Escherichia coli and Dissolved Oxygen Trends in the Upper Llano River Watershed, Texas (2001-2016)

Texas Water Resources Institute | College Station, Texas
Llano River Field Station | Junction, Texas

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List of Abbreviations

cfu colony forming unit

DAR Drainage-Area Ratio

DO dissolved oxygen

E. coli *Escherichia coli*

EPA US Environmental Protection Agency

GAMMs Generalized Additive Mixed Models

L liter

mg milligram

mL milliliter

TCEQ Texas Commission on Environmental Quality

ULRWPP Upper Llano River Watershed Protection Plan

USGS US Geologic Survey

WRTDS Weighted Regressions on Time, Discharge, and Season

Abstract

Trends in *Escherichia coli* (*E. coli*) and dissolved oxygen (DO) at Upper Llano watershed water quality monitoring stations were evaluated for calendar years 2001-2016. *E. coli* concentrations and loads were evaluated with linear regressions and Weighted Regressions on Time, Discharge, and Season (WRTDS). DO concentrations were evaluated with linear regression and generalized additive mixed models (GAMMs). WRTDS and GAMMs were also used to remove effects of year to year variations of flow on *E. coli* and annual variations in mean temperature on dissolved oxygen. Linear regression indicated a statistically significant decrease in *E. coli* concentrations on the main stem of the Llano River immediately downstream of the the North and South Llano River confluence. Linear regression did not indicate statistically significant changes in DO concentrations at any sites in the the watershed. From 2001 through 2016 total *E. coli* loads decreased by 3.99×10^4 million colonies per day on the North Llano and 1.34×10^7 million colonies per day on the Llano River. Flow-normalized loads decreased by 1.27×10^5 million colonies per day and 5.87×10^6 million colonies per day on the North Llano and Llano rivers respectively. Estimated *E. coli* load reductions appear substantial; however, the estimations are considerably biased which decreases the certainty that estimated load reductions are indeed real. Despite uncertainty, it is reasonable to infer that land use practices and changes in the watershed have not contributed to increases in flow-normalized *E. coli* loads between 2001-2016. GAMMs estimate that mean DO concentrations decreased 8.8% on the North Llano and 8% on the Llano during the same time period. While substantial, mean DO concentration remain well within state water quality standards. While GAMMs substantiate underlying trends in DO concetration, they do not provide insight into the driving mechanisms behind the observed responses in DO.

Keywords: Llano River, *E. coli*, Dissolved Oxygen, Water Quality

Introduction

The Upper Llano River Watershed includes the North and South Llano rivers and is located in the central Texas hill country (Figure 1). The North and South Llano are known for healthy water quality that support diverse aquatic communities and sustain locally important recreational opportunities. Although both rivers meet existing water quality standards, local stakeholders voiced concern about changing land uses, proliferation of invasive species, and pressures on water supply as local populations grow. These concerns lead to the formation of local stakeholder organizations and the development of the Upper Llano River Watershed Protection Plan (ULRWPP). The ULRWPP, accepted by the US Environmental Protection Agency (EPA) in Summer 2016, identified and recorded those key concerns raised by local stakeholders. Among the surface water quality issues identified in the plan were concerns about low dissolved oxygen (DO) and the elevated indicator bacteria, *Escherichia coli* (Broad et al., 2016). Since 2016, implementation efforts in the watershed to reduce *E. coli* bacteria loads and potential contributors to depressed DO have expanded. Amongst these efforts are enrolling agricultural producers into Natural Resource Conservation Service conservation plans or prescribed grazing plans intended to reduce runoff, protect riparian areas, increase soil health, and reduce livestock time spent in streams. Although limited water quality sampling has occurred, this report intends to provide an understanding of bacteria and DO trends through 2016. The purpose of this report is to evaluate changes in water quality concentrations over time using simple statistical regression methods and to evaluate potential correlations with DO concentrations.

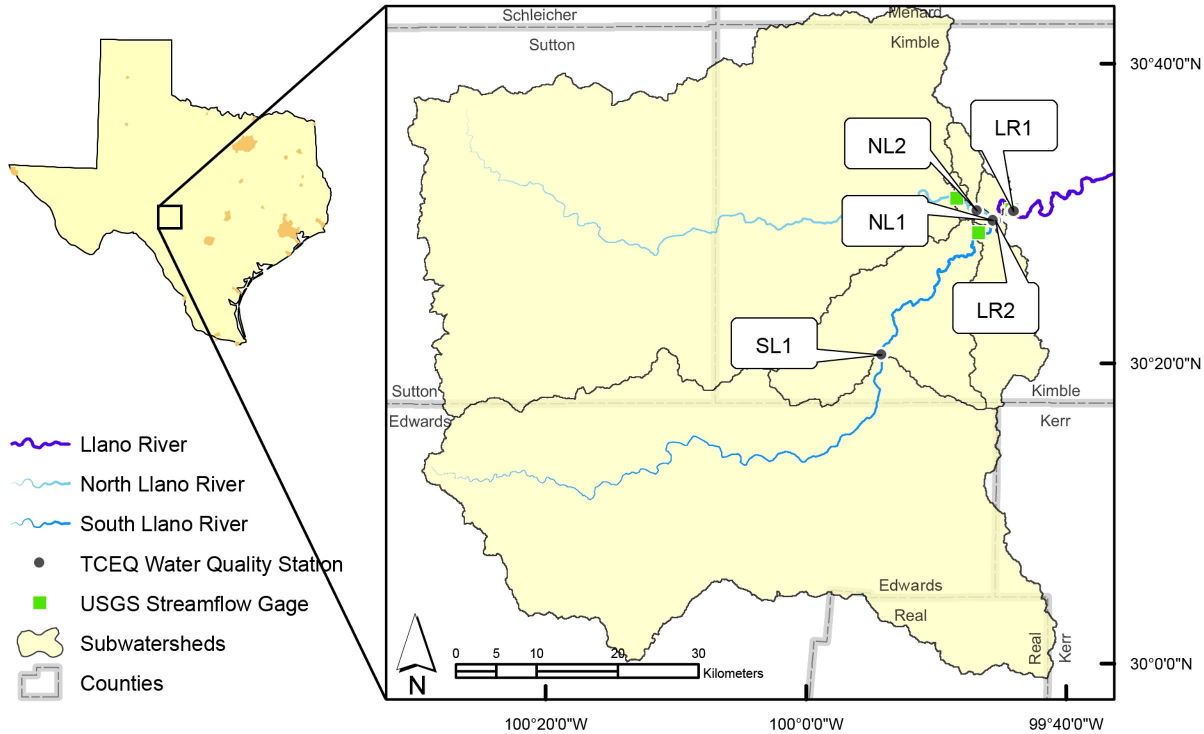

Figure 1: Study area

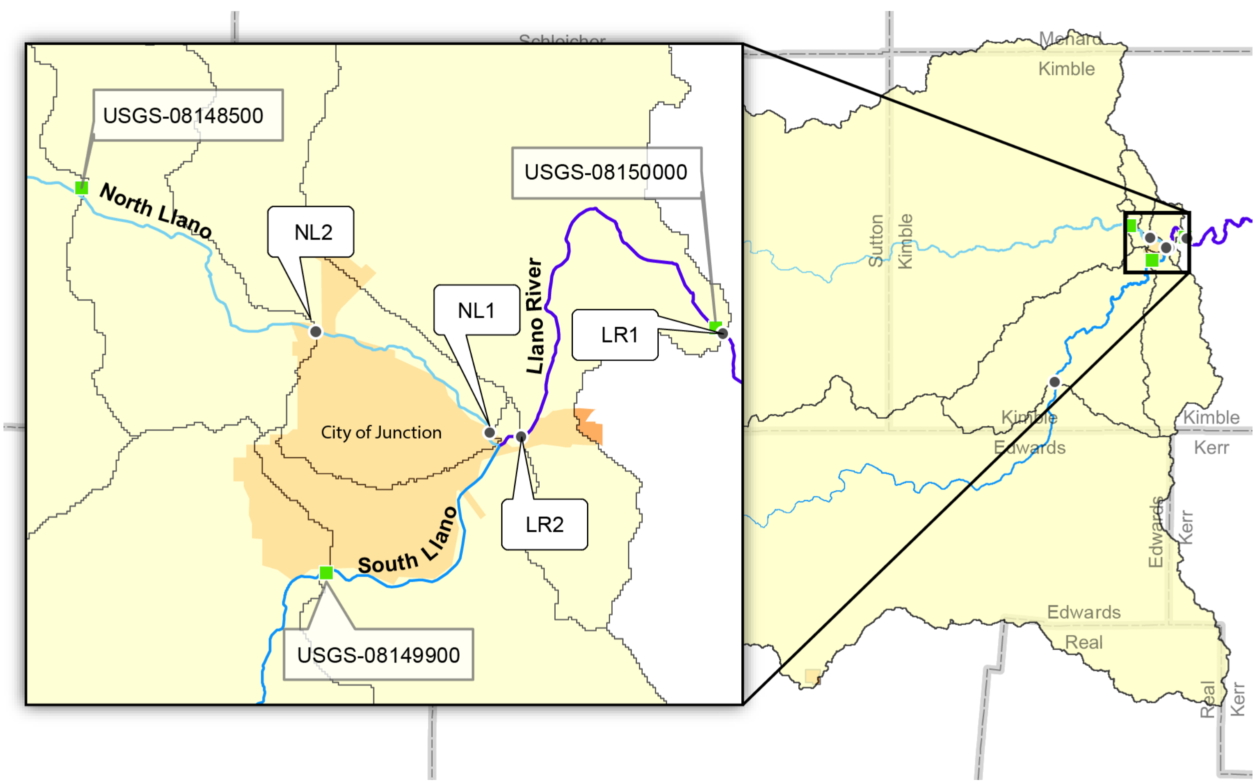

Figure 2: Detailed map of stations near Junction, TX (LR1, LR2, NL1, and NL2)

Table 1: USGS gage summary information

USGS Site No.	Q_{min}*	Q_{max}†	$\bar{Q}$‡	$\tilde{Q}$§	Drainage Area (km^2)	Name	Period of Record
08148500	0.0	13000	37.39	16.0	2332	North Llano River near Junction, TX	2001-2016
08149900	23.4	4160	70.88	49.1	2277	South Llano River at Flat Rock Ln at Junction, TX	2012-2016
08150000	31.8	45900	146.11	95.6	4809	Llano River Near Junction TX	2001-2016

* Minimum flow
† Maximum flow
‡ Mean Flow
§ Median Flow

Table 2: TCEQ water quality monitoring station summary

TCEQ Station Number	Reference Name	Number of Samples	Date Range	Drainage Area	$\frac{A_y}{A_x}$
17471	LR1	16	2011-10-03 : 2015-08-10	4809	NA
21489	LR2	41	2001-08-06 : 2015-02-06	4789	0.996
17425	NL1	35	2001-09-11 : 2014-08-18	2372	1.017
21548	NL2	8	2014-10-13 : 2016-10-18	2342	1.004
18197	SL1	13	2011-04-20 : 2016-10-18	1941	0.852

Methods

Study Location and Data Sources

The Upper Llano Watershed includes the catchments of both the North Llano and South Llano River (Figure 1). For purposes of this report, the watershed was extended downstream to capture water quality trends of the combined flows from both watersheds. The project watershed was 4,809 km² (1,188,330 acres).

We obtained mean daily streamflow from three gages in the study area from the US Geologic Survey (USGS) National Water Information System Figure (1, Figure 2, and Table 1). The Texas Commission on Environmental Quality (TCEQ) water quality data was obtained through the Clean Rivers Program data tool. Although a number of water quality monitoring stations occur within the watershed, stations with less than one year of data were omitted because they could not provide meaningful insight on temporal concentration trends. Records from five water quality stations were included in the analysis (Figure 1, Figure 2, and Table 2). Although NL2 includes a limited number of available sample events, it was included because sampling at the long-term station NL1 was recently discontinued due to site access issues. Water quality monitoring data used in this report were collected by TCEQ, Lower Colorado River Authority, Texas State University, and other Clean Rivers Program Partners. The collection of these data are conducted under respective TCEQ and EPA approved Quality Assurance Project Plans.

Streamflow Estimation

Mean daily streamflows for ungaged water quality monitoring sites (LR2, NL1, NL2, SL1) were developed using the DAR method. DAR provides a simple method of estimating streamflows at ungaged sites, in particular for

Table 3: Reccomended ϕ values for DAR method applied in Texas streams (Asquith et al., 2006)

Streamflow percentile	ϕ	Streamflow percentile	ϕ	Streamflow percentile	ϕ
0	0.885	38	0.893	76	0.934
2	0.885	40	0.894	78	0.934
4	0.885	42	0.895	80	0.933
6	0.886	44	0.897	82	0.933
8	0.886	46	0.899	84	0.931
10	0.887	48	0.902	86	0.927
12	0.887	50	0.905	88	0.920
14	0.887	52	0.908	90	0.906
16	0.887	54	0.912	92	0.890
18	0.888	56	0.916	94	0.865
20	0.888	57	0.920	95	0.850
22	0.888	60	0.924	96	0.830
24	0.889	62	0.927	97	0.806
26	0.889	64	0.930	98	0.773
28	0.889	66	0.932	99	0.737
30	0.890	68	0.934	100	0.700
32	0.890	70	0.935		
34	0.891	72	0.935		
36	0.892	74	0.935		

nested subwatersheds (Hirsch, 1979; Ries and Friesz, 2000; Emerson et al., 2005). DAR calculates streamflow discharge as:

$$Y_i = X_i(\frac{A_y}{A_x})^{\phi}$$

where Y_i is the calculated discharge at the ungaged site at time i, X_i is discharge measured at the gaged site at time i, while $(\frac{A_y}{A_x})$ is the ratio of ungaged and gaged catchment areas. Values used for $(\frac{A_y}{A_x})$ are indicated in Table 2.

In most applications, the exponent, ϕ, is assumed to equal one (Asquith et al., 2006). However, when applied to Texas streams, $\phi = 1$ results in substantial over- or under-estimation at the largest magnitude streamflows (Asquith et al., 2006). Therefore, we applied values for ϕ based on streamflow percentile values as indicated by Asquith et al. (2006) (Table 3). To facilitate interpretation of hydrologic conditions as each station, flow duration curves were plotted and converted to load duration curves overlaid with water quality measurements (Morrison and Bonta, 2008). The load duration curves were developed using Texas's primary contact recreation criterion for *E. coli* bacteria in surface freshwater bodies of 126 colony forming unit (cfu)/100 milliliter (mL). The duration curves provide a visual analysis of flow conditions at stations, as well as an understanding of what flow conditions water quality concentrations and loads exceed established water quality standards.

Correlation Analysis

Monotonic relationships between DO concentrations, log transformed streamflow, and stream temperature were evaluated graphically and with Kendall's Tau (Helsel and Hirsch, 2002). Censored values and outliers were not filtered due to the resistance of rank-methods such as Kendall's Tau to these values. Correlations were considered significant at the $p < 0.05$ level. All statistical analysis and plots were completed in R, versions 3.4.0 (R Core Team, 2016).

Concentration and Load Trends

We evaluated trends in bacteria concentrations collected at each station with simple linear regressions of log transformed concentrations against date (Helsel and Hirsch, 2002). DO concentrations were evaluated using untransformed values regressed against date. The null hypothesis of zero slope over time was rejected at $p < 0.05$. Scatter plots of concentrations and dates are provided to aid result interpretation. We verified linear regression assumptions of normality with qqplots and by plotting model residuals against dates.

LR2 and NL1 were the only stations with enough data to estimate concentrations and loads using regression based techniques. We estimated in-stream mean daily bacteria loads using the Weighted Regressions on Time, Discharge, and Season (WRTDS) approach with the `EGRET` package in `R` (Hirsch et al., 2010; Hirsch and De Cicco, 2015). We used WRTDS outputs to describe trends in concentration and loads over time. WRTDS is a regression-based approach that models log transformed water quality constituents concentrations as a function of time, flow, and season. The functional model is:

$$ln(E.coli) = \beta_0 + \beta_1 T + \beta_2 ln(Q) + \beta_3 cos(2\pi t)$$

Where T is decimal time and included in the model as an annual term (β_1) or seasonal term (β_3, β_4), and Q is flow. WRTDS differs from other regression based estimators, such as LOADEST, by using a weighted regression to estimate coefficients for each combination of Q and t. The coefficients are used to estimate daily concentrations, daily loads, and flow-normalized loads and concentrations. Flow-normalized values are derived from removal of variation in concentration based on random variations in discharge. Further details of WRTDS are outlined in Hirsch et al. (2010). These flow normalized values are useful to understand how changes in land practices over time affect in-stream constituent concentrations and loads. In particular flow normalized values are less influenced by increases in loads solely attributed to increases in stormflow runoff. Flow-normalized values provide useful insight not reflected by regression on concentration values alone, which can be influenced by changes in precipitation and runoff and might not reflect progress in land management activities.

We estimated in-stream DO concentrations at LR and NL1 using Generalized Additive Mixed Models (GAMMs) (Wood, 2008). GAMMs are regression models that use non-linear smooth functions on covariates. DO concentrations were not expected to be a function of flow, but a function of stream temperature, season, long-term trend, and in-stream metabolic processes. Therefore, we utilized GAMMs to describe DO concentration because it allows the inclusion of specified terms and random effects. The generalized GAMMs model used to fit DO concentrations is:

$$DO = \beta_0 + \beta_1 ln(Q) + \beta_2 Year + \beta_3 Month + \beta_4 Temperature$$

Where Q is flow, $Year$ is year term, $Month$ is the seasonal month term, and $Temperature$ is the water temperature term. Appropriateness of GAMMs model fits were evaluated by inspecting model residuals following procedures described by Zuur et al. (2009). GAMMs were developed using the `mgcv` and `nlme` packages in `R`.

Results

Flow Duration Curves

The flow duration curves (Figure 3) indicate the Llano River at stations LR1 and LR2 maintained consistent flows through the period of record (2001-2015). Compared to LR1 and LR2, the flow duration curves of the North Llano at stations NL1 and NL2 depict a flashier stream with periods of no flow. Flows at SL1 indicate a more consistent flow, likely contributing to consistent flows seen downstream at LR1 and LR2. Streamflow records for SL1 are limited because the USGS gage on the South Llano began data collection in 2012.

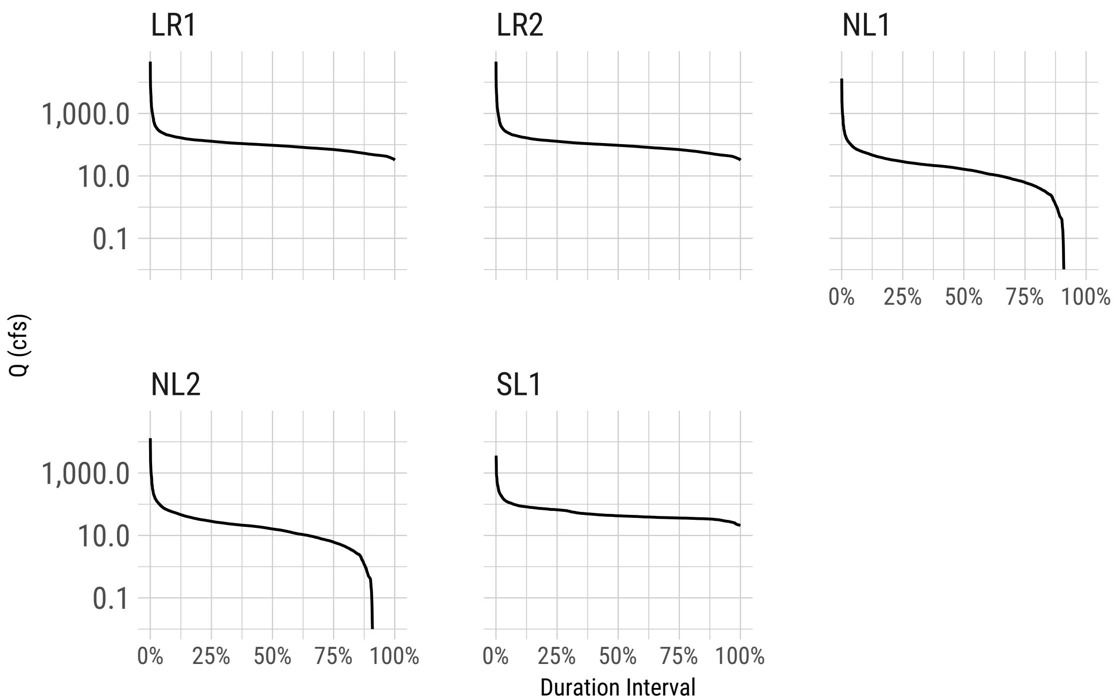

Figure 3: Flow duration curves of estimated mean daily flows at each station. Duration interval indicates the percent of days the mean daily flows is exceeded.

E. coli

E. coli Load Duration Curves

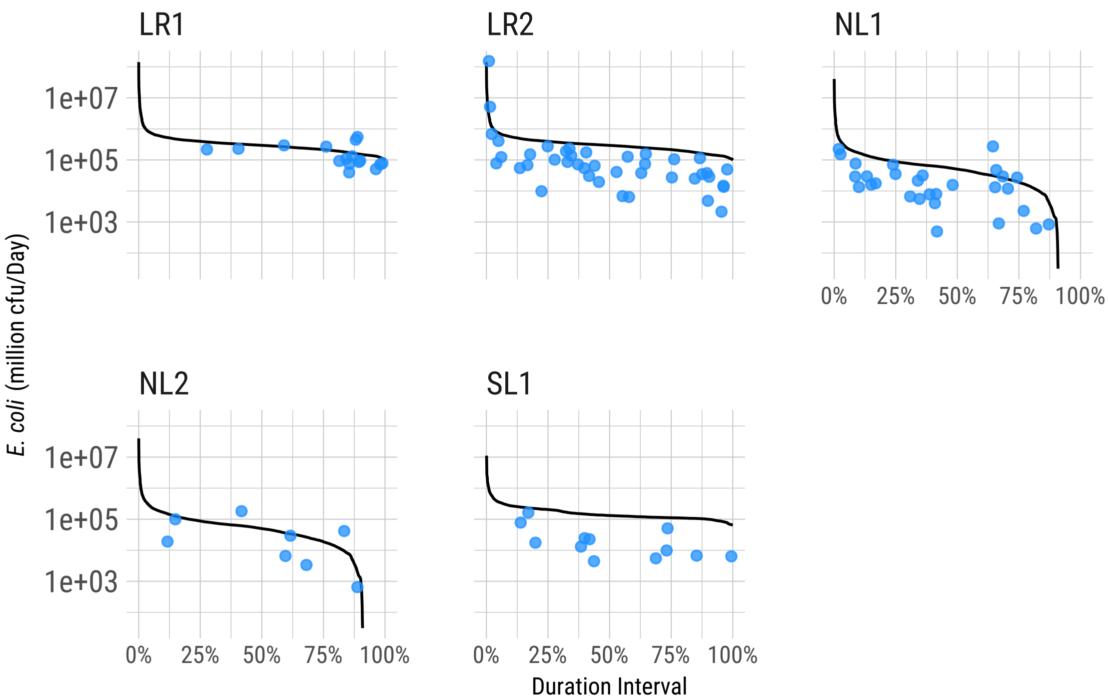

Figure 4: Bacteria load duration curves. The black line indicates allowable bacteria loads based on the 126 colonies per 100 mL water quality criterion. The points indicate measured bacteria concentrations converted to loads based on estimated mean daily flows.

Load duration curves (Figure 4) indicate bacteria concentrations and loads remain below the allowable criterion across all flow conditions at each station. Isolated individual load exceedances, where measured values are above the load duration curve line, occur sporadically across the duration interval.

E. coli Concentration Trends

Linear regression of log transformed bacteria concentrations indicated a significant decrease in *E. coli* concentration at LR2 (T = -2.08, p <0.05) from 2001 through 2016 (Figure 5). The null hypothesis of no increasing or decreasing trend was not rejected at LR1, NL1, NL2, or SL1 over the respective available sample periods.

Figure 6 depicts the WRTDS estimated and flow-normalized bacteria concentrations and loads at LR2 and NL1. *E. coli* concentration at LR2 decreased 74.5 cfu/100mL and load decreased 1.34×10^7 million colonies per day (Table 4, Figure 6). In contrast, daily flow-normalized *E. coli* concentration decreased 33 cfu/100mL and flow normalized loads decreased 5.87×10^6 million colonies per day at LR2 (Table 4, Figure 6). The changes in estimated values are relatively large compared to the flow-normalized values, suggesting some amount of decrease is

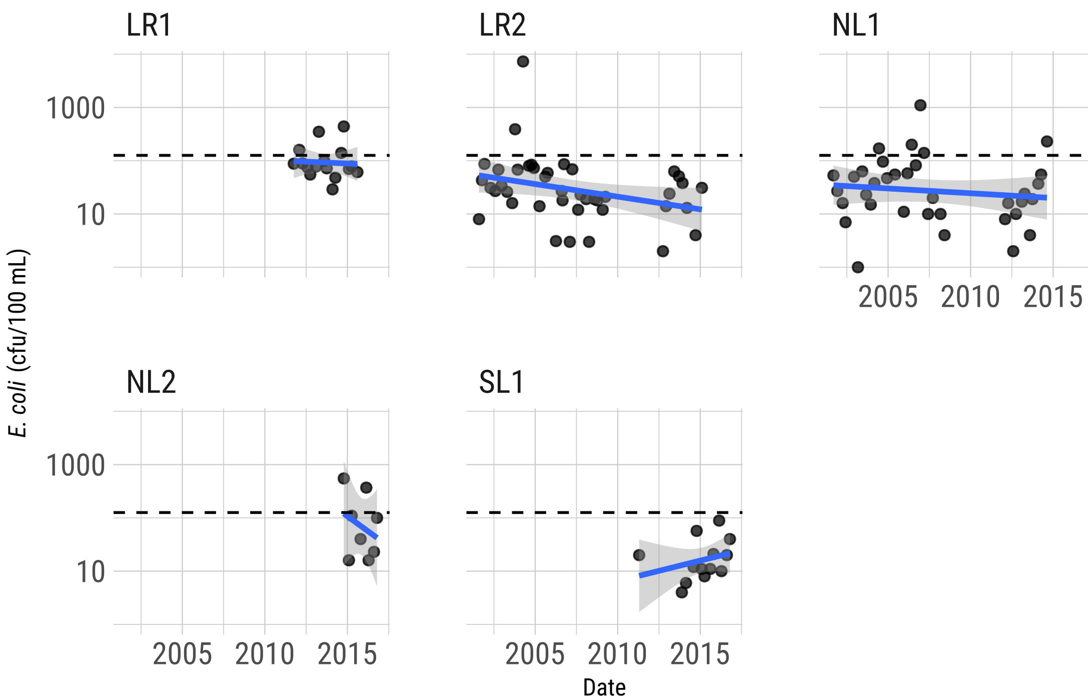

Figure 5: *E. coli* concentrations plotted against time for each station. The solid line is the linear regression through all points, and the shaded area indicates the 95% confidence intervals.

likely the result of changes in streamflow during the sample period and not just a result in changes of on-land practices.

E. coli concentration at NL1 decreased 396.9 cfu/100mL and load decreased 3.99×10^4 million colonies per day. Flow-normalized *E. coli* concentration and load at NL1 decreased by 221 cfu/100mL and 1.27×10^5 million colonies per day from 2002 through 2016. The relatively small change in flow-normalized load in comparision to the estimated change in load suggest that changes in flow at NL1 dampened the observed changes of in-stream loads.

Although substantial, the estimates for both stations exhibit considerable uncertainty with estimated bias statistics of 0.287 for LR2 and 0.138 for NL1. An inspection of model diagnostics indicate samples at both stations are considerably underrepresented at high flow events. The bias reduces certaintity that the estimated loads reductions are true. Furthermore, temporal gaps (2009-2011) in monitoring data decrease confidence in estimated concentration and loads.

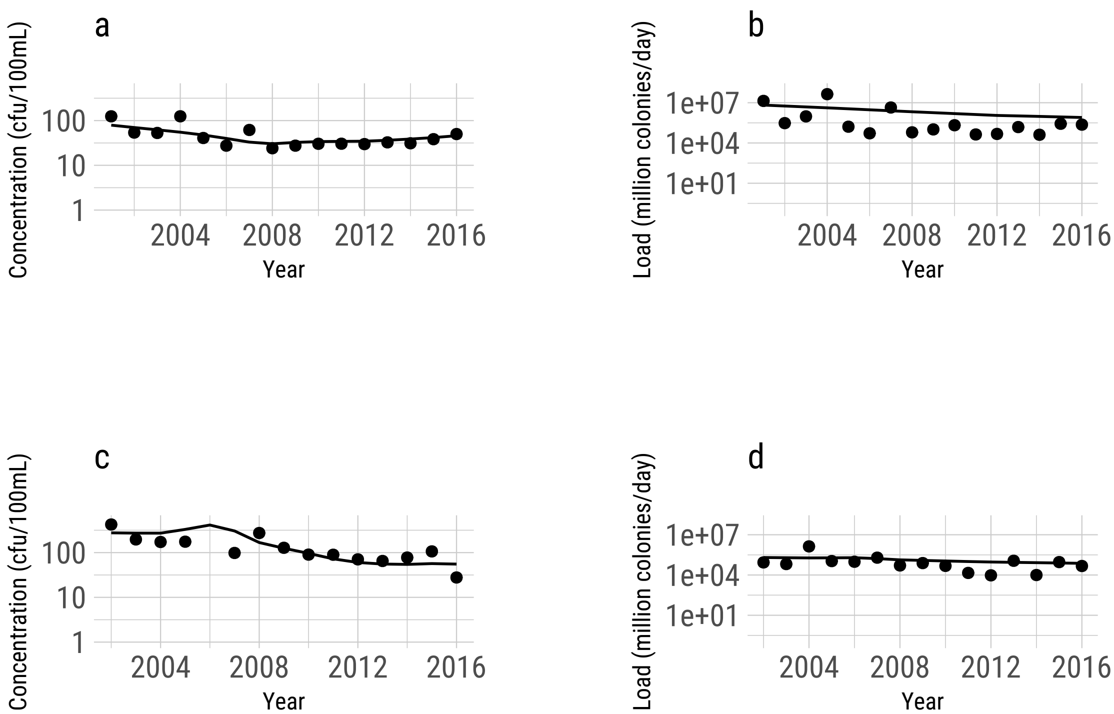

Figure 6: Annual mean estimated (dots) and flow-normalized (lines) *E. coli* concentrations at LR2 (a) and NL1 (b); annual mean estimated and flow normalized *E. coli* loads for LR2 (b) and NL1 (d).

Figure 7 and Figure 8 show the WRTDS estimates of *E. coli* concentration at LR2 and NL1 as a function of discharge over season and year. LR2 is characterized by increases in concentration with increases in discharge. However, in recent years, that relationship is less strong or even negative during spring and early summer months. Estimates at NL1 (Figure 8) indicate elevated concentrations at lower flows which generally decrease with increase discharges. There also appears to be a substantial shift in the concentration-discharge relationship during the June through December time-period with *E. coli* concentrations decreasing more rapidly with increased discharge in recent years.

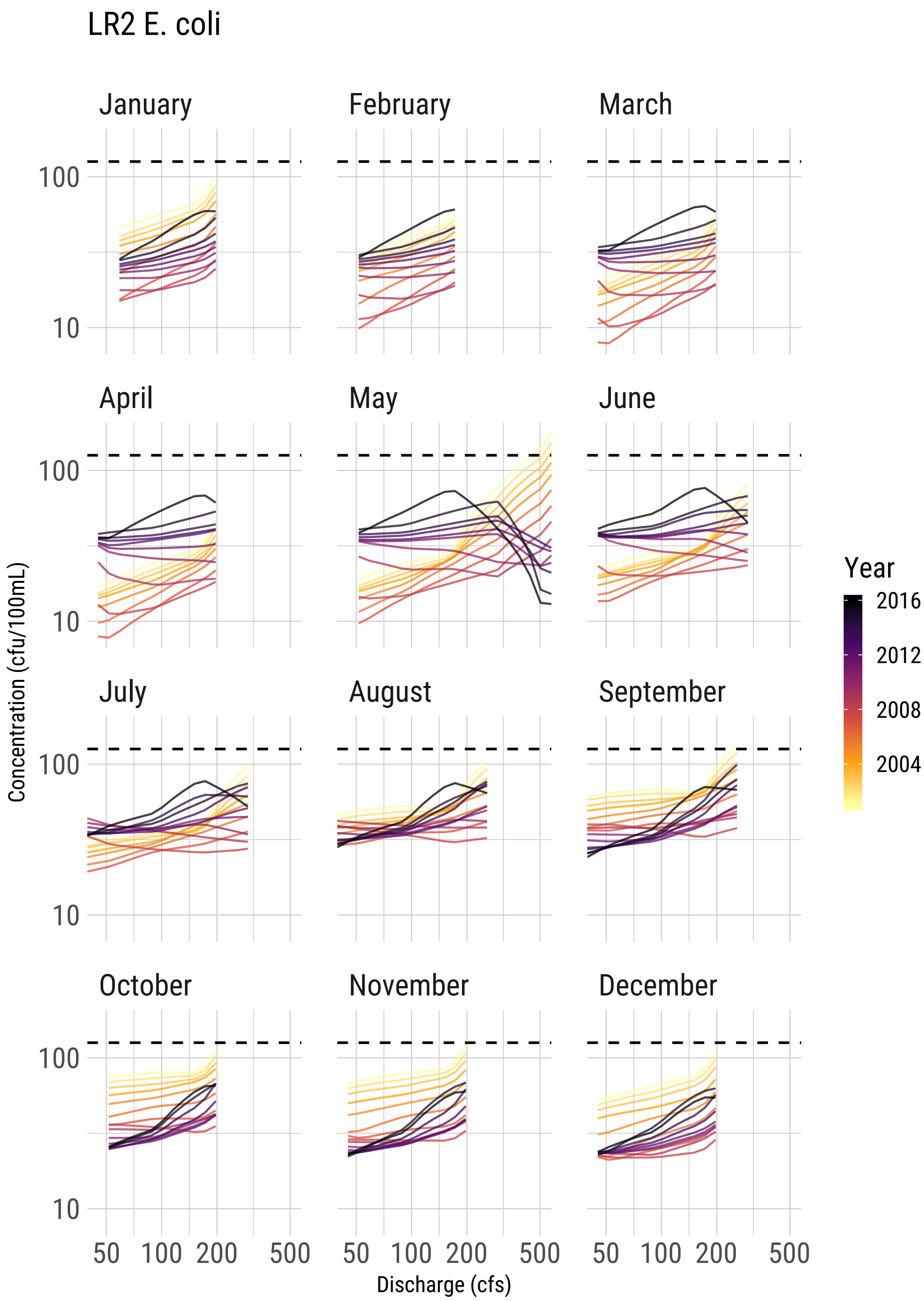

Figure 7: *E. coli* concentration and flow relationship over time and month at LR2.

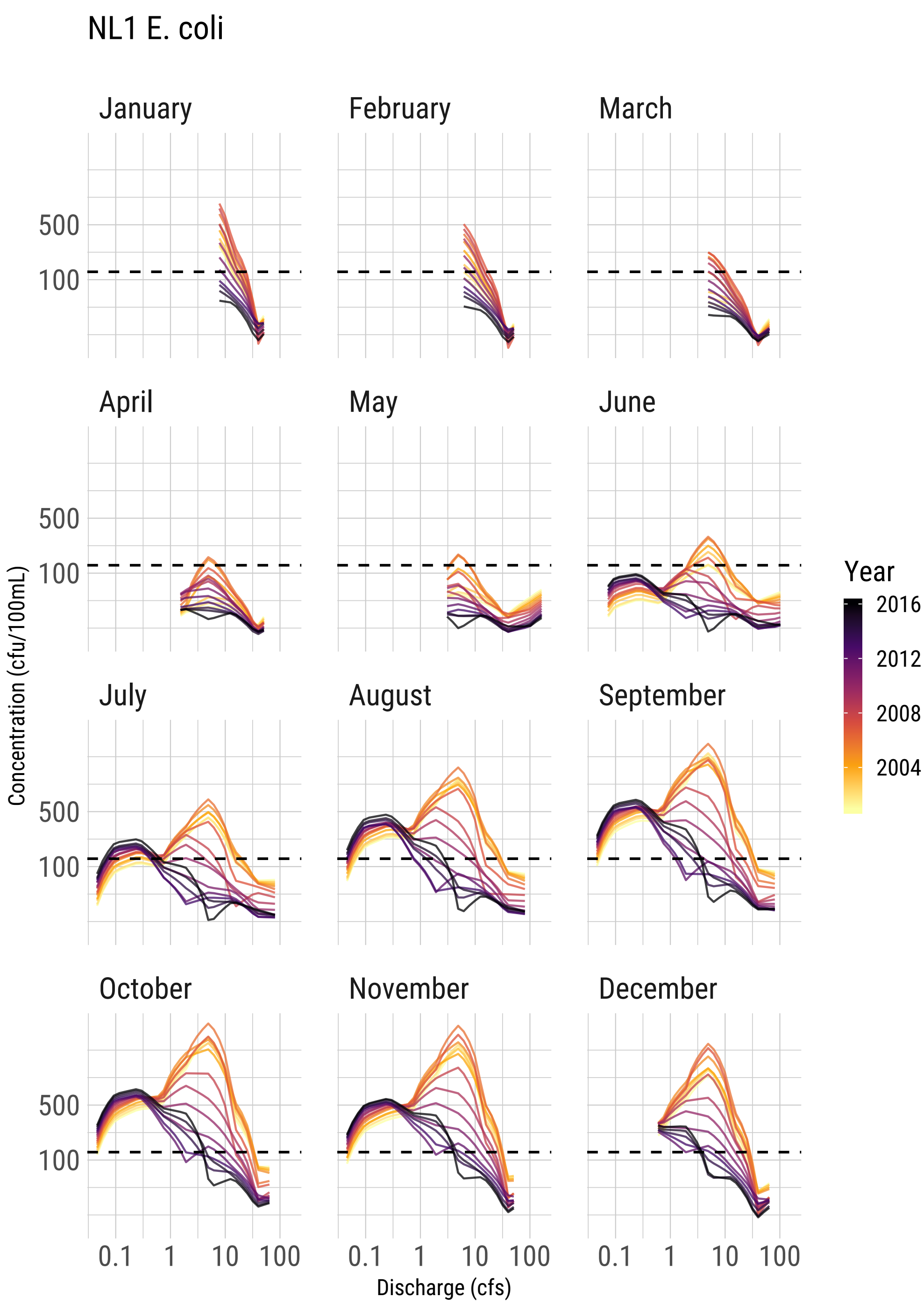

Figure 8: *E. coli* concentration and flow relationship over time and month at NL1.

Table 4: WRTDS estimated changes in flow-normalized mean daily *E. coli* concentrations and loads.

Station	Period	Change in Daily Concentration (cfu/100mL)	Change in Flow Normalized Daily Concentration (cfu/100mL)	Change in Daily Load (million colonies per day)	Change in Flow Normalized Daily Load (million colonies per day)
LR2	2001-2016	-74.5	-33.2	-1.3377644×10^7	-5.869207×10^6
NL1	2002-2016	-396.9	-221.3	-3.9857×10^4	-1.27081×10^5

Dissolved Oxygen

Linear regression did not indicate a statistically significant trend in DO concentration at any of the stations from 2001 through 2016 (Figure 9). Although a handful of samples fell below 5 milligram (mg)/liter (L) (the current TCEQ established standard for minimum DO values in the Upper Llano watershed), long-term trends indicate grab DO concentrations remain well above the established limit.

The correlation matrix (Figure 10) depicts the relationship between DO, temperature, and log flow at each station in the watershed. DO and temperature exhibit a strong relationship at LR1 (τ = -0.81, p < 0.001), LR2 (τ = -0.38, p < 0.001), NL1 (τ = -0.44, p < 0.001), and NL2 (τ = -0.7, p < 0.01). A less strong but still significant relationship between DO and temperature was detected at SL1 (τ = -0.53, p < 0.05). No correlation appears between DO and log flow or temperature and log flow.

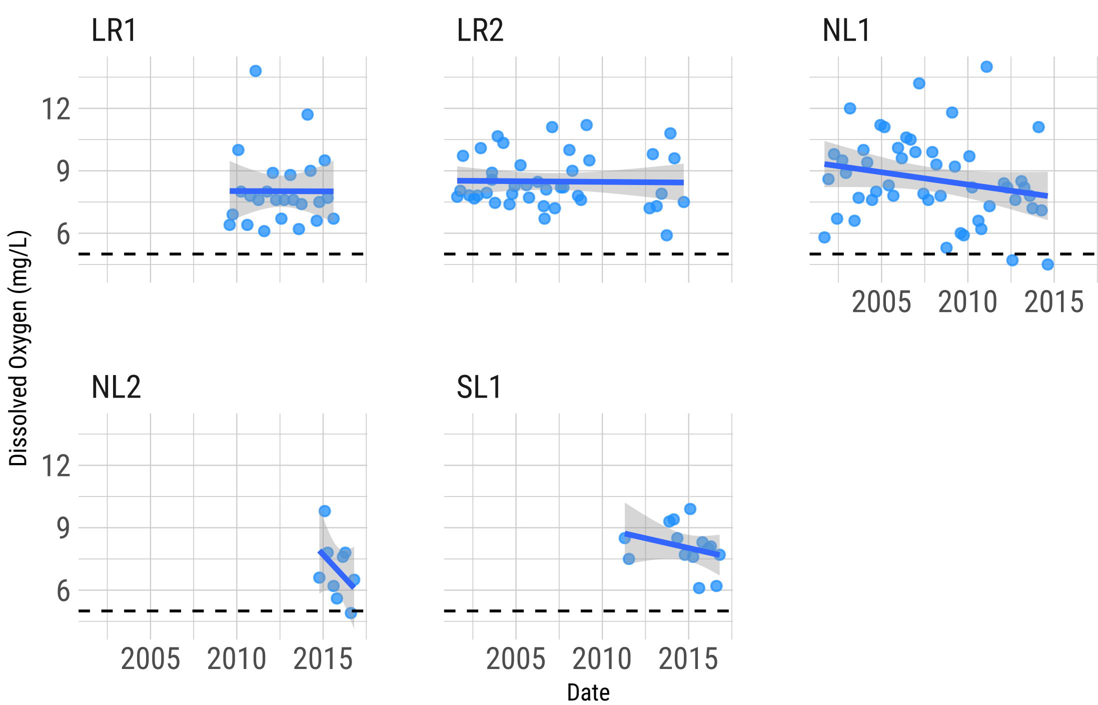

Figure 9: DO concentrations plotted against time for each station. The solid line is the linear regression through all points, and the shaded area indicates the 95% confidence interval.

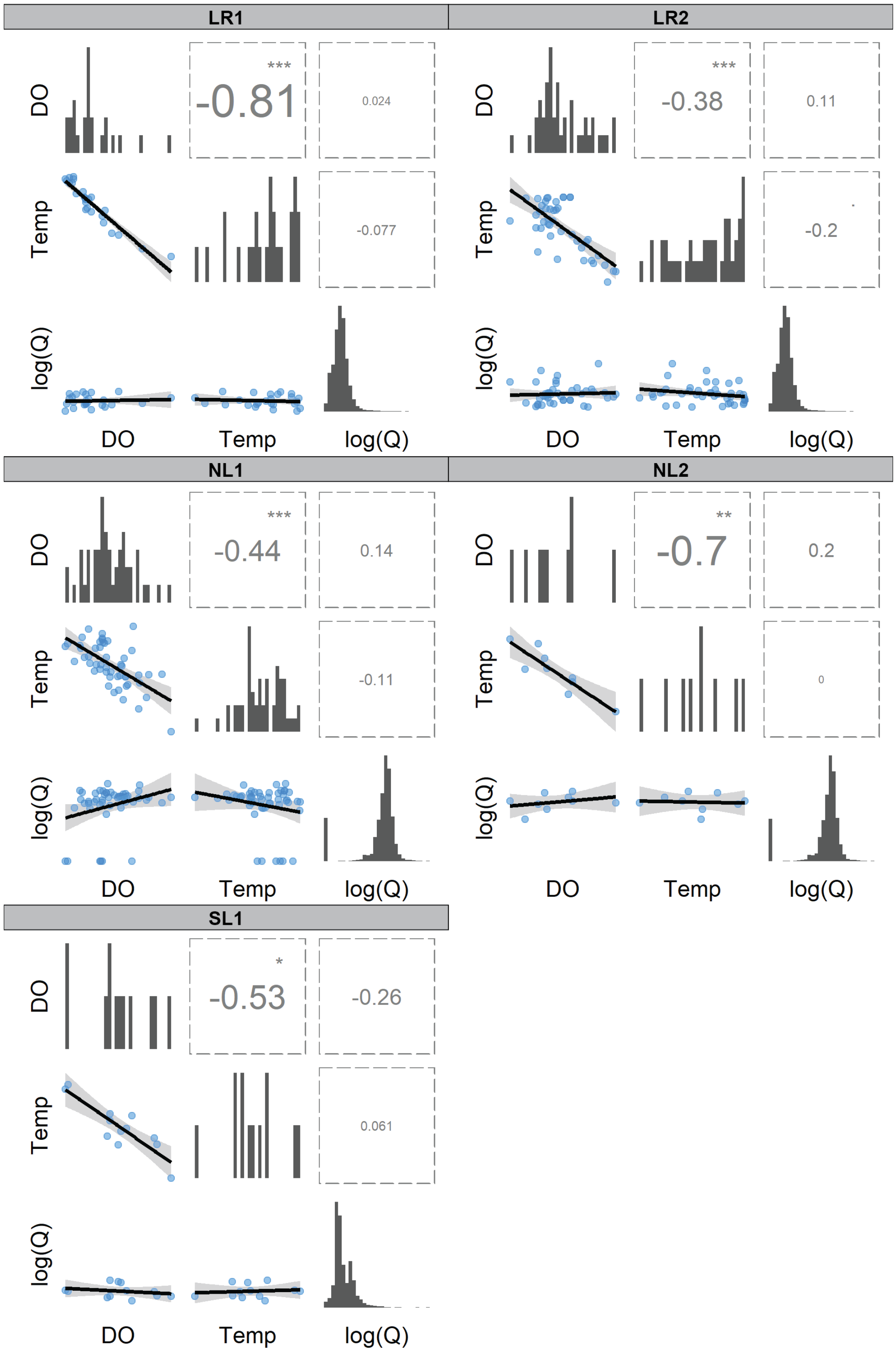

Figure 10: Correlation matrix for project stations. Kendall's Tau values are indicated in the upper right hand section of each matrix, a histogram of indicated values are included along the diagonal plots, and measured values are in the low lower left hand plots. Levels of significance are indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

GAMMs indicate a small but significant decrease in DO concentration at LR2 (F = 5.990, p = 0.02) and NL1 (F = 7.636, p < 0.001) when controlled for temperature and season. Mean DO at LR2 decreased 1.07 mg/L or 8% between 2001 and 2016. The mean DO at NL1 decreased 2.71 mg/L or 8.8% between the maximum value at 2005 and minimum at 2016. The GAMMs fit to DO concentrations demonstrated reasonable certainty with adjusted R-squared values of 0.661 (LR1) and 0.677 (NL1). The adjusted R-squared values describe the amount of variance each model is expected to account for. Figure 11 shows the change in mean DO contributed by the long-term trend term, or what we could expect as a change in mean DO as a function of time, controlled for changes in flow, temperature, and season.

Figure 12, Figure 13 depict the GAMMs estimated dissolved oxygen concentrations as function of water temperature, season (month of year), and year. Both stations reflect a decreasing DO concentration trend over time. Generally, a strong decrease in DO concentration with increasing water temperature occurs as water temperature increase up to 20 degrees Celsius. The GAMMs estimate a leveling off in concentration reductions, with an increase noted at higher temperature at NL1.

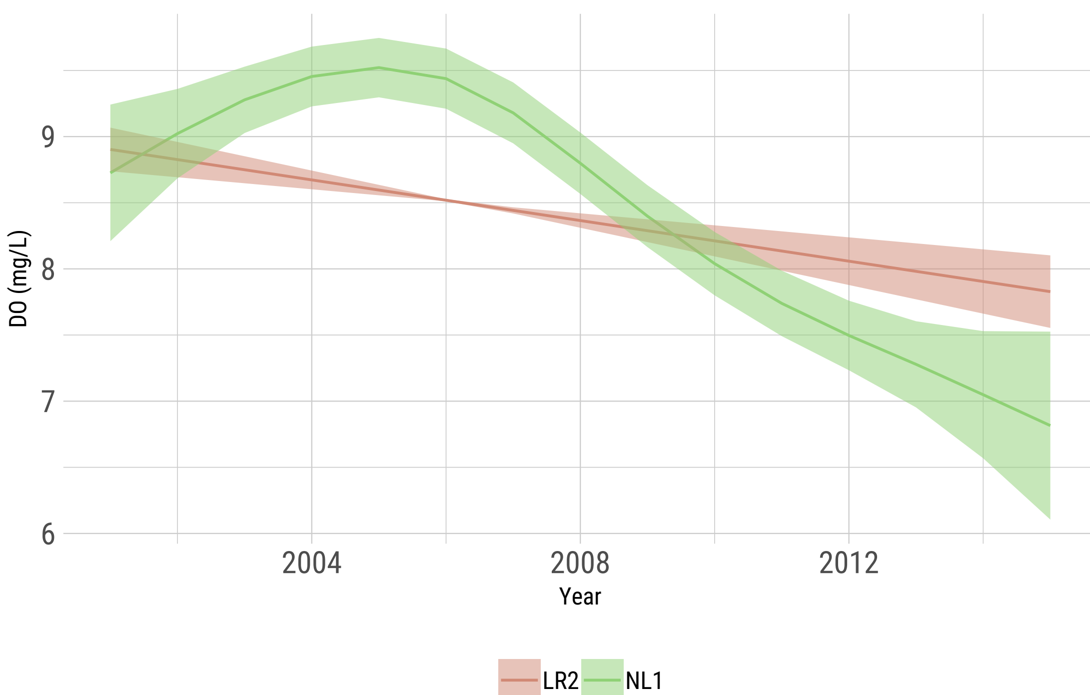

Figure 11: GAMM estimated changes in DO concentration from the long-term trend term. Solid lines are the estimated mean DO concentrations, shaded areas are the estimated standard errors

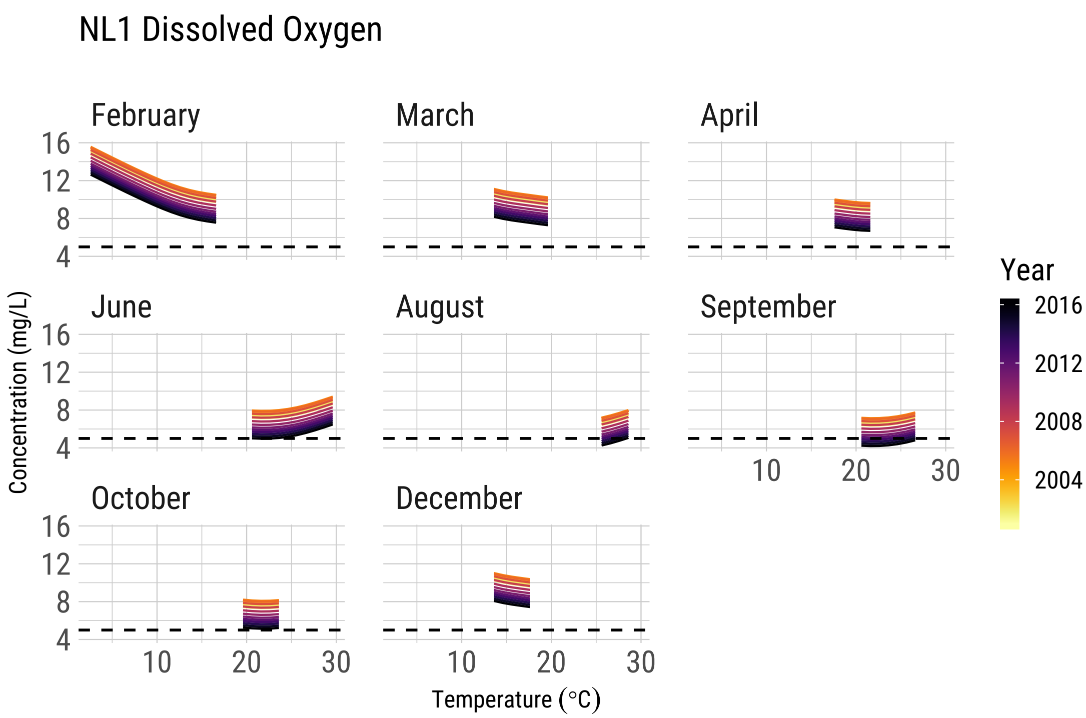

Figure 12: Estimated DO concentration and temperature relationships over time and season at NL1. Months without sampling data are not included in the figure.

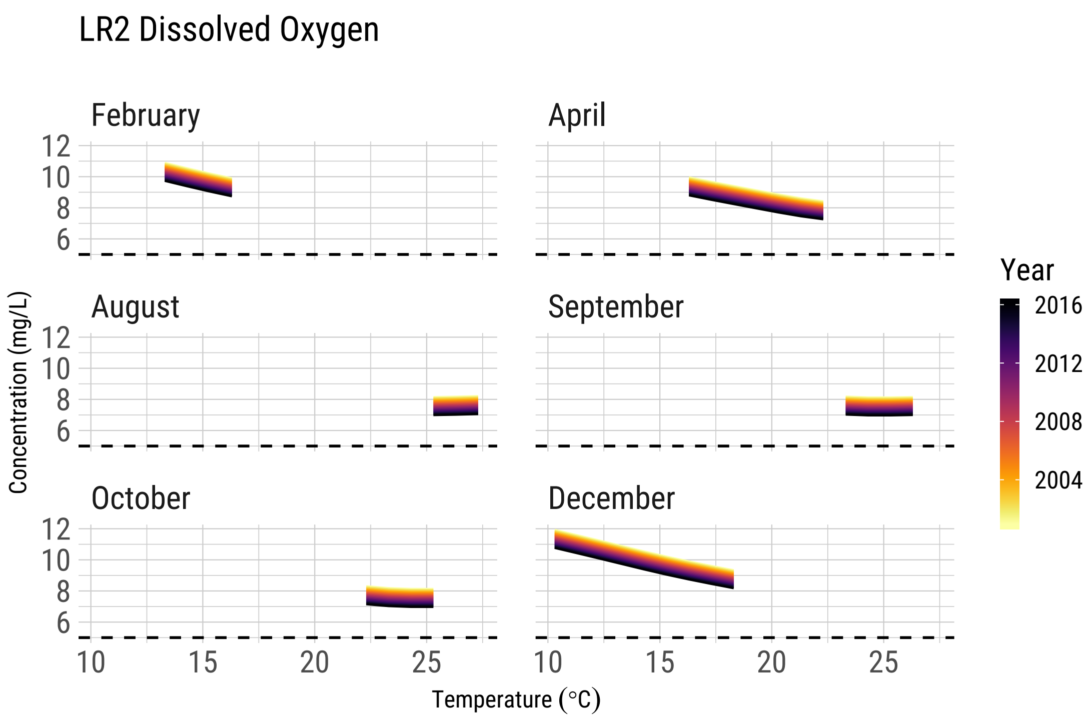

Figure 13: Estimated DO concentration and temperature relationships over time and season at LR2. Months without sampling data are not included in the figure.

Discussion

E. coli

Linear regression indicated a significant decreasing trend in fecal indicator bacteria at station LR2 over the entire sample period. Linear regression did not indicate trends in fecal indicator bacteria at the other sites. These stream concentrations are important to evaluate as indicators of compliance with water quality regulations and stream health. The duration curve and linear regression results indicate general water quality compliance. However, absence of recent sampling at sites with long-term datasets (LR2 and NL1) prevent extrapolation of results to current conditions.

We used the WRTDS regression approach to describe bacteria loads and concentrations controlled for flow, as well as describe bacteria concentration as a function of flow and season. Although WRTDS indicates, substantial decreases in flow normalized *E. coli* concentration and load at LR2 and NL1, considerable bias indicates substantial uncertainty that these regressions are reflections of true instream load reductions. This uncertainty can be attributed to (1) relatively infrequent sampling for load estimation purposes, (2) a substantial data gap in the middle of the data set, (3) and sampling did not capture enough high flow events. Despite the uncertainty, we can reasonably infer that changes in land-use and practices increase bacteria loads since 2001.

An inspection of *E. coli* concentration as a function of flow and time at LR2 (Figure 7), indicates *E. coli* concentration typically increases with flow, as is expected for nonpoint source influenced constituents. Importantly, the seasonal increases (September through December) in concentration that occurred early in the sampling period appear to be reduced at this site. NL2 (Figure 8) shows a completely different relationship, indicating possible flushing and decreased concentration of bacteria as flows increase. The elevated bacteria levels at low flows are muted in recent years.

Dissolved Oxygen

While standard linear regression did not indicate a trend in dissolved oxygen concentrations, GAMMs indicated a small but significant decrease in DO as a function of time controlled for temperature and season. Overall DO concentration remained in compliance over the sampling period; however, the temperature and seasonal controlled trends point to possible underlying changes in mean DO concentration. Importantly, for NL1, periodic seasonal exceedances of the DO water quality standard appear more likely to occur based on the GAMMs (Figure 13). Again, the lack of recent sampling data negatively impacts the confidence of regression results near the end of the sampling period, as reflected in the wide standard errors at NL1 (Figure 11). Increased monitoring frequency and continuity in site sampling effort would certainly increase confidence in regression results going forward. While changes in DO concentrations are informative, these results do not provide insight to the reasons why DO concentrations decreased. Developing an understanding of drivers and mechanisms behind those changes would be valuable for land management decisions. Eutrophication and resulting depressed DO concentrations are typically modelled as a function of nutrient and algal biomass (chlorophyll-a) factors (Biggs, 2000). The sensitivty of Hill Country streams to nutrient enrichment is well documented (Mabe, 2007; Herrington and Scoggins, 2006). Past research suggests that while phosphorus is likely a limiting nutrient in waterbodies like the Llano River system, benthic chlorophyll-a or periphyton is best suited for monitoring potential nutrient enrichment due to the low nutrient characteristics of the watershed (Mabe, 2007; Matlock et al., 1999). The majority of total phosphorus and planktonic chlorophyll-a samples associated with sampling events in this study were censored as less than the laboratory reporting limits which limited possible inclusion for analysis. It is well established that potential drivers of DO concentration and stream eutrophication likely include increased nutrients, particularly phosphorus (Hilton et al., 2006). However in-stream DO and ecosystem responses to eutrophication are not only less studied in riverine systems compared to lentic systems, they are certainly complex and influenced by in-stream metabolic factors, canopy cover, and organic inputs that are outside the evaluation scope of this project

(Hilton et al., 2006; Dodds, 2006).

Conclusion

This report describes trends in *E. coli* and DO in the Upper Llano watershed using regression-based techniques (WRTDS and GAMMs). Examination of the trends indicate that the parameter concentrations are typically well within acceptable ranges for compliance with existing water quality standards. WRTDS indicates daily *E. coli* concentration and load, as well as flow normalized *E. coli* concentration and load have decreased in the North Llano and Llano River. The GAMMs approach identified a decreasing trend in DO concentrations when controlled for variations in season and temperature in the North Llano. Caveats to this report include substantial bias identified in the *E. coli* load estimations attributed to sparse data collection and lack of high flow sampling events that contribute to load estimation uncertaintity. Sampling induced biases can also be compounded by the high uncertanties inherent in *E. coli* monitoring induced by sample collection, lab analysis, and sample preservation/storage (Harmel et al., 2016). In order to provide increasingly reliable load estimates, future sampling efforts need to ensure representativeness of sampling conditions (flow and season) and would improve with increased sampling frequency. Conversely, if insufficient temporal montiroing remains a constraint, inference from statistical models can also be improved by implementing bootstrap simulation techniques to generate confidence and prediction intervals and other measures of statistical accuracy (Efron and Tibshirani, 1986; Hirsch et al., 2015) or by applying Bayesian statistical modeling techniques to quantify uncertainty (Krueger, 2017). Finally, while the GAMMs estimate of DO were reasonable, they do not provide information on what drives responses in DO concentration. While further research on DO concentration might not be warranted since concentrations are well within the established water quality standards, any future work (for example in responses to potential nutrient additions) should be designed with consideration to limitations imposed by the low nutrient environment that is characteristic of Texas Hill Country streams such as the Upper Llano River system.

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