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INSTREAM FLOWS RESEARCH AND VALIDATION METHODOLOGY FRAMEWORK 2016-2017

Colorado and Lavaca Rivers

FINAL REPORT

Prepared for

Texas Water Development Board

Prepared by

Dr. Timothy Bonner (Texas State University)

Dr. Jacquelyn Duke (Baylor University)

BIO-WEST, Inc.

August 15, 2017

PURSUANT TO HOUSE BILL 1 AS APPROVED BY THE 84TH TEXAS LEGISLATURE, THIS STUDY REPORT WAS FUNDED FOR THE PURPOSE OF STUDYING ENVIRONMENTAL FLOW NEEDS FOR TEXAS RIVERS AND ESTUARIES AS PART OF THE ADAPTIVE MANAGEMENT PHASE OF THE SENATE BILL 3 PROCESS FOR ENVIRONMENTAL FLOWS ESTABLISHED BY THE 80TH TEXAS LEGISLATURE. THE VIEWS AND CONCLUSIONS EXPRESSED HEREIN ARE THOSE OF THE AUTHOR(S) AND DO NOT NECESSARILY REFLECT THE VIEWS OF THE TEXAS WATER DEVELOPMENT BOARD.

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Table of Contents

1	Introduction.....	1
1.1	Aquatic	3
1.1.1	Study Objectives.....	4
1.2	Riparian	4
1.2.1	Study Questions and Hypotheses	7
2	Methods and Materials	9
2.1	Aquatics.....	9
2.1.1	Aquatic Field Studies	10
2.1.2	Aquatic Historical Analysis.....	16
2.2	Riparian	16
2.2.1	Colorado Bend Site.....	17
2.2.2	Onion Creek Site	17
2.2.3	Navidad River Site	18
2.2.4	Sandy Creek Site	18
2.2.5	Field Sampling	20
2.2.6	Estimate of Inundation	22
2.2.7	Statistical Analyses.....	22
3	Results, Discussion, and Interdisciplinary Assessment	22
3.1	Aquatics.....	22
3.1.1	Aquatic Field Studies	22
3.1.2	Aquatic Historical Analysis.....	30
3.2	Riparian	34
3.2.1	Colorado Bend.....	34
3.2.2	Onion Creek	43
3.2.3	Navidad River.....	50
3.2.4	Sandy Creek	57
3.2.5	Community and Basin Assessments.....	64
3.2.6	Comparison of Methodologies	64
3.2.7	Conclusions	66
4	Multidisciplinary Evaluation	68
4.1	Summary of Key Ecological Components.....	68
4.1.1	Aquatics.....	68
4.1.2	Riparian	68
4.1.3	Floodplains	69

4.1.4 Ecological Response Summary69

4.1.5 Validation Methodology Assessment Tool70

5 Recommendations for Future Applied Research and Long-term Monitoring..... 70

5.1 Focused Applied Research..... 70

5.2 Long-term Monitoring 71

6 Acknowledgements..... 71

7 References..... 71

List of Figures

Figure 1. Reference map of Colorado River gage locations (taken from the Col/Lav BBASC report). Specific sites used in this study are reported in the text. 11

Figure 2. Reference map of Colorado River tributary gage locations (taken from the Col/Lav BBASC report). Specific sites used in this study are reported in the text. 12

Figure 3. Reference map of Lavaca River basin gage locations (taken from the Col/Lav BBASC report). Specific sites used in this study are reported in the text. 13

Figure 4. An example site showing 75 random points selected within each level..... 19

Figure 5. Example GIS screenshot showing water’s edge, quadrats, mature trees, and elevation contours. 21

Figure 6. Hydrograph from US Geological (USGS) gage # 08173900 on the Guadalupe River at Gonzales from 2011 to 2017 showing Round One (dashed line) and Round Two (dotted line) sampling periods. 23

Figure 7. An nMDS ordination plot for the three river drainages fish communities. 31

Figure 8. Red shiner abundance across percent flow exceedance levels. 32

Figure 9. Swift-water fishes abundance by drainage. 33

Figure 10. Abundance of swift-water fishes across percent flow exceedance levels..... 33

Figure 11. Proportional abundance of moderately swift-water (fluvial) fishes plotted as a response to percent exceedance in the Colorado drainage ($F_{1,6} = 7.527$, $P = 0.03358$) showing best fit line for linear regression model. 34

Figure 12. Overview of Colorado Bend Site showing the three level boundaries (in blue). 35

Figure 13. Colorado Bend Site profile showing general level locations..... 36

Figure 14. An nMDS analysis of Colorado Bend levels community differences. 39

Figure 15. An nMDS analysis of Colorado Bend levels seasonal community differences..... 40

Figure 16. An nMDS analysis of Colorado Bend levels wetland indicator (WI) class plots..... 41

Figure 17. An nMDS analysis of Colorado Bend levels mature tree differences. 42

Figure 18. Overview of Onion Creek Site showing the three level boundaries (in blue). 44

Figure 19. Onion Creek Site profile showing general level locations..... 45

Figure 20. An nMDS analysis of Onion Creek levels community differences. 48

Figure 21. An nMDS analysis of Onion Creek levels seasonal community differences. 49

Figure 22. An nMDS analysis of Onion Creek levels wetland indicator (WI) class differences..... 49

Figure 23. An nMDS analysis of Onion Creek levels mature tree differences. 50

Figure 24. Overview of Navidad Site showing the three level boundaries (in blue). 51

Figure 25. Navidad Site profile showing general level locations..... 52

Figure 26. An nMDS analysis of Navidad levels community differences. 54

Figure 27. An nMDS analysis of Navidad levels wetland indicator (WI) class differences..... 55

Figure 28. An nMDS analysis of Navidad levels mature tree differences. 55

Figure 29. Overview of Sandy Creek Site showing the three level boundaries (in blue). 58

Figure 30. Sandy Creek Site profile showing general level locations..... 59

Figure 31. An nMDS analysis of Sandy Creek levels community differences. 62

Figure 32. An nMDS analysis of Sandy Creek levels seasonal community differences. 62

Figure 33. An nMDS analysis of Sandy Creek levels wetland indicator (WI) class differences..... 63

Figure 34. An nMDS analysis of Sandy Creek levels mature tree differences. 63

List of Tables

Table 1.	Fish and macroinvertebrate data collection per habitat type across basins.	14
Table 2.	Fishes taken from all habitats and basins 2014 through 2017.	25
Table 3.	Number of sites and visits conducted during Round One and Round Two (2014–2017) with breakdown per flow tier.	26
Table 4.	Macroinvertebrates taken overall from 2014 through 2017.	27
Table 5.	Relative abundances of macroinvertebrates taken from Colorado River from 2016 through 2017.	27
Table 6.	Fish and macroinvertebrate data collected per habitat type in the GSA and Brazos basins used in flow tier analysis.	28
Table 7.	Fish and macroinvertebrate community or species response to flow tier and pre-flood vs. post-flood conditions.	29
Table 8.	General site characteristics for sites studied during 2016–2017.	36
Table 9.	Colorado Bend community abundances by season, and mature tree abundances.	37
Table 10.	Stream discharge estimated to inundate Colorado Basin’s Riparian site level based on US Geological Survey (USGS) gage rating curves.	42
Table 11.	Texas Commission on Environmental Quality (TCEQ) flow standards for selected sites in the Colorado Basin.	43
Table 12.	Onion Creek community abundances by season, and mature tree abundances.	46
Table 13.	Navidad community abundances and mature tree abundances.	53
Table 14.	Stream discharge estimated to inundate Lavaca Basin’s Riparian site level based on US Geological Survey (USGS) gage rating curves.	56
Table 15.	Texas Commission on Environmental Quality (TCEQ) flow standards for selected sites in the Lavaca Basin.	57
Table 16.	Sandy Creek community abundances by season, and mature tree abundances.	60
Table 17.	Summary of Ecological Responses for future validation consideration. Check marks indicate an ecological response detected during this project relative to specific TIFP flow categories.	69

List of Appendices

Appendix A Expert Panel Workshop Agendas and Participant List

Appendix B Flow (CFS) on Day of Subsample per Site

Appendix C Habitat Data Summarized by HMU

Appendix D Additional Riparian Data and Analyses

Appendix E Texas Water Development Board Draft Report Comments and Team Responses

List of Acronyms and Abbreviations

Abbreviation	Definition
ANOSIM	Analysis of similarities
ANOVA	Analysis of variance
BBASC	Basin and Bay Area Stakeholder Committee
BBEST	Basin and Bay Expert Science Team
BRAZOS	Brazos Basin
Col / Lav	Colorado and Lavaca Rivers and Matagorda and Lavaca Bays and Basin area
cfs	Cubic feet per second
DBH	Diameter at Breast Height
DEMs	Digital Elevation Models
EFAG	Environmental Flows Advisory Group
EPT	Ephemeroptera-Plecoptera-Tricoptera
FAC	Facultative
FACU	Facultative upland
FACW	Facultative wetland
GSA	Guadalupe, San Antonio, Mission, and Aransas Rivers and Mission, Copano, Aransas, and San Antonio Bays Basin and Bay Area
HEFR	Hydrology-Based Environmental Flow Regime
LCRA	Lower Colorado River Authority
LNRA	Lavaca-Navidad River Authority
nMDS	Non-metric multi-dimensional scaling
NRCS	National Resources Conservation Service
OBL	Obligate
PCA	principal component analysis
PRIMER	Plymouth Routines In Multivariate Ecological Research
SAC	Texas Environmental Flows Science Advisory Committee
SARA	San Antonio River Authority

List of Acronyms and Abbreviations (concluded)

Abbreviation	Definition
SB 2	Senate Bill 2
SB 3	Senate Bill 3
SIMPER	Similarity percentages
TCEQ	Texas Commission on Environmental Quality
TNRIS	Texas Natural Resource Information System
TPWD	Texas Parks and Wildlife Department
TRA	Trinity River Authority
TWDB	Texas Water Development Board
UPL	Upland
USGS	U.S. Geological Survey
WI	Wetland Indicator

EXECUTIVE SUMMARY

Senate Bill 3 (SB 3) established the Colorado and Lavaca Rivers and Matagorda and Lavaca Bays and Basin area (Col/Lav), the regional stakeholder committee (Col/Lav BBASC) and the regional expert science team (Col/Lav BBEST), with the latter two playing key roles in the development of environmental flow recommendations for the Col/Lav. During the SB 3 process, limitations in establishing ecological responses between flow levels and biological components using best-available science arose as a major source of uncertainty in setting environmental flow standards for the Col/Lav and other basins. Typically, when data gaps or uncertainty arose, hydrological surrogates were used as placeholders. Stream flow characteristics were quantitatively defined by a computer program (Hydrology-Based Environmental Flow Regime [HEFR]) for a river reach. Seeking to address this limitation, the Texas Water Development Board (TWDB) commissioned environmental flows validation projects with funds designated by the Texas Legislature to be used in support of SB 3 activities.

The first round of these studies (Round One) took place in 2014–2015 and was targeted at supplementing the available information on flow-ecology relationships in both the Guadalupe/San Antonio (GSA) and Brazos River basins, and informing the development of a methodology with potential future use in evaluating established flow standards. A key focus from the outset of these studies was on determining and evaluating ecological responses to pulse flows. A large amount of data were collected and information acquired along with the development of a framework for testing environmental flow standards. However, the limited time frame of study resulted in inadequate replication of ecological factors across flow tiers and seasons to complete the analysis. As such, TWDB commissioned additional studies in 2016 in support of SB 3 flow validation activities in the Col/Lav, GSA, and Brazos basins. With dynamic characters of stream flow defined in the standards and protected among multiple river reaches, hypotheses about aquatic and riparian community dependencies on stream flows (e.g., Natural Flow Paradigm) were developed and tested in this second round (Round Two) with replication within and across basins.

Eighteen Col/Lav, GSA, and Brazos gage locations were selected for the aquatic assessment specific to the Round Two study. The focus on pulse flows continued during the second round of studies. Sites were selected to represent both tributaries and main-stem reaches. For both rounds of this study, there were 18 sites with 153 visits during 2014–2017, resulting in the collection of more than 43,000 fish and 115,000 macroinvertebrates. Additionally, as part of the investigation, a readably available historical database was compiled from prior BIO-WEST instream flow research across these three basins. The accumulated database served to independently parallel the current research objectives being conducted as part of the SB 3 validation studies. The compiled historical database encompassed 2004 to 2014 with 49 sites within the three basins represented. A total of more than 160,000 fishes were observed from the three drainages with discharge values ranging from 0 cubic feet per second (cfs) to 72,100 cfs.

When evaluating the flow tier analysis specific to this SB 3 study across basins for both fishes and macroinvertebrates, certain ecological responses were evident. Fish community responses were detected within riffle and run habitat while macroinvertebrate responses were detected within riffle habitats. Responses involved changes in densities and/or relative abundance to the entire community or specifically to fluvial specialists. Fish and macroinvertebrate species

responses were associated with specific flow tiers across basins including 1-per-season flow pulses and >1-per-5-year events both having multiple detections of ecological response. The 1-per season flow pulses are less than overbanking conditions, and thus within the range of flows considered by the Texas Commission on Environmental Quality (TCEQ) when setting balanced environmental flow standards. Flows that resulted in overbanking or higher levels of flooding were typically not considered by TCEQ. Overall, the greatest shift in fish communities was observed between pre-flood and post flood in the lower Brazos River. Although a pre-flood and post-flood evaluation using the historical data set was not possible, certain ecological responses of the fish community to flow were evident. Basins with swift-water fishes had positive significant relationships with flow as did fluvial fishes in the Col/Lav drainage.

This riparian study confirmed that with the field and statistical techniques employed, community assemblages could be well-characterized. Three sub-categories of testing (overall community assemblages, Wetland indicator [WI] class groupings, and canopy species) added rich understandings and multi-faceted views of the riparian community. Additionally, community assemblages were shown to differ in varying degrees with an increase in level height/distance to stream. Importantly, this study independently verified Round One observations in the other two basins: that in order to provide continued conservation and maintenance of the current riparian spatial distributions at many Col/Lav sites the existing TCEQ, flow standards (spring and fall) likely need adjustment. Floodplain connectivity investigations focused on the GSA basin in both rounds with no work conducted in the Col/Lav basin.

For intensive ecological data and responses to flow to have meaning to the SB 3 process, they should be collected, analyzed and presented in the context of potential application to the existing TCEQ environmental flow standards. The SB 3 process is by definition designed to be a balance between environmental and human needs, and thus a validation approach is needed to test if maintaining a sound ecological environment can be met over time, or if periodic adjustments to standards may be required. The Draft Report identified key ecological components and described a proposed validation process to assist the Col/Lav BBASC into the future. Examples of the potential application of the validation process were provided in the Draft Report along with a discussion of existing shortcomings and potential future enhancements. The validation methodology assessment tool introduced in the Round One study, highlighted in Round Two Expert Workshops, and presented in detail in the Draft Round Two report was removed from this final report as a TWDB requirement. It is TWDB's professional judgement that insufficient data is available to validate the tool, and thus any practical application of this tool at this time is inappropriate. The project team acknowledges that it is early in the SB 3 adaptive management process and any tools or validation approaches striving to test the scientific defensibility of TCEQ environmental flow standards will need careful vetting and likely further refinement and testing by the BBEST's, BBASC's and TCEQ.

In conclusion, the second phase of this study has contributed to the understanding of flow-ecology responses and taken a step towards addressing questions and concerns raised during the SB 3 process. However, future work could enhance the ability of stakeholders, river managers, and the TCEQ in their roles with respect to validation, application, and adaptive management. Three key areas noted for enhancement include, (1) continued evaluation of fish and macroinvertebrate response to flow tiers; (2) distributional surveys and subsistence, base, and

pulse-flow requirement evaluations of freshwater mussels; and (3) establishing direct ecological responses between channel morphology changes and aquatic organism response. Finally, long-term monitoring remains essential to track ecological condition and more completely and holistically answer this complex validation question over time.

1 Introduction

Senate Bill 3 (SB 3), passed by the 80th Texas Legislature in 2007, amended the existing Texas Water Code §11.1471 and instituted a public, stakeholder-driven, and region-specific process for establishing environmental flow standards for major Texas rivers and bays. This process tasked regional stakeholders and regional scientific experts with developing flow recommendations for each of the 11 designated river drainage and bay regions based on existing data, which would then be submitted to the state.

For the Colorado and Lavaca Rivers and Matagorda and Lavaca Bays (Col/Lav), the regional stakeholder committee (Col/Lav BBASC) was appointed in October 2009. This group then appointed a regional expert science team (Col/Lav BBEST) in March 2010. After numerous meetings and extensive data compilation and analysis, the Col/Lav BBEST submitted their environmental flow recommendations report to the Environmental Flows Advisory Group (EFAG) in March 2011. Then, after a series of meetings and balancing discussions, the Col/Lav BBASC submitted their stakeholder recommendations report to the Texas Commission on Environmental Quality (TCEQ) and the Environmental Flows Advisory Group (EFAG) in August 2011. The TCEQ then adopted environmental flow standards for the Colorado and Lavaca basins, on August 8, 2012.

During the SB 3 process, limitations in establishing ecological responses between flow levels and biological components (e.g., instream, riparian, and estuary components) using existing data was recognized as a major source of uncertainty in setting environmental flow standards for the Col/Lav and other basins. Specifically, findings for certain target components were unavailable at some SB 3 sites, as some sites lacked primary site-specific instream flow and/or freshwater inflow studies. To compensate for these data gaps, the calculations underlying the Col/Lav BBEST environmental flow recommendations necessarily involved various assumptions, as well as the use of surrogate hydrological, ecological or water quality indicators for certain target components. Consequently, the need for improving scientific understanding of key relationships between flow levels and Col/Lav basin ecology (thereby reducing the unwanted uncertainty that these data gaps introduced to the Col/Lav environmental flow standards) emerged as a major point of emphasis following TCEQ rule development. This issue was acknowledged by the Texas Environmental Flows Science Advisory Committee (SAC), the Col/Lav BBASC, and the Texas Water Development Board (TWDB).

Seeking to address these needs, the TWDB commissioned environmental flows validation projects with funds designated by the Texas Legislature to be used in support of SB 3 activities. The first round of these studies took place in 2014–2015 and was targeted at supplementing the available information on flow-ecology relationships in both the Guadalupe-San Antonio (GSA) and Brazos River basins, and informing the development of a methodology with potential future use in evaluating established flow standards. During this first round of studies (Round One) environmental flow experts and biologists from throughout the state were brought together in a series of expert panel workshops to assist the study team in selecting and refining hypotheses to be tested as part of this flow validation process. Selection of final hypotheses was based on: (1) the value of a given response variable in indicating sound ecological environments, (2) that response variable's (e.g. fish, macroinvertebrate, etc.) sensitivity to changes among flow tiers (i.e., subsistence flows, base flows, and 4-per-season, 3-per-season, 2-per-season, 1-per-season,

and 1-per-year pulses), and (3) the length of time required to conduct field research. Following this initial phase of hypothesis selection, an intense period of data collection and analysis focused on multiple ecological indicators (e.g. fish, macroinvertebrates, riparian saplings, etc.) within aquatic, riparian, floodplain, and estuarine communities of these basins and was conducted during fall 2014 and spring 2015. This analysis eventually culminated in detailed final reports for each basin, which were submitted to the TWDB in summer 2015 (SARA et al. 2015, Bonner et al. 2015). These reports summarized the hypothesis selection process, detailed the scientific investigations conducted, and provided preliminary guidance on establishing a validation methodology to evaluate environmental flow standards. However, one of the main limitations of Round One was the limited time frame for data collection (6–9 months). As a result of this limited time frame, many of the ecological indicators evaluated suffered from inadequate replication across flow tiers and seasons.

In 2016, TWDB commissioned additional studies in support of SB 3 flow validation activities in the GSA, Brazos, and Col/Lav river basins. For this current second round of studies (Round Two), a similar team of scientists focused on expanding upon previous work done in the Brazos and GSA basins in Round One, and also added the Col/Lav river basin to further increase available data and replication. As before, expert panel workshops were held to solicit input from academic experts, agency representatives, and others with pertinent expertise. Because the GSA, Brazos, and Colorado / Lavaca basins environmental flows validation projects shared not only the same goals and objectives, but many of the same researchers, as well, joint expert panel workshops were conducted. Workshop agendas and participant lists are provided in Appendix A with a synopsis of the Round two workshops presented below. As stated in the Final Round One report, “the ultimate goal of the second round of workshops will be to refine and finalize a validation methodology and engage scientists and stakeholders throughout the development process.” It was envisioned that a series of three individual workshops be conducted during the Round Two project, but delays in contracting exceeded the Spring and Summer 2016 assumptions specified in the TWDB approved scopes of work for the Brazos and Colorado/Lavaca projects, resulting in only two joint expert panel workshops being conducted during this second round of study.

With a condensed schedule, the first and second workshops were combined and conducted on September 8, 2016 at the Lower Colorado River Authority (LCRA) Dalchau Service Center in Austin. The combined workshop focused on discussing the Round One report, introducing the validation methodology, and soliciting feedback on other considerations for inclusion in focused applied research and long-term monitoring. The attendees list and agenda are provided in Appendix A. In summary, there were excellent comments and guidance provided from academic experts and agency representatives. Several comments focusing on antecedent conditions and aquatic sampling were noted and used to guide the project team in the sampling protocol and determination / classification of flow tiers for analysis. Another major theme at the September 8th workshop was for the project team to focus heavily on additional data collection rather than refinement of sampling methodologies or hypothesis development. There were no written comments from the September 8, 2016 workshop provided by participants to the project team principals.

A second expert workshop was conducted on June 29, 2017 at the San Antonio River Authority main office complex in San Antonio. The attendees list and agenda for this second workshop are provided in Appendix A. The goal of the second workshop was provide a project update and to present and solicit feedback on the development of the tiered validation methodology outlined in the Round One final report and discussed at the September 2016 Expert Panel Workshop. Each project lead (Brazos estuary, floodplains, riparian, and aquatics) provided a detailed project update of methodologies, data analysis and preliminary results. An update presentation on the instream flow validation tool was then given followed by group discussion. During this discussion, it was highlighted that the condensed project schedule eliminated the possibility of a separate validation methodology memorandum as described in the scope of work. However, comments were repeatedly solicited from attendees (both verbal or follow-up written) during this discussion period. It was also noted that the instream validation tool would be described in detail in the Draft Final report submitted to TWDB in August. Finally, Mr. Webster Magnum of the Trinity River Authority (TRA) presented on SB3 funded work that TRA had been conducting in their respective basin. Following this presentation, there was an excellent group discussion on how this additional type of work might be blended into the instream flow validation tool into the future. As with the first workshop, there were no written comments from the June 29, 2017 workshop provided to the project team principals by workshop attendees.

We sincerely thank all participants of the two expert panel workshops for their thought-provoking verbal comments and valuable suggestions.

This report provides an overview of Round Two of the environmental flow validation project within all three of these basins, and specifically addresses studies within the Colorado and Lavaca river basins. Please note that while the focus of this report will be on the Colorado and Lavaca basins, references to and results from other basins will be used in this report to support findings, further develop discussions, and guide future recommendations. A brief introduction to each major instream flow component evaluated is provided below. Section 2.0 provides detailed descriptions of the exact sampling and analysis methods employed. Section 3.0 provides detailed results and discussion related to each major component are provided in Section 3.0. Section 4.0 works towards synthesizing all this information and describes a multidisciplinary evaluation method with which to evaluate environmental flow standards. It is hoped this methodology will be useful to Col/Lav BBASC members by providing some guidance on ways to evaluate/refine environmental flow standards at select sites. Finally, the report closes with recommendations for future applied research and long-term monitoring for consideration by BBASC members and others.

1.1 Aquatic

General aquatic theory suggests that flow alterations cause shifts in fish and macroinvertebrate communities. Typically, swift-water, large-river-type fishes become fewer and generalist fishes become more abundant during periods of altered flow. In the lower Guadalupe River, habitat generalist fishes dominate the fish community, whereas regionally endemic fishes and those with fluvial-adapted spawning strategies decrease during periods of reduced flood frequencies (Perkin and Bonner 2011). In the Brazos River during low-flow conditions, large-river-type fishes, such as small-eye shiners, sharpnose shiners, silverband shiners, and chubs, are replaced with tributary/generalist type fishes, such as red shiners, bullhead minnows, and centrarchids. This generalization is based on historical analyses (Runyan 2007), but also on ecology of other similar

prairie streams. Increases in generalist fishes within main-stem rivers conform to the Native Invader Concept (Scott and Helfman 2001), which states that the first indication of environmental degradation is increases in native, generalists taxa (i.e., native invaders) and can be easily applied to the Biological Gradient Concept (Davies and Jackson 2006), which describes initial resistance followed by rapid changes in fish community structure (i.e., native generalist fishes replacing native specialist fishes) with increases anthropogenic alterations.

1.1.1 Study Objectives

The aquatic study was structured to fill knowledge gaps by targeting aquatic mechanisms of high value to environmental flow standard validation. To this end, we considered the full range of flow tiers, from subsistence flows to high-flow pulses, and asked whether each flow tier benefits river fishes. Aquatic organisms occur and persist in time and space because of a number of interrelated and hierarchically ordered abiotic and biotic processes. Stream flow and variations within directly and indirectly influence occurrences and abundances of aquatic organisms on multiple levels. The goal of the research presented here is to verify ecological services or benefits of recommended flow tiers (i.e., subsistence, base, 4-per-season, 3-per-season, 2-per-season, 1-per-season, 1-per-year, 1-per-2-year, and >1-per-5-year high-flow pulses) with *a priori* predictions. A multitude of hypotheses and predictions from Round One were refined into the following three main objectives:

- **Objective 1.** Quantify relative abundances and densities of fishes in riffle and run habitats between pre-flood and post-flood periods and among flow tiers. Here after, pre-flood period refers to the first year of our work (during a collectively low flow year) and post-flood period refers to the second year of our work.
- **Objective 2.** Quantify densities of macroinvertebrates in riffle and run habitats between pre-flood and post-flood periods and among flow tiers.
- **Objective 3.** Describe fish communities within pools and backwaters, as these habitats were not sampled during Round One studies.

Based on these three objectives, the following three predictions were made:

- **Prediction 1.** Flow tiers will be directly related to relative abundances and densities of riffle fishes and fluvial fishes and inversely related to slack-water fishes in riffle habitats.
- **Prediction 2.** Flow tiers will be directly related to relative abundances and densities of fluvial fishes and inversely related to slack-water fishes in run habitats.
- **Prediction 3.** Flow tiers will be directly related to densities of Ephemeroptera-Plecoptera-Tricoptera (EPT) taxa and inversely related to total macroinvertebrates in riffle habitats.

1.2 Riparian

This study represents a first round of validation/methodology development for the TCEQ environmental flow standards and BBEST/BBASC recommendations along the Col/Lav Basin. The principal investigators for this project previously conducted first-round

validation/methodology development studies on two other basins (in 2014–2015): the Brazos River and GSA basins. The general conclusion from those basins’ studies was that most of the TCEQ flow standards at most sites evaluated did not provide for coverage of 80% or more of riparian species’ distributions. Those studies also suggested that spring and fall are critical times, particularly for the seedling stage of woody riparian vegetation. Without seasonal flows, not only was seed dispersal lessened and lost, but seedling germination and survival were also impacted. Often, replacement occurred only in the near-stream riparian areas that had been inundated by low flows during and in the seasons leading up to the study period. This is a good example of what the future holds if flows are managed at the extremely low levels of 2014. Droughts are cyclic occurrences, but human diversions are not. The years leading up to 2014 provided an excellent view of how a lack of flows along a basin affects riparian reproduction and survival.

The methodology developed in Round One of the GSA and Brazos Basins for testing life stage responses to flow pulses would work well as a focused applied research study. By taking a quick survey of the riparian width and a count and spatial distribution of the three age classes (seedling, sapling, mature) of riparian indicator species, a river manager can discern much about the health and status of the riparian zone, from the immediate/recent flow pulsing to longer-term water inundation into the site. This method could also serve as a form of long-term monitoring because a comparison of any given site using these techniques to the set flow standards will allow a quick analysis of projected riparian persistence, which would provide guidance for future management.

In light of the clear connections of riparian responses to within-season flows, we wanted to expand our work in this study to include additional field testing techniques that could be used to compare with Round One methodologies to further elucidate and characterize riparian community dynamics. A benefit analysis of the permanently located transect method of Round One was conducted, and listed below are listed the pros and cons of this method:

Pros

- Using 3–4 riparian indicator species allows for easy identification and quick, simplified field sampling
- The multi-season approach of tracking individuals in established plots allows for direct comparisons between life stages of individuals and unique flow pulses.
- The method provides for an easily-captured known riparian zone width and distribution of indicator species and their age classes.
- It provides a quick, easily-captured snapshot of the riparian health and indicates whether the flow pulses are meeting the needs of the indicator species.

Cons

- The linkage of individuals (at various life stages) to unique flow events requires multiple sampling events throughout the season.

- The use of an indicator species requires that the indicator species must be present in the zone of interest.
- The method provides limited overall community characterization (including overstory, understory and herbaceous species).
- Tracking community/species-composition temporal changes requires that personnel return to the exact location and duplicate the plot sampling precisely. This can be problematic when channel morphologies change following severe flooding and/or GPS equipment lacks centimeter-resolution accuracy.
- Non-random selection of transects based on indicator species distribution limits statistical analysis of community assemblages.

These limitations (several of which were discussed at the first expert panel workshop of this current round of study) were the focal point for proposing an alternative methodology that would contrast with and enhance the original methodology, one of those methods being the addition of a community characterization of the full species composition present in the zone.

Several studies have used characterization of the understory/herbaceous species in riparian zones to enhance understanding of these unique ecosystems. Naiman et al. (2005) argued that woody plants are of high priority for riparian conservation because they provide sediment and bank stabilization that allow the understory to exist. Azim et al. (2014) argued the disturbances that occur in woody riparian communities create increased riparian habitat complexity and diversity. Common methods for community characterization include cluster and multidimensional scaling ordination analysis of sampled data. These methods lend themselves to comparisons of community assemblages and abiotic variables in the riparian zone. Baker and Wiley (2004) used non-metric multi-dimensional scaling (nMDS) ordination statistics on forest samples to demonstrate discrimination of forest types and tree species in correlation with selected environmental variables. Nicol (2013) compared riparian understory and overstory vegetation using cluster analysis to identify definite communities in relation to location and water resources, but found a lack of differences because the most abundant species were too widespread. Bruno et al. (2014) used these methods in conjunction with analysis of similarities (ANOSIM) and similarity percentages (SIMPER) tests, and showed woody riparian species richness was mainly influenced by flow conditions and valley shape, whereas herbaceous species were more dependent on substrate features. Additionally, they used Bray-Curtis distance matrixes and clustering procedures independently for woody and herbaceous species to characterize the different species assemblages in order to determine within-community dissimilarities of those different groups. Given these demonstrated statistical-based studies, the modifications and refinements made in Round Two aimed at incorporating these techniques in a refined methodology.

This current study marks a culmination of several *flow vs. riparian response* studies related to this and other reaches along multiple basins. It was a goal of the researchers to draw from the building knowledge of these studies, and expand to a multi-basin approach to test questions related to river continuum dynamics, and determine whether these can be discerned in the

riparian zone. As streams flow from headwaters to mouth multiple aspects vary considerably (Vannote et al. 1980). Among them are stream order, flow, sinuosity, soil types, channel width, soil and nutrient deposition, soil and nutrient erosion, etc. This creates heterogeneity along the basin that places unique, localized stressors on the biotic environment. Studying that heterogeneity along a basin's streams may provide clues to predicting riparian community assemblages that respond to those localized conditions. Adoption of these, and the proposed statistical methods intended to streamline a comprehensive characterization of overall riparian communities and community dynamics. The details of the refined methodology can be found in the Hypothesis Development section.

Study questions were developed using results from the first round of validation hypotheses and methodology development along the Brazos and GSA basins. Prior to the first workshop a set of proposed woody riparian variables for testing were generated based on the hypotheses previously developed. They are listed below.

The following list of potential instream processes/characteristics were considered as variables:

Riparian Habitat

- Community mapping
- Distribution, germination, survival, recruitment
 - Seedlings, saplings, mature trees
- Riparian maintenance
 - Tree ring analyses
- Lateral connectivity
 - Seedlings, saplings, mature trees

In addition to discussion of the validation studies conducted in 2014–2015, follow-up hypotheses for select sites were presented and discussed in detail at the first joint Expert Workshop on September 8, 2016. Several study questions and hypotheses related to monitoring the response of processes and characteristics in relation to stream flow were presented. Attendees discussed the pros and cons of using these variables. Based on workshop discussions and suggestions from attendees, the riparian project team modified and refined monitoring protocols and sampling techniques from the 2014/2015 validation study to include randomization of plots and statistical analyses of results. In an effort to maximize conceptual information derived from the two studies when combined, the modifications below were made.

1.2.1 Study Questions and Hypotheses

Whereas Round One of study for Brazos and GSA Basins (validation study) focused on riparian indicator species rather than the community as a whole in order to best determine short-term responses to stream flow, this study focused on the overall community. In order to compare the two methods, the key indicator species concept was not entirely removed, and will be discussed in the results and conclusions sections. Below is a list of the refined riparian questions considered for this current study.

Geomorphological Features

Question 1: Can we categorize sites by general geomorphological characteristics?

Hypothesis 1: Sites are distinguishable from one another based on unique features related to the following:

- Steepness of bank
- Dominant soil class/type
- Local stream sinuosity
- Stream channel width

Biotic Features within Sites

Question 2: What community abundance percentages exist for various species classes? Secondly, what community abundance percentage of mature trees is riparian obligate (OBL) and facultative wetland (FACW) vs. all other wetland indicator (WI) classes?

Hypothesis 2: Community assemblages can be characterized according to 1) overall plant abundance and 2) mature tree abundance. Two sub-categories of testing will include the following:

- Overall community (overstory and understory/herbaceous combined)
- Limited to mature trees

Question 3: Are there community differences between riparian level?

Hypothesis 3: Community assemblages will differ with an increase in level height/distance. Three sub-categories of testing will include the following:

- Overall community (overstory and understory/herbaceous combined)
- Grouped by WI classes
- Limited to woody vegetation

Question 4: Are there community differences between spring and fall (if data exist for seasons)?

Hypothesis 4: Community assemblages will differ between spring and fall. Three sub-categories of testing will include the following:

- Overall community (overstory and understory/herbaceous combined)
- Grouped by WI classes
- Limited to woody vegetation

Abiotic and Biotic Features between Sites within a Basin

Question 5: Are there community differences between sites across the basin?

Hypothesis 5: Community assemblages will differ between multiple sites within a basin.

Question 6: Do the community differences (if present) result from differences in site characteristics?

Hypothesis 6: Community assemblage differences within a basin will correlate with abiotic factors from Question/Hypothesis 1.

Comparisons across Basins

Question 7: Are there community differences between sites compared across multiple basins? If so, can those be correlated with abiotic features?

Hypothesis 7: Community assemblage differences across three unique basins will correlate with abiotic factors from Question/Hypothesis 1.

Inundation into Sites

Question 8: What stream discharges (in cubic feet per second [cfs]) are needed to inundate the level at each site?

Hypothesis 8: Stream discharges can be estimated using simple hydrological modeling for each site's level and riparian species.

Question 9: Do flow tier recommendations align with needed stream discharges in the riparian zone?

Hypothesis 9: TCEQ flow standards meet the needs of riparian communities.

Comparison of the Two Validation Methods (Round One and Round Two)

Question 10: When comparing statistical (current) method to transect (previous) method, which is more beneficial for long-term monitoring?

2 Methods and Materials

2.1 Aquatics

The Round Two Aquatic component involved two main subtasks. First, additional data collection was conducted at multiple sites within all three drainages (Col/Lav, GSA, and Brazos) following methods similar to those used in Round One. These field assessments were targeted following specific flow tiers to establish flow-ecology responses with fish and macroinvertebrates and build on the existing dataset from Round One. Additionally, a historical analysis of fisheries data collected from all three basins by BIO-WEST for various projects over the last several years was also conducted. Most of these data were collected for various instream flow studies, which were not designed in the same manner as the current study. However, these data were typically collected in a habitat-specific fashion and could, in many cases, be linked back to a nearby gage location with flow standards. The methodology for each subtask is described below.

2.1.1 Aquatic Field Studies

Eighteen Col/Lav, Brazos, and GSA gage locations were selected for the aquatic assessment. Sites were selected to represent both tributaries and main-stem reaches. Five of the 18 sites sampled were from the Col/Lav river basins: one main-stem Colorado River site (Colorado River—San Saba) (Figure 1), two Colorado River tributary sites (San Saba River—San Saba, Onion Creek—Driftwood) (Figure 2), and two Lavaca basin sites (Lavaca River—Edna, and Navidad River—Edna) (Figure 3). Seven of the 18 sites sampled were within the GSA basins: three tributaries (Medina River—Bandera, San Marcos River—Luling, and Cibolo Creek—Falls City) and four main-stem sites (San Antonio River—Goliad and Guadalupe River—Comfort, Gonzales, and Cuero). Six of the 18 sites sampled were from the Brazos River Basin: four tributaries (Leon River—Gatesville, Lampasas River—Kempner, Little River—Little River, and Navasota River—Easterly) and two main-stem sites (Brazos River—Hempstead and Rosharon).

During each season (designated by BBEST recommendations), flows were monitored daily using US Geological Survey (USGS) gaging stations at or near each site. Peak flow of the day (expressed in cfs) determined the classification of the peak flow event as one of following nine flow tiers

1. subsistence
2. base
3. 4-per-season
4. 3-per-season
5. 2-per-season
6. 1-per-season
7. 1-per-year
8. 1-per-2-year
9. >1-per-5-year

Each flow tier is assigned an ordinal number of 1 (subsistence) through 9 (>1-per-5-year), respectively. Sites with subsistence and base tiers were visited seasonally or after 10–15 days of continuously maintaining that tier. Sites with flow pulses were visited up to 15 days following the event but with the condition that flows returned to base tier or below lowest flow tier (e.g., 4-per-season on Brazos and 2-per-season for GSA and Col/Lav; See Appendix B). Therefore, abiotic and biotic samples were taken at subsistence or base-flow conditions and not during a high-flow event, which can cause a dilution effect.

For each site visit, one riffle, and one or more shallow runs were sampled, except at main-stem Brazos River sites (i.e., Hempstead and Rosharon) which lacked riffle habitats. In addition to riffles and runs, one pool and one backwater were selected where available (Table 1).

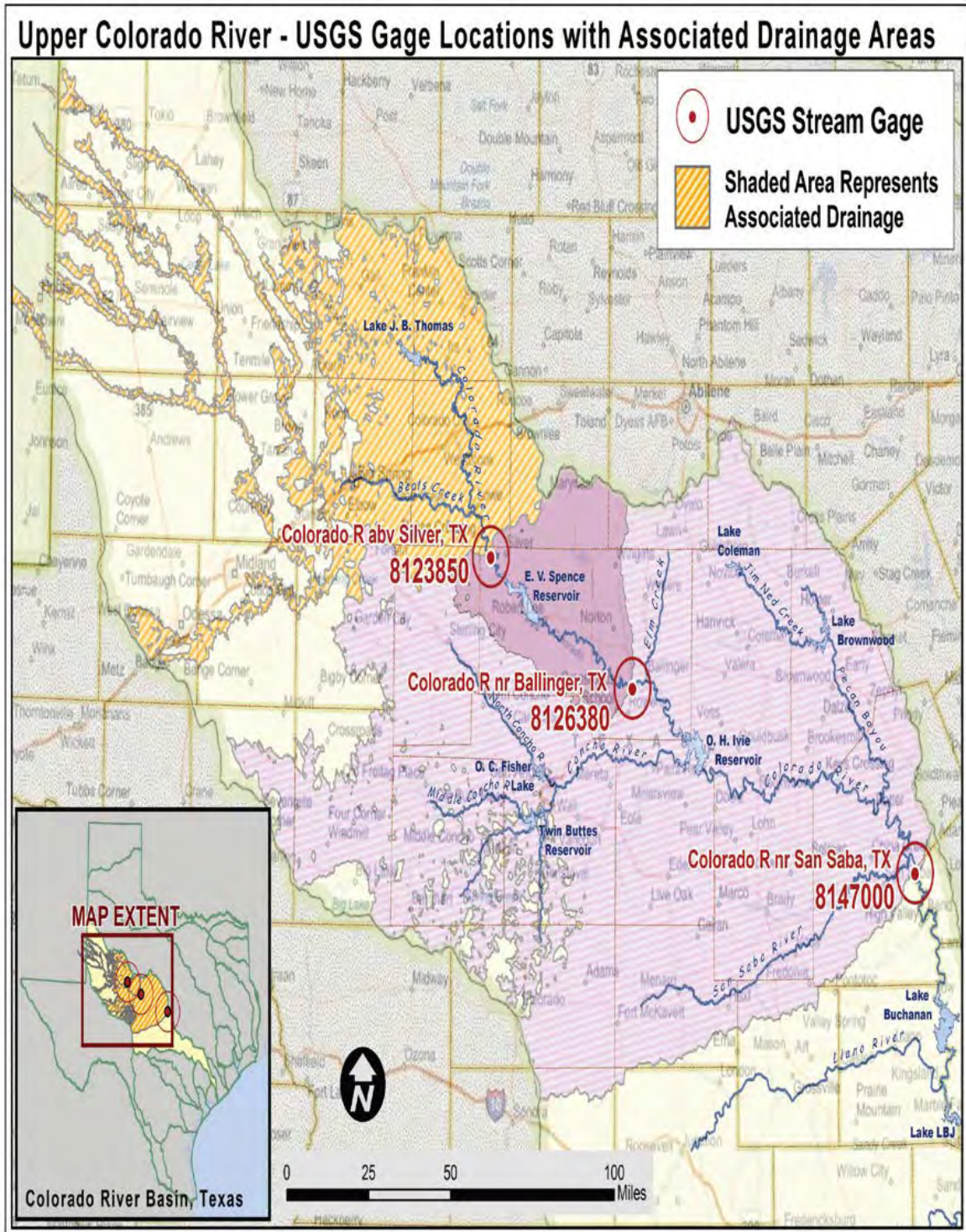


Figure 1. Reference map of Colorado River gage locations (taken from the Col/Lav BBASC report). Specific sites used in this study are reported in the text.

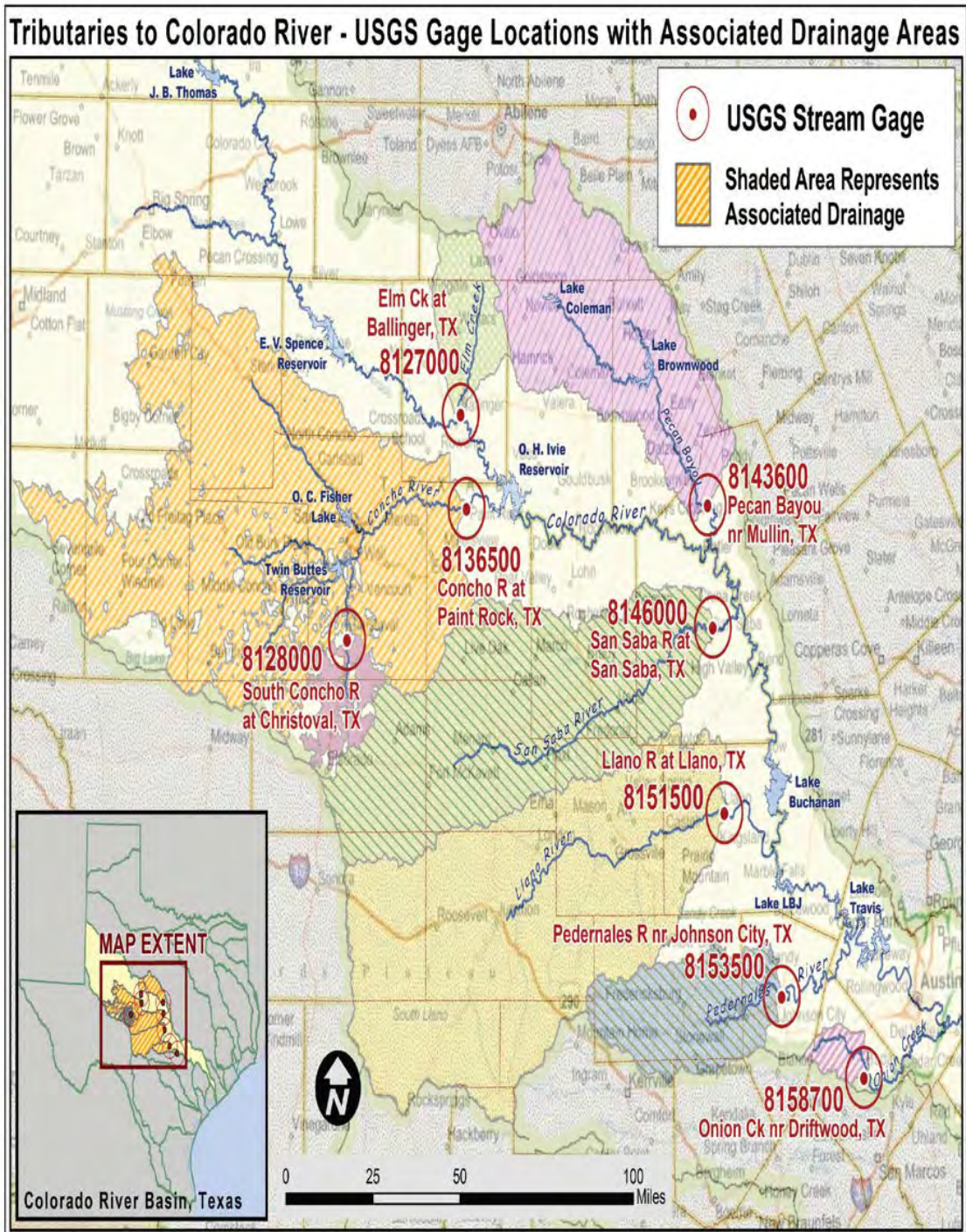


Figure 2. Reference map of Colorado River tributary gage locations (taken from Col/Lav BBASC report). Specific sites used in this study are reported in the text.

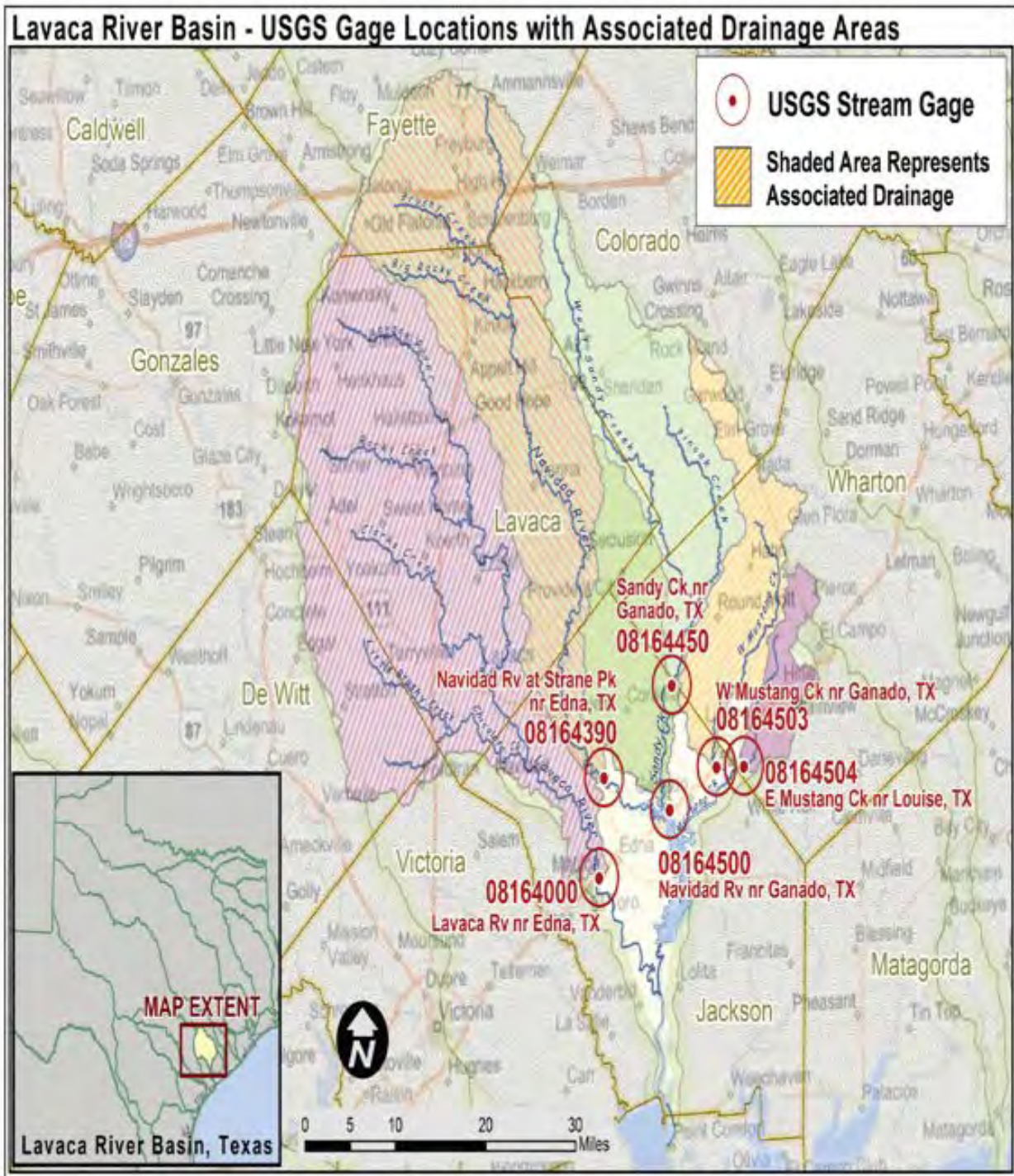


Figure 3. Reference map of Lavaca River basin gage locations (taken from Col/Lav BBASC report). Specific sites used in this study are reported in the text.

Table 1. Fish and macroinvertebrate data collection per habitat type across basins.

Combination / Individual Sites per basin	Fish				Macroinvertebrates
	Riffle	Run	Pool	Backwater	Riffle
GSA					
Medina River—Bandera and Guadalupe River—Comfort	√	√	√	√	√
Guadalupe River—Gonzales and Cuero and San Antonio River—Goliad	√	√	√	√	√
Cibolo Creek—Falls City	√	√			√
San Marcos River—Luling	√	√	√	√	√
Brazos					
Leon River—Gatesville and Lampasas River—Kempner	√	√		√	√
Little River—Little River	√	√	√	√	√
Navasota River—Easterly	√	√	√	√	√
Brazos River—Hempstead and Rosharon		√		√	
Colorado / Lavaca					
San Saba River—San Saba	√	√	√	√	√
Colorado River—San Saba	√	√	√	√	√
Onion Creek—Driftwood	√	√	√	√	√
Lavaca River—Edna	√	√	√	√	√
Navidad River—Edna	√	√	√	√	√

Among riffle habitats, three subsections of the riffle were designated (approximately 30 m²) to capture variability within each riffle habitat (e.g., near shore vs. middle, swifter vs. slacker current velocities, shallower vs. deeper water) and sampled with a barge-mounted or backpack electrofisher. A blocking seine was placed at the downstream end of the subsection with the electrofisher positioned upstream, and the electrofisher was swept side-to-side within the width of seine and moved downstream until coming in contact with the seine. The electrofished area was inspected for any stunned fish. All fish were held in aerated containers, identified to species, enumerated, and released, except for voucher specimens. Voucher specimens were euthanized with MS-222 and fixed in 10% formalin. Following fish collections, a Hess sampler was used to quantify macroinvertebrate community within each riffle subsection. Hess sample contents were preserved in 70% ethanol for subsequent identification in the laboratory. Length, width, standard water quality parameters (water temperature, specific conductance, dissolved oxygen, pH), percent substrate composition, substrate embeddedness (scored 1=<25% embeddedness to 4=100% embeddedness), and percent vegetation were recorded once per riffle subsection. Water depth and current velocity were recorded from three locations within each subsection. At the riffle or from a nearby riffle, up to five individuals of riffle or fluvial specialist species (i.e., *Notropis*, *Macrhybopsis*, Percidae, and juvenile Ictaluridae) were collected, euthanized with MS-222, and fixed in 10% formalin for laboratory quantification of gut fullness, condition, and hepatic-somatic index to be presented in future publications. Among run, pool, and backwater habitats, downstream seining (common or bag seine, depending on water depths) was used to quantify fish occurrence and abundance. Length was usually determined by length of habitat but up to 300 m in long runs such as the lower Brazos River. Fish and habitats were quantified identically to those described for riffle habitats, except Hess samples were not taken and embeddedness was not recorded.

In the laboratory, benthic samples were rinsed using a 250 µm sieve, sorted to order, and enumerated. Fishes taken from riffles were weighed and measured to calculate Fulton Condition

Factor (Anderson and Neumann 1996). For hepatic-somatic index and gut fullness, fish were dissected by exposing the viscera with a longitudinal cut from isthmus to posterior of urogenital vent. The entire gut tract (from esophagus to anus) and other organs were removed from the abdominal cavity. With the use of a dissecting scope, stomachs were removed and separated from the remaining gut tract at the pyloric sphincter muscle. Liver was removed from Percidae only and weighed. Gut fullness (i.e., proportion of stomach filled by contents) were independently assessed by two observers, assigning a number from 0 (empty) to 10 (full) in increments of 1. Discrepancy in number assignment between independent observers required a third observer to assign a number. Due to time restrictions, analyses of gut fullness and hepatic-somatic will be forthcoming.

Total number and density of macroinvertebrates and total number and density of fishes were calculated for each subsection of a riffle and for each run. Total number of macroinvertebrates and fishes and mean density of macroinvertebrates and fishes were calculated from the three subsections and multiple runs (if applicable) to generate a total number and a mean density estimate for one riffle or one run at each site and visit. The riffle or run is the experimental unit that represents the macroinvertebrate community and fish community at each site and visit. Abiotic factors were averaged among subsections or runs to generate an estimate per parameter for one riffle and one run. Therefore 339 riffle subsections were reduced to 130 riffles, and 240 runs were reduced to 153 runs. Abiotic and biotic variables of experimental units were used in subsequent analyses.

Among riffle habitats, total density macroinvertebrates were across flow tiers and before and after the largest flood. Likewise, EPT index was calculated for each riffle by summing densities. Similarly, fishes were grouped along a gradient of swift-water to slack-water specialists following methodologies of Leavy and Bonner (2009). Categories were riffle fishes, fluvial fishes, and slack-water fishes. Density per category per riffle was calculated by summing species within each category. Relative abundance of each category was calculated by summing species abundances within the category, divided by total numbers of fish taken, and multiplied by 100. Among run habitats, density and relative abundance were calculated for each run by the same methodology and similar categories as riffle species. Summaries of abundant species were provided for pool and backwater habitats.

Consequently, two abiotic datasets (one for riffles and one for runs) and three biotic datasets (macroinvertebrates in riffles, fishes in riffles, and fishes in runs) were developed with each row representing an experimental unit and labeled by assigned flow tier (hereafter, “tier”), drainage, season, and peak flow. A series of three-factor analysis of variance was used to test the relationship among response variables (e.g., swift-water fish relative abundances, EPT) and tier (up to 9 levels), drainage (GSA, Brazos, Col/Lav), and season (four seasons in GSA, three seasons in Brazos were converted to a four-seasons scale). With no significant differences in the overall model for swift-water, moderately swift-water, and slack-water fish abundances and densities, tier effects were assessed within sites or a combination of sites (e.g., upper GSA—Medina and Comfort). Replication was deemed adequate if each tier had at least three replicates. Treatment levels with <3 replicates were deleted prior to analyses (e.g., Col/Lav basin). Each one-factor analysis ($\alpha=0.05$) was followed with a Fisher’s LSD test. In addition, one-factor

analysis was used at each site or combination of sites to assess relative abundances and densities between pre-flood and post flood periods (GSA and Brazos riffle and runs only).

2.1.2 Aquatic Historical Analysis

As part of the investigation into the relationship between instream flow and associated ecological communities, data from prior instream flow studies conducted by BIO-WEST were compiled and analyzed keeping a priori predictions data separated by data used for retrospective analysis. This initial dataset included 161,620 fishes collected from 2004 to 2014 and represented 49 sites from the three basins of interest (Col/Lav, GSA, and Brazos). This dataset was refined to match the current study in terms of similar units and response variables. Through this process, data were culled due to lack of information (e.g., no gauge data or abiotic parameters). The resulting refined dataset contained seven GSA basin sites, nine Brazos basin sites, and seven Colorado basin sites, and contained 252 distinct sampling units (i.e., riffle, run pool, backwater) dispersed among drainages (Brazos: 48, Colorado: 8, GSA: 196). For this analysis, percent exceedance flow levels were evaluated instead of flow tiers to evaluate responses to discharge. Using percent exceedance based on the period of record at each USGS gage allowed for comparisons of discharge levels across sites with varying magnitudes of discharge. To evaluate a lag time similar to the current study, we assigned each sampling unit the maximum percent exceedance value from the discharge 15 days prior to the sampling event. This refined dataset was more appropriate and similar to the current study while retaining all pertinent data.

Fishes were grouped along a gradient from swift-water to slack-water specialists accordingly to Leavy and Bonner (2009). Relative abundance of each fish category was calculated by summing species abundances within the category and divided by total numbers of fish. Four datasets were consequently created for analyses: run, riffle, pool, and backwater for each of the three basins. Each row in the dataset represented an experimental unit and was labeled by percent exceedance, drainage, and fish group. Initially, the overall variation in the three drainages (GSA, Brazos, and Colorado) was investigated with the multivariate ordination technique: non-metric multidimensional analysis. We also plotted nMDS ordinations for each of the habitat units (run, riffle, pool, and backwater) for the three river drainages. Subsequently, we used a measure of similarity/dissimilarity (SIMPER) to explore which species were contributing any differences to the observed nMDS plot. Secondly, as performed in the current fish community study, a series of three-factor analysis of variance was used to test the relationship among response variables (e.g., swift-water fish relative abundances) and explanatory variables (e.g., percent exceedance and drainage). If necessary, we explored further using a linear regression model within each basin for the groups of fishes (slack-water, moderately swift-water, and swift-water). Abundance of the most dominant fish species were also evaluated vs. percent exceedance values to parallel the current fish study. All analyses were performed using PRIMER v7 software (Clarke and Gorley 2015) and RStudio (2016).

2.2 Riparian

For the Col/Lav basins, four riparian sites were chosen from the recommended USGS-monitored reaches, two in the Colorado Basin and two in the Lavaca/Navidad. One Colorado Basin site (Colorado Bend Site) was on the main stem of the Colorado River; the other was on a tributary to the Colorado River (Onion Creek Site). One Lavaca/Navidad site was located on the Navidad

River (Navidad Site); the other was on a tributary to the Lavaca River (Sandy Creek Site). A description of each site is provided below.

2.2.1 Colorado Bend Site

The Colorado Bend Site is located in San Saba County at Colorado Bend State Park and is located approximately 48 kilometers downstream from USGS gage #08147000 on the Colorado River near San Saba. The Colorado River originates in the flat western plateau of the Llano Estacado and begins to drop extensively in elevation as it passes through the Edwards Plateau, eventually draining over 31,000 square miles. Groundwater sources also contribute to the river intermittently along its course (Parsons Engineering 1999). At the location of the study site, the Colorado River features exposed bedrock streambed with tall limestone outcroppings on one or both river banks, forming long, tall canyons. Within these canyons the river is restricted to a narrow valley containing multiple smaller exposed-rock outcroppings and sediment banks, which are home to mesic-loving vegetation communities. The narrow valley is bordered by drier, exposed-limestone escarpments and hills dominated by xeric-tolerant vegetation. The land use at the location is protected habitat with no grazing or development; however, the area was at one time in the past an active ranch, most likely with grazing animals. The surrounding area is rural ranching country with very little development. The width of the river at the riparian site generally ranges from 70 to 80 meters. The dominant soil type within the study site is Westola fine sandy loam consisting of some minor soil classes derived from calcareous alluvium. Westola soils are characterized as frequently flooded with 0% to 2% slopes. Historically Westola soils support a tall grass savannah as the climax vegetation community; however, with the increase in grazing and suppression of fire, the community most typically becomes a mixed tree/shrub shade-tolerant herb community. Vine and woody subshrubs increase in density and tall grasses disappear, giving way to cool-season and shade-tolerant species.

2.2.2 Onion Creek Site

The Onion Creek Site is located on private property in Northern Hays County approximately 900 m upstream of USGS gage #08158700 on Onion Creek near Driftwood. Onion Creek, a tributary of the Colorado River, is a typical hill-country stream denoted by exposed limestone bedrock along the upper two thirds of the watercourse. The stream drains 211 square miles and consists of several gaining reaches with spring discharge from the Edwards-Trinity aquifer (Hunt et al. 2016). The land use on and around the site was low-density grazing, and the surrounding area is still rural with some sparse residential development. At the site, Onion Creek crosses the Highway 150 low-water crossing. The width of the stream in this section generally ranges from 10 to 16 m with varying depths. The dominant soil type within the riparian level is Oakalla silty clay loam derived from limestone alluvium. This soil class is characterized as frequently flooded yet well drained with sloping features of 1 to 2%. This soil type historically supports a tall grass savannah community dominated by pecan, live oak, walnut, sycamore, cypress, hackberry, cedar elm, western soapberry, cottonwood, and willow. The understory is composed principally of little bluestem, Indiangrass, switchgrass, eastern gamagrass, southwestern bristlegrass, Virginia wildrye, and perennial forbs, with smaller amounts of shrubs and woody vines, including American beauty berry, hoptree, Mexican buckeye, and roughleaf dogwood. Canopy cover historically ranges from 10 to 30%.

2.2.3 Navidad River Site

The Navidad River Site was located on property owned by the Lavaca Navidad River Authority in central Jackson County approximately 10 km downstream of the USGS gage (#08164390) on the Navidad River at Strane Park near Edna. The Navidad River is a major waterway and shares its basin with a sister river, the Lavaca River, forming the Lavaca-Navidad River basin. The Navidad River arises in Fayette County and flows through Lavaca, Colorado, and Jackson counties. The base flow for the Navidad River is provided entirely by precipitation runoff from the surrounding watershed. No springs are known to contribute (Water Monitoring Solutions 2012). The Navidad River enters Lake Texana, the major reservoir in the watershed, 6.2 km below the study site. The land use around the site was primarily crop farming with some grazing and little development. The riparian study site consisted of non-developed forested land with no crop farming or grazing animals. The width of the Navidad River along the riparian study site ranges from 19 to 30 meters. The site is dominated by Chicolete clay derived from loamy alluvium. Chicolete clay is characterized as deep, moderately well-drained soil that is frequently flooded and has a 0 to 1% slope. This soil class is typically associated with wide, flat floodplains that historically support medium-density woodlands, interspersed with tall grass prairie maintained by an alternating fire and flood regime. Sugar hackberry typically dominates the community along with pecan, cedar elm, live oak, black willow, sycamore, and green ash common closest to the stream bank. In areas with larger amounts of shade and mesic conditions, various sedges and shade-tolerant grasses dominated.

2.2.4 Sandy Creek Site

The Sandy Creek Site was located at the Camp Mauritz property owned and operated by the Lavaca Navidad River Authority in northern Jackson County approximately 12 km downstream of the USGS gage (#08164450) on Sandy Creek near Ganado. Sandy Creek is a major tributary to the Navidad River, arising in Colorado County and draining 289 square miles through parts of Lavaca, Wharton, and Jackson counties. The base flow for Sandy Creek is made up of return irrigation flow from rice fields and other agricultural enterprises in the area. Sandy Creek enters Lake Texana, a major reservoir in the watershed, 8.5 kilometers below the study site. The land use around the site is primarily rural and consists of cleared land for grazing cattle as well as some crop farming. The landscape is typically wooded, especially along the river. Very little development is present in the area although newly constructed gas pipelines and associated infrastructure have contributed slightly to an increase in development. The width of Sandy Creek along the riparian study site generally ranges from 35 to 50 meters. The site was dominated by two soil types, Kuy sand derived from sandy alluvium and Navidad fine sandy loam. Kuy sand is characterized as deep sand that is rarely flooded and has a 1 to 5% slope. Kuy sand is primarily associated with upland hills and berms adjacent to small streams. These soils are made up of very deep sands that are excessively drained and at times quite droughty (Web Soil Survey 2017). The vegetation community associated with this soil type is historically a tall/mid-height grass savannah produced from alternate cycles of drought, ample rainfall, fire, and moderate grazing. Dominant species included big bluestem, little bluestem, Indiangrass, switchgrass and Florida paspalum. Historically, live oak and post oak trees would occur intermittently throughout the community, but with the introduction of intensive grazing practices and the elimination of fire, this grassy savannah community has transitioned to a wooded community with dense canopy cover. Navidad fine sandy loam is characterized as frequently flooded with 0 to 1% slope. This soil class is typically associated with broad, gently sloping bottomlands, which act as

drainage ways during flood events. It historically supports a woody grassland community with more mesic tolerant species. The makeup of this plant community is also dictated by flooding, which can reduce woody cover. Dominant species historically found within this community include eastern gamagrass, big bluestem, little bluestem and giant cane. Dominant woody species included hackberry, live oak, and pecan, with green ash and black willow in the lowest areas. As with other plant communities, the introduction of intensive grazing can cause a transition toward a different plant community, one typically dominated by woody species.

Initial site visits were made to get a general idea of the layout and habitat quality of the site. After initial field visits to the area, Digital Elevation Models (DEMs) /aerial photos and overall site coordinates were used to create three parallel-to-stream corridor transects per site. Although the topography varied at each site, in general a lower level (Level 1) was placed along the stream edge, a middle level (Level 2) was placed along the rising bank and an upper level (Level 3) was placed at the slope crest. Each level was formed based on field and image observations; and though they did not necessarily cover the same amount of area, the total area of each of the survey sites was kept similar. The boundaries of each level were digitized in ArcGIS to create shapefiles. Using the random point generator in ArcGIS a shapefile of 75 random points was created for each level and for each sampling period (Figure 4). These shapefiles were then loaded onto a Trimble GPS unit for use in the field.

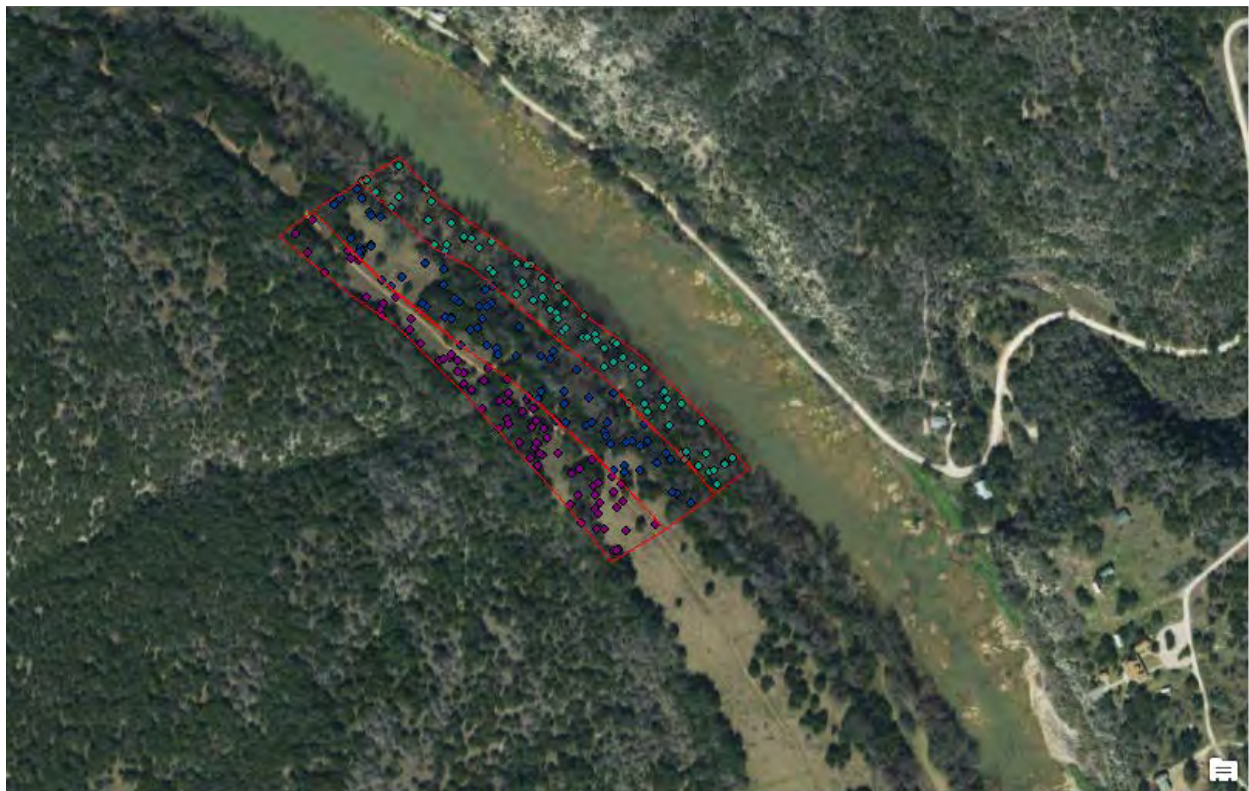


Figure 4. An example site showing 75 random points selected within each level. (Image source: Google Earth.)

2.2.5 Field Sampling

Riparian sites in the Lavaca and Colorado basins were sampled twice, once in Fall 2016 and then again in Spring 2017.

Lavaca/Navidad Basin

Sandy Creek site	December 6, 2016 and May 1, 2017
Navidad River site	December 8, 2016 and May 3, 2017

Colorado Basin

Onion Creek site	November 10, 2016 and June 5, 2017
Colorado Bend State Park site	November 16, 2016 and May 16, 2017

In the field, the point shapefile for each level was loaded onto the Trimble GPS unit so that the randomly generated points could be viewed. From the 75 random points, 35 points were located within each level for data collection. Once a point was located with the Trimble GPS unit, a 2x2 m quadrat constructed of PVC was set in place with the Trimble GPS unit located in the middle of the quadrat. The latitude and longitude of the point were recorded using the Trimble GPS unit while biological data were recorded on data sheets.

Woody vegetation individuals were counted, classed into WI (see wetland indicator explanations below) and grouped according to the following noted size classes:

- Seedling. Just sprouted or less than 1 cm diameter and less than 50 cm in height
- Sapling. 1–5 cm in diameter and greater than 50 cm in height
- Overstory (mature). >5 cm

The wetland indicator (WI) classes are as follows:

- Wetland obligate, almost always found in very wet locations—symbol: OBL
- Facultative wetland, usually found in wet locations—symbol: FACW
- Facultative, found in both wet and non-wet locations—symbol: FAC
- Facultative upland, usually found in non-wet locations—symbol: FACU
- Upland, almost always found in upland, non-wet locations—symbol: UPL

The woody species in this basin that fall into the OBL class are buttonbush and water hickory. Those considered FACW are green ash, bald cypress, black willow, box elder, Possomhaw holly, sycamore, and swamp oak.

For mature trees the Diameter at Breast Height (DBH), which is measured approximately 1.37 m from the ground) was recorded using an arborists' thinline and recorded for each trunk larger than 5cm. Understory/herbaceous vegetation were identified to genus (or to species if possible), counted, and classed into wetland indicators. Herbaceous species were limited to the six most-prevalent species in the 2x2 m quadrats.

A second, independent mature tree sampling recorded overall riparian mature tree counts. It was conducted within circular plots with a radius of 11.27 m measured from a random point within each level. Within these plots all mature trees (those with a DBH of 5cm or greater) were identified to species and their DBH was recorded. If a multi-trunked tree had more than one

trunk larger than 5 cm in diameter, each DBH measurement was recorded as well. The latitude and longitude of each tree were recorded using a Trimble GPS unit.

After field visits the collected biological data were combined with the GPS coordinates to create an attribute table for each plot. Five-foot DEM contours downloaded from the Texas Natural Resource Information System (TNRIS 2017) were combined to provide elevation data for each plot. The distance to each plot from the river's edge was calculated from the mapped water's edge collected at the time of field visits (Figure 5).

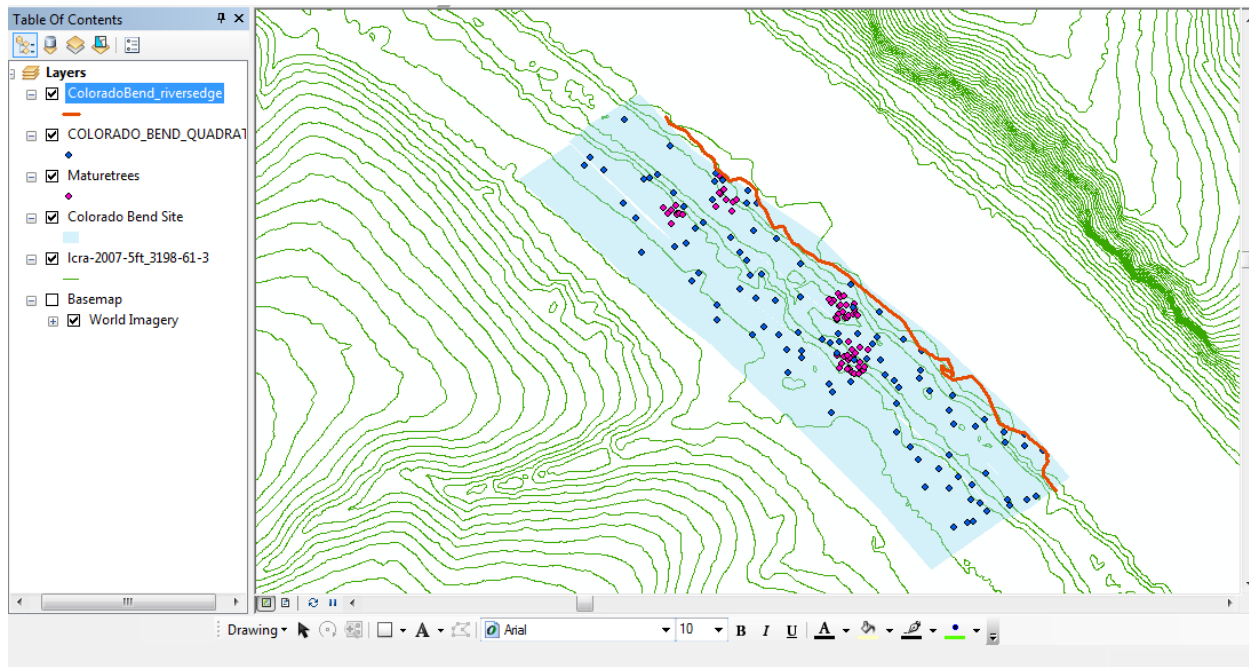


Figure 5. Example GIS screenshot showing water's edge, quadrats, mature trees, and elevation contours.

Each site's general geomorphological features were recorded, including the following variables:

- **Steepness of bank**, calculated as the perpendicular rise (m) over run (m) from water's edge to the riparian outer boundary.
- **Dominant soil order**. National Resources Conservation Service (NRCS) Soil Orders of Texas was used for mapping (NRCS 2017).
- **Dominant soil type** (sandy, clay, loam), categorized as: Silty=1, Sandy=2, Clay=3, Silt/Sand=4, Silt/Clay=5, Clay/Sand=6, Loam=7 (equal mix of all). Web Soil Survey (2017) was used for mapping soil types.
- **Local stream sinuosity**, categorized as straight=1, low (cutbank side) =2, low (point bar side) =3, high (cutbank side) =4, high (point bar side) =5.
- **Stream channel width**, recorded in meters.

2.2.6 Estimate of Inundation

Flood inundation values were estimated using available DEM data available for each site. These data ranged temporally from 2007–2014. Utilizing the USGS Rating Curve tool (USGS 2017), a rating curve was created using the nearest upstream USGS gauge for each site. This rating curve was then applied respectively to each site for level and individual point calculations. The highest point of elevation within each level was estimated (using field GPS points) and then applied to the rating curve, using the shoreline elevation as the start of the curve. The rating curve was also applied to the elevation of each mature tree or quadrat elevation, again using the shoreline elevation for each site as the starting elevation. Discharge levels were estimated using the rating curve and provided the approximate discharge amount needed to inundate the associated elevation of each level, quadrat, and mature tree.

2.2.7 Statistical Analyses

Questions 3 through 7 were designed to be tested statistically. Plymouth Routines In Multivariate Ecological Research (PRIMER) statistical software was used for analysis of data related to these questions (Clarke and Gorley 2015). To answer Question 3 an ordinate (nMDS) test based on Bray-Curtis matrix and clustering techniques was run for each site's level and plots to visualize species composition differences. A first run included the entire community assemblage by individual species, a second run included the entire community grouped by WI class, and a third run included the mature-trees-only dataset by individual species. This test was followed by an ANOSIM for each site/level, duplicating each of the three runs above, and a SIMPER test was used to show which species were most contributing to similarities and/or dissimilarities between groups. Question 4 was removed from analysis because ultimately only one seasonal sampling event was permitted in the study. To answer Question 5, these same tests were run by combining each site's entire community and testing each against the other. Additionally, Level 1 of one site was compared against Level 1 of all other within-basin sites, etc.

Question 6 was addressed by testing the outcomes of Question 5 against abiotic factors in Question 1 using principal component analysis (PCA) of the correlation variance between the abiotic factors and riparian communities. In addition to overall community assemblages, this analysis was performed on the riparian canopy, using the mature tree datasets from each site.

To answer Question 7, the same tests for Questions 5 and 6 were repeated for all sites *across basins*. The basins of interest and their respective sites were: GSA Basin, with Goliad and Gonzales sites; the Brazos Basin, with Hearne and Brazos Bend sites; the Colorado-Lavaca Basin with Onion Creek, Colorado Bend, Sandy Creek, and Navidad River sites.

3 Results, Discussion, and Interdisciplinary Assessment

3.1 Aquatics

3.1.1 Aquatic Field Studies

Aquatic sampling as part of Round One of this study occurred from summer 2014 through spring 2015 following a multi-year period of relatively dry conditions throughout most of Texas. During much of this period, most of the state was in an extreme drought condition. This dry pattern had a strong influence on hydrologic conditions and resulted in few pulse-flow events

being captured during Round One of this study. The lack of pulse-flow events leading up to and during Round One is evident in the example hydrograph below from the Guadalupe River at Gonzales (Figure 6). However, in late spring 2015, as Round One data collection was winding down, intense and relatively widespread rain events brought massive flooding to many areas of central Texas. The remaining portion of 2015 was wet, with another large flood event experienced in fall 2015. Although variable across basins and sites, this wet pattern generally continued through 2016. Data collection for Round Two which included the Col/Lav basin began

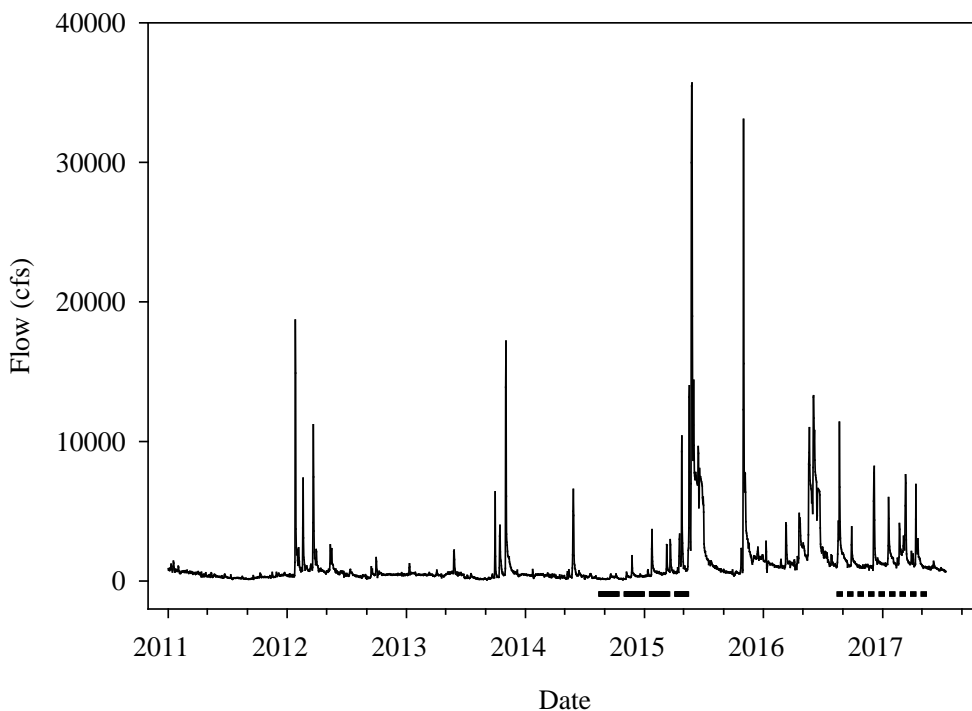


Figure 6. Hydrograph from US Geological (USGS) gage # 08173900 on the Guadalupe River at Gonzales from 2011 to 2017 showing Round One (dashed line) and Round Two (dotted line) sampling periods.

in late summer 2016 during a much wetter period following the large flood events of 2015. Although this allowed for capturing additional pulse-flow conditions at some sites, relatively continuous high flows hampered sampling at others. However, this also allowed for a comparison of pre-flood to post-flood conditions in the GSA and Brazos basins in addition to flow-tier analysis, as presented in the results below.

Overall Fish Community

Totals of 59 species and 43,804 fishes were recorded from Col/Lav (N of species=31), GSA (40), and Brazos (48) basins among all habitats between 2014 and 2017 (Table 2). Total number of site visits was 153. Among the 153 site visits, flow tiers were subsistence (N=4), base (48), 4-per-season (6), 3-per-season (9), 2-per-season (25), 1-per-season (40), 1-per-year (10), 1-per-2-year (2), and >1-per-5-year (9) (**Error! Reference source not found.**). A total of 362 habitats was sampled (130 riffle, 153 run, 23 pool, and 56 backwater). Although the analysis below

focuses on response to hydrologic parameters, a summary of habitat parameters for riffle, runs, pools, and backwaters are provided in Appendix C.

In Round Two of the study (2016–2017), total number of sites was 18, and total number of site visits was 84. Among the 84 site visits, flow tiers were base (12), 4-per-season (4), 3-per-season (9), 2-per-season (17), 1-per-season (27), 1-per-year (5), 1-per-2-year (2), and >1-per-5-year (8). A total of 224 habitats was sampled (66 riffle, 79 run, 23 pool, and 56 backwater). Results of Round One and Round Two were combined for flow-tier analysis.

Table 2. Fishes taken from all habitats and basins 2014 through 2017.

Species Name	Fluvial Category	2014–2017 Relative Abundance (%)	GSA Relative Abundance (%)	Brazos River Relative Abundance (%)	Colorado River Relative Abundance (%)
<i>Atractosteus spatula</i>	Slack	<0.1	<0.1	<0.1	
<i>Lepisosteus oculatus</i>	Slack	<0.1			<0.1
<i>Anguilla rostrata</i>	Slack	<0.1	<0.1		
<i>Brevoortia patronus</i>	Slack	0.14		0.24	<0.1
<i>Dorosoma cepedianum</i>	Slack	0.23		0.42	
<i>Dorosoma petenense</i>	Slack	1.8		3.3	
<i>Anchoa mitchilli</i>	Slack	<0.1	<0.1	0.14	
<i>Camptostoma anomalum</i>	Swift	1.3	2.9	0.55	<0.1
<i>Carpoides carpio</i>	Slack	<0.1		<0.1	<0.1
<i>Cyprinella lutrensis</i>	Moderate	40.0	30.5	46.0	40.1
<i>Cyprinella hybrid</i>	Moderate	<0.1	<0.1	<0.1	<0.1
<i>Cyprinella venusta</i>	Moderate	17.4	8.1	19.3	38.1
<i>Hybognathus nuchalis</i>	Slack	<0.1		<0.1	
<i>Lythrurus fumeus</i>	Slack	0.43		0.77	<0.1
<i>Macrhybopsis hyostoma</i>	Swift	0.87		1.6	
<i>Macrhybopsis marconis</i>	Swift	0.26	0.75	<0.1	
<i>Notropis amabilis</i>	Swift	8.4	24.3		
<i>Notropis buchmanii</i>	Slack	2.3	1.1	3.5	
<i>Notropis shumardi</i>	Swift	2.9	<0.1	5.3	
<i>Notropis texanus</i>	Slack	<0.1			0.30
<i>Notropis volucellus</i>	Moderate	6.1	15.8	0.97	1.1
<i>Pimephales vigilax</i>	Moderate	5.7	2.4	7.9	5.3
<i>Moxostoma congestum</i>	Moderate	<0.1	0.20	<0.1	<0.1
<i>Astyanax mexicanus</i>	Swift	<0.1	0.21	<0.1	
<i>Ictalurus furcatus</i>	Swift	0.33	<0.1	0.60	
<i>Ictalurus punctatus</i>	Swift	1.2	1.1	1.4	0.60
<i>Noturus gyrinus</i>	Slack	<0.1	<0.1	<0.1	<0.1
<i>Pylodictis olivaris</i>	Swift	0.16	0.25	<0.1	0.45
<i>Mugil cephalus</i>	Slack	<0.1		<0.1	0.13
<i>Labidesthes sicculus</i>	Slack	<0.1		<0.1	
<i>Menidia audens</i>	Slack	<0.1	<0.1	<0.1	<0.1
<i>Fundulus notatus</i>	Slack	0.38		0.69	
<i>Gambusia affinis</i>	Slack	3.1	1.7	2.7	9.2
<i>Poecilia formosa</i>	Slack	<0.1	0.13		
<i>Poecilia latipinna</i>	Slack	<0.1	0.16	<0.1	
<i>Morone saxatilis</i>	Moderate	<0.1		<0.1	
<i>Lepomis auritus</i>	Slack	0.11	0.11	<0.1	0.22
<i>Lepomis cyanellus</i>	Slack	<0.1	<0.1	<0.1	<0.1
<i>Lepomis gulosus</i>	Slack	<0.1		<0.1	<0.1
<i>Lepomis humilis</i>	Slack	<0.1	<0.1	<0.1	
<i>Lepomis macrochirus</i>	Slack	0.15	0.15	0.12	0.34
<i>Lepomis megalotis</i>	Slack	0.69	0.45	0.61	1.9
<i>Lepomis microlophus</i>	Slack	<0.1		<0.1	<0.1
<i>Lepomis miniatus</i>	Slack	<0.1			<0.1
<i>Micropterus dolomieu</i>	Moderate	<0.1	<0.1		
<i>Micropterus punctulatus</i>	Slack	<0.1	0.16	<0.1	<0.1
<i>Micropterus salmoides</i>	Slack	<0.1	<0.1	<0.1	0.19
<i>Micropterus treculii</i>	Moderate	<0.1	0.13	<0.1	
<i>Pomoxis annularis</i>	Slack	<0.1	<0.1	<0.1	
<i>Etheostoma chlorosoma</i>	Slack	<0.1		<0.1	
<i>Etheostoma gracile</i>	Slack	0.18	<0.1	0.32	
<i>Etheostoma lepidum</i>	Swift	0.19	0.56		
<i>Etheostoma spectabile</i>	Swift	2.9	4.3	2.5	0.22
<i>Percina apristis</i>	Swift	0.24	0.68		
<i>Percina carbonaria</i>	Swift	0.45	1.0	<0.1	0.60
<i>Percina sciera</i>	Swift	0.18		0.24	0.43
<i>Percina shumardi</i>	Swift	0.71	2.0		
<i>Aplodinotus grunniens</i>	Slack	<0.1		<0.1	
<i>Herichthys cyanoguttatus</i>	Slack	0.14	0.40		<0.1
N of species		59	40	48	31
N of individuals		43,804	15,121	24,037	4,645

Table 3. Number of sites and visits conducted during Round One and Round Two (2014–2017) with breakdown per flow tier.

	GSA	Brazos	Colorado	Total
Sites	7	6	5	18
Visits	59	68	26	153
Subsistence	1	3	0	4
Base	21	16	11	48
Flow Pulses	37	49	15	103
4 / season	-	6	-	6
3 / season	-	9	-	9
2 / season	5	12	8	27
1 / season	22	14	4	40
1 / year	5	2	3	10
1 / 2 year	1	1	0	2
1 / 5 year	4	5	0	9

Data from the Col/Lav basin were not included in pre-flood vs. post-flood analysis, or flow-tier analysis because no pre-flood data were available and there was insufficient replication to analyze by flow tier. Instead, these analyses focused on Brazos and GSA gage locations where more data were available across a range of hydrologic conditions. Additional data collection in the Col/Lav basin will allow for these same analyses to be conducted once a more robust dataset is gathered. Below is a summary of fish communities documented at Col/Lav sites, followed by a summary of the pre-flood vs. post-flood and flow-tier analyses conducted within the GSA and Brazos basins. Results from these analyses in other basins can provide guidance in assessing environmental flow recommendations within the Col/Lav basin, as described in Section 4.0.

Colorado/Lavaca Fish Community

San Saba River—San Saba

A total of 632 fishes was recorded from six sampling events and three flow tiers (base, 2-per-season, and 1-per-season). Most abundant fishes were *Cyprinella lutrensis* (N=201), *Cyprinella venusta* (193), and *Gambusia affinis* (93).

Colorado River—San Saba

A total of 2007 fishes was recorded from six sampling events and three flow tiers (base, 2-per-season, and 1-per-season). Most abundant fishes were *Cyprinella lutrensis* (N=1,608), *Pimephales vigilax* (163), and *Cyprinella venusta* (145).

Onion Creek—Driftwood

A total of 587 fishes was recorded from four sampling events and two flow tiers (2-per-season and 1-per-year). Most abundant fishes were *Cyprinella venusta* (N=542), *Gambusia affinis* (18), and *Lepomis megalotis* (13).

Lavaca River—Edna

A total of 379 fishes was recorded from five sampling events and three flow tiers (base, 2-per-season, and 1-per-season). Most abundant fishes were *Cyprinella venusta* (N=313), *Lepomis megalotis* (24), and *Notropis texanus* (13).

Navidad River—Edna

A total of 1,040 fishes was recorded from five sampling events and two flow tiers (base, and 2 per season). Most abundant fishes were *Cyprinella venusta* (N=577), *Gambusia affinis* (302), and *Pimephales vigilax* (61).

Macroinvertebrates

Totals of nine orders and 115,228 individuals were recorded from Col/Lav (N of individuals=21,796), GSA (41,990), and Brazos (51,442) basins among all habitats between 2014 and 2017 (Table 4). In the second year of the study (2016–2017), totals of nine orders and 65,000 individuals were recorded. Site-specific macroinvertebrate data from the Col/Lav drainage are provided in Table 5.

Table 4. Macroinvertebrates taken overall from 2014 through 2017.

Species	Total N	Mean Density	Percent Density
<i>Coleoptera</i>	18,762	49.63	16.33
<i>Diptera</i>	20,159	53.19	17.49
<i>Ephemeroptera</i>	44,502	117.42	38.62
<i>Hemiptera</i>	819	2.16	0.71
<i>Lepidoptera</i>	290	0.77	0.25
<i>Megaloptera</i>	485	1.28	0.42
<i>Odonata</i>	2,169	5.72	1.88
<i>Plecoptera</i>	1,318	3.48	1.14
<i>Tricoptera</i>	26,724	70.51	23.19
Total	115,228	304.03	

Table 5. Relative abundances of macroinvertebrates taken from Colorado River from 2016 through 2017.

Macroinvertebrate Order	San Saba San Saba	Colorado Bend	Onion Creek Driftwood	Lavaca Edna	Navidad Strane Park
Ephemeroptera	56.73	29.86	39.62	34.27	59.21
Tricoptera	9.38	43.17	29.77	4.11	14.47
Diptera	11.08	10.99	13.93	60.5	21.93
Coleoptera	19.52	14.98	3.78	1.13	0
Odonata	1.9	0.35	7.52	0	0
Plecoptera	0.5	0.16	4.86	0	4.39
Hemiptera	0.19	0.03	0	0	0
Megaloptera	0.47	0.28	0.52	0	0
Lepidoptera	0.22	0.17	0	0	0
EPT	66.61	73.19	74.25	38.38	78.07
Richness	9	9	7	4	4
Total N	7,229	13,793	447	289	38

Across Basin Summary

The following section summarizes results of flood and flow-tier analyses across the GSA and Brazos basins for both fishes and macroinvertebrates. As described in the methods section, with no significant differences in the overall model for swift-water, moderately swift-water, and slack-water fish abundances and densities, tier effects were assessed within sites or a combination of sites (e.g., upper GSA, Medina River—Bandera and Guadalupe River—Comfort). Additionally, as previously described, insufficient replication at this time prevented the use of Col\Lav fish and macroinvertebrate data in flow-tier analysis.

Table 6 below shows the sites or combination of sites evaluated and available data collected per habitat type used in the flow-tier analysis.

Table 6. Fish and macroinvertebrate data collected per habitat type in the GSA and Brazos basins used in flow tier analysis.

Combination/Individual Sites per basin	Fish		Macroinvertebrates
	Riffle	Run	Riffle
GSA			
Medina River—Bandera and Guadalupe River—Comfort	√	√	√
Guadalupe River—Gonzales and Cuero and San Antonio River—Goliad	√	√	√
Cibolo Creek—Falls City	√	√	√
San Marcos River—Luling	√	√	√
Brazos			
Leon River—Gatesville and Lampasas River—Kempner	√	√	√
Little River—Little River	√	√	√
Navasota River—Easterly	√	√	√
Brazos River—Hempstead and Rosharon		√	

Seven sites/combinations had riffle data for both fish and macroinvertebrates with data collected for run habitats at eight sites/combinations. Ecological responses were detected within riffle habitats among all sites or combination of sites (N=7) and were detected within run habitats among four of the eight sites or combination of sites.

Species responses were associated with flow tiers in five of the eight sites or combination of sites (Table 7). Within the upper GSA, >1-in-5-year flow tier was associated with greater relative abundances of *C. venusta* and lower relative abundances of *C. anomalum* in riffles, when compared to base flow. Within the lower GSA, 1-per-season flow tier was associated with greater densities fluvial specialist *M. marconis* and lower relative abundances of fluvial specialist *Percina* in riffles, when compared to base flow. Within the San Marcos River, 1-per-season flow tier was associated with greater abundances and densities of *C. lutrensis* in riffles, greater abundances of *C. lutrensis* in runs, and greater densities of *P. vigilax* in runs, when compared to Table 7 summarizes where ecological responses were documented relative to base-flow conditions for fish and macroinvertebrate communities or individual species. Ecological responses of both community and individual species were documented between pre-flood and post-flood conditions, whereas only species-specific responses were noted per individual flow tiers.

Species responses were associated with flow tiers in five of the eight sites or combination of sites (Table 7). Within the upper GSA, >1-in-5-year flow tier was associated with greater relative abundances of *C. venusta* and lower relative abundances of *C. anomalum* in riffles, when compared to base flow. Within the lower GSA, 1-per-season flow tier was associated with greater densities fluvial specialist *M. marconis* and lower relative abundances of fluvial specialist *Percina* in riffles, when compared to base flow. Within the San Marcos River, 1-per-season flow tier was associated with greater abundances and densities of *C. lutrensis* in riffles, greater abundances of *C. lutrensis* in runs, and greater densities of *P. vigilax* in runs, when compared to base. With the lower Brazos River, 2-per-season and 1-per-season flow tiers were associated with lower relative abundances of *C. lutrensis* in runs, when compared to base and 3-per-season flow tiers. Among predications, *M. marconis* response (densities positively associated with flow tiers) and *C. lutrensis* response (relative abundances negatively associated with flow tiers, in the lower Brazos River only) were predicted a priori. Negative association with flow tiers observed with *C. anomalum* and *Percina* were opposite of predicted. Positive association with flow tiers observed for *C. lutrensis* (i.e., San Marcos River), *C. venusta*, and *P. vigilax* were opposite of predicted. Macroinvertebrate response was associated with flow tiers within lower GSA with total macroinvertebrate densities being greater at base than 1-per-season.

Table 7. Fish and macroinvertebrate community or species response to flow tier and pre-flood vs. post-flood conditions.

Combination / Individual Sites per basin	Fish and Macroinvertebrate response (Community or species)							Pre-flood vs. post-flood
	4/S	3/S	2/S	1/S	1/Y	1/2Y	1/5Y	
GSA								
Medina River—Bandera and Guadalupe River—Comfort							√	√
Guadalupe River—Gonzales and Cuero and San Antonio River—Goliad				√				
Cibolo Creek—Falls City								√
San Marcos River—Luling				√				√
Brazos								
Leon River—Gatesville and Lampasas River—Kempner								√
Little River—Little River								√
Navasota River—Easterly							√	√
Brazos River—Hempstead and Rosharon			√	√				√

Analysis of pre-flood and post-flood conditions revealed that densities of total fishes decreased at upper GSA sites (riffle) and lower Brazos River (run), increased in Navasota River (riffle), Leon and Lampasas rivers (run), and San Marcos River (run). Relative abundances or densities of at least one riffle specialist (i.e., *C. anomalum*, *Etheostoma*, and *Percina*) decreased at four of the seven sites or combination of sites. Relative abundances or densities of at least one *Cyprinella* increased within riffles at five of the seven sites or combination of sites. Relative abundances or densities of *Cyprinella* increased in runs among three of the eight sites or combination of sites and decreased in the lower Brazos River. Relative abundances and densities of fluvial specialists (i.e., *N. shumardi* and *M. hyostoma*) increased in runs of the lower Brazos River. Densities increased for *N. volucellus* and *P. vigilax* each within one site or combination of sites.

Greatest shift in fish communities was observed between pre-flood and post-flood lower Brazos River. Pre-flood fish community was dominated by *C. lutrensis* and *P. vigilax* (mean relative abundance: 85% \pm 1 SE:7.0) and few fluvial specialists *N. shumardi* and *M. hyostoma* (1.1% \pm 0.25). The post-flood fish community was dominated, as predicted, by fluvial specialist *N. shumardi* and *M. hyostoma* (60% \pm 8.7) and fewer *C. lutrensis* and *P. vigilax* (20% \pm 4.9). Mechanisms underlying the shifts are being assessed but likely represent two factors: (1) displacement of *C. lutrensis* and *P. vigilax* and (2) increased reproductive success of *N. shumardi* and *M. hyostoma* during an extended period of high flows. Shift in the lower Brazos River community was not detected among flow tiers, except for *C. lutrensis*. Combining *N. shumardi* and *M. hyostoma* relative abundances and densities among flow tiers pre-flood and post-flood periods produces large variation within treatment. As such, separating communities between pre-flood and post-flood periods and then assessing differences among flow tiers, when observations are available into the future, would provide a more logical assessment of the flow tiers.

In the Navasota River, a “wash-in” event was observed. *Dorosoma petenense* was not observed at the Navasota River—Easterly site between August 2014 and March 2017. Following a >1-per 5-year event, *D. petenense* comprised 94% of the fish community. The source of the wash-in was likely Lake Limestone, located upstream of the Navasota River site. The observation is relevant for tier-validation methodologies in that displacement of some fishes (e.g., wash-out of slack-water fishes) is expected with high flow pulses but might be compensated by increases of some slack-water fishes by a wash-in.

Macroinvertebrate responses were detected within riffle habitats among three of seven sites or combination of sites. Total macroinvertebrate densities decreased within lower GSA and increased in Leon and Lampasas rivers between pre-flood and post-flood periods. EPT densities increased at Leon and Lampasas rivers and at Cibolo Creek between pre-flood and post-flood periods.

3.1.2 Aquatic Historical Analysis

A total of 105,151 fishes representing 67 species were recorded in the final historical dataset. It should be noted that the aquatic historical analysis did not include any information from the Lavaca/Navidad basin. Run habitats were sampled 77 times, riffle habitats 55 times, pool habitats 53 times, and backwater habitats 67 times. The most abundant species in the dataset were Red Shiner *Cyprinella lutrensis*, (N=49,326), Bullhead Minnow *Pimephales vigilax* (13,839), Western Mosquitofish *Gambusia affinis* (10,160), and Blacktail Shiner *Cyprinella venusta* (N=5,903).

The nMDS multivariate ordination plot shows the Colorado drainage fish community to be distinct from the GSA and Brazos drainages within this dataset (Figure 7). A SIMPER analysis showed that the Colorado drainage had higher abundance of several species including: River Carpsucker *Carpiodes carpio*, Gizzard Shad *Dorosoma cepedianum*, Guadalupe Bass *Micropterus treculii*, Texas Logperch *Percina carbonaria*, Blue Sucker *Cycleptus elongatus*, and Dusky Darter *Percina sciera* compared to the other drainages which contributed to the observed differences in the overall community analysis. However, it should be pointed out that sampling methodologies differed slightly among collections and these data were not collected to evaluate differences in fish communities between the basins.

Using the full dataset, abundance of the four dominant species listed above were evaluated vs. percent flow exceedance level. As described in the methods section, percent flow exceedance levels were evaluated instead of flow tiers to evaluate responses to discharge. Using percent exceedance based on the period of record at each USGS gage allowed for comparisons of discharge levels across sites with varying magnitudes. An example graph for Red Shiner is provided in Figure 8. No significant relationships were observed.

Among basins, swift-water fishes were more abundant in the Colorado dataset (Figure 9). Using the complete dataset from all basins, swift-water fish abundance increased with percent exceedance level ($F_{3, 248} = 3.843, P = 0.01025$) (Figure 10). No other differences were detected among or within basins for each habitat type (riffle, run, pool, and backwater) using the three-factor analyses.

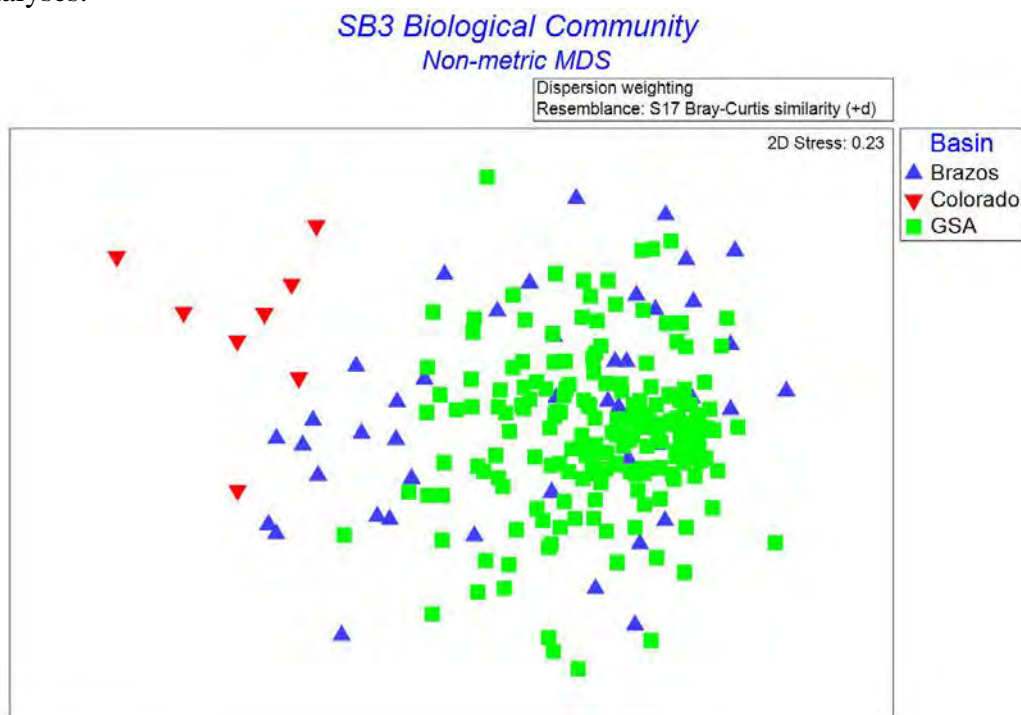


Figure 7. An nMDS ordination plot for the three river drainages fish communities.

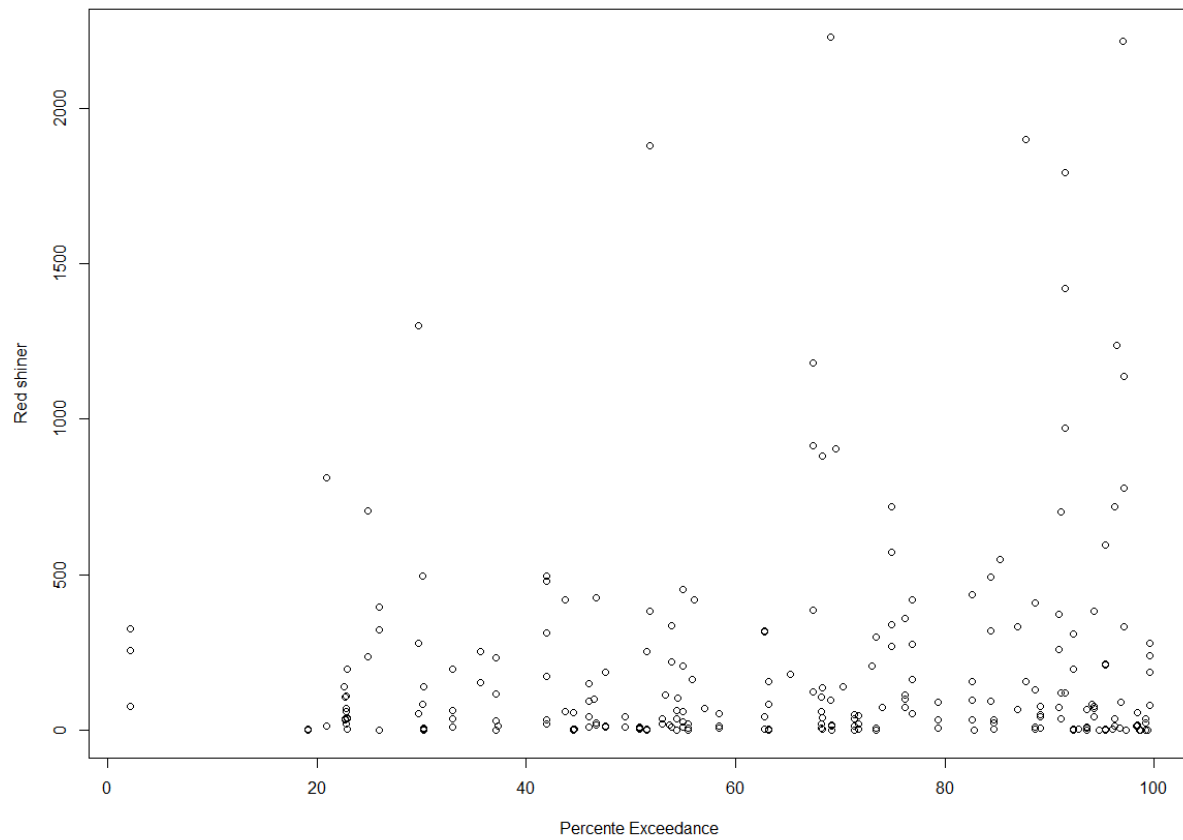


Figure 8. Red shiner abundance across percent exceedance levels.

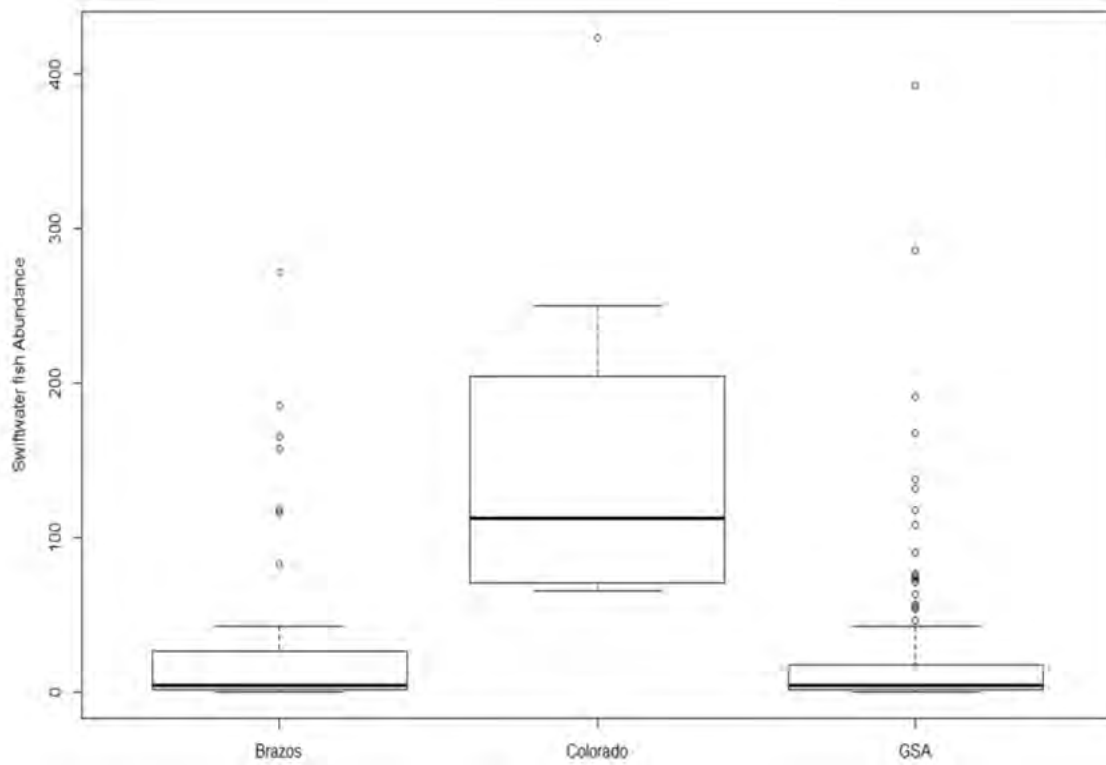


Figure 9. Swift-water fishes abundance by drainage.

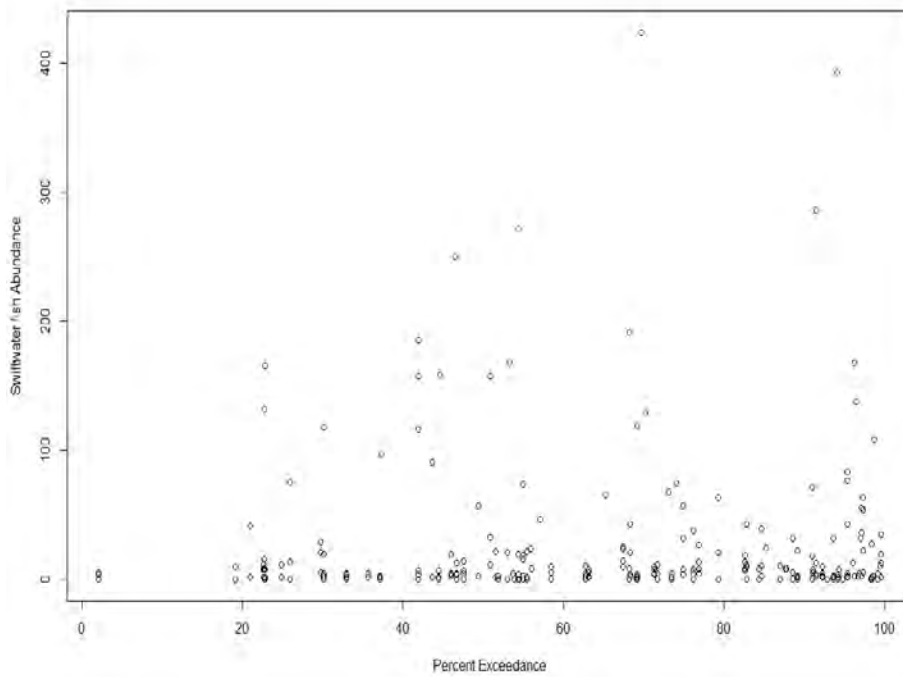


Figure 10. Abundance of swift-water fishes across percent exceedance levels.

Linear regression within each basin revealed that the proportion of moderately swift water fishes to the total number of fishes increased with percent in the Colorado drainage ($F_{1,6}=7.527$, $P=0.03358$) (Figure 11). No other relationships were noted among fish groupings within basins.

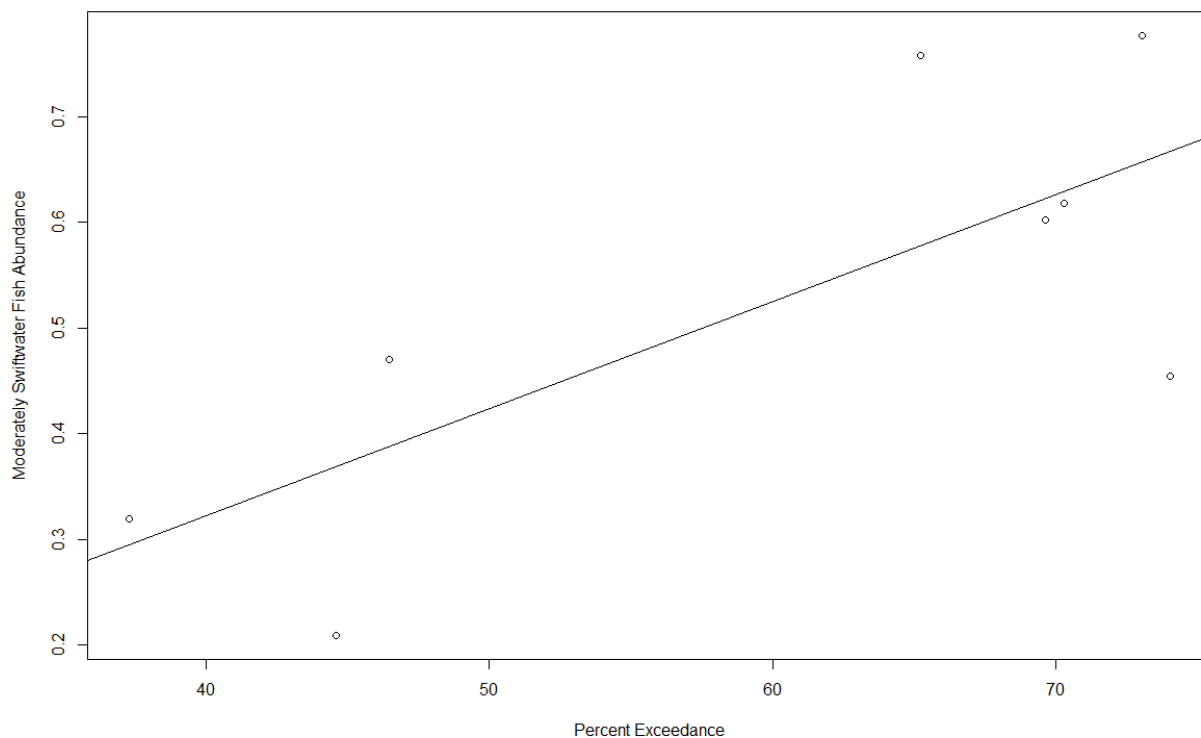


Figure 11. Proportional abundance of moderately swift-water fishes plotted as a response to percent exceedance in the Colorado drainage ($F_{1,6}=7.527$, $P=0.03358$) showing best fit line for linear regression model.

3.2 Riparian

3.2.1 Colorado Bend

Data at this location were collected in the late fall (December 2016) and late spring (May 2017). The riparian levels were diverse in topography. Level 1 was the most diverse level in topography and vegetation, with communities ranging from aquatic to mesic to upland (Figure 12). Level 1 included a narrow fringe wetland adjacent to the water. Here, more aquatic species such as Emory's sedge and rice cutgrass thrive in deposited sediment banks. A steeply sloped initial bank provided areas for woody species such as green ash, sycamore, and black willow to dominate. Multiple mesic grasses and forbs were present along this slope as well. At the crest of the initial slope, there was a wide, flat bank dominated by inland seaots and Virginia wild rye. Ashe juniper and yaupon were the dominant woody species here.

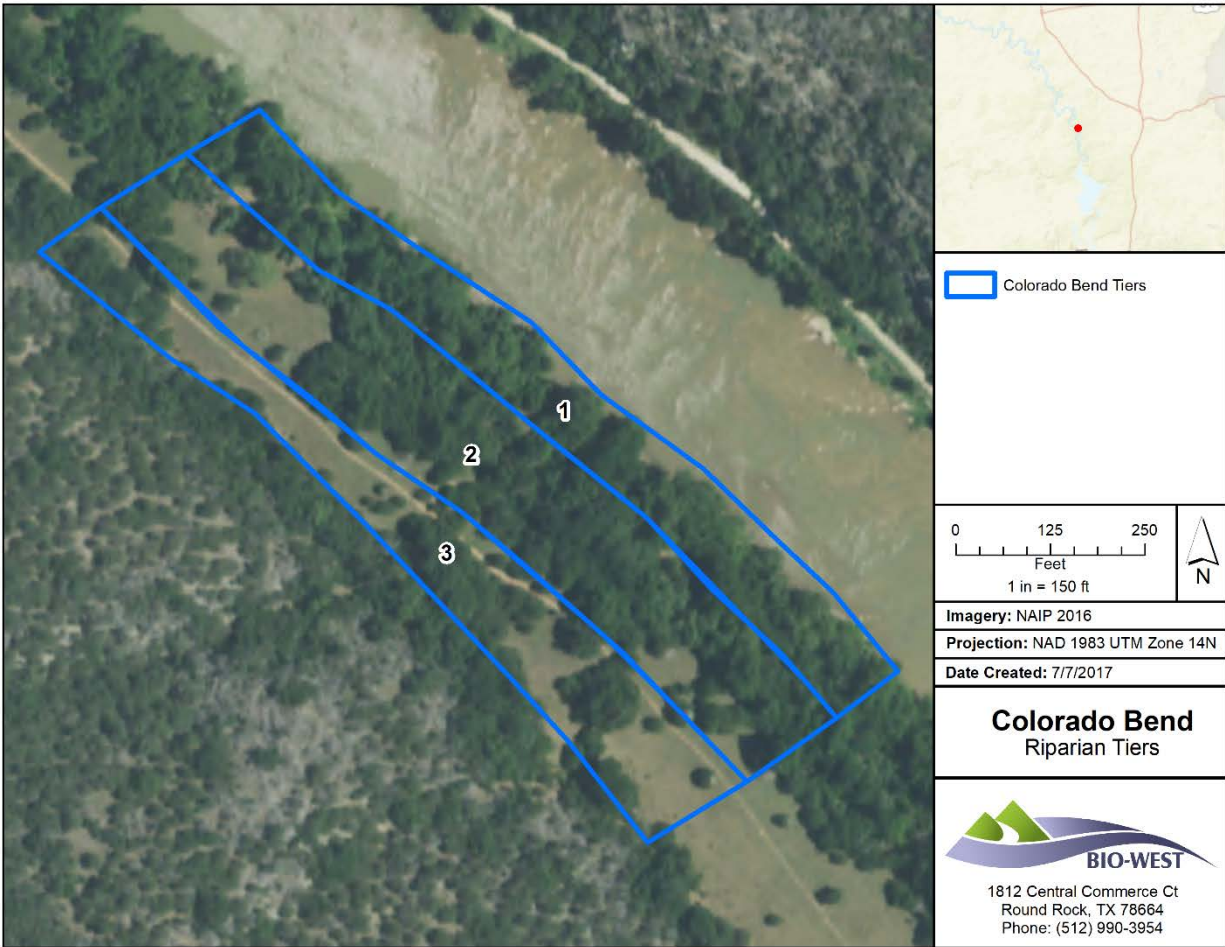


Figure 12. Overview of Colorado Bend Site showing the three level boundaries (in blue).

Level 2 consisted of a second, higher-elevation slope dominated by larger trees such as red oak, cedar elm, and dense Ashe juniper. This level was more shaded with dense canopy cover (over 50%) and exhibited an increase in bare ground. Herbaceous species and ground cover were limited to areas of open canopy. Level 3 began at the crest of the second slope and extended across flat terrain dominated by prairie grasses and forbs such as silver bluestem and King Ranch bluestem. Ashe juniper and cedar elm were the dominant woody species, with an occasional black walnut.

A representative profile (Figure 13) shows the slope from river's edge to the uppermost extent is has an overall site steepness factor of 0.11 (Table 8). Table 9 shows fall 2016 and spring 2017 community abundances and mature tree abundances, respectively. In fall, seaots were most abundant at 40% of the community. Green ash was 1.7% of the community and the only riparian species in the sampled plots. In spring, cedar elm seedlings and mature trees comprised 44% of the overall community and are by far the most prevalent species (up from only 6.5% the fall season before). Black willow and green ash were present, but each represented only 0.3% of the community. The total number of species increased from 35 to 50, and the total number of individual plants more than doubled from 1,272 in fall to 2,975 the following spring. This shows there are distinct differences in the community between seasons. Unfortunately, because of the

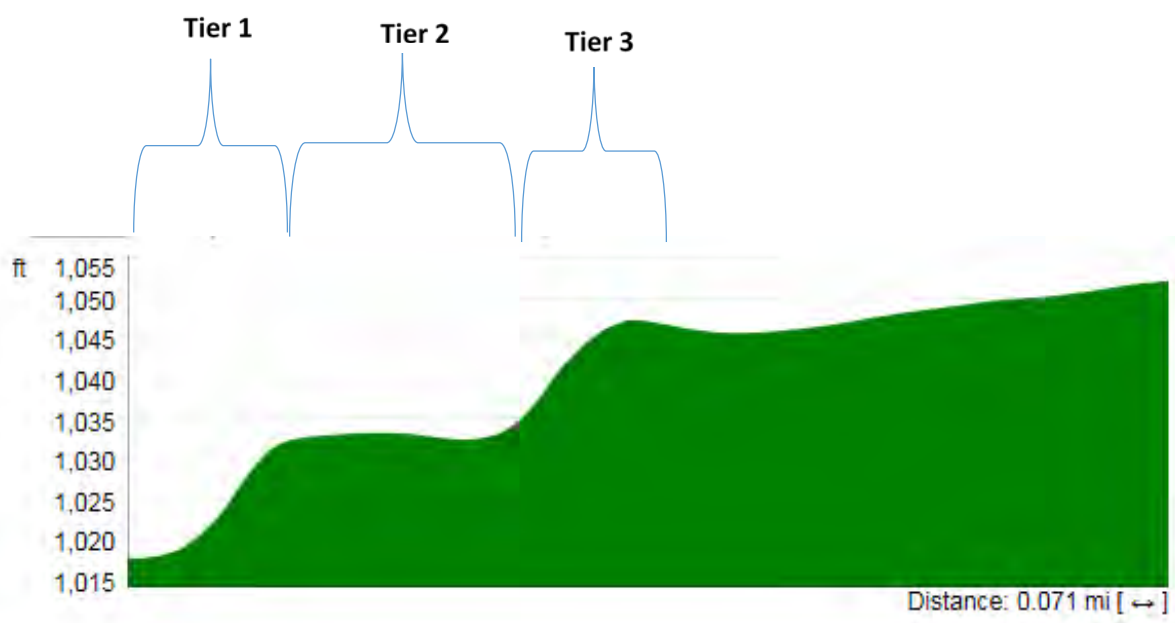


Figure 13. Colorado Bend Site profile showing general level locations.

Table 8. General site characteristics for sites studied during 2016–2017.

Site	Basin	Steepness of Zone	Dominant Soil Type	Dominant Soil Order	Sinuosity Factor	Channel Width (m)
Onion Creek	COLN	0.03	5	Mollisol	1	17
Colorado Bend	COLN	0.11	4	Alfisol	1	88.5
Sandy Creek	COLN	0.03	2&4	Vertisol	3	36.52
Navidad River	COLN	0.01	5	Vertisol	1	24.67
Brazos Bend	Brazos	0.13	2	Alfisol	3	50.45
Hearne	Brazos	0.04	7	Alfisol	3	73.23
Gonzales	GSA	0.05	7	Alfisol	5	41.87
Goliad	GSA	0.10	7	Mollisol	1	25.29

timing of the study (which did not extend through the entire growing season), it cannot be determined whether 2017 was a boom year for many species or whether this is a common seasonal pattern: spring counts well outnumbering fall. Grazing, dry summer months, annual life cycles, periodic flow pulses, etc., likely cause a natural attrition, but those cannot be discerned from this dataset. Of note is that, with the reduction of many herbaceous species, the percentage of riparian species within the community increased in fall samplings (there were far fewer other species, so riparian counts carry more weight in the community). This would be a consideration if sampling of a site were to be conducted only once a year. Ashe juniper and cedar elm top the list of mature trees at a combined 62%. Green ash were less than 5% of canopy species. There were no black willow in the sampling. Because these trees do cover this zone, this result may be a relic of randomized sampling that missed important indicator species by chance. The dominance by Ashe juniper and cedar elm are driving the high percentage of FAC and UPL (78% collectively).

An nMDS 2-dimensional ordination plot of level shows a slight progression of community assemblage dissimilarities from Level 1 to Level 3 (Figure 14), although the ANOSIM statistics in the figure indicate those differences are moderately low. For the riparian assessment, these two statistical approaches were chosen for a visual representation of variation (nMDS) as well as an investigation of the significance of the differences (ANOSIM) in vegetation community. An examination of the dissimilarities between those level using SIMPER tests (Appendix D, Table 1) shows that, even though the dissimilarities between the level are moderately low, neither are there clearly distinctive species that are well represented in all level. Each level has a variety of species most-contributing to the site as a whole, yet they are mostly unique from each another. No riparian species made the rankings in any of the level.

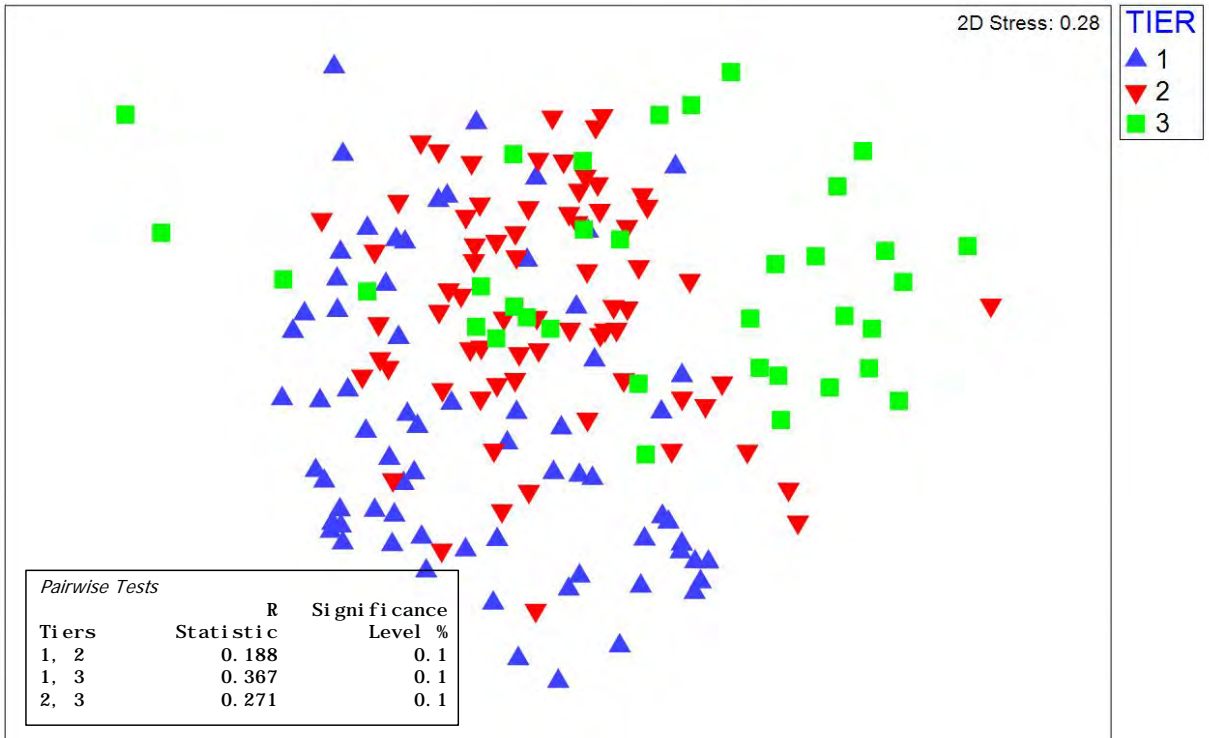


Figure 14. An nMDS analysis of Colorado Bend levels community differences. Inset box shows the ANOSIM statistic for level differences; P=.1%.

Between seasons, there are variations between many of the plots, yet there is still considerable overlap in the community assemblages (Figure 15). The SIMPER tests (Appendix D, Table 2) for similarity show that just as in the overall community, seaots are major contributors to the homogeneity between the seasons, even though their counts were considerably lower in fall. Cedar elm, the highly abundant woody species, also contributed to the similarity between seasons, again even with the large differences in counts between them. A comparison of the dissimilarities between the seasons (Appendix D, Table 3) also ranks seaots as the largest contributor. This fact underscores how differences in abundances due to sampling timing can create large heterogeneity in the community assemblages that do not necessarily reflect environmental influences. In other words, when attempting to distinguish one community assemblage from another, variation in sampling periods between sites could potentially create transient (temporal) differences that mask actual (spatial) differences.

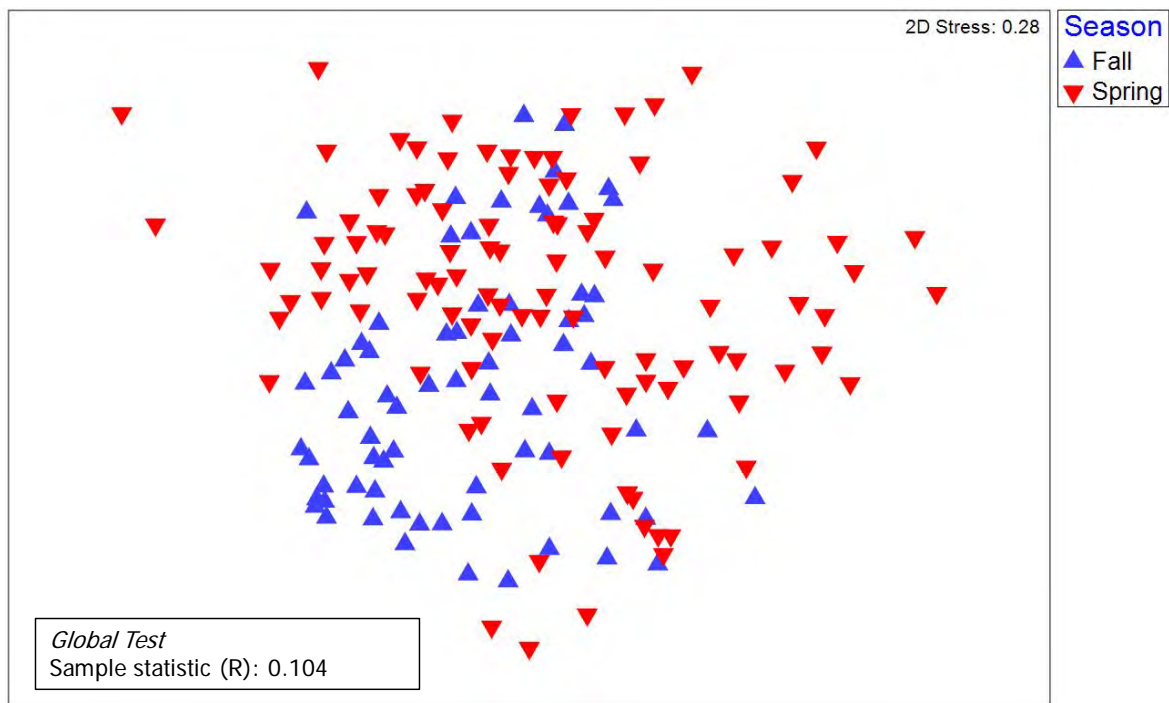


Figure 15. An nMDS analysis of Colorado Bend levels seasonal community differences. Inset box shows the ANOSIM Global test statistic.

When grouped by WI classes (Figure 16), the progression of heterogeneity from Level 1 to Level 3 becomes less distinguishable, which is supported by the low ANOSIM values. This is explained by the loss of OBL species beyond Level 1 and an increase in UPL species in Level 2 and 3 (Appendix D, Table 4).

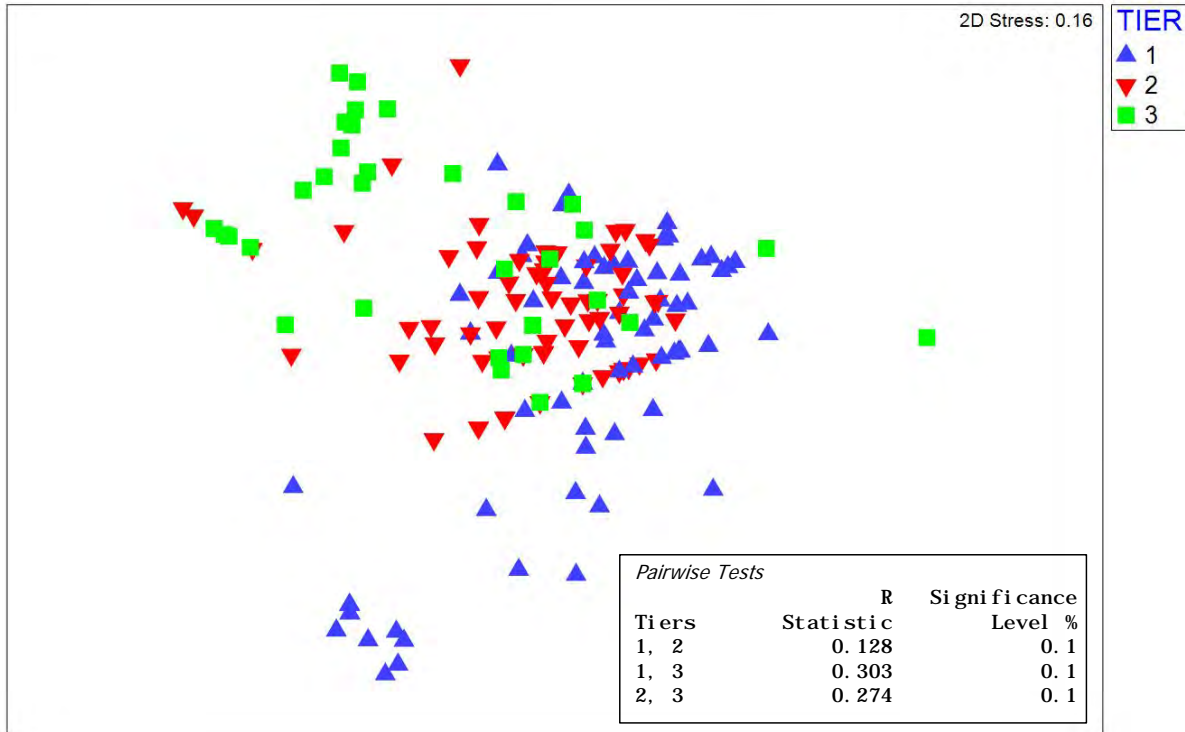


Figure 16. An nMDS analysis of Colorado Bend levels wetland indicator (WI) class plots. Inset box shows the ANOSIM statistic for level differences; $p=0.1\%$.

When limiting the data to the mature-trees dataset (Figure 17), only Level 1 and 2 (which did show variation) had canopy trees in the randomly selected plots (Level 3 randomly-selected plots lacked woody vegetation). Additionally, the sample sizes for Level 1 and 2 were so low that beyond the nMDS ordination no significant ANOSIM statistics could be generated. This reflects a limitation to using small numbers of randomly pre-selected plots to sample canopy vegetation – there is the risk that no canopy trees will be present in those plots. In order to investigate the dissimilarity seen in Figure 17, a SIMPER test was run (Appendix D, Table 5). Cedar elm topped the list of contributors to dissimilarity because of its higher abundance in Level 2. Ashe juniper had higher abundance in Level 1, and the riparian species green ash was completely missing in Level 2. Bumelia and red oak were ranked in Level 2 but missing in Level 1, and dogwood was ranked in Level 1 but missing from Level 2. Clearly, this site had a mixture ranging from OBL to UPL species all co-existing within the site.

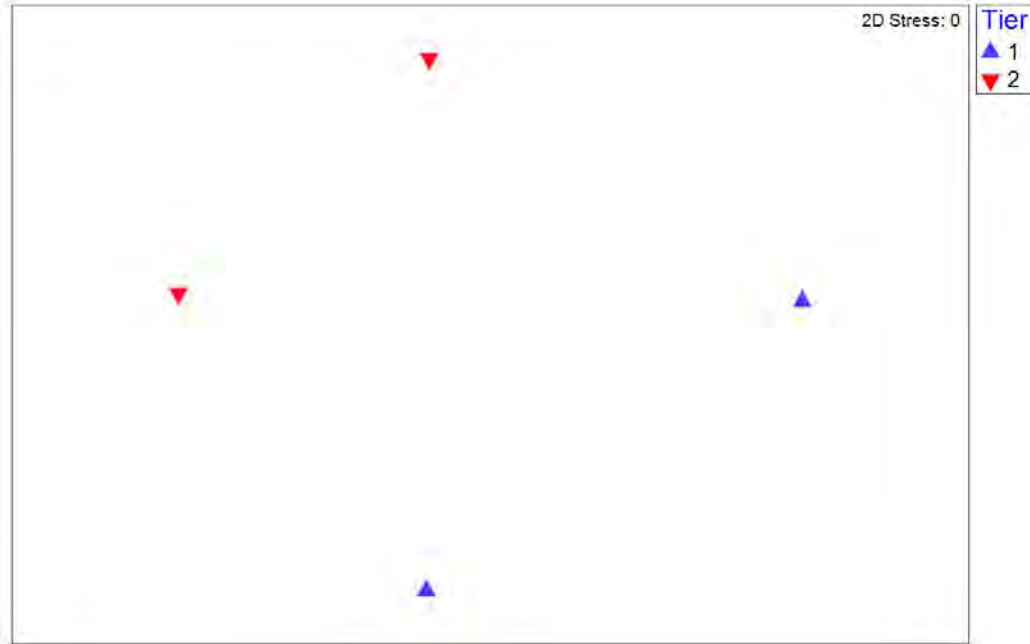


Figure 17. An nMDS analysis of Colorado Bend levels mature tree differences.

Analyses of the estimated stream discharge levels to inundate this site are shown in Table 10. The discharge estimated to inundate all of Level 1 is approximately 20,000 cfs. Level 2 inundation would require approximately 45,000 cfs and Level 3 inundation is beyond the rating curve. Table 11 shows that the spring small and large season TCEQ flow standards flows and the annual pulse inundate portions of Riparian level 1 and 2.

Table 10. Stream discharge estimated to inundate Colorado Basin’s Riparian site level based on US Geological Survey (USGS) gage rating curves.

Riparian Site	Strata	Estimated Inundation Flow Rate (cfs)
Colorado Bend State Park	1	20,000
	2	45,000
	3	Off the rating curve
Onion Creek	1	5,000
	2	6,000
	3	6,500

Table 11. Texas Commission on Environmental Quality (TCEQ) flow standards for selected sites in the Colorado Basin. Taken from: TCEQ 2014.

Gauge Location	Study Site	Season / Time Period	Subsistence (cfs)	Hydrologic Condition	Base (cfs)	Small Season Pulse (cfs)	Large Season Pulse (cfs)	Annual Pulse (cfs)
San Saba	Colorado Bend	Winter	50	Severe	95	520	1600	18,900
		Winter		Dry	95	520	1600	18,900
		Winter		Avg	150	520	1600	18,900
		Winter		Wet	210	520	1600	18,900
		Spring	50	Severe	120	5800	11000	18,900
		Spring		Dry	120	5800	11000	18,900
		Spring		Avg	190	5800	11000	18,900
		Spring		Wet	360	5800	11000	18,900
		Summer	30	Severe	72	510	1400	18,900
		Summer		Dry	72	510	1400	18,900
		Summer		Avg	120	510	1400	18,900
		Summer		Wet	210	510	1400	18,900
		Fall	30	Severe	95	890	3800	18,900
		Fall		Dry	95	890	3800	18,900
		Fall		Avg	150	890	3800	18,900
Driftwood	Onion Creek	Winter	1	Severe	2	N/A	170	1,200
		Winter		Dry	2	N/A	170	1,200
		Winter		Avg	6	N/A	170	1,200
		Winter		Wet	26	N/A	170	1,200
		Spring	1	Severe	4	200	620	1,200
		Spring		Dry	4	200	620	1,200
		Spring		Avg	12	200	620	1,200
		Spring		Wet	34	200	620	1,200
		Summer	1	Severe	1	N/A	N/A	1,200
		Summer		Dry	1	N/A	N/A	1,200
		Summer		Avg	3	N/A	N/A	1,200
		Summer		Wet	7	N/A	N/A	1,200
		Fall	1	Severe	1	18	120	1,200
		Fall		Dry	1	18	120	1,200
		Fall		Avg	3	18	120	1,200
Fall	Wet	7		18	120	1,200		

3.2.2 Onion Creek

Data at this location were collected in November 2016 as a fall sampling event and April 2017 as a spring sampling event. Level 1 was the most topographically diverse level (Figure 18). It consisted of a bank crest, steeply sloping bank, and very narrow fringe bank located immediately adjacent to the water’s edge. This well-shaded fringe bank consisted mostly of wetland adapted vegetation including bald cypress, sycamore, and buttonbush. These species were exclusively limited to the narrow fringe and did not extend up-slope. Level 1’s slope was dominated by mesic yet shade-adapted species such as Texas aster and Lyre leaf salvia, while the crest of the bank began to give away to more upland species such as Ashe juniper and yaupon mixed with various grasses and forbs.

Level 2 consisted mostly of flat topography with little to no slope and was dominated by shrubs such as hoptree, American beautyberry and red buckeye intermixed with grasses and forbs. While Level 2 was located well above the stream terrace, there were obvious signs of high water reaching this level. Multiple woody debris piles were present well into Level 2.

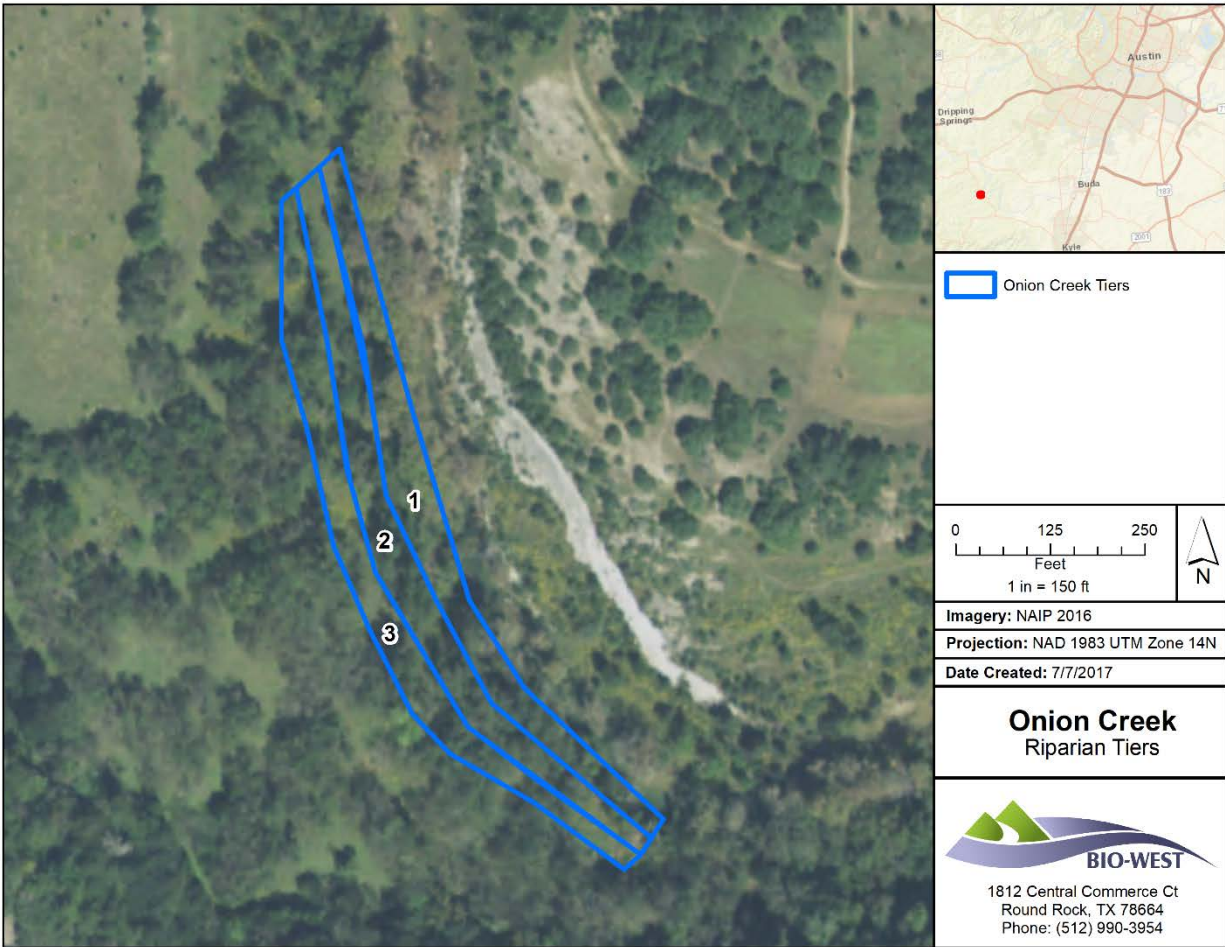


Figure 18. Overview of Onion Creek Site showing the three level boundaries (in blue).

Level 3 again consisted mostly of flat topography with little to no slope and a few side depressions. This level was mostly open with few understory shrubs. Pecan was the dominant woody species with short grasses and forbs forming the ground cover. This level was most- obviously impacted by grazing, although cattle had open access throughout the study area.

A representative profile (Figure 19) shows the slope from river’s edge to the uppermost extent has an overall site steepness factor of 0.03 (Table 8). Table 12 shows fall 2016 and spring 2017 community abundances and mature tree abundances, respectively. In both fall and spring, seaots were the dominant species, though their abundance decreased in spring. In fall, sycamore, box elder, and green ash were less than 1% (collectively) of the community. In spring they were still only 0.4%. Pecan dominated the canopy at 38%, followed by Ashe juniper and bald cypress, which were each at 17%. The bald cypress canopy contribution underscores how a riparian species can be an important component in the community, yet when overall community assemblages are sampled, its abundance is a fraction of 1%. The UPL species (17%) are just as abundant as the FACW species (17%) in this site underscoring the mixed community observed in the field.

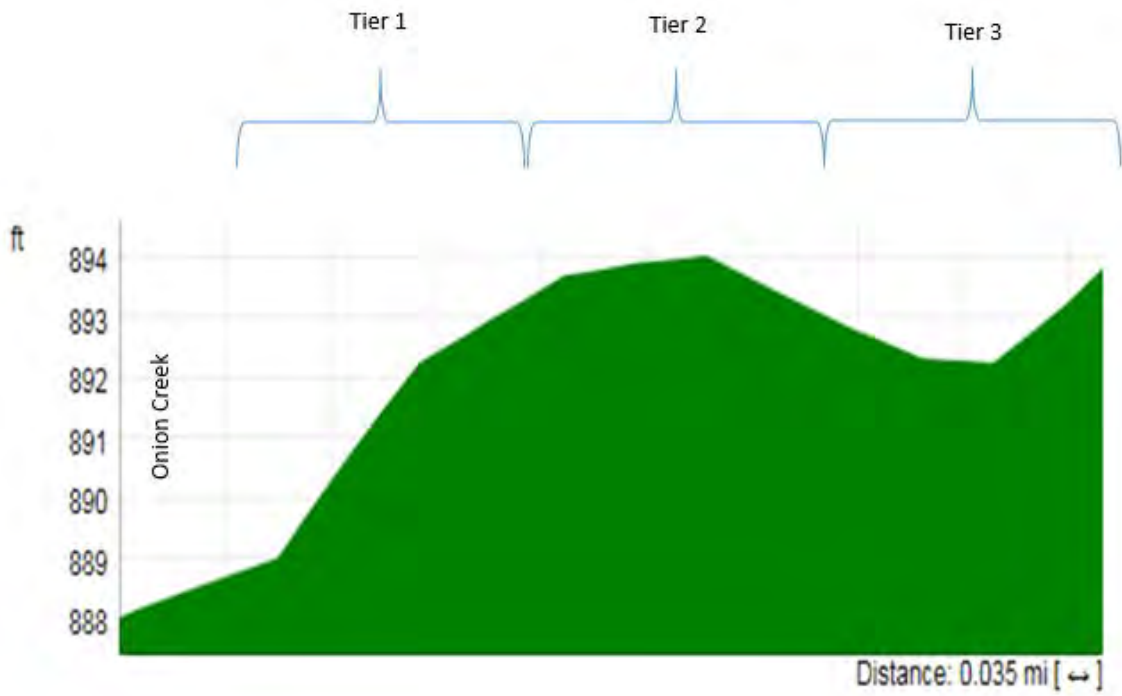


Figure 19. Onion Creek Site profile showing general level locations.

Table 12. Onion Creek community abundances by season, and mature tree abundances.

Fall Plots		Spring Plots		Mature Trees	
Species	% of Total	Species	% of Total	Species	% of Total
Inland seaoats	44.2	Inland seaoats	24.7	Pecan	37.9
Cedar elm	11.2	Frostweed	14.3	Ashe juniper	17.2
Hackberry	7.9	Goldeneye daisy	12.4	Bald cypress	17.2
Frostweed	5.2	Hackberry	9.1	Hackberry	10.3
Plains bristlegrass	4.6	Cedar elm	8.5	Sycamore	5.2
Horse briar	3.5	Emory sedge	4.0	Texas persimmon	5.2
Goldeneye daisy	3.0	Horse briar	3.2	Red mulberry	3.4
Yaupon holly	2.3	American beautyberry	3.0	American elm	1.7
Maxamillion sunflower	2.3	Wafer ash	2.6	Red buckeye	1.7
American beautyberry	2.1	Maxamillion sunflower	2.1	N=58	
Wafer ash	1.5	Stickywilly	1.8		
Turkscap	1.0	Texas persimmon	1.6	FAC	50.0
Switchgrass	0.9	Common nettle	1.5	UPL	17.2
Ashe Juniper	0.8	Texas aster	1.3	FACU	15.5
Gum bumelia	0.8	Yaupon holly	1.3	FACW	17.2
Emory sedge	0.8	Gum bumelia	1.0	OBL	0.0
Wood fern	0.8	Buttonbush	0.9	Invasive	0.0
Pecan	0.8	Red buckeye	0.8		
Gamma grass	0.8	Soapberry	0.6		
Buttonbush	0.6	Switchgrass	0.6		
Texas persimmon	0.5	Pecan	0.5		
Red buckeye	0.4	Brook weed	0.5		
Sycamore	0.4	Gamma grass	0.4		
Brook weed	0.4	Wood fern	0.4		
American elm	0.4	Goldencrown grass	0.3		
Red mulberry	0.4	Ashe Juniper	0.2		
Box elder	0.3	Red mulberry	0.2		
Dewberry	0.3	Rosinweed	0.2		
Wood violets	0.3	Bald cypress	0.2		
Bald cypress	0.2	Green ash	0.2		
Bluemist flower	0.2	Live Oak	0.2		
Poison ivy	0.2	Sycamore	0.2		
Roughleaf dogwood	0.2	Frogfruit	0.2		
Green ash	0.1	Carolina ponyfoot	0.2		
Virginia creeper	0.1	Swamp sweetscent	0.2		
Agarita	0.1	White boneset	0.2		
Chinaberry	0.1	American elm	0.1		
Elbow bush	0.1	Texas aster	0.1		
Soapberry	0.1	Black walnut	0.1		
Texas mulberry	0.1	Elbow bush	0.1		
Bull nettle	0.1	Roughleaf dogwood	0.1		
Indian grass	0.1	Horseherb	0.1		
N=1814		Turkscap	0.1		
		N=1642			

An nMDS ordination plot of Onion Creek's level shows that, because of large variation among each level, little heterogeneity between them can be statistically represented. This is supported by the ANOSIM R values (Figure 20). This within-level variation is seen particularly in Level 1, which has a spread that encompasses the variability of the other two level. Table 6 in Appendix D shows the major contributors to dissimilarity. It is apparent that, with the only species not present in all level being plains bristle grass, this site has much homogeneity throughout all level.

Figure 21 plots the dissimilarity in the community between seasons. There are distinctions between the two, but with a low ANOSIM R statistics (see figure) of 0.29, these differences are statistically low. In other words, as is displayed in Appendix D, Table 7, the majority of the change that is occurring in this site is shifting abundances of the same groups of species.

Grouping species by WI classes does little to refine community assemblage differences because of the extreme variation across all level (Figure 22). An examination of the dissimilarity (Appendix D, Table 8) shows FACU and FAC dispersed throughout the zone. As was noted in the abundance tables, these species are pervasive in this site and generally outnumber the riparian species. FACW and OBL species' counts were so low that they are completely absent from similarity (not shown) and dissimilarity rankings at this site.

There are apparent differences among the mature trees (Figure 23), but the sample size was too small to produce significant ANOSIM results. Level 1 has a mixture of bald cypress, sycamore, Ashe juniper, and pecan; Level 2 contains Ashe juniper, pecan, Texas persimmon, and red buckeye; Level 3 contains only Texas persimmon abundantly enough to contribute in rankings (Appendix D, Table 9).

Overall community assemblages at this site showed much overlap between level and significant inhabitation in all level by non-riparian-associated species. Because the mature tree sampling's woody riparian classes were so sparsely represented, this would indicate that the herbaceous/understory assemblages are so diverse and abundant that woody riparian species' contribution cannot be discerned within the larger community. The limitation of randomly selected species also made characterization of the riparian community difficult.

Analyses of the stream discharge approximations for this site are shown in Table 10. The discharge estimated to inundate all of Level 1 is approximately 5,000 cfs. Level 2 inundation would require approximately 6,000 cfs and Level 3 requires approximately 6,500 cfs. Table 11 shows that none of the TCEQ flow pulses would inundate large portions of this riparian zone.

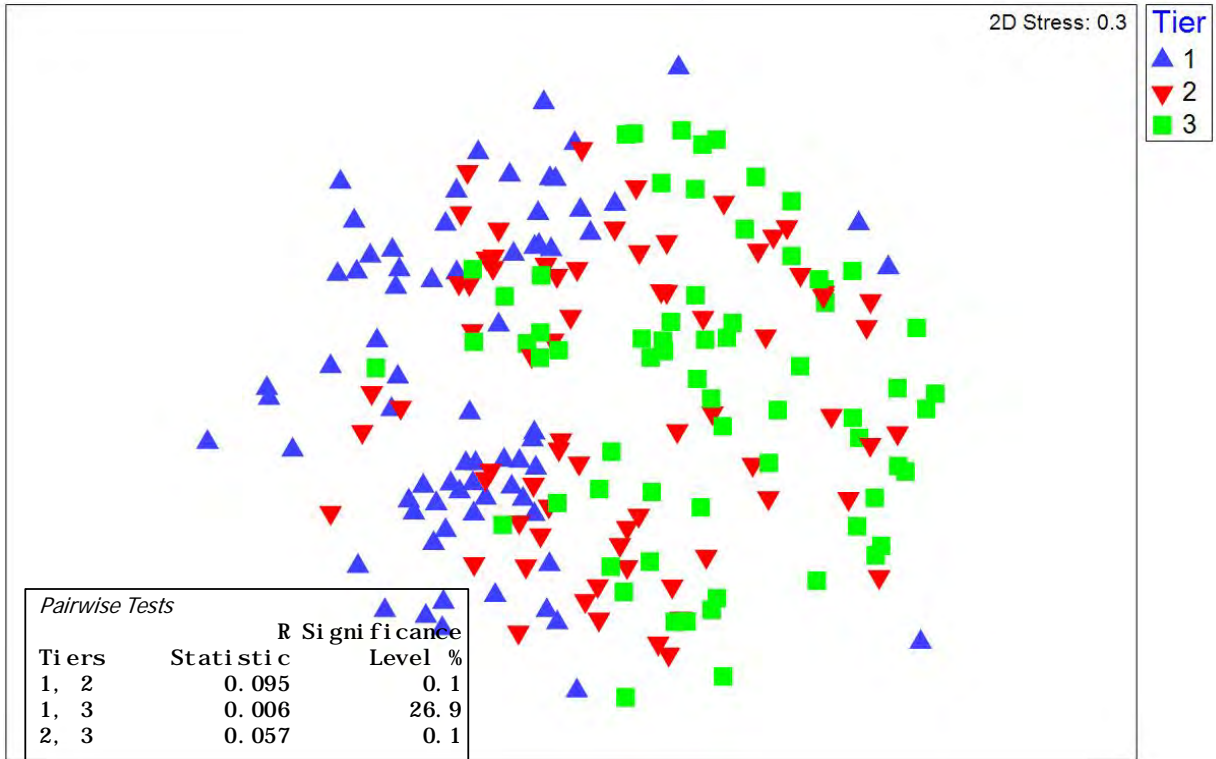


Figure 20. An nMDS analysis of Onion Creek levels community differences. Inset box shows the ANOSIM results; p=.1%.

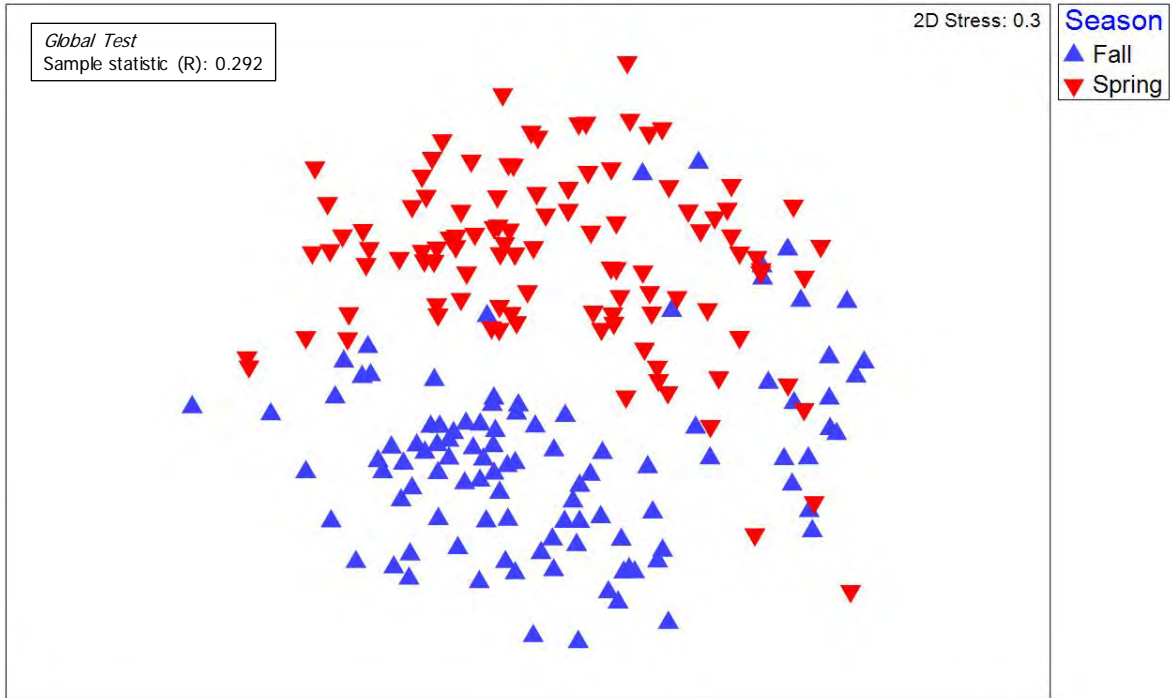


Figure 21. An nMDS analysis of Onion Creek levels seasonal community differences. Inset box shows the ANOSIM results; $p=0.1\%$.

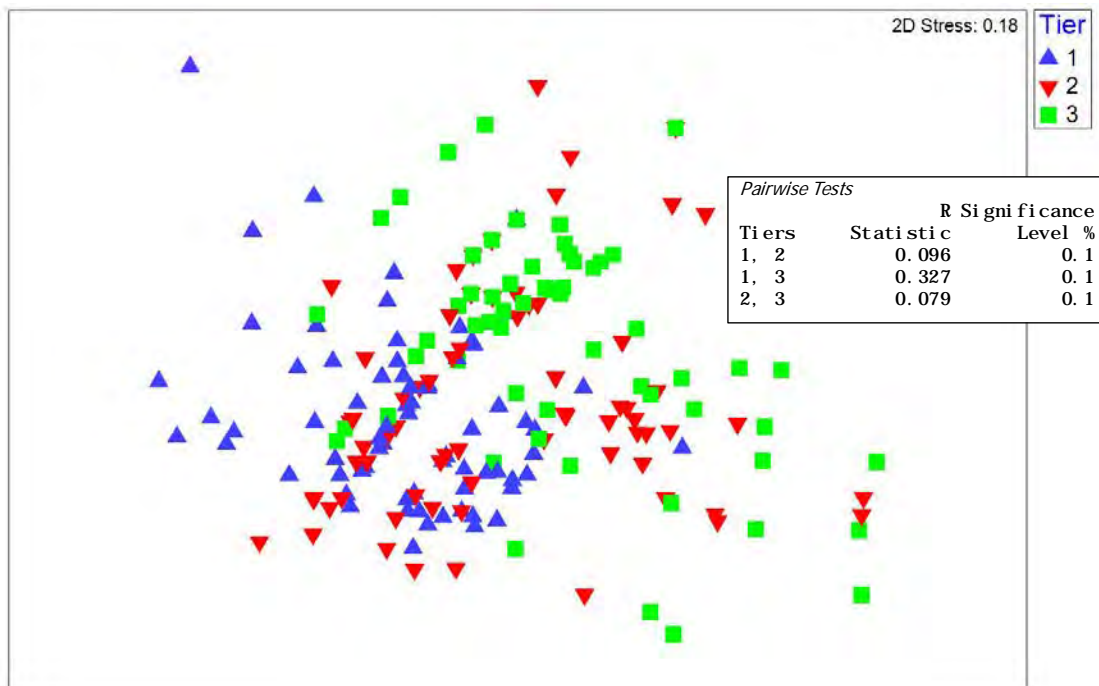


Figure 22. An nMDS analysis of Onion Creek levels wetland indicator (WI) class differences. Inset box shows the ANOSIM results; $p=0.1\%$.

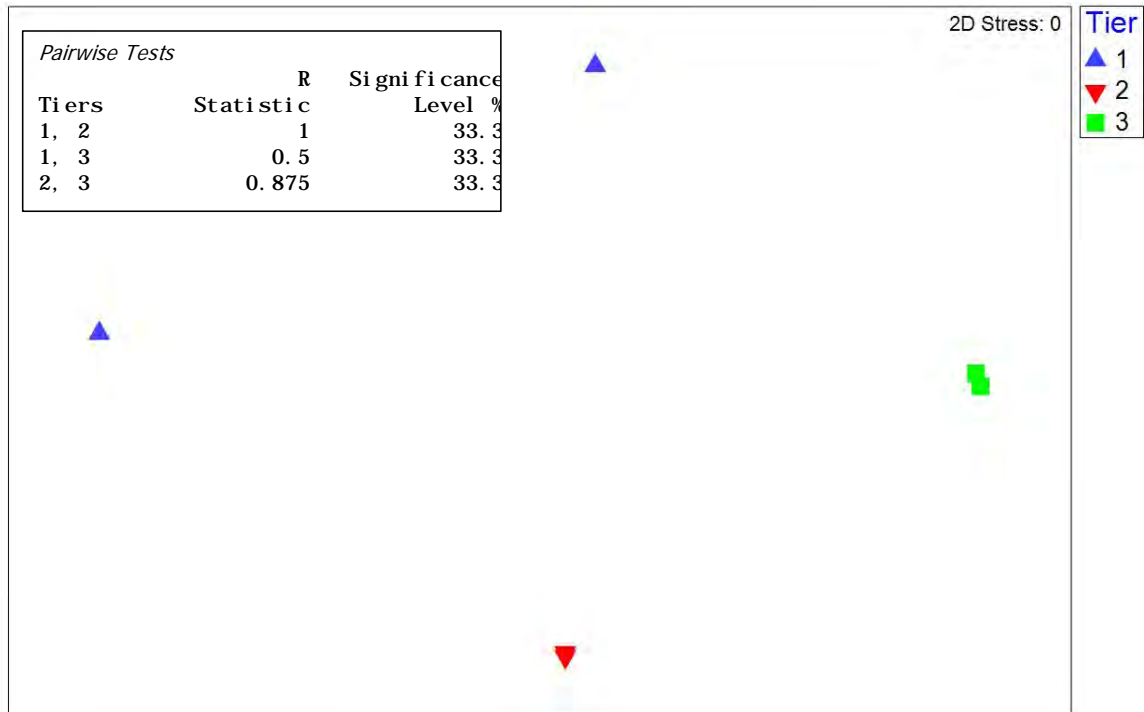


Figure 23. An nMDS analysis of Onion Creek levels mature tree differences.

3.2.3 Navidad River

Data at this location were collected in December of 2016 and May of 2017. The site topography was relatively flat, with only one steep slope along the river’s edge located in Level 1 (Figure 24). This vertical bank rose generally 3-4 meters from water’s edge to the floodplain. Level 1 had a thinner canopy cover with box elder, cedar elm, and green ash dominating. Inland seaots were the dominant herbaceous species along with various sedges. Level 2 was dominated by dense canopy cover such as box elder and green ash, and brushy species including yaupon, trifoliolate orange, and soapberry; herbaceous vegetation was limited. Level 3 was characterized by a much taller canopy containing larger, more mature trees. The open areas were dominated by herbaceous vegetation. Seasonal wetland depressions were common in Level 3 and provided habitat for mesic and wetland plant species, including obedient plant, creeping burrhead, and crowfoot sedge. Box elder and green ash were common in this level as well.

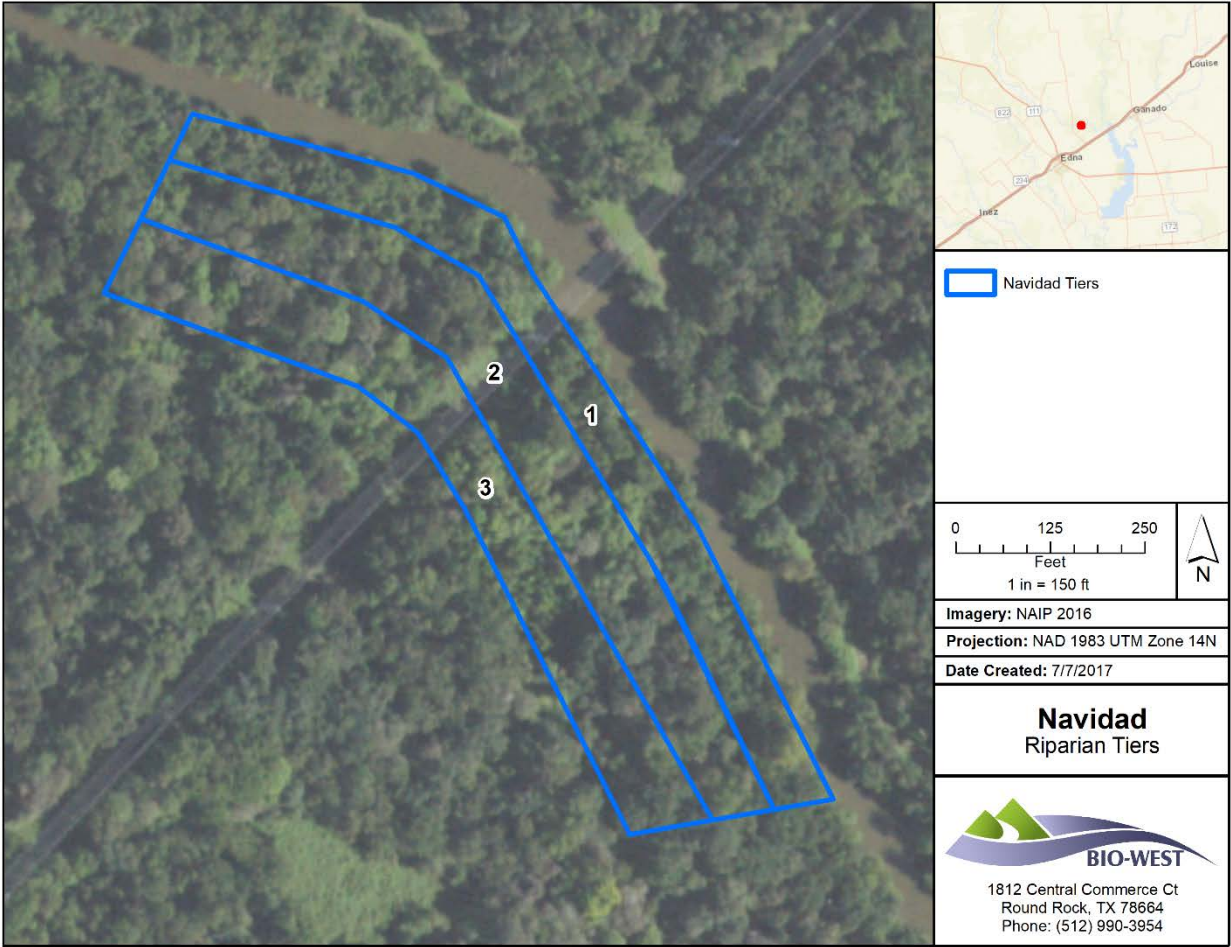


Figure 24. Overview of Navidad Site showing the three level boundaries (in blue).

A representative profile (Figure 25) shows the slope from river's edge to the uppermost extent has an overall site steepness factor of 0.01 (see Table 8). Table 13 shows spring 2017 community abundances and mature tree abundances. Fall 2017 samples were taken, but because of a corruption of the dataset they were excluded from analysis. In this community assemblage, more than 4,600 individuals were counted. Cedar elm dominates both the overall community assemblage and the canopy with abundances of 37% and 27%, respectively. The two most herbaceous species are Carolina sedge and inland sea oats. In the overall community sample, green ash comprises 0.5% and is the only riparian woody species represented. In the mature trees, box elder made up 12% of the community, and green ash are over 5%. FAC, FACU and UPL woody species strongly dominated with a combined abundance of ~79%. FACW were present though, and accounted for 17% of the canopy.

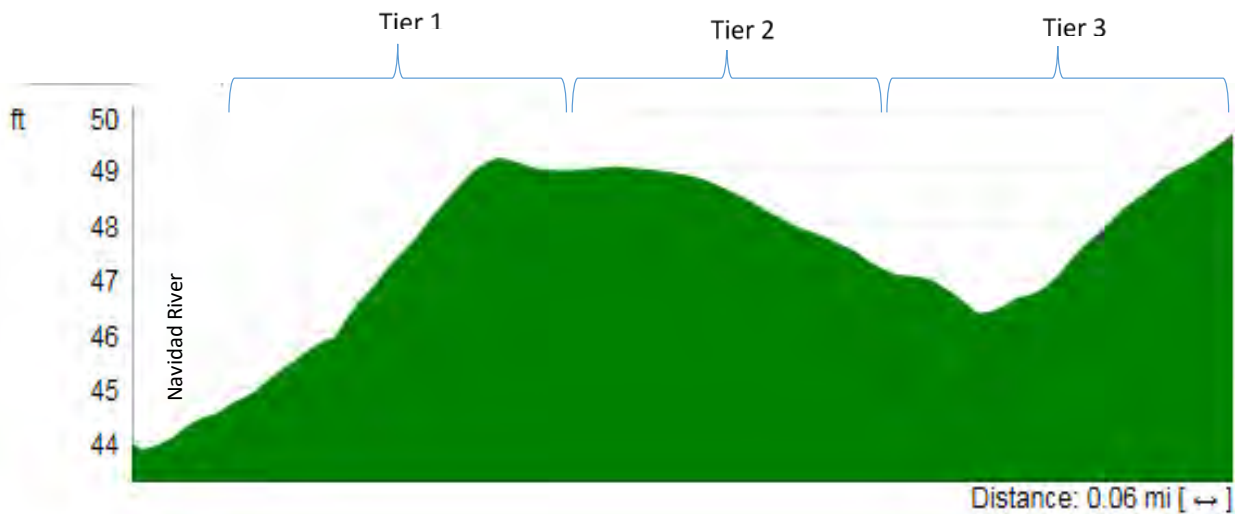


Figure 25. Navidad Site profile showing general level locations.

Table 13. Navidad community abundances and mature tree abundances.

Spring Plots		Mature Trees	
Species	% of Total	Species	% of Total
Cedar elm	37.0	Cedar elm	26.6
Carolina sedge	19.8	Yaupon holly	17.0
Inland seaoats	12.6	Box elder	11.7
Barnyard grass	7.1	Hackberry	9.6
Box elder	3.6	American elm	7.4
Virginia creeper	2.8	Pecan	7.4
Trifoliolate orange	2.1	Green ash	5.3
Trumpet creeper	1.4	Anacua	4.3
Yaupon holly	1.4	Water oak	3.2
Poison ivy	1.4	Hercules club	2.1
Turkscap	1.2	Water hickory	2.1
Hackberry	1.0	Chinaberry	1.1
Frostweed	1.0	Chinese tallow	1.1
Crowsfoot sedge	1.0	Dogwood	1.1
Soapberry	0.8		
Wild onion	0.7	N=94	
Green ash	0.5	FAC	62.8
Horse briar	0.4	UPL	4.3
Obedient plant	0.4	FACU	11.7
Slippery elm	0.3	FACW	17.0
Pepper vine	0.3	OBL	2.1
Pecan	0.3	Invasive	2.1
Dewberry	0.3		
Virginia wildrye	0.3		
Stickywilly	0.3		
Chinese tallow	0.2		
Roughleaf dogwood	0.2		
Rosette grass	0.2		
Live Oak	0.2		
Wild rose	0.2		
Pepper vine	0.1		
Anacua	0.1		
Alabama supplejack	0.1		
Yellow woodsorrel	0.1		
Coralberry	0.1		
Gum bumelia	0.1		
Southern red oak	0.1		
Water oak	0.1		
Creeping burrhead	0.1		
Goldenrod	0.1		

N=4695

An nMDS ordination plot and ANOSIM test (Figure 26) of Navidad's level show that though a small separation exists between Level 1 and Level 2 and 3, there is little difference between Level 2 and 3. Table 10 in Appendix D shows the major contributors to dissimilarity, where it becomes apparent that, even though there are differences between Level 1 and the other level, other than barnyard grass, the same species are present through all three, just in different abundances. The biggest contributors to dissimilarity are seaoats (highly abundant in Level 1) and cedar elm (more abundant in Level 2). The nine contributing species in Level 2 and 3 are present in both, and shifts in their abundances are the explanation for the (very small) differences that did exist.

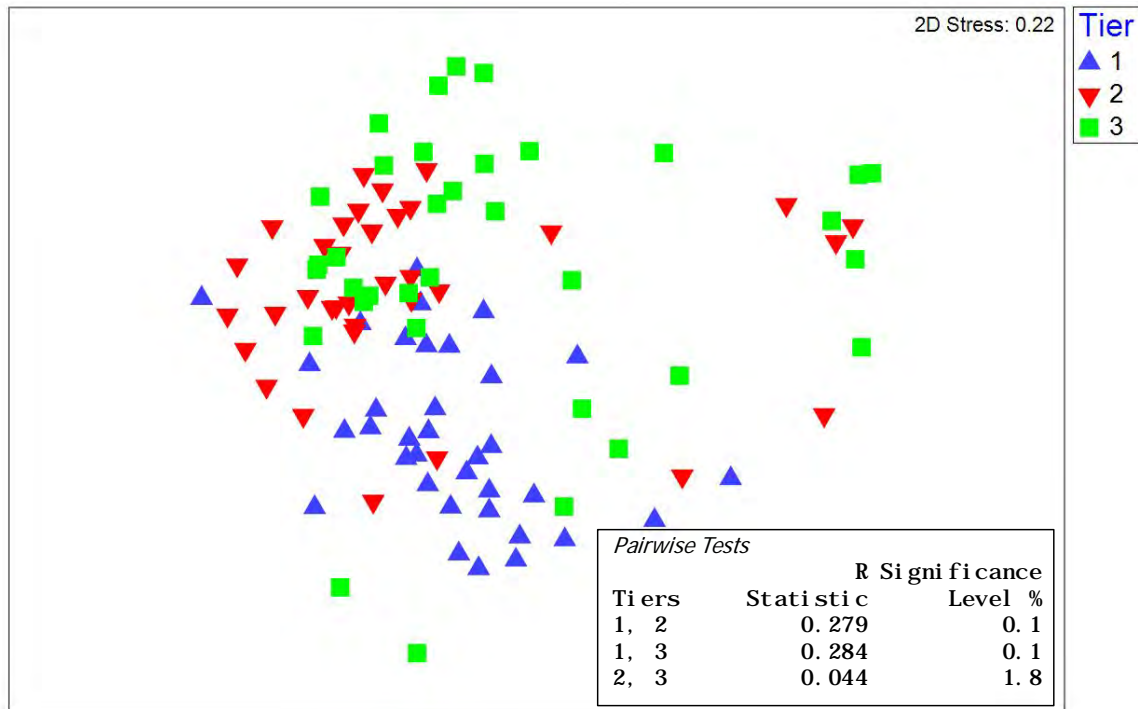


Figure 26. An nMDS analysis of Navidad levels community differences. Inset box shows the ANOSIM results; p=.1%.

Grouping species by WI classes does little to refine community assemblage differences because of the extreme variation within all level (Figure 27). An examination of that dissimilarity (Appendix D, Table 11) shows OBL, FACW, and FAC species are dispersed throughout the zone. Because OBL species' abundances were still seen and even increased in Level 3, this calls into question whether the level boundaries captured the full extent of these species' distribution.

There are apparent differences among the mature trees within the three level (Figure 28), but the sample size was too low to produce significant ANOSIM results. The greatest contributor to differences between Level 1 and the other two level is box elder (Appendix D, Table 12), which was present only in Level 1. Green ash and pecan were present as well in Level 1, and though missing in Level 2, they were found again in Level 3. The wetland depression seen in the site profile may account for this spatial distribution.

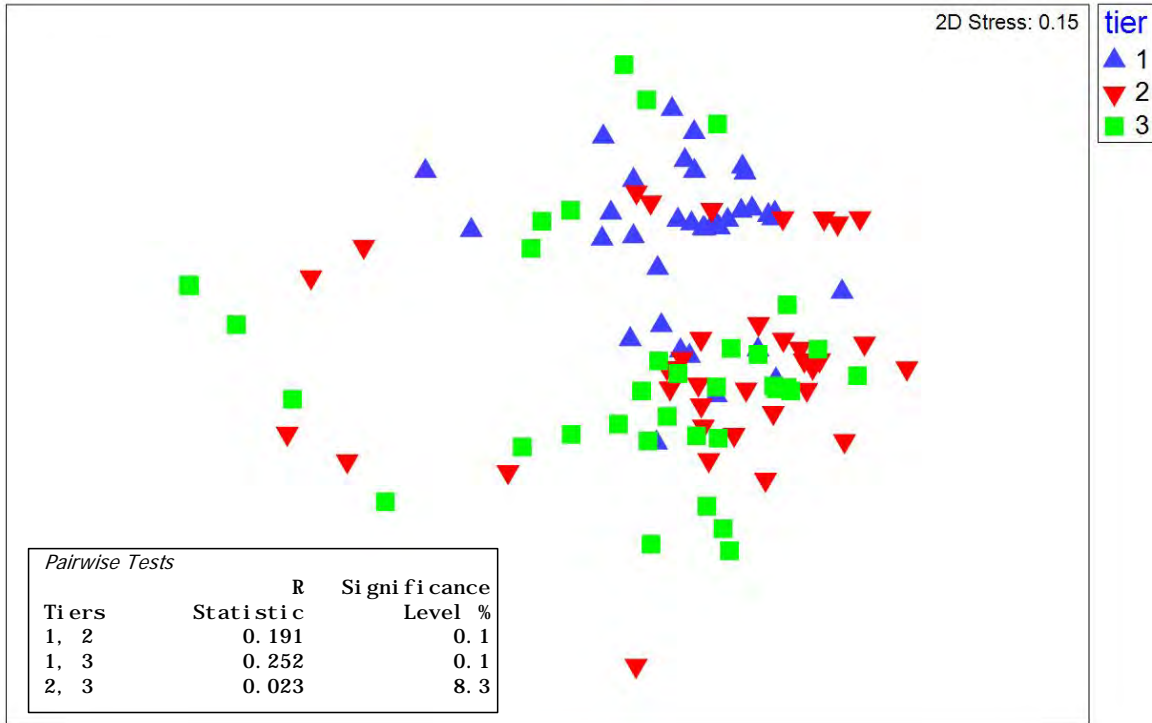


Figure 27. An nMDS analysis of Navidad levels wetland indicator (WI) class differences. Inset box shows the ANOSIM results; p=.1%.

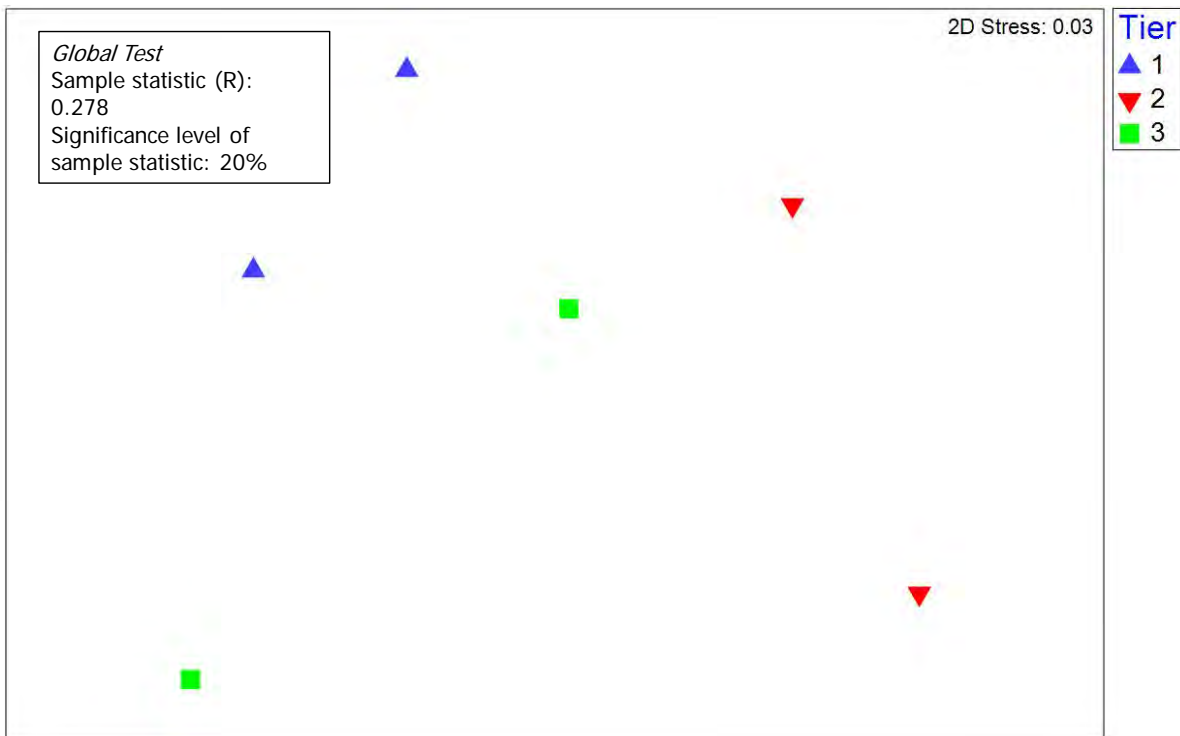


Figure 28. An nMDS analysis of Navidad levels mature tree differences. Inset box shows the ANOSIM results; p=.1%.

Overall community assemblages at this site showed much overlap between level and significant inhabitation in all level by both riparian and non-riparian-associated species. Because of the limited canopy tree sampling counts in the randomly selected plots, characterization of the riparian community was not statistically feasible.

Analyses of the stream discharge estimates for this site are shown in Table 14. The discharge estimated to inundate all of Navidad Level 1 is approximately 50 cfs (Table 14). Levels 2 and 3 inundation would require approximately 1,000 cfs. Table 15 shows that all small and large season pulses (except summer), and the annual pulse will inundate 100% of the riparian distribution.

Table 14. Stream discharge estimated to inundate Lavaca Basin’s Riparian site level based on US Geological Survey (USGS) gage rating curves.

Riparian Site	Strata	Estimated Inundation Flow Rate (cfs)
Navidad	1	50
	2	1,000
	3	1,000
Sandy Creek	1	10,000
	2	11,000
	3	4,000

Table 15. Texas Commission on Environmental Quality (TCEQ) flow standards for selected sites in the Lavaca Basin. Taken from: TCEQ 2014.

Gauge Location	Study Site	Season / Time Period	Subsistence (cfs)	Hydrologic Condition	Base (cfs)	Small Season Pulse (cfs)	Large Season Pulse (cfs)	Annual Pulse (cfs)
Edna	Navidad	Winter	1	Severe	14	2,000	2,500	2,500
		Winter		Dry	14	2,000	2,500	2,500
		Winter		Avg	35	2,000	2,500	2,500
		Winter		Wet	71	2,000	2,500	2,500
		Spring	2.8	Severe	18	2,500	2,500	2,500
		Spring		Dry	18	2,500	2,500	2,500
		Spring		Avg	35	2,500	2,500	2,500
		Spring		Wet	71	2,500	2,500	2,500
		Summer	1.2	Severe	24	200	610	2,500
		Summer		Dry	24	200	610	2,500
		Summer		Avg	47	200	610	2,500
		Summer		Wet	84	200	610	2,500
		Fall	2.2	Severe	17	2,000	2,500	2,500
		Fall		Dry	17	2,000	2,500	2,500
		Fall		Avg	35	2,000	2,500	2,500
		Fall		Wet	71	2,000	2,500	2,500
Ganado	Sandy Creek	Winter	1	Severe	5	800	1,800	2,200
		Winter		Dry	5	800	1,800	2,200
		Winter		Avg	14	800	1,800	2,200
		Winter		Wet	30	800	1,800	2,200
		Spring	1	Severe	5	1,400	2,200	2,200
		Spring		Dry	5	1,400	2,200	2,200
		Spring		Avg	14	1,400	2,200	2,200
		Spring		Wet	30	1,400	2,200	2,200
		Summer	1	Severe	9	91	260	2,200
		Summer		Dry	9	91	260	2,200
		Summer		Avg	21	91	260	2,200
		Summer		Wet	39	91	260	2,200
		Fall	1	Severe	9	630	1,800	2,200
		Fall		Dry	9	630	1,800	2,200
		Fall		Avg	21	630	1,800	2,200
		Fall		Wet	39	630	1,800	2,200

3.2.4 Sandy Creek

Data at this location were collected in November 2016 (as a fall sampling event) and May 2017 (as a spring sampling event). Level 1 (Figure 29), was dominated by Kuy sands and showed the greatest topographical relief. A narrow shelf along the stream edge was dominated by mesic and aquatic species such as sedges and grasses along with green ash, buttonbush, and box elder. Well above the creek bed, this narrow band gave way to a steep sandy berm, which was dominated by woody upland species such as cherry laurel, yaupon, and American beautyberry, and these were intermixed with woody vines, including greenbriar and grape. Few understory forbs or grasses occurred as canopy cover was very thick.



Figure 29. Overview of Sandy Creek Site showing the three level boundaries (in blue).

Level 2 was located on the downslope of the sand berm, where the vegetation community switched to more open canopy with an increase in understory forbs and grasses. While cherry laurel was still a common woody species, forbs such as inland seaots, frostweed, and turkscap were more common. This change in vegetation may be attributed to a switch from Kuy sands to Navidad sandy loam, as well as a maintained right-of-way for access by the property owners, which increased light penetration.

Level 3 was dominated by lower-lying topography evident by broad side channels and pooled standing water, which provided wetland habitat for mesic loving species. Due to the poor drainage and obvious signs of inundation, Navidad sandy loam soils were most likely the dominant soil type. Dominant woody vegetation here consisted of hackberry and cedar elm with water hickory and water oak in the lower lying wetted areas. Forbs included inland seaots, turkscap and frostweed along with various unidentified grasses and sedges.

A representative profile (Figure 30) shows the slope from river's edge to the uppermost extent has an overall site steepness factor of 0.01, the lowest slope of all sites sampled (Table 8), and driven in part because of the large slump in the site. Table 16 shows fall 2016 and spring 2017 community abundances and mature tree abundances, respectively. Between fall 2016 and spring

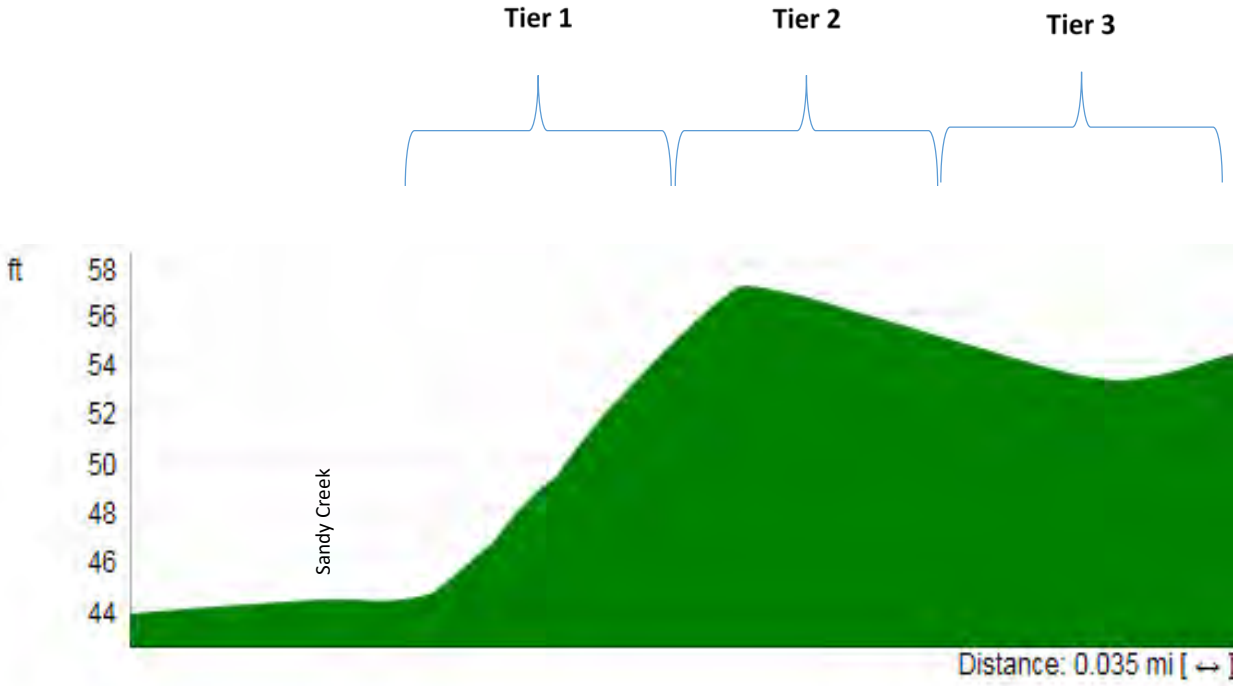


Figure 30. Sandy Creek Site profile showing general level locations.

Table 16. Sandy Creek community abundances by season, and mature tree abundances

Fall Plots		Spring Plots		Mature Trees	
Species	% of Total	Species	% of Total	Species	% of Total
Cherry laurel	46.4	Cedar elm	19.6	Yaupon holly	23.31
Inland seaoats	17.0	Cherry laurel	19.2	Cherry laurel	21.80
Yaupon holly	13.6	Carolina sedge	16.9	Cedar elm	11.28
Soapberry	4.0	Inland seaoats	14.9	Water oak	9.02
Horse briar	3.8	Hellers rosettegrass	6.3	Chinaberry	6.77
Frostweed	2.7	Wood sorel	5.2	Sycamore	6.77
Turkscap	2.5	Yaupon holly	2.3	Water hickory	6.77
Yellow woodsorrel	1.7	Green ash	1.7	American elm	5.26
Water pepper	1.5	Southern red oak	1.6	Hackberry	2.26
Water oak	0.9	Turkscap	1.5	Box elder	1.50
Green ash	0.8	Frostweed	1.5	Slash pine	1.50
Beautyberry	0.6	Bee balm	1.0	Shumard red oak	1.50
Black willow	0.6	Virginia creeper	0.8	Swamp oak	1.50
Buttonbush	0.6	Soapberry	0.8	Green ash	0.75
Chinaberry	0.6	Horse briar	0.7		
American elm	0.4	Pepper vine	0.6	N=33	
Southern red oak	0.4	Poison ivy	0.5	FAC	57.14
Slippery elm	0.4	Hackberry	0.5	UPL	0.00
Sycamore	0.4	Smartweed	0.5	FACU	27.07
Pepper vine	0.4	Bermuda grass	0.3	FACW	2.26
Box elder	0.2	Oneflower flatsedge	0.3	OBL	6.77
Dwarf palmetto	0.2	Box elder	0.3	Invasive	6.77
Slash pine	0.2	Americian beautyberry	0.3		
Water hickory	0.2	Chinese tallow	0.3		
Dewberry	0.2	Pecan	0.3		
N=528		Canadian germander	0.2		
		Dewberry	0.2		
		Snailseed	0.2		
		Slippery elm	0.1		
		Water oak	0.1		
		Muscadine grape	0.1		
		American elm	0.1		
		Buttonbush	0.1		
		Roughleaf dogwood	0.1		
		Purpleleatherflower	0.1		
		Wildrose	0.1		
		Rice cutgrass	0.1		
		Black willow	0.1		
		Dwarf palmetto	0.1		
		Sycamore	0.1		
		Coralberry	0.1		
		N=2781			

2017 the community assemblage counts jumped fivefold—from 528 individuals to 2,781. As in the Colorado Bend site, this shows distinct differences within the community between seasons. Unfortunately, because of the timing of the study (which did not extend through the entire growing season), it cannot be determined whether 2017 was a boom year for many species or if spring counts well outnumbering fall is merely a common seasonal pattern. Grazing, dry summer months, annual life cycles, periodic flow pulses, etc., likely cause a natural attrition, but those cannot be discerned from this dataset.

In fall, cherry laurel alone makes up more than 46% of the community, with seaoats at 17%. In spring, cherry laurel is outnumbered by cedar elm (20%) and drops to 20% of community abundance. Green ash is 0.8% of the community in fall, increasing to 1.7% in spring, which indicates seed dispersal was prolific between the two sampling dates. Box elder was 0.2% in fall and 0.3% in spring. Black willow was 0.6% in fall and only 0.1% in spring. Yaupon holly dominate the canopy at 23%, followed by cherry laurel at 22% and cedar elm at 11%. Sycamore are 7%, box elder are 1.5%, and green ash are least abundant at 0.8%. FAC species dominate the canopy at 57%, followed by FACU at 27%. Collectively, FACW and OBL make up 4% of the tree community.

An nMDS ordination plot of Sandy Creek's level shows that, because of large variation among each, little heterogeneity between them can be statistically represented. This is supported by the ANOSIM R values (Figure 31). The major contributors to dissimilarity between Level 1 and 2 are the abundances of cherry laurel, yaupon, and seaoats (Appendix D, Table 13). Between Level 1 and 3, those species are cherry laurel and seaoats, and between Level 2 and 3 they are cherry laurel and Carolina sedge. This explains the very low overall differences between the level. No woody riparian species are ranked as contributors to either similarity (not shown) or dissimilarity in these levels.

Figure 32 plots the differences in community between seasons. There is moderate heterogeneity between the two, as verified the ANOSIM R statistics (in figure) of 0.46. As shown in the abundance table (Table 16) the greatest difference between these two seasons is the number of plants present, with spring being vastly more populated than the previous fall. The major contributors to dissimilarity between the seasons are the same species seen found throughout the level: cherry laurel, seaoats, and Carolina sedge (Appendix D, Table 14).

Grouping species by WI classes does little to refine community assemblage dissimilarities because of the extreme variation across all level (Figure 33). An examination of the contributors to dissimilarity (Appendix D, Table 15) shows FACU and FAC dispersed throughout the zone. As was noted in the abundance tables, these species are pervasive in this site and generally outnumber the riparian species. FACW and OBL species' counts were so low that they are completely absent from similarity (not shown) and dissimilarity rankings at this site.

There are apparent differences among the mature trees (Figure 34), but the sample size was too low to produce significant ANOSIM results. Level 3 had too few sampled canopy trees to statistically analyze. Level 1 and 2 both had a mixture of cherry laurel, yaupon, water oak, sycamore, chinaberry (one of the few invasive canopy species sampled), and water hickory (Appendix D, Table 16).

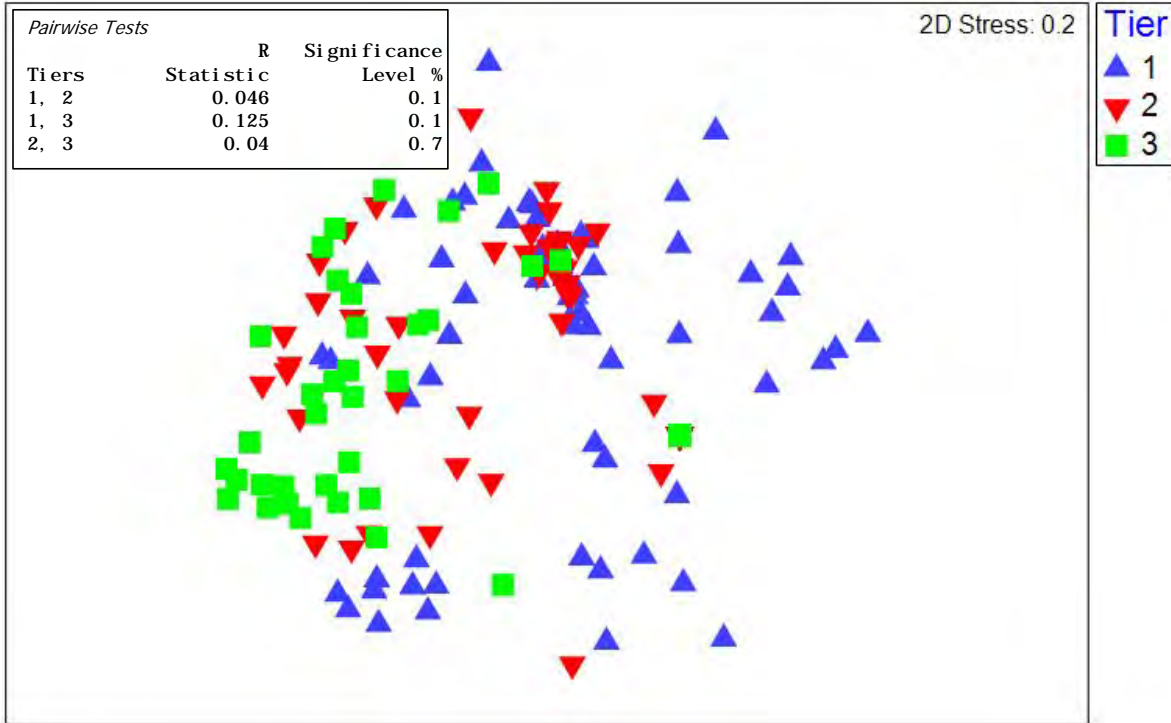


Figure 31. An nMDS analysis of Sandy Creek levels community differences. Inset box shows the ANOSIM results; p=.1%.

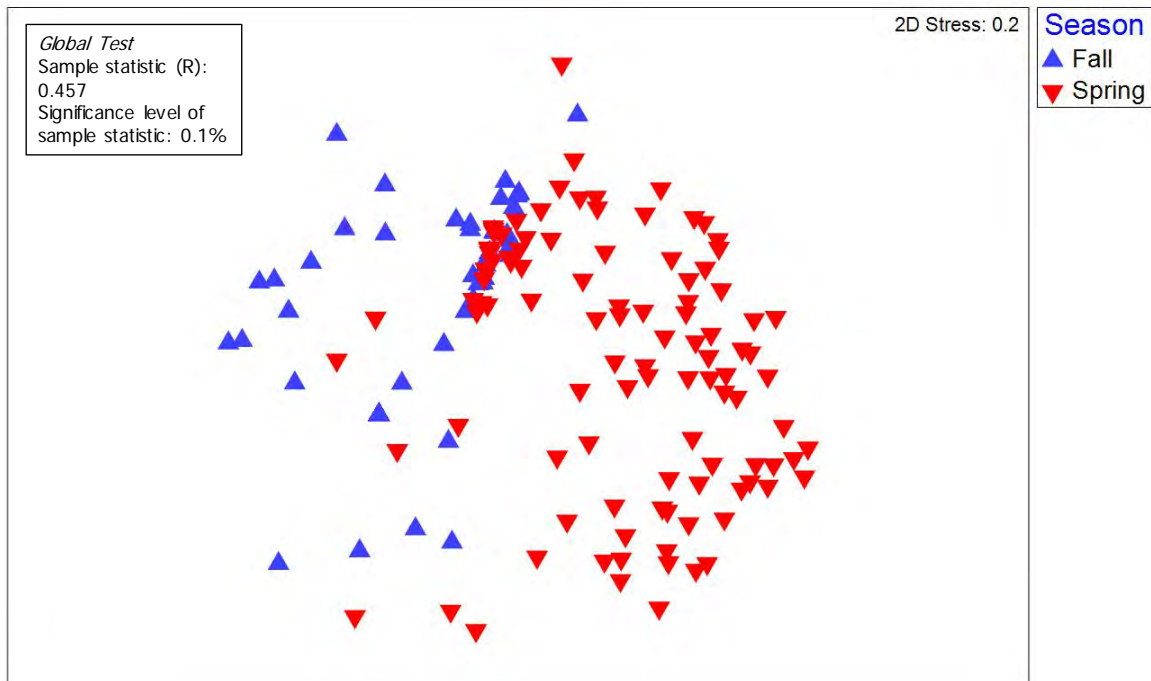


Figure 32. An nMDS analysis of Sandy Creek levels seasonal community differences. Inset box shows the ANOSIM results; p=.1%.

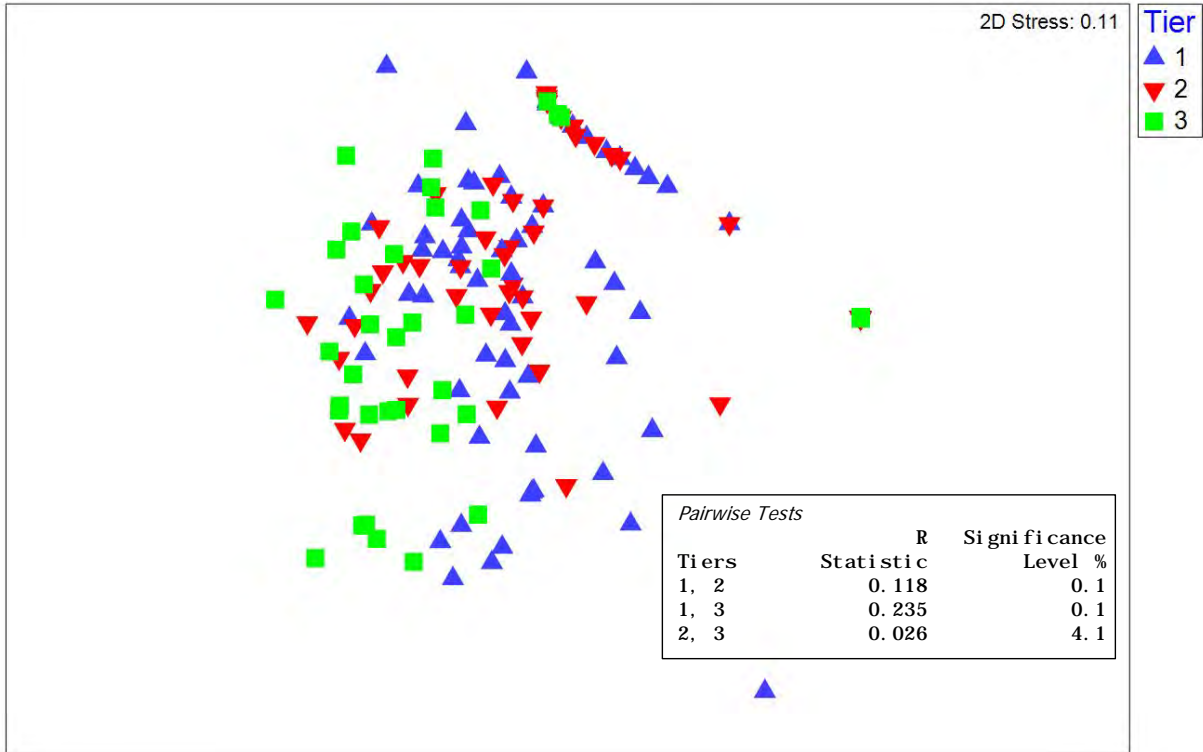


Figure 33. An nMDS analysis of Sandy Creek levels wetland indicator (WI) class differences. Inset box shows the ANOSIM results; p=.1%.

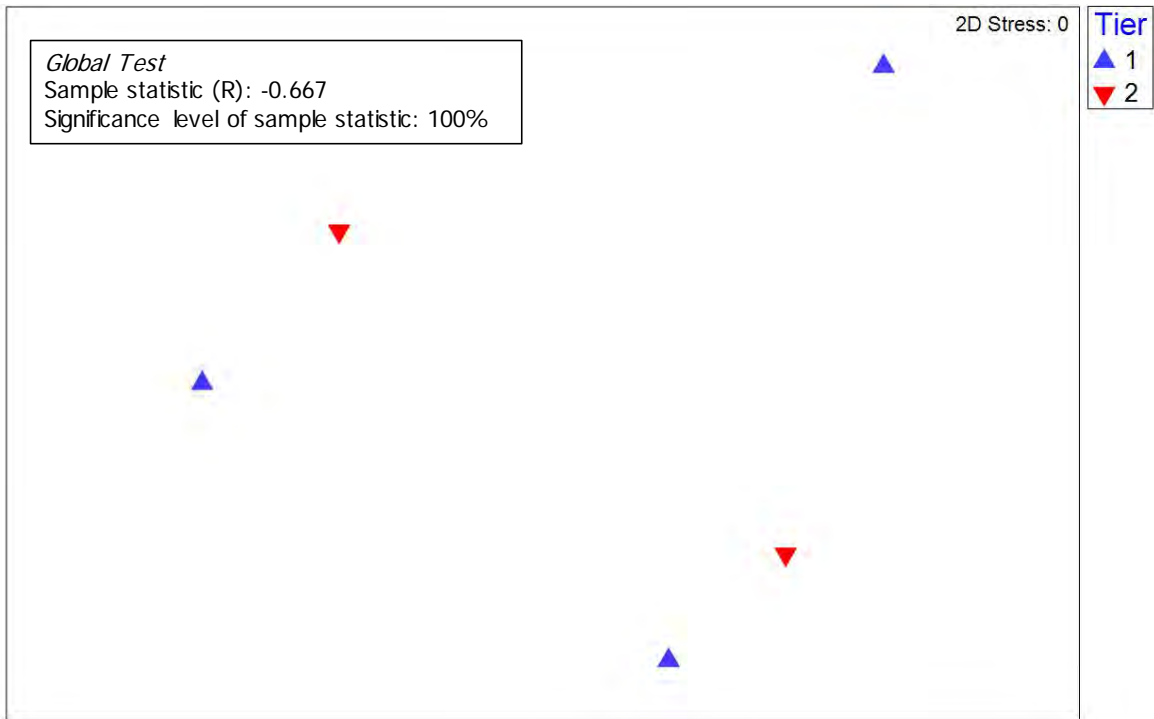


Figure 34. An nMDS analysis of Sandy Creek levels mature tree differences. Inset box shows the ANOSIM results; p=.1%.

Overall community assemblages at this site showed much overlap between level and significant inhabitation in all level by non-riparian-associated species. Because even the mature tree sampling lacked many of the riparian trees, characterization of the riparian community was difficult.

Analyses of the stream discharge estimates for this site are shown in Table 14. The discharge estimated to inundate all of Level 1 is approximately 10,000 cfs (Table 14). Level 2 inundation would require approximately 11,000 cfs and Level 3 needs approximately 4,000 cfs to inundation estimation. As is common among this basin, a low-lying floodplain in Level 3 gives erroneous discharge estimates because the pulse would still have to crest Level 2; thus, the inundation need is actually better represented by Level 2 needs. Table 15 shows that no TCEQ standards flow pulses inundate large portions of the riparian distribution.

3.2.5 Community and Basin Assessments

One of the important questions this study aimed to explore was the homogeneity of sites within the basin, or lack thereof. Even though this study had a sample size of two sites, it marks an important beginning to exploring the river continuum as another aspect of riparian community influencers. A detailed community assessment within the Col/Lav basin is provided in Appendix D.

Another important question for consideration regarding validation and monitoring methodologies being developed by this study was ‘Are there riparian community differences related to unique site characteristics that could be applied across basins?’ If such a scenario were to exist this would provide yet one more methodology for river managers to employ when considering rivers, and stretches of rivers, outside the scope of this study. A detailed across-basin assessment of riparian habitats within the Col/Lav, GSA, and Brazos basins is provided in Appendix D.

Overall, data indicate that currently there is a lack of distinct correlation by community groupings, by site, or by basin to any one abiotic factor that would allow easily distinguishable community assemblage responses to known variables. However, this is a first effort, and improvements can be made to the methodology. Given there were distinct differences in this study’s outcomes, further investigation of these relationships—using increased sampling sites and sampled plots/trees within those sites—is warranted. Suggestions for further refinement are given in the Comparison of Methodologies section, below.

3.2.6 Comparison of Methodologies

Returning to the discussion of the pros and cons of the “transect methodology” that was previously employed in SB3 flow studies, there were clear advantages and disadvantages to that method (as shown in the Introduction section). The current study’s alternate technique, the “corridor methodology” sought to address some of the previous methodologies’ shortcomings while also exploring new techniques that could be applied to riparian flow investigations. Below are the pros and cons of the corridor methodology as discovered through this study.

Pros

- Studying the overall community assemblages gives a more robust understanding of community species composition with a statistically significant number of repeat sample events, rather than focusing only on riparian woody indicators.
- Having a secondary mature-tree sampling remedies the problematic difficulty of randomly selecting sites that may completely miss riparian species.
- As long as future samplings are scheduled in a comparable season, this method will allow for comparison of community dynamics from previous studies and also increase characterizations with subsequent visits.
- Coupled with site channel properties and USGS gauging information, the method can provide a quick (though generalized) snapshot of whether the flow needs are meeting the needs of the indicator species.
- Ease of use and freedom from a known transect provide beneficial versatility to field sampling.
- Randomization allows for statistical analysis of data.
- A potential benefit (though not yet realized with the initial attempt) is that community assemblages may exhibit responses to localized stream characteristics, enabling river managers to more broadly apply these methods to future stream reaches.

Cons

- The linkage of individuals (at various life stages) to unique flow events cannot be described with this method.
- The corridor sampling technique requires a secondary mature-tree sampling (see above) to ensure riparian species are captured in analysis, and so that riparian functioning can be quantified. The lack of mature-tree sample sizes made statistics problematic for many sites. This was even more problematic when trying to analyze woody riparian species only.
- The methodology needs to be further refined and modified if the final “pro” bullet point above is to be realized.
- Using general level boundaries to estimate inundation needs is not recommended; instead, known indicator species are necessary to more accurately estimate flow needs.

Overall, this technique worked well in some selected riparian areas, and less so in others. Overall it did bring increased understanding to riparian sites within this basin, and even across basins. It holds promise as a methodology that can continue to build on this ever-increasing knowledge base if refinements are made to ensure that the riparian community and full distribution can be better represented and extrapolated for analysis. Below are some recommendations for future improvement.

Rather than select one or the other technique (transect vs. corridor) a hybridized methodology would circumvent some problematic issues with each individual technique. While employing the randomized sampling, modification of the secondary mature-tree sampling is recommended to include seedlings and saplings, and to increase sampling size. The small number of random plots chosen was often inadequate in achieving sample sizes large enough to ensure robust statistical

analysis. Increasing this sampling better facilitates a subtest in which the “noise” of understory/herbaceous plants are removed to examine the canopy component; current datasets are severely limited here. This also allows statisticians to extrapolate by age classes—a very valuable component that may yield much in riparian characterization.

Including a perpendicular-to-stream assessment of OBL and FACW species distributions with an added size class attribute is recommended. Size-class analyses will allow for the detection and monitoring of the spatial aspect of ongoing riparian species recruitment. The characterization of OBL and FACW species ensure that the full extent of those stream-constricted species is included in long-term monitoring datasets, allowing for future detection of encroachment, constriction, and/or expansion studies, etc. Having known distributions of riparian-restricted species also allows for greater accuracy in estimating needed inundation of flow pulses into the zone. If full distributions of the riparian vegetation are not included in estimated inundation needs, then there is very real danger that modifications based on erroneous flow needs could do harm to these already fragile systems.

Future statistical tests should add a level that removes from analyses pervasive species that may be obscuring less-prevalent but more keystone-functioning species that, if detected, could bring success to the early attempts at creating community assemblages linked to localized environmental variables. As mentioned, Nicol (2013) compared riparian understory and overstory vegetation using cluster analysis to identify definite communities in relation to location and water resources, but found a lack of differences because the most abundant species were too widespread. An example of this scenario within the current study may be the wide-spread hackberry in these basins. Their seedlings dominated datasets and analyses, yet offered little useful assemblage-distinguishing value. With their exclusion, it may allow for the detection of distribution patterns in the less-prevalent species. There were a number of species (e.g., cherry laurel seaots, ragweed) to which this may apply. These plants may be transient pioneer residents (or early seedlings) that temporarily flourish between flow cycles, yet obscure datasets aimed at monitoring persistent species. Using statistical analyses to detect their effects when included vs. removed may lend valuable insight that is missing in this round.

3.2.7 Conclusions

Several questions and hypotheses were considered in this study. In response to the first hypothesis, that sites would be distinguishable from one another based on unique features related to various abiotic features: the study showed that steepness of bank, dominant soil class/type, local stream sinuosity, and stream channel width were candidates for consideration because these did vary across sites and basins. The limitation to this was that with only 2–4 sites per basin and eight total sites across three basins, variation in this small sample size was also limited, which is problematic when larger variation is needed in order to make sound conclusions.

This study confirmed that, with the field and statistical techniques employed, community assemblages could be well characterized, but improvements are needed to ensure that riparian species are well represented. Three sub-categories of testing (overall community assemblages, WI class groupings, and canopy species) added rich understandings and multi-faceted views of the riparian community, even though they did not allow for distinctions between sites. Large seasonal differences were found in two of three sites that were investigated by season. There is a

potential for temporal heterogeneity in community assemblage to mask spatial differences among sites. Because of this, if sampling is to occur only once in the growing season, it is recommended that (1) all sites be sampled in the same season and as close to one another in time as possible, and (2) when riparian species are the focus, sampling should occur in fall. This is because riparian species' abundances decrease during spring as herbaceous plants are flourishing.

Community assemblages were confirmed to show heterogeneity between multiple sites within a basin, and though there were sometimes strong correlations to various abiotic factors no clear direct response of community assemblage-to-environmental variable could be inferred. Correspondingly, similar conclusions were made regarding community assemblage differences across the three unique basins. There are commonalities between all sites. There is heterogeneity. Whether and how that heterogeneity can be linked to local environments remains undescribed at this time and certainly warrants further investigation.

A simplified estimation of stream discharges allowed general approximation of each site's level and riparian species inundation needs, and a comparison of those to TCEQ flow standards revealed the following:

1. Using level boundaries gives a gross estimation that often over-estimates needed discharges. Individual species' distributions need to be quantified to refine the needs-assessment.
2. The TCEQ flow standards are inconsistent in meeting the needs of the riparian zone. Furthermore, additional research is recommended to clarify riparian needs so that managers can make the most-informed decisions possible regarding the future of these zones.

Importantly, this study independently verifies previous flow studies' outcomes: that in order to provide continued conservation and maintenance of the current riparian spatial distributions at many sites the existing TCEQ flow standards (spring and fall) need further research and possible adjustment. Without seasonal flows along the Colorado, Navidad and Lavaca Rivers, and their tributaries, riparian zones may face longitudinal and perpendicular constriction in most cases.

Finally, one limitation (of this and previous studies) is the extremely truncated (and awkward, from a riparian perspective) time period. Because no investigations have spanned an entire (intact) growing season, little can be said about the summer season or the seasonal changes that occur from spring to fall in a single season.

4 Multidisciplinary Evaluation

As previously reported, for intensive ecological data and responses to flow to have meaning to the SB 3 process, it must be collected, analyzed and presented in the context of potential application to the existing TCEQ environmental flow standards. The SB 3 process is by definition designed to be a balance between environmental and human needs, and thus a validation approach is needed to test if the environmental goal of maintaining a sound ecological environment can be met over time or if periodic adjustments may be required. This section provides a summary of key ecological components that have been studied in detail via this effort. It is acknowledged that it is early in the SB 3 adaptive management process and any tools or validation approaches striving to test the scientific defensibility of TCEQ environmental flow standards will need careful vetting and likely further refinement and testing by the BBESTs, BBASCs and TCEQ.

4.1 Summary of Key Ecological Components

4.1.1 Aquatics

As previously described, there was insufficient replication from the first round of sampling in the Col/Lav basin to conduct specific flow tier analysis. However, when evaluating the flow tier analysis across the GSA and Brazos basins for both fishes and macroinvertebrates, certain ecological responses (defined as statistical differences in relative abundance or diversity caused by flow) were evident. Fish community responses were detected within both riffle and run habitat and macroinvertebrate responses were detected within riffle habitats. Responses involved changes in densities and/or relative abundance to the entire community or specifically to fluvial specialists. Fish and macroinvertebrate species responses were associated with specific flow tiers across both basins as described in the Results section above. In summary, 1-per-season flow pulses and >1-per-5-year events had multiple detections of ecological responses of fish and/or macroinvertebrates at the community or species level. The 1-per-season flow pulses are within the range of the TCEQ flow standards whereas, the >1-per-5-year event consists of an overbanking event not captured in the TCEQ standards.

Overall, the greatest shift in fish communities was observed between pre-flood and post-flood in the lower Brazos River. As such, separating communities between pre-flood and post-flood periods and then assessing differences among flow tiers, when observations are available into the future, proffers a logical assessment of the flow tiers. Although a pre-flood and post-flood evaluation using the historical data set was not possible, certain ecological responses of the fish community to flow were evident. Basins with swift-water fishes including the Colorado basin had positive significant relationships with flow which lends supports to flow-ecology relationships described during this SB 3 study.

4.1.2 Riparian

This riparian study confirmed that, with the field and statistical techniques employed, community assemblages could be well characterized. Three sub-categories of testing (overall community assemblages, wetland indicator class groupings, and canopy species) provided multi-faceted views of the riparian community. Additionally, community assemblages (using the same three sub-categories) were shown to differ in varying degrees with an increase in level height/distance to stream. Importantly, this study independently verifies Round One outcomes in the GSA and

Brazos basins: that in order to provide continued conservation and maintenance of the current riparian spatial distributions at many sites the existing TCEQ flow standards (spring and fall) need adjustment.

4.1.3 Floodplains

As previously discussed, there were no floodplain connectivity studies conducted during Round One sampling in the Col/Lav basins. As such, any reference to floodplain connectivity below should be referenced back to the GSA report (SARA et al. 2017).

4.1.4 Ecological Response Summary

Overall, Round Two field investigations coupled with Round One preliminary results led to the detection of ecological responses specific to flow categories (Table 19).

Table 17. Summary of Ecological Responses for future validation consideration. Check marks indicate an ecological response detected during this project relative to specific TIFP flow categories.

Ecological Component	Texas Instream Flow Program (TIFP) Flow Categories			
	Subsistence	Base	Pulses	Overbank
Main Channel—Fish and Macroinvertebrates	√	√	√	√
Riparian Community			√	√
Floodplain Connectivity			√	√

The Round Two effort expanded our understanding of ecological responses between main-stem fish and macroinvertebrates (statistical differences in relative abundance or diversity caused by flow) and flow pulses. Ecological responses to fish and macroinvertebrate communities and fluvial specialists were detected with respect to flow tiers in the 1-per-season and >1-per-5-year event categories. It was evident that major flooding shaped the aquatic communities at several locations, but the flows required to do this were well above any TCEQ environmental flow standard. Time ran out on this study before it could be seen if flows within the range of the TCEQ environmental flow standards may serve as protective flows to maintain these reshaped aquatic communities into the future. However, at this point, it is premature to treat the previous statement in any way other than a hypothesis for future testing as the SB 3 process moves forward. It is also important to note that a considerable amount of work is presently being conducted for freshwater mussels in the State of Texas. It may very well be that freshwater mussels will offer a main-stem aquatic response to pulse-flow validation within the range of TCEQ standards. Again, this is another topic for future evaluation, as freshwater mussels were not studied during this effort.

At present, fish and macroinvertebrate community data from this study is recommended for use in assessing subsistence, base, and pulse-flow standards. We recommend focusing on native fish assemblages and fluvial specialists. The floodplain connectivity and riparian data are recommended for use in evaluating pulse-flow standards both in terms of timing, frequency, and duration. We again recommend focusing on native fish communities in the floodplains as well as native tree species in the riparian zone.

4.1.5 Validation Methodology Assessment Tool

The validation methodology assessment tool introduced in the Round One study, highlighted in Round Two Expert Workshops, presented in detail in the draft Round Two report, and subsequently presented to both the Brazos and GSA BBASC's upon completion of the draft report has been removed from the final report as a TWDB requirement. It is TWDB's professional judgement that insufficient data is available to validate the tool, and thus any practical application of this tool at this time is inappropriate.

5 Recommendations for Future Applied Research and Long-term Monitoring

The second phase of the overall SB 3 validation studies across basins (including this first round of sampling in the Col/Lav basin) has contributed to the understanding of flow-ecology responses, a key question raised during the SB 3 process. However, it is acknowledged that future work could enhance the ability of stakeholders, river managers, and the TCEQ relative to validation, application, and adaptive management. This section describes recommendations for additional focused research as well as the establishment of targeted locations for long-term monitoring. Focused applied research remains necessary to answer questions or provide guidance in the short-term relative to establishing ecological responses to flow and informing the continued development of the validation methodology. Additionally, long-term monitoring is needed to track ecological condition over time in a way amenable to “validate” said short-term answers.

5.1 Focused Applied Research

Focused applied research into the future should include the following key topics:

- ***Continued aquatic community assessments.*** Similar to the situation faced in the GSA and Brazos basins after completion of Round One, the Col/Lav basin suffers from inadequate replication to directly use in flow tier analysis. Therefore, it is recommended that aquatic applied research in the Col/Lav basin build on existing data and focus on documenting baseline conditions and sampling after flow pulses over the course of the upcoming Round 3 efforts.
- ***Freshwater mussels.*** Little information is available on the abundance and distribution of freshwater mussels in the Lavaca/Navidad drainage, and in lower Colorado River tributaries. Several species which potentially inhabit these areas are currently under review for potential endangered species listing. Better distributional information on freshwater mussels in these areas will be important information for river managers in these basins in the coming years.

Evaluate subsistence, base, and pulse-flow requirements of freshwater mussels in the context of water quantity needs. It is anticipated that this work would build upon the ongoing SB 2 and other State funded initiative currently evaluating freshwater mussels.

- ***Channel morphology.*** Establishing direct ecological responses between channel morphology changes and organismal response.

5.2 Long-term Monitoring

Because aquatic components are quite dynamic, it is recommended that long-term monitoring occur at select sites at least annually in the spring, with an additional trip considered during high, summertime temperatures. It is recommended that all habitat types (riffle, run, pool and backwater) be monitored.

A major limitation of both rounds of riparian studies was the extremely truncated (and awkward, from a riparian perspective) time periods. Because no investigations have spanned an entire (intact) growing season, little can be said about the summer season or the seasonal changes that occur from spring to fall in a single season. It is recommended that a few representative sites be selected to track riparian conditions over time (including the full growing season) using a combination of the community and indicator approach.

Long-term monitoring of select floodplain features is recommended on an annual or every-other-year basis to assess the maintenance of ecological function and establish the range of variability in connection with the elevation anticipated in the unique floodplain features.

6 Acknowledgements

The project team would like to thank the numerous property owners throughout the Col/Lav basin that made this study possible. A special thanks to the Lavaca Navidad River Authority for providing access to multiple riparian sites. Stakeholders such as these allow for protection of natural resources that are important for people and natural systems. A sincere thank you goes out to TWDB for managing this contract and for their support and guidance during this accelerated process. We graciously thank all participants of the two expert panel workshops for their thought-provoking comments and valuable suggestions. Acknowledgments would not be complete without recognizing the resource agency scientists and Col/Lav BBEST and BBASC members who provided time, guidance, and sharing of expertise both in front of and behind the scenes during this exciting project.

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Appendix A. Expert Panel Workshop Agendas and Participant List

GSA / BRAZOS / COLORADO ENVIRONMENTAL FLOWS VALIDATION PROJECT
2016 WORKSHOP #1 AGENDA
September 8, 2016

- 9:00 to 9:15 Welcome and Introductions – **LCRA**
- 9:15 to 11:00 Overview of Previous Studies
- **INTRO – Oborny**
 - **AQUATIC – Bonner**
 - **RIPARIAN – Duke**
 - **FLOODPLAIN – Littrell**
 - **BRAZOS ESTUARY – Guillen**
 - **APPLICATION - Oborny**
- 11:00 to 11:15 Break
- 11:15 to 12:00 **BRAZOS ESTUARY – Guillen**
- Proposed Plan
 - Site Selections (maps and pictures)
 - Sampling Protocols and Procedures
 - Expert Panel Feedback
- 12:00 to 1:00 Lunch: On-site
- 1:00 to 1:30 **FLOODPLAIN - Littrell**
- Proposed Plan
 - Site Selections (maps and pictures)
 - Sampling Protocols and Procedures
 - Expert Panel Feedback
- 1:30 to 2:00 **RIPARIAN – Duke**
- Proposed Plan
 - Site Selections (maps and pictures)
 - Sampling Protocols and Procedures
 - Expert Panel Feedback
- 2:00 to 2:30 **AQUATIC – Bonner**
- Proposed Plan
 - Site Selections (maps and pictures)
 - Sampling Protocols and Procedures
 - Expert Panel Feedback
- 2:30 to 3:00 **PROJECT SCHEDULE – Team**
- 3:00 to 4:00 **EXPERT PANEL DISCUSSION**
- 4:00 Adjourn



SAN ANTONIO
RIVER AUTHORITY
Leaders in Watershed Solutions

Expert Science Workshop
Sept. 8, 2016

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GSA / BRAZOS / COLORADO ENVIRONMENTAL FLOWS VALIDATION PROJECT
2017 WORKSHOP AGENDA
June 29, 2017

10:00 to 10:15	Welcome and Introductions – SARA
10:15 to 10:30	Introduction - Oborny <ul style="list-style-type: none">• Expert panel interaction and feedback welcome throughout• Study Goals and Objectives• Project Components and Researchers• Validation Framework Methodology
10:30 to 11:00	BRAZOS ESTUARY – Guillen <ul style="list-style-type: none">• Sites and Methods• Results and Conclusions• Paths forward
11:00 to 11:30	FLOODPLAIN - Littrell <ul style="list-style-type: none">• Sites and Methods• Results and Conclusions• Paths forward
11:30 to 12:00	RIPARIAN – Duke <ul style="list-style-type: none">• Sites and Methods• Results and Conclusions• Paths forward
12:00 to 1:00	Lunch – on site
1:00 to 1:30	AQUATIC – Bonner <ul style="list-style-type: none">• Sites and Methods• Results and Conclusions• Paths forward
1:30 to 1:45	Instream Flow Validation Tool – Oborny <ul style="list-style-type: none">• Work in progress – general framework• Ecological components• Additional components for consideration
1:45 to 2:00	Invited Presentation on Trinity River Activities – Webster Mangham
2:00 to 3:00	EXPERT PANEL DISCUSSION
3:00	Adjourn



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Environmental Flows Expert Panel Workshop
San Antonio River Authority

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Appendix B. Flow (CFS) on Day of Subsample per Site

Appendix C. Habitat Data Summarized by HMU

Table C1. Description of riffle habitats taken from GSA, Brazos, and Colorado basins.

	N	Mean	SD	Min	Max
Riffle	130				
Area (m ²)	12,407	31.17	19.07	6.60	198.00
Tier (1 = subsistence; 9 = >1 in 5 year)				1	9
Peak Flow (cfs)		3530	8852	4	83800
Season					
Summer	17				
Fall	34				
Winter	31				
Spring	48				
Water Temperature (°C)		20.1	6.2	7.8	32.4
Dissolved Oxygen (mg/l)		8.9	2.3	4.2	15.9
Specific Conductance (µS/cm)		653.9	295.1	233.0	1881.0
pH				6.9	9.5
Current Velocity (m/s)		0.7	0.4	0.0	2.8
Depth (m)		0.3	0.2	0.1	1.4
Vegetation (%)		10.5	22.7	0.0	100.0
Substrate					
Silt (%)		1.4	6.8	0.0	70.0
Sand (%)		11.7	15.7	0.0	100.0
Gravel (%)		42.1	25.9	0.0	95.0
Cobble (%)		29.4	26.6	0.0	100.0
Boulder (%)		7.8	17.7	0.0	90.0
Bedrock (%)		7.0	22.2	0.0	100.0

Table C2. Description of run habitats taken from GSA, Brazos, and Colorado basins.

	N	Mean	SD	Min	Max
Run	153				
Area (m ²)	35,344	148	250	12	2,915
Tier (1 = subsistence; 9 = >1 in 5 year)				1	9
Peak Flow (cfs)		7,121	19,033	4	157,000
Season					
Summer	19				
Fall	41				
Winter	41				
Spring	52				
Water Temperature (°C)		20.5	6.2	7.8	32.6
Dissolved Oxygen (mg/l)		8.8	2.1	4.6	15.9
Specific Conductance (µS/cm)		648.8	268.9	202.0	1881.0
pH				5.2	9.5
Current Velocity (m/s)		0.3	0.2	0.0	1.4
Depth (m)		0.6	0.2	0.1	1.3
Vegetation (%)		5.4	17.4	0.0	98.0
Substrate					
Silt (%)		15.8	24.5	0.0	100.0
Sand (%)		38.1	37.2	0.0	100.0
Gravel (%)		23.9	23.9	0.0	90.0
Cobble (%)		10.4	19.4	0.0	80.0
Boulder (%)		3.5	11.8	0.0	95.0
Bedrock (%)		7.8	22.5	0.0	100.0

Table C3. Description of pool habitats taken from GSA, Brazos, and Colorado basins.

	N	Mean	SD	Min	Max
Pool	23				
Area (m ²)	780	31	25	9	135
Tier (1 = subsistence; 9 = >1 in 5 year)				2	9
Peak Flow (cfs)		5,489	8,835	23	31,300
Season					
Summer	1				
Fall	4				
Winter	7				
Spring	11				
Water Temperature (°C)		20.6	4.9	12.7	27.7
Dissolved Oxygen (mg/l)		7.9	1.9	4.7	13.2
Specific Conductance (µS/cm)		561.8	220.9	232.0	1043.0
pH				7.0	9.5
Current Velocity (m/s)		0.1	0.1	0.0	0.3
Depth (m)		0.8	0.3	0.2	1.6
Vegetation (%)		1.7	6.4	0.0	30.0
Substrate					
Silt (%)		22.6	31.0	0.0	80.0
Sand (%)		35.8	38.7	0.0	100.0
Gravel (%)		20.7	24.4	0.0	80.0
Cobble (%)		15.0	23.0	0.0	80.0
Boulder (%)		4.4	10.8	0.0	50.0
Bedrock (%)		0.8	4.0	0.0	20.0

Table C4. Description of backwater habitats taken from GSA, Brazos, and Colorado basins.

	N	Mean	SD	Min	Max
Backwater	56				
Area (m ²)	2,532	44	89	9	630
Tier (1 = subsistence; 9 = >1 in 5 year)				2	9
Peak Flow (cfs)		10,259	19,940	23	112,000
Season					
Summer	2				
Fall	17				
Winter	16				
Spring	21				
Water Temperature (°C)		20.8	4.8	11.8	31.4
Dissolved Oxygen (mg/l)		7.7	1.8	4.6	12.8
Specific Conductance (µS/cm)		640.6	229.8	235.0	1271.0
pH				7.2	9.4
Current Velocity (m/s)		0.0	0.0	0.0	0.2
Depth (m)		0.5	0.2	0.2	1.4
Vegetation (%)		6.2	17.7	0.0	90.0
Substrate					
Silt (%)		63.2	32.1	0.0	100.0
Sand (%)		17.1	24.0	0.0	100.0
Gravel (%)		10.5	20.4	0.0	80.0
Cobble (%)		3.9	10.7	0.0	50.0
Boulder (%)		2.6	11.1	0.0	70.0
Bedrock (%)		2.3	12.1	0.0	70.0

Appendix D. Additional Riparian Data and Analyses

Table 1. SIMPER similarity analysis for Colorado Bend tiers. Seoats are likely inland seoats but a definitive species identification could not be made.

Tier 1

Average similarity: 16.40

Species	Av. Abund	Av. Si m	Si m/SD	Contri b%	Cum. %
Sea0ats	1.34	3.42	0.30	20.86	20.86
Emory sedge	0.84	2.83	0.25	17.23	38.08
Horse briar	0.75	2.49	0.37	15.18	53.26
Inlandseoats	0.95	2.09	0.22	12.77	66.03
Cedar elm	0.65	1.71	0.35	10.45	76.48

Tier 2

Average similarity: 18.06

Species	Av. Abund	Av. Si m	Si m/SD	Contri b%	Cum. %
TX persimmon	0.79	5.63	0.55	31.19	31.19
Wildrye	0.90	4.11	0.39	22.74	53.93
Horse briar	0.58	2.33	0.34	12.90	66.84
Cedar elm	0.76	2.19	0.31	12.11	78.94

Tier 3

Average similarity: 12.24

Species	Av. Abund	Av. Si m	Si m/SD	Contri b%	Cum. %
TX persimmon	0.55	2.49	0.34	20.35	20.35
Mexicanhat	0.62	1.88	0.33	15.38	35.72
Cedar elm	1.60	1.13	0.23	9.25	44.97
KRbluestem	0.52	1.09	0.21	8.94	53.91
Bushcrotton	0.65	1.01	0.18	8.28	62.19
Ashe Juniper	0.31	0.87	0.23	7.13	69.32
Wildrye	0.44	0.76	0.18	6.20	75.52

Table 2. SIMPER similarity analysis for Colorado Bend tiers between seasons.

Tier Fall

Average similarity: 17.50

Species	Av. Abund	Av. Si m	Si m/SD	Contri b%	Cum. %
SeaOats	1. 53	4. 90	0. 38	28. 02	28. 02
TX persimmon	0. 67	3. 64	0. 43	20. 82	48. 84
Cedar elm	0. 59	2. 32	0. 36	13. 25	62. 10
Horse briar	0. 71	2. 01	0. 31	11. 51	73. 60

Tier Spring

Average similarity: 12.05

Species	Av. Abund	Av. Si m	Si m/SD	Contri b%	Cum. %
TX persimmon	0. 46	2. 01	0. 33	16. 70	16. 70
Wildrye	0. 57	1. 92	0. 30	15. 92	32. 62
Horse briar	0. 46	1. 60	0. 29	13. 32	45. 93
Cedar elm	1. 08	1. 52	0. 28	12. 64	58. 57
Inlandseaoats	0. 72	1. 38	0. 20	11. 49	70. 05

Table 3. SIMPER dissimilarity analysis for pairwise tests between Colorado Bend tier community assemblages between seasons.

Tiers Fall & Spring

Average dissimilarity = 90.08

Species	Tier Fall	Tier Spring	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund				
SeaOats	1. 53	0. 00	9. 90	0. 67	10. 99	10. 99
Cedar elm	0. 59	1. 08	8. 16	0. 68	9. 06	20. 05
Wildrye	0. 48	0. 57	6. 77	0. 68	7. 52	27. 56
Horse briar	0. 71	0. 46	6. 51	0. 82	7. 22	34. 79
TX persimmon	0. 67	0. 46	6. 21	0. 88	6. 90	41. 69
Inlandseaoats	0. 00	0. 72	5. 12	0. 45	5. 69	47. 37
Emory sedge	0. 25	0. 39	4. 79	0. 43	5. 31	52. 69
Ashe Juniper	0. 20	0. 31	3. 20	0. 66	3. 56	56. 24
Frostweed	0. 24	0. 29	3. 16	0. 56	3. 50	59. 75
Hackberry	0. 35	0. 12	2. 87	0. 60	3. 19	62. 93
Bumelia	0. 29	0. 00	2. 08	0. 56	2. 31	65. 24
Bushcrotton	0. 00	0. 28	1. 82	0. 26	2. 02	67. 27
Roughleaf dogwood	0. 15	0. 12	1. 65	0. 39	1. 83	69. 10
Soapberry	0. 17	0. 08	1. 62	0. 38	1. 80	70. 90

Table 4. SIMPER dissimilarity analysis for pairwise tests between Colorado Bend tiers' WI classes.

Tiers 1 & 2

Average dissimilarity = 56.41

Species	Tier 1 Av. Abund	Tier 2 Av. Abund	Av. Di ss	Di ss/SD	Contrib%	Cum. %
FAC	3.92	2.81	25.03	1.45	44.38	44.38
FACU	0.81	0.86	9.51	1.07	16.85	61.23
OBL	0.92	0.00	9.40	0.56	16.67	77.90

Tiers 1 & 3

Average dissimilarity = 70.88

Species	Tier 1 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contrib%	Cum. %
FAC	3.92	2.60	28.19	1.45	39.78	39.78
UPL	0.24	2.22	17.91	1.15	25.27	65.05
FACU	0.81	1.50	12.18	1.11	17.18	82.23

Tiers 2 & 3

Average dissimilarity = 60.11

Species	Tier 2 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contrib%	Cum. %
FAC	2.81	2.60	24.50	1.35	40.75	40.75
UPL	0.81	2.22	20.77	1.18	34.56	75.32

Table 5. SIMPER dissimilarity analysis for pairwise tests between Colorado Bend tiers' mature trees.

Tiers 1 & 2

Average dissimilarity = 65.72

Species	Tier 1 Av. Abund	Tier 2 Av. Abund	Av. Di ss	Di ss/SD	Contrib%	Cum. %
Cedar elm	0.50	2.51	10.85	1.73	16.51	16.51
Ashejuniper	2.44	1.87	8.39	2.32	12.77	29.27
Green ash	1.21	0.00	7.12	4.55	10.84	40.11
Bumelia	0.00	1.00	5.95	5.50	9.06	49.17
Americanelm	1.00	0.71	4.47	1.62	6.80	55.97
Dogwood	0.71	0.00	4.43	0.85	6.74	62.71
Red oak	0.00	0.87	4.39	0.86	6.69	69.40
Possumhaw	0.71	0.50	4.10	1.03	6.24	75.64

Table 6. SIMPER dissimilarity analysis for Onion Creek tiers.

Tiers 1 & 2

Average dissimilarity = 82.52

Species	Tier 1 Av. Abund	Tier 2 Av. Abund	Av. Di ss	Di ss/SD	Contrib%	Cum. %
SeaOats	1.45	0.50	11.67	0.80	14.14	14.14
Inlandseaoats	1.15	0.49	8.37	0.83	10.15	24.29
Hackberry	0.93	0.62	6.68	1.10	8.10	32.39
Goldeneye	0.50	0.77	6.61	0.82	8.01	40.40
Frostweed	0.54	0.73	6.32	0.83	7.65	48.05
Cedar elm	0.92	0.50	6.28	1.03	7.60	55.65
Beautyberry	0.37	0.24	3.90	0.59	4.73	60.38
Wafer ash	0.30	0.29	3.14	0.70	3.80	64.18
Horse briar	0.32	0.21	2.97	0.56	3.60	67.78
maxamillion sunflower	0.26	0.11	2.52	0.38	3.05	70.83

Tiers 2 & 3

Average dissimilarity = 81.85

Species	Tier 2 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contrib%	Cum. %
SeaOats	0.50	1.28	9.47	0.92	11.57	11.57
Frostweed	0.73	0.80	8.70	0.87	10.63	22.20
Goldeneye	0.77	0.46	7.63	0.78	9.32	31.52
Cedar elm	0.50	1.08	7.63	1.07	9.32	40.84
Hackberry	0.62	0.93	7.44	1.07	9.10	49.93
Inlandseaoats	0.49	0.25	5.06	0.58	6.18	56.11
Wafer ash	0.29	0.31	3.73	0.70	4.55	60.66
Horse briar	0.21	0.39	3.53	0.60	4.31	64.98
Beautyberry	0.24	0.20	3.20	0.56	3.91	68.89
Plainsbristgrass	0.32	0.00	2.58	0.30	3.15	72.04

Tiers 1 & 3

Average dissimilarity = 77.81

Species	Tier 1 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contrib%	Cum. %
SeaOats	1.45	1.28	11.35	0.92	14.59	14.59
Inlandseaoats	1.15	0.25	7.16	0.79	9.20	23.79
Cedar elm	0.92	1.08	6.72	1.18	8.64	32.43
Frostweed	0.54	0.80	6.07	0.84	7.80	40.22
Hackberry	0.93	0.93	5.82	1.11	7.48	47.71
Goldeneye	0.50	0.46	4.76	0.71	6.12	53.83
Horse briar	0.32	0.39	3.34	0.67	4.29	58.12
Beautyberry	0.37	0.20	3.19	0.61	4.10	62.22
Wafer ash	0.30	0.31	2.96	0.71	3.80	66.02
Yaupon	0.26	0.38	2.84	0.74	3.65	69.67
Maxamill s. flower	0.26	0.03	1.81	0.34	2.33	72.00

Table 7. SIMPER dissimilarity analysis for pairwise tests between Onion Creek tier community assemblages between seasons. Note: Inland seaoats and seaoats were separated because of uncertainty of subspecies identification.

Tiers Fall & Spring

Average dissimilarity = 82.94

Species	Tier Fall	Tier Spring	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund				
SeaOats	1.20	0.00	10.74	1.22	12.95	12.95
Inland seaoats	0.00	0.76	6.58	0.86	7.93	20.89
Hackberry	0.65	0.67	6.53	1.02	7.87	28.76
Frostweed	0.34	0.61	6.51	0.86	7.84	36.60
Cedar elm	0.66	0.59	6.47	1.04	7.80	44.41
Goldeneye	0.25	0.57	6.18	0.79	7.46	51.86
Wafer ash	0.25	0.32	4.07	0.72	4.91	56.77
Beautyberry	0.26	0.21	3.78	0.63	4.56	61.33
Horse briar	0.28	0.19	3.30	0.62	3.98	65.31
TX persimmon	0.09	0.22	2.64	0.55	3.19	68.49
Yaupon	0.24	0.14	2.54	0.61	3.06	71.55

Table 8. SIMPER dissimilarity analysis for pairwise tests between Onion Creek tiers' WI classes.

SIMPER

Tiers 1 & 2

Average dissimilarity = 42.88

Species	Tier 1	Tier 2	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund				
FAC	4.20	3.24	19.17	1.44	44.71	44.71
FACU	1.91	1.92	10.08	1.24	23.50	68.21
UPL	0.62	0.87	7.65	0.98	17.84	86.04

Tiers 1 & 3

Average dissimilarity = 49.91

Species	Tier 1	Tier 3	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund				
FAC	4.20	1.75	23.55	1.74	47.17	47.17
FACU	1.91	1.46	10.86	1.26	21.76	68.93
UPL	0.62	0.80	8.80	0.90	17.62	86.55

Tiers 2 & 3

Average dissimilarity = 48.98

Species	Tier 2	Tier 3	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund				
FAC	3.24	1.75	22.15	1.32	45.22	45.22
FACU	1.92	1.46	14.61	1.21	29.83	75.06

Table 9. SIMPER dissimilarity analysis for pairwise tests between Onion Creek tiers' mature trees.

Tiers 1 & 2

Average dissimilarity = 53.29

Species	Tier 1 Av. Abund	Tier 2 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Bal dcypress	2.12	0.00	15.31	2.88	28.72	28.72
Sycamore	1.21	0.00	8.77	6.20	16.45	45.17
Ashej uni per	1.00	1.71	7.51	1.14	14.10	59.27
Pecan	1.37	2.32	6.78	1.85	12.73	72.00

Tiers 1 & 3

Average dissimilarity = 54.88

Species	Tier 1 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Bal dcypress	2.12	0.00	18.01	2.77	32.82	32.82
Sycamore	1.21	0.00	10.33	5.13	18.83	51.65
Ashej uni per	1.00	0.00	8.22	0.86	14.97	66.62
Txpersi mmon	0.50	0.71	5.94	1.16	10.83	77.45

Tiers 2 & 3

Average dissimilarity = 38.23

Species	Tier 2 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Ashej uni per	1.71	0.00	17.08	5.04	44.69	44.69
Txpersi mmon	0.00	0.71	6.20	0.86	16.21	60.90
Redbuckeye	0.50	0.00	5.38	0.85	14.07	74.96

Table 10. SIMPER dissimilarity analysis for pairwise tests between Navidad tier community assemblages

Tiers 1 & 2

Average dissimilarity = 72.50

Species	Tier 1 Av. Abund	Tier 2 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Cedar elm	2.01	4.28	13.43	1.30	18.52	18.52
Inland seaoats	3.07	1.08	9.94	1.38	13.71	32.23
Carolina sedge	0.88	2.60	9.37	1.20	12.93	45.15
Virg creeper	1.16	0.50	4.43	1.13	6.11	51.27
Box elder	1.03	0.48	4.38	0.96	6.04	57.31
Tri fol i ate orange	0.30	0.82	3.26	0.92	4.49	61.80
Trumpet creeper	0.41	0.52	2.74	0.82	3.78	65.58
Yaupon	0.38	0.54	2.71	0.89	3.74	69.31
Barnyardgrass	0.00	0.41	2.21	0.29	3.05	72.36

Tiers 1 & 3

Average dissimilarity = 78.37

Species	Tier 1 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Inland seaoats	3.07	0.81	10.97	1.43	13.99	13.99
Carolina sedge	0.88	2.80	10.82	1.19	13.81	27.80
Cedar elm	2.01	2.16	9.95	1.18	12.70	40.50
Box elder	1.03	0.67	5.03	1.03	6.41	46.91
Virgini a creeper	1.16	0.26	4.87	1.09	6.22	53.13
Barnyardgrass	0.00	0.92	4.62	0.36	5.90	59.02
Yaupon	0.38	0.27	2.25	0.65	2.87	61.90
Tri fol i ate orange	0.30	0.36	2.20	0.70	2.81	64.70
Trumpet creeper	0.41	0.16	2.20	0.63	2.80	67.51
Poi son i vy	0.35	0.15	2.19	0.44	2.80	70.30

Tiers 2 & 3

Average dissimilarity = 72.26

Species	Tier 2 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Cedar elm	4.28	2.16	14.42	1.28	19.96	19.96
Carolina sedge	2.60	2.80	9.55	1.14	13.21	33.17
Barnyardgrass	0.41	0.92	5.79	0.46	8.02	41.19
Inland seaoats	1.08	0.81	4.84	0.99	6.69	47.88
Box elder	0.48	0.67	3.65	0.76	5.05	52.93
Tri fol i ate orange	0.82	0.36	3.32	0.93	4.60	57.53
Virgini a creeper	0.50	0.26	2.48	0.73	3.43	60.97
Yaupon	0.54	0.27	2.46	0.81	3.40	64.37
Trumpet creeper	0.52	0.16	2.33	0.65	3.22	67.59
Hackberry	0.35	0.46	2.23	0.85	3.09	70.67

Table 11. SIMPER dissimilarity analysis for pairwise tests between Navidad tiers' WI classes.

Tiers 1 & 2

Average dissimilarity = 44.31

Species	Tier 1 Av. Abund	Tier 2 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
FAC	4.50	4.82	15.68	1.31	35.39	35.39
OBL	0.91	2.80	13.18	1.31	29.74	65.14
FACW	1.09	0.95	7.54	0.89	17.02	82.16

Tiers 1 & 3

Average dissimilarity = 49.07

Species	Tier 1 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
FAC	4.50	3.16	15.42	1.47	31.44	31.44
OBL	0.91	3.04	14.62	1.34	29.80	61.23
FACW	1.09	1.78	11.07	0.83	22.57	83.80

Tiers 2 & 3

Average dissimilarity = 47.93

Species	Tier 2 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
FAC	4.82	3.16	17.27	1.38	36.04	36.04
OBL	2.80	3.04	11.36	1.19	23.71	59.75
FACW	0.95	1.78	10.62	0.82	22.16	81.91

Table 12. SIMPER dissimilarity analysis for pairwise tests between Navidad tiers' mature trees.

Tiers 1 & 2

Average dissimilarity = 67.23

Species	Tier 1	Tier 2	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund				
Boxel der	2.08	0.00	10.21	1.90	15.19	15.19
Yaupon	2.34	0.50	9.62	2.54	14.31	29.50
Pecan	1.57	0.00	8.01	20.02	11.92	41.42
Green ash	1.00	0.00	5.14	9.02	7.64	49.06
Ameri canel m	1.00	0.71	5.03	1.16	7.49	56.54
Anacua	0.00	1.00	4.93	0.86	7.33	63.87
Hackberry	1.37	0.50	4.51	1.19	6.70	70.58

Tiers 1 & 3

Average dissimilarity = 52.57

Species	Tier 1	Tier 3	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund				
Boxel der	2.08	0.00	9.94	1.83	18.90	18.90
Yaupon	2.34	1.00	7.40	1.06	14.07	32.97
Cedar elm	1.87	1.12	6.05	1.18	11.51	44.48
Ameri canel m	1.00	0.50	4.73	1.13	9.00	53.47
Pecan	1.57	0.71	4.71	1.00	8.96	62.43
Green ash	1.00	0.87	4.24	9.80	8.07	70.50

Tiers 2 & 3

Average dissimilarity = 68.87

Species	Tier 2	Tier 3	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund				
Cedar elm	2.50	1.12	13.58	1.03	19.71	19.71
Green ash	0.00	0.87	8.16	0.86	11.85	31.56
Water oak	0.00	1.00	7.87	4.15	11.43	42.99
Anacua	1.00	0.00	7.36	0.84	10.69	53.69
Hackberry	0.50	1.37	7.13	1.15	10.36	64.04
Yaupon	0.50	1.00	7.00	1.26	10.17	74.21

Table 13. SIMPER dissimilarity analysis for pairwise tests between Sandy Creek tier community assemblages.

Tiers 1 & 2

Average dissimilarity = 85.90

Species	Tier 1 Av. Abund	Tier 2 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Cherry laurel	1.59	1.27	21.30	0.83	24.79	24.79
Yaupon	0.53	0.39	8.03	0.66	9.35	34.14
Inland seaoats	0.64	0.52	7.06	0.64	8.22	42.36
SeaOats	0.42	0.00	5.12	0.35	5.96	48.32
Wood sorel	0.37	0.12	3.86	0.39	4.50	52.82
Horse briar	0.25	0.13	3.83	0.38	4.46	57.28
Carolina sedge	0.09	0.50	3.33	0.44	3.87	61.15
Soapberry	0.23	0.09	2.91	0.41	3.39	64.54
Cedar elm	0.07	0.42	2.68	0.43	3.12	67.67
Hrosettegrass	0.15	0.26	2.45	0.41	2.85	70.51

Tiers 1 & 3

Average dissimilarity = 93.02

Species	Tier 1 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Cherry laurel	1.59	0.59	21.40	0.75	23.01	23.01
Inland seaoats	0.64	0.86	8.19	0.72	8.81	31.82
Carolina sedge	0.09	1.19	7.49	0.65	8.05	39.87
Yaupon	0.53	0.15	7.11	0.55	7.65	47.51
Cedar elm	0.07	1.24	6.80	0.66	7.31	54.83
SeaOats	0.42	0.00	5.31	0.34	5.71	60.54
Wood sorel	0.37	0.27	4.55	0.44	4.89	65.43
Horse briar	0.25	0.00	3.28	0.30	3.53	68.95
Hsettegrass	0.15	0.40	3.00	0.47	3.23	72.18

Tiers 2 & 3

Average dissimilarity = 91.04

Species	Tier 2 Av. Abund	Tier 3 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Cherry laurel	1.27	0.59	22.51	0.76	24.73	24.73
Carolina sedge	0.50	1.19	12.56	0.84	13.79	38.52
Cedar elm	0.42	1.24	10.73	0.83	11.79	50.31
Inland seaoats	0.52	0.86	9.67	0.92	10.62	60.93
Yaupon	0.39	0.15	6.56	0.48	7.20	68.13
Hrosettegrass	0.26	0.40	4.39	0.54	4.83	72.96

Table 14. SIMPER dissimilarity analysis for pairwise tests between Sandy Creek tier community assemblages between seasons

Tiers Fall & Spring

Average dissimilarity = 92.71

Species	Tier Fall	Tier Spring	Av. Di ss	Di ss/SD	Contrib%	Cum. %
	Av. Abund	Av. Abund				
Cherry laurel	0.76	1.54	19.89	0.78	21.46	21.46
Inland seaots	0.00	1.35	10.90	0.88	11.76	33.21
Crolina sedge	0.00	1.19	9.40	0.64	10.14	43.35
Cedar elm	0.00	1.16	7.84	0.64	8.46	51.81
Yaupon	0.33	0.38	6.35	0.57	6.85	58.66
Wood sorel	0.00	0.51	4.86	0.46	5.24	63.90
Hrosettegrass	0.00	0.54	3.84	0.48	4.14	68.04
Horse briar	0.11	0.15	2.79	0.30	3.01	71.05

Table 15. SIMPER dissimilarity analysis for pairwise tests between Sandy Creek tiers' WI classes.

Tiers 1 & 2

Average dissimilarity = 66.41

Species	Tier 1	Tier 2	Av. Di ss	Di ss/SD	Contrib%	Cum. %
	Av. Abund	Av. Abund				
FACU	2.36	1.84	29.04	1.04	43.73	43.73
FAC	2.07	1.25	24.85	1.09	37.42	81.15

Tiers 1 & 3

Average dissimilarity = 78.14

Species	Tier 1	Tier 3	Av. Di ss	Di ss/SD	Contrib%	Cum. %
	Av. Abund	Av. Abund				
FACU	2.36	1.21	32.36	1.05	41.41	41.41
FAC	2.07	1.75	28.76	1.15	36.81	78.21

Tiers 2 & 3

Average dissimilarity = 80.78

Species	Tier 2	Tier 3	Av. Di ss	Di ss/SD	Contrib%	Cum. %
	Av. Abund	Av. Abund				
FACU	1.84	1.21	34.83	1.11	43.11	43.11
FAC	1.25	1.75	27.39	1.28	33.90	77.02

Table 16. SIMPER dissimilarity analysis for pairwise tests between Sandy Creek tiers' mature trees.

Tiers 1 & 2

Average dissimilarity = 50.18

Species	Tier 1	Tier 2	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund				
Cherry laurel	1.78	2.37	6.60	1.40	13.15	13.15
Yaupon	1.67	3.15	6.27	3.20	12.50	25.64
Water oak	1.05	1.22	5.38	1.48	10.72	36.36
sycamore	0.75	1.00	4.66	0.93	9.29	45.66
Chinaberry	0.94	0.50	4.58	0.96	9.13	54.79
Water hickory	0.33	0.87	4.10	1.08	8.17	62.96
American elm	0.82	0.00	3.71	0.64	7.40	70.36

Community-Wide

One of the important questions this study aimed to explore was the homogeneity of sites across the basin, or lack thereof. With four separate sites, this study marks an important beginning to exploring the river continuum as another aspect of riparian community influencers. This section will discuss results of that focus, with the multi-basin section to follow.

Figure 1 shows a 2-D (on the left) and 3-D (on the right) ordination plot of the Lavaca-Colorado Basin's four sites. The plot indicates there were differences between the overall communities. The ANOSIM stats show those differences are moderate, and there exists commonalities between the sites. The greatest dissimilarities were between Onion Creek and Sandy Creek (0.571 R statistic) and Onion Creek and Navidad River (R statistic 0.543). This makes sense given this is between an upstream tributary and two reaches near the river's mouth. The greatest similarity existed between those same two downstream reaches (Navidad River and Sandy Creek), which were relatively near one another. Table 17 shows cedar elms are a major commonality between most sites, as are seaoats. Cherry laurel topped the list for Sandy Creek, Texas persimmon topped Colorado Bend's list, hackberry was Onion Creek's top contributor, and cedar elm was the top contributor to similarity for Navidad River: all woody species. Table 18 shows the dissimilarities lists between those sites were populated by the same species that topped the similarities lists. Of those, only cherry laurel and Texas persimmon were not found in every site: cherry laurel was missing from Colorado Bend and Onion Creek, and Texas persimmon was missing from Sandy Creek.

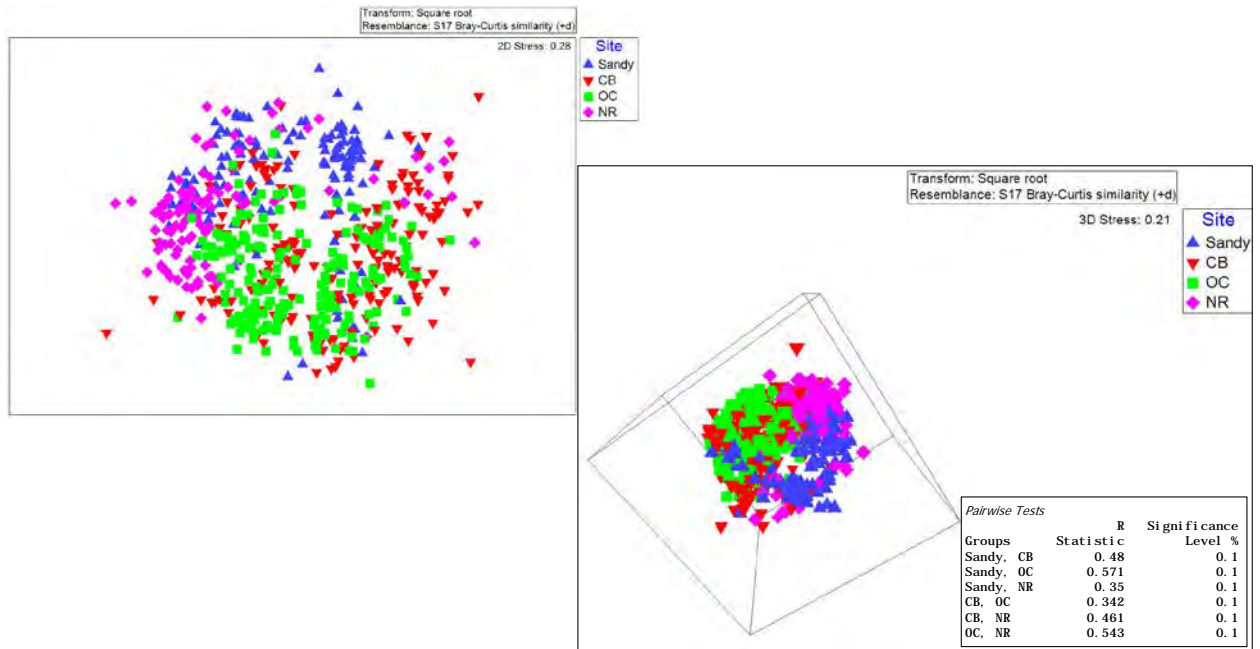


Figure 1. nMDS analysis of the Colorado-Lavaca Basin's community assemblages for all four sites.

Table 17. SIMPER similarity analysis for the Colorado-Lavaca communities between fall and spring.

Group Sandy

Average similarity: 11.58

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
Cherry laurel	1.15	6.68	0.43	57.69	57.69
Inland seaoats	0.67	1.32	0.27	11.43	69.12
Yaupon	0.35	0.99	0.21	8.54	77.67

Group CB

Average similarity: 11.88

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
TX persimmon	0.54	2.64	0.37	22.18	22.18
Cedar elm	0.89	1.81	0.31	15.23	37.41
Horse briar	0.56	1.76	0.30	14.78	52.20
Wildrye	0.53	1.46	0.23	12.26	64.45
Ashe Juniper	0.26	0.81	0.22	6.80	71.25

Group OC

Average similarity: 19.83

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
Hackberry	0.82	4.27	0.60	21.54	21.54
Cedar elm	0.82	3.24	0.52	16.36	37.89
SeaOats	1.08	3.22	0.34	16.26	54.15
Frostweed	0.69	2.25	0.33	11.34	65.49
Goldeneye	0.58	1.94	0.28	9.76	75.25

Group NR

Average similarity: 27.84

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
Cedar elm	2.82	8.43	0.79	30.28	30.28
Carolina sedge	2.09	6.44	0.58	23.14	53.42
Inland seaoats	1.65	5.49	0.62	19.71	73.14

Table 18. SIMPER dissimilarity analysis for pairwise tests between the Colorado-Lavaca community assemblages between fall and spring.

Groups Sandy & CB

Average dissimilarity = 96.78

Species	Group Sandy		Group CB	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund	Av. Abund				
Cherry laurel	1.15	0.00	0.00	9.17	0.69	9.48	9.48
Cedar elm	0.58	0.89	0.89	8.21	0.61	8.49	17.97
inlandseaoats	0.67	0.43	0.43	6.73	0.55	6.96	24.92
TX persimmon	0.00	0.54	0.54	5.86	0.55	6.05	30.98
SeaOats	0.14	0.61	0.61	5.80	0.41	6.00	36.97
Horse briar	0.13	0.56	0.56	5.58	0.58	5.77	42.74
Wildrye	0.00	0.53	0.53	5.26	0.44	5.43	48.17
Emory sedge	0.00	0.34	0.34	3.55	0.28	3.67	51.84
carolinasedge	0.59	0.00	0.00	3.30	0.42	3.41	55.25
Yaupon	0.35	0.05	0.05	3.13	0.48	3.23	58.48
Ashe Juniper	0.00	0.26	0.26	2.73	0.38	2.82	61.30
Frostweed	0.10	0.27	0.27	2.70	0.43	2.79	64.09
Hackberry	0.06	0.21	0.21	2.10	0.42	2.17	66.26
hellersrosettegrass	0.27	0.08	0.08	1.99	0.37	2.06	68.32
Soapberry	0.12	0.12	0.12	1.77	0.35	1.83	70.15

Groups Sandy & OC

Average dissimilarity = 95.26

Species	Group Sandy		Group OC	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund	Av. Abund				
SeaOats	0.14	1.08	1.08	9.23	0.61	9.69	9.69
Cherry laurel	1.15	0.00	0.00	9.02	0.67	9.46	19.16
Cedar elm	0.58	0.82	0.82	7.81	0.85	8.20	27.35
inlandseaoats	0.67	0.64	0.64	7.51	0.67	7.88	35.24
Hackberry	0.06	0.82	0.82	6.88	0.82	7.22	42.45
Frostweed	0.10	0.69	0.69	6.35	0.57	6.67	49.12
Goldeneye	0.00	0.58	0.58	5.66	0.47	5.94	55.06
Yaupon	0.35	0.22	0.22	3.67	0.58	3.85	58.91
carolinasedge	0.59	0.00	0.00	3.25	0.42	3.41	62.32
Horse briar	0.13	0.30	0.30	3.01	0.48	3.16	65.47
Beautyberry	0.04	0.27	0.27	2.99	0.38	3.14	68.62
Wafer ash	0.00	0.30	0.30	2.96	0.41	3.11	71.73

Groups CB & OC

Average dissimilarity = 90.47

Species	Group CB		Group OC	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund	Av. Abund				
SeaOats	0.61	1.08	1.08	9.37	0.72	10.36	10.36
Cedar elm	0.89	0.82	0.82	7.69	0.78	8.50	18.86
inlandseaoats	0.43	0.64	0.64	6.23	0.60	6.89	25.75
Hackberry	0.21	0.82	0.82	5.72	0.95	6.32	32.07
Frostweed	0.27	0.69	0.69	5.55	0.71	6.14	38.21
Horse briar	0.56	0.30	0.30	4.91	0.70	5.43	43.64
TX persimmon	0.54	0.16	0.16	4.55	0.71	5.03	48.67
Goldeneye	0.00	0.58	0.58	4.28	0.56	4.73	53.40
Wildrye	0.53	0.00	0.00	3.97	0.48	4.39	57.78
Emory sedge	0.34	0.10	0.10	3.13	0.34	3.46	61.25
Ashe Juniper	0.26	0.09	0.09	2.33	0.55	2.57	63.82
Wafer ash	0.01	0.30	0.30	2.21	0.54	2.44	66.26
Beautyberry	0.00	0.27	0.27	2.10	0.43	2.32	68.58
Yaupon	0.05	0.22	0.22	1.57	0.45	1.73	70.31

Groups Sandy & NR

Average dissimilarity = 91.22

Species	Group Sandy		Group NR	Av. Di ss	Di ss/SD	Contri b%	Cum. %
	Av. Abund	Av. Abund	Av. Abund				
Cedar elm	0.58	2.82	2.82	14.57	1.04	15.97	15.97
carolinasedge	0.59	2.09	2.09	11.72	0.94	12.85	28.82
inlandseaoats	0.67	1.65	1.65	10.06	0.91	11.03	39.85
Cherry laurel	1.15	0.01	0.01	6.30	0.70	6.91	46.76
Box elder	0.04	0.73	0.73	4.65	0.61	5.10	51.86
Virginia creeper	0.06	0.64	0.64	4.04	0.65	4.43	56.29
barnyardgrass	0.00	0.45	0.45	3.65	0.25	4.00	60.29
Yaupon	0.35	0.40	0.40	3.51	0.61	3.84	64.13
trifoliatorange	0.00	0.49	0.49	2.64	0.56	2.89	67.02
trumpetcreeper	0.00	0.37	0.37	2.12	0.46	2.33	69.35
Hackberry	0.06	0.34	0.34	2.06	0.58	2.26	71.61

When plotted by tier (Figure 2) those differences are lessened as each tier level across the basin has similarities with all other tiers. This is verified with ANOSIM statistics in the figure. A SIMPER test (Table 19) shows that all sites' major tier similarities arise from the presence of seaoats, cedar elm, hackberry, and cherry laurel across the basin.

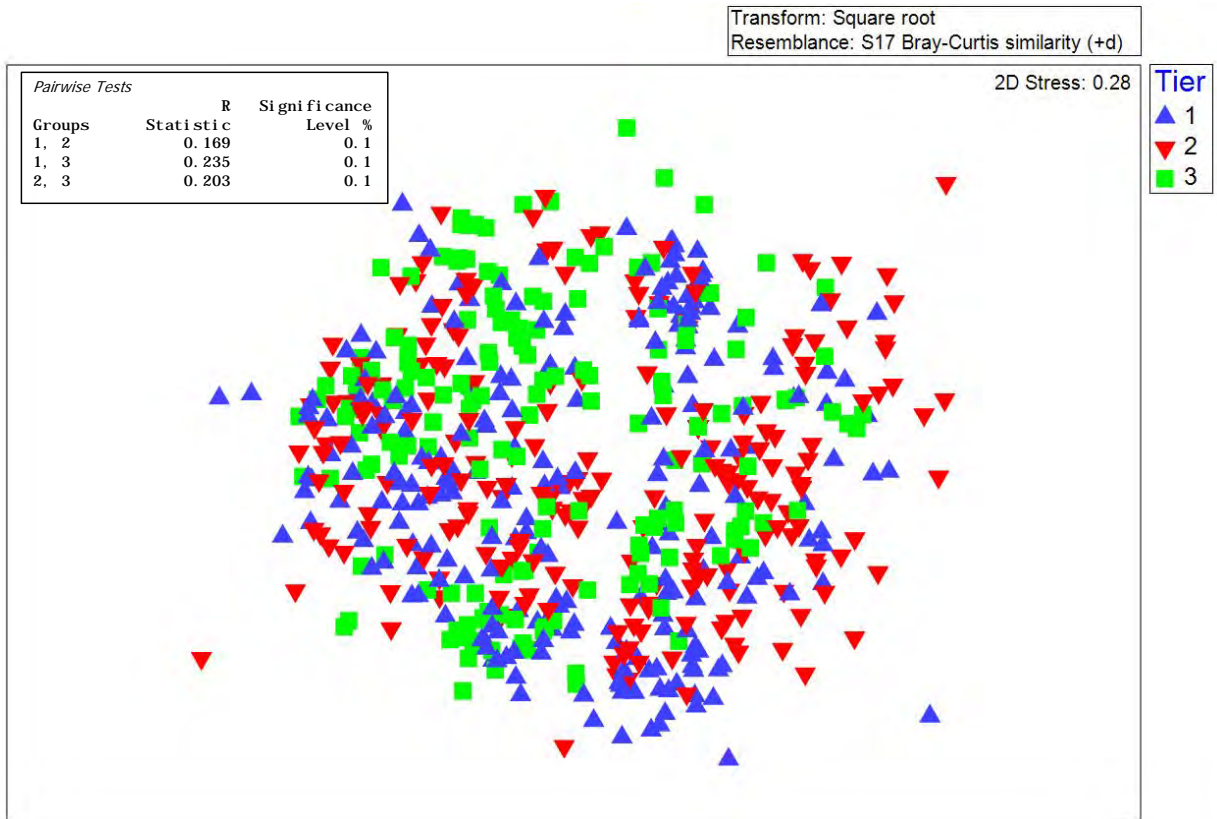


Figure 2. nMDS analysis of the Colorado-Lavaca Basin's community assemblages across comparable tiers.

Table 19. SIMPER similarity analysis for the Colorado-Lavaca communities across comparable tiers.

*Examines Tier Tiers
(across all Season Tiers)
Tier 1*

Average similarity: 16.64

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
SeaOats	0.92	3.95	0.33	23.71	23.71
Cedar elm	1.20	3.20	0.44	19.24	42.95
Inlandseaoats	0.65	2.23	0.36	13.40	56.35
Cherry laurel	0.52	1.32	0.16	7.95	64.31
Hackberry	0.43	1.14	0.31	6.86	71.17

Tier 2

Average similarity: 11.24

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
Cedar elm	1.10	2.80	0.42	24.87	24.87
Carolinasedge	0.71	1.74	0.25	15.44	40.31
Inlandseaoats	0.54	1.54	0.30	13.73	54.04
Frostweed	0.41	0.79	0.20	7.05	61.09
TX persimmon	0.29	0.76	0.19	6.74	67.83
Hackberry	0.31	0.74	0.24	6.54	74.37

Tier 3

Average similarity: 13.79

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
Inlandseaoats	1.12	5.87	0.50	42.58	42.58
Cedar elm	0.86	1.93	0.37	13.97	56.55
Hackberry	0.37	0.83	0.23	6.03	62.59
SeaOats	0.41	0.82	0.19	5.92	68.51
Horse briar	0.31	0.64	0.19	4.65	73.16

Figure 3, an ordination plot of the combined-sites' seasons shows that while there is considerable overlap of many species in plots that persist throughout the year, there are also many distinct spring plots that reflect the 'boom' of species and counts seen in Colorado and Sandy Creek sites. Table 20 shows the major contributors to similarity were the same species as the overall community assemblages: the prolific seaoats, cedar elm, hackberry, and cherry laurel. When grouped by WI classes and plotted by site and tier (Table 21), the two basin sites showed low heterogeneity because of large variation within all the sites' tiers.

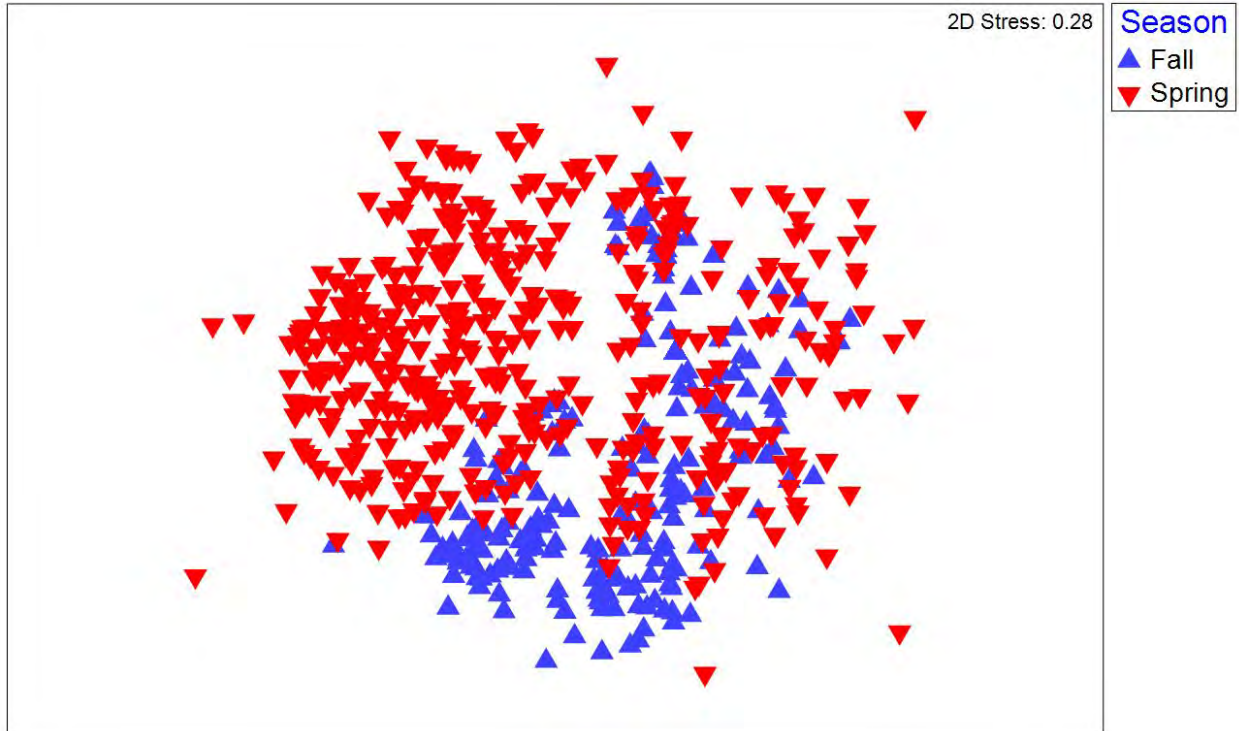


Figure 3. nMDS analysis of the Colorado-Lavaca community assemblages differences between fall and spring.

Table 20. SIMPER similarity analysis for the Colorado-Lavaca communities between fall and spring.

*Examines Season Tiers
 (across all Tier Tiers)*

Tier Fall

Average similarity: 12.98

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
SeaOats	1.29	6.22	0.44	47.89	47.89
Cedar elm	0.49	1.20	0.27	9.28	57.16
Hackberry	0.39	1.09	0.29	8.38	65.54
cherry laurel	0.29	0.97	0.13	7.46	73.00

Tier Spring

Average similarity: 14.32

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
Inlandseaoads	1.25	4.21	0.46	29.42	29.42
Cedar elm	1.45	3.35	0.47	23.37	52.79
carolinasedge	0.82	1.35	0.23	9.42	62.21
Hackberry	0.36	0.84	0.25	5.85	68.06
Frostweed	0.40	0.75	0.19	5.21	73.26

Table 21. ANOSIM analysis of the Colorado-Lavaca Basin's WI differences across sites.

*Tests for differences between unordered Site Tiers
(across all Season x TIER Tiers)*

Global Test

Sample statistic (Average R): 0.354

Significance level of sample statistic: 0.1%

Number of permutations: 999 (Random sample from a large number)

Number of permuted statistics greater than or equal to Average R: 0

Pairwise Tests

Tiers	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Sandy, CB	0.371	0.1	Very large	999	0
Sandy, OC	0.457	0.1	Very large	999	0
Sandy, NR	0.194	0.1	Very large	999	0
CB, OC	0.17	0.1	Very large	999	0
CB, NR	0.402	0.1	Very large	999	0
OC, NR	0.412	0.1	Very large	999	0

The canopy trees for Colorado-Lavaca were plotted (Figure 4) and the results reveal that Onion Creek and Colorado Bend tend to have unique canopy assemblages while Navidad and Sandy Creek exhibit some overlap (ANOSIM R statistics displayed in the figure). Variation by tiers showed no consistent pattern between sites. The SIMPER similarity test (Table 22) shows the prevalence of cedar elm and hackberry make them major contributors. The only woody riparian species contributing to similarity were green ash in Tier 1 and water hickory in Tier 2. Those trees most responsible for heterogeneity between all sites' tier levels (Table 23) were yaupon, Ashe juniper, cedar elm, and cherry laurel – the same species that generally account for homogeneity as well. Box elder was missing from all sites' Tier 2 plots but found in both Tier 1 and Tier 3 in all sites. This is likely just an effect of random sampling, though several of the sites had a Tier 2 slump in their profiles which may partially explain this anomaly.

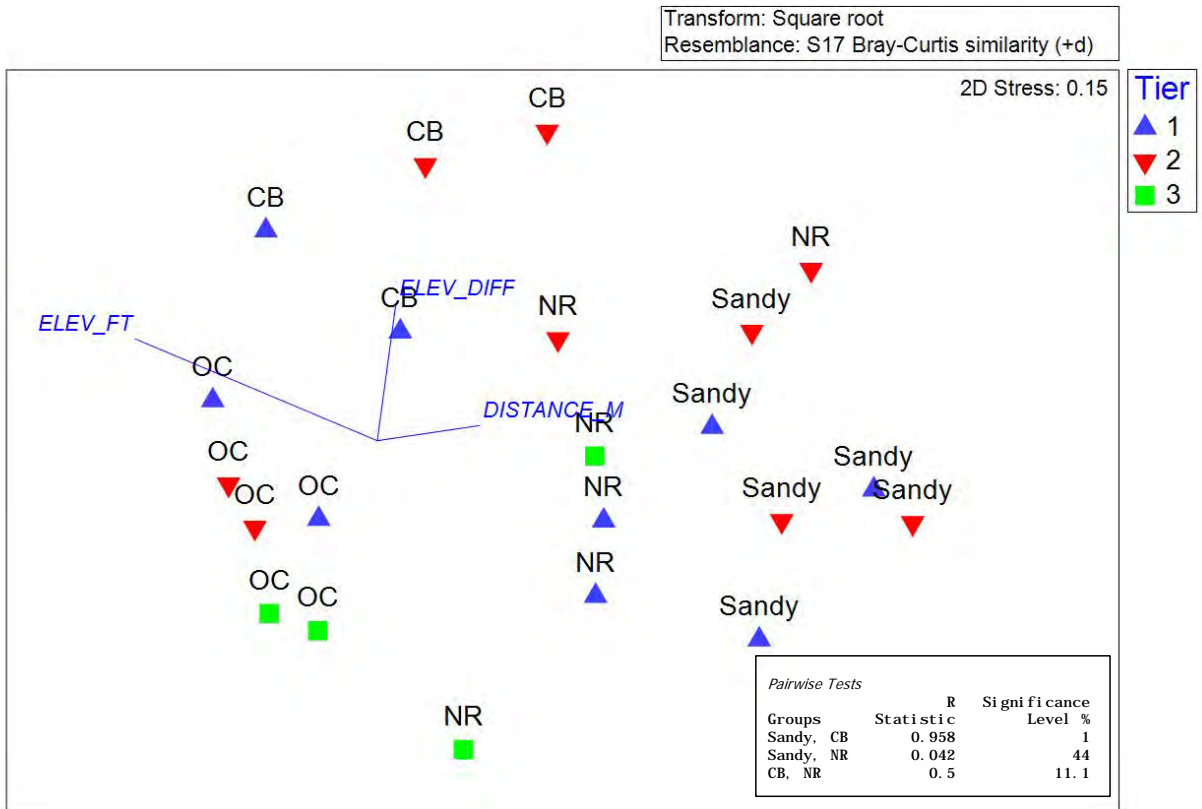


Figure 4. nMDS analysis of the Colorado-Lavaca Basin's mature tree differences across comparable tiers.

Table 22. SIMPER similarity analysis for the Colorado-Lavaca Basin's mature trees across comparable tiers.

*Examines Tier Tiers
(across all Season Tiers)
Tier 1*

Average similarity: 32.48

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
Cedar elm	0.97	6.94	1.35	21.37	21.37
Yaupon	1.08	6.28	0.81	19.33	40.70
Hackberry	0.86	4.41	0.95	13.56	54.27
Green ash	0.60	3.96	0.88	12.19	66.46
Americanelm	0.83	2.88	0.58	8.88	75.34

Tier 2

Average similarity: 33.54

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
Cedar elm	1.65	18.34	1.92	54.69	54.69
Yaupon	0.97	3.95	0.56	11.77	66.46
water hickory	0.55	2.06	0.38	6.13	72.60

Tier 3

Average similarity: 47.71

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum. %
Pecan	1.29	21.26	0.71	44.57	44.57
Hackberry	1.18	19.36	2.64	40.58	85.15

Table 23. SIMPER dissimilarity analysis for pairwise tests between the Colorado-Lavaca Basin's mature trees across comparable tier groups.

Tiers 1 & 2

Average dissimilarity = 69.81

Species	Group 1 Av. Abund	Group 2 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Yaupon	1.08	0.97	6.36	1.29	9.11	9.11
ashej uni per	0.76	0.79	5.56	0.81	7.97	17.08
cedar elm	0.97	1.65	5.51	1.10	7.89	24.97
cherry laurel	0.59	0.53	4.92	0.77	7.04	32.01
ameri canel m	0.83	0.43	4.62	1.15	6.62	38.63
green ash	0.60	0.00	3.67	1.26	5.26	43.89
sycamore	0.63	0.22	3.67	0.77	5.25	49.14
Water oak	0.46	0.38	3.62	0.91	5.18	54.32
Water hi ckory	0.11	0.55	3.51	0.81	5.02	59.35
boxel der	0.62	0.00	3.33	0.69	4.77	64.12
hackberry	0.86	0.44	3.25	1.07	4.65	68.77
pecan	0.76	0.52	3.22	0.89	4.61	73.38

Tiers 1 & 3

Average dissimilarity = 62.01

Species	Group 1 Av. Abund	Group 3 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
ashej uni per	0.76	0.00	5.13	0.60	8.27	8.27
cedar elm	0.97	0.56	4.95	1.13	7.99	16.26
Yaupon	1.08	0.50	4.93	0.84	7.94	24.20
ameri canel m	0.83	0.25	4.73	0.99	7.63	31.83
sycamore	0.63	0.00	4.55	0.81	7.34	39.17
pecan	0.76	1.29	4.05	1.12	6.53	45.70
bal dcypress	0.47	0.00	4.00	0.49	6.46	52.16
green ash	0.60	0.43	3.90	1.03	6.29	58.44
Water oak	0.46	0.50	3.34	1.21	5.39	63.83
cherry laurel	0.59	0.00	3.22	0.49	5.20	69.03
hackberry	0.86	1.18	3.21	0.77	5.17	74.20

Tiers 2 & 3

Average dissimilarity = 65.16

Species	Group 2 Av. Abund	Group 3 Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
cedar elm	1.65	0.56	8.34	0.92	12.81	12.81
ashej uni per	0.79	0.00	6.78	0.82	10.41	23.21
Yaupon	0.97	0.50	6.37	0.95	9.78	32.99
hackberry	0.44	1.18	5.95	1.06	9.13	42.13
green ash	0.00	0.43	5.40	0.75	8.28	50.41
Water oak	0.38	0.50	4.77	1.28	7.33	57.73
pecan	0.52	1.29	4.11	1.01	6.30	64.03
Water hi ckory	0.55	0.25	4.01	0.78	6.16	70.19

Overall, the communities of the four Colorado-Lavaca sites (Figure 5) show Colorado Bend is most strongly influenced by steepness and channel width. This site has both a short/steep slope and wide channel. Onion Creek and Navidad sites are most strongly influenced by elevation and

are both along slopes with low elevations. Sandy creek is strongly influenced by sinuosity, and was the only site in the basin to have a low point bar. The ANOSIM statistics show that the dissimilarities between each of the sites except Onion Creek and Navidad (which are both influenced most strongly by elevation) are very large.

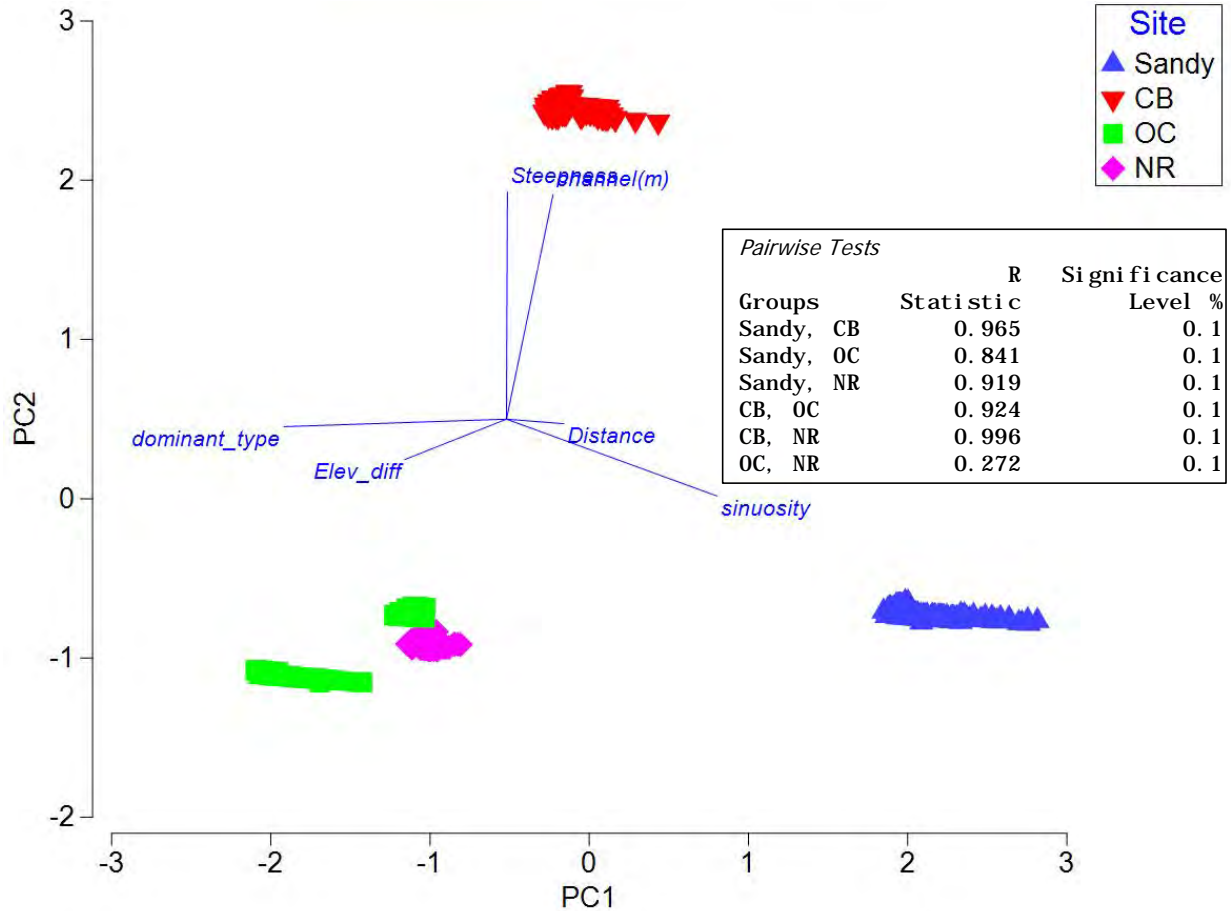


Figure 5. Principal component analyses (PCA) of community assemblages for the Colorado-Lavaca Basin, associated among sites and abiotic factors. Steepness and channel width are overlain in the plot. Inset box shows the ANOSIM statistic for differences; p=.1%.

A PCA plot of the Colorado-Lavaca Basin associated by site and season (Figure 6) shows that the spring samples tend to have less variation in their plots in comparison to the fall plots. So even though the spring plots often had much greater species richness and diversity, they were more strongly related to abiotic variables than their fall counterparts. The ANOSIM result (in the figure) displays low values, indicating the differences are minimal statistically.

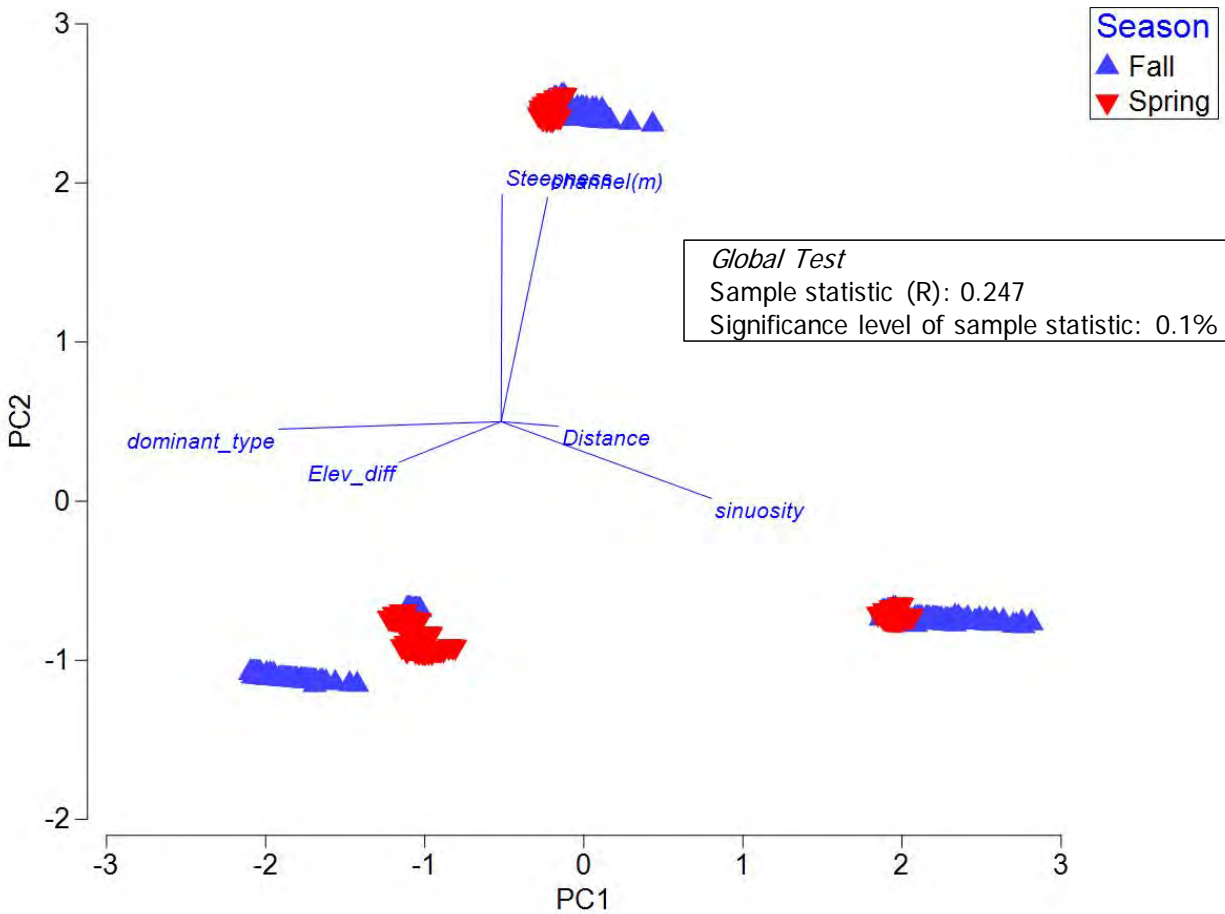


Figure 6. Principal component analyses (PCA) of the Colorado-Lavaca Basin community, associated among site, seasons, tiers, and abiotic factors. Steepness and channel width are overlain in the plot. Inset box shows the ANOSIM statistic for differences; $p=0.1\%$.

A PCA of mature trees for the Colorado-Lavaca Basin (Figure 7) shows that Onion Creek and Navidad canopy communities are strongly influenced by dominant soil type, and both contain silty/clay mixtures. Colorado Bend trees are influenced by steepness in a site that had the greatest steepness factor. Sandy Creek was influenced by sinuosity (similar to the overall community assemblage), and distance.

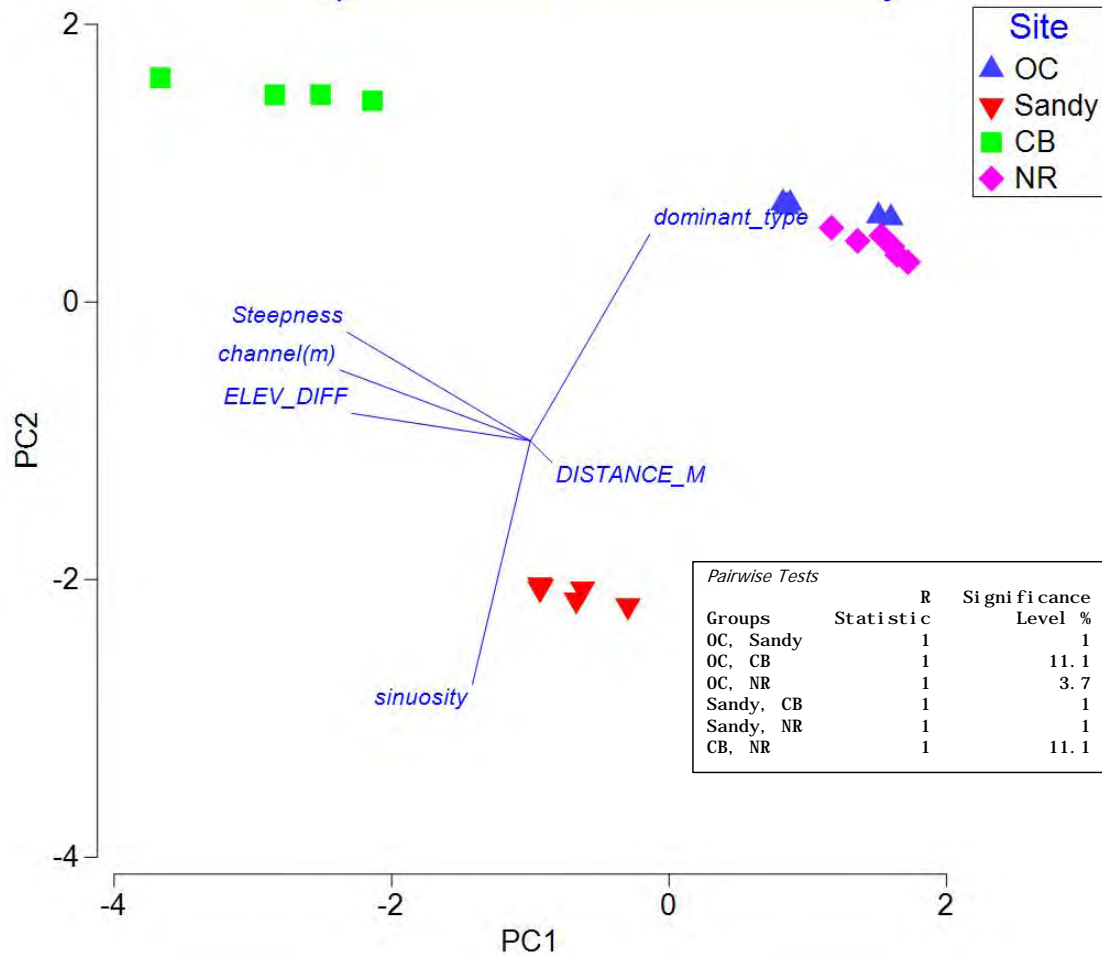


Figure 7. Principal component analysis (PCA) of the mature trees for the Colorado-Lavaca Basin associated among sites and abiotic factors. Inset box shows ANOSIM results; $p=0.1\%$

Basin-Wide

One of the questions for consideration regarding validation and monitoring methodologies being developed by this study was ‘Are there riparian community differences related to unique site characteristics that could be applied across basins?’ If such a scenario were to exist this would provide yet one more methodology for river managers to employ when considering rivers, and stretches of rivers, outside the scope of this study.

Figure 8 shows an nMDS 3-dimensional ordination plot of the community assemblages for all three basins – GSA, Colorado-Lavaca, and Brazos. There are noticeable differences between the basins, although the ANOSIM results show these are moderately low. The greatest dissimilarity exists between GSA and Brazos, while GSA and Colorado-Lavaca are most similar. When

grouped by tier (Figure 9) those dissimilarities dissolve as shown in the figure and verified by the ANOSIM results. An examination of the dissimilarity between basins (see below) sheds light on the overall community assemblages' contributing species.

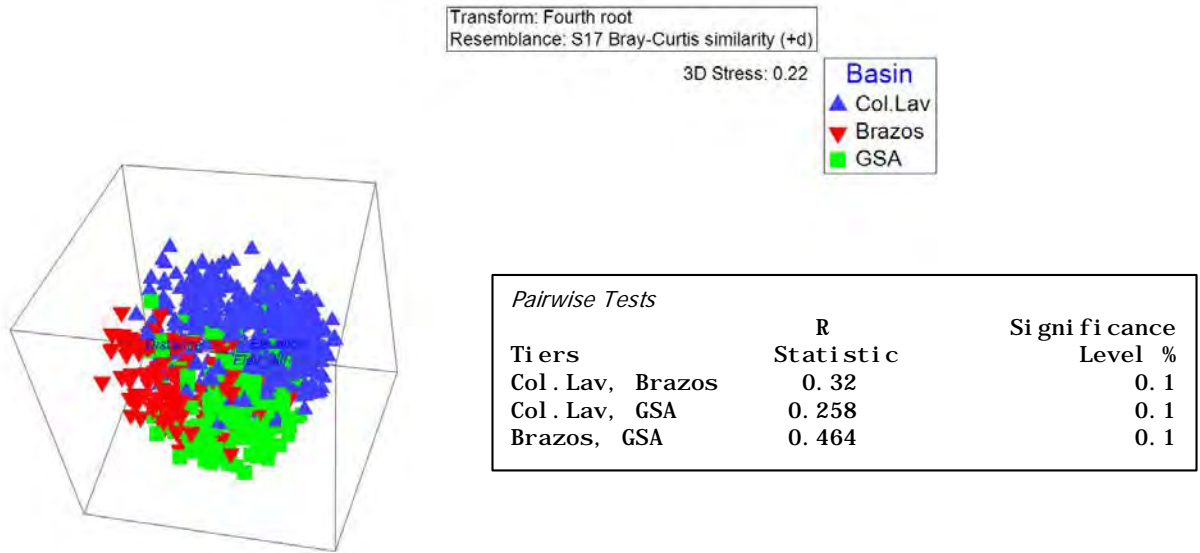


Figure 8. nMDS 3-D analysis of the community assemblage differences across all the GSA, Brazos and Colorado-Lavaca basins. The inset box shows the ANOSIM statistic for differences; $p=0.1\%$.

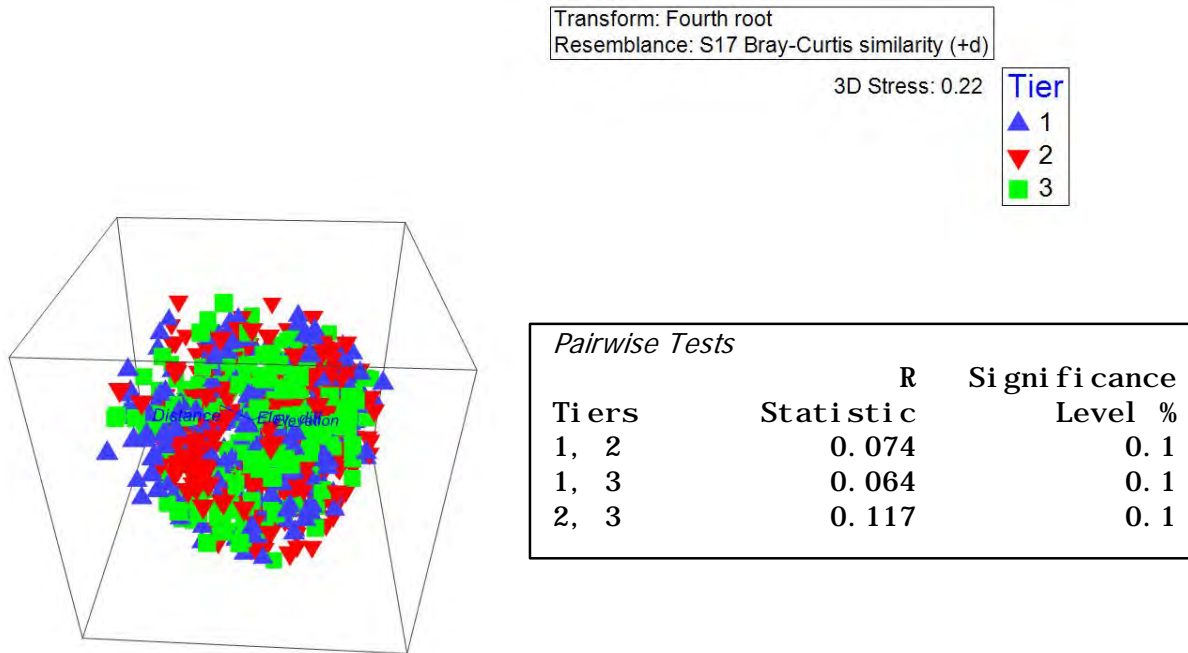


Figure 9. nMDS 3-D analysis of the community assemblage differences across tiers from all three basins. The inset box shows the ANOSIM statistic for differences; $p=.1\%$.

Table 24 (comparing Colorado-Lavaca to Brazos) indicates that a total of 22 species combined contribute to 71% of the dissimilarity between the two basins. Of those, 13 species are present in both basins. The major contributor to dissimilarity is seaots, yet they are present in both basins. Cedar elm, which contributes 6% to the dissimilarity, is the second-ranked species and is only located in significant numbers in Colorado-Lavaca yet virtually absent in Brazos sites. The only riparian canopy species in the rankings are black willow and sycamore, though they are present in both basins but with different abundance percentages. Table 25 (comparing Colorado-Lavaca to GSA) shows 20 species contribute 71% of the dissimilarity between these two basins. Giant ragweed, the major contributor to dissimilarity, was absent in the Colorado-Lavaca basin. The riparian canopy species' dissimilarity contributors were box elder and green ash, though they were present in both basins, so again it was largely a matter of abundance differences for widely dispersed species.

Table 24. SIMPER dissimilarity analysis for pairwise tests between Colorado-Lavaca and Brazos Basins' community assemblages.

Col.Lav & Brazos
Average dissimilarity = 97.64

Species	Col . Lav Av. Abund	Brazos Av. Abund	Av. Di ss	Di ss/SD	Contrib%	Cum. %
Inland seaoats	0.44	0.18	6.51	0.61	6.67	6.67
Cedar elm	0.60	0.00	5.93	0.71	6.08	12.74
Box elder	0.10	0.40	5.39	0.62	5.52	18.26
Cocklebur	0.00	0.37	4.77	0.38	4.88	23.15
Hackberry	0.31	0.14	4.48	0.60	4.58	27.73
Roughleaf dogwood	0.05	0.24	3.52	0.49	3.61	31.34
Black willow	0.01	0.28	3.36	0.40	3.44	34.78
Horse briar	0.23	0.06	3.35	0.45	3.44	38.22
SeaOats	0.28	0.00	3.15	0.40	3.22	41.44
Trumpet creeper	0.04	0.25	3.10	0.49	3.18	44.62
Cherry laurel	0.21	0.00	3.09	0.32	3.17	47.78
Pepper vine	0.03	0.25	2.98	0.49	3.05	50.84
Sycamore	0.02	0.25	2.93	0.43	3.00	53.84
Frostweed	0.24	0.00	2.78	0.40	2.85	56.70
Yaupon	0.20	0.00	2.29	0.38	2.35	59.04
Carolin sedge	0.25	0.00	2.20	0.38	2.26	61.30
TX persimmon	0.16	0.00	2.04	0.33	2.08	63.38
Wildrye	0.10	0.07	1.90	0.32	1.95	65.33
Goldeneye	0.12	0.00	1.57	0.27	1.61	66.94
Virginia creeper	0.10	0.05	1.52	0.35	1.56	68.50
Giant ragweed	0.00	0.12	1.21	0.29	1.24	69.74
Emory sedge	0.06	0.00	1.19	0.15	1.22	70.96

Table 25. SIMPER dissimilarity analysis for pairwise tests between Colorado-Lavaca and GSA basins' community assemblages.

Col.Lav & GSA
Average dissimilarity = 92.35

Species	Col . Lav Av. Abund	GSA Av. Abund	Av. Di ss	Di ss/SD	Contrib%	Cum. %
Giant ragweed	0.00	0.79	6.91	0.82	7.48	7.48
Inland seaoats	0.44	0.46	6.15	0.82	6.66	14.14
Poison ivy	0.03	0.55	5.59	0.66	6.06	20.20
Cedar elm	0.60	0.25	5.32	0.83	5.76	25.96
Horse briar	0.23	0.50	4.97	0.77	5.38	31.34
Hackberry	0.31	0.39	4.59	0.78	4.97	36.32
Dewberry	0.03	0.47	4.09	0.70	4.43	40.74
Virginia creeper	0.10	0.33	3.44	0.62	3.73	44.47
Box elder	0.10	0.26	2.65	0.53	2.87	47.34
Wildrye	0.10	0.23	2.57	0.48	2.78	50.12
SeaOats	0.28	0.00	2.37	0.39	2.57	52.69
Cherry laurel	0.21	0.00	2.12	0.34	2.30	54.98
Frostweed	0.24	0.02	2.07	0.45	2.24	57.23
stickwilly	0.01	0.26	2.01	0.43	2.17	59.40
purple leatherflower	0.00	0.18	1.86	0.40	2.02	61.42
Green ash	0.08	0.16	1.84	0.43	2.00	63.42
Pecan	0.06	0.15	1.81	0.44	1.96	65.38
Carolina sedge	0.25	0.00	1.76	0.37	1.91	67.29
Yaupon	0.20	0.02	1.76	0.43	1.90	69.19
TX persimmon	0.16	0.00	1.45	0.37	1.57	70.76

Table 26 (comparing Brazos and GSA) shows 16 species contributed 72% of the dissimilarity between the two basins. These two had the greatest dissimilarity between them so it makes sense that fewer species contributed a cumulative equal amount of dissimilarity as the other basins'

comparisons. Giant ragweed, the major contributor, was present in both but had different abundances between the basins. Only one herbaceous plant (cockleburr) was absent in GSA and only cedar elm was absent in the Brazos rankings. Box elder, sycamore, and green ash were present in both basins, so it was largely variation in their abundances that created dissimilarity rather than heterogeneity of species richness.

Table 26. SIMPER dissimilarity analysis for pairwise tests between Brazos and GSA basins' community assemblages.

Brazos & GSA

Average dissimilarity = 93.81

Species	Brazos	GSA		Diss/SD	Contrib%	Cum. %
	Av. Abund	Av. Abund	Av. Diss			
Giant ragweed	0.12	0.79	8.51	0.84	9.08	9.08
Poison ivy	0.07	0.55	5.93	0.68	6.32	15.39
Box elder	0.40	0.26	5.86	0.76	6.24	21.64
Inland sea oats	0.18	0.46	5.74	0.70	6.11	27.75
Horse briar	0.06	0.50	5.23	0.65	5.57	33.33
Dewberry	0.06	0.47	4.98	0.72	5.31	38.63
Hackberry	0.14	0.39	3.99	0.63	4.26	42.89
Cockleburr	0.37	0.00	3.87	0.41	4.12	47.01
Black willow	0.28	0.04	3.61	0.37	3.85	50.86
Virginia creeper	0.05	0.33	3.49	0.59	3.72	54.58
Sycamore	0.25	0.12	3.43	0.51	3.65	58.23
Wildrye	0.07	0.23	2.75	0.43	2.93	61.17
Roughleaf dogwood	0.24	0.10	2.71	0.53	2.88	64.05
Pepper vine	0.25	0.07	2.70	0.52	2.87	66.92
Cedar elm	0.00	0.25	2.39	0.50	2.55	69.48
Green ash	0.03	0.16	2.26	0.39	2.40	71.88

Analyses for the WI classes across basins yielded few differences to investigate, for both overall community assemblages (on the right in Figure 10) and grouped by tiers (on the left in the figure). Based on these results and low ANOSIM R statistics, no further analyses were performed on this grouping technique. A comparison (verified by both nMDS and ANOSIM) of the mature canopy across basins (Figure 11) indicates that the Colorado-Lavaca basin is most dissimilar to the Brazos, and less-so to the GSA basin. GSA and Brazos had the least amount of dissimilarity (an opposite finding to the overall community assemblages above). These dissimilarities were all moderately low as shown by the ANOSIM results in the figure. Grouped by tier (Figure 12), these differences diminish (as in the overall community assemblages above). Although individual tiers do differ, the large variation among each site's tiers creates too much overlap when sites' datasets are all plotted together.

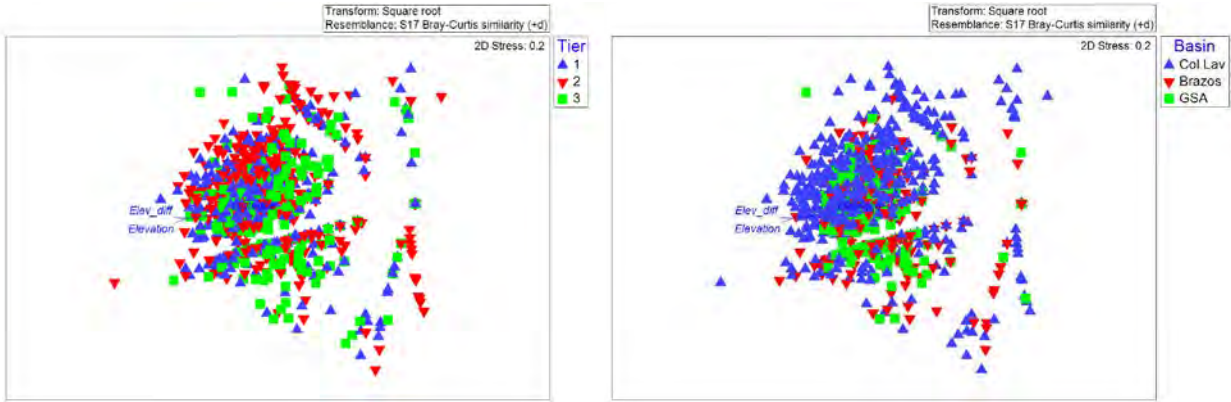


Figure 10. nMDS analysis of the community assemblage differences across all three basins' WI classes. On the left the WI classes are grouped by tier, on the right are the overall community assemblages.

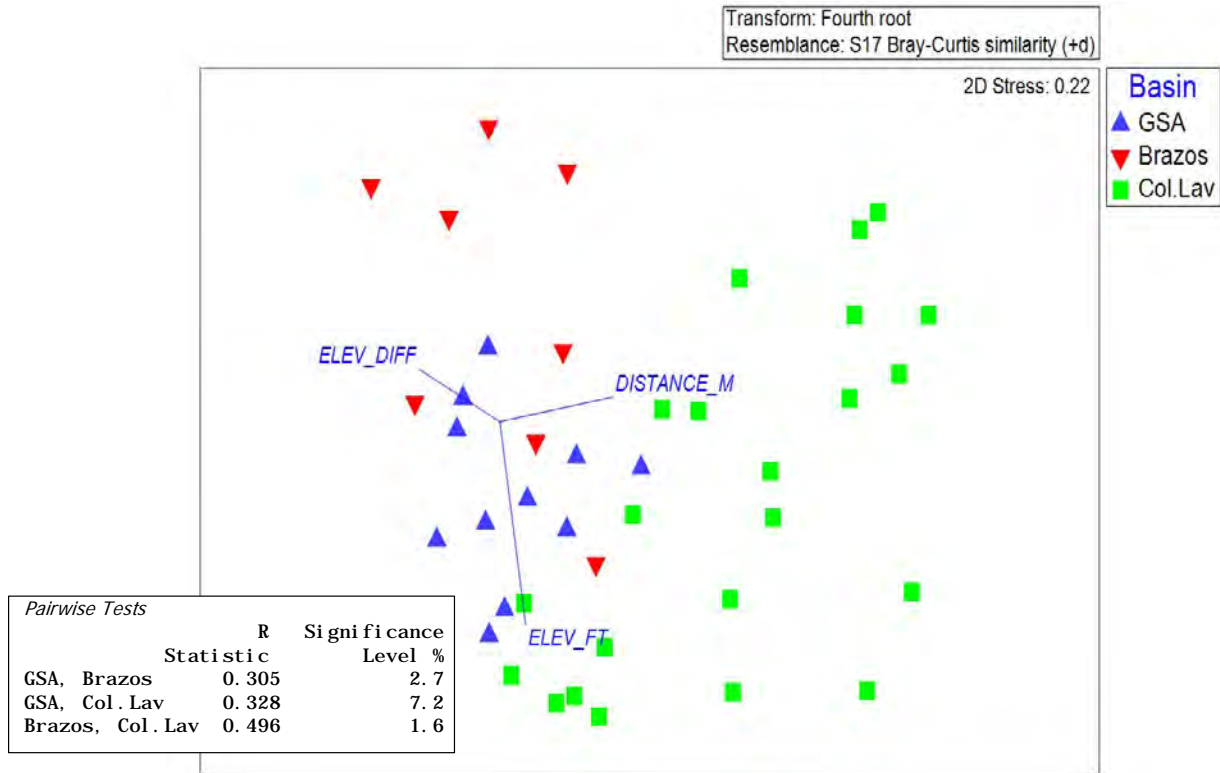


Figure 11. nMDS analysis of the GSA Basin's mature tree differences across all sites. The inset box shows the ANOSIM results; p=.1%.

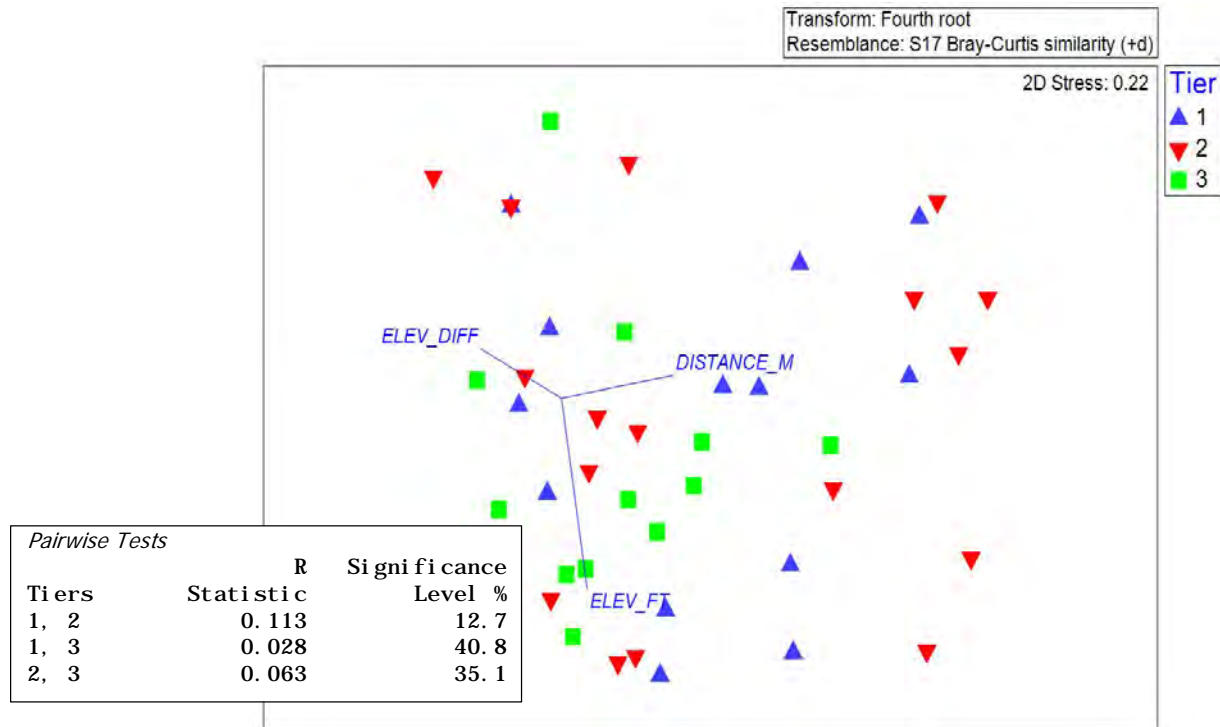


Figure 12. nMDS analysis of the GSA Basin’s mature tree differences across comparable tiers. Inset box shows the ANOSIM results; p=.1%.

An examination of the dissimilarity between basins (Table 27) sheds light on the mature trees’ contributing species. Seven species contribute 75% of dissimilarity between GSA and Brazos; however, all species are present in both basins, just in different abundances. Between GSA and Colorado-Lavaca Basins 11 species contribute a combined 73% of dissimilarity. Hackberry tops the rankings for both between-basin comparisons. Missing species from the GSA basin rankings (but present in Colorado-Lavaca) are yaupon, Ashe juniper, and water oak. No species were present in GSA but lacking in Colorado-Lavaca rankings. Between Brazos and Colorado-Lavaca basins, 11 species contribute 72% of the dissimilarity. Again, hackberry ranks high. This is likely an indicator of just how prevalent this highly adaptable species is – it is prevalent across most sites and all basins, and seen highly ranked in many similarity and dissimilarity tests presented throughout this study because of its widespread, pervasive presence. Species missing from the Brazos rankings (but present in Colorado-Lavaca) are cedar elm, yaupon and Ashe juniper. While a cursory glance would mark these species as possible community assemblage indicators, the lack of these species from some basins’ assemblages may be more a relic of the random sampling method than true indicators, as these are species known to be present across many ecosystems across Texas. However, this may warrant further investigation to narrow how prevalently they exist in various riparian sites and what conditions would make them proliferate there. Black willow and slippery elm were missing from Colorado-Lavaca but present in the

Brazos Basin. However, again this does not justify those species as an indicator of localized uniqueness as these are well-known riparian inhabitants across Texas.

Table 27. SIMPER dissimilarity analysis for pairwise tests for all basins' mature trees across sites.

GSA & Brazos
Average dissimilarity = 71.30

Species	GSA		Brazos		Av. Diss	Diss/SD	Contrib%	Cum. %
	Av. Abund	Av. Abund	Av. Abund	Av. Abund				
Hackberry	1.11	0.59	11.11	1.08	15.58	15.58		
Pecan	1.15	0.35	9.45	1.42	13.25	28.83		
Boxelder	0.71	1.19	9.14	1.08	12.81	41.64		
Green ash	0.99	0.38	8.30	0.99	11.65	53.29		
Sycamore	0.45	0.31	5.26	0.76	7.38	60.67		
Blackwillow	0.29	0.38	5.14	0.71	7.21	67.88		
Cottonwood	0.09	0.42	4.76	0.83	6.68	74.56		

GSA & Col.Lav
Average dissimilarity = 74.79

Species	GSA		Col. Lav		Av. Diss	Diss/SD	Contrib%	Cum. %
	Av. Abund	Av. Abund	Av. Abund	Av. Abund				
Hackberry	1.11	0.71	7.10	1.33	9.49	9.49		
Green ash	0.99	0.30	6.94	1.12	9.28	18.78		
Pecan	1.15	0.58	6.45	1.42	8.63	27.40		
Cedar elm	0.18	0.85	6.38	1.15	8.53	35.94		
Boxelder	0.71	0.18	5.80	1.30	7.75	43.69		
Americanelm	0.31	0.47	4.51	0.92	6.03	49.72		
Yaupon	0.00	0.64	4.43	0.85	5.92	55.63		
Ashejuniper	0.00	0.44	3.70	0.65	4.95	60.58		
Sycamore	0.45	0.28	3.45	0.82	4.62	65.20		
Red mulberry	0.21	0.09	2.89	0.64	3.86	69.06		
Water oak	0.00	0.37	2.75	0.64	3.68	72.74		

Brazos & Col.Lav
Average dissimilarity = 90.27

Species	Brazos		Col. Lav		Av. Diss	Diss/SD	Contrib%	Cum. %
	Av. Abund	Av. Abund	Av. Abund	Av. Abund				
Boxelder	1.19	0.18	10.73	1.33	11.89	11.89		
Cedar elm	0.00	0.85	9.06	1.03	10.03	21.92		
Hackberry	0.59	0.71	7.52	1.26	8.34	30.26		
Pecan	0.35	0.58	5.87	0.78	6.50	36.76		
Americanelm	0.47	0.47	5.68	0.91	6.29	43.05		
Yaupon	0.00	0.64	5.31	0.78	5.88	48.93		
Ashejuniper	0.00	0.44	5.09	0.62	5.64	54.57		
Blackwillow	0.38	0.00	4.40	0.67	4.88	59.44		
Green ash	0.38	0.30	3.55	0.73	3.94	63.38		
Sycamore	0.31	0.28	3.53	0.57	3.91	67.29		
Slippery elm	0.40	0.00	3.49	0.73	3.87	71.16		

The community differences between sites and basins, although moderately low, warranted an attempt at examination of the biotic community-to-environmental variables. Table 28 shows the PCA statistics for the community assemblages in all three basins associated among basin, site, and abiotic factors. Figure 13 is a visual representation of the PCA for community assemblages across all three basins associated among basin and abiotic factors. Also shown in the figure are the ANOSIM statistical outcomes. The Colorado-Lavaca Basin's pattern of sites were scattered across the plot. The Brazos Basin showed strong association with sinuosity. The GSA Basin was influenced by both sinuosity and dominant soil type. The influence by dominant soil type is surprising, given the two sites within that basin had limited correlation with that variable, as

shown above. However it can be explained: whereas within-basin dominant soil type was less important than other variables, when compared across basins, steepness and sinuosity were minor, but soil had more of an effect. Overall, the R statistic showed the visual differences between basins' environmental influences had very low correlations. This further supports that the current methodology has not yet been able to assign distinct assemblages to set variables that hold up at all spatial scales.

Table 28. Principal component analyses (PCA) of the community assemblages for the GSA, Brazos and Colorado-Lavaca Basins associated among basin, site and abiotic factors.

Eigenvectors
(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3	PC4	PC5
Distance	0.034	-0.620	0.343	-0.314	-0.562
Elev_diff	0.336	-0.538	-0.200	-0.391	0.529
steepness	-0.606	-0.228	-0.319	-0.080	0.363
dominant_type	0.218	0.387	-0.532	-0.633	-0.292
sinuosity	-0.123	0.351	0.670	-0.530	0.353
channel(m)	-0.676	-0.033	-0.090	-0.247	-0.249

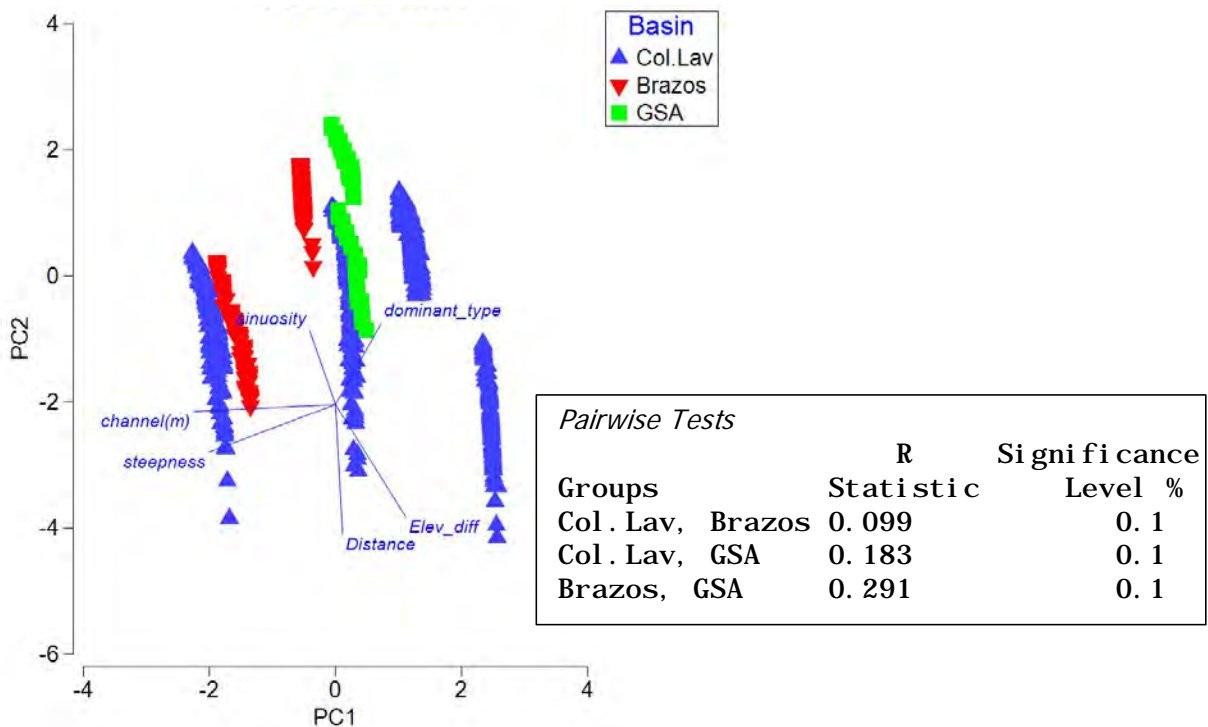


Figure 13. Principal component analysis (PCA) of the community assemblages for the GSA, Brazos, and Colorado-Lavaca Basins associated among basin and abiotic factors. Inset box shows the ANOSIM results; p=.1%.

When associated among individual site and environmental factors (Figure 14), Onion Creek shows the strongest correlation with elevation differences while Colorado Bend more strongly associates with channel width as does one of the Brazos Bend sites. Gonzales is most strongly associated with a combination of sinuosity and dominant soil type, although several other sites are as well. The ANOSIM shows varying amounts of homogeneity emerge, but no clear associations emerge.

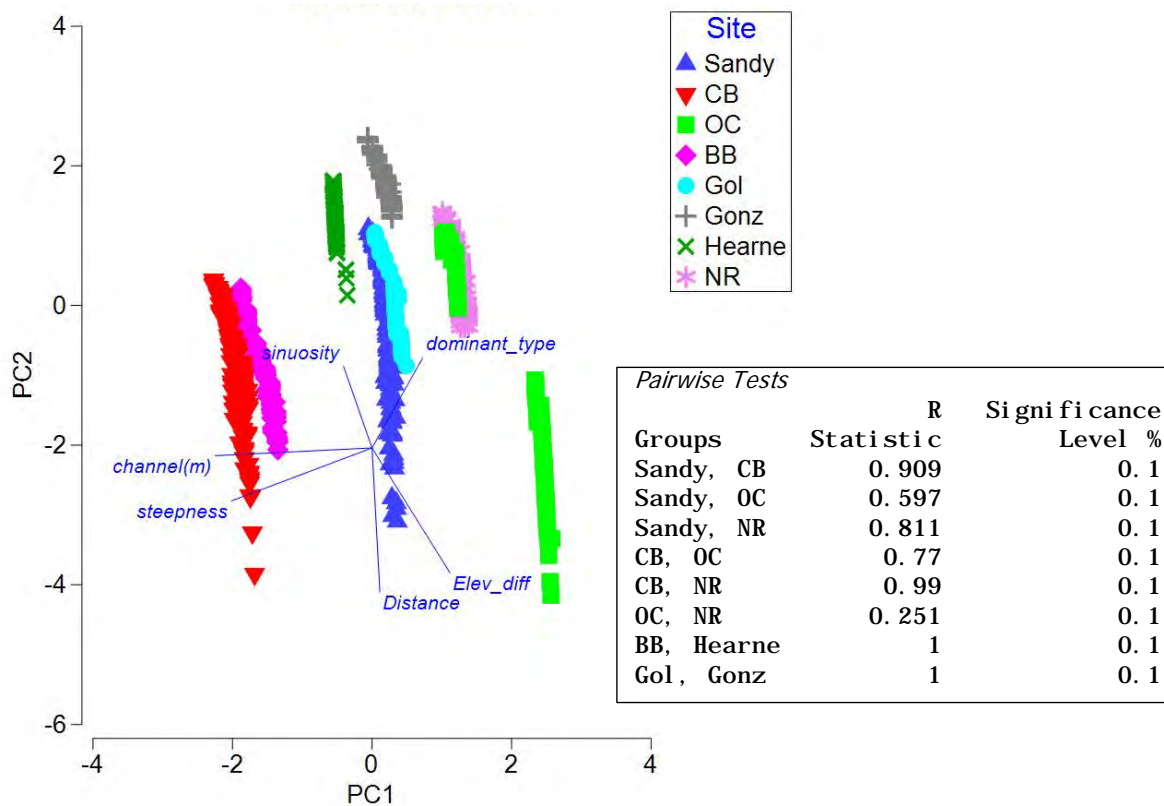


Figure 14. Principal component analysis (PCA) of the community assemblages for the GSA, Brazos, and Colorado-Lavaca Basins associated among site and abiotic factors. Inset box shows the ANOSIM results; $p=0.1$.

When associated by tier (Figure 15), distinctions between the tiers of each site, and their association with environmental factors is once again observed: variation exists among tier levels. Interestingly Tier 1 seems to be intermediate between Tiers 2 and 3 in most sites. Perhaps this is explained by the community assemblages of both the water's edge groups and the far-removed groups being strongly influenced by alterations in environmental variables, whereas the mid-slope community residents are typically a mixture of species that naturally have much greater adaptability. This is similar to the conclusions of Rood *et.al.* (2010), who showed that whereas the facultative species are more resilient to river regulation and variability, obligates are highly vulnerable. This study would support that those plants in the furthest edges of the zone likely represent the transition to upland communities, and being at the edge of this riparian

ecotone, those species may also be highly influenced by environmental factors that limit their distributions to varying scales.

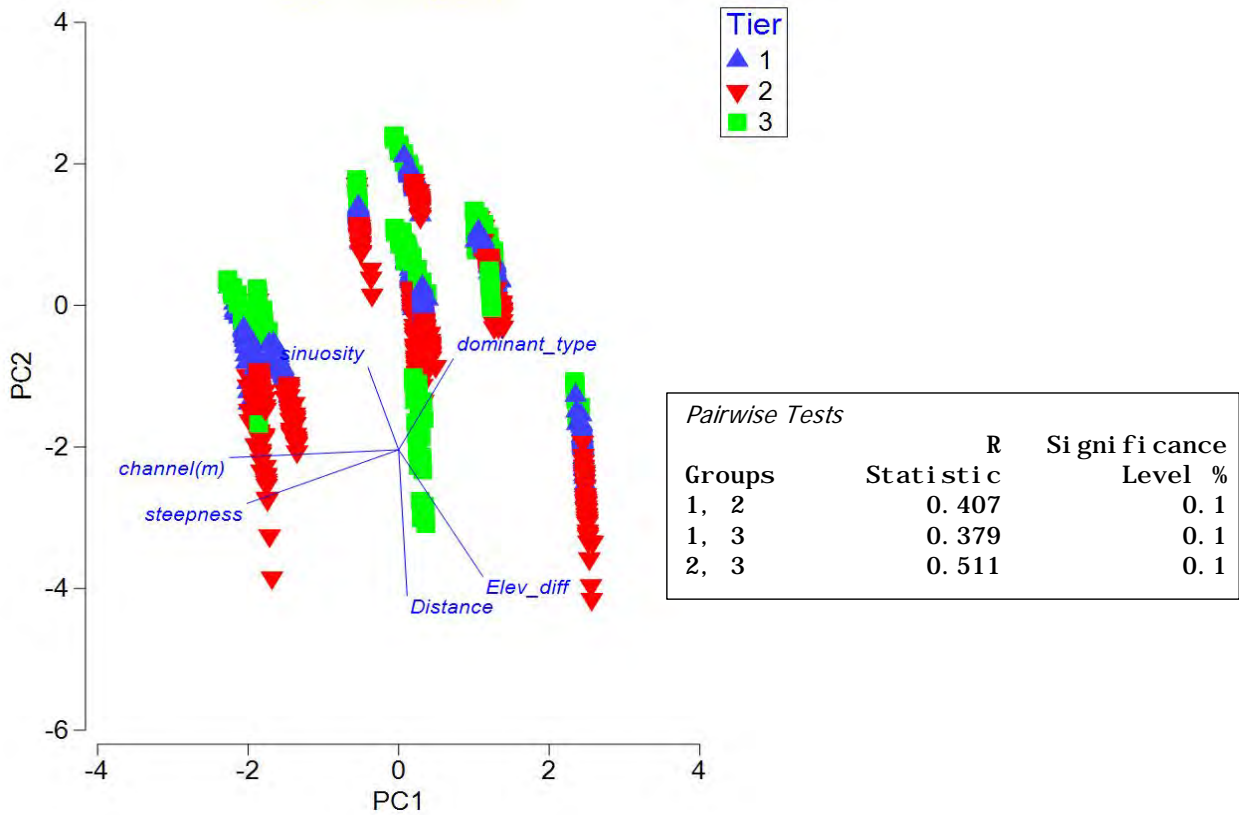


Figure 15. Principal component analysis (PCA) of the community assemblages for the GSA, Brazos, and Colorado-Lavaca Basins associated among site, tiers, and abiotic factors. Inset box shows the ANOSIM results; p=.1%.

The mature trees' correlations to abiotic variables across the three basins are shown in Table 29. Figure 16 shows the Colorado-Lavaca canopy trees are more strongly influenced by distance to stream than other basins. Canopy trees in the GSA basin are more strongly associated with sinuosity and dominant soil type while the Brazos trees are divided among dominant soil type and elevation differences. Figure 17 groups the trees by site. The division in Brazos Basin sites' influences can now be seen as: those trees influenced by dominant soil type were Hearne canopy trees; those more strongly influenced by elevation differences were Brazos Bend sites.

Table 29. Principal component analysis (PCA) of the community assemblages for the GSA, Brazos, and Colorado-Lavaca Basins associated among basin, site and abiotic factors.

Eigenvectors

(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3	PC4	PC5
DISTANCE_M	0.151	-0.686	0.277	0.109	0.623
ELEV_DIFF	-0.587	-0.085	-0.233	-0.336	0.374
steepness	-0.611	-0.022	-0.357	0.218	0.057
dominant_type	0.240	0.602	-0.202	0.352	0.643
sinuosity	-0.237	0.398	0.681	-0.449	0.192
channel(m)	-0.383	0.025	0.487	0.709	-0.134

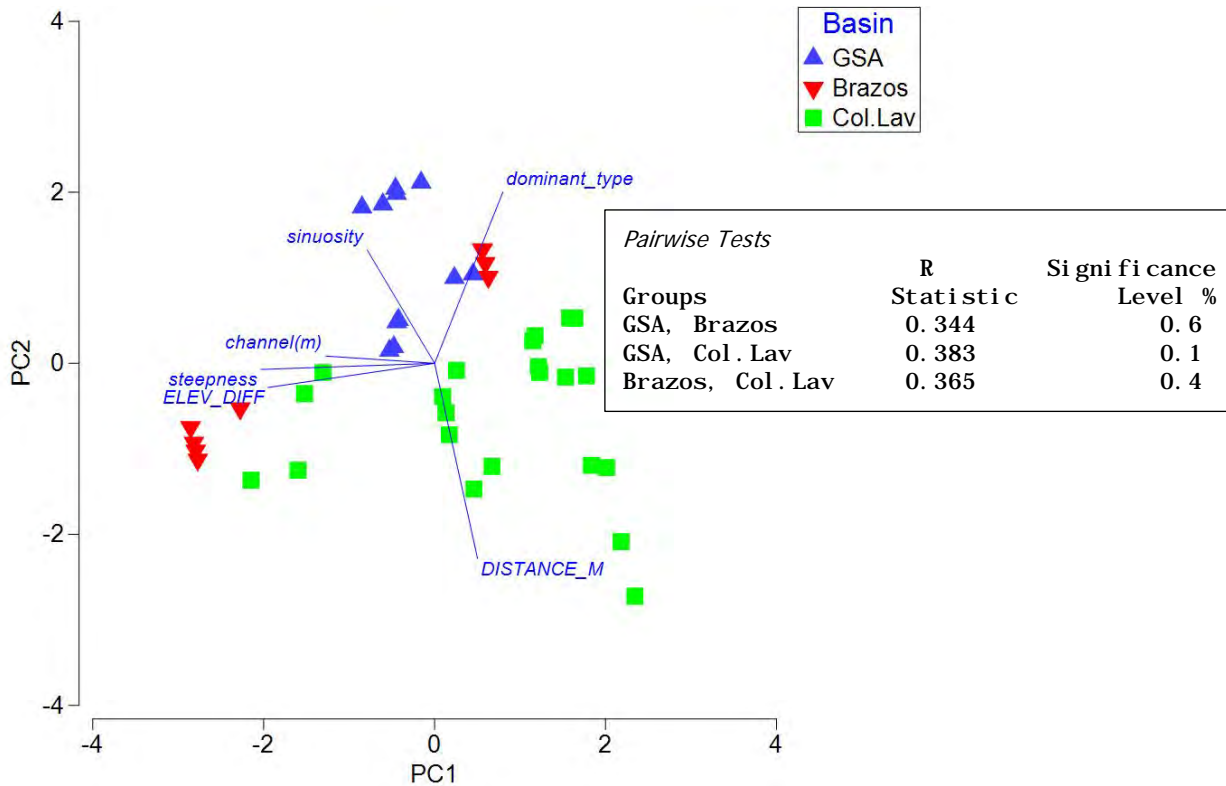


Figure 16. Principal component analysis (PCA) of the mature tree dataset for the GSA, Brazos, and Colorado-Lavaca Basins associated among basin and abiotic factors. The inset box shows the ANOSIM results; p=.1%.

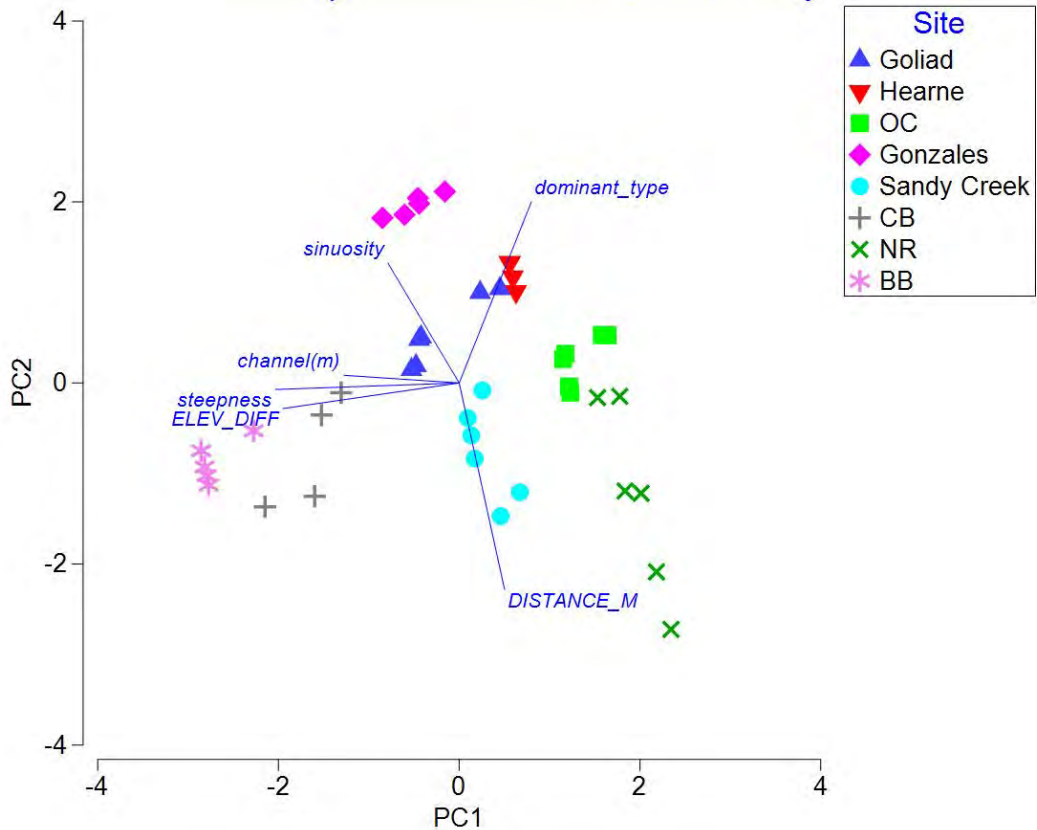


Figure 17. Principal component analysis (PCA) of the mature tree datasets for the GSA, Brazos, and Colorado-Lavaca Basins associated among site and abiotic factors.

Table 30 is a summary of abiotic variables' influence on each site. The top half of the table displays within-basin correlations; the bottom half displays all basins (across-basins) combined. Within the Colorado-Lavaca Basin low elevation was more influential than high elevation. This relationship generally held across the basins, though Navidad River showed a stronger across-basin correlation to another variable. Those sites with the greatest steepness factor (quickest rise over a set distance) were generally most influenced by it; though the Brazos Bend's correlation lessened across basins. There appears to be much heterogeneity among dominant soil types. Many sites had strong correlations with this attribute but no particular soil type appears to be most often associated with those sites. While the within-basin patterns for sinuosity seem to favor point bars over straight reaches, this relationship does not hold up across basins. Within each basin those reaches with the widest channels had the greatest influence on their stream's communities. In summary, lower elevation, greater channel width, and greater steepness had increased levels of influence on community assemblages; sinuosity and dominant soil types failed to show distinct patterns.

Table 30. Summary of abiotic influences both within each basin and across each basin. Each attribute identified in the Within Basin and All Basins Combined column is highlighted on the right. Solid lines group sites into basins.

Site	Within Basin	Elev (m)	Steepness	Dominant Soil	Sinuosity	Channel Width
Onion Creek	Elev, Dominant Soil	2	0.03	Silt/Clay	Straight	17
Colorado Bend	Steepness, Channel Width	9	0.11	Silt/Sand	Straight	88.5
Sandy Creek	Sinuosity	2	0.03	Silt/High Sand	Low Point Bar	36.52
Navidad River	Dominant soil, elev	1	0.01	Silt/Clay	Straight	24.67
Brazos Bend	Steepness,	10	0.13	Sandy	Low Point Bar	50.45
Hearne	Channel width, Dominant Soil	3	0.04	Loam	Low Point Bar	73.23
Gonzales	Channel width, Sinuosity	4	0.05	Loam	High Point Bar	41.87
Goliad	Steepness	8	0.10	Loam	Straight	25.29

Site	All Basins Combined	Elev (m)	Steepness	Dominant Soil	Sinuosity	Channel Width
Onion Creek	1 Dominant soil, 2) elev	2	0.03	Silt/Clay	Straight	17
Colorado Bend	Steepness, Channel Width	9	0.11	Silt/Sand	Straight	88.5
Sandy Creek	Relatively independent	2	0.03	Silt/High Sand	Low Point Bar	36.52
Navidad River	Dominant Soil	1	0.01	Silt/Clay	Straight	24.67
Brazos Bend	1) Dominant soil, 2) Sinuosity	10	0.13	Sandy	Low Point Bar	50.45
Hearne	Sinuosity, dominant soil	3	0.04	Loam	Low Point Bar	73.23
Gonzales	Sinuosity	4	0.05	Loam	High Point Bar	41.87
Goliad	Dominant soil, sinuosity	8	0.10	Loam	Straight	25.29

Overall these and the biotic statistics indicate that currently there is a lack of distinct correlation by community groupings, by site, or by basin to any one abiotic factor that would allow easily-distinguishable community assemblage linkages to known variables. However, this is a first effort, and improvements can be made to the methodology. Given there were distinct differences in this study's outcomes, further investigation of these relationships, using increased sampling sites and sampled plots/trees within those sites, is warranted.

**Appendix E. Texas Water
Development Board Draft Report
Comments and Team Responses**

Instream Flows Research and Validation Methodology Framework 2016-2017

Nolan Raphelt - Contract Manager

Contract numbers 1600012009, 1600012010, 1600011937

TWDB/BBASC Comments to Final Report

REQUIRED CHANGES

Thank you for the thorough review! Our Project Team Responses for Contract numbers 1600012010 and 1600011937 are provided below in Blue. Responses for Contract number 160002009 are provided under separate cover specifically associated with the Brazos River and Estuary report.

General Draft Final Report Comments:

1. The Texas Water Development Board (TWDB) is providing review comments in this document for contract numbers 1600012009, 1600012010, 1600011937. The majority of comments from the Texas Water Development Board staff, Texas Parks and Wildlife Department (TPWD) staff, The Guadalupe, San Antonio, Mission, and Aransas Rivers and Mission, Copano, Aransas Basin and Bay Area Stakeholder Committee, The Colorado and Lavaca Basin and Bay Area Stakeholder Committee, and the Brazos River and Associated Bay and Estuary System Basin and Bay Area Stakeholder Committee focused on the Aquatics section of all three reports. Reviewers generally considered the riparian sections of the reports to be the strongest aspect of this work. The riparian study design is well explained and justified, and the approach has the potential to evaluate environmental standards. The Brazos Estuary sections received positive comments considering the amount of environmental and biological data collected. Several reviewers commented that the collected data will set the stage for more detailed research designed to evaluate the ecological response to flow variation.

No response necessary.

2. Reviewers commented that the riparian research is the strongest aspect of the report. The study design is well explained and justified, and the approach has good potential to evaluate environmental standards. Essentially, this approach substitute's space for time by evaluating riparian tree species at different elevations on riverbanks. This makes sense, because trees are very long-lived, and it would be extremely difficult (and expensive, and time consuming) to track the fates of individual trees in response to an extended flow history. By knowing which flow tiers inundate various elevation tiers of the riverbanks, fairly robust inferences can be made about how trees respond. The most frequently flooded zones should support few upland tree species and be dominated by riparian specialist species, particularly young trees. Higher tiers should be dominated by stands of older trees among the riparian specialists, with young trees recruiting only under certain flow conditions that probably occur infrequently.

No response necessary.

3. As will become evident as one reads the remainder of this document, the Aquatics sections of this report will require major revision or complete rewriting prior to being submitted as a final report.

No response necessary.

4. Project Scopes of Work required three expert panel/stakeholder workshops in association with these projects. In addition, the Scopes of Work specified that the final report include both a “summary of the meetings” and a “synopsis of the three expert panel workshops.” Please summarize the results of the workshops in the body of the reports with some discussion of how they influenced hypothesis selection, evaluation of proposed parameters, sites, hypotheses, etc. Also, please include complete summaries/meeting notes as appendices.

The Guadalupe / San Antonio contract specifies that only two Expert Panel workshops were to be conducted. The Brazos and Colorado / Lavaca basins contracts both specify that three Expert panel workshops will be conducted if schedules meet certain assumptions. Those assumptions laid out in each contracts scope of work state that the first workshop for the Brazos Basin would need to occur in Spring 2016 to be effective and the first workshop in the Colorado / Lavaca basin would need to occur in Summer 2016 to be effective. Unfortunately, both these latter two contracts were not signed until Fall 2016. Text has been added to the Introduction to explain the contracting delays and resulting consequence of only two workshops.

Text has been added to the Introduction to summarize the results of both the September 8, 2016 and June 29, 2017 Joint Expert Panel workshops. Final agendas and attendance lists have been added to the Appendices. There were no written comments received by the project principals from any participants from either workshop.

5. Several reviewers commented that the report, specific to aquatics, is critically flawed in terms of the underlying sampling strategies used to test the hypotheses and inadequate analytical approach(s) to analyze the data. The aquatics are fundamentally descriptive in nature and lack even a cursory linkage to the broader literature on ecological flow regimes and expected responses in fish or macroinvertebrate communities. The report provides no inference on fish species population structure and corresponding implications on recruitment success under the different periods of antecedent hydrologic regimes covering the study (and historical data collection) period. Changes in relative abundance or density, in and of themselves, especially in light of the sampling strategy employed, do not provide adequate inference to the responses of the fish or macroinvertebrate community to the antecedent flow regime. Please respond.

The 2016 – 2017 report is a continuation of a study that began in 2013. Our 2013 – 2014 report (for GSA and BRA only) describes the study in more detail and provides context to the current report. The 2016-2017 report, therefore, should be viewed as a summary of work to date, work in progress, and preview of upcoming publications. Timeframe of

this final report (field sampling from Sept 2016 to May 2017, draft report due July 2017) only allowed time to summarize some of the major findings.

Comments on study design, statistics used, value of fish densities/relative abundances are welcomed but difficult to interpret and argue until the data are fully analyzed and assessed relative to study objectives and stream theory.

Introducing stream theory, hypothesis development, well-defined objectives, detail methodologies, statistical models used, detailed study results, and a full discussion on how our findings support or not current theory, synthesis with existing literature will be provided in upcoming publications.

As a reminder, our primary task is to develop a methodology to validate TCEQ flow standards and BBASC/BBEST recommendations. Our vision of the method to monitor the value of flow standards and recommendations will be ongoing, much in the same way water quality standards are monitored into the future. Based on findings so far with the aquatics section, we're confident that we are on the right path to provide an unbiased assessment of flow standards/recommendations.

6. Reviewers expressed concern that it is infeasible to ascertain population level responses in the fish community based on the study methodology using a 15-day lag in sampling after a pulse event. It is well documented in the literature that in riffle substrates are mobilized during an event, that recolonization and subsequent density of the macroinvertebrate community takes longer than two weeks. The report summarily ignores the implications of substrate disturbance (or lack thereof) during the sequence of sampling events. Even a cursory examination of the site hydrographs show that Phase I was best characterized as reflecting drought conditions versus the Phase II sampling during a wet period. The report fails to consider the structure of the fish and macroinvertebrate community in light of drought conditions that preceded the Phase I sampling. Even the incorporation of additional sample data from BioWest (2004-2014) fails to address the fish community structure in response to drought versus the Phase II flow regime characteristics. The methodology does not appear to acknowledge the significant potential that assessing sampling 15 days after flows were within a particular flow tier does not provide a meaningful evaluation of the flow tier without careful consideration of antecedent conditions. That approach apparently would assess a flow of 300 cfs the same regardless of whether it occurred after an extended period of flows of 5 cfs or of 500 cfs. The validity of that aspect of the approach is far from evident. It is not clear how the methodology is able to meaningfully incorporate reproduction and recruitment effects, or food supply effects, resulting from antecedent flows. Please respond.

15-day lag time might or might not be an adequate time period for fish and invertebrates at all sites. As part of methodology development, we will make adjustments based on our findings. However, "It is well documented in the literature that in riffle substrates are mobilized during an event" highlights the types of questions we are addressing. What part of the flow standards/recommendations (i.e., flow tiers: 3 per season, 2 per season, 1

per year) does the riffle bed become mobilized and what are the benefits to long-term sustainability of the fish and macroinvertebrate communities (comprised of swiftwater forms and slackwater forms)?

“The report fails to consider the structure of the fish and macroinvertebrate community in light of drought conditions”: Our work documented the fish and macroinvertebrate community during a low flow period. A large flood followed. We documented this as well and compared the two, so “drought” conditions were considered. At some of our sites, we didn’t detect much of a change; we did at other sites. Next year, perhaps flows will neither be during a “drought” period or following large-scale flooding. We’ll compare those communities to assess if fish communities changed or not. We use historical information as a context to predict directionality of change. When historical information is lacking, we can use “reference conditions”. Very similar to the approach used to assess water quality standards.

Riverine community response to various flow tiers might or might not depend on antecedent conditions before floods and before droughts. We find little value in arguing this point now, since our methodologies will document these communities under a variety of options (as nature provides them).

The validity of that aspect of the approach is far from evident. It is not clear how the methodology is able to meaningfully incorporate reproduction and recruitment effects, or food supply effects, resulting from antecedent flows. Partly because our work is in progress and it takes a long-term vision to see the big picture. Think about it this way, what is an alternative to validating instream flow standards and recommendations? Note that the question is not “alternatives to developing instream flow standards/recommendations?”.

Assume a simple example: an unregulated stream reach with variable flows. Assume the instream flow standard is set at 100 cfs for subsistence, 500 cfs for base with a one per year flow pulse of 1,000 cfs. What are the steps to validating that this recommendation will maintain a Sound Ecological Environment (SEE) (with some concept of SEE if based on historical fish collections and knowledge that the system is currently an intact and pristine system)? We don’t believe we should wait until all of the water, except for the flow standard, be taken out of the system in order to assess if sufficient to maintain SEE. Instead, we would target individual flow components:

- does subsistence flow (100 cfs) support the community for brief periods between base flows? Should it be higher or lower?
- Are base flow and the one per year flow pulse sufficient to maintain SEE. Can it be higher or lower?

Target sampling (less than, equal to, or greater than subsistence, base, high flow pulse) will provide a quantification on how fish and macroinvertebrate communities respond to the various flow components of a flow standard. Changes (e.g., adjustments [up or down] to recommendations) can be made based on community responses and our understanding of likely mechanisms before the remaining water is allocated for other

uses. Through monitoring of this river reach, reproductive success and feeding at the various flow tiers can be assessed directly and indirectly (densities of fluvial specialists are greater each year with a 1,000 cfs flow pulse than years without a 1,000 cfs flow pulse).

7. Reviewers commented that the report compiles data and develops examples of decision making scenarios based on study results from not only the Colorado-Lavaca, but also the Brazos and Guadalupe-San Antonio basins. It is not clear if results from other basins and/or locations within basins are transferable. A number of variables could influence biotic community response to flow events including the size of watershed and drainage area, number of upstream tributaries, stream morphology, temperature, length of time between pulse flow events, water quality, and others. Though the information gathered in the study is helpful in understanding the flow-ecology relationships of the stream segments studied, data is insufficient and the results are inconclusive for establishing relationships between long-term biological community change in a given stream segment and individual flow regime components. Ecological disruption after a pulse event may produce a temporary shift in community structure, but any changes as reflected in species abundance may be short in duration and not represent community equilibrium. In summary, there are concerns about the uncertainty in report analyses due to the limited timeframe, potential confounding causal factors at play, site effects, and the (in)ability to detect and attribute measurable biological and ecological responses to individual flow events. Please respond.

Initially, our vision was to validate TCEQ standards and BBASC recommendations at a few sites, in order to draw inference into the ecological responses and flow tiers among all sites (and basins). Bases for this was that BBEST and BBASC flow tiers by site were calculated without regard to stream order, stream morphology, water temperature, etc. In addition, the number of tiers recommended each season are the same, although cfs of the tier differs.

With our validation methodology, we can assess if using the same flow recommendations by site has equal ecological benefits across all sites (and season).

With two years of data, early indication is that the answer is “likely not”. Our statistical design allows us to test if, for example, densities of slackwater fishes in riffle habitats are reduced following a 1 per year flow pulse from base flow conditions (hypothesis developed from work by Minckley and Deacon 1991 and mentioned in Poff et al. 1997). We used a 3-factor ANOVA (flow tier, basin, season) to assess main effects and interactive effects. In the first round of study, we found few differences between response variables and flow tier. We also found a few basin, season, and interactive effects, but our sample sizes were not sufficient to maintain replication when exploring by tier, basin, and season.

This second round of study, we again ran 3-factor ANOVA (flow tier, basin, and season) on fluvial fish relative abundances and densities, slackwater fish relative abundances and densities, and macroinvertebrate abundances and densities, with and without Colorado

River data since we only had data for one year. These analyses failed to reject the null. However, we had more replication at site (or among similar sites) and were able to explore how communities of fishes and macroinvertebrates responded among flow tiers.

Lower Brazos River sites responded as predicted (e.g., fluvial fishes increased in densities and abundances, slackwater fishes decreased in densities and abundances), whereas upper Upper Guadalupe River and Medina River sites did not following a 1 and 5-year event.

It is not clear if results from other basins and/or locations within basins are transferable—We agree, and part of our validation methodology is to assess transferability, in whole or in part, among sites and basins. Perhaps upper reach sites respond more similarly across basins than upper and lower reaches within the same basin. We're considering these as we continue to analyze our data and as we prepare to collect more data.

Though the information gathered in the study is helpful in understanding the flow-ecology relationships of the stream segments studied, data is insufficient and the results are inconclusive for establishing relationships between long-term biological community change in a given stream segment and individual flow regime components. We agree; however, we're encouraged that we are on the right path to ultimately detect ecological benefits of flow tiers by stream reach, if and when they exist. Data are insufficient for several reasons (one year was low flows, second year followed high flows) at this point; hence, our request/proposal to continuing to gather new data.

Ecological disruption after a pulse event may produce a temporary shift in community structure, but any changes as reflected in species abundance may be short in duration and not represent community equilibrium. Or no shift in community structure. Fish and macroinvertebrate community responses following a pulse event is a fundamental question being addressed by this study. Based on Natural Flow Paradigm, we can predict that the ecological integrity of a river community depends on the natural streamflow variability with natural streamflow variability defined by BBASC and BBEST as subsistence, base, and high flow pulses in a stream reach calculated from historical central tendencies by season. Streamflow is the master variable (Poff et al. 1997). One of our objectives is to quantify how a community changes (or not) with frequently occurring but low magnitude flow pulses with or without duration (e.g., 3 per season event) and non-frequently, high magnitude with or without duration (e.g., 1 per year). Both types of flow pulses are recommended because we think they are important based on existing literature, including the same literature used to formulate the Natural Flow Paradigm. We seek to specifically address how these flow pulses affect the riverine community. Temporary shifts might or might not have a lasting effect on the ecological integrity. We (or others) can address these issues using our validation methodologies, but we need to obtain more replications per flow tiers and under a range of climatic conditions and seasons (e.g., wet years and dry years, wet years during the summer, dry year during the summer, after large floods, before and after droughts).

8. Please provide a systematic discussion of the life-span and reproductive strategies of the fish community and how these could relate to the 'response' or lack thereof observed between Phase I, Phase II, or in general given the different hydrologic regimes observed as illustrated in the hydrographs (see Appendix A and Figure 5 in the GSA and Brazos reports, Figure 6 in Colorado – Lavaca report). Based on the ecological literature, one would expect a differential response between different reproductive guilds given the large changes in both base flows and flood events between the antecedent hydrologic conditions prior to Phase II sampling. The report only provides one instance of any reference to changes in fish community based on reproductive strategy.

We chose to assess guilds based on habitat guilds (fluvial specialists vs. slackwater specialists). Within a family of fishes, there's sufficient correspondence between habitat guilds and reproductive guilds (fluvial specialists tend to broadcast spawn, more slackwater types tend to substrate spawn) that conducting analyses on both would be redundant. Through time, we plan to bundle life-history traits, eco-morphology, feeding guilds, and other traits/characteristics in order to understand how, why, and when (define the meaning of "flows are important" relevant to our basins) some species are benefited by dynamic flows, others are not, and how all of this relates to maintaining SEE with e-flow standards/recommendations. But first, we have to test validity of our predictions to determine which species are affected by dynamic flows. We think we know, hence our predictions, but now we are testing. Reproductive strategies of species then might or might not explain the how.

The report only provides one instance of any reference to changes in fish community based on reproductive strategy. Is this a reference to *N. shumardi*, *M. hyostoma*, and *M. marconis*? Likely all three are broadcast spawning fishes. We observed greater numbers following 1 per 5-year flow pulse but not at smaller flow pulses. Others (e.g., *P. vigilax* and *C. lutrensis*; substrate spawners although *C. lutrensis* has reported to broadcast and substrate spawn) were less abundant in the lower Brazos River following a 1 per 5-year flow pulse but not at other sites with a similar flow tier.

9. The methodology as discussed in these three reports appears to attempt to assess the components of the overall flow regime independent of their role as part of the overall regime. For example, under the methodology, if conditions were found to be acceptable in terms of species presence at a baseflow of 300 cfs and, separately, at a subsistence flow of 60 cfs, it appears the overall flow regime might be deemed acceptable, regardless of whether the stream being sampled had actually experienced flows limited to the regime being evaluated. In other words, just because the stream experienced those flows on particular days, sampling results do not necessarily evaluate the adequacy of the overall regime if, for most of the time during the study period and even before, the stream was experiencing flows quite different from those protected by the flow standards. That may, or may not, have been the case, but the information to understand the overall flow pattern appears to be absent from the report. Please respond.

See Response 6. Testing if the overall standard/recommendation e-flow maintains SEE (the purple line in the GSA BBEST report) cannot be conducted until flows above and beyond standards/recommendations are removed. Early on, this limitation in testing e-flow standards/recommendations was an impediment in developing a validation methodology. Assuming water is allocated to other uses and taken out of the system, it might be too late for corrective action (e.g., reclaim water previously allocated for other uses), if the e-flow standards/recommendations were not maintaining SEE. To start the validation sooner than later, we decided to assess components of the e-flow standards/recommendation. One benefit of assessing ecological values associated with flow tiers is that it would be easier to make adjustments.

For example, assume a 2 per season tier is 500 CFS and a 1 per season tier is 1,000 CFS. We categorically define a flow pulse ≥ 500 CFS and $<1,000$ CFS as 2 per season tier. One event might be 550 CFS and another 800 CFS. Using ANOVA, the treatment level would be 2 per season. However, we also assess all dependent variables vs. flow with linear regression. A response might not occur at 550 CFS but it might occur at 800 CFS. Under this scenario, BBASC has the option to increase “2 per season event” from 500 to 800 CFS.

10. The Brazos estuarine research suffers from the same basic limitation as the aquatic research in this report. The research is descriptive, with fishes, macroinvertebrates and environmental data surveyed at various locations on various dates having various discharges. This is very valuable information to set the stage for more detailed research designed to evaluate ecological responses to flow variation. But in and of itself, these descriptive data do not allow us to make decisions about the need for flows of specific magnitudes, frequencies and durations.

Please see Brazos Estuary report responses.

11. Everyone knows that more freshwater flowing into an estuary will reduce salinity and favor freshwater species to move further downstream. We know that less freshwater flowing into an estuary will push freshwater species out and allow more marine species to occupy zones further upstream. This is logical and well documented worldwide. The lower reaches of the Brazos River conform to this well-known dynamic. So, the descriptive research conducted during the first and second TWDB contracts was very informative, and shows us the species involved in this dynamic. It also shows spatial and temporal variation in abiotic environmental parameters, which is useful background information to have in order to move on to more detailed studies. However, the information gained by these descriptive studies does not allow the workgroup to make any decisions about how much freshwater needs to be delivered to the lower reaches and coast, and for how long, and when it should be delivered. This might be a value judgment, but it also likely is the case that estuarine and marine species already have extensive habitats all along the Gulf coast that is available to support stocks; whereas, many freshwater species in the Brazos River (several threatened minnow species, Alligator gar, etc.) have much more restricted geographic ranges and limited available habitats. At any rate, the study design adopted in this report fails to provide any specific

recommendations regarding the suitability of current environmental flow standards. Like the aquatics section, this section makes no attempt at specific numerical recommendations for flow components in the standards. It is difficult to perceive how this could be attempted based on the information generated for this report. Please respond.

Please see [Brazos Estuary report responses](#).

12. Issues that deserve special consideration in estuaries is the influence of river discharge on sediment and nutrient dynamics. The importance of sediment and nutrient delivery to coastal habitats is discussed with literature references included. This is an important topic, and it would be beneficial if future projects could research sediment and nutrient dynamics in the lower-most reaches of the Brazos River channel as well as coastal marshes located to the southwest of the Brazos River mouth that are supported by sediments and nutrients that wash out during flow pulses. The research reported here includes measurements of dissolved inorganic nitrogen and phosphorus, but these measurements do not allow us to understand nutrient dynamics.

Please see [Brazos Estuary report responses](#).

13. In section 3.1.1, the pre- and post- flood comparison is not appropriate, and certainly not with relative abundance data. We would be interested in seeing literature support on why an assessment between pre and post flood is not appropriate especially with relative abundance data. Minckley and Meffe (1987, used in Poff et al. 1997 to build their argument for the Natural Flow Paradigm) used relative abundances to assess differential selection of fishes by flood magnitude. We used relative abundances (along with diversity, richness, and several other community indices in Round 1 and again will be assessed with Round 2 data added) and densities to assess differential selection. This means that a high flow pulse could decrease densities of all species (fluvial specialists, slackwater specialists) but the remaining community could be dominated by fluvial specialists (assessed with relative abundances). Hence differential selection occurred.

What is needed is analysis of how prior flow history (windows of varying time spans) correlate with densities of fishes in various habitat types. We targeted riffles and runs in Round 1. This was done to assess how flowing water habitats and the fishes therein responded to flow pulses. In Round 2, we included pools and backwater habitats. We're not sure of the meaning of "prior flow history" but see additional comments below.

Even this would be a very tenuous analysis, because a sufficiently long time series of data would be needed, and those periods would need to encompass a variety of flow conditions -- intra- and inter-annual. We agree and this is included as part of our validation methodology. Before any samples were collected, we anticipated that changes in fish communities (e.g., maintaining historically-documented fish community, comprised of primarily fluvial specialist and some slackwater forms) would be easily detected with small (e.g., 3 per season) to large flows (e.g., 1 per year). But to our surprise as adherents to the Natural Flow Paradigm based on the literature and our own observations in the field, we detected few changes. When we did, as in the lower Brazos River, the level of

flow was greater than anticipated. As such, validating flow standards/recommendations wouldn't be a quick process. Our first question was what factors could contribute to not rejecting the null. Was it study design? We are open to improving our study design and analyses, but our study design worked to detect community shift towards a dominance of fluvial specialists in the lower Brazos River (more similar to its historical community). Was it "drought conditions" in Round 1 followed by massive floods at most of our sites in Round 2? Maybe, except that we didn't detect many differences at most of our sites. Is flow really the master variable at all sites? Is the Natural Flow Paradigm an accurate view of how stream fish communities are assembled and maintained? Is the quantification of Natural Flow Paradigm by BBEST and BBASC (use of flow tiers) an accurate interpretation of the Natural Flow Paradigm?

Moving forward, more community information would be beneficial until we see a wide range of water years (replicated high flow years, low flow years, average flow years). Gaining more replications is advantageous for at least two reasons. One, it can provide greater understanding of the eco-flow relationships (provide the longer term data set, but taken at the scale necessary to inform standards/recommendations). Two, it can be used as a biomonitoring to ensure that SEE is being maintained (similar to the Biological Condition Gradient; Davies and Jackson 2006).

Again, what matters to fish ecology (and river ecology in general) is not just the flow on the date of sampling (or a single date a few days prior), but the flow components (e.g., timing, magnitude, duration of flow pulses) during an extended period prior to when the survey was done. In theory, yes, but we are testing this theory (defining "what matters") across a number of sites in order to replicate. However, we are not comparing fish community to a flow on a single date. We established fish community (richness, densities, relative abundances, and many others) at base flow (usually multiple samples because we do this for each season). A rain event produces a flow event that we can categorize into one of the flow tiers (1 per season event, magnitude and duration; timing is already set by season). Flows subside back to or near base flow and we sample again to assess changes in the community (e.g., richness, densities, relative abundances of community guilds for both fishes and macroinvertebrates, feeding, reproduction). Given that the work is in progress, Round 1 had several flow pulses for various tiers (based on magnitude) but duration was not met. We still sampled because we also want to assess the effect of duration. In Round 2, again several flow tiers (based on magnitude) occurred and duration. Though we have limited replication, we can now compare community responses at a magnitude but when duration was and wasn't met. To develop an extend period, one must get started. As for the part of the previous flows that can be related to a fish community on Day X, our context is the flow standards/recommendations. Is a 1 per season flow of no value because the previous six months were at subsistence? Maybe! But the resolution of our data (quantifying communities during all flow tiers) will enable us to assess these questions.

Also, in section 3.1.1, it is assumed that "pre-flood" is the dataset from TWDB contract 1, and "post-flood" is the dataset from the 2nd contract. This comparison and terminology is very misleading. What was observed, was a relatively dry year (not a severe drought)

followed by a relatively wet year. Correct. Round 1 was a below average flow year and Round 2 corresponded with >1 per 5 year flow pulses at our sites. We'll review the report and ensure that this is understood. However, we provided 5 year hydrographs for each of our sites, so that each individual reader can generate their own descriptors of the water flows during each year.

But there were variable flows during both periods (a variety of tiers). What is needed is an analysis of the ecological processes that influence the populations of fluvial specialists that are indicators of the condition of the ecosystem. Such as? Using the lower Brazos River as an example, *M. hyostoma* and *N. shumardi* are what we considered fluvial specialists. Based on historical analyses, *C. lutrensis* and *P. vigilax* have increased in abundance within the lower Brazos River. Increases in generalist species, such as *C. lutrensis* and *P. vigilax*, are consistent with modified river flows. The exact mechanisms are unknown (successful recruitment of larvae under modified river flows, these two species are no longer displaced downstream because flow magnitudes have decreased). Ecological processes that influence populations of fluvial specialists are largely known (enough to develop the Natural Flow Paradigm and instream flow recommendations), although there are gaps in the understanding. It is time now to directly test the relationships. Thinking about and considering various processes have merit. However, this study concentrated on the direct relationship between aspects of flow (e.g., base, flow pulses) and biota using the standards/recommendation as context. With this structure in place, we have the ability to continue assessing and considering all of the processes that lead to observed patterns because we are now documenting the patterns at the appropriate scale.

There is no need to worry about the status of red shiners or green sunfish, for example. Actually, most of the common species that were the focus of the analysis are not good indicator species. The research should have targeted the fluvial specialists, as was advised by various environmental flow experts and many scientific and agency reports. We target all species within the fish community, fluvial specialists and otherwise. We disagree with "no need to worry about...red shiners". Fluvial specialists might obtain very little from flow pulses, but the community stays intact because the flow pulses negatively affect the non-fluvial specialists (i.e., differential selection). Understanding how some fishes are negatively affected by flow is equally as important to understanding how some fishes are positively affected by flow.

Community-level analyses could be useful for tracking the status of rivers over the long term - over decades – to determine if major changes to the flow regime have caused significant shifts in the fauna (such as the Sabine River below Toledo Bend Reservoir where it was shown that *Cyprinella lutrensis* has largely replaced *Cyprinella venusta*, etc.). Please respond.

E-flow standards/recommendations are set and will be used into the foreseeable future. Are they doing the job as intended (maintaining SEE)? As long as we have e-flow standards/recommendations, we should be monitoring to ensure that the intentions are being met, similar to water quality standards. We can't just be satisfied with producing

standards/recommendations, no matter how much time we put into the development. What if we are wrong? What if we were right? Documenting this is the logical next step and one mandated by the SB III process.

Community and population level analyses are useful and currently are being done within our validation methodology. We're a bit confused by some of the comments. For one, isn't *Cyprinella venusta* replacing *C. lutrensis* in the Sabine River? This interaction, if true, might reveal greater understanding of the eco-flow relationships and sounds like a good indicator species/relationship for assessing Sabine River standards and recommendations. Second and previous to this statement, we were advised to 'not worry' about Red Shiners (*C. lutrensis*). This underscores the need fully understand how our riverine communities (fishes and macroinvertebrates) are responding to flow tiers, using the context of the SB III process to provide replications within and between basins for flow tiers.

14. In section 3.1.1, all the graphs show virtually no relationship with flow tiers, which is what would be anticipated given the approach taken. Significant correlations would not be expected when the analysis is done in this way. We are interested in reviewing any supporting evidence for this claim? During early stages of proposal development and expert science meetings, we anticipated an effect. Flows are "important" in maintaining SEE. At some flow tier (e.g., 2 per season, 1 per year), something (e.g., increases in fluvial specialists, decreases in slackwater forms) would be detectable. We found few effects. So, maybe it was basin dependent or season dependent (we tested these). Now with two rounds worth of data, we had enough replication as sites (upper reach sites GSA, lower reach sites GSA, upper reach sites BRA, lower reach sites BRA) to look reach/site scales. Lower Brazos River fish community responded as predicated (a change was detected). No change was detected among several of our other sites, despite a >1 in 5-year event. Very surprising, but now we are in a much better position to understand why predicated changes were not detected. Our steps are consistent with typical analyses.

What needs to be examined is the flow conditions during periods of appropriate length that precede collection of a biological data point, and the best indicators of ecological response would be processes such as fish reproduction, recruitment (survival of young), foraging success and growth rate. We are quantifying various aspects of reproduction, recruitment, and foraging success in context of the flow standards/recommendations. We assessed this in Round 1 and again in Round 2. What we reported for Round 2 was our community level assessment because of the high flow events (>1 per 5 year) between Rounds 1 and 2. Rather than look at subtle differences in the communities (e.g., foraging success), we were anxious to see if the fish and macroinvertebrate community differed before and after the large flood events. If they do not (but they did on the lower Brazos River), then the opportunity gives us a chance to understand why and what other factors to quantify in order to assess e-flow relationships. Or, flows are not the master variable in maintaining SEE, which is logical in some of our upper reach sites, especially upper GSA where groundwater contributes to majority of the surface flows.

“Appropriate length” What is the appropriate length and how would one start to think about this? We are relating community composition (and feeding, reproduction, etc.) to previous events, such as a reach under base flow for >45 days, and a reach following a flow pulse within 15 days return to base flow. Does community composition matter that the flows prior to base (or whatever flow tier was related) were at subsistence or had a >1 per 5-year event? Maybe, but there is a way to know. Converting our validation methodology into a biomonitoring protocol, we would have sufficient replication to assess preceding flow conditions through time.

When there is a high flow pulse, fish move around to seek the appropriate habitat given the options presented by environmental conditions. What we know and what we think we know can be different. I would like to see quantification of “fish move around”. I suspect some fluvial forms seek out flow refugia (near the banks, we’ve observed this before). However, Minckley and Meffe (1987) and many others report a wash out of some species. Given a regional species pool, slackwater (or maybe tributary forms) can reinvade but the time scale is important. Much like a fire through a forest. Regional species pool dedicate what returns but the length of time and repeatable of fire are selection processes associated with a community in time and space.

Fish may be absent from a riffle during one day, but return several weeks later when conditions improve. We are quantifying this. In the lower Brazos, Red Shiners and Bullhead Minnows are returning but slowly and over a period of a year. Central Stonerollers have not returned to riffle habitats in upper GSA within a year following >1 per 5-year flow pulse. Fish communities are dynamic through time and space, attributed to many factors. We’re attempting to understand the variability of communities and species attributed primarily or in part to flow events. Once patterns are documented among flow tiers (and not simply thought to occur in a certain fashion), then we can explore and test specific mechanisms. Take a flow river reach and build a dam. Fish community will change upstream from the dam. Slackwater species become more abundant, swift water specialists become less abundant (at least some, but not all). Why? Is it related to lack of flow variability? If so, how? Instead of building a dam, dewater the stretch to <75% of base flow. Are riffle fishes simply moving around and we can’t find them, hence low densities and low relative abundances? Or, did processes change (abiotic and biotic—competition with slackwater species) and species vacate the reach through dispersion or death?

If all fishes simply move to flow refugia during a high flow pulse then return within a week or so (i.e., no differential selection as suggested so far by our upper GSA and BRA sites), then perhaps our thoughts on the value of flow pulses are incorrect and the flow standards/recommendations are unnecessary. Through time, we can address these issues with our validation methodology.

The same is true for other kinds of habitats. And some species recruit strongly in oxbows and other kinds of slackwater habitats, and then enter the river channel following a high flow pulse that connects habitats. They may not seem abundant during the high flow conditions but they will appear in certain habitats in greater numbers when flows decline.

For clarification, we are not sampling during a high flow pulse. We sampled at base flows once the flow pulse passed.

So, this analysis cannot deal with such dynamics, because it only examines fish abundance and flow conditions on a single date at a given site (and it is unclear how that single date was selected to characterize flow rate – this is discussed further below).
Correction: fish and macroinvertebrates densities and abundances are quantified during subsistence and base and following flow pulses, involving numerous dates, sites, reaches, and basins. For the above scenario (slackwater type that uses oxbows or slackwater mainstem habitats for spawning, as an example gar), we can deal with this dynamic using validation methodology protocols, allowing quantification with sufficient replication. Through time, we could theorize based on available literature that gar populations are benefited by having access to oxbows more so than if gar only spawn in slackwater habitats of a mainstem. Flow pulses of 1 per year (for example, this is known but simplifying for this example) allow gar access to oxbows during the spring/early summer. Prediction could be that more juvenile will occur during late summer in the mainstem lower Brazos River during a summer with >1 per year high flow pulse event, than in summers with <1 per year high flow event (no access to oxbows). We would need this to be replicated and it might take many years to adequately “replicate” (more rivers would be better, but we could replicate the same reach through time), given that we don’t have complete control of flows in the lower Brazos River. So we would target sample years in late summers with spring/early flows <1 and >1 per year. Target sampling to document flow tier effects (using the common language of standards/recommendations) is what we are doing.

Abundance data are very difficult to standardize in rivers with conditions that change with flow level. We agree and the reason why we allow flows return to base (or near to base, we still exploring how close to base we can sample) to avoid dilution effect.

A change in local abundance doesn't mean the population has declined or increased in abundance – the fish move around. If true, then how do some fishes become extirpated by rivers and reaches of rivers? In the lower Brazos River, historical community analyses indicate *N. oxyrinchus* comprised 22% of the fish community (1939 – 1969), 4% of the fish community (1970 – 1994), and 0.04% of the community (1995 – 2006). One possible explanation is that this is normal dynamics of a riverine community. Another is that the population is declining. Will *N. oxyrinchus* bounce back (supports normal dynamics) or not (supports a true decline)? However, there’s plenty of literature support that documents extirpation events in other reaches and for other stream fishes. On a smaller scale (within a year), how and why fish and macroinvertebrate communities (including species) change relative to flow (within and among subsistence, base, high flow pulses) are our primary questions. If communities do not change (or bounce back quickly), then what are the values of dynamic flows to aquatic organism? Next question, how would one test the other values (thinking about and stating likely values are different than testing them).

But each species needs certain habitat conditions all the time, and the flow regime must provide for those. We generally agree with this statement. A species of fish will need a few things in order to live (water sufficient to support physiological processes, such as enough oxygen or within their temperature tolerances). Additional requirements depend on life stage, reproductive strategies, and feeding guilds. However, our validation methodology is not designed to assess what each species needs. We are not attempting to create a zoo, where all fishes have the “right” flow regime to provide the “right” habitat conditions all of the time. Instead, we are assessing if flow standards/recommendations maintain SEE, meaning that some fishes will be positively affected by high flows and others will be negatively affected by high flows. In this way, we maintain the natural heterogeneity found within a basin, since not all species are homogeneously distributed within all reaches of a basin.

Please explain how data collection and analysis procedures account for changes in fish location when computing fish abundance. [Discussed above.](#)

15. In section 3.1.1 of the Colorado – Lavaca basin report, a suggestion that a more robust data set is needed to analyze flow-ecology relationships seems appropriate. Data currently available is insufficient/inconclusive to make recommendations for changes to the environmental flow standards or to suggest a valid strawman for any changes.

We agree with this statement at this time. We only have one-year worth of data for Colorado-Lavaca basin and two years for GSA and BRA. However, flow standards/recommendations (with a few exceptions) are about the same in all three basins. Given this, part of our validation methodology is to assess ubiquity (or the lack thereof and why) of processes (flow tiers) and patterns. Conducting this work in multiple basins will help to understand the ubiquity or not, so we do agree that a more robust data set is needed. If we find value to, for example, a 2 per season flow event at all sites in GSA and BRA, then this can be used, if only by some, to inform the value of 2 per season flow event in the Colorado-Lavaca basin.

16. Changes in apparent abundance of *Dorosoma petenense* could be a result of these fish migrating into the river from floodplain habitats or from the mainstem river. This issue of lateral connectivity was not examined in this report. Even though lateral connectivity was not studied under this contract, this issue remains relevant to interpretations of patterns from surveys conducted exclusively in the river channel. There is considerable information about lateral connectivity and flows for the lower Brazos, most of which was discussed and referenced in the Brazos BBEST recommendation document that was cited in the final project report. Please discuss how results may have been influenced by lateral connectivity.

The report includes a paragraph on the increase of *D. petenense* within the upper reaches of the Navasota River. We pasted the paragraph below. Wash ins, which lead to a change in the riverine communities, were observed and can have a confounding effect on our study results. We would predict that *D. petenense* densities and abundances would be less after a high flow tier, which tier is to be determined. However, we observed an

increase but only at one site. Through time and based on our observations before the high flow pulse, we predict that *D. petenense* relative abundances will be lower. Perhaps being flushed into a small stream habitat and outside of the reservoir could be a sink.

This is the type of information that we are attempting to document and quantify—how fish and macroinvertebrate communities change across flow tiers.

As for lateral connectivity in the lower Brazos and Guadalupe rivers, changes in fish communities could be attributed to wash ins at low magnitude flow pulses from oxbows (assuming oxbows are connected at this point). We are mindful of this as one of several possible mechanisms involving why our findings might not support our a priori hypotheses. To date, we've analyzed patterns in abundant fishes per reach. As our work is still in progress, we still might detect a likely lateral connectivity influence on the mainstem fish community following flow pulses.

“In the Navasota River, a “wash in” event was observed. *Dorosoma petenense* was not observed at the Navasota River – Easterly site between August 2014 and March 2017. Following a >1 per 5-year event, *D. petenense* comprised 94% of the fish community. Source of the wash in was likely Lake Limestone, located upstream of the Navasota River site. The observation is relevant for tier validation methodologies in that displacement of some fishes (e.g., wash out of slackwater fishes) is expected with high flow pulses but might be compensated by increases of some slackwater fishes by a wash in.”

17. In Section 4.1.4, the statement in the paragraph below Table 24 seems too bold, and their veracity could be questioned. Nowhere in the report are results showing that,

“Direct ecological responses of fish and macroinvertebrate communities and fluvial specialists were detected with respect to flow tiers in the 1-per-season and >1-per5-year event categories.”

Please see Summary under Aquatic Biota section. Statistical tests are provided to support this statement about 1 per season and >1 per 5 year events.

The scatterplots showing taxon density or relative abundance in relation to flow all had large scatter revealing little relationship.

We agree, except for the relationships reported in the above sentence.

Also, it is important to bear in mind that patterns of correlation are not equivalent to evidence of causation between one variable and another. A strong relationship in such plots does not allow one to infer that the taxon does or does not benefit from higher or lower flows on the date of the survey, or a date during the 15-day interval prior. Please respond to these concerns.

Our work and procedures are more than “correlation”. We’re using a scientific methodology to advance knowledge and understanding.

Here is our approach:

SB III process used theory to establish e-flow recommendations. Specially, the Natural Flow Paradigm (Poff et al. 1997): ecological integrity of river ecosystems depends their natural dynamic character. Or dynamic character “causes” ecological integrity to be maintained. Side note: Theory does describe causation and can be bold.

One measure of ecological integrity: densities and abundances of fluvial specialists

Dynamic Character: maintained with e-flow standards/recommendations: subsistence, base, and several high flow pulses.

If Natural Flow Paradigm theory is correct, then we predict that a fish community dominated historically by fluvial specialists will show a positive relationship (at least with relative abundance) with flow tiers, realizing that a wash out might occur.

We tested this prediction and other aspects (e.g., single species) of this prediction.

Due to the current lack of replication within the Brazos River basin, we were limited to assess pre-flood fish communities versus post flood fish communities. Therefore, tested relative abundances and densities with a t-test (or one factor F-test with only two levels of a single treatment).

Using fluvial specialist *M. hyostoma*, relative abundance increased ($F_{1, 18} = 8.5, P < 0.01$) and densities increased ($F_{1, 18} = 5.3, P < 0.03$) between pre-flood and post flood (about 150,000 cfs went through the systems and flows stayed elevated for about a year). Therefore, we detected responses. Our results supported, or were consistent with the theory. This is not a bold statement.

We cannot control nor are responsible for what “one” can or cannot “infer” from our work. Even at times with overwhelming support for various scientific theories, some remain unconvinced. Being critical and unconvinced has merit. Even adherents of a theory can still be skeptical. This is the strength of science...not everyone has to agree on the processes responsible for observed patterns.

However, we are interested in hearing all view points and encourage all to continue this discussion. Specifically, what evidence would convince you that the e-flow standards/recommendations are necessary “as is” in maintaining SEE? Note that our work is not to show benefits of high flow pulses. We’re past this because the standards/recommendations are in place. But rather, our work is to show value of the specific standards/recommendations (and above and below, so adjustments can be made), which explicitly defines the different types of high flow pulses giving all of us a common language.

A priori predictions and testing with replication (meeting basic experimental design requirements) will provide the information necessary to support or change current e-flow standards/recommendations. Should we be assessing other dependent variables? Probably so and happy to discuss all suggestions. Will the standards/recommendations be changed based on evidence generated by our work? Some will say yes and others will say no. According to the SB III process, change or not begins with BBASC.

18. Section 4.1.4, the statement that freshwater mussels might be a better indicator than fish or aquatic insects seems a weak excuse. This project could have focused more intensely on those fish and aquatic insect species that are fluvial specialists, and therefore known to be sensitive to changes in flow regime. Please note again the reference to "flow regime" which implies the various flows that occur during various time intervals leading up to a given survey date, and not the single flow recorded on the survey date or a few days prior. This is an important point, because species that are opportunistic strategist, or r-strategists, can persist in systems with frequent high flow pulses because they are good at recolonizing disturbed habitats where species that are superior competitors have their densities reduced periodically. The simple correlation method employed under this contract for the aquatics component has very little capability to discover such relationships. Please respond to these concerns.

With the TWDB required deletion of Sections 4.2 and 4.3, Section 4.1.4 was considerably shortened. This modification resulted in the deletion of the statement of concern referenced in this comment.

19. The Sections, 4.2 and 4.3, shall be removed from the report. They do not materially address the validation approach needed to assess the efficacy of an ecological flow regime. Another reason these Sections should be removed is a review of over 200 journal articles revealed consistent evidence that fish are sensitive to changes in flow regime. When flow regimes change: fish abundance, assemblage structure, and diversity were all negatively affected by both increases and decreases in flow regime components. Fish responses were also negatively affected by reductions in discharge and by both increases and decreases in frequency of high-flow events (Webb et al, 2013).

A large number of studies do report the relationship between high flow pulses and changes in fish communities. Hence our surprise when our work failed to detect many changes!

So why the disconnect? We're still pondering this, but here are a few items to consider:

1) most of the studies are observational and lack sufficient replication. Often, we're sampling the aquatic communities and a big flood occurs. We document pre and post events and surmise the value of the flood pulse in maintaining the community.

Among science literature, there is a difference between "here's what we saw" type publications vs. "here's our theory, our predications, properly replicated and how our

predictions support or not the theory”. How many of the 200 mentioned journal articles tested predictions with replication? Consider the value of Poff et al. (1997) The Natural Flow Regime—paradigm for river conservation and restoration. A valuable resource for adherents of the Natural flow Paradigm. How does one know if ecological integrity of river ecosystems depends on their natural dynamic character? How does one know if BBEST/BBASC/TCEQ adequately captured “natural dynamic character” with their standards/recommendations?

Poff et al. (1997) synthesized compelling information to support keeping dynamic river flows in river (low flows, high flows if all part of their dynamic nature). It’s a great theory, but where has it been tested with replication among the previously mentioned 200 journal articles?

2) Maybe our experiment design is not sufficient to detect changes. However, we found that “fish are sensitive to changes in flow regime”, and “fish abundance, assemblage structure, and diversity were all negatively affected by both increases and decreases in flow regime components” in the lower Brazos River with a >1 per 5-year flow pulse.

Why did our experimental design not work at other sites? Statements like “fish are sensitive to changes in flow regime” and “fish abundance, assemblage structure, and diversity were all negatively affected by both increases and decreases in flow regime components” underscore our collective problem with the lack of a common language. So far, we’ve demonstrated that fishes are sensitive to changes in flow regime (>1 per 5-year in the lower Brazos) and have not detected a sensitivity to changes in flow regime (3 per season flow pulse in the lower Brazos). Are these conflicting statements? No, if we use a common language and recognize that not all flow pulses are equal. They differ in magnitude, frequency, and duration. Among the 200 mentioned journals, what were the range of flows where fish community changes were observed?

3) A number of the community-flow relationship articles are conducted downstream from a dam and in areas of extensive anthropogenic alterations. We’re working in areas with minimal to moderate levels of anthropogenic alterations based on historical assessments (parts of the GSA and BRA) and based on reference sites (regional IBIs). Perhaps “flow is the master variable” and “dynamic character interpreted to be a series of high flow pulses” aren’t accurate at all sites and basins within the range of conditions observed and with the current fish community. Thinking about hierarchical nature of habitat associations, suppose the breadth of flows are minimal okay to support the current fish community. Seasonal flow pulses at various magnitudes, timing, and duration might have little regulatory benefits.

With our validation methodology, we are testing specific predications. As more contextual monitoring continues, we’ll have a better grasp on how flow tiers support SEE, but our techniques will enable us to develop other theories on how processes affecting patterns in our fish communities.

With two years of data, we're not ready to reject that the Natural Flow Paradigm is inaccurate. We are excited to build and modify our understanding between aquatic communities and flows. We encourage others to become involved as well, because the proper management of our aquatic resources depend on the exact nature of flows and biota. Develop new studies that can benefit the BBASC and TCEQ standards/recommendations using the existing structure. See for yourself if and when a community changes with recommended subsistence, base, and high flow pulses.

The validation procedures offer no guidance on how to pick flow regimes that do not cause changes in fish abundance, assemblage structure and diversity or how determine what the resulting loss of fish abundance, assemblage structure and diversity will be with selection of a particle flow value

Correct. Our validation methodology is not designed to offer guidance on how to pick flow regimes. It was designed to validate the established flow regimes.

In our opinion, this commenter appears to have been expecting a predictive ecological model, not a TCEQ environmental flow standards assessment tool. The project team feels that the assessment tool approach was laid out in the Round One final reports for the GSA and Brazos basins, discussed at both Round Two expert workshops, and presented in detail in all three Draft Reports provided to TWDB on August 15, 2017. The project team never intended to develop nor did the TWDB approve scopes of work referencing a predictive tool capable of offering guidance on "how to pick flow regimes that do not cause changes in fish abundance, assemblage structure and diversity or how determine what the resulting loss of fish abundance, assemblage structure and diversity will be with selection of a particle flow value."

The project team does not disagree that the literature proffers that flow regimes are important to aquatic communities. Where the literature is limited or often silent is on specific ecological responses that can be tied to specific flow tiers. The assessment of the individual components of a "flow regime" was the goal of this project.

Finally, per TWDB's requirement, the sections 4.2 and 4.3 from the GSA Draft Report (included at the conclusion of these responses) were removed in their entirety. The following text was inserted in the main body of Chapter 4 of the report to replace the entirety of Section 4.2 and 4.3.

"The validation methodology assessment tool introduced in the Round One study, highlighted in Round Two Expert Workshops, presented in detail in the draft Round Two report, and subsequently presented to both the Brazos and GSA BBASC's upon completion of the draft report has been removed from the final report as a TWDB requirement. It is TWDB's professional judgement that insufficient data is available to validate the tool, and thus any practical application of this tool at this time is inappropriate."

20. Throughout the report there are occasional use of terms that are either ill-defined, or used in a way that is confusing or potentially redundant. Examples are: inadequate replication of ecological indicators, response variable, aquatic mechanisms of high value, and hypotheses vs. predictions. These terms need to be defined and where overlapping, explained.

We reviewed the document and looked to improve clarity where practical.

21. The term "direct ecological linkages" is used frequently in this report. This terminology is quite vague; please define "direct ecological linkages" in terms of something the reader can clearly understand.

To reduce confusion "Direct ecological linkages" was uniformly changed throughout the final document to "ecological response" which references a biological response to an environmental driver, in the case of this report that driver being "flow".

22. Please include the following statement on the front cover of each report:

PURSUANT TO HOUSE BILL 1 AS APPROVED BY THE 84TH TEXAS LEGISLATURE, THIS STUDY REPORT WAS FUNDED FOR THE PURPOSE OF STUDYING ENVIRONMENTAL FLOW NEEDS FOR TEXAS RIVERS AND ESTUARIES AS PART OF THE ADAPTIVE MANAGEMENT PHASE OF THE SENATE BILL 3 PROCESS FOR ENVIRONMENTAL FLOWS ESTABLISHED BY THE 80TH TEXAS LEGISLATURE. THE VIEWS AND CONCLUSIONS EXPRESSED HEREIN ARE THOSE OF THE AUTHOR(S) AND DO NOT NECESSARILY REFLECT THE VIEWS OF THE TEXAS WATER DEVELOPMENT BOARD.

The required text above was inserted on the front cover of each report.

Specific Draft Final Report Comments

1. Executive Summary, 1st page: Text says:

"Stream flow characteristics were quantitatively defined by a computer program (Hydrology-Based Environmental Flow Regime [HEFR]) to calculate mean magnitude and duration for each flow tier (e.g., subsistence, base, high-flow pulse) for a river reach."

HEFR considered magnitude, but not duration, aspects related to subsistence and base flow tiers. Please revise to more accurately portray the computation procedure used and output produced by HEFR.

Text was modified to state, "Stream flow characteristics were typically quantitatively defined by a computer program (Hydrology-Based Environmental Flow Regime [HEFR]) for a river reach."

2. Executive Summary, 1st page (Brazos Report Only): The text states:

“Typically, when data gaps or uncertainty arose, hydrological surrogates were used as placeholders in accordance with the Natural Flow Paradigm.”

As noted by reviewers, this may perpetuate a misconception regarding the influence of the Natural Flow Paradigm on the SB3 process and the adopted flow regimes. The Natural Flow regime paradigm is a statistical analysis of pre-regulation/minimally altered stream records that can be used to identify the most important characteristics of the flow regime, which have created, over a long time frame, the geomorphic and ecological systems upon which the biological community developed, and these statistics can be used to estimate the magnitudes, durations, frequencies and timing of critical components of the flow regime that should be protected from future diversion, if the goal is to ensure a sound environment. The “hydrological surrogates” used by the Brazos BBEST were derived from heavily regulated records, in most cases where the majority of the record occurred after more than 50% of contributing drainage area had been impounded by upstream reservoirs. Of the eight sites selected for analysis in the current study, only one (Lampasas River near Kempner) should be considered as having a pre-regulation/minimally altered stream record in accordance with the Natural Flow Paradigm. Procter Lake, constructed in 1963, impounded over 50% of the drainage of the USGS gage Leon River near Gatesville, whose flow statistics were based on 1951–2010 records. Belton and Lake Limestone, and other reservoirs, had similar effects on the flows recorded at USGS gages on the Little River near Cameron and Navasota River near Easterly, respectively. The mainstream gages on the Brazos have been altered by major projects on the Brazos including, Possum Kingdom, Whitney and Granbury which have impacted more than one-third of their drainage areas for most, if not all, of the periods of record for which there is historic flow data. Please note in the text that flow data used to calculate hydrologic surrogates included already “altered” flows and that the process was therefore not strictly an application of the Natural Flow Paradigm.

Text was modified to state, “Typically, when data gaps or uncertainty arose, hydrological surrogates were used as placeholders.”

3. Executive Summary, 1st page: Text says,

“However, the limited time frame of study resulted in inadequate replication of ecological indicators across flow tiers and seasons to complete the analysis [emphasis added].”

The use of “ecological indicators” here is confusing. It is believed that “ecological indicators” in broad scientific use is generally used to refer to a measure of either ecological status or function, such as abundance, health, reproduction. Please clarify if the authors are really referring to an inadequate number of samples to adequately examine presumed ecological relationships.

Ecological indicators are our dependent/response variables. Inadequate replication means that we had insufficient replication ($N < 3$) for a flow tier in a season.

4. Executive Summary, 2nd page: Text states:

“Overall, the greatest shift in fish communities was observed between pre-flood and post-flood in the lower Brazos River.”

This finding does provide some validation to the concept that flow regimes can impact biological communities and it may be useful to analyze the conditions, both hydrological and biological, that preceded these time frames to better understand how these responses conform or do not conform to outcomes that would be expected by general aquatic theories.

We agree.

The use of the labels “pre-flood” and “post-flood” should be reconsidered. Especially “pre-flood”, which since the system had not yet experienced the flood, is not particularly informative with respect to the collections/observations. A better approach would be to use the concepts used in SB3 flow standards which include different recommendations for subsistence, dry, average and wet conditions and consider what states are best defined by the antecedent flow priori to collections. An important hypothesis of SB3 was the need for variability in both base flow and pulse requirements. Please respond as to the merits of a pre- and post-flood approach as opposed to a ‘subsistence,’ “dry,” “average,” and “wet” approach.

We did both (and mentioned previously in our responses). In the results section, under Riffle habitats, we state “Patterns in relative abundances for slackwater fishes, moderately swift water fishes, and swift water fishes in riffle habitats were not detected ($P > 0.05$) among flow tiers or discharge (Figure 1).”

Now with more data, we have some replication to assess community responses at smaller groupings. We provided findings “as usual” (i.e., by flow tier) when significant (example: “Density differed among flow tiers for *M. marconis* ($F_{1, 10} = 15.1, P < 0.01$) with densities at 1 per season tier greater than base”). As mentioned in this comment, we assessed pre-flood vs. post flood fish communities, regardless if statistical differences were detected or not. We feel these labels are appropriate because 1) they are accurate descriptors of the events, and 2) to emphasis community change did or did not occur following >1 per 5 season events. If changes in the fish community did not occur at 50,000 cfs in the lower GSA, then why would we expect a change at 10,000 cfs (for example, as in a smaller magnitude but more frequent high flow pulse). Something we’re still pondering.

5. Executive Summary, 2nd page: Text states:

“The 1-per season flow pulses are within the cfs range for the Texas Commission on Environmental Quality (TCEQ) environmental flow standards...”

The environmental flow standards specify a single flow trigger for 1-per season pulses. Please clarify the meaning of the phrase “cfs range.”

Text was modified to state, “The 1-per season flow pulses are less than overbanking conditions, and thus within the range of flows considered by the Texas Commission on Environmental Quality (TCEQ) when setting balanced environmental flow standards. Flows that resulted in overbanking or higher levels of flooding were typically not considered by TCEQ.”

6. Introduction: In order to compare fish densities in habitats, the surveys must be conducted under the same flow conditions so that the collecting gear efficiency is comparable between surveys. Ideally, all surveys would be conducted under very similar base-flow conditions. Then data analyses can examine how fish densities in those habitats were influenced by the flow regimes during the days and weeks prior (variable time windows can be analyzed). This is the only way to standardize the surveys.

We agree. All collections were made at base flow condition. We’re evaluating how far above base flow (but below the next flow tier) that can be assessed without dilution effect. This will give us greater ability to sample before the next flow tier occurs.

One cannot make inferences about the quality of the environment for fishes within a given area of stream channel based on fish surveys conducted under very different flow conditions.

We are not sampling while a flow pulse is occurring.

This is because the amount of habitat changes, the relative locations of habitats shift with flow conditions, and fishes move around to seek the conditions they need depending on flow conditions and the distribution of habitats in space and time. For example, during a high flow pulse, most fishes will abandon what used to be a shallow run habitat (which is now a roaring torrent of water) and move higher up the littoral zone to find current velocities, depths, and substrates that allow them to survive. The fishes do not disappear during these high flow pulses, they simply move around. They return to their preferred habitats, often at a different location, when the flow pulse subsides. Of course, some fishes spawn during high flow pulses (gars, certain minnow species), and they move to particular areas to do so. Other fishes spawn during base flow conditions (e.g., sunfishes, bass).

We would like to review your evidence to support these statements. Or, is this a conceptual model on what you believe will occur? Our conceptual model differs from your model. With our narrative, we’re predicting that fish communities will change with flow pulses, maintaining high abundances of fluvial specialists in the system (and suggested based on historical assessments...fluvial specialists will dominate).

Furthermore, we predict that removal of all flow pulses will not maintain SEE in our river reaches.

Under your above described scenario, one predication might be that the community will not change across flow tiers. Fishes of a community are temporarily displaced but will return in equal abundance as before the flow pulse. Other factors are responsible for the heterogeneity observed in fish communities along a longitudinal gradient within and across drainages (headwaters to gulf).

Based on what we've observed so far, both of our conceptual models are wrong. Or, both models are correct but it depends. Or, we haven't seen enough to tentatively accept one conceptual model over the other. We're leaning towards "it depends and more information will be beneficial so we understand what is influencing why some communities change and others do not with flow pulses". Maybe high flow pulses benefit lower reaches of rivers and not so much headwater reaches.

The methods section describes that this project's aquatics surveys were conducted under subsistence and base-flow conditions. When there was a high flow pulse, surveys were conducted only after the flow had fallen back to base-flow conditions, after a period of 1-15 days. Confusing...why state (above) "One cannot make inferences about the quality of the environment for fishes within a given area of stream channel based on fish surveys conducted under very different flow condition", then acknowledge here that surveys were conducted only after flow had return to base flow?

Presumably a given sample was associated with the peak of the previous flow pulse, but it is very unclear how samples were matched with a single discharge value (an associated flow tier). Our procedure is described in Methods. As an example, flow reached 7,000 cfs (which was classified as a one per year event). We waited until flows reached base flow before sampling. It was a little bit tricky after >1 per 5 year events occurred. Base flows were not reached before several smaller flow pulses went through the system. Here, we chose to represent the highest flow pulse observed between our sampling events. Therefore, our first sample in the lower Brazos River (and GSA) was linked to the >1 per 5 year event. Since we are developing a methodology to validate (along with validating), our procedures are not set in stone. One could argue that our first time to sample lower Brazos River should be tied to the most recent flow pulse observed (3 per season event) than the >1 per 5 years event. We would disagree with this for several reasons, but there is always flexibility in our approach.

At any rate, the reviewers feel it is not appropriate to analyze fish or macroinvertebrate abundance data in relation to a single discharge value, whether that value was recorded on the date of the survey or a certain date within a 15-day window prior to the survey date.

Addressed above. Analyses using relative abundances are established in the literature. And we used densities because we realize that relative abundances have limitations.

Leaving aside for a moment the issue of whether or not abundance at a given site is a good response variable for making inferences, what would be required is analysis of the flow regime during longer periods prior to the survey. Please respond

What in particular would be analyzed in the flow regime for longer periods? We could create a large number of summary statistics. So, what do any of the reviewers want to see and how would it relate to our findings? Even a simple example would be beneficial to understand the concern. All reviewers have access to USGS stations used in this study. We'll be happy to share our data with anyone wanting to "analyze flow regime during longer periods prior to the survey".

7. Introduction: Reviewers noted that the report does not discuss one of the most problematic issues that were raised by reviewers and other participants at the Expert Workshops: the fundamental difficulty of using biologic field data in research. All the Predictions made herein rely on an approach to relate biologic state variables (abundance, diversity, and etc.) to the single abiotic variable of flow condition as was present at the site some number of days previous to sampling. However, such biologic metrics are subject to innumerable influences related to habitat quality, predator-prey interactions, competition, disease progressions, food quantity and quality, previous spawning success, etc. In scientific parlance, these would be characterized as "antecedent conditions" and "uncontrolled variables." These matter immensely as to whether a relationship would be expected between the biologic measure and flow tier at a single point in time on the day of sampling. For instance, in this research, two samples of any given species of fish that were measured after a specific flow tier (e.g. 1 per season high-flow pulse), were treated the same, whether or not that flow occurred on the heels of a six-month drought or only a week after another high-flow pulse. Please respond.

Using Crozier et al. (2016; Antecedent Conditions in Encyclopedia of Natural Hazards (https://link.springer.com/referenceworkentry/10.1007%2F978-1-4020-4399-4_13), "Antecedent conditions represent a temporary state within dynamic natural and social systems that precedes and influences the onset and magnitude of a hazard and its consequences. They are distinct from, but influenced by, what are commonly referred to as preconditions (preexisting conditions). Preconditions are generally static or slow changing and influence the inherent (as opposed to temporary) susceptibility of an area. For example, in natural systems, rock type, soil structure, and topographic geometry are common preconditions that affect susceptibility to landslide occurrence, whereas groundwater level, soil moisture content, and under certain circumstances, vegetation cover are dynamic factors representing influential antecedent conditions for landsliding."

"Examples of antecedent conditions for specific hazards include tidal phase (tsunami and storm surge), vegetation moisture levels (forest fire), humidity (heat waves), groundwater level (liquefaction and flooding), wind direction and strength (volcanic eruption), temperature and freeze/thaw history of snow packs (snow avalanching), and amount of debris accumulated in source areas (debris flow). Antecedent conditions can also be represented by hazard history. For instance, forest fires can induce hydrophobic conditions in soils that favor the development of debris flows during heavy rainfall, and foreshocks may weaken natural and man-made structures causing amplified damage in subsequent earthquakes."

In our aquatic communities, we have preexisting conditions and antecedent conditions. How do we know if “these matter immensely” or not and if “innumerable influences related to habitat quality, predator-prey interactions, competition, disease progressions, food quantity and quality, previous spawning success, etc.” influence (or not) patterns quantified in this study?

We quantify them. Our work had to have a beginning, so we started. At all of our sites, we have a general understanding on what fish to expect and their numbers (relative abundances, not so much densities). We made collections and then updated our understanding while measuring changes related to flow tiers. A >1 per 5-year flow pulses inundated our reaches in GSA and BRA basins. We compared preexisting conditions to what we found after the high flow pulse. As part of validation methodology (future monitoring with respect to flow tiers), we’ll eventually obtain numerous preexisting conditions and be able to distinguish between preexisting conditions and antecedent conditions. In the meantime, one might believe that nothing can be known because of innumerable influences. This is an individual perspective and one that we cannot argue against. We respect anyone’s right to this opinion. For others, we believe our findings to date, though counter to expectations, are simple to interpret. We found evidence to support that flow pulses do matter at times (relationships are statistically significant) and not at other times under the conditions observed to date (failure to reject the null). We’re very interested in how preexisting conditions and antecedent conditions might or might not influence the patterns observed with flow pulses. As such, we recommend collecting more information.

8. In the end, these researchers ended up partially acknowledging the role of ‘antecedent conditions’ implicitly with the efforts at “pre-flood” and “post-flood” segregation of the data and analyses. The authors are clearly acknowledging the potential for that flood event to have constituted an important antecedent condition for the Round Two work. The text suggests that the antecedent condition for Round One was the drought (Section 3.1.1), but it is only cited as a limitation on the number of samples that could be collected.

Addressed above. Some are more concerned about “antecedent conditions” than us. We’re not concerned about it. In time, we’ll understand its influence at least in part and look forward to unlocking the mystery.

9. This report needs some forthright discussion of the realistic expectation of this research to uncover trends given the potential for uncontrolled variables and antecedent conditions.

As mentioned above, our work is in progress. We’ve explained our findings (and the various caveats) to BBASC in presentations.

10. Introduction, 1st page, 3rd paragraph: Clarify what is meant by “regional ecology” and its relation to environmental flow standards for specific streams and/or reaches of streams.

Revised for clarity in the report.

11. Introduction 1st page, 4th paragraph: Selection of hypothesis discussion. Most of the aquatics results present in Section 3.1 appear to be based on dominate species rather than those that are of greatest ecological significance or are most sensitive to flows. Please explain why selection of indicator species was not made based the stated criteria and also discuss why dominate species were used and not species most sensitive to flows.

All species are considered (see Round 1 reports). In the second round (this report), our goal was to describe how the fish community changed between pre-flood and post flood because this was a unique opportunity. However, we still assessed fluvial specialists (those considered sensitive to flow), which in some of our reaches were the dominant species.

12. Introduction, 1st page, 4th paragraph: The sentence beginning with “*Selection of final hypotheses...[in Round One]*” has several terms that are not defined and the interrelationships amongst them is unclear. Please define the terms “response variable” and “ecological indicators”.

Revised for clarity in the report.

13. Introduction, 1st page, 4th paragraph: In this paragraph, background information is provided regarding SB3 and the need for additional research. At the bottom of page one, the following is stated:

“Selection of final hypotheses was based on: (1) the value of a given response variable in indicating sound ecological environments, (2) that response variable’s sensitivity to changes among flow tiers (i.e., subsistence flows, base flows, and 4-per-season, 3-per- season, 2-per-season, 1-per-season, and 1-per-year pulses), and (3) the length of time required to conduct field research.”

Item 1 is an important point, because one does not want to waste time and money investigating response variables that cannot inform us about the functions of environmental flow components. Please explain why so many analyses were performed on species such as mosquitofish, red shiners, and many others that are expected to have little sensitivity to flow variation in terms of population dynamics. These species are common in rivers and streams throughout much of the state, and therefore are very poor candidates for study.

Explained above (differential selection). Each basin has a set number of species (let’s call it the regional pool). They are not equally distributed among all river reaches and at equal abundance. Some species are not found at all sites (local species pool), and some species are more abundant than others at some sites. Are species and their abundances therefore randomly distributed? No, based on general stream theory. Various abiotic and

biotic filters influence species occurrences and abundances. One in particular, known as the master variable, is flow.

From headwaters to lowland reaches, flow and many other factors are associated with species occurrences and abundances. Take a lower reach and make the flow like a nearby tributary reach. A safe prediction is that the lower reach fish community would shift and look like the tributary fish community. Understanding how and why some common, non-fluvial specialist species would increase is as important as understanding how and why fluvial specialists would decrease.

All fishes are expected to have sensitivity to flow variation; fluvial specialists are thought to have the least sensitivity (meaning they can withstand highly fluctuating flow). Western mosquitofish might be considered the most sensitive to flow variation. Understanding and documenting this is part of the puzzle. Also, Western Mosquitofish are mentioned specifically in the Natural Flow Regime (Poff et al. 1997) and were formative to development of the theory.

With our work, we are analyzing the responses of all species. Ideally, by guild (e.g., fluvial specialists), but by species to fully explore and understand our results.

14. Introduction, 2nd page, 1st paragraph: Note that “ecological indicators” as used here appears to comport with the general scientific use of the term as a measure of ecological status. This does not appear to be the same as “inadequate replication of ecological indicators” as uses in the Executive Summary, as commented on above. Please clarify.

Meaning explained above.

15. Introduction, 2nd page, 3rd paragraph: The following is stated:

“Please note that while the focus of this report will be on the Brazos/GSA/Colorado-Lavaca basin(s), references to and results from other basins may be used in this report to support findings, further develop discussions, and guide future recommendations.”

The report compiles data and develops examples of decision making scenarios based on study results from not only the Colorado-Lavaca, but also the Brazos and Guadalupe-San Antonio basins. It is not clear if results from other basins and/or locations within basins are transferable. A number of variables could influence biotic community response to flow events including the size of watershed and drainage area, number of upstream tributaries, stream morphology, temperature, length of time between pulse flow events, water quality, and others. Please discuss how using data from outside a particular river basin helps to evaluate flow standards for a given stream reach/gage. Responses to flow variation are always local, with some biological responses being rapid (short term) and others having various lag times (long term). Please explain why it is appropriate to merge datasets from different basins to evaluate responses to flow variation.

We selected a few reaches out of all with e-flow recommendations within the GSA, BRA, and Col to draw inferences. Our experimental design is sufficient to inform if there are general tendencies in biotic responses to flow tiers across all basins and reaches or not. In our full model (3-factor ANOVA), one factor is basin. If basin in an interaction term is significant, then we assess within basin. Ideally, we would show the value of a flow tier (e.g., 1 per season) across all of our reaches. If so, then this finding would be meaningful to other reaches that we're not testing and even outside our targeted basins. As we gather more information, perhaps we'll find that e-flow recommendations should be validated by reach. This is possible but not probable based on the information we've gathered to date.

16. Section 1.1: The report states:

“General aquatic theory suggests that flow alterations cause shifts in fish and macroinvertebrate communities. Typically, swift-water, large-river-type fishes become fewer and generalist fishes become more abundant during periods of altered flow.”

and later:

“In the Brazos River during low flow conditions, large-river-type fishes, such as smalleye shiners, sharpnose shiners, silverband shiners, and shoal chubs, are replaced with tributary/generalist type fishes, such as red shiners, bullhead minnows, and centrarchids. This generalization is based on historical analyses (Runyan 2007), but also on ecology of other similar prairie streams.”

The first above, in referring to flow alterations is referring to long term changes in flow regimes, for example those that might be observed downstream of a reservoir where pulses are muted and low flows elevated and made more constant. General aquatic theory predicts that these alterations in flow regime will cause predict community shifts.

The second sentence above seems to suggest that when the flow rate in a river drops during low flow conditions, there is a shift in species relative abundances. This is not what is intended in Runyan (the museum study was also describing long term flow regime shifts) but this does highlight a central assumption of this study, namely that one should expect to detect species level population shifts in response to short term changes in flow and that detection of these shifts is how flow standards should be validated. Several reviewers objected to this assumption. Please provide citations to relevant literature to support this assumption.

Conceptual models (theories) do not have to be universally accepted. Conceptual models are developed in order to develop testable predictions. Multiple narratives can be developed. We can argue back and forth on which ones are better, but the argument can't be advanced without testing of model predictions.

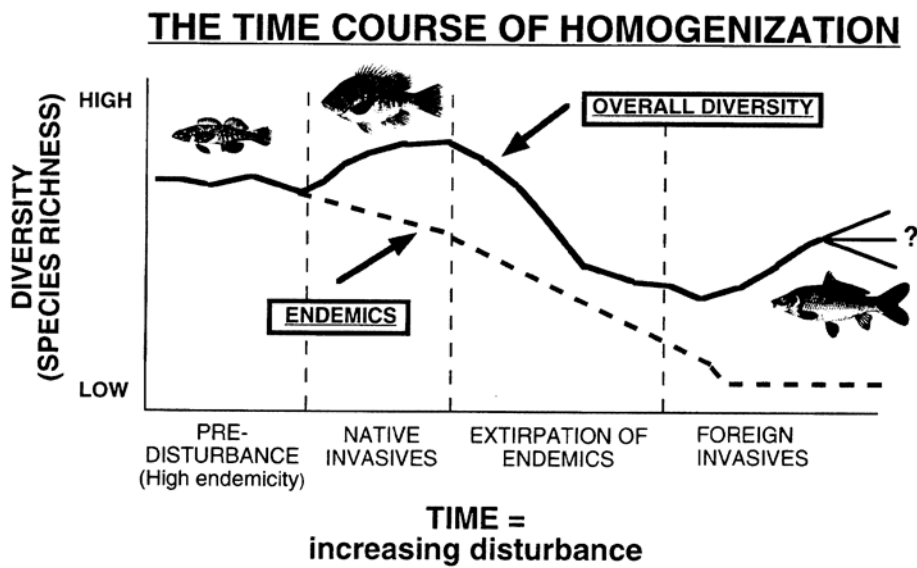
Testing occurs and, based on results, the narrative is supported, and can be revised and (hopefully) becomes more accurate, or the narrative is discarded.

However, how does a long term shift in fish communities occur? Does it begin with short term win/loss by some species? Can we detect evidence for this by assessing intra-annual patterns? We think so, therefore part of our narrative.

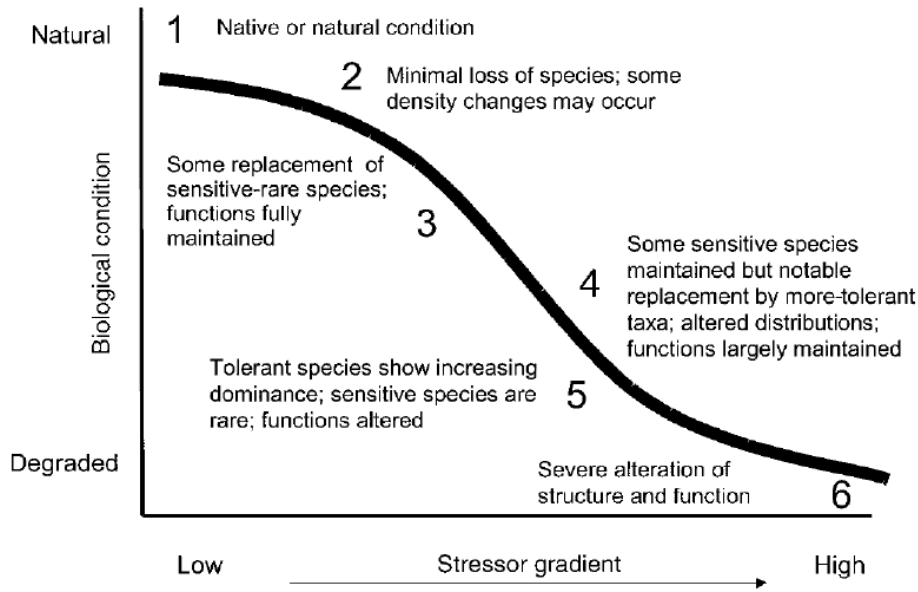
In contrast to our narrative, what are the other narratives that explain long-term changes in fish communities? What are the testable predictions? How can these be tested in the context of existing standards and recommendations?

As for citations related to our conceptual models, we recommend the following:

Scott M. C. and G. S. Helfman. 2001. Native invasion, homogenization, and the mismeasures of integrity of fish assemblages. *Fisheries* 26:6-15.



Davies, S. P. and S. K. Jackson. 2006 The biological condition gradient: a descriptive model for interpreting change in aquatic ecosystems. *Ecological Applications* 16:1251-1266.



17. Section 1.1, (Brazos Report Only): Please clarify if the characterization of changes in the large-river fish community refers to the entirety of the Brazos River or is more specific to the Brazos River system upstream of Possum Kingdom.

Our study reach is the lower Brazos River basin.

18. Section 1.1: The Native Invader Concept may be applicable, but its initial description (Scott and Helfman 2001) was related to habitat homogenization from deforestation and loss of riparian cover resulting in replacement of fish species adapted for lower temperatures and low sediment substrates by native species more suited for higher temperatures and sediments. Scott and Helfman (2001) suggest that “such invasion should be recognized as an early warning sign of the homogenization process.” Please clarify that the Native Invader Concept is applicable to this study.

See comment above. Our work is testing the applicability to the Native Invader Concept.

19. Section 1.1.1: Further explanation or examples of “...aquatic mechanisms of high value to environmental flow standard validation” is needed to allow the reader to better understand study objectives, hypotheses, and methods.

The statement is the topic sentence of the paragraph. Following explanation and examples (Objectives) follow.

20. Section 1.1.1., Objective 1: Explanation is needed on the correlations of biological responses to various lag times. This is because the biological responses to flow changes are not instantaneous.

High flow pulses passed through the system. We had a standardized procedure to sample afterwards.

21. Section 1.1.1, Prediction 1: This prediction seems rather naïve, and for the reason stated by the authors above on p. 3 (“Aquatic organisms occur and persist in time and space because of a number of interrelated and hierarchically ordered abiotic and biotic processes. Stream flow and variations within directly and indirectly influence occurrences and abundances of aquatic organisms on multiple levels”). As the authors noted, there are both direct and indirect effects of flow changes on biota, and also a hierarchy of responses. To this one can add the issue of differential time lags of response. Please respond.

What is meant by time lags? Lag times for processes or to observe patterns?

Otherwise, one can add several specific examples to “abiotic and biotic” and “direct and indirect”.

22. Section 1.1.1., Prediction 2: Several reviewers disagree with this prediction, especially regarding fishes in shallow run habitats. Most of the time, fluvial specialists and other kinds of fishes will attain peak per-unit-area densities in their preferred habitats during periods of low flows. Maybe, depends on how “low flow” is defined and conditions therein (e.g., a day from complete drying? at subsistence? at base? Is water quality sufficient to support life? Is there “preferred habitat” available in this low flow scenario? River drying into pools “at low flows” will not have shallow water run habitats).

Yet they require high flow pulses to create the environmental conditions in those habitats that they require for success in the longer term -- e.g., substrate scouring to create foraging habitat (not supported by our work so far) and to promote prey availability (no support for this so far); to stimulate spawning (as a synchronizing cue? No support for this in the literature for North American fishes and no support in this study); to enhance recruitment (how?, our previous work detected increase gut fullness related to a flow pulse, so maybe. How would this be tested with respect to standards/recommendations?); and to facilitate sediment suspension (causing increased turbidity that may reduce predation by visual predators; for how long?). Please provide citations to relevant literature to support this prediction.

As described above, each observer is free to develop his/her own conceptual models, predictions, and study design. We can discuss if predictions are correct or not. Plus, it's pretty easy to argue against a prediction after evidence is gathered and the prediction wasn't met. As such, we set predictions *a priori*, then conduct the research.

Disagreeing with a prediction (asking the wrong question) after testing has merit. This leads to refining theory (or selecting a new one), developing additional predictions, and further testing. But, one can't ignore the findings by saying “we didn't agree with the prediction”.

Question: If prediction 1 is acceptable, then why aren't the same filters occurring in run habitats?

As for literature support, see Scott and Helfman 2001.

As an example: Assume the x-axis in the below graph ranges from unregulated river reduced down to a ditch.

In an unregulated river (left side of x-axis, flows pulse through a system. For a species type or guild of species, densities and relative abundances before a flow pulse (base condition, assuming this is what is meant by "low flows") at "pre-disturbance" can be less than, equal to, or greater than the densities after a flow pulse.

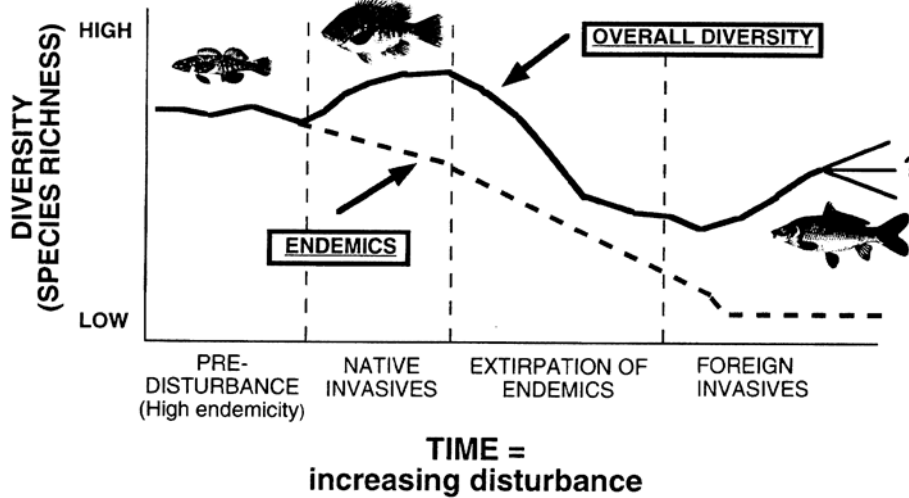
Assuming "fishes will attain peak per-unit-area densities in their preferred habitats during periods of low flows", our methodologies are comparing peak density to peak density. However, consider the possible outcomes:

If all fishes and guilds are equally abundant (density and relative abundances), then we fail to reject the null hypothesis "relative abundances and densities of fluvial fishes and slack-water fishes in run habitats are independent of flow tiers". How can others interpret these findings? As mentioned previously, failure to reject the null is like a hung jury. We don't know if flows are related to the abundances of fishes within a community. However, how many times will failure to reject the null have to happen before someone decides to abandon the hypothesis? As for our work, it's too early in the process to claim that standards/recommendation have no ecological value although we've failed to reject the null of several predictions. Also, it's too early to claim that we've disproven the Natural Flow Paradigm (as interpreted to set standards/recommendations).

If fluvial specialists' densities are the same (in a short time period, maybe increased due to recruitment over long time periods like extended flows for over a year in the lower Brazos River) but relative abundances are greater, whereas generalist or slackwater densities and relative abundances are lower, then we reject the null, the prediction was realized (ecological integrity is dependent on the natural dynamic characters).

What exactly is disagreeable about Prediction 2?

THE TIME COURSE OF HOMOGENIZATION



23. Introduction, Section 1.1.1., Prediction 3: Reviewers commented as with the fishes, this is only true if one analyzes data using appropriate hydrological variables that integrate flow components over variable time intervals. What matters most is not the discharge on the day of the survey, but the discharge on the days leading up to and including the day of the survey. Please respond.

As mentioned previously, what are appropriate hydrological variables, what does it mean to integrate flow components, what are the time intervals of interest? Do any of these matter? Maybe...we can test it with enough replication.

Discharge on the day of the survey only matters if those flows are at base flow condition (or close to base, we're trying to determine "how close" is close). We do not use "flow on the day of survey" in our analyses. Our validation method stipulates that we sample at base, watch a flow tier pass, then sample at base again.

24. Section 1.1.1, 1st paragraph: The text starts with,

"The aquatic study was structured to fill knowledge gaps by targeting aquatic mechanisms of high value to environmental flow standard validation."

The term "aquatic mechanism" is undefined. It is surmised that the authors may intend to write something like "relationships of ecological status to flow". Whatever the definition, which is needed, any such mechanism would seem to warrant the qualifying adjective "presumed" ahead of it. That would seem to be an underlying precept for couching everything to be examined as a hypothesis as was done in Round One. Please define "aquatic mechanism."

See response to #19.

25. Section 1.1.1, 1st paragraph: The list of Objectives uses the terms “pre-” and “post-flood” without a definition. Please define “pre-” and “post-flood” as directed in the Executive Summary comments.

Definition added.

26. Section 1.1.1, Objective 3: This section seems to only be about the GSA. Please clarify if this objective relates to other basins as well.

Text revised in the report.

27. Section 1.1.1, In order to assess whether the sampling approach and data analysis utilized are appropriate, reviewers requested additional detail regarding each study objective. Please provide additional detail on the presumed ecological linkage to the flow tiers to put objectives into context. Similarly, please provide additional discussion of the ecological linkages/relationships that are forming the basis of predictions.

Basic information is provided. Fuller context and discussion will be forthcoming in future publications.

28. Section 1.2, 4th bullet item under Pros: The reference to flow “needs” meeting the needs of the indicator species is confusing. It is not clear what concept is actually intended. Please clarify.

“Flow needs” was changed to “flow pulses” in the text to clarify.

29. Section 1.2, 2nd bullet item under Cons: The concept stated here is somewhat unclear. It would seem that the absence of the indicator species also might be of importance. Presumably, the intended point is that the use of indicator species requires the ability to sample the indicator species, but more explanation is warranted. Please clarify.

Text was modified as follows: ‘The indicator species must be present in order to focus on only those select species’ to: ‘The use of an indicator species requires that the indicator species must be present in the zone of interest.’

30. Section 1.2, the last bulleted statement under Cons states that “Observed changes cannot be statistically represented because of the non-random selection of transects when focusing on indicator species distribution.” Reviewers commented that this does not actually pose a problem. Depending on the question and study design, there should be appropriate statistical options that should be explored. Please respond.

Text was modified as follows: ‘Observed changes cannot be statistically represented because of the non-random selection of transects when focusing on indicator species distribution’ to ‘Non-random selection of transects based on indicator species distribution limits statistical analysis of community assemblages.’

31. Section 1.2, 4th paragraph: Section 1.2, 4th paragraph: It is unclear if “this” study refers to the current study or to one of the studies discussed in the previous paragraph. Please clarify and, if the referring to previous studies, please include some discussion of the previous “flow vs. riparian response” studies related to “this and other reaches” along multiple basins. Citation to, and some discussion of, those studies would be helpful.

Text was modified as follows: ‘this study’ to ‘this current study’.

32. Section 1.2.1: In the subsection “Biotic Features within Sites” there are a series of Questions and corresponding Hypotheses listed. Hypothesis 2 as stated “Community assemblages can be characterized” is a very weakly formulated hypothesis statement. Please discuss how these can be tied into the classifications found in Question 2.

Text was modified as follows: Hypoth 2 to: ‘Community assemblages can be characterized according to 1) overall plant abundance and 2) mature tree abundance.’

33. Section 1.2.1, Hypothesis 1: Please discuss why neither elevation relative to normal streamflow nor some measure of flow volume are included as distinguishing features. Given the important role assigned that process in the scope of work, it seems important to have some summary of that process included.

The focus of the riparian assessment in the Round 2 study was to evaluate methods for long-term monitoring and validation. Collecting the inquired about information for the riparian component was beyond the scope of this work, and thus the reason we used nearby USGS gauges to estimate flow pulse inundation.

34. Section 1.2.1, Hypothesis 2: The reference here to “tiers” is confusing. Other areas of the report refer to “tiers” as the flow tiers. Presumably, the reference here is intended to refer to the subparts of the riparian corridor. Please use a different term, such as “zone,” in the context of riparian habitat to reduce the potential for confusion.

Riparian “tiers” were changed to “level” throughout the document.

35. Section 1.2.1: In the subsection “Biotic Features within Sites” with regard to Question 3 & Hypothesis 3 – Please clarify if the report is referring to ‘flow tiers’ here. If so, this language would appear to be aimed at addressing community differences that may exist in response to varying patterns of inundation from different flow tiers, which in turn is a function of distance from the stream and elevation, etc. This language should be made clearer. If there is an explicit flow-spatial extent correspondence intended, a reference to the other section in which that correspondence was made is essential. If there is not, a different terminology rather than “Tier” should be used in the Riparian context. The idea of using bank elevation as a proxy for exposure to various flow tiers is sound science. This should provide an efficient (economical) means to test the flow tiers based on long-lived, sessile organisms (trees).

See point 34 above. The 'tier' *does* refer to within-zone tiers and was modified to the new nomenclature (level).

36. Section 1.2.1: Determination of the flow rate that inundates different forest communities is a straight forward data gap that this study clearly addresses. Please provide data addressing what duration and frequency of such events would be need to maintain the desired forest community.

This study does not allow for a duration/frequency estimate beyond the general recommendation of no longer than ~4 days and in spring and fall (as was stated and defended in our conclusions). Further elucidation of this will entail long-term observational data to determine what flows and frequencies benefit/diminish the zone through time, and is a much larger project than the current focus.

37. Section 1.2.1: Question/Hypothesis 9 – Reviewers cautioned that the appropriate time scale should be used in responding to this question/hypothesis. Young tree recruitment will show a faster response than larger trees, but even evidence of change in young tree abundance may require several years to see an effect of a change in flow regime (i.e., having a sufficiently large database to find a pattern). Please comment on how time scales were considered in the riparian analysis.

This question/hypothesis was addressed by estimating the flow pulse inundations necessary to reach the elevations associated with mature tree distributions. Because the longest-lived life stage was used, this focus automatically provides for all life stage needs, as longevity (mature tree presence) indicates younger life stages survived.

38. Section 2.1.1, 3rd paragraph: Samples were collected from sites with flow pulses up to 15 days following a pulse event. Depending on the size of the pulse event, any changes in aquatic community composition could be temporary and not representative of a changed community due to flow alteration. Assessing changes to community structure in dynamic systems and relating changes to a particular event/disruption requires more than point of time sampling. Please explain how you determined changes in the fish community were a result of flow alteration.

Comment addressed in previous responses regarding lag times, sampling and analysis.

39. Section 2.1.1: Though the sampling methods for riffle and run habitats are described, there is no information on the methods used in backwater and pool habitats. Please add a discussion of sampling methods used in backwater and pool habitats, also include the seining protocol.

Text was revised in the report.

40. Section 2.1.1: Please provide the following information (summarized in the text and complete in an appendix) which is considered standard and required to be collected and

reported by TCEQ in its biological monitoring procedures manual and is used for calculating indices of biological integrity.

- A. Identify the dates when the sites were sampled.
- B. Describe the surface area and depth of sediment sampled with the Hess sampler.
- C. Describe how many seine hauls were made at each site, the length of the seine hauls, and types of habitat sampled with seines.
- D. Describe the habitat characteristics at each sample site, substrate type, types of instream cover, stream widths, depths, and flows.
- E. Describe the water quality when sampling was conducted.

This information was given in the Round 1 reports. We prioritized our time this year by documenting evaluating the effects of the large flood events.

41. Section 2.1.1: Reference to stunned fish on the “benthos” is confusing. The intended reference seems to be to fish and benthic organisms. Please clarify.

Text revised in the report.

42. Section 2.1.1: Please clarify how the fish gut analyses will be incorporated in the study results and when the results will be made available. Esophagus is misspelled.

Text revised in the report. We’re still processing gut contents down to lowest practical taxa. Results will be presented in future publications.

43. Section 2.1.1: After a pulse event, new riffle habitats are formed/inundated/available that may not reflect a well-established benthic macroinvertebrate (BMI) community if moderate to significant scouring forces occurred or if substantial drift was induced. The “duration of existence” of the riffles is an important factor for the establishment of macroinvertebrate communities. A recently-scoured riffle may not have recovered or reestablished BMI populations. This “minimum period of existence of riffle” needs to be taken into consideration before sampling the riffles for flow validation. Further, one BMI sample from each representative riffle sample may not be adequate to accurately capture the characteristics of the BMI assemblage given the patch dynamics of these organisms and the spatial hydraulic diversity of riffles. Please provide data/information on “duration of existence” of the riffles and also clarify how it was determined that one BMI sample was sufficient to capture the characteristics of the BMI assemblage.

This is part of the story that we are quantifying. New riffles form at some sites and riffles persist at others, after >1 per 5 year event. We did not find a relationship between “duration of existence” since densities were largely not different. However, we’re taking taxonomy to family level, in order for a more robust assessment. Results will be presented in future publications. Also, we quantified multiple subsamples (N = 3) for each riffle.

44. Section 2.1.1: Reviewers commented that the paragraph starting with “*In the laboratory, benthic samples, were rinsed...*” is unclear. This paragraph seems to refer to how macroinvertebrates were compared and combined. Please clarify.

See below

45. Section 2.1.1 Section 2.1.1: The paragraph that begins with “*Among riffle habitats, total density...*” discusses how relative abundance of each category (riffle fish, fluvial fish, and slackwater fish) was calculated.

Text describes how relative abundance was calculated: “Categories were swift-water fishes, moderately swift-water fishes, and slack-water fishes. Density per category per riffle was calculated by summing species within each category. Relative abundance of each category was calculated by summing species abundances within the category, divided by total numbers of fish taken, and multiplied by 100.”

It is not clear if relative abundance for each category was calculated based on the category's concentration in riffles, runs and slackwater separately or if relative abundance for these categories was based only on their presence in riffles.

Relative abundances of swift-water fishes, moderately swift-water fishes, and slack-water fishes were calculated separately for riffles, runs, pools, and backwaters.

It is not clear if this approach takes into account the size of riffles. A small riffle may be less likely to have representatives of all three categories than a large river riffle just because of the size of the riffle. A base flow riffle that is only a foot deep and 15 feet wide will not accommodate as many fish as a 2-3 feet deep and 100 foot wide riffle regardless of flow tier. Please clarify as to whether riffle size was accounted for in the analysis.

As stated in text, “Among riffle habitats, three subsections of the riffle were designated (approximately 30 m²) to capture variability within each riffle habitat (e.g., near shore vs. middle, swifter vs. slacker current velocities, shallower vs. deeper water)”.

We standardized samples based on area. We were not sampling a large riffle and making comparisons to a small riffle. Instead, we compared subsamples of riffles to subsamples of riffles. In addition, we calculated relative abundance. Even if size of riffle was influential, relative abundance of categories would be independent of riffle size.

46. Sections 2.1.1: Please clarify if the classification of low tiers is based on either BBEST, BBASC, or TCEQ adopted standards levels of flow magnitude.

We are tracking all of them.

Since the research did not apparently track the duration (only magnitude) of high-flow pulses, it is quite possible that many of the samples were taken on the heels of events that are so-called “non-qualifying” high-flow pulses due to insufficient duration. The duration of flow conditions to provide a complete characterization can be retrieved from the same USGS records utilized for the tier assignments. Please fully characterize the pulse events and consider this information in the analyses.

Duration is known and readily available via USGS station for each site. In Round 1, none of the durations were met. In Round 2, most flow pulses met duration. We’re starting to have sufficient replication for flow tier magnitude. In time, we’ll have sufficient information to assess flow tier magnitude-duration met or not. This is part of validation methodology, where we assess seasonality, magnitude, and duration. However, we can only assess as the events occur.

47. Section 2.1.1 No data is presented to verify the actual flows at the time of field data collection. It is unclear whether flows had returned to baseflow or the lower tier at the time samples were collected.

As mentioned in the text, we sampled at base flow conditions. However, we assessed fish communities in the lower GSA before flows reached base flows. At the time, we were anxious to get some insight into the fish community following the >1 per 5-year event. Since we developing a methodology along with validating flow tiers, we’ll continue to assess if flows must return to base flow or some level above base flow in order to increase sampling efforts.

The report fails to fully characterize pulse flow events (duration, for example, can make a very large difference in ecological responses... We would be interested in viewing your evidence for this claim. Claims like this are the reason why we feel validation is so important. We suspect magnitude is more important than duration; however, we could be wrong. With a validation methodology in place, these types of questions can be addressed with a priori predications and replication) or provide a quantitative assessment of the antecedent flow conditions prior to sampling, such as number of events or tiers that occurred between sampling events. Please add a more complete description of the flow conditions preceding and during sampling to the report.

Flow records preceding our collections dates are of public record. Anyone believing antecedent conditions might have an influence can readily access and explore antecedent conditions. We are happy to share our information in any form, so others can explore with our data.

As mentioned above, we’re not detecting a lot of differences, so “what are the antecedent conditions” is not a high priority at this time. Our main priority is to assess “preexisting” conditions, hence we are reporting upper reaches GSA, upper reaches BRA, and so forth. Through time and replication and data taken at the correct scale to inform standards/recommendations, we’ll have a robust data set to explore numerous scenarios.

Perhaps a naïve perspective of our team, we envisioned that various levels of flow pulses would quickly show ecological responses in fish and macroinvertebrate community. We found this as predicted but only at the highest of flow pulse (and cfs) going through the lower Brazos River. Not surprisingly, our perspectives were inaccurate. But, we are curious to know why. We hope that it will be as easy as “duration wasn’t met”.

48. Section 2.1.2, 1st paragraph: It is not clear why this “historical” data was analyzed separately from the other fish data. This data was “refined” to match the current study framework so one would expect it to be included with the current fish data. Also, please explain why the data was analyzed differently utilizing a “percent exceedance” approach rather than the flow tier approach.

Text revised in the report to say...keeping a priori predictions data separated by data used for retrospective analysis.

49. The explanation of how the percent exceedance categorization was completed is incomplete. It is not clear what value is being exceeded. As this a critical aspect in evaluating the validity of the comparisons, please provide a more detailed description of this process.

Text was modified for clarity.

50. Section 2.2: For each riparian study site, please provide some explanation of the selection process for the site, including a characterization of the extent to which the site is considered to be representative of any particular portion of the overall basin. Also, please describe why riparian sites which were different from the fish/macroinvertebrate sites were chosen for sampling.

Text is present in this paragraph that explains site selection, “Each of these sites was chosen because they were included in Round One, monitoring of them began prior to this study, and each has a historical riparian community characterized through multiple previous studies.”. Riparian sites could not be coupled with fish/macroinvertebrates because the local geomorphology, etc. that make a stream reach ideal for one biological entity do not inherently make it ideal for all others. Riparian selection required that we have riparian vegetation present, therefore it was necessary for each team to independently locate sites.

51. For each riparian study site, some explanation of the selection process for the site should be provided, including a characterization of the extent to which the site is considered to be representative of any particular portion of the overall basin.

See #50 above.

52. Section 2.2, Colorado – Lavaca Report Only: The Sandy Creek Site is referred to as a tributary of the Lavaca River. Later in the report it is referred to as a tributary of the Navidad River. Please clarify which is correct.

Sandy Creek is a tributary of the Navidad River.

53. Section 2.2, Colorado – Lavaca Report Only: Please include figures showing the locations of the riparian sample sites at a larger scale, particularly since the Navidad River and Sandy Creek sites may be close enough to Lake Texana to be influenced by the reservoir water levels and the Colorado Bend site may be close enough to the headwaters of Lake Buchanan to be influenced by reservoir water levels.

Each of the riparian sites are downstream of the USGS gages depicted in earlier figures. Text descriptions are provided but specific figures depicting landowner properties were not presented in this public report out of respect to the respective landowners.

54. Section 2.2.1, Figure 3 in Brazos and GSA Reports, Figure 5 in Colorado – Lavaca Report: Using the Colorado Bend image with 5ft LCRA contours developed from LiDAR data drastically over-states the accuracy of the elevation data used from USGS DEM grids with granularity of 32-ft (10m) grid. Please discuss the accuracy differences between these data types.

The focus of Round 2 riparian research was to evaluate and compare procedures for effective long-term monitoring. A secondary goal was to provide an estimate of inundation for new Round 2 riparian study sites. The estimation approach used for Round 2 was by default not as accurate as if this would have been the primary study goal. Text was modified in this section to better highlight the estimation level assessment conducted as opposed to a more thorough assessment using higher resolution aerial imagery and detailed water surface elevational data.

55. Section 2.2.3, Colorado – Lavaca Report Only: This section references historical canopy cover but provides no context or reference to a source to provide that context. Please clarify how this discussion and the reference to grass species that do not appear in Table 13 are intended to inform understanding of the site.

Text was modified to clarify.

56. Section 2.2.4 Colorado – Lavaca Report Only: The reference for the statement about the source of base flow for Sandy Creek is unclear. Brune’s Springs of Texas (1981) is cited, but the context is questionable. First, even streams without significant spring contributions may have base flow supported by rainfall in addition to irrigation return flows. The reference to Springs of Texas, which was published 36 years ago, regarding diminution of seep and spring flow “over the last 40 years” is questionable. The conclusion may be correct, but a more current source should be used. Please clarify the use of this citation.

The statement was deleted from the text as it was simply background site description information.

57. Figure 4, Colorado – Lavaca Report Only: Suggest using a different color to indicate the randomly selected points in Tier 2. The dark purple points are difficult to distinguish on color printouts.

We appreciate the comment.

58. Section 2.2.1 Brazos and GSA, Section 2.2.5, Colorado – Lavaca Report: The first sentence appears to state there was only one sample event which was conducted in the spring. Because the text describes two sample dates for each location, please revise to clarify that an additional sampling event occurred during a different season. Also, please clarify whether the same randomly selected points were sampled on both dates or if new randomly selected points were identified for the second sample date. Please include the following in either the text or an appendix:
- A. Identify sample dates.
 - B. Describe how the length of the tiers was determined.
 - C. Describe how the 35 points were selected from the 75 randomly selected points.
 - D. Describe how the circular plots for mature tree counts were randomly selected.

Text was modified in each report for clarity.

A. Identify sample dates.

Sites in the GSA (Goliad State Park and Gonzales) and Brazos (Brazos Bend State Park and Hearne) basins were sampled only in spring 2017 for “verification” since these four sites already had two or more years of ongoing riparian sampling conducted by the project team. Verification data was compared back to previous years’ data and all data was incorporated into this research. All other sites in the Lavaca, and Colorado basins were sampled twice, once in Fall 2016 and then again in Spring 2017. These sites were new and had no previous riparian data collected.

Lavaca/Navidad Basin

Sandy Creek site	December 6, 2016 and May 1, 2017
Navidad River site	December 8, 2016 and May 3, 2017

Colorado Basin

Onion Creek site	November 10, 2016 and June 5, 2017
Colorado Bend State Park site	November 16, 2016 and May 16, 2017

Guadalupe/ San Antonio River Basin

Goliad State Park site	May 4, 2017
Gonzales, Texas site	June 1, 2017

Brazos River Basin

Brazos Bend State Park site	May 10, 2017
Hearne, Texas site	June 8, 2017

- B. Describe how the length of the tiers was determined.

The length of the tiers “levels” was based on a large enough size so as to encompass enough of the river riparian ecosystem for sufficient data collection yet a suitable size for sampling within a day’s timeframe. Also, accessibility by foot along the entire length of the tier was important, and physical features (e.g. ravines, impenetrable brush, steep gradients, property fence lines) sometimes determined the beginning or ending boundaries.

C. Describe how the 35 points were selected from the 75 randomly selected points.

Random points were selected in ARC GIS using the random point creator in ARC GIS toolbox. Once a tier boundary was created in ARC GIS the program can create any number of random points within the boundary. Many more random points were created than were necessary for data collection since the team anticipated many points would be inaccessible due to thick brush or rough terrain. Once the point shapefile was created it was loaded onto a Trimble gps unit so that points could be located in the field. The 35 points selected as sites for data collection were selected in the field. We started at one end of the tier and navigated to one of the 75 random points on the shapefile. If that location was accessible e.g. no steep drop offs, thick brush, etc. for data collection then data was collected at the point. Then we navigated to the next point and made the same determination until we collected data at 35 points. We also took into account the proximity of points so that we did not collect data at points too close to each other. This ensured we were able to gather data across the entire tier and prevented data “clumping”. New randomly selected points were created for each tier for each sampling event.

D. Describe how the circular plots for mature tree counts were randomly selected.

The circular plots were selected based on random points created in ARC GIS as discussed above. Initially 75 random points were created in each tier per site. Many more than necessary. In the field, we navigated to one of those points, selecting a point that was oriented toward the middle of the tier and accessible (e.g. no impenetrable brush or ravines) and made that point the center of the circular plot. If a point was not considered accessible due to any number of reasons we navigated to another random point and made the same determination.

59. Section 2.2.2, Brazos and GSA Reports, Section 2.2.6, Colorado – Lavaca Report: It is not clear that the method used to determine inundation elevations is valid. The rating curve for different points along a river will vary greatly, depending on the slope of the stream, channel configurations and other factors. In addition, the shoreline is not the start of the rating curve for USGS gages. The elevation associated with a certain flow could be determined by the use of streamflow modeling. The elevations should be presented as highly speculative. Please include the rating curves and a discussion of their accuracy.

We understand the limitation and ball park nature of the estimation approach used in the Round 2 study. As previously described, the focus of Round 2 riparian research was to evaluate and compare procedures for effective long-term monitoring. A secondary goal was to provide an estimate of inundation for new Round 2 riparian study sites. The

estimation approach used for Round 2 was by default not as accurate as if this would have been the primary study focus. It was encouraging however, that this estimation approach provided similar results for the Round 1 sites measured previously. Text was modified in this section to better highlight the estimation level assessment conducted as opposed to a more thorough assessment using higher resolution aerial imagery and detailed water surface elevational data.

60. Section 2.2.2, Brazos and GSA Report, Section 2.2.6, Colorado – Lavaca Report: No inundation modeling actually occurred as indicated in the report. Please change section 2.2.2 heading from “Inundation Modeling” to something more indicative of method used, like “Inundation Prediction” or “Estimate of Inundation.” If this interpretation is incorrect and modeling was performed, please clearly identify the model used.

Excellent point and the title has been modified to “Estimate of Inundation”.

61. Section 2.3.2, Brazos Report Only: The report states “we downloaded hourly and monthly average stream flow estimates.” Hourly statistics are not available on the USGS site and flow statistics are only available up through the water year ending October 2016. It is unclear if calculated averages or downloaded statistics are used in this study. Daily mean discharges were used in development of the SB3 rules. Please clarify what data was used in this analysis.

Please see Brazos Estuary report responses.

62. Section 3.1.1: It is unclear what is being assigned “Pre-flood period” and “Post-flood period” here. Is this comparing the TWDB contract-1 dataset with the contract-2 dataset? Or was there a particular flow event that nicely divided the contract-2 data into a before and after period? It is impossible to discern this from the hydrograph. Please clarify.

The former is correct.

63. Section 3.1.1: This section appears to address one or more of the formal Predictions postulated in Section 1.1.1. However, on several levels this discussion fails to effectively communicate the evidence to support/not support the Predictions. There are innumerable citations of species names and trends in relative abundance or other measures as a function of flow tiers and meso-habitat type, but in the end, it is quite chaotic. Please rewrite the section for clarity making several changes: restating the Predictions, tying the specific trend (e.g. “Negative association with flow tiers observed with *C. anomalum* and *Percina* were opposite of predicted.”) to a Prediction, discussing the support/non-support, and discussion of caveats.

This work is “in progress” and will continue pending funding. Our report provides an update on the work to date, and what could be assembled within two months of our last collection (contract obligations). As part of the update, we assessed the larger questions (changes in community pre and post flood), which we agree seems chaotic, but trends are

starting to merge (see across basin summary). We have a lot more data to analyze and interpret. This information will be forthcoming in the form of future publications.

64. Section 3.1.1: Section 3.1.1: Most of the results indicate relative abundances were not different among flow tiers or flow tier lacked sufficient replication to assess differences in relative abundances. Please provide some discussion of how this data is useful for flow validation.

See Table 6 and discussion in Across-basin Summary.

65. Section 3.1.1: The text states:

“Mechanisms underlying the shifts are being assessed but likely represent two factors: displacement of C. lutrensis and P. vigilax and increase reproductive success of N. shumardi and M. hyostoma during an extended period of high flows.”

Please explain if this comment is supported by data collection or analysis in this study, professional opinion, literature, or some other source.

Explained in more detail above (differential selection).

66. Section 3.1.1: Please explain why only the 3 or 4 most abundant species for each combination of sites for riffle, run, and pool were analyzed. We assessed community responses, using the most abundant species at each reach. This was our first pass of the data set. Rare species might be informative (where still assessing trends), but catching a few and none among samples pre-flood and catching a few and none post-floods yield insignificant results. Also, please explain why the data was only analyzed by longitudinal groupings between basins rather than assessing each basin individually. Reaches within basins were assessed in order to detect commonality in responses since overall model (including basin effect) were not significant. Please explain why fluvial specialist species were not assessed individually. Fluvial specialists were assessed individually (Percina, Etheostoma, Macrhybopsis, Notropis shumardi)

67. Section 3.1.1: The very low number of subsistence tiers represented in the site visits raises questions about how well the data reflect the impacts of subsistence flow conditions. Please discuss how this affects the ability to evaluate the overall adequacy of flow standards and/or how this could be addressed with additional future evaluation. Subsistence flows lacked sufficient replication and were dropped from statistical analyses (although included in some figures). Our subsistence flow data shouldn't be censored; the information gives a view of the community. But more information is needed at all sites at subsistence flow in order for us to understand the value of subsistence flow standards/recommendations. Value to future evaluation: We're excited about this and hence the value of our validation methodology. We now have a tremendous data set (central tendencies and breadth of variability of species and community densities and relative abundances for fish and macroinvertebrates) taken at times to reflect base

conditions (more would be better!) and following several flow tiers, including a >1 per 5-year event. We will be able to quantify the effects of fish and macroinvertebrate community (and species) shifts (or not) at subsistence flow (and less than subsistence flows) than can inform the standards/recommendations.

68. Section 3.1.1: The term “ecological responses” is used repeatedly. Please provide a definition and discussion of the term, including temporal elements. Please also provide a similar discussion for the term “species response” if it encompasses more than a change in density and relative abundance.

Ecological response, species responses, response variables, and dependent variables might be used interchangeably. Language was standardized via text modification as deemed appropriate.

69. Section 3.1.1, Colorado – Lavaca Only: The reference to “Table 6” appears to be intended to be a reference to Table 5. Please check and correct if needed.

Text modified.

70. Section 3.1.1, Colorado – Lavaca Only: It appears the reference should be to “Table 6” because Table 7 is part of the riparian assessment. Please correct.

Text modified.

71. Section 3.1.1: Reviewers had several questions regarding this section. It is unclear if an assessment was done to identify ecological responses for other variables besides “pre-flood” and “post-flood” conditions. Addressed above. Given our time frames, we chose to concentrate the results on pre and post flood effects. If eco-flow relationships to maintain SEE exist, then they should be most evident at the highest of flow tiers. We also provided information on flow tiers, at least the ones where we found significant results. It is also unclear what would constitute an “ecological response” in the context of a species-specific evaluation of flow tier data. See comment above. Are there other flow-related factors that could explain the “ecological response” other than the distinction between pre-flood and post-flood conditions? This is part of our inquiry. Do high flow pulses (e.g., 1 per season) affect all aquatic communities similarly? (now, we can say, with evidence, “no”). Since no, we are in the early stages of evaluating the role of other factors (flow related or not), such as stream order, adventitious streams, community type (e.g., spring fish community vs non-spring fish community). If so, how was that factor identified as the appropriate one on which to focus? We’re quantifying a lot of factors that might or might not correlate to shifts in communities related to flow. This is part of the exploratory nature of our work, since eco-flow relationships were not easily detected. So far, we’re observing that spring-dominated fish communities (upper GSA) are shifting less than lower reach fish communities. Therefore, eco-flow relationships might depend on additional factors (community type). Is this appropriate? It depends on the repeatability of the observation. If repeatable (after sufficient replication), then it becomes predictable. If we predict that a fish community will look a certain way after

various flow pulses in a spring-dominated fish community (using an additional factor) and the prediction is met, then we would have confidence in the appropriateness of the additional factor. For example, the hydrograph shown in Figure 6 appears to show much more frequent pulses as well as higher base flows in the most recent sampling period. Our methodology is design to assess these effects. We need to see more conditions. So far, we've had a dry year, followed by a >1 per 5-year flow event. More years will provide a greater range in flow conditions. Hence our call to develop a "water quantity" biomonitoring protocols, similar to "water quality" biomonitoring protocols to ensure that our standards/recommendations are doing the job as intended. With more information, but more importantly, taken at the scale necessary to inform standards/recommendations, how two, 2-per season events back to back without dropping to base (so we couldn't sample) compares to how a single 2-per season event affects aquatic community. Any other imaginable scenario can be entertained with data generated by a water quantity biomonitoring, as long as the scenario has occurred (but even if not occurring, our information could be informative). For example, if someone has the desire to assess the value of 3, back to back, 1 per season flow events, then one would watch flow gage for this particular event to occur. One documentation isn't sufficient (but could be informative), so more of the same events would have to be quantified and at different sites and conditions (e.g., upper reaches, lower reaches, spring season, summer season). How was the relative role of those changes evaluated? Please respond.

72. Section 3.1.1: It is noted that potential increased reproductive success for two fish species during an extended period of high flows is one explanation for fish community changes. The issue of duration of high flows sufficient to trigger changes seems to be an issue of potential importance. However, it is not obvious that duration of flows is being evaluated in the study. Please include some discussion of the issue of the role of high flow pulse duration.

Addressed above

73. Section 3.1.1: The text in the Overall Fish Community says:

"Among the 84 site visits, flow tiers were base (12), 4-per-season (4), 3-per-season (9), 2-per-season (17), 1-per-season (27), 1-per-year (5), 1-per-2-year (2), and >1-per-5-year (8)."

Please clarify that the sampling did not take place during the high-flow pulse events, but after a time delay for flows to return to base or subsistence levels.

Addressed above and mentioned in the report. "Sites with flow pulses were visited up to 15 days following the event but with the condition that flows returned to base tier or below lowest flow tier (e.g., 4-per-season on Brazos and 2-per-season for GSA and Colorado). Therefore, abiotic and biotic samples were taken at subsistence or base flow conditions and not during a high-flow event, which can cause a dilution effect."

74. Section 3.1.1: In the “Across Basin Summary Section” it appears that the data collected in the Colorado – Lavaca River is not included in the analysis. Please clarify why this data was not included in the across basin summary.

Colorado River basin wasn't sampled in Round 1. We had funding in Round 2 to start the process of gathering data at the scales necessary to inform standards/recommendations. Since we chose to concentrate on community responses following the highest of flows, we concentrated on the sites (GSA and BRA) with pre and post data.

75. Section 3.1.1, Across Basin Summary subsection: The foundation for the summary conclusion about ecological responses is not apparent. Please clarify what responses are being referenced here. Please explain and provide references on the validity of combining the data from the Brazos and GSA basins and then perform a statistical analysis of the combined data. See above. We revised the text to improve clarity with “responses”. Ideally, the value of flow tiers will be ubiquitous across basin and reach. Establishing universal trends, like the Natural Flow Paradigm, would provide confidence in how we manage our systems. Therefore, step 1 of our design is to test Y (e.g., densities of fluvial specialist) among flow tiers, basin, and season. Flow tiers and seasons are our main question, but we thought basin might be influential as well. If interaction between basin and tier (or season) was significant, then we split analysis and assessed response variable by basin (See Sokal and Rohlf. 1981. Biometry, 2nd Edition). Therefore, we would combine across basins, if interaction was not significant.

In Round 2, we started with our overall full model (tier, basin, season) for various dependent variables. We didn't find significance, which was counter to our expectations based on stream theory. As such, we wanted to understand why. With a decent amount of data accumulated at this point, we went deeper into the data set (by reach, by basin, effects of pre and post).

76. Section 3.1.1: Section 3.1.1: Figure 5 in the GSA and Brazos reports, Figure 6 in Colorado – Lavaca report and corresponding figures in Appendix A. Several reviewers expressed the desire for figures that show the actual dates of collection for both the historical data sets analyzed as well as the Phase I and Phase II data sets. Please add additional figures to the appendix that show antecedent flow conditions for several weeks/months prior to collection.

We provided hydrographs (previous 5 years) that show previous flow conditions. Dates and flow at time of sample are provided in the appendix

77. Section 3.1.1: Please provide a table that shows the actual flows during which the sampling occurred.

See response to #76.

78. Section 3.1.1: As noted in the historical fish analysis section, the maximum exceedance flow in the 15 days prior to sampling was used to establish the antecedent flow tier. Please justify the use of 15 days as the single maximum value versus other flow metrics.

We used the same time interval to be consistent with the aquatic biota study. As mentioned previously, we are in the processing of evaluating the 15-day interval.

79. Section 3.1.1: The analysis on flow ecology responses should be conducted on a site-by-site basis and not rely mostly on the combined across all sites approach. This is evident when examination of the minimum and maximum flows reported in Appendix B which range for example from 4 to over 83,000 cfs for riffles. Please provide summary flow and water quality data at each river site sampled.

See our response above. Round 2 analyses included across all sites and then at site level (or grouped by a few sites, as in lower GSA) to explore patterns in the data set. We are not done with the data set yet. We're continuing to analyze our results. Flow information was added. Water quality information was provided in Round 1 report. Additional Round 2 information will be forthcoming in future publications.

80. Section 3.1.1: Please provide a systematic discussion of the life-span and reproductive strategies of the fish community and how the 'response' or lack thereof between Phase I, Phase II, or in general given the different hydrologic regimes observed as illustrated in the hydrographs (see Appendix A and Figure 5 in the GSA and Brazos reports, Figure 6 in Colorado – Lavaca report). Based on the ecological literature, one would expect a differential response between different reproductive guilds given the large changes in both base flows and flood events between the antecedent hydrologic conditions prior to Phase II sampling.

Addressed above.

81. Section 3.1.2: It is not apparent how an analysis showing different species composition in different river basins helps to determine if current environmental flow standards for segments of the Brazos, Colorado – Lavaca, and GSA basins are appropriate. Please provide a discussion and references of how mixing data from different basins is appropriate for determining environmental flow standards.

Addressed above.

82. Section 3.1.2: The interpretation of the data reported is that the aquatic historical analysis did not include any information from the Lavaca/Navidad basin. It may be helpful to explicitly state that is the case (if it is).

Good point. Text was modified in this section to highlight that point.

83. Section 3.1.2: The sentence starting with "*Linear regression within each basin*" is confusing. Please reword for clarity. Suggest rewording to read, "Linear regression

within each basin revealed that the proportion of moderately swift water fishes to the total number of fishes increased with percent exceedance....” (Assuming that is the intent of the sentence).

Text was modified for clarity as requested.

84. Section 3.2: For each of the riparian sites, the text describes the discharge needed to inundate all riparian species and then describes a flow that will inundate 80% of the riparian distribution. In each case the flow to inundate 80% of the riparian distribution appears to be a mathematical calculation of 0.8 times the flow estimated from the rating curve to inundate the entire riparian distribution. It seems the flow needed to inundate 80% of the riparian distribution will be the flow needed to inundate the elevation covering 80% of the riparian distribution and not 80% of the flow needed to inundate the entire riparian distribution. For an example, see discussion in the Colorado – Lavaca report on p. 61 which refers to a flow of 1,000 cfs to fully inundate all riparian species and a flow of 800 cfs to inundate 80% of the riparian distribution. It appears this is a mathematically derived estimate and not one based on elevations over which riparian vegetation are distributed. Please clarify the process used to determine flows that inundate 80% of the riparian areas and include (in an appendix) the rating curves on all riparian sites included in the three reports and provide a discussion of their accuracy.

All reference to 80% inundation for the riparian zone was removed from the report. With the TWDB required deletion of the Validation Assessment Tool (4.2) and application (4.3) sections, this discussion was rendered irrelevant.

85. Section 3.2.1: In order to better inform BBASC evaluations, please provide a simple explanation of the statistical approach and guidance on how to interpret the results. For the typical BBASC member, terms like nMDS and ANOSIM statistic are not particularly meaningful.

Text added in the report

86. Section 3.2.1, Colorado – Lavaca only: In Table 9 page 40, it is not clear why a different flow level is required to inundate the various “tiers” of riparian habitat during different seasons. Because the ground elevation does not change, it is not obvious why the amount of flow needed to produce inundation changes. Please provide an explanation of the methodology employed to develop the inundation flow levels needed to make the seasonal variations in inundation flow understandable. The same issue arises for the Onion Creek results in this table.

These tables were in error and have been corrected in the final report. There are no seasonal differences in inundation level at any site.

87. Section 3.2.3, Colorado – Lavaca only, Navidad River: Green ash is referred to as the only riparian woody species represented. Pecan is also present. Please clarify why Pecan is or is not considered to be a riparian species.

Pecan is classified as a FAC species. We limited to OBL and FACW.

88. Section 3.2.4, Colorado – Lavaca Only, Sandy Creek: References to tables should be corrected to refer to tables 14 and 15. Please explain the dramatically different results for inundation flows by season, varying by almost 3,000 cfs. The same comments apply for the variation in results shown in Table 14 for this site.

References to Tables 14 and 15 have been corrected. Additionally, the errors in these tables have been corrected in the final report. There are no seasonal differences in inundation level at any site.

89. Section 3.2.5, Brazos and GSA Reports, Section 3.2.7, Colorado – Lavaca Report: Regarding the statement:

“Existing TCEQ flow standards need adjustment”

This is contradictory to the statement,

“...additional research is recommended to clarify riparian needs so that managers can make the most-informed decisions possible.”

Please modify or reconcile these statements.

The first statement has been modified to say “Existing TCEQ flow standards may need adjustment based on existing information and future research”. It was not the intent of the project team to make recommendations but rather provide data for the BBASC’s and BBEST’s to conduct their own assessments.

90. Section 3.2.6 Colorado – Lavaca, 3.2.4 Brazos and GSA, Comparison of Methodologies: This section describes future statistical tests being applied to the data with some species excluded. Please describe why that approach was not applied to these data.

This study was specifically designed to examine overall community assemblages. The methods were developed for this goal. The reason we did not perform analyses of less-prevalent but more keystone-functioning species (as we suggest future studies do) was that the sampling was not intended to allow for that. In the appendixes were our attempts to do this very function and it was noted that a lack of robust sampling of the less-prevalent species prevented satisfactory statistical outcomes. That’s why we suggested a follow-up study that takes such a focus.

91. Section 3.2.7 Colorado – Lavaca, 3.2.5 Brazos and GSA, Conclusions: This section states,

“...there were sometimes strong correlations to various abiotic factors...”

Please clarify which strong correlations this is referring to.

These refer to the extensive PCA statistics found and discussed extensively in the appendixes.

92. Section 3.2.7 Colorado – Lavaca, 3.2.5 Brazos and GSA, Conclusions: For clarity, please repeat the study questions and hypotheses (from Section 1.2.1) and provide brief answers and conclusions.

We feel this is redundant and encourage the reader to refer back to Section 1.2.1 if interested.

93. Section 3.3.3, GSA Report Only: The text repeatedly used the terms “recommended” and “recommendations” however these terms as used here are not clear in meaning. In the SB3 context, “recommended” has generally taken on the meaning of a set of recommendations from either the BBEST or BBASC and is contrasted to the “adopted” values of TCEQ or in the “standards”. Tables 14 and 15 which are referenced makes use of “adopted” values. Please clarify the intent here.

Text was modified to clarify comparisons are being made to TCEQ adopted standards.

94. Section 3.3.3, GSA Report Only: The text discusses the frequencies at which oxbow connectivity occurs. Presuming that the text here is referring to flows that may be expected under the adopted standards [see previous comment], the reviewers do not agree with this statement “recommendations [of frequencies under the adopted standards] generally protect annual connection frequencies similar to those experienced historically for these particular habitats (Table 15).” The reviewers disagree with this statement on several levels. The first disagreement is with the numerical values presented in the column “Number of Annual Connection Events Protected by TCEQ Flow Standards. This is because in the Adopted Standards, Section 298.375 (d) (6), for sites on the Guadalupe River, states that “if a pulse flow requirement for a large seasonal pulse is satisfied for a particular season, one of the smaller pulse requirements is also considered to be satisfied.” Therefore, while Table 14 accurately portrays which seasonal pulses would connect floodplain habitats, the reviewers do not agree that the tally of “Number of Annual Connection Events Protected by TCEQ Flow Standards” presented here is accurate. At Gonzales, for example, “protected” events, if all candidate events covered by the standards occurred in a single year, could range from a low of 4 to the maximum of 5 listed. At Cuero, the range of similar “protected” events would strictly range from 6-8. The more strenuous objection to the comparison made in the last two columns of Table 15 relates to the appropriateness of comparing a single theoretical ideal year of pulses that are protected and could potentially occur [column label “Number of Annual Connection Events Protected by TCEQ Flow Standards”] with the frequencies that did occur under the long-term historical record. This objection is more fully explained in the Required Changes, Tables and Figures Comments section (Table 15). Please provide

hydrologic analysis that supports the statement that annual connection frequencies will be similar to historic frequencies.

Table 15 was deleted as its contents were not used in the validation methodology. Only the flow level necessary to connect these floodplain features (as shown in Table 14) was used in the Validation Assessment tool presented in the Draft report.

95. Section 4.1.2: The sentence,

“Importantly, this study independently verifies Round One outcomes in the Brazos and GSA basins: that in order to provide continued conservation and maintenance of the current riparian spatial distributions at many sites the existing TCEQ flow standards (spring and fall) likely need adjustment.”

seems to be an understatement. This study demonstrated that the flow magnitudes included in the standards are too low to inundate certain riparian species at the elevations at which they were observed. High flow magnitudes are necessary but not sufficient, they must occur at the right times of the year, last for sufficient durations and occur with sufficient frequency. Please discuss why the magnitude, duration, and timing of pulses is required to maintain the existing riparian habitat.

Based on existing information, the project team agrees with this comment, but it was not the intent of the project team to make recommendations but rather provide data for the BBASC's and BBEST's to conduct their own assessments.

96. Section 4.1.4: It is stated in this section,

“We recommend focusing on native fish assemblages and fluvial specialists.”

And later,

“A potential ecological goal for subsistence and base flow evaluations would be to maintain the densities and relative abundance of native fishes as a community or individual species (e.g., fluvial specialists) with no less than a 25% reduction from recent (past 10 years) or historical (past 50 years) conditions.”

It is difficult to determine where this information comes from. Acceptable deviations from current conditions (25%) are put forward without justification or citations. Reviewers agreed that the focus should be on native fish assemblages and fluvial specialists and the pulse flow analysis should consider time, frequency and duration. Please clarify why the current study did not focus on native fish assemblages and fluvial specialists and why 25% is considered an acceptable reduction.

This is simply a hypothetical example to show that a quantifiable biological goal needs to be set in order for a meaningful assessment to be conducted. In our opinion, comparing to SEE is not appropriate or accomplishable. This hypothetical scenario is not supported

by any documentation and by no means was ever implied to be an “acceptable reduction”. The project team actually used 10% as a “potential” goal in the original Draft Report, Section 4.1.4 and could have just as easily chosen the hypothetical situation of 0%.

97. Section 4.1.1: This section was difficult to understand, particularly because the term “responses” was not defined. Please be clear about what kinds of responses are being referenced here. For example, be more specific about what is implied by “positive significant relationships.” The relevance of the fish community findings to environmental flows standards is not clear. Please reword for clarity.

Text was modified in this section to clarify that responses are statistical differences in relative abundance or diversity caused by flow. A positive response refers to increase in one or both parameters for swift-water fishes.

98. Section 4.1.2: Define for the reader what is meant by “WI class groupings”. The following text is not informative, “... *added rich understanding and multi-faceted views of the riparian community.*” Simply provide the major findings and conclusions in easy-to-understand language. Please include the evaluation of any existing flow standards and provide any resulting recommendations. The report should be very clear about this. If there are no specific recommendations about flows feasible at this time, then please explain why, and under what circumstance specific recommendations would be feasible.

This summary statement was adjusted to read, “Three sub-categories of testing (overall community assemblages, wetland indicator class groupings, and canopy species) provided multi-faceted views of the riparian community.” This is only meant to be a summary statement. Results as requested in the remainder of the comment are provided in Section 3.2. As for recommendations, it was not the charge of the independent scientists conducting the work to provide “recommendations” but rather provide data, analysis and a potential assessment tool for the BBASC’s and BBEST’s to use to formulate their own recommendations.

99. Section 4.1.4: There is no obvious support for the ecological goal of a 25% reduction of densities and relative abundance of native fishes in the Brazos and Colorado – Lavaca Basins and a 10% reduction in the GSA Basin. Because these back-of-the-envelope numbers can easily become benchmarks for future work there should be very clear guidance given on how to determine acceptable reduction in densities and relative abundance of native fishes. The reports as written now provide no guidance or references on streams that have successfully been managed to achieve given reductions and densities of abundance of native species. Please provide data supporting these goals or remove them from this report.

Please see response to Comment 96 above. These are simply hypothetical examples to show that quantifiable biological goals need to be set in order for a meaningful assessment to be conducted.

100. Section 4.1.3, GSA Report Only, Floodplains: The paragraph that concludes with the sentence:

“Overall, when comparing to the TCEQ environmental flow standards, considering recommended frequencies, if the appropriate seasonal flows occur, the standards generally protect annual connection frequencies similar to those experienced historically for these particular habitats.”

There are several problems with the wording of this sentence. The word “recommended” is confusing; presumably the intent is to refer to the adopted TCEQ standards values (see Section 3.3.3 comments above). More fundamentally, the conclusion that “the standards generally protect annual connection frequencies similar to those experienced historically” is not supported. Please revise as necessary.

This statement was deleted.

101. Section 4.1.4: Referring to the sentence:

“Although the focus of this study (both rounds) was on pulse-flow responses...”

That focus did not appear to be clearly stated at the beginning of the project description. If it was the focus, please state so clearly at the beginning of the report and discuss the reason why pulse-flow responses were selected as the focus for this work.

Text was modified in the Introduction to highlight that pulse flows were the focus of both rounds of study.

102. Section 4.1.4: The potential ecological goal appears to be poorly phrased. It seems likely that the intended test is to have no more than a 25% reduction rather than no less than that reduction. In either articulation, the basis for the test requires discussion. Please clarify if the goal is intended to apply on both a community and an individual species basis or just one of the two. Please clarify if the goal is intended to apply both to data for the last 10 years and past 50 years or only one of the two. A 25% reduction allowed every 10 years, would cause the fish community to almost disappear in only a few decades. The description of the pulse flow potential goal is difficult to follow. Please clarify if it is intended to focus solely on the 1-per-season pulse. Also, please clarify what is meant by a “1-per-season ecological response” and how it would be measured. If these tests were discussed at the expert/stakeholder workshop, please provide some summary of the discussion.

The hypothetical goals discussion in Section 4.1.4 was designed to introduce the proposed validation methodology assessment tool in Sections 4.2 and 4.3. With the TWDB required deletion of Section 4.2 and 4.3, there no longer any need for an introduction to the tool. As such, all references to hypothetical goals in Section 4.1.4 have been removed.

103. Section 4.1.4: This section references timing, frequency, and duration of pulses. There is almost no discussion of the duration component of pulses in the methodology so the basis for a duration recommendation is unclear. The basis for the recommendation of a focus just on native tree species is unclear. Please clarify the basis for the duration recommendation. It would be helpful to have some discussion of the roles played by inundation and how duration might affect those roles.

The hypothetical goals discussion (including duration) in Section 4.1.4 was designed to introduce the proposed validation methodology assessment tool in Sections 4.2 and 4.3. With the TWDB required deletion of Section 4.2 and 4.3, there no longer any need for an introduction to the tool. As such, all references to hypothetical goals including duration in Section 4.1.4 have been removed.

104. Section 4.1.4: Referring to the sentence:

“A potential ecological goal for recent floodplain features in the GSA basin would be to have semiannual connectivity in the spring and fall with a period of connection of up to a week.”

Please provide supporting documentation to the necessity of the Spring and Fall connectivity and citations that support connectivity of one-week provides for sufficient time for ecological functions of oxbow lakes.

The hypothetical goals discussion in Section 4.1.4 was designed to introduce the proposed validation methodology assessment tool in Sections 4.2 and 4.3. With the TWDB required deletion of Section 4.2 and 4.3, there no longer any need for an introduction to the tool. As such, all references to hypothetical goals in Section 4.1.4 have been removed.

105. Section 4.1.4: The last sentence states,

“A potential ecological goal...would be to inundate approximately 80% of the existing native riparian species...”

Please describe the basis for the 80% goal and provide citation(s).

The hypothetical goals discussion in Section 4.1.4 was designed to introduce the proposed validation methodology assessment tool in Sections 4.2 and 4.3. With the TWDB required deletion of Section 4.2 and 4.3, there no longer any need for an introduction to the tool. As such, all references to hypothetical goals in Section 4.1.4 have been removed.

106. Section 4.1.4: The last sentence refers to,

“...an ecological assessment based on the flows that have occurred since implementation of SB 3 standards.”

The meaning of that statement is a bit unclear. Please define what is meant by “implementation.” Some permits have been issued with flow conditions informed by SB3 flow standards, but it is unclear, and unlikely, that any of those permits have actually significantly affected flow levels. Flows of a particular magnitude occurring before “implementation” of SB3 standards are not really any different than flows of a similar magnitude occurring after “implementation.” As noted previously, this study does not appear to be evaluating the potential effects of the patterns of flows protected by the SB 3 standards but rather just conditions during a snapshot of time when a particular flow level is occurring.

The assessment tool proposed was purposely designed to be in real time, not some unknown future condition. The assessment is predicated on the following two assumptions, 1) as long as the river is staying healthy (as defined by the quantifiable goals established by the BBASC and not “sound ecological environment”) then the adopted standards are acceptable, and 2) long-term monitoring is actively being conducted in order to determine trends in those goals over time. The first provides an assessment in real-time while the second provides the warning system for adaptive management into the future.

However, with the assessment tool section of the draft report being deleted per TWDB requirement, this paragraph is no longer relevant and was deleted from the final report.

107. Section 4.1.4: It is not clear that an overriding concern of the BBASC and SAC was to “...know what the ecology needs, not just what it has seen in the past.” Some context is needed. It is also not clear that sufficient time has elapsed since adoption of the flow standards to produce/detect any ecological changes related to the flow standards. Please clarify.

With the entire assessment tool section of the draft report being deleted per TWDB requirement, this statement is no longer relevant and was deleted from the final report.

108. Sections 4.2 and 4.3 shall be removed from the report. Several reviewers recommended these sections be removed from the report. One reviewer commented that continuing to sample as proposed will not provide useful information on the relationships between ecological flow regimes and responses in either the fish or macroinvertebrate communities. A second reviewer recommended deleting this section, because it largely falls outside the scope of work for the contract. A third reviewer recommended that this section should be removed. It does not add much value, relies on standards for acceptable alteration that are not supported by data or references and proposes strategies which are clearly beyond the scope.

As stated in the response to Comment #19, these sections have been removed in the final report as a requirement of TWDB.

The following text was inserted in the main body of Chapter 4 of the report to replace the entirety of Section 4.2 and 4.3.

“The validation methodology assessment tool introduced in the Round One study, highlighted in Round Two Expert Workshops, presented in detail in the draft Round Two report, and subsequently presented to both the Brazos and GSA BBASC’s upon completion of the draft report was removed from the final report as a TWDB requirement. It is TWDB’s professional judgement that insufficient data is available to validate the tool, and thus any practical application of this tool at this time is inappropriate.”

The project team respectfully disagrees with the first reviewer’s professional opinion.

The second reviewer apparently did not have access to the scopes of work as each scope had a statement similar to the GSA statement that reads, *“Following data collection, and in conjunction with advice from the Expert Panel Workshops, the objective is to complete the validation methodology and provide the GSA BBASC with a working tool for TCEQ standards evaluation.”* Additionally, had this reviewer read the Round One final reports or attended the Round Two Expert Panel workshops, there would be no question to whether this approach was within the bound of the scope of work for this contract.

The third reviewer appears to be judging the assessment tool on its merit to be a predictive ecological model, which it was never intended or promoted to be. Additionally, this third reviewer must not have had access to the TWDB approved scopes of work or attended any of the Round Two expert workshops based on their assertion that this is “clearly” beyond the scope of work. Section 4.2 and 4.3 directly apply to the scope statement quoted in the previous paragraph.

109. Section 4.3 in Brazos and GSA Reports, Section 4.2.5 in Colorado – Lavaca Report: Page 108 - Please define what meant by a recent oxbow. Please provide a reference that identifies the need for a minimum of 75% of oxbows to be connected for two consecutive days. Please discuss how the aquatic community is affected if 85% of the oxbows are connected and what is lost if only 60% are connected and how the aquatic community is affected if 4, 8, 16, or 30 consecutive days of connectivity occur.

No response needed as TWDB required that Section 4.3 be removed in its entirety.

110. Section 4.3.1, Brazos Report Only: Brazos River-Rosharon, page 110. The reference to fall wet season pulse standards should be winter. Please correct.

No response needed as TWDB required that Section 4.3 be removed in its entirety.

111. Section 4.3.1, Brazos Report Only: Brazos River-Bryan, page 111. The reference to fall pulse standards should be winter. Please correct.

No response needed as TWDB required that Section 4.3 be removed in its entirety.

112. Section 4.2.4: Reviewers did not see a need for comment on the broader issues of SB2 and 3 in this report and recommended that the ideas about how the SB3 process should play out in the future should be deleted -- it is not the concern of this research team. If the research team has specific recommendations about future research that can help in the adjustment of environmental flow recommendations from an ecological standpoint, then those should be offered in a clear and succinct manner.

No response needed as TWDB required that Section 4.2 be removed in its entirety.

113. Section 4.2.5 in Colorado – Lavaca Report, Section 4.3.1 in Brazos and GSA Reports: It is not valid to increase flow values for a given frequency event. The standards would then require events to occur at a frequency not supported by historical data. If a change is needed, the valid approach would be to go to a less frequent event with higher flow. Assuming that a 1-per season flow of 27,000 cfs is needed, the flow of 27,000 cfs could be provided by a 1-per season pulse in winter (25,700 cfs) and spring (33,700 cfs). It would not occur with a frequency of 1-per season in summer (13,300 cfs). (BBEST report.)

No response needed as TWDB required that Section 4.2 be removed in its entirety.

114. Section 4.2.5 in Colorado Lavaca Report, Section 4.3.1 in Brazos and GSA Reports: There is a recommendation to reduce durations of pulse flows because existing durations in environmental flow standards may drown seedlings and saplings. Please provide citation(s) to support this recommendation.

No response needed as TWDB required that Section 4.2 be removed in its entirety.

115. Section 5: This section editorializes the level of success accomplished by the work, and the importance of the steps taken. Please delete text that is editorial in nature.

This section was modified to delete editorial text although the authors stand behind the success of both rounds of studies.

116. Section 5.1: *Post-flood aquatic community shift dynamics*: Extensive review by TPWD, TWDB, and outside experts from Public and Private entities are not encouraged that this is a useful approach and disagree with the assertion.

We appreciate the comment, but this section reflects the professional opinion of the independent instream flow scientists hired to conduct this work.

117. Section 5.1: *Post-flood aquatic community shift dynamics*: Please explain how a “post-flood” aquatic community assessment and sampling under the current framework will be used to validate flow tiers.

The post-flood aquatic community assessment will inform as to whether the ecological responses observed during Round 2 of studies was temporary or more permanent (i.e. necessary for the resetting of conditions in the stream). Thus, it allows for a temporal assessment of the TCEQ standards based on longer term antecedent conditions.

118. Section 5.1: *Channel morphology*: This guidance is beyond the area of expertise of the study team, beyond the scope of work, and quite vague. Please delete.

We appreciate the comment, but this section reflects the professional opinion of the independent instream flow scientists hired to conduct this work.

119. Section 5.2: The phrase “Biological Condition Gradient” first appears in this section of the reports. Please define and state its relevance to the analysis in terms readily understood by BBASC members and other readers.

Please refer to earlier comment responses on this topic.

120. Section 5.2: This section refers to development of an IBI Water Quantity approach and to an existing IBI Water Quality approach. However, the state’s current IBI is not a Water Quality approach. The state’s current IBI focuses on relationships between ecological health of fish and benthic macroinvertebrate communities with habitat, including flow, water quality, and other factors that may be relevant on a site-specific basis. Please correct this section since there is not currently an IBI Water Quality approach.

The paragraph regarding IBI’s was deleted from the report.

Figures and Tables Comments

1. Section 2.2.4 Figure 4 Colorado-Lavaca Report only: Please use a different color to indicate the randomly selected points in Tier 2. The dark purple points are difficult to distinguish on color printouts.

We appreciate the comment.

2. Section 3.1.1 Table 2 Colorado – Lavaca Report and GSA Report, Table 6 in Brazos Report: Please add a table showing the species’ abundance, density, and relative abundance for each sample date for each sample site in each basin.

This is not a table but the data set. Release of this information will be forthcoming in future publications.

- Section 3.1.1, Table 4, Colorado-Lavaca Report only: Please add an additional table showing the orders' density for each sample site in the Colorado-Lavaca basin.

Release of this information will be forthcoming in future publications.

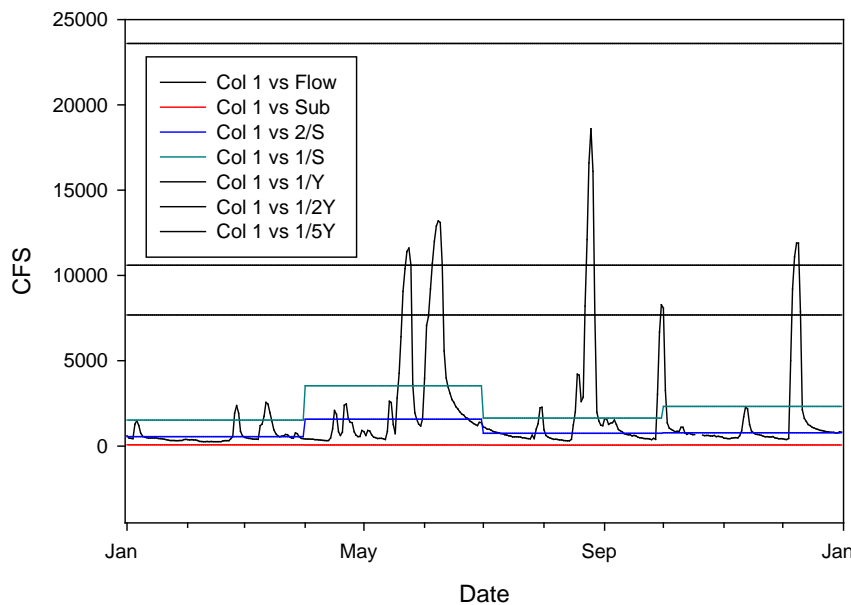
- Section 3.1.1, Table 6 in GSA and Colorado – Lavaca Reports, Table 10 in Brazos Report: This table indicates that there was a response for 1/5Y at Navasota River – Easterly but that effect can't be found in the results, descriptions, or figures. Please correct the table.

See Brazos River Report. Riffle and run responses are provided.

- Section 3.1.1, Table 6 in GSA and Colorado – Lavaca Reports, Table 10 in Brazos Report: It would be helpful to know which species are considered flow dependent. Please add a column indicating whether species are considered generalist or fluvial specialist. See fluvial category column. We labeled them as slack, moderate, and swift. The term “generalist” includes slack and moderate.

- Section 3.1.1, Figure 5 in Brazos and GSA Reports, Figure 6 in Colorado – Lavaca Report: Please include some delineation illustrating what constitutes a “flood event” in these figures.

Flow tier magnitude for each site and seasons are provided in the BBASC reports and TCEQ standards. Visualization of this is difficult to view on a single graph (see below example), primarily because 3 per season, 2 per season (2/S), 1 per season (1/S) differ among seasons.



7. Section 3.1.1, Figure 6 Colorado – Lavaca Report only: The figure included in this report contains data from the Guadalupe River at Gonzales. Please explain why data from the Guadalupe River is included rather than data from the Colorado – Lavaca basin in the caption.

Consequence of generating three separate reports from a single study, one of which (Colorado-Lavaca) began two years later. Fig 6, as stated in the text, is an example graph to illustrate pre and post evaluation period. It doesn't make sense if viewing Colorado-Lavaca as an independent study. It is not. Due to how recommendations/standards were developed in Colorado, GSA, and BRA, all three basins can be assessed to add greater replication (and wider range of conditions observed).

8. Section 3.1.2, Figure 9 in Colorado – Lavaca Report, Figure 24 in GSA Report, Figure 26 in Brazos Report: As described in the text, this graph compares abundance of Swift-water fish in the three basins. However, this is a box plot and it is not clear what the parts of the boxes represent and why “Percent Exceedance” is on the x-axis. Please clarify.

“Percent Exceedance” was removed from the X-axis as it was an error.

9. Section 3.2.1, Table 8 in Colorado – Lavaca and GSA Reports, Table 12 in Brazos Report: Please include a column showing which species are herbaceous, woody, and their wetland indicator status.

Thank you for the comment. These tables were provided to show basic community assemblage data. The requested data can be compiled by interested reviewers at their discretion using the published literature they are most comfortable with.

10. Section 3.2.1, Table 9 in GSA Report: The reason for the difference in inundation flow by season requires explanation. The “tier” max elevations listed here do not appear to match the elevations shown in Figure 30. Please explain or correct the discrepancy.

Text and tables were corrected in the final report. There are no seasonal differences in inundation level at any site.

11. Section 3.2.1, Table 13 in Brazos Report, Table 9 in GSA Report: Please provide more explanation about how recommended flows were derived. There was only a broad assumption that water level changes at the gage site are the same at the transect site. Please provide data and analyses that confirm the assumption that water surface at each site were the same as at the USGS gage. This assumption is not intuitively obvious. Explain how the tier max elevation was derived. Using USGS DEM data, for example in the Brazos report on page 64, it can be argued that 49.56 ft is the same as 50.14 ft which is approximately 50ft. Additionally, there is no substantial difference between 42,602.48 cfs and 43,561.22 cfs; they could both be rounded to 43,000 cfs....or to a range 40-45k cfs, based on the methods used to derive those numbers.

We agree with the comment and rounding interpretation. The riparian inundation estimates were intended to be just that, estimates. Estimated inundation values were rounded to the nearest 50, 100 or 500 cfs as applicable for display understanding that larger scale rounding could also be applied.

12. Section 3.2.1, Table 13 in Brazos Report, Table 9 in Colorado – Lavaca and GSA Reports: Please provide more explanation of the rating curve development for each site, and include the gage datum.

Addressed above.

13. Section 3.2.1, Figures 14-17 in Colorado-Lavaca Report, Figures 29-30 in Brazos Report, Figures 31-33 in GSA Report: In order to fulfill the study objective of informing BBASC evaluations, please provide additional explanation of the statistical approach used to create these figures and of potential interpretation of the results.

The general methodology is provided in the methods section. Please refer to the riparian appendix for further descriptions and application.

14. Section 3.2.1, Tables 8 and 10 Colorado – Lavaca Report only: Explanation of the methodology for determining inundation flows is required. Please explain the relationship between Tables 8, 9, and 10 and the discussion on page 39 as the numbers do not match. Please clarify how the flow sufficient to inundate 80% of distribution was calculated. Species, such as possumhaw holly and black willow, which are listed in Table 8 as occurring at the site, are not included in Table 10. NRCS describes them as FACW species. The Colorado-Lavaca Report states that these species will be fully inundated at 7,200 cfs and Table 9 shows a value of 22,408 cfs for full inundation. Please explain how the inundation flow of 4 cfs for sycamores was calculated at Colorado Bend State Park and 1 cfs at Onion Creek. These same comments apply to all sites discussed in the Colorado – Lavaca Report.

Addressed above.

15. Section 3.2.1, Tables 8 and 10 GSA Report only: The above comments also apply to the GSA report, please clarify the discussion and tables. Also, please provide analysis to clarify how 80% of the full distribution of all riparian species is inundated at 8,000 cfs (Goliad Site). This appears to be a straight mathematical determination of the fully inundation flow of 10,000 cfs.

Addressed above.

16. Section 3.2.1, Tables 12 and 14 Brazos Report only: The above comments also apply to the Brazos report; please clarify the discussion and tables.

Addressed above.

17. Section 3.2.2, Figure 19 Colorado-Lavaca Report only: Please provide elevation and distance in feet rather than meters in order to allow comparison to other information.

Change made.

18. Section 3.2.3, Table 15 Colorado-Lavaca Report only: The results listed here for the Navidad site all appear to be anomalous. Because species occurrence is not listed by “Tier,” the extent of errors is difficult to define. However, the text on page 51 expressly states that green ash occurs in “Tier” 3. If that is true, a flow of 26 cfs will not inundate any portion of “Tier” 3 and cannot represent the high elevation flow for that species.

As described in the report, the mature tree plots are a separate dataset from the tier/plot methods. So, a presence of green ash in Level 3 in one sampling technique (mature tree) cannot automatically be added to the level/plot (community) datasets. The random sampling method can/does miss important trees that are present but not encountered in random collection. Because in the random sampling green ash were only observed in Level 1, our discussion of the inundation estimate (correctly) underestimates that need given the dataset. But had we captured the mature green ash located uphill in the random sampling we would have indicated that in Table 16 which is what the commenter appears to have been expecting given the mature tree dataset.

19. Section 3.2.4, Figure 30 Colorado-Lavaca only: The elevations depicted in this figure do not match the elevations shown for this site in Table 14. For example, the highest elevation shown in Figure 30 is about 57 feet while the highest elevation shown in Table 14 for this site is slightly above 65.5 feet.

Text and table were modified for clarity.

20. Section 3.3.3, Table 15 GSA Report only: It is not clear how the “Number of Annual Connection Events Protected by TCEQ Flow Standards” column was populated, especially with the caveat of “if all the flow standards occur”. Since TCEQ’s implementation guidelines of the standards do not require the 2-per-season pulse level for a season if a 1-per-season pulse already occurred in that season. Please clarify.

Table 15 was deleted.

21. Section 3.3.3 Table 15 GSA Report only: The information and labels utilized in this table present a misleading comparison of expected connection frequency of the floodplain habitats under the adopted TCEQ standards. The associated text referencing this Table is therefore also misleading. In fact, the values for Victoria would lead a non-hydrologist (or BBASC member) to believe connection frequencies may even increase over historical levels. Even with the correction spelled out in the previous comments, the table will still mislead when it compares a single theoretical ideal year of pulses that are protected and could potentially occur [column label “Number of Annual Connection Events Protected by TCEQ Flow Standards”] with the frequencies that did occur under the long-term historical record. The problem with the comparison as given is that the column “Number

of Annual Connection Events Protected by TCEQ Flow Standards” represents a maximum ‘protected’ connection frequency for one or more years, which may actually never occur. The values in the table do not represent a long-term expectation compared to those of the last column and therefore are not “apples-to-apples” in common parlance. The table ignores a significant long-term outcome under the adopted standards: high-flow pulses, especially the smaller magnitudes, will go down in frequency after the implementation of new project(s) that are complying with the SB3 standards for high-flow pulses. That is an unequivocal result that was widely acknowledged during the SB3 process by BBEST and the SAC based on explicit simulations of theoretical SB3-compliant projects. The degree of alteration will depend on project particulars and the streamflow behavior, but the potential is that a highly-altered connection frequency over the long term may emerge. That potential is not evident whatsoever in the table, which paints the opposite picture. To illustrate this further, consider that the last column of Table 15, “Historical Connection Frequency” is a long-term average for a variety of years ranging from those in which connection frequencies were low (potentially none) through those in which it was high. The column before that [Number of Annual Connection Events Protected by TCEQ Flow Standards] is again a theoretical single year. To get at a long-term expectation for the “Protected by TCEQ Flow Standards” column, consider streamflows after the implementation of a new SB3-compliant project. For the years in which the connection frequency would already be low before the project, in the range of 0-3 high-flow pulses of connecting magnitude per season, the frequency will be lower but similar with the project, due to the protections of the standards. The big change would be for the years in which connection frequency before the project would have been higher than the standards’ requirements. Any pulses over and above the protected level could be removed, depending on the capacity of the project. So, in a year with many connecting-magnitude high-flow pulses coming down the river above a new project, the project would only have to pass the minimum number required by the standards. For example, if in a particular year, there were 10 high-flow pulses in each season of connecting magnitude above a project, below the project this could fall to between 2 and 3 required pulses per season, depending on the project, pulse sizes, and order of occurrence. So, in this theoretical year the connection frequency would drop from a historical value of 40 to between 8 and 12. Granted, there is no a priori number which can be derived as the ‘post-project / protected by Standards’ connection frequency over the long-term to make the “apples-to-apples” comparison that Table 15 strives to present. A long-term connection frequency value with SB3 protection depends not only on the project specifics but also on the nature of the high-flow pulses magnitudes and timing [if heavily concentrated in certain seasons, this yields the lowest values for post-project connection frequency]. The only solution here is to heavily caveat the comparison with appropriate expansion of the accompanying text linked to the table, modified column labels, and footnotes. The very minimal parenthetical text in the label for Table 15 “(if all flow standards occur)” is not at all adequate to alert the reader to the embedded assumptions and limited comparability of the last two columns. Please either delete this table and accompanying text or perform the necessary hydrologic analysis to provide the reader with a realistic value of events protected by the TCEQ flow standards.

[Good points, Table 15 and associated text was deleted.](#)

22. Section 4.1.4, Table 24 in Brazos Report, Table 19 in Colorado – Lavaca Report, Table 16 in GSA Report: It is unclear what the checkmarks represent. Please define what the check marks represent in the caption.

Text was added to the caption to explain that checkmarks represent an ecological response to flow.

23. Section 4.2.5 Table 20 in Colorado – Lavaca Report: It is unclear what the checkmarks represent. Please define what the check marks represent in the caption. Table 20 appears twice, please revise.

No response needed as TWDB required that Section 4.2 be removed in its entirety.

24. Section 4.3 Table 25 in Brazos Report, Table 17 in GSA Report: It is unclear what the checkmarks represent. Please define what the check marks represent in the caption.

No response needed as TWDB required that Section 4.3 be removed in its entirety.

References

1. Given the difficult challenge of devising an approach to validate environmental flow standards, and the rather unstructured discussion of the topic in this report, a major omission is citation of the most influential and current literature dealing with this topic. This is a rapidly evolving field of research, and there are many points of view represented in a large literature. Please provide the reader with citations that confirm the approach taken in these reports. Of special interest to the BBASC would be projects that reduce the frequency of pulses based on the fact that the pulses show no ecological benefits and that were successful in maintaining the aquatic biota diversity.

The project team does not disagree that there is a wealth of literature on instream flow science and particularly, how important flow regimes are in supporting aquatic communities. Where the literature is limited or often silent is on specific ecological responses that can be tied to specific flow tiers. The assessment of the individual components of a “flow regime” was the goal of this project. As such, this is new science and is not presently supported or refuted in current literature. We look forward to publishing and starting to enhance the literature available on this specific component of instream flow science.

2. Much of the literature referenced in the report deals with the riparian and estuarine components, and there is relatively little supportive information regarding ideas and options for how to determine environmental flows for instream biota. Only Poff et al. 1997 is cited to provide general guidance here. No supportive information from the scientific literature is provided for specific guidance. Please provide the reader with

citations regarding determination of environmental flows and instream biota that confirm the approach taken in these reports.

Literature support was discussed in the previous comment and has been described and discussed elsewhere (e.g., workshops). As work is in progress, future publications will contain the traditional organization of published findings.

SUGGESTED CHANGES

General Draft Final Report Comments

1. Please consider including a list of acronyms. There are many acronyms in the report that are not readily understood by likely readers and users of the report outside of the research scientific community, such as most members of the BBASC.

List of acronyms have been included.

2. Section 5: Regarding the sentence:

“However, it is acknowledged that future work could enhance the ability of stakeholders, river managers, and the TCEQ relative to validation, application, and adaptive management.”

Yes, this has been stated multiple times in this report, which takes up space that could be used to better explain the findings and how they can be used to make specific recommendations about environmental flow standards. Suggest deletion of all of sections 4 and 5, because the text is very redundant and not directly relevant to the contract scope of work.

Please see comment responses above.

Specific Draft Final Report Comments

1. Executive Summary: Regarding the sentence

“Hypotheses and goals were kept the same so that accumulated historical database could be compared to the current research investigation.”

It is not clear what is meant by “the same” since the Executive Summary earlier states that,

“hypotheses... were developed and tested in this second round...”

Please clarify in what ways the hypotheses and goals were or were not the same as those in the first round.

Text was modified.

2. Section 1.3 Brazos Estuary, Brazos Report Only: The estuary sections present a great deal of descriptive data. The objectives and hypotheses seem reasonable although expected species population level responses, like the majority of the species responses in the aquatic section, will likely not be detectable and would benefit for a more comprehensive time series analysis.

Please see Brazos Estuary report responses.

3. Section 3.1.1: For greater clarity, please consider rewriting to quote specific predictions in question should and the results which are consistent with and opposite of the predictions listed. Thus “It was predicted that.... The increasing density of ___ with higher flow pulses was consistent with this prediction, while the decreasing density of ___ and ___ with higher flow pulses was the opposite of the prediction.

We revised the documents to improve clarity as much as possible. Comments like these are helpful to improve clarity.

4. Section 3.1.1: The findings would be clearer to the reader if the species cited were identified as fluvial specialists or generalists.

This information is contained in the species table.

5. Section 3.2 Riparian (including Subsections 3.2.1 and 3.2.2): This Section uses the term “Tier” to refer to a spatial subdivision of the floodplain whereas the use of the term ‘tier’ in other sections, especially related to “Aquatics,” refers to one of nine flow rate magnitudes of the environmental flow regime (e.g. subsistence or 1/year high-flow pulse as defined in Section 2.1.1). Initially it was thought that in this Riparian section this was a clever shorthand for linking the flow magnitude tier to a corresponding spatial extent of inundation at that flow. However, upon further reading, this potential linkage appears to not be the case or at least one has not found that linkage within the report. Evidence pointing to a lack of correspondence is in table where the Tiers (spatial) and flows to inundate appear. The flows are not in increasing order for example at Goliad due to some topographical features, so they would not appear to be related to flow tiers which uniformly increase.

See points 34 and 35 above, which changed the nomenclature of within-zone ‘tiers’ to “level”.

6. Section 3.2.1 Brazos Bend and Hearne Sites, Brazos Report Only: Reviewers commented that using sites for assessment where the adjacent/opposite bank is severely eroding due to poor land management practices and is not representative of the reach or of a healthy

riparian area. The sampled side likely experiences increased bar aggradation and migration, and the riparian species are reflective of this (more willows). It is understood that accessibility is problematic and where you can obtain landowner permission is not always ideal but please consider acknowledging the limitations of sites in general and these sites specifically in the report.

It is our experience that along these large rivers the long term downcutting that has occurred has left much of the river banks devoid of a healthy, well-connected riparian zone. In short, some of the best (often the only) reaches with riparian connectivity remaining are the sand bars. Yes, owner permission is a definite limitation, but even more so is the dearth of riparian vegetation along the river continuum. Each of the Brazos sites represent sand bars where the opposite bank is a cut bank, and these sites' characteristics are not reflective of poor land management practices. (E.g. willows will thrive on sand bars irrespective of the land management along a sand bar.) Instead, they reflect ecologically expected successional communities along just such a stream reach. What we will concede is that land owner permission definitely limits across-bank studies, as that opposite bank is usually not owned by the same person/entity.

7. Section 3.2.1, Brazos Report Only, page 59, last paragraph, last sentence: In the second part of sentence is an assumption that is countered by literature indicating black willow inundation survival of up to 30 days. Recommend removing assumption.

Assumption was removed.

8. Section 3.2.4, Brazos and GSA Reports, Section 3.2.6, Colorado – Lavaca Report: Regarding the *Pros* and *Cons* bullet list, Pro #1: Reviewers suggested including the phrase “with a statistically significant number of repeat sample events.”

We agree and text was modified as indicated.

9. Section 3.2.4, Brazos and GSA Reports, Section 3.2.6, Colorado – Lavaca Report: Regarding the *Pros* and *Cons* bullet list, Con #1 needs some clarification because conclusion from previous discussion it was thought that the same could be said for the corridor method.

The transect method, which established plots wherein all species and life stages were collected allowed for the linkage of survival and recruitment of those individuals to be tracked over time and in response to specific flow pulses. The corridor method (which is being discussed in this section) does not (as is stated). It appears the reviewer may be confusing the two methods.

10. Section 3.2.4, Brazos and GSA Reports, Section 3.2.6, Colorado – Lavaca Report: Regarding the *Pros* and *Cons* bullet list, specifically Con #2: Reviewers suggested indicating how many repeat corridor sampling events over what time-frame are necessary to have statistical significance and to ensure changes measured between sampling events are significant. If it is not time and effort causing repeat corridor sampling to be a con,

clearly discuss why secondary corridor sampling is a con. If it is time and effort causing repeat corridor sampling to be a con, it is not really a con; it is just what it takes to gather data necessary to assess long-lived communities like forests.

We don't foresee a 'magic number' for repeated samplings; rather there is an increase in the statistical output with each successive sampling (as more of the community is gradually encountered via random plots). It was stated within the report that this first round of sampling revealed extremely truncated datasets, for riparian-functional species in particular. Repeating the methodology builds that dataset through time. And yes, we considered time/effort/funding as a con, given there is no guarantee future funding/studies will be performed on any given project. But with those resources, repeat sampling becomes a pro rather than a con. We like the way the reviewer stated it: it is just what it takes to gather data necessary to assess long-lived communities like forests.

11. Section 3.2.5, Brazos and GSA Reports, Section 3.2.7, Colorado – Lavaca Report: In the last paragraph, the reviewers disagree with the statement suggesting multiple sampling trips per season are needed to document adequately. Reviewers suggest species recruitment and successful individual maturity provides the information this paragraph indicates is missing. Please respond regarding this alternative approach.

Multiple sampling trips over successive seasons (spring, summer, etc.) provide information regarding survival and recruitment within a growing season. But we agree that multiple sampling trips *per season* (e.g. fall) are not necessary. If the focus is within a growing season, then sampling seasonal changes give a more robust dataset than a single sampling event. If longevity is the focus, then fewer within growing season samplings are needed.

12. Section 4.1.4: In the Brazos and Colorado – Lavaca reports: The recommended 25% reduction goal and 10% in the GSA seems arbitrary. Please describe the basis for the desirability of these percentages of reduction in relative abundance of native fishes.

They are arbitrary and simply provided to provide the BBASC and BBEST something to start the discussions.

13. Section 4.1.4: The riparian zone is not well defined; therefore, please clarify if the recommended 80% inundation just includes the three tiers in the studies or whether it includes areas outside of the tiers. The goal of twice per year inundation is not clearly supported by the data analysis as presented in this report. Based on the data presented the twice per year frequency recommendation seems to be arbitrary.

Again, these are arbitrary goals to stimulate BBASC and BBEST discussion.

14. Section 4.2, Validation Approach, in its entirety: While Section 4.1 is a “Summary” and is a valuable portion of the deliverable to satisfy the Scope of Work; Section 4.2 appears

to be entirely the presentation of a ‘brainstormed / possible’ path forward to pursue a refined version of this research in the future.

[No response necessary.](#)

15. Section 4.3, Potential Application, in its entirety: Section 4.3 appears to be entirely the presentation of a new “balancing” approach for environmental flow needs in light of the expanded findings of this research. The presented ideas for how a BBASC might approach goal setting for Aquatics, Floodplain connectivity, and Riparian is clearly outside of the Scope of Work for this project.

[Please see previous responses regarding the Round 1 final reports, Round 2 expert workshops, and contractual scopes of work.](#)

16. Section 5.0 Brazos Report Only: Please clarify if there any “goals” associated with the estuary work like there were for the instream work and add discussion similar to the instream flow work.

[Please see Brazos Estuary report responses.](#)

Figures and Tables Comments:

[We appreciate the following comments. It is comments like these that assist authors in improving the present document and future publications. Changes suggested below were incorporated as deemed appropriate by the authors.](#)

1. Section 2.2 Figure 2 in Brazos and GSA Reports, Figure 4 in Colorado – Lavaca Report: The colors of the random points selected in Tiers 2 and 3 make the points all but invisible. Lighter colors should be used, as in Tier 1.
2. Section 2.3.2, Brazos Report Only, page 23, table 3: The Rosharon station number and station name is incorrect. Rosharon is referred to as Romayer in the text and the table. Search the document for Romayer in multiple places.
3. Section 3.1.1, Figure 6 in Brazos and GSA Reports only: It is not clear if the flow represented in figure is antecedent flow associated with the pulse or flow on the day of fish sampling. Please clarify.
4. Section 3.1.1, Table 2 in Colorado – Lavaca and GSA Reports, and Table 6 in Brazos Report: This table, with the listing of only the formal species names, is extremely difficult to utilize even for an expert. It is likely meaningless to BBASC members or other non-specialist. Adding the common names would be a great aid to accessibility.
5. Section 3.1.1, Table 3 in Colorado – Lavaca and GSA Reports, and Table 7 in Brazos Report: This table would be easier to understand if the ‘-’ symbol were replaced with

“N/A” the symbol for not applicable. It isn’t clear that the ‘-‘ is different than ‘0’ and is only implicit if one knows that 4/season pulses are not part of the standards.

6. Section 3.1.2, Figures 22, 29, 30, 31, 34, 35, and 36 in the Brazos Report, Figures 7, 14, 15, 16, 17, 20, 21, 22, 23, 26, 27, 28, 31, 32, and 33 in the Colorado – Lavaca Report and Figures 25, 31, 32, 33, 36, 37, and 38 in the GSA Report: It is not clear what each point represents, it could be fish communities at different sample collections or something else. The n-MDS ordination plot is not widely used or widely understood. An explanation of the meaning with each table would be useful. For example, the explanation might be “Points that are close together on the graph represent [insert what is being plotted] that are similar, while points that are far apart represent [insert what is being plotted] that are less similar.”
7. Section 3.3.2, Figure 44, page 86: There is so much information on this figure that it is impossible to read. Ideally, a separate figure should be created for each river kilometer. Alternately, two river kilometer points could be represented on each figure. A less ideal solution would be to use color as well as shape to differentiate the river kilometers on one figure.
8. Section 3.1.2, Figure 9 in Colorado – Lavaca Report, Figure 24 in Brazos Report, and Figure 26 in GSA report: The “Percent Exceedance” label on the X-axis should be omitted.
9. Section 3.2.1, Table 7 in Colorado – Lavaca and GSA Reports, Table 11 in Brazos Report: The Steepness of Zone in the table header appears to be slope. Please use slope as it will be more readily understood by a wider audience.
10. Section 3.2.1, Table 9 in GSA Report, Table 13 in Brazos Report, and Tables 9 and 14 in Colorado – Lavaca Report: It is not clear that the method used to determine inundation flow rates is valid and flows do not be carried to the nearest 10th of a cfs.
11. Section 3.2.1, Brazos Report Only, Table 15, page 64: Flow standards are for sites in the Brazos Basin, not the GSA basin. Also, pulse flows should indicate the frequency (1 per season, 2 per season, or three per season).
12. Section 3.3.3, Brazos Report Only, Figure 50, page 96: The cluster symbols along the X-axis are unreadable at the current scale.