooplankton, with *Acartia tonsa* more abundant in summer and fall, and *Acartia hudsonica* more abundant in winter and spring (Deevey 1956).

The San Francisco Bay region is characterized by a Mediterranean climate, with two distinct seasons differing mostly in their amount of precipitation. Our observations in the San Francisco Bay system were focused on the Sacramento–San Joaquin River Delta outflow, as this area provides critical habitat to the delta smelt. This region is hydrologically complex and highly impacted by activities on the surrounding watersheds as well as in the Delta itself. A pelagic organism decline has been noted in the SFB system overall (Cloern 2007). In contrast to many estuarine systems, including LIS, although nutrient concentrations are high, phytoplankton blooms are rare in SFB. This has been attributed to several causes including high turbidity, high ammonium concentrations, and very dense populations of the introduced clam *Corbula amurensis* (Alpine and Cloern 1988; Alpine and Cloern 1992; Thompson 2000; Wilkerson et al. 2006; Dugdale et al. 2007). The mesozooplankton is dominated by introduced copepods, including species of *Limnoithona*, *Pseudodiaptomus*, *Acartiella*, and *Eurytemora* (Kimmerer 2004; Bouley and Kimmerer 2006).

Sampling in these two sites provided us with the opportunity to compare the roles of microzooplankton in estuaries having widely differing conditions of salinity, climate, nutrients, and mesozooplankton (predator) assemblage. We expected that differences in phytoplankton abundance and composition associated with these factors would affect the roles of microzooplankton in the two systems.

Methods

Sampling We made a total of 44 estimates of microzooplankton grazing, 25 in LIS and 19 in SFB, using the dilution technique of Landry and Hassett (1982; Table 1). The method was applied in a similar way in both systems (see details below), except for the addition of nutrients. We never added nutrients in the SFB experiments because nutrients in this system are always high (dissolved inorganic nitrogen (DIN) >15 μM, PO_4^{3-} >1.5 μM; Wilkerson et al. 2006) and because previous work showed that it takes several days to deplete nutrients during incubations of undiluted water (Dugdale et al. 2007). In addition, previous dilution experiments in SFB did not amend with nutrients and found little change in nutrient concentrations during incubations (Murrell and Hollibaugh 1998). On the other hand, phytoplankton biomass is generally higher, and in situ nutrient concentrations are sometimes low in LIS, especially

Table 1 Experimental conditions

Date	Estuary	No. of experiments	Size fractionated	Flow cytometry	Nutrients added
Mar 2005	LIS	5	N	N	Y
Jul 2005	LIS	12	Whole, <10 μm	7 experiments	Y
Mar 2006[a]	LIS	4	Whole	N	Y
Jun 2006	LIS	4	Whole, <10 μm	N	Y
Mar/Apr/May 2006	SFB	6	N	N	N
Jul/Aug 2006	SFB	4	N	N	N
Apr 2007[b]	SFB	3	N	N	N
Jul 2007[b]	SFB	3	N	N	N
Jul 2008	SFB	3	Whole, <5 μm (1 experiment)	N	N

[a] Size fractionated experiments were done, but results were not replicated well within treatments, so the data were not analyzed further.

[b] Experiments were conducted using the 2-point method.

in summer (DIN <5 μM, PO_4^{3-}<2 μM; Anderson and Taylor 2001; Capriulo et al. 2002), so we always included nutrient and non-nutrient treatments there.

Sampling in LIS was conducted during March and July 2005 and March and June 2006. Salinity of experimental water ranged from 25 to 26. Water temperature during the March sampling was 1°C to 2°C, while in the summer, it was 20°C to 24°C in 2005 and 18°C in 2006. Forty liters of water were collected at either sunrise or sunset by Niskin bottles on a rosette. Samples were collected from the surface (1 m) or from the shallow chlorophyll maximum when present (typically between 3 and 6 m in the 15–20-m water column). Samples were incubated shipboard in an insulated incubator equipped with a rotating wheel to keep particles from settling (Crocker and Gotschalk 1997). Temperature was maintained with flowing surface seawater. One layer of neutral density screening was used to maintain light at a level equivalent to 50% surface intensity. Nevertheless, it is not likely that the light field experienced by phytoplankton in either the SFB or LIS experiments was as low as in situ levels. This is a problem common to incubation experiments in all turbulent, turbid systems where phytoplankton are mixed vertically across a range of light levels throughout the day (Falkowski and Wirick 1981; McManus 1995). In most of the LIS experiments, we made size-fractionated estimates of phytoplankton growth (separate whole and <10-μm chlorophyll measurements from the same incubation bottles), and in seven experiments from LIS in summer of 2005, we also measured picophytoplankton using flow cytometry. Fractionated chlorophyll samples from March 2006 (four experiments) all showed very poor reproducibility within treatments and were omitted from further analysis.

In SFB, samples were collected in March, April, May, July, and August 2006, April and July 2007, and July 2008. Sampling was typically conducted at a salinity of 2, so sampling locations varied up and downstream over time, depending on freshwater flow. Nearly all were in Suisun Bay, ca. 35–40 km downstream of the Delta proper. Suisun Bay is shallow, with about 30% of its area being 2 m or less in depth, with a narrow navigation channel (ca. 15 m; Foxgrover et al. 2007). The water temperature was lowest in March and highest in July and ranged from 10 to 23°C. Surface water was collected from a small vessel by submerging an inverted carboy with open spigot to allow air to escape while filling. Samples were typically collected before 1000 h and were transported in the dark to the laboratory at the Romberg Tiburon Center (RTC) in Tiburon, CA. Bottles were incubated along the seawall of the RTC in the same incubator setup as the LIS experiments.

Experimental setup Except for six experiments from SFB that used a two-point method (April and July 2007; see below), the basic dilution procedure was the same for all experiments. All water from a site was combined in a 60-L container, then siphoned through a submerged 200-μm mesh to gently exclude larger grazers. Particle-free water was prepared by gravity filtration through a 0.2-μm capsule filter or a 142-mm diameter glass fiber filter (Gelman A/E). A series of 1-L polycarbonate bottles with 100%, 50%, 25%, and 10% treatments was prepared by combining seawater with the appropriate volume of particle-free water. We added inorganic nutrients to all diluted treatments in the LIS experiments to a level 1% of Guillard's F/2 with Si (i.e., F/200) using Sigma-concentrated medium. For the undiluted treatments, triplicate nutrient and non-nutrient bottles were included to control for possible nutrient stimulation of growth in the dilution series (Andersen et al. 1991; Landry 1993). The bottles were sealed without air using Parafilm to prevent bubbles from forming in the headspace.

Initial and final (24 h) chlorophyll *a* samples were collected on Whatman GFF filters. Volumes filtered ranged from 100 to 500 mL, depending on treatment. We also

filtered for smaller chlorophyll *a* size fractions for some experiments (Table 1). For these, chlorophyll was measured in an additional set of samples that had been passed through a 10- (LIS) or 5-μm (SFB) mesh (initial water samples and from each bottle at the end of the incubations). Filters were immediately frozen and later analyzed by fluorometry (Parsons et al. 1984).

Initial samples of undiluted water were preserved in 5% acid Lugol's solution for microzooplankton abundance counts. Either 100 (LIS) or 50 mL (SFB) subsamples were settled down to 5 mL, transferred to tissue culture well plates, resettled, and counted on an inverted microscope. A minimum of 200 cells per sample was counted. Two-dimensional shapes and linear dimensions were recorded for biovolume calculations, except for copepod nauplii, for which length–weight regressions from the literature were used to calculate biomass (Uye 1991; Mauchline 1998). For non-tintinnid ciliates, a factor of 0.19 $pgC\,\mu m^{-3}$ was used to convert biovolume to carbon mass (Putt and Stoecker 1989). For tintinnids, we measured lorica volume and used a conversion factor of 0.072 $pgC\,\mu m^{-3}$. This is equal to the conversion factor 0.053 $pgC\,\mu m^{-3}$ for tintinnids measured for formaldehyde-preserved samples (Verity and Langdon 1984), increased by 35% to account for the greater shrinkage with Lugol's preservation (Putt and Stoecker 1989). The smallest ciliates (10–15 μm) were enumerated and sized, but nanoflagellates were not. For dinoflagellates, we used a factor of 0.14 $pgC\,\mu m^{-3}$ to convert biovolume to carbon mass (Lessard 1991). With Lugol's preservation, it is not possible to discriminate between heterotrophs and autotrophs, so dinoflagellate data are reported separately from the microzooplankton. Our microzooplankton counts, which focused on ciliates and copepod nauplii, thus provide a minimum estimate of microzooplankton abundance. We regularly observed the ciliate *Myrionecta rubra* in both systems but did not include it in calculations of microzooplankton biomass or abundance because it appears to function mostly as an autotroph (Dolan et al. 2000).

For seven experiments from LIS in July 2005, phytoplankton were analyzed by flow cytometry on a BD BioSciences FACScan flow cytometer. Using the forward scatter and two fluorescence channels, we binned autofluorescent particles into three categories: <2-μm phycoerythrin (PE)-containing (i.e., *Synechococcus*-like), <2-μm non-PE, and >2 μm. The >2-μm category effectively ranged from 2 to 10 μm because larger cells were extremely rare at the volumes seen by the instrument. Cell concentrations and sizes were calibrated with 2-μm fluorescent latex beads and cultured phytoplankton of known size.

Analysis In the dilution method, phytoplankton apparent growth rate (k, the net result of growth and mortality) is measured in unaltered sample water and in samples diluted with filtered seawater. Intrinsic phytoplankton growth rate (μ, independent of mortality) and microzooplankton grazing rate (g) can be estimated in various ways from apparent growth in the various dilution treatments, as discussed in a number of papers (e.g., Landry 1993; Gallegos 1989; Redden et al. 2002). Productive waters such as estuaries can provide challenges in interpreting dilution experiments due to the occurrence of saturated grazing and potential nutrient depletion due to high biomass; a number of methods have been proposed to deal with these complications.

To analyze our experiments, we first examined the results to detect significant grazing, saturation, and nutrient effects. Grazing rates were calculated for experiments in which there was either a significant difference (t test) between undiluted and most diluted treatments or a significant regression among all treatments. Saturation was determined by lack of significant difference between whole and 50% dilute treatments (t test). Nutrient effects (LIS only) were evaluated by t test between nutrient and no-nutrient treatments.

When nutrient and saturation effects were not significant, we determined g and μ as slope and intercept, respectively, of a line fit to all treatments as in the original procedure of Landry and Hassett (1982). When nutrient effects were significant, we calculated g and μ according to Landry (1993), and we accounted for saturation effects as in Redden et al. (2002).

In April and July 2007, we evaluated along-estuary variations in grazing in SFB by conducting experiments at three stations with a range of salinities (0.5, 2, and 5) using a two-point method (quadruplicate undiluted and 95% filtered seawater treatments only; Murrell et al. 2002; Strom and Fredrickson 2008). By eliminating the intermediate dilution treatments, we were able to set up and break down experiments at different salinities almost simultaneously. This version of the method preserves the basic assumption that net growth in the undiluted treatment represents growth minus grazing. Because we used a very high dilution (95% filtered seawater) for the second treatment, we assumed that its net growth could be used as an approximation of intrinsic growth, or μ. Grazing is thus the difference between the treatments.

Results

Long Island Sound In March 2005, during the annual spring diatom bloom, three out of five dilution experiments showed significant grazing rates (Table 2), ranging from 0.2 to 0.8 day^{-1}. One experiment showed evidence of saturation. In contrast, of the four LIS dilution experiments run in March 2006, only one showed a significant grazing effect,

Table 2 Results from dilution experiments in Long Island Sound

Date	Experiment	Size fraction	Saturated	Chl *a* ($\mu g L^{-1}$)	Microzooplankton ($\mu g C L^{-1}$)	*g* (day^{-1})	μ (day^{-1})
Mar 2005	1	Whole	No	7.22	9.54	0.23	0.35
	2	Whole	NA	13.05	11.12	0	0.05
	3	Whole	NA	6.51	7.64	0	0.03
	4	Whole	Yes	9.94	6.43	0.83	0.24
	5	Whole	No	14.73	7.90	0.25	0.44
Jul 2005	6	Whole	NA	21.30	42.42	0	0.47
	6	<10	No	8.45		1.47	0.92
	7	Whole	NA	17.56	33.74	0	−0.81
	7	<10	No	9.01		0.93	0.30
	8	Whole	Yes	16.65	55.20	2.83	2.57
	8	<10	NA	8.52		0	−0.31
	9	Whole	Yes	30.51	33.3	0.65	0.02
	9	<10	Yes	13.21		1.42	0.97
	10	Whole	Yes	28.01	3.30	1.05	0.45
	10	<10	NA	12.07		0	−0.74
	11	Whole	No	18.33	10.57	0.60	0.41
	11	<10	No	8.21		1.20	0.90
	12	Whole	No	31.21	75.71	0.87	0.37
	12	<10	No	10.28		2.38	2.21
	13	Whole	NA	18.1	31.79	0	0.46
	13	<10	No	5.62		0.37	0.26
	14	Whole	No	22.24	27.04	1.22	0.41
	14	<10	NA	6.91		0	−0.23
	15	Whole	No	36.38	13.49	1.61	0.99
	15	<10	No	6.22		2.19	2.56
	16	Whole	NA	8.51	24.45	0	−0.22
	16	<10	NA	4.80		0	0.40
	17	Whole	NA	16.36	24.95	0	−0.62
	17	<10	No	5.66		0.71	0.48
Mar 2006	18	Whole	No	5.69	ND	0.88	1.13
	19	Whole	NA	9.02	2.32	0	−0.18
	20	Whole	NA	5.30	5.01	0	0.01
	21	Whole	NA	9.38	2.73	0	0.02
Jun 2006	22	Whole	Yes	7.98	56.81	0.52	0.52
	22	<10	Yes	2.94		1.32	1.39
	23	Whole	Yes	9.44	13.67	0.72	1.31
	23	<10	NA	3.24		0	0.69
	24	Whole	No	6.98	9.99	0	1.02
	24	<10	NA	3.13		0	0.61
	25	Whole	Yes	8.71	29.42	0.55	−0.10
	25	<10	No	2.89		1.53	0.80

Chlorophyll *a* and microzooplankton refer to values at the start of experiments.

NA not applicable (grazing not significant), *ND* no data.

with a grazing rate of 0.9 day^{-1} (Table 2). Phytoplankton growth ranged from 0.0 to 0.4 day^{-1} in 2005 and −0.2 to 1.1 day^{-1}in 2006. Chlorophyll *a* concentrations were similar in both years, ranging from 6.5 to 14.7 $\mu g L^{-1}$ and 5.3 to 9.4 $\mu g L^{-1}$ in 2005 and 2006, respectively, while microzooplankton biomass was somewhat higher in 2005 (6.4 to 11.1 $\mu g C L^{-1}$) compared to 2006 (2.3 to 5.0 $\mu g C L^{-1}$). Based on the minimal response to nutrient additions

(Fig. 2), nutrient limitation was not apparent in either year. Dinoflagellate abundance was relatively low in both winter sampling periods (Table 3), averaging 8.4 μgCL^{-1} in 2005 and 0.9 μgCL^{-1} in 2006.

In July 2005 and June 2006, we conducted dilution experiments with both whole water and <10-μm size fractions. In 2005, seven of 12 whole water experiments showed significant grazing, and of these, three were saturated (Table 2). Similarly, in 2006, three of four experiments showed significant grazing, and all of these indicated saturated grazing (Table 2). For the whole water experiments, grazing rates were up to 2.8 day^{-1} in 2005 and 0.7 in 2006. For the <10-μm fraction, eight of the 2005 experiments had significant grazing, with rates ranging from 0 to 2.4 day^{-1}, and with one showing a saturated grazing response, while in 2006, there was significant grazing in two of the experiments; grazing rates in 2006 were 0–2.5 day^{-1}. Microzooplankton biomass ranged from an anomalously low value of 3.3 μgCL^{-1} up to 75.2 $\mu gC L^{-1}$ in 2005 and from 10 to 56.8 μgCL^{-1} in 2006. In summer 2005, chlorophyll *a* ranged from 9.6 to 36.4 μgL^{-1} in the whole fraction and 4.8 to 13.2 in the <10-μm fraction, whereas in 2006 the range was from 7.0 to 9.4 $\mu g L^{-1}$ and 2.9 to 3.1 μgL^{-1} in the whole and <10-μm size fractions, respectively. Phytoplankton growth rates were extremely variable, ranging from −0.8 to 2.6 day^{-1} and −0.1 to 1.3 day^{-1} for the whole water fraction and −0.7 to 2.6 day^{-1} and 0.6 to 1.4 day^{-1} for the <10-μm fraction, in 2005 and 2006, respectively. The very low values appear to be associated with nutrient limitation in situ, as there were large differences in growth between nutrient and non-nutrient treatments (Fig. 2). Dinoflagellate biomass differed markedly between the 2 years; it was 345.8 μgCL^{-1} in 2005 and 27.2 μgCL^{-1} in 2006.

Results from the seven experiments from July 2005 that were analyzed by flow cytometry are summarized in Table 4. Picophytoplankton containing a phycoerythrin fluorescence signature (PE; i.e., *Synechococcus*-like cells) represented 43–90% of the total <2-μm autofluorescent cells (mean 57%). These cells were grazed significantly in all seven experiments in July 2005. Grazing rates ranged from 0.4 to 1.1 day^{-1} (mean 0.7; Table 4). In five of the seven experiments, they showed evidence of saturation. Nutrient additions led to significantly increased growth in only two of the seven experiments. μ for PE-containing cells averaged 0.8 day^{-1} but was more variable than *g*.

Non-phycoerythrin picophytoplankton was significantly grazed in five of the seven experiments. When significant, grazing was substantially higher than for PE cells (0.8–1.9 day^{-1}; mean 1.4; Table 4). In the two experiments for which grazing rates could not be calculated, the slope of *k* vs. dilution was strongly positive. These were the only two dilution plots in the study that showed a significant positive slope. In contrast to PE cells, saturation was evident in only one of the five significant grazing experiments, and nutrient addition stimulated growth in five of seven experiments. As with PE cells, growth was highly variable, ranging from −0.9 to 1.6 day^{-1}.

The >2-μm fraction of the flow cytometer counts consisted of autofluorescent cells ranging up to about 10 μm. Fewer than half were cryptophytes, as indicated by few cells with a PE signal in this size fraction. Grazing was significant in five of seven experiments and showed evidence of saturation in three of the significant five (Table 4). Grazing was somewhat less than that on the <2-μm cells and ranged from 0.3 to 0.8 day^{-1} (mean 0.6; Table 4). Growth of these cells ranged from 0.1 to 1.1 day^{-1} (mean 0.7) and was stimulated by nutrients in six of seven experiments ($p \cong 0.06$ for the non-significant experiment). In common with both categories of <2-μm cells, grazing and growth rates were not significantly correlated.

San Francisco Bay For the two dilution experiments in late winter (March) 2006 in SFB, our sampling caught two markedly different situations. In mid-March, phytoplankton, microzooplankton, and dinoflagellate biomass were low (3.2 μgL^{-1}, 1.8 μgCL^{-1}, and 0.1 μgCL^{-1}, respectively; Table 5), while 1 week later they were all over three times higher. We did not observe significant grazing on either date and phytoplankton growth rates were also low (0.2–0.3 day^{-1}).

Our spring dilution experiments showed significant, saturated, microzooplankton grazing responses in three out of four experiments, with grazing ranging from 0.2 to 0.7 day^{-1} (Table 5). Phytoplankton growth rates were typically higher, ranging from 0.6 to 1.0 day^{-1}. While chlorophyll *a* in this area typically ranges from 1 to 2 $\mu g L^{-1}$ (Wilkerson et al. 2006) during April and May, we found concentrations between 3.1 and 10.8 μgL^{-1}. Microzooplankton biomass ranged from 1.2 to 3.1 μgCL^{-1} and dinoflagellate biomass from 0 to 0.1 μgCL^{-1}.

On four dates in summer 2006, we measured significant grazing only once (1.3 day^{-1}). Chlorophyll *a* concentrations were from 3.2 to 6.6 μgL^{-1}, and microzooplankton biomass was between 16.5 and 53.4 μgCL^{-1}. Dinoflagellate biomass was much lower, between 0.1 and 1.7 μgCL^{-1}. Phytoplankton growth rates were 0.6 to 1.5 day^{-1}. In contrast, in summer 2008 we measured significant grazing on three dates ranging from 0.3 to 0.9 day^{-1} in whole water and 1.8 day^{-1} in the <5-μm size fraction. Phytoplankton growth rates were always higher than grazing rates, ranging from 0.4 to 2.2 day^{-1}. Chlorophyll *a* concentration was 2.2–3.6 μgL^{-1}, whereas microzooplankton biomass was more variable, from 6.5 to 35.6 μgCL^{-1}. Dinoflagellate biomass was uniformly low, averaging 0.3 μgCL^{-1}.

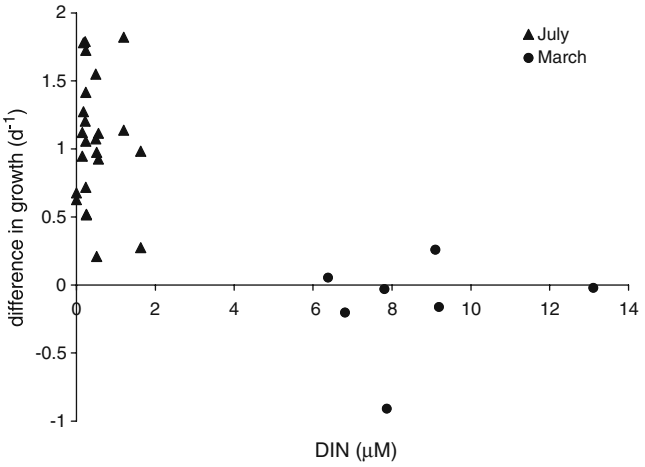

Fig. 2 Effect of nutrient additions on phytoplankton growth in LIS experiments, expressed as the difference between nutrient and no-nutrient treatments (undiluted water) in net growth rates (*k*), in summer (July, only 2005) and winter (March 2005 and 2006) experiments, as a function of initial DIN concentration

In spring and summer 2007, we ran a series of two-point dilutions to investigate variation with salinity in the upper SFB estuary. On both dates, we found increasing phytoplankton growth rates and microzooplankton grazing rates with increasing salinity (Fig. 3 and Table 5). Chlorophyll *a* concentration was in the normal range for this system, averaging 2.0±0.2 μgL^{-1} in April and 1.6±0.2 μgL^{-1} in July. Microzooplankton abundance was somewhat higher in April compared with the previous year and lower in July than either 2006 or 2008 (Table 5).

Discussion

Comparison with Other Systems Grazing rates we measured in LIS and SFB were similar to those found in other estuaries (LIS 0.7±0.2 day^{-1}, SFB 0.7±0.2 day^{-1}, averaged for summer experiments). In Mobile Bay, a river-dominated estuary with strong salinity gradients, grazing measured through different seasons averaged 0.6 day^{-1} (Lehrter et al. 1999). At the mouth of Mobile Bay, grazing was higher, averaging 1.3 day^{-1}. In Chesapeake Bay, grazing in the oligohaline zone was −0.2, 0.8, and 1.6 day^{-1} in April, May, and August (McManus and Ederington-Cantrell 1992), and in the mesohaline, it was 0.2 and 0.1 in April, 0.4 in May, and 0.2 in August. Grazing in a coastal lagoon in southwest Western Australia ranged from 0 to 0.7 day^{-1} and averaged 0.4±0.3 in summer and 0.3±0.3 in all other seasons (Paterson et al. 2008). In a much more broad comparison, Calbet and Landry (2004) found estuarine grazing rates averaging 0.5±0.0.

Size Fractions and Picophytoplankton Since the original work of Malone (1971a, b), many workers have observed differences in abundance, growth, and grazing in different size fractions of phytoplankton. Juhl and Murrell (2005), for example, found that in Pensacola Bay, USA, the larger size fraction (>5-μm) grew faster than either the <5-μm fraction (as chlorophyll) or microscopically counted populations of unicellular *Cyanobacteria*, though both large and small phytoplankton were grazed at similar rates. Strom et al. (2007) and Strom and Fredrickson (2008) also found that larger phytoplankton grew faster but that grazing was less than growth in the larger fractions. The idea that growth and grazing are more closely coupled in the smaller phytoplankton size fractions has become widely accepted in plankton ecology (Fenchel 1988). In the present study, for 16 size-fractionated experiments in LIS, the <10-μm fraction contained 39% (±9.8) of the total chlorophyll. In general, our results for growth and grazing were not concordant between the whole and <10-μm size fractions in LIS. We found that growth in the smaller size fraction

Table 3 Seasonal averages of chlorophyll *a*, microzooplankton (not including dinoflagellates) biomass, microzooplankton abundance, dinoflagellate biomass, dinoflagellate abundance, phytoplankton growth, and microzooplankton grazing for LIS and SFB

	Initial chlorophyll *a* (μgL^{-1})	Microzooplankton biomass (μgCL^{-1})	Microzooplankton abundance (L^{-1})	Dinoflagellate biomass (μgCL^{-1})	Dinoflagellate abundance (L^{-1})	Growth (day^{-1})	Grazing (day^{-1})
LIS winter	8.98 (1.08)	6.58 (1.04)	5,238 (1,023)	5.59 (1.83)	3,714 (1,240)	0.23 (0.13)	0.18 (0.10)
LIS summer	18.58 (2.28)	30.36 (4.88)	12,361 (1,831)	266.13 (62.59)	175,303 (50,566)	0.42 (0.20)	0.66 (0.19)
SFB winter	6.85 (3.68)	4.68 (2.85)	2,910 (1,770)	0.16 (0.08)	190 (70)	0.24 (0.08)	0.00 (0.00)
SFB spring	4.68 (1.44)	4.52 (1.26)	2,880 (467)	0.53 (0.35)	363 (216)	0.79 (0.12)	0.43 (0.17)
SFB summer	3.23 (0.55)	26.27 (5.86)	14,086 (3,030)	0.86 (0.26)	777 (277)	1.04 (0.18)	0.65 (0.18)

Values in parentheses are standard error. All values are for whole water samples and experiments.

Table 4 Results of the seven experiments from July 2005 (LIS) in which flow cytometer measurements were made

Experiment	<2 non-phycoerythrin		<2 phycoerythrin		>2	
	μ	g	μ	g	μ	g
9	0.06	1.62	1.19	0.55[a]	0.91	0.84[a]
11	1.48	0.61[a]	0.89	0.39[a]	1.14	0.26[a]
13	0.58	ND	0.85	0.71[a]	1.00	0
14	1.47	1.86	1.01	1.11	0.93	0.68
15	1.63	1.82	0.75	0.95[a]	0.51	0.68
16	−0.36	ND	0.73	0.75	0.11	0
17	−0.87	0.83	0.27	0.67[a]	0.47	0.73[a]
Mean	0.57	1.35	0.81	0.73	0.72	0.46
SD	1.00	0.59	0.29	0.24	0.37	0.36

Phytoplankton were binned into <2 μm with and without phycoerythrin autofluorescence and >2-μm categories.

ND indicates positive slope to dilution plot.

[a] Indicates saturation of grazing

was higher than that of the whole community in 10 of 16 experiments (mean 0.85 and 0.51 day^{-1} for <10 and whole fractions, respectively). Grazing was also generally higher for the small size fraction, also exceeding that of the whole community in 10 of 16 experiments (mean 0.70 and 0.37 day^{-1} for <10 and whole fractions, respectively). This result is also seen in the flow cytometer data, where the <2-μm PE-containing cells grew and were grazed faster than the >2-μm ones (Table 4), but the differences between the different size classes were never statistically significant.

Saturation One common feature of measurements of microzooplankton grazing in estuaries is the observation of saturation, indicated by failure to produce an increase in

Table 5 Results from dilution experiments in San Francisco Bay

Date	Experiment	Size fraction	Saturated	Chl *a* (μgL^{-1})	Microzooplankton (μgCL^{-1})	g (day^{-1})	μ (day^{-1})
March 2006	1	Whole	NA	3.17	1.83	0	0.16
	2	Whole	NA	10.54	7.53	0	0.32
April 2006	3	Whole	Yes	9.54	1.79	0.67	0.57
	4	Whole	NA	3.08	1.16	0	0.62
May 2006	5	Whole	Yes	3.50	2.09	0.25	1.02
	6	Whole	Yes	10.76	3.10	0.21	0.91
July 2006	7	Whole	Yes	3.20	53.37	1.26	1.54
	8	Whole	NA	3.19	36.17	0	0.68
Aug 2006	9	Whole	NA	6.58	42.26	0	0.49
	10	Whole	NA	5.75	16.48	0	0.31
April 2007	11	Whole	NA	1.87	6.26	0	0.39
	12	Whole	NA	2.35	9.79	0.60	0.68
	13	Whole	NA	1.68	7.47	1.28	1.32
July 2007	14	Whole	NA	1.97	ND	0.82	1.37
	15	Whole	NA	1.39	5.70	1.23	1.62
	16	Whole	NA	1.43	ND	1.56	1.92
July 2008	17	Whole	No	3.03	35.64	0.26	0.38
	18	Whole	Yes	2.17	6.45	0.90	1.33
	19	Whole	No	3.64	12.08	0.52	0.80
	19	<5	Yes	2.43		1.77	2.22

Chlorophyll *a* and microzooplankton refer to values at the start of experiments.

NA not applicable (two-point experiments or grazing not significant), *ND* no data.

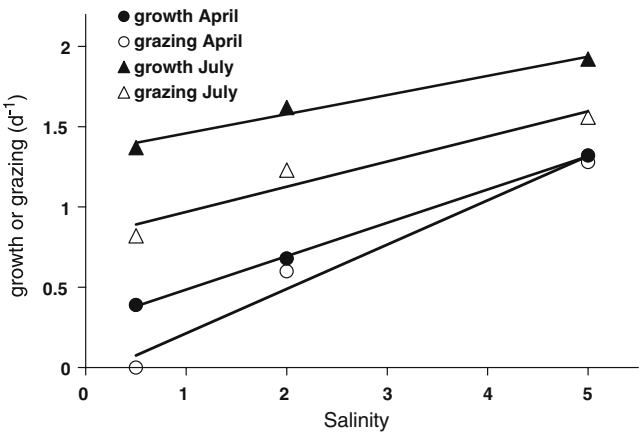

Fig. 3 Results of the experiments comparing growth and grazing in SFB across the salinity gradient from 0.5 to 5

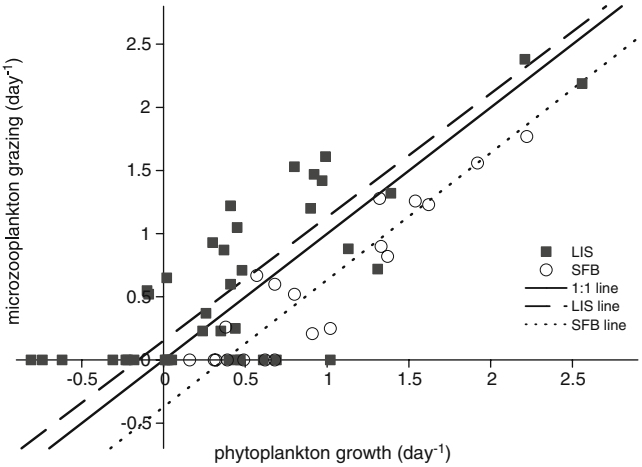

Fig. 5 Grazing versus growth rates for all experiments. 1:1 line is the *solid line*. Model II regression lines were calculated for LIS (y=0.98x +0.15) and SFB (y=1.01x−0.37)

phytoplankton growth in the first one or two dilutions in the series (Gallegos 1989; Redden et al. 2002). We observed evidence of saturation in 10 out of 25 significant grazing experiments in LIS and six of eight experiments (excluding two-point experiments) in SFB. When saturation is indicated, it is possible to calculate the concentration of phytoplankton above which saturation will occur in situ (Redden et al. 2002). Chlorophyll concentrations in these experiments were usually well above the threshold for saturation (Fig. 4), and we sometimes found no dilution effect in both the 50% and 25% dilution treatments.

Growth: Grazing In both SFB and LIS, microzooplankton grazing was strongly correlated with phytoplankton growth rates (Fig. 5). Model II regressions for the two systems had similar slopes (1.01 and 0.98 for SFB and LIS, respectively), but the LIS data had an intercept of 0.15 day^{-1}, while growth almost always exceeded grazing (one exception) in SFB. The growth data are not strictly comparable, however, because phytoplankton growth is difficult to extrapolate over the entire water column based on our surface measurements and because differences in the two systems make comparing such integrations problematic. For example, SFB is shallow, well-mixed except in times of high river flow, highly turbid, and strongly influenced by benthic grazing. The vertical light gradient limits integrated productivity, while benthic grazers keep biomass low (Alpine and Cloern 1988, 1992). LIS is deeper, less turbid, and tends to be stratified, especially in late spring and summer. Light is limiting in spring, while dissolved inorganic nutrients, especially nitrogen, are usually limiting in summer (Conover 1956; Anderson and Taylor 2001), and benthic grazers have a smaller impact on growing phytoplankton, especially during the summer period of hypoxia. Thus, while Fig. 5 may indicate potential fate of phytoplankton production in the surface water, it cannot be used to infer the balance over the whole water column.

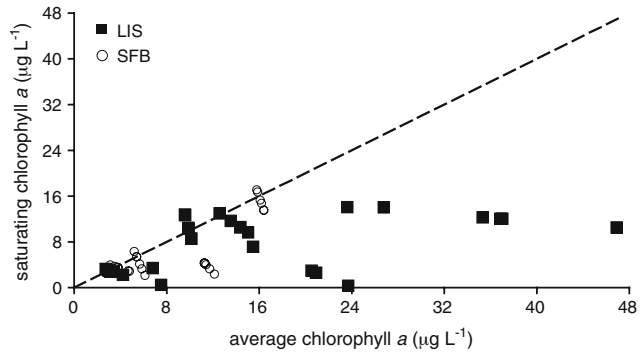

Fig. 4 Chlorophyll *a* concentration required to saturated microzooplankton grazing as a function of average (of initial and final) chlorophyll *a* concentration in incubation bottles, for experiments that were saturated, calculated using the method of Redden et al. (2002)

Role of Dinoflagellates The biomass and abundance of dinoflagellates differed between the two estuaries (Table 3). In SFB, dinoflagellate biomass rarely exceeded microzooplankton biomass. In contrast, particularly in summer 2005, dinoflagellate biomass was often several orders of magnitude greater than microzooplankton biomass in LIS. Given the ambiguity of dinoflagellate trophic mode, it is difficult to speculate on their impact on phytoplankton mortality. There was no clear relationship between dinoflagellate biomass or abundance and grazing rates in our study. Putland and Iverson (2007) also did not find a clear trend between herbivory and dinoflagellate abundance. We did find the highest microzooplankton grazing rates *and* phytoplankton growth rates, when dinoflagellates made up the highest proportion of possible grazers during summer in LIS.

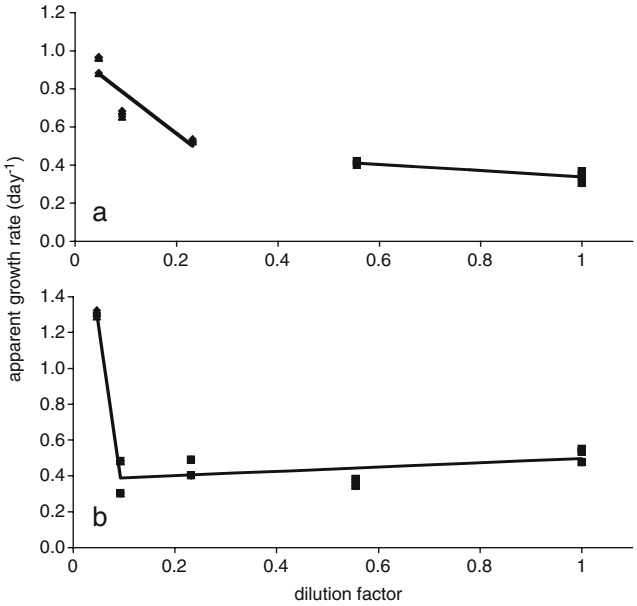

Fig. 6 Results of size-fractionated dilution experiments from SFB in July 2008. An extra, highly dilute (95%) treatment was added to discriminate between insignificant and highly saturated grazing. **a** shows results from the whole water fraction, and **b** is the <5-μm fraction

Non-significant Grazing Another common feature of dilution experiments in productive waters is the observation of non-significant grazing (i.e., no effect of dilution on phytoplankton growth; Murrell and Hollibaugh 1998). Kamiyama (1994) performed 36 size-fractionated dilution experiments in Japan's Hiroshima Bay. He found no significant dilution effect nearly half of the time (33 out of 72 observations, or 46%, including size fractions). Based on its salinity and nutrient concentrations, Hiroshima Bay is more similar to LIS than SFB, where we found insignificant grazing in 17 out of 41 observations (41%). Neither whole nor size-fractionated treatments were more likely to show insignificant grazing, and about half the time in both studies when one size fraction showed no grazing the other fraction was significant. It is especially curious that grazing was not detectable in LIS in seven of the eight experiments in which the phytoplankton intrinsic growth rate was negative (Table 2).

Murrell and Hollibaugh (1998) conducted a series of dilution experiments throughout northern SFB. They observed insignificant effects of dilution 84% of the time. Of the seven light-incubated experiments they performed near our study area (Suisun Bay), none showed significant grazing. We found insignificant grazing in seven of 20 experiments. Because they did observe significant dilution effects in some experiments that were incubated in the dark, Murrell and Hollibaugh (1998) suggested that changes in pigment content of cells due to higher light in diluted samples may have led to flat dilution curves. To evaluate this, we measured light in triplicate whole and 95% diluted samples from SFB in the laboratory, using the same bottles

Table 6 Values for saturation reported in the literature for two dinoflagellates and three ciliates

Grazer	Food	Saturation concentration (μgCL^{-1})
Protoperidinium spp.[a]	*Ditylum* sp.	80–280
Luciella masanensis[b]	*Amphidinium carterae*	336
	Cryptophyte	86
	Perch blood cells	21
	Heterosigma akashiwo	18
Tintinnopsis dadayi[c]	Small flagellates	200–400
Strobilidium spiralis[c]	Small flagellates	200–400
Strombidinopsis sp.[d]	*Cochlodinium polykrikoides*	322
	Gymnodinium sanguineum	266
	Prorocentrum minimum	164
	Lingulodinium polyedrum	566
	Scrippsiella trochoidea	270

All are measurements of ingestion saturation, except that for *Protoperidinium*, for which only saturation of growth was reported. Saturation of growth and grazing should be the same if growth efficiency does not vary at saturating food levels.

[a] From Menden-Deuer et al. (2005); saturation concentration is for growth

[b] From Jeong et al. (2007); half-saturation constant in cells per milliliter converted from reported cell volumes and multiplied by 2 to approximate saturation level.

[c] From Verity (1991)

[d] From Jeong et al. (1999); reported half-saturation constant multiplied by 2 to approximate saturation level.

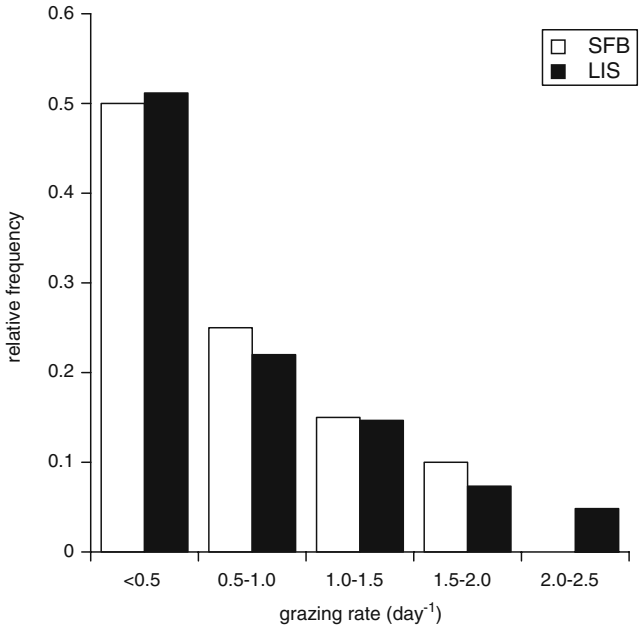

Fig. 7 Frequency distributions of grazing values in the two estuaries

employed in the experiments. We found no significant difference (*t* test) in measurable light within the bottles. We thus conclude that grazing was very low during those experiments or that some experimental artifact other than a light gradient led to our inability to measure grazing in some cases.

Because low or zero grazing observations can occur in close proximity in space or time to experiments with high levels of grazing (e.g., experiments 10 and 11 in LIS; Table 2), or when microzooplankton biomass is high (e.g., experiment 9, August 2006; Table 5), and because of the frequent observation of saturated grazing in experiments conducted in productive waters, we wondered if an extremely high degree of saturation might be misinterpreted as zero grazing in some cases. To evaluate this possibility, we conducted an experiment in SFB in July 2008 in which an additional treatment, 5% whole seawater, was added. Both the whole and <5-μm size fractions showed evidence of saturation, but the smaller size fraction only showed a significant dilution effect in the 5% treatment (Fig. 6). If we had not diluted past 10%, that experiment would have been a "zero" for grazing.

The dilution plot for the <5-μm size fraction suggests that grazing is saturated even at 10% of the initial concentration, or about 0.24 $\mu g chl L^{-1}$. Table 6 summarizes some literature values for ingestion saturation in laboratory cultures of microzooplankters. Although there are some lower values, most observations are greater than 100 $\mu g C L^{-1}$. At a C:Chl of about 20, our 10% dilution treatment would have had only about 5% of this value as phytoplankton carbon. It thus seems unlikely that phytoplankton concentration alone would have been saturating for microzooplankton ingestion in situ. However, given the high concentrations of particulate suspended matter, including organic matter, it is possible that particle-rich estuaries like LIS and SFB may provide challenges to microzooplankters such as ciliates and dinoflagellates that encounter food items individually and may have to spend a large amount of time sorting through non-nutritious particles prior to ingestion. Such an effect would lead to apparent saturation of ingestion even at low concentrations of edible food. It would not indicate true saturation in the sense of abundant food, but rather only saturation of the feeding mechanism. This possible explanation for non-significant dilution experiments deserves further experimental investigation.

Overall, the physical and hydrographic differences in these two estuaries were reflected in strong differences in factors associated with phytoplankton. Chlorophyll *a* concentrations were distinctly lower in turbid SFB compared with LIS (Table 3; *t* test, $p<0.001$). Although nutrient loading to western LIS is high (Hu et al. 1998), unlike SFB we found strong indications of nutrient limitation of phytoplankton growth there. The change in phytoplankton growth rate with added nutrients increased with decreasing ambient nutrient concentrations (Fig. 2). In SFB, we always observed positive growth of phytoplankton, although we did not amend bottles with added nutrients, while in LIS net growth was typically negative in summer without added nutrients.

Given the differences in phytoplankton and physical factors, we were surprised to find that microzooplankton dynamics were remarkably similar in these systems. We found no differences in microzooplankton abundance or biomass between the two sites in winter or summer and, in spite of statistically significant differences in chlorophyll *a* concentrations in summer, grazing rates between the two locations did not differ significantly (Table 3; *t* test, $p=0.40$). Except for the few observations of very high grazing in LIS (>2.0 day^{-1}), the frequency distribution of grazing values was very similar in the two sites (Fig. 7).

In summary, despite strong differences in hydrography, nutrients, and phytoplankton abundance, among other factors, LIS and SFB showed similar levels of grazing on phytoplankton by microzooplankton. Microzooplankton biomass was similar in the two estuaries, while phytoplankton abundances were lower in SFB. This suggests that microzooplankton may also derive nutrition from non-phytoplankton organic matter in this particle-rich estuary (Froneman and McQuaid 1997). We suggest that the presence of non-phytoplankton food, both nutritious and non-nutritious, may lead to the frequent observation of apparent saturation of feeding during dilution experiments in estuaries and recommend further study of this issue.

Acknowledgments We thank colleagues at the Romberg Tiburon Center/San Francisco State University for hosting us during the SFB experiments, especially Wim Kimmerer, Anne Slaughter, and Toni Ignoffo. Don Schoener helped with experiments in both locations, and Gary Wikfors of the NOAA Milford Laboratory kindly provided access to the flow cytometer. We also thank Penny Vlahos for sharing unpublished nutrient data from Long Island Sound. Funding was provided by the CALFED Bay-Delta Science Program (award SCI-05-C107), by the NOAA Coastal Services Center (award NA04NOS4730256), and by the University of Connecticut.

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