

Phosphorus regulates stream injury by filamentous green algae, thresholds, DO, and pH

R. J. Stevenson¹, Brian J. Bennett,² Donielle N. Jordan,² and Ron D. French

¹Center for Water Sciences, Department of Zoology, Michigan State University, East Lansing,
Michigan 48824 USA

²CDM, 100 North Tucker Blvd. Suite 550, Saint Louis, MO 63101 USA

Email: rjstev@msu.edu; bennettbj@cdm.com; jordandn@cdm.com; frenchrd@cdm.com

Abstract

Nutrient concentrations, benthic algal biomass, dissolved oxygen, and pH were measured in 70 or more streams during spring and summer in the Illinois River Watershed (IRW) to determine injury to streams that was related to spreading poultry waste on fields. Nutrient concentrations were independently related to poultry house density in watersheds and percent urban land use in watersheds and were unusually high compared to regions with similar geology and hydrology. Molar N:P ratios were high and indicated that phosphorus was the most likely limiting nutrient. Phosphorus concentrations, as well as poultry house density and urban land use, were related to algal biomass during spring, but were less related during summer. A threshold response in cover of stream bottoms by filamentous green algae (FGA: *Cladophora*, *Rhizoclonium*, and *Oedogonium*) during spring was observed at 27 $\mu\text{g TP/L}$, with increases from averages of 4 to 36 percent cover in streams with TP less than and greater than the TP threshold. Average

concentrations of dissolved oxygen concentration (DO), variability in DO, and pH during spring were positively related to TP, chlorophyll a, and FGA cover. Minimum DO during spring and early morning DO during summer were negatively related to TP concentration. Spring pH and summer DO frequently violated water quality standards for protecting biodiversity. We conclude that poultry house operations as well as urban activities, independently and interactively, pollute IRW streams with phosphorus that results in an injury to aesthetic condition and potential for injury of biodiversity.

Keywords: phosphorus, poultry waste, benthic algae, *Cladophora*, streams

Introduction

Nutrient pollution has long been recognized as a widespread and important contaminant of streams and rivers (MacKenthun, 1968; Carpenter et al., 1998; Smith et al., 1999; USEPA, 2007). Low concentrations of nutrients occur naturally in water and are essential for the growth of algae, which supports the production of invertebrates and fish. Nutrient pollution, the loading of nutrients from human activities in streams and rivers, causes many instream as well as downstream problems. Recreation and biodiversity are two valued ecological attributes, more recently called ecosystem services (Millennium Assessment, 2005), that are affected by nutrient pollution and excessive growths of algae.

Excess algae resulting from nutrient pollution can dramatically reduce the desirability of recreational use of streams when algae on the stream bottom exceed 150 mg/m^2 of chlorophyll a (Suplee et al., 2008). Multiple lines of evidence show that the natural balance of stream flora and

fauna (*sensu* Karr and Dudley, 1981), herein referred to as biodiversity, are diminished by nutrient pollution. Correlations between human alterations of watersheds and nutrient concentrations measured in stream surveys, experiments, and process-based models show that nutrients from urban and agricultural activities move from land to waterways via surface and groundwater (Allan et al., 1997; Johnson et al., 1997; Sharpley et al. 2007; Tesoriero et al., 2009). Stream surveys, experiments, and models also confirm that nutrients stimulate growth (Bothwell, 1985; Dodds et al., 1997; Biggs, 2000; Rier and Stevenson, 2006) with both N and P being potential limiting factors in streams (Francoeur, 2001). The biodiversity of algae, invertebrates, and fish have also been related to nutrient concentrations (Miltner and Rankin, 1997; Wang et al., 2007; Stevenson et al., 2008).

Additional research is needed to sufficiently refine and quantify relationships among human activities, nutrients, and ecological response for application in environmental management. Quantitative relationships among these variables are needed to evaluate tradeoffs between costs and benefits of reducing nutrient pollution. Thresholds in ecological responses along environmental gradients help develop consensus among stakeholders for management actions (Muradian 2001). Thresholds in algal biomass and biodiversity along nutrient gradients have been hypothesized and observed (Dodds et al., 1997; Stevenson, 1997; Stevenson et al., 2008). The biodiversity and response of streams to nutrient pollution varies with climate, geology, stream size, and hydrogeomorphology (Biggs et al., 1995; Riseng et al., 2004; Dodds and Oakes, 2004). Thus, regional studies are needed for regional applications to better understand variability in these relationships caused by interactions with natural factors (e.g., climate and geology) and anthropogenic factors (e.g., nutrient sources and hydrologic modifications) that vary among regions and stream types (e.g., size, geomorphology).

This study was initiated because the Attorney General of the State of Oklahoma filed a lawsuit against numerous poultry companies. Results of this study were designed to quantitatively relate nutrient pollution to decreases in ecosystem services (i.e., injury) and evaluate whether poultry waste application to fields was a significant contributor to that injury. These evaluations would be used to determine whether the application of poultry wastes to fields should be stopped and to assign monetary damages for the ecological injury caused by poultry wastes. Additional studies were conducted to document sources and fates of nutrients in the IRW, model the proportion of nutrient pollution from different sources (e.g., municipal waste water treatment plants, septic systems, cattle, crop fertilization), determine downstream effects on a reservoir, and quantify the value of ecosystem services and injury to them.

The specific objectives of the research presented in this paper were to measure physical, chemical, and algal conditions in approximately 70 streams during spring and summer seasons, as well as land use in these watersheds. Then we evaluated injury of IRW streams resulting from phosphorus pollution, poultry manure application, and other phosphorus sources. We tested a set of hypotheses to evaluate the direct and indirect relationships among phosphorus sources (as indicated by land use), nutrient concentrations, algal biomass, and chemical stressors (DO and pH) of stream biodiversity. In particular, we were interested in evaluating relationships for thresholds which could be used to establish benchmarks for injury. Based on these relationships, the likely effects of poultry manure spreading on aesthetics and biodiversity were determined.

Methods

Study area

The Illinois River Watershed is a 4328 km² watershed in the Ozark Highlands ecoregion flowing from Arkansas to Oklahoma (Figure 2). The watershed is underlain by karst geology with gneiss and limestone bedrock covered by thin soils. Therefore, rainfall travels to streams via groundwater and surficial runoff resulting in a channel with riffle and pool sequences in which riffles are dominated by cobble or simply exposed bedrocks. Stream discharge is usually highest during winter and early spring and may drop to expose riffles from late spring through fall. Rains may occur anytime during the year and generate sufficient flow to fill the channel. Based on this flow regime, we would expect that spates and drought limit secondary production and allow accumulations of high biomasses of diatoms as well as filamentous green algae given sufficient nutrients and time for algal colonization (Riseng et al., 2004; Stevenson et al., 2006).

Urban, agricultural, and forested land comprise 8.8, 43.7, and 46.0% respectively, of the land cover in the Illinois River Watershed. Most (99.7%) of the agricultural land is pasture, with a very small proportion planted with row crops. There are 3226 poultry houses in the Watershed in which poultry are or have been raised and manure is produced within the Illinois River Watershed. Sixty percent of these poultry houses are active, 26% are inactive, and others are abandoned (11%), removed (3%), or suspected to be poultry houses (9%)(Fisher, 2008). Three small urban centers are located at the head of two of the five major tributaries. Municipal wastewater treatment plants are co-located with these urban areas. A phosphorus mass balance (Engel 2008) indicates that 76% of the phosphorus additions to the IRW result from import of poultry feed for poultry operations with other sources greater than 1% being commercial fertilizers (7.5%), dairy cattle (5.2%), humans (3.2%), swine (2.9%), industrial sources (mostly poultry processing facilities, 2.7%), and beef cattle (1.7%).

Sampling, sample analysis, and map analysis

To evaluate the injury of the IRW resulting from poultry waste application to fields, we first had to quantify relationships among indicators of all major types of human activities, nutrient pollution, algal biomass, and chemical stressors of biodiversity (low DO and high pH) in the IRW (Figure 1). We used percentage of different urban and agricultural land uses in watersheds and poultry house density to characterize human activities and test hypotheses about relationships between sources of phosphorus and phosphorus concentration. We then tested the hypotheses that nutrient pollution, particularly phosphorus concentrations are related to poultry house operations, algal biomass, dissolved oxygen concentrations and pH. If relationships are statistically significant, we determine the magnitude of effects (injury) that are statistically related to poultry house operations and phosphorus pollution. Throughout this process, assumptions were tested statistically and alternative hypotheses were explored thoroughly.

Approximately 70 stream sites were selected for sampling to develop relationships illustrated in Figure 1 using a random stratified sampling design. Seventy sites were selected because in a previous study (Stevenson et al., 2006) using similar sampling methods, 70 sites were determined to be adequate for characterizing relationships among land use, nutrients, and algal biomass. Because our goal was to evaluate poultry house operations effects on nutrient pollution, and poultry operations were the dominant source of phosphorus in the watershed (Engel, 2008), we randomly selected sites from five groups of potential sampling sites (the strata). Sites assigned to these five groups based on poultry house density and geographic location in the IRW. The range in poultry houses was split evenly in five sub-ranges or quintiles.

All potential sampling sites were assigned to these groups. Then even numbers of sampling sites were selected from each group (Figure 2). Our goal in selecting sites by groups was to get a full range of nutrient conditions associated with poultry house operations and to have relatively even spacing geographically across the watershed. We expected to get a range of phosphorus and nitrogen concentrations that would bridge benchmarks for limiting concentrations of total phosphorus (TP), soluble reactive phosphate (SRP), total nitrogen (TN), and nitrate-nitrite (NO_x). Based on past experiments and surveys, we expected these limiting nutrient concentrations to be approximately 0.020-0.030 mg P/L for TP and SRP, 0.200 mg NO_x /L, and 0.500 mg TN/L (Bothwell, 1988; Dodds et al., 1997; Rier and Stevenson, 2006).

Two field sampling campaigns were conducted during summer 2006 and spring 2007. Spring and summer were selected as sampling periods because the potential for: 1) high algal biomass related to filamentous algal growth is greatest during the spring and 2) low dissolved oxygen is usually greatest during the warm-water, low-flow periods during summer. Land use, habitat characteristics, water chemistry, algal biomass, dissolved oxygen, and pH were determined for all sites. During the summer 2006 sampling campaigns, each site was visited and sampled one time. During the spring 2007 campaign, each site was sampled weekly for eight weeks. We sampled streams weekly during the spring 2007 campaign from mid-March through mid-May to characterize average conditions and the variation in conditions during a period when we expected spring rains to cause high temporal variability in water chemistry and algal biomass. In addition, we sampled streams in a haphazard order from week to week to eliminate bias that could be caused by diurnal variation in temperature, light, and nutrient concentrations. This repeated sampling of sites at times throughout the day provided the opportunity to examine

variability at a site that could be related to a systematic diurnal cycle associated with algal photosynthesis and respiration.

The sampling reach in each stream was defined as two or more riffles. Percent canopy cover was determined with a canopy densitometer. The pH, conductivity, and temperature of streams were measured in the field with an Oakton model 300® multimeter during each site visit. DO was measured colorimetrically in the field with a Chemetrics V-2000® photometer, vacu-vials, and reagents during each site visit. Water samples for analysis of TP and SRP concentrations were collected during each of the eight visits during the spring 2007 campaign. Water samples for TN, NO_x, and ammonia (NH₃-N) were collected once from each site during each season. Water samples were stored on ice and shipped via overnight courier to a laboratory for nutrient chemistry analysis. In the laboratory, standard methods (APHA 1998) were used to assay nutrient concentrations.

Benthic algal samples were collected one time during each field campaign to determine ash-free dry mass and chlorophyll a (chl a) concentrations of all algae in the streams during the summer 2006 and spring 2007. Algae were sampled by scraping algae from known areas of rocks in five 3-rock clusters that were randomly selected from the stream bottom. Sampling occurred during base-flow periods after at least of week without high discharge events. Scrapings from the five 3-rock clusters were kept separate for chl a analysis to provide an estimate of the mean and variance in periphyton at sites. Samples from the 3-rock clusters were filtered and frozen the day of sampling and shipped to a commercial laboratory for chl a determination. There, chl a was measured fluorometrically (APHA 1998). Areas of rocks scraped and sub-sample volumes were recorded to enable calculation of chl a per unit area of stream bottom ($\mu\text{g chl a/cm}^2$).

Cover of the stream bottom by filamentous green algae is an indicator of algal biomass and aesthetic problems caused by these algae. We estimated the percent cover of stream bottoms with visual observations (Stevenson and Bahls, 1999; Stevenson et al., 2006). An area of stream was delimited using a viewing bucket or frame in which a grid of 50 points was arranged. The grid helped quantify percent cover of the stream bottom by filamentous algae. Twenty observations were made along 5 to 10 transects in riffles of the sampling reach. The percent cover of the stream bottom by filamentous algae and type of filamentous algae composing the cover was recorded. Based on a preliminary stream survey, field crews were trained to identify *Cladophora*, *Rhizoclonium*, Zygnematalean filaments (*Spirogyra*, *Mougeotia*, and *Zygnema*) *Tetraspora*, *Draparnaldia*, *Vaucheria*, and *Batrachospermum* based on growth form, color, and feel. Subsamples of macroalgae were collected for testing identifications and verification. Macroalgal testing showed that *Oedogonium* also occurred in streams and was difficult to distinguish from *Cladophora* and *Rhizoclonium*. They were difficult to distinguish from each other because they all feel rough in the field and branching patterns can be variable and difficult to observe. Since algae in these genera (*Oedogonium*, *Cladophora*, and *Rhizoclonium*) pose the greatest problems for nuisance accumulations in IRW streams and they were difficult to distinguish, we grouped them in a category that we refer to as filamentous green algae (FGA).

Land-use categories were determined as a percentage of basin area using land use classifications and maps from the 2001 National Landcover Dataset, which was assembled by the MultiResolution Land Characteristics Consortium and is derived from 30-meter resolution Landsat satellite imagery. We selected percent urban and agricultural land use in watersheds for indicators of the effects of those human activities on stream condition, which is a common approach for relating land uses to stream condition (Allan et al., 1997; Johnson et al., 1997). In

addition, poultry house density (houses/mi²) was determined for each watershed plus a 2 mile buffer around watersheds. The 2 mile buffer around watersheds was included as a source of poultry waste to a watershed because poultry waste is transported relatively short distances from the house and may be across watershed boundaries (Bert Fisher, personal communication). Thus, poultry houses outside the watershed of a stream could be contributing to P loading. That hypothesis was tested by relating P concentration in a stream to the density of poultry houses in the watershed when poultry houses within the watershed only were counted and poultry houses within 2 miles of the watershed were counted as being in the watershed. The correlation with stream P was greatest when poultry houses within 2 miles of the watershed were included in the number of poultry houses in the watershed (Engel, 2008). We used total poultry house density as an indicator P contamination of watershed soils because inactive and abandoned houses were past sources of waste, and phosphorus can persist in soils long after waste application.

Statistical methods

To test hypotheses systematically, we grouped variables into four categories according to their causes and effects in the conceptual model (Figure 1). These categories were: 1) land use and land cover, 2) nutrients, 3) algal biomass, and 4) chemical stressors. The direct and indirect effects among the 4 categories of variables were then related by regression analysis to quantify relationships. For example, first we related direct effects of land use on nutrient concentrations and we characterized N:P ratios to determine the most limiting nutrient. Then we related algal biomass to nutrient concentrations, a direct relationship, followed by relating land use to algal

biomass, an indirect relationship. Finally we quantified relationships between DO and pH with algal biomass, nutrient concentrations, and land use, successively.

The rationale for this approach is direct relationships between cause-effect factors should be the most precise, and they help evaluate plausible causes. Indirect relationships were valuable for two reasons. One, some independent variables like land use vary less over time than nutrient concentrations in streams that vary with time of day and weather. Therefore, land use could provide a more precise and accurate characterization of nutrient concentrations affecting benthic algae than actual measured nutrient concentrations in waters of the stream. Two, independent variables help us determine the causal pathway. If poultry house density in the watershed is related to nutrients, algal biomass, and DO or pH, then it is plausible that poultry operations caused these alterations of IRW streams, even if they are not all related.

At each successive step in the causal pathway the range of conditions was characterized to provide a foundation for expected effects and for comparison with other regions. We used regression analyses to test hypotheses about relationships to determine whether they were statistically significant (unlikely to be observed by chance) and whether they were biologically significant (had a large effect).

All variables were transformed if necessary to meet the assumptions of parametric statistical techniques. Distribution of each variable was plotted in a results file and examined to determine whether they were skewed. We used logarithmic, square root, and positive power transformations (e.g. X^2 or X^{10}) to normalize distributions of variables. Distributions of transformed variables were also plotted and transformed using another power until their distributions were not skewed. Variables with too many zeros were not included in the statistical analyses. In addition to plotting distributions of variables before statistical tests, residuals were

plotted and examined after statistical tests to ensure they were near-normally distributed. Outliers were removed from analyses in some cases. In no case were results of the statistical tests substantially different before and after outlier removal. If they were, the cause of the outlier being unusual would be recorded and discussed.

Thresholds in relationships were identified using CART (classification and regression tree analyses) analysis. Thresholds were evaluated by comparing the variance explained by the non-linear model identified by CART analysis and linear model from regression. The ratio between variances explained by CART and linear models was used as a positive indicator of the biological and political significance of thresholds. All analyses were conducted using SYSTAT 11.0.

For data collected during spring, analyses were restricted to a single observation per site using a summary statistic, such as averages, minima, maxima, or standard deviations. The use of one estimate from each site maintained the independence in observations in the dataset, which can be a problem when repeated measures are collected from sites and used in statistical analyses.

Results

Land Use and Nutrient concentrations

A broad range of phosphorus concentrations was observed in rivers of the IRW, with slightly higher TP and SRP concentrations observed during summer than spring ($p < 0.05$). TP concentrations ranged from less than 0.010 to more than 0.648 mg TP/L during both spring and

summer, with median concentrations of 0.057 and 0.076 mg TP/L during spring and summer, respectively (Figure 3). SRP concentrations ranged from less than or equal to 0.002 to more than 0.059 mg SRP/L during both spring and summer, with median concentrations of 0.034 and 0.057 mg SRP/L during spring and summer, respectively. Thus TP concentrations ranged from less than our benchmark for phosphorus limitation to greater than that benchmark (0.030 mg SRP/L).

Nitrogen concentrations as TN were higher during spring than summer ($p < 0.05$), ranging from less 0.800 to more than 20.4 mg TN/L in both spring and summer, with median TN concentrations of 4.0 and 2.8 mg/L during spring and summer, respectively. NO_x concentrations were not different in spring and summer, ranging from 0.1 to more than 7.5 mg $\text{NO}_x\text{-N/L}$ in both spring and summer, with median TN concentrations of 1.8 mg/L during both spring and summer. Where as TN concentrations were above concentrations that we would expect to be limiting benthic algal growth, NO_x concentrations were slightly below our benchmark for nitrogen limitation of algal growth.

N:P ratios indicated that phosphorus was more likely limiting than nitrogen, and were higher during spring than summer. N:P ratios ranged from 20.9 to 1890 during spring with a median of 261 and from 12.8 to 1024 during summer with a median of 152. Temperature and conductivity were also lower during spring than summer (Figure 3). Median water temperatures were 17°C during spring and 25°C during summer. Median conductivities were 294 and 449 mmhos/cm during spring and summer, respectively.

Regression analyses indicated that both TP and TN concentrations in IRW streams during spring were related to poultry house density as well as the percent urbanized land use in watersheds (Figure 4A & B). Forward stepwise multiple linear regression with land use attributes and watershed area produced a TP model with significant relationships between TP and both

poultry house density (PHD) and urban land use ($p \leq 0.001$, Table 2). The resulting TP model predicted that natural background TP concentration, when percent urban land use and poultry house density both equal 0, was 0.007 mg/L. Forward stepwise multiple linear regression with land use attributes and watershed area produced a TN model with significant relationships between TN and both poultry house density and urban land ($p = 0.019$ and < 0.001 , respectively). The spring TN model predicted that natural background TN concentration, when urban and poultry house density both equal 0, was 1.15 mg/L.

TP concentrations in IRW streams during summer 2006 were related to poultry house density, but effects were masked statistically by high TP in waters downstream from urban land use (Figure 4C and D). TP concentrations were very weakly related to poultry house density in a multiple regression model ($r^2 = 0.223$) that included both poultry house density and the percent of watersheds that were urbanized (Table 2). The -4.292 intercept in this model indicated the natural background concentration of TP was 0.014 mg TP/L. If analysis was restricted to a subset of data in which urban land use was less than 10 percent of the watershed, poultry house density was significantly related to TP concentration ($p = 0.014$, Figure 4D). Multiple regression showed that TN was not related to poultry house density during summer 2006, even if the dataset was constrained to streams with low urban activity ($p = 0.369$); but TN during summer was significantly related to urban land use ($p < 0.001$),

Algal biomass

Benthic algae in the IRW were composed mostly of diatoms, filamentous green algae, the red alga *Batrachospermum*, and the filamentous xanthophyte *Vaucheria*. *Cladophora*, *Rhizoclonium*,

and *Oedogonium* were the most common filamentous green algae observed in streams.

Spirogyra, *Mougeotia*, *Zygnema*, and *Draparnaldia* were also observed, but less frequently.

Algal biomass was higher during spring 2007 than summer 2006. Benthic algal chl *a* was greater during spring 2007 than summer 2006 (Figure 5). During spring, chl *a* ranged from 0.2 to 33.5 $\mu\text{g}/\text{cm}^2$ with a median of 4.9 $\mu\text{g}/\text{cm}^2$. During summer, chl *a* ranged from 0.007 to 13.8 $\mu\text{g}/\text{cm}^2$ with a median of 3.79 $\mu\text{g}/\text{cm}^2$. During spring 2007, FGA cover ranged from 0 to 91 percent of the bottom of streams with a median of 20.8 percent FGA. During summer, FGA cover ranged from 0 to 85.8 percent of the bottom of streams with a median of 3.1 percent during the summer.

Benthic algal chl *a* and FGA during spring were significantly related to TP concentration ($p < 0.001$) (Figure 5, Table 2). Chlorophyll *a* and FGA were more highly correlated with P concentrations than nitrogen (Table 2). Both measures of algal biomass were also more highly correlated with TP than with SRP or TDP. Benthic chl *a* and FGA during spring was significantly related to poultry house density ($p = 0.001$) and percent urban land use ($p < 0.001$) in a model determined by stepwise multiple linear regression (Figure 6, Table 2). For both measures of algal biomass, agricultural land use was also related to benthic algal biomass, but not after variation associated with poultry house density was accounted for.

Thresholds were indicated in chl *a* and FGA cover along the TP gradient, but only the FGA CART model explained substantially more variation than the linear model. A changepoint in benthic chlorophyll *a* was identified at 0.029 mg TP/L, with an average biomass of 1.3 and 5.4 $\mu\text{g chl a}/\text{cm}^2$ below and above the changepoint (Figure 5). The CART relationship explained 32.8 percent of the variance in benthic algal biomass. This compared to 39.1 percent of explained variance in benthic chlorophyll *a* using a linear regression model with TP concentration. A

CART changepoint in spring FGA cover was identified at 0.027 mg TP/L, with an average FGA cover of 4 and 36 percent in TP concentrations less than and greater than the TP changepoint, respectively (Figure 5). The CART relationship explained 42.7 percent of the variance in FGA cover compared to 28.7 percent of variance explained by a linear regression model with TP concentration.

The proliferations of benthic algae were related to canopy cover during the spring, but positively rather than negatively as predicted.

Benthic algal biomass was significantly related to TP concentrations during summer ($p < 0.05$, Figure 5). Summer biomass of FGA was not related to TP concentrations in streams ($p = 0.313$). Benthic algal biomass was related to urban land use ($p = 0.013$), but not to poultry house density during summer 2006 ($p = 0.429$, Figure 6). Cover of the stream bottom by FGA was not related to poultry house density or urban land use during summer 2006 ($p > 0.190$, Figure 6). The lack of relationships between either measure of algal biomass and poultry house density was true even if data analyses were restricted to sites with low urban activity to reduce statistical effects of sites with high urban activity.

DO and pH

DO concentrations in streams over the 8-week spring 2007 sampling period were higher than during summer, with an average of 10.1 mg/L, a minimum of 7.6, and a maximum of 13.4 (Figure 7). Average DO concentration in streams during the summer 2006 sampling period was 5.2 mg/L with a minimum of 0.9 and a maximum of 9.2. DO concentration varied with time of day that sites were sampled during spring. Sites sampled early and late during the day had lower

DO than during mid-day. The standard deviation in stream DO during the 8-week spring 2007 sampling period averaged 1.8 mg/L with a minimum of 0.4 and a maximum of 3.7 mg/L. The minimum DO observed in each stream over the eight week period averaged 7.6 with the lowest value being 4.1 and the highest minimum being 9.7 mg/L.

Average DO measured in a stream during spring was positively related to benthic algal biomass ($p=0.003$), FGA cover ($p<0.001$), TP concentration ($p=0.05$), and poultry house density and percent urban land use in watersheds ($p\leq 0.002$) (Figure 8). The standard deviation in DO of streams during the eight weeks of sampling was positively related by multiple linear regression to chl *a* and FGA cover ($p<0.001$) as well as total phosphorus, poultry house density, and percent urban land use in watersheds upstream from the sites sampled ($p<0.05$, Figure 9). Minimum DO concentrations over the 8 week period were not clearly linked to higher algal biomass or poultry house density, but they were related negatively ($p=0.001$) to TP concentrations in streams (Figure 10).

DO in streams during summer was not related to TP concentration when all streams were included in the analysis (Figure 11). However, when we limited the streams in the data set to streams with a low percentage of urban land use in their watersheds, DO was significantly negatively related to TP concentration. Summer DO was complexly related to land use (Figure 11). In a two factor multiple regression analysis, DO was positively related to urban land use ($p<0.05$) and not significantly related to poultry house density. Again, the statistical masking of poultry house effects on DO by urban effects became evident; DO was negatively related ($p=0.075$) to poultry house density if the dataset was limited to streams with less than 10 percent urban land use in watersheds

The median of the 8-week spring average of pH in IRW streams was 7.8, with a 6.9 minimum, and a maximum of 9.1 (Figure 7). The maximum pH in an IRW stream during the spring 2007 had an average value of 8.8 with the lowest maximum of 7.2 and the highest pH maximum of 10.6. These maximum values of pH are higher than 9.0 more than 25 percent of the time. Median pH during the summer was 7.6 with a minimum of 6.1 and maximum of 8.3.

The high pH in IRW streams during spring was not due to natural causes. Average pH at sites increased with increasing benthic algal biomass ($p=0.118$), FGA cover ($p=0.001$), TP concentration ($p=0.013$), urban land use ($p=0.03$), and probably poultry house density ($p=0.166$) (Figure 12). Maximum pH observed at sites was better correlated with these causal variables than average pH. Maximum pH at sites increased with increasing benthic algal biomass ($p<0.001$), FGA cover ($p=0.005$), TP concentration ($p=0.004$), and urban land use ($p<0.001$), and poultry house density ($p=0.037$) (Figure 13). We did not observe significant variation in pH with time of day during spring as we did DO concentration. During summer, one time measurements of pH were not related to TP concentration, poultry house density, or urban land use.

Discussion

Nutrients and land use

Nutrient pollution was relatively high in the IRW watershed versus other watersheds in the US with similar geology and hydrogeomorphology. Nutrient concentrations in minimally disturbed watersheds (*sensu* Stoddard et al., 2006) were predicted to average 0.010 mg TP/L in the IRW based on regression models relating nutrient concentration to urban land use and poultry house

density in watersheds. Dodds and Oakes (2004) observed variation in predicted minimally disturbed conditions among ecoregions from 0.010 to 0.070 mg TP/L. Phosphorus concentrations in minimally disturbed watersheds in the Knobs ecoregion of Kentucky-Indiana and the mid-Atlantic Highlands, which had similar geology, soils, and stream hydrology as the IRW, were 0.010 mg TP/L (Stevenson et al. 2006, 2008), which is the same as the IRW. Given similar minimally disturbed conditions in these regions, we compared the 25th and 75th percentiles of TP concentrations in streams of the three regions. We calculated these percentiles using data from the previously studied regions and found for the streams of the IRW during spring, Kentucky-Indiana Knobs region during spring, and the mid-Atlantic Highlands during early summer, that the 25th percentiles of TP concentrations were respectively 0.030, 0.010, and 0.010 mg/L and the 75th percentiles were 0.127, 0.027 and 0.030 mg/L. We did not review TN concentrations in the three regions, because N:P ratios were greater than 16:1 in almost all streams. A 16:1 molar N:P ratio indicated that phosphorus was the most likely limiting nutrient (Redfield 1958), if nutrients were sufficiently low to limit algal growth.

Elevated phosphorus and nitrogen concentrations were related to poultry house operations in the IRW, as well as urban activities. First, a large portion of the phosphorus entering the IRW was related to poultry operations according to mass balance models (Engel 2008). Nutrients in poultry waste are readily transported via runoff and percolation to groundwater sources of stream flow in hydrogeological settings like the IRW (Sharpley et al., 2007). Many cattle occur in the IRW, but the phosphorus in their manure can be attributed to grass they eat, which used the phosphorus that has been applied to fields in the form of poultry waste (Tarkalson and Mikkelsen, 2003). Relatively little addition of phosphorus is imported into the IRW for fertilizing fields and feeding cattle. In addition, nutrients were statistically related to

poultry house density in watersheds and better related to poultry house density than percent agricultural lands in the watershed even though the litter is applied to pastures. Finally, many watersheds in the IRW have high poultry house density, high phosphorus, and low urban land use, leaving poultry waste as the only reasonable source of phosphorus in those streams.

Algal biomass

High phosphorus pollution associated stimulated accrual of benthic algae in IRW streams during spring and summer, but in different forms. Stimulation of algal growth across the range of phosphorus conditions in the IRW has been observed in numerous other studies (Dodds et al., 1997; Biggs, 2000; Stevenson et al., 2006). Chlorophyll a and FGA cover were both positively related to TP concentration, as well as TDP and SRP concentrations, during spring. Only benthic chlorophyll a was related to TP during summer. FGA densities were low and unrelated to TP during summer, probably because optimal temperatures for FGA growth occur during spring. *Cladophora*, the most common taxon observed in FGA, is widely recognized to have a spring growing season in this climatic zone (Dodd and Gudder, 1992).

A threshold in the relationship between FGA and TP was observed during spring at 27 $\mu\text{g TP/L}$. This threshold is supported by our comparison of the variance explained by non-linear and linear models with the CART model explaining 50% more variance than the linear model. Thresholds in algal biomass response to nutrients have often been observed close to this TP concentration. Peak biomass of diatoms and filamentous Zygnematales in stream-side experiments was saturated at 30 $\mu\text{g TP/L}$ (Bothwell, 1988; Rier and Stevenson, 2006), i.e. biomass increased greatly in treatments with TP concentrations ranging near zero to 30 $\mu\text{g/L}$ and

increased little when TP was greater than 30 $\mu\text{g/L}$. Dodds et al. (1997) found that accrual of benthic algae from streams throughout the world, measured as chlorophyll a, was also saturated at approximately 30 $\mu\text{g TP/L}$.

We re-evaluated data on *Cladophora* cover and TP concentrations in >100 streams from Kentucky and Michigan (Stevenson et al. 2006) using CART analysis; these streams were sampled using the same methods as in this IRW stream study, per stream rapid periphyton assessments and water chemistry sampling with 4-8 sampling times per stream in an 8-week period. CART analysis of the Kentucky-Michigan stream data showed a threshold in TP concentration at 0.023 mg TP/L (Figure 14). The variance explained by the CART model was twice as great as a linear model for the *Cladophora*-TP relationship. *Cladophora* cover was predicted to increase from 1 to 10 percent at this threshold, although the 10% underestimates potential problems with higher TP. Many streams with higher TP had no *Cladophora*, indicating that some other, non-nutrient factor prevented *Cladophora* from growing and reducing estimates of average *Cladophora* cover in high TP streams. If streams with zero cover were not included in the calculation, average *Cladophora* cover in streams with TP greater than 0.023 mg TP/L was 16 or 22 percent (depending upon whether cover was square-root transformed or not, respectively, when calculating the average). Note, *Cladophora* cover was almost always less than 5 percent in Michigan and Kentucky streams when TP concentration was less than 0.010 mg/L.

Phosphorus pollution stimulated an increase in algal biomass in IRW streams to what has been characterized to be a nuisance level. FGA cover in minimally disturbed, low P streams usually had less than 10 percent FGA cover. When TP was greater than 0.27 $\mu\text{g/L}$, FGA cover averaged 36% cover. Welch et al. (1988) synthesized results of literature and considered

nuisance algal biomass to be greater than 20 percent FGA cover or 100 to 150 mg chl *a*/cm². Surveys of Montana voters and recreational users of the Clark Fork River showed desirability for use decreased dramatically (threshold response) when chlorophyll *a* exceeded 150 mg /m² (=15 µg chl *a*/cm²) (Suplee et al. 2008). In addition, the percent FGA cover of IRW streams with FGA was higher than in Kentucky and Michigan streams (Stevenson et al. 2006). Less than 3 percent of streams in Kentucky and Michigan streams had a seasonal average greater than 50 percent FGA cover. In the IRW, about 25 percent of the sampled streams had greater than 50 percent of the stream bottom covered by FGA.

We relied on FGA cover, rather than chl *a*, to assess the magnitude of impact of phosphorus pollution on benthic algae because we believe our chl *a* measurements of biomass were low compared to FGA cover measurements due to a laboratory error. Estimating FGA cover by visual assessment is relatively error-proof compared to assessing benthic algal chl *a*, so we put more credibility into the FGA measurements. In addition, conversations with the analytical laboratory after sample and data analyses uncovered that the subsampling techniques that the laboratory used before chl *a* assays probably under-sampled the FGA because a small bore pipette was used in subsampling and filaments were not cut into easily sampled pieces. In addition, we made a comparison of the relationships between chl *a* and FGA cover for the IRW and Kentucky streams, where similar diatom and FGA assemblages were observed to develop on substrata. The model for chl *a* as a function of FGA cover in the IRW was:

$$\ln(\text{chl } a) = -0.088 + 0.465 * \ln(\text{FGA}+1)$$

The model for chlorophyll *a* as a function of FGA cover in Kentucky streams was:

$$\ln(\text{chl } a) = 1.137 + 0.642 * \ln(\text{FGA}+1)$$

In both models, chlorophyll *a* (chl *a*) and FGA were natural log-transformed. 1 was added to FGA to accommodate zero values.. Chlorophyll *a* was measured as $\mu\text{g}/\text{cm}^2$ and the units for FGA are percent cover. Both intercept and slopes in the Kentucky model were significantly greater ($p < 0.05$ and < 0.10 , respectively) than the IRW model. According to the IRW model, chl *a* with 35 percent FGA cover was $4.7 \mu\text{g}/\text{cm}^2$ ($= 47 \text{ mg}/\text{m}^2$), which is a relatively low value when compared to the potential range of benthic chl *a* for so much FGA. According to the Kentucky model, chl *a* with 35 percent FGA cover was $30 \mu\text{g}/\text{cm}^2$ ($= 300 \text{ mg}/\text{m}^2$), which is well above the $150 \text{ mg}/\text{m}^2$ nuisance level established by Welch et al. (1988) and Suplee et al. (2008). Based on the more reasonable predictions of the Kentucky model than IRW model, we assume that laboratory error caused an underestimation of chl *a* in our samples. A reason that our chl *a* measures were correlated to urban land use, poultry house density, and TP is probably that the microalgal fraction assayed (not associated with FGA) was also related to TP and prevalence of its sources. For example, the density of microalgae in samples should increase with FGA, because many microalgae are epiphytes on FGA and would fall off during processing.

DO and pH

Nutrient pollution in the IRW indirectly increased average DO and the variability in DO at a site during spring and negatively affected DO concentration during summer. Few field surveys such as ours report the relationship between DO and nutrient concentrations, perhaps because diurnal variability makes resolving relationships difficult when sampling takes place at different times of day. In our study, we controlled for time of day by sampling early in the morning during summer. During spring, we sampled each site many times and at different times

of day, so we could characterize variability and the range in DO conditions over an eight week period.

The different effects of nutrients on DO during the two seasons were probably related to discharge levels and covarying factors. During spring, discharge was sufficiently high that reaeration of water may have prevented minimum DO from getting too low. At no time were dissolved oxygen concentrations less than 7.6 during spring. Variability in DO was related to TP and algal biomass, thus the potential for DO depletion was developing with increasing algal biomass. In one case during spring April 2006, discharge was low and an estimated 1,343 fish (*Camptostoma anomalum*) were killed in a 2 km segment of the river (Oklahoma Water Resources Board, personal communication). Extensive growths of FGA were observed across the bottom of the Illinois River at a site with dead fish. We monitored the depletion of DO the day after the fish kill was reported and observed that DO was greater than 8 mg/L before 6 PM on April 19 and decreased to as low as 0.9 between 5:30 and 8:00 AM. It seems highly likely that the low DO concentrations killed the fish and were caused by FGA, which in turn resulted from high TP concentrations.

During summer, the negative relationships between DO and TP as well as poultry house density were only observed in streams with low urban activities upstream. DO was less than 5.0 mg/L, the Oklahoma state water quality criterion, in 45% of IRW streams during summer. Lack of flow augmentation by waste water treatment plants may explain why TP had a negative effect on DO during the low flow, high temperature summer period in streams with high TP and urban land use. It is also possible that allochthonous organic matter loading was correlated with TP loading; and during low flows of summer in rural streams, when reaeration could not compensate for the oxygen demand, low early morning DO concentrations developed.

pH was positively related to algal biomass during spring, but not during summer. Increases in pH with algal biomass are probably related to algal uptake of CO₂ and a shift in the carbonate equilibrium, thereby alkalizing the water. pH was elevated to greater than 9.0, which is the Oklahoma state water quality criterion for protection of biodiversity. Thus, elevated pH concentrations related to high algal accumulations may have had a negative effect on biodiversity during spring. No survey studies documenting relationships between nutrients and algae with pH are known.

Conclusions about causation

Both urban activities and poultry house operations had substantial effects on phosphorus pollution and benthic algal biomass during spring, resulting in injury to aesthetics and potentially to biodiversity. Spring pH was commonly elevated about 9.0, which may affect biodiversity of algae, which are highly sensitive to pH (Pan et al., 1996), as well as invertebrates and fish. Punctuated DO stress may result during spring when discharge is low. Altered physical habitat by FGA can affect species composition of invertebrates (Dudley et al., 1986). During summer, low DO related to elevated phosphorus and poultry house density may negatively affect biodiversity in streams with low urban activity.

Multiple lines of evidence (e.g. Beyer 1998) support attribution of injury to aesthetics and elevated chemical stressors to spreading of poultry wastes on fields. Phosphorus, the most likely limiting nutrient in these streams, was related to poultry house density, increases in algal biomass and pH during spring, and decreases in DO during summer in IRW streams. These observations are consistent with predictions of an *a priori* model and results of phosphorus enrichment in

other studies in which either phosphorus was manipulated experimentally or measured in large numbers of streams during surveys. Poultry operations are the dominant source of phosphorus in the IRW. Phosphorus concentrations and FGA were higher in the IRW than other regions in which similar minimally disturbed conditions would be expected, extensive alteration of lands by humans exists, and in which little poultry industry activity occurs. Finally, effects of phosphorus pollution occurred in many streams in which there was no other plausible source that could cause the elevated phosphorus concentrations.

Acknowledgements

This research was funded by the Office of the Attorney General of Oklahoma. The design and data analysis of the project were benefited by discussions with David Page, Bernie Engel, Roger Olsen, and Bert Fisher. Julie Heinlein, Jan Seidler, Renee Mulcrone, Tony Gendusa, and Karthic Poosekar helped with algal sample analysis at Michigan State University and field sampling by CDM, Inc.

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Table 1. Regression models relating land use, poultry house density, nutrient concentrations, and benthic algal biomass. The standard deviation and attained significance of the constant, first, and second dependent variables of models are reported. PCURB=percent urban land use. PHD=poultry house density (houses per mi²). DV=dependent variable. Chl_{acm2}= benthic algal biomass (chlorophyll a/cm²).

Season	Model	LU Range	Standard Error			Attained Significance		
			Constant	1st DV	2nd DV	Constant	1st DV	2nd DV
Spring	$\ln(TP) = -4.924 + 0.581*\ln(PCURB) + 0.580*\ln(PHD)$	all sites	0.366	0.118	0.159	<0.001	<0.001	0.001
Spring	$\ln(TN) = 0.14 + 0.367*\ln(PCURB) + 0.273*\ln(PHD)$	all sites	0.266	0.084	0.114	0.602	<0.001	0.019
Summer	$\ln(TP)=-4.292+0.566*\ln(PCURB)+0.278*\ln(PHD)$	all sites	0.453	0.125	0.184	<0.001	<0.001	0.134
Summer	$\ln(TP)=-3.90+0.548*\ln(PHD)$	low urban	0.287	0.208		<0.001	0.014	
Summer	$\ln(TN)=0.102+0.353*\ln(PCURB)$	all sites	0.192	0.069		0.596	<0.001	
Spring	$\text{sqr}(FGA)=9.034+1.430*\ln(TP)$	all sites	0.833	0.273		<0.001	<0.001	
Spring	$\text{sqr}(FGA)=0.288+1.515*\ln(PHD)+1.220*\ln(PCURB)$	all sites	1.04	0.452	0.334	0.783	0.001	0.001
Spring	$\ln(\text{chlacm2})=3.109+0.640*\ln(TP)$	all sites	0.327	0.107		<0.001	<0.001	
Spring	$\ln(\text{chlacm2})=-1.024+0.560*\ln(PHD)+0.706*\ln(PCURB)$	all sites	0.374	0.162	0.121	0.008	0.001	<0.001

Figure Captions

Figure 1. Variables grouped into four categories according to their causes and their effects in the conceptual model. Solid arrows indicate direct effects. Dashed arrows indicate indirect effects.

Figure 2. Sampling locations during the summer 2006 field sampling program.

Figure 3. Ranges in physical and chemical conditions in IRW streams during spring 2007 and summer 2007. Boxes delineate the interquartile range, median, and whiskers with 1.5 times the interquartile range.

Figure 4. Relationships between nutrient concentrations, poultry house density (PHD), and percent urban land use (PCURB). A and B) All sites during spring 2007. C) All sites during summer 2006. D) Sites with percent urban land use less than 10% during summer 2006.

Figure 5. Relationships between total phosphorus benthic algal biomass as chlorophyll a/cm² and FGA cover for spring (A & C) and summer (B & D).

Figure 6. Relationships between benthic algal biomass (as chlorophyll a/cm² and FGA cover) and total phosphorus benthic algal biomass during spring (A & C) and summer (B & D).

Figure 7. Ranges in chemical stressors in IRW streams during spring 2007 and summer 2007. Boxes delineate the interquartile range, median, and whiskers with 1.5 times the interquartile range.

Figure 8. Relationships between average and standard deviation in dissolved oxygen concentrations measured weekly in a stream for eight weeks as related to benthic algal biomass and filamentous green algal cover during spring 2007.

Figure 9. Relationships between the minimum of dissolved oxygen concentrations measured weekly in a stream for eight weeks and algal biomass, filamentous green algal cover, poultry house density, percent urban land use, and total phosphorus concentrations during spring 2007.

Figure 10. Relationships between average and maximum pH measured weekly in a stream for eight weeks and benthic algal biomass, filamentous green algal cover, total phosphorus concentration, urban land use, and poultry house density during the spring of 2007.

Figure 11. Change point determined by CART in the *Cladophora* cover of Kentucky and Michigan streams Redrawn from Stevenson et al. (2006).

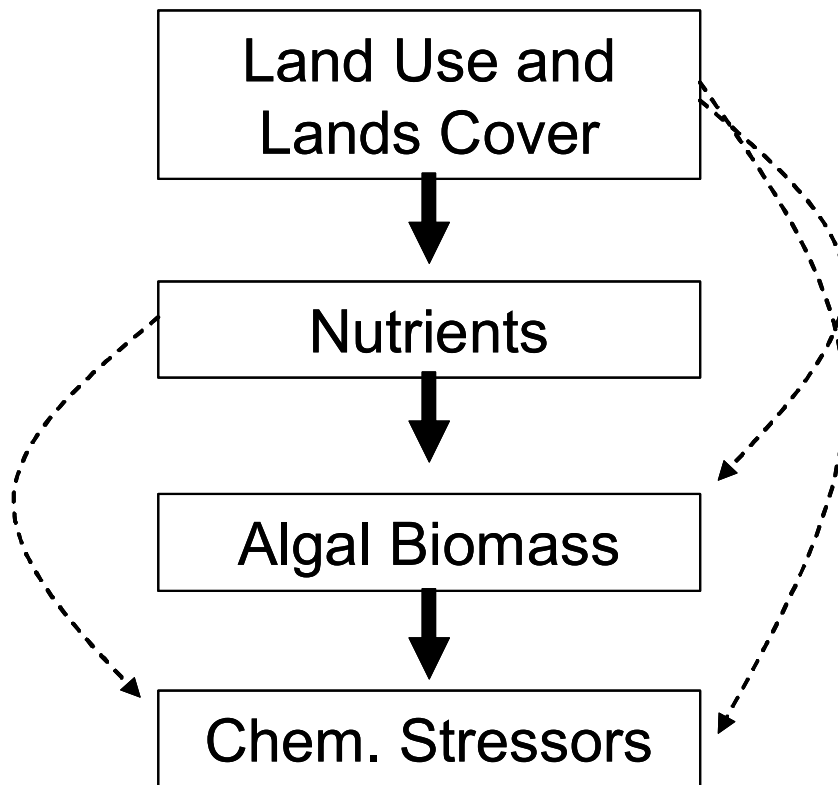


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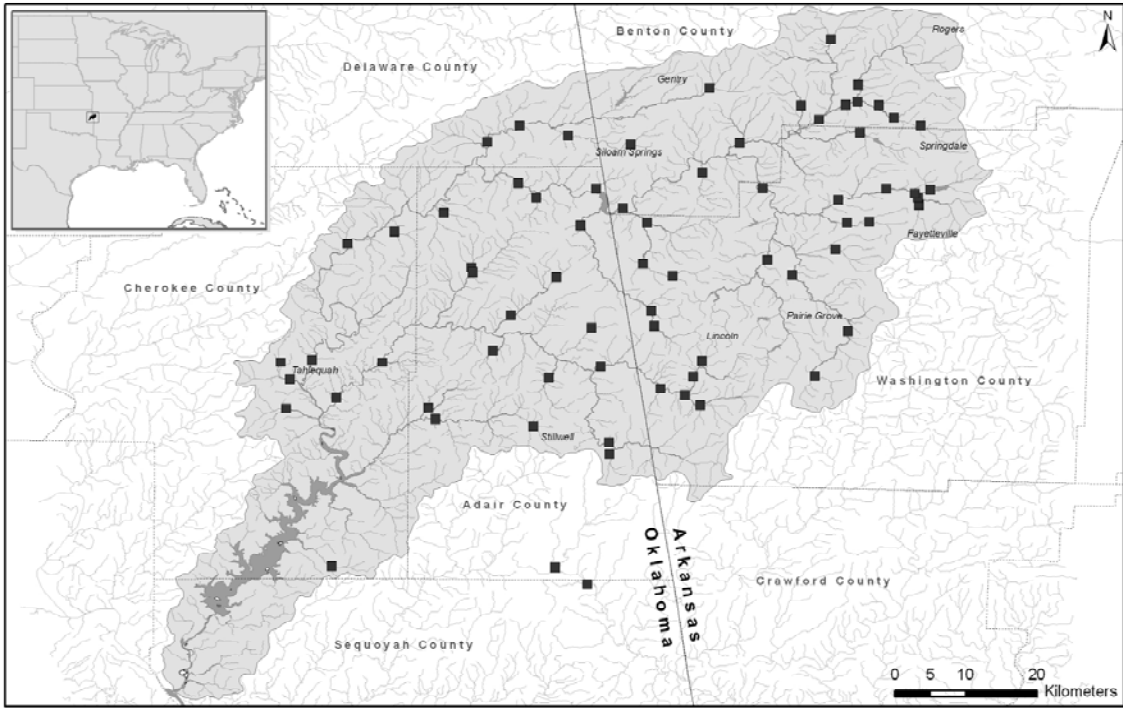


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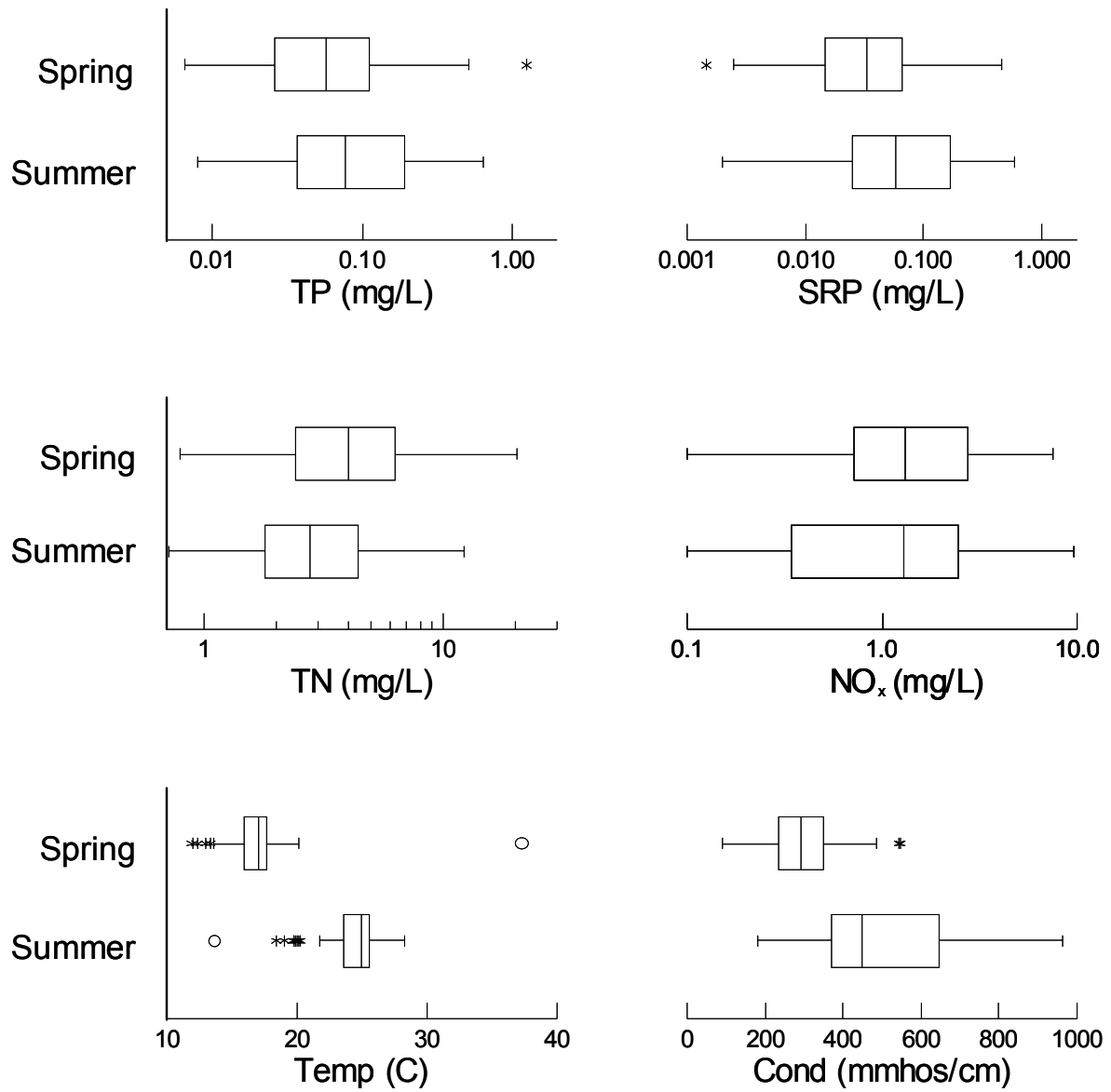


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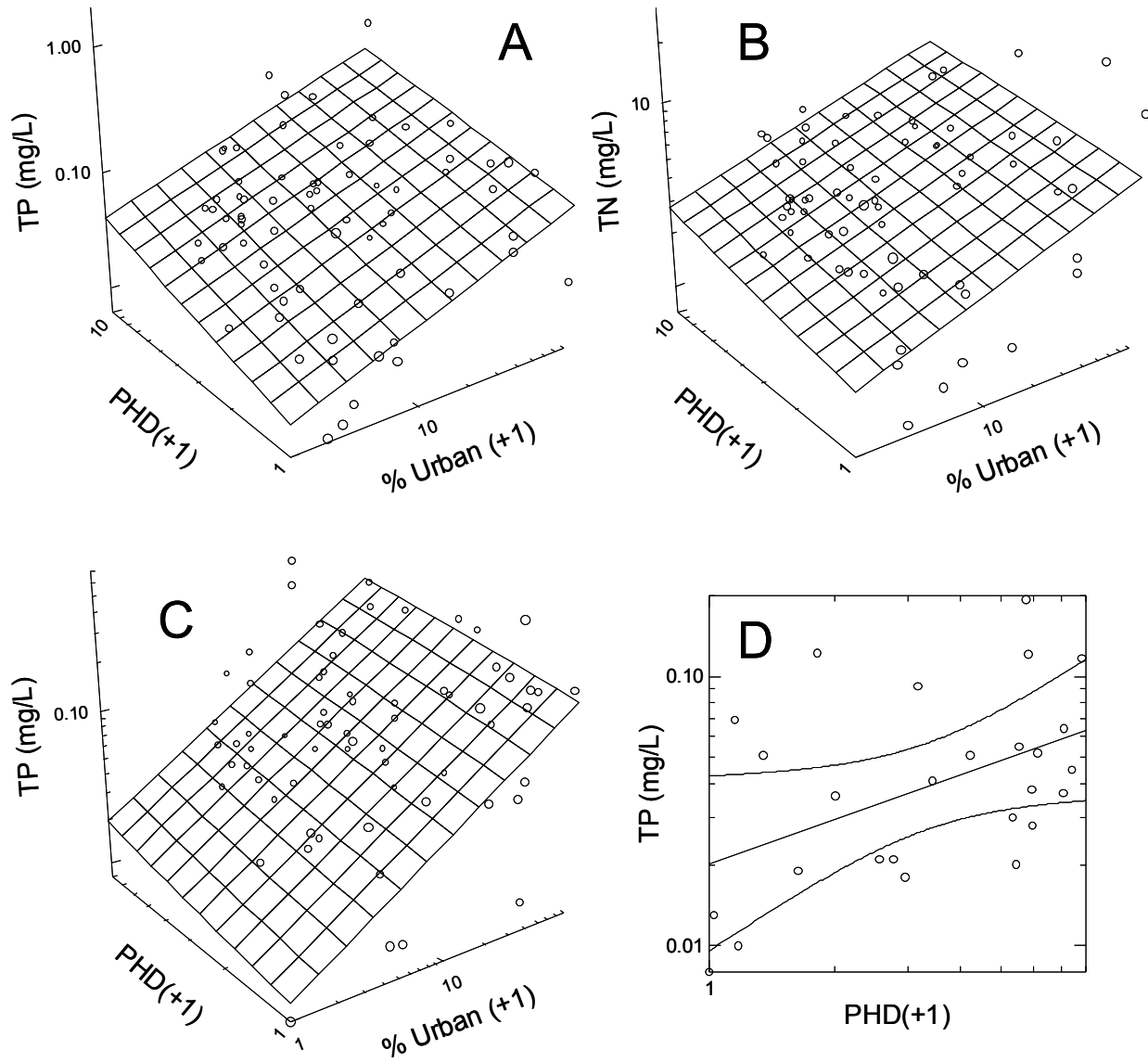


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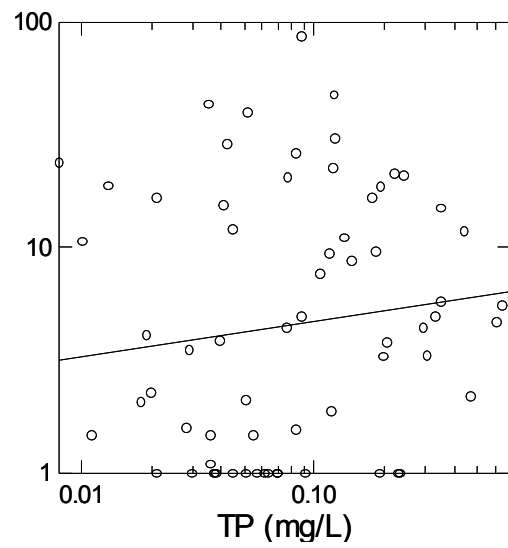
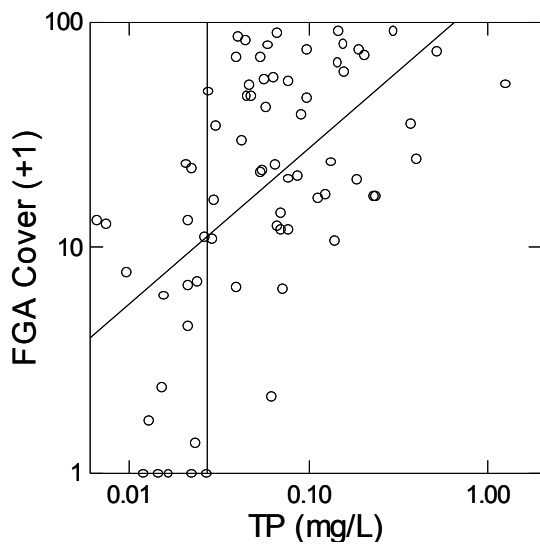
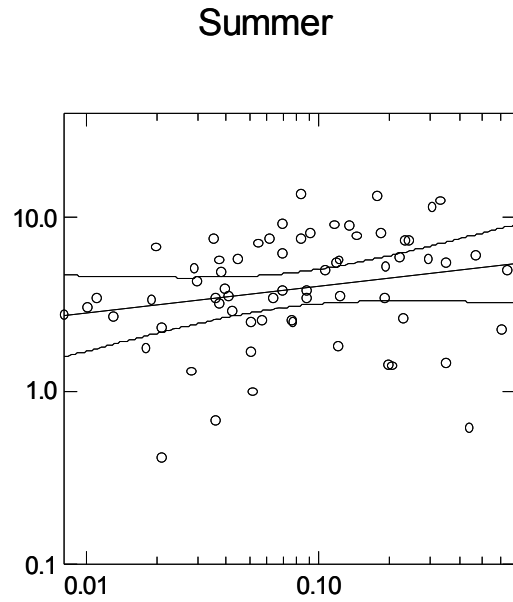
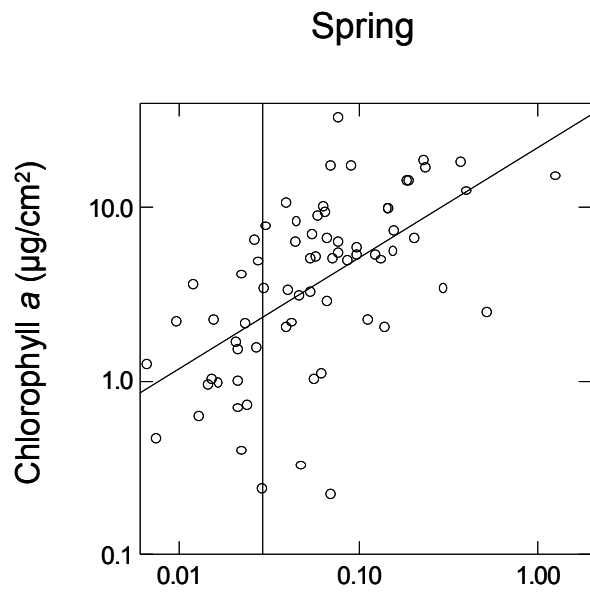


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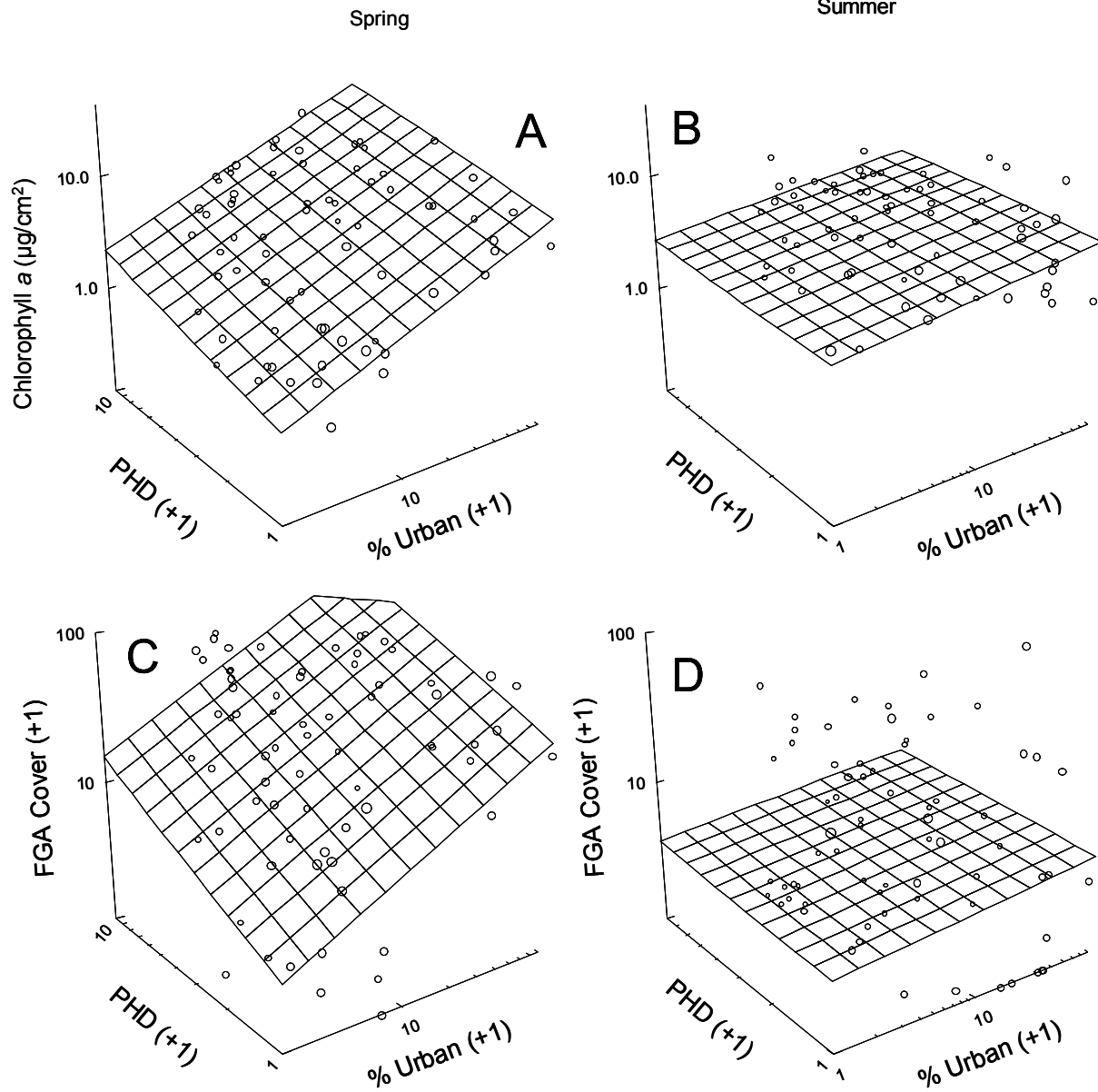


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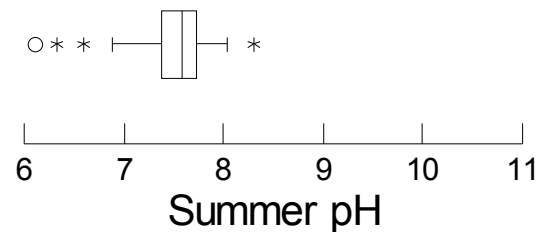
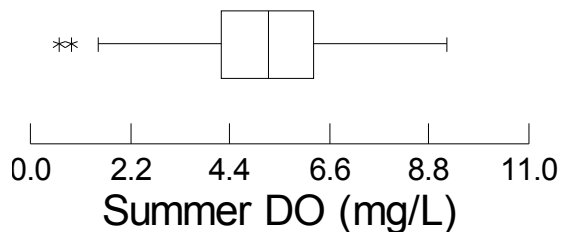
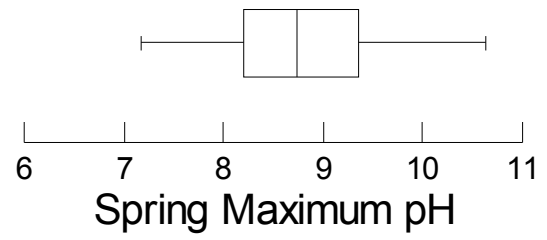
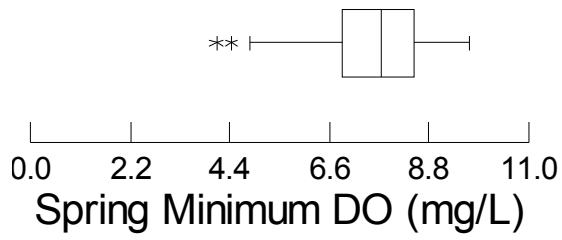
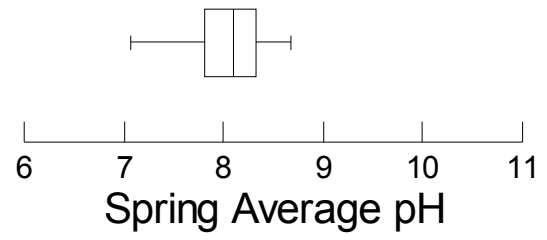
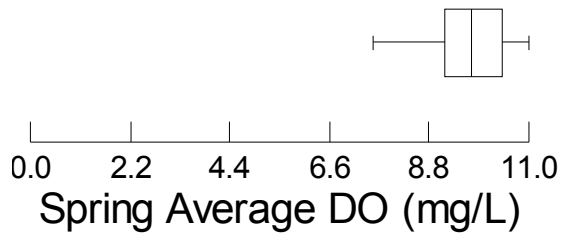


Figure 7

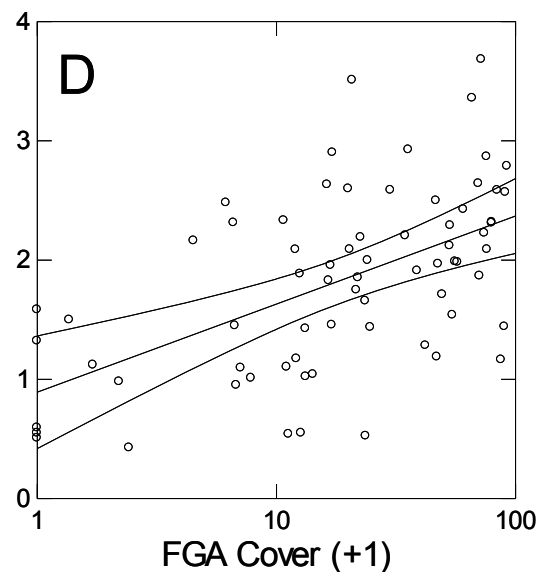
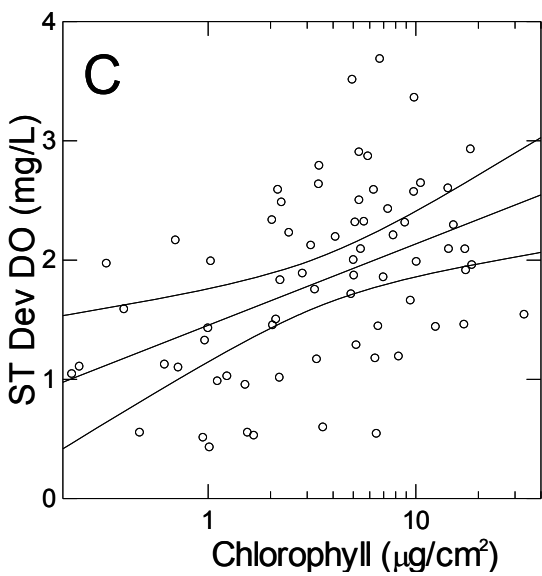
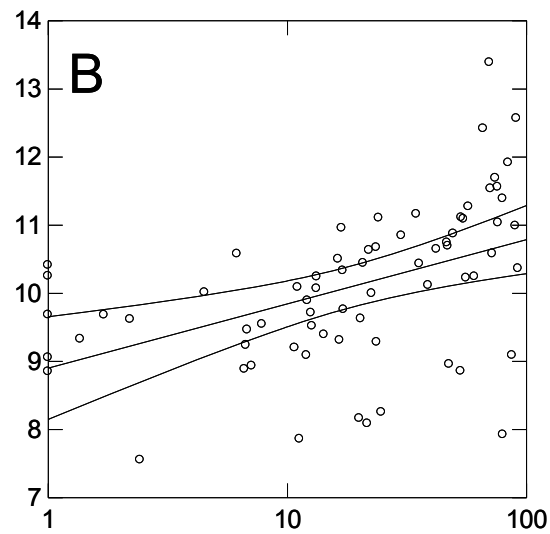
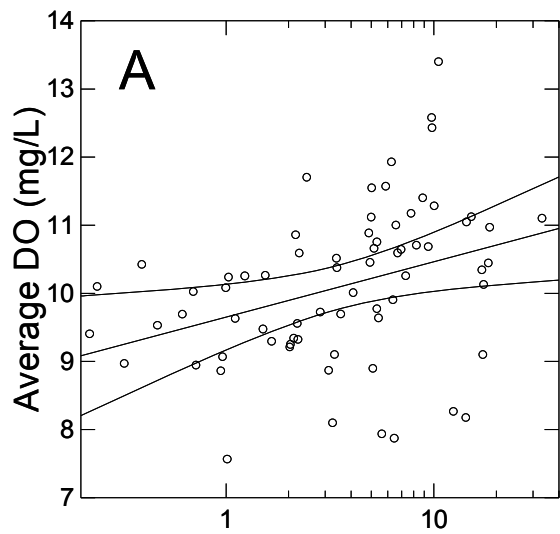


Figure 8

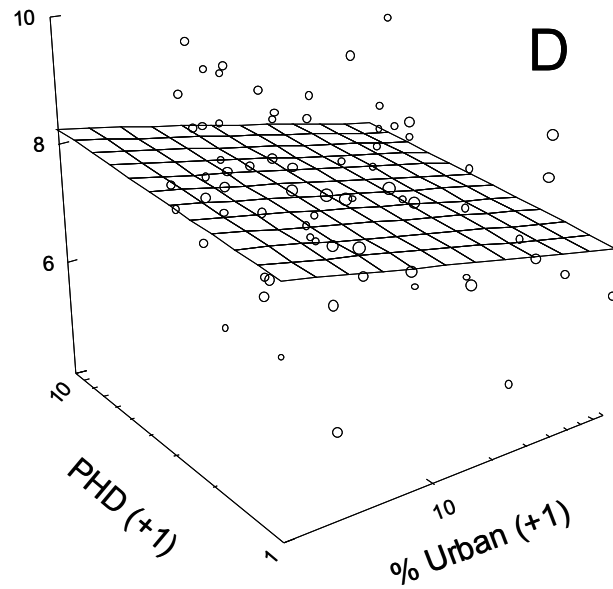
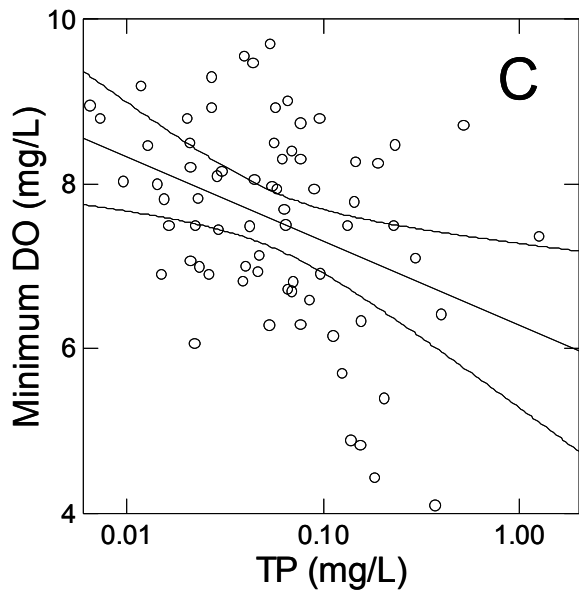
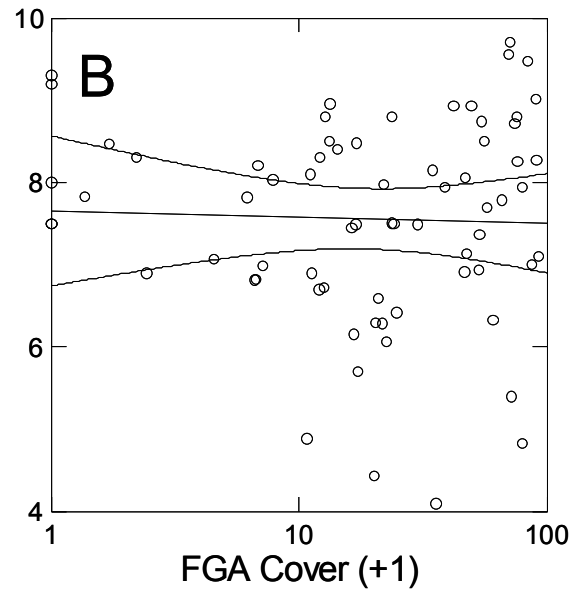
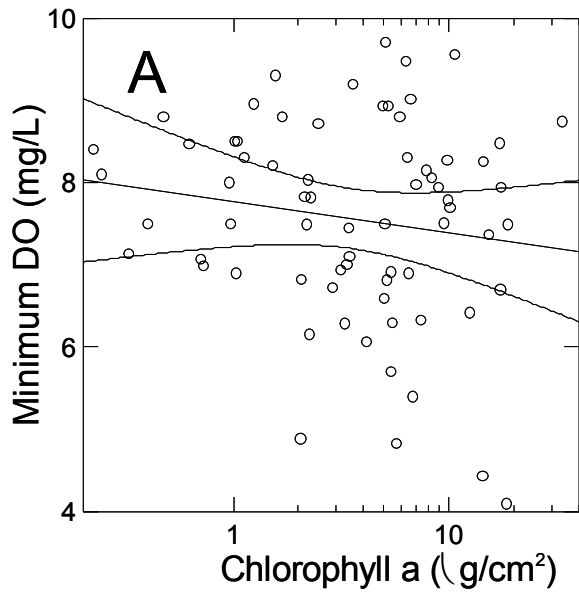


Figure 9

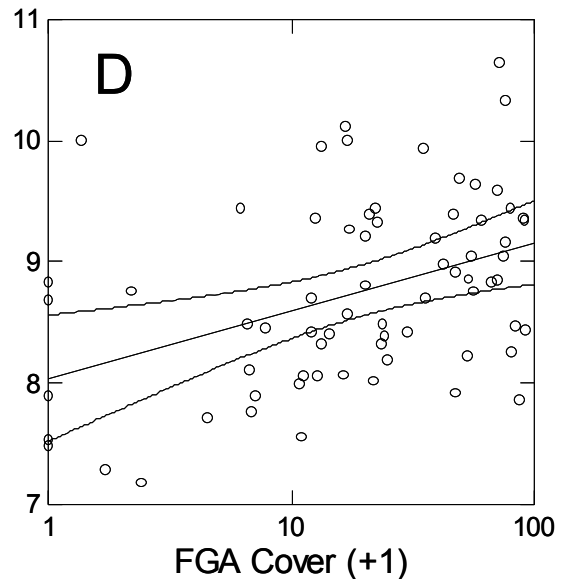
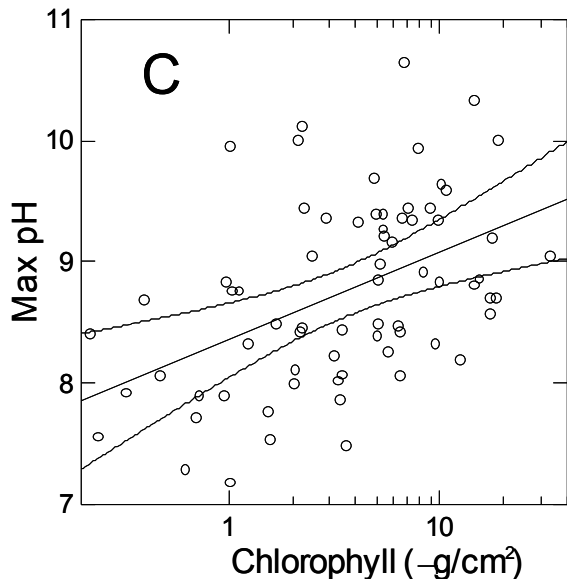
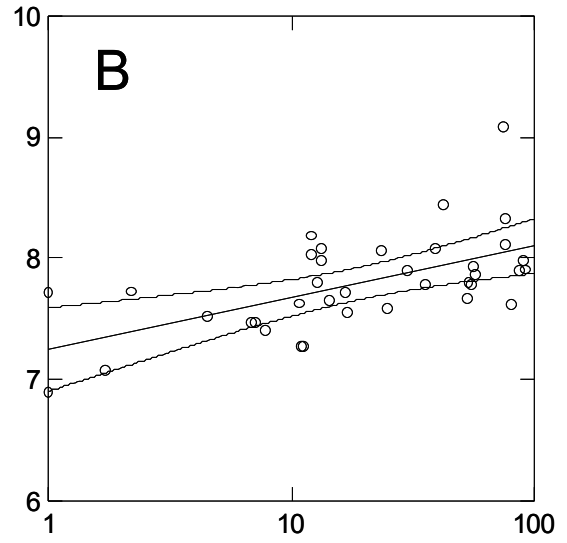
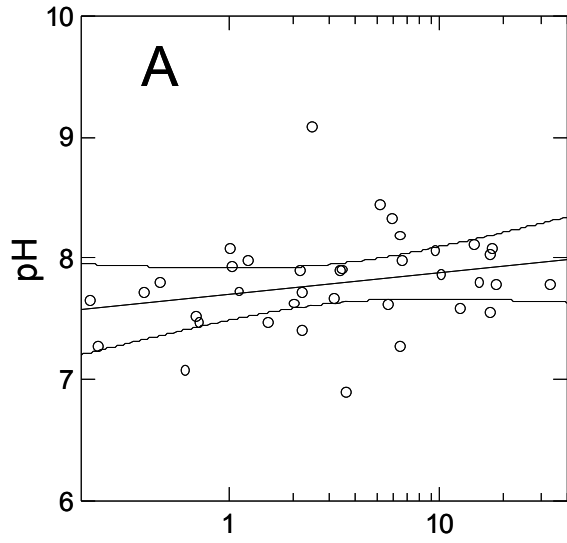


Figure 10

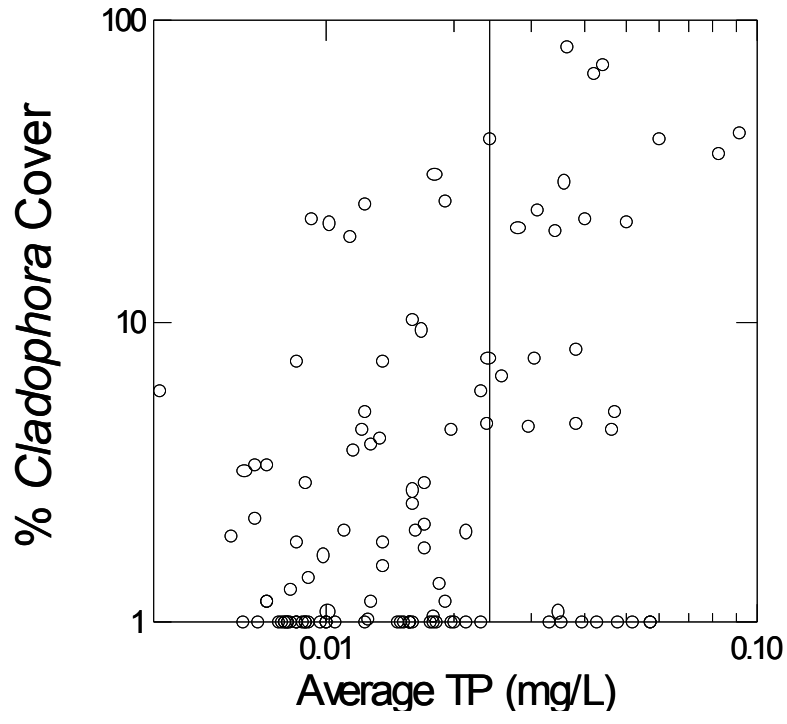


Figure 11

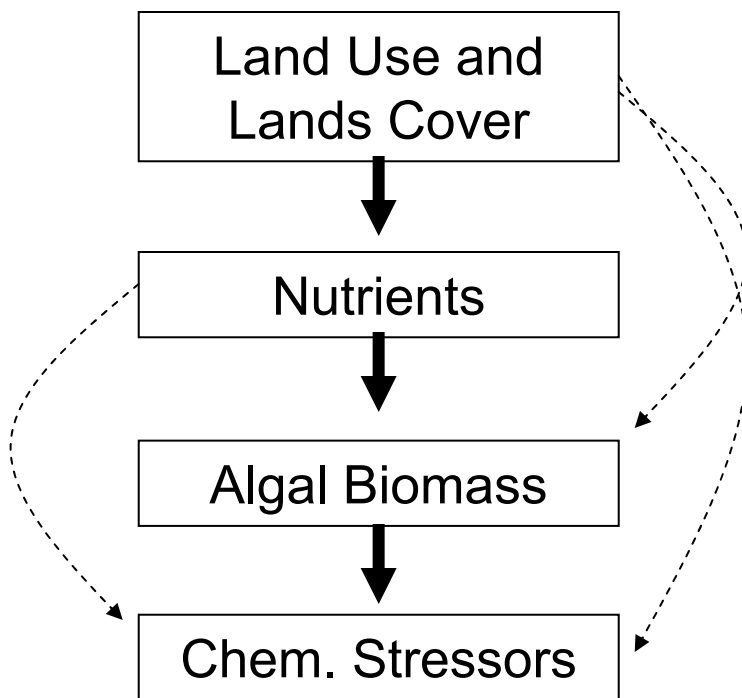


Figure 1

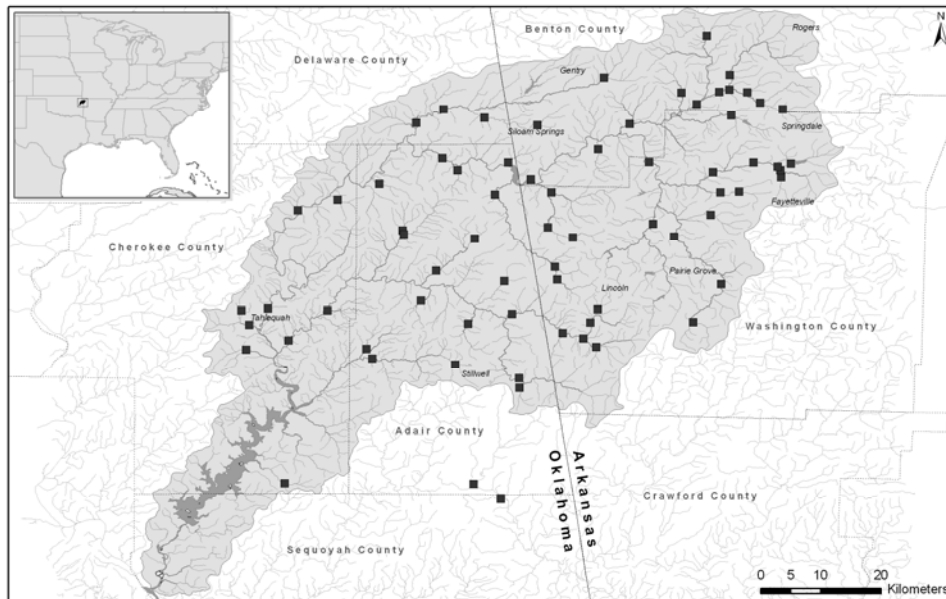


Figure 2

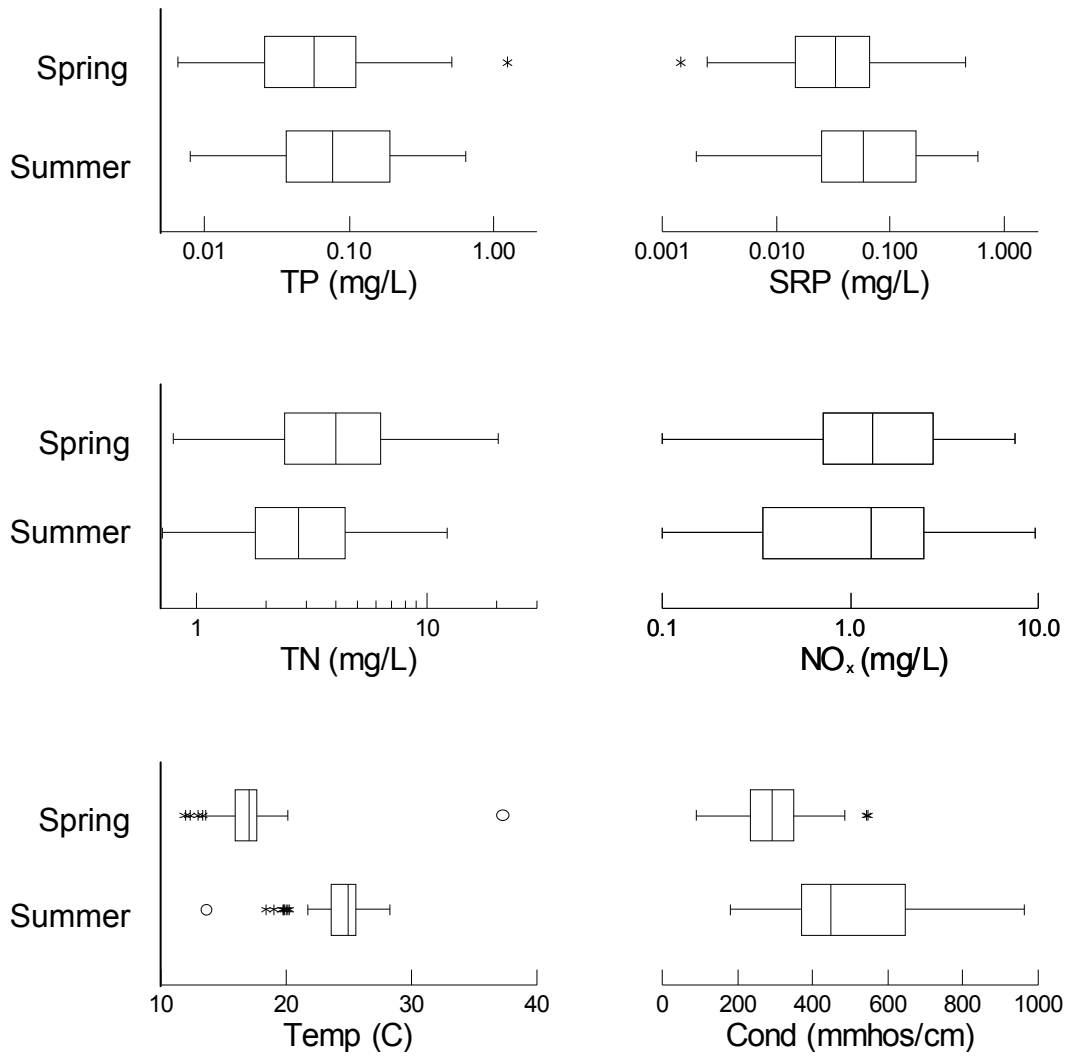


Figure 3

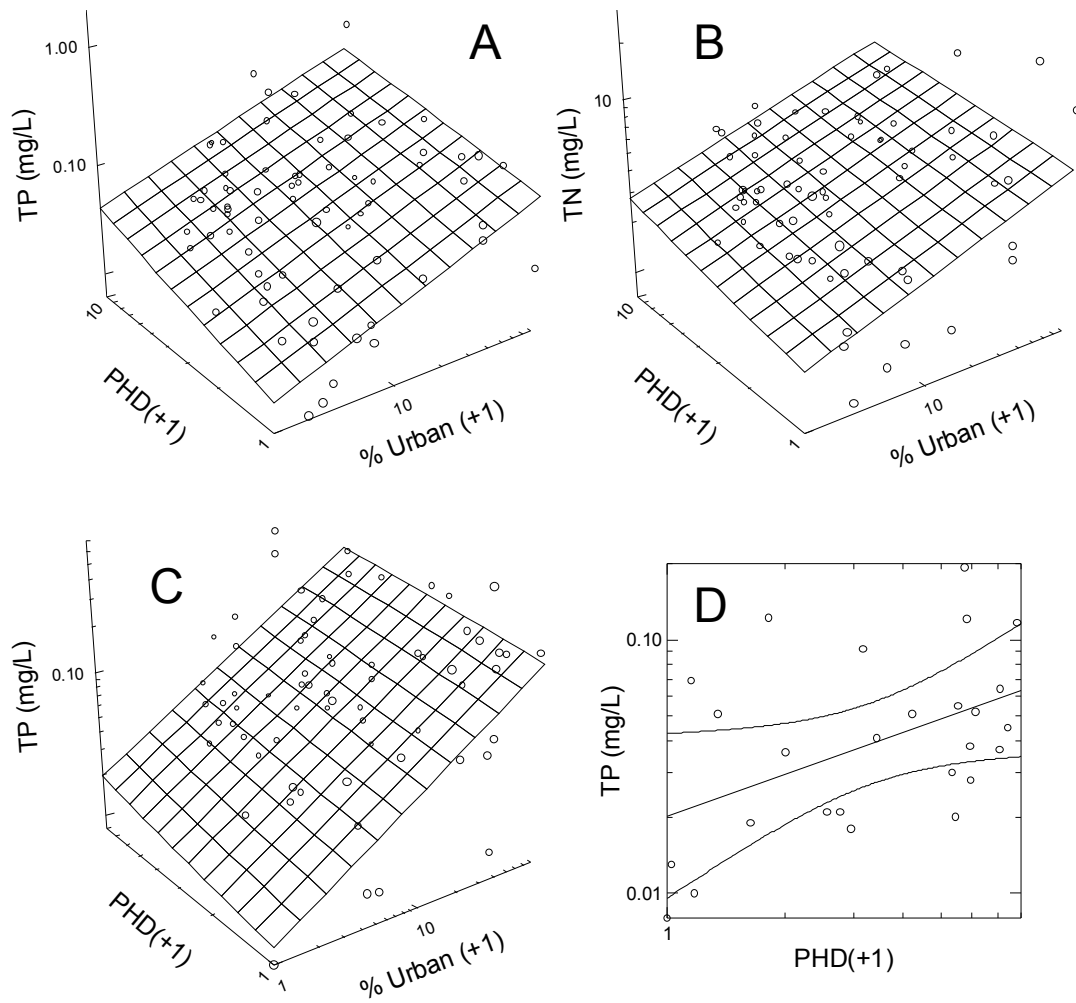


Figure 4

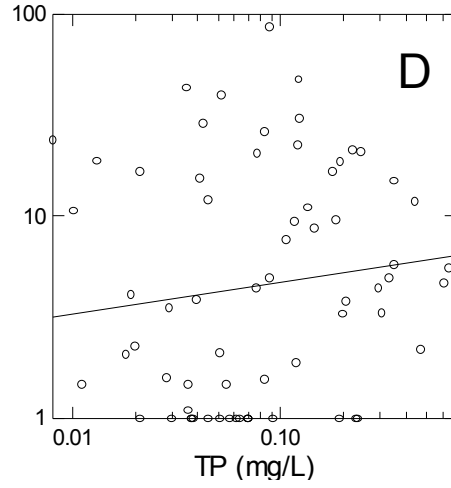
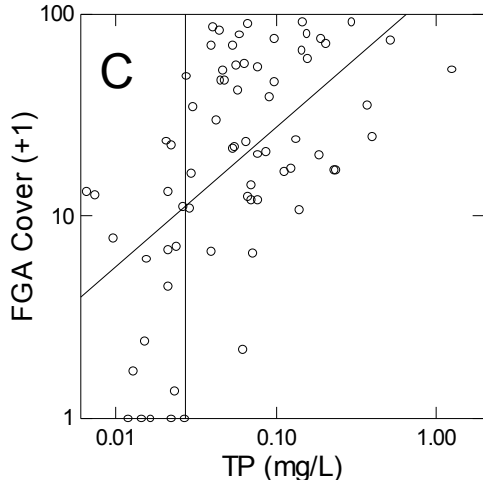
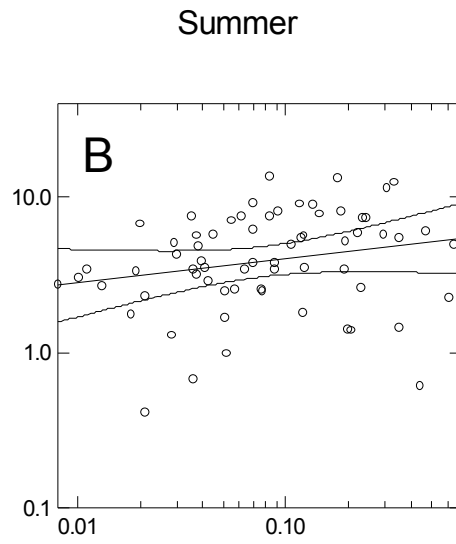
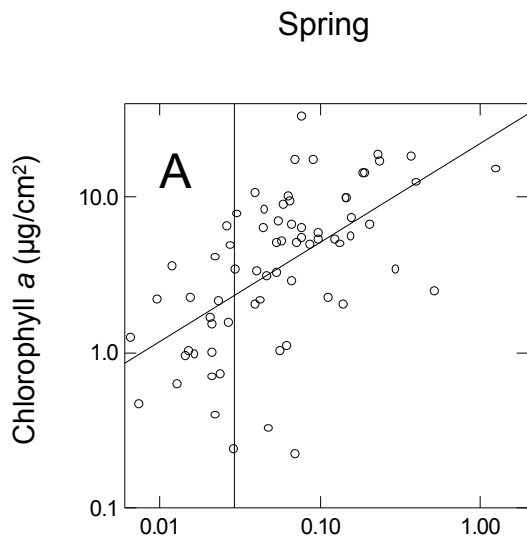


Figure 5

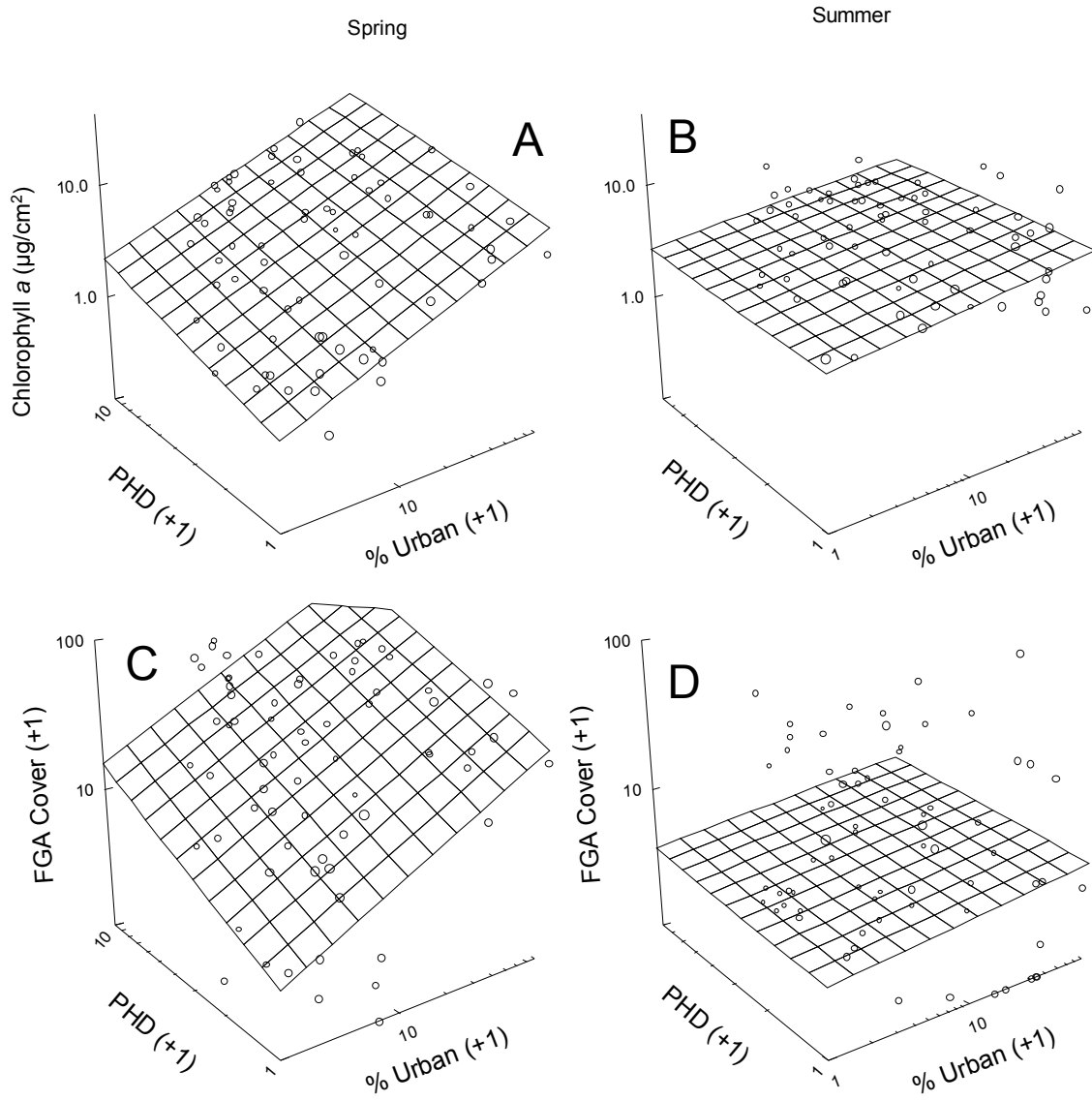


Figure 6

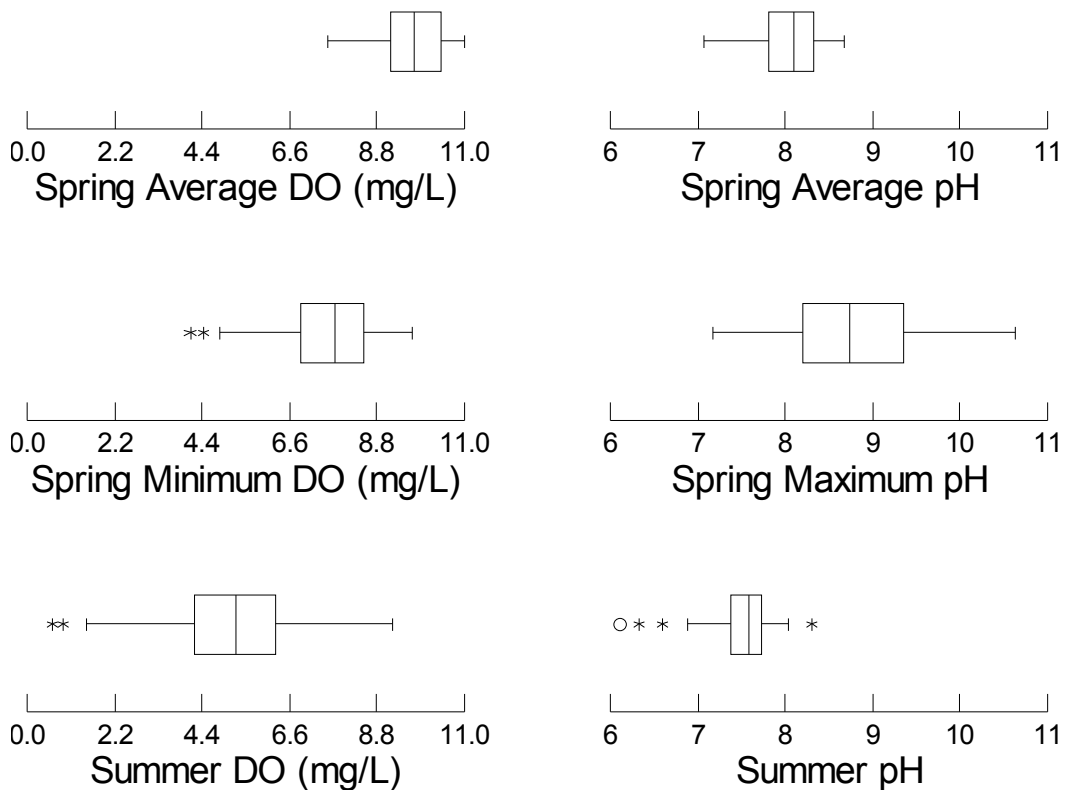


Figure 7

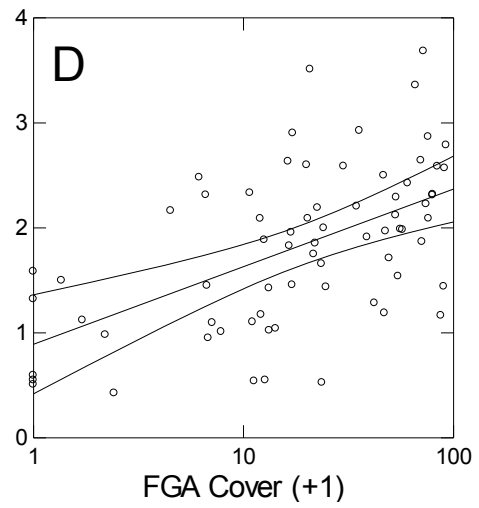
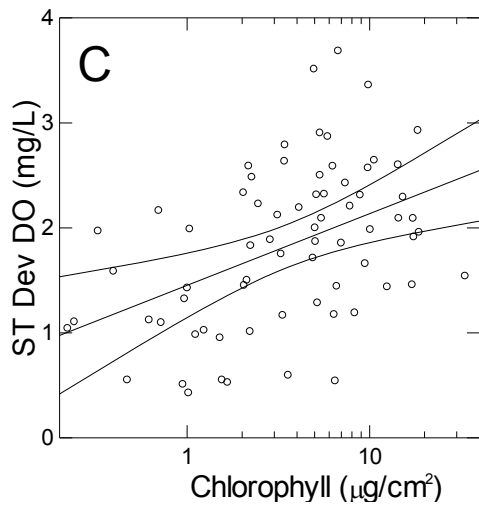
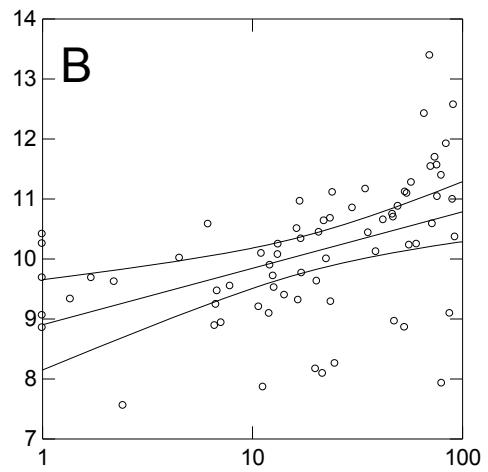
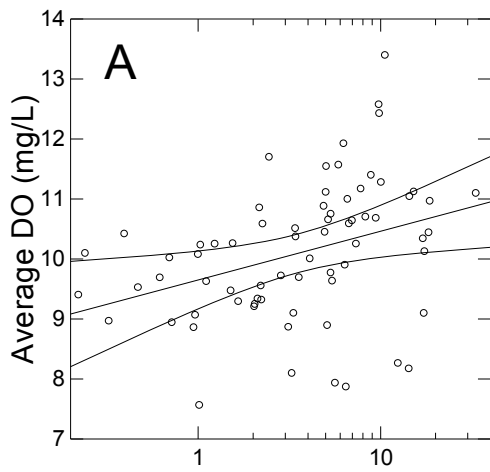


Figure 8

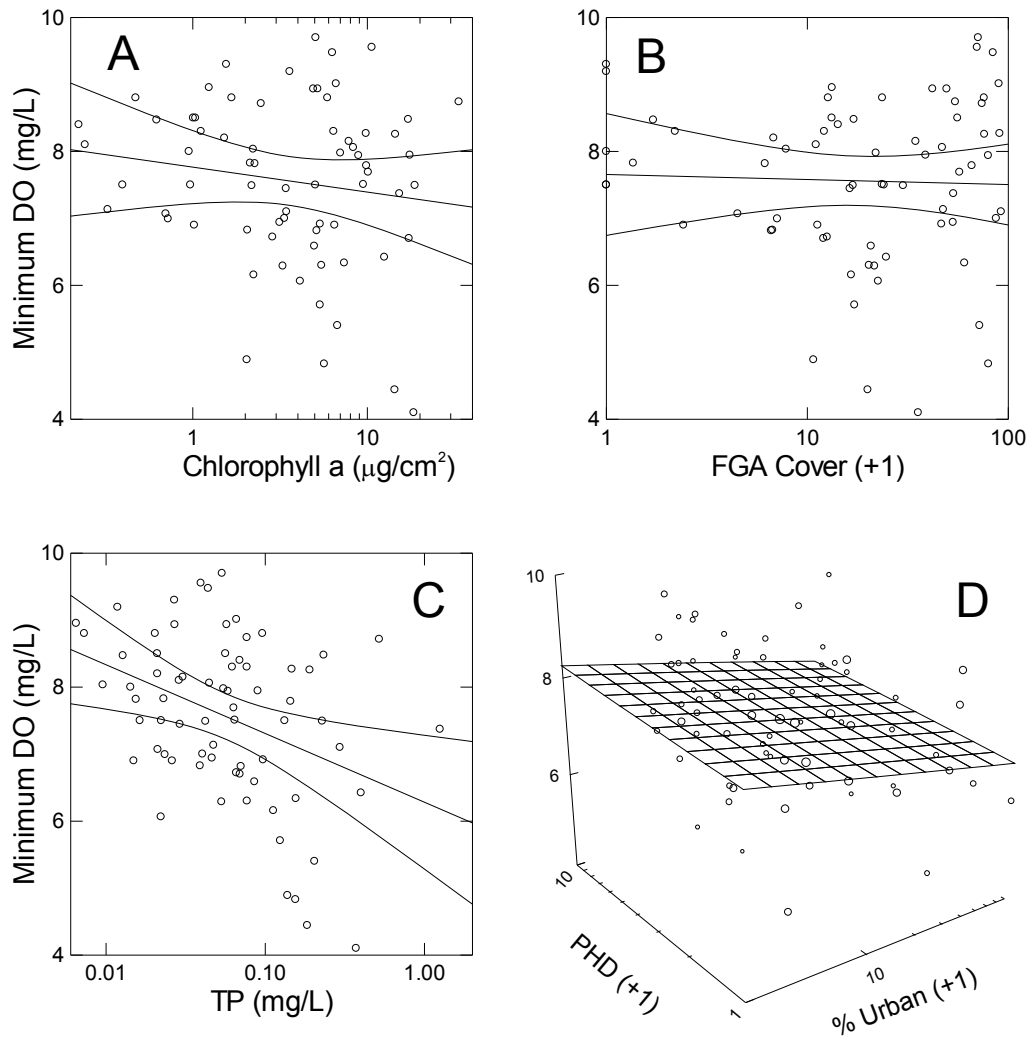


Figure 9

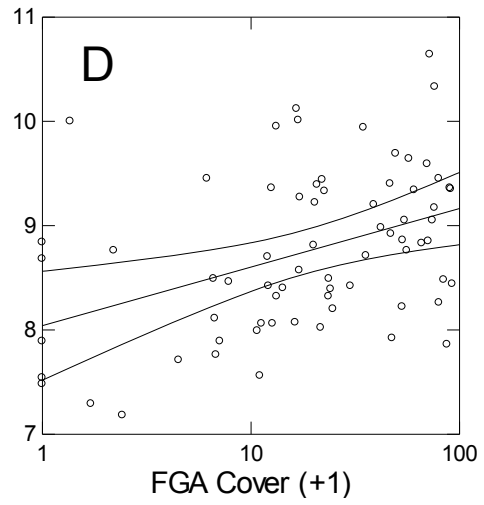
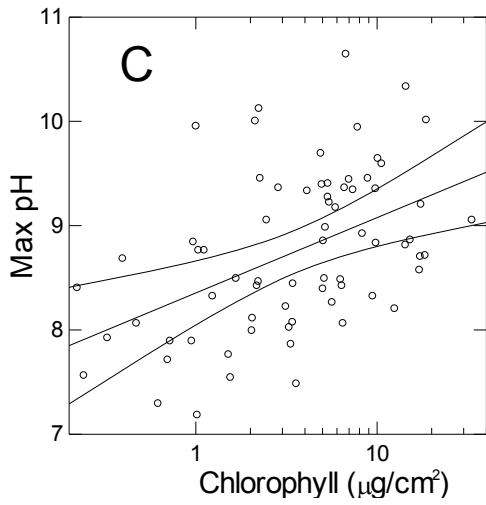
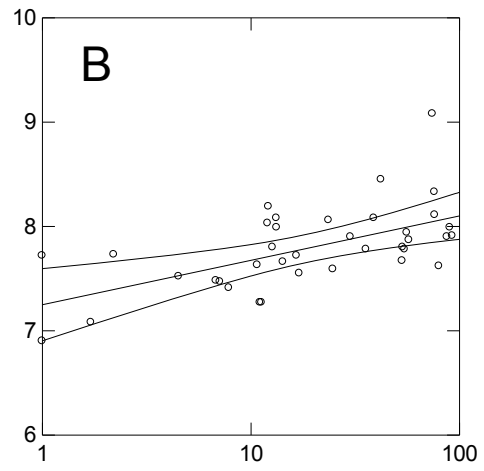
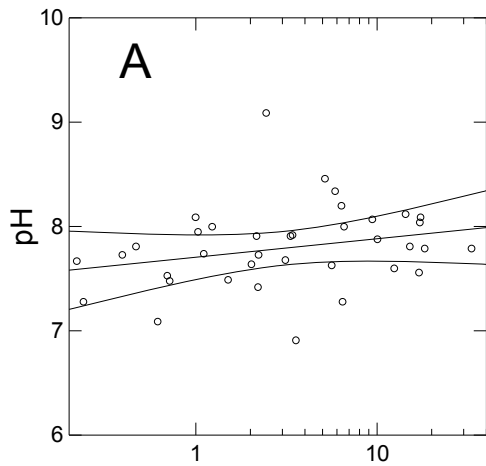


Figure 10

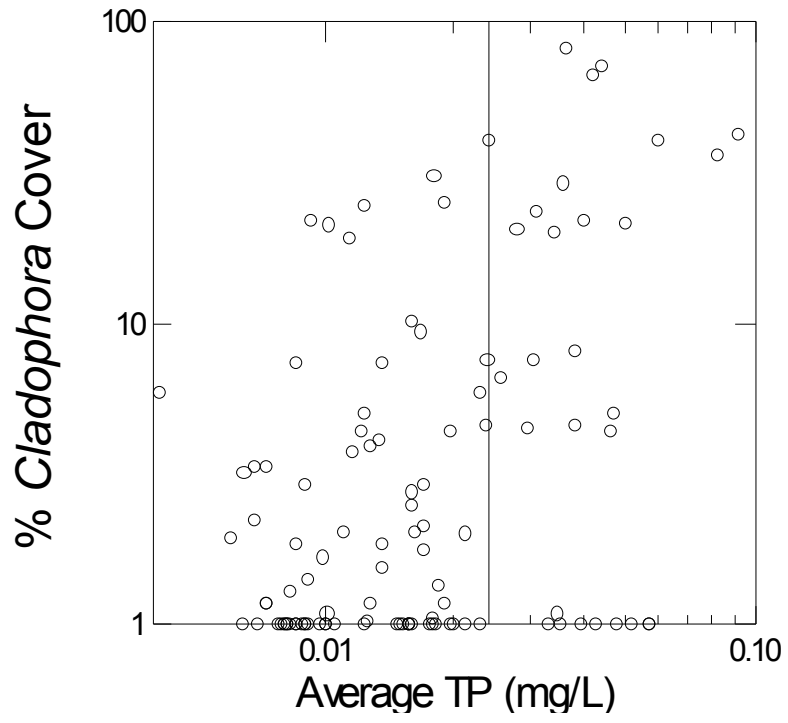


Figure 11