THE RELATIVE IMPORTANCE OF HETEROTROPHIC BACTERIA TO PELAGIC ECOSYSTEM DYNAMICS VARIES WITH TROPHIC STATE OF RESERVOIRS

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ABSTRACT

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Previous research in pelagic ecosystems suggests that nutrient sequestration and carbon flow through heterotrophic bacteria is relatively high in oligotrophic systems but decreases as trophic state increases. I assessed this hypothesis and mechanistic explanations in 17 reservoirs in Texas and Ohio, USA. I measured bacterial production (BPr), primary production (PPr), particulate nutrients, dissolved organic carbon (DOC), soluble reactive phosphorus (SRP), and dissolved inorganic nitrogen (DIN). The % of particulate carbon (C), nitrogen (N), and phosphorus (P) in the bacterial size fraction

(< 1-μm) was highest in oligotrophic reservoirs but decreased as trophic state increased. Comparison of particulate nutrient data to Minnesota natural lakes suggests a similarity in nutrient sequestration by bacteria in reservoirs and natural lakes. BPr and PPr ranged from 17 – 172 and from 41 – 1,695 μg C L^{-1} d^{-1}, respectively. BPr:PPr decreased as our measure of trophic state, chlorophyll, increased ($r^2 = 0.42$). DOC, DOC:SRP, and DOC:DIN did not improve predictions of BPr:PPr over that based solely upon chlorophyll. These variables may have limited use as predictors of BPr:PPr across a broad range of trophic state. BPr:PPr was better predicted by DOC:particulate carbon (PC) ($r^2 = 0.47$) and mixing depth (Z_{mix}) ($r^2 = 0.61$) than by chlorophyll. However, the relationship between BPr:PPr and DOC:PC was primarily driven by the relationship between BPr:PPr and PC ($r^2 = 0.41$), which was a surrogate for trophic state. Unexpectedly, the strength of Z_{mix} as a predictor BPr:PPr did not primarily reflect differences in the light environment. My results support the hypothesis that the relative importance of heterotrophic bacteria is highest in oligotrophic systems and decreases as trophic state increases, but I found limited support for several of the proposed mechanisms of this pattern. My results suggest that patterns in microbial dynamics are similar in reservoirs and natural lakes.

I. INTRODUCTION

Our understanding of the role that heterotrophic bacteria play in pelagic ecosystems has changed dramatically over the last three decades. The classical view of planktonic food webs saw bacteria primarily as decomposers and recyclers of nutrients. That view began to change with the recognition of the so-called microbial loop, a food web made of heterotrophic bacteria, picoplankton, and their protistan predators (Pomeroy 1974; Azam et al. 1983). It is now recognized that heterotrophic bacteria are not only decomposers in pelagic ecosystems but also are competitors for nutrients and a critical trophic link between dissolved organic carbon (DOC) and the classic autotroph-grazer food web. Due to large surface area to volume ratios, bacteria can out-compete phytoplankton for phosphorus (P), especially in systems where P supply is low or dissolved organic carbon (DOC) is relatively abundant (Currie and Kalff 1984; Joint et al. 2002; Danger et al. 2007). Heterotrophic bacteria often play a large role in carbon (C) cycling by dominating plankton community respiration, making many oligotrophic pelagic ecosystems net heterotrophic (del Giorgio and Peters 1994; González et al. 2003; but see Carignan et al. 2000). In addition, bacteria can consume a substantial fraction of primary production in the form of DOC released from living or dying algal cells (Cole et al. 1982; Baines and Pace 1991). Heterotrophic bacteria and their primary predators, heterotrophic nanoflagellates (HNF), also serve as critical links in pelagic food webs by returning C and nutrients to the autotroph-grazer portions of the food web (Azam et al. 1983; Vargas et al. 2007). Furthermore, mixotrophic phytoplankton can consume

bacteria, presumably as a source of nutrients and energy (Bird and Kalff 1986; Sanders et al. 1989).

The relative importance of heterotrophic bacteria in pelagic ecosystems is hypothesized to be greatest in oligotrophic systems and to decrease with increased trophic state (del Giorgio and Peters 1994; Biddanda et al. 2001; Cotner and Biddanda 2002). del Giorgio and Peters (1994) showed that the ratio of photosynthesis to community respiration was largely a function of trophic status in 20 southern Quebec lakes. Biddanda et al. (2001) found that the percentage of particulate nutrients in the bacterial size fraction and the ratio of bacterial respiration to total planktonic respiration were highest in oligotrophic Minnesota lakes but decreased in more eutrophic lakes. Several explanations for these patterns have been proposed. In oligotrophic systems, greater relative abundance of DOC and dissolved organic nutrients versus dissolved inorganic nutrients should give heterotrophic bacteria a competitive advantage over phytoplankton, leading to a greater role of bacteria in carbon (C) and nutrient cycling (Cotner and Biddanda 2002; Joint et al. 2002). Thus, phytoplankton production is thought to be partially constrained by competition with bacteria for nutrients when nutrient supply is low and DOC is relatively high (Currie and Kalff 1984; Joint et al. 2002). In oligotrophic systems, a higher ratio of DOC to particulate organic carbon (POC) should favor bacteria in competition with eukaryotic phagotrophs for organic C (Cotner and Biddanda 2002). In addition, viral infection rates of bacteria (Weinbauer et al. 1993) and bacterivory by protozoa (Sanders et al. 1992; Auer et al. 2004) increase with trophic state, reducing the direct role of bacteria in carbon and nutrient cycling in eutrophic systems.

Most evidence of a greater role for heterotrophic bacteria in oligotrophic systems comes from studies of marine ecosystems and natural lakes (see review in Cotner and Biddanda 2002). Few studies have addressed the relative importance of phytoplankton and heterotrophic bacteria in reservoirs (but see Chrzanowski and Hubbard 1988; Auer et al. 2004). Reservoirs share many structural and functional characteristics with natural lakes including pelagic or open-water habitats and similar planktonic species (Kalff 2003), and rates of bacterial and primary production are consistent with natural lakes (Chrzanowski and Hubbard 1988; Knoll et al. 2003). In addition to their similarities with other pelagic systems, the widespread distribution and abundance of reservoirs makes them important ecosystems for study. Globally, there are over 50,000 reservoirs with dams higher than 15 m, and reservoirs now temporarily retain ~ 20% of the runoff from world watersheds (Kalff 2003). Despite their similarities with natural lakes and their abundance, relatively little is known about reservoirs as regards broader ecological questions. Furthermore, the great variation both within and between reservoirs in physical habitat structure, nutrient loading, hydrologic characteristics, and irradiance levels, make them ideal systems for exploring how these environmental factors affect bacteria and phytoplankton (Kimmel et al. 1990; Kalff 2003; Knoll et al. 2003).

In this study, we investigated the role of heterotrophic bacteria in the pelagic zone of reservoirs from two geographically and climatically distinct regions: Ohio and Texas, USA. We had two main goals: First, we wanted to test the general hypothesis that the relative importance of heterotrophic bacteria decreases with trophic state in pelagic ecosystems (Biddanda et al. 2001; Cotner and Biddanda 2002). Specifically, we hypothesized that: a) the percentage of particulate nutrients in the bacterial size fraction

(< 1.0 μm) and b) the ratio of bacteria production (BPr) to primary production (PPr) would decrease as trophic state increased. Second, we wanted to test some of the potential mechanisms that may determine the relative importance of bacteria in pelagic ecosystems, including the relative abundance DOC versus inorganic nutrients, the relative abundance of DOC versus particulate carbon (PC), and the underwater light environment (Cotner and Biddanda 2002). Specifically, we hypothesized that bacteria's importance, as measured by BPr:PPr, would increase as a) DOC:soluble reactive phosphorus, b) DOC:dissolved inorganic nitrogen, c) DOC:PC, and d) mixed layer depth increases. To explore these questions, we measured BPr, PPr, and nutrients in two sets of reservoirs in Ohio and Texas.

II. MATERIALS AND METHODS

Study sites

We sampled eight reservoirs in Ohio, located throughout the state, and nine reservoirs in central Texas (Table 1). These regions have north temperate and sub-tropical climates, respectively. Reservoirs were selected to encompass a wide range in productivity and trophic state in each region (Ground and Groeger 1994; Knoll et al. 2003).

Ohio reservoirs were generally smaller and shallower, with surface area ranging from 232 to 1,650 hectares (Table 1). Texas reservoirs were on average larger but also encompassed a wider range in size, with surface area ranging from 166 to 9,330 hectares. As expected, mixed layer depth (Z_{mix}) was largely a function of surface area (Sterner et al. 1997) with the larger reservoirs having greater Z_{mix}. Granger Lake (Texas) was the only reservoir that was not stratified, and therefore Z_{mix} for Granger is also the maximum depth.

Reservoirs were sampled during the summer and early fall growing season: Ohio reservoirs between May and September, 2005 and Texas reservoirs between July and October, 2006. Each reservoir was sampled a minimum of two times, with three Ohio reservoirs (Acton, Burr Oak, and Pleasant Hill) sampled 9, 8, and 7 times, respectively. The first sampling date for Lake Dunlap (Texas) was excluded from analyses, because there was a storm water runoff event at the time of sampling that strongly affected lake conditions, such as dissolved nutrients and non-volatile suspended solids. With the

exception of Lake Dunlap, reported results for each reservoir represent the mean of all sampling dates. Reservoirs from Ohio and Texas were sampled with the same methods, and samples were analyzed using the same techniques unless noted otherwise.

Field sampling

Reservoirs were sampled at a site near the dam, the deepest point in most reservoirs. We estimated the mixed layer depth of each reservoir from profiles of temperature, dissolved oxygen, and specific conductance measured with an YSI Model 85D (Texas) or Model 58 (Ohio). Photosynthetically available radiation (PAR) was measured at 0.5 to 1-m intervals using a LiCor model 1935A light meter fitted with a 4π sensor. We measured Secchi depth with a black and white disk. Integrated water samples were collected from the upper mixed layer using a weighted Tygon tube and an electric bilge pump. The tube was continuously moved up and down through the mixed layer as the pump ran, and water was collected in Nalgene high density polyethylene bottles. Samples were immediately placed in coolers and held at lake temperature until analyses were completed.

Water chemistry

The following water chemistry variables were quantified for each reservoir: chlorophyll-*a* (chlorophyll), alkalinity, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), total phosphorus (TP), total nitrogen (TN), soluble reactive phosphorus (SRP), ammonium (NH_4^+), nitrate (NO_3^-), total particulate nutrients (C, N, and P), and < 1 μm particulate nutrients (C, N, and P < 1 μm). All variables were determined from duplicate (alkalinity, DIC, DOC, < 1 μm particulate nutrients) or triplicate (all other variables) analyses of depth-integrated water samples. Chlorophyll

samples were filtered onto Gelman A/E glass fiber filters, frozen, extracted with acetone, and measured using a Turner TD-700 (Ohio) or a Turner Designs Trilogy fluorometer (Texas). Alkalinity and DIC were determined by Gran titrations (Wetzel and Likens 2000).

TP and TN were determined from unfiltered water samples. TP was measured as PO_4^{3-}, after digestion with potassium persulfate, using the molybdenum blue method (Wetzel and Likens 2000). TP was measured on a Lachat QuikChem® FIA+ 8000 Series autoanalyzer (Ohio) or a Varian Cary 50 UV-Vis spectrophotometer (Texas). TN samples were digested with alkaline potassium persulfate and analyzed as NO_3^- on a Varian Cary 50 UV-Vis spectrophotometer using second derivative UV spectroscopy (Crumpton et al. 1992).

Dissolved nutrients were determined from water filtered through pre-ashed Gelman A/E filters. Ohio samples were measured with a Lachat QuikChem® FIA+ 8000 Series autoanalyzer, and Texas samples were measured with a Varian Cary 50 UV-Vis spectrophotometer. SRP was determined with the molybdenum blue method (Wetzel and Likens 2000). NH_4^+ was determined with the phenate method (Solorzano 1969). For Ohio, NO_3^- was determined using the cadmium reduction method (Wetzel and Likens 2000), and for Texas, NO_3^- was determined with second derivative UV spectroscopy (Crumpton et al. 1992). For both sets of reservoirs, DOC samples were filtered though pre-ashed Whatman GF/F filters, and the filtrate was analyzed on a Shimadzu TOC-Vcsh total organic carbon analyzer.

Total particulate C and N samples were filtered onto pre-ashed Whatman GF/F filters (nominal pore size = 0.7 μm), dried at 60°C for 48 h, frozen, and analyzed on

either a Perkin Elmer CHN analyzer (Ohio) or a CE Elantech CN analyzer (Texas). For particulate C and N < 1 μm, water was first filtered through 1-μm Nulcleopore filters and then treated the same as total particulate C and N. Total particulate P samples were filtered onto Whatman GF/F filters, digested with hydrochloric acid, and measured as PO_4^{3-} using the molybdenum blue method. For particulate P < 1 μm, samples were first filtered through 1-μm Nulcleopore filters and then treated the same as total particulate P.

Primary production

For all reservoirs, PPr estimates were performed within 36 hours of sample collection (Knoll et al. 2003). Integrated samples of lake water were transported to the laboratory in dark 2-liter Nalgene bottles and stored at mixed layer temperature until estimates were made. We measured primary production using the ^{14}C method as described by Knoll et al. (2003). Briefly, this method consists of three basic steps. First, a photosynthesis-irradiance (P-I) curve is generated by measuring ^{14}C uptake by phytoplankton over a range of light intensity (Fee 1990; Knoll et al. 2003). Second, the P-I curve and lake chlorophyll concentration are used to estimate the parameters α^B (the slope of the P-I curve at low PAR levels) and P^B_M (the rate of photosynthesis at optimal PAR levels) (Fee 1990). Both of these photosynthetic parameters are biomass-corrected for chlorophyll concentration. Finally, depth-specific PAR measured in each lake, chlorophyll concentrations, α^B, and P^B_M are used to estimate PPr.

In the laboratory, lake water was transferred to a 1-liter dark glass bottle and 2.5-10 μCi of $Na^{14}COH$ was added. After mixing, four 0.5-mL subsamples were transferred to scintillation vials to determine initial ^{14}C. The mixture was then poured into 12 60-mL Pyrex glass bottles, which were incubated for 2-3 hours in a photosynthetic chamber.

The chamber consisted of a water bath maintained at mixed layer temperatures and illuminated with a 1,000 W metal halide bulb. After incubation, samples were filtered through Gelman A/E glass-fiber filters to collect phytoplankton. Each filter was rinsed with dilute HCl to remove any ^{14}C-DIC, placed in a scintillation vial, and dried overnight at 60 °C. Ten mL of Scintiverse BD was added to each vial and radioactivity was measured with a Beckman LS 6000IC scintillation counter. We used 10 light intensities and two dark bottles to generate the P-I curve for each reservoir on each date. ^{14}C uptake rates in light bottles were corrected for nonphotosynthetic uptake measured in dark bottles.

The computer program PSPARMS (Fee 1990) was used to estimate α^B and P^B_M from the P-I curve and chlorophyll concentrations. Estimates of simulated cloud-free areal PPr were generated for the mixed layer of each reservoir on each date with the computer program DPHOTO (Fee 1990). We report volumetric PPr, which was calculated by dividing areal PPr by Z_{mix}. Field measurements of PAR and the computer program FITSOLAR were used to estimate the attenuation of solar PAR by local atmospheric effects (Fee 1990). For Texas, the average attenuation value of 0.44 (i.e., 44% of solar PAR reaches the lake surface), was used for all dates, except for one date in October (Lake Medina) when an attenuation constant of 0.54 was estimated. For Ohio reservoirs, an attenuation constant of 0.3852 was used for all dates. These values were substituted in DPHOTO for the default atmospheric attenuation value of 0.325 (Fee 1990).

Bacterial production

BPr was measured using the micro-centrifuge ^{3}H-leucine method (Simon and Azam 1989; Smith and Azam 1992; Pace et al. 2004). Four 1.5-mL water samples were incubated in microcentrifuge tubes with ^{3}H-leucine for approximately one hour. The same brand and type of microcentrifuge tube was used for Ohio and Texas (Pace et al. 2004). ^{3}H-leucine uptake was stopped by adding 300 µl of cold 50% trichloroacetic acid (TCA) at the end of the incubation. Samples were centrifuged at 14,000 rpm for ten minutes, and the supernatant was removed with an aspirator. A wash of 1.5 mL of cold 5% TCA was added to each tube, tubes were centrifuged, and liquid was removed again. Finally, 1.5 mL of Scintiverse BD was added to each tube, and tubes were placed in glass scintillation vials and counted on a Beckman LS 6000IC scintillation counter. We corrected each BPr estimate for uptake measured in duplicate "killed" controls that were prepared by adding 300 µl of 50% TCA prior to addition of ^{3}H-leucine but otherwise treated as described above.

Statistics

We used the software program SPSS 15.0 to perform all statistical analyses. Ordinary least-squares regressions were used to test hypotheses and describe the strength of relationships between PPr, BPr, and environmental variables. In particular, we were interested in how the sequestration of particulate nutrients and the ratio of BPr:PPr varied with trophic state. We used chlorophyll-*a* as our measure of trophic state because it is: 1) commonly used for this purpose, 2) easily and often measured in limnological studies, and 3) used by Cotner and Biddanda (2001) as their measure of trophic state. Preliminary analyses revealed that most relationships conformed most closely to a power curve rather

than a linear relationship. All data were log-transformed before statistical analyses to linearize relationships and to satisfy the assumption of homoscedasticity. Regression parameters are given for simple linear regressions of the form $\log(y) = b \log(x) + c$, where y is the dependent variable, x the independent variable, b the slope, and c the y-intercept. A correction factor (CF) was calculated for each regression from the standard error of the estimate (SEE) according to Sprugel (1983). Regressions can be converted to power curves of the form $y = (d\, x^b)$ CF, where d is the antilog of c. We constructed figures using untransformed data so that the reader may more easily understand the form of relationships and the magnitude and range of values presented. The equation for the power curve is given with each figure. Analysis of covariance (ANCOVA) was used to test for differences of regression slopes and y-intercepts between categories (e.g., N versus P) with the independent variable as a covariate and the category as a factor variable. Alpha was set at 0.05 for all tests of significance.

We used both simple and multiple regressions to examine some of the potential mechanisms that may determine the relative importance of bacteria in pelagic ecosystems. We initially ran simple linear regressions on BPr:PPr versus DOC:SRP, DOC:DIN, DOC:PC, and Z_{mix} to determine if these variables explained more variance than chlorophyll. If these "mechanistic" variables were weaker predictors than chlorophyll (based on r^2 values), then we ran multiple regressions with both the "mechanistic" variable and chlorophyll as predictor variables. The r^2 value, F-statistic, and standard error of the estimate (SEE) of the multiple regressions were compared to the simple linear regression of BPr:PPr versus chlorophyll to examine whether these "mechanistic" variables improved predictions over that based solely on trophic state.

III. RESULTS

Effect of trophic state on sequestration of particulate nutrients

As a cumulative data set, Texas and Ohio reservoirs encompassed a large range in trophic state (1.3 – 73.8 μg chlorophyll L^{-1}). As predicted, the % particulate C, N, and P in the < 1-μm size fraction all decreased as chlorophyll increased (Fig. 1a-c). The % particulate C, N, and P < 1 μm ranged from 10–34%, 7– 40%, 8–52%, respectively. The inverse relationship with chlorophyll was strongest for N ($r^2 = 0.82$, $p < 0.001$) and weakest for P ($r^2 = 0.37$, $p = 0.009$) (Fig. 1b,c). The % particulate P < 1 μm appeared to decline to more slowly than the % particulate N < 1 μm as chlorophyll increased (Fig. 1b,c), but the slopes of the two relationships were not significantly different (ANCOVA, $F = 2.878$, $p = 0.10$).

The possibility exists that our data represent two different relationships (Texas versus Ohio) rather than one larger pattern, because there was limited overlap in values of chlorophyll between the two states (Table 1). However, two lines of evidence support the argument that one relationship can describe both Texas and Ohio reservoirs: 1) where chlorophyll concentrations overlap, the % of particulate nutrients in the < 1-μm size fraction are similar between Texas and Ohio (Fig. 1), and 2) when our data is plotted with that of Biddanda et al. (2001), there is a further suggestion one larger and robust pattern. We plotted the particulate nutrient data on Minnesota kettle lakes and Lake Superior (Biddanda et al. 2001) with our data on Texas and Ohio reservoirs, and the relationships are remarkably similar (Fig. 1a-c). Using ANCOVA, we found no significant difference

between the two data sets in the relationship of chlorophyll to the % of C ($p = 0.066$ and 0.964 for comparison of the intercept and slope, respectively), N ($p = 0.430$ and 0.466), or P ($p = 0.804$ and 0.839) in the < 1-μm size fraction (Fig. 1a-c).

Relationship of trophic state, BPr, and PPr

Texas and Ohio reservoir PPr ranged from 41 to 1,695 μg C L^{-1} d^{-1} and was strongly related to chlorophyll levels ($r^2 = 0.84$, $p < 0.001$, Fig. 2a) BPr ranged from 17 to 172 μg C L^{-1} d^{-1} and exhibited a strong relationship with chlorophyll ($r^2 = 0.88$, $p < 0.001$, Fig. 2b) and PPr ($r^2 = 0.89$, $p < 0.001$, Table 2). The slope of the PPr-chlorophyll relationship (0.78) was greater than the slope of the BPr-chlorophyll relationship (0.55), indicating that PPr increased with trophic state at a much faster rate than BPr (Fig. 2 a,b). Where chlorophyll concentrations overlapped between Texas and Ohio reservoirs, PPr and BPr estimates were similar (Fig. 2a,b).

As predicted, the ratio of BPr:PPr decreased with increasing chlorophyll ($r^2 = 0.42$, $p = 0.005$, Fig. 3). BPr:PPr ranged from 0.45 (chlorophyll = 1.3 μg L^{-1}) to 0.10 (chlorophyll = 49.09 μg L^{-1}). BPr:PPr varied widely from 0.16 – 0.45 when chlorophyll was < 10 μg L^{-1}, but appeared to reach an asymptote and decreased more slowly at higher chlorophyll concentrations (Fig. 3).

Potential mechanisms controlling the relative importance of bacteria

Contrary to predictions, the ratios of DOC:inorganic nutrients were generally weak predictors of the relative importance of bacteria in pelagic C cycling, as measured by BPr:PPr. DOC:SRP was not significantly related to BPr:PPr ($r^2 = 0.10$, $p = 0.220$). DOC:DIN was significantly related to BPr:PPr ($r^2 = 0.34$, $p = 0.013$), but explained less variance than chlorophyll ($r^2 = 0.42$). When we included DOC:SRP and DOC:DIN with

chlorophyll in multiple regressions predicting BPr:PPr, there was little or no improvement in r^2 values ($\leq 6\%$ additional variance explained), SEE, or F-statistics relative to the regression with only chlorophyll as a predictor (Table 2). Similarly, including DOC as an additional independent variable in a multiple regression model explained only an additional 3% of the variance in BPr:PPr (Table 2).

Two other two explanatory variables, DOC:PC and Z_{mix}, exhibited stronger relationships with BPr:PPr and explained more variance than chlorophyll (Table 2). DOC:PC explained a little less than half of the variance in BPr:PPr ($r^2 = 0.47$, $p = 0.002$). Of the four explanatory variables, Z_{mix} was the best predictor of BPr:PPr ($r^2 = 0.61$, $p < 0.001$). For comparative purposes, we also regressed BPr:PPr against its components. PPr was a better a predictor ($r^2 = 0.70$, $p < 0.001$) of the BPr:PPr ratio than any of the explanatory hypotheses tested above, but BPr explained much less of the variance in this ratio ($r^2 = 0.40$, $p = 0.007$).

IV. DISCUSSION

Effect of trophic state on sequestration of particulate nutrients

Our results strongly support the hypothesis that bacteria constitute a relatively large fraction of the total particulate nutrient pool in oligotrophic systems and that their contribution declines as trophic state increases (Biddanda et al. 2001; Cotner and Biddanda 2002). We found significant inverse relationships between chlorophyll and % particulate nutrients < 1 μm for C, N, and P. In oligotrophic reservoirs, the < 1-μm size fraction accounted for as much 34, 40, and 52 % of the total particulate C, N, and P, respectively but declined rapidly as chlorophyll increased. Inverse relationships between chlorophyll and the % of particulate nutrients in the < 1-μm size fraction are consistent with evidence that the ratio of heterotrophic to autotrophic biomass decreases as trophic state increases (Gasol et al. 1997).

The trends in sequestration of planktonic nutrient data are further supported by the comparison of our reservoirs to the lakes of Biddanda et al. (2001). Together these two independent data sets lend strong support to the idea that a large fraction of the particulate nutrient pool is attributable to bacteria in oligotrophic systems and that this fraction declines rapidly with increasing trophic state (Biddanda et al. 2001; Cotner and Biddanda 2002). The remarkable similarity between these two data sets also implies that pelagic ecosystem dynamics in reservoirs are similar to natural lakes.

The decreasing contribution of the < 1-μm size fraction to total particulate C, N, and P as chlorophyll increases is explained by the rates at which the total and the < 1-μm

particulate nutrients increase with trophic state. Total particulate C, N, and P increase approximately 9, 14, and 6 times faster, respectively than < 1-μm C, N, and P as chlorophyll increases. The possible mechanisms behind the inverse relationship between the relative importance of bacteria (as measured by biomass, respiration, or production) and trophic state have been thoroughly outlined elsewhere (see review in Cotner and Biddanda 2002). They include increased competition with phytoplankton for nutrients, greater predation on bacteria by HNF, and higher mortality from viral infection in more eutrophic systems (Currie and Kalff 1984; Sanders et al. 1992; Weinbauer et al. 1993)

Another pattern that emerges from the combined data set of our reservoirs and natural lakes is that the % particulate N < 1 μm decreases significantly faster than the % particulate P < 1 μm as chlorophyll increases (ANCOVA, $F = 7.775$, $p = 0.007$) (Fig. 4b,c). This results in predictions that the < 1-μm size fraction will account for nearly equal percentages of particulate N and P when chlorophyll equals 1 μg L^{-1} but will account for almost two times more of particulate P (~ 20 %) than of particulate N (~ 10 %) at a chlorophyll concentration of 50 μg L^{-1} (Fig. 4b,c). The differences in sequestration of P and N between size classes as trophic state increases is also reflected in the stoichiometry of the > 1-μm and the < 1-μm size fractions. We calculated molar C:P and C:N ratios for both size fractions for our reservoirs and the lakes of Biddanda et al. (2001). C:P ratios of the two size fractions decreased at similar rates with increasing chlorophyll (ANCOVA, $F = 0.376$, $p = 0.542$), but there was a significant difference in the slopes of C:N versus chlorophyll for the two size fractions (ANCOVA, $F = 4.776$, $p = 0.033$). C:N of the > 1-μm size fraction decreased with increasing chlorophyll ($r^2 = 0.48$, $p = 0.001$), but C:N of the < 1-μm size fraction did not vary significantly with chlorophyll

($r^2 = 0.02, p = 0.423$). Thus, both size fractions become richer in P per unit C and at similar rates as trophic state increases, but only the larger size fraction becomes richer in N with increasing trophic state. This suggests that the algal fraction but not the bacterial fraction utilizes increased N supply in eutrophic systems and is consistent with findings that as trophic state and P supply increase phytoplankton are more likely to become N limited (Downing and McCauley 1992; Danger et al. 2007). The relatively consistent C:N of the < 1-μm size fraction across a broad range of chlorophyll concentrations also implies that P rather than N may be limiting for bacteria over a wide range of trophic state (Davies et al. 2004).

Relationship of trophic state, BPr, and PPr

Our results support the hypothesis that BPr:PPr decreases as trophic state increases. This is in agreement with several studies that have found that the relative importance of heterotrophic to autotrophic processes decreases with trophic state. In lakes studied by del Giorgio and Peters (1994), the ratio of community respiration to photosynthesis decreased as chlorophyll increased. Biddanda et al. (2001) found that the ratio of bacterial respiration (BR) to total community respiration also decreased as chlorophyll increased. The decrease of BPr:PPr as chlorophyll increased is also consistent with regressions of BPr versus PPr with slopes less than one, indicating that BPr increases with PPr but at slower rate (Cole et al. 1988; this study).

There is debate over whether many oligotrophic pelagic ecosystems are net autotrophic or heterotrophic (del Giorgio et al. 1997; Carignan et al. 2001). We cannot address this question directly with our data, but we can estimate the fraction of bacterial carbon demand (BCD = BPr + BR) that could be supported solely by PPr in the

reservoirs we studied. We used the following regression model from del Giorgio and Cole (1998) to estimate BR from our BPr measurements:

$$BR = 3.70\ BPr^{0.41}$$

where, both BR and BPr are expressed in $\mu g\ C\ L^{-1}\ h^{-1}$. The fraction of BCD that could be supported by PPr (PPr/BCD) ranged from 0.43 to 4.55, with BCD exceeding PPr in four of the 17 reservoirs. In all cases where BCD exceeded primary production, PPr was < 120 $\mu g\ C\ L^{-1}\ d^{-1}$. This is consistent with the estimates of del Giorgio et al. (1997) who predicted that BR should generally exceed net production when PPr is ~95 $\mu g\ C\ L^{-1}\ d^{-1}$. Although we compared BCD instead of BR to PPr, most BCD (70 – 84%) in these four reservoirs was accounted for by BR. Given these estimates, there are two main conclusions that can be reached. First, in our more oligotrophic reservoirs (< 100 $\mu g\ C\ L^{-1}\ d^{-1}$) pelagic BCD exceeds PPr and therefore must be met by carbon sources other than phytoplankton-derived carbon. In reservoirs, the most likely alternate C sources are allochthonous-C inputs from inflowing rivers or littoral-derived C from macrophytes or periphyton (Rooney and Kalff 2003). Secondly, in the majority of our reservoirs, where production was >300 $\mu g\ C\ L^{-1}\ d^{-1}$, PPr exceeds BCD by a factor of approximately two or more. This implies that bacteria are likely not C-limited in most of these reservoirs and that some other factor must be controlling BPr.

The relationship of BPr:PPr with chlorophyll was generally more variable (r^2 = 0.42), especially in our more oligotrophic reservoirs, than would be expected from the strong relationships of BPr and PPr with chlorophyll (r^2 = 0.84 and 0.89, respectively). In their study of five oligotrophic lakes on the Faroe Islands, Palsson et al. (2005) found that areal BPr:PPr also ranged widely (0.21 – 0.95). The higher variance of BPr:PPr in

more oligotrophic systems could be caused by bottom-up control of BPr, whereas top-down control may be more common in eutrophic systems. For example, if BPr is more often limited by DOC or P supply in oligotrophic systems then BPr:PPr may vary widely in these systems because of intersystem differences in the quantity and quality of DOC or P. In eutrophic systems, top-down control by predation (Sanders et al. 1992) or viral infection (Weinbauer et al. 1993) may be a more consistent limitation on BPr and therefore dampen intersystem differences in BPr:PPr. This hypothesis is also consistent with higher variance of bacterial growth efficiency (BGE) in oligotrophic versus eutrophic lakes (Biddanda et al. 2001).

Our estimates of PPr are generally similar to previous studies. Using our chlorophyll versus PPr regression equation we predicted PPr over a range of chlorophyll concentrations. When simulated chlorophyll is between 20 and 70 $\mu g\ L^{-1}$, our estimates are within ~ 20% of those predicted by two extensive reviews of volumetric PPr in lakes (Smith 1979; del Giorgio and Peters 1993) (Fig. 4a). At lower chlorophyll concentrations, our estimates of PPr become progressively greater than those of Smith (1979) and del Giorgio and Peters (1993) (Fig. 4a). This is somewhat expected because we integrated our PPr values over the mixing depth. Smith (1979) used euphotic depth for all estimates of PPr, and del Giorgio and Peters' (1993) data set contained many points that had been integrated over euphotic depth. Volumetric PPr integrated over the euphotic depth will likely underestimate PPr in the mixed layer of oligotrophic lakes and overestimate PPr in eutrophic lakes (del Giorgio and Peters 1993). Therefore, volumetric PPr per unit chlorophyll in our Texas and Ohio reservoirs are similar to other studies, but

our PPr estimates at low chlorophyll values will likely exceed estimates that integrate PPr over the euphotic depth instead of the mixing depth.

Our estimates of BPr per unit chlorophyll are much higher those of Cole et al. (1988), but our estimates of BPr per unit PPr are very similar to theirs (Fig. 4b,c). Since we integrated measurements over the mixed layer and Cole et al. (1988) reviewed only studies that integrated measurements over the euphotic zone, methodological differences may explain some of the discrepancy between the two estimates based on chlorophyll. PPr may be a more consistent predictor than chlorophyll of BPr. If PPr per unit chlorophyll in the studies reviewed by Cole et al. (1988) was consistently much lower than in our reservoirs, then this could explain the paradox that our estimates based on PPr are very similar but our estimates based on chlorophyll are very different. This implies a fundamental difference in the chlorophyll-BPr relationship between our reservoirs and the pelagic systems reviewed by Cole et al. (1988), which could be the result of several causes, including climatic, plankton community composition, or methodological differences.

Potential mechanisms controlling the relative importance of bacteria

Our results do not support the hypothesis that the relative importance of bacterial metabolism is dependent on DOC concentrations (del Giorgio and Peters 1994; Jansson et al. 2000) and offer only weak support that the ratios of DOC:inorganic nutrients have similar effects (Cotner and Biddanda 2002). DOC:SRP was not significantly related to BPr:PPr, and DOC:DIN was a weaker predictor than chlorophyll. Additionally, multiple regressions with chlorophyll and either DOC, DOC:SRP, or DOC:DIN did not

substantially improve predictions of BPr:PPr as compared to the model based on chlorophyll alone.

There are several reasons why DOC and the ratios DOC:SRP and DOC:DIN may not be good predictors of the relative importance of bacterial metabolism in the reservoirs we studied and in general. First, DOC concentrations measured across broad trophic ranges probably do not reflect the quantity of DOC directly available to bacteria. There are many different sources and forms of DOC and these differ in their availability to bacteria (del Giorgio and Cole 1998; Lennon and Pfaff 2005). For example, the more labile forms of DOC, such as cell exudates from phytoplankton, are a variable and often small proportion of total DOC and are not reflected by bulk DOC measurements (Benner 2003). A related point is that DOC, DOC:SRP, nor DOC:DIN may not be good indicators of the nutritional quality of substrates used by bacteria in natural systems, because DOC derived from different sources can differ greatly in its nutrient composition and in its effect on bacterial production, respiration, and growth efficiency (del Giorgio and Cole 1998; Lennon and Pfaff 2005). Finally, observed relationships between DOC with the magnitude of bacterial metabolism versus primary production (del Giorgio and Peters 1994; Jansson et al. 2000) may be due to the shading effect of humic DOC, as opposed to a direct effect of increased DOC availability on bacteria. del Giorgio and Peters (1994) found that DOC was related to the ratio of respiration to photosynthesis but that this effect was mostly due to the suppression of PPr as DOC increased, likely from increased shading. If highly colored lakes are included in studies, then researchers may find a relationship between DOC and the relative importance of bacteria. In systems with

more moderate concentrations of DOC or just lower concentrations of humic substances, such as the reservoirs we studied, researchers may find no such relationship.

Cotner and Biddanda (2002) hypothesized that in oligotrophic systems relatively high dissolved organic matter versus particulate organic matter should favor bacteria in competition with phagotrophic heterotrophs for C but that as the relative abundance of particulate organic matter increased with trophic state phagotrophs would be favored. This could explain the decreased contribution of bacteria to community biomass and metabolism in more eutrophic systems (del Giorgio and Peters 1994; Gasol et al. 1997). In our study, the relative contribution of bacteria to community metabolism, as measured by BPr:PPr, increased with DOC:PC. However, this relationship can be explained by alternative mechanisms to those proposed by Cotner and Biddanda (2002). The strength of the relationship between DOC:PC and BPr:PPr is primarily due to the negative relationship between PC and BPr:PPr ($r^2 = 0.418$, $p = 0.005$) rather than the relationship between DOC and BPr:PPr ($r^2 = 0.14$, $p = 0.134$). PC is strongly related to chlorophyll ($r^2 = 0.94$, $p < 0.001$) and likely a surrogate for trophic state. Thus, it is possible that the relationship between DOC:PC and BPr:PPr is caused by increased levels of PC in eutrophic systems supporting larger numbers of phagotrophic heterotrophs that out compete bacteria for detrital PC. However, it also leaves open any of the other explanations for decreasing importance of bacterial biomass and metabolism with trophic state, such as increased rates of predation or viral infection (Sanders et al. 1992; Weinbauer et al. 1993).

While DOC concentrations and DOC:inorganic nutrients were weak predictors of BPr:PPr, Z_{mix} was a stronger predictor of BPr:PPr than chlorophyll. Cotner and Biddanda

(2002) suggest that light-dependent nutrient uptake by phytoplankton could explain an inverse correlation between Z_{mix} and the ratio of phytoplankton to bacterial biomass. This explanation assumes that Z_{mix} is inversely related to mean light in the mixed layer. This was not the case in our study. Mean irradiance in the mixed layer (I_m), calculated as a fraction of surface light (Sterner et al. 1997), was not significantly related to Z_{mix} in our reservoirs ($r^2 = 0.03$, $p = 0.545$). Therefore, the relationship of Z_{mix} with BPr:PPr cannot be explained solely by light environment. Z_{mix} was a good indicator of trophic state because it was inversely correlated with TP ($r = -0.63$), TN ($r = -0.65$), and PPr ($r = -0.86$), but was not as strongly correlated with these trophic measures as chlorophyll ($r = 0.85$, 0.86, and 0.92 for TP, TN, and PPr, respectively). Z_{mix} was more strongly correlated than chlorophyll with reservoir size ($r = 0.80$ and -0.59, respectively). Furthermore, when we excluded the three most oligotrophic reservoirs, which had relatively high I_m, there was a moderately strong inverse correlation between Z_{mix} and I_m ($r = -0.59$). The strength of Z_{mix} as a predictor of BPr:PPr may be because it is partially correlated with nutrient supply, reservoir size, and I_m all of which may directly or indirectly affect PPr and BPr.

Conclusions

Previous work in pelagic habitats of natural lakes and the ocean have led to the hypothesis that the relative importance of heterotrophic bacteria to ecosystem structure and function is high in oligotrophic systems but decreases as trophic state increases (del Giorgio and Peters 1994; Gasol et al. 1997; Biddanda et al. 2001). Our study of 17 reservoirs that varied greatly in trophic state strongly supports this hypothesis. Two

measures of the importance of bacteria, the % of particulate nutrients < 1 μm and BPr:PPr, were high in oligotrophic reservoirs but decreased as trophic state increased.

The patterns of particulate nutrients sequestration and stoichiometric differences between size fractions are consistent with a change in the competitive relationship between bacteria and phytoplankton as trophic state increases. In oligotrophic systems, the large proportion of particulate nutrients sequestered in the < 1-μm size fraction implies that competition with bacteria is a major factor limiting phytoplankton growth in these systems. This is consistent with experimental evidence that bacteria dominate nutrient uptake when supply is low (Currie and Kalff 1984). Furthermore, the consistently lower C:P stoichiometry of the < 1-μm size fraction and the relatively high % of particulate P < 1 μm indicates that even in eutrophic systems bacteria are effective competitors for P.

Our study offers only weak support for the hypotheses that DOC or the relative abundance of DOC versus inorganic nutrients are major factors controlling the contribution of bacteria to pelagic ecosystem function. There are several reasons why DOC or its relative abundance to inorganic nutrients may not consistently be strong predictors of the relative importance of bacterial metabolism. Similarly, the significant relationship between DOC:PC and BPr:PPr was due to PC being a surrogate for trophic state and may have little to do with the relative importance of DOC as bacterial substrate. We acknowledge that DOC can stimulate bacterial metabolism and have indirect negative effects on phytoplankton in small-scale experiments (e.g., Joint et al. 2002), but at an ecosystem scale, it is likely that the size of this effect will be small unless measured DOC is very high or PPr is very low.

The reservoirs we studied seem to be similar to other pelagic habitats in terms of the relative importance of bacteria to particulate nutrient pools and ecosystem function. PPr per unit chlorophyll was similar to estimates made in natural lakes, and BPr per unit PPr was similar to data from natural lakes and marine habitats. We believe the economic feasibility of studying reservoirs, their widespread distribution, and the similarities between reservoirs and other pelagic habitats make a strong case that increased effort should be made to address important ecological questions in these ecosystems.

Table 1. Reservoir characteristics. Location and mean values of chlorophyll, total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC), Secchi depth (Secchi), surface area (SA), and mixed layer depth (Z_{mix}) for two sets of reservoirs in Ohio and Texas. For Granger, which was never stratified, Z_{mix} is also the maximum depth.

Reservoir	Lat/Long (°N, °W)	Chlorophyll ($\mu g\ L^{-1}$)	TP ($\mu g\ L^{-1}$)	TN ($\mu g\ L^{-1}$)	DOC ($mg\ L^{-1}$)	Secchi (m)	SA (ha)	Z_{mix} (m)
Texas								
Canyon	29.88, 98.26	1.30	4.9	161.7	3.3	4.63	3330	10
Stillhouse Hollow	31.02, 97.53	1.50	6.5	227.7	4.2	4.60	2600	8.5
Medina	29.54, 98.93	1.51	6.4	171.7	2.9	3.23	2260	8.5
Buchanan	30.75, 98.42	7.53	12.3	377.8	4.8	1.93	9330	7.5
Bastrop	30.16, 97.29	8.34	28.8	819.5	11.5	2.00	367	6
LBJ	30.55, 98.34	11.23	15.7	405.8	5.1	1.58	2580	4
Inks	30.73, 98.37	12.27	29.1	416.8	4.9	1.65	320	2.5
Granger	30.70, 97.32	15.07	33.8	473.8	3.9	0.48	1780	6.75
Dunlap	29.65, 98.07	16.46	35.0	1075	3.1	0.75	166	2
Ohio								
Burr Oak	39.54, 82.06	15.09	17.9	355	2.8	1.72	266	3.0
Berlin	41.05, 81.00	15.34	26.0	456	4.3	0.96	1560	4.5
Hoover	40.11, 82.88	20.57	32.4	740	4.1	0.95	1335	4.3
Dillon	39.99, 82.08	30.11	312.7	1111	1.6	1.01	536	3.5
Tappan	40.35, 81.23	46.65	36.0	639	2.3	0.83	964	4.8
Pleasant Hill	40.62, 82.32	49.09	56.7	938	1.8	0.92	312	4.1
Acton	39.56, 84.74	73.64	74.3	4682	2.8	0.76	232	2.6
Delaware	40.36, 83.07	73.81	81.6	1824	4.2	0.65	453	4.0

Table 2. Regression parameters. Linear regression parameters relating chlorophyll (μg L^{-1}), % of particulate nutrients < 1μm (% C, N, and P < 1), bacterial production (BPr, μg C L^{-1} d^{-1}), primary production (PPr, μg C L^{-1} d^{-1}), BPr:PPr, mixing depth (Z_{mix}, m), DOC (mg L^{-1}), and molar ratios of DOC:soluble reactive phosphorus (SRP), DOC:dissolved inorganic nitrogen (DIN), and DOC: particulate carbon (PC). Simple linear regressions are of the form $\log(y) = b \log(x) + c$, where y is the dependent variable, x the independent variable, b the slope, and c the intercept. The standard error of the slope and intercept are given in parentheses. The correction factor (CF) was calculated from the standard error of the estimate (SEE) according to Sprugel (1983). Simple linear egressions can be converted to power curves of the form $y = (d\ x^b)$ CF, where d is the antilog of c.

y	x	b		c		r^2	SEE	CF	F	p
% C < 1	Chl	-0.230	*(0.048)*	1.469	*(0.060)*	0.60	0.106	1.03	22.75	< 0.001
% N < 1	Chl	-0.353	*(0.043)*	1.619	*(0.054)*	0.82	0.097	1.03	67.15	< 0.001
% P < 1	Chl	-0.212	*(0.071)*	1.617	*(0.088)*	0.37	0.156	1.07	8.90	0.009
PPr	Chl	0.777	*(0.087)*	1.626	*(0.108)*	0.84	0.191	1.10	78.52	< 0.001
BPr	Chl	0.553	*(0.052)*	1.125	*(0.064)*	0.88	0.114	1.03	114.18	< 0.001
BPr	PPr	0.654	*(0.060)*	0.111	*(0.153)*	0.89	0.112	1.03	118.30	< 0.001
BPr:PPr	Chl	-0.217	*(0.066)*	-0.479	*(0.082)*	0.42	0.146	1.06	10.63	0.005
BPr:PPr	DOC:SRP	0.092	*(0.072)*	-1.047	*(0.258)*	0.10	0.181	1.09	1.64	0.220
BPr:PPr	DOC:DIN	0.219	*(0.078)*	-1.114	*(0.145)*	0.34	0.155	1.07	7.85	0.013
BPr:PPr	DOC:PC	0.309	*(0.084)*	-0.849	*(0.048)*	0.47	0.139	1.05	13.52	0.002
BPr:PPr	Z_{mix}	0.709	*(0.146)*	-1.191	*(0.101)*	0.61	0.119	1.04	23.65	< 0.001
BPr:PPr	PPr	-0.332	*(0.056)*	0.108	*(0.142)*	0.70	0.104	1.03	35.07	< 0.001
BPr:PPr	BPr	-0.361	*(0.114)*	-0.092	*(0.203)*	0.40	0.148	1.06	9.94	0.007
Multiple regressions										
BPr:PPr	Chl	-0.196	*(0.070)*	-0.608	*(0.154)*	0.45	0.146	1.06	5.80	0.015
	DOC	0.192	*(0.194)*							
BPr:PPr	Chl	-0.216	*(0.078)*	-0.484	*(0.297)*	0.42	0.151	1.06	4.96	0.024
	DOC:SRP	0.001	*(0.069)*							
BPr:PPr	Chl	-0.154	*(0.080)*	-0.760	*(0.228)*	0.48	0.142	1.05	6.45	0.010
	DOC:DIN	0.118	*(0.089)*							

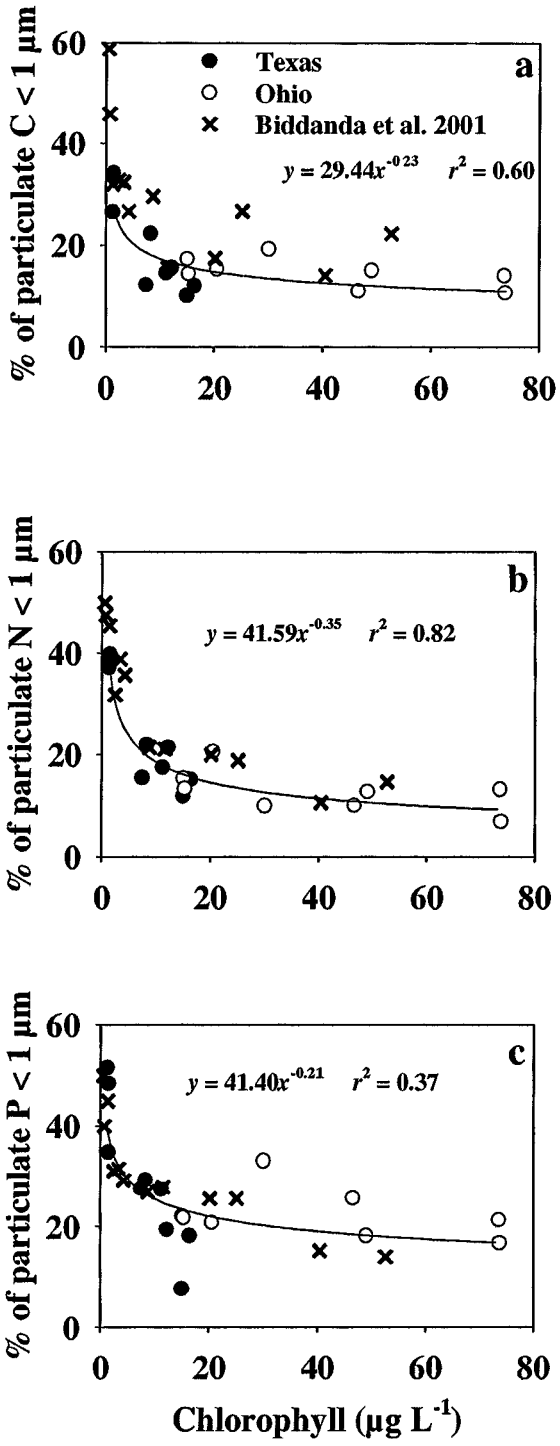

Fig. 1. Particulate nutrients. Percentage of particulate (a) carbon, (b) nitrogen, and (c) phosphorus in the < 1-µm size fraction as a function of chlorophyll in Texas and Ohio reservoirs and in Minnesota lakes (Biddanda et al. 2001). Regression lines and equations are for Texas and Ohio reservoirs only.

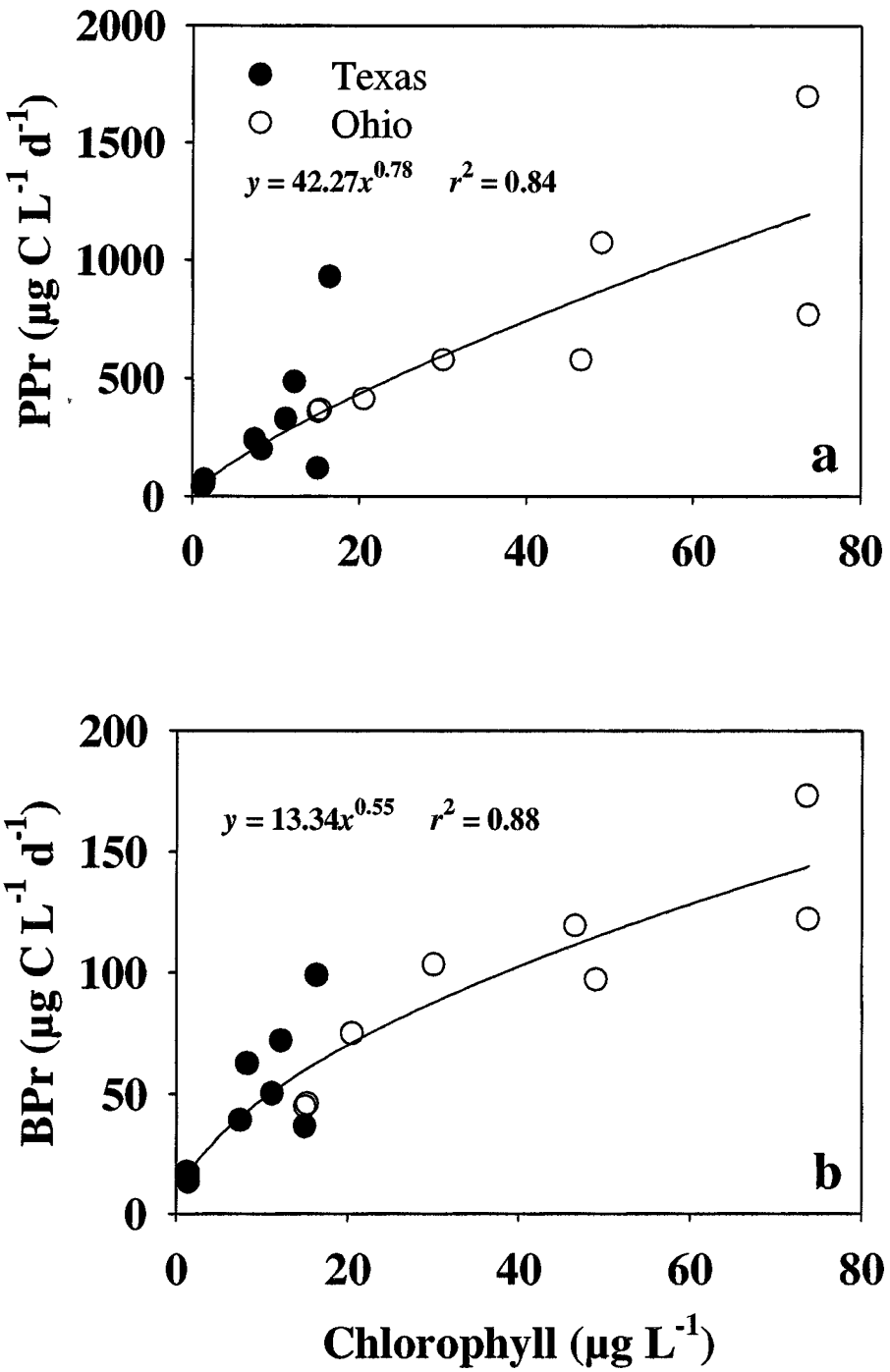

Fig 2. Primary production and bacterial production. Relationship of (a) primary production (PPr) and (b) bacterial production (BPr) with chlorophyll concentration in Texas and Ohio reservoirs (n=17).

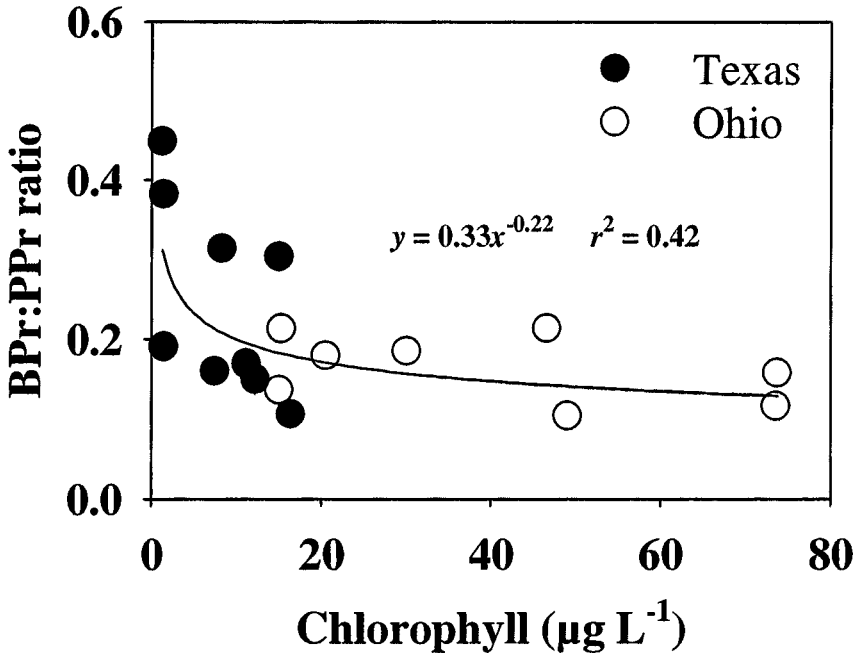

Fig. 3. BPr:PPr ratio. The ratio of bacterial production to primary production (BPr:PPr) as a function of chlorophyll concentration in Texas and Ohio reservoirs (n=17).

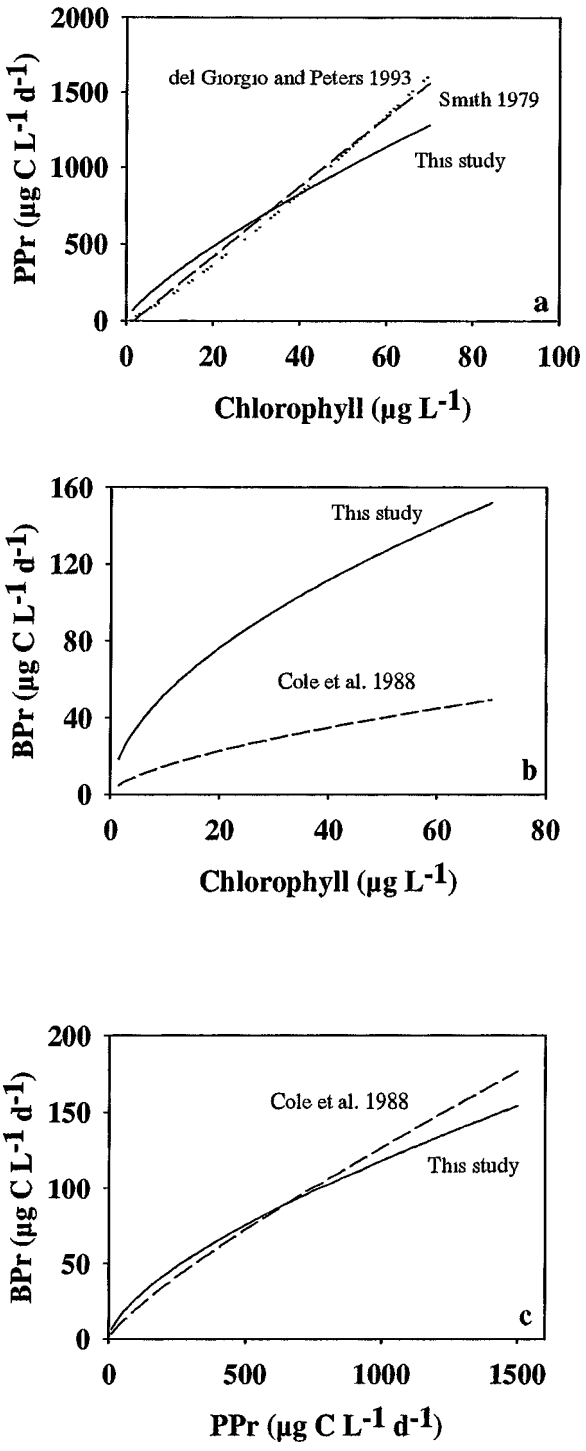

Fig. 4. Comparison of PPr and BPr with other estimates. Comparison of our model predicting a) primary production (PPr) from chlorophyll to those of Smith (1979) and del Giorgio and Peters (1993) and comparisons of our models predicting bacterial production (BPr) from b) chlorophyll and c) PPr to those of Cole et al. 1988.

V. LITERATURE CITED

Auer, B., U. Elzer, and A. Hartmut. 2004. Comparison of pelagic food webs in lakes along a trophic gradient and with seasonal aspects: influence of resource and predation. Journal of Plankton Research **26**:697-709.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series **10**:257-263.

Benner, R. 2003. Molecular indicators of the bioavailability of dissolved organic matter. Pages 121-137 *in* S. E. G. Findlay and R. L. Sinsabaugh, editors. Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press, San Diego.

Baines, S. B., and M. L. Pace. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. Limnology and Oceanography **36**:1078-1090.

Biddanda, B. A., M. Ogdahl, and J. B. Cotner. 2001. Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. Limnology and Oceanography **46**:730-739.

Bird, D. F., and J. Kalff. 1986. Bacterial grazing by planktonic lake algae. Science **231**:493-495.

Carignan, R., D. Planas, and V. Chantal. 2000. Planktonic production and respiration in oligotrophic Shield lakes. Limnology and Oceanography **45**:189-199.

Chrzanowski, T. H., and J. G. Hubbard. 1988. Primary and bacterial production in a southwestern reservoir. Applied and Environmental Microbiology **54**:661-669.

Cole, J. J., S. Findlay, and M. L. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Marine Ecology Progress Series **43**:1-10.

Cole, J. J., G. E. Likens, and D. L. Strayer. 1982. Photosynthetically produced dissolved organic carbon: an important carbon source for planktonic bacteria. Limnology and Oceanography **27**:1080-1090.

Cotner, J. B., and B. A. Biddanda. 2002. Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems **5**:105-121.

Crumpton, W. G., T. M. Isenhart, and P. D. Mitchell. 1992. Nitrate and organic N analyses with second-derivative spectroscopy. Limnology and Oceanography **37**:907-913.

Currie, D. J., and J. Kalff. 1984. The relative importance of bacterioplankton and phytoplankton in phosphorus uptake in freshwater. Limnology and Oceanography **29**:311-321.

Danger, M., C. Oumarou, D. Benest, and G. Lacroix. 2007. Bacteria can control stoichiometry and nutrient limitation of phytoplankton. Functional Ecology **21**:202-210.

Davies, J.-M., W. H. Nowlin, and A. Mazumder. 2004. Temporal changes in nitrogen and phosphorus codeficiency of plankton in lakes of coastal and interior British Columbia. Canadian Journal of Fisheries and Aquatic Sciences **61**:1538-1551.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics **29**:503-541.

del Giorgio, P. A., J. J. Cole, and A. Cimbleris. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. Nature **385**:148-151.

del Giorgio, P. A., and R. H. Peters. 1993. Balance between phytoplankton production and plankton respiration in lakes. Canadian Journal of Fisheries and Aquatic Sciences **50**:282-289.

del Giorgio, P. A., and R. H. Peters. 1994. Patterns planktonic P:R ratios in lakes: influence of lake trophy and dissolved organic carbon. Limnology and Oceanography **39**:772-787.

Downing, J. A., and E. McCauley. 1992. The nitrogen:phosphorus relationship in lakes. Limnology and Oceanography **37**:936-945.

Fee, E. J. 1990. Computer programs for calculating in situ phytoplankton photosynthesis. Canadian Technical Report of Fisheries and Aquatic Sciences 1740. Department of Fisheries and Oceans.

Gasol, J. M., P. A. del Giorgio, and C. M. Duarte. 1997. Biomass distribution in marine planktonic communities. Limnology and Oceanography **42**:1353-1363.

González, N., R. Anadón, and L. Viesca. 2003. Carbon flux through the microbial community in a temperate sea during summer: role of bacterial metabolism. Aquatic Microbial Ecology **33**:117-126.

Ground, T. A., and A. W. Groeger. 1994. Chemical classification and trophic characteristics of Texas reservoirs. Lake and Reservoir Management **10**:189-201.

Jansson, M., A.-K. Bergstrom, P. Blomqvist, and S. Drakare. 2000. Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. Ecology **81**:3250-3255.

Joint, I., P. Henriksen, G. A. Fonnes, D. Bourne, T. F. Thingstad, and B. Riemann. 2002. Competiton for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. Aquatic Microbial Ecology **29**:145-159.

Kalff, J. 2003. Limnology: inland water ecosystems. Prentice Hall.

Karlsson, J., M. Jansson, and A. Jonsson. 2002. Similar relationships between pelagic primary and bacterial production in clearwater and humic lakes. Ecology **83**:2902-2910.

Kimmel, B. L., O. T. Lind, and L. J. Paulson. 1990. Reservoir primary production. Pages 133-193 *in* K. W. Thornton, B. L. Kimmel, and F. E. Payne, editors. Reservoir limnology: ecological perspectives. Wiley.

Knoll, L. B., M. J. Vanni, and W. H. Renwick. 2003. Phytoplantkon primary production and photosynthetic parameters in reservoirs along a trophic gradient of watershed land use. Limnology and Oceanography **48**:608-617.

Lennon, J. T., and L. E. Pfaff. 2005. Source and supply of terrestrial organic matter affects aquatic microbial metabolism. Aquatic Microbial Ecology **39**:107-119.

Pace, M. L., P. A. del Giorgio, D. Fischer, R. Condon, and H. Malcom. 2004. Estimates of bacterial production using the leucine incorporation method are influenced by differences in protein retention of microcentrifuge tubes. Limnology and Oceanography: Methods **2**:55-61.

Palsson, C., E. S. Kritzberg, K. Christoffersen, and W. Graneli. 2005. Net heterotrophy in Faroe Islands clear-water lakes: causes and consequences for bacterioplankton and phytoplankton. Freshwater Biology **50**:2011-2020.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. Bioscience **24**:499-504.

Rooney, N., and J. Kalff. 2003. Submerged macrophyte-bed effects on water-column phosphorus, chlorophyll-*a*, and bacterial production. Ecosystems **6**:797-807.

Sanders, R. W., D. A. Caron, and U.-G. Berninger. 1992. Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. Marine Ecology Progress Series **86**:1-14.

Sanders, R. W., K. G. Porter, S. J. Bennet, and A. E. DeBiase. 1989. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in freshwater planktonic community. Limnology and Oceanography **34**:673-687.

Simon, M., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Marine Ecology Progress Series **51**:210-213.

Smith, D. C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Marine Microbial Food Webs **6**:107-117.

Smith, V. H. 1979. Nutrient dependence of primary productivity in lakes. Limnology and Oceanography **24**:1051-1064.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography **14**:799-801.

Sprugel, D. G. 1983. Correcting for bias in log-transformed allometric equations. Ecology **64**:209-210.

Sterner, R. W., J. J. Elser, E. J. Fee, S. J. Guildford, and T. H. Chrzanowski. 1997. The light:nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. The American Naturalist **150**:663-684.

Vargas, C. A., R. A. Martinez, L. A. Cuevas, M. A. Pavez, C. Cartes, H. E. Gonzalez, R. Escribano, and G. Daneri. 2007. The relative importance of microbial and classical food webs in a highly productive coastal upwelling area. Limnology and Oceanography **52**:1495-1510.

Weinbauer, M. G., D. Fuks, and P. Peduzzi. 1993. Distribution of viruses and dissolved DNA along a coastal trophic gradient in the northern Adriatic Sea. Applied and Environmental Microbiology **59**:4074-4082.

Wetzel, R. G., and G. E. Likens. 2000. Limnological Methods, 3rd edition. Springer-Verlag, New York.

VITA

Brad Caston was born in Beaumont, TX on September 4, 1975 to Byron and Dianna Caston. He grew up in the piney woods near Kirbyville with his three siblings Eric, Stephanie, and Spencer. After graduation from high school, Brad moved away to Austin, TX to play tennis and study at Concordia University but later transferred to and graduated from the University of Texas. He worked in Austin for several years as an environmental consultant before moving to San Marcos to pursue graduate studies at Texas State University-San Marcos.

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