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Algal Biomass Accrual in Relation to Nutrient Availability along a Longitudinal Gradient in the Upper Green River, Kentucky

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ALGAL BIOMASS ACCRUAL IN RELATION TO NUTRIENT AVAILABILITY ALONG A LONGITUDINAL GRADIENT IN THE UPPER GREEN RIVER, KENTUCKY

A Thesis
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Of the Requirements for the Degree
Master of Science

By
Mary Douglas Penick

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ALGAL BIOMASS ACCRUAL IN RELATION TO NUTRIENT AVAILABILITY ALONG A LONGITUDINAL GRADIENT IN THE UPPER GREEN RIVER, KENTUCKY

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Mary Douglas Penick August 2010 46 pages

Directed by: Dr. Scott Grubbs, Dr. Albert Meier, and Dr. Michael May

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Nutrient limitation in aquatic ecosystems results from a deficiency in nitrogen or phosphorus levels relative to cellular growth needs. Nutrient limitation of freshwater systems is a function of biotic and abiotic factors. Biotic factors include vascular and nonvascular plant community composition. Abiotic factors include underlying bedrock and land-use activities (e.g. agriculture, septic systems). Nutrient availability directly affects growth, productivity, and community structure of primary producers. The purpose of this study was two-fold: (1) to assess the relationship between ambient algal biomass and in-stream nutrient levels along the longitudinal course of a river through a transition from weak to well-developed underlying karst bedrock, and (2) experimentally assess if periphyton was nitrogen or phosphorous limited between weak and well-developed karst sites. Sestonic and filamentous biomass (= chlorophyll-α) levels increased monthly along the longitudinal gradient. In contrast, periphyton biomass levels increased minimally monthly and displayed no longitudinal pattern. Nitrate and soluble reactive phosphorus levels exhibited distinct longitudinal increases, whereas total phosphorous displayed minimal change and ammonia levels decreased in the downstream direction. Total nitrogen (TN) levels increased upstream but decreased sharply in the well-developed downstream karst sites. The nutrient limitation assays revealed that the highest periphyton levels were with N + P treatments at the most upstream sites. Overall, in Kentucky's Green River algal biomass accrual appears to be mainly P-limited but likely also by TN availability during late summer.

Introduction

Nutrient limitation occurs across a broad variety of ecosystems. Limitation occurs when a nutrient is in short supply relative to cellular growth requirements (Dodds et al. 2002), and therefore, limits the productivity and biomass accrual of primary producers. The most common limiting macronutrients are nitrogen and phosphorus (Elser 2001, Dodds 2002). While nitrogen (N) is the main limiting nutrient in terrestrial ecosystems (Grimm et al. 2003), aquatic ecosystems are usually limited by N and/or phosphorus (P). Convention asserts that freshwater systems are typically P limited and marine systems N limited (Hecky and Kilham 1988, Dodds & Welch 2000, Grimm et al. 2003). This assumption, however, is imperfect and disputed. In an extensive study of literature on 200 temperate streams from North America and New Zealand, Dodds et al. (2002) found that N limitation, P limitation, and N and P colimitation each could occur, and thereby affect periphyton biomass accrual. Nutrient research in the U.S. often focuses on the effects of N loading on streams and lakes, and many freshwater systems are classified as P-limited. Conversely, streams in Queensland, Australia are classified as N-limited, so nutrient control research in that region focuses on P loading (Mosisch et al. 2001). Dodds and Welch (2000) compiled data from 158 bioassays, of which 13% were stimulated solely by N, 18% by P, 44% by both N and P, and 25% by neither nutrient. Rosemond et al. (1993) found that within a single system the limiting nutrient varied annually and small changes in the concentration of one limiting nutrient induced limitation by the other.

Nutrient availability is influenced by multiple factors, including underlying geology and watershed-scale land-use patterns. Lohman et al. (1991) investigated stream nutrient levels across the U.S.A. and found that P limitation was common east of the

Mississippi River and N limitation in the Pacific Northwest. Grimm et al. (2003) asserted that the old view of P limitation in freshwater was a result of geographically-biased studies. Much of the research had been conducted in the eastern U.S. in areas with low-P parent geology and excessive N inputs from agriculture.

Weathering of stream geomorphology was once considered a minor contributor to nutrient availability (Grimm et al. 2003). Holloway (1998), however, reported that exposed sedimentary rocks contain 20% of the global N inventory and when stream bedrock has high nutrient (e.g., NO_3) levels the underlying geology becomes a major contributor of that nutrient to the stream channel. Dodds and Welch (2000) found that phosphate-rich rocks may provide sufficient P to prevent limitation. Groundwater may also contribute to in-stream nutrient levels. In karst geologic regions, nutrient-enriched groundwater can mix with surface water via springs and fractures in the bedrock (Notestein et al. 2003). Thus, nutrient limitation can differ according to base geology and watershed characteristics.

Primary producers in lotic systems are either suspended in the water column (i.e., sestonic) or attached to substrates (i.e., benthic). Benthic algae are able to colonize most submerged substrates and are classified by habitats (Lowe and LaLiberte 2006). Benthic microfloral growth is referred to as periphyton (Wetzel 1983), which includes microscopic algae plus bacteria and fungi (Stevenson 1996). Filamentous algae are single cells that form long chains or filaments (AQUAPLANT 2009), often found attached to benthic substrates via holdfasts or floating as dense mats on the water's surface and include the globally ubiquitous *Cladophora* (Dodds and Gudder 1992).

Primary productivity and algal biomass accrual are influenced by nutrient availability (e.g., Lohman et al. 1992, Dodds 2002). Periphyton accrual rates are higher in areas of greater nutrient availability. For example, flood events scour periphyton from natural substrates and recolonization is rapid in highly enriched areas (Lohman et al. 1992). Nutrients also influence algal community structure and individual species may have different nutrient requirements (Hecky and Kilham 1988, Borchardt 1996). The same ecosystem could exhibit P limitation one year and N limitation the next due to alterations of algal community structure, which may explain why N and P colimitation occurs (Tank and Dodds 2003).

Short life spans and high turnover rates of benthic and sestonic algae allow for species replacement during periods of nutrient limitation. If a lake becomes N limited, N-fixing cyanobacteria can alleviate this deficiency by maintaining productivity proportional to P load (Vitousek and Howarth 1991). The types of algal species present are also affected by nutrients. Baysinger-Daniel (1989) found filamentous species were more common in sites that were high in nutrients and moderately enriched, while low-nutrient sites were dominated by diatom and cyanobacteria assemblages (Lohman et al. 1992). Interactions between species of algae may exist. *Cladophora* is found associated with N-fixing cyanobacteria. The nitrogen released by cyanobacteria allows *Cladophora* to survive in N-deficient waters (Dodds and Gudder 1992).

Nutrients levels may become a secondary factor regulating algal growth, however, if light availability becomes a limiting factor (Hecky and Kilham 1988, Mosisch et al. 2001, Carey et al. 2007). Light levels also shape algal community structure. Mosisch et al. (2001) found that communities were dominated by filamentous algae in open canopy sites and by diatoms in shaded sites. Grazer population density can influence community structure. Communities with dense grazer populations were P limited, while those with few grazers were N limited (McCormick and Stevenson 1989, McCormick 1990). Additionally, current velocity can influence algal biomass accrual (Busse et al. 2006, Rinke et al. 2001). The tough, flexible thallus of *Cladophora* spreads out at low velocities and streamlines with increasing flow, allowing it to withstand higher flow velocities (Dodds 1991, Dodds and Gudder 1992). Periphyton communities are often stratified vertically, and overstory taxa limit understory growth by maintaining a light barrier and competitively taking up nutrients from the water column. Grazers may remove the overstory periphyton layer, however, and allow light and nutrients to penetrate to the understory (McCormick and Stevenson 1991).

Algal cells have a molar N:P ratio of 16:1 (i.e., Redfield Ratio; Redfield 1958). Determining which nutrient limits growth often involves calculating an N:P ratio. The form of nutrient used, however, affects the ratio outcome. Dodds (2003) found that dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) were poor predictors of total nitrogen (TN) and total phosphorus (TP) and were weakly correlated with periphyton biomass. Dodds (2003) argued that TN and TP more accurately reflect nutrient supply and are more closely correlated with periphyton biomass. Busse et al. (2006) used TN/TP and the following criteria to predict the limiting nutrient: $N/P > 20$ suggested P limitation, $N/P < 10$ suggested N limitation, and $10 < N/P < 20$ suggested colimitation for both N and P or neither.

The purpose of this study was two-fold: (1) to assess the relationship between ambient algal biomass and in-stream nutrient levels along the longitudinal transition of a river from weak to well-developed underlying karst bedrock geology, and (2) experimentally assess if periphyton was nitrogen or phosphorous limited between weak and well-developed karst sites.

Methods

Study site descriptions

This research occurred in seven sites positioned along the Green River between the Green River Lake (GRL) and Mammoth Cave National Park, Kentucky, USA (Fig. 1–2). The Green River flows in a westerly direction from source before emptying into the Ohio River, passing through Level III Ecoregions 71 (Interior Plateau) and 72 (Interior River Valley and Hills) (Woods et al., 2002). The five most upstream sites (1–5) are positioned in the Eastern Highland Rim Level IV Ecoregion, characterized by Mississippian limestone, shale, sandstone, undulating plains, hills, and poorly - developed karst topography. The most upstream sites are underlain by Fort Payne formation limestone that transitions longitudinally to Salem-Warsaw-Harrodsburg formation limestone. Soils surrounding the upstream sites in Green and Taylor Counties include Frederick, Garmon, Monongahela, Mountview, and Shelocta silt loams. Surface layers (A horizons) of each soil exhibit P concentrations of 3.7, 2.1, 4.6, 60, and 1.7 ppm, respectively. Phosphorus concentrations of the base soil horizons range from 0.4 to 1.0 ppm (USDA 1982). Downstream reaches in Hart County are underlain by Ste. Genevieve and St. Louis limestone formations that surround the Salem-Warsaw-Harrodsburg formations along the river. Upon entering Hart County, the river is bordered by Nolichucky-Canmer and Frederick-Crider soils that transition to Nolichucky-Canmer, Baxter-Crider, and Caneyville-Fredonia-Hagerstown soils. The two downstream sites are located in the Western Pennyroyal Karst Plain (site 6) and Crawford-Mammoth Cave Upland (site 7) Level IV Ecoregions, respectively. These ecoregions are underlain by Mississippian-age limestone and Chesterian age bedrock formations, as well as fractured bedrock with low surface stream density and N-rich groundwater (Kentucky Geological Survey 2010, Woods et al. 2002). These sites are bordered by Caneyville-Fredonia-Hagerstown soils that transition longitudinally to Jefferson-Lily-Wellston soils. Baxter, Frederick, and Canmer soils possess 70, 260, and 10 p/m P in the A horizon and 3, 5, and 5 p/m P in the most basal horizon, respectively (USDA 1993). Each site was characterized by open canopy and shallow run habitats underlain by cobbles and small boulders. All sampling occurred in 10–50 cm deep water within a 100-m reach.

Field and laboratory methods

Longitudinal trends – environmental parameters

Quantification of in-stream environmental parameters occurred during baseflow conditions in July–August 2008 and July, August and October 2009 (Fig. 3). Sampling did not occur in September 2009 due to repeated precipitation events leading to high river flow conditions. Current velocity was measured using a Marsh-McBirney Flo-Mate velocity meter at each site during each sampling event to calculate discharge. Dissolved oxygen (DO), pH and temperature were measured with a Hach HQ40d digital meter. Water samples for the quantification of ambient nutrient levels were collected midstream (*sensu* Dodds 2003). Either three (2008) or four (2009) replicate samples were analyzed from each site. Total phosphorus (TP; acid persulfate digestion), soluble reactive phosphorus (SRP; ascorbic acid method), nitrate (cadmium reduction method), ammonia (salicylate method), and total nitrogen (TN; persulfate digestion method) levels were quantified spectrophotometrically. Nutrient levels were compared against a set of standards.

Longitudinal trends – algal biomass

Algal biomass levels were quantified similarly during summer baseflow conditions in July–August 2008 and June–August 2009 (Fig.3). Specifically, periphyton and sestonic algal biomass levels were quantified in 2008, and periphyton, sestonic, and filamentous algal biomass in 2009. River flow levels were too high in October 2009 to permit effective quantification of all algal biomass measures.

Ten replicate samples were taken for each algal type across both years. Grab samples were collected from each site at midstream for the quantification of sestonic algal biomass levels. Periphyton and filamentous algae samples were taken from 0.5 m-wide, cross-stream quadrats. Ten quadrats were selected from a transect spanning the sample area using a random numbers table. Periphyton samples were taken from naturally-occurring cobble-sized rocks found in the downstream left portion of the quadrat. A new quadrat was selected at random if a quadrat did not contain a cobble-sized rock. A penny was used to establish a 2.2 cm diameter (area = 3.8 cm^2) circle at the center of each rock. Periphyton within that circle was scraped from the rock using a scalpel (Lamberti & Resh 1983) and rinsed with stream water into a small pan. Residual periphyton was scrubbed from the circle using a clean toothbrush (Steinman and Lamberti 1996). Samples were rinsed from the pan into acid-washed, sterile 275 mL bottles and filled with stream water (Dodds 2003, Moschish et al. 2001). A new transect was established 1–2 m upstream and ten new quadrats were sampled for filamentous algae. If present, algae were clipped from a 75-cm^2 area located in the upstream right corner of the quadrat, placed in a plastic bag, and frozen upon returning to the lab. Algae sample bottles and bags were wrapped in aluminum foil to prevent exposure to light. All samples were stored on ice in a cooler before being returned to the lab for refrigeration and filtration. Algae samples were refrigerated up to 24 hours prior to filtration.

Nutrient limitation of weak and well-developed karst systems

Nutrient-diffusing substrates (NDS) were deployed during August-September 2009 at two sites to evaluate algal nutrient needs according to ecoregion. The NDS were constructed according to Carey et al. (2007). Forty LDPE vials were filled with 10 replicates each of four nutrient solutions (control, N = 87.5 mg/L NO_3^- using 632 mg/L KNO_3, P = 12 mg/L PO_4^- using 103.8 mg/L Na_2HPO_4, and N+P) and attached to a floating platform in a randomized pattern. Nutrients diffused out through a glass fiber filter (Whatman, 24 mm diameter, 0.7 μm pore size) and a membrane filter (Millipore, 25 mm, 0.45 μm pore size). Glass fiber filters served as growth surfaces for algae. The NDS were anchored to the streambed and retrieved after 15 days. Glass fiber filters were frozen and analyzed for chlorophyll-α concentration to determine algal biomass per unit area (mg/m^2).

Laboratory Methods

Periphyton and sestonic algae samples were vacuum-filtered onto 47 mm diameter, 0.7 μm pore size Whatman GF glass fiber filters (Taylor 2004). Filtered samples were transferred to individual petri dishes, wrapped in foil and frozen in the dark for up to 14 d. Algae and NDS samples were analyzed for chlorophyll-α concentration using USGS methods (Yin 2005). A filtered sample was placed in a 50 mL centrifuge tube with 10 mL of a 50:50 solution of dimethyl sulfoxide (DMSO) and acetone plus 3–6 glass beads. The tube was vortexed for 30 s, stored overnight in the dark at 4°C for chlorophyll-α extraction, centrifuged 10 min at 3000 x G (4300 rpm), and the supernatant was transferred to a new tube. A second 10 mL of DMSO:acetone was added to the original sample and the overnight extraction, centrifugation, and removal of supernatant sequence was repeated. The two supernatant liquids were combined. Five mL of the DMSO:acetone solution was added to the original sample and the process was repeated. A total of 25 mL of DMSO:acetone was added to each sample over a period of three days. The resulting supernatant was centrifuged and 5 mL of the liquid were transferred to a glass tube and analyzed with a Shimadzu RF-5301 PC spectrofluorometer. Samples were measured against set standards. Samples with high intensity readings were diluted and re-analyzed.

Chlorophyll standards were produced from an initial standard of 239 ppb, and a series of five 20% serial dilutions of 47.60 ppb, 9.52 ppb, 1.90 ppb, and 0.38 ppb. Additionally, a minimum detectable limit (MDL) was calculated for standards monthly. The lowest standard was measured seven times, and the mean value was multiplied by 3.14 to produce the MDL. A linear regression performed between intensities and concentration values of the chlorophyll standards established a standard curve used to calculate chlorophyll-α concentrations (mg/L) of each algae sample. Chlorophyll-α concentrations were used to calculate sestonic (mg/L), periphyton and filamentous algal biomass (mg/m^2) using Standard Methods 10-200 H (APHA 1998).

Analytical Methods

Nutrient and algal biomass levels were plotted on a monthly basis to assess for increasing, decreasing, or stable longitudinal trends. Decreasing or stable nutrient levels implied limitation. Nutrient and algal biomass levels were also compared across months to assess for nutrient limitation throughout the growing season. Biomass levels were compared to nutrient levels using linear regression analysis to determine if relationships between biomass and nutrients existed along longitudinal and temporal gradients. TN:TP ratios for each site were calculated and compared to the Redfield ratio. A two-way factorial ANOVA compared NDS periphyton biomass levels between the control and the three nutrient addition (N, P, and N + P) treatments to assess limitation. The critical α level was reduced as 0.05/28 (= 0.00179) due to the total number of pair-wise comparisons. Linear regressions and the ANOVA were performed using Statistica 7.0 (StatSoft®, Tulsa, OK, U.S.A.).

Results

Temporal trends – environmental parameters and algal biomass

Mean stream temperature and discharge decreased from July to August during 2008 (Table 1). DO levels increased with decreasing temperature and stream depth. While mean TP, TN and NO_3 values decreased, mean SRP, NH_3, and TN:TP ratio values increased. Sestonic and periphytic algal biomass similarly increased from July to August during 2008 (Table 1).

Temperature increased from July to August of 2009, then decreased in October (Table 2). Mean DO levels increased from July to October, while pH decreased slightly. Discharge decreased from July to August. Mean TP concentrations fell from July to August and exhibited little change in October. SRP levels were relatively stable in July and August, but increased in similar fashion to TP in September and returned to the previous level in October. TN decreased from July through August and then increased in October. NO_3 showed a similar pattern, as did TN:TP ratios. Conversely, mean NH_3 increased through August, then decreased significantly in October. Mean sestonic algal biomass remained constant from July through August, then increased in October. Periphytic biomass increased from July to August. Filamentous algal levels increased markedly from July through August (Table 2).

Longitudinal trends – environmental parameters

During July 2008, TP levels varied little longitudinally from non-karst to the karst region (Fig. 4a). July TP levels were high at the upstream-most site, then dropped and increased longitudinally until stabilizing in the downstream karst region. A similar pattern was displayed in August, except for a near three-fold increase at the most downstream site and lower overall TP concentrations. During 2009 TP levels exhibited minimal changes longitudinally during each month. These TP levels increased upon entering the downstream karst region during July, but were stable during other months. Overall TP concentrations were higher in July than in August (Fig. 4b). SRP levels followed a pattern similar to TP values in 2008, differing only in a marked decrease upon entering the downstream karst region during August (Fig. 5a). SRP levels increased steadily along the longitudinal gradient in 2009 (Fig. 5b).

No clear longitudinal patterns were displayed with TN, though July and August TN displayed similar trends during both years. Initial TN levels dropped with distance from the most upstream site. Total nitrogen levels generally increased along the longitudinal gradient during summer 2008, although the most downstream site was mainly lower during August (Fig. 6a). Total nitrogen levels in July 2009 increased longitudinally then decreased upon entering the karst, while August TN increased until entering the karst region. In the downstream karst region TN diminished longitudinally. Overall TN levels were lower during August. October TN levels followed a similar pattern as August, but with elevated TN values (Fig. 6b).

Nitrate displayed a consistent pattern of increasing longitudinally during both years, with the downstream karst sites having the highest levels (Figs. 7a–b). Nitrate levels exhibited minor differences between months. Of all nutrients measured, only nitrate followed a longitudinal pattern of continuous increase.

Ammonia levels showed no clear longitudinal patterns during 2008 (Fig. 8a). In contrast, during 2009 ammonia levels decreased with increasing distance from the Green

River dam and generally exhibited the lowest concentrations in the downstream karst region (Fig. 8b). Ammonia concentrations were highest directly downstream of the Green River Lake (GRL), but displayed little longitudinal change and minimal monthly variability. Downstream ammonia levels were relatively stable, with little variation or pattern.

A similar seasonal pattern was exhibited in 2009. July TN:TP values increased longitudinally in 2008. August TN:TP decreased longitudinally into the downstream karst region, then increased along the longitudinal gradient. Upstream TN:TP values were higher in August than July, but were lower in the downstream karst region. With the exception of the spike during August, TN:TP ratios changed little longitudinally during 2008 (Fig. 9a). A similar longitudinal pattern of TN:TP was present in 2009. July TN:TP levels were lower in the upstream reaches than the downstream karst reaches. August levels were greater in the upstream reaches and dropped longitudinally. October TN:TP levels followed the same pattern as July, but with slightly lower overall values. There was a broader range in TN:TP ratio values in 2008 compared to 2009. TN:TP ratios during 2008 ranged between 12 and 333 (Fig. 9a), while in 2009 TN:TP ratios ranged between 7 and 47 (Fig. 9b).

Longitudinal trends – algal biomass

Sestonic algal biomass levels remained relatively constant longitudinally during July 2008 (Fig. 10a). In August 2008, sestonic biomass levels were higher compared to July and increased to the upstream karst region and then decreased longitudinally. During 2009 there was a consistent pattern of the highest biomass levels at the upstream end that declined longitudinally (Fig. 10b). The lowest levels were typically at the most downstream karst site. Periphyton biomass levels were highly variable during 2008 and 2009, both longitudinally and between successive months (Figs. 11a-b). Filamentous algal biomass levels generally increased longitudinally during 2009 (Fig. 12). Filamentous levels increased from July to August with the biomass peaking at the most downstream karst site.

Nutrient limitation between weak and well-developed karst systems

Mean chlorophyll-α values from NDS treatments were generally greater at the upstream weakly-developed karst site (Table 3). The highest accrual of periphyton biomass occurred on the upstream N + P addition treatment. There were two significant, pair-wise differences. Periphyton biomass on the upstream N + P (0.46 mg/L chlorophyll-α) treatment was significantly higher than both the downstream control (0.29 mg/L; Bonferonni adjusted p-value = 0.02) and downstream N (0.30 mg/L; Bonferonni adjusted p-value = 0.03) addition treatments (Table 3). Growth on upstream N+P also exceeded growth on other upstream treatment vials, but not to a significant degree. The same was true for the downstream N+P treatment compared to downstream control, N, and P treatments. While the upstream P vials amassed the least amount of periphyton (0.32 mg/L), the downstream P vials yielded chlorophyll levels of 0.39 mg/L that were almost as high as that of the N+P treatment (0.40 mg/L).

Discussion

Longitudinal Trends

Clear longitudinal patterns of some nutrient and algal biomass levels were exhibited during low flow conditions in both 2008 and 2009. Ammonia displayed its highest concentrations directly downstream of the Green River Lake (GRL), but displayed little change longitudinally and minimal monthly variability. TP and SRP exhibited minimal longitudinal change and small differences between months. Nitrate levels displayed distinct increases longitudinally during both years, yet with minor differences between months. No clear longitudinal patterns were displayed with TN.

Filamentous algae biomass levels increased longitudinally and across months, but were not observed at the upstream sites during July and August. In July 2008, sestonic algal biomass varied minimally along the longitudinal gradient. Overall biomass in August was greater than July and increased up to the karst region, where it declined quickly. Sestonic algal biomass in 2009 was greatest at the upstream-most site and exhibited sharp declines to the next site each month. Sestonic algal biomass exhibited minimal longitudinal change during July and August, while October levels declined. Periphyton biomass displayed the same seasonal trends during 2008 and 2009. Biomass levels were low in July and much higher during August. Biomass increased slightly longitudinally, but overall changes were seen between months rather than sites.

Both N and P are considered primary production - limiting nutrients in lotic waters, and algal biomass diminishes when water column nutrients are below certain threshold levels. Algal biomass accrual is related to breakpoints of 30 μg/L TP and 40 μg/L TN (Dodds et al. 2002). Green River TP values ranged from 100-200 μg/L and TN levels from 100-400 µg/L, well above the proposed thresholds for algal growth. In this study sestonic algal biomass had an inverse relationship with TP and SRP concentrations in 2008, but this relationship was less obvious in 2009. Sestonic biomass increased monthly in conjunction with decreasing TP and SRP levels. This relationship differs from that found in a freshwater lake in Sweden where high increases in TP were associated with increases in N-fixing cyanobacteria in the water column (Vrede et al 2008). Similar to sestonic algal biomass, periphyton biomass was greater when TP and SRP levels were low. Total nitrogen followed the same seasonal pattern as phosphorus, while ammonia levels mirrored the periphyton growth. Sestonic algae experienced minimal monthly changes relative to the dramatic increases in and filamentous algae. As periphyton and filamentous biomass levels increased across months, TN levels decreased. Filamentous algal decreases coincided with the increase in nitrogen levels. Therefore, these forms of algal biomass may be factors in the nitrogen shifts. In general, algal biomass was lowest during July, and as it increased from month to month phosphorus decreased. Nitrogen showed dramatic fluxes in concentrations between months, but TP and SRP changed relatively little over time. The minimal changes in phosphorus levels across months, in comparison to the large shifts in temporal and longitudinal TN values, suggest the system is primarily phosphorus-limited and seasonally limited by nitrogen.

Longitudinal gradients of stream nutrients result from in-stream uptake and groundwater inputs (Mulholland & Rosemond 1992) as well as point source inputs. The River Continuum Concept asserts that upstream communities influence downstream communities via effects on the quality and quantity of material in transport (Vannote et al. 1980). Continuous nutrient additions from karst flow tributaries, groundwater and in-stream sources indicate that nutrient levels could increase longitudinally. Nitrate and filamentous algal levels followed this trend, increasing longitudinally. In contrast, the other algal and nutrient levels exhibited comparatively more subtle patterns. These patterns are similar to those found by Hecky and Kilham (1988) that phosphate occurs in relative proportions similar to, or less than, the proportions required by phytoplankton. Other nutrients (e.g., nitrate) accumulate because they are available in excess. Algae take up and sequester surplus P, which maintains homeorhetic P levels along the longitudinal gradient. Uptake and sequestration of N affects concentrations of TN in the Green River as well. Nutrient concentrations may also be affected by turnover rates within the system and high turnover rates result in lower nutrient supply (Dodds 2003).

Vegetative growth of *Cladophora* results from basal cells on bedrock remaining after scour events. *Cladophora* form large, conspicuous floating mats in wide channels with open canopy. The algae are colonized by epiphytes, some of which are N-fixing taxa. Filamentous algae and epiphytes take up dissolved N from the water column, but N-fixing epiphytes on *Cladophora* may be a source of N (Power et al. 2009). Epiphyte assemblages change over time, and these shifts are indicated by color changes in the host mat. A whole-mat shift to a rusty color coincided with epiphytic succession by diatoms with N-fixing endosymbionts, and the presence of N-fixing epiphytes led to a spike in downstream dissolved N levels (Power et al. 2009). A similar trend was noted in a freshwater lake study, in which decreases in N availability caused a shift in the phytoplankton community. The community shifted from being dominated by diatoms to one dominated by N-fixing cyanobacteria, which increased available N (Vrede et al. 2008). In the case of the Green River, the downstream accumulation of nitrate and large populations of filamentous algae cannot be considered unrelated. With the large potential habitat for N-fixing cyanobacteria provided by filamentous algae, one could propose that large populations of cyanobacteria fix high amounts of N_2 into NH_3. The NH_3 is then taken up by nitrifying bacteria in the water column, where nitrification yields nitrate. While nitrate levels increased longitudinally, levels of NH_3 in the Green River had minimal longitudinal changes. This may not be an uncommon occurrence. Grimm et al. (2003) cited findings that small streams draining forests may contain low levels of NH_3 but still exhibit high rates of nitrification. Ammonia is also preferred over nitrate by phytoplankton as an N source (Dortch 1990, cited by Maberly et al. 2002). Therefore, nitrate levels may be attributed to contributions of N-fixing epiphytes inhabiting *Cladophora* mats and nitrifying bacteria downstream. Additionally, ammonia levels may be influenced by the nutrient preferences of phytoplankton and algae.

Nitrate may also be linked to groundwater and karst-flow tributaries feeding into the Green River. The permeable karst geology provides multiple pathways for groundwater to mix with surface waters (Katz et al.1997). Sources of nitrate in groundwater include commercial and residential fertilizers, and nitrate enrichment of groundwater has been documented in Florida streams (Notestein 2003). Land adjacent to the Upper Green River is primarily in agriculture, so row crop and livestock operations, in conjunction with springs, may be a source of nitrate.

Green River nutrient levels across sites and months were low relative to springhead and tributary inputs (Upper Green River Watershed Watch Program, 2009). Nutrient levels in tributaries and springs followed a similar longitudinal pattern as the Green River. Nitrate and phosphate levels were lower in upstream regions of the Green

River and its tributaries. Upon reaching the karst region, however, levels of nitrate and phosphate increased dramatically in the tributaries. One surface tributary (i.e., Brush Creek) displayed comparatively high nitrate (11.11–11.14 mg/L) and phosphate (0.55–0.76 mg/L) levels. Two karst springheads of varying sizes, however, exhibited contrasting nutrient levels. Gardner Spring (very small) exhibited nitrate and phosphate concentrations of 0.82 mg/L and 0.22 mg/L, respectively. Downstream of Gardner Spring is McCoy Bluehole (large), with high nitrate (4.57 mg/L) yet low phosphate (0.09 mg/L) levels. Although Green River nitrate levels from this study increased longitudinally, in-stream concentrations never exceeded 1.4 mg/L. Hence, surface-flowing tributaries and large springheads contribute markedly higher nutrient loads than riverine levels.

Soils and bedrock can be natural sources of nutrients. Runoff from surrounding soils and groundwater passing through bedrock accumulate different forms of N and P. Surface runoff from topsoil and organic matter contribute P to the river. Weathering and dissolution of bedrock by groundwater flow is also a source of P. The bedrock in the study region is mainly Upper Mississippian-age limestone, and soils surrounding the upstream sites in Green and Taylor Counties include Frederick, Garmon, Monongahela, Mountview, and Shelocta silt loams. Surface layers (A horizons) of each soil exhibit P concentrations of 3.7, 2.1, 4.6, 60, and 1.7 ppm, respectively (USDA 1982). Phosphorus levels in the Hart County soils were much higher than those of Green and Taylor Counties. Baxter, Frederick, and Canmer soils possess 70, 260, and 10 p/m P in the A horizon and 3, 5, and 5 p/m P in the most basal horizon, respectively (USDA 1993). Phosphorus concentrations in the Green River increased each month upon entering the karst region. Phosphorus elevations along the longitudinal gradient in the Green are similar to differences between Hart County and Green and Taylor Counties, lending credence to the possibility of topsoil P contributions. Potential P inputs from bedrock and soil increase longitudinally, yet monthly levels of TP stabilize in the downstream reaches. Algal biomass and in-stream P uptake may explain this pattern. In July 2009, filamentous algal levels were very low and found only in the downstream reaches. August filamentous levels were considerably greater. In contrast, July TP concentrations were higher than August levels. As algal biomass increased monthly, overall TP levels decreased. These trends suggest that larger populations of filamentous algae take up and sequester greater amounts of P and thereby maintain stable longitudinal P concentrations.

The longitudinal nitrate accumulation may be due solely to weathering of bedrock and groundwater contributions. Hill (1981) noted that saltpeter (KNO_3) deposits in Mammoth Cave originated from nitrates leached from limestone bedrock. Nitrate is leached from exposed limestone and sinkholes, as well as organic soils, during rainfall and transported as runoff to streams. Holloway (1998) found that a California watershed with high bedrock concentrations of N contributed up to 90% of the nitrates in downstream reservoirs. Therefore, in regions with high geological N, great amounts of nitrate may be available to lotic systems.

Waters from deep-release reservoirs often contain high nutrient concentrations relative to waters influent to the dam (Wright 1967, Knight et al. 1976, Marcus 1980). The nutrient content of these waters enriches the immediate downstream environment and can promote eutrophication. Chlorophyll production by periphyton communities directly downstream of a Utah reservoir exceeded that of communities above the dam (McConnell and Sigler 1959). Marcus (1980) found that periphyton directly below the

Hyalite Reservoir in southern Montana responded mainly to variations in nitrogen availability, and benthic algae quickly depleted nitrogen levels. Nitrogen levels were therefore lower in the downstream reaches, limiting periphyton growth. This effect is not uncommon. Periphyton communities directly below a dam typically take up nutrients from effluent waters and thereby limit growth in the downstream region (Cooper and Wilhm 1975). The Green River exhibits this same pattern with TN. Concentrations are high directly below the dam, but are depleted rapidly by algal communities over the course of ca 30 river kilometers. Eutrophication does not appear to be of concern directly downstream of the Green River dam. Sestonic algal levels are highest immediately below the lake, suggesting lentic taxa are released even during summer baseflow conditions, and decline along the longitudinal course of the river. Periphyton and filamentous algal growth experience seasonal changes, but neither exhibit comparatively high levels immediately below or near the Green River Lake. In particular, filamentous algae are absent from the upstream reaches and become a prominent primary producer only in the downstream karst reaches.

Nutrient limitation of weak and well-developed karst systems

Data from the longitudinal study indicated P-limitation in the upstream reaches and co-limitation downstream. At the time of NDS deployment, upstream P levels had experienced little change and upstream N levels had increased from July to August. Nitrogen and phosphorus levels in the karst region had decreased from the previous month. Periphyton biomass accrual in the upstream reaches does not appear to be limited by N or P because the most chlorophyll came from the N+P treatment. Downstream, however, algae responded to P addition. This can be attributed to the longitudinal differences in P from July to August. The minimal P level change monthly in the upstream reaches did not influence periphyton biomass accrual, but the decrease in P levels in the karst region resulted in greater algal biomass accrual of P and N+P vials compared to N and control vials.

Addition of P has stimulated periphyton biomass accrual in other studies. Notestein et al. (2003) found that additions of P increased periphyton abundance in a spring-fed karst river system. They also concluded that increases in NO_3^- had no significant effect on periphyton growth, but N + P additions together stimulated growth. A nutrient diffusing substrate study in a southeastern coastal blackwater stream found periphyton growth was primarily limited by light and secondarily limited by P and N+P treatments (Carey et al. 2007). These findings are congruent with Lohman's (1991) assertion that east-draining streams in the U.S. are P-limited. Peterson et al. (1983), however, noted the same trend in an arctic tundra stream in Alaska. Maberly et al. (2002) found both N and P limitation in small upland lakes in Scotland. Many lakes were limited by a single nutrient, but with seasonal progression shifted to co-limitation. In accordance with the findings of Vrede et al. (2008), the transition to co-limitation was attributed to seasonal alternation of periphyton species composition (Maberly et al. 2002).

Nutrient criteria

While the U.S. EPA does not have national criteria regarding TP and SRP in agricultural watersheds, nuisance algal growth often occurs at a level of 0.1 mg/L P. Recent data suggest a range of only 0.010–0.075 mg/L P for nuisance algal growth

(USEPA 2008). A study by the USGS showed that 70% of 97 streams in agricultural or urban areas exhibited TP levels >0.1 mg/L (USGS 1999), and the U.S. EPA reported that nearly 47% of streams had TP levels between 0.1 and 0.3 mg/L (USEPA 2008). The results reported in this study during 2008 (0.01–0.09 mg/L) and 2009 (0.08–0.21 mg/L) suggested the Green River possesses moderately low TP levels. The U.S. EPA also reported that 41% of streams exhibited SRP levels from 0.1–0.3 mg/L, which is consistently higher than the levels reported from the Green River during 2008 (0.01–0.09 mg/L) and 2009 (0.02–0.10 mg/L).

Ambient stream N levels vary across the U.S., differing by ecoregion and type of land use. Nitrogen levels in aquatic systems are usually low, but in watersheds where land is mainly used for agriculture, nitrogen from fertilizer and animal waste increases ambient N levels. Of all streams monitored in a U.S. EPA study, 5.3% displayed TN levels < 1 mg/L, while 16.5% fell within the range of 1–<2 mg/L and 46.6% ranged from 2–<6 mg/L (USEPA 2008). The USGS report showed that 83% of agricultural and urban streams exhibited >1 mg/L TN and the highest TN concentrations were found in southeastern streams (USGS 1999). During 2008, TN levels in the Green River ranged from 0.5–3.3 mg/L, and from 0.6–4.3 mg/L in 2009. The U.S. EPA reported that recommended water quality levels of TN range from 0.12 – 2.20 mg/L. Green River TN levels reported in this study exceed the minimum appropriate TN level, but the trend of high TN concentrations is consistent with streams in the southeastern U.S.

Nitrate levels in the Green River ranged from 0.10 to 0.93 mg/L in 2008, and from 0.20 to 1.38 during 2009. Of the streams studied by the U.S. EPA, 22.3% displayed less than 1 mg/L NO_3^- and 17.7% of streams displayed NO_3^- levels from 1 to < 2 mg/L

(USEPA 2008). These results indicate nitrate levels in the Green are similar to those of 40% of U.S. streams in agriculture-oriented watersheds.

Measurements of TN and TP are used along with the Redfield ratio to predict which nutrient limits system primary productivity. Following the criteria set by Busse et al. (2006), TN:TP ratios > 20 suggest P-limitation. In 2008, TN:TP levels indicated clear P-limitation in the Green River. Data from 2009, however, shows P-limitation in upstream sites only and throughout the sampling area individually during July and October. August data indicates that while the upstream sites are P-limited, downstream sites are co-limited (TN:TP ratios between 10 and 20). Phosphorus limitation may be continuous longitudinally, but co-limitation takes place seasonally.

The Upper Green River is nutrient-rich relative to other streams throughout the U.S. Like many streams throughout the southeastern U.S., it displays high levels of N and P that can be attributed to soil, bedrock, and groundwater inputs. The Green River also exhibits P limitation that transitions to N & P co-limitation on a seasonal basis. Nutrient and algal data from both the longitudinal and NDS studies support these conclusions.

Information and models for control of nutrient concentrations and algal growth are available for lakes and reservoirs, but such models for streams are less accurate. Efforts to establish whole-watershed nutrient criteria are currently under way through partnerships between the U.S. EPA and individual researchers. Because streams differ so broadly across ecoregions, no single standard for N or P content will suffice to maintain water quality and stream integrity. Instead, standards must be set for each watershed, and it is through research such as this that such a goal may be achieved.

Tables and Figures

Table 1. Mean ± 1 standard error (S.E.) and range of environmental parameters values during July–August 2008.

	July		August	
Parameter	Mean ± 1 S.E.	Range	Mean ± 1 S.E.	Range
Temp (°C)	26.0 ± 0.21	24.1 – 28.3	25.0 ± 0.70	22.5 – 27.2
DO (mg/L)	7.9 ± 0.18	6.6 – 10.3	8.5 ± 0.64	6.4 – 11.2
Stream width (m)	26.7 ± 2.40	7.6 – 51.0	24.4 ± 5.11	8.3 – 41.0
Mean stream depth (m)	0.2 ± 0.01	0.1 – 0.3	0.2 ± 0.03	0.1 – 0.3
Mean velocity (m/s)	0.6 ± 0.04	0.1 – 0.9	0.4 ± 0.07	0.2 – 0.7
Discharge (m^3/s)	2.4 ± 0.32	0.6 – 6.2	1.5 ± 0.31	0.6 – 2.7
Mean TP	0.04 ± < 0.01	0.02 – 0.05	0.03 ± 0.01	0.01 – 0.09
Mean SRP	0.02 ± < 0.01	0.01 – 0.04	0.03 ± 0.01	0.01 – 0.09
Mean TN	1.90 ± 0.13	0.83 – 3.17	1.24 ± 0.38	0.5 – 3.33
Mean NO3	0.45 ± 0.04	0.30 – 0.88	0.30 ± 0.12	0.10 – 0.93
Mean NH3	0.14 ± 0.01	0.09 - 0.21	0.15 ± 0.01	0.08 – 0.19
TN:TP	62.3 ± 3.2	44.4 – 100.0	100.3 ± 41.0	11.3 – 333.3
Seston Chl-a (mg/L)	0.0001 ± < 0.0001	0.0001 – 0.0001	0.0008 ± 0.0001	0.0005 – 0.0015
Periphyton Chl-a (mg/m^2)	0.0006 ± < 0.0001	0.0003 – 0.0010	1.1371 ± 0.4459	0.2431 – 3.6994

Table 2. Mean ± 1 standard error (S.E.) and range of environmental parameters values during July–October 2009. NA = data not obtained.

	July		August	
Parameter	Mean ± 1 S.E.	Range	Mean ± 1 S.E.	Range
Temp °C	26.1 ± 0.49	24.4 – 28.5	26.6 ± 0.37	25.7 – 28.4
DO (mg/L)	7.7 ± 0.15	7.0 – 8.2	NA	NA
pH	7.9 ± 0.03	7.8 – 8.0	7.7 ± 0.06	7.5 – 7.9
Stream width (m)	26.1 ± 5.16	11.5 – 44.3	20.9 ± 3.82	7.80 – 35.0
Mean Stream Depth (m)	0.2 ± 0.02	0.2 – 0.3	0.2 ± 0.03	0.11 – 0.38
Mean Velocity (m/s)	0.7 ± 0.12	0.2 – 1.1	0.7 ± 0.10	0.15 – 0.89
Discharge (m^3/s)	4.3 ± 1.31	1.3 – 10.0	2.3 ± 0.61	0.93 – 5.07
Mean TP	0.14 ± 0.02	0.09 – 0.21	0.11 ± 0.01	0.08 – 0.13
Mean SRP	0.06 ± 0.01	0.02 – 0.09	0.07 ± 0.01	0.03 – 0.10
Mean TN	3.01 ± 0.56	0.70 – 4.40	2.30 ± 0.32	1.35 – 3.43
Mean NO_3	0.50 ± 0.13	0.20 – 1.20	0.50 ± 0.12	0.20 – 1.13
Mean NH_3	0.10 ± 0.01	0.07 – 0.12	0.11 ± 0.02	0.03 – 0.19
TN:TP	21.3 ± 4.1	7.8 – 40.0	22.6 ± 3.7	12.3 – 33.8
Sestonic Chl-a (mg/L)	0.0002 ± 0.0001	0.0000 – 0.0007	0.0002 ± 0.0001	0.0001 – 0.0005
Periphyton Chl-a (mg/m^2)	0.0451 ± 0.0059	0.0261 – 0.0578	0.2191 ± 0.0442	0.0653 – 0.3920
Filamentous Chl-a (mg/m^2)	0.0382 ± 0.0257	0.0000 – 0.1662	0.6107 ± 0.3989	0.0000 – 2.4175

Table 2. Continued

	October Mean ± 1 S.E.	Range
Temp °C	13.8 ± 0.27	12.8 – 16.9
DO (mg/L)	9.7 ± 0.12	9.2 – 10.1
pH	7.8 ± 0.08	7.4 – 8.0
Stream width (m)	NA	NA
Mean Stream Depth (m)	NA	NA
Mean Velocity (m/s)	NA	NA
Discharge (m^3/s)	NA	NA
Mean TP	0.1 ± 0.01	0.08 – 0.13
Mean SRP	0.07 ± 0.01	0.04 – 0.08
Mean TN	2.84 ± 0.51	0.58 – 4.53
Mean NO_3	0.62 ± 0.15	0.30 – 1.38
Mean NH_3	0.08 ± 0.03	0.02 – 0.25
TN:TP	27.8 ± 5.5	7.2 – 46.4
Sestonic Chl-a (mg/L)	0.0003 ± 0.0001	0.0000 – 0.0010
Periphyton Chl-a (mg/m^2)	NA	NA
Filamentous Chl-a (mg/m^2)	NA	NA

Table 3. Pair-wise Bonferroni-adjusted test results of the factorial ANOVA comparing NDS chlorophyll-α periphyton biomass levels (mg/m^2) between control and nutrient addition treatments. U = upstream, D = downstream, C = control, N = nitrogen, P = phosphorous, N+P = nitrogen + phosphorous. Significant differences are indicated by bold type.

		Pair-wise probabilities						
Site: treatment	Mean ± 1 S.E. Chl-a biomass levels	U:N	U:P	U:N+P	D:C	D:N	D:P	D:N+P
U:C	0.41 ± 0.03291	1.00	1.00	1.00	0.38	0.55	1.00	1.00
U:N	0.37 ± 0.03117		1.00	1.00	1.00	1.00	1.00	1.00
U:P	0.32 ± 0.01259			0.09	1.00	1.00	1.00	1.00
U:N+P	0.46 ± 0.02622				**0.02**	**0.03**	1.00	1.00
D:C	0.29 ± 0.04512					1.00	1.00	0.63
D:N	0.30 ± 0.04019						1.00	0.90
D:P	0.39 ± 0.02739							1.00
D:N+P	0.40 ± 0.04198							

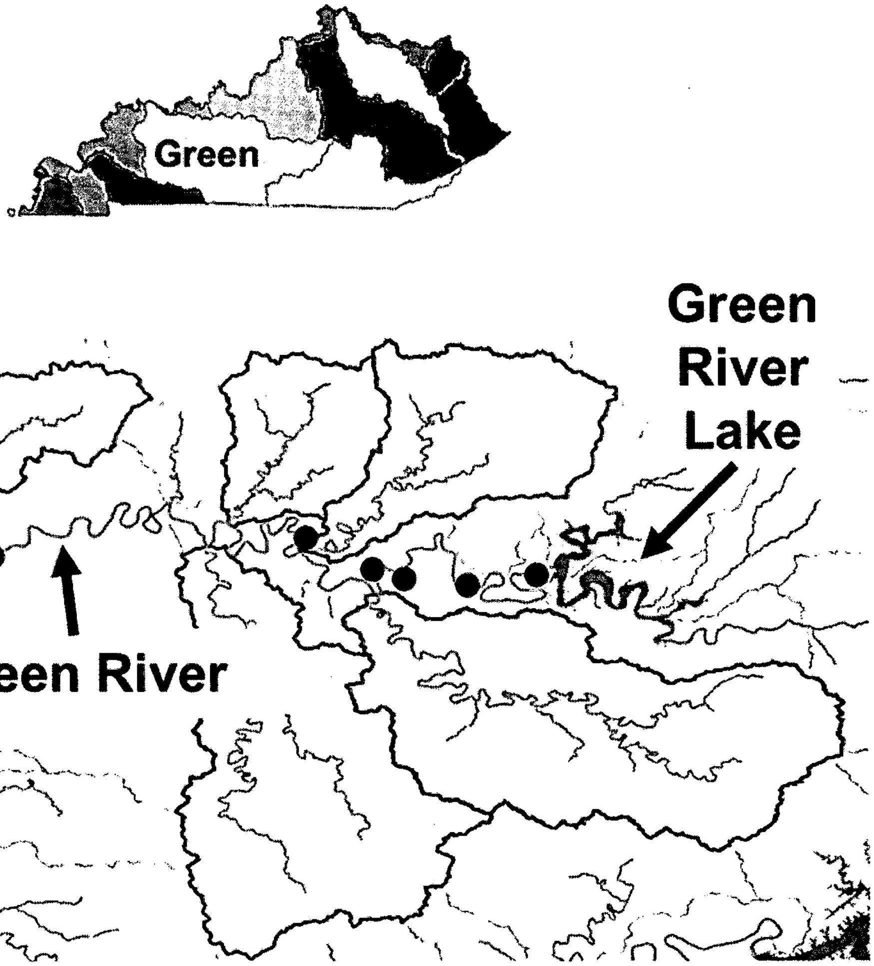

Fig. 1. Map depicting location of the Green River Basin, including the mainstem Green River and Green River Lake, in Kentucky. Sampling locations are marked with solid black circles.

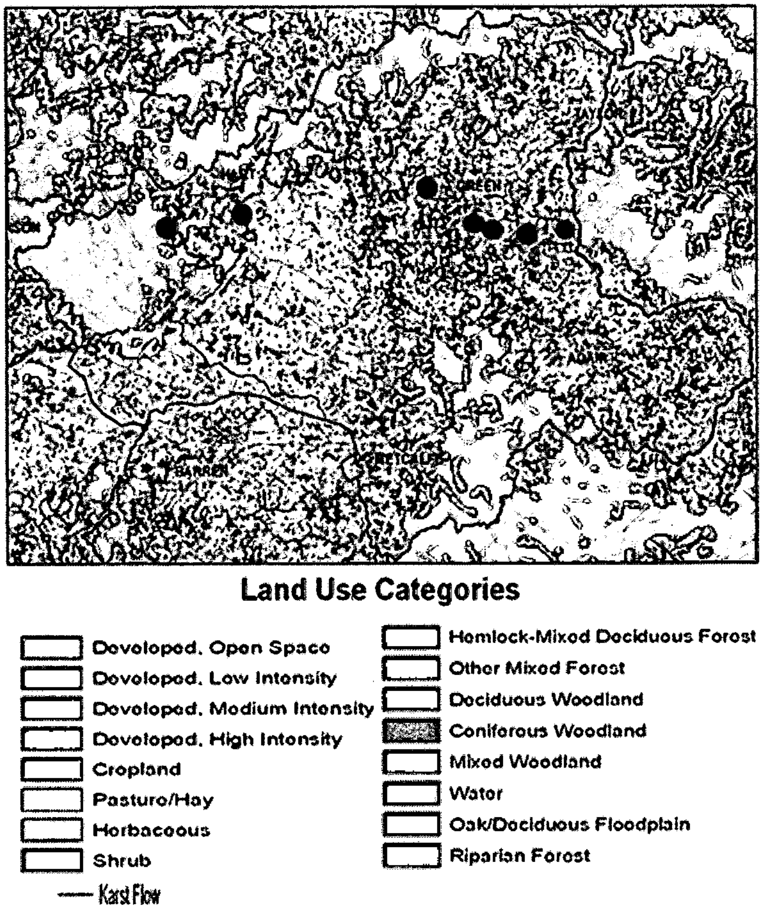

Fig. 2. Map depicting major land-use categories within the Upper Green River Basin, Kentucky. Sampling locations are marked with solid black circles.

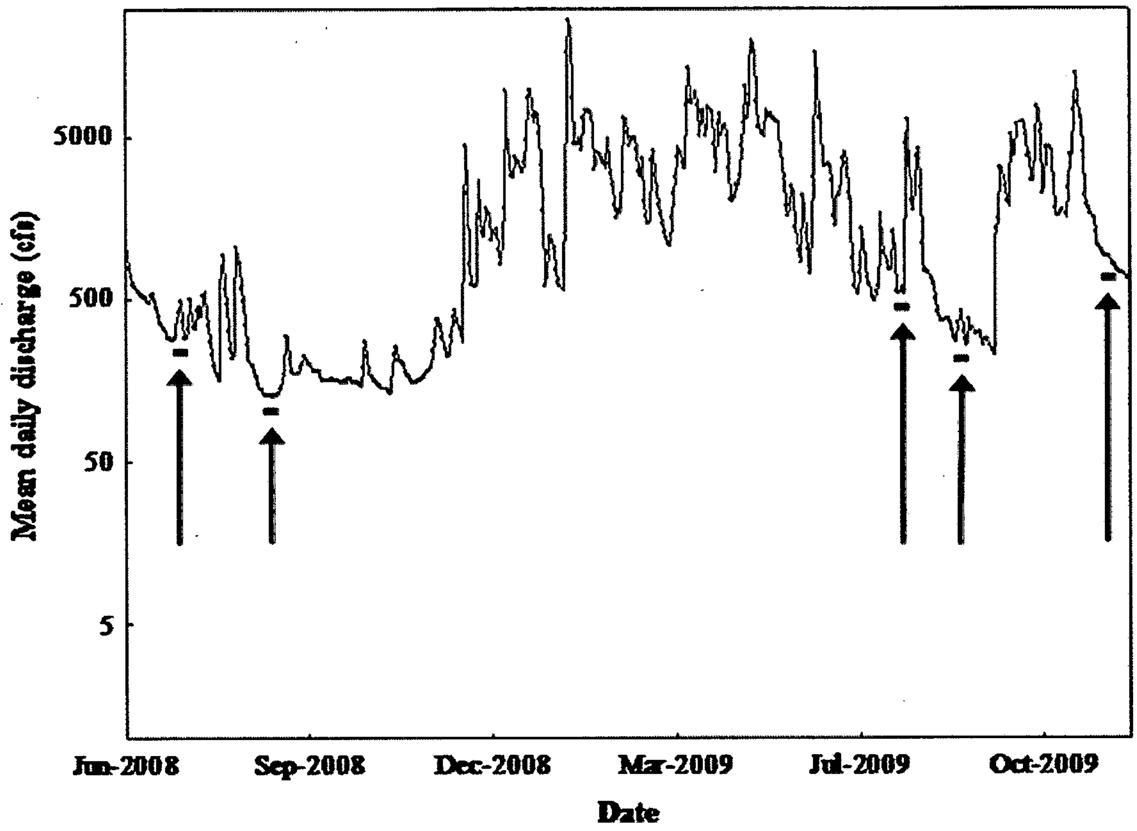

Fig. 3. Mean daily discharge for the Green River at the Munfordville, Kentucky gauging station (USGS site 00308550) during the study period. Vertical arrow and corresponding bars refer to sampling periods.

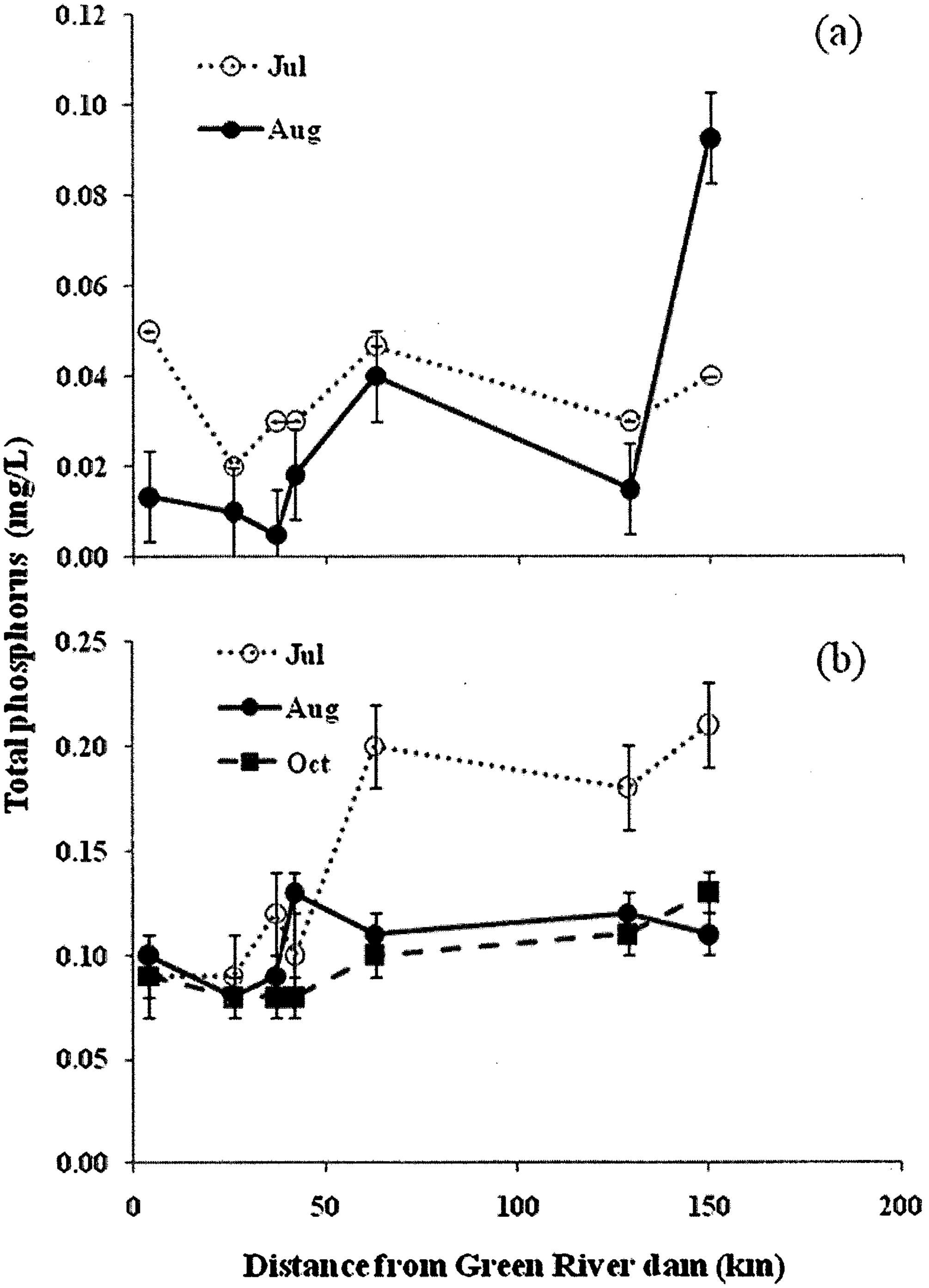

Fig. 4. Total phosphorus levels (mg/L) with increasing distance from dam (km) during (a) 2008 and (b) 2009.

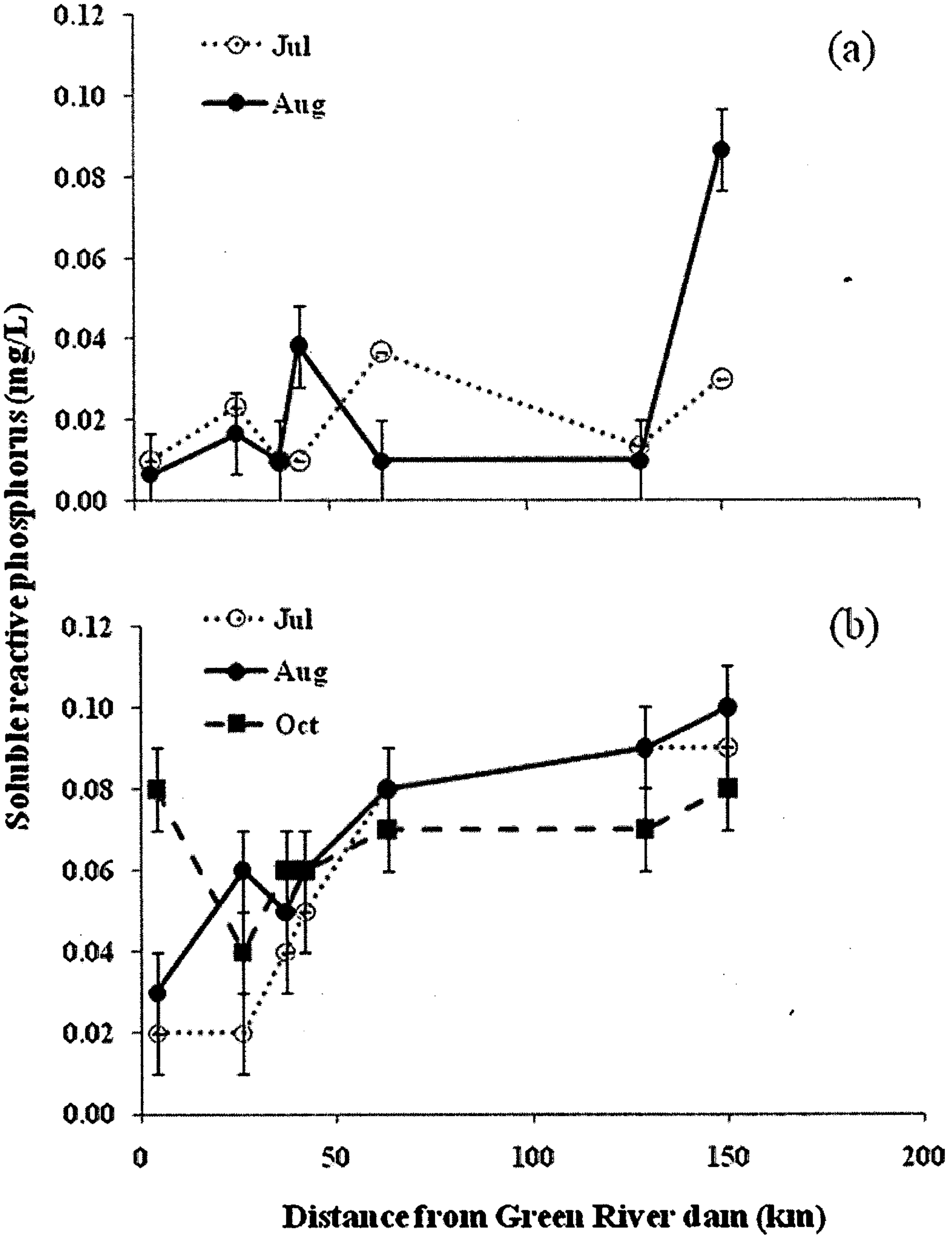

Fig. 5. Soluble reactive phosphorus levels (mg/L) with increasing distance from dam (km) during (a) 2008 and (b) 2009.

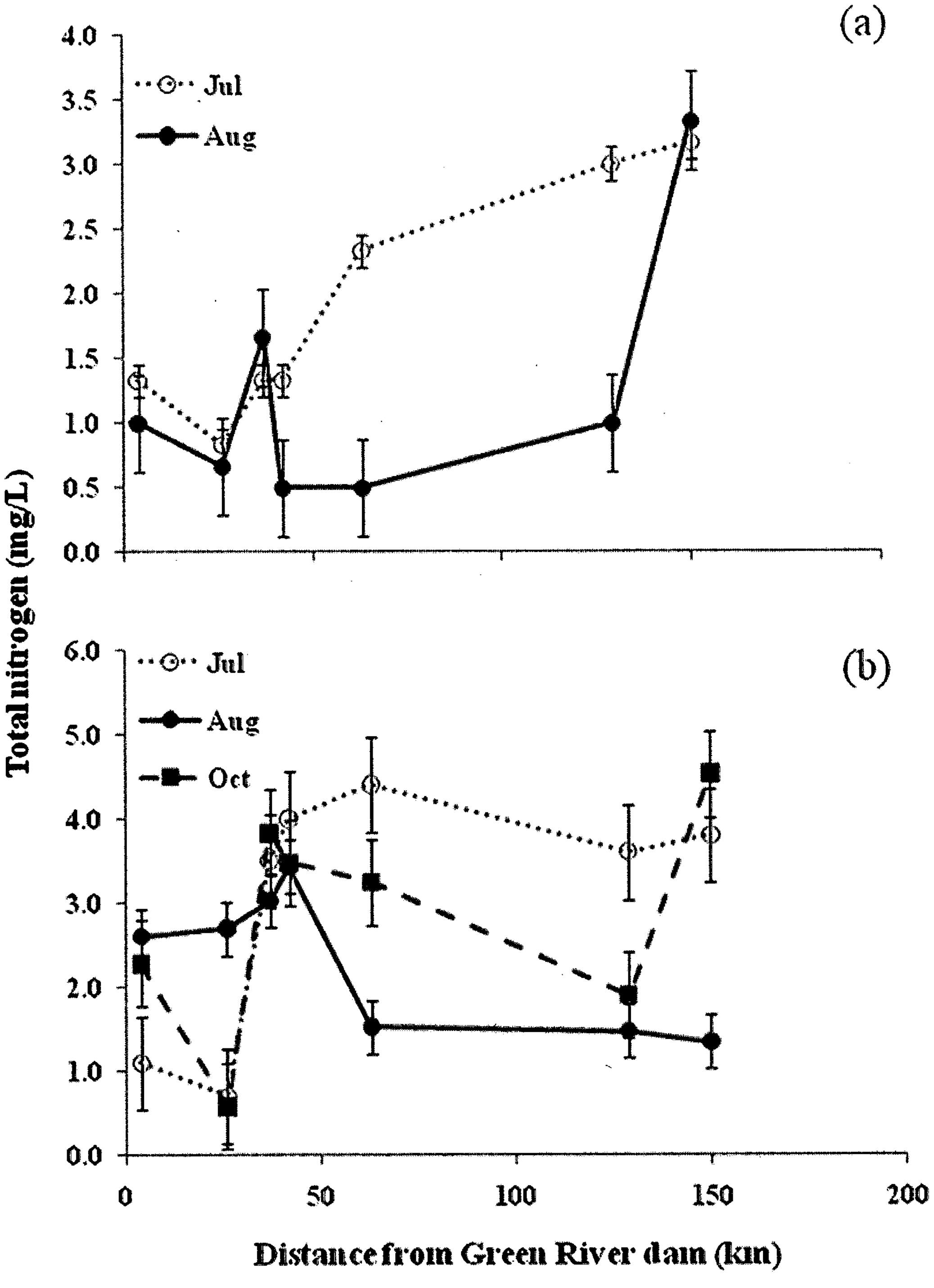

Fig. 6. Total nitrogen levels (mg/L) with increasing distance from dam (km) during (a) 2008 and (b) 2009.

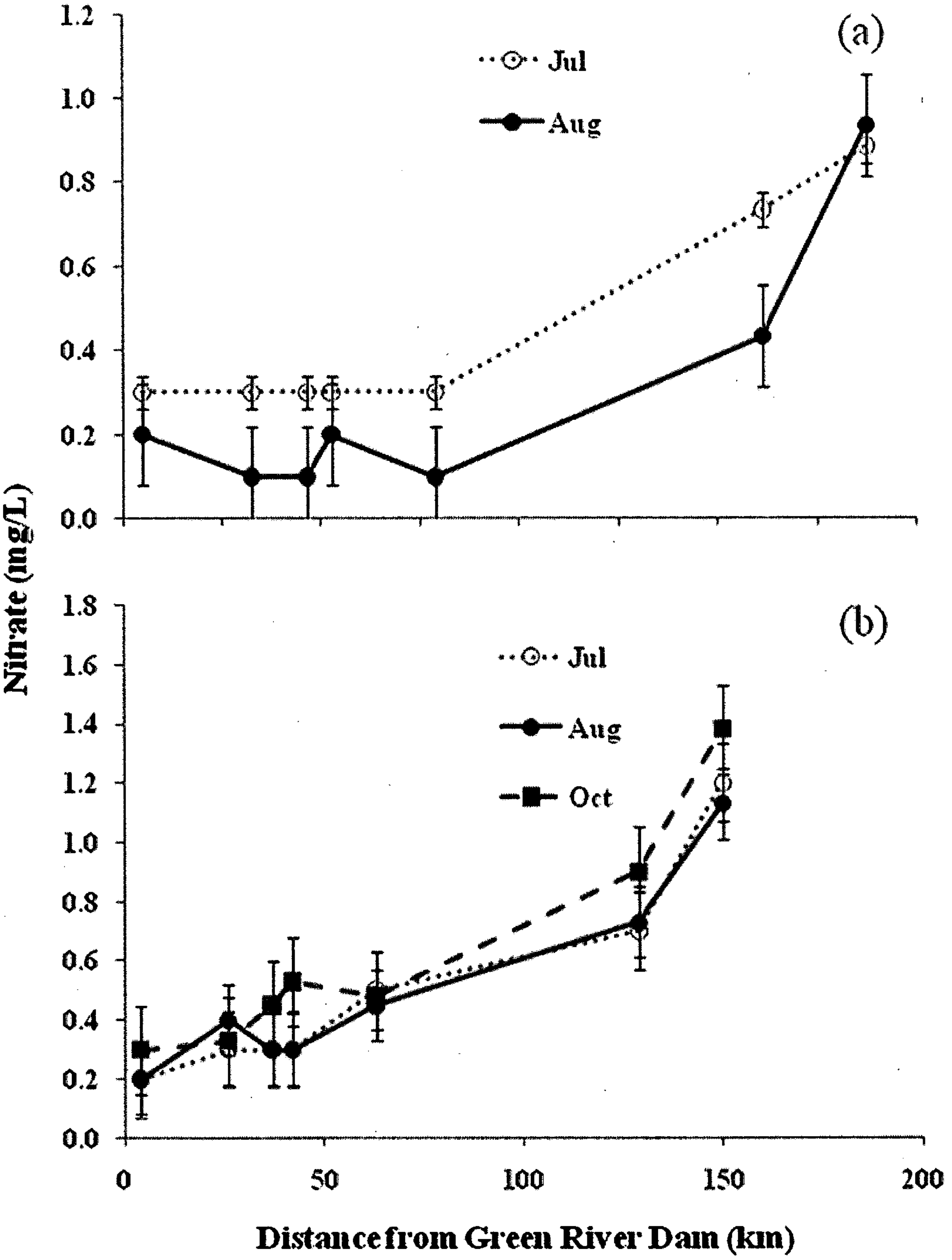

Fig. 7. Nitrate levels (mg/L) with increasing distance from dam (km) during (a) 2008 and (b) 2009.

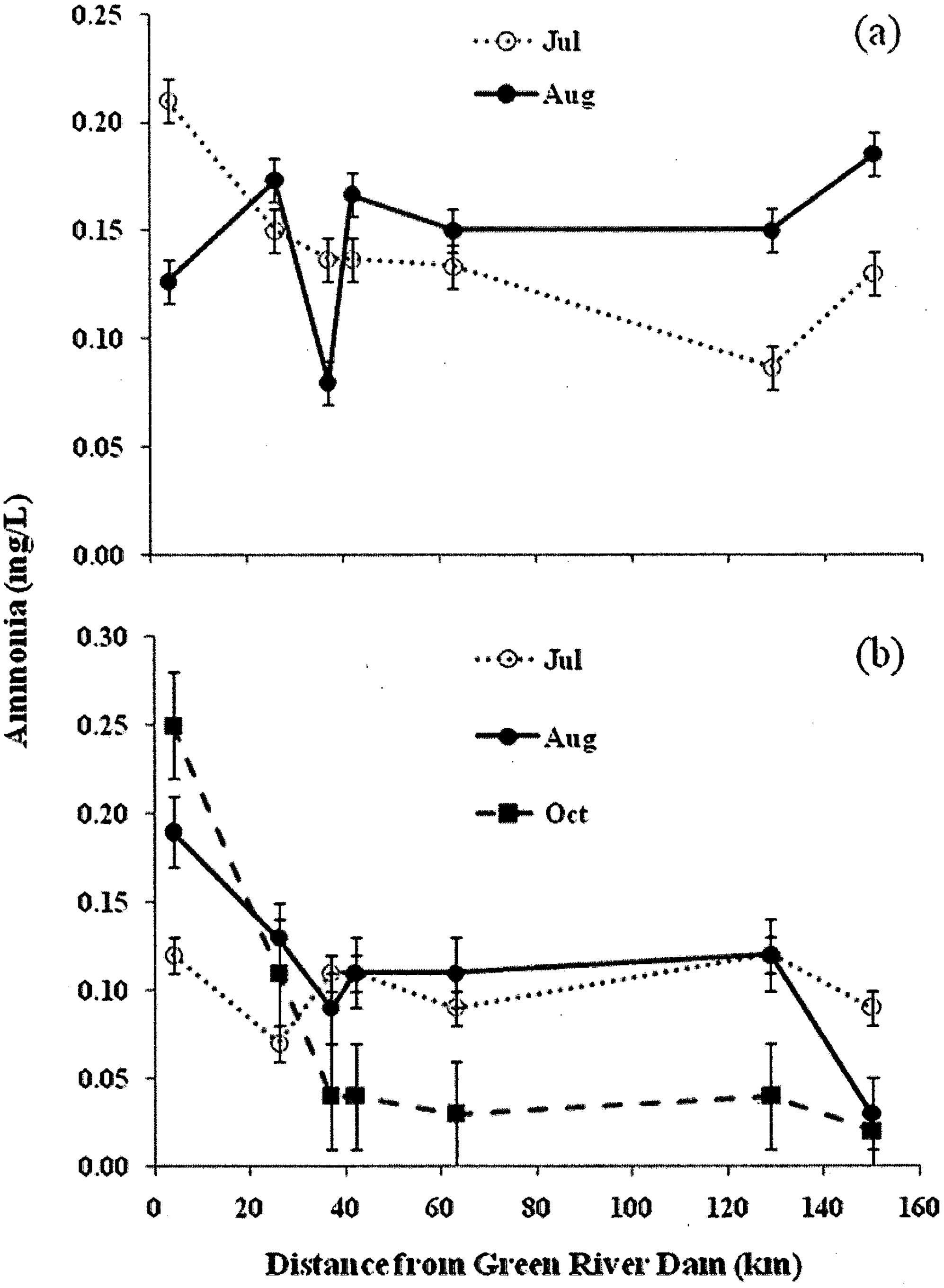

Fig. 8. Ammonia levels (mg/L) with increasing distance from dam (km) during (a) 2008 and (b) 2009.

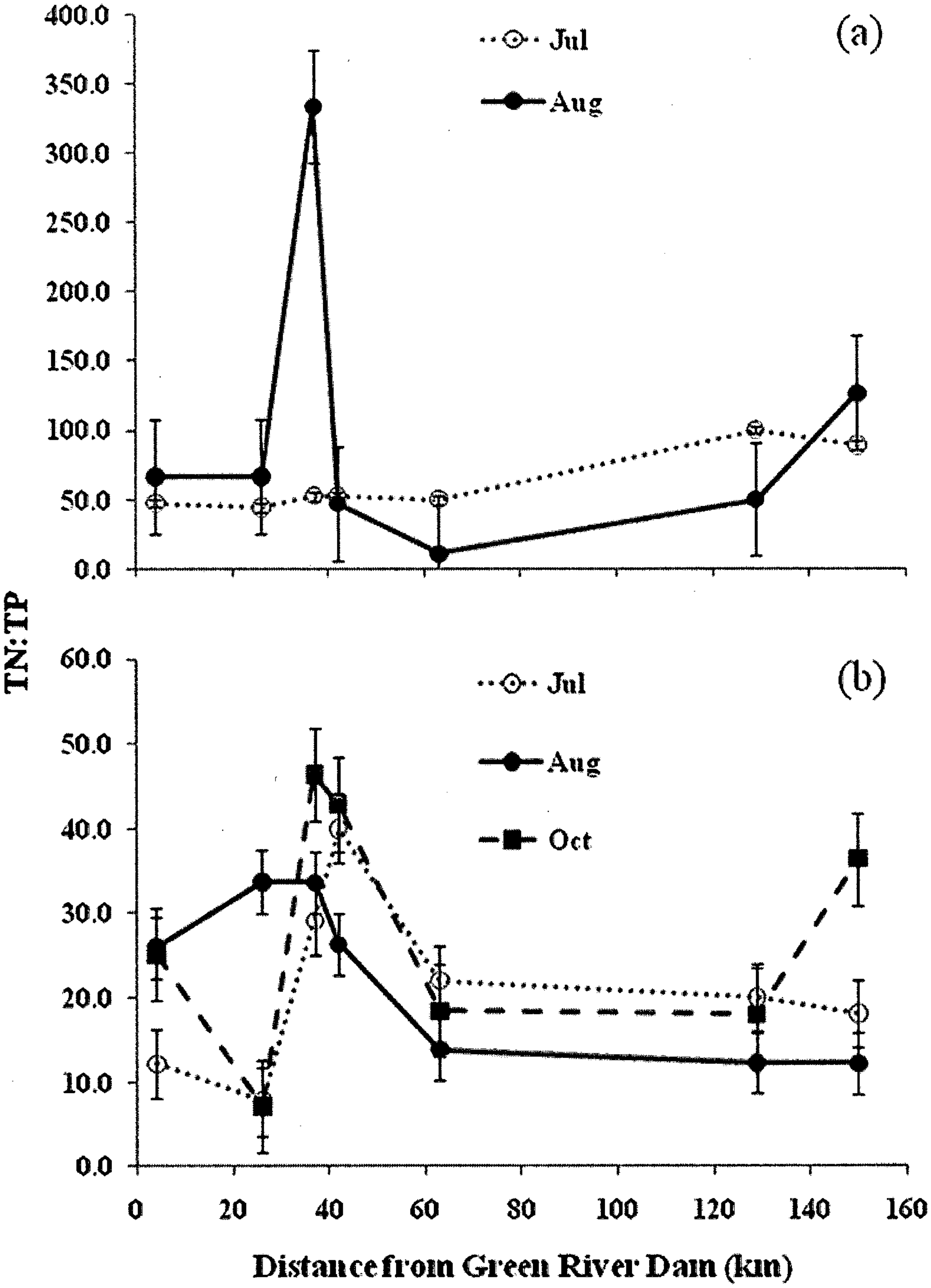

Fig. 9. Total nitrogen to total phosphorus ratios levels (TN:TP) with increasing distance from dam (km) during (a) 2008 and (b) 2009.

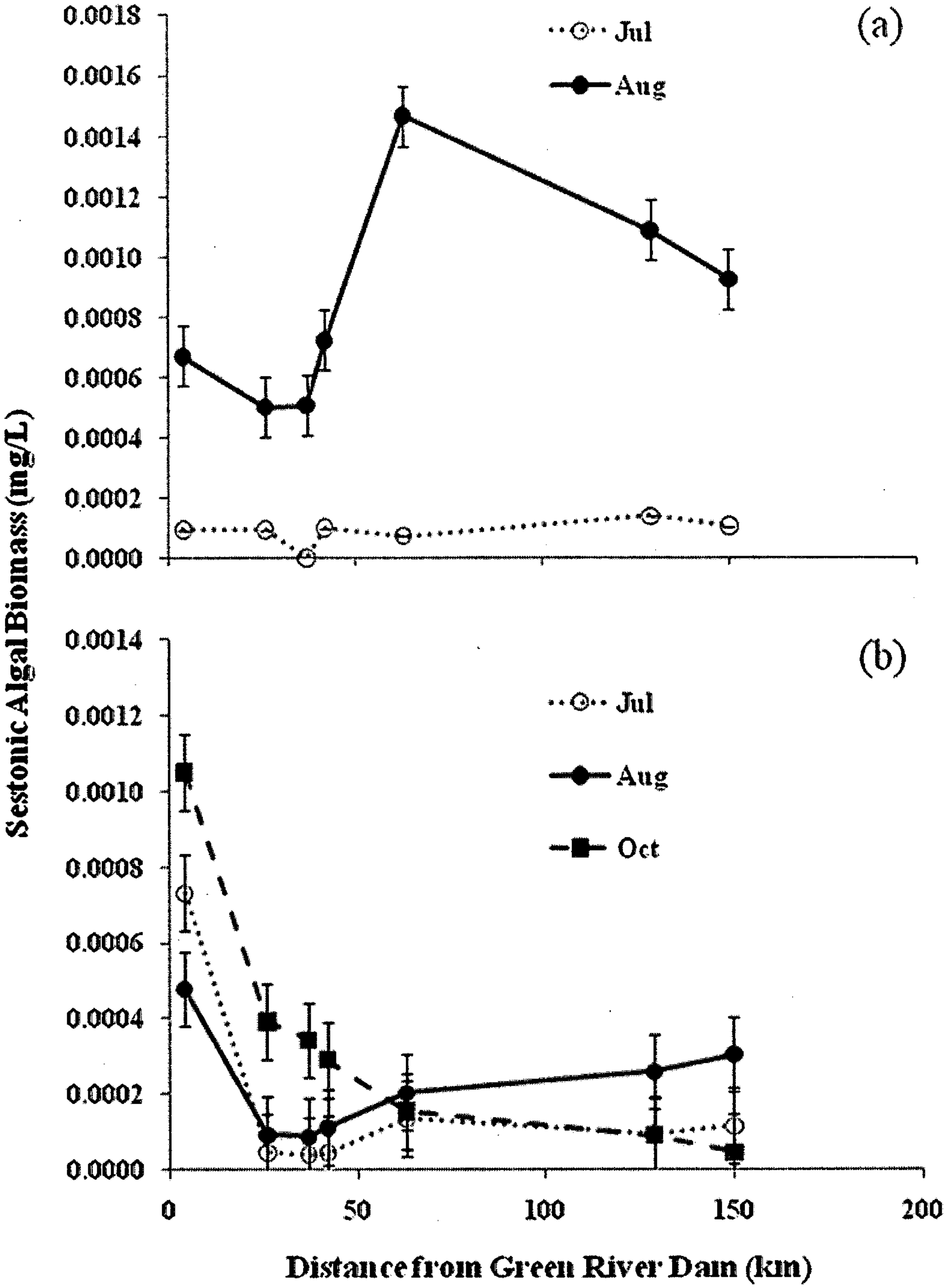

Fig. 10. Sestonic algal biomass (mg/L) with increasing distance from dam (km) during (a) 2008 and (b) 2009.

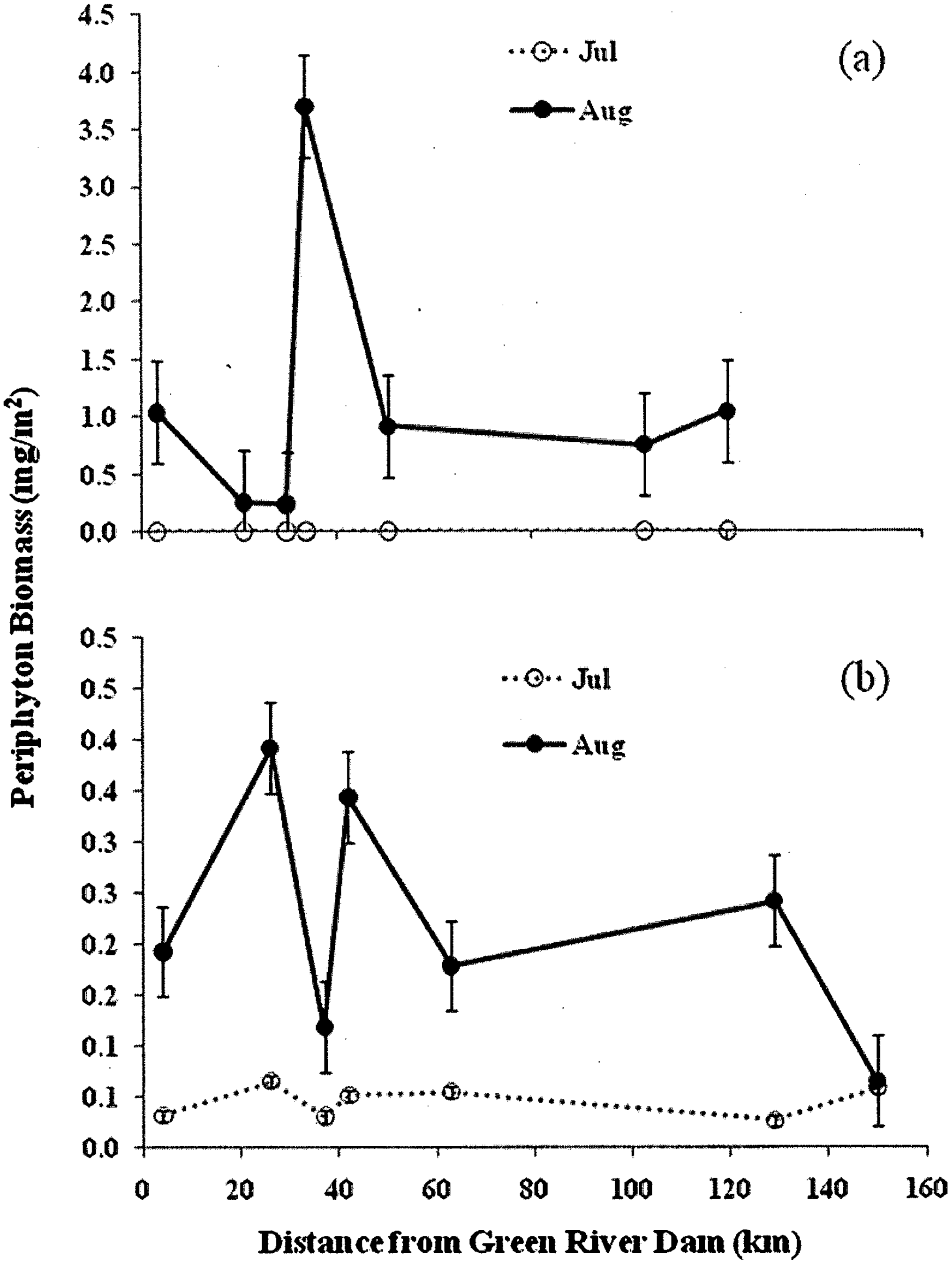

Fig. 11. Periphyton biomass (mg/m^2) with increasing distance from dam (km) during (a) 2008 and (b) 2009.

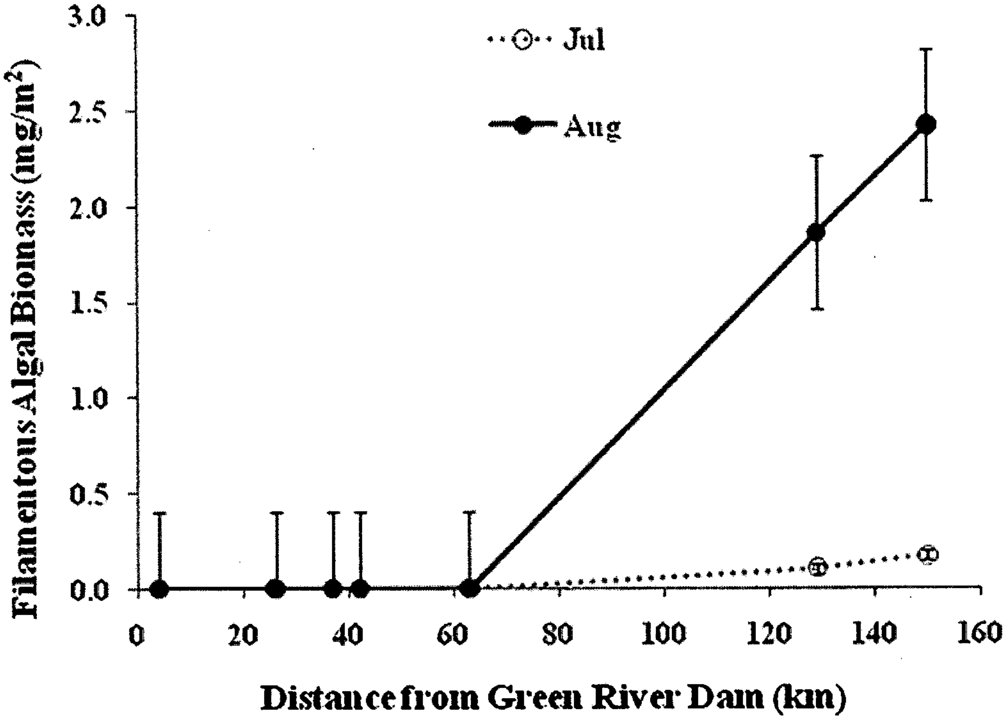

Fig. 12. Filamentous algal biomass (mg/m^2) with increasing distance from dam (km) during 2009.

Literature Cited

American Public Health Association. 1998. Standard methods for the analysis of water and wastewater. 20th edition. APHA, Washington, D.C.

AQUAPLANT. 2009. Filamentous algae. URL address http://aquaplant.tamu.edu/database/algae/filamentous_algae.htm. Accessed October 2009.

Baysinger-Daniel, C. 1989. Some factors affecting benthic algal community structure in Ozark Border streams. M.S. thesis, University of Missouri, Columbia, MO. 158 p.

Borchardt, M.A. 1996. Nutrients *in*: R.J. Stevenson, M.L. Bothwell and R.L. Lowe (editors). Algal Ecology. Academic Press, California.

Busse. L., J. Simpson, and S. Cooper. 2006. Relationships among nutrients, algae, and and use in urbanized southern California streams. Canadian Journal of Fisheries and Aquatic Sciences 63:2621–2638.

Carey, R.O., G. Vellidis, R. Lowrance, and C. Pringle. 2007. Do nutrients limit algal periphyton in small blackwater streams? Journal of the American Water Resources Association 43:1–11.

Clark, G.M., D.K. Mueller, and M.A. Mast. 2000. Nutrient concentrations and yields in undeveloped stream basins of the United States. Journal of the American Water Resource Association 36: 849–860.

Cooper, J.M. and J. Wilhm. 1975. Spatial and temporal variation in productivity, species diversity, and pigment diversity of periphyton in a stream receiving domestic and oil refinery effluents. Southwestern Naturalist 19:413–428.

Dodds, W.K. 1991. Micro-environmental characteristics of filamentous algal communities in flowing freshwaters. Freshwater Biology 25:199–209.

Dodds, W.K. and D.A. Gudder. 1992. The ecology of *Cladophora*. Journal of Phycology 28:415–427.

Dodds, W.K. and E.B. Welch. 2000. Establishing nutrient criteria in streams. Journal of the North American Benthological Society 19:186–196.

Dodds, W.K., V.H. Smith and K. Lohman. 2002. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Canadian Journal of Fisheries and Aquatic Sciences 59:865–874.

Dodds, W.K. 2003. Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. Journal of the North American Benthological Society 22:171–181.

Dortch, Q. 1990. The interaction between ammonium and nitrate uptake by phytoplankton. Marine Ecology Progress Series 61: 183–201.

Elser, J.J., K. Hayakawa, and J. Urabe. 2001. Nutrient limitation reduces food quality for zooplankton: daphnia response to seston phosphorus enrichment. Ecology 82:898–903.

Grimm, N.B., S.E. Gergel, W.H. McDowell, E.W. Boyer, C.L. Dent, P. Groffman, S.C. Hart, J.Harvey, C. Johnston, E. Mayorga, M.E. McClain, and G. Pinay. 2003. Merging aquatic and terrestrial perspectives of nutrient biogeochemistry. Oecologia 442:485–501.

Guildford, S.T. and R.E. Hecky. 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? Limnology and Oceanography 45:1213–1223.

Hecky, R.E. and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. Limnology and Oceanography 33(4): 796–822.

Hill, C. 1981. Origin of cave saltpeter. Journal of Geology 89:252–259.

Holloway, J.M., R.A. Dahlgren, B. Hansen, and W.H. Casey. 1998. Contribution of bedrock nitrogen to high nitrate concentration in stream water. Nature 395:785–788.

Katz, B.G., R.S. DeHan, J.J. Hirten and J.S. Catches. 1997. Water-quality and hydrology of the Homosassa, Chassahowitzka, Weeki Wachee, and Aripeka spring complexes, Citrus and Hernando Counties, Florida (Origin of Increasing Nitrate Concentrations). Ambient Ground-Water Quality Monitoring Program. Southwest Florida Water Management District. Brooksville, FL: 1-167.

Lamberti, G.A. and V.H. Resh. 1983. Stream periphyton and insect herbivores: An experimental study of grazing by a caddisfly population. Ecology 64:1124–1135.

Lohman, K., J.R. Jones, and C. Baysinger-Daniel. 1991. Experimental evidence for nitrogen limitation in a northern Ozark stream. Journal of the North American Benthological Society 10:14–23.

Marcus, M.D. 1980. Periphytic community response to chronic nutrient enrichment by a reservoir discharge. Ecology 61:387–399.

McConnell, W.J. and W.F. Sigler. 1959. Chlorophyll and productivity in a mountain river. Limnology and Oceanography 4:335–351.

McCormick, P.V. 1990. Direct and indirect effects of consumers on benthic algae in an ephemeral stream food web. Canadian Journal of Fisheries and Aquatic Sciences 47:2057–2065.

McCormick, P.V. and R.J. Stevenson. 1989. Effects of snail grazing on benthic algal community structure in different nutrient environments. Journal of the North American Benthological Society 8:162–172.

McCormick, P.V. and R.J. Stevenson. 1991. Grazer control of nutrient availability in the periphyton. Oecologia 86:287–291.

Mosisch, T.D., S. Bunn, and P. Davies. 2001. The relative importance of shading and nutrients on algal production in subtropical streams. Freshwater Biology 46:1269–1278.

Mulholland, P.J. and A. Rosemond. 1992. Periphyton response to longitudinal nutrient depletion in a woodland stream: evidence of upstream-downstream linkage. Journal of the North American Benthological Society 11:405–419.

Notestein, S.K., T.K. Frazer, M.V. Hoyer, and D.E. Canfield, JR. 2003. Nutrient limitation of periphyton in a spring-fed, coastal stream in Florida, USA. Journal of Aquatic Plant Management 41:57–60.

Power, M., R. Lowe, P. Furey, J. Welter, M. Limm, J. Finlay, C. Bode, S. Chang, M. Goodrich, and J. Sculley. 2009. Algal mats and insect emergence in rivers under Mediterranean climates: towards photogrammetric surveillance. Freshwater Biology 54:2101–2115.

Pringle, C.M. and F.J. Triska. 2006. Effects of nutrient enrichment on periphyton. Pages 743–757 *in* F.R. Hauer and G.A. Lamberti (editors). Methods in Stream Ecology. Academic Press, San Diego.

Redfield, A.C. 1958. The biological control of chemical factors in the environment. American Scientist 46:205–221.

Rosemond, A.D., P.J. Mulholland, and J.W. Elwood. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology 74:1264–1280.

Steinman, A.D. and Lamberti, G.A. 1996. Biomass and pigments of benthic algae. Pages 295–313 *in* F.R. Hauer and G.A. Lamberti (editors). Methods in Stream Ecology. Academic Press, San Diego.

Stevenson, R.J. 1996. Algal ecology in freshwater benthic habitats. Pages 3–30 *in* R.J. Stevenson, M.L. Bothwell, and R.L.Lowe (editors). Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego.

Tank, J.L. and W.K. Dodds. 2003. Nutrient limitation on epilithic versus epixylic biofilms. Freshwater Biology 48:1031–1049.

Taylor, S.L., S.C. Roberts, C.J. Walsh, B.E. Hatt. 2004. Catchment urbanization and increased benthic algal biomass in streams: linking mechanisms to management. Freshwater Biology 49:835–851.

U.S. Department of Agriculture. Soil Conservation Service. 1982. Soil survey of Green and Taylor Counties, Kentucky. National Cooperative Soil Survey.

U.S. Department of Agriculture. Soil Conservation Service. 1993. Soil survey of Hart County, Kentucky. National Cooperative Soil Survey.

U.S. Environmental Protection Agency. 2008. Nitrogen and phosphorus in streams in agricultural watersheds *in* Report on the environment. URL address http://cfpub.epa.gov/eroe/index.cfm?fuseaction=detail.viewInd&ch=47&subtop=200&lv=list.listByChapter&r=188223. Accessed May 2010.

U.S. Geological Survey. 1999. The quality of our nation's waters – nutrient and pesticides. U. S. Geological Survey Circular.

Vitousek, P.M. and R.W. Howarth. 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 57:87–115.

Vrede, T., A. Ballantyne, C. Mille-Lindblom, G. Algesten, C. Gudasz, S. Lindahl, and A.K. Brunberg. 2008. Effects of N : P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. Freshwater Biology 54: 331–344.

Wetzel, R.G. 1983. Limnology, 2nd ed. Saunders College Publishing, Philadelphia, PA.

Woods, A.J., J.M. Omernik, W.H. Martin, G.J. Pond, W.M. Andrews, S.M. Call, J.A. Comstock, and D.D. Taylor. 2002. Ecoregions of Kentucky (color poster with map, descriptive text, summary tables, and photographs): Reston, VA, U.S. Geological Survey (map scale 1:1,000,000).

Yin, S. 2005. USGS Upper Midwest Environmental Sciences Center Modification of SM 10-200 H *in* American Public Health Association. Standard methods for the analysis of water and wastewater. APHA, Washington, D.C.