The relative importance of heterotrophic bacteria to pelagic ecosystem dynamics varies with reservoir trophic state

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Abstract

We tested the hypothesis that nutrient sequestration and carbon flow through heterotrophic bacteria is relatively highest in oligotrophic systems and decreases with trophic state in 17 reservoirs in Texas and Ohio, U.S.A. The percentage of particulate carbon (C), nitrogen (N), and phosphorus (P) in bacteria ($< 1 \mu m$) was highest in oligotrophic reservoirs and decreased with chlorophyll *a*, our measure of trophic state. Patterns of nutrient sequestration in bacteria were very similar to Minnesota (U.S.A.) natural lakes. Bacterial production (BPr) and primary production (PPr) ranged from 13 μg C L^{-1} d^{-1} to 172 μg C L^{-1} d^{-1} and from 41 μg C L^{-1} d^{-1} to 1695 μg C L^{-1} d^{-1}, respectively, and the BPr : PPr ratio decreased with trophic state. Inclusion of variables such as dissolved organic carbon (DOC), DOC : soluble reactive phosphorus, and DOC : dissolved inorganic nitrogen did not improve predictions of BPr : PPr over that based solely upon chlorophyll *a*. BPr : PPr was better predicted by DOC : particulate carbon (PC) and mixing depth (Z_{mix}) than by chlorophyll *a*. The relationship between BPr : PPr and DOC : PC was primarily driven by the relationship between BPr : PPr and PC, 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. Our results support the hypothesis that the relative importance of heterotrophic bacteria is highest in oligotrophic systems and decreases with trophic state, but provide limited support for proposed mechanisms of this pattern. Patterns in microbial nutrient dynamics are similar in reservoirs and natural lakes.

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 nutrient recyclers, but that view changed with the recognition of the microbial loop composed 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 but also are competitors with phytoplankton for nutrients and a critical trophic link between dissolved organic carbon (DOC) and the classic autotroph–grazer food chain. Due to large surface area : volume ratios, bacteria can outcompete phytoplankton for inorganic nutrients (in particular phosphorus) especially in systems where inorganic nutrient supply is low or DOC is 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 and making many oligotrophic systems 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 as DOC released from 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 pathways (Azam et al. 1983; Vargas et al. 2007). Furthermore, mixotrophic phytoplankton can consume bacteria 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 trophic state (del Giorgio and Peters 1994; Biddanda et al. 2001; Cotner and Biddanda 2002). This prediction comes from several lines of evidence. First, many oligotrophic systems are net heterotrophic, exhibiting phytoplankton photosynthesis to plankton community respiration ratios ($P : R$) $<$ 1, and $P : R$ tends to increase with trophic status across systems (del Giorgio and Peters 1994). Second, the proportion of total planktonic respiration accounted for by bacterioplankton is highest in oligotrophic systems and decreases with trophic status (Biddanda et al. 2001), indicating a relatively greater role of bacteria in less productive systems. Third, the ratio of pelagic bacterioplankton biomass to phytoplankton biomass is highest in oligotrophic systems and declines with trophic status (del Giorgio and Gasol 1995; Gasol et al. 1997). Finally, the percentage of total particulate nutrients sequestered in the bacterioplankton size fraction is greatest in oligotrophic systems and declines with lake trophic state (Biddanda et al. 2001).

Several mechanistic explanations have been proposed for the negative relationship between the importance of heterotrophic bacteria and trophic state. In oligotrophic systems, the greater relative abundance of DOC and dissolved organic nutrients vs. dissolved inorganic nutrients should provide heterotrophic bacteria a competitive advantage over phytoplankton, leading to a greater role of bacteria in carbon and nutrient cycling (Cotner and Biddanda 2002; Joint et al. 2002). Thus, phytoplankton

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production is thought to be partially constrained by competition with bacteria when inorganic nutrient supply is low and DOC is high (Currie and Kalff 1984; Joint et al. 2002). In oligotrophic systems, a higher ratio of DOC to particulate organic carbon should favor bacteria in competition with eukaryotic phagotrophs for organic C (Cotner and Biddanda 2002). In addition, viral infection of bacteria (Weinbauer et al. 1993) and protozoan bacterivory (Sanders et al. 1992; Auer et al. 2004) increase with trophic state, reducing the direct nutrient cycling role of bacteria in eutrophic systems.

Most evidence of a greater role for heterotrophic bacteria in oligotrophic systems comes from studies of marine ecosystems and natural lakes (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). 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 $\sim$ 20% of the runoff from world watersheds (Kalff 2003). The amount of organic carbon that sediments in reservoirs each year is estimated to be as much as one and a half to four times the amount that is buried in the world's oceans (Dean and Gorham 1998; Downing et al. 2008). Therefore, reservoir ecosystem processes potentially affect global carbon cycling.

Despite the importance of reservoirs in global hydrologic and nutrient cycling, relatively little is known about reservoirs with regard to fundamental ecological questions about pelagic nutrient cycling. Reservoirs share many structural and functional characteristics with natural lakes, but also are different in many aspects. Similarities include the presence of pelagic and littoral habitats, the occurrence of similar planktonic species (Kalff 2003), and rates of bacterial and primary production are often similar (Chrzanowski and Hubbard 1988; Knoll et al. 2003). However, reservoirs display substantial differences with natural lakes in physical habitat structure, nutrient loading, hydrologic characteristics, and irradiance levels (Thornton 1990*a*; Kalff 2003; Knoll et al. 2003). Differences between reservoirs and natural lakes and the importance of reservoirs on a global scale dictates that we examine the role of heterotrophic bacteria across a diversity of reservoirs and determine which environmental factors affect their relative importance.

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, U.S.A. We had two primary goals. 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 the percentage of particulate nutrients in the bacterial size fraction ($< 1~\mu$m) and that the ratio of bacteria production (BPr) to primary production (PPr) would decrease as trophic state increased. Also, we wanted to examine some of the potential mechanisms that determine the relative

importance of bacteria in pelagic systems, including the relative abundance of DOC vs. inorganic nutrients, the relative abundance of DOC vs. particulate carbon (PC), and the underwater light environment (Cotner and Biddanda 2002). Specifically, we hypothesized that the importance of bacteria, as measured by BPr:PPr, would increase as (1) DOC:soluble reactive phosphorus (SRP), (2) DOC:dissolved inorganic nitrogen, (3) DOC:PC, and (4) mixed-layer depth increases. To explore these questions, we measured BPr, PPr, and nutrients in reservoirs in Ohio and Texas. Few previous studies have simultaneously examined nutrient sequestration, BPr, PPr, and potential factors affecting the relative importance of heterotrophic bacteria in pelagic ecosystems.

A secondary goal of our study was to compare algal–bacterial nutrient sequestration patterns, BPr, and PPr between natural lakes and reservoirs. Reservoirs often exhibit greater turbidity due to higher concentrations of suspended sediments than in natural lakes (Thornton 1990*a*) and these conditions may lead to suppression of phytoplankton production and an enhancement of the role of bacteria in pelagic nutrient cycles. In addition, the much tighter coupling of reservoirs with the terrestrial landscape (e.g., increased allochthonous C inputs) may further lead to an increased role of bacteria in reservoirs when compared to natural lakes.

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 subtropical climates, respectively. Reservoirs were selected to encompass a wide range in productivity and trophic state within each region (Ground and Groeger 1994; Knoll et al. 2003).

Ohio reservoirs were generally smaller and shallower than Texas reservoirs, with surface area ranging from 2.3 km^2 to 15.6 km^2 (Table 1). While Texas reservoirs were on average larger, they also encompassed a wider range in size, with surface area ranging from 1.7 km^2 to 93.3 km^2. As expected, the larger reservoirs had greater mixed-layer depth (Z_{mix}; Sterner et al. 1997), and Z_{mix} was correlated with reservoir surface area (Pearson $r = 0.59$). Granger Lake (Texas) was the only reservoir that was not stratified; therefore, Z_{mix} for Granger is also the maximum depth.

Reservoirs were sampled during the summer–early autumn 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 nine, eight, and seven 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 nonvolatile suspended solids (NVSS). With the exception of Lake Dunlap, reported results for each reservoir represent the mean of all sampling dates.

Table 1. Location, Chl *a*, total phosphorus (TP), total nitrogen (TN), primary production (PPr), bacterial production (BPr), Secchi depth (Secchi), surface area (SA), and mixed-layer depth (Z_{mix}) for two sets of reservoirs in Ohio and Texas, U.S.A. For Granger, which was never stratified, Z_{mix} is also the maximum depth.

Reservoir	Lat, Long (°N, °W)	Chl *a* ($\mu g\ L^{-1}$)	TP ($\mu g\ L^{-1}$)	TN ($\mu g\ L^{-1}$)	PPr ($\mu g\ C\ L^{-1}\ d^{-1}$)	BPr ($\mu g\ C\ L^{-1}\ d^{-1}$)	Secchi (m)	NVSS (mg L^{-1})	SA (km²)	Z_{mix} (m)
Texas										
Canyon	29.88, 98.26	1.30	4.9	162	41.40	17.38	4.63	0.39	33.3	10.0
Stillhouse Hollow	31.02, 97.53	1.50	6.5	228	49.99	17.24	4.60	0.03	26.0	8.5
Medina	29.54, 98.93	1.51	6.4	172	69.02	13.18	3.23	0.80	22.6	8.5
Buchanan	30.75, 98.42	7.53	12.3	378	237.86	39.00	1.93	0.07	93.3	7.5
Bastrop	30.16, 97.29	8.34	28.8	820	198.75	62.33	2.00	0.01	3.7	6.0
LBJ	30.55, 98.34	11.23	15.7	406	325.88	49.89	1.58	0.21	25.8	4.0
Inks	30.73, 98.37	12.27	29.1	417	481.25	71.55	1.65	0.12	3.2	2.5
Granger	30.70, 97.32	15.07	33.8	474	117.73	36.62	0.48	11.53	17.8	6.75
Dunlap	29.65, 98.07	16.46	35.0	1075	926.50	98.63	0.75	3.31	1.7	2.0
Ohio										
Burr Oak	39.54, 82.06	15.09	17.9	355	357.33	44.54	1.72	1.21	2.7	3.0
Berlin	41.05, 81.00	15.34	26.0	456	364.08	45.71	0.96	4.62	15.6	4.5
Hoover	40.11, 82.88	20.57	32.4	740	410.22	74.59	0.95	1.01	13.4	4.3
Dillon	39.99, 82.08	30.11	312.7	1111	574.82	103.08	1.01	2.93	5.4	3.5
Tappan	40.35, 81.23	46.65	36.0	639	574.65	119.24	0.83	0.13	9.6	4.8
Pleasant Hill	40.62, 82.32	49.09	56.7	938	1071.68	96.90	0.92	1.54	3.1	4.1
Acton	39.56, 84.74	73.64	74.3	4682	1695.88	172.94	0.76	2.12	2.3	2.6
Delaware	40.36, 83.07	73.81	81.6	1824	766.50	122.08	0.65	6.50	4.5	4.0

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 a Yellow Springs Instruments Model 85D (Texas) or Model 58 (Ohio). Photosynthetically available radiation (PAR) was measured at 0.5–1-m intervals using a Li-Cor 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* (Chl *a*), alkalinity, dissolved inorganic carbon (DIC), DOC, total phosphorus (TP), total nitrogen (TN), 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. Chl *a* 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 titration (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 in Ohio and a Varian Cary 50 Ultraviolet-Visible light (UV-Vis) spectrophotometer in 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 preashed 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 preashed Whatman GF/F filters, and the filtrate was analyzed on a Shimadzu TOC-V_{CSH} total organic carbon analyzer. NVSS concentrations were determined by filtering water onto precombusted, preweighed Gelman A/E filters and drying filters at 60°C for 48 h and reweighing to determine total suspended solids. Filters were subsequently ashed and reweighed to calculate NVSS (Knoll et al. 2003).

Total particulate C and N samples were filtered onto preashed 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 Carbon–Hydrogen–Nitrogen analyzer (Ohio) and a CE Elantech Carbon–Nitrogen analyzer (Texas). For particulate C and N < 1 μm, water was first filtered through 1-μm Nuclepore 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 concentrated HCl at 100°C, and measured as PO_4^{3-} using the molybdenum blue method. For particulate P < 1 μm, samples were first filtered through 1-μm Nuclepore filters and then treated the same as total particulate P. Measurement of C, N, and P in total and < 1-μm-size seston fractions likely includes particulate material that is not classified as 'phytoplankton' or 'bacteria' (i.e., detritus, sediments); however, we assume that the nutrient content and ratios in the > 1-μm and < 1-μm size fractions are reflective of those in phytoplankton and bacteria (Sterner and Elser 2002).

Primary production—For all reservoirs, PPr estimates were performed within 36 h 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 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 Chl a 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 Chl a concentration. Finally, depth-specific PAR measured in each lake, Chl a 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 h in a photosynthetic chamber consisting of a water bath maintained at mixed-layer temperatures and illuminated with a 1000-W metal halide bulb. After incubation, samples were filtered through Gelman A/E glass-fiber filters to collect phytoplankton. Use of A/E filters (nominal pore size = 1 μm) for PPr estimates may have led us to exclude the contribution of a portion of the autotrophic picoplankton (< 3 μm) to overall PPr (Agawin et al. 2000; Bell and Kalff 2001 [but *see* Fahnenstiel et al. 1994]). Each filter was rinsed with dilute HCl to remove ^{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 Photosynthesis Parameters (Fee 1990) was used to estimate α^B and P^B_M from the P–I curve and Chl a concentrations. Estimates of simulated cloud-free areal PPr were generated for the mixed layer of each reservoir on each date with the computer Program Day Photosynthesis (DPHOTO; Fee 1990). We report volumetric PPr, which was calculated by dividing areal PPr by Z_{mix}. We used both field measurements of PAR from our sites in Texas and Ohio and the computer Program FITSOLAR, which calculates the empirical constant that describes the net effect of atmospheric factors on incoming PAR to estimate the attenuation of solar PAR (Fee 1990). Based upon these comparisons, we decided to use empirically derived attenuation values rather than the DPHOTO default atmospheric attenuation value of 0.33 (Fee 1990). For Texas, the 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 0.39 was used for all dates.

Bacterial production—BPr was measured using the microcentrifuge ^3H-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 ^3H-leucine for ~ 1 h. The same brand and type of microcentrifuge tube was used for Ohio and Texas (Pace et al. 2004). ^3H-leucine uptake was stopped by adding 300 μL of cold 50% trichloroacetic acid (TCA) at the end of the incubation. Samples were centrifuged at 16,684 g for 10 min, 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 ^3H-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 Chl 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 (2002) as their measure of trophic state, and we wished to explicitly compare our relationship with theirs. 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 readers may more easily understand the form of relationships and the magnitude and range of values presented. Analysis of covariance (ANCOVA) was used to test for differences of regression slopes and *y*-intercepts between categories (e.g., N vs. 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 vs. DOC : SRP, DOC : DIN, DOC : PC, and Z_{mix} to determine if these variables explained more variance than Chl *a* alone. If these 'mechanistic' variables were weaker predictors than Chl *a* (based on r^2 values), then we ran multiple regressions with both the mechanistic variable and Chl *a* as predictor variables. The r^2 value, *F*-statistic, and SEE of the multiple regressions were compared to the simple linear regression of BPr : PPr vs. Chl *a* to examine whether these mechanistic variables improved predictions over that based solely on trophic state.

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 Chl *a* L^{-1}; Table 1). As predicted, the percentage of particulate C, N, and P in the < 1-μm size fraction all decreased as Chl *a* increased (Fig. 1a–c; Table 2). The percentage of particulate C, N, and P < 1 μm ranged from 10% to 34%, 7% to 40%, 8% to 52%, respectively. The inverse relationship with Chl *a* 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; Table 3). The percentage of particulate P < 1 μm appeared to decline more slowly than the percentage of particulate N < 1 μm as Chl *a* increased (Fig. 1b,c; Table 3), but the slopes of the two relationships were not significantly different (ANCOVA, $F_{1,30} = 2.878$, $p = 0.10$).

The possibility exists that our data represent two different relationships (Texas vs. Ohio) rather than one larger pattern, because there was limited overlap in values of Chl *a* between the two states (Table 1). However, two lines of evidence support the argument that one relationship can describe both Texas and Ohio reservoirs: where Chl *a* concentrations overlap, the percentage of particulate nutrients in the <1-μm size fraction are similar between Texas and Ohio (Fig. 1), and when our data are plotted with that of Biddanda et al. (2001), there is a further suggestion of one larger robust pattern. We plotted the particulate nutrient data on Minnesota, U.S.A., natural lakes and Lake Superior (Biddanda et al. 2001) with our
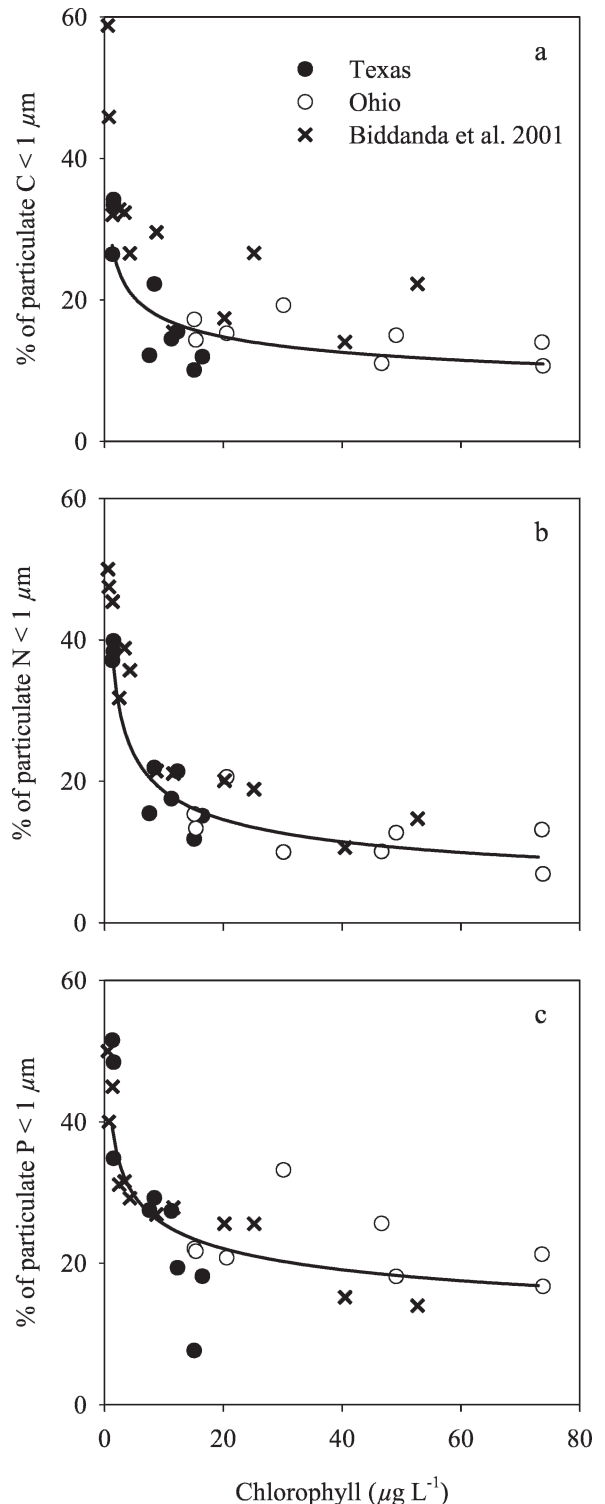

Fig. 1. Percentage of particulate (a) carbon, (b) nitrogen, and (c) phosphorus in the < 1-μm size fraction as a function of Chl *a* in Texas and Ohio, U.S.A., reservoirs (*n* = 17) and in Minnesota, U.S.A., lakes (*n* = 12; Biddanda et al. 2001). Regression lines are for Texas and Ohio reservoirs.

Table 2. Dissolved organic carbon (DOC), soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), total particulate nutrients, and particulate nutrients in the < 1-μm size fraction in Texas and Ohio, U.S.A., reservoirs. All values are in units of μmol L^{-1}.

Reservoir	DOC	SRP	DIN	Total C	<1 μm C	Total N	<1 μm N	Total P	<1 μm P
Texas									
Canyon	274	0.040	0.99	34.14	8.81	3.80	1.30	0.13	0.06
Stillhouse Hollow	347	0.046	2.31	30.52	10.31	3.91	1.49	0.15	0.07
Medina	240	0.040	2.52	32.04	10.43	3.58	1.36	0.18	0.06
Buchanan	403	0.045	3.08	105.47	12.85	13.53	2.06	0.36	0.10
Bastrop	957	0.046	7.92	98.21	21.16	14.90	3.18	0.65	0.19
LBJ	424	0.036	3.77	125.72	18.25	17.22	2.99	0.50	0.14
Inks	411	0.057	3.62	119.40	19.13	14.36	3.18	0.90	0.18
Granger	326	0.175	4.38	165.29	16.47	21.21	2.51	1.13	0.09
Dunlap	255	0.111	64.11	187.05	22.39	27.25	4.12	1.35	0.25
Ohio									
Burr Oak	230.8	0.101	3.30	102.25	16.47	11.97	1.66	0.57	0.13
Berlin	356.2	0.053	3.94	81.89	11.64	13.39	2.10	0.86	0.18
Hoover	342.7	0.060	14.17	155.40	23.69	14.54	2.55	1.12	0.23
Dillon	136.3	3.851	28.72	152.89	26.37	22.11	1.66	2.47	0.58
Tappan	192.3	0.092	2.53	242.53	26.69	37.20	3.73	1.41	0.36
Pleasant Hill	147.4	0.170	17.77	252.67	37.78	33.69	4.26	1.88	0.34
Acton	229.8	0.183	279.42	348.04	48.12	50.51	6.44	2.31	0.49
Delaware	345.7	0.109	60.89	323.52	34.41	43.46	3.24	2.96	0.50

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 Chl *a* to the percentage of C ($F_{1,25}$ = 3.703, p = 0.066 and $F_{1,25}$ = 0.002, p = 0.964 for comparison of the intercept and slope, respectively), N ($F_{1,25}$ = 0.642, p = 0.430 and $F_{1,25}$ = 0.554, p = 0.463), or P ($F_{1,25}$ = 0.022, p = 0.884 and $F_{1,25}$ = 0.042, p = 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 μg C L^{-1} d^{-1} to 1695 μg C L^{-1} d^{-1} and was strongly related to Chl *a* levels (r^2 = 0.84, p < 0.001; Fig. 2a; Table 1). BPr ranged from

Table 3. Linear regression parameters relating Chl *a* (μg L^{-1}), percentage of particulate nutrients < 1 μm (% 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 regressions 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*	df	*p*
%C < 1	Chl *a*	−0.230 (0.048)	1.469 (0.060)	0.60	0.106	1.03	22.75	1,16	<0.001
%N < 1	Chl *a*	−0.353 (0.043)	1.619 (0.054)	0.82	0.097	1.03	67.15	1,16	<0.001
%P < 1	Chl *a*	−0.212 (0.071)	1.617 (0.088)	0.37	0.156	1.07	8.90	1,16	0.009
PPr	Chl *a*	0.777 (0.087)	1.626 (0.108)	0.84	0.191	1.10	78.52	1,16	<0.001
BPr	Chl *a*	0.553 (0.052)	1.125 (0.064)	0.88	0.114	1.03	114.18	1,16	<0.001
BPr	PPr	0.654 (0.060)	0.111 (0.153)	0.89	0.112	1.03	118.30	1,16	<0.001
BPr : PPr	Chl *a*	−0.217 (0.066)	−0.479 (0.082)	0.42	0.146	1.06	10.63	1,16	0.005
BPr : PPr	DOC : SRP	0.092 (0.072)	−1.047 (0.258)	0.10	0.181	1.09	1.64	1,16	0.220
BPr : PPr	DOC : DIN	0.219 (0.078)	−1.114 (0.145)	0.34	0.155	1.07	7.85	1,16	0.013
BPr : PPr	DOC : PC	0.309 (0.084)	−0.849 (0.048)	0.47	0.139	1.05	13.52	1,16	0.002
BPr : PPr	Z_{mix}	0.709 (0.146)	−1.191 (0.101)	0.61	0.119	1.04	23.65	1,16	<0.001
BPr : PPr	PPr	−0.332 (0.056)	0.108 (0.142)	0.70	0.104	1.03	35.07	1,16	<0.001
BPr : PPr	BPr	−0.361 (0.114)	−0.092 (0.203)	0.40	0.148	1.06	9.94	1,16	0.007
Multiple regressions									
BPr : PPr	Chl *a*	−0.196 (0.070)	−0.608 (0.154)	0.45	0.146	1.06	5.80	2,16	0.015
	DOC	0.192 (0.194)							
BPr : PPr	Chl *a*	−0.216 (0.078)	−0.484 (0.297)	0.42	0.151	1.06	4.96	2,16	0.024
	DOC : SRP	0.001 (0.069)							
BPr : PPr	Chl *a*	−0.154 (0.080)	−0.760 (0.228)	0.48	0.142	1.05	6.45	2,16	0.010
	DOC : DIN	0.118 (0.089)							

13 μg C L^{-1} d^{-1} to 172 μg C L^{-1} d^{-1} and exhibited a strong relationship with Chl *a* (r^2 = 0.88, p < 0.001; Fig. 2b) and PPr (r^2 = 0.89, p < 0.001; Table 3). The slope of the PPr–Chl *a* relationship (0.78) was > the slope of the BPr–Chl *a* relationship (0.55), indicating that PPr increased with trophic state at a much faster rate than BPr (Fig. 2a,b; Table 3). Where Chl *a* 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 Chl *a* (r^2 = 0.42, p = 0.005; Fig. 2c). BPr:PPr ranged from 0.45 (Chl *a* = 1.3 μg L^{-1}) to 0.10 (Chl *a* = 49.09 μg L^{-1}). BPr:PPr varied widely from 0.16 to 0.45 when Chl *a* was < 10 μg L^{-1}, but appeared to reach an asymptote and decreased at a much slower rate at higher Chl *a* concentrations.

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; Table 3). DOC:DIN was significantly related to BPr:PPr (r^2 = 0.34, p = 0.013), but explained less variance in BPr:PPr than Chl *a* (r^2 = 0.42). When we included DOC:SRP and DOC:DIN with Chl *a* in multiple regressions predicting BPr:PPr, there was little or no improvement in r^2 values (≤ 6% additional variance explained), SEE, or *F*-statistics relative to the regression with only Chl *a* as a predictor (Table 3). 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 3).

Two other explanatory variables, DOC:PC and Z_{mix}, exhibited stronger relationships with BPr:PPr and explained more variance than Chl *a* (Table 2). DOC:PC explained a little < half of the variance in BPr:PPr (r^2 = 0.47, p = 0.002). However, 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).

Discussion

Effect of trophic state on sequestration of particulate nutrients—Our results strongly support the hypothesis that bacteria constitute a larger fraction of the total particulate nutrient pool in oligotrophic systems than in more productive systems (Biddanda et al. 2001; Cotner and Biddanda 2002). We found significant inverse relationships between Chl *a* and the percentage of particulate nutrients < 1 μm for C, N, and P. In our most 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 Chl *a* increased. Inverse relationships between Chl *a* and the percentage of particulate
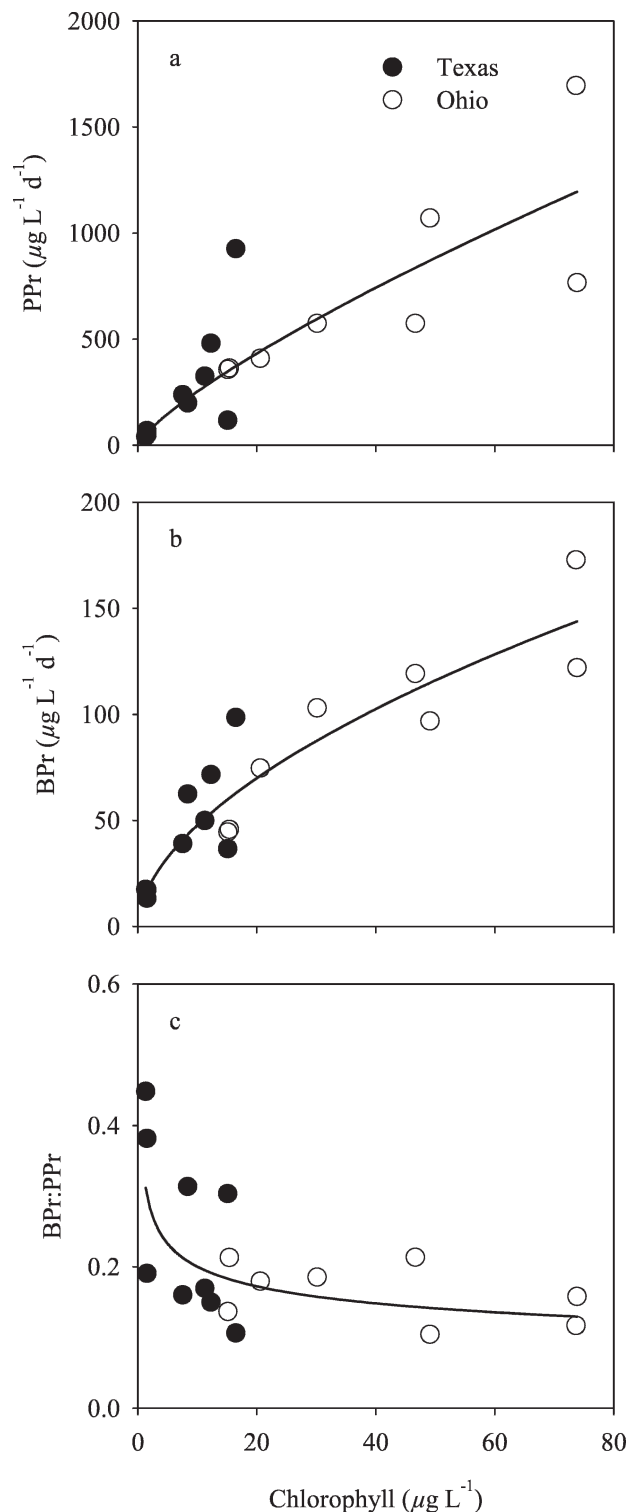

Fig. 2. Relationship of (a) primary production (PPr), (b) bacterial production (BPr), and (c) the ratio of bacterial production to primary production (BPr:PPr) as a function of Chl *a* concentration in Texas and Ohio, U.S.A., reservoirs (*n* = 17).

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).

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 these aspects of pelagic ecosystem dynamics are similar in reservoirs and natural lakes.

The decreasing contribution of the < 1-μm size fraction to total particulate C, N, and P as Chl a increases is explained by the rates at which the absolute quantities of the total and < 1-μm size fractions change with trophic state. Both size fractions increase with Chl a, but total particulate C, N, and P increase ~ 9, 14, and 6 times faster, respectively than < 1-μm C, N, and P (Table 3). 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 HNFs, 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 percentage of particulate N < 1 μm decreases significantly faster with Chl a than the percentage of particulate P < 1 μm (ANCOVA, $F_{1,54} = 7.775$, $p = 0.007$; Fig. 1b,c). This results in predictions that the < 1-μm size fraction will account for nearly equal percentages of particulate N and P when Chl a equals 1 μg L^{-1} but will account for almost two times more of particulate P ($\sim 20\%$) than of particulate N ($\sim 10\%$) at a Chl a concentration of 50 μg L^{-1} (Fig. 1b,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 natural lakes of Biddanda et al. (2001). Molar C : N and C : P ratios in Texas and Ohio reservoirs ranged from 6 to 12 and 60 to 295, respectively, for the > 1-μm size fraction and from 6 to 15 and 48 to 206, respectively, for the < 1-μm size fraction. For Minnesota lakes, molar C : N and C : P varied from 6 to 23 and 61 to 332, respectively, for the > 1-μm size fraction, and from 5 to 16 and 85 to 253, respectively, for the < 1-μm size fraction. C : P ratios of the two size fractions decreased at similar rates with increasing Chl a (ANCOVA, $F_{1,54} = 0.376$, $p = 0.542$), but there was a significant difference in the slopes of C : N vs. Chl a for the two size fractions (ANCOVA, $F_{1,54} = 4.776$, $p = 0.033$). C : N of the > 1-μm size fraction decreased with increasing Chl a ($r^2 = 0.48$, $p = 0.001$), but C : N of the < 1-

μm size fraction did not vary significantly with Chl a ($r^2 = 0.02$, $p = 0.423$). 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 may utilize increased inorganic N supply in eutrophic systems and this prediction 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). Alternately, declining phytoplankton C : N ratios could be a consequence of increasing nitrogen containing biochemical constituents in algal cells such as protein; protein content of algal cells has been shown to change in response to environmental parameters, which often vary along productivity gradients, such as light intensity and dissolved inorganic nutrient ratios (Leonardos and Geider 2004). Relatively consistent C : N of the < 1-μm size fraction across a broad range of Chl a concentrations also suggests that P rather than N may be limiting for bacteria over a wide range of trophic state (Davies et al. 2004) and that bacteria may utilize the increased P supply causing a decline in their cell C : P ratios. However, bacteria exhibit much greater homeostatic control of C : nutrient ratios than phytoplankton along nutrient availability gradients (Chrzanowski and Kyle 1996; Sterner and Elser 2002) and our observed changes in bacterial C : P along a productivity gradient may be related to other factors that vary with ecosystem productivity, such as cellular growth rates, RNA content, and the dominant growth strains of bacteria between systems (Makino et al. 2003; Cotner et al. 2006).

An alternative explanation for the decrease in particulate C : P ratios with trophic state is that there is an increase in inorganic particulate P, such as P-containing clay or suspended sediments, as trophic state increases. Suspended solids increase with agricultural or urban land use (Jones and Knowlton 2005), which also influence the trophic state of reservoirs (Knoll et al. 2003). We did not determine the contribution of inorganic particulate P to total or < 1-μm particulate P, but we hypothesize that it is unlikely that suspended sediments strongly affected the overall pattern particulate C : P ratios in our reservoirs. Suspended sediments mostly strongly affect the riverine zone of reservoirs during base flow conditions, because most riverine-derived suspended sediments settle out of the mixed layer before reaching the downstream or lacustrine zone (Kimmel et al. 1990). All of our samples were taken during base flow conditions at the downstream end of reservoirs near the dam, and only one reservoir (Granger Lake) was not thermally stratified at the time of sampling. Therefore, it is unlikely that the pattern of decreasing particulate C : P ratios with increasing trophic state observed in our study are due to P-containing suspended sediments.

Relationship of trophic state, BPr, and PPr—Our results support the hypothesis that BPr : PPr decreases as trophic state increases, in agreement with several other studies. In lakes studied by del Giorgio and Peters (1994), the ratio of community respiration to photosynthesis decreased with

Chl *a*. Biddanda et al. (2001) found that the ratio of bacterial respiration (BR) to total community respiration also decreased as Chl *a* increased. The decrease of BPr : PPr as Chl *a* increased is also consistent with regressions of BPr vs. PPr with slopes < 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 (bacterial carbon demand = BPr + bacterial respiration) 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 bacterial respiration (BR) from our BPr measurements:

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

where both BR and BPr are expressed in $\mu g \ C \ L^{-1} \ h^{-1}$. This equation (Model I) from del Giorgio and Cole (1998) was selected because the authors state that it is most comparable to previous studies and provides the best predictive model of BR from BPr. The fraction of bacterial carbon demand (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 ~ 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 Chl *a* 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 Chl *a* ($r^2 = 0.84$ and 0.89, respectively). In their study of 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 (Granéli et al. 2004) 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. Conversely, reduced variation in BPr : PPr with increasing productivity might be due to a shift toward consistently more labile substrates for bacterial growth, leading to less variability in BPr. However, both of these hypotheses are consistent with observed patterns of lower bacterial growth efficiency in oligotrophic vs. eutrophic lakes (Biddanda et al. 2001).

Our estimates of PPr are generally similar to previous studies. Using our Chl *a* vs. PPr regression equation (Table 3) we predicted PPr over a range of Chl *a* concentrations. When simulated Chl *a* is between 20 $\mu g \ L^{-1}$ 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. 3a). At lower Chl *a* concentrations, our estimates of PPr become progressively > those of Smith (1979) and del Giorgio and Peters (1993). This might be expected because we integrated our PPr values over mixing depth. Smith (1979) used euphotic depth for all estimates of PPr, and del Giorgio and Peters' (1993) data set contained estimates 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 Chl *a* in our Texas and Ohio reservoirs are similar to other studies, but our PPr estimates at low Chl *a* values will likely exceed estimates that integrate PPr over the euphotic depth instead of the mixing depth.

Our estimates of BPr per unit Chl *a* are much higher those of the review by Cole et al. (1988), but our estimates of BPr per unit PPr are very similar to theirs (Fig. 3b,c). We integrated measurements over the mixed layer, but Cole et al. (1988) reviewed only studies that integrated measurements over the euphotic zone and methodological differences may explain some of the discrepancy between the two estimates based on Chl *a*. Thus, PPr may be a more consistent predictor than Chl *a* of BPr. If PPr per unit Chl *a* 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 Chl *a* are very different. This implies a fundamental difference in the Chl *a*–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 factors, plankton community composition, or methodological differences.

While our estimates of pelagic BPr and PPr are similar to those observed in natural lakes, BPr and PPr in reservoirs may be more strongly influenced by suspended sediments and particle-associated bacteria than natural lakes because turbulent mixing and sediment resuspension is generally higher in reservoirs (Thornton 1990*b*). Increased sediment resuspension could affect rates of pelagic BPr and PPr through several mechanisms: it could increase BPr by increasing the number of benthic particle-associated bacteria in the water column, it could increase BPr by
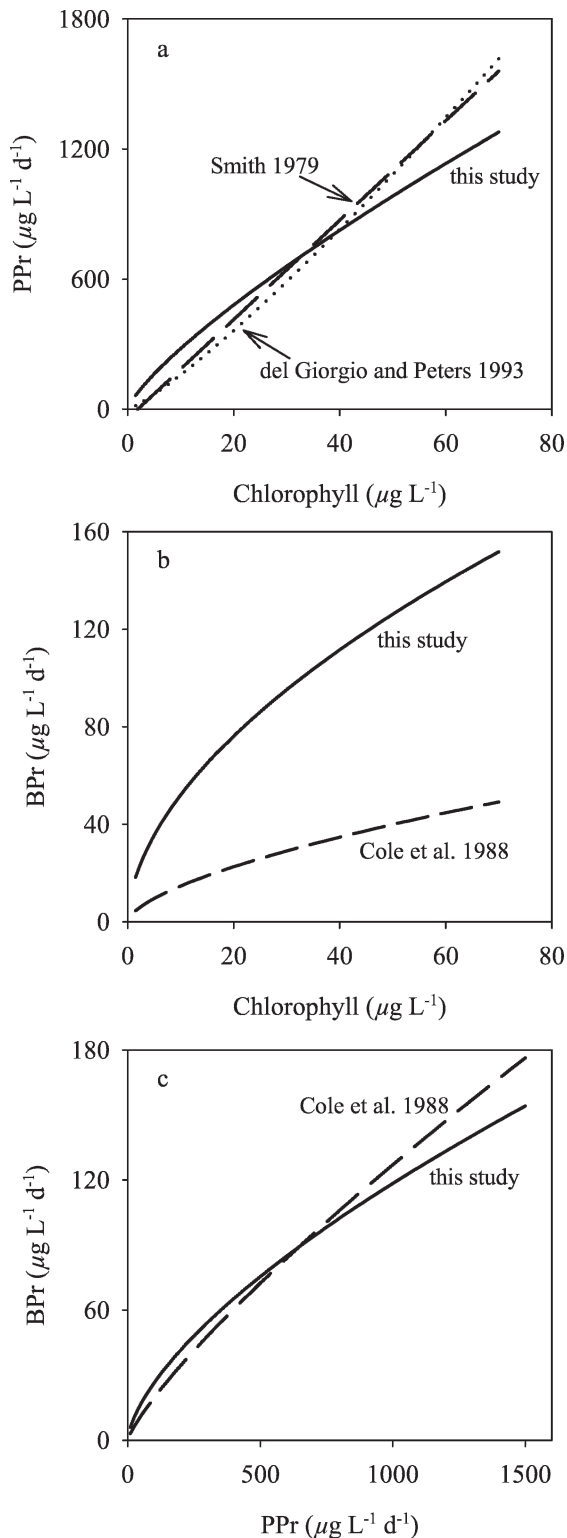
Fig. 3. Comparison of our model predicting (a) primary production (PPr) from Chl *a* to those of Smith (1979) and del Giorgio and Peters (1993) and comparisons of our models predicting bacterial production (BPr) from (b) Chl *a*, and (c) PPr to those of Cole et al. (1988).

increasing available benthically derived C and nutrients for heterotrophic bacteria, and it could decrease PPr through increased shading of phytoplankton (Cotner et al. 2000; Biddanda and Cotner 2002). In the present study, we did not perform direct measurements of particle-associated bacteria, but we believe the contribution of particle-associated bacteria to BPr and influence of resuspended sediments on the observed relationships between Chl *a* and BPr : PPr are probably minimal in our reservoirs for several reasons. As stated previously, our sampling locations within reservoirs (at the dam) and time period of sampling (during mid- to late-summer stratification) make it less likely that suspended sediments and particle-associated bacteria strongly influenced our results. Second, it might be predicted that resuspension of sediments would increase with deeper Z_{mix}, and, thus, increase the contribution of particle-associated bacteria to pelagic BPr in reservoirs with deeper Z_{mix} (i.e., the larger less productive reservoirs in our study). This was not observed; there is not a significant relationship between Z_{mix} ($r^2 = 0.12$, $F_{1,16} = 2.00$, $p = 0.178$) or reservoir surface area ($r^2 = 0.05$, $F_{1,16} = 0.731$, $p = 0.406$) and NVSS, an often-used measure of suspended sediments (Jones and Knowlton 1995). In addition, mean irradiance in the mixed layer (I_m), calculated as a fraction of surface light (Sterner et al. 1997), was not a significant function of NVSS ($r^2 = 0.16$, $F_{1,16} = 2.95$, $p = 0.107$), indicating that water column sediment concentrations and the underwater light environment were not substantially related to each other in our reservoirs. Finally, we did not detect a significant relationship between NVSS and BPr ($r^2 = 0.06$, $F_{1,16} = 0.910$, $p = 0.355$), PPr ($r^2 = 0.12$, $F_{1,16} = 2.04$, $p = 0.174$), or BPr : PPr ($r^2 = 0.15$, $F_{1,16} = 2.62$, $p = 0.127$). Therefore, in our study, it seems unlikely that resuspended sediments or particle-associated bacteria had a strong effect on BPr, PPr, or the relationship between trophic state and BPr : PPr. However, there is little doubt that suspended sediments and particle-associated bacteria can play a potentially substantial role in the light environment and production dynamics of the reservoirs in our study at other locations within the reservoirs (i.e., the upstream riverine zone) and at other time periods (i.e., after a runoff event).

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 on the relative importance of bacteria (Cotner and Biddanda 2002). DOC : SRP was not significantly related to BPr : PPr, and DOC : DIN was a weaker predictor than Chl *a*. Additionally, multiple regressions with Chl *a* and either DOC, DOC : SRP, or DOC : DIN did not substantially improve predictions of BPr : PPr as compared to the model based on Chl *a* 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 lentic systems in general. First,

bulk 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 lability and availability to bacteria (del Giorgio and Cole 1998; Lennon and Pfaff 2005). For example, the more labile forms of DOC, such as phytoplankton cell exudates, can be a highly variable and often small proportion of total DOC (Benner 2003). Ostapenia et al. (2009) examined the lability of DOC in a set of six lakes from around the world that varied in trophic state (2.3–50.6 μg chlorophyll L^{-1}) and determined that, irrespective of lake trophic status, $\sim$ 80% of the DOC pool was refractory. Second, bulk DOC measurements or DOC : dissolved inorganic nutrients may not serve as 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). Last, the degree of biological utilization and lability of DOC in lentic systems can be dependent upon lake and reservoir hydrological characteristics, such as water residence time and amount of runoff (Cimbleris and Kalff 2003; Pace and Cole 2002). Thus, we conclude that the use of bulk DOC or ratios of DOC : dissolved inorganic nutrients are not likely to be consistently reliable predictors of the relative importance of bacteria in the nutrient cycling across a diversity of lakes and reservoirs.

In addition to issues related to variability in lability and nutritional quality of DOC, the optical characteristics of the DOC pool may also affect the utility of DOC as a predictor of the relative importance of bacterial metabolism in lakes and reservoirs. Observed relationships between DOC and BPr : PPr (del Giorgio and Peters 1994; Jansson et al. 2000) may be due to the shading effects of humic or colored DOC, rather than the direct effects 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. Indeed, other studies have noted a similar suppression of algal production due to shading by increased DOC concentrations (Carpenter et al. 1998). Thus, if highly colored lakes are included in studies examining the relative importance of bacteria metabolism in lakes and reservoirs, then researchers may find a relationship between bulk DOC and the relative importance of bacteria. However, we hypothesize that 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 relative to particulate organic matter should favor bacteria in competition with phagotrophic heterotrophs for C. They further predicted that as the relative abundance of particulate organic matter increased with trophic state, phagotrophs would be favored. This mechanism could explain the decreased contribution of bacteria to community biomass and metabolism in 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 Chl a ($r^2 = 0.94$, $p < 0.001$) 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 Chl a. 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) 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 (Pearson $r = -0.63$), TN (Pearson $r = -0.65$), and PPr (Pearson $r = -0.86$), but was not as strongly correlated with these trophic measures as Chl a ($r = 0.85$, 0.86, and 0.92 for TP, TN, and PPr, respectively). Z_{mix} was more strongly correlated than Chl a with reservoir size ($r = 0.80$ and 0.59, respectively). However, when we excluded the three most oligotrophic reservoirs (Canyon, Stillhouse Hollow, and Medina) all of which had relatively high I_m, there was an inverse correlation between Z_{mix} and I_m ($r = -0.59$). Thus, the strength of Z_{mix} as a predictor of BPr : PPr may be because it is partially associated with a number of factors that may directly or indirectly affect PPr and BPr, including nutrient supply, reservoir size, and I_m.

Previous work in pelagic habitats of natural lakes and marine systems have led to the hypothesis that the relative importance of heterotrophic bacteria to ecosystem structure and function is high in oligotrophic systems but decreases with trophic state (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 percentage of particulate nutrients < 1 μm and BPr : PPr, were high in oligotrophic reservoirs but decreased with trophic state.

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 percentage of particulate P < 1 μm indicates that even in eutrophic systems bacteria may be effective competitors for P.

Our study offers only weak support for the hypotheses that DOC or the relative abundance of DOC vs. inorganic nutrients are major factors controlling the contribution of bacteria to pelagic ecosystem function, at least when we consider all forms of DOC pooled together. 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 (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.

Because of their widespread distribution, abundance, and economic importance it is important to determine if reservoirs systematically differ from other pelagic ecosystems. The patterns we observed in reservoirs were similar to other pelagic habitats in terms of the relative importance of bacteria to particulate nutrient pools and ecosystem function. PPr per unit Chl a was similar to estimates made in natural lakes, and BPr per unit PPr was similar to data from natural lakes and marine habitats. Our study suggests that reservoirs are similar in these aspects to other pelagic ecosystems and our findings should aid in efforts to understand the general and global importance of C cycling in aquatic systems.

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