Bacterial abundance in Missouri (USA) reservoirs in relation to trophic state and global patterns

Anthony P. Thorpe and John R. Jones

Introduction

The increase in bacterioplankton abundance (BA) with lake phytoplankton and nutrients is a generally accepted pattern in aquatic microbial ecology (AIZAKI et al. 1981, BIRD & KALFF 1984, GASOL & DUARTE 2000). As a contribution to regional limnology, cross-sectional data from Missouri reservoirs are used to describe patterns between BA and metrics of lake trophic state and attributes of the seston. Also, the Missouri data are combined with the published literature to show global patterns between BA and algal chlorophyll and phosphorus. Using these global relations, transition values are proposed for lake trophic state categories based on BA.

Key words: Bacteria, phosphorus, algae, chlorophyll, empirical studies, trophic state

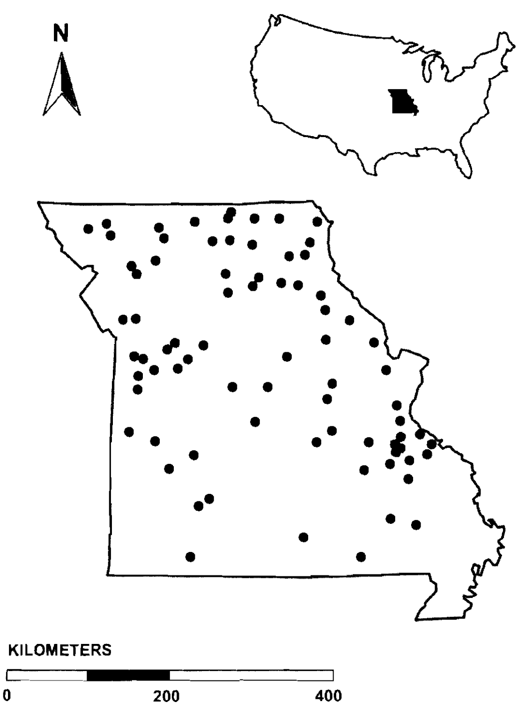

Fig. 1. Locations of sampled reservoirs.

Methods

A total of 78 reservoirs were sampled on four occasions during 1999 and 2000. Forty-two reservoirs were sampled both summers, and the other 36 were sampled one summer. Sampling commenced in mid-May with each collection circuit lasting three weeks (the period required to collect one sample from each reservoir, about 20 per week). This schedule was repeated four times such that seasonal collection periods corresponded to late spring (15 May to 3 June), early summer (5 June to 21 June), mid-summer (26 June to 28 July, and late summer (31 July to 18 August). Samples were collected from the surface layer, typically near the dam, and were processed by standard methodology (KNOWLTON & JONES 2000). Measurements include total phosphorus (TP), total nitrogen (TN), algal chlorophyll (Chl), Secchi transparency, volatile suspended solids (VSS), non-volatile suspended solids (NVSS), nephalometric turbidity (Turb), filtrate turbidity (f-Turb, turbidity remaining in filtrate passed through Whatman 934-AH glass fiber filters used to measure VSS and NVSS), and dissolved organic carbon (DOC). Absorbance of filtrate at 440 nm (f-440Abs) is the absorbance of glass fiber filtrate in a 5 cm cuvette. The f-440Abs is influenced by both color materials (KIRK 1983, PACE & COLE 2002) and small particles, including clay minerals (KNOWLTON & JONES 2000). This measurement was included because clay materials are known to combine with DOC to form aggregates which support bacterioplankton (LIND et al. 1997). Units are given in Table 1.

Sub-samples (60 mL) for bacterial enumeration were preserved with Lugol's solution at 1 mL preservative/100 mL sample, but a few samples were preserved with buffered formalin. Subsamples (≤ 1 mL)

0368-0770/05/0029-0239 $ 2.00

were pipetted into a Millipore 25 mm glass filter column, to which 5 mL of bacteria-free deionized water was added. This solution was filtered onto a 0.2 mm membrane filter and stained with Acridine Orange (HOBBIE et al. 1977). Filters were examined under a Zeiss Axiophot microscope with a 100X objective, a 1.25X optibar, and a 10X ocular lens. Bacteria were counted in at least 10 fields, with an ideal target of 35 observed cells per field. A total of 350–400 cells/sample were counted and the results were expressed as $*10^6$ cells mL^{-1}. No distinction was made between attached and free-floating bacteria. Representative fields were photographed and cell dimensions were measured by using software calibrated with fluorescent beads of known size. Bacterial Biomass was estimated by applying the equation $CC = 120 * V^{0.72}$ (POSCH et al. 2001, where CC is fg carbon per cell and V is volume in μm^3) to the mean cell size and multiplying by the number of cells.

Literature data provided by GERTRUDE NÜRNBERG (NÜRNBERG & SHAW 1998) and from BIRD & KALFF (1984), SHORTREED & STOCKNER (1986), DEL GIORGIO & SCARBOROUGH (1995), GASOL et al. 1995, LIND et al. (1997), ZINABU & TAYLOR (1997), BOUVY et al. (1998), WEISSE and MACISSAC (2000), and BETTAREL et al. (2003) were aggregated to lake means for BA, Chl and TP (n = 67 for Chl and 53 for TP); in 20 lakes this involved averaging across several studies. Lakes with only one sample were omitted from the analysis. Data of AIZAKI et al. (1981) were excluded due differences in methodology (BIRD & KALFF 1984).

Data were log-transformed prior to being analyzed: at the level of individual observations (samples from a given date and reservoir); within a given collection period (four per summer); and as reservoir means (geometric means across all collection dates, n = 78). Least squares regression and standard linear tests were performed using SAS software. Significance was set at $p < 0.01$ unless otherwise stated. Akaike's Information Criteria (AIC) was used to assess relative support for competing regression models (BURNHAM & ANDERSON 2002).

Results

Reservoir mean values of algal biomass and plant nutrients ranged from oligotrophic to hypereutrophic (Table 1) and represent the continuum of reservoir resources within Missouri (JONES & KNOWLTON 1993). Non-algal seston is a prominent feature of many Missouri reservoirs (JONES & KNOWLTON 1993, KNOWLTON & JONES 2000), and the fraction retained on fiber filters, NVSS, ranged from 0.6 to 28.3 mg L^{-1}. Whole-water turbidity ranged from 1 to 92 NTU (Table 1). Particulates in the filtrate, measured as f-Turb and f-440Abs (see methods), spanned about two orders of magnitude (Table 1). The organic seston retained on fiber filters, VSS, ranged from 0.5 to 22.1 mg L^{-1}.

Table 1. Summary statistics and units reported. Filtrate 440 Absorbance measured at λ = 440 nanometers.

Variable	Units	Mean	Median	Min	Max
Bacterial Abundance (BA)	$*10^6$ cells mL^{-1}	7.1	7.3	1.6	24.6
Total Phosphorus (TP)	$\mu g\ L^{-1}$	32	40	5	205
Total Nitrogen (TN)	$\mu g\ L^{-1}$	618	643	182	1978
Chlorophyll (CHL)	$\mu g\ L^{-1}$	12.1	15.8	1.0	116.1
Secchi	m	1.1	1.0	0.1	5.9
Non-volatile Suspended Solids (NVSS)	mg L^{-1}	2.9	3.0	0.6	28.3
Volatile Suspended Solids (VSS)	mg L^{-1}	2.5	2.7	0.5	22.1
Turbidity (Turb)	NTU	5.2	5.2	1.1	92.2
Filtrate Turbidity (f-Turb)	NTU	1.4	1.3	0.3	54.8
Filtrate 440 Absorbance (f-440 Abs)	Absorbance Units	0.038	0.036	0.008	0.742
Dissolved Organic Carbon (DOC)	mg L^{-1}	4.5	4.8	1.0	11.1

On average, algal biomass composed some 27% of VSS (assuming Chl is 2% of algal biomass). Mean DOC levels ranged from 1 to 11 mg L^{-1}, which encompasses the lower half of the worldwide range (Xenopoulos et al. 2003). Reservoir mean values (n = 78) of all measured parameters in Table 1 (excluding BA) were strongly inter-correlated. All 45 pairings were significant (r = 0.43 to 0.96, as absolute values), and over half of the r values were >0.8.

Bacterial abundance (BA) was log-normally distributed among the individual samples (n = 464); counts varied by two orders of magnitude, from 0.7 to $56.5*10^6$ cells mL^{-1}. Only 10% of the counts were > $20*10^6$ cells mL^{-1}. In some 75% of reservoirs sampled both summers (n = 42), BA varied by less than a factor of five between the minimum and maximum counts (median = 3.7-fold), which is consistent with variation encountered in other temperate lakes (Kalff 2002). Across all samples (log-transformed), the coefficient of variation for BA (41%) was similar to Chl (49%), larger than TP (26%), TN (9%) or DOC (30%), and only about half the value for NVSS and VSS (90 and 93%, respectively). Reservoir mean BA values

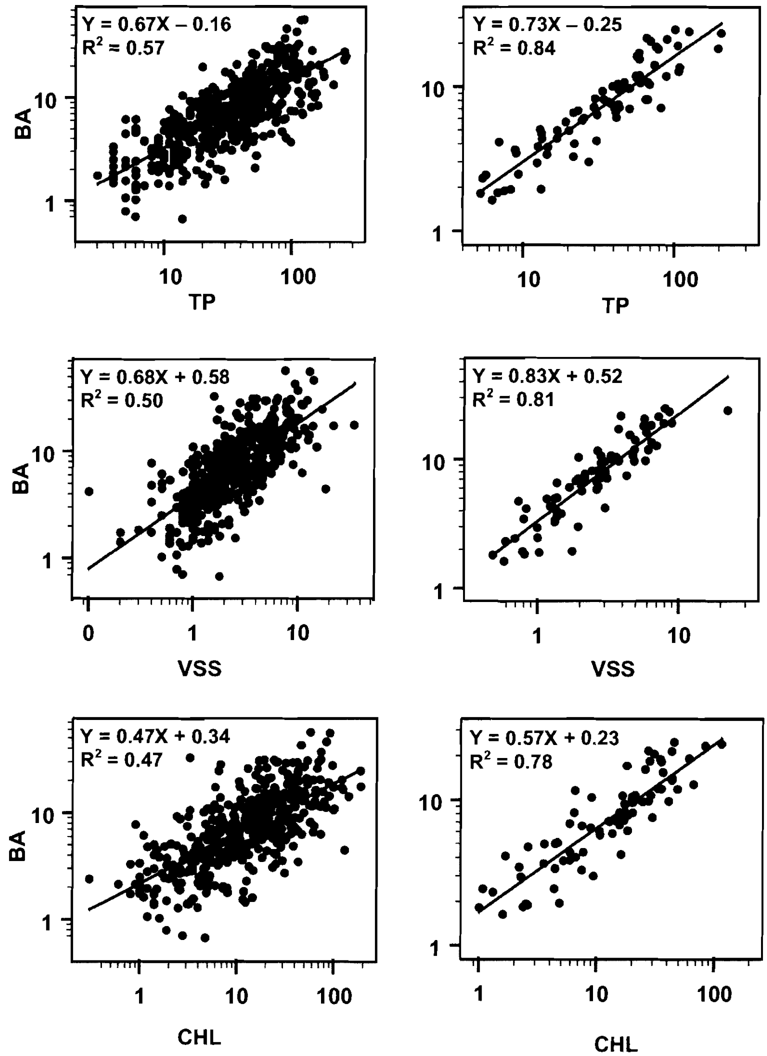

Fig. 2. Relations of BA with TP, VSS and CHL in Missouri reservoirs using log transformed discrete (left column, n = 464) and geometric mean data (right column, n = 78).

ranged 15-fold, from 1.6 to 24.6*10^6 cells mL^{-1} (Table 1). Bacterial biomass exhibited a larger range than BA (13 to 293 μg C L^{-1}) among reservoir means, with a median of 72 μg C L^{-1}.

In a cross-system analysis using individual samples, TP accounted for somewhat more variation in BA (r^2 = 0.57, Fig. 2) than surrogate measures of the organic substrate, measured as VSS or Chl (r^2 = 0.50 and 0.47, respectively, Fig. 2). Multiple regression showed VSS entered as a second variable with TP to explain 60% of the variation in BA (equation 1, Table 2). But two-variable models including both TP and measures of the seston (such as Chl or Turb), or measures of particulates and/or color in the filtrate (such as f-Turb, f-440Abs and DOC), were significant and resulted in r^2 values of 0.57 to 0.59. When TP and TN were eliminated from the analysis (to assess patterns with measures of resource availability), two-variable models, including a measure of the organic substrate retained on a filter (VSS or Chl) and a measure of particles/color in the filtrate (f-Turb, f-440Abs, or DOC), accounted for some 49 to 54% of BA variability (models not shown). Among these competing models, Akaike's Information Criteria (BURNHAM & ANDERSON 2002) suggests the two-variable model based on VSS and f-440Abs has the strongest support (W_i = 0.999; equation 2, Table 2). In temperate lakes temperature is known to account for variation in BA as a second variable with TP (CURRIE 1990), and while this was the case in the 2000 Missouri data set (range 20 to 31 C, models not shown), temperature was not significant in 1999 or when the summers were combined.

An examination of cross-system patterns among the 42 reservoirs sampled both summers showed the strength of BA-water quality couplings differed temporally. For example, BA-VSS relations were stronger in both seasons during mid- and late-summer collections (r^2 = 0.59 to 0.65) than during the two earlier collections (r^2 = 0.23 to 0.35). BA-Chl relations showed this same temporal pattern. Within half of the eight collection periods TP showed the strongest relation with BA (r^2 = 0.58 to 0.77), but in some cases NVSS (r^2 = 0.59, late spring 1999), Chl (r^2 = 0.43, early summer 1999), TN (r^2 = 0.43, early summer 2000), and VSS (r^2 = 0.65, mid-summer 1999) were more tightly related. These findings highlight that BA covaries with such factors as grazing, organic substrates, nutrients, and non-algal particulates (CURRIE 1990, LIND & DÁVALOS-LIND 1991), and the importance of these bivariate couplings vary with time. Time lags between variables and long sampling intervals also influence these comparisons (GASOL & DUARTE 2000).

Aggregating the data to the level of the reservoir means (n = 78) is a smoothing function (JONES et al. 1998) that reduced the variation in BA, thereby strengthening the overall regression relations with measures of lake trophic state and the seston (r^2 = 0.78 to 0.84, Fig. 2). For example, among individual samples there was a 15-fold range in BA at any given level of TP, but among reservoir means this range was reduced to 3-fold (Fig. 2). Regressions based on aggregated data show r^2 values some 0.3 units larger than with unaggregated data (Fig. 2). Even though regressions are stronger with aggregated data, models based on reser-

Table 2. Two-variable models, for bacterial abundance (BA) regressed on select limnological variables. All variables were log transformed. See Table 1 for variable descriptions.

Relation	R^2	SE
Individual Samples (n = 464)		
1. BA = 0.05 + 0.46 TP + 0.28 VSS	0.60	0.217
2. BA = 0.88 + 0.60 VSS + 0.19f-440Abs	0.54	0.231
Reservoir Means (n = 78)		
3. BA = 0.04 + 0.44 TP + 0.37 VSS	0.86	0.115
4. BA = 0.34 + 0.71 VSS + 0.34 DOC	0.84	0.124

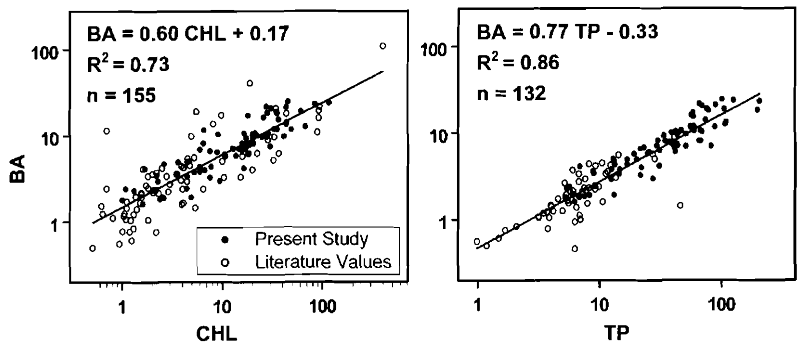

Fig. 3. Global relations of BA with CHL and TP (using log transformed geometric means, Missouri and literature data combined).

voir mean TP, VSS, and Chl did not differ from relations based on individual observations (p>0.05, using a standard linear test). This finding is consistent with the hypothesis that the cross-sectional pattern between BA and trophic state in Missouri reservoirs is the same at each level of aggregation.

In multiple regression, based on reservoir means, VSS entered as a second variable with TP to explain 86% of the variation in BA (equation 3, Table 2). When TP was omitted from the analysis, VSS entered in stepwise regression, and a two variable model with VSS and DOC accounted for 84% of the variation in BA (equation 4, Table 2). When paired with VSS, f-turb and f-440Abs accounted for somewhat less variation than did DOC. The r^2 for models using Chl as the first variable were similar to those based on VSS (models not shown). Among competing models with either VSS or Chl, Akaike's Information Criteria (Burnham & Anderson 2002) provides the strongest support to VSS and DOC (W_i = 0.958; equation 4, Table 2).

The Missouri reservoir BA-Chl relation based on aggregated data (Fig. 2) did not significantly differ from the lake pattern from the published literature (Fig. 3). When data from these sources were combined, the r^2 for the global BA-Chl relation was 0.73 (Fig. 3). Likewise, Missouri and literature BA-TP relations did not significantly differ, and the r^2 for the global BA-TP relation was 0.86 (Fig. 3).

Using published boundaries for TP to distinguish lake trophic state (Forsberg & Ryding 1980) and the global BA-TP regression (Fig. 3), we calculated transition values separating lakes into trophic state categories based on BA (Table 3). The same approach was taken using the global BA-Chl regression (Fig. 3 and Table 3) resulting in a similar outcome. Both approaches closely match the lake classification criteria for BA proposed by Kalff (2002).

Discussion

Measurements of BA in Missouri reservoirs are within the mid-range for aquatic systems reported in the published literature (Gasol & Duarte 2000). The strong coupling of BA with several surrogate measures of resource availability (TP, VSS, and Chl, Fig. 2) is in keeping with the undisputed pattern that heterotrophic bacteria depend on the overall organic base in lakes, thereby extending this generalization to reservoir systems in the mid-continent region of North America. The strength of the BA-TP relation in Missouri reservoirs (Fig. 2) likely results from the regulation of algal abundance by TP (Jones & Knowlton 1993) and the direct positive effect of P on bacteria (Currie 1990, Smith & Prairie 2004), but in comparative analyses such influences can not be distinguished from the strong correlation of TP with all measures of available carbon and particles in these systems. Interestingly, bacterioplankton in Missouri reservoirs covaries more strongly with VSS, a broad measure of both autochthonous and allochthonous particulate organic materials (Jones & Knowlton 1993, Knowlton & Jones 2000), than with autochthonous production measured as algal Chl. Also, the slope of the regression relation

Table 3. Trophic state criteria for phosphorus and chlorophyll (after FORSBERG & RYDING 1980), and trophic state transition values based on Bacterial Abundance (BA) calculated using global BA-TP and BA-Chl regressions (Fig. 3). Upper boundaries and 95% confidence intervals (CI) are shown for each category.

	Upper Bounds			Upper Bounds		
	TP $\mu g\ L^{-1}$	BA $*10^6$ cells mL^{-1}	95% CI	Chl $\mu g\ L^{-1}$	BA $*10^6$ cells mL^{-1}	95% CI
Oligotrophic	10	2.8	2.1–3.6	3	2.9	2.3–3.5
Mesotrophic	25	5.6	4.0–7.7	7	4.8	3.7–6.1
Eutrophic	100	16.2	11.0–24.0	40	13.5	9.4–19.4

(Fig. 2) is steeper with VSS (0.83) than with Chl (0.57). Entry of measures of particulates and/or color as a second variable with VSS (Table 2) is consistent with the finding that BA increases with the heterotrophic pathway provided by clay-organic aggregates and nonliving organic particulates in filtrate (LIND & DÁVALOS-LIND 1991, LIND et al. 1997, CARRIAS et al. 2002). The implication of these findings is that allochthonous carbon, in various size fractions, supports BA in Missouri reservoirs and accounts for cross-system variation not explained by algal Chl. Allochthonous inputs are an important characteristic of reservoir seston (JONES & BACHMANN 1978). Missouri reservoirs are constructed in valleys previously modified by human development and receive nonpoint source inputs of nutrients and materials from diverse land use practices in their watersheds. Overall, lake fertility and suspended solids increase with the proportion of cropland agriculture and decline with forest cover (JONES et al. in press).

Relations between BA and resource supply, measured as Chl or TP, have been demonstrated in worldwide studies (NÜRNBERG & SHAW 1998, KALFF 2002), and our comparison shows Missouri reservoirs fit within cross-system patterns covering a broad range of fresh water systems (Fig. 3). Slope coefficients of the combined global BA-Chl (slope = 0.60, Fig. 3) and BA-TP (slope = 0.77) relations are similar to many of the power increases observed by others (CURRIE 1990, GASOL & DUARTE 2000, KALFF 2002) and support the widely held finding that BA does not increase proportionally with these measures of lake fertility. This pattern is reflected in the trophic state boundaries used to classify lakes (Table 3). An order of magnitude increase occurs in Chl and TP between the upper limit for both oligo- and eutrophic lakes in classic trophic state classification, but across this range BA increases only ~ 5-fold (Table 3). The Missouri analysis suggests broad measures of allochthonous carbon substrates, in various size fractions, could further describe mechanisms influencing BA in lakes and reservoirs on a global scale.

Acknowledgements

This project was supported by the Missouri Department of Natural Resources and the University of Missouri. We are grateful to DAN OBRECHT, JOSH MILSPAUGH, LAURA DÁVALOS-LIND and MATT KNOWLTON for comments on the manuscript and to BARRETT VAHLE and CHRIS RADCLIFFE for field assistance.

References

AIZAKI, M., OTSUKI, A., FUKUSHIMA, T., HOSOMI, M. & MURAOKA, K., 1981: Application of Carlson's trophic state index to Japanese lakes and relationships between the index and other parameters. – Verh. Internat. Verein. Limnol. **21**: 675–681.

BETTAREL, Y., SIME-NGANDO, T., AMBLARD, C., CARRIAS, J.F. & PORTELLI, C., 2003: Virioplankton and microbial communities in aquatic systems: a seasonal study in two lakes of differing trophy. – Freshw. Biol. **48**: 810–822.

BIRD, D.F. & KALFF, J., 1984: Empirical relationship between bacterial abundance and chlorophyll concentration in fresh and marine waters. – Can. J. Fish. Aquat. Sci. **41**: 1015–1023.

BOUVY, M., ARFI, R., CECCHI, P., CORBIN, D., PAGANO, M., SAINT-JEAN, L. & THOMAS, S., 1998: Trophic coupling between bacterial and phytoplanktonic compartments in shallow tropical reservoirs (Ivory Coast, West Africa). – Aquat. Microbial Ecol. **15**: 25–37.

BURNHAM, K.P. & ANDERSON, D.R., 2002: Model selection and multi-model inference: a practical information-theoretic approach. – Springer-Verlag, New York.

CARRIAS, J.F., SERRE, J.P., SIME-NGANDO, T. & AMBLARD, C., 2002: Distribution, size, and bacterial colonization of pico- and nano-detrital organic particles (DOP) in two lakes of different trophic status. – Limnol. Oceanogr. **47**: 1202–1209.

CURRIE, D.J., 1990: Large-scale variability and interactions among phytoplankton, bacterioplankton and phosphorus. – Limnol. Oceanogr. **35**: 1437–1455.

DELGIORGIO, P.A. & SCARBOROUGH, G., 1995: Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production rates. – J. Plankton Res. **17**: 1905–1924.

FORSBERG, C. & RYDING, S.O., 1980: Eutrophication parameters and trophic state indices in 30 Swedish waste-receiving lakes. – Arch. Hydrobiol. **89**: 189–207.

GASOL, J.M., SIMONS, A.M. & KALFF, J., 1995: Patterns in the top-down versus bottom-up regulation of heterotrophic nanoflagellates in temperate lakes. – J. Plankton Res. **17**: 1879–1903.

GASOL, J.M. & DUARTE, C.M., 2000: Comparative analyses in aquatic microbial ecology: how far do they go? – FEMS Microbiol. Ecol. **31**: 99–106.

HOBBIE, J.E., DALEY, R.J. & JASPER, S., 1977: Use of nucleopore filters for counting bacteria by fluorescent microscopy. – Appl. Environ. Microbiol. **33**: 1225–1228.

JONES, J.R. & BACHMANN, R.W., 1978: Phosphorus removal by sedimentation in some Iowa reservoirs. – Verh. Internat. Verein. Limnol. **20**: 1576–1580.

JONES, J.R. & KNOWLTON, M.F., 1993: Limnology of Missouri Reservoirs: An Analysis of Regional Patterns. - Lake and Reserv. Managem. **8**: 17–30.

JONES, J.R., KNOWLTON, M.F. & KAISER, M.F., 1998: Effects of aggregation on chlorophyll-phosphorus relations in Missouri reservoirs. – Lake and Reserv. Managem. **14**: 1–9.

JONES, J.R., OBRECHT, D.V., KNOWLTON, M.F. & COOK, E.A., 2004: Reservoir water quality in Missouri: a statewide analysis to determine the importance of nonpoint source inputs on trophic state. – in press.

KALFF, J., 2002: Limnology. – New Jersey: Prentice Hall, 592 pp.

KIRK, J.T.O., 1983: Light and photosynthesis in aquatic ecosystems. – Cambridge Univ. Press: Cambridge, 416pp.

KNOWLTON, M.F. & JONES, J.R., 2000: Seston, light, nutrients and chlorophyll in the lower Missouri River, 1994–1998. – J. Freshwater Ecol. **15**: 283–297.

LIND, O.T. & DÁVALOS-LIND, L., 1991: Association of turbidity and organic carbon with bacterial abundance and cell size in a large, turbid, tropical lake. – Limnol. Oceanogr. **36**: 1200–1208.

LIND, O.T., CHRZANOWSKI, T.H. & DÁVALOS-LIND, L., 1997: Clay turbidity and the relative production of bacterioplankton and phytoplankton. – Hydrobiologia **353**: 1–18.

NÜRNBERG, G.K. & SHAW, M., 1998: Productivity of clear and humic lakes: nutrients, phytoplankton, bacteria. – Hydrobiologia **382**: 97–112.

PACE, M.L. & COLE, J.J., 2002: Synchronous variation of dissolved organic carbon and color in lakes. – Limnol. Oceanogr. **47**: 333–342.

POSCH, T., LOFERER-KRÖßBACHER, M., GAO, G., ALFREIDER, A., JERNTHALER, J. & PSENNER, R., 2001. Precision of bacterioplankton biomass determination: a comparison of two fluorescent dyes, and of allometric and linear volume-to-carbon conversion factors. – Aquat. Microb. Ecol. **25**: 55–63.

SHORTREED, K.S. & STOCKNER, J.G., 1986: Trophic status of 19 subarctic lakes in the Yukon Territory. – Can. J. Fish. Aquat. Sci. **43**: 797–805.

SMITH, E.M. & PRAIRIE, Y.T., 2004: Bacterial metabolism and growth efficiency in lakes: the importance of phosphorus availability. – Limnol. Oceanogr. **49**: 137–147.

WEISSE, T. & MACISAAC, E., 2000: Significance and fate of bacterial production in oligotrophic lakes in British Columbia. – Can. J. Fish. Aquat. Sci. **57**: 96–105.

XENOPOULOS, M.A., LODGE, D.M., FRENTRESS, J., KREPS, T.A., BRIDGHAM, S.D., GROSSMANN, E. & JACKSON, C.J., 2003: Regional comparisons of watershed determinants of dissolved organic carbon in temperate lakes form the Upper Great Lakes region and selected regions globally. – Limnol. Oceanogr. **48**: 2321–2334.

ZINABU, G.M. & TAYLOR, W.D., 1997: Bacteria-chlorophyll relationships in Ethiopian lakes of varying salinity: are soda lakes different? – J. Plankton Res. **19**: 647–654.

Authors' address:

A. P. THORPE and J. R. JONES, Department of Fisheries and Wildlife, 302 ABNR Building, University of Missouri, Columbia, MO 65211, USA.