Hydrobiologia **481:** 19–31, 2002.

Inter-annual, seasonal and spatial variability in nutrient limitation of phytoplankton production in a river impoundment

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Received 6 October 2001; in revised form 2 May 2002; accepted 14 May 2002

Key words: phytoplankton, reservoirs, primary production, nutrient limitation

Abstract

We characterize seasonal and spatial patterns in phytoplankton abundance, production and nutrient limitation in a mesotrophic river impoundment located in the southeastern United States to assess variation arising from inter-annual differences in watershed inputs. Short-term (48 h) in situ nutrient addition experiments were conducted between May and October at three sites located along the longitudinal axis of the lake. Nutrient limitation was detected in 12 of the 18 experiments conducted over 2 years. Phytoplankton responded to additions of phosphorus alone although highest chlorophyll concentrations were observed in enclosures receiving combined (P and N) additions. Growth responses were greatest at downstream sites and in late summer suggesting that those populations experience more severe nutrient limitation. Interannual variation in nutrient limitation and primary production corresponded to differences in the timing of hydrologic inputs. Above average rainfall and discharge in late-summer (July–October) of 1996 coincided with higher in-lake nutrient concentrations, increased production, and minimal nutrient limitation. During the same period in 1995, discharge was lower, nutrient concentrations were lower, and nutrient limitation of phytoplankton production was more pronounced. Our results suggest that nutrient limitation is common in this river impoundment but that modest inter-annual variability in the timing of hydrologic inputs can substantially influence seasonal and spatial patterns.

Introduction

Advective environments (estuaries, rivers, river impoundments) are characterized by downstream directionality of water flow or, in the case of estuaries, by tidal-influenced bidirectional flow. As a consequence of their hydrogeomorphic position, these systems experience short water residence time (WRT) and large inputs of nutrients and suspended particulate matter. Aquatic ecologists have traditionally viewed advective systems as nutrient-saturated because light limitation and short WRT were thought to suppress algal abundance and maintain high per capita nutrient availability (Alpine & Cloern, 1992; Cole et al., 1992; Smith et al., 1999). This perspective has lingered despite historical (Kofoid, 1903) and more recent studies showing that these systems are capable of supporting large resident plankton populations and experiencing substantive nutrient depletion (Garnier et al., 1995; Reynolds & Descy, 1996; Wehr & Thorp, 1997).

River impoundments constitute a diverse group of waterbodies that occupy an intermediate position between natural lakes and free-flowing rivers in terms of their water residence time (Kennedy, 2001). Soballe & Kimmel (1987) analyzed data from over 600 waterbodies in the United States and found that algal abundance per unit of phosphorus increased as a function of water residence time. Similar correlations have been reported from a number of site-specific studies

(Jordan et al., 1991; Dokulil, 1994; Basu & Pick, 1996). These findings suggest that short WRT limits nutrient utilization and biomass accrual in river impoundments and results in lower phytoplankton abundance relative to natural lakes with comparable nutrient levels. Similar studies have also documented positive associations between chlorophyll and phosphorus among regional groups of river impoundments (Hoyer & Jones, 1983; Soballe et al., 1992). Thus correlational analyses preclude characterizing impoundments as nutrient-saturated systems but rather, suggest that the importance of nutrient limitation is dependent upon site-specific factors influencing WRT and nutrient availability.

Assessment of nutrient limitation in river impoundments is complicated by temporal variability arising from seasonal and inter-annual fluctuations in watershed inputs and by spatial complexity associated with longitudinal gradients in channel morphology (Kennedy & Walker, 1990). A general model of reservoir primary production proposed by Kimmel et al. (1990) predicts that phytoplankton should be light-limited in the upper reaches of the impoundment due to their proximity to riverine inputs of nutrients and suspended particulate matter. Downstream (near the dam), cross-sectional area and WRT increase, resulting in lake-like and nutrient-limited conditions particularly during summer base flow. Few studies have quantified nutrient limitation in impoundments at spatial and temporal scales needed to resolve in-lake gradients, seasonal changes and inter-annual variability. Groeger & Kimmel (1988) reported that phytoplankton growing in the near-dam region experienced more severe N-limitation in comparison to upstream communities. In contrast, Sterner (1994) did not detect consistent differences in the severity of nutrient limitation between near-dam and mid-lake sites in a Texas reservoir. Knowlton & Jones (1996) reported phytoplankton growth in a turbid Missouri reservoir was frequently limited by both light and nutrient availability. Poor temporal resolution inherent in many nutrient limitation studies (Elser et al., 1990) may be a particularly important shortcoming in studies of river impoundments which generally exhibit low ratios of N:P in comparison to natural lakes (Soballe & Kimmel, 1987). Complex seasonal patterns such as shifts from P- to N-limitation may arise from variation in watershed inputs and internal cycling in systems near co-limitation (Effler & Bader, 1998).

In this study, we quantify seasonal and spatial variability in phytoplankton abundance, production and nutrient limitation in a mesotrophic river impoundment. We used in situ enclosures to measure phytoplankton responses to nutrient amendments seasonally and along the longitudinal axis of the impoundment. Our objectives were: (1) to assess the effects of inter-annual variation in hydrologic inputs on seasonal and spatial patterns in phytoplankton growth and biomass accrual, and (2) to compare the frequency and severity of nutrient limitation during 2 years with contrasting flow regimes. Spatial and temporal complexity in algal growth responses is interpreted in the context of changing nutrient levels, light availability and community dominants.

Materials and methods

Study area

Herrington Lake is an impoundment of the Dix River located in north-central Kentucky, USA (Fig. 1). The lake's watershed (113 700 ha) is comprised of 71% agricultural, 26% silvicultural and 3% urban areas (Kentucky Division of Water, 1984). The lake basin lies within a steep and narrow canyon formed by the Dix River. As a result, the lake is deep (mean and maximum depths of 24 and 76 m, respectively) with an average width of only 0.2 km (length=56 km, surface area=1190 ha). Along most of the shoreline, the lake is protected by steep bluffs. The average retention time is 9.2 months.

Five sampling sites were selected to characterize in the upstream (riverine), middle (transition), and downstream (lacustrine) sections of the lake (Fig. 1). Sites 1, 2, 3 and 5 were within the mainstem of the lake, whereas site 4 was located in an embayment (Cane Run Creek). Thermal stratification, light attenuation (PAR), nutrient concentrations, and phytoplankton (biomass, production, species composition) were monitored at 1–4 week intervals from April to October in 1995 and 1996.

Temperature and light

Temperature and dissolved oxygen were measured at 1-m intervals using a YSI 6000 water quality meter. Light attenuation (PAR) profiles were measured using a Protomatic photometer equipped with upward and downward spherical sensors exposed as hemispheres. The wavelength response of this meter is similar to the ideal quantum response for PAR but with a slight bias to underestimate light energy at longer wavelengths

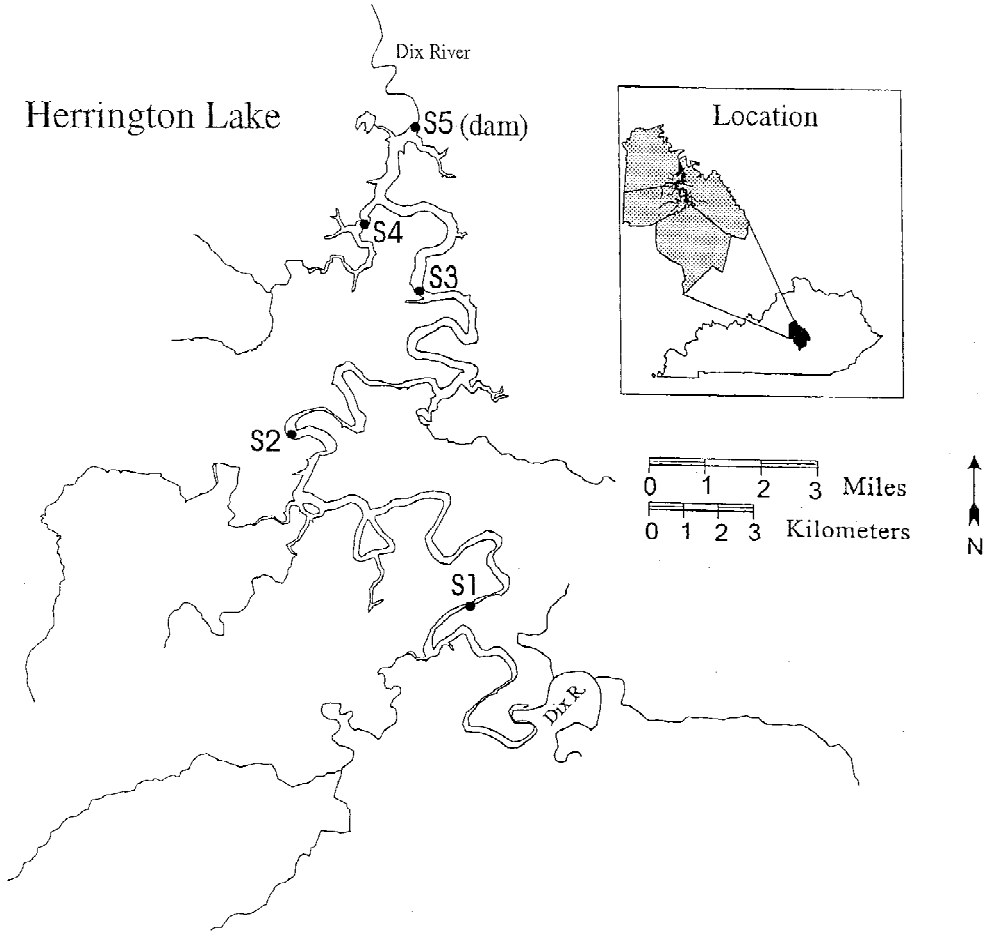

Figure 1. Map of Herrington Lake showing location of sampling sites.

(>650 nm; Bukaveckas & Robbins-Forbes, 2000). Profiles were taken at 0.5 m intervals from the surface to $z_{1\%}$ between 1000 and 1400 h. The attenuation coefficient for downwelling irradiance (K_d) was determined from a linear regression of the natural logarithm of downwelling irradiance against depth (Kirk, 1994). Correlation coefficients derived from fitting least squares linear regressions to irradiance data were uniformly high ($R^2 > 0.98$). The standard error of the slope was less than 5% for 90% of the regressions. For dates when *in situ* nutrient addition experiments were performed, mean light levels at the depth where enclosures were incubated (z=1 m) were estimated as follows:

$$I_{\text{enclosure}} = I_0 * \mathrm{e}^{(-Kd*z)},$$

where I_0 is the total daily solar radiation (PAR) at the lake surface. To quantify light availability experienced by phytoplankton in the lake, light levels within the mixed zone (z_{mix}) were estimated using the equation of Gosselain et al. (1994):

$$I_{\text{mix}} = I_0/(Kd * z_{\text{mix}}).$$

Chlorophyll a

Water samples for chlorophyll analyses were taken at three equally spaced depths between the surface and $z_{1\%}$ using a 2.5 l Kemmerer water sampler. Samples were stored in 1 l polyethylene bottles on ice, and processed within 1 to 2 h of collection by filtration through 0.45 μm Gelman A/E glass fiber filters. The filters were subsequently frozen and processed within 2–7 days. Filters were macerated in 10.0 ml of 90% buffered acetone (buffering agent: $MgCO_3$) and allowed to extract for 12–16 h at 4 °C. Following centrifugation, the extracts were analyzed

spectrophotometrically to determine chlorophyll *a* and pheophytin *a*. Extracts were analyzed using a Varian DMS 70 dual beam spectrophotometer equipped with long pathlength (4 cm) cells and narrow (1 nm) bandwidth. Optical densities were measured at 664 nm and 750 nm before acidification with 0.1 N HCl and at 750 nm and 665 nm after acidification. Chlorophyll *a* concentrations were corrected for pheophytin *a* using the Lorenzen equations as modified by Speziale et al. (1984).

Phytoplankton production

Phytoplankton production was measured monthly using the isotope technique described by Vollenweider (1969). Samples were collected from 3 equally spaced depths within the photic zone. Two light bottles and one dark bottle (60 ml BOD) from each of the three depths were inoculated with 1 μCi of [^{14}C]$NaHCO_3$ (310.80 MBq-mmol) and incubated for 2 h (1200–1400). After incubation, all samples were filtered through 0.45 μm Millipore membrane filters. The filtration pressure applied did not exceed 300 mmHg (Pregnall, 1991). Filters were dissolved in 6.5 ml of Aqua-sol and radioactivity was determined using a Tri-Carb 1900 TR liquid scintillation analyzer. Quenching was corrected using an external unquenched ^{14}C standard with known activity.

Dissolved inorganic carbon samples were collected in 60 ml acid-washed plastic syringes and stored on ice. Samples were analyzed within 1–2 days on a Shimadzu Total Carbon Analyzer (Model TOC-5050A) using the combustion/non-dispersive infrared gas analysis method (APHA, 1992).

Photosynthesis-irradiance curves were modeled using the following equation:

$$P = P_{\max} \tanh(\alpha I / P_{\max}),$$

where P is the biomass-specific rate of production (per unit chlorophyll) at irradiance I (Jassby & Platt, 1976). Alpha (α) is the slope of the light-saturation curve which measures the efficiency of inorganic carbon assimilation at low light levels. $P_{\max}$ is the maximum photosynthetic rate at optimal illumination levels as described by the plateau of the line. We compared *P–I* models for data aggregated by site (all months) versus by month (all sites) and found that the models derived for monthly-aggregated data accounted for a greater proportion of variability (R^2=0.79 to 0.92). These models were used to estimate production on sampling dates when only chlorophyll and light attenuation were measured (N=6). In addition, the models were used to derive estimates of whole-lake production for July–October based on daily solar radiation and lake bathymetric data. The period of July to October was chosen because of comparable sampling frequencies for both years.

Phytoplankton community composition

Samples for phytoplankton enumeration were preserved with 2.25-ml of M3 fixative (APHA, 1992) and stored in the dark at room temperature until analysis. Subsamples were filtered through 0.45 μm Millipore membrane filters, cleared in glutaraldehyde and mounted with EUPARAL. Identification and enumeration of phytoplankton was made with an Olympus BH-2 microscope at 500× and 1250×, under Nomarski differential interference contrast. Cell measurements were made on 15–20 randomly selected cells of predominant species. Volumes of the cells were estimated by comparing cells to simple geometric shapes (Orlik et al., 1998). The mean cell volumes for the common species were multiplied by population density to derive an estimate of species biovolume.

Nutrient enrichment experiments

Nutrient enrichment experiments were conducted using phytoplankton collected from the upstream, middle, and downstream sections of the lake (sites 1, 3, 4; respectively). Experiments were performed on six occasions (August, September, October, 1995; May, July, September, 1996). Experimental design and data analysis followed the protocol described in Bukaveckas & Shaw (1998). Water was pumped from 1-m below the surface, transferred through a 150 μm zooplankton net and collected in a large polyethylene mixing container (128 l). Water was distributed into 12 10-l polyethylene containers which were subsequently assigned to one of four treatment groups: control (no nutrient addition), nitrogen addition, phosphorus addition, and combined nitrogen and phosphorus (three replicates in each group). Inorganic nitrogen was added as $NaNO_3$ (200 μg N l^{-1} in 1995 and 400 μg N l^{-1} in 1996) and phosphorus was added as NaH_2PO_4 (40 μg P l^{-1}). The containers were incubated for 48 h at a depth of 1-m. Chlorophyll and nutrient concentrations were measured at the beginning and end of each experiment to quantify phytoplankton growth responses and rates of nutrient assimilation. Phytoplankton growth responses to nutrients were quantified

using the following formula:

$$\text{nutrient response} = \frac{\text{CHL}_{(\text{trmt})} - \text{CHL}_{(\text{control})}}{\text{CHL}_{(\text{control})}},$$

where CHL_{trmt} is the mean chlorophyll concentration after 48 h among three replicates receiving nutrient additions (+N, +P or +NP). $\text{CHL}_{\text{control}}$ is the mean chlorophyll concentration after 48 h among replicates receiving no nutrients. Nitrate concentrations were determined using the automated cadmium reduction method (APHA, 1992) and performed on an autoanalyzer (Skalar San Plus). Phosphorus (soluble reactive) concentrations were analyzed on unfiltered samples using the manual ascorbic acid method (APHA, 1992). Ammonium concentrations were checked routinely but typically did not exceed 10 μg l^{-1}.

Statistical analyses

Statistical comparisons of interannual variability in selected parameters (nutrients, chlorophyll, phytoplankton production, and biomass) were performed using a one-way analysis of variance (ANOVA) accompanied by tests for normality and heterogeneity of variance. For each nutrient enrichment experiment, we determined significant treatment effects using a one-way ANOVA and Student–Newman–Keuls pairwise comparisons. If there was a significant response to P- or N-addition alone, then the assemblage was considered P-limited or N-limited, respectively. If there was a statistically significant response to both P- and N-addition, or, if the response to the combined addition was significantly greater than the response to the single nutrient, then the assemblage was considered co-limited (+NP). Statistical significance was evaluated at the $p<0.05$ level and performed using SIGMASTAT (Version 2.0, 1992–1995).

Results

Hydrology and nutrient chemistry

Discharge measurements from the primary inflow and rainfall data were used to characterize seasonal and inter-annual differences in water and material inputs to the impoundment. Total rainfall for the period March–October was similar in 1995 and 1996 (89 and 88 cm, respectively) but the timing of inputs differed (Fig. 2). In 1995, highest precipitation and inflow occurred early in the growing season while July–October was

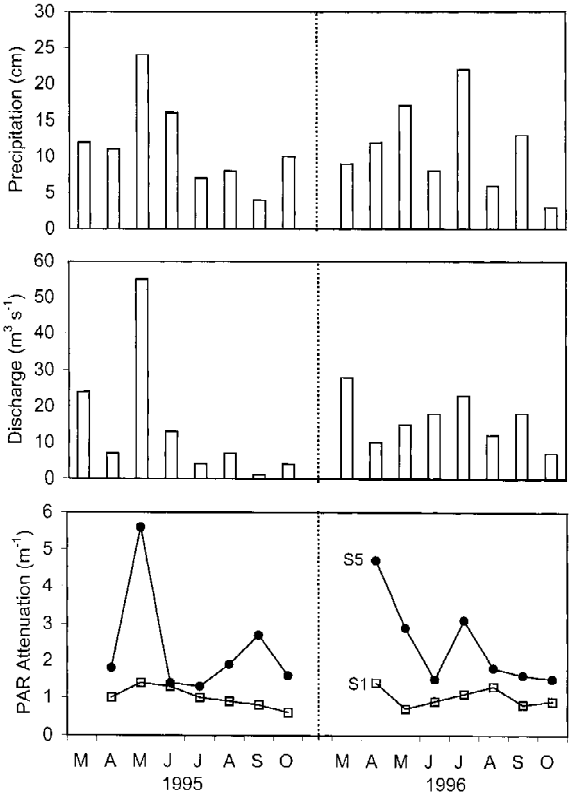

Figure 2. Monthly precipitation at the outlet of Herrington Lake, average discharge of the primary inflow to Herrington Lake (Dix River), and average monthly PAR attenuation coefficients (Kd) at upstream (S1) and downstream (S5) sampling locations.

characterized by low precipitation (<10 cm monthly) and low inflow (<10 m^3 s^{-1}; Fig. 2). The same period in 1996 was characterized by more variable precipitation (3–22 cm monthly) and inflow (7–23 m^3 s^{-1}). As a result, cumulative inflow during July–October was higher in 1996 (equivalent to 45% of lake volume) as compared to 1995 (14% of lake volume). Elevated inflows during 1996 were associated with higher PO_4–P and NO_3–N concentrations throughout the lake (Table 1). Average PO_4–P and NO_3–N concentrations among sites were four-fold and five-fold higher (respectively) in 1996 and between-year differences were statistically significant (p=0.004 and p=0.017, respectively). In both years, PO_4–P and NO_3–N concentrations were highest at the upstream site and decreased toward the dam. Longitudinal gradients within the lake were particularly apparent in 1995 when PO_4–P and NO_3–N concentrations at the upstream site (S1) were two-fold and three-fold higher (respectively) compared to the downstream site (S4).

Table 1. Soluble reactive phosphorus (PO_4–P) and nitrate (NO_3–N) concentrations ($\mu g\ l^{-1}$) in Herrington Lake during 1995 and 1996. Data shown are averages for samples collected on dates corresponding to enclosure experiments

Site	1995		1996	
	PO_4–P $\mu g\ l^{-1}$	NO_3–N $\mu g\ l^{-1}$	PO_4–P $\mu g\ l^{-1}$	NO_3–N $\mu g\ l^{-1}$
1	16.6	174	50.2	524
3	10.1	41	43.1	287
4	8.1	51	47.8	285
Mean	11.6	89	47.0	366

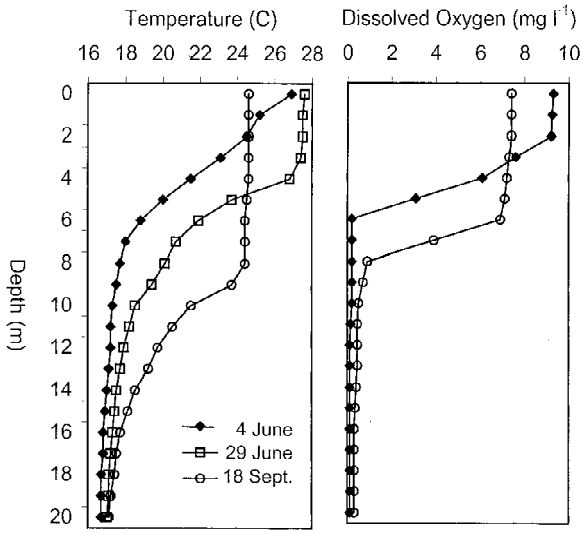

Figure 3. Depth profiles of temperature and dissolved oxygen at the downstream sampling location (Site 5) illustrating the deepening of the upper mixed layer between 4 June and 18 September 1995.

Light attenuation and thermal stratification

Light and temperature gradients within the water column were used to characterize the light climate and define the upper mixed layer. Longitudinal differences in light attenuation and thermal stratification were apparent in both years. Light attenuation was greatest at the upstream site (S1) and decreased toward the dam (Fig. 2). The depth of the photic zone ($z_{1\%}$) ranged from 0.5 to 3 m at the upstream site and increased to 5–8 m at the downstream site (S5). Seasonal and spatial variation in light attenuation was attributed to differences in the scattering of light by suspended particulate matter (data not shown). The effects of suspended matter on attenuation were most

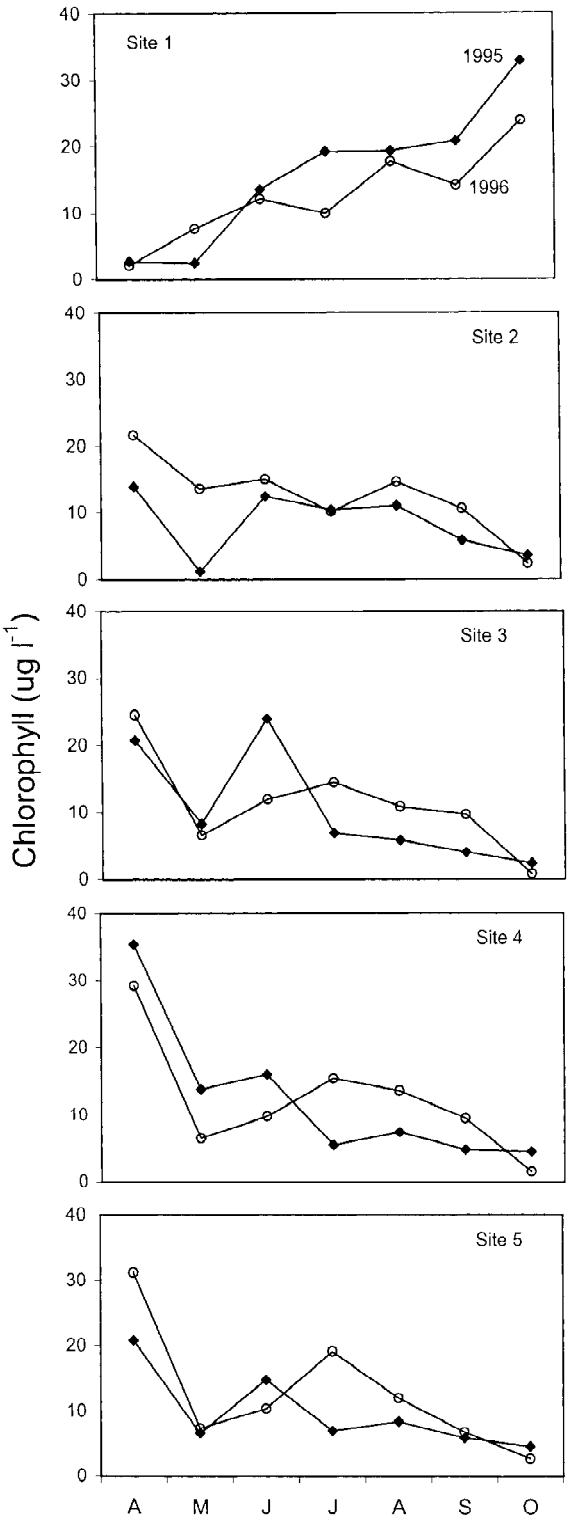

Figure 4. Average monthly chlorophyll concentrations for Sites 1 (most upstream) through 5 (near dam) during 1995 and 1996.

apparent at the upstream site during periods of elevated discharge. Attenuation coefficients (averaged across sites) were not significantly different between years. At the upstream site (S1), temperature gradients between the surface and bottom were less than 10 °C and the mixing depth was poorly defined. Stronger thermal stratification was observed at the downstream site (S5) where temperature ranged from 10 to 20 °C between the surface and z_{max}. At this site, mixing depths were well defined on some dates with z_{mix} ranging from 4 m in June to 8 m in September (Fig. 3). On most dates, however, z_{mix} could not be adequately resolved from thermal profiles (e.g., 4 June; Fig. 3) since temperature declined gradually (ca. 1 °C m^{-1}) within the upper 10 m. Temperature gradients of 2 to 6 ° C were common even in the upper 5 m of the lake. A consistent oxycline was observed at depths of 3–4 m during June–August and gradually deepened to 6–8 m by September–October (Fig. 3). Dissolved oxygen profiles were used to infer mixing depths and to estimate ambient light levels within the upper mixed layer.

Chlorophyll and primary production

We measured chlorophyll and primary production to characterize seasonal and spatial patterns in relation to inter-annual differences in inflow. Monthly averages of chlorophyll *a* concentrations ranged from 2 to 33 μg l^{-1} and exhibited similar seasonal and spatial patterns in both years (Fig. 4). At the upstream site, highest chlorophyll concentrations were observed in late-summer (July–October). At the mid-lake and downstream sites, highest concentrations occurred in early summer (April–May). A transitional period was evident in June when chlorophyll was relatively uniform throughout the lake. Comparisons of average chlorophyll concentrations at each site during 1995 and 1996 did not reveal any significant differences between years. Chlorophyll concentrations within the water column were variable even within the mixed zone (upper 5 m) with highest concentrations typically occurring within 1–2 m of the lake surface. Highest near-surface concentrations occurred when temperature gradients in the upper 5 m were ca. 5 °C.

Primary production was generally highest at the upstream site (S1) where depth-averaged rates within the mixed zone attained 125 and 310 mg C m^{-3} h^{-1} in 1995 and 1996, respectively (Fig. 5). Production rarely exceeded 60 mg C m^{-3} h^{-1} at the downstream sites. Seasonal patterns were similar at all sites with

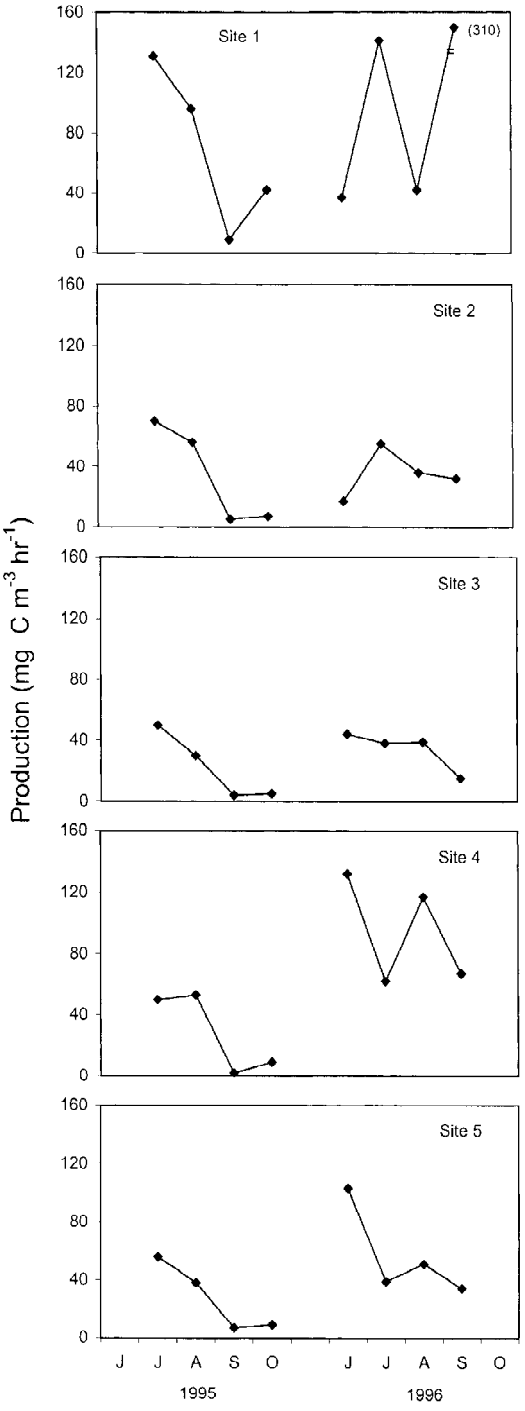

Figure 5. Average monthly volumetric production for Sites 1 (most upstream) through 5 (near dam) during 1995 and 1996.

Table 2. Percent of lake volume and whole-lake production within assigned reservoir segments (site numbers refer to Fig. 1; MC=main channel). Production was calculated for the period of July to October

	S1–S2 %	S2–S3 %	S3–S5 %	S4–MC %	S5–Dam %
Lake volume	22.5	43.3	31.5	1.7	1.0
Production 1995	35.6	43.8	18.1	1.8	0.6
Production 1996	27.2	41.8	27.9	2.2	0.9

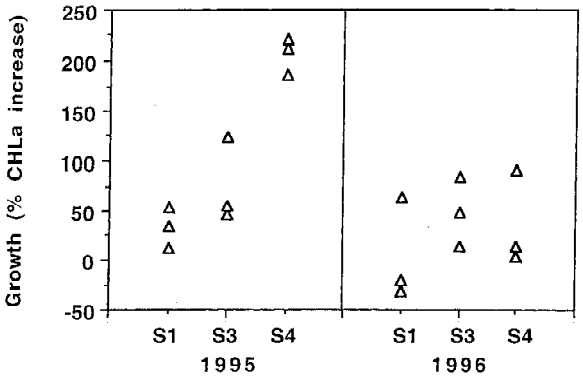

Figure 6. Phytoplankton growth responses (as % increase in chlorophyll relative to Control enclosures) to nutrient amendments at Sites 1 (upstream), 3 (mid-lake) and 4 (downstream) during 1995 and 1996.

values typically ranging from 30 to 130 mg C m^{-3} h^{-1} in July–August but rarely exceeding 15 mg C m^{-3} h^{-1} during September–October. The embayment (S4) exhibited high production, particularly in 1996, but accounted for a small proportion of the lake's volume (2%) and a correspondingly small fraction of whole-lake production. In general, the proportion of whole-lake production occurring within segments delineated by the five sampling sites corresponded with the proportion of lake volume represented by each segment (Table 2). However, during low flows in 1995, a disproportionate fraction of lake production (relative to volume) occurred in the upstream segment (S1–S2). During higher flows in 1996, a greater proportion of phytoplankton production shifted downstream to the lacustrine zone (S3–S5). Whole-lake production for July–October was higher in 1996 compared to 1995 (102 and 86 g C m^{-2}, respectively).

Nutrient limitation experiments

Phytoplankton responses to nutrient amendments were used to quantify the frequency and severity of nutrient limitation. A total of 18 experiments (three sites on six dates) were conducted during 1995 and 1996. Of the 18 experiments, eight showed P-limitation, one showed N-limitation, three showed co-limitation by N and P, and six showed limitation by some factor other than N or P (Table 3). One of the 1996 experiments gave equivocal results in that we detected a significant response to P addition but not the combined N+P addition. Nutrient limitation was observed more frequently at the mid- and down-stream sites (nine of 12 experiments) than at the upstream site (three of six experiments). Among the 1995 experiments, phytoplankton growth responses to N+P addition were consistently greater at the furthest downstream site (ca. 200% increase in CHLa) in comparison to the upstream and mid-lake sites (Fig. 6). In 1996, growth responses were lower overall (<100%) and no consistent differences among sites were observed (Fig. 6). The incidence of nutrient limitation was greater in 1995 (eight of nine experiments) than in 1996 (four of nine experiments).

Phytoplankton community dominants

Cyanophytes dominated the algal community comprising on average 76% of algal biomass (all sites and dates). From July through October cyanophytes accounted for more than 90% of algal biomass at all sites. Dominant taxa included *Aphanocapsa delicatissima* (19%), *Oscillitoria planktonica* (11%), *Gomphosphaerium lacustris* (8%), *Aphanothece nidulans* (6%) and *Merismopedia tennuissima* (3%; percentages denote average across all dates and sites). Diatoms (*Cyclotella pseudostelligera, Stephanodiscus hantzschii*) and chlorophytes (*Pandorina morum, Ankistrodesmus convolutus*) were abundant only in early summer. Diatoms comprised 22% of algal biomass in May and June samples collected at the upstream site but did not exceed 5% of biomass at any of the downstream sites. Chlorophytes represented 32% of algal biomass in May and June of 1995 but never exceeded 10% of biomass in 1996. None of the diatom or chlorophyte taxa accounted for more than 5% of biomass when averaged across all sites and dates. We analyzed phytoplankton responses to nutrient addition (1995 only) by comparing rates of change in species composition (using scores from Canonical Correspondence Analyses) with rates of change in

Table 3. Mean chlorophyll concentrations (±sd) in experimental enclosures receiving no nutrient addition (Control), P addition, N addition or N and P combined addition. Response type indicates statistically significant responses ($p<.05$)

Date	Site	Control $\mu g\,l^{-1}$	P Addition $\mu g\,l^{-1}$	N Addition $\mu g\,l^{-1}$	P+N Addition $\mu g\,l^{-1}$	Response
August, 1995	1	16.9±4.7	18.6±2.9	19.3±3.8	16.8±1.3	None
	3	2.9±0.2	2.7±0.2	3.7±0.5	6.5±1.0	N+P
	4	2.9±0.5	3.2±0.1	3.9±0.1	9.1±0.6	N+P
Sept., 1995	1	40.2±3.3	51.3±7.6	44.2±9.9	61.9±5.4	P
	3	3.0±0.6	3.7±0.7	4.5±0.5	4.5±0.6	N
	4	2.2±0.2	6.8±0.9	3.6±0.3	6.0±1.0	P
October, 1995	1	28.8±0.1	37.8±3.8	28.2±4.3	38.5±1.2	P
	3	4.6±0.2	7.1±0.9	5.6±0.6	6.5±0.6	P
	4	3.1±0.4	6.7±0.5	3.9±0.7	8.9±0.2	P, N+P
May, 1996	1	11.3±2.8	17.9±2.8	8.2±1.7	18.5±1.3	P
	3	16.1±1.5	30.0±3.4	15.8±3.0	29.6±2.9	P
	4	21.2±2.2	40.3±5.0	19.0±2.6	40.4±3.9	P
July, 1996	1	17.5±0.4	11.9±0.4	12.5±1.9	9.7±2.2	None
	3	32.3±3.5	48.1±2.4	37.7±1.3	32.0±2.0	P
	4	27.4±3.2	29.0±7.8	24.4±9.0	28.3±4.5	None
Sept., 1996	1	21.4±1.4	15.5±2.2	13.3±1.2	17.0±1.2	None
	3	7.9±0.9	7.7±0.8	8.9±1.8	6.9±2.3	None
	4	9.9±2.6	8.5±1.5	11.4±1.9	8.1±1.0	None

community biomass. We did not observe a correlation between these two measures of community response. Shifts in species dominance occurred in some enclosures despite little change in community abundance, while in others, community biomass increased but species dominance remained unchanged.

Discussion

Kimmel et al.'s (1990) model of reservoir primary production predicts that nutrient availability decreases and light availability increases from upstream (near inflow) to downstream (near dam). As a result, phytoplankton production should be highest mid-lake (where light and nutrients are optimal) and decrease upstream (light limitation) and downstream (nutrient depletion). Longitudinal gradients in light availability, nutrient concentrations and phytoplankton production in Herrington Lake were generally consistent with the predictions of this model. Light penetration was lowest at the upstream site and increased with distance downstream. Nutrient concentrations (PO_4–P and NO_3–N) decreased downstream presumably because greater light penetration combined with longer water residence allowed phytoplankton to utilize nutrients more efficiently. Based on longitudinal gradients observed in August–October of 1995, outflow nutrient concentrations (measured near-dam) were depleted to 50% (PO_4–P) and 30% (NO_3–N) of concentrations measured at the most upstream site. Similar longitudinal gradients have been reported for other reservoirs (Cooke et al., 1993; Effler & Bader, 1998). As we did not measure dissolved organic and particulate fractions we are unable to estimate overall retention of N and P but our findings suggest that retention of inorganic fractions was higher during the low-flow year (Garnier et al., 1999).

Seasonal and spatial variability in chlorophyll and primary production were consistent with hypothesized effects of light availability and nutrient limitation. During high flow periods (April–May), chlorophyll was lowest upstream presumably because high turbidity and short water residence time precluded phytoplankton community development. The opposite trend was observed during summer base flow when the upper reaches of the lake accounted for a disproportionate fraction of whole-lake primary production and the upstream site exhibited consistently higher chloro-

phyll concentrations. Direct measurement of nutrient limitation by in situ bioassays showed that phytoplankton growth responses to nutrient additions were higher near the dam. This finding was consistent with our hypothesis of greater nutrient limitation downstream and with observed gradients in chlorophyll and primary production.

Rainfall was similar during the summers of 1995 and 1996 but differences in the timing of discharge events had a substantial influence on longitudinal gradients within the lake. In 1995, typically low rainfall and discharge during July–October were associated with low nutrient concentrations and severe nutrient limitation of phytoplankton growth. In contrast, higher rainfall and discharge during the same period in 1996 resulted in substantially higher nutrient concentrations throughout the lake. This increase in nutrients coincided with increased production and minimal nutrient limitation of phytoplankton growth in the lacustrine zone. Significant responses to nutrient addition were detected in only four of nine experiments (1996) compared to eight of nine during the previous year. These results suggest that modest increases in discharge during the growing season may alleviate nutrient limitation. Chlorophyll levels were comparable in both years despite higher flushing rates in 1996 (45 vs. 14% of lake volume) suggesting that export of phytoplankton biomass from the lake was ca. three-fold greater in 1996. Greater availability of autochthonous particulate organic matter may stimulate secondary production in downstream areas but we know of no studies that have explicitly linked organic carbon export from river impoundments to downstream food-webs over time scales that address seasonal or interannual variations in discharge.

Our results suggest that nutrient limitation is common in this river impoundment (e.g., eight of nine experiments in 1995) despite constraints imposed by light limitation. Our findings were similar to those reported from more turbid and eutrophic reservoirs in Missouri (Knowlton & Jones, 1996) and Texas (Sterner, 1994). By comparing phytoplankton growth rates at ambient and enriched nutrient concentrations, Sterner (1994) determined that nutrients limited realized growth rates to half or less of potential growth rates when epilimnetic temperatures exceeded 15 °C. In our experiments, water temperatures ranged from 15 to 29 °C and we observed two- to three-fold increases in chlorophyll at downstream sites within 48 h following the addition of nitrogen and phosphorus. Using the same experimental design, we quantified nutrient limitation of phytoplankton and bacterioplankton in an impoundment of the Tennessee River (Kentucky Lake) where WRT was shorter (ca. 20 d) and thermal stratification was absent (Bukaveckas et al., in press). By August, phytoplankton growth rates at ambient nutrient levels were 25% or less of nutrient-saturated rates.

Highest chlorophyll concentrations were observed in enclosures receiving combined (+PN) additions. Co-limitation of algal production by nitrogen and phosphorus has been demonstrated from a variety of lakes and reservoirs (Dodds & Priscu, 1990; Knowlton & Jones, 1996) and may arise when phytoplankton are close to being limited by the 'non-limiting' nutrient (Vanni & Temte, 1990). Thus, a community (or a subset of its species) experiencing P limitation receives an addition of P (no N) but fails to increase in biomass due to the rapid onset of N limitation (Suttle & Harrison, 1988). Co-limitation may be a common feature among reservoirs since they generally exhibit lower ratios of N:P in comparison to natural lakes (Soballe & Kimmel, 1987). Nitrogen was found to limit algal growth at the community level (Sterner & Grover, 1998) and at the taxon level (Grover et al., 1999) in two mid-latitude reservoirs exhibiting comparable N and P levels as those reported in our study. Epilimnetic N:P ratios in Herrington Lake (atomic ratios of NO_3–N to PO_4–P) were consistently below the Redfield ratio and were typically less than 10:1. Recent studies have shown that nutrient regeneration by zooplankton and fish can account for a significant fraction of algal demand and lead to differential recycling of N and P (Sterner et al., 1992; Hassett et al., 1997). Cyanophytes dominate the summer algal community of this and other mid-latitude lakes (Phlips et al., 1997; Grover et al., 1999) and as these are generally considered to be a poor food source for consumers (DeMott, 1998) the importance of consumer-driven recycling may be reduced. Further research is needed to link differential recycling of N and P by grazers with shifts in N vs. P limitation of phytoplankton in river impoundments and other lakes where co-limitation is common.

Algal responses to nutrient additions are determined in part by light levels within enclosures (Saunders et al., 2000). Enclosures incubated at a fixed depth may over-estimate growth responses following nutrient addition if phytoplankton within enclosures experience higher light conditions compared to phytoplankton in the lake. We calculated light levels experienced by phytoplankton within enclosures based on daily

solar radiation and water column light attenuation (see 'Methods'). Ambient light levels experienced by phytoplankton can be derived from daily solar radiation and light attenuation provided that the mixing depth is known. The occurrence of weak thermal stratification in the upper reaches of the lake and poorly defined epilimnia downstream, complicate the determination of z_{mix} in our system. Therefore, we rearranged the equation of Gosselain et al. (1994; see 'Methods') to solve for the mixing depth simulated by incubating the enclosures at 1 m (z_{mix} where $I_{mix} = I_{enclosure}$).

Mixing depths simulated by the enclosures ranged from 1.2 m (upstream site) to 3.5 m (mid- and downstream site). For the upstream site, the simulated mixing depth is likely to be conservative with respect to the actual (realized) mixing in the water column. Therefore, light levels experienced by phytoplankton in enclosures would be greater than those experienced by phytoplankton in the lake. Despite this, nutrient limitation was observed in only two of six experiments and growth responses were weaker than those observed downstream. These results were consistent with the hypothesis that light rather than nutrient limitation constrains phytoplankton growth in the riverine portion of the lake. At the mid- and down-stream sites, the simulated mixing depth (3.5 m) may approximate actual mixing experienced by phytoplankton in the lake during June–August (thermocline and oxycline ca. 4 m). The simulated mixing depth may not have been representative of mixing conditions during the September–October experiments when deeper (ca. 8 m) thermo- and oxy-clines were observed. However, temperature gradients were frequently observed in the upper 5 m of the water column suggesting that turbulence was insufficient to maintain a uniform mixed zone. Furthermore, the presence of chlorophyll gradients suggest phytoplankton growth rates exceeded vertical mixing rates. Huisman et al. (1999) have argued that phytoplankton blooms can be induced by two fundamentally different mechanisms: (1) if a shallow, mixed layer forms by means of thermal stratification (Sverdrup's 'critical depth' concept), or (2) if weak turbulent mixing allows phytoplankton growth rates near the surface to exceed vertical mixing rates. Although 'critical turbulence' values are thought to be low in turbid systems (ca. 0.31 $cm^2\ s^{-1}$ for our downstream site), the morphometry of Herrington Lake (small fetch, steep shoreline) may favor the formation of weak thermal gradients allowing phytoplankton to take advantage of higher light conditions near the surface.

In summary, our findings support the generalized model of reservoir primary production proposed by Kimmel et al. (1990). Short-term in situ enclosure experiments replicated both spatially and temporally allowed us to delineate longitudinal gradients in the severity of nutrient limitation and to assess the effects of inter-annual climatic variability. Our results suggest that modest interannual variability in hydrodynamics substantially alters nutrient-algal relations. Modest increases in discharge during the growing season mitigated nutrient limitation and resulted in threefold greater export of algal-C. Our findings have implications for understanding localized (in-lake) processes that govern nutrient-algal relations and for landscape-scale considerations of C, N and P flux from large watersheds. An important challenge for aquatic ecologists is to document the effects of climate-driven hydrodynamics on the transport of nutrients and organic carbon from proximal to distant (downstream) food webs (e.g., Polis et al., 1996; Rabalais et al., 1998). The dynamics of these linkages are central to our understanding of large-scale spatial organization of aquatic ecosystems.

Acknowledgements

This research was supported, in part, by the U.S. Geological Survey through the U.S. Environmental Protection Agency Clean Lakes Program. Additional support was provided by grants from the Kentucky Water Resources Research Institute and the Kentucky Institute of Environment and Sustainable Development. We are grateful to L. Jarrett for advice during early stages of the project, R. McCandless and D. Metzmeier for assisting in the field, S. Rier and R. Schultz for analysis of nutrient samples and A. St. Amand for help with algal taxonomy.

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