NOTE

The Role of Protistan Microzooplankton in the Upper San Francisco Estuary Planktonic Food Web: Source or Sink?

Gretchen Rollwagen-Bollens · Scott Gifford · Stephen M. Bollens

Abstract Decline of native pelagic species in estuarine systems is an increasing problem, especially for native fishes in the San Francisco Estuary and Delta (SFE-D). Addressing these losses depends on understanding trophodynamics in the food web that supports threatened species. We quantified the role of microzooplankton (heterotrophic–mixotrophic protists <200 μm) in the food web of the upper SFE-D. We sampled protist plankton abundance and composition at two sites (Suisun Bay and Grizzly Bay) approximately monthly from February 2004 to August 2005 and conducted dilution experiments during spring and summer of both years in Suisun Bay. Heterotrophs dominated the protist community in Suisun Bay and Grizzly Bay, particularly in the <20 μm size range, and peaks in protistan microzooplankton biomass were associated with high phytoplankton biomass. In both years, microzooplankton grazing rates were high (0.5–0.7 day^{-1}) during the spring and lower (~0.2 day^{-1}) during summer. Phytoplankton growth rates peaked in April 2004 (~0.7 day^{-1}) but were much lower (<0.1 day^{-1}) in spring 2005, despite relatively high abundance. Thus, microzooplankton grazing consumed as much as 73% of phytoplankton standing stock during spring and ~15% of standing stock during summer of both years. Combined

with earlier results, we conclude that microzooplankton can be important mediators of carbon and energy flow in the upper SFE-D and may be a "source" to the metazoan food web.

Keywords Microzooplankton · Grazing · San Francisco Estuary · Dilution experiment · Protist plankton · Grazing impact

Introduction

A serious problem facing coastal and estuarine systems worldwide is the increasing loss of abundance and biodiversity of aquatic species, particularly fishes (Lotze et al. 2006; Worm et al. 2006). This problem is especially acute in the San Francisco Estuary/Sacramento–San Joaquin River Delta (SFE-D), which has experienced dramatic declines in the abundance of native fishes such as chinook salmon (*Oncorhynchus tshawytscha*) and the threatened delta smelt (*Hypomesus transpacificus*; Brown and Moyle 2005; Feyrer et al. 2007).

Various inter-related factors have been put forth to explain declines in fish populations in the SFE-D, ranging from freshwater diversion and loss of habitat to species introductions (Brown and Moyle 2005; MacNally et al. 2010). However, many of these factors also affect fish prey resources; therefore, successful restoration requires a quantitative understanding of the trophic relationships at the base of the food web and how energy is transferred to fish.

Previous studies of plankton dynamics in the SFE-D have traditionally focused on the components of the classical grazing food chain, from phytoplankton (e.g., Cloern et al. 1985; Jassby et al. 2002) to mesozooplankton,

G. Rollwagen-Bollens (✉) · S. M. Bollens
School of Earth and Environmental Sciences and School of Biological Sciences, Washington State University,
14204 NE Salmon Creek Ave,
Vancouver, WA 98686, USA
e-mail: rollboll@vancouver.wsu.edu

S. Gifford
Department of Marine Science, University of Georgia,
Athens, GA, USA

such as copepods and cladocerans (e.g., Ambler et al. 1985; Bollens et al. 2002, in review; Mueller-Solger et al. 2002), and macrozooplankton, such as mysids and shrimp (Gewant and Bollens 2005).

However, microzooplankton (here defined as heterotrophic–mixotrophic protist plankton <200 μm, e.g., ciliates and flagellates) can also be highly abundant in the SFE-D (Rollwagen-Bollens and Penry 2003; Rollwagen-Bollens et al. 2006) and are substantial consumers of phytoplankton carbon in open ocean and coastal environments (reviewed in Calbet and Landry 2004), as well as in some estuaries (e.g., Juhl and Murrell 2005; Leising et al. 2005). In turn, microzooplankton are the preferred prey for mesozooplankton (e.g., copepods) over a wide range of aquatic environments, from estuaries to the open ocean (e.g., Campbell et al. 2008; Dagg et al. 2009; Olson et al. 2006).

Microzooplankton may also be an important food resource for larval fish, based on observations in the field (Bollens and Sanders 2004; de Figueiredo et al. 2007) and in the laboratory (Fukami et al. 1999; Friedenberg et al., in review). Thus, the degree to which microzooplankton graze phytoplankton and are further consumed by copepods and other metazoans can have very significant implications for the amount of material and energy that ultimately gets transferred to at-risk fish.

Our primary goal in this study was to quantify the role of protistan microzooplankton in the planktonic food web in the upper saline reaches of the SFE-D. We examined the abundance and taxonomic composition of the protistan plankton at two contrasting sites, the Suisun Bay channel and the Grizzly Bay shoals, on an approximately monthly basis over 2004 and 2005. In addition, we experimentally assessed microzooplankton grazing in the Suisun Bay channel over the same time period. Finally, we combine these results with a concurrent study of mesozooplankton (copepod and cladoceran) feeding in Suisun Bay (Gifford et al. 2007) to discuss the potential trophic role of microzooplankton in the upper SFE planktonic food web.

Study Location

Suisun and Grizzly Bays together comprise the most inland embayment of the upper SFE and are the gateway into the lower estuary from the Sacramento–San Joaquin River Delta, which drains over 40% of the land area of California. Grizzly Bay is characterized by shallow (~1–3 m) subtidal shoals bounded by mudflats and wetlands to the north and opens into Suisun Bay to the south (Fig. 1). Suisun Bay is also fairly shallow (~2–6 m) but has a relatively deep (~12–

Fig. 1 Map of upper San Francisco Estuary, California, USA. *Filled circles* show locations of the sampling stations in the Suisun Bay channel and Grizzly Bay shoals
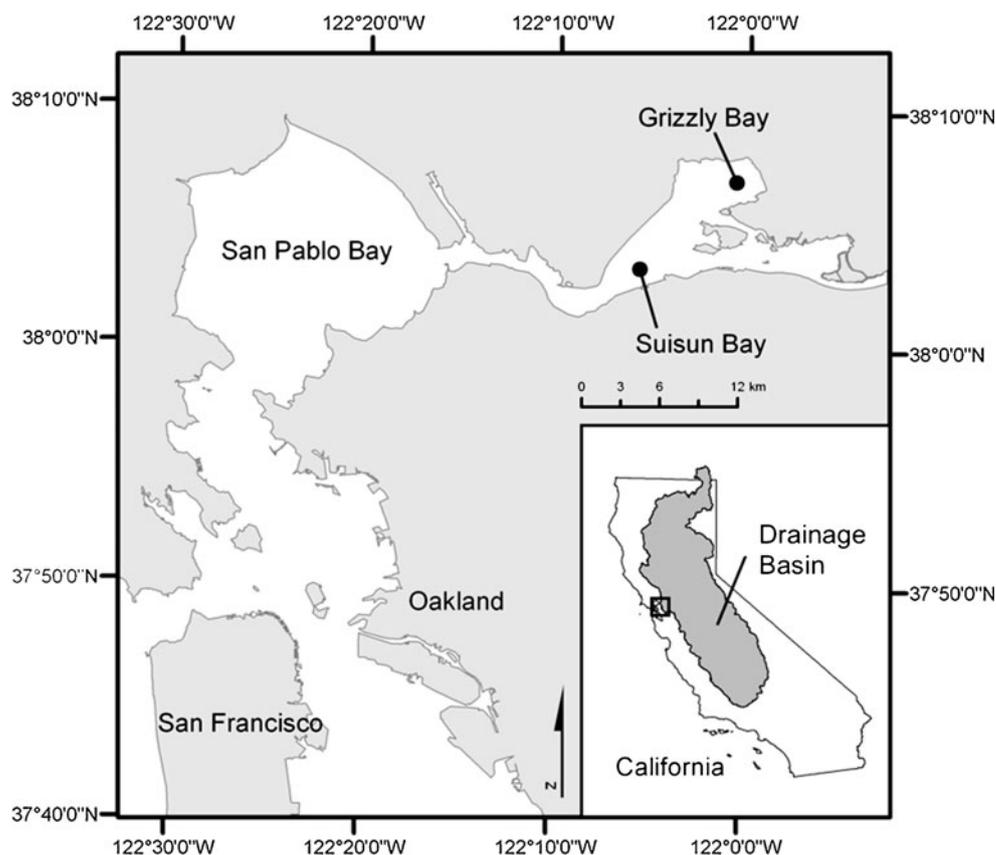
15 m) channel that runs along its southern edge. Suisun and Grizzly Bays receive considerable freshwater inflow during the winter and spring due to rainfall and meltwaters from the Sierra Nevada mountains, with freshwater input roughly one third as high during the summer and fall (California Department of Water Resources "DayFlow" program: http://www.water.ca.gov/dayflow/output/). They thus act as a partially mixed estuary through winter and spring, with short water residence times and high turbidity, and become more well-mixed during summer and fall when freshwater flows reach their annual minima (Cloern et al. 1985; Conomos et al. 1985).

Materials and Methods

Plankton Sampling in Suisun and Grizzly Bays

We sampled at two locations in the upper SFE: the Suisun Bay channel (depth=15 m) and the Grizzly Bay shoals (depth <2 m; Fig. 1). Our Suisun Bay channel location corresponded to a site regularly occupied by the US Geological Survey (USGS) San Francisco Bay Water Quality monitoring program (see http://sfbay.wr.usgs.gov/access/wqdata for details), USGS station 07. We sampled over an 18-month period from February 2004 to August 2005 on a monthly or bi-monthly basis (Table 1). Profiles of temperature and salinity were obtained at each sampling time and location, using a Seabird SBE19 conductivity–temperature–depth recorder. In addition, water was collected from three depths at the channel station: surface, mid-depth (7–9 m), and near bottom (12–14 m) and from just below the surface at the shoal station using 2.5-l Niskin bottles equipped with Teflon springs to avoid metal contamination.

Subsamples (200 ml) for microscopical analyses of community composition were gently siphoned from the Niskin bottles and preserved in 10% acid Lugol's solution for enumeration of organisms 20–200 μm ("microplankton"; Gifford 1988) and 1% glutaraldehyde for enumeration of organisms 2–20 μm ("nanoplankton"; Sherr et al. 1993). Additional subsamples (100 ml) were filtered onto GF/F glass fiber filters, extracted in 90% acetone, and analyzed via fluorometry for chlorophyll *a* concentration (Strickland and Parsons 1972).

Microplankton were enumerated and identified using the Utermöhl method (Throndsen 1978). Aliquots of 25–50 ml were settled overnight into counting chambers and the entire chamber examined at 100–200× using an inverted microscope. All protistan cells were sized and identified to the lowest possible taxonomic level, typically to genus. Carbon biomass was calculated from cell biovolume using conversion factors in Menden-Deuer and Lessard (2000). Nanoplankton samples were processed within 24 h of

Table 1 Dates and locations of sampling and temperature and salinity measurements obtained between February 2004 and August 2005 in Suisun and Grizzly Bays

Location	Year	Date	Temperature (°C)	Salinity (PSU)
Suisun Bay channel (38°03′ N, 122°06′ W)	2004	February 4	10.6	8.38
		March 2	11.0	0.09
		March 15	15.7	0.34
		April 5	15.1	3.76
		April 19	15.6	0.34
		May 10	18.1	9.24
		June 9	19.1	11.85
		September 7	22.2	12.89
		November 30	11.6	13.05
	2005	January 25	n.d.	n.d.
		March 8	14.4	1.13
		March 30	13.6	0.12
		April 19	15.2	3.64
		May 6	17.5	1.77
		May 16	18.5	1.95
		June 6	16.3	n.d.
		August 1	21.0	6.37
Grizzly Bay shoals (38°06′ N, 121°58′ W)	2004	February 4	11.0	3.48
		April 5	14.9	3.76
		April 19	15.4	0.16
		May 10	19.0	3.45
		June 9	n.d.	n.d.
		September 7	21.9	n.d.
		November 30	11.1	10.01
	2005	January 25	n.d.	n.d.
		March 8	14.9	0.41
		March 30	14.5	0.39
		April 19	15.8	1.49
		May 6	17.7	0.97
		May 16	19.3	0.24
		June 6	18.9	0.08
		August 1	21.1	5.93

All samples measured from ~0.5 m below the surface

n.d. no data available

collection; 10–25 ml aliquots were stained with fluorescein isothiocyanate (FITC), filtered onto 1-μm black membrane filters, mounted on glass slides, and stored at −20°C (Sherr et al. 1993). Filters were examined using epifluorescence microscopy under blue excitation at 400×. The first 150 protistan organisms observed were sized and identified to the lowest taxonomic level and characterized as being pigmented or non-pigmented. Carbon biomass was calculated as described above using Menden-Deuer and Lessard (2000). Data from the three depth-specific

samples in the Suisun Bay channel were averaged to yield a single water column mean for each date.

Microzooplankton Dilution Experiments

Dilution experiments (Landry and Hassett 1982) to quantify microzooplankton community grazing rates upon phytoplankton were conducted using water and organisms collected from the Suisun Bay channel in March, April, May, and August/September of each year, following modified protocols in Murrell and Hollibaugh (1998). Natural seawater was collected from mid-depth (7–9 m) using a large volume Niskin bottle equipped with Teflon springs and transferred to an acid-washed carboy. We did not pre-filter the experimental water, so as to avoid any potential damage to fragile protist grazers. Seawater for dilutions was collected similarly but was then gravity-filtered through GF/F filters into clean carboys. Each dilution experiment was set up with three replicates at each of five dilution levels (10%, 25%, 50%, 75%, and 100%) of natural seawater with filtered seawater. An additional three bottles were filled for each dilution level and then immediately sampled for initial values. All incubations were conducted using acid-washed 1-l polycarbonate bottles. Initial bottles were subsampled for chlorophyll a, microplankton, and nanoplankton as described above. Treatment bottles were incubated for 24 h (12:12 h ratio of light to dark) on a plankton wheel (0.5 rpm) in a temperature-controlled room, set to ambient temperature (11–22°C; Table 1). Light levels were kept low and constant over the 12-h light period using low-wattage incandescent bulbs, which approximated the light available at the sampling depths in the Suisun Bay channel. At the end of the incubation, all treatment bottles were sampled for chlorophyll a concentration and micro- and nanoplankton abundance as described above. Following the approach in Murrell and Hollibaugh (1998), incubation bottles were not supplemented with additional nutrients. Inorganic nitrogen, phosphate, and silicate are rarely if ever limiting in the SFE (Cloern and Dufford 2005), and nitrate + nitrite concentrations at our sampling station averaged 27 μM and never fell below 19 μM throughout 2004–2005 (USGS data available at http://sfbay.wr.usgs. gov/access/wqdata). Moreover, Murrell and Hollibaugh (1998) found no significant changes in nitrogen concentrations over the course of their 24-h incubations.

Net phytoplankton growth rate in each treatment bottle was calculated using the change in chlorophyll a concentration over the 24-h incubation, assuming exponential growth. Intrinsic phytoplankton growth rates (per day) and microzooplankton community grazing rates (per day) were estimated as the y-intercept and slope (respectively) of the regression of net growth rate in the treatment bottles versus the fraction of undiluted seawater in each experiment (Landry and Hassett 1982).

Finally, the impact of microzooplankton grazing on phytoplankton was assessed by calculating the % of chlorophyll a standing stock consumed by protist grazers per day (Calbet and Landry 2004). Briefly, the microzooplankton community grazing rate was multiplied by the geometric mean of initial and final chlorophyll a concentrations in the undiluted treatment of each experimental incubation, multiplied by 100, and divided by the concentration of chlorophyll a initially present in the environment.

Results

Abundance, Biomass, and Taxonomic Composition of Protist Plankton

Suisun Bay Channel Mean water column chlorophyll a concentrations peaked in April or May of both 2004 and 2005 (5.5 and 5.3 μg Chl a l^{-1}, respectively), due primarily to increased abundance of both large (>20 μm) diatoms (*Amphiprora* sp.) and small (~5 μm) chain-forming diatoms (*Skeletonema* sp.; Table 2). In terms of relative abundance, nanoplankton dominated the protist plankton in Suisun Bay. However, other than substantial peaks in small diatoms during April/May of both years, there was little seasonal pattern in abundance of the <20-μm protist community (Table 2).

The carbon biomass of microzooplankton, including the mixotrophic ciliate *Myrionecta rubra* (= *Mesodinium rubrum*), also showed maxima during the spring chlorophyll peaks in Suisun Bay. Loricate ciliates (primarily *Tintinnopsis* sp.) dominated in May 2004 and heterotrophic aloricate ciliates (*Strombidium* spp.) dominated in April/May 2005 (Fig. 2b). Notably, biomass of microzooplankton was relatively high (~15 μg C l^{-1}) during summer/early autumn of both years, with ciliates (*Strombidium* spp., *M. rubra*) and <20-μm gymnodinoid dinoflagellates dominating the community (Fig. 2b, c).

Grizzly Bay Shoals Peaks in chlorophyll a concentration were also observed in the Grizzly Bay shoals during April or May of 2004 and 2005, although the maximum in May 2005 (18 μg Chl a l^{-1}) was substantially higher than spring 2004 (Fig. 3a). In contrast to the Suisun Bay channel, in Grizzly Bay, only diatoms (*Amphiprora* sp., *Skeletonema* sp.) reached high abundance during spring of 2004 and 2005, while microzooplankton >20 μm in size were in relatively low abundance throughout the sampling period (Table 3). The heterotrophic protists were instead dominated by small (<20 μm) flagellates, whose abundance varied without any consistent seasonal pattern (Table 3). Similar to the Suisun Bay channel, carbon biomass of >20 μm micro-

Table 2 Mean (+ 1 SE) water column abundances of major taxonomic groups of microplankton (20–200 μm) and nanoplankton (2–20 μm) collected in the Suisun Bay channel between February 2004 and August 2005

Year	Date	Microplankton abundance (cells ml⁻¹)						Nanoplankton abundance (cells ml⁻¹)			
		Loricate ciliates	Aloricate ciliates	Myrionecta rubra	Diatoms	Dinoflagellates	Flagellates	Diatoms	Dinoflagellates	Autoflagellates	Heteroflagellates
2004	February 4	0.6 (0.3)	0.6 (0.1)	1.1 (0.5)	0.9 (0.0)	0.1 (0.0)	0.1 (0.0)	115 (63)	22 (13)	305 (155)	1,622 (754)
	March 2	0.9 (0.5)	1.8 (0.9)	0.5 (0.0)	1.6 (0.3)	0.0 (0.0)	0.6 (0.2)	74 (45)	0 (0)	2,262 (870)	1,001 (592)
	March 15	0.9 (0.1)	2.0 (0.5)	0.4 (0.0)	3.2 (1.2)	0.9 (0.3)	0.2 (0.1)	820 (419)	45 (22)	1,267 (6)	530 (84)
	April 5	1.2 (0.2)	2.4 (0.1)	0.4 (0.2)	2.0 (0.1)	0.0 (0.0)	2.3 (0.9)	2,712 (213)	0 (0)	551 (82)	3,147 (460)
	April 19	0.4 (0.1)	2.1 (0.1)	0.2 (0.0)	137.8 (16.5)	0.0 (0.0)	28.8 (6.3)	636 (156)	0 (0)	577 (65)	2,076 (154)
	May 10	6.5 (1.0)	1.3 (0.1)	2.5 (0.4)	2.8 (0.6)	0.9 (0.2)	28.1 (4.8)	201 (84)	30 (4)	212 (22)	737 (67)
	June 9	1.4 (0.4)	1.0 (0.3)	0.7 (0.5)	2.8 (0.5)	0.4 (0.4)	81.6 (27.1)	197 (98)	41 (19)	327 (39)	234 (51)
	September 7	0.8 (0.2)	6.7 (2.3)	6.3 (3.1)	1.8 (0.5)	5.9 (2.8)	14.8 (2.6)	126 (54)	97 (39)	1,726 (109)	975 (81)
	November 30	0.3 (0.1)	1.7 (0.3)	4.7 (0.8)	5.1 (1.3)	0.0 (0.0)	7.3 (1.0)	290 (78)	30 (15)	1,570 (227)	2,790 (341)
2005	January 25	5.2 (2.2)	2.4 (0.4)	1.1 (0.2)	3.1 (0.7)	0.0 (0.0)	4.6 (1.1)	268 (13)	37 (7)	722 (238)	2,314 (478)
	March 8	1.5 (0.3)	5.0 (0.2)	2.7 (0.7)	4.0 (0.7)	0.2 (0.1)	3.2 (0.1)	82 (32)	7 (7)	2,053 (1,242)	2,686 (787)
	March 30	1.3 (0.3)	2.0 (0.2)	1.0 (0.2)	16.2 (9.6)	0.0 (0.0)	2.9 (1.9)	238 (39)	7 (7)	342 (45)	1,153 (30)
	April 19	1.4 (0.2)	19.2 (4.9)	2.9 (0.7)	5.7 (1.0)	1.8 (0.7)	1.3 (0.3)	4,062 (1,553)	67 (39)	774 (60)	4,018 (692)
	May 6	1.4 (0.3)	11.4 (1.5)	1.6 (0.3)	98.3 (7.8)	0.3 (0.1)	0.0 (0.0)	573 (173)	7 (7)	670 (59)	2,001 (351)
	May 16	3.8 (2.8)	11.3 (3.3)	1.1 (0.4)	36.2 (7.0)	0.1 (0.1)	0.0 (0.0)	260 (116)	22 (22)	848 (123)	2,634 (123)
	June 6	0.7 (0.2)	4.5 (0.8)	0.5 (0.0)	10.7 (0.4)	0.0 (0.0)	1.3 (0.3)	789 (54)	0 (0)	684 (7)	1,518 (34)
	August 1	6.9 (0.5)	4.8 (1.6)	2.2 (0.1)	9.9 (0.9)	0.3 (0.3)	0.0 (0.0)	655 (166)	476 (161)	402 (59)	7,016 (719)

Fig. 2 Mean water column values of **a** chlorophyll *a* concentration, **b** microplankton carbon biomass, and **c** nanoplankton carbon biomass in the Suisun Bay channel, upper San Francisco Estuary, from February 2004 to August 2005. *Stars* indicate dates of microzooplankton community grazing (*dilution*) experiments. *Error bars* = 1 SE
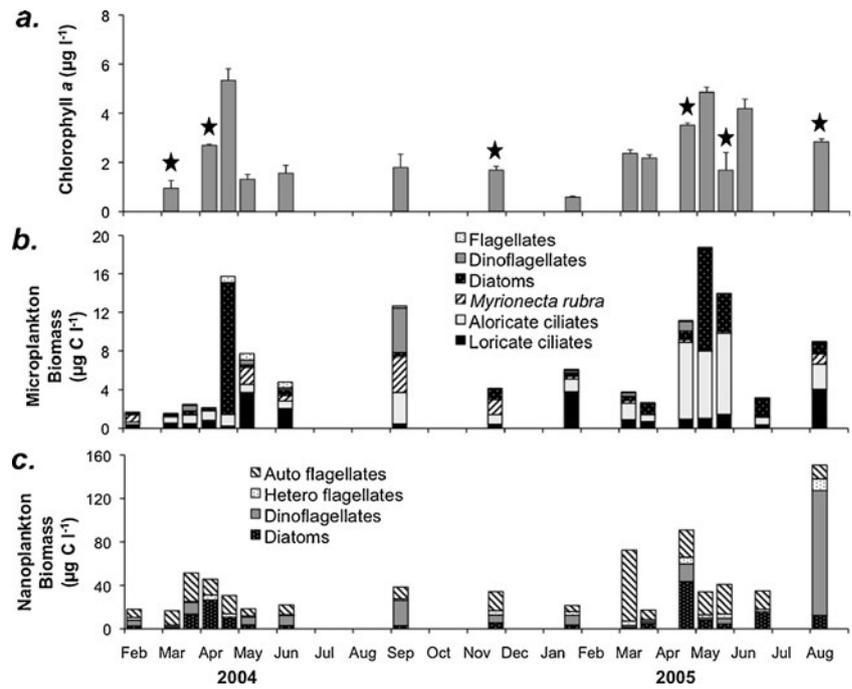
zooplankton (primarily *Strombilidium* sp. ciliates) was the highest during the summer/autumn of both 2004 and 2005, despite these taxa being relatively low in abundance (Fig. 3b).

Phytoplankton Growth Rates and Microzooplankton Community Grazing Rates

Of nine dilution experiments conducted between March 2004 and August 2005, six experiments resulted in

significant ($p<0.05$) regressions of net phytoplankton growth rate to dilution level (Table 4; Fig. 4). In spring 2004, phytoplankton growth rates were positive, with maximal growth (0.67 day^{-1}) observed in April. Microzooplankton grazing rates nearly balanced phytoplankton growth in March and April 2004 (Fig. 5). In November 2004, phytoplankton growth rate was near zero; however, microzooplankton grazing rates were observed to be "negative," i.e., the regression slope was positive (Figs. 4 and 5). However, in spring and summer 2005, phytoplankton growth

Fig. 3 Water column values of **a** chlorophyll *a* concentration, **b** microplankton carbon biomass, and **c** nanoplankton carbon biomass in the Grizzly Bay shoals, upper San Francisco Estuary, from February 2004 to August 2005
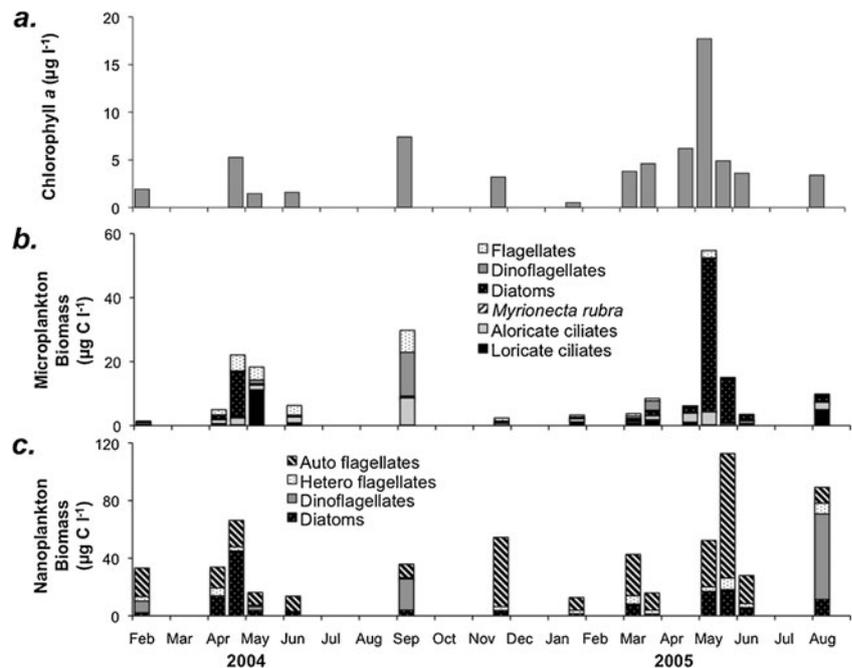
Table 3 Water column abundances of major taxonomic groups of microplankton (20–200 μm) and nanoplankton (2–20 μm) collected in the Grizzly Bay shoals between February 2004 and August 2005

Year	Date	Microplankton abundance (cells ml⁻¹)						Nanoplankton abundance (cells ml⁻¹)			
		Loricate ciliates	Aloricate ciliates	Myrionecta rubra	Diatoms	Dinoflagellates	Flagellates	Diatoms	Dinoflagellates	Autoflagellates	Heteroflagellates
2004	February 4	0.8	1.2	0.2	2.2	0.0	0.3	89	33	603	2,087
	April 5	0.9	3.3	0.3	98.4	0.0	19.9	1,060	0	558	3,594
	April 19	0.5	4.0	0.4	217.8	0.1	63.9	1,864	0	569	1,931
	May 10	19.9	1.9	3.8	2.8	2.0	23.1	156	11	290	625
	June 6	1.3	2.1	1.2	8.9	0.0	36.7	223	0	324	290
	September 7	0.3	18.1	41.3	17.4	24.2	75.4	223	89	1,585	625
	November 30	0.2	1.6	8.4	5.6	0.0	12.0	156	0	1,986	1,696
2005	January 25	1.7	2.9	1.2	6.6	0.1	4.5	89	0	1,741	1,585
	March 8	1.5	1.3	2.0	31.4	0.1	8.7	357	0	870	3,839
	March 30	3.3	4.9	4.9	19.1	0.5	5.9	112	0	424	1,741
	April 19	1.9	9.0	4.0	15.2	0.2	0.1	0	0	0	0
	May 6	0.5	6.7	1.7	499.5	0.0	4.2	1,094	0	1,138	2,120
	May 16	0.4	4.1	0.2	112.8	0.0	1.1	1,161	0	2,656	5,312
	June 6	0.9	5.9	1.9	9.3	0.0	2.0	268	0	692	1,875
	August 1	8.8	4.3	2.2	17.9	0.6	0.5	513	246	335	4,933

Table 4 Phytoplankton growth rates and microzooplankton community grazing rates estimated from dilution experiments conducted using water collected from the Suisun Bay channel between March 2004 and August 2005

Year	Date	Depth (m)	Temp (°C)	Sal (PSU)	Growth rate (day⁻¹)	Grazing rate (day⁻¹)	r^2	p value
2004	March 2	7	11.1	0.85	0.25	0.27	0.41	0.02*
	April 5	6	15.1	4.62	0.67	0.47	0.91	<0.001**
	May 10	5	18.0	9.51	−0.17	−0.25	0.21	0.09
	September 7	5	21.9	15.58	−0.54	0.04	0.00	0.83
	November 30	6	12.0	14.71	0.01	−0.21	0.68	<0.001**
2005	March 8	6	14.3	1.82	0.06	0.00	0.00	0.91
	April 19	7	15.3	7.82	0.08	0.24	0.93	<0.001**
	May 16	6	18.3	6.42	0.07	0.71	0.87	<0.001**
	August 1	7	21.3	6.52	−0.08	0.17	0.80	<0.001**

Temp temperature, *Sal* salinity

*$p<0.05$ (regression slope significantly different from zero); **$p<0.001$ (regression slope highly significantly different from zero)

rates were consistently low (−0.08 to 0.08 day⁻¹) while microzooplankton grazing rates were comparable to spring 2004 (Table 4; Fig. 5).

To further explore the potential causes of the variable phytoplankton growth and microzooplankton grazing rates, we examined the abundance and composition of the microzooplankton grazer community initially present during the dilution experiments (Fig. 6). The microzooplankton grazer community differed substantially between experiments conducted in 2004 and 2005. During the March and April 2004 experiments, abundance of potential microzooplankton consumers was relatively low

(<8 cells ml⁻¹) and dominated mainly by loricate (*Tintinnopsis* sp.) and aloricate (*Strombidium* spp.) ciliates and a range of colorless flagellates. In November 2004, the one date when a "negative" microzooplankton community grazing rate was observed, the abundance of potential microzooplankton grazers was somewhat higher (~10 cells ml⁻¹) with a substantial proportion of the community consisting of the mixotrophic ciliate *M. rubra* (Fig. 6).

In spring 2005, the abundance of microzooplankton consumers present in dilution experiments was nearly fourfold higher than in 2004, reaching 38 cells ml⁻¹ in

Fig. 4 Linear regressions of net phytoplankton growth rates (per day) to the fraction of undiluted seawater in 24-h incubation experiments conducted in **a** 2004 and **b** 2005, with water collected from the Suisun Bay channel
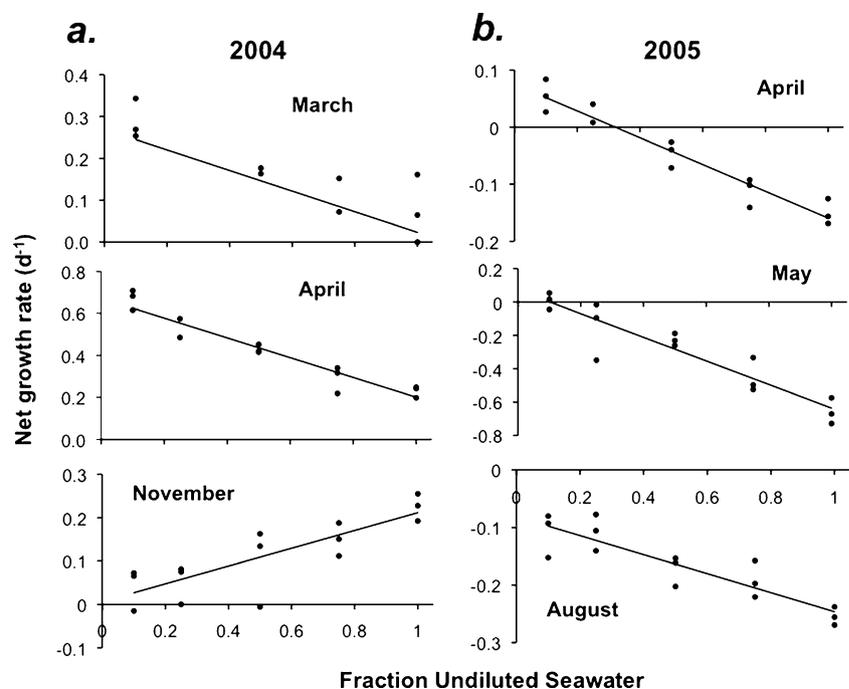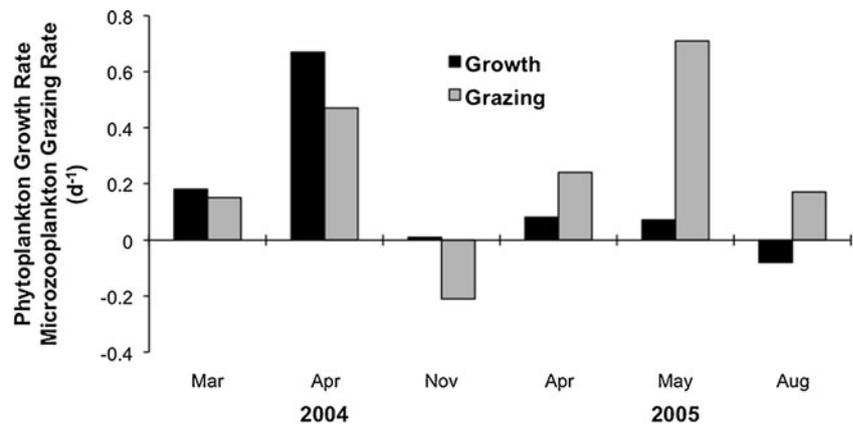
Fig. 5 Phytoplankton growth rates (per day) and microzooplankton community grazing rates (per day) calculated from dilution experiments conducted from March 2004 to August 2005, using water collected in the Suisun Bay channel

Discussion

April. The grazer community in 2005 was also dominated to a much larger degree by aloricate (*Strombidium* sp.) ciliates in April, with additional loricate ciliates (*Stenosemella* sp.) present in May. Abundance of microzooplankton grazers in August 2005 was lower than earlier in the year but slightly higher than observed in autumn of 2004. Notably, the microzooplankton community was strongly dominated by large loricate ciliates (*Tintinnopsis* sp.) in August 2005 (Fig. 6).

Microzooplankton grazing impact in Suisun Bay was substantial, with 55–73% of chlorophyll *a* standing stock consumed per day in spring of both 2004 and 2005. Grazing impact was lower during early spring and summer, averaging ~15% of standing stock per day (Fig. 7). In November 2004, grazing impact was calculated to be "negative," due to the "negative" value of microzooplankton grazing rate, suggesting that the presence of microzooplankton during this experiment may have enhanced phytoplankton growth (Fig. 7).
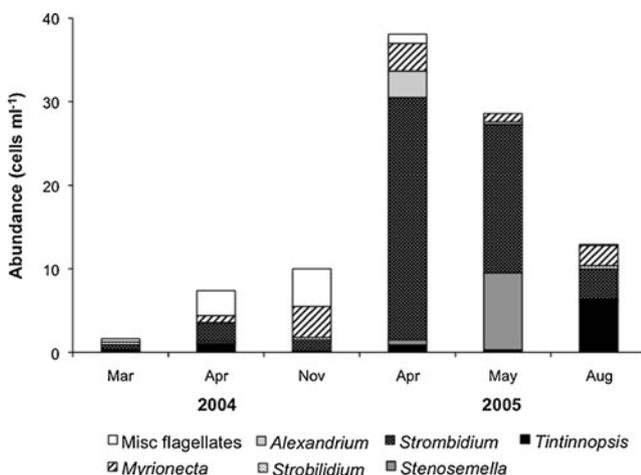
Our results clearly show that microzooplankton are a significant component of the plankton in Suisun and Grizzly Bays and at times may be the dominant organisms (>2 μm in size) in terms of both abundance and biomass. The diversity of microzooplankton in Suisun and Grizzly Bays is very similar to that observed further downstream in San Pablo Bay (Rollwagen-Bollens and Penry 2003; Rollwagen-Bollens et al. 2006), and the overall abundance is comparable although somewhat (~5–15%) lower than in other temperate and subtropical estuaries (e.g., Dolan and Coats 1990; Muylaert et al. 2000; Sakka Hlaili et al. 2007).

The total biomass and taxonomic composition of protist plankton was also very similar between the deep Suisun Bay channel and the shallow Grizzly Bay shoals throughout the annual cycle. However, during the spring of 2004 and 2005, the biomass of large (>20 μm) diatoms was substantially higher in Grizzly Bay, likely contributing to the higher chlorophyll concentration observed at this location in May 2005. This result aligns with the results of earlier studies of the SFE-D that demonstrate that phytoplankton production is often higher in shoal areas vs. deep channels (Lucas et al. 1999a, b) and that shoal regions may contribute phytoplankton biomass to channels (Cloern 2007; Cloern et al. 1985; Lucas et al. 2009).

Moreover, in both Suisun and Grizzly Bays, there is a strong relationship (Pearson's correlation=0.7; $p<0.001$) between peaks of autotrophic plankton and peaks in biomass of microzooplankton. This pattern is similar to that observed further downstream in San Pablo Bay (G. Rollwagen-Bollens, unpublished data) as well as in other temperate and subtropical estuaries (Johnson et al. 2003; Sime-Ngando et al. 1995; Urrutxurtua et al. 2003). The strong positive relationship between peaks of autotrophic and heterotrophic protist biomass suggests a trophic link between phytoplankton and microzooplankton, which is borne out by our experimental results.

Fig. 6 Abundance (cells per milliliter) of potential microzooplankton grazer taxa present during six dilution experiments conducted in Suisun Bay from March 2004 to August 2005
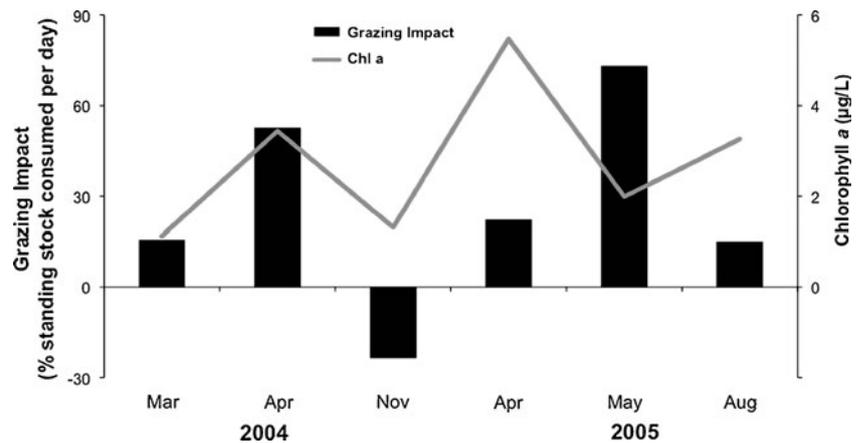
Fig. 7 Microzooplankton grazing impact, measured as % standing stock of chlorophyll *a* consumed per day, and initial chlorophyll *a* concentration, determined from six dilution experiments conducted in the Suisun Bay channel from March 2004 to August 2005

Microzooplankton community grazing rates on phytoplankton chlorophyll in Suisun Bay were consistently high (up to 0.7 day^{-1}) over our nearly 2-year sampling period. In spring of both years, these high microzooplankton grazing rates resulted in microzooplankton consuming as much as 73% of phytoplankton standing stock. Similarly high microzooplankton grazing rates have been observed in a wide range of estuarine (Leising et al. 2005; Sakka Hlaili et al. 2007; Sime-Ngando et al. 1995) and coastal/open ocean environments (Calbet and Landry 2004).

Interestingly, microzooplankton grazing rates were similar in spring of both years, but phytoplankton growth rates were nearly fourfold lower in spring 2005 compared to spring 2004 and on one occasion (August 2005) were negative. The imbalance between phytoplankton growth (low) and microzooplankton grazing (high) seen during 2005 could not be sustained by a population for very long without it eventually declining in size, yet phytoplankton abundance and biomass was actually somewhat higher in 2005 than in 2004.

Low phytoplankton growth rates concurrent with high standing stocks have been observed previously in Suisun Bay (Cloern et al. 1985) and could be reconciled in several ways. First, higher phytoplankton growth and/or lower grazing could have occurred during periods between our experiments. Second, local phytoplankton abundance could have been supplemented by an external source (from upstream or shoals) as has been observed and modeled in the lower SFE (Lucas et al. 1999b). Finally, water residence time could have increased enough to allow accumulation of cells in Suisun Bay, as suggested by Cloern et al. (1985).

What is more unusual, or at least underreported in the literature, is the "negative" microzooplankton grazing rate in November 2004. Negative grazing rates have been observed from dilution experiments in other studies (e.g., Pearce et al. 2008) and could have been the result of a "trophic cascade" effect within the dilution experiment treatment bottles. The assemblage of planktonic protist grazers is often highly diverse with respect to taxonomy, size, relative abundance, and trophic complexity (Reckermann and Veldhuis 1997). If the protist community in the dilution treatments during November 2004 consisted of a three-trophic-level system consisting of a small number of large micrograzers (e.g., ciliates) feeding on smaller, more abundant nanograzers (e.g., heterotrophic flagellates) feeding on even more abundant pico- or nanoautotrophs, then the dilution process could have effectively reduced the impact of micrograzers, allowing the smaller nanograzers to increase and exert higher grazing pressure on pico- or nanophytoplankton compared to less dilute treatments. Indeed, such "top-down" trophic interactions within the protist community were recently modeled to occur in dilution experiments by First et al. (2009).

Notably, the microplankton community during the November 2004 dilution experiment was dominated by the mixotrophic ciliate *M. rubra*, and the abundance of other heterotrophic ciliates and dinoflagellates was extremely low. Possibly the dilution process removed these large, chlorophyll-rich ciliates such that the most dilute treatments no longer had the contribution of *M. rubra* to overall phytoplankton apparent growth, leading to higher apparent growth in the undiluted treatment during November 2004.

Overall, while the number of dilution experiments conducted in this study was modest and there could have been inconsistencies between the laboratory and field conditions (e.g., light levels), our results demonstrate microzooplankton in Suisun Bay to have a significant role in consuming algal production during periods of both high and low chlorophyll concentration. And a substantial proportion of phytoplankton carbon may be moving through the microzooplankton community, as evidenced by microzooplankton grazing impacts as high as 73% of phytoplankton standing stock per day.

This is in contrast to the conclusions of Murrell and Hollibaugh (1998), who conducted a series of dilution

experiments in Suisun Bay over 9 months in 1993–1994, of which only three resulted in a significant slope between apparent chlorophyll-based phytoplankton growth rate and level of dilution. Based on these results, they concluded that microzooplankton exerted little control over phytoplankton growth in Suisun Bay and discussed the problems with the dilution method and the potential complications of turbidity, light availability, and a diverse grazer community for interpreting experimental results (e.g., Dolan and McKeon 2004, but see response by Landry and Calbet 2005, and more recent lively discussion, e.g., Agis et al. 2007; Teixeira and Figueiras 2009). Nevertheless, the three valid experiments conducted by Murrell and Hollibaugh (1998) each resulted in microzooplankton grazing rates that exceeded phytoplankton growth rates, and their grazing rates in May 1994 (Table 2 in Murrell and Hollibaugh 1998) were very similar to our results in April 2005. We therefore believe their data complement our own results and serve to reinforce the argument that microzooplankton grazing is important in Suisun Bay.

This argument is further supported by the observation, made concurrently with our study, that heterotrophic protists were the dominant component of mesozooplankton predator diets in Suisun Bay in 2004–2005, regardless of season, predator species, or size (Gifford et al. 2007). Clearance and ingestion rates of copepods (*Acartia* sp., *Oithona davisae*, *Limnoithona tetraspina*) and cladocerans (*Daphnia* sp.) feeding on natural assemblages of planktonic prey were the highest for ciliates in seven experiments conducted over 18 months (March 2004 to August 2005), demonstrating a strong preference by mesozooplankton for protistan microzooplankton over diatoms and other autotrophic cells (Gifford et al. 2007).

Thus, we conclude from these results that microzooplankton have a substantial grazing impact on phytoplankton in Suisun Bay during spring and summer and are further consumed by the dominant mesozooplankton predators. Microzooplankton are therefore a key component of the planktonic food web in Suisun Bay. An important question, then, is what impact microzooplankton may have on the amount of production that is available to higher trophic levels.

Microzooplankton: Source or Sink?

Consumption of microzooplankton, particularly ciliates, by metazoan predators may serve to trophically complement phytoplankton biomass and thereby enhance metazoan growth (Huo et al. 2008; Klein Breteler et al. 1999; Tang and Taal 2005). Phytoplankton abundance in the SFE-D is often low, except during brief spring blooms (Cloern 1996). Thus, consuming microzooplankton may serve to augment a diet of algae, resulting in microzooplankton acting as a "source" of production to the planktonic food web.

Conversely, the addition of a trophic step between phytoplankton and mesozooplankton can mean lower trophic efficiency of the food web, which could result in microzooplankton serving as a "sink" for production in the food web. However, experimental and field evidence demonstrates that many heterotrophic planktonic protists, particularly small oligotrich ciliates such as we found in Suisun Bay (e.g. *Strombidium*, *Strombilidium*), effectively and consistently consume bacterioplankton (Artolozaga et al. 2002; Sherr et al. 1989). Moreover, in the northern SFE, organic carbon available for bacterial growth is primarily of allochthonous origin (Jassby et al. 1993; Murrell and Hollibaugh 2000). Therefore, microzooplankton could be re-packaging and contributing carbon otherwise unavailable to the classic metazoan food web, which could counterbalance losses from reduced trophic efficiency.

In conclusion, our results, in combination with those of Gifford et al. (2007), show that microzooplankton may consume a substantial amount of phytoplankton production and are an important prey resource to copepods and cladocerans in Suisun Bay, especially when algal biomass is low. With such strong trophic connections between phytoplankton, microzooplankton, and mesozooplankton in Suisun Bay, these links need to be taken into account when quantifying the pathways for materials and energy that support higher trophic levels, including at-risk fish species such as Delta smelt.

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