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Size-Dependent Top-Down Control On Phytoplankton Growth By Microzooplankton In Eutrophic Lakes

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Size-dependent top-down control on phytoplankton growth by microzooplankton in eutrophic lakes

Wai Hing Wong · Nancy N. Rabalais ·
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Abstract We hypothesized that the grazing on phytoplankton by the microzooplankton community is size-dependent and, therefore, the top-down control on phytoplankton by microzooplankton community could be one possible mechanism explaining why small phytoplankton become less abundant than large phytoplankton in eutrophic waters. We tested this hypothesis using the dilution method to measure microzooplankton grazing rates and phytoplankton growth rates in the eutrophic waters of the Barataria estuary, southeastern Louisiana. Microzooplankton grazing rates on the slower growing, small phytoplankton ($<5\ \mu\text{m}$) were higher than on the large phytoplankton ($>20\ \mu\text{m}$) which had relatively faster growth rates. The proportional loss of the small,

medium, large phytoplankton, and total phytoplankton community by microzooplankton grazing was 44, 53, 0, and 29%, respectively. The relative weakness of top-down grazing control on large phytoplankton by microzooplankton, and the relatively fast growth of large phytoplankton, may be why the average size of phytoplankton, whether isolated cells or colonies, tend to increase in these eutrophic waters and elsewhere.

Keywords Microzooplankton · Grazing · Phytoplankton growth · Eutrophication

Introduction

Although small phytoplankton, such as picoplankton, may be quantitatively important in some productive waters (Carrick & Schelske, 1997), many studies have demonstrated that phytoplankton size structure shifts from smaller cells to larger-sized phytoplankton and/or colonies when waters become eutrophic (Watson & Kalff, 1981; Harris, 1986; Duarte et al., 1990; Chisholm, 1992; Duarte & Canfield, 1992; Gin et al., 2000). The results from experiments suggest that there is a physiological basis for this pattern (Legendre et al., 1993; Tamigneaux et al., 1999). Cermeño et al. (2005), for example, demonstrated that both light-saturated, chlorophyll-specific photosynthesis, and the maximum photosystem (II) photochemical efficiency, were significantly higher in the $>20\ \mu\text{m}$ size class than in smaller size classes (<5 , and $5\text{--}20\ \mu\text{m}$). This result

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may explain why large phytoplankton become dominant in eutrophic waters (Cerniño et al., 2005), but more work remains to be done (Raven, 1998). For example, the relationships between grazer and prey may also be an important influence on the size structure of phytoplankton communities (Thatcher et al., 1993; Haga et al., 1995). Microzooplankton grazers (<200 µm) can quickly respond to changes in the abundance of their phytoplankton prey, and thereby contribute to the dynamic equilibrium in the biomass of each prey (Fenchel, 1982; Sherr & Sherr, 1984; Goldman & Caron, 1985). It is recognized that the active grazing by microzooplankton in marine systems is a common phenomenon that plays a more crucial role than mesozooplankton (>200 µm) grazing in structuring the whole phytoplankton community, even though phytoplankton are the most important dietary component of mesozooplankton (Strom et al., 2001; Calbet & Landry, 2004; Liu et al., 2014). Meanwhile, based on estimation of metabolic rates (i.e., feeding and growth) of individual zooplankton species, it has been found that the grazing efficiency of different groups (i.e., nano-, micro-, and mesozooplankton) is different (Hansen et al., 1997). However, the size selectivity data are collected based on individual species under laboratory conditions (Hansen et al., 1997 and references therein). In the present study, we hypothesized that the grazing on phytoplankton by the microzooplankton community is size-dependent and, therefore, the top-down control on phytoplankton by microzooplankton community could also be a possible mechanism explaining why small phytoplankton become less abundant than large phytoplankton in eutrophic/hypereutrophic waters. We tested this hypothesis by making seasonal estimates of microzooplankton grazing rates and phytoplankton growth rates in three eutrophic freshwater and brackish water lakes in southeastern Louisiana, USA.

Materials and methods

Sampling stations

We collected water over four seasons for plankton samples from three shallow (<2.5 m) coastal lakes (Lake Cataouatche, Lake Salvador, and Lac des Allemands, Fig. 1) in the upper Barataria estuary of

southeastern Louisiana (Table 1). The locations for water collection were 29°49.730N and 90°11.000W in Lake Cataouatche, 29°45.990N and 90°10.990W in Lake Salvador, and 29°53.580N and 90°34.750W in Lac des Allemands (Fig. 1). The brackish lakes Salvador and Cataouatche, whose salinities are occasionally about 7.6 psu (Ren et al., 2009), are influenced by microtidal exchange with the lower Barataria Bay and Gulf of Mexico (i.e., the tidal change is not as significant as the typical spring and neap tides). These lakes are intermittently influenced by flow from the U.S. Army Corps of Engineers Mississippi River water diversion at Davis Pond that enters Lake Cataouatche on the north, and that flows into Lake Salvador to the south. Lac des Allemands is a freshwater lake with no influence from the Mississippi River diversion, and has chlorophyll *a* concentrations that occasionally rise above 200 µg l⁻¹ (Table 2). Lake water salinity, dissolved oxygen, and temperature were measured with a Hydrolab H₂O Surveyor 3 (Hydrolab-Hach Company, Loveland, Colorado; Table 1). A minimum of one hundred L of water from each lake was collected at 20–30 cm below the water's surface at 16:30 h, carefully poured into 75 l carboys so as not to damage the delicate microzooplankton, and transported to a laboratory by 18:30 h.

Microzooplankton sampling and counting

We collected zooplankton samples with a 0.34 m diameter × 2.5 m long, 37 µm mesh plankton net towed 5 cm below the water's surface for 30–90 s (the tow length depended on the water turbidity) (Pagano & Saintjean, 1989; Liu & Dagg, 2003). The volume of water filtered through the net was measured with a calibrated General Oceanics flowmeter mounted in the net opening. The towing distance and volume filtered were calculated based on the equations provided by General Oceanics, where

$$\text{Distance} = (\text{No. of rotations of flowmeter}) \times \text{Rotor constant}/999,999$$

and,

$$\text{Water volume} = \text{Distance} \times \pi \times (\text{radius of net})^2.$$

Two aliquots of plankton net-filtered water samples were collected at four sites from each of the three lakes in October 2003, January to February 2004, March to

Fig. 1 Locations of Lake Cataouatche, Lake Salvador, and Lac des Allemands in the upper Barataria estuary

Table 1 Surface water characteristics of the lakes and experimental incubators for the twelve experiments

Season/date	Water body	Lake temperature (°C)	DO (mg l ⁻¹)	Salinity (psu)	Incubator temperature (°C)
Autumn					
10/13/2003	Lake Cataouatche	25.4	9.0	0.7	N/A
10/21/2003	Lake Salvador	N/A	N/A	N/A	23.0
10/28/2003	Lac des Allemands	21.7	9.8	0.1	21.5
Winter					
01/12/2004	Lake Cataouatche	11.8	14.6	0.4	11.5
01/20/2004	Lake Salvador	12.5	10.5	0.7	12.0
02/03/2004	Lac des Allemands	12.0	11.9	0.1	12.0
Spring					
03/30/2004	Lake Cataouatche	23.3	9.6	0.3	22.0
04/05/2004	Lake Salvador	21.7	11.0	0.3	21.0
04/12/2004	Lac des Allemands	21.0	7.8	0.1	20.0
Summer					
06/28/2004	Lake Cataouatche	N/A	N/A	N/A	31.0
07/05/2004	Lake Salvador	31.2	7.8	0.1	31.0
07/11/2004	Lac des Allemands	29.7	7.5	0.2	30.5

N/A no data available

April 2004, and June to July 2004. One aliquot was preserved with formalin (final concentration 2%) to quantify the density of small rotifers (<202 µm) (Garza-Mourino et al., 2005). The other aliquot was preserved with Lugol's (final concentration 3%) and used to enumerate ciliates (Bulit et al., 2003). A 10 ml subsample was placed in an Utermöhl sedimentation chamber to settle for 8–12 h before being counted with

an inverted microscope (Nikon-TMD 300) at ×200 or 400 magnification. The abundance of microzooplankton was only estimated as the sum of the density of all small rotifers + ciliates (<202 µm), because smaller microzooplankton such as heterotrophic dinoflagellates and small ciliates (<37 µm) were not counted, and because nauplii (usually >202 µm), large rotifers, and mesozooplankton were removed. Water sample

Table 2 Chlorophyll *a* content ($\mu\text{g l}^{-1}$) and microzooplankton density (individual l^{-1} ; mean $\pm$ 1 SD) in the lakes by season

Season	Lake Cataouatche	Lake Salvador	Lac des Allemands
Chlorophyll <i>a</i>			
Autumn 2003	7.41 $\pm$ 0.14	3.56 $\pm$ 0.07	18.13 $\pm$ 0.28
Winter 2004	47.43 $\pm$ 5.27	7.61 $\pm$ 0.42	184.92 $\pm$ 2.47
Spring 2004	6.76 $\pm$ 0.00	24.23 $\pm$ 1.41	17.12 $\pm$ 0.21
Summer 2004	8.92 $\pm$ 0.19	6.76 $\pm$ 0.11	26.91 $\pm$ 0.28
Average	17.63	10.54	61.77
Microzooplankton			
Autumn 2003	2032.6 $\pm$ 743.1	1158.7 $\pm$ 690.8	5187.5 $\pm$ 1553.9
Winter 2004	2188.2 $\pm$ 272.0	2522.9 $\pm$ 496.8	4125.4 $\pm$ 1438.2
Spring 2004	4248.5 $\pm$ 1495.0	2340.0 $\pm$ 658.6	3829.2 $\pm$ 735.6
Summer 2004	974.7 $\pm$ 282.5	2352.0 $\pm$ 519.8	4877.3 $\pm$ 829.5
Average	2361.0	2093.4	4504.9

were screened with a 202 μm mesh before being used in the microzooplankton grazing experiments and the microzooplankton density was determined. The samples were diluted before being placed in Utermöhl sedimentation chambers if the microzooplankton density was too high to count.

Microzooplankton grazing experiments

We determined microzooplankton grazing on phytoplankton and phytoplankton growth by using the dilution method (Landry & Hassett, 1982) in twelve microzooplankton grazing experiments conducted from Autumn 2003 to Summer 2004. All experimental bottles, tubing and other containers were washed with 10% HCl and rinsed twice with distilled and de-ionized water before use. Water samples were slowly and carefully screened with a 202 μm mesh net under gravity to remove mesozooplankton. The dilution medium was prepared from screened water (40 l) that was pre-filtered with a peristaltic pump (Millipore, MA, USA) through a GF/F filter (0.5 μm), and then gravity-filtered with a 0.2 μm in-line AcroPakTM 500 filter capsule (Pall Corporation, Ann Harbor, Michigan). Screened water (202 μm) was diluted with the dilution medium and put into plastic containers to create the following concentration of the original unfiltered water: 6.25, 12.5, 25, 50, and 100%. Inorganic nutrients—N (NO_3^-), P (H_2PO_4^-), and Si (SiO_4^{2-}) at Redfield Ratios (molar ratio = N:P:Si::16:1:16)—were added to each container in sufficient amount to support doubling the initial chlorophyll *a* concentration in 2 days (Table 3 in Ren et al., 2009), so that no nutrient deficiencies would

occur during incubations. A sixth container was filled with 100% lake water that had no nutrient additions and was screened through a 202 μm mesh. The four experimental replicates were then dispensed into narrow-mouth 2 l polycarbonate (NALGENE) bottles, sealed without air bubbles, and randomly placed in a large outdoor Plexiglas incubator (length $\times$ width $\times$ depth = 1.45 $\times$ 0.76 $\times$ 0.38 m^3) set up on the Louisiana Universities Marine Consortium dock. Near-surface marina waters were pumped through this incubator to maintain ambient temperature. There were no differences in water temperature between the incubator and the lakes (Table 1, paired *t* test, $P > 0.05$, $n = 9$). One layer of neutral screen was applied to reduce the light level to that at 0.5 m below the water surface. The flowing water maintained the 2 l containers in continuous suspension, but they were also physically and randomly relocated every 4 h during the 24 h incubations.

Three to 250 ml of each dilution were filtered at 0 and 24 h using a 20 μm polycarbonate filter (Osmonics), a 5 μm polycarbonate filter (Whatman), and a GF/F glass fiber filter (Whatman, 0.7 μm). The volume filtered depended on the chlorophyll *a* concentration and the size of the filter paper being used. The filters were extracted with DMSO in a 40:60 mixture with 90% acetone for 10–12 h, and the fluorescence of the extract was measured with a Turner Design fluorometer (10-AU, Sunnyvale, California) before and after acidification. The chlorophyll *a* concentrations from the three size-fractionated phytoplankton (<5, 5–20, and >20 μm) were calculated. Total content of chlorophyll *a* of two raw water

samples from each lake in each season was also quantified (Table 2).

We followed established protocols to calculate phytoplankton growth rates and zooplankton grazing rates on phytoplankton. Nutrient-amended dilution treatments were used to calculate zooplankton grazing rates, and treatments without nutrient addition were used to estimate phytoplankton growth rate (Landry et al., 1998; Calbet & Landry, 2004). The phytoplankton apparent growth rate (k day⁻¹) was calculated as the variation of chlorophyll *a* concentration during the 24 h incubation, $k = \ln(P_t/P_0)$, where P_0 (μg l⁻¹) is the initial chlorophyll *a* content, and P_t (μg l⁻¹) is the chlorophyll *a* content at the end of the 24 h incubation. The phytoplankton grazing mortality rate (m : day⁻¹) was calculated by linear regression of the apparent growth rate (k_n) versus the dilution factor (D : fraction of undiluted water) from the nutrient-enhanced dilution treatments. The instantaneous growth rate (μ₀ day⁻¹) without nutrient addition was estimated as:

$$\mu_0 = k_0 + m,$$

where k_0 is the apparent growth rate of phytoplankton in the treatments without nutrient additions. When saturation grazing occurred (Gallegos, 1989), k_n from greater than half dilution treatments ($D < 0.5$) were used in the linear regression to obtain μ_n , the phytoplankton instantaneous growth rate under the condition of nutrient enrichment (Redden et al., 2002). Microzooplankton grazing mortality rate (m) was therefore calculated as

$$m = \mu_n - k_n$$

(Liu & Dagg, 2003). Some negative estimates are expected because of photoacclimation responses of phytoplankton to experimental incubation conditions or because day-to-day variations in light levels might produce offsetting positive and negative errors in the growth rate estimates (Calbet & Landry, 2004). A negative instantaneous growth rates (μ₀), occurring mostly between -0.01 and 0 day⁻¹, was changed to +0.01 day⁻¹ to facilitate the calculations (Calbet & Landry, 2004).

Four sediment samples were taken from each lake in late summer 2004 using an Ekman grab. The surface sediment from each sample was carefully transferred into a glass vial and extracted with 90% acetone. The content of chlorophyll *a* and phaeopigments in the

sediments was done using the acidification method described earlier and a Turner Design fluorometer. The dry weight of the sediment was estimated after being dried at 60°C for 24 h. The overlying water samples for each sediment sample were also collected and extracted with 90% acetone to measure the content of chlorophyll *a* and phaeopigments.

Results

Chlorophyll *a* content of different size-fractionated phytoplankton

The chlorophyll *a* concentration (μg l⁻¹) ranged from 6.8 to 47.4 in Lake Cataouatche, 3.6–24.2 in Lake Salvador, and 17.1–184.9 in Lac des Allemands (Table 2). The annual average chlorophyll *a* concentration (μg l⁻¹) was 10.5 in Lake Salvador, 17.6 in Lake Cataouatche, and 61.8 in Lac des Allemands. A two-way analysis of variance demonstrated that there was a significant difference in chlorophyll *a* concentration among the three lakes ($P < 0.05$). The highest concentration was in winter, the lowest in autumn, and there was no significant difference in the chlorophyll *a* concentration between spring and summer. Small phytoplankton were more abundant when the chlorophyll *a* concentration was lower, and the percentage of medium and large-sized phytoplankton increased as chlorophyll *a* concentration increased (Fig. 2). There was a significant non-linear relationship between the contribution of each size group (measured as %) and the total chlorophyll *a* concentration (μg l⁻¹) (Fig. 2).

Microzooplankton grazing and phytoplankton growth

Rotifers are the dominant microzooplankton in south-east Louisiana brackish waters. Stone et al. (1980), for example, found 67 species of rotifers in Lake Pontchartrain and these same rotifers were also the dominant taxon in our study. The major microzooplankton found in the lakes we sampled were rotifers, such as *Polyarthra* sp., *Branchionus* sp., *Keratella* sp., *Filinia longiseta* (Ehrenberg, 1834), and ciliates. Although the raw water (screened with 202 μm mesh) was used for the microzooplankton grazing experiment, it is possible that smaller ciliates <37 μm were strained from the sample because of the large mesh

Fig. 2 Relationship between total phytoplankton chlorophyll *a* content and percentage of size-fractionated phytoplankton; the following regressions fit the curves of phytoplankton <5 µm, 5–20 µm, and >20 µm: $Y = -11.58 \ln(X) + 76.94$, $R = 0.68$,

$P < 0.01$; $Y = 7.33 \ln(X) + 21.12$, $R = 0.64$, $P < 0.01$; $Y = 4.25 \ln(X) + 1.93$, $R = 0.48$, $P < 0.05$. The X-axis is the total chlorophyll *a* content (µg l⁻¹); the Y-axis is the % of total chlorophyll within each size class

size (37 µm) used for the microzooplankton collection and quantification. An analysis of variance showed that Lac des Allemands had a higher concentration of microzooplankton than the other two lakes ($P = 0.001$), but that there was no seasonal differences in microzooplankton abundance ($P = 0.75$; Table 2). Among the three lakes, the microzooplankton grazing rates were highest in Lake Salvador (Table 3), which also had the lowest chlorophyll *a* concentration (Table 2). There was no grazing in the hypereutrophic Lac des Allemands during the winter or summer when the chlorophyll *a* biomass was 184.9 ± 2.5 and 26.9 ± 0.3 µg l⁻¹, respectively (mean $\pm$ 1 SD). There was no significant relationship found between either microzooplankton grazing and microzooplankton abundance or between microzooplankton grazing and chlorophyll *a* content (either by size fraction or total phytoplankton).

The instantaneous phytoplankton growth rates increased with phytoplankton size, but not significantly, while the apparent growth rate of phytoplankton >20 µm was greater than those of both the <5 µm and the 5–20 µm size fractions (Table 4). The microzooplankton grazing rate was higher on small phytoplankton and decreased when phytoplankton size increased (Table 4). The variability in grazing rates

was high in all four seasons. We determined the relationship between microzooplankton grazing and phytoplankton growth and found that the proportional loss of the small, medium, and large phytoplankton, and the total phytoplankton community by microzooplankton grazing was 44, 53, 0, and 29%, respectively.

Discussion

Based on the chlorophyll *a* concentrations in Lake Cataouatche, Lake Salvador, and Lac des Allemands, the trophic status of these three lakes was mostly meso- to hypereutrophic (Bricker et al., 2003). The nutrient loading to the three lakes includes loading from local runoff and atmospheric deposition. Fertilizer runoff from sugar cane cropland surrounding Lac des Allemands is a likely source of the nutrients stimulating chlorophyll *a* accumulation. The primary nutrient source for Lake Cataouatche is from the Davis Pond Diversion, which receives water from a Mississippi River diversion. Some of this diverted water enters Lake Salvador. The Atchafalaya River is a source of some water brought to Lake Salvador via the Intracoastal Waterway, which is located on its southwestern and southeastern border.

Table 3 Microzooplankton grazing rates on phytoplankton (day^{-1}) determined using the dilution method ($n = 24$ for each experiment) in the lakes by season

Water body/season/phytoplankton size	Linear regression equation	<i>R</i>	<i>P</i> value	Grazing rate (day^{-1})
Lake Cataouatche				
Autumn 2003				
<5 μm	$y = -0.33x + 0.72$	0.69	<0.01	0.33
5–20 μm	*			0.00
>20 μm	*			0.00
Total phytoplankton	$y = -0.07x + 0.88$	0.44	<0.01	0.07
Winter 2004				
<5 μm	$y = -0.57x + 0.35$	0.77	<0.01	0.57
5–20 μm	*			0.00
>20 μm	*			0.00
Total phytoplankton	$y = -0.22x + 0.34$	0.84	<0.01	0.23
Spring 2004				
<5 μm	**			0.23
5–20 μm	**			0.67
>20 μm	*			0.00
Total phytoplankton	*			0.00
Summer 2004				
<5 μm	*			0.00
5–20 μm	$y = -0.90x + 1.84$	0.84	<0.01	0.91
>20 μm	*			0.00
Total phytoplankton	**			0.04
Lake Salvador				
Autumn 2003				
<5 μm	$y = -0.85x + 0.85$	0.87	<0.01	0.85
5–20 μm	$y = -0.34x + 1.02$	0.74	<0.01	0.34
>20 μm	$y = -0.19x + 0.10$	0.44	<0.01	0.19
Total phytoplankton	$y = -0.63x + 0.89$	0.89	<0.01	0.63
Winter 2004				
<5 μm	$y = -0.74x + 0.30$	0.84	<0.01	0.74
5–20 μm	*			0.00
>20 μm	$y = -0.18x + 0.43$	0.55	<0.01	0.18
Total phytoplankton	$y = -0.16x + 0.26$	0.69	<0.01	0.16
Spring 2004				
<5 μm	$y = -0.46x + 0.47$	0.52	<0.01	0.46
5–20 μm	*			0.00
>20 μm	$y = -0.64x + 0.89$	0.54	<0.01	0.64
Total phytoplankton	$y = -0.44x + 0.97$	0.85	<0.01	0.44
Summer 2004				
<5 μm	$y = -0.56x + 1.30$	0.95	<0.01	0.56
5–20 μm	$y = -0.37x + 1.63$	0.81	<0.01	0.37
>20 μm	$y = -0.44x + 1.39$	0.73	<0.01	0.44
Total phytoplankton	$y = -0.47x + 1.39$	0.95	<0.01	0.47

Table 3 continued

Water body/season/phytoplankton size	Linear regression equation	<i>R</i>	<i>P</i> value	Grazing rate (day ⁻¹)
Lac des Allemands				
Autumn 2003				
<5 µm	$y = -0.18x + 0.74$	0.49	<0.01	0.18
5–20 µm	$y = -0.36x + 0.85$	0.85	<0.01	0.36
>20 µm	$y = -0.37x + 0.73$	0.54	<0.01	0.37
Total phytoplankton	$y = -0.23x + 0.75$	0.87	<0.01	0.23
Winter 2004				
<5 µm	*			0.00
5–20 µm	*			0.00
>20 µm	*			0.00
Total phytoplankton	*			0.00
Spring 2004				
<5 µm	$y = -0.31x + 0.15$	0.84	<0.01	0.31
5–20 µm	*			0.00
>20 µm	*			0.00
Total phytoplankton	$y = -0.14x + 0.12$	0.75	<0.01	0.14
Summer 2004				
<5 µm	*			0.00
5–20 µm	*			0.00
>20 µm	*			0.00
Total phytoplankton	*			0.00

* No grazing impact was found

** Grazing saturation was found

Table 4 Average microzooplankton grazing ($n = 12$) and phytoplankton growth rate ($n = 48$) in the upper Barataria estuary (mean $\pm$ 1 SE)

Phytoplankton size	Microzooplankton grazing rate, m (day ⁻¹)	Phytoplankton instantaneous growth rate, μ_0 (day ⁻¹)	Phytoplankton apparent growth rate, k_0 (day ⁻¹)
<5 µm	0.35 $\pm$ 0.09	0.17 $\pm$ 0.06	-0.24 $\pm$ 0.06 ^a
>5 to <20 µm	0.22 $\pm$ 0.09	0.28 $\pm$ 0.06	0.06 $\pm$ 0.05 ^a
>20 µm	0.15 $\pm$ 0.07	0.33 $\pm$ 0.06	0.21 $\pm$ 0.06 ^b
Total phytoplankton	0.20 $\pm$ 0.06	0.26 $\pm$ 0.03	0.01 $\pm$ 0.04

Data with different letters in the same column means that there are significant differences (ANOVA, $P < 0.05$)

The growth data indicate that large phytoplankton outpace the growth of small phytoplankton in these eutrophic waters. As a result, the total phytoplankton chlorophyll *a* content increased as the percentage of small phytoplankton (<5 µm) decreased logarithmically, while the percentage of large phytoplankton

(>20 µm) and medium phytoplankton (5 < 20 µm) increased logarithmically. These results are consistent with the observation that long-chain diatoms and colonial cyanobacterial blooms occur on a regular basis in these lakes (Rabalais, 2005). Others have also reported that there is a tendency toward replacement of

small taxa by large taxa as phytoplankton biomass increases, or a tendency toward an increased size of particular taxa (Duarte et al., 1990; Duarte & Canfield, 1992). The causes of these size distributions could be the result of micro- or mesoplankton grazing, benthic filter-feeders, size-selective sedimentation, or different photosynthesis rates of different size classes of phytoplankton (Cermeño et al., 2005).

The microzooplankton community in these lakes is composed mainly of planktonic rotifers (42–202 μm), copepod nauplii, and a few ciliates. Hansen et al. (1997) summarized the size selectivity spectra of multiple individual zooplankton predators collected from feeding studies and concluded that the linear size ratio between the suspension filter-feeding rotifers and their optimal prey is 18:1. We used this ratio and determined that the optimum size for rotifer prey would be between 2.3 and 11.2 μm , which is in the size range of the phytoplankton group mostly impacted by microzooplankton grazing demonstrated in our present study. Although the data used by Hansen et al. (1997) were mostly collected using laboratory cultured zooplankton predators and preys (microalgae), our experiment using the undivided microzooplankton community provides solid evidence that microzooplankton grazing on phytoplankton is size-dependent. Cermeño et al. (2005) discovered that the higher photochemical efficiency of phytoplankton $>20 \mu\text{m}$ is a key reason why large phytoplankton are dominant in eutrophic waters. The present research demonstrates that the stronger top-down control on smaller phytoplankton, but weaker impact on larger phytoplankton by microzooplankton community grazing, could also be a possible mechanism explaining why small phytoplankton become less abundant than large phytoplankton in eutrophic/hypereutrophic waters. The data interpretation seems appropriate for the range of chlorophyll *a* values examined (annual average from 10.5 to 61.8 μg chlorophyll *a* l^{-1}), but may not be appropriate for situations with lower chlorophyll *a* concentrations, in colder climates, or in situations with different turbidity levels, etc.

An average of 67 and 60% of the phytoplankton growth in marine and coastal systems, respectively, is controlled by microzooplankton grazing (Calbet & Landry, 2004). The grazing on the total phytoplankton in our present experiment is less than half of these rates (Table 4). Phytoplankton cells generally decrease in size from inshore to offshore (Mouw & Yoder, 2005),

and the phytoplankton in the open ocean are usually consist of picophytoplankton that dominate chlorophyll biomass (Odate, 1996). Because small phytoplankton in Lake Salvador are more dominant than in Lake Cataouatche or Lac des Allemands, the impact of grazing from microzooplankton in Lake Salvador was more significant than in the other two lakes. The majority of phytoplankton in Lake Cataouatche and Salvador were diatoms at most times, while cyanobacteria were the dominant group with 56% in autumn 2003 and up to 96% in other seasons (Ren et al., 2009). This difference may also partially explain the limited effect of microzooplankton grazing in Lac des Allemands, especially in winter, spring, and summer, because cyanobacteria are usually not preferred food items by many zooplankton groups such as rotifers (Sellner et al., 1993). Nonetheless, our study provides direct evidence that, in these eutrophic waters, microzooplankton grazing provides little top-down control of phytoplankton growth, and almost no control on the largest phytoplankton (Table 4). According to Cermeño et al. (2005), large phytoplankton have a higher maximum photosynthetic efficiency among different size-fractionated phytoplankton. In addition, because smaller microzooplankton such as flagellates and small ciliates ($<37 \mu\text{m}$) were not quantified in the study, the role of these grazers should be evaluated in these eutrophic waters in the future (Nejstgaard et al., 2001).

The mesozooplankton in the three lakes we studied was composed mainly by *Acartia tonsa* Dana, 1849, *Eurytemora affinis* (Poppe, 1880), *Daphnia lumholtzi* G.O. Sars, 1885, large rotifers ($>202 \mu\text{m}$), and large copepod nauplii ($>202 \mu\text{m}$). However, two experiments on mesozooplankton grazing conducted in summer 2005 did not show a significant impact on

Fig. 3 The relationship between the amount of chlorophyll *a* per microzooplankton ($\text{pg chlorophyll } a \text{ no}^{-1}$) in all three lakes. The data are from Table 2

Fig. 4 Relationship between water chlorophyll *a* content and sediment chlorophyll *a* (open symbols) and phaeopigment content (dark symbols) (microgram per gram dry weight of sediment) in Lac des Allemands, Lake Salvador, and Lake

Cataouatche. The regression line (dotted line) for chlorophyll *a* is $Y = 0.63X - 2.24$ ($n = 12$, $R = 0.83$, $P < 0.001$) and the regression line (solid) for phaeopigments is $Y = 2.14X + 8.04$ ($n = 12$, $R = 0.72$, $P < 0.005$)

phytoplankton growth in Lake Salvador and Lac des Allemands (Wong et al., unpublished data). The density of zooplankton did not increase in proportion to the rise in chlorophyll *a* biomass (Fig. 3) although mesozooplankton were present in these waters, and were sometimes even abundant. This result suggests that mesoplankton were unable to compensate for higher phytoplankton growth of larger phytoplankton in the upper Barataria estuary and is consistent with what is reported in the scientific literature. For example, it appears that mesozooplankton have a minimal role in controlling total phytoplankton biomass in highly productive estuarine and coastal areas (Strom et al., 2001; Calbet et al., 2003; Chan et al., 2004; Lionard et al., 2005) and there is a decline of relative importance of mesozooplankton grazing with increasing primary production in marine ecosystems, and the effect of mesozooplankton on primary production (% consumed per day) is moderate in most of the studies (Calbet, 2001). Alternative food sources (e.g., protozoans) could represent an important component of mesozooplankton diet in unproductive ecosystems (Calbet, 2001).

Zooplankton, therefore, appear to have a limited impact on phytoplankton growth (i.e., limited impact on small phytoplankton from microzooplankton from

the present study and no effect on phytoplankton from preliminary mesozooplankton grazing study) in these lakes, and they appear to be unable to control phytoplankton there.

The other phytoplankton grazers in this system include benthic suspension feeders, such as *Rangia* clams or multiple species of freshwater sponges, that may impede the development of microalgae blooms because of their relatively high filtration rates (Buskey et al., 1997; Murrell & Hollibaugh, 1998; Abadie & Poirrier, 2000; Bologna et al., 2005; Wong et al., 2010). Losses by advection of phytoplankton biomass from these coastal lakes are minimal because there are no strong currents and water residence times are high (pers. comm., E. Swenson). Sinking may be the fate for most of the phytoplankton in these three lakes. Support for this possibility is provided by the strong relationship between the chlorophyll *a* biomass in overlying waters and the sediment chlorophyll *a* and phaeopigment concentrations in each lake in summer 2004 (Fig. 4). These experimental lakes are turbid (high chlorophyll *a* concentrations in Table 2), e.g., it is not possible to see the bottom of these shallow lakes (<2.5 m). Benthic microalgal growth in these lakes should be limited by light because the secchi disk is <1 m (Ren et al., 2009).

The relative weakness of microzooplankton top-down grazing control on the largest phytoplankton, and the relatively fast growth of large phytoplankton observed in our experiments, may be why the average size of phytoplankton tends to increase when phytoplankton become more abundant in these meso- and hypereutrophic waters, and elsewhere. Size-selective grazing of predators on prey may also drives changes in the algal size structure of oligotrophic waters, which is something to quantify with additional research, and result in quite different outcomes, or not.

Conclusions

We found that microzooplankton grazing rates on small phytoplankton ($<5\ \mu\text{m}$) were higher than on the large phytoplankton ($>20\ \mu\text{m}$) in these eutrophic waters. The proportional loss of the small, medium, large phytoplankton, and total phytoplankton community by microzooplankton grazing was 44, 53, 0, and 29%, respectively. The grazing impacts of microzooplankton on phytoplankton in eutrophic waters are size specific. The relatively strong control on smaller phytoplankton and weakness of top-down grazing control on large phytoplankton by microzooplankton, as well as the relatively fast growth of large phytoplankton, may explain why the average size of phytoplankton, whether as isolated cells or as colonies, tends to increase in eutrophic waters.

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