

# Microzooplankton grazing and the control of phytoplankton biomass in the Suwannee River estuary, USA

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**Abstract** Microzooplankton grazing can have significant impacts on the distribution and abundance of phytoplankton, thereby influencing the frequency and duration of algae blooms. Observations of high ciliate abundances in the Suwannee River estuary, Florida, suggest a significant potential for top-down pressure on the phytoplankton community by microzooplankton. We examined the composition of the microzooplankton and determined grazing mortality losses for phytoplankton within the Suwannee River estuary from 2001 to 2002. Our results indicated grazing mortality rates of  $1.4 \text{ d}^{-1}$ , equivalent to a loss of up to 76% of phytoplankton standing crop and up to 83% of total daily primary production. The microzooplankton community was primarily composed of ciliates, dinoflagellates, and copepod nauplii. The densities of ciliates in the estuary were comparable to densities reported in highly eutrophic ecosystems (9,400–72,800 ciliates  $\text{l}^{-1}$ ). Grazing pressure on small phytoplankton may be further enhanced because ciliates and small dinoflagellates have growth rates similar to those of phytoplankton, and therefore can keep up with surges in abundance.

**Keywords** Suwannee River · Estuary · Microzooplankton grazing · Protozoan · Ciliates · Gulf of Mexico

## Introduction

Nutrient loading to coastal environments has significantly increased world-wide, resulting in increases in harmful algal blooms, diminished invertebrate populations, changes in the dynamics of local food webs, and expanding problems with hypoxia (Nixon, 1995; Valiela et al., 1997; Paerl et al., 1998). Historically, research on eutrophication has focused on the impact of nutrient availability on algal production and composition (Ingrid et al., 1996). However, algal dynamics can also be strongly influenced by loss processes, such as biomass transport, dilution, sinking, mortality and grazing (Banse, 2002). A change in the magnitude in any of these factors can enhance or diminish the potential for algal blooms, given the availability of sufficient nutrients and favorable hydrologic conditions.

In the open ocean, grazing plays a large role in the control of phytoplankton standing crop, and rates of phytoplankton growth and grazer-induced mortality can nearly balance each other (Banse, 1992). High rates of phytoplankton loss due to grazing have also been observed in many estuarine and coastal environments (Dagg & Turner, 1982; Burkill et al., 1987;

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Kamiyama, 1994; Strom & Strom, 1996). In the northern Gulf of Mexico, micro-zooplankton has been shown to remove up to 100% of phytoplankton production per day (Dagg, 1995). As in freshwater lakes, micro-zooplankton grazing may induce cascading effects on the trophic structure of coastal systems impacted by nutrient loading, including changes in the composition of higher organisms, nutrient uptake, energy transfer, community respiration, and decomposition of organic matter (Carpenter & Kitchell, 1996). The potential for micro-zooplankton control of phytoplankton biomass is strengthened by the fact that some micro-zooplankton grazers have growth rates similar to phytoplankton (Montagnes, 1996), which enables them to exploit pulses of phytoplankton growth.

This study examined the role of zooplankton grazing on phytoplankton in the Suwannee River estuary, located in the Northern Gulf of Mexico. Prior studies of the Suwannee River estuary suggest that phytoplankton dynamics may be subject to “top-down” controls as well as chemical and physical forcing factors (Bledsoe & Philips, 2000). This suggestion is based on observations of high densities of micro-zooplankton, particularly ciliates. The goal

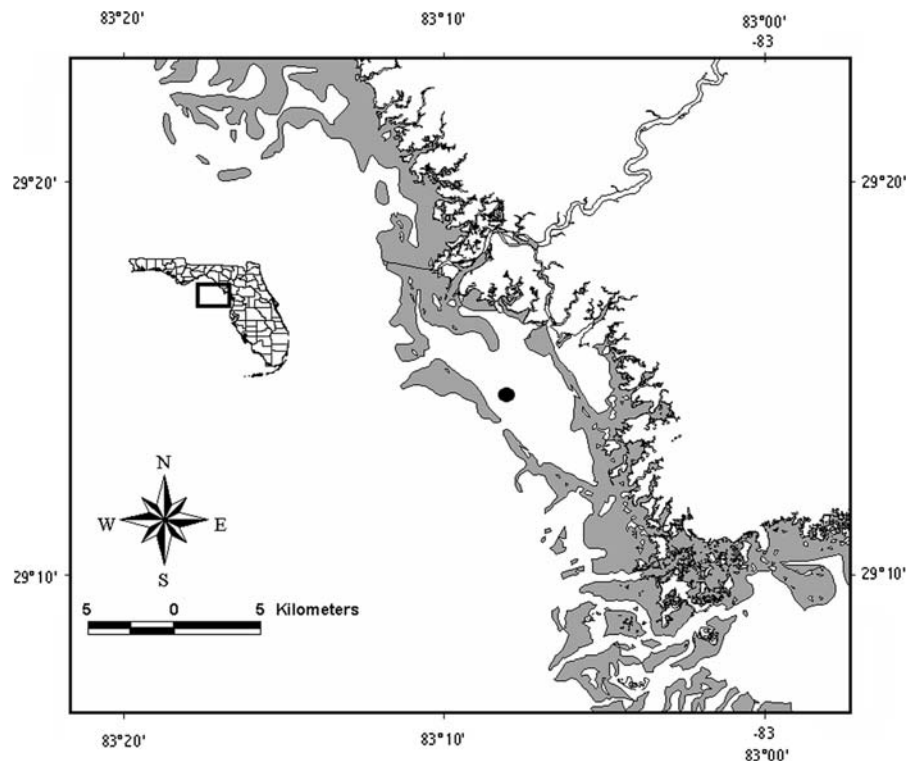
of this study was to determine seasonal trends in the impact of micro-zooplankton ( $>5\text{--}200\ \mu\text{m}$ ) grazing on phytoplankton biomass in the Suwannee River estuary. Micro-zooplankton grazing rates were determined using dilution experiments (Landry & Hassett, 1982) and compared to the growth rates of natural phytoplankton communities in the Suwannee River estuary.

## Materials and methods

### Study site description

Micro-zooplankton grazing experiments were conducted monthly from January 2001 to January 2002 using whole water samples collected from the Suwannee River estuary (Fig. 1). Located at approximately latitude  $29^{\circ}13.60'N$  and longitude  $83^{\circ}8.10'W$ , the Suwannee River estuary includes a delta region near the river mouth, which is characterized by a network of oyster reefs. The coastal region is generally well mixed due to its shallow depth ( $<3\ \text{m}$ ), although it can become vertically stratified depending on tide, wind, and river discharge (Bledsoe & Philips, 2000).

**Fig. 1** Map of the Suwannee River and its estuary, Florida, USA. Filled circle indicates sampling location for micro-zooplankton grazing experiments conducted from January 2001 to January 2002. Oyster reefs and mud flats ( $<1\ \text{m MLW}$ ) are indicated in light gray



## Experimental procedure

Micro-zooplankton grazing rates and phytoplankton growth rates were estimated under laboratory conditions using a dilution technique described by Landry & Hassett (1982). Seawater (approximately 80–90 l) was collected with a water column integrating tube (DeVries & Stein, 1991), which captures water from the surface to 0.1 m above the sediment, and transported in acid-washed carboys to the laboratory. Half of the seawater was filtered through a 0.2- $\mu\text{m}$  Millipore filter with a peristaltic pump and used as the dilution medium. The remaining seawater was combined with filtered seawater in dilutions of 100, 80, 60, 50, 40, and 30% for a total volume of 2 l. High densities of large gelatinous zooplankton (e.g., ctenophores) in autumn were excluded from all dilutions using a 1,000  $\mu\text{m}$  mesh-screen. Removal of gelatinous zooplankton was necessary due to difficulty in replicating treatments, when these large organisms were present in significant numbers. Experiments were conducted in 3 l polycarbonate incubation containers in duplicate. A second series of dilution experiments was performed monthly from August 2001 to January 2002 with samples collected at the same time and station. The second series of samples were pre-filtered with a 202  $\mu\text{m}$  mesh Nitex screen to exclude meso-zooplankton before incubation.

Incubations were conducted in a temperature-controlled room set at field water temperatures recorded on each sampling date (Table 1). Full spectrum light intensity was fixed between 100 and 120  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Photoperiod was 12/12 dark/light h, respectively, from October through March and 10/14 dark/light h from April through September. Since nutrient limitation has been observed in this region of the estuary (Bledsoe & Phlips, 2000), excess nutrients ( $\text{KNO}_3$ ,  $\text{K}_2\text{HPO}_4$ , and  $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ ) were added to each container to yield final concentrations of 400  $\mu\text{g N l}^{-1}$ , 40  $\mu\text{g P l}^{-1}$ , and 400  $\mu\text{g Si l}^{-1}$ , to prevent nutrient limitation of phytoplankton growth. The dilution containers were incubated for 24 h under gentle mixing using stir bars (78  $\times$  12 mm) at 60 rpm.

## Sample analysis

Water samples were subdivided on site into aliquots for analysis of chlorophyll *a*, phytoplankton

**Table 1** Environmental conditions for the dilution experiments within the Suwannee River estuary, Florida, USA

Sampling date	Total depth (m)	Temperature ( $^{\circ}\text{C}$ )	Salinity (psu)
1/24/01	1.5	11.2	22.3
2/26/01	2.1	18.9	25.5
3/26/01	2.0	17.3	20.2
4/23/01	2.8	23.4	25.1
5/21/01	2.5	26.3	28.9
6/18/01	2.4	29.3	26.5
7/23/01	2.3	29.4	15.7
8/20/01	2.1	30.5	14.6
9/24/01	1.4	28.9	25.2
10/23/01	1.8	23.8	27.0
11/27/01	2.6	22.3	30.6
1/28/02	2.5	8.9	23.0
1/9/02	2.4	9.0	28.8

composition (Quinlan & Phlips, 2007), nutrient concentrations (APHA, 1995), and micro-zooplankton composition.

Fluorescence microscopy of unpreserved samples was used to enumerate picoplanktonic cyanobacteria and chlorophytes (<5  $\mu\text{m}$ ) (Quinlan & Phlips, 2007). Lugol's preserved phytoplankton and zooplankton samples were characterized microscopically using a Utermöhl settling chamber at 100 $\times$  and 400 $\times$  magnification (Utermöhl, 1958). Larger zooplankton (>50  $\mu\text{m}$ ) were concentrated from 1,000 to 100 ml using a 41- $\mu\text{m}$  mesh Nitex screen and enumerated by a complete count of a known volume at 100 $\times$ . Counts were completed upon reaching 100 individuals of the dominant taxa.

Zooplankton and phytoplankton biovolumes ( $\mu\text{m}^3$ ) were obtained by assigning combinations of geometric shapes to fit the characteristics of individual taxa (Smayda, 1978). Mean volume per taxa was determined from specific dimensions measured for a minimum of 30 organisms per taxa. Equivalent spherical diameters (ESD) were used to express predator and prey sizes and defined as the diameter of a sphere with equal [ESD = (biovolume/0.523) 0.33] (Hansen et al., 1994).

Nutrient limitation bioassay experiments were performed each month to determine the potential limitations on phytoplankton growth, as described in Bledsoe & Phlips (2000). Under nutrient-limiting

conditions, the addition of that nutrient, or combination of nutrients, resulted in significantly greater growth than the control ( $P < 0.05$ ). When algal growth in the control treatment increased relative to the initial control, it was concluded that surplus bioavailable nutrients were present at the time of sampling. Relationships between different variables and treatments were determined using ANOVA analysis.

Phytoplankton growth and grazing mortality were determined using the exponential growth equation of Landry & Hassett (1982) shown in Eq. 1.

$$P_t = P_0 \exp [(k - g)t] \quad (1)$$

where  $k$  and  $g$  are the instantaneous phytoplankton growth and grazing mortality rate ( $\text{day}^{-1}$ ), respectively,  $t$  is the time in days,  $P_t$  and  $P_0$  are the final and the initial chlorophyll  $a$  concentration, based on initial whole water chlorophyll  $a$  concentration and dilution factor. Values of  $k$  and  $g$  were determined from linear regression of the change in phytoplankton biomass over time, as estimated from chlorophyll, against the fraction of undiluted seawater. Linearity of this relationship was subjected to a lack-of-fit test at the 0.05 level (Evans & Paranjape, 1992). The percent loss of phytoplankton standing crop per day ( $P_i$ ) and the percent loss of phytoplankton production per day ( $P_p$ ) were determined from instantaneous growth ( $k$ ) and grazing rates ( $g$ ) applied to the initial chlorophyll  $a$  concentrations ( $C_0$ ) shown in Eqs. 2 and 3.

$$P_i = (C_0 - C_0 e^g) / C_0 \times 100 \quad (2)$$

$$P_p = [(C_0 e^k - C_0) - (C_0 e^{(k-g)} - C_0)] / (C_0 e^k - C_0) \times 100 \quad (3)$$

## Results

### Environmental conditions

Water column depths on the thirteen sampling dates ranged from 1.4 to 2.8 m (mean =  $2.2 \pm 0.4$  m). Water temperatures ranged from 8.9 to 30.5°C (mean =  $21.5 \pm 7.8$ °C) and salinities ranged from 14.6 to 30.6 psu (mean =  $24.1 \pm 4.9$  psu) (Table 1). The large salinity range reflects variations in the flow rates of the Suwannee River. The lowest salinities

were observed during periods of relatively high freshwater discharge (July and August 2001).

Nitrate + nitrite concentrations ranged from 0 to 181  $\mu\text{g N l}^{-1}$ , ammonium ranged from 9 to 211  $\mu\text{g N l}^{-1}$ , and soluble reactive phosphorus from 1 to 46  $\mu\text{g P l}^{-1}$ . Due to proximity of the Suwannee River outflow to the study site, the highest concentrations of bioavailable nutrients were observed during periods of high river flow, as reflected by the negative relationship between salinity and nutrient concentration. Nutrients were limiting on several dates, primarily during periods of low river discharge and nutrient input (Table 2). On all dates when nutrient limitation was observed, nitrogen was the primary limiting nutrient.

### Phytoplankton community

Phytoplankton biomass, estimated by chlorophyll  $a$ , ranged from 1.7 and 21.1  $\mu\text{g Chl } a \text{ l}^{-1}$  (Table 3). Phytoplankton biomass peaked in May and June and then again from August to October 2001. Microscopic analysis of water samples indicated that diatoms accounted for the highest percentage (>70%) of total phytoplankton biovolume ( $\mu\text{m}^3 \text{ ml}^{-1}$ ) (Fig. 2). Common taxa included *Chaetoceros* spp., *Thalassionema nitzschioides* (Grunow), *Rhizosolenia* spp., *Leptocylindrus danicus* (Cleve), and centric diatoms 5–10  $\mu\text{m}$  in diameter. Picoplanktonic cyanobacteria 1–2  $\mu\text{m}$  in diameter and chlorophytes 2–4  $\mu\text{m}$  (c.f. *Nannochloropsis* sp.) in diameter accounted for up to 15% and 55% of total phytoplankton biovolume, respectively, during summer and autumn (Fig. 2). The latter species dominated the phytoplankton community in terms of density (Fig. 2). Phytoflagellates accounted for up to 30% of the total phytoplankton biovolume in spring. Overall, the phytoplankton community was numerically dominated by taxa <5  $\mu\text{m}$  equivalent spherical diameter (ESD), which accounted for 91–99% of total phytoplankton abundance (Fig. 4). Dinoflagellates were generally low in density and represented less than 10% of the total phytoplankton biovolume. For simplicity, all dinoflagellates observed in this study were classified under mixotrophic dinoflagellates due to the ability of a majority of these organisms to switch from photoautotrophy to osmotrophy to phagotrophy depending upon environmental conditions.

**Table 2** Initial field nutrient concentrations and limiting nutrients measured for the dilution experiments in the Suwannee River estuary, Florida, USA

Sampling date	Nitrate ( $\mu\text{g N l}^{-1}$ )	Nitrite ( $\mu\text{g N l}^{-1}$ )	Ammonium ( $\mu\text{g N l}^{-1}$ )	Phosphorus ( $\mu\text{g P l}^{-1}$ )	Silica ( $\mu\text{g Si l}^{-1}$ )	Limiting nutrient
1/24/01	65*	–	25	5	2.4	U
2/26/01	7*	–	9	4	1.3	N
3/26/01	52*	–	46	7	1.1	U
4/23/01	0*	–	55	3	0.3	N
5/21/01	2*	–	9	1	0.9	N
6/18/01	0*	–	17	3	1.2	N
7/23/01	181*	–	211	46	5.7	U
8/20/01	163	37	180	43	6.1	U
9/24/01	0	2	30	5	1.5	N
10/23/01	16	4	49	12	0.9	N
11/27/01	36	4	49	12	0.9	U
1/9/02	61	2	30	12	2.3	U
1/28/02	25	2	22	11	2.0	U

Note: The (\*) indicates the nitrogen measurement of  $\mu\text{g Nitrate} + \text{Nitrite-N l}^{-1}$ . The limiting nutrient is based on nutrient limitation bioassays, where U indicates that nutrients were not limiting during the bioassays; N indicates nitrogen was the primary limiting nutrient, P as phosphorus limited and Si as silica limitation

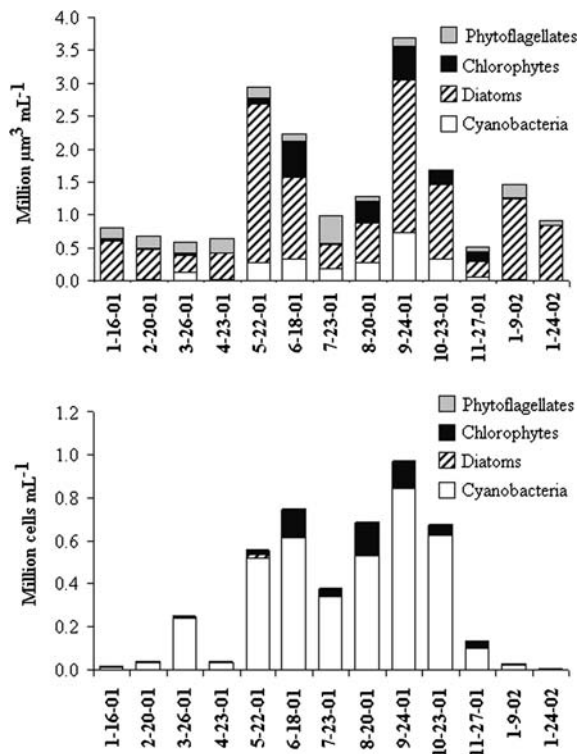
**Table 3** Dilution experiment estimates of initial phytoplankton biomass as chlorophyll *a* (Chl *a*,  $\mu\text{g l}^{-1}$ ), phytoplankton growth coefficients (*k*,  $\text{d}^{-1}$ ), grazing mortality coefficients (*g*,  $\text{d}^{-1}$ ), standing stock of phytoplankton biomass removed

daily ( $P_i$ , %  $\text{d}^{-1}$ ), and phytoplankton production grazed daily ( $P_p$ , %  $\text{d}^{-1}$ ) in the Suwannee River estuary, Florida, USA, obtained using linear regression based on relationship between rate of change of chlorophyll and the dilution factor

	Whole seawater dilutions					<202 $\mu\text{m}$ Seawater dilutions				
	Chl <i>a</i>	<i>k</i>	<i>g</i>	$P_i$ (%)	$P_p$ (%)	Chl <i>a</i>	<i>k</i>	<i>g</i>	$P_i$ (%)	$P_p$ (%)
1/24/2001	4.7	0.41	0.31	27	79	–	–	–	–	–
2/26/2001	8.2	0.45	0.12	11	31	–	–	–	–	–
3/26/2001	7.5	0.81	0.27	24	43	–	–	–	–	–
4/23/2001	7.3	2.34	1.34	74	82	–	–	–	–	–
5/21/2001	18.7	2.74	1.33	74	79	–	–	–	–	–
6/18/2001	10.7	2.37	1.41	76	83	–	–	–	–	–
7/23/2001	7.7	2.49	1.02	64	70	–	–	–	–	–
8/20/2001	12.3	2.47	0.917	60	66	11.8	2.76	1.34	74	79
9/24/2001	21.1	0.89	0.37	31	52	20.1	1.74	1.45	77	93
10/23/2001	16.2	1.24	0.32	27	39	14.8	1.95	1.33	74	86
11/27/2001	7.2	–	–	–	–	7.17	1.51	0.9	59	76
1/9/2002	1.7	1.51	0.605	45	58	1.32	1.25	0.62	46	65
1/28/2002	2.9	1.45	0.96	62	81	2.78	1.76	0.55	42	51

Rates of nutrient-enhanced phytoplankton growth (*k*) ranged between 0.41 and  $2.74 \text{ d}^{-1}$  (or 0.59 to 3.95 doublings per day), peaking from April to August (Table 3). Phytoplankton growth rates (based on changes in chlorophyll *a*) in the experimental

containers were generally lower in January, February, and March 2001 (mean =  $0.56 \text{ d}^{-1}$  or 0.8 doublings per day). Nutrient-enhanced growth rates ( $\text{d}^{-1}$ ) for major genera of phytoplankton were variable (Table 4). Diatom growth rates ( $\text{d}^{-1}$ ) were positive,



**Fig. 2** Phytoplankton biovolume (million  $\mu\text{m}^3 \text{mL}^{-1}$ ) (top) and density (million cells  $\text{mL}^{-1}$ ) (bottom) by taxa in the Suwannee River estuary from January 2001 to January 2002

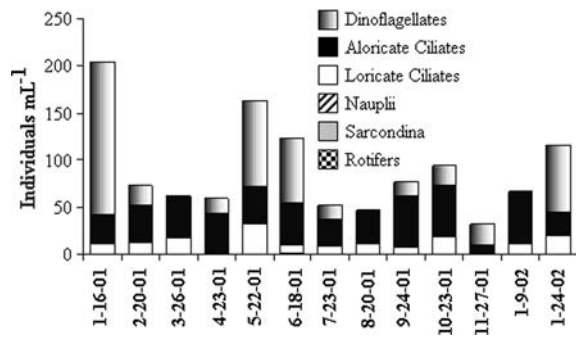
with the exception of January and September 2001, and were highest in the spring and autumn. Cyanobacteria had positive nutrient-enhanced growth rates in January, February, and April, but negative rates the rest of the year. Chlorophytes had positive nutrient-enhanced growth rates most of the year except June, August and September. Phytoflagellates had positive growth rates most of the year, except January, February, and July.

#### Micro-zooplankton populations

Aloricate and loricate ciliates (tintinnids) and dinoflagellates dominated the micro-zooplankton community in terms of numbers throughout the year (Fig. 3). The most common taxa included oligotrichs of the genera *Strombidium*, *Strobilidium*, and *Halteria*, with densities ranging from 32 to 56 individuals  $\text{mL}^{-1}$  (Fig. 3). Tintinnid genera were also numerous throughout the year and included the genera *Eutintinnus*, *Salpingella*, *Amphorella*, *Favella*, and *Tintinnopsis*. The most frequently occurring dinoflagellates included those in the genera *Gymnodinium*, *Gyrodinium*, *Torodinium*, *Protoperidinium*, and *Prorocentrum*. High densities of *Heterocapsa rotundata* (Lohmann) and *Katodinium glaucum* (Lebour) were also observed

**Table 4** Individual growth rates ( $\text{d}^{-1}$ ) for phytoplankton and micro-zooplankton in the Suwannee River estuary, Florida, USA

	Cyanobacteria	Diatoms	Phytoflagellates	Chlorophytes	Dinoflagellates	Loricata ciliates	Aloricate ciliates	Nauplii
1/24/2001	1.03	-1.28	-0.10	1.75	-0.98	0.98	0.21	0.43
2/20/2001	0.25	0.32	-0.24	1.58	-4.63	1.32	1.71	0.35
3/26/2001	-1.17	0.40	0.59	0.82	-	1.9	1.13	0.05
4/23/2001	0.91	2.02	1.21	1.06	0.02	0.69	1.06	0.32
5/21/2001	-1.39	2.77	0.30	0.83	0.70	1.64	0.69	0
6/18/2001	-0.62	1.99	0.70	-0.71	0.69	2.47	1.07	-0.25
7/23/2001	-0.58	1.68	-0.06	0.79	1.37	1.51	1.87	-0.23
8/20/2001	-0.23	1.61	1.48	-0.35	-	2.5	0.77	0.55
9/24/2001	-1.47	-0.04	0.64	-0.62	-2.29	0.79	1.6	0.03
10/23/2001	-0.58	2.72	3.19	1.09	-4.07	0.69	0.55	0.18
11/27/2001	-0.03	2.41	1.66	0.70	-0.41	0.92	0.09	0.27
1/9/2002	0.66	1.57	0.00	1.85	-2.25	0.95	0.1	0.95
1/24/2002	1.65	1.13	1.10	0.47	0.01	0.1	0.7	1.05



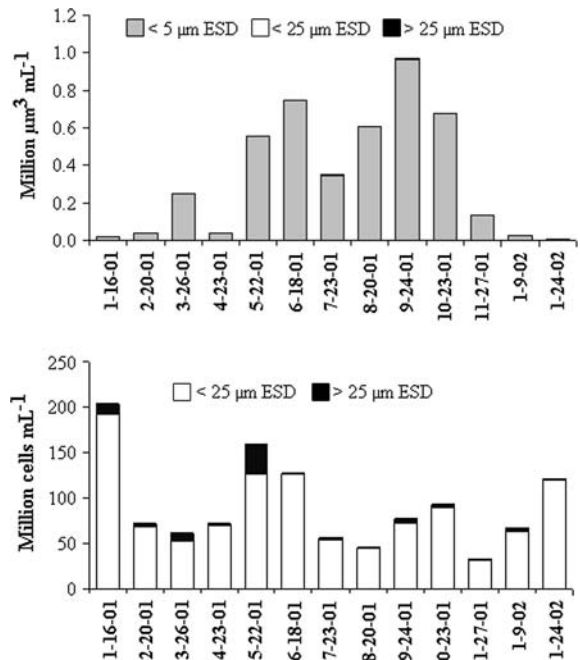
**Fig. 3** Micro-zooplankton densities (individuals  $\text{mL}^{-1}$ ) by taxonomic group in the Suwannee River estuary, Florida from January 2001 to January 2002. Dinoflagellates indicate a mixed population of heterotrophic, phagotrophic, and mixotrophic dinoflagellates

during January 2001 and 2002 (700 individuals  $\text{L}^{-1}$ ). Copepod nauplii and copepodites were commonly observed, with highest densities observed during June 2001 (860 individuals  $\text{L}^{-1}$ ) (Fig. 3). Overall, the composition of the micro-zooplankton community was dominated by taxa  $<25 \mu\text{m}$  equivalent spherical diameter (ESD), which accounted for 78–99% of total micro-zooplankton abundance (Fig. 4). Ciliates (including tintinnids) and dinoflagellates comprised the majority of micro-zooplankton within this size class.

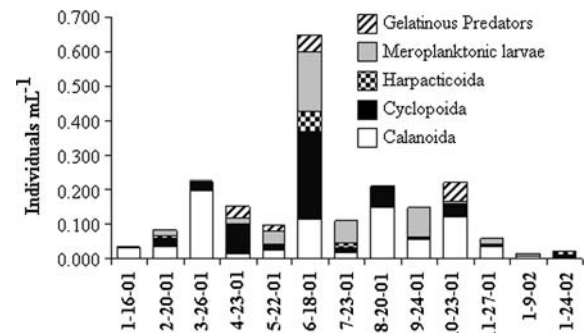
#### Meso-zooplankton populations

Meroplanktonic larvae (e.g., echinoderm, bivalve, polychaete, and gastropod) were observed in many of the water samples used for the dilution experiments, and were most numerous during the summer (Fig. 5). Gelatinous zooplankton were prevalent during the spring and fall, and included larvaceans, medusa, chaetognaths, and salps. All taxa were observed in both the whole and pre-filtered ( $202 \mu\text{m}$  mesh) samples during the initial and final counts. Ctenophores (e.g., *Mnemiopsis* sp.) occurred in high densities during the fall and were removed from all samples collected from August to November 2001.

The abundance of adult copepods varied throughout the year. The three major groups of copepods were represented. Calanoids were dominated by *Acartia* spp., cyclopoids were dominated by *Oithona* spp., and harpacticoids were dominated by *Microsetella* spp. The highest densities were observed during



**Fig. 4** Initial phytoplankton densities (million cells  $\text{mL}^{-1}$ ) in the Suwannee River estuary, Florida, USA (top) and initial microzooplankton densities (individuals  $\text{mL}^{-1}$ ) (bottom) from January 2001 to January 2002 based on equivalent spherical diameters ( $\mu\text{m}$  ESD)



**Fig. 5** Initial concentrations (individuals  $\text{mL}^{-1}$ ) of meroplanktonic larvae, gelatinous zooplankton, copepods in the Suwannee River estuary, Florida, USA

June 2001, due to the presence of large numbers of *Oithona* spp. (Fig. 5).

#### Microzooplankton growth and grazing mortality rates

The results of micro-zooplankton grazing experiments are summarized in Table 3. Grazing mortality

rate coefficients ( $g$ ) ranged from 0.31 to 1.41  $d^{-1}$ , corresponding to 11–76% of daily phytoplankton biomass and 31–82% of daily phytoplankton production in the whole water samples (Table 3). The removal of the meso-zooplankton resulted in grazing mortality rate coefficients 24–98%, somewhat higher than the whole water grazing experiments. In the January 2002 experiment, removal of meso-zooplankton decreased the grazing coefficient by 68%. The highest phytoplankton grazing mortality rates occurred during the spring and early summer experiments of April, May, June, and July (mean = 1.27  $d^{-1}$ ) and corresponded to grazing impacts of 64–76% of phytoplankton standing crop per day and 70–83% of the phytoplankton production per day.

Growth rates of the individual micro-zooplankton taxa were calculated from microscopic counts and ranged from  $-0.6$  to 2.5  $d^{-1}$  (or doublings of  $-0.87$  to 3.6 per day) (Table 4). Ciliates (aloricate and loricate) showed exceptionally high growth rates ( $>1 d^{-1}$ ). Growth rates were significantly correlated to initial densities of pico-phytoplankton (Pearson  $r^2 = 0.58$ ,  $P < 0.001$ ). Ciliate growth rates were highest during the warmest months. Dinoflagellates showed negative rates of increase in most months except May, June, and July (Table 4), while copepod nauplii had negative growth rates during this same period. Overall, grazing mortality rate coefficients ( $g$ ) increased significantly with instantaneous phytoplankton growth ( $k$ ) (Pearson  $r^2 = 0.84$ ,  $P < 0.001$ ).

## Discussion

### Importance of grazing in the Suwannee estuary

High densities of microzooplankton in the Suwannee River estuary have a significant influence on phytoplankton standing crops, with experimentally derived grazing loss rates of up to 76% per day. The ranges for maximum phytoplankton growth ( $k$  values from 0.4 to 2.7  $d^{-1}$ ) and grazing ( $g$  values from 0.12 to 1.4  $d^{-1}$ ) observed in this study are within the ranges reported for other estuarine/nearshore around the world (Murrell et al., 2002). However, the potential impact of grazing on phytoplankton biomass in situ may be underestimated by the experimentally derived values. In the experiments, the phytoplankton communities were maintained under conditions of surplus nutrient

and light, yet, phytoplankton growth in the Suwannee River estuary is often subject to nutrient limitation (Table 2). In addition, ambient light availability in the Suwannee River estuary can fall below levels needed to saturate phytoplankton growth, due to sediment resuspension or introduction of high levels of colored dissolved organic matter (CDOM) from the river (Bledsoe & Philips, 2000). All of these factors can reduce rates of phytoplankton growth, thereby increasing the potential impact of grazing on phytoplankton biomass (Berges & Falkowski, 1998). In addition, benthic invertebrates (e.g., *Crassostrea virginica* (Gmelin) and *Mercenaria mercenaria* (Linnaeus), which are commonly found in the estuary, are likely to contribute to the loss of phytoplankton standing crop. Møhlenberg (1995) found reduced phytoplankton biomass in the lower water column related to mussel beds in the Roskilde Fjord, Denmark. Similar findings were reported for San Francisco Bay, USA (Cloern, 1982).

While the results of this study indicate that micro-zooplankton grazing has the potential to significantly impact phytoplankton biomass in the Suwannee River estuary, the specific magnitude of the effect is dependent on a range of physical, chemical, and biological conditions that regulate phytoplankton growth rates, as well as zooplankton growth and grazing rates. It is also clear from other studies that different species of phytoplankton may be more or less sensitive to grazing pressures, leading to periodic blooms of grazing-resistant species, such as toxic dinoflagellates (Turner, 2006; Philips et al., 2006; Badyalak and Philips, 2008).

### The importance of ciliates in top-down control of phytoplankton

The composition of the micro-zooplankton community of the Suwannee River estuary was dominated by ciliates, hetero/mixotrophic dinoflagellates, and nauplii, which accounted for up to 99% of total abundance (Fig. 3). Total ciliate abundance (9,400–72,800  $l^{-1}$ ) was exceptionally high by comparison to several well-studied marine ecosystems, such as Narragansett Bay, USA (5–2,800  $l^{-1}$ ; Verity, 1986), the Mediterranean (200–20,000  $l^{-1}$ ; Sherr et al., 1989), Oslofjord, Norway (2,000–10,500  $l^{-1}$ ; Paasche & Kristiansen, 1982), and the California Current (2,400–18,000  $l^{-1}$ ; Beers & Stewart, 1971). The annual mean abundance of ciliates



within the Suwannee River estuary ( $53,000\text{ l}^{-1}$ ) is comparable to densities observed in eutrophic environments such as Dalnee Lake, USSR ( $18,000\text{--}42,000\text{ l}^{-1}$ ; Sorokin & Paveljeva, 1972), Damariscotta estuary, USA ( $20\text{--}46,000\text{ l}^{-1}$ ; Sanders, 1987), eutrophic Florida lakes (annual mean  $55,000\text{ l}^{-1}$ ; Beaver & Crisman, 1982), and Tokyo Bay, Japan ( $10,000\text{--}100,000\text{ l}^{-1}$ ; Kume, 1979).

High ciliate abundance in blackwater systems of Florida has been linked to high dissolved organic carbon (Beaver & Crisman, 1982; Findlay et al., 1986). Similarly, Carlough & Meyer (1989) reported that ciliate concentrations in the Ogee River of Georgia are correlated to dissolved-organic carbon and river discharge. Colored dissolved organic matter in the Suwannee River is derived from the leaching of humic substances from the soils within the watershed. While CDOM levels in the Suwannee River estuary are often high, they are not correlated to ciliate densities. High CDOM levels within the Suwannee River are often correlated with higher riverine flow conditions, limiting the residence time needed for the increase in ciliates numbers in the river, and increasing the flushing out of marine ciliates from the estuary. Microzooplankton density (including ciliates) decreased in July and August 2001, a period of high river discharge, as indicated by the low salinities. The latter observation suggests that hydrodynamic conditions in the Suwannee River influence microzooplankton composition and grazing pressure on the phytoplankton community.

The high densities of ciliates in the Suwannee River estuary may be a function of the food-web structure and prey availability or prey-size preference. Hansen et al. (1994) showed that planktonic predators have a range of prey-size preferences. A significant amount of the microzooplankton community within the Suwannee River estuary has an ESD of  $<25\text{ }\mu\text{m}$ . Based on the composition of the microzooplankton community in the Suwannee River, the preferred prey-size probably falls within the  $<5$  to  $<25\text{ }\mu\text{m}$  size class (Hansen et al., 1994), which includes smaller phytoplankton, such as pico-cyanobacteria, pico-chlorophytes, and  $5\text{--}10\text{ }\mu\text{m}$  centric diatoms. Many ciliates and heterotrophic flagellates prefer to consume pico-cyanobacteria rather than bacteria (Callieri et al., 2002). This differentiation is likely due to the larger average cell volume of picoplankton compared to the average cell volume of

pelagic bacteria (Weisse and Kenter, 1991). This is of particular importance to the Suwannee River estuary where the phytoplankton community is regularly dominated by pico-cyanobacteria and small spherical green algae ( $2\text{--}4\text{ }\mu\text{m}$ ), particularly during warmer months (Quinlan & Phlips, 2007). High densities of microzooplankton may produce an increase in the grazing pressure on the smaller size fraction of phytoplankton. This relationship appears evident as pico-cyanobacteria growth rates were low to negative from May to September 2001 (Table 4).

Grazing pressure on small phytoplankton may be further enhanced because ciliates and small dinoflagellates have growth rates similar to those of phytoplankton and therefore can keep up with surges in abundance (Montagnes, 1996). Ciliate and dinoflagellate growth rates, in addition to initial densities, increased with increasing concentrations of diatoms, picoplankton, and phytoflagellates in this study, suggesting a response by the ciliates to increasing prey availability. In contrast, when ciliate densities were lowest, growth rates of picoplanktonic cyanobacteria and chlorophytes were positive. Similar responses have been observed in the tropical Pacific (Beers & Stewart, 1971) and in experimental manipulations (Fulton, 1984).

Ciliates may also be an important link in the incorporation of carbon from picoplankton community to higher trophic levels. For example, ciliates feeding on pico-phytoplankton are utilizing a prey source that is difficult for larger grazers (i.e., copepods) to consume (Nival & Nival, 1976; Porter et al., 1979), thereby linking the most numerically abundant food supply in the Suwannee River estuary, picoplankton, to higher trophic levels (Gifford & Dagg, 1988; Sherr et al., 1989; Pierce & Turner, 1992). The importance of ciliates as prey extends to the benthic invertebrate community in the Suwannee River estuary. Many adult bivalves do not efficiently retain pico-phytoplankton ( $<2\text{ }\mu\text{m}$ ) as an energy source (Pouvreau et al., 1999). However, LeGall et al. (1997) reported significant retention and ingestion of ciliates by oysters, supporting the role of protozoa as a realistic trophic link between pico-phytoplankton and higher organisms. Future studies within the Suwannee River estuary may further elucidate the trophic link between the phytoplankton, microzooplankton, and benthic bivalve community.

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