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Phytoplankton pigment specific growth and losses due to microzooplankton grazing in a northern Gulf of Mexico estuary during winter/fall

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GULF AND CARIBBEAN

R E S E A R C H

PHYTOPLANKTON PIGMENT SPECIFIC GROWTH AND LOSSES DUE TO MICROZOOPLANKTON GRAZING IN A NORTHERN GULF OF MEXICO ESTUARY DURING WINTER/FALL

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ABSTRACT: Microzooplankton dilution grazing experiments were carried out on 6 dates, over a 3 month period at 2 locations in the Bay of St. Louis, MS (BSL) to determine phytoplankton pigment specific growth rates under natural (μ_0) and replete (μ_n) nutrient conditions and microzooplankton grazing. We hypothesized that diatoms would be the largest portion of the phytoplankton composition due to the winter/fall season and that these organisms would have the highest growth/grazing rates. We suspected that river flow from the Jourdan River would adversely affect growth and grazing rates of all phytoplankton classes. Growth rates of 5 phytoplankton accessory pigments (peridinin, fucoxanthin, alloxanthin, zeaxanthin, chlorophyll b) were identified. Intrinsic growth rates (μ_0) were often zero or negative (range: −0.46 to 0.56/d) at the location nearest the Jourdan River, particularly for alloxanthin (e.g., cryptophytes) and peridinin (e.g., dinoflagellates). Significant grazing of chlorophyll *a* was observed on 3 of 6 dates while grazing on marker pigments was variable. The phytoplankton community appeared nutrient limited during all but one experiment ($\mu_0 < \mu_n$). Intrinsic growth and grazing rates were correlated ($p < 0.05$, Spearman Rank Order correlation). Peridinin and alloxanthin—based growth and grazing rates were positively correlated with salinity, suggesting a river influence on these 2 phytoplankton pigment classes. We conclude that in the BSL microzooplankton preferentially grazed on the phytoplankton class which had the highest intrinsic growth rate. We show that this is greatly affected by riverine input into the estuary and nutrient limitation.

KEY WORDS: Phytoplankton ecology, Subtropical Estuary, Dilution Technique, Grazing, Nutrient limitation

INTRODUCTION

The primary source of phytoplankton mortality in coastal and estuarine systems is grazing by microzooplankton (< 200 μm), which can represent an average loss of 60% of phytoplankton production (Calbet and Landry 2004). Grazing has been shown to control not only the abundance of phytoplankton in a population, but also the composition of the population through selective grazing on different phytoplankton classes (Porter 1977; Burkill et al. 1987; Strom and Welshmeyer 1991). The Landry and Hassett (1982) dilution technique is the most widely used method for the simultaneous estimation of phytoplankton growth and microzooplankton grazing rates in marine waters with minimal manipulation of the community. Application of this technique has enabled the examination of microzooplankton grazing and its impact on phytoplankton biomass and composition in a wide range of ocean systems (Calbet and Landry 2004; Schmoker et al. 2013).

The dilution technique was adapted by Burkhill et al. (1987) to give growth and grazing rates of individual phytoplankton taxa by coupling it with HPLC pigment analysis. Utilizing taxon—specific marker pigments, grazing and growth rates varied by phytoplankton taxa and were often significantly correlated, with faster growing phytoplankton classes grazed at the highest rates (Burkill et al.1987; Strom and Welschmeyer 1991; Latasa et al. 1997). There are limited data available on applications of the dilution technique in subtropical estuaries in comparison to other locations, thus representing a major knowledge gap (Schmoker et al. 2013).

Since estuaries are directly affected by urbanization, it is important to understand phytoplankton growth and losses, since nutrient loading can lead to an increase in biomass and/or blooms. Studies examining phytoplankton growth and microzooplankton grazing rates in subtropical estuaries have shown a strong top—down control of the phytoplankton community (Juhl and Murrell 2005; Palomares—García et al. 2006; Putland and Iverson 2007). In these studies, the rates of growth and grazing were of similar magnitude and microzooplankton proved to be major consumers of phytoplankton production. In other studies, growth rates were often greater than grazing rates, suggesting other factors controlled the population such as viral lysis, physical factors, and/or environmental conditions (Chevez et al. 1991; Landry et al. 1995; Murrell et al. 2002; Calbet et al. 2011; Ortmann et al. 2011).

The biological communities in the Bay of St. Louis (BSL) presented an opportunity to increase our understanding of the interaction between phytoplankton growth, microzooplankton grazing, and nutrient limitation. Recent studies indicate that the N/P ratio was lower than the Redfield ratio and given the low concentration of dissolved inorganic nitrogen (DIN), the BSL was considered nitrogen—deficient (Cai et al. 2012; Camacho et al. 2014). Dissolved inorganic nitrogen concentrations ranged from <1 μM to 12 μM and were highest during high river discharge (Sawant 2009; Cai et al. 2012; Camacho et al. 2014). Orthophosphate (PO_4^{3-}) concentrations were generally low with mean concentrations

of < 0.5 μM, and increase with increasing salinity (Phelps 1999; Sawant 2009; Cai et al. 2012).

The taxonomic composition of the phytoplankton assemblage and its relation to measured environmental parameters has been previously examined in the BSL (Holtermann 2001; Molina 2011). In Holtermann (2001), the phytoplankton was comprised of diatoms, cyanobacteria, and chlorophytes during summer and by diatoms during winter. During the winter, chlorophyll *a* (chl *a*) was dominated by diatoms. A bloom of dinoflagellates occurred during the spring and fall at the mouth of the Jourdan River (JR), while during the rest of the year dinoflagellates contributed little to total chl *a*. Molina (2011) examined one station near the mouth of the BSL, finding diatoms were the dominant taxa during the study period (September 2007 to November 2009) and there was no clear indication of any seasonal trends in composition.
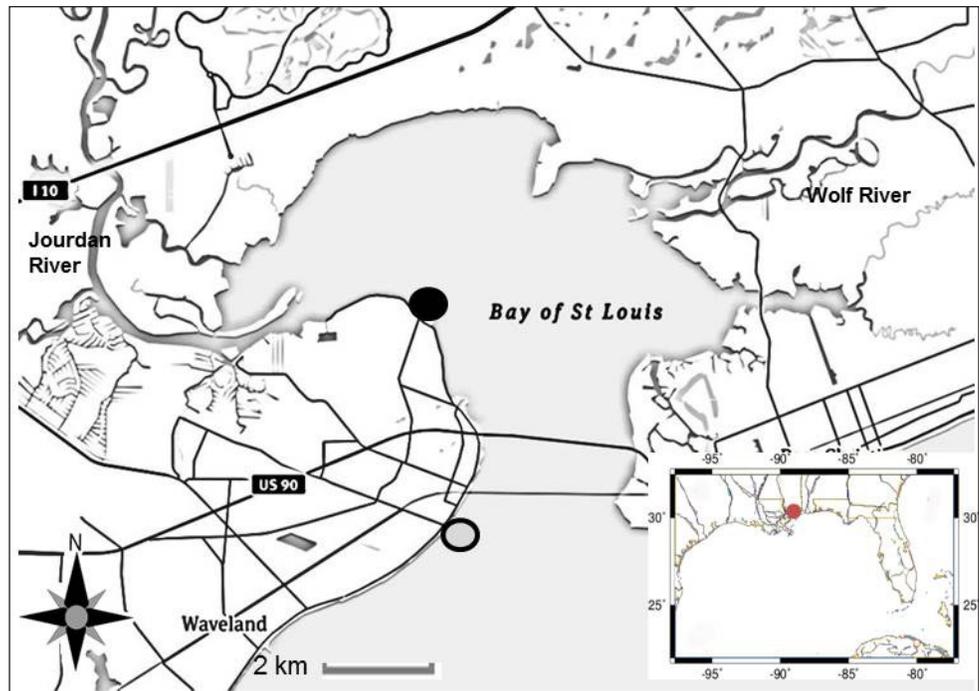

FIGURE 1. Map of the Bay of St. Louis with the location of the Dunbar Street and Washington Street piers marked (solid and open circles, respectively). Inset: location of the sampling area along the northern Gulf of Mexico.

No studies have investigated microzooplankton grazing on phytoplankton in the BSL. The purpose of this study was to fill a knowledge gap in phytoplankton ecology about the dynamics of phytoplankton growth and microzooplankton grazing in this nutrient limited subtropical estuary. We hypothesized that diatoms would be the most prevalent species during winter samplings, and that they would have high growth rates and therefore high grazing rates in the BSL. We also believe that environmental factors, particularly salinity, will affect the growth and grazing rates of all phytoplankton pigment classes.

Materials and Methods

Site Description

The BSL is a small (area = 40 km²; Eleuterius 1984), shallow (~1.5 m mean depth) semi–enclosed estuary located on the Gulf of Mexico coast of Mississippi (MS) connected to the Mississippi Sound (a large barrier island estuary that spans 145 km between MS and Alabama (AL)) through an inlet that is about 3 km wide and 300 m long (Figure 1). Two rivers provide freshwater input to the BSL: the JR to the west (historical mean discharge rate: 23.5 m³/s) and the Wolf River (WR) to the east (historical mean discharge rate: 20 m³/s; Eleuterius, 1984). The range of salinity in the BSL is 0–26 and is lower in the winter due to increased river runoff (Phelps 1999; Sawant 2009; Cai et al. 2012). Water temperature ranges from 9.9–33.2°C annually (Phelps 1999; Sawant 2009; Cai et al. 2012). Annual chl *a* concentration ranges from 0.12–56.08 μg/L (Sawant 2009).

Sample Collection and Processing

Samples were collected from the Washington Street (WS) pier located near the mouth of the bay and the Dunbar Street (DS) pier located near the mouth of the JR (Figure 1). Sampling was conducted once monthly for 3 months (November 2013 through January 2014) at each location. The WS location was sampled and processed first, the DS location was sampled 2 days later.

All incubation bottles, filtration flask, and filter holders used in the study were washed with 10% HCl and triple rinsed with nanopure water. Sampling carboys were triple rinsed with BSL water prior to filling. On sampling days, 50 L of surface water was collected and environmental parameters (temperature (°C), salinity, turbidity (formazine turbidity units, FTU)) were measured using an In–Situ® Multi–Parameter Troll 9500 WQP–100 (In–Situ Inc.) profiling device. After returning to the laboratory, the water sample was filtered through 200 μm mesh to remove the larger zooplankton and detritus.

Initial samples were taken from the carboy for analysis of pigment composition (μg/L), nutrient concentrations (μM), particulate organic carbon (POC; mg/L), and particulate nitrogen (PN; mg/L). Triplicate whole seawater samples (WSW) were prepared in 2 L trace metal–clean polycarbonate bottles (Fitzwater et al. 1982). The bottles were soaked in Micro–90 cleaning solution (Sigma Chemical Company)

for a total of 5 days, rinsed with nanopure water, soaked for 2 days in nanopure water, and finally soaked in 10% HCL to ensure removal of trace metals. A carboy containing 26 L of WSW was spiked with nutrients to a final concentration of 16 µM NO_3^- and 1.6 µM PO_4^-. Particle free seawater (PFSW) was prepared by filtering half the spiked sample through a 142 mm diameter Gelman A/E glass fiber filter followed by filtration through a 0.2 µm Whatman POLY-CAP TC filter capsule to ensure removal of all organisms (Li and Dickie 1985). The dilution series included triplicates of 100%, 70%, 40%, and 10% WSW diluted with PFSW. The bottles were incubated for 24 h at *in situ* temperature in a Sanyo MLR–351H plant growth chamber using a 12:12 light:dark cycle. The light levels in the incubators were measured to be about 300 micromoles quanta/m²/sec using a Biospherical Instruments, Inc. QSL–100 Quantum Scalar Photosynthetically Available Radiation (PAR) Irradiance Sensor. Incubator conditions were monitored throughout the experiment using an Onset HOBO Data Logger with temperature and PAR sensors.

HPLC Analysis

For pigment analysis, about 350–1600 mL of sample was filtered onto 47 mm Whatman GF/F filters, then placed into a cryotube, and stored in liquid nitrogen until HPLC analysis. Prior to HPLC analysis, samples were freeze dried to remove excess water, which allowed for better extraction of the pigments (Hagerthey et al. 2006). Pigments were then extracted from the filters overnight in 90% acetone and filtered through a 0.2 µm PTFE syringe filter to remove particles. A 1:1 mixture of extracted sample and ion pairing agent (IPA: 0.5 M Ammonium Acetate at pH 7.2) was prepared for injection. The HPLC method of Wright et al. (1991) was used for detection of pigments using an Alltech Alltima High Purity C–18 column on a Waters 600 Controller and Pump HPLC connected to a Waters 2996 Photodiode Array Detector. The method was modified as follows: solvent B was changed to 100% acetonitrile with 0.01% 2,6–di–*tert*–butyl–4–methylphenol. The external standard equation of Mantoura and Repeta (1997) was used to calculate pigment concentration of the sample.

Particulate Organic Carbon and Nitrogen Analysis

About 50 mL of WSW was filtered onto a combusted (450°C, 6 h) 21 mm Whatman GF/F filter for the determination of POC and PN. The samples were dried (60°C, 24 h), folded and placed into tin boats, and analyzed using a Costech ECS 4010 elemental analyzer. The concentration of the sample was determined from a standard linear regression using acetanilide constructed for each run of 3 runs (r^2 ranged from 0.998–0.999 for N and 0.999–1.00 for C).

Nutrient Analysis

About 50 mL of sample were filtered through a pre–rinsed Whatman 25 mm GF/F filter for nutrient analysis.

The filtrate was stored frozen (−4°C) in acid cleaned (10% HCL) 250 mL polyethylene sample bottles until analysis. Samples were analyzed fluorometrically (nitrogen species) and colormetrically (PO_4^- and $Si(OH)_4$) using an Astoria Pacifica A2+2 nutrient auto–analyzer (Method #A179, A027, A205, and A221; Astoria–Pacific International, Oregon USA).

Calculations

Growth and grazing rates (/d) of pigments were calculated based on the method of Landry et al. (1995). The apparent growth rate (k) is defined as growth in the incubation bottles in the presence of grazing pressure and calculated by: $k = (1/t)\ln[N_t/(N_o \times D)]$, where N_t and N_o are the final and initial pigment concentrations (µg/L), respectively, D is the proportion of WSW, and t is duration of incubation (h). The grazing rate (m) was calculated as the slope of the model II regression between k and dilution factor; if the slope was not significantly different from zero (p > 0.05), then m was assumed zero (0/d). The intrinsic growth rate (μ_o), growth in the absence of added nutrients and grazing, was calculated as k in non–diluted, non–nutrient amended bottles plus grazing rate ($\mu_o = k + m$). The nutrient–replete growth rate (μ_n, defined as growth in the presence of added nutrients and absence of grazing) was estimated as the Y–axis intercept of the linear regression (model II) between k (y axis) and dilution factor (D), for cases when the slope of the regression was significantly different from zero (p < 0.05). When the slope of the regression was not significantly different from zero, μ_n was calculated as the mean k of all nutrient–replete dilutions.

Nutrient limitation was explored using the Nutrient Limitation Index (NLI; Landry et al. 1998). This metric is the ratio of the growth rate in the absence of nutrients (μ_o) to the growth rate in the presence of nutrients (μ_n). When NLI is <1, the phytoplankton class is considered to be nutrient limited during the incubation.

Statistics

Significance and the 95% confidence interval of the model II (standard major axis: SMA; one–tailed; 99 permutations) regression were determined using the lmodel2 package in the statistical program R (Legendre 2008; R Core Team 2013). To compare growth and grazing rates to selected measured environmental parameters, a Spearman correlation (r_s, two–tailed; H0: There is no association between the two variables) was performed using SPSS v22.

Results

Conditions in the Bay

Temperature at all sampling locations ranged from 4.8–15.4°C, while salinity ranged from 10.9–23.1 (Table 1). Inorganic nitrogens (NO_2, NO_3, and NH_4) were low during all samplings (< 2 µM). Chlorophyll *a* ranged from 4.2–9.7

TABLE 1. *Measured environmental conditions (mean ± sd) at the Washington St. (WS) location and the Dunbar St. (DS) location. FTU = formazine turbidity units.*

Site	Date	Temp (°C)	Salinity	Turbidity (FTU)	C:N	NO_2 (µM)	NO_3 (µM)	NH_4 (µM)	PO_4 (µM)	SiO_4 (µM)	Chl *a* (µg/L)
WS	11 Nov 2013	15.4	18.7	7	7.7 ± 0.4	0.03	0.17	0.23	0.71	37.55	4.25 ± 0.08
	10 Dec 2013	12.5	23.1	2.9	6.5 ± 0.3	0.05	0.35	1.77	0.52	28.62	5.1 ± 0.07
	09 Jan 2014	4.8	13.6	6.6	11.4 ± 1.5	0.04	0.25	1.60	1.02	50.33	7.2 ± 0.15
DS	14 Nov 2013	10.8	17.6	25.8	9.5 ± 0.2	0.02	0.19	0.31	0.70	39.94	7.0 ± 0.1
	12 Dec 2013	10.8	10.9	19.7	9.2 ± 1.2	0.07	1.27	0.56	0.11	60.54	9.7 ± 0.17
	13 Jan 2014	10.7	10.9	2	8.4 ± 0.8	0.09	0.61	0.63	0.88	66.78	5.0 ± 0.19

µg/L, being highest on 12 December 2013 and lowest on 11 November 2013.

A total of 6 marker pigments were identified in the samples (Table 2). Fucoxanthin was found at the highest concentration indicating the bay was diatom dominated (Figure 2). Peridinin and alloxanthin were also prevalent, indicative of dinoflagellates and cryptophytes. Chlorophyll *b* was found in low concentrations (range 0–0.71 µg/L), whereas lutein and prasinoxanthin were often present at very low concentrations (< 0.13 µg/L). This made it impossible to examine

TABLE 2. *Diagnostic pigments used in this study.*

Pigment	Abbreviation	Taxonomic Group
Peridinin	Per	Dinoflagellates
Fucoxanthin	Fuc	Diatoms
Zeaxanthin	Zea	Cyanobacteria
Alloxanthin	Alx	Cryptophytes
Chlorophyll *b*	Chl *b*	Green Algae
Chlorophyll *a*	Chl *a*	All photosynthetic groups

chlorophytes and prasinophytes separately and they were therefore grouped as green algae. Zeaxanthin was detected during November indicating the presence of cyanobacteria.

Growth and grazing rates

The phytoplankton community (measured as chl *a*) had nutrient–replete growth rates (μ_n) that ranged from 0.14–0.79/d (Tables 3 and 4). The intrinsic community growth rates (μ_0) ranged from −0.11 to 0.44/d and were always lower than or similar to the nutrient replete growth rates (μ_n). The lowest growth rates (μ_0 and μ_n) were observed on 14 November 2013 and the highest were observed on 11 November 2013. Significant grazing (m) at the community level (chl *a*) was observed only during three of the samplings (Tables 3 and 4; range 0–0.49/d). During the three samplings in which significant grazing on the community (chl *a*) was observed, grazing rates were lower than or similar to

nutrient–replete growth rates (μ_n).

The nutrient–replete growth rate (μ_n) for marker pigment classes at the WS location ranged from 0–1.0/d (Table 3). Diatoms (fucoxanthin) had the highest growth rates (μ_n) in November and December (1.01 and 0.93/d, respectively); while in January diatoms and green algae had similar rates (0.36 and 0.33/d, respectively). The intrinsic growth rates (μ_0), estimated using marker pigments, ranged from 0–0.73/d and varied for all pigment classes (Table 3). The intrinsic growth rates (μ_0) were less than or similar to the nutrient replete growth rates, except for alloxanthin during December, when μ_0 (0.73/d) was greater than μ_n (0.58/d).

The growth rates observed at the DS location often showed extreme nutrient limitation (NLI < 1), which varied by pigment class. The nutrient–replete growth rate (μ_n) ranged from 0–0.67/d at the DS location (Table 4). Large negative intrinsic growth rates were observed for peridinin and alloxanthin (−0.46 and −0.32/d, respectively) in December due to nutrient limitation within the incubation bottle.

Grazing rates (m) for marker pigment classes ranged from 0–0.88/d during the study (Tables 3 and 4). Selective grazing on specific pigments was observed in 5 out of 6 experiments. In one experiment significant grazing was observed for all marker pigment classes (11 November 2013 sampling, WS). In another experiment (12 December 2013; DS) no significant grazing was observed for any marker pigment.

Possible Controls on Phytoplankton Growth and Microzooplankton Grazing

Three possible controls of phytoplankton growth and microzooplankton grazing were explored to gain an understanding of these processes: nutrient limitation during the incubation, coupling between growth and grazing rates, and correlations with measured environmental variables. Nutrient limitation during the incubation was observed during all experiments based on the NLI (Tables 3 and 4). The large values in the table are due to nutrient–replete growth rates (μ_n) close to 0/d and large negative values for μ_0. Alloxanthin in January at the WS location had no NLI value due to μ_n = 0/d.
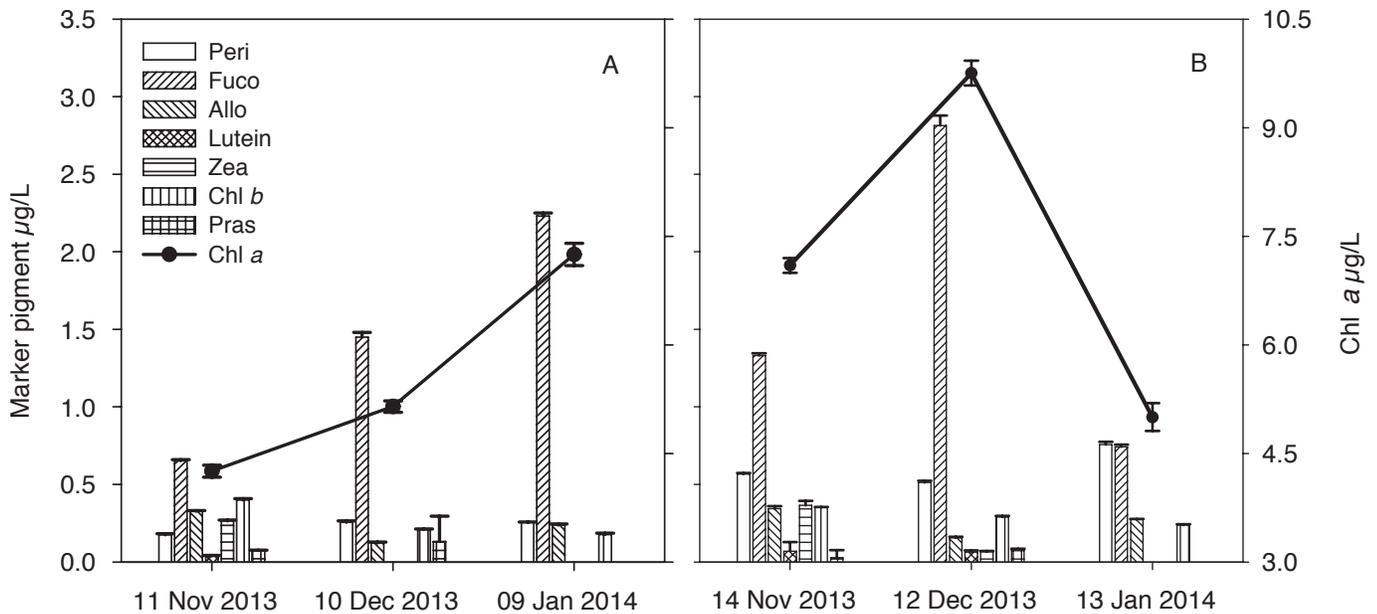
FIGURE 2. *Mean (± sd) marker pigment and chl a concentrations of the Bay of St. Louis for the Washington Street (A) and Dunbar Street (B) sampling locations during all sampling periods. Per = peridinin, Fuc = fucoxanthin, alx = alloxanthin, Zea = zeaxanthin, Chl b = chlorophyll b, Pras = prasinoxanthin, Chl a = chlorophyll a.*

A reported mechanism to explain selective grazing of microzooplankton on the phytoplankton community is that microzooplankton selectively graze on the phytoplankton classes which demonstrate the highest growth rates. The intrinsic growth rate (μ_o) was significantly correlated to grazing rates at the WS location (r_s = 0.698, p = 0.003) and the DS location (r_s = 0.773, p < 0.001; Figure 3). The nutrient–replete growth rate was not correlated with the grazing rates at either location. To determine if microzooplankton grazed on phytoplankton classes that experienced the least amount of nutrient limitation, grazing rates were compared to NLI. The NLI was correlated to grazing rates only at the DS location (r_s = 0.873, p = 0.001; Figure 3B). The negative values obtained for the nutrient limitation index were removed from this analysis since they were a product of the calculation and are not an accurate measure of the phytoplankton dynamics. A comparison of biomass (µg/L of pigment) and grazing rates showed no correlation (p > 0.05) indicating that phytoplankton classes were not selectively grazed due to high abundance.

Measured environmental conditions at the time of sampling were correlated to some phytoplankton pigment classes. Peridinin and alloxanthin growth and grazing rates were shown to be correlated significantly with salinity of the bay (Table 5; for peridinin r_s = 0.899 for μ_o, r_s = 0.841 for μ_n and r_s = 0.941 for m; for alloxanthin r_s = 0.986 for μ_o and 0.955 for m; all p < 0.05). The correlation analysis also showed that silicate was correlated inversely to salinity within the BSL (r_s = −0.986, p < 0.01 data not shown). Nutrient–replete growth rates and grazing rates for alloxanthin were

also correlated significantly with temperature (Table 5, r_s = 0.899 for μ_n and 0.832 for m; both p < 0.05). Fucoxanthin and chl a nutrient–replete growth rates were correlated inversely (r_s = −0.829, p < 0.05) to the C:N ratio of particulate organic matter at the time of sampling.

DISCUSSION

This study demonstrated that microzooplankton grazing and environmental conditions may play an important role in controlling phytoplankton composition in the BSL. Nutrient limitation was observed during 5 out of 6 of the samplings, as evident by higher growth rates in nutrient replete incubation bottles and the low observed nutrient concentrations. Microzooplankton grazers selected the phytoplankton classes that had the highest intrinsic growth rate, therefore exerting a degree of control on phytoplankton composition. Growth and grazing rates of cryptophytes (alloxanthin) and dinoflagellates (peridinin) were correlated with measured environmental parameters (e.g. salinity, silicate, and temperature) indicating the importance of fresh water inflow in controlling phytoplankton composition.

The community growth rates (chl a) were similar to rates measured in other Gulf of Mexico estuaries: the Suwannee River estuary in Florida (Jett 2004) and Mobile Bay in Alabama (Lehrter et al. 1999; Ortmann et al. 2011). The growth rates were also similar to those found in estuaries in other regions (Murrell and Hollibaugh 1998; Calbet and Landry 2004; York et al. 2010). Calbet and Landry (2004) summarized results from dilution experiments in 66 studies from coastal, oceanic, and estuarine habitats and found that

in estuarine systems the mean community (chl *a*) growth rate was 0.97 ± 0.07/d and the mean grazing rate was 0.53 ± 0.04/d (n=136). The rates measured during the current study only included winter samplings, which may explain why the rates observed were lower than those observed in other studies. It is expected that summer growth and grazing rates would be higher than winter rates due to seasonal factors; this has been shown in other studies at multiple locations (Strom et al. 2001; Gutierrez–Rodriguez et al. 2011; Lawrence and Menden–Deuer 2012). The low ambient nutrient concentration in the BSL is likely also a factor in the low rates observed where nitrogen limitation was suggested.

Even though fucoxanthin was always the dominant pigment, it was not always the pigment with the highest growth rate. This finding indicates that some mechanism controls the biomass of the fastest growing classes, which in this study was shown to be selective grazing by microzooplankton and environmental factors (e.g. salinity, silicate, and temperature). The strong correlation between intrinsic growth and grazing rates suggested that microzooplankton play a large role in controlling phytoplankton biomass, but only for certain phytoplankton classes. This correlation between rates demonstrates the strong ecological coupling between these two groups of organisms and has been demonstrated in numerous studies, including this one (Burkill et al. 1987; Strom and Welschmeyer 1991; Latasa et al. 1997; Murrell et al. 2002). When the phytoplankton in this study were supplemented with nutrients this ecological coupling appeared to break down as evidenced by the lack of correlation between nutrient replete growth rates and microzooplankton grazing rates.

The connection between top—down and bottom—up controls on phytoplankton biomass was previously noted in Pensacola Bay, FL, another Gulf of Mexico estuary (Juhl and Murrell 2005). In Pensacola Bay, the intrinsic growth rates (μ_o) of the phytoplankton were matched by equal grazing rates due to nutrient limitation of the phytoplankton community. In the BSL, grazing rates were higher than intrinsic growth rates (μ_0) for 38.4% of pigments tested. Nutrient—replete growth rates (μ_n) proved to be variable in relation to grazing rates. Negative and zero μ_o were observed when grazing rates were 0/d during 66.6% of the experiments; this was also observed in Long Island

TABLE 3. *Pigment specific regression statistics, growth/grazing rate, and nutrient limitation index (NLI; μ_o/μ_n) for the Washington Street location.*

	Pigment	Model r²	m/d (95% CI)	μ_n/d (95% CI)	μ_o/d	NLI
11 Nov 13	Chlorophyll a	0.72**	0.49 (0.34, 0.70)	0.79 (0.71, 0.91)	0.35	0.44
	Peridinin	0.73**	0.61 (0.43, 0.87)	0.60 (0.50, 0.74)	0.23	0.39
	Fucoxanthin	0.50**	0.52 (0.31, 0.83)	1.01 (0.89, 1.17)	0.53	0.53
	Zeaxanthin	0.58**	0.32 (0.20, 0.49)	0.81 (0.74, 0.91)	0.73	0.9
	Alloxanthin	0.87**	0.65 (0.51, 0.83)	0.70 (0.62, 0.80)	0.34	0.49
	Chlorophyll b	0.67**	0.53 (0.36, 0.79)	0.87 (0.77, 1.01)	0.52	0.59
10 Dec 13	Chlorophyll a	0.09	0	0.69 (0.61, 0.77)	0.11	0.16
	Peridinin	0.49**	0.37 (0.23, 0.60)	0.44 (0.36, 0.57)	0.24	0.56
	Fucoxanthin	0.13	0	0.93 (0.84, 1.01)	0.32	0.35
	Zeaxanthin	–	–	–	–	–
	Alloxanthin	0.40*	0.88(0.52, 1.48)	0.58 (0.38, 0.91)	0.73	1.26
	Chlorophyll b	0.08	0	0.57 (0.47, 0.67)	0.13	0.24
09 Jan 14	Chlorophyll a	0.32*	0.21 (0.06, 0.93)	0.25 (0.23, 0.37)	0.21	0.77
	Peridinin	0.52**	0.13 (0.08, 0.21)	0.07 (0.04, 0.11)	0.02	0.4
	Fucoxanthin	0.44**	0.28 (0.17, 0.47)	0.36 (0.30, 0.46)	0.31	0.87
	Zeaxanthin	–	–	–	–	
	Alloxanthin	0	0	0	−0.06	
	Chlorophyll b	0.63*	0.47 (0.31, 0.71)	0.33 (0.24, 0.46)	0.33	1.01

*Modell II regression, ** shows significance at p < 0.01, * shows significance at p < 0.05. m = grazing rate, μ_n = nutrient replete growth rate, μ_o= apparent growth rates, CI =Confidence Interval*

TABLE 4. *Pigment specific regression statistics, growth/grazing rate, and nutrient limitation index (NLI; μ_o/μ_n) for the Dunbar Street location.*

	Pigment	Model r²	m/d (95% CI)	μ_n/d (95% CI)	μ_o/d	NLI
14 Nov 13	Chlorophyll *a*	0.08	0	0.14 (0.10, 0.18)	−0.11	−0.8
	Peridinin	0.37*	0.34 (0.20, 0.58)	0.27 (0.19, 0.40)	0.21	0.77
	Fucoxanthin	0.05	0	0.19 (0.15, 0.23)	−0.01	−0.07
	Zeaxanthin	0.01	0	0.12 (0.08, 0.15)	0.03	0.26
	Alloxanthin	0.56*	0.35 (0.22, 0.54)	0.39 (0.32, 0.50)	0.16	0.41
	Chlorophyll *b*	0.05	0	0.23 (0.17, 0.28)	−0.04	−0.17
12 Dec 13	Chlorophyll *a*	0.17	0	0.50 (0.45, 0.59)	0.13	0.27
	Peridinin	0.14	0	−0.01 (−0.08, 0.05)	−0.46	36.27
	Fucoxanthin	0.09	0	0.67 (0.59, 0.76)	0.34	0.51
	Zeaxanthin	–	–	–	–	
	Alloxanthin	0	0	0.02 (−0.06, 0.10)	−0.32	−15.89
	Chlorophyll *b*	0.14	0	0.46 (0.38, 0.54)	0.07	0.15
13 Jan 14	Chlorophyll *a*	0.45**	0.49 (0.30, 0.82)	0.47 (0.36, 0.65)	0.44	0.94
	Peridinin	0.24	0	0.25 (0.17, 0.33)	0.07	0.31
	Fucoxanthin	0.58**	0.53 (0.34, 0.82)	0.54 (0.44, 0.70)	0.52	0.97
	Zeaxanthin	–	–	–	–	
	Alloxanthin	0.08	0	0.25 (0.07, 0.43)	−0.11	−0.44
	Chlorophyll *b*	0.53**	0.59 (0.37, 0.94)	0.57 (0.45, 0.76)	0.56	0.99

*Modell II regression, ** shows significance at p < 0.01, * shows significance at p < 0.05. m = grazing rate, μ_n = nutrient replete growth rate, μ_o= apparent growth rates, CI = confidence interval*
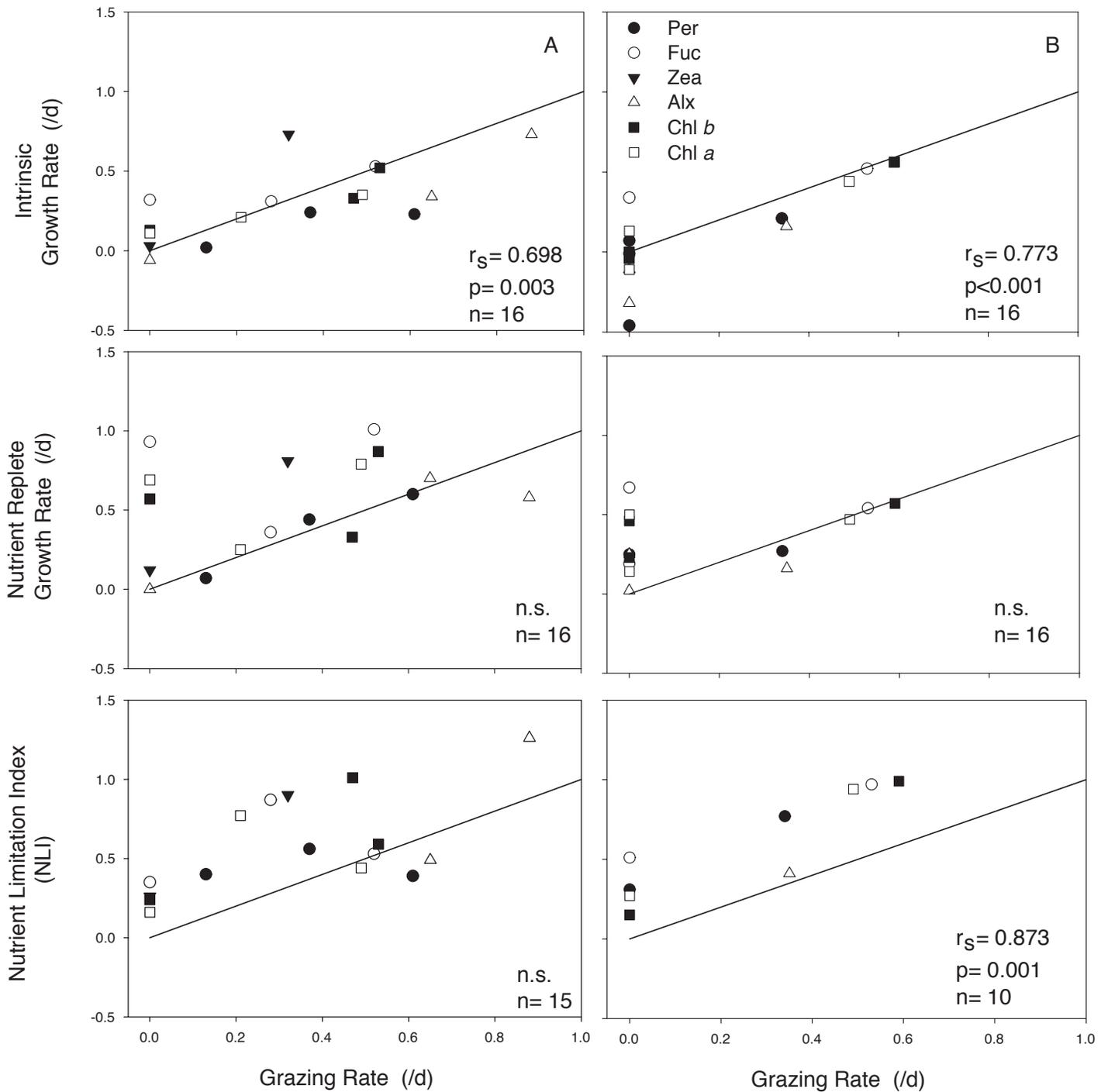
FIGURE 3. *Relationship between grazing rates and intrinsic growth rates (µo), nutrient replete growth rates (µn), and nutrient limitation index (NLI) at the Washington Street (A) and Dunbar Street (B) locations. Per = peridinin, Fuc = fucoxanthin, alx = alloxanthin, Zea = zeaxanthin, Chl b = chlorophyll b, Pras = prasinoxanthin, Chl a = chlorophyll a. r_s , Spearman Rank Order Correlation.*

Sound, New York and Tuggerah Lake, Australia (York et al. 2010; Sanderson et al. 2012). When nutrient limitation was minimal in the BSL, growth and grazing rates became more similar to one another, suggesting that nutrient limitation played a large role in controlling phytoplankton composition. Non–significant grazing was also more frequently observed at the DS location (69.2% vs. 23% of test), which suggests that the Jourdan River may decouple microzooplankton from their phytoplankton prey. This may be due

to the hydrodynamics of the river mouth, lowered salinity, low residence time, and/or higher turbidity.

The correlations between salinity and growth/grazing rates for peridinin and alloxanthin suggested that river flow was a factor in controlling the dynamics of these two phytoplankton classes. The data in this study suggest that during times of low river flow (high salinity) or when sampling further from the JR, dinoflagellates and cryptophytes grew faster and were grazed at a higher rate than the other

TABLE 5. *Significant correlations (r_s, Spearman Rank Order Correlation; $p < 0.05$) between growth rate (μ) or grazing rate (m) with measured environmental parameters (n=6).*

Pigment	Rate	Environmental Parameter	r_s
Peridinin	m	Salinity	0.941
	μ_o	Salinity	0.899
	μ_n	Salinity	0.841
Alloxanthin	m	Temperature	0.832
	m	Salinity	0.955
	μ_o	Salinity	0.986
	μ_n	Temperature	0.899
Fucoxanthin	μ_n	C:N	−0.829
Chl *b*	μ_n	Chl *a* (µg/L)	−0.829
	μ_o	Chl *a* (µg/L)	−0.829
Chl *a*	μ_n	C:N	−0.829

phytoplankton taxa. In Galveston Bay a greater biomass of diatoms was observed near the riverine inputs while dinoflagellates were found in areas of the bay where hydrologic displacement or nutrient loading from the river were not important (Dorado et al. 2015). Previous studies in the BSL have shown that the DIN concentrations were highest during times of high river flow (Sawant 2009; Cai et al. 2012; Camacho et al. 2014). As discussed by Dorado et al. (2015), it is possible that diatoms utilize these nutrient inputs, while they may be unimportant to dinoflagellates and cryptophytes since these organisms may be able to utilize alternative methods to acquire nutrients (i.e., mixotrophy, phagotrophy). Mesocosm studies of estuarine phytoplankton communities have shown that phytoflagellates were more abundant in static waters than waters which were being actively mixed, supporting hydrologic displacement as a factor adversely affecting cryptophytes and dinoflagellates (Pinckney et al. 1999). Further studies are needed in the BSL to further understand the correlation between river flow and growth/grazing rates.

The lack of correlation between ambient nutrient concentrations and phytoplankton growth during the current study is interesting, but not surprising, given the small sample number (n=6). Given the low ambient nutrient concentrations in the BSL it is likely that any new nutrients put into the system will be readily taken up by the phytoplankton community. Therefore, ambient nutrient concentration may be a misleading parameter for relating nutrients to phytoplankton growth. It may be more important to measure nutrient concentrations entering the system (i.e. from the

Jourdan River). The difference in growth rates between the intrinsic and nutrient—replete growth rates supports that the community was most likely nutrient deficient and gives a better idea of the effects of nutrients on this population.

Many of the experiments in this study had slopes of apparent growth rate vs. dilution factor regressions that were not different significantly than zero, indicating no measurable grazing. This result has been observed in many studies using the dilution method in a variety of environments (Landry and Hassett 1982; Landry et al. 1984; Paranjape 1987; Gifford 1988; Kamiyama 1994; Murrell and Hollibaugh, 1998; Kim et al. 2007; York et al. 2010). Non—significant grazing has been attributed to high variability in the method due to the small sample number used in the dilution series (Schmoker et al. 2013). It has been suggested that the high variability masks low grazing rates. The data from the current study suggested that the 0/d grazing rates were the result of a decoupling between phytoplankton growth and microzooplankton grazing, possibly due to environmental factors such as temperature and/or river flow affecting the growth of the phytoplankton. It has been suggested that when phytoplankton lack the nutritional compounds required for grazers, growth and grazing can become uncoupled since the phytoplankton are no longer a viable food source for the microzooplankton (Murrell and Hollibaugh 1998; Strom 2002). Given the small sample number in the current study, more information is needed to further examine the effect of environmental parameters on grazing rates.

In summary, this study investigated how phytoplankton growth rates, microzooplankton grazing rates, and environmental conditions affected phytoplankton composition in the BSL, MS, in the northern Gulf of Mexico. We observed very low and often negative intrinsic growth rates (μ_0), selective grazing (m) by the microzooplankton community, and extensive nutrient limitation of the phytoplankton community. The low growth rates observed are likely attributable to the season which the study was conducted (winter/fall). Even though fucoxanthin was the most abundant pigment, microzooplankton grazed on the phytoplankton pigment classes which had the highest intrinsic growth rate in the BSL at the time of sampling. We also observed salinity influence on growth and microzooplankton grazing rates on only two pigment classes of dinoflagellates (peridinin) and cryptophytes (alloxanthin). These factors together have been shown to influence the composition of the phytoplankton community in the BSL.

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