DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

January 2012

AWRC

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The Red River is a trans-boundary, multi-jurisdictional basin, where water-quality standards often change at the state lines. The state agencies with USEPA Region VI focused resources to organize water-quality data from within this basin and have it statistically analyzed to evaluate the relationships between nutrients and sestonic chlorophyll-a (chl-a). There were 152 sites within the Red River Basin that had nutrient and sestonic chl-a data, and these sites were narrowed down to 132 when a minimum number of observations was required. Sestonic chl-a levels increased with increasing nutrient concentrations; these significant regressions were used to predict nutrient concentrations at 10 µg chl-a L^{-1}. Total nitrogen (TN) and phosphorus (TP) concentrations (at 10 µg chl-a L^{-1}) varied across the Red River Basin and its eco-regions from 0.10-0.22 mg TP L^{-1} and 0.75-2.11 mg TN L^{-1}. Nutrient thresholds were also observed with sestonic chl-a at 0.14 mg TP L^{-1} and 0.74 mg TN L^{-1} using categorical and regression tree analysis (CART). CART analysis also revealed that hierarchical structure was important when attempting to predict sestonic chl-a from TN, TP and conductivity. The ranges of TN and TP concentrations that resulted in chl-a concentrations which exceeded 10 µg chl-a L^{-1} were similar in magnitude to the threshold in TN and TP that resulted in increased sestonic chl-a. This corroborating evidence provides useful guidance to the states with jurisdiction within the Red River Basin for establishing nutrient criteria, which might be similar when the Red River and its tributaries cross political boundaries.

ABBREVIATIONS: CART, categorical and regression tree analysis; DP, dissolved phosphorus; NO_3-N, nitrate-nitrogen; TN, total nitrogen; TP, total phosphorus; USEPA, U.S. Environmental Protection Agency.

INTRODUCTION

Nutrient pollution from both point sources (e.g., industrial discharge and effluent waste-water) and non-point sources (e.g., agriculture and urban) is a major stressor of aquatic ecosystems worldwide (Burkart and James, 1999; Turner and Rabalais, 2004; Sharpley et al., 2009). The Water Quality Report to U.S. Con-gress in 2004 indicated that over 36 percent of assessed stream and river miles were impaired due to nutrients (USEPA, 2004). An important water quality issue in the U.S. includes the hypoxic zone in the Gulf of Mexico, which has been linked to anthropogenic nutrient exports from the Atchafalaya and Mississippi River Basins in the central and southern U.S. In this hypoxic zone, elevated primary productivity caused by excess nutrients allows the seasonal accumulation of organic matter that decom-poses and consumes oxygen (Diaz and Rosenberg, 1995; Diaz, 2001; Rowe, 2001). Nutrients have become a major concern in other major U.S. estuarine and coastal environ-ments, where a variety of ecosystem functions are impacted (Cooper and Brush, 1991; Paerl, 1997; Yu et al., 2008; Brush, 2009).

Effectively managing the landscape and in-fluential subwatersheds within large river basins is essential for both improving aquatic con-ditions at local or regional scales and reducing impacts from nutrient enrichment further downstream (e.g., coastal and marine zones). For example, management decisions at regional and local scales in the Mississippi River Basin that covers 41% of total land area of the conterminous U.S. ultimately affects water quality in the Gulf of Mexico; efforts have been made to identify nutrient sources to prioritize areas for best management practices (David et al., 2010). While protection of aquatic eco-system services (e.g., water quality, biodiver-sity, and fisheries) has become a paramount concern of watershed management programs at the state and tribal level, there are few nu-

meric criteria that can be used to guide these programs. To address this limitation, the USEPA has charged states and tribes with establishing numeric criteria so that nutrient threat and or impairment can be detected and waterbody conditions assessed routinely (USEPA, 2000). However, establishing criteria has proven to be no easy task, and only a few states have recently promulgated nutrient criteria specific to streams and rivers (e.g., New Jersey, Hawaii, Vermont, Oregon, Montana and Florida). Many of these states with specific nutrient criteria developed these values for select streams and not as statewide criteria.

Water quality and habitat degradation from anthropogenic sources influence the ecological properties of complex, natural systems. This complexity makes it difficult to establish nu-trient criteria in part because of the high level of environmental heterogeneity and the wide array of nutrient sources across watersheds. However, the link between nutrients and bio-logical conditions has been determined in numerous studies (Wang et al., 2007; Stevenson et al., 2008; Evans-White et al., 2009; Black et al, 2011; Chambers et al., 2011a), and these relationships provide strong evidence showing that increasing nutrient concentrations have an effect on aquatic life. Furthermore, several sta-tistical techniques have been used in recent years to relate nutrient thresholds and some biological attribute (Qian et al., 2003). Accord-ingly, state and tribal agencies should use a variety of statistical to provide evidence to support nutrient criteria development. Mul-tiple lines of evidence are often used in criteria development, including (1) statistical distri-butions of available nutrient data, (2) statistical relations between nutrients and various bio-oplogical attributes, and (3) a comprehensive literature review on criteria development.

The purpose of this study was to investigate the relationships between sestonic chlorophyll-a (chl-a) and nutrients in the multi-jurisdictional

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Red River Basin that spans five states, including Arkansas, Louisiana, New Mexico, Oklahoma, and Texas. A previous study has established the statistical distributions of median nutrient concentrations across 589 stations on the Red River and its tributaries; these site specific data have been compared to USEPA recommended nutrient criteria for 14 nutrient ecoregions (Longing and Haggard, 2010). We seek to build on that project providing another line of evidence to assist the five states in the Red River Basin when developing nutrient criteria. The two specific objectives of this study are to establish relationships between nutrients and sestonic chl-a using bivariate regression and to detect thresholds where deviation in chl-a concentrations change along the nutrient concentration gradient.

METHODS

Study Area

The Red River is located in the South Central USA and is the southernmost major watershed of the Mississippi River Basin that drains portions of five states (Arkansas, Oklahoma, Louisiana, New Mexico, and Texas) and ultimately enters the Gulf of Mexico. The headwaters of the Red River drain the Texas panhandle and eastern New Mexico, and the river flows east where its banks become the boundary between Oklahoma and Texas, except where it is impounded to form Lake Texoma. The river continues east and then south where it forms the boundary between Texas and Arkansas, and then flows into Louisiana. The Red River merges with the Atchafalaya River, and it flows into through the Atchafalaya Delta and Bay into the Gulf of Mexico. We designated Alexandria, Louisiana as the downstream point used to delineate the Red River Basin (Longing and Haggard, 2010). A variety of landscapes exist in the watershed, from agricultural land-use across the majority of the basin to a portion containing the Ouachita Mountains that ex-

tends from western Arkansas into Oklahoma. The Red River Basin includes four aggregate eco-regions, including the Great Plains Grasses and Shrublands (GPGS), Central Eastern and Forested Uplands (CEFU), South Central Cultivated Great Plains (SCCGP), and Southeastern Temperate Forested Plains and Hills (STFPH).

Data Sources

Sestonic chlorophyll-a (chl-a) data were compiled concurrently with other water quality parameters for the time period 1996–2006 for the Red River Basin, which was described in detail by Longing and Haggard (2010). Although a total of 23 water quality parameters were provided across six sources, there were limited biological response parameters (e.g., chl-a) for that time period in the Red River Basin. Sestonic chl-a data was measured from stations across two of the six data sources, Oklahoma Water Resources Board (OWRB) and Texas Commission on Environmental Quality (TCEQ). All data were prepared following a quality assurance project plan that detailed use of secondary data from our various state-level sources. Therefore, the specifics of data collections depend on the individual SOPs of each data source, and therefore besides some data screening (see Longing and Haggard, 2010) and ensuring that parameter units were similar across sources, there were no further data manipulations. Water quality parameters were combined into one spreadsheet and assigned unique station identification. The watersheds were delineated from station GPS coordinates and digital elevation models and then watershed area and land-use composition were determined for each watershed.

Data Analyses

The compiled dataset containing chl-a data (OWRB and TCEQ) included 152 individual stations having at least one chl-a measurement. Principal components analysis (PCA via PC–ORD,

MjM, Glenedon Beach, Oregon) was used to initially explore patterns in the data and to determine potential outliers that might influence relationships of nutrients and chl-a. PCA is a multivariate ordination procedure that transforms a set of correlated parameters into uncorrelated variables or synthetic axes (i.e., principal components) that explains the most variation among sites. We conducted outlier analysis by flagging stations that fell greater than two standard deviations from the centroid of the dataset using Euclidean distances (McCune and Grace, 2002). Additionally, we extracted a "qualifier" database that was limited to sites that had at least four sestonic chl-a observation over the time period evaluated. These procedures resulted in a total of 20 stations being removed from the overall dataset, so that the number of sites reduced from 152 to 132 in the qualifier dataset.

For all analyses, we explored the relationship between nutrient concentrations (dissolved phosphorus (DP), total P (TP), nitrate-nitrogen (NO_3-N), total N (TN)), electricial conductivity, and chl-a. Linear regression analysis was used to determine relationships between nutrient concentrations and sestonic chl-a using both raw median data and transformed median data (log_{10}) from both datasets. The log-log regressions were used to provide an equation from which we estimated what the nutrient concentration would be corresponding to 10 µg sestonic chl-a L^{-1}. Because re-transformation from log-log regression equations often results in bias, we also used a simplified bias correction factor (BCF, Helsel and Hirsch, 2002) to adjust the estimated nutrient concentration:

$$BCF = \frac{\sum 10^{e_i}}{n}$$

where n is the number of observations and e_i is the difference between the measured and estimated value in log_{10} units, and the re-transformed value can be multiplied by the BCF.

Because biological response to nutrient gradients may be subtle and difficult to detect with linear regression analysis, categorical and regression tree (CART) analysis was used to determine thresholds at which median chl-a variation changed across median nutrient concentrations or conductivity. CART analysis is very useful for resolving nonlinear, hierarchical, and high-order interactions among predictor variables (De'Ath and Fabricius, 2000) and for detecting numerical values that lead to ecological changes (Qian et al., 2003). CART models use recursive partitioning to separate data into subsets that are increasingly homogeneous. CART analyses were performed using MVPART library in R 2.8.1 (http://www.r-project.org/), requiring a minimum of 20 observations to be used in any single split and that each terminal node in the model had a minimum of ten observations. CART analysis is insensitive to missing data and outliers, so this analysis was conducted on the complete dataset that included median values from all 152 stations.

RESULTS

Sestonic chl-a increased within increasing nutrient concentrations, showing positive correlations with DP, TP, NO_3-N and TN (Table 1, P<0.01). When using the database that contained all sites (requiring only one observation per site), nutrients explained from 6% (NO_3-N) to 31% (TP) of the variability in sestonic chl-a concentrations (Figure 1). The number of observations used in regression analysis varied with the individual nutrients, ranging from 79 (TN) to 132 (TP). The log-log regression was used to estimate the nutrient concentration corresponding to 10 µg chl-a L^{-1}, resulting in re-transformed concentrations from 0.10 mg DP L^{-1} to 2.03 mg TN L^{-1}. BCFs ranged from 1.61 (TP) to 2.03 (TN), showing how re-transformation from log_{10} potentially underestimated nutrient concentrations corresponding to 10 µg chl-a L^{-1}. When using the qualifier dataset, the number of

Table 1. Regression statistics and nutrient concentrations at a sestonic chlorophyll-a (chl-a) concentration of 10 µg L^{-1} based on the log-log regressions using all median values from the dataset representing the Red River Basin.

Nutrient	Slope	Intercept	n	r^2	P	X (mg L^{-1}) at 10 µg chl-a L^{-1})	BCF
DP	0.48	1.47	120	0.21	<0.001	0.10	1.68
TP	0.68	1.47	132	0.31	<0.001	0.20	1.61
NO$_3$-N	0.30	0.93	119	0.06	0.007	1.70	1.76
TN	0.91	0.70	79	0.27	<0.001	2.11	2.03

n is the number of source stations used for data analysis; X is the nutrient concentration when Y equals 10 µg chl-a L^{-1} based on retransformation from the log-log regression; BCF is the bias correction factor that can be used to correct nutrient concentrations for any bias related to retransformation from log$_{10}$.

observations decreased substantially for all nutrient regressions. The number of sites with paired median chl-a and nutrient concentrations varied from 40 (TN) to 81 (TP), showing how few stations had multiple sestonic chl-a measurements. However, even with reduced numbers of observations sestonic chl-a was positively correlated with DP, TP, NO$_3$-N and TN (Table 2, P<0.01). The amount of variation in sestonic chl-a explained by nutrients varied from 12% (NO$_3$-N) to 37% (TP), where the r^2 increased with the qualifier dataset relative to all data (except for DP). The re-transformed nutrient concentrations corresponding to 10 µg cl-a L^{-1} with the qualifier dataset were less than those observed with all data, ranging from 0.09 mg DP L^{-1} to 1.56 mg TN L^{-1} (Figure 2). BCFs ranged from 1.37 (TN) to 1.51 (DP), suggesting that re-transformation bias might have underestimated nutrient concentrations by as much as 50%.

Table 2. Regression statistics and nutrient concentrations at a sestonic chlorophyll-a (chl-a) concentration of 10 µg chl-a L^{-1} based on the log-log regressions using the "qualifier" dataset representing the Red River Basin.

Nutrient	Slope	Intercept	n	r^2	P	X (mg L^{-1}) at 10 µg chl-a L^{-1})	BCF
DP	0.31	1.32	67	0.15	0.001	0.09	1.51
TP	0.58	1.48	81	0.37	<0.001	0.15	1.39
NO$_3$-N	0.30	1.08	70	0.12	0.003	0.53	1.49
TN	0.95	0.82	40	0.35	<0.001	1.56	1.37

n is the number of source stations used for data analysis; X is the nutrient concentration when Y equals 10 µg L^{-1} based on retransformation from the log-log regression; BCF is the bias correction factor that can be used to correct nutrient concentrations for any bias related to retransformation from log$_{10}$.
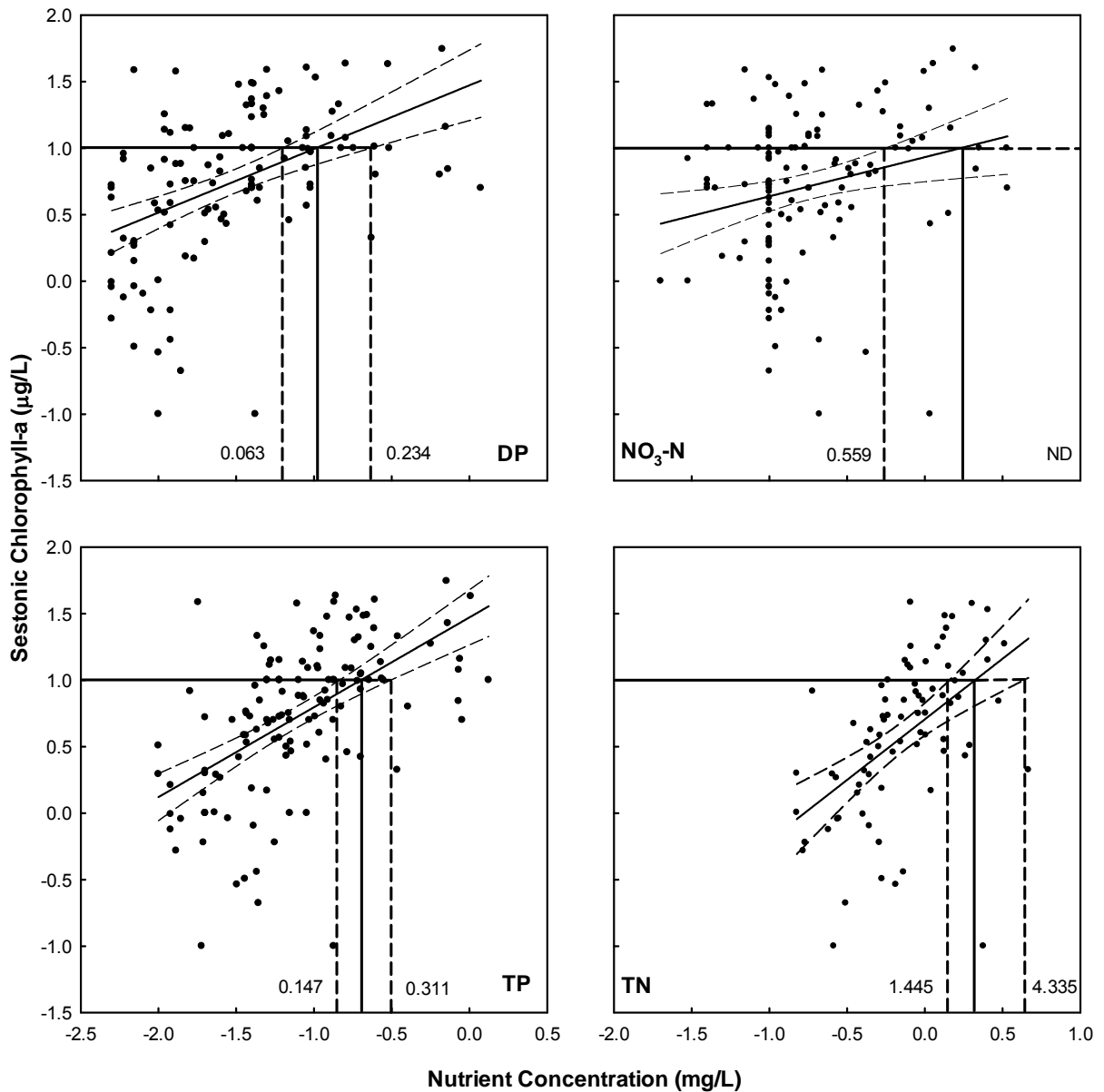
Figure 1. The relation between log-transformed nutrients and sestonic chlorophyll-a (chl-a) using all median values from the dataset representing the Red River Basin, where the dropdown dashed lines represent concentrations corresponding to 10 µg L^{-1} at the upper and lower 95% confidence interval about the slope.
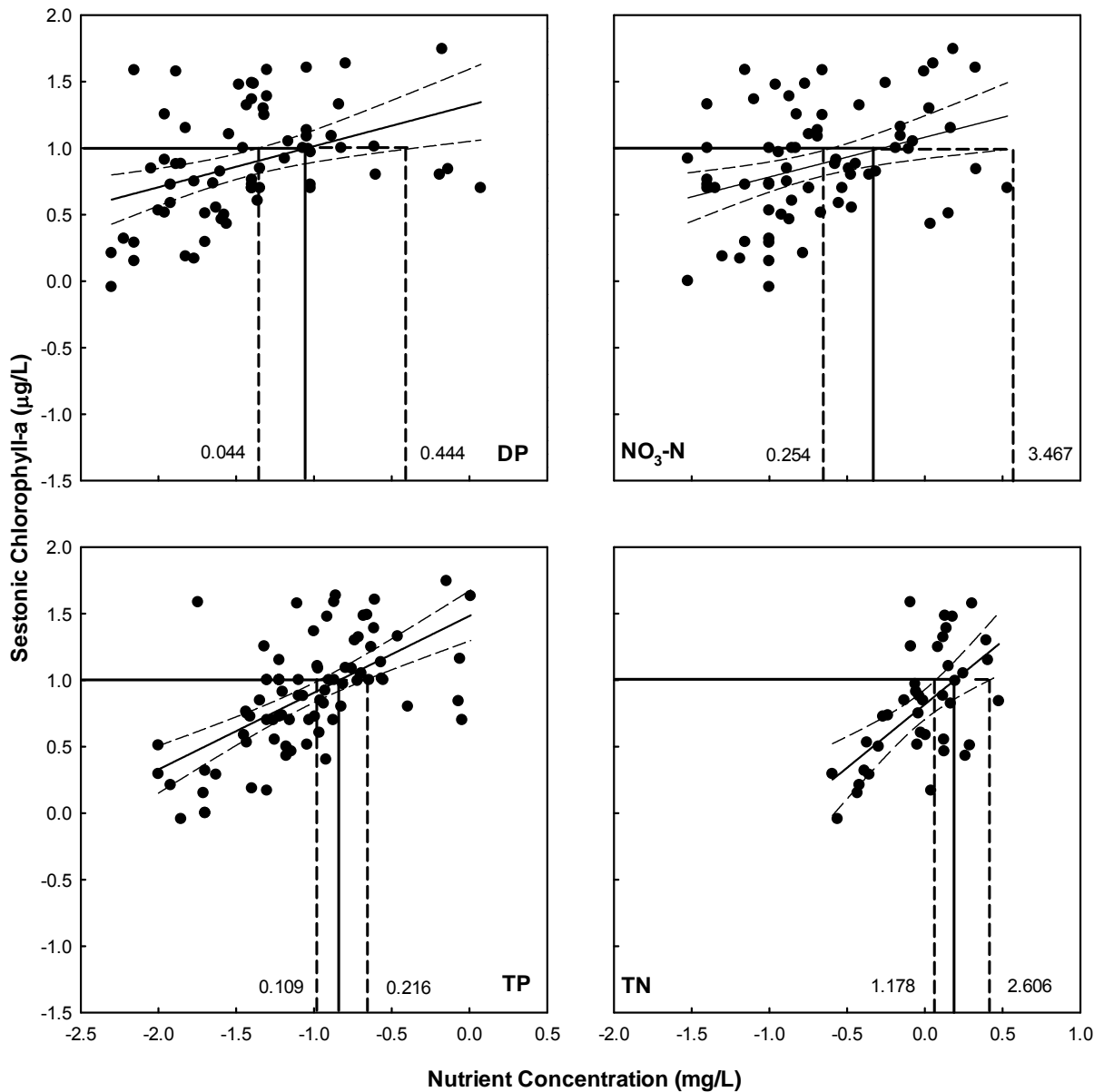
Figure 2. The relation between log-transformed nutrients and sestonic chlorophyll-a (chl-a) using the "qualifier" dataset representing the Red River Basin, where the dropdown dashed lines represent concentrations corresponding to 10 µg L^{-1} at the upper and lower 95% confidence interval about the slope.

Table 3. Regression statistics and nutrient concentrations at a sestonic chlorophyll-a (chl-a) concentration of 10 µg L^{-1} based on the log-log regressions using the "qualifier" dataset representing the aggregate ecoregions across the Red River Basin.

Nutrient	Slope	Intercept	n	r^2	P	X (mg L^{-1}) at 10 µg chl-a L^{-1}	BCF
Great Plains Grasses and Schrublands							
DP	-	-	11	<0.01	0.966	-	-
TP	0.57	1.451	16	0.29	0.032	0.16	1.79
NO$_3$-N	-	-	12	0.13	0.250	-	-
TN	-	-	3	0.36	0.592	-	-
Central and Eastern Forested Uplands							
DP	0.60	1.58	7	0.50	0.078	0.11	1.09
TP	0.73	1.49	7	0.64	0.030	0.22	1.06
NO$_3$-N	-	-	7	0.15	0.39	-	-
TN	1.27	0.80	7	0.61	0.038	1.44	1.07
South Central Cultivated Great Plains							
DP	0.24	1.36	35	0.10	0.065	0.03	1.38
TP	0.46	1.44	44	0.23	0.001	0.11	1.29
NO$_3$-N	-	-	37	0.06	0.130	-	-
TN	0.71	0.84	23	0.21	0.028	1.68	1.27
Southeastern Temperate Forested Plains and Hills							
DP	-	-	14	0.04	0.482	-	-
TP	0.44	1.28	17	0.31	0.040	0.23	1.22
NO$_3$-N	-	-	14	0.01	0.758	-	-
TN	2.08	1.13	7	0.98	0.000	0.87	1.01

n is the number of source stations used for data analysis; X is the nutrient concentration when Y equals 10 µg chl-a L^{-1} based on retransformation from the log-log regression.

Log-log regressions were also explored with qualifier data specific to the four aggregate eco-regions across the Red River Basin, including GPGS, CEFU, SCCGP, and STFPH (Table 4). Themajority of the data was from sites within the SCCGP, and log-log regressions were significant for OP, TP and TN within this eco-region. The other eco-regions had less than 20 stations with median values, but several significant log-log- regression were observed between nutrients and sestonic chl-a, except for NO$_3$. The retransformed nutrient concentra-tions that corre-sponded to 10 µg chl-a L^{-1} ranged from 0.03 to 0.11 mg L^{-1} for DP, 0.11 to

Table 4. Threshold values for individual nutrient concentrations and conductivity with sestonic chlorophyll-a concentrations using regressions using all median values from the dataset representing the Red River Basin.

Variable	Units	Threshold Value	Variance Explained (r^2)	Sestonic Chlorophyll-a Mean ± Standard Error [n]	
				Below Threshold	Above Threshold
DP	mg L^{-1}	0.03	0.17	5.72±0.96 [59]	14.8±1.52 [61]
TP	mg L^{-1}	0.14	0.24	6.71±0.76 [94]	18.2±2.14 [38]
NO$_3$-N	mg L^{-1}	0.49	0.14	7.82±0.85 [97]	17.9±3.16 [22]
TN	mg L^{-1}	0.75	0.30	2.90±0.43 [39]	12.5±1.61 [40]
Conductivity	µS cm^{-1}	1040	0.18	5.69±0.72 [69]	14.7±1.65 [63]

n denotes the number of observations used to estimate the chl-a mean and standard error.

0.23 mg L^{-1} for TP, and 1.44 to 1.68 mg L^{-1} for TN across the eco-regions of the Red River Basin. These concentrations increase relative to the BCF for each individual regression, where BCFs ranged from 1.01 for TN in the STFPH eco-region to 1.79 for TP in the GPGS eco-region.

While the log-log regressions suggested that TP explained the most variance in sestonic chl-a, CPA on individual independent variables (including DP, TP, NO$_3$-N, TN and conductivity) showed that TN explained more variance in sestonic chl-a than TP. Each nutrient and conductivity showed significant change points with sestonic chl-a (Table 4, $P<0.05$), where variance explained was in this order: TN (30%), TP (24%), conductivity (18%), DP (17%), and NO$_3$-N (14%). The change points for TN and TP were 0.75 and 0.14 mg L^{-1}, where median chl-a concentrations were above generally above 10 µg L^{-1} when total nutrient concentrations exceeded these concentrations.

CART analyses indicated that that hierarchical structure existed between nutrients, conductivity and sestonic chl-a (Figure 3). First, TP (0.14 mg L^{-1}, r^2=0.24) was the best predictor of sestonic chl-a. The data that had TP concentrations less than 0.135 mg L^{-1} split again with TN (0.74 mg L^{-1}, r^2 = 0.14), and that above 0.14 mg TP L^{-1} also split again with conductivity (1470 µS cm^{-1}, r^2=0.18). The regression tree explained 56% of the variation in sestonic chl-a

and identified three distinct groups of data, such as (1) sites with low chl-a (2.79 µg L^{-1} on average) where TP and TN were less than 0.14 and 0.74 mg L^{-1}, respectively, (2) sites with high chl-a (26.6 µg L^{-1} on average) where TP and conductivity exceeded 0.14 mg L^{-1} and 1470 µS cm^{-1}, respectively, and (3) sites with chl-a near 10 µg L^{-1} on average. The third group was observed under two scenarios, when TP was less than 0.14 mg L^{-1} but TN was greater than 0.74 mg L^{-1} and when TP was greater than 0.14 mg L^{-1} but conductivity less than 1470 µS cm^{-1}.

DISCUSSION

The USEPA (2000) stated that the frequency distributions of median nutrient concentrations provides a starting point for which nutrient criteria can be derived, based on the 25th percentile of all data or the 75th percentile of reference conditions. Reference condition has many interpretations, but Stoddard et al. (2006) suggested that this term should be reserved to imply the absence of human disturbance in streams. It is difficult to establish where reference conditions may exist because humans have altered the landscape extensively, and others might argue that reference conditions should not apply to streams draining catchments with human-altered landscapes. Specific to the Red River Basin, Longing and Haggard
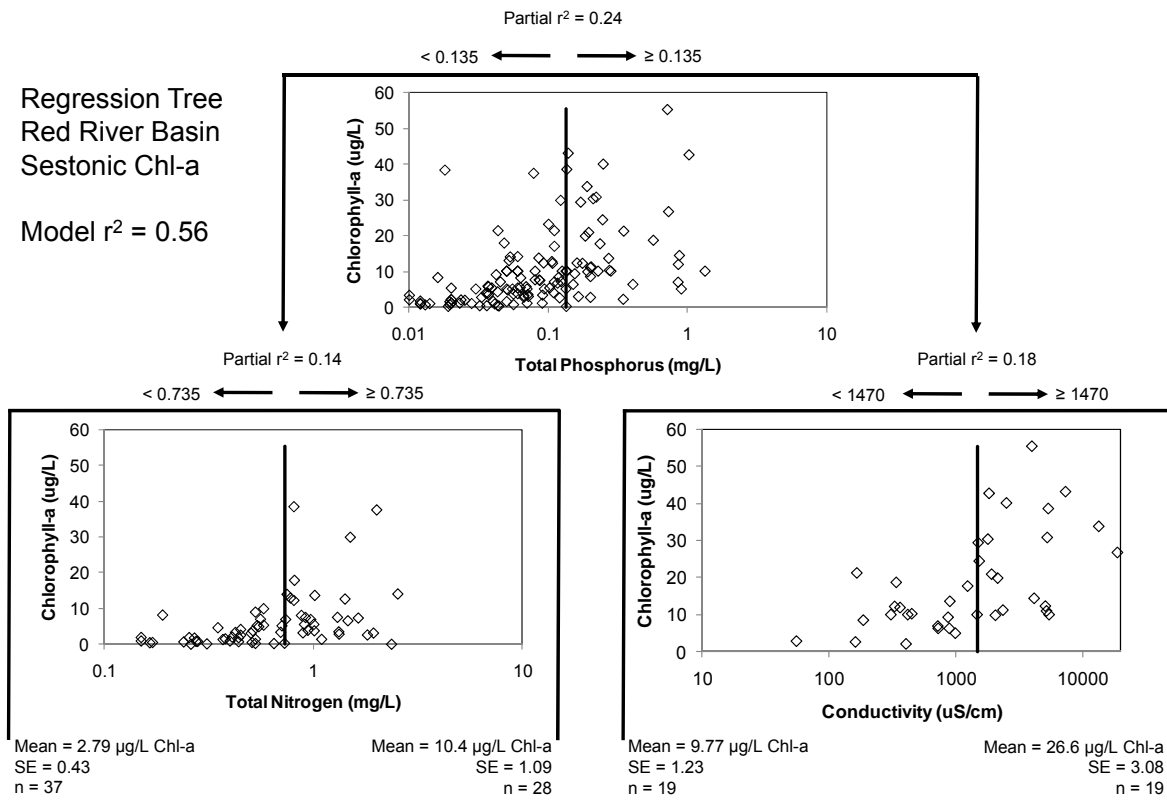
Figure 3. Hierarchical structure and threshold values in nutrients, conductivity and sestonic chlorophyll-a using all median values from the dataset across the Red River Basin.

(2010) presented 25th percentiles of median nutrient concentrations and compared those to values suggested in the USEPA aggregate eco-region approach (USEPA, 2002). The 25th percentiles were variable between the eco-regions within the Red River Basin and that compiled for the aggregate eco-regions. For example, the number of medians that exceeded the USEPA recommended numeric criteria was over 97% for TP in CEFU, and it was less than 45% for TN in this eco-region (Haggard and Longing, 2010). The 25th percentile of sestonic chl-a concentrations in the Red River Basin ranged from 0.75 to 10 µg chl-a L^{-1} across the different eco-regions (Longing and Haggard, 2010), which generally exceeded the values observed by USEPA (2000) for these ecoregions.

Similar to lakes, boundary conditions that separate oligo-mesotrophic and meso-eutrophic stream conditions have been derived from relationships between TP, TN, and sestonic chl-a for some streams and rivers (Dodds et al., 1998). Several studies have shown positive linear or curvi-linear relations between sestonic chl-a and nutrients in rivers (Van Nieuwenhuyse and Jones, 1996; Lohman and Jones, 1999; Royer et al., 2008; Chambers et al., 2011), especially when sites downstream from effluent discharges were excluded in the analysis (Morgan et al., 2006). The database developed by Longing and Haggard (2010) was limited by the availability of sestonic chl-a, but analysis of the available data showed a statistically significant linear regression between sestonic

chl-a and nutrients across the Red River Basin and within individual eco-regions. Thus, sestonic chl-a would likely be an important indicator of biological response in slow flowing, larger rivers like the Red River and its larger tributaries, as suggested by Royer et al. (2008) for larger rivers in Illinois.

Several studies have used these observed relations between nutrients and sestonic chl-a in a predictive manner, estimating either variable based on a given value. For example, Dodds (2006) suggested that 0.071 mg TP L^{-1} (representing the upper third of reference conditions from Smith et al., 2003) corresponded to 11.9 µg chl-a L^{-1} (based on nutrient-chl relation in Van Nieuwenhuyse and Jones, 1996). We presented nutrient concentrations that correspond to 10 µg chl-a L^{-1} based on the significant log-log regressions, showing the 95% confidence intervals of the regression. We initially used the 10 µg chl-a L^{-1} was an arbitrary value selected by the states within the Red River Basin, but we found corroborating evidence supporting this number from the literature (Dodds, 2006) and more importantly from the regression tree analysis derived in this study. The nutrient concentrations corresponding to 10 µg chl-a L^{-1} were variable for TN (0.87-2.11 mg L^{-1}) and TP (0.11-0.23 mg L^{-1}) across the Red River Basin and its eco-regions (Tables 1-3). The confidence interval about these concentrations varied from a doubling to an order of magnitude change between the low and high ends (Figures 1 and 2). These linear regressions were significant across the Red River basin and its eco-regions, but the amount of variation explained by nutrients was between 6 and 98% depending on the number of observations. The regressions using the qualifier dataset across the whole basin showed that nutrients explained between 12 and 37% of the variability in sestonic chl-a. There is variability not accounted for that needs to be considered when using these regressions, such as catchment area, time since last high flow

event, and other well-known physical controls on biological activity in streams (Dodds and Whiles 2010).

Sestonic chl-a may provide a reliable basis for evaluating the influence of total nutrient concentrations (TP and TN) in temperate streams, but the relationship between nutrients and sestonic chl-a can vary with catchment area. Van Nieuwenhuyse and Jones (1996) found that sestonic chl-a response to nutrients (i.e., TP) was 2.3 fold greater with a two-order-magnitude increase in catchment area. Catchment area was significantly related to sestonic chl-a across the Red River Basin, where variation in chl-a was significantly different above and below 11,600 km^2 (r^2 = 0.17, data not shown). This relationship was complicated by high chl-a concentrations observed in smaller streams with relatively high conductivity, as shown in the regression tree analysis (Figure 3). When we limited the regression to streams within a selected range of catchment area, it was evident that the nutrient concentrations corresponding to 10 µg chl-a L^{-1} were greater for streams with smaller catchment and decreased to the values observed for larger database (Figure 4). In these streams, average nutrient concentrations that yielded approx.-imately 10 µg chl-a L^{-1}were 0.14 mg TP L^{-1} and 1.56 mg TN L^{-1} (Table 2). These threshold values were similar in magnitude to those derived from the complete dataset, which suggests that the larger catchments had the most influence on the linear regressions.

The importance of non-linear stressor-response relationships has been observed in several recent studies on the effects of nutrient enrichment on aquatic ecosystems (Qian et al., 2003; Chambers et al., 2011a). CART analyses indicated that sestonic chl-a across the Red River Basin were generally greater when TP was greater than 0.14 mg L^{-1}, which is greater than most TP-chl-a thresholds reported from other rivers. For example, Royer et al. (2008) sug-
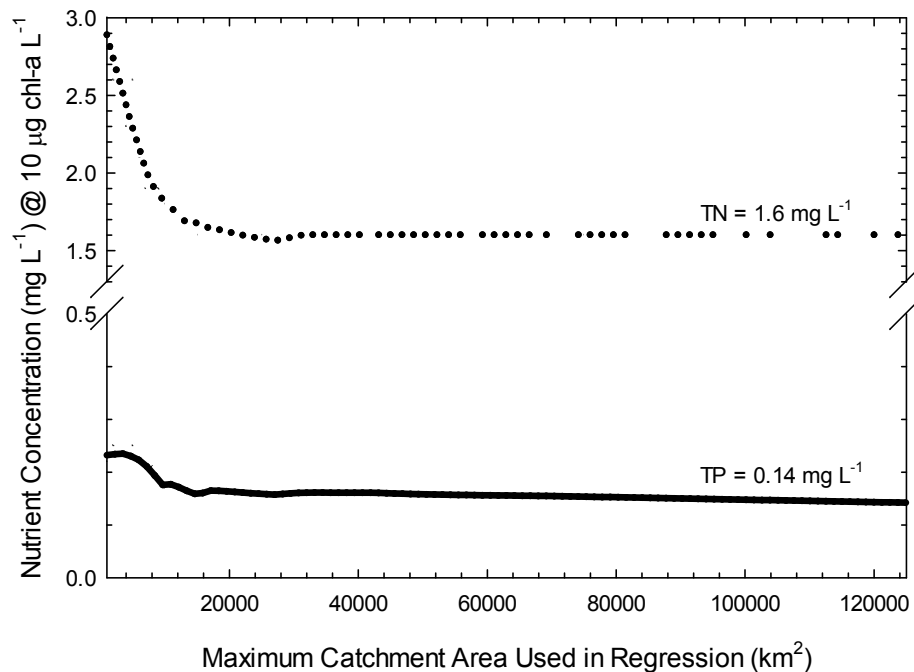
Figure 4. Variation in the nutrient concentrations corresponding to a sestonic chlorophyll-a concentration of 10 µg L^{-1} with changes in catchment area, based on linear regressions.

gested an apparent threshold of 0.07 mg TP L^{-1} was observed in the relationship between sestonic chl-a and TP in Illinois streams, when the stream had an less than 25% open canopy and TP was less than 0.20 mg L^{-1}. Literature TP thresholds for other biological variables (e.g., benthic chl-a, diatom species, and macroin-vertebrate indices) generally varied from <0.01 to 0.10 mg L^{-1} (Evans-White et al., 2009; Stevenson et al., 2008; Wang et al., 2007). The threshold for TN (0.75 mg L^{-1}) at the Red River Basin was relatively similar to values reported in the literature for different biological response variables (0.3 to ~2 mg L^{-1}; e.g., see Black et al., 2011; Evans-White et al., 2009; Chambers et al., 2011a).

We showed that hierarchical structure existed with sestonic chl-a and TN, TP and conductivity (r^2=0.56), and the thresholds in the regression tree model were similar to that observed when

assessed individually. Although CART analysis is inherently designed to identify hierarchy in predictor variables in large datasets (De'Ath and Fabricius, 2000), this capability has rarely been applied in analyses of nutrient threshold data-sets. However, information gleaned from this hierarchy could be potentially useful in considering criteria. For example, in the Red River basin at least ten sites had median chl-a concentrations that exceeded 10 µg L^{-1} when TP concentrations were less than 0.14 mg L^{-1} (Figure 3 top panel). However, all ten of these sites had TN concentrations that exceeded 0.75 mg L^{-1} (Figure 3 lower left panel). As a result, the combination of slightly lower TP concen-trations, perhaps those more similar to those observed in other locations (0.05-0.1 mg L^{-1} TP), and TN concentrations exceeding 0.75 mg L^{-1} resulted in median chl-a concentrations that approached or exceeded 10 µg L^{-1} in more than 50% of sites. Therefore, utilizing hierarchical

structure as a tool for understanding large datasets may further inform the process of nutrient criteria development.

The overall goal of this project (including Longing and Haggard, 2010) was to provide the statistical analysis to help guide the state agencies responsible for water quality standards and numeric nutrient criteria. The first step established in Longing and Haggard (2010) reported frequency distributions of TN, TP and sestonic chl-a at various spatial levels (ecoregions to hydrologic unit code 8), where the 25th percentiles varied 0.02-0.07 mg L^{-1} for TP and 0.21-0.86 mg L^{-1} for TN. The next step was the relation between nutrients and biological response (sestonic chl-a) in this study, using simple linear regression and regression tree models, i.e. CART. The nutrient concentrations corresponding to 10 µg chl-a L^{-1} or threshold response were generally greater than the 25th percentiles for TP and TN recommended by the (USEPA, 2002) and reported by Longing and Haggard (2010), although the TN ranges slightly overlaped. Recently, the USEPA (2010, 2011) have released guidance on using nutrient (stresssor) and biological response with which this project generally followed those procedures (when applicable).

The Red River is a transboundary, multi-jurisdictional basin, where water-quality standards often change at political boundaries and numeric nutrient criteria do not currently exist. The stressor-response relationships explored in this study suggest that numeric nutrient criteria within the ranges of 0.10-0.22 mg TP L^{-1} and 0.75-2.11 mg TN L^{-1} may control sestonic chl-a concentrations in the basin in the range of 10 µg L^{-1} or less. However, this assumes that the average physical conditions within the basin which may also influence algal biomass remain similar to the conditions which were present during the time of monitoring. Total P thresholds for the Red River Basin were likely greater than other studies because of the relatively turbid nature of this system, and this should be taken into consideration in the development of numeric nutrient criteria for this multi-jurisdictional basin. However, it appears that TN thresholds in the Red River Basin were within the range of thresholds reported in the literature. The numbers reported in this study are applicable to the streams and rivers within this larger basin, and are not necessarily protective of other aquatic systems, such as Lake Texoma or the Gulf of Mexico. The downstream transport of nutrients and potential effects in reservoir and estuary systems might require reduced numeric nutrient criteria relative to flowing waters.

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