

## Gulf of Mexico Science

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Volume 27  
Number 2 *Number 2*

Article 4

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2009

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DOI: 10.18785/goms.2702.04

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### Recommended Citation

Putland, J. N., B. Mortazavi and R. L. Iverson. 2009. Changes in Phytoplankton and Bacterioplankton Biomass and Rate Processes in Apalachicola Bay, Florida, in Response to Reduction in River Discharge. *Gulf of Mexico Science* 27 (2). Retrieved from <https://aquila.usm.edu/goms/vol27/iss2/4>

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# Changes in Phytoplankton and Bacterioplankton Biomass and Rate Processes in Apalachicola Bay, Florida, in Response to Reduction in River Discharge

JENNIFER N. PUTLAND, BEHZAD MORTAZAVI, AND RICHARD L. IVERSON

**Bacterioplankton abundance and chlorophyll concentration and the factors that control them (temperature, nutrient concentrations, and rates of growth, grazing, and export) were studied in Apalachicola Bay, FL, during two summers with contrasting river discharge. A reduction in river discharge from the summer of 2003 (S03) to the summer of 2004 (S04) led to a reduction in estuarine concentrations of dissolved inorganic nitrogen and rates of export, phytoplankton and bacterioplankton growth, and microzooplankton grazing on phytoplankton and bacterioplankton. Bacterioplankton abundance and chlorophyll concentration during S03 were not significantly different from those during S04. Neither the growth rates and abundances of ciliates nor the egg production rates and abundances of *Acartia tonsa* were significantly affected by the reduction in rate processes of bacterioplankton or phytoplankton. The proposed diversion of freshwater from the Apalachicola River during summer months may lead to substantial changes in the rate processes of bacterioplankton and phytoplankton, but little change in the standing stocks of bacterioplankton, phytoplankton, ciliates, *A. tonsa*, or ciliate growth rates and *A. tonsa* egg production rates.**

## INTRODUCTION

Estuaries are critical nursery habitats to many commercial and recreational fish species. Estuarine food webs are primarily fueled by autochthonous algal production (Sullivan and Moncreiff, 1990; Chanton and Lewis, 2002). Bacterioplankton are a secondary source of food supporting estuarine microbial food webs (Iriarte et al., 2003; Putland and Iverson, 2007a).

Worldwide, many of the largest rivers have been diverted from the estuaries into which they discharge (Postel and Richter, 2003). Managing estuaries and the resources they support requires an understanding of how lower trophic levels, such as bacterioplankton and phytoplankton, respond to river water diversion.

Determining the impact of river water diversion on the estuarine biomass of bacterioplankton and phytoplankton (hereafter, bacterioplankton or phytoplankton are interchangeably referred to as “prey”) is complex because of the lack of long-term databases on growth and loss processes:  $B_{final} = B_{initial} e^{(\mu - g - S - \epsilon) \times t}$ , where  $\mu$ ,  $g$ ,  $S$ , and  $\epsilon$  represent daily rates of prey growth, zooplankton grazing, sedimentation, and export from the estuary, respectively, and where  $B$  represents prey biomass and  $t$  represents days (Cloern et al., 1985; Mortazavi et al., 2000b). Changes in river discharge can alter export (Eyre, 2000) from the estuary and bacterioplankton and phytoplankton growth rates (Murrell,

2003; Bledsoe et al., 2004) through changes in the estuarine light and nutrient environment. Mounting evidence indicates that microzooplankton (in practice defined as grazers <202  $\mu\text{m}$  in size) are the main grazers of bacterioplankton (Sherr and Sherr, 2002) and phytoplankton (Calbet and Landry, 2004) in oceanic and estuarine waters. Although microzooplankton grazing is typically coupled with prey growth (Calbet and Landry, 2004), microzooplankton grazing can decouple from prey growth, particularly during periods characterized by high nutrient regimes and/or high river discharge. Microzooplankton are more capable of ingesting small autotrophs (Irigoiien et al., 2005), and, therefore, an increase in nutrient input, which can lead to the predominance of large algae (Örnólfsson et al., 2004; Hoover et al., 2006), can decouple microzooplankton grazing from phytoplankton growth (Juhl and Murrell, 2005). Microzooplankton grazing can also decouple from prey growth if microzooplankton abundances are reduced as a result of heavy top-down control (Reaugh et al., 2007) and/or export out of the estuary (Murrell and Loes, 2004; Jyothibabu et al., 2006; Murrell et al., 2007).

Apalachicola Bay, FL, is an economically and ecologically important estuary located in the northern Gulf of Mexico. It is a short-residence time, well-mixed, river-dominated subtropical estuary. The Apalachicola River is the main source of freshwater and nutrients to the estuary

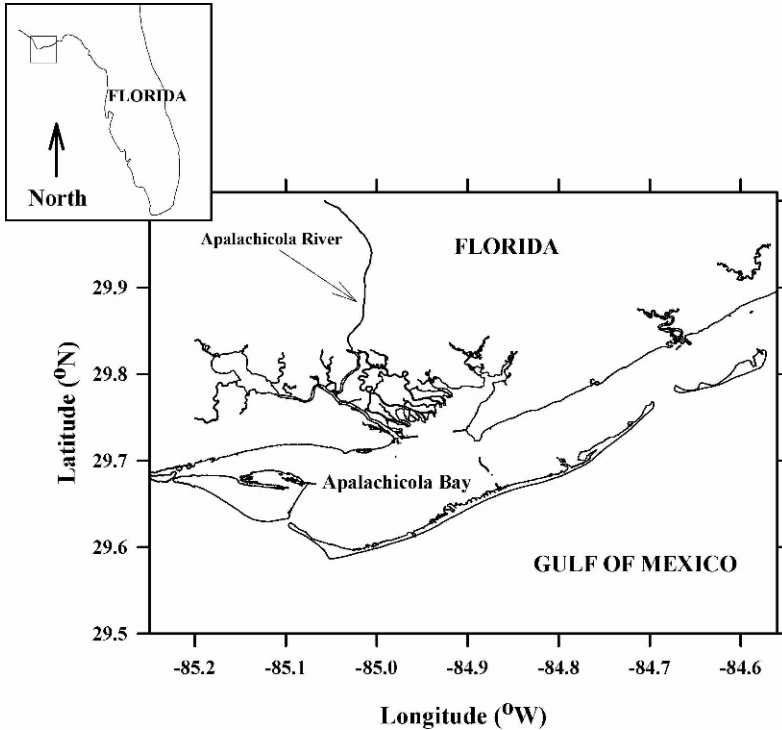


Fig. 1. Study site, Apalachicola Bay, FL.

(Mortazavi et al., 2000a). Diversion of water from headwaters of the Apalachicola River during summer has been proposed to satisfy upstream freshwater requirements (Lewis, 1997). Ecology of microzooplankton bacterivory and herbivory (Putland and Iverson, 2007a), the copepod *Acartia tonsa* (Putland and Iverson, 2007b), and phytoplankton (Putland and Iverson, 2007c) were studied in Apalachicola Bay during a 2-yr period that coincided with above-average river discharge during summer 2003 and a natural drought during summer 2004. In the present study, the data acquired from these studies (bacterial abundances, chlorophyll concentration, and rates of prey growth and microzooplankton grazing), in addition to estimates of export and nutrient concentration, were synthesized to examine the potential impact of river water diversion on bacterioplankton abundance and chlorophyll concentration and the main factors that control them (growth, zooplankton grazing, and export; Mortazavi et al., 2000b). A secondary objective of the present study was to determine if higher trophic levels may be affected by a reduction in river discharge. Ciliates are typically important grazers of bacterioplankton and phytoplankton, while *A. tonsa* graze on phytoplankton and microzooplankton. Therefore, we specifically examined whether ciliate abundance and growth

rates and *A. tonsa* abundance and egg production rates differed between the summer of 2003 and the summer of 2004.

#### MATERIALS AND METHODS

**Study site.**—Samples were collected from Apalachicola Bay, FL (Fig. 1), throughout 2003 and 2004. In estuaries, a variety of variables can be related to salinity (Fisher et al., 1988; Kimmerer, 2002). Therefore, to examine changes in variables in the estuary it was necessary to sample across the salinity gradient in Apalachicola Bay. Physical, chemical, and biological variables at fixed stations in the estuary can be highly variable because of daily variations in river flow, wind speed, and tides (e.g., Chauhan et al., 2009). As a result, sampling at fixed stations might not have allowed us to sample across the salinity gradient. Therefore, instead of sampling at fixed stations, on each sampling date a total of three to six samples was collected across the salinity gradient (between salinity measures of 0 and 35). Samples were generally collected in the morning and were not collected at any specific point during the tidal cycle.

Water samples were collected from a depth of 0.5 m below the sea surface with a darkened polycarbonate carboy. Irradiance was measured

at the surface and depth of collection with a model 192SA Li-Cor underwater quanta sensor attached to a handheld meter. Immediately after collection temperature and salinity (measured on the Practical Salinity Scale) of collected water was measured with an YSI salinometer, and water was subsampled from the carboy for concentrations of nutrients and chlorophyll and abundance of bacterioplankton and ciliates. The remaining collected seawater was used to estimate rates of growth for bacterioplankton, phytoplankton, and ciliates as well as microzooplankton grazing rates on bacterioplankton and phytoplankton.

Copepods were collected with a conical 202- $\mu\text{m}$  nylon-mesh net equipped with a closed cod end. The net had a 6:1 ratio of filtering area to mouth area. The net was towed horizontally for short periods ( $<2$  min) and filtered water from the surface to 0.5 m in depth. The first tow at the sampling site was preserved in acid Lugol's (10% final concentration) and was used to estimate the abundance of *A. tonsa*. The second tow at the sampling site was gently rinsed into a cooler containing surface-collected seawater and was used to estimate *A. tonsa* egg production rate.

*Nutrient concentration.*—Seawater collected for nutrient analyses was stored on ice in polyethylene bottles for a maximum of 4 hr prior to being filtered through a 0.2- $\mu\text{m}$  surfactant-free cellulose acetate filter. The filtrate was analyzed for nitrate, nitrite, ammonium, and soluble reactive phosphorus (SRP). Concentrations of ammonium and SRP were determined spectrophotometrically using the methods of Bower and Holm-Hansen (1980) and Murphy and Riley (1962), respectively. Spectrophotometric measurements were made with an ultraviolet/Vis Cary 1 Bio spectrophotometer. The concentration of nitrite plus nitrate was determined with the Vanadium (III) reduction chemiluminescence detection method (Braman and Hendrix, 1989). The resulting chemiluminescence was measured with a Model 42 Thermo Environmental chemiluminescence  $\text{NO}_x$  analyzer connected to a 3396 Series II HP integrator. Hereafter, the sum of nitrate, nitrite, and ammonium is referred to as dissolved inorganic nitrogen (DIN).

*Standing stocks.*—Seawater for chlorophyll analysis was stored on ice in polyethylene bottles for a maximum of 4 hr prior to being filtered through 47-mm GF/F filters at  $<117$  mm Hg in vacuum pressure. Filtered samples (250 ml) were stored in darkness at  $-20^\circ\text{C}$  and analyzed within 1 wk of sample collection. Chlorophyll *a* was extracted from filters in 90% acetone for 24 hr in darkness

at  $-20^\circ\text{C}$ . The concentration of chlorophyll *a* was measured fluorometrically with a Model 10 Turner Designs fluorometer equipped with filter sets for optimal sensitivity of chlorophyll *a* in the presence of chlorophyll *b* (Welschmeyer, 1994).

Seawater samples for bacterioplankton analyses were preserved with glutaraldehyde (2% final concentration) and stored in darkness at  $4^\circ\text{C}$ . Bacteria were enumerated within 2 wk of sample collection. Samples were filtered ( $<117$  mm Hg vacuum pressure) onto 0.2- $\mu\text{m}$  black Poretics polycarbonate filters and stained with Acridine Orange (Hobbie et al., 1977). Filters were mounted with Cargille type-B immersion oil onto glass slides. A BH Olympus epifluorescence microscope equipped with a blue excitation filter set (U-MWIB, excitation, 460–490 nm; emission, 515–700 nm) was used to visualize bacteria. Cells were counted at a total magnification of  $\times 1,875$ . At least 100 cells (Hobro and Willen, 1977) were counted per sample in a minimum of 10 random fields.

Seawater samples for ciliates were preserved in acid Lugol's (2% final concentration), stored in darkness at  $4^\circ\text{C}$ , and enumerated within 1 mo of sample collection. Samples (10–50 ml) were settled for 24 hr with Utermohl settling chambers. Cells were viewed at a total magnification of  $\times 200$  through phase-contrast light microscopy with a M40 Wild inverted microscope. Heterotrophic and mixotrophic ciliate cells were identified with the online guide of Ströder-Kypke et al. (<http://www.liv.ac.uk/ciliate/intro.htm>). Concurrently collected, glutaraldehyde-preserved samples mounted on slides (Putland and Iverson, 2007c) were examined with epifluorescence microscopy to determine if ciliates contained chloroplasts. For each settled sample, at least 100 cells were counted (Hobro and Willen, 1977). Cells were counted from the settled samples in transects.

Samples for determination of *A. tonsa* abundance were counted within 1 wk of collection. The volume filtered for net tows was estimated using a General Oceanics flowmeter that was mounted on the inside of the net. Another General Oceanics flowmeter was mounted on the outside of the net to estimate net filtration efficiency. Abundances of adult (male and female) *A. tonsa* were only estimated for tows for which filtration efficiency measured  $\geq 50\%$ . Of all tows conducted, 22% were discarded because the filtration efficiency was below 50%. Samples were split (with a Folsom plankton splitter) between two and five times, after which approximately 300 adult *A. tonsa* were counted per sample with an Olympus stereomicroscope.

*Rates of prey growth and microzooplankton grazing.*—Rates of growth and grazing for bacterioplankton and phytoplankton were estimated with the dilution method (Landry and Hassett, 1982). One dilution assay was conducted for each water sample collected. Experimental set-up and equations to estimate rates of growth and grazing are described in detail in Putland and Iverson (2007a). Briefly, each dilution assay consisted of a dilution gradient of seven target dilutions (one bottle per dilution) of 95%, 85%, 75%, 65%, 55%, 45%, 35%, and 0% diluent (<0.2- $\mu\text{m}$  filtered seawater). Appropriate volumes of diluent were added to 2-liter polycarbonate incubation bottles. The <202- $\mu\text{m}$  seawater was then added to bottles by dispensing 0.5-m collected seawater through silicon tubing that was equipped with a 202- $\mu\text{m}$  Nitex screen. In Apalachicola Bay, silicate does not limit phytoplankton growth. While phosphorus tends to limit phytoplankton productivity in lower salinity waters (<20 psu), wind mixing alleviates phosphorus limitation (Fulmer, 1997). Only nitrogen (as ammonium chloride) was added to the incubation bottles because it is the nutrient that most frequently limits phytoplankton productivity in this estuary (Fulmer, 1997). One additional 0% diluent bottle per dilution assay did not receive the nitrogen enrichment and therefore served as a control. Bottles were placed on a plankton wheel (~0.5 rpm) and incubated in an outdoor incubator flushed continuously with seawater from the estuary. Incident irradiance was attenuated with neutral density screening to simulate the light energy from the collection site. Samples were taken for chlorophyll *a* and bacterioplankton immediately after preparation of dilution treatments and again after 24 hr. Samples for bacterioplankton and chlorophyll *a* were preserved, stored, and analyzed according to the procedures previously mentioned.

Rates of growth and grazing mortality were estimated with Model I linear regressions of apparent growth rate (*AGR*) vs actual dilution factor (*ADF*). The *ADF* for each bottle was calculated as follows:

$$ADF = [T_o \text{ chl } a (X_i)] \times [T_o \text{ chl } a (X_o)]^{-1}, \quad (1)$$

where  $T_o \text{ chl } a (X_i)$  is the time-zero chlorophyll *a* concentration at target dilution factor  $X_i$  and  $T_o \text{ chl } a (X_o)$  is the time zero chlorophyll *a* concentration of the 0% diluent treatment. The prey *AGR* ( $\text{d}^{-1}$ ) in each incubation bottle was calculated as

$$AGR = [t^{-1}] \times [\ln(P_t \times P_o^{-1})], \quad (2)$$

where  $t$  is the duration of the incubation (in days) and  $P_o$  and  $P_t$  refer to initial and final prey concentrations, respectively. The y-intercept of the linear regression is the nutrient-enriched rate of prey growth ( $\mu_{NE}$ ,  $\text{d}^{-1}$ ) in the absence of grazing, while the absolute value of the negative slope is the rate of microzooplankton grazing ( $g$ ,  $\text{d}^{-1}$ ). Rates of non-nutrient enriched prey growth ( $\mu$ ,  $\text{d}^{-1}$ ) were calculated as the sum of the apparent growth rate in the control bottle and the rate of microzooplankton grazing.

Applying linear regression analysis to dilution plots of *AGR* vs *ADF* can result in errors in variable estimates if the relationship between *AGR* and *ADF* is nonlinear as a result of feeding thresholds or saturated feeding (Gifford, 1988; Gallegos, 1989; Dolan et al., 2000; Moigis, 2006). In cases in which the relationship between *ADF* and *AGR* was nonlinear and indicative of saturated grazing, piecewise linear regression (Redden et al., 2002) was used to estimate the rates of prey growth and microzooplankton grazing. The dilution plot was split into two regions. The region corresponding to relatively high dilution and significant negative slope was used to estimate the nutrient-enriched prey growth. In this region, *AGR* was regressed against *ADF*, and the y-intercept was taken as the estimate of the nutrient-enriched phytoplankton growth. The region corresponding to relatively low dilution and slope not significantly different from zero was used to estimate the mean *AGR* ( $AGR_{mean}$ ). The rate of microzooplankton grazing for nonlinear dilution plots was then estimated as

$$g = \mu_{NE} - AGR_{mean}. \quad (3)$$

*Ciliate growth rate.*—Rates of growth for heterotrophic and mixotrophic ciliates were estimated by incubating seawater (<202  $\mu\text{m}$ ) for 24 hr. Seawater was added to two 1L incubation bottles through silicon tubing that was equipped with 202- $\mu\text{m}$  Nitex screening. The silicon tubing was kept submerged below the water line in the bottles to reduce damage to delicate protists. All bottles were placed on a plankton wheel (~0.5 rpm) and incubated for 24 hr in an outdoor incubator flushed continuously with seawater from Apalachicola Bay. Incident irradiance was attenuated with neutral density screening to simulate the light energy from the collection site. Ciliates were sampled from bottles at time zero and again after 24 hr. Samples for ciliates were preserved, stored, and analyzed according to the procedures previously mentioned. Rates of growth for ciliates were estimated as follows,

$$\text{Ciliate } \mu = [t^{-1}] \times [\ln(C_t \times C_o^{-1})], \quad (4)$$

where  $t$  is the duration of the incubation (in days) and  $C_o$  and  $C_t$  refer to initial and final ciliate abundances, respectively.

*Acartia tonsa* egg production rate.—Experimental set-up and equations to estimate the rate of egg production are described in detail in Putland and Iverson (2007b). Briefly, seawater (<20  $\mu\text{m}$ ) was added to 1-liter polyethylene incubation bottles through silicon tubing that was equipped with 20- $\mu\text{m}$  Nitex screening. Three bottles were filled per station. A total of five to 20 adult copepods (males plus females) were added to each bottle. All bottles were incubated in an outdoor incubator flushed continuously with seawater from Apalachicola Bay. Incident irradiance was attenuated with neutral density screening to simulate the light energy from the collection site.

After an incubation period of 24 hr, copepods were removed from the bottles by filtering the contents of the bottles through a 202- $\mu\text{m}$  Nitex screen. Filtered copepods were washed into a dish of filtered seawater. Copepods that were alive were counted with an Olympus stereomicroscope. Copepods were preserved in acid Lugol's (10% final concentration) and counted immediately. Bottles containing the <202- $\mu\text{m}$  filtrate were reincubated in the outdoor incubator. Nauplii were removed from the bottles after 24 hr by filtering the contents of the bottles through a 20- $\mu\text{m}$  Nitex screen. Filtered nauplii were gently washed into a bottle, preserved with acid Lugol's (10% final concentration), and counted immediately with an Olympus stereomicroscope. The viable egg production rate was then calculated as follows:

$$\text{EPR} = [t^{-1}] \times [\text{No. nauplii} \times \text{No. females}^{-1}], \quad (5)$$

where  $t$  is in the initial incubation period,  $\text{No. Nauplii}$  is the number of nauplii, and  $\text{No. Females}$  is the number of alive adult female *A. tonsa* were counted at the end of the first incubation period.

*Export rate*.—Estuarine total water residence time can be calculated by dividing the estuary's volume by the sum of the total water imported (i.e., freshwater and tidal) into the estuary (Mortazavi et al., 2000a). These estimates of total water residence time (2.5–12.5 d) in Apalachicola Bay are comparable to freshwater residence times in Apalachicola Bay estimated from naturally occurring geochemical tracers (6–12 d) (Dulaiova and Burnett, 2008) or the

freshwater fractionation method (3–10 d) (Huang and Spaulding, 2002). The theoretical freshwater residence time, estimated as the quotient of the estuary's volume and freshwater discharge from the Apalachicola River, is also comparable to estuarine total water residence time for Apalachicola Bay (Dulaiova and Burnett, 2008). Huang and Spaulding (2002) also found strong correlations ( $r = 0.94$ ) between Apalachicola River discharge and freshwater residence time in Apalachicola Bay. Therefore, the average daily rate of export from the estuary to the Gulf of Mexico can be approximated to a first order as the quotient of average daily river discharge into the estuary [acquired from the U.S. Geological Survey (<http://waterdata.usgs.gov>)] and the estuary's volume.

*Calculated ratio of prey standing stock*.—A previous study (Mortazavi et al., 2000b) calculated that phytoplankton biomass in Apalachicola Bay is primarily determined by growth ( $\mu$ ,  $\text{d}^{-1}$ ), microzooplankton grazing ( $g$ ,  $\text{d}^{-1}$ ), and export ( $\epsilon$ ,  $\text{d}^{-1}$ ). The calculated average ratio of summer 2004 (S04) to summer 2003 (S03) standing stock ( $B_{\text{calc}}$ ) was estimated with average daily rates of field-estimated prey growth, microzooplankton grazing, and export for each summer and assuming exponential cell growth, thus:

$$B_{\text{calc}} = [(e^{\mu-g-\phi})^{\times 1 \text{ day}} \text{S04}] \times [(e^{\mu-g-\phi})^{\times 1 \text{ day}} \text{S03}]^{-1}. \quad (6)$$

*Statistical analyses*.—Paired-sample t-tests were used to determine if variables were significantly different among the months of S03 and S04. The differences between the two data sets were tested for normality (with the Kolmogorov-Smirnov test). In cases in which the assumption of normality was not met, Wilcoxon paired-sample, nonparametric tests were performed. For all statistical analyses, a  $P$ -value of less than 5% was used to determine significance (Sokal and Rohlf, 1995).

## RESULTS

*Temperature and river discharge*.—Average surface temperature was lowest (<26°C) between Nov. and April and highest (>26°C) between May and Oct. (Fig. 2a). Data collected between May and Oct. were grouped and hereafter are referred to as summer data. The average temperature during S03 was slightly lower, although not significantly so (Table 1), than that measured during S04.

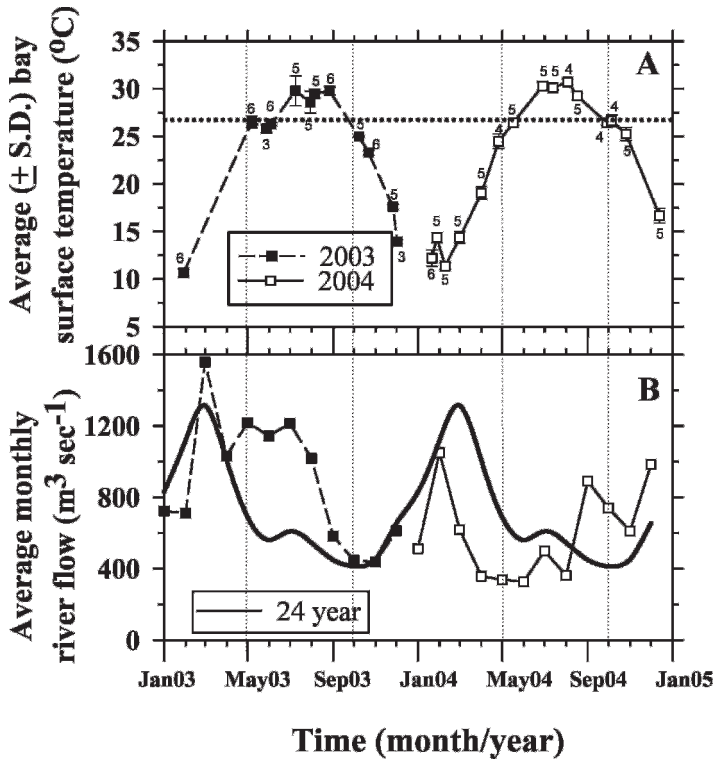


Fig. 2. (A) Average surface temperature in Apalachicola Bay, FL, throughout the salinity gradient on each date the estuary was sampled. Number of salinity "stations" sampled for each date is shown above or below error bars in this and subsequent figures. Horizontal dotted line demarcates 26°C. Vertical dotted lines demarcate summer (>26°C, May to Oct.). (B) Average monthly flow from the Apalachicola River and for the 24-yr average (source: <http://waterdata.usgs.gov>).

The 24-yr average Apalachicola River discharge (Fig. 2b) peaks around 1,300 m<sup>3</sup> sec<sup>-1</sup> in approximately March (source: <http://waterdata.usgs.gov>). Discharge declines thereafter and reaches a minimum of 400 m<sup>3</sup> sec<sup>-1</sup> in the fall. In 2003, river discharge also peaked in March (at 1,600 m<sup>3</sup> sec<sup>-1</sup>). River discharge was nearly twice the 24-yr average discharge, at about 1,100 m<sup>3</sup> sec<sup>-1</sup>, throughout S03, before declining to 400 m<sup>3</sup> sec<sup>-1</sup> in the fall. In contrast, in 2004 river discharge peaked in Feb., at 1,000 m<sup>3</sup> sec<sup>-1</sup>, and discharge during S04 was about 64% of the 24-yr average summer value. During fall 2004, discharge increased to about twice the 24-yr average fall discharge. Export during S03 was about twice that of S04. The rate of export during S03 was significantly higher than during S04 (Table 1).

**Nutrient concentration.**—The average concentrations of DIN and SRP in the estuary declined from 2003 to 2004 (Fig. 3a,b). While the concentration of SRP during S03 was not significantly different from that of S04, the

concentration of DIN was significantly higher during S03 than during S04 (Table 1).

**Standing stocks.**—Average chlorophyll concentration (Fig. 4a) and bacterioplankton abundance (Fig. 4b) peaked during summer. Bacterioplankton abundance and chlorophyll concentration during S03 were not significantly different from those during S04 (Table 1). Similarly, ciliate and *A. tonsa* abundances during S03 were not significantly different from those during S04 (Table 1).

**Biological rate processes.**—The average rates of growth for phytoplankton (Fig. 5a) and bacterioplankton (Fig. 5b) declined from 2003 to 2004. For both phytoplankton and bacterioplankton, the S03 growth rates were significantly greater than the S04 growth rates (Table 1). The nutrient-enriched phytoplankton growth rates were also significantly higher during S03 than during S04 (Table 1). The nutrient-enriched bacterial growth rates were not significantly different between S03 and S04. The average

TABLE 1. Average  $\pm$  standard error (SE) values (n) for physical, chemical, and biological variables during summer 2003 and 2004 in Apalachicola Bay, FL. Averages were estimated from all data collected on all dates (see figures for dates) within each summer. Variables included temperature ( $T_p$ , °C), dissolved inorganic nitrogen ( $DIN$ ,  $\mu\text{g N liter}^{-1}$ ), soluble reactive phosphorus ( $SRP$ ,  $\mu\text{g P liter}^{-1}$ ), phytoplankton growth rate ( $Chl a \mu$ ,  $\text{d}^{-1}$ ) ( $\mu_{NE}$  refers to nutrient-enriched growth rate), bacterial growth rate ( $Bact \mu$ ,  $\text{d}^{-1}$ ), ciliate growth rate ( $Cil \mu$ ,  $\text{d}^{-1}$ ), *Acartia tonsa* egg production rate ( $EPR$ , eggs female $^{-1} \text{d}^{-1}$ ), microzooplankton grazing rate on phytoplankton ( $Chl a g$ ,  $\text{d}^{-1}$ ) and bacteria ( $Bact g$ ,  $\text{d}^{-1}$ ), and export rate (Export,  $\text{d}^{-1}$ ). Standing stocks for chlorophyll ( $Chl a$ ,  $\mu\text{g liter}^{-1}$ ), bacterioplankton ( $Bact$ , cells  $\times 10^9 \text{ liter}^{-1}$ ), ciliates (*Ciliate*, cells  $\text{ml}^{-1}$ ), and adult *A. tonsa* (*A. tonsa*, No.  $\text{liter}^{-1}$ ). Results of paired t-tests testing for differences between months of summer 2003 and 2004 are denoted as \*\*  $P < 0.01$  and \*  $P < 0.05$ ; ns indicates not significant ( $P > 0.05$ ).

Variable	Summer 2003	Summer 2004	Significance
Physical/chemical			
$T_p$	27.2 $\pm$ 0.35 (36)	28.2 $\pm$ 0.35 (28)	ns
$DIN$	378 $\pm$ 51 (33)	144 $\pm$ 22 (28)	**
$SRP$	4.5 $\pm$ 0.68 (33)	2.1 $\pm$ 0.31 (27)	ns
Rates			
$Chl a \mu$	1.19 $\pm$ 0.10 (13)	0.18 $\pm$ 0.15 (9)	*
$Chl a \mu_{NE}$	1.23 $\pm$ 0.10 (13)	0.45 $\pm$ 0.15 (10)	*
$Bact \mu$	0.85 $\pm$ 0.07 (13)	0.29 $\pm$ 0.05 (9)	*
$Bact \mu_{NE}$	0.87 $\pm$ 0.07 (13)	0.35 $\pm$ 0.07 (10)	ns
$Cil \mu$	0.17 $\pm$ 0.27 (11)	-0.10 $\pm$ 0.18 (21)	ns
$EPR$	24 $\pm$ 5 (15)	31 $\pm$ 8 (13)	ns
$Chl a g$	1.23 $\pm$ 0.10 (13)	0.45 $\pm$ 0.08 (10)	**
$Bact g$	1.04 $\pm$ 0.08 (13)	0.54 $\pm$ 0.09 (10)	ns
Export	0.16 $\pm$ 0.02 (6)	0.09 $\pm$ 0.02 (6)	* <sup>a</sup>
Biomass			
$Chl a$	5.33 $\pm$ 0.32 (36)	5.06 $\pm$ 0.42 (28)	ns
$Bact$	2.54 $\pm$ 0.14 (36)	2.94 $\pm$ 0.24 (28)	ns
<i>Ciliate</i>	25 $\pm$ 3 (36)	16 $\pm$ 3 (28)	ns
<i>A. tonsa</i>	3 $\pm$ 1 (12)	0.6 $\pm$ 0.22 (10)	ns

<sup>a</sup> Wilcoxon paired-sample test.

rates of microzooplankton grazing on phytoplankton (Fig. 6a) and bacterioplankton (Fig. 6b) declined from 2003 to 2004. The rates of grazing on phytoplankton were significantly higher during S03 than during S04. The rate of grazing on bacteria was not significantly different between S03 and S04. On average, phytoplankton and bacterioplankton growth rates during S04 were 15% and 34%, respectively, of those measured during S03 (Table 2). Microzooplankton grazing rates on phytoplankton and bacterioplankton during S04 were, on average, 37% and 52%, respectively, of those measured during S03 (Table 2). Ciliate growth rates and *A. tonsa* egg production rates during S03 were not significantly different from those during S04 (Table 1).

The calculated average ratio of S04 to S03 prey biomass ( $B_{calc}$ ) was similar to the measured ratio of S04 to S03 prey biomass ( $B_m$ ) (Table 2). For example, chlorophyll concentration during S04 was 85% of that during S03, based on average rates of prey growth, microzooplankton grazing, and export ( $B_{calc}$ ). Based on measured chlorophyll concentrations ( $B_m$ ), chlorophyll concen-

tration during S04 was 95% of that measured during S03. Bacterioplankton abundance during S04 was 101% of that during S03, based on average rates of growth, grazing, and export. Based on measured bacterioplankton abundances, bacterioplankton abundance during S04 was 116% of that measured during S03.

## DISCUSSION

*Rates of prey growth, microzooplankton grazing, and export.*—The rates of growth for bacterioplankton and phytoplankton, microzooplankton grazing, and export in Apalachicola Bay were comparable to those found in other estuaries (Jochem, 2003; Murrell, 2003; Calbet and Landry, 2004; Murrell et al., 2007). River discharge directly affected export and indirectly affected rates of growth and grazing. Average export during S03, when river discharge was above the 24-yr average, was approximately twice that during S04 (Tables 1, 2). On average, phytoplankton and bacterioplankton growth rates during S04 were 15% and 34%, respective-



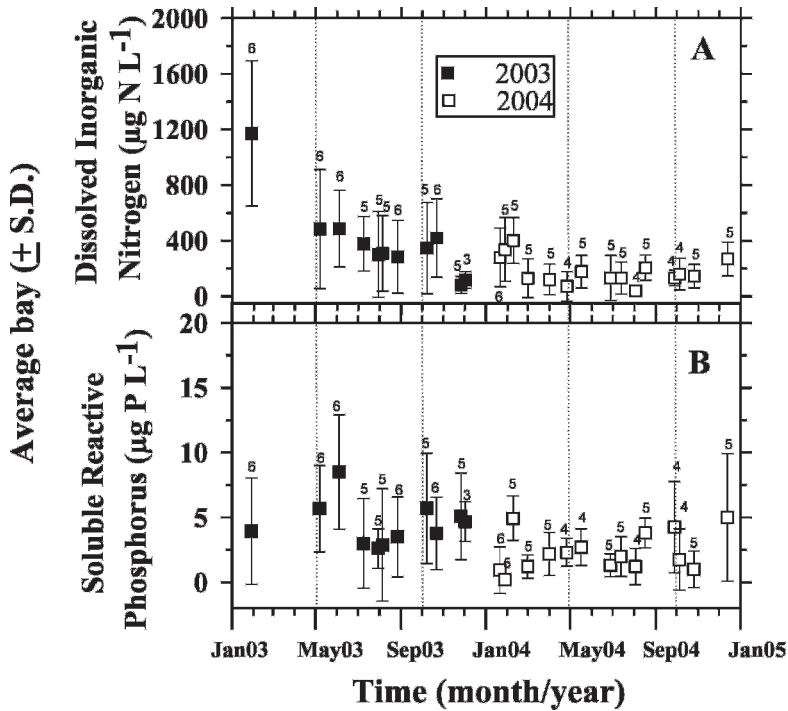


Fig. 3. Average (A) dissolved inorganic nitrogen concentration (DIN) and (B) dissolved soluble reactive phosphorus (SRP) concentration in Apalachicola Bay, FL, throughout the salinity gradient on each date the estuary was sampled. Vertical dotted lines demarcate summer (>26°C, May to Oct.).

ly, of those during S03 (Table 2). Growth rates of phytoplankton and bacteria are influenced by temperature and substrate supply (Eppley, 1972; Shiah and Ducklow, 1994). It is unlikely that temperature led to the reduction in growth rates, as there was no significant temperature change from S03 to S04 (Table 1). Bacterioplankton and phytoplankton depend on inorganic nitrogen and phosphorus for growth, and bacterioplankton also depend on organic carbon for growth (Revilla et al., 2000; Iriarte et al., 2003; Murrell, 2003). During summer in Apalachicola Bay, phytoplankton nitrogen demand is primarily supported by regenerated nitrogen, followed by new nitrogen from the Apalachicola River and benthic flux (Mortazavi et al., 2000a). The reduction in bacterioplankton and phytoplankton growth rates from S03 to S04 may have been due to the reduction in river discharge from S03 to S04, which led to a reduction in substrate concentrations, namely inorganic nutrients (Fig. 3; Table 1) and possibly allochthonous organic carbon (Revilla et al., 2000; Iriarte et al., 2003; Murrell, 2003). On average, microzooplankton grazing rates on phytoplankton and bacterioplankton during S04 were 37% and 52% of those during S03 (Table 2). In a fashion similar to that of conditions in other estuaries

(McManus and Ederington-Cantrell, 1992; Lehrter et al., 1999; Juhl and Murrell, 2005), microzooplankton grazing is positively correlated to prey growth in Apalachicola Bay (Putland and Iverson, 2007a). Therefore, it was not surprising that, like the rates of growth for bacterioplankton and phytoplankton, the rates of microzooplankton grazing declined from S03 to S04 (Table 1).

The reported rates of export from Apalachicola Bay to the Gulf of Mexico are first-order approximations, calculated as the quotient of average daily river discharge into the estuary and the estuary's volume. The rates of prey growth and microzooplankton grazing were determined with the dilution method of Landry and Hassett (1982). Despite its limitations, this procedure is widely used to estimate rates of prey growth and microzooplankton grazing (Calbet and Landry 2004). The dilution method assumes that prey growth is exponential and constant across the dilution gradient, that micrograzers are not food satiated, and that grazing varies with the density of micrograzers. Because nutrient concentrations are generally high in estuaries, nutrients are generally not added to incubation bottles of dilution experiments conducted in estuarine waters.

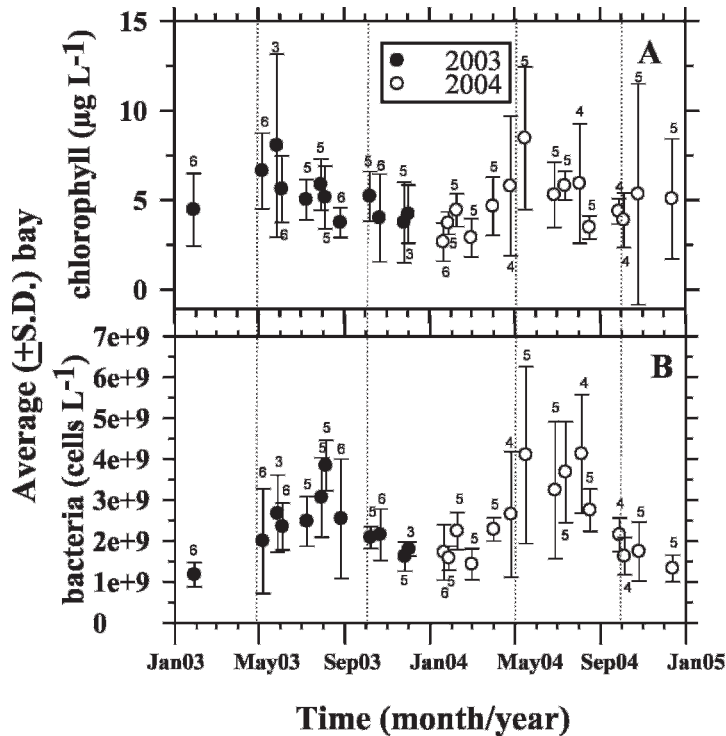


Fig. 4. Average (A) chlorophyll concentration and (B) bacterioplankton abundance in Apalachicola Bay, FL, throughout the salinity gradient on each date the estuary was sampled. Vertical dotted lines demarcate summer ( $>26^{\circ}\text{C}$ , May to Oct.).

In Apalachicola Bay, wind mixing tends to alleviate phosphorus limitation of phytoplankton, and nitrogen frequently limits phytoplankton (Fulmer, 1997). Therefore, in the present study, we added nitrogen to the incubation bottles. Although it is possible that prey growth rates were limited by phosphorus in the dilution experiments, the fact that none of the dilution plots had positive slopes suggests that prey growth rates were not nutrient limited in the dilution experiments. The light level among the incubation bottles of the dilution experiments is another factor that may have influenced phytoplankton growth rates. Apalachicola Bay is a turbid estuary. As a result, incubation bottles with the most diluent appeared to have the least amount of color. Although light levels were not measured among the incubation bottles, it is possible that light levels were greatest in bottles with the most diluent. Higher light levels in incubation bottles with the most diluent may have caused phytoplankton growth rates to be overestimated.

With respect to grazing rates, we utilized piecewise linear regression (Redden et al., 2002) to estimate rates of prey growth and microzooplankton grazing when dilution plots

exhibited saturated feeding. However, grazing rates were not corrected for micrograzer growth and therefore may be overestimates (Gallegos, 1989; Dolan et al., 2000). The degree to which the aforementioned factors influenced the rates of prey growth and microzooplankton grazing is unknown. However, we suspect that the reported rates are reasonable approximations of the rates occurring in situ. First, the observed trends in rates of prey growth, microzooplankton grazing, and export followed ecological concepts (as discussed above). Second, when used to predict the ratio of biomass, together the rates of prey growth, microzooplankton grazing, and export yielded a ratio of S04 to S03 prey biomass similar to that measured directly (see "Standing Stocks" below).

*Standing stocks.*—Bacterioplankton abundances and chlorophyll concentrations in Apalachicola Bay were comparable to those found in other estuaries (Boynton et al., 1982; Revilla et al., 2000; Iriarte et al., 2003). The magnitude of bacterioplankton and chlorophyll standing stocks results from the interaction of the rates of prey growth, microzooplankton grazing, and export. Despite the decline in the rates of

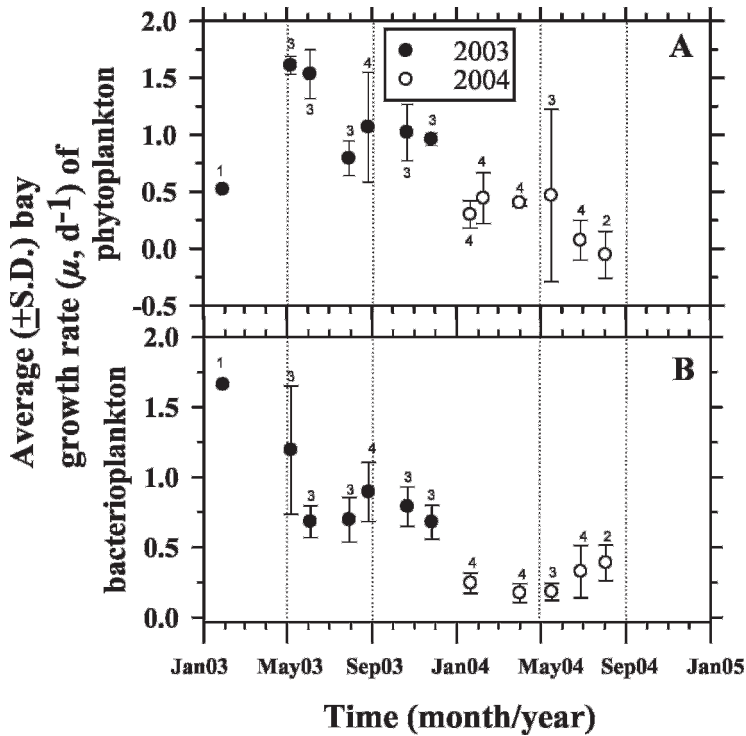


Fig. 5. Average growth rates of (A) phytoplankton and (B) bacterioplankton in Apalachicola Bay, FL, throughout the salinity gradient on each date the estuary was sampled. Vertical dotted lines demarcate summer (>26°C, May to Oct.).

growth, grazing, and export from S03 to S04, resulting from reduced river discharge and nutrient concentrations, there was no significant change from S03 to S04 in the standing stocks of bacterioplankton and chlorophyll (Table 1). The ratio of S04 to S03 standing stocks, whether measured ( $B_m$  in Table 2) or calculated ( $B_{calc}$ ), did not indicate a substantial change in standing stocks of bacterioplankton and chlorophyll.

The conclusion that a reduction in river discharge will not lead to a substantial change in prey biomass is based upon data collected from 2 yr, one relatively high river discharge year and one relatively low river discharge year. We were interested in whether this conclusion is robust when there are different magnitude reductions in river discharge and phytoplankton growth rate accompanied by a similar increase in top-down control (defined as  $g \times \mu^{-1}$ ) by microzooplankton. Top-down control by microzooplankton on bacterioplankton and phytoplankton was, on average, two to three times greater during S04 than during S03 (Table 2). Figure 7 is a hypothetical example predicting the change in phytoplankton biomass in response to different scenarios of reduced export and phytoplankton growth. In this example microzooplankton grazing averages 100% of phy-

toplankton growth during higher river discharge and increases to 250% of phytoplankton growth during lower river discharge. The initial phytoplankton growth rate was set as 0.5, 1.0, or 3.0  $d^{-1}$ . Figure 7a represents the scenario in which there is a relatively small (21%) decline in average summer river discharge (from 540  $m^3 sec^{-1}$  to 425  $m^3 sec^{-1}$ ). Figure 7b represents the scenario in which there is a larger (74%) reduction in average summer river discharge (from 540  $m^3 sec^{-1}$  to 140  $m^3 sec^{-1}$ , the proposed minimum discharge for the Apalachicola River) (Lewis, 1997). Figure 7c represents the scenario in which there is an extreme (93%) change in river discharge. For this scenario we used above-average summer river discharge (935  $m^3 sec^{-1}$ ) and assumed it was reduced to 70  $m^3 sec^{-1}$ . The hypothetical example demonstrates that during scenarios in which reduced river discharge leads to reduced rates of export and prey growth and an increase in microzooplankton top-down control, the only time during which there is little (<20%) change in prey biomass is when the initial phytoplankton growth rate is  $\leq 1 d^{-1}$  and when there is a substantial (>60%) reduction in phytoplankton growth rate.

The hypothetical example might explain why river water diversion leads to lower estuarine

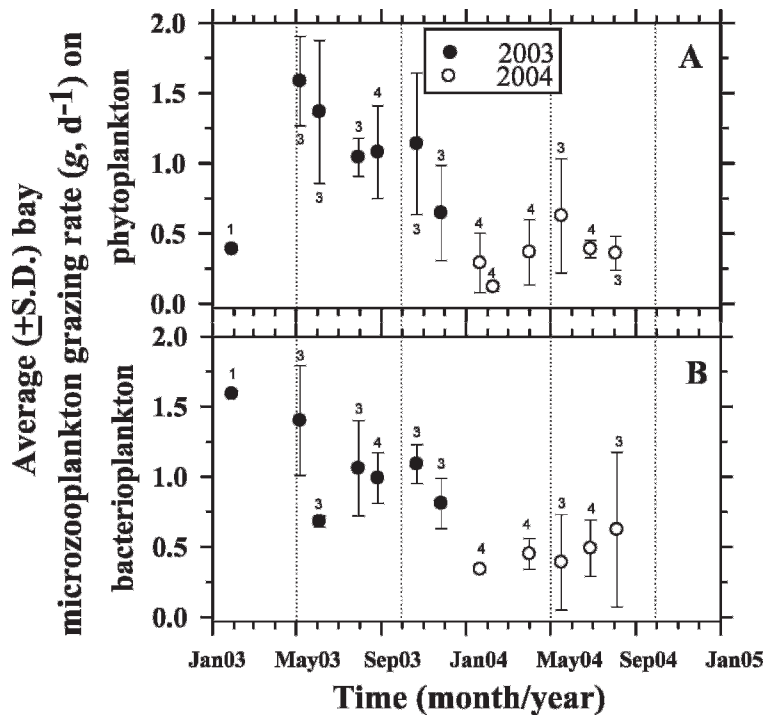


Fig. 6. Average microzooplankton grazing rates on (A) phytoplankton and (B) bacterioplankton in Apalachicola Bay, FL, throughout the salinity gradient on each date the estuary was sampled. Vertical dotted lines demarcate summer ( $>26^{\circ}\text{C}$ , May to Oct.).

chlorophyll concentrations in estuaries such as the San Francisco estuary (Nichols et al., 1986) and the Kariega estuary (Froneman, 2000). In these estuaries the decline in chlorophyll standing stocks resulting from reduced river discharge may, in part, be due to a reduction in export, a relatively small ( $<60\%$ ) reduction in phytoplankton growth rate, and an increase in top-down control (defined as  $g \times \mu^{-1}$ ) by microzooplank-

ton. In both the Kariega estuary (Froneman, 2000) and Apalachicola Bay (J. N. Putland, unpubl. data), small autotrophs dominate the phytoplankton community during periods of reduced river discharge. This may cause top-down control to increase because small autotrophs are typically more easily grazed by microzooplankton than are large autotrophs (Irigoiien et al., 2005).

TABLE 2. Average values  $\pm$  standard error (SE) values (n) during summer 2003 (S03) and 2004 (S04) in Apalachicola Bay, FL, for phytoplankton and bacterioplankton standing stock ( $B$ ) and daily rates of growth ( $\mu$ ), grazing ( $g$ ), and export ( $\varepsilon$ ). Standing stock is chlorophyll concentration ( $\mu\text{g liter}^{-1}$ ) and bacterioplankton abundance ( $\text{cells} \times 10^9 \text{ liter}^{-1}$ ).  $B_m = \text{variable (S04)} \times \text{variable (S03)}^{-1}$  and represents the measured ratio of variable ( $B$ ,  $\mu$ ,  $g$ , or  $\varepsilon$ ) during summer 2004 to summer 2003.  $B_{calc} = [(e^{\mu-g-\varepsilon}) \times 1 \text{ day S04}] \times [(e^{\mu-g-\varepsilon}) \times 1 \text{ day S03}]^{-1}$  and represents (based on the average daily rates of growth, grazing, and export for S03 and S04) the calculated ratio of standing stock during summer 2004 to summer 2003.

Taxa	Variable	Summer 2003	Summer 2004	$B_m$	$B_{calc}$
Phytoplankton	$B$	$5.33 \pm 0.32$ (36)	$5.06 \pm 0.42$ (28)	0.95	0.85
	$\mu$	$1.19 \pm 0.10$ (13)	$0.18 \pm 0.15$ (9)	0.15	
	$g$	$1.23 \pm 0.10$ (13)	$0.45 \pm 0.08$ (10)	0.37	
	$\varepsilon$	$0.16 \pm 0.02$ (6)	$0.09 \pm 0.02$ (6)	0.56	
Bacterioplankton	$B$	$2.54 \pm 0.14$ (36)	$2.94 \pm 0.24$ (28)	1.16	1.01
	$\mu$	$0.85 \pm 0.07$ (13)	$0.29 \pm 0.05$ (9)	0.34	
	$g$	$1.04 \pm 0.08$ (13)	$0.54 \pm 0.09$ (10)	0.52	
	$\varepsilon$	$0.16 \pm 0.02$ (6)	$0.09 \pm 0.02$ (6)	0.56	

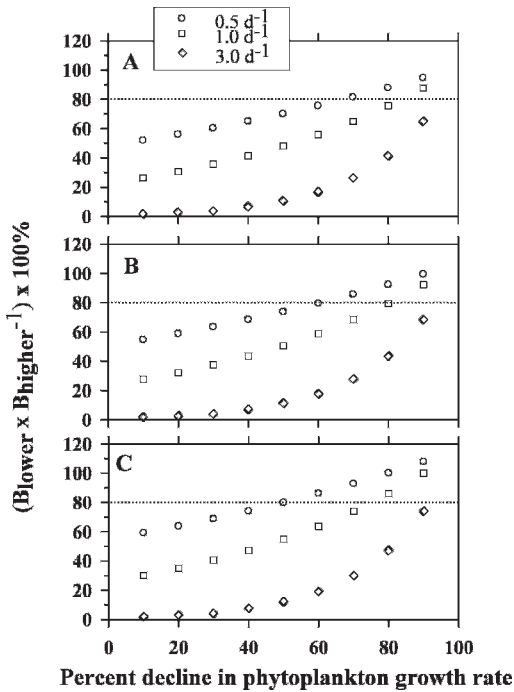


Fig. 7. Hypothetical example of how a decrease in export and phytoplankton growth rate accompanied by microzooplankton grazing will impact phytoplankton biomass. The initial phytoplankton growth rate ( $\mu$ ) was assumed to be 0.5, 1.0, or 3.0  $\text{d}^{-1}$ . Grazing rate ( $g$ ) averages 100% of phytoplankton growth during higher river discharge and increases to 250% of phytoplankton growth during lower river discharge. The average daily rate of export ( $\epsilon$ ) was calculated as the quotient of average daily river flow into the bay and bay volume. The 24-yr average summer flow of 540  $\text{m}^3 \text{sec}^{-1}$  (source: <http://waterdata.usgs.gov>) represents an export of 0.09  $\text{d}^{-1}$ . In (A), export declined by 0.02  $\text{d}^{-1}$  from the 24-yr average summer flow, to 425  $\text{m}^3 \text{sec}^{-1}$ . In (B), export declined by 0.07  $\text{d}^{-1}$  from average summer flow to the proposed minimum flow of 140  $\text{m}^3 \text{sec}^{-1}$ . In (C), export declined by 0.15  $\text{d}^{-1}$  from above average summer river flow of 935  $\text{m}^3 \text{sec}^{-1}$  to 70  $\text{m}^3 \text{sec}^{-1}$ . The ratio of biomass for the lower to the higher river flow period was calculated as  $(B_{\text{lower}} \times B_{\text{higher}}^{-1}) \times 100\%$ , where  $B_{\text{lower}} = (e^{(\mu-g-\epsilon) \times 1 \text{ day}})_{\text{lower flow}}$  and  $B_{\text{higher}} = (e^{(\mu-g-\epsilon) \times 1 \text{ day}})_{\text{higher flow}}$ . The horizontal dashed lines denote  $B_{\text{lower}}$  is 80% of  $B_{\text{higher}}$ .

**Higher trophic levels.**—Bacterioplankton and phytoplankton are primary prey for ciliates, while ciliates and phytoplankton are major prey items for *A. tonsa*. During the present study, neither the growth rates of ciliates nor the egg production rates of *A. tonsa* were significantly affected by the reduction in rate processes of their prey (Table 1). Ciliate growth rates (Jeong et al., 2004) and *A. tonsa* egg production rates (Kjørboe et al., 1985) are a function of prey concentration. The lack of change in ciliate

growth rates and *A. tonsa* egg production rates between S03 and S04 is therefore not surprising because of the lack of significant changes in bacterioplankton and ciliate abundance and chlorophyll concentration. The absence of significant changes in the abundance of ciliates and *A. tonsa* (Table 1) may, in part, be due to the lack of change in ciliate growth rates and *A. tonsa* egg production rates, respectively, between S03 and S04. However, like bacterioplankton and phytoplankton, additional processes (e.g., grazing and export) influence the abundance of ciliates and *A. tonsa*. Deciphering the absence of significant changes in abundance of either ciliates or *A. tonsa* will not only require information on their respective rates of increase (e.g., growth rates for ciliates, egg production rates for *A. tonsa*) but will also require knowledge of their respective loss processes.

#### CONCLUSION

The present study synthesized data collected during 2 yr, one relatively high river discharge year and one relatively low river discharge year, in order to examine the potential impact of river water diversion on bacterioplankton abundance and chlorophyll concentration as well as the factors that control them. The study demonstrated that monitoring prey biomass, specifically chlorophyll concentration and bacterial abundance, does not necessarily reflect the substantial changes in rate processes that can occur with changes in river discharge. Even though the reduction in Apalachicola River discharge did not lead to significant changes in chlorophyll concentration or bacterial abundance, there were substantial reductions in estuarine nutrient concentrations and rates of prey growth and microzooplankton grazing (Table 1). For example, the average reductions in phytoplankton and bacterioplankton growth rates were 85% and 66%, respectively, and microzooplankton grazing rates on phytoplankton and bacterioplankton declined, on average, by 63% and 48%, respectively (Table 2). Neither the growth rates and abundances of ciliates nor the egg production rates and abundances of *A. tonsa* (Table 1) were affected by the reduction in rate processes of their prey. However, a reduction in river discharge that leads to reduced rates of export and prey growth and an increase in microzooplankton top-down control can also lead to a reduction in prey biomass (Fig. 7). Moreover, it is possible for prey growth and microzooplankton top-down control to respond differently to a reduction in river discharge. For example, microzooplankton top-down control might de-

crease if reduced river discharge leads to an increase in predation pressure on microzooplankton. Assuming the results from this study are representative of summers with lower and higher river discharge, then the proposed diversion of freshwater from the Apalachicola River during the summer months may lead to substantial changes in rate processes of bacterioplankton and phytoplankton but little change in the standing stocks of bacterioplankton, phytoplankton, ciliates, and *A. tonsa* or in ciliate growth rates and *A. tonsa* egg production rates.

#### ACKNOWLEDGMENTS

This research was conducted with a Graduate Research Fellowship award (to JNP) from the Estuarine Reserves Division, Office of Ocean and Coastal Resource Management, National Ocean Service, National Oceanic and Atmospheric Administration. The authors thank the Department of Statistics at Florida State University (FSU) for providing statistical consultation, the Department of Biology at FSU for use of their epifluorescence microscope, and L. Edmiston at the Apalachicola Bay National Research Reserve for providing assistance with sampling. We would like to thank two anonymous referees for their insightful comments.

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