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FACTORS LIMITING BENTHIC ALGAL ABUNDANCE IN VIRIGINA STREAMS
OF THE COASTAL PLAIN.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science at Virginia Commonwealth University.

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Abstract

FACTORS LIMITING BENTHIC ALGAL ABUNDANCE IN VIRGINIA STREAMS OF THE COASTAL PLAIN.

By Michael Patrick Brandt, M.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2009

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Algae are important components of stream food webs and often used in biomonitoring assessments. Little is known regarding the factors that limit their abundance in streams of the VA Coastal Plain. The surficial geology of the Coastal Plain is predominately sandy deposits which comprise the dominant substrate in streams of this region. In a comparative study of five streams located near the VCU Rice Center, we quantified substrate composition, light availability, and nutrient concentrations to assess their relative importance in determining benthic algal abundance. The proportion of stream area comprised of hard substrates was a significant predictor of variation in benthic algal abundance ($r^2=0.66$). An experimental component comparing algal colonization on artificial hard substrates (tile) to the natural substrate reinforced the importance of substrate stability. Hard substrates which included gravel and aggregated clay likely provided greater stability for algal colonization relative to sand and silt deposits, resulting in lower mortality from scouring

and sedimentation. Incident solar radiation was a secondary factor affecting algal abundance with shaded streams exhibiting lower benthic chlorophyll. Where substrate and light conditions were favorable, relationships between benthic algal abundance and dissolved phosphorus concentrations were observed. Seasonal fluctuations were ameliorated by high light conditions and constant disturbances at sites lacking hard substrates which kept CHLa at consistently low levels. The mean proportion of FBOM C derived from benthic algae ranged from 10 to 24%. In spite of the consistently low observed benthic algal abundance at sandy unstable Coastal Plain streams, benthic algae are an important source of benthic organic matter.

CHAPTER 1 Introduction

An understanding of the factors limiting benthic algal production is vital in comprehending energy flow in stream ecosystems (Dodds et al. 2002). Through photosynthesis benthic algae supply organic matter necessary to support grazers and higher trophic levels (Slavik et al. 2004). The proportion of algal production that is not directly utilized by grazers contributes to the accumulation of fine benthic organic matter (FBOM; organic matter less than 1mm). Heterotrophic microbes living in association with FBOM provide additional food resources supporting secondary production. In this way, benthic algae contribute to both the herbivore and detritivore pathways in stream food webs.

Benthic algal growth is regulated by a complex interplay of factors that determine growth (light, nutrients, temperature) and mortality (substrate stability, discharge, and grazing). The river continuum concept outlines the physical environment expected in low order forested streams and the factors constraining autotrophic production in them (Vannote et al. 1980). The riparian forest provides organic matter inputs that supports heterotrophic production but also limits photosynthetically active radiation to the autotrophic community. Light has been shown in multiple studies to affect benthic algal production in streams (Biggs 1988, Duncan and Blinn 1989). Seasonal changes in canopy cover often parallels patterns in benthic algal abundance (Duncan and Blinn 1989, Junior et al. 1991). The increased

light and temperature associated with early spring pre-leaf out conditions promote benthic algae growth.

There is a complex relationship between light and nutrient limitation of autotrophic production in streams. Nitrogen and phosphorus levels in streams are frequently high where anthropogenic sources are present (Cerc and Seitzinger 1997; Allan and Castillo 2007). Light availability in most low order streams is limited by the riparian zone and thus nutrients may be present in excess of plant needs. Nutrient enrichment experiments performed in densely shaded streams failed to stimulate benthic algal production (Greenwood and Rosemond 2005). If riparian disturbance results in greater incident solar radiation, nutrient availability may be a limiting factor for benthic algal production. Multiple studies have demonstrated responses in algal biomass to nutrient additions including: phosphorus alone, nitrogen alone, and combined N and P additions (Pringle and Bowers 1984, Flecker et al. 2002, Tank and Dodds 2003, Sabater et al. 2005).

In addition to light and nutrients, mortality associated with disturbance (scour events) may also affect benthic algal abundance. Substrate stability has been shown to be an important factor determining the sensitivity of benthic algal communities to high discharge events (Murdock and Dodds 2007). Coastal streams have a unique sand based geology and these fine substrates have lower stability than coarse substrates (e.g., gravel, cobble) and are therefore more prone to disturbance. Substrate size is generally a good predictor of stability although substrates consisting of clay, which has a smaller particle size than sand, can exhibit high stability because of electrostatic cohesion

among particles (Xia et al. 2008). Benthic algae growing on sand substrates are likely to experience higher loss rates due to scour effects than in comparison to hydrologically similar streams with more stable substrates. The combined effects of periodic high discharge and substrate instability may limit the accumulation of benthic algal biomass even when light and nutrient conditions are favorable.

In this study I explored relationships between benthic algal abundance, substrate stability, incident solar radiation, and nutrient concentrations in streams of the Virginia Coastal Plain. The objective was to assess whether geologic characteristics (unstable, predominantly sandy substrates) are more important determinates of benthic algal abundance than light and nutrient availability in streams dominated by fine substrates. I hypothesized that benthic algal abundance could be predicted based on substrate composition with abundance increasing as a function of the availability of hard substrates. The alternative hypothesis was that substrate effects were of minor or secondary importance due to constraints imposed by light and nutrient availability. To test these hypotheses I compared benthic algal abundance among five streams differing in substrate composition, riparian cover and nutrient availability. In addition to the survey approach I also conducted an experiment to compare algal biomass accumulation on hard substrates (ceramic tiles) vs. naturally occurring substrates in two streams differing in substrate composition (high vs. low proportion of hard substrates).

CHAPTER 2 Methods

Benthic algae were sampled in five Coastal Plain streams located in proximity to the VCU Rice Center, Charles City County, VA: Powell Creek, Courthouse Creek, Herring Creek, Crump Creek and Kimages Creek (Table 1). A permanent study reach of approximately 200 meters in length was established at each site. Two study reaches were located at Kimages Creek in order to characterize benthic algal abundance in both reference and restored sections. The restored section was recently re-established as a flowing stream following the drawdown of an impoundment (Lake Charles). The reference section was located upstream of the restored section and was not previously inundated. The reaches were shaded to varying degrees by a forested riparian zone except for the restored section of Kimages Creek where it flowed through an open (treeless) area of the former Lake Charles (Table 1). Data were collected monthly for 6-12 months over the period of a year during 2008 to characterize seasonal variation. Sampling began in February at Crump Creek, in March at Kimages Creek, and in January at all other sites. Sampling concluded in August at Crump and Courthouse Creeks, in October at Kimages Creek and in December at Powell and Herring Creeks. Sampling ceased at Kimages Creek in October due to beaver dams that made the creek

non-wadeable and prevented benthic algae sampling. A total of 53 monthly collections were made at the six study reaches within the twelve month period.

Benthic algae were collected at approximately 20 meter intervals along each of the study reaches. Sampling consisted of driving a bottomless pail (bottom area = 0.07 m^2) 10 cm into the substrate (Bott et al. 2006). The substrate was hand mixed to a depth of 5 cm until suspended. A 1L sub-sample was removed and sieved (1mm) to ensure only FBOM was sampled. The samples were kept in dark bottles and on ice until filtered in the lab. Materials collected on filters were analyzed to determine its chlorophyll (CHLa), C and N content. Filters for C-N analysis were oven dried at 60°C for 48 hours and then stored in a desiccator. C and N content was determined using a Perkin-Elmer CHN analyzer and expressed per unit area (FBOM C and FBOM N as g/m^2). Filters for CHLa analysis were cold stored in a sealed test tube for a maximum of thirty days. CHLa was extracted using 10ml of 90% acetone for 24 hours and measured with a Turner Designs Model AU fluorometer (APHA et al 1992). CHLa concentrations were also expressed per unit of stream bottom (mg/m^2).

Environmental data collected on each sampling date included incident solar radiation, nutrient concentrations, discharge and temperature. Photosynthetically active radiation was measured at each site where CHLa samples were collected using a Li-Cor sensor. Four sets of light measurements were taken along the study reach at identical times each day. Samples for determination of dissolved inorganic nitrogen ($\text{DIN} = \text{NH}_4 + \text{NO}_3$) and soluble reactive phosphorus (PO_4) were collected at the top and bottom of the study reach and a whole reach average was used for testing relationships with

benthic CHLa. Nutrient concentrations were determined colorimetrically using a Skalar segmented flow analyzer (APHA et al 1992). Discharge was measured based on velocity (determined with a flow meter) and cross-sectional area (measured at 0.5 meter intervals across the channel) at the most upstream sampling site. The temperature in Celsius was measured using a Hydrolab Minisonde 4a. The percentage of substrate types (sand, silt, clay, pebble and gravel, (<1/2" -1") and rock (>1")) were quantified by visual estimation on a single date by placing a 0.5 m² frame at five random spots at each sampling site along the study reach (where CHLa and PAR were measured). Clay, pebble, gravel, and rock were categorized as hard substrates.

A colonization experiment utilizing artificial substrates was conducted during January to March 2009. The accumulation of CHLa was measured on unglazed ceramic tiles (to mimic hard substrates) and on natural substrates. The experiment was designed to measure accumulation of CHLa on hard substrates at a site where hard substrates were prevalent (Herring Creek) and a site where they were largely absent (Powell Creek). We hypothesized that if the availability of hard substrates limited benthic algal abundance, CHLa accumulation on tiles should exceed that of natural substrates at the site where hard substrates were lacking (Powell Creek). Herring Creek served as a control where we expected CHLa on tiles to be similar to the natural (predominately hard) substrates. Two 4"x4" tiles were attached with silicone to concrete blocks (to reduce incident of burial or tile loss) and were placed at each sampling site along the study reach. Tiles were deployed on January 1st sampled on February 4th, then redeployed and sampled again on March 7th. Data from the two

sampling dates were averaged for use in subsequent analysis. Velocity and depth were measured at each tile location. Tiles were processed in the field to remove attached algae by brushing and rinsing with tap water. Samples were put on ice and returned to the lab in the absence of light. CHLa determination for tile samples followed the same procedure as was utilized for the FBOM survey component.

CHAPTER 3 Statistical Analysis

Statistical analyses were performed using SAS V9.1 (SAS Institute, Cary, North Carolina). ANOVAs were performed to partition variance attributable to intra-site (reach scale), inter-site (among streams) and seasonal effects. These analyses revealed that intra-site effects (i.e. location within the reach) were not a significant predictor of variation in CHLa. Subsequent analyses used whole-reach average values and a two way ANOVA with stream and season as model components. Two way ANOVAs were conducted utilizing pairwise comparisons of the least squared means. Seasons were categorized as follows: Summer (June, July, August), Fall (September, October, November), Winter (December, January, February) and Spring (March, April, May).

Relationships between the predictor variables (PAR, DIN, SRP, discharge, % hard substrate, and temperature) and the dependent variable (benthic CHLa) were also tested utilizing univariate and multivariate linear regressions. Regressions were also performed with transformed variables to determine whether non-linear models would explain a greater proportion of the variance in benthic CHLa. Univariate and multivariate regressions were performed on pooled data from all sites as well as individual sites. Data from Kimages Creek were excluded from the pooled analysis owing to the lack of substrate data for this site. Parameters measured at multiple locations within reaches (CHLa, % hard substrate, FBOM C and N) yielded a large

dataset for testing relationships (6 reaches * 7-12 months * 3-11 sites/reach =440 measurements). Parameters that were measured at the reach scale (nutrients, discharge, PAR, temperature) required CHLa values to be aggregated at this scale and yielded a smaller dataset to test relationships (6 reaches * 7-12 months=53 measurements). Relationships between CHLa and FBOM composition were tested to determine whether the C and N content of FBOM was correlated with benthic algal abundance.

Analyses of the experimental component utilized a one-way t-test comparing the ratios of tile CHLa to natural substrate CHLa at measurement locations along the study reach. The ratios were generated to normalize each tile CHLa value relative to its paired natural substrate value to take into account potential effects arising from within reach variation (e.g., PAR, water velocity). The one-way t-test was designed to test the hypothesis that the ratio of tile: natural substrate CHLa would be higher at the site lacking hard substrates (Powell Creek).

CHAPTER 4 Results

A. Seasonal and Inter-Site Patterns

Mean and monthly values were compared within and among sites to characterize seasonal and inter-stream variation in benthic CHLa, PAR, nutrients, substrate, discharge and temperature. Mean CHLa (all months) ranged from 11 to 196 mg/m² among the 6 reaches (Table 2, Figure 1). Site and season were significant predictors of variation in benthic CHLa ($p < .0001$) and together accounted for 41% of the variation. Kimages Creek exhibited the highest CHLa (reference=124 mg/m²; restored=196 mg/m²), followed by Herring Creek (73 mg/m²). CHLa concentrations were low and not significantly different at Crump, Courthouse and Powell Creeks (all < 30 mg/m²). For all sites, summer (103 mg/m²) and fall (79mg/m²) had higher mean CHLa relative to winter (32 mg/m²) and spring (24 mg/m²) (Table 3). There was a significant interaction (p -value $< .0001$) between site and season. This interaction reflects that the high CHLa sites (Kimages and Herring Creeks) exhibited large seasonal variation which was not observed at the low CHLa sites. One way ANOVAs verified the results from the two way ANOVAs.

Inter-site and seasonal differences in average CHLa followed trends in incident solar radiation (Table 2, Figure 1). Kimages Creek exhibited the highest PAR (reference=528 $\mu\text{E}/\text{m}^2/\text{s}$; restored=838 $\mu\text{E}/\text{m}^2/\text{s}$), followed by Herring Creek (340 $\mu\text{E}/\text{m}^2/\text{s}$). No significant differences in PAR were detected among Crump, Powell and

Courthouse Creeks (all $<315 \mu\text{E}/\text{m}^2/\text{s}$). For all sites, spring ($414 \mu\text{E}/\text{m}^2/\text{s}$) and summer ($397 \mu\text{E}/\text{m}^2/\text{s}$) had higher mean PAR compared to winter ($296 \mu\text{E}/\text{m}^2/\text{s}$) and fall ($285 \mu\text{E}/\text{m}^2/\text{s}$, Table 3). Site and season were significant predictors of PAR (site $p < .0001$; season $p = .002$) and together accounted for 83% of the variation. A significant interaction of site and season was not detected. Inter-site differences in CHLa also followed trends in substrate composition among the four sites where data were available (excluding Kimages). Herring Creek (90%) had a higher mean percent hard substrate than Courthouse Creek (36%) which was higher than Powell Creek (22%), and Crump Creek (3%; Table 1). Mean CHLa at each site increased in direct relation to the mean percent hard substrate available for colonization.

Seasonal and spatial patterns in benthic CHLa did not follow trends in dissolved inorganic nutrient concentrations (Table 2, Figure 1). Kimages, Crump, and Courthouse Creeks all exhibited high dissolved inorganic nitrogen ($\text{DIN} > 0.350 \text{ mg}/\text{L}$) though only Kimages exhibited elevated CHLa. Powell and Herring Creeks exhibited similar and low DIN ($< 0.115 \text{ mg}/\text{L}$) whereas benthic CHLa concentrations were two-fold higher at Herring. For all sites, spring ($0.276 \text{ mg}/\text{L}$) and winter ($0.248 \text{ mg}/\text{L}$) had higher mean DIN relative to summer ($0.231 \text{ mg}/\text{L}$) and fall ($0.192 \text{ mg}/\text{L}$, Table 3). Site and season were significant predictors of variation in DIN (site $p < .0001$; season $p = .004$) and together accounted for 80% of the variation. There was a significant interaction ($p = .002$) between site and season. This interaction was due to the high DIN sites exhibiting seasonal variation which did not occur at low DIN sites. A one way ANOVA determined that season was not a significant predictor of variation in DIN

when site was not included in the model. A one way ANOVA verified site was a significant predictor of variation in DIN ($<.0001$) but accounted for only 53% of the variation.

Mean SRP concentrations (all months) exhibited low variability among sites (range=0.019 to 0.030 mg/L), and inter-site differences were not statistically significant (Table 2, Figure 1). Season was a significant predictor of variation in SRP ($p=.0004$) and accounted for 56% of the variation. Summer (0.037 mg/L) and fall (0.029 mg/L) had higher mean SRP compared to winter (0.015 mg/L) and spring (0.014 mg/L, Table 3). A significant interaction was not detected.

Inter-site differences in CHLa generally did not follow trends in discharge or temperature. Mean discharge ranged from 28 to 266 (L/s) among the 6 reaches (Table 2, Figure 1). Herring Creek exhibited the highest discharge (266 L/s), followed by Crump and Powell Creeks (185 L/s and 170 L/s, respectively). Discharge at Kimages and Courthouse Creeks averaged less than 75 L/s. For all sites winter (253 L/s) and spring (216 L/s) had higher mean discharge compared to fall (83 L/s) and summer (57 L/s, Table 3). Site and season were significant predictors of variation in discharge (site $p=.001$; season $p=.02$) and accounted for 67% of the variation. A significant interaction was not detected. Mean temperatures ranged from 16.4 to 22.3 °C and were not significantly different among the 6 reaches (Table 2, Figure 1). Mean temperatures ranged from 7.1°C to 25.2°C during winter to summer (Table 3). Season was a significant predictor of variation in temperature (season $p<.0001$) and accounted for 88% of the variation. A significant interaction was not detected.

B. Regression Models

A pooled data set (excluding Kimages) was used to test for relationships between CHLa and substrate composition. The percentage of hard substrates was the most powerful predictor of CHLa ($r^2=0.66$, $P<0.0001$, Figure 2). Weaker but significant and positive relationships were also found with PAR ($p=.0006$, $r^2=0.21$), SRP ($p=.0003$, $r^2=0.19$) and temperature ($p=.0015$, $r^2=0.19$). There was a significant, negative and non linear relationship between discharge and CHLa ($p=.0021$, $r^2=0.17$). Because the univariate regressions revealed a number of variables that potentially influence benthic CHLa, multivariate models were also tested. For the four-site data set, a model including substrate, SRP, discharge and PAR (in order of importance) accounted for 80% of the variation in benthic CHLa (Table 4; Kimages Creek excluded due to lack of substrate data). For the six-site data set (Kimages included but substrate excluded as a predictor), a multivariate model based on PAR and SRP explained 47% of the variation in CHLa ($p<.0001$; Table 4).

Univariate and multivariate regression models were also developed using site-specific datasets to assess inter-stream differences among factors affecting benthic CHLa. Substrate composition was not included in the site specific models because within-site variation in percent hard surfaces was minimal and did not correlate with CHLa. The influence of incident solar radiation and nutrient availability differed among sites. PAR was a significant predictor of benthic CHLa at one of the three sites exhibiting low average irradiance (Courthouse Creek, $r^2=0.87$, Figure 3). Among the high irradiance sites, SRP was a significant predictor of benthic CHLa at Herring Creek

and the restored section of Kimages Creek; ($r^2=0.86$ and $r^2=0.70$, respectively; Figure 4). Temperature was a significant predictor at the reference section of Kimages Creek ($r^2= 0.69$, $P=0.04$) and at Courthouse Creek ($r^2= 0.68$, $P=0.01$). Discharge was significantly and negatively related to benthic CHLa at Herring Creek ($r^2= 0.61$, Figure 5). Multivariate regression models accounted for a greater proportion of the variation in benthic CHLa at three of the sites (Table 4). The combination of PAR and discharge explained 97% of the variation in benthic CHLa at Courthouse Creek. DIN and PAR explained 92% of the variation at Crump Creek. SRP and discharge accounted for 93% of the variation in benthic CHLa at Herring Creek.

C. Substrate Experiments

The accumulation of CHLa on natural (FBOM) and artificial (tile) substrate was measured to experimentally assess the importance of substrate composition for benthic algal abundance. At the site where hard substrates were lacking (Powell Creek) mean benthic CHLa was higher on tile than the natural substrate (80.6 ± 23.9 mg/m² and 56.6 ± 13.4 mg/m², respectively). At the site where hard substrates were prevalent (Herring Creek) mean benthic CHLa was lower on tile than on natural substrate (29.9 ± 5.2 mg/m² and 61.1 ± 14.3 mg/m², respectively). A one way t-test determined that the ratio of CHLa on tile relative to natural substrate was significantly higher at Powell Creek (mean= 5.12 ± 2.4) than Herring Creek (mean= 0.86 ± 0.18 ; $p<0.0001$). Results from this experiment support the hypothesis that the lack of hard substrates limited benthic CHLa at Powell Creek.

D. Algal Contributions to FBOM

CHLa was a significant predictor of variation in the C and N content of FBOM ($p < .0001$, $r^2 = 0.37$ and $p < .0001$, $r^2 = 0.42$, respectively, Figure 6). To estimate the proportion of FBOM C that was derived from benthic algae, CHLa concentrations are converted to benthic algal C using a fixed C: CHLa ratio of 50 (Kim et al. 2007). The mean proportion of FBOM C ranged from 10 to 24%, highest at Herring Creek.

CHAPTER 5 Discussion

Our results suggest that in the Coastal Plain of Virginia inter-stream variation in benthic algal abundance was determined in part by the availability of hard substrates. Among the four sites where substrate composition was quantified, Herring Creek exhibited the highest average CHLa concentration and greatest proportion of hard substrates. At Crump, Powell, and Courthouse Creeks, the low proportion of hard substrate likely resulted in low substrate stability and a physical environment that was not conducive to the accumulation of benthic algae. Greater algal biomass was observed at Herring Creek despite higher discharge and lower dissolved inorganic N concentrations. Results from the experimental component of this study also support the hypothesis that substrate composition is an important factor in determining algal biomass. At Powell Creek the ratio of CHLa was significantly higher on hard substrate (tile) than on the natural sand substrate. At Herring Creek the natural substrate performed statistically similar to the artificial hard substrate (tile). Light and nutrient conditions were generally similar at these two sites. The experimental findings suggest that the availability of stable colonization sites was an important factor influencing benthic algal abundance.

Although substrate composition at Kimages Creek was not quantified as part of this study, high CHLa concentrations at this site are consistent with the hypothesis that substrate stability is an important determinant of benthic algal abundance. Substrate

composition at Kimages Creek differed from the other sites in that it was comprised of fine grain particles (clay and silt) which were formerly lake deposits (K. Cannatelli, University of Virginia, pers. Comm.). Although fine-grained, clay particles exhibit greater cohesion and stability than sand or silt (Denef et al. 2002, Xia et al. 2008) which may in part account for high levels of benthic CHLa at Kimages Creek. Substrate composition was a useful predictor of intra-stream (reach-scale) variation in CHLa as has been reported in some studies (Potapova and Charles 2005; Townsend and Gell 2005). At my study sites the proportion of hard substrates was either minimal (3 to 36%) or predominant (>90%) thus the explanatory power at a given site was low.

The findings suggest that substrates are an important determinant of benthic CHLa in these streams but other factors, specifically light and nutrient availability cannot be ruled out. Light effects were only weakly detected in the pooled dataset ($r^2=0.23$) but were strongly related to CHLa at one site (Courthouse Creek, $r^2=0.87$). This site had a dense riparian zone which is typical of many low order Coastal Plain streams. These results suggest that PAR levels at the upper ranges detected in this study (>250 $\mu\text{E}/\text{m}^2/\text{s}$) were not likely limiting to benthic algal production but that light limitation may occur at the low PAR sites. At the highest PAR site (Kimages Creek) CHLa exceeded 100 mg/m^2 , an indication of stream impairment (Dodds et al. 1997). The restored section of Kimages Creek had mean PAR comparable to unshaded agricultural streams and similar levels of algal biomass (Quinn et al. 1997).

Evidence for nutrient limitation at the study sites was equivocal. Dissolved inorganic N concentrations varied widely and included two sites (Herring and Powell)

with very low levels (<0.15 mg/L). Despite this variability, DIN was not a useful predictor of variation in benthic CHLa. Tank and Dodds (2003) found multiple streams with DIN concentrations below 0.055 mg/L which did not exhibit N limitation. DIN concentrations measured in this study were as much as seven times higher and suggest that N was not a limiting nutrient in Coastal Plain streams. SRP concentrations, though less variable, were significantly correlated with benthic CHLa at two sites where light and substrate conditions were favorable (Kimages and Herring). SRP concentrations at these sites were near the threshold where limitation effects have been observed (0.025 mg/L; Hill et al. 2009; Biggs 2000). These findings suggest that when light and substrate conditions are favorable, P availability may be a secondary limiting factor in Coastal Plain streams. A study of 620 North American streams reported that TP concentrations were a useful predictor of benthic algal biomass (Dodds et al. 2002). Yangdong et al. (1999) also found a strong correlation ($r^2=0.69$) between CHLa and TP in the Coastal Plain. TP was not measured in this study and it is possible that the potential for P limitation may be underestimated from measurements of the soluble fraction (SRP).

FBOM in low order streams is largely the byproduct of terrestrial inputs (Smock, 1997; Findlay et al. 2002). Terrestrial inputs include mineral particulates (sand, clay) and partially decomposed plant materials which are characterized by low C and N content. Autochthonous sources are comparatively rich in C and N though their importance to FBOM C and N content is unknown. CHLa was a significant predictor of FBOM C and N ($r^2=0.37$ and $r^2=0.42$, respectively) suggesting that benthic algae may

be important in influencing the quality of FBOM despite the quantitative dominance of terrestrial inputs. Using a literature estimate of the C:CHLa for benthic algae we estimated that benthic algae contribute on average 17% of the C content of FBOM in Virginia Coastal streams (Table 5). The proportion of FBOM C derived from benthic algae indicates that autochthonous inputs are higher than might be expected in low order streams.

Overall, the findings suggest that sand substrates typical of Coastal Plain streams create unstable habitat for benthic algal colonization but where hard (Herring) or stable (Kimages) substrates are available benthic algal abundances are comparable to those observed in other ecoregions. The range of CHLa values obtained in this study is similar to that observed in Piedmont streams (Figure 7). The comparatively high mean CHLa at Kimages Creek could be explained by the high light conditions in the former lake bed, but many of the Piedmont streams are in agricultural areas which also lack forested riparian zones (McTammany et al. 2007). At the restored section of Kimages Creek the physical conditions, including low discharge, open canopy and stable substrate, most likely played an important role in the high mean algal abundance. A study of New Zealand streams demonstrated that algal abundance is largely formulated by basin-scale factors such as geology and local-scale variables such as water velocity (Biggs and Gerbeaux 1993). The same was true for Virginia streams of the Coastal Plain. Coastal streams have unique characteristics that shape the community structure. This follows the idea of ecoregional classification which identifies several components, such as geology, soils, anthropogenic land changes, and vegetation which help define

the region's abiotic characteristics (Yangdong et al. 1999). Once identified these ecoregional characteristics can explain relationships between benthic algal populations and the local abiotic forces (Stevenson 1997). The most important ecoregional characteristic of the Coastal Plain streams was geology and the resulting unstable sand substrate. Streams consisting of a high proportion of stable substrates are less prone to disturbance and better reflect the influence of environmental conditions other than substrate composition.

Stream health assessment models often utilize benthic algal biomass as indicators of nutrient enrichment (Smith 2003). Coastal Plain streams are the primary streams of the Chesapeake Bay watershed and contribute to nutrient loads that result in harmful algal blooms (Boesch et al. 2000). Identifying streams impacted by non point sources is difficult. In Coastal Plain streams with sand-based substrates, low algal biomass does not necessarily indicate low nutrient enrichment concentrations. It would be useful to create an assessment tool which can adequately estimate benthic algae in respect to the geologic (particle size distribution) and hydrologic (slope, velocity, discharge) conditions affecting substrate stability. Artificial substrates are commonly utilized to reduce variability in benthic algae stream assessments (Ponader et al. 2008), but models would benefit from identifying how substrate composition (particle size distribution) and stability control benthic algal abundance. This would improve assessment precision, remove the need for utilizing artificial substrates and add to the understanding of stream ecology.

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Literature Cited

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Tables

Table 1. Overview of six study reaches located in proximity to the VCU Rice Center. Substrate coverage not quantified at Kimages Creek.

Reaches	Stream Order	Reach Length	Sampling Locations	Substrate Type	Canopy	Tributary River
Crump	2 nd	200	11	97% Sand, 3% Pebble, Gravel, or Rock	Moderate	York
Courthouse	2 nd	190	11	64% Sand, 36% Pebble, Gravel, or Rock	Dense	James
Powell	3 rd	216	8	78% Sand, 22% Pebble, Gravel or Rock	Moderate	James
Herring	3 rd	180	11	10% Sand, 90% Pebble Gravel, or Rock	Moderate	James
Kimages Reference	2 nd	70	4	Sand, Silt, Clay	Moderate	James
Kimages Restored	2 nd	330	9	Sand, Silt, Clay	Open	James

Table 2. Mean values of benthic CHLa, incident solar radiation (PAR), nutrients (DIN, SRP), discharge and temperature for five streams of the VA coastal plain. Means are based on data collected monthly for 6-12 months over the period of a year during 2008. Restored and reference sections of Kimages Creek are shown separately. Statistical results are based on two way ANOVAs using site and season as model components. R-square denotes the proportion of variance explained by the model terms. Abbreviations: Photosynthetically active radiation (PAR), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus, Crump Creek (Cp), Powell Creek(P), Courthouse Creek(Ct), Herring (H), Kimages Creek reference (Kref), Kimages Creek restored (Krest). Summer (Su), Fall (F), Winter (W), and Spring (S).

	Benthic CHLa (mg/m²)	PAR (μE/m²/s)	DIN (mg/L)	SRP (mg/L)	Discharge (L/s)	Temperature °C
Crump	11.4±1.8	310.3±52.7	0.375±0.079	0.025±0.004	185±75	16.6±2.1
Powell	24.6±4.3	244.6±37.4	0.110±0.017	0.024±0.004	173±33	17.6±1.8
Courthouse	28.3±7.9	134.5±41.9	0.392±0.088	0.019±0.006	42±10	16.4±2.9
Herring	72.9±16.2	340.1±39.4	0.075±0.021	0.029±0.008	266±72	17.5±2.5
Kimages REF	123.7±29.7	528.3±65.3	0.408±0.077	0.022±0.007	33±9	22.1±2.1
Kimages REST	196.2±48.9	837.7±73.4	0.395±0.042	0.030±0.005	28±8	22.3±2.0
DF	19,440	19,33	19,31	19,31	19,33	19,31
Site P-values	<.0001	<.0001	<.0001	NS	0.001	NS
Differences	Krest>Kref > H>P=Ct>C p	Krest>Kref> H=Cp=P>Ct	Krest=Kref= Ct=Cp>P=H	None	H=Cp=P>Ct = Krest=Kref	None
Season P-Values	<.0001	0.002	Two way: (0.004) One way: (NS)	0.0004	0.02	<.0001
Differences	Su=F>W=S p	Sp=Su>W=F	None	Su=F>W=S p	W=Sp>F=Su	All Different
Interaction	<.0001	NS	0.002	NS	NS	NS
R-square	0.41	0.83	Two way: (0.80) One Way: (0.53)	0.56	0.67	0.88

Table 3. Monthly and seasonal averages of benthic CHLa, incident solar radiation (PAR), nutrients (DIN, SRP) discharge and temperature for the six study reaches. Abbreviations: Photosynthetically active radiation (PAR), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP).

Season	Month	Benthic CHLa (mg/m ²)	DIN (mg/L)	SRP (mg/L)	PAR (μE/m ² /s)	Discharge (L/s)	Temperature °C
Winter	Dec	28.8	0.079	0.023	194	323	No Data
	Jan	41.1	0.319	0.009	306	211	6.0
	Feb	25.2	0.346	0.012	389	226	8.2
	Average	31.7	0.248	0.015	296	253	7.1
Spring	Mar	39.8	0.276	0.014	417	95	12.2
	Apr	20.3	0.225	0.018	367	286	14.6
	May	10.3	0.326	0.012	459	268	17.4
	Average	23.5	0.276	0.014	414	216	14.7
Summer	Jun	71.5	0.292	0.037	455	87	25.6
	Jul	109.7	0.290	0.037	361	45	25.5
	Aug	128.4	0.111	0.036	374	39	24.3
	Average	103.2	0.231	0.037	397	57	25.2
Fall	Sept	57.6	0.269	0.013	416	75	24.2
	Oct	126.9	0.248	0.046	279	70	21.0
	Nov	51.2	0.060	0.029	160	104	12.0
	Average	78.6	0.192	0.029	285	83	19

Table 4. Multivariate models used to predict variation in benthic CHLa for streams in the Coastal Plain of Virginia. Model r-square denotes proportion of variation described by the full model. Model components listed in order of importance. Abbreviations: Photosynthetically active radiation (PAR), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP).

Site	Y	X	Model r²	p-value
Crump	CHLa	DIN, PAR	0.92	0.007
Courthouse	CHLa	PAR, Discharge	0.97	0.0002
Herring	CHLa	SRP, Discharge	0.93	<.0001
Four Sites	CHLa	Substrate, SRP, Discharge, PAR	0.80	<.0001
All Sites	CHLa	PAR, SRP	0.47	<.0001

Table 5. Proportion of mean and seasonal fine benthic organic matter (FBOM) Carbon derived from benthic algae based on concurrent monthly measurements (6-12 months) obtained during 2008 at six study reaches utilizing a C:CHLa ratio of 50.

% Algae Carbon	Crump	Courthouse	Powell	Herring	Kimages
mean	10±0	14±1	11±1	24±2	23±4
winter	9±1	23±3	11±2	19±4	No Data
spring	10±1	15±2	15±2	23±3	10±2
summer	10±0	6±0	8±1	33±5	32±7
fall	No Data	No Data	8±2	18±4	21±4

Figures

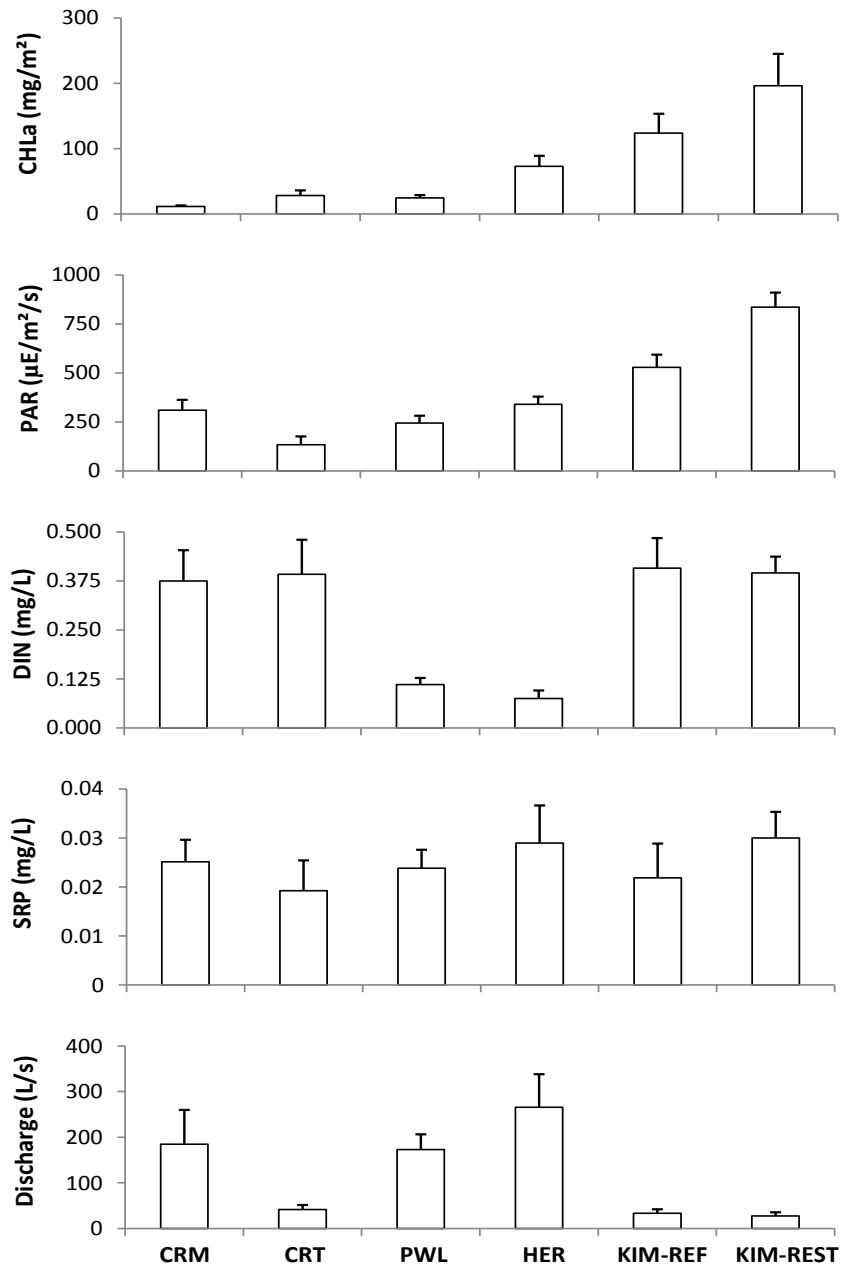


Figure 1. Inter-site comparison of mean CHLa, Incident solar radiation (PAR), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), and discharge based on data collected monthly for 6-12 months over the period of a year during 2008. Bars represent standard error. Abbreviations: Crump (CRM), Courthouse (CRT), Powell (PWL), Herring (HER), Kimages reference (KIM-REF), Kimages restored (KIM-REST).

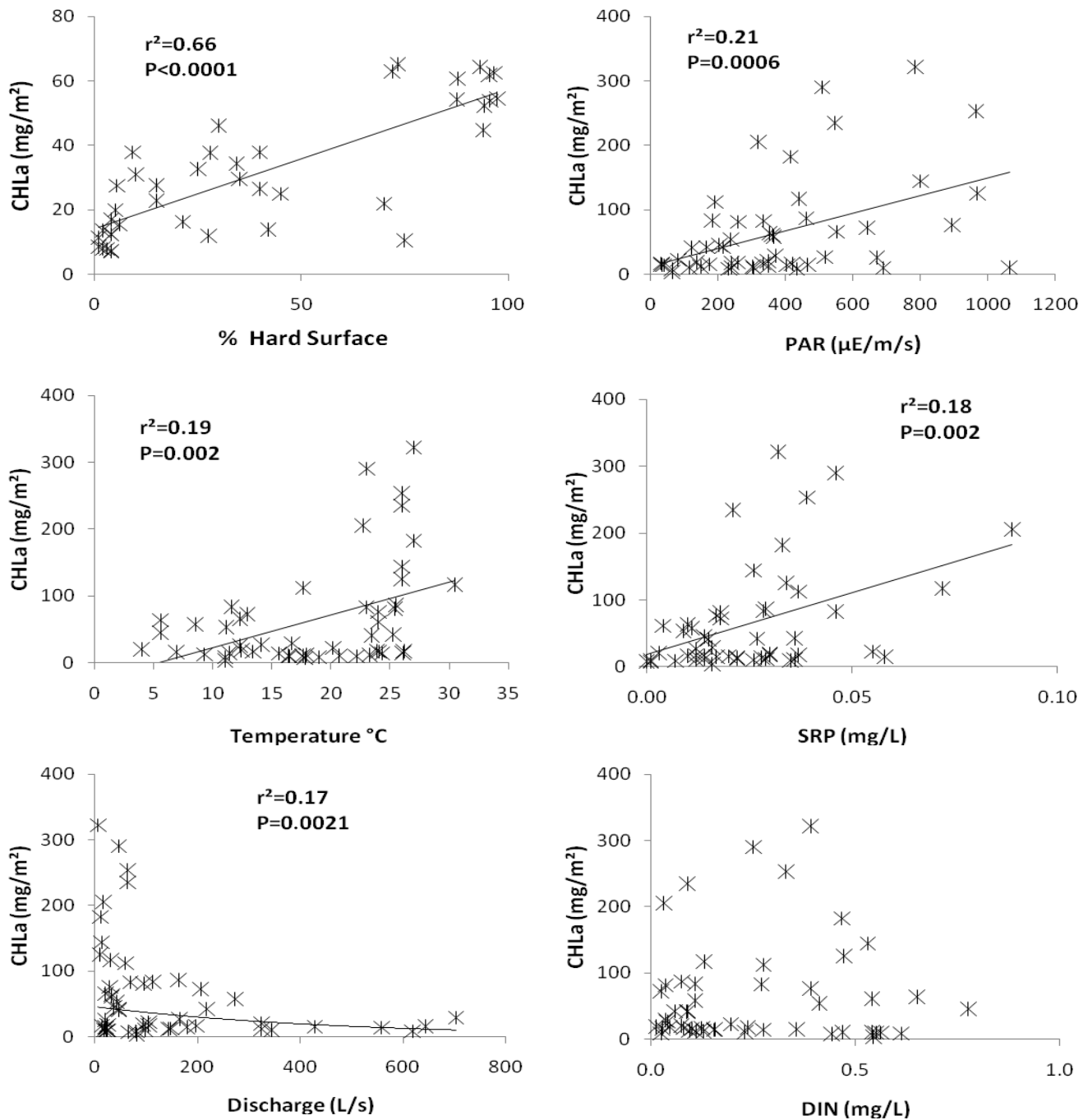


Figure 2. Relationships between benthic CHLa and discharge, water temperature, incident solar radiation (PAR), dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) based on data collected monthly for 6-12 months over the period of a year during 2008 from the six study reaches. Statistical results are based on univariate linear regression, except discharge. R-square denotes the proportion of variance explained by the predictor variables. Percent hard substrate analysis performed without Kimages Creek.

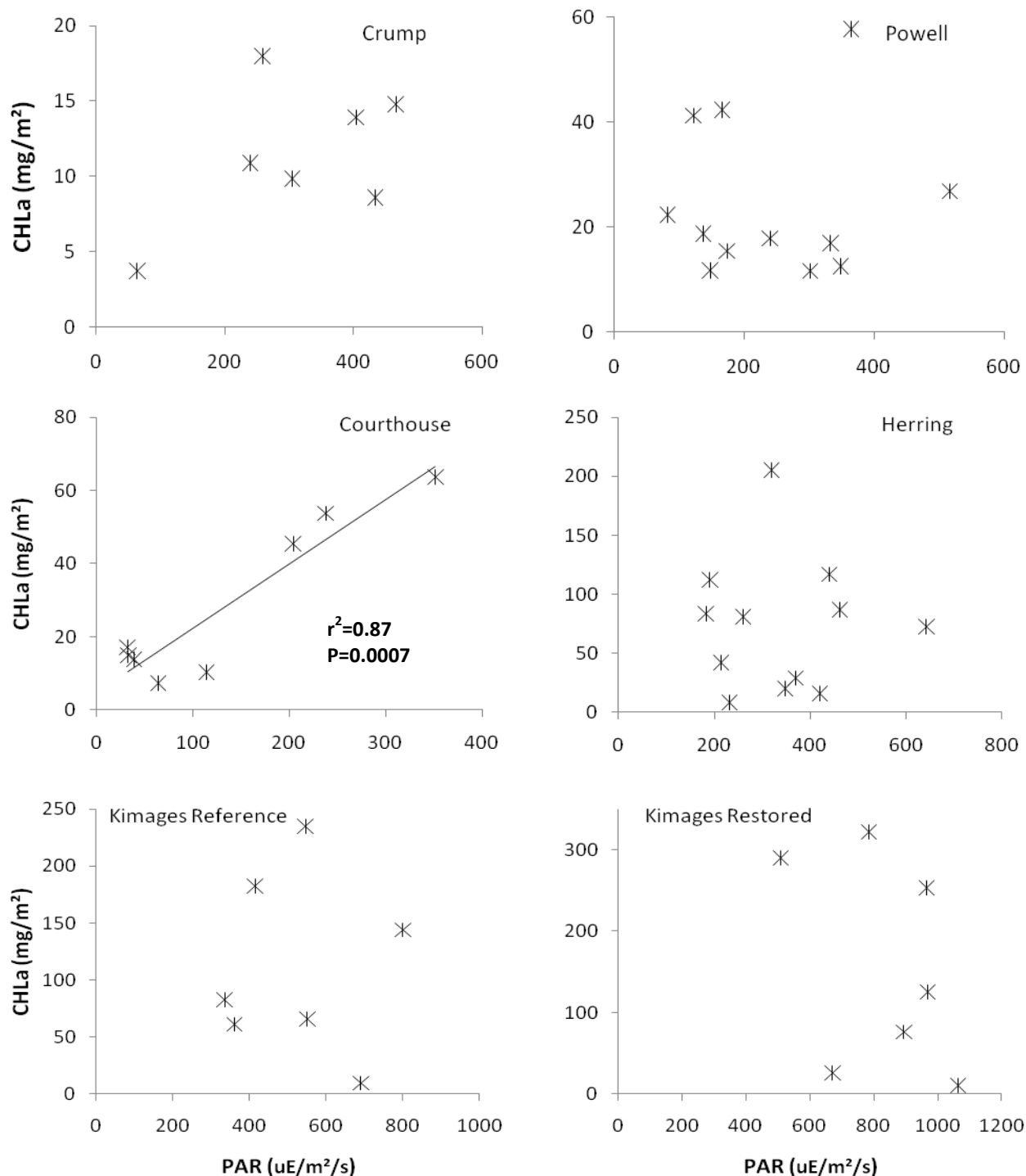


Figure 3. Relationship between CHLa and incident solar radiation (PAR) at six study reaches. Data shown are monthly values obtained in 2008 (Note the difference in X and Y axis scales). Regression lines depicted where significant relationships were found. R-square denotes the proportion of variance in CHLa explained by PAR.

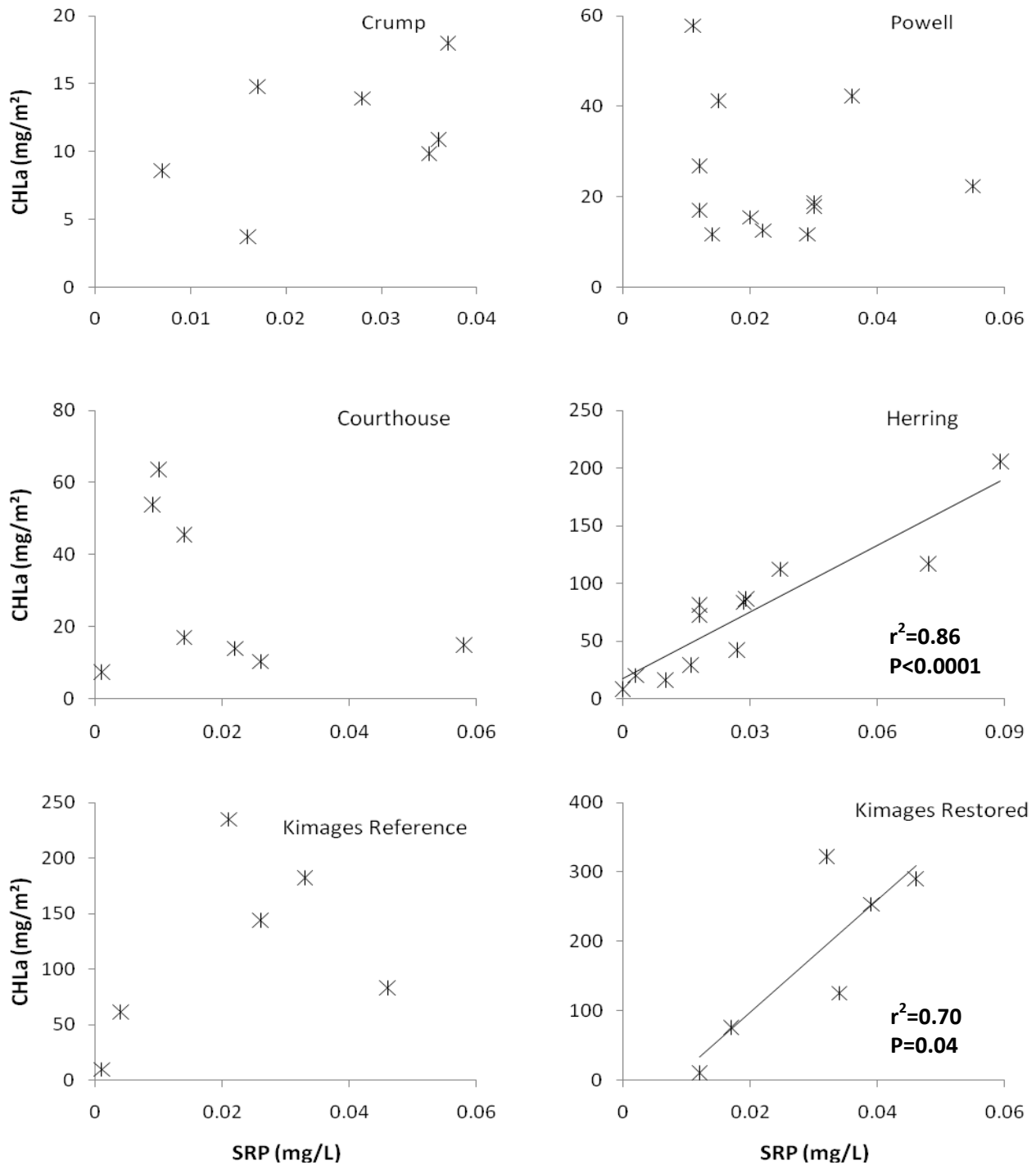


Figure 4: Relationship between CHLa and soluble reactive phosphorus (SRP) at six study reaches. Data shown are monthly values obtained in 2008 (Note the difference in X and Y axis scales). Statistical results pulled from multivariate regression from each reach. Regression lines depicted where significant relationships were found. R-square denotes the proportion of variance in CHLa explained by SRP.

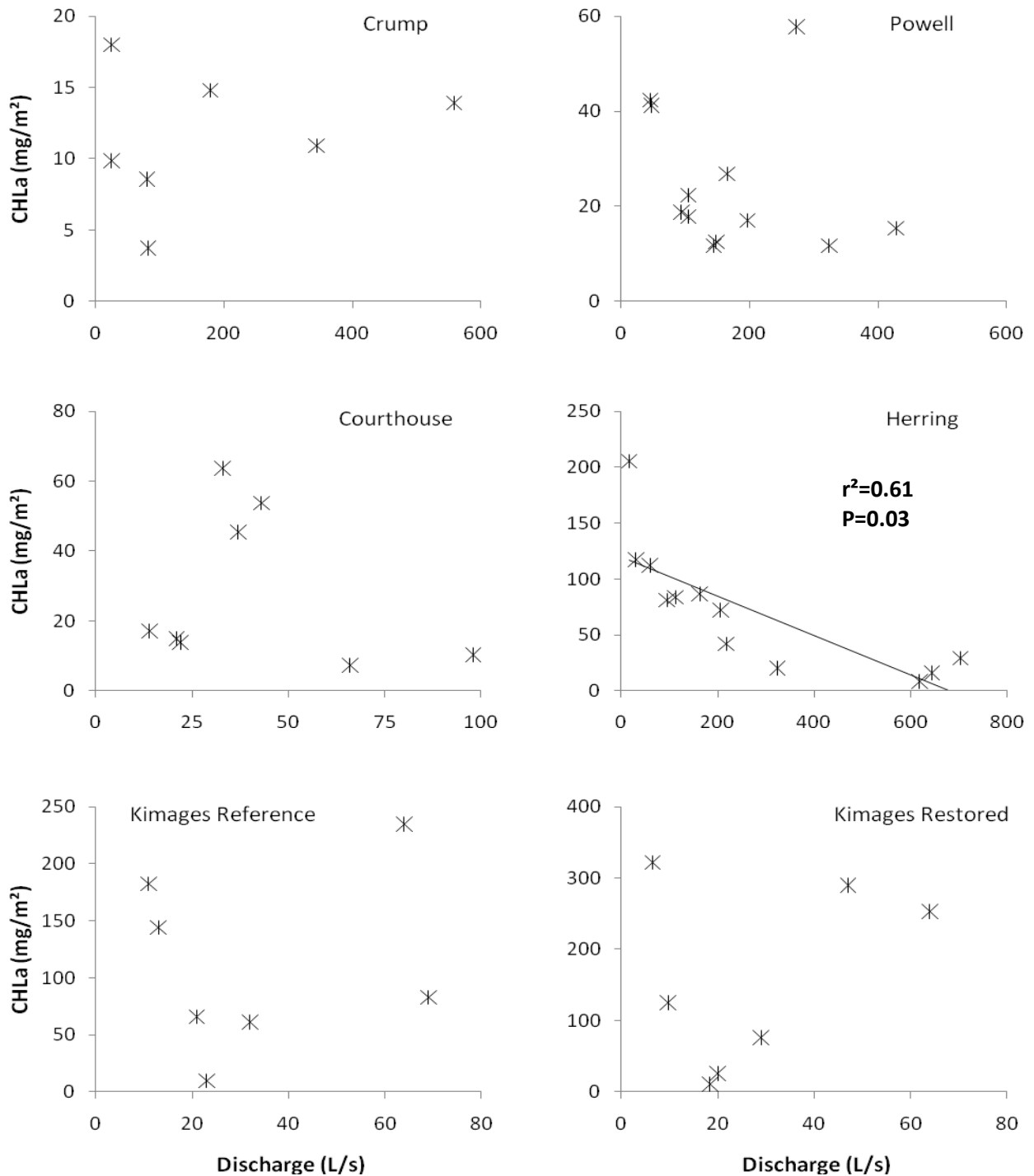


Figure 5. Relationship between CHLa and discharge at six study reaches. Data shown are monthly values obtained in 2008 (Note the difference in X and Y axis scales). Statistical results pulled from multivariate regression from each reach. Regression lines depicted where significant relationships were found. R-square denotes the proportion of variance in CHLa explained by discharge.

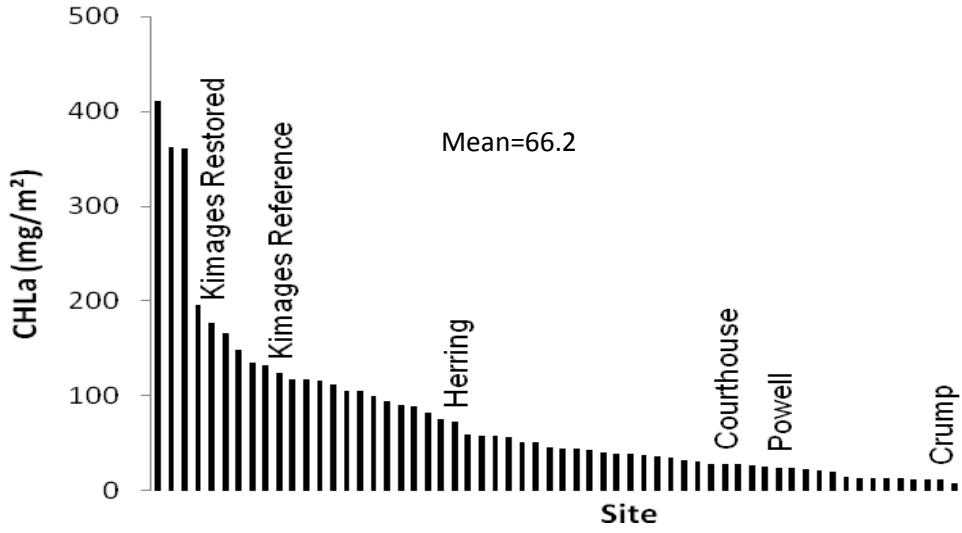


Figure 6. Mean CHLa of streams of the Piedmont and mountain regions of Virginia based on single measurements during 2008 (Data provided by VADEQ). Data from this study added to the figure for comparison.

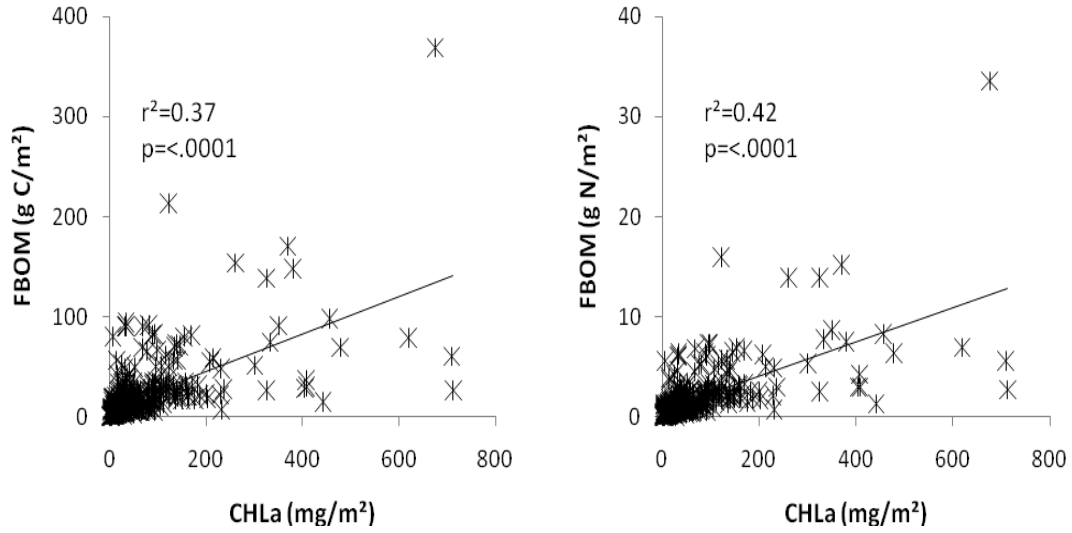


Figure 7. Relationship between mean CHLa and fine benthic organic matter (FBOM) C (g/m²) and N (g/m²). Based on concurrent monthly measurement obtained during 2008 (6-12 months) at six study reaches. Statistical results based on univariate regression. R-square denotes the proportion of variance in FBOM C (g/m²) and FBOM N (g/m²) explained by CHLa.

Appendix A

Appendix 1. Mean values of benthic CHLa, incident solar radiation (PAR), dissolved organic nitrogen (DIN) and soluble reactive phosphorus (SRP), discharge and temperature at Crump Creek. Means are based on data collected monthly for 6-12 months over the period of a year during 2008.

Month	Benthic CHLa (mg/m²)	DIN (mg/L)	SRP (mg/L)	PAR (μE/m²/s)	Discharge (L/s)	Temperature °C
Feb	8.6	0.612	0.007	434	80	11.1
Mar	3.7	0.544	0.016	64	82	11.0
Apr	14.8	0.356	0.017	466	179	11.4
May	13.9	0.274	0.028	404	558	15.6
Jun	10.9	0.230	0.036	240	344	20.7
Jul	9.9	0.562	0.035	305	25	22.2
Aug	18.0	0.046	0.037	259	25	23.9

Appendix 2. Mean values of benthic CHLa, incident solar radiation (PAR), dissolved organic nitrogen (DIN) and soluble reactive phosphorus (SRP), discharge and temperature at Powell Creek. Means are based on data collected monthly for 6-12 months over the period of a year during 2008.

Month	Benthic CHLa (mg/m²)	DIN (mg/L)	SRP (mg/L)	PAR (μE/m²/s)	Discharge (L/s)	Temperature °C
Jan	57.8	0.108	0.011	365	273	8.5
Feb	12.5	0.094	0.022	349	148	9.3
Mar	17.0	0.124	0.012	333	197	13.4
Apr	26.9	0.040	0.012	517	165	14.1
May	11.7	0.128	0.029	302	324	17.9
Jun	17.9	0.236	0.030	240	105	26.2
Jul	42.3	0.090	0.036	166	46	25.3
Aug	41.3	0.088	0.015	122	47	23.5
Sept	11.7	0.109	0.014	148	144	23.3
Oct	22.3	0.195	0.055	82	105	20.2
Nov	18.8	0.012	0.030	137	94	12.4
Dec	15.4	0.100	0.020	174	428	No Data

Appendix 3. Mean values of benthic CHLa, incident solar radiation (PAR), dissolved organic nitrogen (DIN) and soluble reactive phosphorus (SRP), discharge and temperature at Courthouse Creek. Means are based on data collected monthly for 6-12 months over the period of a year during 2008.

Month	Benthic CHLa (mg/m²)	DIN (mg/L)	SRP (mg/L)	PAR (μE/m²/s)	Discharge (L/s)	Temperature °C
Jan	45.5	0.776	0.014	204	37	5.6
Feb	63.7	0.650	0.010	352	33	5.6
Mar	53.8	0.412	0.009	238	43	11.1
Apr	10.4	0.468	0.026	114	98	16.4
May	7.3	0.442	0.001	64	66	17.8
Jun	13.9	0.154	0.022	39	22	24.4
Jul	15.0	0.156	0.058	33	21	26.1
Aug	17.1	0.080	0.014	32	14	24.0

Appendix 4. Mean values of benthic CHLa, incident solar radiation (PAR), dissolved organic nitrogen (DIN) and soluble reactive phosphorus (SRP), discharge and temperature at Herring Creek. Means are based on data collected monthly for 6-12 months over the period of a year during 2008.

Month	Benthic CHLa (mg/m²)	DIN (mg/L)	SRP (mg/L)	PAR (μE/m²/s)	Discharge (L/s)	Temperature °C
Jan	20.1	0.074	0.003	348	324	4.0
Feb	16.2	0.028	0.010	420	643	6.9
Mar	72.4	0.024	0.018	642	206	12.9
Apr	29.1	0.036	0.016	370	703	16.7
May	8.5	0.024	0.000	232	618	19.0
Jun	117.1	0.130	0.072	440	31	30.5
Jul	86.8	0.075	0.029	462	163	25.5
Aug	205.5	0.031	0.089	319	17	22.7
Sept	81.3	0.036	0.018	260	95	25.4
Oct	112.2	0.275	0.037	191	60	17.7
Nov	83.6	0.108	0.028	183	113	11.6
Dec	42.1	0.058	0.027	214	218	No Data

Appendix 5. Mean values of benthic CHLa, incident solar radiation (PAR), dissolved organic nitrogen (DIN) and soluble reactive phosphorus (SRP), discharge and temperature at the reference section of Kimages Creek. Means are based on data collected monthly for 6-12 months over the period of a year during 2008.

Month	Benthic CHLa (mg/m²)	DIN (mg/L)	SRP (mg/L)	PAR (μE/m²/s)	Discharge (L/s)	Temperature °C
Mar	66.0	No Data	No Data	551	21	12.3
May	9.7	0.550	0.001	690	23	16.5
June	144.1	0.530	0.026	800	13	26.0
July	182.4	0.467	0.033	415	11	27.0
Aug	235.0	0.089	0.021	546	64	26.0
Sept	61.3	0.540	0.004	361	32	24.0
Oct	83.0	0.270	0.046	335	69	23.0

Appendix 6. Mean values of benthic CHLa, incident solar radiation (PAR), dissolved organic nitrogen (DIN) and soluble reactive phosphorus (SRP), discharge and temperature at the restored section of Kimages Creek. Means are based on data collected monthly for 6-12 months over the period of a year during 2008.

Month	Benthic CHLa (mg/m²)	DIN (mg/L)	SRP (mg/L)	PAR (μE/m²/s)	Discharge (L/s)	Temperature °C
Mar	25.7	No Data	No Data	671	20	12.3
May	10.8	0.540	0.012	1064	18	17.6
June	125.3	0.472	0.034	969	10	26.0
July	321.9	0.390	0.032	785	7	27.0
Aug	253.4	0.330	0.039	965	64	26.0
Sept	76.2	0.390	0.017	894	29	24.0
Oct	290.1	0.250	0.046	509	47	23.0