

INSTREAM FLOWS RESEARCH AND VALIDATION METHODOLOGY FRAMEWORK 2016–2017

Brazos River and Associated Bay and Estuary System

FINAL REPORT

Prepared for

Texas Water Development Board

Prepared by

Dr. Timothy Bonner (Texas State University)

Dr. Jacquelyn Duke (Baylor University)

Dr. George Guillen (Environmental Institute of Houston - University of Houston Clear-Lake) 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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Abbreviation Definition ADCP Acoustic Doppler Current Profiler 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 CF **Coastal Fisheries** cfs Cubic feet per second DBH Diameter at Breast Height DEMs **Digital Elevation Models** DO Dissolved oxygen **EFAG** Environmental Flows Advisory Group EPA U.S. Environmental Protection Agency 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 ICWW Intracoastal waterway LCRA Lower Colorado River Authority Lavaca-Navidad River Authority LNRA mg/L Milligrams per liter Ν Number of samples nMDS Non-metric multi-dimensional scaling NO₂ Nitrite NO₃ Nitrate NRCS National Resources Conservation Service NTU Nephlometric turbidity unity OBL Obligate

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LIST OF ACRONYMS AND ABBREVIATIONS

Abbreviation Definition Р Phosphorous PCA Principal component analysis PHDI Palmer Hydrological Drought Index POM Particulate organic matter Parts per billion ppb psu Practical salinity units PRIMER Plymouth Routines In Multivariate Ecological Research **River kilometers** rkm SAC Texas Environmental Flows Science Advisory Committee SARA San Antonio River Authority **SB** 2 Senate Bill 2 SB 3 Senate Bill 3 SE Standard error SIMPER similarity percentages TCEQ Texas Commission on Environmental Quality TKN Total Kjehldahl nitrogen TNRIS Texas Natural Resource Information System TPWD Texas Parks and Wildlife Department TRA Trinity River Authority TSS Total suspended solids TWDB Texas Water Development Board UPL Upland USGS U.S. Geological Survey WI Wetland Indicator WSE Water Surface Elevation °C **Degrees** Celsius $\Delta ft/hr$ deviation in successive hourly water surface level measurements ΔS Vertical salinity gradient

LIST OF ACRONYMS AND ABBREVIATIONS (CONTINUED)

EXECUTIVE SUMMARY

Senate Bill 3 (SB 3) established the Brazos River and Associated Bay and Estuary area (Brazos), the regional stakeholder committee (Brazos BBASC) and the regional expert science team (Brazos BBEST), with the latter two playing key roles in the development of environmental flow recommendations for the Brazos. 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 Brazos 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 was 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 too much 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 Brazos, GSA, and Colorado/Lavaca (Col/Lav) 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 Brazos, GSA, and Col/Lav 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 dataset 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 Brazos 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 being conducted in the Brazos basin.

The Brazos estuary component built upon the database established in Round One and further described water quality and nekton community patterns. It also quantified estuary salinity regime, nutrients, suspended solids, and utilization by estuarine-dependent nekton. Discharge data collected at Rosharon was used to initiate development of predictive models that relate environmental conditions in the estuarine zone to flow tier recommendations. Discharge levels measured at the site at river kilometer 42 agreed with upstream gage readings. The best use of the estuary models described herein would involve conducting future sampling to assess conditions within the lower estuary across all seasons and flow tiers, thereby increasing the number of samples used to populate these predicted models. Once abiotic and biotic responses are more fully understood, environmental flow recommendations can be validated or adjusted to maintain a sound ecological environment within the estuary. Without these additional data, we will continue to have an incomplete understanding of the response of the estuarine zone of the Brazos River to the adopted environmental flow standards.

For intensive ecological data and responses to flow to have meaning to the SB 3 process, it 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 Brazos BBASC in 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, BBASC, and TCEQ.

In conclusion, the second phase of this study has contributed to the understanding of flowecology 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; (3) establishing direct ecological responses between channel morphology changes and aquatic organism response; and (4) continuation of flow-response driven characterization of the Brazos estuary ecology. 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 Brazos River basin and associated bay and estuary system (BRA), the regional stakeholder committee (BRA BBASC) and the regional expert science team (BRA BBEST) were formed in 2011. After numerous meetings and extensive data compilation and analysis, the BRA BBEST submitted their environmental flow recommendations report to the BRA BBASC in March 2012. Then, after a series of meetings and balancing discussions, the BRA BBASC submitted their stakeholder recommendations report to the Texas Commission on Environmental Quality (TCEQ) and the Environmental Flows Advisory Group (EFAG) in September 2012. Following a public comment period, the TCEQ then adopted environmental flow standards for the BRA, effective March 6, 2014.

During the SB 3 process, limitation 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 BRA 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 BRA 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 BRA flow levels and lower Brazos basin ecology (thereby reducing the unwanted uncertainty that these data gaps introduced to the BRA 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 BRA 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 BRA and Guadalupe-San Antonio (GSA) 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 (e.g. fish, macroinvertebrate, etc.) 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. 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. 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 BRA, GSA, and Colorado/Lavaca (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 BRA 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 the Brazos basin. Please note that while the focus of this report will be on the Brazos basin, references to and results from other basins may 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 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 BRA 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 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. 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 in 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-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 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 preflood 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

Round One 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 or lost, but seedling germination and survival were also impacted. The methodology developed in Round One for testing life stage responses to flow pulses worked 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 indicator species. This information allows a river manager to discern much about the health and status of the riparian zone, and provides a method

for a quick analysis of projected riparian persistence with respect to inundation provided by the flow standards. In light of the clear connections of riparian responses to within-season flows (or lack thereof), we wanted to expand our work in Round Two to include additional field testing techniques that could be used in comparison 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 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 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

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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) ordinance 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 the described statistical methods was intended to streamline a comprehensive characterization of overall riparian communities and community dynamics.

In addition to discussion of the validation study conducted in 2014–2015 (SARA et al, 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/characteristics in relation to stream flow were presented by the riparian project team. 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 focused on riparian indicator species rather than the community as a whole in order to best determine short-term responses to stream flow, Round Two focused on the overall community composition. 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 the second round of study.

Geomorphological Features

Ouestion 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? Secondarily, 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 tier 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

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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?

1.3 Brazos Estuary

Estuaries are classified based on multiple criteria including salinity regime, tidal influence, freshwater inflow, and geomorphology (Savenije 2005, Day et al. 2013). Many of the Texas Bays exhibit a lagoon-type morphology that contain rivers discharging near the upstream end, shallow series of primary and secondary bays, oyster reefs, fringing wetlands, and several tidal passes that connect them to the Gulf of Mexico. In contrast, the Brazos River estuary is unique in that it is one of the few "riverine" estuaries found along the Texas coast (Palmer et al. 2011, Orlando1993, Savenije 2005, Engle et al. 2007). It also serves as a tidal inlet between the Gulf of Mexico and coastal estuaries including adjacent waterbodies. Tidal inlets are a very important

feature of coastal areas. The exchange of water between the ocean and the inner estuary facilitates the exchange of sediment, nutrients and biota between the ocean and estuary. Depending on the amount of freshwater inflow, depth, and tidal regime many riverine type estuaries can experience large lateral (upstream to downstream) and vertical changes in salinity. During low freshwater inflow, upstream density currents (reinforced by flood tides) can transport far upstream bottom marine water that is more saline, colder, and denser (Orlando 1993). Although tidal predictions are based on regional or global information, local geographical and hydrological effects induced by storm systems, droughts, and floods can significantly alter the manifestation of astronomical tides (Dwyer 1997). Water levels within estuaries along the Texas Gulf coast are frequently more sensitive to meteorological and hydrological forces due to the shallow depths and the small astronomical tides that normally occur in this region.

A useful conceptual model that describes the primary relationships between freshwater inflow and geomorphology, physicochemistry, and biological attributes was first proposed by Alber (2002). Similar to the natural flow paradigm and river continuum concept for rivers, the estuarine model predicts that the discharge of freshwater under natural varying conditions creates a predictable optimal salinity gradient for the assemblage of organisms that have evolved within an estuary (Vannote 1980, Poff et al 1997, Alber 2002). This gradient is manifested both laterally and vertically in tidal rivers in the form of physical variation in current speed and direction, salinity, suspended solids, and nutrients that ultimately influences the geometry, stability, and location of resulting pycnocline and turbidity maximum zone (Wolanski 2007). Interactions of these processes result in additional ecosystem services including delivery of delta-forming sediments and nutrients that support primary producers in estuaries (Alber 2002, Wolanski 2007). Lack of flow pulses and sustained periods of low freshwater inflow during warmer months can lead to a stable pycnocline in tidal rivers like the Brazos River (Lin et al. 2006, Hagy and Murrell 2007). Formation of a stable pycnocline limits vertical mixing and promotes the formation of hypoxic or anoxic conditions along tidally influenced river bottoms (Kuo et al. 1991).

As with many estuarine systems, a significant amount of primary production in the Brazos River and nearshore Gulf of Mexico is driven by the export of upstream nutrients and detritus including high levels of nitrogen, phosphorus, and particulate organic matter (POM) (Day et al. 2013). These nutrients support both phytoplankton and benthic and planktonic heterotrophic protozoa, which are in turn fed upon by larval and juvenile estuarine organisms immigrating into the Brazos River (Day et al. 2013). However, if nutrient levels exceed the assimilative capacity of the receiving marine waterbody, eutrophic conditions may develop that ultimately lead to hypoxia and mortality of aquatic organisms. A classic extreme example of this phenomenon is the "dead zone" in the central Gulf of Mexico that periodically develops during late spring and summer months in response to excessive discharges of nutrients from the Mississippi River (Rabalais et al. 2002, Diaz and Rosenberg 2008, Dodds 2006). There is now sufficient evidence that Brazos River discharges have been associated with nearshore hypoxia events in the past (DiMarco et al. 2008, DiMarco et al. 2012).

Hypoxia within Gulf Coast estuaries has been linked with (1) seasonally high temperatures that increase biochemical oxygen demand, (2) neap-spring tidal cycles, (3) salinity and/or temperature stratification that limit vertical mixing and reaeration, (4) eutrophication, and (5)

diurnal cycling of dissolved oxygen (Engle et al. 1999). Increased vertical stratification is highly correlated with incidents of hypoxia and anoxia resulting in loss of habitat and related fish kill events. Park et al. (2007) in their study of Mobile Bay found that despite a large velocity shear, stratification was strong enough to suppress vertical mixing most of the time. Bottom dissolved oxygen was closely related to the vertical salinity gradient (Δ S). Hypoxia seldom occurred when Δ S (over 2.5 m) was <2 practical salinity units (psu) and occurred almost all the time when Δ S was >8 psu in the absence of extreme events like hurricanes (Park et al. 2007). Past studies of the Brazos River have detected hypoxia in the lower tidal portion of the river (Kirkpatrick 1979, Emitte 1983).

Riverine estuaries, such as the lower Brazos River, exhibit short hydrological residence times and high turnover rates (Engle et al. 2007). The productivity of riverine estuaries or tidal rivers is dependent on maintaining natural hydrographic variation because the majority of nutrient input is dependent on upstream sources (Orlando et al. 1993, Engle et al. 2007). Part of this natural variability includes alternating periods of drought that reduces bank vegetation and large, highflow pulses that are important for maintaining the river delta geomorphology (Orlando et al. 1993, Gibeaut et al. 2000, Fraticelli 2006). The current Brazos River delta is an arcuate, wavedominated delta that protrudes 2 km into the Gulf of Mexico (Gibeaut et al. 2000). Large flood events are mostly responsible for deposition and delta enlargement (Rodriguez et al. 2000). There is also evidence that these plumes of sediment and associated nutrients are responsible for providing trophic subsidies (i.e., organic material and nutrients) to the nearshore environment (Connolly et al. 2009). During large flood events, motile estuarine organisms unable to tolerate low salinities can be displaced downstream into the Gulf of Mexico. These organisms will either return as salinity increases or experience increased mortality. Less-mobile marine stenohaline benthic organisms, such as brittle stars, cannot tolerate large declines in salinity and high mortality is the likely outcome (Day et al. 2013). In contrast, some species of benthic organisms such as Rangia cuneata will increase in number due to their preference for oligohaline conditions (Montagna et al. 2008).

During drought conditions, bottom salinity in the Brazos River and other riverine estuaries can increase significantly and extend far upstream (Orlando et al. 1993). During these periods, estuarine and marine organisms can move far upstream displacing many freshwater species. If drought conditions persist for an extended period, the structure and function of the estuary could be altered resulting in sustained periods of vertical stratification, bottom hypoxia, reduced fishery production and harvest, and shift to more marine species in the lower reaches of the estuary (Orlando et al. 1993, Livingston 1997, Gillson 2011).

The ability to predict changes in salinity, water quality, and biota of the lower Brazos River estuary has been limited due to the lack of routine biological monitoring. The Texas Parks and Wildlife Department (TPWD) Coastal Fisheries (CF) Monitoring Program has not collected and currently does not collect data within the lower Brazos River (Robinson pers. comm.). The current TPWD CF independent monitoring program relies on the use of otter trawls, large bag seines, gill nets, and oyster dredges (Martinez-Andrade 2015). With the exception of the otter trawl, the other gear types are very inefficient or impossible to deploy in flowing rivers with steep narrow shorelines.

There have been very few studies of the aquatic biota of the lower Brazos River (Palmer et al. 2011; Emitte 1983; Kirkpatrick 1979). Kirkpatrick (1979) conducted water quality, hydrology and biological sampling of the lower Brazos River during March 16–17, June 29, and August 17, 1977 at sites located at river miles 0.6, 6.2, 13.7 and 24.9 (river kilometers [rkms] 1, 10, 22 and 40 km). During these dates, average daily flow at the Rosharon gage was reported at 8,900, 4,290 and 1,170 cfs, respectively. Biological sampling on these dates included monitoring of nekton with a 12-foot otter trawl towed for 10 minutes, and a 125x8-foot experimental gillnet (with 0.75–2.5-inch bar mesh) deployed overnight. During the month of March, he reported that otter trawl catches at rkm 22 and 40 were dominated by River Prawn (Macrobrachium ohione) and Blue Catfish (Ictalurus furcatus). In contrast, he failed to capture any nekton with otter trawls at rkm 22 or 40 during August 1977 (Kirkpatrick 1979). Gillnet capture rates were highest in the lower river sites (rkm 1 and 10) during all three months. Nekton was not captured at rkm 22 or 40 during June or August 1977. Highest nekton diversity was observed at the lowest river site. Kirkpatrick (1977) reported that the strong halocline and hypoxia observed during summer months on the bottom of the river were the primary causes for the absence or low numbers of nekton.

Emitte (1983) conducted a study of the nekton in the lower Brazos River during 1982. Bottom nekton was collected using a 20-foot otter trawl towed three times at each site for a total of 10 minutes per site. During that study trawling was conducted at river miles 3, 6, 8 and 9.5 (rkms 4.8, 9.7, 12.9, and 15.3) during February, May, August, and November 1982. Although the online website for the US Geological Survey (USGS) gage at Rosharon does not provide discharge data for these dates, Emitte (1983) reported that river flows on these dates were 1,867, 5,656, 2,620, and 1,266 cfs, respectfully. During his study, he found that the highest diversity and catch rates were generally observed during February. Higher diversity and numbers of nekton were collected at the most downstream sites. Atlantic Croaker (*Micropogonias undulatus*), Blue Crab (*Callinectes sapidus*), Blue Catfish (*Ictalurus furcatus*), White Shrimp (*Litopenaeus setiferus*), Ohio Shrimp (*Macrobrachium ohione*), Threadfin Shad (*Dorosoma petenense*), and Sand Seatrout (*Cynoscion arenarius*) represented the highest number of nekton captured during his study (Emittee 1983).

A comparative study of Texas benthic communities between various types of estuaries was conducted during 2001–2005 by Palmer et al. (2011). They found that the lower Brazos River benthos exhibited similar community structure and responses to freshwater inflow as other river (San Bernard and Rio Grande) and secondary bay (Lavaca Bay) estuaries. They further concluded that much of the research performed on the benthic macrofauna of major bays of Texas is directly comparable and thus of value in assessing the environmental flow needs of rivers and lagoons.

The only historical comprehensive study that has been performed on the nekton of the lower Brazos River was conducted by Johnson (1977) using otter trawls during February 1973 to January 1975. He documented strong seasonal and latitudinal gradients in nekton influenced by changes in freshwater inflow and resulting changes salinity. Unfortunately, little information was provided on site by seasonal nekton community structure and individual species catch rates. No information was provided on individual collections. Furthermore, little data was provided on hydrological, water quality, or biological data collected for each individual collection. Replicate catch data is also lacking and the dates when collections were made are not provided in his report. Therefore, it was impossible to directly compare his data with more recent studies using more rigorous quantitative methods. The general lack of sufficient replication precludes the use of formal statistical methods to compare seasonal and latitudinal trends.

More recent data on the nekton of the lower Brazos River was collected by Miller (2014) and the first phase of this study reported in Bonner et al. (2015). Miller (2012) found that nekton assemblages at the mouth of the Brazos River exhibited 60% similarity on an annual basis with nekton communities sampled at the same site by during the 1970s (Johnson 1977). He also found that nekton community diversity was highest near the mouth of the Brazos River. Nekton communities sampled during 2014–2015 by Bonner et al. (2015) found that intrusion of freshwater species into the lower river occurred in response to 1-per-season pulses. Both studies relied primarily on otter trawls and shallow water beam trawls to sample the nekton population. These studies also used the same amount of sampling efforts (replicates, time). Therefore, these datasets are directly comparable and provide us with the opportunity to examine the influence of season and hydrological conditions on nekton community structure. These past data were incorporated into our current analysis and are discussed in more detail within this current report.

The primary objectives of the Brazos estuary study were as follows:

- 1. To use relevant historical and new data collected within the tidal portion of the lower Brazos River to:
 - a. characterize flow regime and tidal dynamics,
 - b. describe the response of salinity regime to varying flow,
 - c. assess water quality and nutrient patterns, and
 - d. characterize nekton abundance, diversity, and community composition.
- 2. To investigate and begin development of potential models that predict the relationship between discharge, flow tiers, seasonality, salinity, nutrients and nekton composition including estuarine species within the lower tidal portion of the Brazos River.

It was hypothesized that at higher flow tiers and discharge:

- 1. salinity levels in the Brazos River estuary would decline rapidly;
- 2. the lateral extent and vertical stability of the pycnocline would decline;
- 3. nutrient and suspended solid levels would increase;
- 4. the occurrence and density of estuarine dependent species would decline; and
- 5. under moderately high flows, vertical mixing and reaeration would increase, leading to higher abundances of nekton in trawl samples.

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 (GSA, Brazos, Col/Lav) methods similar to those used in Round One. These specific 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 decade 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 collected in a habitat-specific fashion and could, in many cases, be linked back to a nearby gage location with TCEQ environmental flow standards. The methodology for each subtask is described below.

2.1.1 Aquatic Field Studies

Eighteen Brazos, GSA, and Colorado gage locations were selected for the aquatic assessment. Sites were selected to represent both tributaries and main-stem reaches (the numbers included in the following reach names correspond to gage locations shown in Figure 1). Six of the 18 sites sampled were from the Brazos River Basin: four tributaries (11-Leon River—Gatesville, 12-Lampasas River—Kempner, 13-Little River—Little River, and 17-Navasota River—Easterly) and two main-stem sites (18-Brazos River—Hempstead and 20- Brazos River—Rosharon). Seven of the 18 sites sampled were within the GSA basins: three tributaries (Medina River— Bandera, San Marcos River—Luling, Cibolo Creek—Falls City) and four main-stem sites (San Antonio River—Goliad and Guadalupe River—Comfort, Gonzales, and Cuero). Five of the 18 sites sampled were from the Col/Lav river basins: one main-stem Colorado River site (Colorado River—Bend), two Colorado River tributaries (San Saba River—San Saba, Onion Creek— Driftwood), and two Lavaca basin sites (Lavaca River—Edna, and Navidad River—Edna).

During each season (designated by BBEST recommendations), flows were monitored daily using USGS gaging stations at or near each site. Peak flow (expressed in cfs) of the day 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 the base tier or below lowest flow tier

(e.g., 4-per-season on Brazos, and 2-per-season for GSA and Colorado; See Appendix B). Therefore, abiotic and biotic samples were taken at subsistence or base flow conditions and not during a high-flow event, preventing a dilution effect.

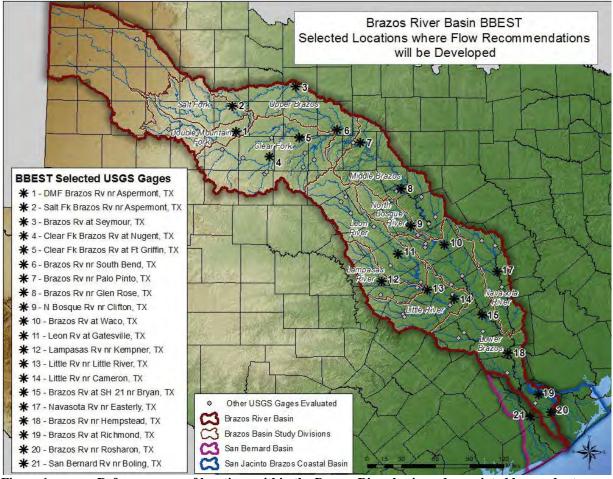


Figure 1.Reference map of locations within the Brazos River basin and associated bay and estuary
system (taken from BRA BBEST report). Specific sites used in this study are reported in the
prose.

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).

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 potential 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. Within the main-stem Brazos River, seine hauls were taken from point-sand bar habitats. Fish and habitats were quantified identically to those described for riffle habitats, except Hess samples were not taken and embeddedness was not recorded.

Combination / Individual Sites		Fish			Macroinvertebrates		
per basin	Riffle	Run	Pool	Backwater	Riffle		
GSA							
Medina River—Bandera and Guadalupe River—Comfort							
Guadalupe River—Gonzales and Cuero	2			al	2		
and San Antonio River—Goliad	N	N	N	N	V		
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				\checkmark			

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

In the laboratory, benthic samples were rinsed using a 250 μ m sieve, sorted to order, and enumerated. 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 nine 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, moderate, 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 (α =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 was 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 (GSA, Brazos, and Colorado). 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. 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 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 or overall community structure. 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 plotted vs. percent exceedance values to parallel the current fish study. All analyses were performed using PRIMER v7 software (Clarke and Gorely 2015) and RStudio (2016).

2.2 Riparian

The Brazos River drains nearly 45,000 square miles from New Mexico to the Gulf Coast. The basin can be divided into two distinct halves. The northern half, generally considered the area from Waco, Texas, northwestward, is characterized by an arid environment. Upstream of Possum Kingdom Reservoir multiple major tributaries provide combined inflows to create the main stem of the Brazos River. In this portion of the basin, flows for the Brazos River rely heavily on runoff from the surrounding watershed, with only minor input from groundwater or springs. Typically, most minor tributaries are seasonal. Below Possum Kingdom Reservoir the river flows become heavily manipulated from the multiple major reservoirs along this stretch (Baldys and Schalla 2016).

The southern half of the Brazos River basin is very different. South of Waco, the watershed is much more temperate with larger perennial tributaries. Gaining reaches become more common as the river passes over several major aquifers, with the most notable being stretches of the river that run across the Carrizo-Wilcox aquifer in Milam County and Robertson County and the Gulf Coast aquifer (Turco et al. 2007). For Round Two riparian studies, data collection was focused at two sites on the lower Brazos River with well-developed riparian zones.

The Brazos Bend site is located on the property of Brazos Bend State Park in southern Fort Bend County approximately 7.3 km upstream from the USGS gage (#08116650) on the Brazos River near Rosharon (see Figure 1 for general location of gage). The land use around the site is mostly rural with small-acreage home sites, small farms, and some cropland, though urban development in this area is increasing. River width along the study site generally ranges from 77 to 88 meters. The exclusive soil type within the study site is sandy alluvial deposits. This soil is characterized by steep sloping gradients, and soil that is moderately erodible, occasionally flooded, and somewhat poorly drained. This soil historically supported a changing composite of vegetation

due to its highly erodible characteristic. The community ranges from tallgrass prairie to densely wooded forest.

The Hearne riparian site is located on private property along the main stem of the Brazos River in eastern Milam County approximately 5 km upstream of the USGS gage (#08108700) near Bryan, Texas (see Figure 1 for general location of gage). The land use around the site is entirely dedicated to large-scale commercial farming of corn, sorghum, and cotton. Most fields are irrigated, with water being produced from the underlying Carrizo-Wilcox aquifer. River width along the study site generally ranges from 70 to 82 meters. The exclusive soil type within the study site is Gaddy fine sandy loam. This soil is characterized as having 0 to 1% slope and is excessively well drained, yet frequently flooded. This soil historically supported a wildrye/switchgrass complex savannah community with cottonwood interspersed, but as fire and grazing have been suppressed, the community has quickly transformed into a woodland complex dominated by green ash, elm, and pecan, with remnants of cottonwood. Shade-tolerant and mesic grasses and forbs such as seaoats, ironweed and various sedges dominate.

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 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 2). These shapefiles were then loaded onto a Trimble GPS unit.

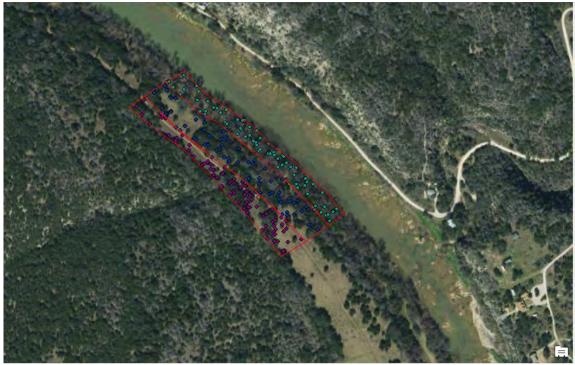


Figure 2.

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

2.2.1 Field Sampling

Riparian sites in the Brazos basin (Brazos Bend State Park [May 10, 2017] and Hearne [June 8, 2017]) were sampled only in 2017 for "verification" since these 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. 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 5 cm 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 3).

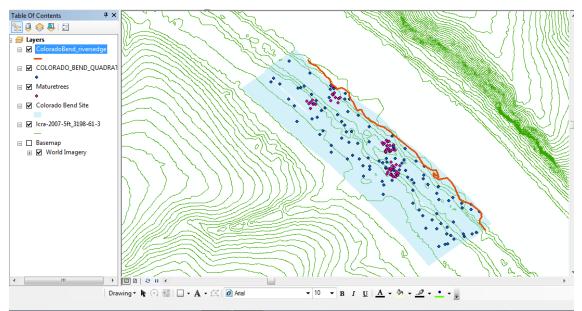


Figure 3. Example GIS screenshot showing water's edge, quadrats, mature trees, and elevation contours.

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.

2.2.2 Estimate of Inundation

Flood inundation values were estimated using the 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 calculated (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. Discharge levels were estimated using the rating curve and provided an approximate discharge amount needed to inundate the associated elevation of each level, quadrat, and mature tree.

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.

2.3 Brazos Estuary

2.3.1 Study Area

The tidal portion¹ (TCEQ segment 1201) of the Brazos River is classified as the first 25 miles (40.2 km) from its confluence with the Gulf of Mexico in Freeport, Texas, to a point about 100 m upstream of SH 332 in Brazoria County (TCEQ 2004). The tidal portion of the Brazos River can be described as a riverine or deltaic type estuary (Dyer 1997, Savenije 2005). The lower Brazos River exhibits oligohaline (low salinity-freshwater) conditions with significant variation associated with freshwater inflow (Orlando et al. 1993). The tidal portion of the Brazos River is currently classified as an unimpaired waterbody with a high rating for aquatic life use (State of Texas 2014a). The riparian ecosystem of the lower Brazos River is defined by low coastal plain vegetation transitioning from freshwater bottomland hardwoods in the upper reach to primarily saltmarsh vegetation in the lower reach (Vines 1984, Dahm et al. 2005). The channel is relatively wide (>50 m along most of its length) with the average depth gradually increasing from the mouth (4.65 m) to the upper reach (42 km) of the sampling area (7.23 m) (Miller 2014, Bonner et al. 2015).

During November 2014 to May 2015 and December 2016 to May 2017, we conducted a total of eight and six sampling events, respectively, in the lower Brazos River at multiple monitoring sites (Figure 4). This included five primary monitoring sites located (approximately) at river miles 0.6, 6.2, 13.7 and 24.9 (rkms 1, 10, 22, 31, and 42) upstream from the mouth (sites B01, B10, B22, B31, and B42, respectively (Table 2). Most of these sites corresponded with locations of the previous nekton surveys of the lower Brazos River conducted in 2012 (Miller 2014) and 1974–1975 (Johnson 1977). In general, during each sampling event, each primary site was sampled for water quality, nutrients, and nekton. Additionally, four secondary monitoring sites were established at approximately 5, 15, 25, and 35 rkm upstream from the mouth (sites B05, B15, B25, and B35, respectively). Instantaneous water quality variables were recorded at each secondary site during event as described below. In addition, continuous monitoring sites were established at rkms 10, 21, and 35 upstream of the mouth.

¹ The term "lower" is used interchangeably with "tidal" in this report unless noted.

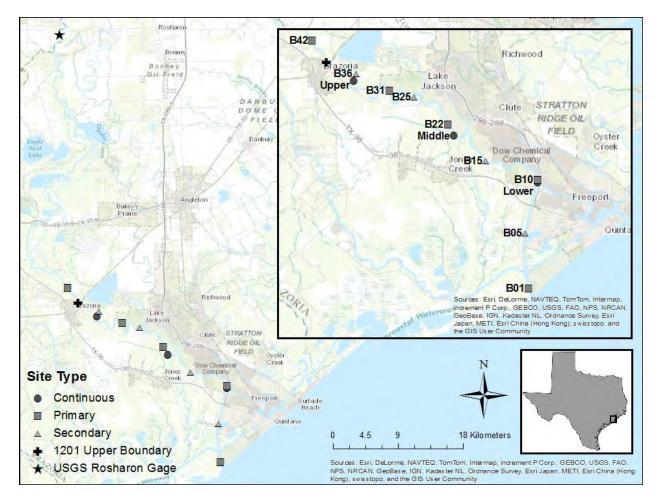


Figure 4. Site map of the lower Brazos River depicting the locations of continuous, primary, and secondary sampling sites.

C' 4-	Distance	T (*/ 1	T	Water Quality ^a			Nekton			
Site	From Gulf (rkm)	Latitude	Longitude	WQ	Nutrients	HOBO	ES	BT	ОТ	Historical Sampling
B01	1	28.88368	-95.38227	Х	X		Xc	Χ	Х	Miller (2014)
B05	5	28.92592	-95.38534	Χ						
Lower	10	28.96457	-95.37428			Х				
B10	10	28.96682	-95.37464	Χ	Х		Х	Χ	Х	Miller (2014)
B15	15	28.98117	-95.41979	Χ						
Middle	21	29.00054	-95.44773			X ^d				
B22	22	29.00908	-95.45314	Χ	Х		Х	Χ	Х	Miller (2014)
B25	25	29.02987	-95.48269	Х						
B31	31	29.03473	-95.50422	Х	Х		Х	Χ		
B34	34	29.03582	-95.53136						Xe	
Upper	35	29.04218	-95.53557			Х				
B36	36	29.04785	-95.53343	Х						
B42	42	29.07288	-95.57167	Х	X		Х	Х	Х	Miller (2014)

Table 2.Sampling sites including distance from mouth of river at Gulf of Mexico (rkm), GPS
coordinates, and type of data collected at each site on the lower Brazos River from
November 2014 to May 2015 and December 2015 to May 2016.

^a WQ=field measurement of water temperature, specific conductance, salinity, pH, dissolved oxygen, and Secchi disk transparency. HOBO=stationary temperature, conductivity and dissolved oxygen sondes including water depth at middle site.

^b ES=electroshocking. BT=beam trawl. OT=otter trawl.

 $^{\rm c}$ Electroshocking conducted at B01 when conductivities were <18,000 μ S/cm. Sampling was not conducted during 2016-17.

^d Relative water depth was also measured at this site during February to August 2015 and December 2016 to May 2017 using paired Level Troll and BaroTroll pressure transducers.

^e Otter trawl sampling for B31 conducted at B34 due to snags at original location

2.3.2 Hydrology and Water Quality

In order to assess the potential influence of flow regime and adopted environmental flow tiers for the lower Brazos River, we downloaded continuous (15 minute recording interval), daily average, and monthly average stream flow estimates from USGS gage #08116650 in Rosharon, Texas, for the duration of the study (2014–2017) and previous (2011–2012) historical data (Table 3). Daily average values were used to estimate monthly values for more recent months (post October 2016). Top of the hour (e.g. 8:00, 9:00, 10:00 etc.) measurements or those closest to top of the hour values were extracted from the continuous data to facilitate direct comparison with similarly extracted hourly data generated from automated water quality monitors that record at intervals that differ from the USGS gage. Since the intent of these comparisons was to illustrate potential co-variation between variables at the sub-day recording interval it was necessary to reduce the data set to comparable time intervals. However, it was not necessary to examine sub-hourly variation. We also utilized data from the USGS gage #08114000 located at Richmond, Texas. Discharge data from the Richmond site was used as an independent variable in a linear regression model that was developed to estimate discharges at the Rosharon gage (dependent variable) during long periods when the gage was not in operation.

Independent estimates of river discharge were also made at site B42 (river kilometer 42) during surveys conducted in 2014-15 and 2016 and 17. Estimates of net river discharge were made at site B42 using a RiverSurveyor S5/M9 Acoustic Doppler Current Profiler (ADCP) (Sontek 2011). The ADCP was deployed according to standardized protocol by attaching to a floating hydroboard and towing it across the river roughly perpendicular to the flow multiple times to estimate the net river discharge at the point of measurement (SonTek 2011; TCEQ 2012). These measurements were conducted to determine if significant differences in flow occurred between the Rosharon gage and the upper part of the tidal portion of the Brazos River.

Station ID	Site Code	Latitude	Longitude	Data Acquired	Agency	Sources and Comments
KTXLAKEJ7 Private Weather Gage	PW	29.035	-95.462	daily precipitation	Weather Underground	www.wunderground.com/personal- weather-station/dashboard ?ID=KTXLAKEJ7#history/s2017060 1/e20170701/month
42019 TABS Buoy 60 nautical miles south of Freeport	WI	27.907	-95.352	wind speed and direction (hourly)	NOAA National Data Buoy	www.ndbc.noaa.gov/station_history.p hp?station=42019
8772447 Tide Gage at Freeport	TG	28.94333	-95.3017	water level and predicted tide	NOAA	tidesandcurrents.noaa.gov/stationhom e.html?id=8772447
08114000 Brazos R. at Richmond	RI	29.58222	-95.7575	annual, monthly, daily, and 15 minute discharge	USGS	waterdata.usgs.gov/nwis/nwismap/?sit e_no=08114000&agency_cd=USGS Upstream of Rosharon. Used to estimate Rosharon discharge using regression model during periods of missing data.
08114000 Brazos River at Rosharon	RO	29.34944	-95.5822	annual, monthly, daily, and 15 minute discharge	USGS	https://waterdata.usgs.gov/nwis/dv/?sit e_no=08116650&agency_cd=USGS& amp;referred_module=sw Environmental Flow Compliance point.

Table 3.	Automated hydrological and meteorological monitoring data sources used during this study.

Sampling events were classified as occurring during the winter, spring, and summer seasons and classified by flow tier according to adopted environmental flow standards for the lower Brazos River and methods outlined in State of Texas (2014b). Sampling events were assigned a flow-tier status ranging from subsistence flow to various flow pulses (Table 4). The assignment of flow tiers begins with the determination of the season of the sampling event, which is defined and illustrated in Table 5 (State of Texas 2014b). Once the season is determined, a weighted Palmer Hydrologic Drought Index (PHDI) is calculated from the individual PHDI estimates for the various climatic divisions of Texas, and the geographic weight assigned to these estimates based on where a USGS gage is located (e.g. upper, middle, or lower basin). These data are obtained for the last month of the previous season (winter, spring, summer). The PHDI data were obtained from the TWDB *Water for Texas* website located at:

(https://waterdatafortexas.org/drought/phdi/monthly?time=2017-01).

Table 4.Environmental flow standards for Brazos River estuary based on flow at the United States
Geological Survey Gage (USGS) 08116650, Brazos River near Rosharon. Source: (State of
Texas 2014b) Figures 30 TAC §298.470(b); 30 TAC §298.470(c); TAC §298.480(19).

Season	Subsistence	Hydrologic Condition	Base	Dry Condition Seasonal Pulse	Average Condition Seasonal Pulse	Wet Condition Seasonal Pulse
		Dry	1,140 cfs	1-per-season Trigger: 9,090	3-per-season Trigger:	2-per-season Trigger:
Winter (Nov.–Jun.)	430 cfs ^a	Average	2,090 cfs	cfs Volume: 94,700 af ^b	9,090 cfs Volume: 94,700 af	13,600 cfs Volume: 168,000 af
		Wet	4,700 cfs	Duration: 12 days	Duration: 12 days	Duration: 16 days
		Dry	1,250 cfs	1-per-season Trigger:	3-per-season Trigger:	2-per-season Trigger:
Spring (Mar.–Jun.)	430 cfs	Average	2,570 cfs	6,580 cfs Volume: 58,500 af	6,580 cfs Volume: 58,500 af	14,200 cfs Volume: 184,000 af
		Wet	4,740 cfs	Duration: 10 days	Duration: 10 days	Duration: 18 days
		Dry	930 cfs	1-per-season Trigger:	3-per-season Trigger:	2-per-season Trigger:
Summer (Jul.–Oct.)	430 cfs	Average	1,420 cfs	2,490 cfs Volume: 14,900 af	2,490 cfs Volume:	4,980 cfs Volume: 39,100 af
		Wet	2,630 cfs	Duration: 6 days	14,900 af Duration: 6 days	Duration: 9 days

Weighted Palmer Hydrological Drought Index (PHDI)=sum of (geographic weight * climatic division PHDI value from last month of previous season)

= (0.619 * North Central PHDI) + (0.147*East Texas PHDI) + (0.057*Edwards Plateau PHDI) + (0.132 * South Central PHDI) + (0.045* Upper Coast PHDI).

Resulting Assigned Hydrologic Condition:

Lower Basin Dry=Weighted PHDI<-1.73

Lower Basin Average=Weighted PHDI=-1.73- 2.13

Lower Basin Wet=Weighted PHDI>2.13

Data source: waterdatafortexas.org/drought/phdi/monthly?time=2017-01

^a cfs=cubic feet per second.

^b af=acre-feet.

Table 5.Texas Council on Environmental Quality (TCEQ) adopted environmental flow tiers for the
Brazos River estuary that were sampled in the past and during the current study. Events 1-
12 (Miller 2014); Events 13–20 (Bonner et al. 2015); Event 21 was an independent survey
conducted by coauthor G. Guillen EIH lab; Events 22–27 current study. Dates represent
primary date of sampling. In some cases, data was also collected 1–2 days before or after the
primary date. Biological data from events 1, 4 and 5 were not used due to data being
collected over a wide range of dates vs. 1 or 2 dates.

Event	Date	Season	Avg. Daily Flow cfs ^a	Flow Tier	Lag Time (days)	Field Water Quality	Nutrients	Otter and Beam Trawl
1	01/18/12	Winter	1280	Dry-Base ^b	8	Х		Х
2	02/14/12	Winter	7,470	Dry-Base	6	Х		Х
3	03/12/12	Spring	11,500	Dry-Base	19	Х		Х
4	04/11/12	Spring	10,400	Dry-1ps ^c	16	Х		Х
5	05/08/12	Spring	1,390	Dry-1ps	43	Х		Х
6	06/12/12	Spring	304	Dry-Sub ^d	2	Х		Х
7	07/10/12	Summer	380	Dry-Sub	59	Х		Х
8	08/14/12	Summer	475	Dry-Sub	21	Х		Х
9	09/11/12	Summer	710	Dry-Sub	58	Х		Х
10	10/16/12	Summer	920	Dry-Sub	94	Х		Х
11	11/13/12	Winter	275	Dry-Sub	122	Х		Х
12	12/13/12	Winter	350	Dry-Sub	152	Х		Х
13	11/11/14	Winter	1,220	Avg-Sub	55	Х	Х	Х
14	12/09/14	Winter	1,050	Avg-Sub	11	Х	Х	Х
15	01/06/15	Winter	4,230	Avg-Base	14	Х	Х	Х
16	02/04/15	Winter	5,740	Avg-Base	8	Х	Х	Х
17	02/18/15	Winter	2,090	Avg-Sub	22	Х	Х	Х
18	04/01/15	Spring	7,080	Avg-3ps	18	Х	X	Х
19	04/29/15	Spring	13,100	Avg-3ps	10	Х	Х	Х
20	05/07/15	Spring	9,280	Avg-3ps	18	Х	Х	Х
21	8/12/15	Summer	6,120	Wet-2ps	73	Х		Х
22	12/1/16	Winter	3,250	Wet-Sub	16	Х		Х
23	12/20/16	Winter	3,670	Wet-Sub	13	Х		Х
24	1/31/17	Winter	9,670	Wet-Base	9	Х		Х
25	3/15/17	Spring	6,200	Wet-Base	20	Х		Х
26	5/1/17	Spring	9,650	Wet-Base	14	Х		Х
27	5/24/17	Spring	3,150	Wet-sub	37	Х		Х

^a Avg. daily flow = discharge at Rosharon gage

^b Base=base flow

^c ps=pulse/season

^dSub=subsistence flow

Factors that can influence streamflow, water level, and vertical stratification include astronomical tides, meteorology, and estuarine/river geomorphology (Ward and Montague 1996; Savenijie 2005). To document the potential influence of these factors on streamflow and discharge we also collected information on predicted astronomical tide levels and observed water level data from the NOAA tide station FCGT2-8772447 located at the US Coast Guard station in Freeport, TX. The site is owned and maintained by NOAA's National Ocean Service. The gage is located at 28.943 N 95.302 W (28°56'36" N 95°18'9" W). The water surface level and predicted tides were obtained via electronic download from the web link below. https://tidesandcurrents.noaa.gov/waterlevels.html?id=8772447.

These data were used to evaluate overall changes in water level due to the combined effects of astronomical tides, wind speed and direction, and freshwater inflow. Available hourly data was used to estimate average, minimum, maximum, and ranges in daily tide levels. Meteorological data (wind speed and direction, precipitation) was not consistently reported at the NOAA tide gage. Therefore, we supplemented readings at this site with wind speed and direction data collected from the Weather Underground private meteorological monitoring network website available through the website below. <u>https://www.wunderground.com/personal-weather-station/dashboard?ID=KTXLAKEJ7#history/s20141225/e20150101/mweek</u>. We selected this precipitation site for a couple of reasons including prior use by Miller (2014) and the location close to sites B22, which is near the middle of our study area. These data were only used as a very coarse measurement of precipitation patterns in the lower watershed. It was not used in any hydrological modeling or analysis.

Vertical profiles of water temperature (expressed in degrees Celsius [°C]), salinity (expressed in psu), dissolved oxygen (expressed in mg/L), pH, and turbidity (expressed in nephelometric turbidity unit [NTU]) were recorded at the thalweg of each primary and secondary sampling site using a YSI 600XLM multiprobe sonde (YSI, Inc., of Yellow Springs, Ohio). Prior to and after sampling, the sonde was calibrated according to TCEQ Surface Water Quality Monitoring quality assurance standards (TCEQ 2012). The value of each water-quality variable measured at the surface (0.3m), 25% of total depth, 50% of total depth, 75% of total depth, and bottom (0.3m above the bottom substrate) was recorded while conducting water quality profiles. Additionally, total depth was recorded at each site and Secchi disk transparency (depth m) was recorded at all primary sites.

Surface water grab samples were collected at primary sites during each sampling event. These samples were submitted to Eastex Environmental (of Houston, Texas). Nitrate and nitrite nitrogen (Nitrate+Nitrite; mg/L) were analyzed using EPA method SM 4500-NO₃ E &F. Total Kjehldahl nitrogen (TKN) measured in mg/L was analyzed using US Environmental Protection Agency (EPA) methods SM4500 SM 4500-N_{org} B or C and SM 4500-NH₃ B. Total phosphorous (total P) measured in mg/L was analyzed using EPA method SM 4500-PE. Total suspended solids (TSS) measured in mg/L were analyzed using EPA method SM 2540 D. During 2016 and 2017 samples were submitted to Eastex Environmental laboratory for quantitative determination of chlorophyll-a levels at the primary sites.

Continuous monitoring sites were equipped with temperature and conductivity U26-001 HOBO data loggers (Onset Computer Corporation of Bourne, Massachusetts) (Table 2 and Figure 4).

Data loggers were downloaded monthly and checked for battery life, fouling, and damage. Conductivity values were converted to salinity using HOBOware (v3.7.2), which implements the practical salinity scale (PSS-78) algorithm (Lewis and Perkin 1978).

Automated paired pressure transducer sensors were used to collect barometric pressure compensated measurements of water depth relative to the position of the submerged depth sensor. Paired In-Situ model Level TROLL 300 and BaroTROLL instruments were deployed at the middle site (Figure 4 and Table 2). The water depth probe, Level TROLL 300, was deployed near the bottom of a pier, while the barometer, BaroTROLL was deployed above the water surface at the same location. These units were deployed in a 1-inch PVC tube attached to 4-inch PCV piling with a zip tie. The Level TROLLS were equipped with a long metal cable and wrapped in household plastic wrap while deployed. The Level TROLL 300 instrument uses barometric pressure readings from the co-located BaroTROLL thermometer and barometer to correct depth readings obtained with the Level TROLL 300 (In-Situ 2013). This was conducted using WinSitu BaroMerge software.

Water quality variables from vertical profiles (surface and bottom) were summarized by mean ± 1 standard error (SE), range, and number of samples (N) across all sites by flow tier. Results of visual and statistical analyses conducted on water quality and hydrological variables were compared against the current environmental flow hypotheses and conditions predicted by conceptual models with regard to critical functions (e.g., nursery habitat, salinity regime, nutrients) provided by various components of the flow regime. Regression and ANOVA models were used to describe potential relationships between river inflow and water quality, and primary production as measured by chlorophyll-*a*.

Linear regression models that utilized daily average discharge and river kilometer and first order interactions as independent variables and water quality variables including surface and bottom salinity and dissolved oxygen, chlorophyll-a, nitrate-nitrite nitrogen, TKN, TSS and total P) as dependent variables were utilized to test for relationships between flow regime and location in the estuary and these variables. A two-factor analysis of variance (ANOVA) with interaction term was used to test for differences (α =0.05) in surface and bottom salinity and dissolved oxygen concentrations as well as nutrients (NO₃-, NO₂-, TKN, Total P), chlorophyll-a and TSS between flow tiers and river kilometer sites. If significant differences were detected between flow tiers or sites, then a post-hoc multiple comparison test was performed to identify individual differences between tiers or sites. Tukeys multiple comparison test was used post-hoc to assess pair-wise differences among tiers and sites when statistically significant.

Interpolated salinity contours for the entire river reach were created by plotting percent total depth of vertical profile salinity measurements by site (rkm). Additionally, salinity values for surface, middle, and bottom readings were plotted against flow tier and discharge to assess the relationship of salinity to instream flow recommendations. Dissolved oxygen concentrations were grouped by surface, middle, and bottom readings and graphed by site to describe spatial relationships of water profiles. Continuous salinity values for the upper, middle, and lower reach were compared against the hydrograph and tide data to visually assess the relationship of freshwater inflow and tides on salinity regime.

2.3.3 Nekton

Demersal nekton were collected in the thalweg at all primary sites with an otter trawl (3.1 m wide, 38.2mm stretch mesh, 6.1mm net fitted within cod end) towed for 5-minutes per replicate. A total of three replicate tows were made. Trawls were performed counter to flow (facing upriver) at an average speed of 2.5 knots and equipped with a 30-m tow line. In instances where snags prevented the full trawling allotment, catch was released and the trawl was redeployed upstream of the hazard (snag) location.

Shoreline nekton were collected at all primary sites using a modified 6.4 mm mesh Renfro beam trawl manufactured by Sea-Gear Corporation of Melbourne, Florida (Renfro 1963). Triplicate hauls were pulled parallel to shore for 15.2 m/haul on one bank per site (alternating sides during each sampling event and at each site).

As previously described during 2014–2015, larger nekton were collected using a boat-mounted 9.0 GPP electrofishing unit (Smith-Root of Vancouver, Washington) for a total of 20 minutes shock time per site (Bonner et al. 2015). Electrofishing was conducted at sites B10, B22, B31, and B42, and opportunistically at site B01 depending on surface conductivity. Due to difficulties and reduced sampling efficiency associated with high conductivity, turbidity, and flows, this type of sampling was not conducted during 2016–2017. Electrofishing catch data from 2014–2015 is therefore not discussed or presented in much detail.

Collected nekton was identified to the lowest possible taxonomic level and counted. Nekton includes mobile finfish and invertebrates such as shrimp, swimming crabs, and squid. Any specimen unidentifiable in the field was anesthetized in MS-222, preserved in 10% formalin and brought back to the lab for subsequent identification and enumeration. Laboratory and field identification was conducted using expert knowledge and taxonomic keys and reported using common and scientific names from most current nomenclature adopted by the American Fisheries Society (Hoese and Moore 1998, Turgeon et al 1998, Turgeon et al. 2005, Cairns et al. 2003, Hubbs et al. 2008, Thomas et al. 2007, Merrit et al. 2008, Merryman et al. 2012, Page and Burr 2011, Page et al. 2013, Voshell 2002, Rothschild 2004, Heard 1979, Perry and Larsen 2017, Felder 1973, Williams 1984, Price 1982, Perry and Larsen 2017, Ditty and Alvardo-Bremer 2011, Wallus and Simon 2008, Richards 2005, Balcer et al. 1984, Auer 1982). All sampling techniques were reviewed and approved by the UHCL Institutional Animal Care and Use Committee (IACUC protocol #14.002-S) and are covered under Texas Parks and Wildlife Scientific Collection Permit #SPR-0504-383 and subsequent revisions.

The number of individuals per taxa was tallied for each gear type and replicate sample. Nekton taxa were further categorized into life history salinity preference groups of freshwater, estuarine, or marine based on published literature (Nelson 1992, Hoese and Moore 1998, Kells and Carpenter 2011). Species classified as estuarine were those that regularly utilize estuaries to fulfill a significant portion of at least one stage of their life cycle. This data was used to construct various nekton community metrics. The total number of nekton, and nekton taxa richness, total number of nekton classified as estuarine or marine, and total number of estuarine/marine taxa richness were calculated for each replicate sample (Magurran 1998 and 2004). We analyzed each sampling gear data set separately with statistical models due to differences in habitat and sampling gear efficiency. A two-way analysis of variance with

interaction terms was used to test for differences in total catch, number of taxa, total catch of estuarine taxa, number of estuarine taxa by gear type between flow tiers and sites. Tukeys multiple comparison test was used post-hoc to assess any pair-wise differences among flow tiers and/or sites. Linear regression models were used to describe potential relationships between daily average discharge measured at the Rosharon gage and river kilometer including interactions as independent variables and the multiple nekton community metrics by gear type as dependent variables.

Spatial and flow tier mediated effects on nekton community composition were analyzed using PRIMER 7 statistical package (Clarke and Warwick 2001). We compared each sampling gear data set separately due to differences in habitat and sampling gear efficiency. Prior to analysis nekton abundance data were square root transformed. A Bray-Curtis resemblance matrix was constructed on the transformed data to measure the similarity of nekton community composition between collections (sites X dates). Subsequently, classification and ordination of the communities were conducted using cluster analysis and non-metric multi-dimensional (nMDS) scaling using the default program settings (exception: 50 restarts, minimum stress=0.001). One-way ANOSIM was conducted to test for significant (α =0.05) differences in species assemblages between collections reclassified into flow tiers and river kilometer site.

2.3.4 Historical Data

A pilot study was performed in 2012 on the lower Brazos River following many of the same protocols as described above (Miller 2014). Data collected by Miller (2014) included nekton captured with identical trawl gear and with the original design beam trawl (Renfro 1963, Guillen and Landry 1979) using the same effort. The original beam trawl design included a plankton net 0.2 m diameter wide by 0.6 m long, constructed of 0.38 mm nitex netting in the cod end. As a result, a smaller range of nekton would likely be captured in comparison to our modified beam trawl, which has a 6.4 mm bar nylon netting. The species composition should be very similar. Because these data were compared using square root transformed data, the effect due to gear differences should be trivial.

Water quality (temperature, salinity, and dissolved oxygen data from vertical profiles) and nekton data collected by Miller (2014) were combined with our dataset to expand the scope of our assessment. This allowed us to better examine the relationships to discharge, flow tier, water quality, and nekton communities (otter trawl and beam trawl data) by increasing our sample size. These data were subjected to the same univariate and multivariate methods previously described, which increased our replication of flow tiers and allowed us to include other tiers not sampled during the 2014–2017 study period. Site B31 was not sampled and nutrient samples were not collected at any site during 2012 study period. Historical events 9, 12, and 13 were excluded from nekton analysis due to prolonged time lapses between samples collected for the same event. A complete summary of methods used to monitor water quality and nekton is provided in Miller (2014).

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 Brazos River at Hempstead (Figure 5; hydrographs from other sites are provided in Appendix C). 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 began in late summer 2016 during a much wetter period following the large flood events of 2015/16. 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 addition to flow-tier analysis, as presented in the results below.

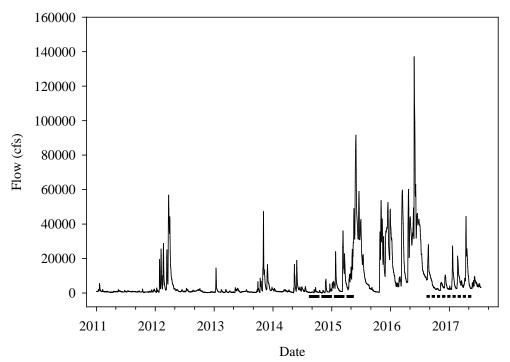


Figure 5. Hydrograph from US Geological Survey (USGS) gage # 08111500 on the Brazos River at Hempstead from 2011 to 2017 showing Round One (dashed line) and Round Two (dotted line) sampling periods.

Overall Fish Community

Totals of 59 species and 43,804 fishes were recorded from Brazos (N of species=48), GSA (40), and Colorado (31) basins among all habitats between 2014 and 2017 (Table 6). 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) (Table 7). 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 D.

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 are combined below for riffle and run habitats.

Atractosteus spatula Lepisosteus oculatus Anguilla rostrata Brevoortia patronus Dorosoma cepedianum Dorosoma cepedianum Dorosoma petenense Anchoa mitchilli Campostoma anomalum Carpiodes carpio Cyprinella lutrensis Cyprinella hybrid Cyprinella venusta Hybognathus nuchalis Lythrurus fumeus Macrhybopsis hyostoma Macrhybopsis marconis Notropis shumardi Notropis texanus Notropis volucellus Pimephales vigilax Moxostoma congestum Astyanax mexicanus Noturus gyrinus Pylodictis olivaris Mugil cephalus Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia latipinna Morone saxatilis Lepomis duritus Lepomis duritus Lepomis humilis Lepomis humilis	Slack Slack Slack Slack Slack Slack Slack Swift Slack Moderate Moderate Moderate Slack Swift Swift Slack Swift Slack Swift Slack Moderate Moderate Moderate Moderate Moderate Sight Slack	$\begin{array}{c} (\%) \\ <0.1 \\ <0.1 \\ <0.1 \\ 0.23 \\ 1.8 \\ <0.1 \\ 1.3 \\ <0.1 \\ 1.3 \\ <0.1 \\ 40.0 \\ <0.1 \\ 17.4 \\ <0.1 \\ 0.43 \\ 0.87 \\ 0.26 \\ 8.4 \\ 2.3 \\ 2.9 \\ <0.1 \\ 6.1 \\ 5.7 \\ <0.1 \\ 0.1 \\ \end{array}$	(%) <0.1 <0.1 <0.1 2.9 30.5 <0.1 8.1 0.75 24.3 1.1 <0.1 15.8 2.4	(%) <0.1 0.24 0.42 3.3 0.14 0.55 <0.1 46.0 <0.1 19.3 <0.1 0.77 1.6 <0.1 0.77 1.6 <0.1 0.77 0.77 0.77 0.97	(%) <0.1 <0.1 <0.1 <0.1 <0.1 40.1 <0.1 38.1 <0.1 0.30 1.1
Lepisosteus oculatus Anguilla rostrata Brevoortia patronus Dorosoma cepedianum Dorosoma petenense Anchoa mitchilli Campostoma anomalum Carpiodes carpio Cyprinella lutrensis Cyprinella lutrensis Cyprinella venusta Hybognathus nuchalis Lythrurus fumeus Macrhybopsis hyostoma Macrhybopsis shumardi Notropis suchanani	Slack Slack Slack Slack Slack Slack Swift Slack Moderate Moderate Slack Slack Swift Swift Slack Swift Slack Swift Slack Moderate Moderate Moderate Moderate Swift Slack	$\begin{array}{c} < 0.1 \\ < 0.1 \\ 0.14 \\ 0.23 \\ 1.8 \\ < 0.1 \\ 1.3 \\ < 0.1 \\ 40.0 \\ < 0.1 \\ 0.1 \\ 0.43 \\ 0.87 \\ 0.26 \\ 8.4 \\ 2.3 \\ 2.9 \\ < 0.1 \\ 6.1 \\ 5.7 \\ < 0.1 \\ \end{array}$	<0.1 <0.1 2.9 30.5 <0.1 8.1 0.75 24.3 1.1 <0.1 15.8	$\begin{array}{c} 0.24 \\ 0.42 \\ 3.3 \\ 0.14 \\ 0.55 \\ < 0.1 \\ 46.0 \\ < 0.1 \\ 19.3 \\ < 0.1 \\ 0.77 \\ 1.6 \\ < 0.1 \\ 0.77 \\ 1.6 \\ < 0.1 \\ \hline \end{array}$	<0.1 <0.1 <0.1 40.1 <0.1 38.1 <0.1 0.1
Anguilla rostrataBrevoortia patronusDorosoma cepedianumDorosoma petenenseAnchoa mitchilliCampostoma anomalumCarpiodes carpioCyprinella lutrensisCyprinella lutrensisCyprinella hybridCyprinella venustaHybognathus nuchalisLythrurus fumeusMacrhybopsis hyostomaMacrhybopsis hyostomaNotropis shumardiNotropis shumardiNotropis volucellusPimephales vigilaxIctalurus furcatusIctalurus gyrinusPylodictis olivarisMugil cephalusLabdesthes sicculusFundulus notatusGambusia affinisPoecilia formosaPoecilia latipinnaMorone saxatilisLepomis gulosusLepomis gulosusLepomis humilis	Slack Slack Slack Slack Slack Swift Slack Moderate Moderate Slack Slack Swift Slack Swift Slack Swift Slack Moderate Moderate Moderate Swift Slack	$\begin{array}{c} < 0.1 \\ 0.14 \\ 0.23 \\ 1.8 \\ < 0.1 \\ 1.3 \\ < 0.1 \\ 40.0 \\ < 0.1 \\ 0.1 \\ 0.43 \\ 0.87 \\ 0.26 \\ 8.4 \\ 2.3 \\ 2.9 \\ < 0.1 \\ 6.1 \\ 5.7 \\ < 0.1 \\ \end{array}$	<0.1 2.9 30.5 <0.1 8.1 0.75 24.3 1.1 <0.1 15.8	$\begin{array}{c} 0.42 \\ 3.3 \\ 0.14 \\ 0.55 \\ < 0.1 \\ 46.0 \\ < 0.1 \\ 19.3 \\ < 0.1 \\ 0.77 \\ 1.6 \\ < 0.1 \\ \hline 0.77 \\ 1.6 \\ < 0.1 \\ \hline 0.77 \\ 5.3 \\ \hline 0.97 \\ \hline \end{array}$	<0.1 <0.1 <0.1 40.1 <0.1 38.1 <0.1 0.1
Brevoortia patronus Image: Consoma cepedianum Dorosoma petenense Image: Consoma petenense Anchoa mitchilli Image: Consoma anomalum Campostoma anomalum Image: Consoma consolum Carpiodes carpio Image: Consoma consolum Cyprinella lutrensis Image: Consoma consolum Cyprinella venusta Image: Consoma consolum Macrhybopsis hyostoma Image: Consoma consolum Notropis subuchanani Image: Consoma congestum Notropis volucellus Image: Consoma congestum Astyanax mexicanus Image: Consoma congestum Notropis volucellus Image: Consoma congestum Notropis volucellus Image: Consoma congestum Astyanax mexicanus Image: Consoma congestum Noturus gyrinus Image: Consoma congestum	Slack Slack Slack Slack Swift Slack Moderate Moderate Slack Swift Swift Slack Swift Slack Swift Slack Moderate Moderate Moderate Swift Swift Swift	$\begin{array}{c} 0.14 \\ 0.23 \\ 1.8 \\ < 0.1 \\ 1.3 \\ < 0.1 \\ 40.0 \\ < 0.1 \\ 17.4 \\ < 0.1 \\ 0.43 \\ 0.87 \\ 0.26 \\ 8.4 \\ 2.3 \\ 2.9 \\ < 0.1 \\ 6.1 \\ 5.7 \\ < 0.1 \\ \end{array}$	<0.1 2.9 30.5 <0.1 8.1 0.75 24.3 1.1 <0.1 15.8	$\begin{array}{c} 0.42 \\ 3.3 \\ 0.14 \\ 0.55 \\ < 0.1 \\ 46.0 \\ < 0.1 \\ 19.3 \\ < 0.1 \\ 0.77 \\ 1.6 \\ < 0.1 \\ \hline 0.77 \\ 1.6 \\ < 0.1 \\ \hline 0.77 \\ 5.3 \\ \hline 0.97 \\ \hline \end{array}$	<0.1 <0.1 40.1 <0.1 38.1 <0.1 0.30
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Dorosoma petenense Anchoa mitchilli Campostoma anomalum Carpiodes carpio Cyprinella lutrensis Cyprinella hybrid Cyprinella hybrid Cyprinella venusta Hybognathus nuchalis Lythrurus fumeus Macrhybopsis hyostoma Macrhybopsis marconis Notropis amabilis Notropis shumardi Notropis texanus Seguenta Moxostoma congestum Astyanax mexicanus	Slack Slack Swift Slack Moderate Moderate Slack Slack Swift Slack Swift Slack Swift Slack Moderate Moderate Swift Swift Swift	$\begin{array}{c c} 1.8 \\ <0.1 \\ 1.3 \\ <0.1 \\ 40.0 \\ <0.1 \\ 17.4 \\ <0.1 \\ 0.43 \\ 0.87 \\ 0.26 \\ 8.4 \\ 2.3 \\ 2.9 \\ <0.1 \\ 6.1 \\ 5.7 \\ <0.1 \\ \end{array}$	2.9 30.5 <0.1 8.1 0.75 24.3 1.1 <0.1 15.8	$\begin{array}{c} 3.3 \\ 0.14 \\ 0.55 \\ < 0.1 \\ 46.0 \\ < 0.1 \\ 19.3 \\ < 0.1 \\ 0.77 \\ 1.6 \\ < 0.1 \\ \hline \\ 3.5 \\ 5.3 \\ \hline \\ 0.97 \\ \end{array}$	<0.1 40.1 <0.1 38.1 <0.1 0.1
Anchoa mitchilli Campostoma anomalum Carpiodes carpio Cyprinella lutrensis Cyprinella hybrid Cyprinella hybrid Cyprinella venusta Hybognathus nuchalis Lythrurus fumeus Macrhybopsis hyostoma Macrhybopsis marconis Notropis amabilis Notropis shumardi Notropis texanus Notropis texanus Notropis texanus Notropis texanus Ictalurus furcatus Ictalurus furcatus Noturus gyrinus Pylodictis olivaris Mugil cephalus Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis quosus Lepomis gulosus Lepomis humilis	Slack Swift Slack Moderate Moderate Slack Slack Swift Swift Slack Swift Slack Moderate Moderate Moderate Swift Swift Swift	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	2.9 30.5 <0.1 8.1 0.75 24.3 1.1 <0.1 15.8	$\begin{array}{c} 0.14 \\ 0.55 \\ < 0.1 \\ 46.0 \\ < 0.1 \\ 19.3 \\ < 0.1 \\ 0.77 \\ 1.6 \\ < 0.1 \\ \hline \\ 3.5 \\ 5.3 \\ \hline \\ 0.97 \\ \end{array}$	<0.1 40.1 <0.1 38.1 <0.1 0.1
Campostoma anomalum Carpiodes carpio Cyprinella lutrensis Cyprinella hybrid Cyprinella hybrid Cyprinella venusta Hybognathus nuchalis Lythrurus fumeus Macrhybopsis hyostoma Macrhybopsis marconis Notropis amabilis Notropis shumardi Notropis volucellus Pimephales vigilax Moxostoma congestum Astyanax mexicanus Ictalurus furcatus Notrous gyrinus Pylodictis olivaris Mugil cephalus Labidesthes sicculus Fundulus notatus Gambusia affinis Poecilia latipinna Morone saxatilis Lepomis cyanellus Lepomis quosus Lepomis gulosus Lepomis humilis	Swift Slack Moderate Moderate Slack Slack Swift Swift Slack Swift Slack Moderate Moderate Swift Swift Swift	$\begin{array}{c c} 1.3 \\ <0.1 \\ 40.0 \\ <0.1 \\ 17.4 \\ <0.1 \\ 0.43 \\ 0.87 \\ 0.26 \\ 8.4 \\ 2.3 \\ 2.9 \\ <0.1 \\ 6.1 \\ 5.7 \\ <0.1 \\ \end{array}$	2.9 30.5 <0.1 8.1 0.75 24.3 1.1 <0.1 15.8	$\begin{array}{c} 0.55 \\ <0.1 \\ 46.0 \\ <0.1 \\ 19.3 \\ <0.1 \\ 0.77 \\ 1.6 \\ <0.1 \\ \end{array}$ $\begin{array}{c} 3.5 \\ 5.3 \\ 0.97 \\ \end{array}$	<0.1 40.1 <0.1 38.1 <0.1 0.1
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Lythrurus fumeusMacrhybopsis hyostomaMacrhybopsis marconisNotropis amabilisNotropis buchananiNotropis buchananiNotropis shumardiNotropis shumardiNotropis volucellusPimephales vigilaxMoxostoma congestumAstyanax mexicanusIctalurus furcatusIctalurus gyrinusPylodictis olivarisMugil cephalusLabidesthes sicculusFundulus notatusGambusia affinisPoecilia latipinnaMorone saxatilisLepomis qulosusLepomis gulosusLepomis gulosusLepomis humilis	Slack Swift Swift Slack Swift Slack Moderate Moderate Moderate Swift Swift	$\begin{array}{c} 0.43 \\ 0.87 \\ 0.26 \\ 8.4 \\ 2.3 \\ 2.9 \\ < 0.1 \\ 6.1 \\ 5.7 \\ < 0.1 \end{array}$	24.3 1.1 <0.1 15.8	0.77 1.6 <0.1 3.5 5.3 0.97	0.30
Macrhybopsis hyostoma Macrhybopsis marconis Notropis amabilis Notropis buchanani Notropis buchanani Notropis buchanani Notropis shumardi Notropis shumardi Notropis cexanus Notropis volucellus Pimephales vigilax Moxostoma congestum Astyanax mexicanus Ictalurus furcatus Ictalurus gyrinus Pylodictis olivaris Mugil cephalus Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis gulosus Lepomis gulosus	Swift Swift Slack Swift Slack Moderate Moderate Moderate Swift Swift	$\begin{array}{c} 0.87 \\ 0.26 \\ 8.4 \\ 2.3 \\ 2.9 \\ < 0.1 \\ 6.1 \\ 5.7 \\ < 0.1 \end{array}$	24.3 1.1 <0.1 15.8	1.6 <0.1 3.5 5.3 0.97	0.30
Macrhybopsis marconis Notropis amabilis Notropis buchanani Notropis shumardi Notropis shumardi Notropis shumardi Notropis shumardi Notropis shumardi Notropis texanus Pimephales vigilax Moxostoma congestum Astyanax mexicanus Ictalurus furcatus Ictalurus gyrinus Pylodictis olivaris Mugil cephalus Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis gulosus Lepomis gulosus	Swift Swift Slack Swift Slack Moderate Moderate Moderate Swift Swift	$\begin{array}{r} 0.26 \\ 8.4 \\ 2.3 \\ 2.9 \\ < 0.1 \\ 6.1 \\ 5.7 \\ < 0.1 \end{array}$	24.3 1.1 <0.1 15.8	<0.1 3.5 5.3 0.97	
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Notropis buchanani Notropis shumardi Notropis texanus Notropis volucellus Pimephales vigilax Moxostoma congestum Astyanax mexicanus Ictalurus furcatus Ictalurus furcatus Notrurus gyrinus Pylodictis olivaris Mugil cephalus Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia latipinna Morone saxatilis Lepomis quisus Lepomis gulosus Lepomis gulosus Lepomis humilis	Slack Swift Slack Moderate Moderate Swift Swift Swift	2.3 2.9 <0.1 6.1 5.7 <0.1	1.1 <0.1 15.8	5.3	
Notropis shumardi Notropis texanus Notropis volucellus Pimephales vigilax Moxostoma congestum Astyanax mexicanus Ictalurus furcatus Ictalurus punctatus Noturus gyrinus Pylodictis olivaris Mugil cephalus Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia latipinna Morone saxatilis Lepomis cyanellus Lepomis gulosus Lepomis functius	Swift Slack Moderate Moderate Swift Swift Swift	2.9 <0.1 6.1 5.7 <0.1	<0.1	5.3	
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Moxostoma congestum Astyanax mexicanus Ictalurus furcatus Ictalurus punctatus Noturus gyrinus Pylodictis olivaris Mugil cephalus Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia latipinna Morone saxatilis Lepomis cyanellus Lepomis gulosus Lepomis fullosus	Moderate Swift Swift Swift	<0.1	2.4		
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Ictalurus punctatus Noturus gyrinus Pylodictis olivaris Mugil cephalus Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia formosa Morone saxatilis Lepomis auritus Lepomis gulosus Lepomis humilis	Swift	< 0.1	0.21	< 0.1	
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Pylodictis olivaris Mugil cephalus Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia formosa Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis gulosus Lepomis fullosus Lepomis gulosus Lepomis humilis	<u>01</u> 1	1.2	1.1	1.4	0.60
Mugil cephalus Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia formosa Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis gulosus Lepomis fullosus Lepomis gulosus Lepomis humilis	Slack	< 0.1	<0.1	<0.1	< 0.1
Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia formosa Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis cyanellus Lepomis gulosus Lepomis humilis	Swift	0.16	0.25	<0.1	0.45
Labidesthes sicculus Menidia audens Fundulus notatus Gambusia affinis Poecilia formosa Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis cyanellus Lepomis gulosus Lepomis humilis	Slack	<0.1		<0.1	0.13
Fundulus notatus Gambusia affinis Poecilia formosa Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis cyanellus Lepomis gulosus Lepomis humilis	Slack	< 0.1		<0.1	
Fundulus notatus Gambusia affinis Poecilia formosa Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis cyanellus Lepomis gulosus Lepomis humilis	Slack	< 0.1	<0.1	< 0.1	< 0.1
Gambusia affinis Poecilia formosa Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis cyanellus Lepomis gulosus Lepomis humilis	Slack	0.38		0.69	
Poecilia formosa Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis cyanellus Lepomis gulosus Lepomis humilis	Slack	3.1	1.7	2.7	9.2
Poecilia latipinna Morone saxatilis Lepomis auritus Lepomis cyanellus Lepomis gulosus Lepomis humilis	Slack	<0.1	0.13		
Morone saxatilis Lepomis auritus Lepomis cyanellus Lepomis gulosus Lepomis humilis	Slack	<0.1	0.16	<0.1	
Lepomis auritus Lepomis cyanellus Lepomis gulosus Lepomis humilis	Moderate	<0.1		<0.1	
Lepomis cyanellus Lepomis gulosus Lepomis humilis	Slack	0.11	0.11	< 0.1	0.22
Lepomis gulosus Lepomis humilis	Slack	<0.1	<0.1	<0.1	<0.1
Lepomis humilis	Slack	<0.1		<0.1	<0.1
	Slack	<0.1	<0.1	<0.1	
	Slack	0.15	0.15	0.12	0.34
Lepomis megalotis	Slack	0.69	0.45	0.61	1.9
Lepomis microlophus	Slack	<0.1	0.45	<0.1	<0.1
Lepomis miniatus	Slack	<0.1		<0.1	<0.1
Micropterus dolomieu	Moderate	<0.1	<0.1		<u></u>
Micropterus punctulatus	Slack	<0.1	0.16	<0.1	<0.1
Micropterus salmoides	Slack	<0.1	<0.1	<0.1	0.19
Micropterus treculii	Moderate	<0.1	0.13	<0.1	0.17
Pomoxis annularis	Slack	<0.1	<0.1	<0.1	
Etheostoma chlorosoma	Slack	<0.1	<u>\U.1</u>	<0.1	
Etheostoma chiorosoma Etheostoma gracile	Slack	0.18	<0.1	0.32	
				0.32	
Etheostoma lepidum	Swift	0.19	0.56	25	0.22
Etheostoma spectabile	Swift	2.9	4.3	2.5	0.22
Percina apristis	Swift	0.24	0.68	.0.1	0.70
Percina carbonaria	Swift	0.45	1.0	<0.1	0.60
Percina sciera	Swift	0.18	2.0	0.24	0.43
Percina shumardi	Swift	0.71	2.0		
Aplodinotus grunniens	Slack	<0.1		<0.1	
Herichthys	Slack	0.14	0.40		<0.1
cyanoguttatus				40	
N of species N of individuals		59	40 15,121	48 24,037	<u>31</u> 4,645

Table 6.Fishes taken from all habitats and basins 2014 through 2017.

	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

Table 7.Number of sites and visits conducted during Round One and Round Two (2014–2017) with a
breakdown per flow tier.

Riffle Habitats

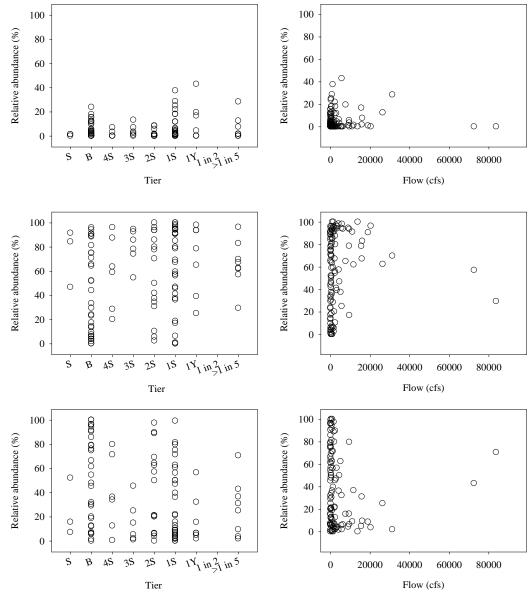
Patterns in relative abundances for slack-water fishes, moderately swift-water fishes, and swiftwater fishes in riffle habitats were not detected (P > 0.05) among flow tiers or discharge (Figure 6). Subsequent analyses were made at a site or at a combination of sites, grouped by geographic, hydrologic, or community similarity. Only results for the Brazos basin sites are presented in this section, with results from across all basins summarized in Section 3.1.1.7.

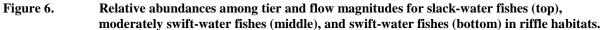
Leon River-Gatesville and Lampasas River-Kempner

A total of 3,313 fishes was recorded from 22 sampling events and seven flow tiers (subsistence to >1-per-5-year). One riffle was censored from relative abundance analysis because only one fish was captured. Most abundant fishes were *Cyprinella lutrensis* (N=1,833), *Cyprinella venusta* (674), *Ictalurus punctatus* (243), *Pimephales vigilax* (156), *Etheostoma spectabile* (147), and *Campostoma anomalum* (96).

Relative abundances decreased for *C. anomalum* (F _{1,19} =4.7, P=0.04) and were not different for *C. lutrensis*, *C. venusta*, *P. vigilax*, and *E. spectabile* (P>0.05) between pre-flood and post-flood periods (Figure 7). Relative abundances were not different (P>0.05) among flow tiers for *C. lutrensis*, *C. venusta*, *P. vigilax*, *E. spectabile*, and *C. anomalum*.

Densities decreased for *E. spectabile* (F $_{1, 20}$ =5.0, P=0.04) and were not different (P>0.05) for total fishes, *C. lutrensis*, *C. venusta*, *P. vigilax*, and *C. anomalum* between pre-flood and post-flood periods (Figure 8). Densities were not different (P>0.05) among flow tiers for total fishes, *C. lutrensis*, *C. venusta*, *P. vigilax*, *E. spectabile*, and *C. anomalum*.





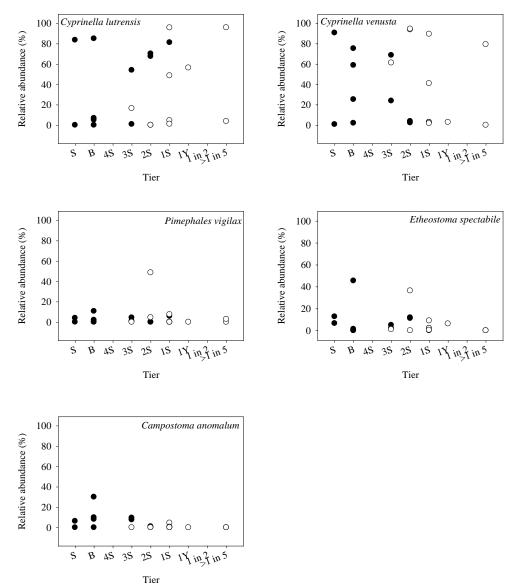
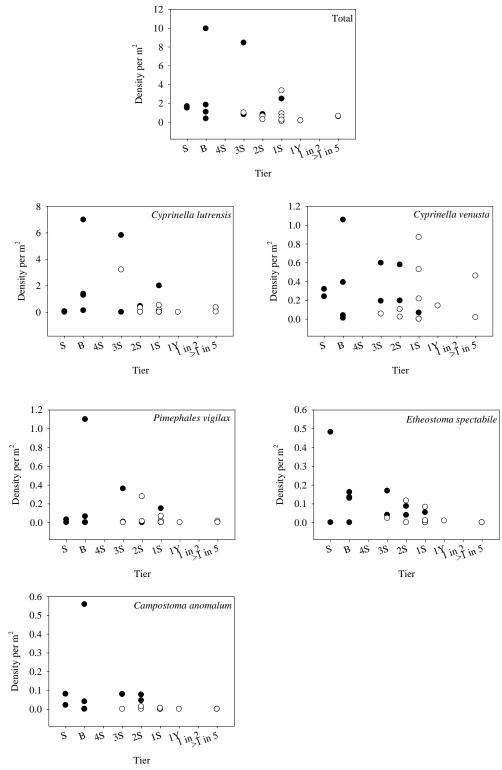
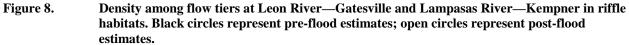


Figure 7. Relative abundances among flow tiers at Leon River—Gatesville and Lampasas River— Kempner in riffle habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.





Instream Flows Research and Validation Methodology Framework TWDB 2016–2017

Little River—Little River

A total of 1,135 fishes was recorded from 14 sampling events and six flow tiers (base to >1-per-5-year). Most abundant fishes were *Etheostoma spectabile* (N=-442), *Cyprinella venusta* (309), *Cyprinella lutrensis* (240), *Ictalurus punctatus* (48), *Pimephales vigilax* (32), and *Campostoma anomalum* (23).

Relative abundances increased for *C. lutrensis* (F _{1, 12} =22.9, P<0.01), decreased for *C. anomalum* (F _{1, 12} =7.4, P=0.02), and were not different for *E. spectabile*, and *C. venusta* between pre-flood and post-flood periods (Figure 9). Relative abundances were not different (P>0.05) among flow tiers for *E. spectabile*, *C. lutrensis*, *C. venusta*, and *C. anomalum*.

Densities decreased for *E. spectabile* (F _{1, 12} =14.1, P<0.01), decreased for *C. anomalum* (F _{1, 12} =8.1, P=0.01), and were not different for total fishes, *C. lutrensis*, and *C. venusta* between pre-flood and post-flood periods (Figure 10). Densities were not different (P>0.05) among flow tiers for total fishes, *E. spectabile*, *C. lutrensis*, *C. venusta*, and *C. anomalum*.

Navasota River-Easterly

A total of 1,470 fishes was recorded from 12 sampling events and seven flow tiers (base to >1-per-5-year). Most abundant fishes were *Cyprinella venusta* (N=792), *Pimephales vigilax* (397), *Dorosoma petenense* (72), *Etheostoma gracile* (62), *Cyprinella lutrensis* (55), and *Percina sciera* (37).

Relative abundances were not different (P>0.05) for *C. venusta*, *P. vigilax*, *D. petenense*, and *P. sciera* between pre-flood and post-flood periods (Figure 11). Flow tiers lacked sufficient replication to assess differences in relative abundances.

Densities increased for total fishes (F $_{1,10}$ =8.5, P=0.02), increased for *C. venusta* (F $_{1,10}$ =6.4, P=0.03), and were not different (P>0.05) for *P. vigilax*, *D. petenense*, and *P. sciera* between pre-flood and post-flood periods (Figure 12). Flow tiers lacked sufficient replication to assess differences in densities.

Run Habitats

Patterns in relative abundances for slack-water fishes, moderately swift-water fishes, and swift-water fishes in run habitats were not detected (P>0.05) among flow tiers or discharge (Figure 13). Subsequent analyses were made at site or at a combination of sites.

Leon River—Gatesville and Lampasas River—Kempner

A total of 6,414 fishes was recorded from 22 sampling events and seven flow tiers (subsistence to >1-per-5-year). One run was censored from relative abundance analysis because no fish was captured. Most abundant fishes were *Cyprinella lutrensis* (N=3,336), *Cyprinella venusta* (2,195), *Pimephales vigilax* (464), *Notropis buchanani* (129), *Gambusia affinis* (98), and *Notropis volucellus* (88).

Relative abundances were not different (P>0.05) for *C. lutrensis*, *C. venusta*, *P. vigilax*, *N. buchanani*, *G. affinis*, and *N. volucellus* between pre-flood and post-flood periods or among flow tiers (Figure 14).

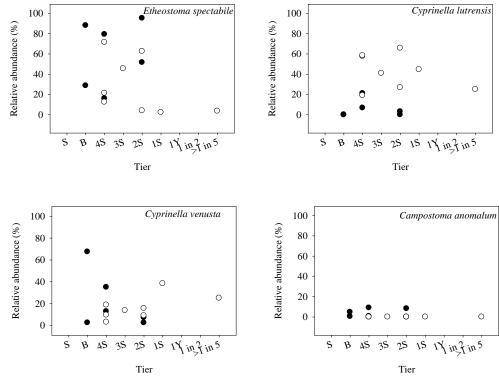
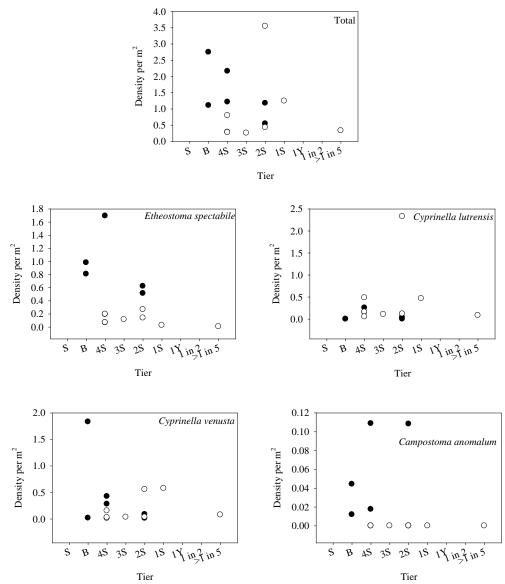


Figure 9. Relative abundances among flow tiers at Little River—Little River in riffle habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.





Density among flow tiers at Little River—Little River in riffle habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

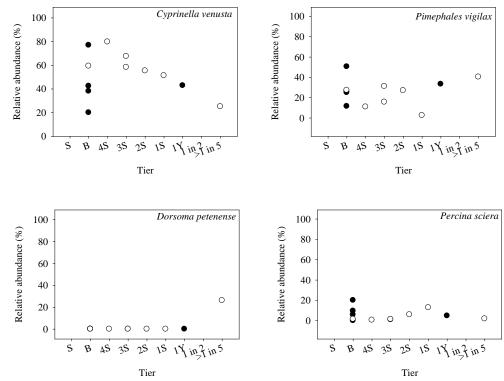


Figure 11. Relative abundances among flow tiers at Navasota River—Easterly in riffle habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

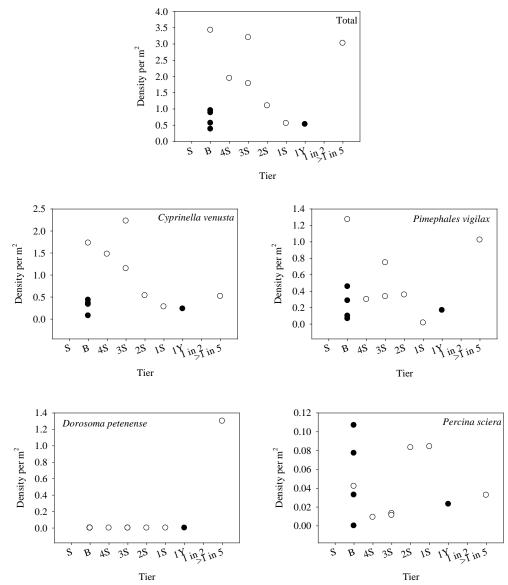


Figure 12. Density among flow tiers at Navasota River—Easterly in riffle habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

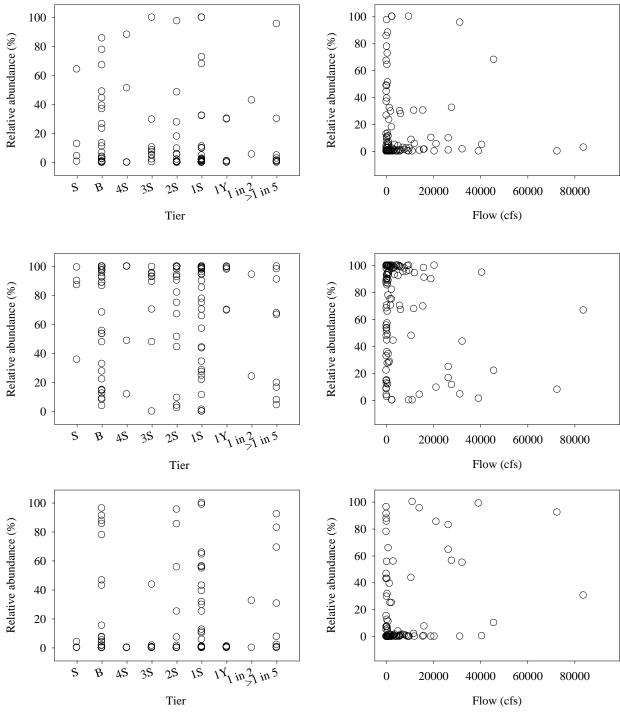


Figure 13. Relative abundances among tier and flow magnitude for slack-water fishes (top), moderately swift-water fishes (middle), and swift-water fishes (bottom) in run habitats.

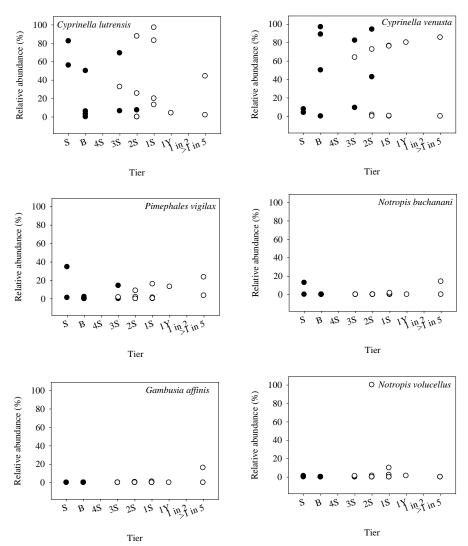


Figure 14. Relative abundances among flow tiers at Leon River—Gatesville and Lampasas River-Kempner in run habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

Densities increased for *C. venusta* (F _{1, 20} =4.5, P=0.047), increased for *N. volucellus* (F _{1, 20} =6.0, P=0.02), increased for total fishes (F _{1, 20} =5.5, P=0.03), and were not different for *C. lutrensis*, *P. vigilax*, *N. buchanani*, and *G. affinis* between pre-flood and post-flood periods (Figure 15). Densities were not different (P>0.05) for *C. lutrensis*, *C. venusta*, *P. vigilax*, *N. buchanani*, *G. affinis*, and *N. volucellus* among flow tiers.

Little River—Little River

A total of 268 fishes was recorded from 13 sampling events and six flow tiers (base to >1-per-5year). One run was censored from relative abundance analysis because no fish was captured. Most abundant fishes were *Cyprinella venusta* (N=171) and *Cyprinella lutrensis* (48).

Relative abundances and densities were not different (P>0.05) for *C. venusta* and *C. lutrensis* between pre-flood and post-flood periods and among flow tiers (Figure 16). Densities were not different (P>0.05) for total fishes between pre-flood and post-flood periods (Figure 17).

Navasota River—Easterly

A total of 1,146 fishes was recorded from 12 sampling events and seven flow tiers (base to >1 - per-5-year). Most abundant fishes were *Dorosoma petenense* (N=597), *Cyprinella venusta* (224), *Lythrurus fumeus* (128), and *Pimephales vigilax* (118).

Relative abundances were not different (P>0.05) for *D. petenense*, *C. venusta*, *L. fumeus*, and *P. vigilax* between pre-flood and post-flood periods (Figure 18). Flow tiers lacked sufficient replication to assess differences in relative abundances.

Densities increased for *C. venusta* (F_{1,10}=6.9, P=0.03) and were not different (P>0.05) for total fishes, *D. petenense*, *L. fumeus*, and *P. vigilax* between pre-flood and post-flood periods (Figure 19). Flow tiers lacked sufficient replication to assess differences in densities.

Brazos River-Hempstead and Rosharon

A total of 7,944 fishes was recorded from 20 sampling events from seven flow tiers (subsistence to >1-per-5-year). Most abundant fishes were *Cyprinella lutrensis* (N=5,275), *Notropis shumardi* (647), *Notropis buchanani* (604), *Pimephales vigilax* (598), *Macrhybopsis hyostoma* (349).

Relative abundances decreased for *C. lutrensis* (F _{1, 18} =51.4, P<0.01), increased for *N. shumardi* (F _{1, 18} =21.0, P<0.01), increased for *M. hyostoma* (F _{1, 18} =8.5, P<0.01), and were not different (P>0.05) for *N. buchanani* and *P. vigilax* between pre-flood and post-flood periods (Figure 20). Relative abundance differed among flow tiers for *C. lutrensis* (F _{3, 13} =4.8, P=0.02) with relative abundances at base and 3-per-season less than those at 2-per-season and 1-per-season. Relative abundances were not different (P>0.05) among flow tiers for *N. shumardi*, *N. buchanani*, *P. vigilax*, and *M. hyostoma*.

Densities decreased for *C. lutrensis* (F _{1, 18} =10.3, P<0.01), increased for *N. shumardi* (F _{1, 18} =6.9, P=0.02), increased for *M. hyostoma* (F _{1, 18} =5.3, P<0.03), decreased for total fishes (F _{1, 18} =5.7, P<0.03), and were not different (P>0.05) for *N. buchanani* and *P. vigilax* between pre-flood and post-flood periods (Figure 21). Densities were not different (P>0.05) among flow tiers for total fishes, *C. lutrensis*, *N. shumardi*, *N. buchanani*, *P. vigilax*, and *M. hyostoma*.

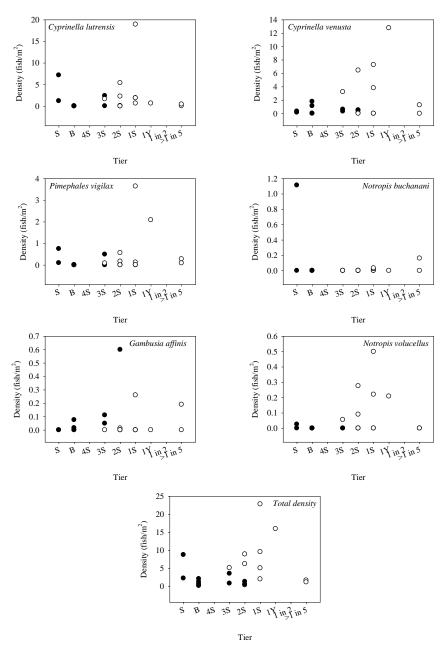


Figure 15. Density among flow tiers at Leon River—Gatesville and Lampasas River—Kempner in run habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

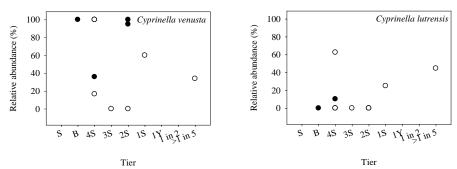


Figure 16. Relative abundances among flow tiers at Little River—Little River in run habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

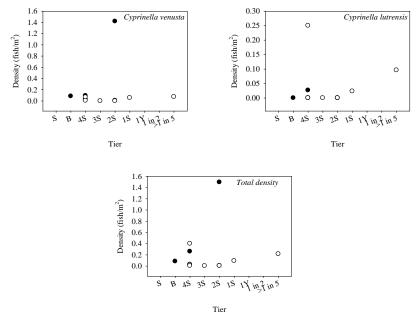


Figure 17. Density among flow tiers at Little River—Little River in run habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

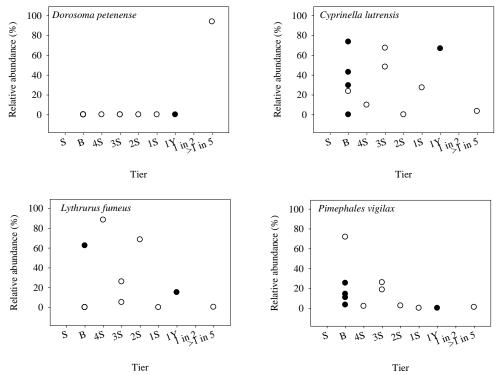


Figure 18. Relative abundances among flow tiers at Navasota River—Easterly in run habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

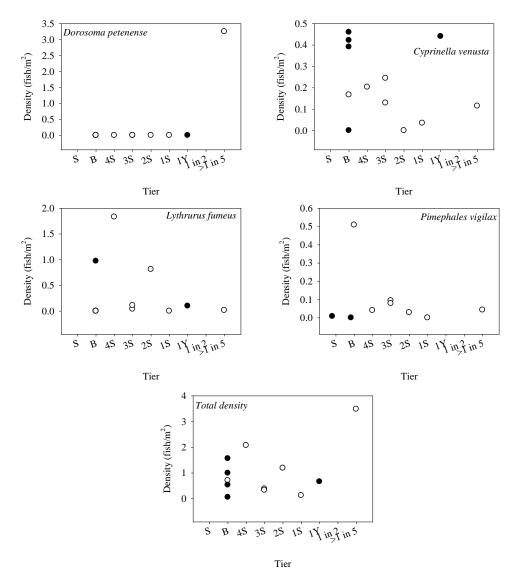


Figure 19. Density among flow tiers at Navasota River—Easterly in run habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

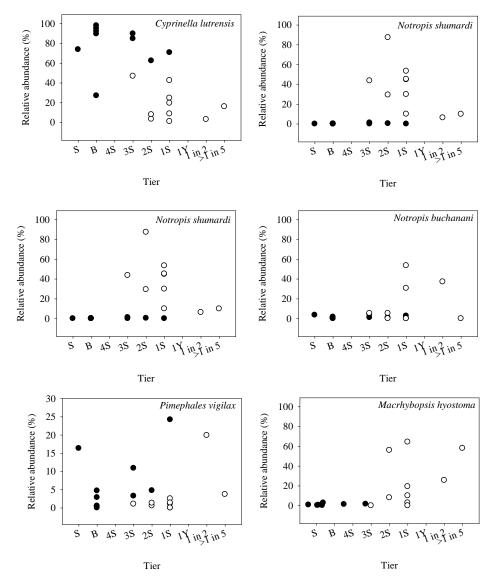


Figure 20. Relative abundances among flow tiers at Brazos River—Hempstead and Rosharon in run habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

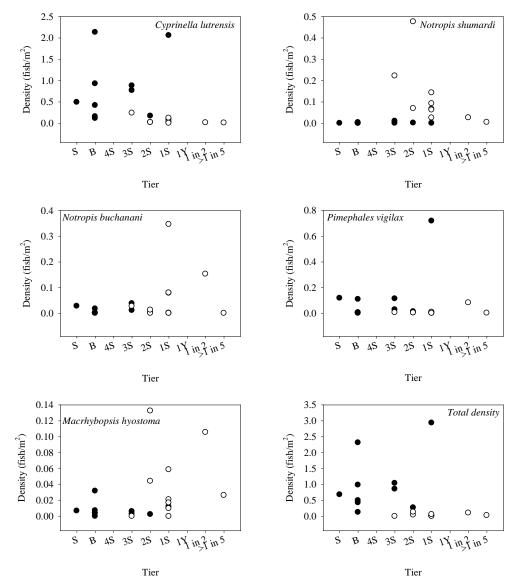


Figure 21. Density among flow tiers at Brazos River—Hempstead and Rosharon in run habitats. Black circles represent pre-flood estimates; open circles represent post-flood estimates.

Pool Habitats

Across all sites and basins, a total of 759 fishes was recorded from 25 sampling events and seven flow tiers (base, 4-per-season, 3-per-season, 2-per-season, 1-per-season, 1-per-year and >1-in-5-year). Most abundant fishes were *Cyprinella lutrensis* (N=345), *Cyprinella venusta* (172), *Notropis volucellus* (73) and *Lythrurus fumeus* (53).

Pool habitats were not present at all sampling sites. During Round One (2014–2015), pool habitats were not assessed; therefore, insufficient replication and lack of pre-flood condition preclude analyses of fish community response to flow tiers within pool habitats. Below, we provide a summary of fish collections from pool habitats within the Brazos basin.

Leon River—Gatesville and Lampasas River - Kempner Pools were not available.

Little River—Little River Academy

A total of 73 fishes was recorded from three sampling events and three flow tiers (4-per-season, 2-per-season, and >1-per-5-year). Most abundant fishes were *Notropis volucellus* (N=31), *Cyprinella venusta* (29), and *Cyprinella lutrensis* (12).

Navasota River-Easterly

A total of 156 fishes was recorded from six sampling events and six flow tiers (base, 4-perseason, 3-per-season, 2-per-season, 1-per-season, and >1-in-5-year). Most abundant fishes were *Cyprinella venusta* (N=76), *Lythrurus fumeus* (N=53), and *Pimephales vigilax* (N=17).

Brazos River—Hempstead and Rosharon

Pools were not available.

Backwater Habitats

Across all sites and basins, a total of 3,744 fishes was recorded from 58 sampling events and seven flow tiers (base, 4-per-season, 3-per-season, 2-per-season, 1-per-season, 1-per-year and >1-in-5-year). Most abundant fishes were *Gambusia affinis* (N=987), *Notropis shumardi* (629), *Cyprinella venusta* (247), and *Pimephales vigilax* (210).

Backwater habitats were not present at all sampling sites. During Round One (2014–2015), backwater habitats were not assessed; therefore, insufficient replication and lack of pre-flood condition preclude analyses of fish community response to flow tiers within backwater habitats. Below, we provide a summary of fish collections form backwater habitats within the Brazos basin.

Leon River-Gatesville and Lampasas River - Kempner

A total of 392 fishes was recorded from four sampling events and three flow tiers (2-per-season, 1-per-season, and >1-in-5-year). Most abundant fishes were *Gambusia affinis* (N=169), *Fundulus notatus* (121), *Cyprinella lutrensis* (57), and *Pimephales vigilax* (25).

Little River—Little River Academy

A total of 169 fishes was recorded from six sampling events and four flow tiers (4-per-season, 3-per-season, 1-per-season, and >1-per-5-year). Most abundant fishes were *Gambusia affinis* (76), *Cyprinella lutrensis* (44), and *Cyprinella venusta* (19).

Navasota River—Easterly

A total of 468 fishes was recorded from eight sampling events and six flow tiers (base, 4-perseason, 3-per-season, 2-per-season, 1-per-season, and >1-per-5-year). Most abundant fishes were *Cyprinella venusta* (N=132), *Gambusia affinis* (81), and *Pimephales vigilax* (71).

Brazos River-Hempstead and Rosharon

A total of 1,081 fishes was recorded from eight sampling events and four flow tiers (3-perseason, 2-per-season, 1-per-season, and >1-per-5-year). Most abundant fishes were *Notropis shumardi* (N=629), *Gambusia affinis* (145), *Cyprinella* lutrensis (126), and *Notropis buchanani* (88).

Macroinvertebrates

Totals of nine orders and 115,228 individuals were recorded from Brazos (N of individuals=51,442), GSA (41,990), and Colorado (21,796) basins among all habitats between 2014 and 2017 (Table 8). In Round Two (2016–2017), totals of nine orders and 65,000 individuals were recorded. Macroinvertebrate abundances by site are provided in Appendix E.

Table 8. Macroinvertebrates taken overall from 2014 through 2017.

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

Leon River—Gatesville and Lampasas River—Kempner

A total of 9,749 macroinvertebrates was recorded from 21 sampling events and seven flow tiers (subsistence to >1-per-5-year). Densities increased for total macroinvertebrates (F_{1,19}=15.5, P<0.01) and EPT (F_{1,19}=16.2, P<0.01) between pre-flood and post-flood periods. Densities were not different (P>0.05) among flow tiers for total macroinvertebrates and EPT.

Little River—Little River

A total of 4,483 macroinvertebrates was recorded from 13 sampling events and six flow tiers (base to >1-per-5-year). Densities were not different (P>0.05) for total macroinvertebrates or for EPT between pre-flood and post-flood periods and among flow tiers.

Navasota River—Easterly

A total of 2,899 macroinvertebrates was recorded from nine sampling events and five flow tiers (base to >1-per-5-year). Densities were not different (P>0.05) for total macroinvertebrates or for EPT between pre-flood and post-flood periods. Flow tiers lacked sufficient replication to assess differences in densities.

Across-Basin Summary

Little River—Little River Navasota River—Easterly

Brazos River-Hempstead and Rosharon

Although only data from the Brazos basin are presented above, the following section summarizes results of flow-tier analysis across Brazos and GSA 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., lower Brazos, Brazos River—Hempstead and Brazos River—Rosharon). Table 9 shows the sites or combination of sites evaluated and available data collected per habitat type at each site used in the flow tier analysis.

in flow tier analysis.				
	Fi	sh	Macroinvertebrates	
Combination/Individual Sites per basin	Riffle	Run	Riffle	
GSA				
Medina River—Bandera and Guadalupe River—Comfort		\checkmark		
Guadalupe River—Gonzales and Cuero and San Antonio River—Goliad				
Cibolo Creek—Falls City	\checkmark	\checkmark	\checkmark	
San Marcos River—Luling			\checkmark	
Brazos				
Leon River—Gatesville and Lampasas River—Kempner		\checkmark		

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Table 9.Fish and macroinvertebrate data collected per habitat type in the GSA and Brazos basins used
in flow tier analysis.

As shown in Table 9, 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. Table 10 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.

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

Table 10.Fish and macroinvertebrate community or species response to flow tier and pre-flood vs. post-flood (S=season, Y=year).

Species responses were associated with flow tiers in five of the eight sites or combination of sites (Table 10). Within the upper GSA, the >1-per-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, the 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, the 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, the 2-per-season and 1-per-season flow tiers were associated with lower relative abundances of C. lutrensis in runs, when compared to the base and-3-perseason 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 predictions. Positive association with flow tiers observed for C. lutrensis (i.e., San Marcos River), C. venusta, and P. vigilax were opposite of predictions. Macroinvertebrate response was associated with flow tiers within lower GSA with total macroinvertebrate densities being greater at base than 1-per-season.

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 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%, ± 1 SE: 7.0) and few fluvial specialists *N. shumardi* and *M. hyostoma* (1.1% \pm 0.25). Post-flood fish community was dominated, as predicted, by fluvial specialist *N. shumardi* and *M. hyostoma* (60% ± 8.7) and fewer *C. lutrensis* and *P. vigilax* (20% ± 4.9). 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. 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 and post-flood periods 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. 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. Densities of EPT 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. 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 22). 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 that 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.

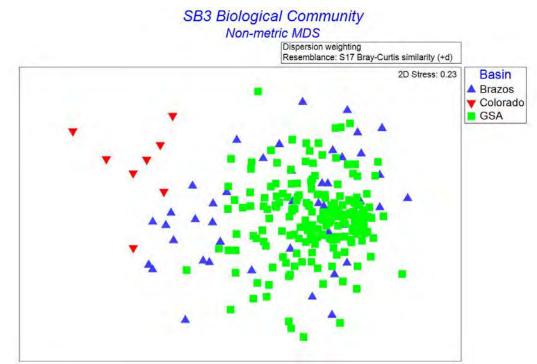


Figure 22. An n-MDS ordination plot for the three river drainages fish communities.

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 23. No significant relationships were observed for the four species.

Among basins, swift-water fishes were more abundant in the Colorado dataset (Figure 24). 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 25). No other differences were detected among or within basins for each habitat type (riffle, run, pool, and backwater) using the three-factor analyses.

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 26). No other relationships were noted among fish groupings within basins.

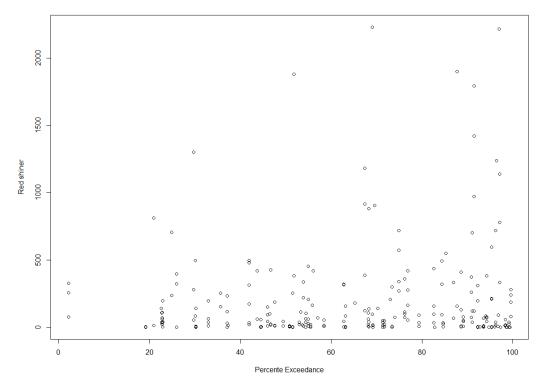


Figure 23. Red Shiner abundance across percent exceedance levels.

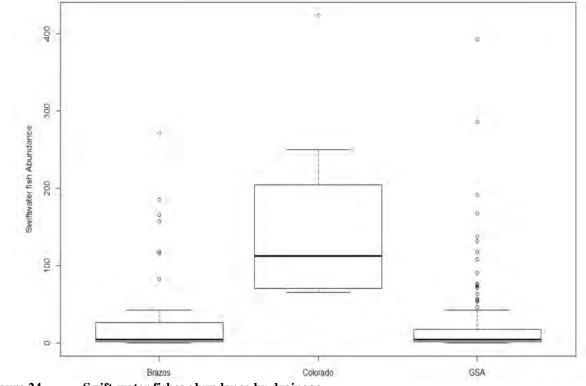


Figure 24. Swift-water fishes abundance by drainage.

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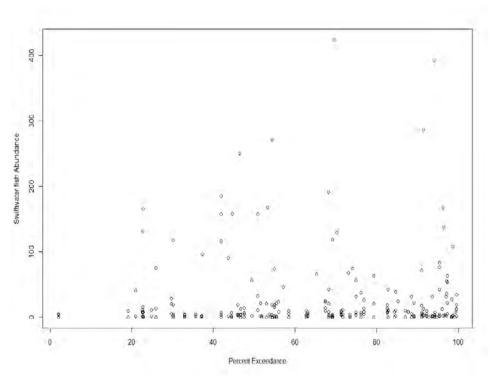


Figure 25. Abundance of swift-water fishes across percent exceedance levels.

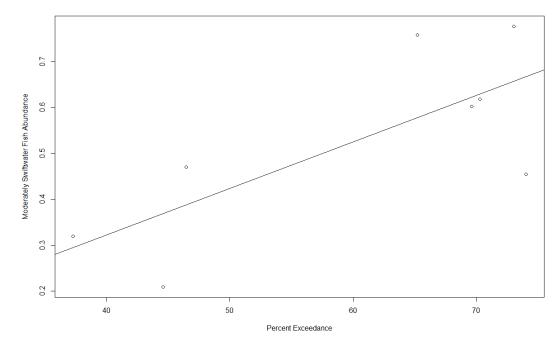


Figure 26. Proportional abundance of moderately swift-water (fluvial) fishes plotted as a response to percent exceedance in the Colorado drainage (F 1, 6=7.527, P=0.03358) showing best fit line for linear regression model.

3.2 Riparian

Results and discussion of outcomes will be discussed by individual site first and then by community, with basin-wide results to follow.

3.2.1 Brazos Bend Site

Data at this location were collected in May 2017 as a spring sampling event. There were noticeable differences in the vegetation communities between level (Figure 27). Level 1 consisted mostly of a wide, flat sand bar that was mostly devoid of vegetation, with only a few small herbaceous species emerging and some woody riparian tree (e.g., black willow) seedlings. During very high flows from June 2015 to November 2016, all of Level 1 was completely submerged numerous times. This submergence scoured vegetation that was previously established, and it also relocated sediment and soils. Only recently have water flows been low enough for the re-establishment of pioneer vegetation.

Level 2 was located on a steeply rising sandy slope, which rose nearly 8 vertical meters in elevation from Level 1. As Level 2 increased in elevation the presence of vegetation increased. While a majority of Level 2 was submerged along with Level 1 during the floods, the duration of submergence was possibly less, allowing for herbaceous vegetation to survive or recolonize quickly. Level 2 was mostly dominated by cocklebur and other weedy herbaceous species with black willow saplings numerous at the highest elevations within the level.

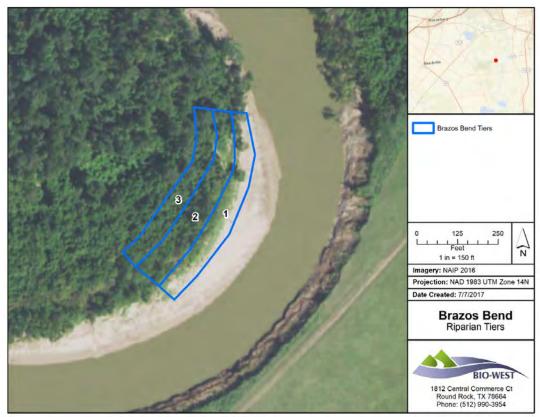


Figure 27. Overview of the Brazos Bend site showing the three level boundaries (in blue).

Level 3 was situated well above Level 1. Its topography was flat with some small berms and shallow depressions. Level 3 was dominated by large, mature trees such as box elder and sycamore along with numerous seedlings of these and other tree species. In Level 3 the woody canopy was quite dense with very little understory vegetation. It was evident that even this level has been flooded recently because of an observed layer of newly deposited silt on the forest floor.

A representative profile (Figure 28) shows that while Level 1 and Level 3 are nearly horizontal; the slope in Level 2 is a steep ~0.40 (meters rise/meters run). When taken together the overall site steepness factor is 0.13 (Table 11). The overall sampled community included 2721 individuals and the canopy tree sampling had 80 trees (Table 12). The most prevalent species in the site was cedar sedge at 41% abundance. Three riparian species also topped the community species' composition: sycamore at 15%, black willow at 14% and box elder at 11%. Dominating the mature trees were box elder at 76%, which contributed largely to the 80% dominance of FACW species in the canopy.



Figure 28.

Brazos Bend Site profile showing general level locations.

Table 11.	General site characteristics for sites studied during 2016–2017.
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Site	Basin	Steepness of Zone	Dominant Soil Type	Dominant Soil Order	Sinuousity 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

Species% of TotalCedar sedge40.7Sycamore15.4Black willow14.1Box elder10.8beakedcornsalad2.9Virginia wildrye2.5Cowitchvine2.5Obedientplant2.4Fiddledock1.5Greenbriar1.2Roughleaf dogwood1.1Waterhyssop1.0
Sycamore15.4Black willow14.1Box elder10.8beakedcornsalad2.9Virginia wildrye2.5Cowitchvine2.5Obedientplant2.4Fiddledock1.5Greenbriar1.2Roughleaf dogwood1.1
Black willow14.1Box elder10.8beakedcornsalad2.9Virginia wildrye2.5Cowitchvine2.5Obedientplant2.4Fiddledock1.5Greenbriar1.2Roughleaf dogwood1.1
Box elder10.8Box elder10.8beakedcornsalad2.9Virginia wildrye2.5Cowitchvine2.5Obedientplant2.4Fiddledock1.5Greenbriar1.2Roughleaf dogwood1.1
beakedcornsalad2.9Virginia wildrye2.5Cowitchvine2.5Obedientplant2.4Fiddledock1.5Greenbriar1.2Roughleaf dogwood1.1
Virginia wildrye2.5Cowitchvine2.5Obedientplant2.4Fiddledock1.5Greenbriar1.2Roughleaf dogwood1.1
Cowitchvine2.5Obedientplant2.4Fiddledock1.5Greenbriar1.2Roughleaf dogwood1.1
Obedientplant2.4Fiddledock1.5Greenbriar1.2Roughleaf dogwood1.1
Fiddledock1.5Greenbriar1.2Roughleaf dogwood1.1
Greenbriar 1.2 Roughleaf dogwood 1.1
Roughleaf dogwood 1.1
Waterhyssop 1.0
Switchgrass 1.0
Horseweed 0.4
Creeping burclover 0.4
Carolina sedge 0.3
Oxalis sp. 0.2
Salt cedar 0.2
Crabgrass 0.2
Goldencrown grass 0.2
Bermuda grass 0.1
Black medick 0.1
Hackberry 0.1
Gamma grass 0.1
Johnson grass 0.1
Brazilian verbena 0.1
Mexican hat 0.1
N=2721

Table 12. Brazos Bend community and mature tree abundances.

Mature Trees	
Species	% of Total
Box elder	76.3
Sycamore	10.0
Cottonwood	7.5
Blackwillow	3.8
Pecan	2.5
N=80	
FAC	20.0
UPL	0.0
FACU	0.0
FACW	80.0
OBL	0.0
Invasive	0.0

An nMDS ordination plot of the site's level shows a distinct dissimilarity between Levels 1 and 3, but much overlap of each with Level 2 (Figure 29). This is verified by the ANOSIM statistics in the figure. 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 species resulting in dissimilarities between those levels (Appendix F, Table 1) shows that other than clover in Level 3, the species present in both 1 and 3 are similar, just in different abundances. Interestingly, the pattern of riparian woody vegetation shows only black willow (in low abundance) exists in Level 1, while Level 2 includes that as well as box elder and sycamore. Level 3 still shows heavy dispersal. So rather than the riparian canopy diminishing with increasing levels, it is still present and even flourishing with higher elevation. One explanation may be the very broad point bar at

this site. Because of the spring floods, much of the existing canopy was removed; skewing the abundances by level (personal observation from previous studies). Another explanation is that even though these are very high elevation slopes, the shear amount of water coming through this stretch (so near the river mouth) inundates well up into those reaches. When grouped by WI classes (Figure 30), the differences between Level 1 and Level 3 become less distinguishable. The differences that do exist between the levels is the already-noted increased in FACW species with increasing level number, as well as the presence of FACU species in Level 3 (see Appendix F, Table 2).

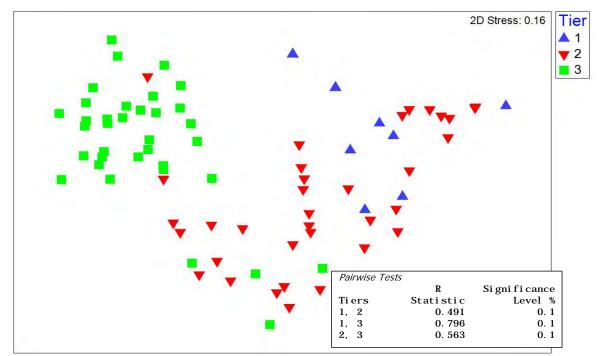


Figure 29. An nMDS analysis of Brazos Bend levels' community differences. The inset box shows ANOSIM results; p=.1%.

Analysis of the separate mature-trees-only dataset (Figure 31) shows all three levels are distinctly dissimilar to one another, as shown by the nMDS ordination; however, the small sample size in Level 1 was problematic when ANOSIM statistics were attempted (Figure 31). Because of this, no SIMPER tests could be performed, but in general the mature-tree dataset reflects a similar increase in riparian species similar to the overall community assemblages (sparse in Level 1 and increasing in upper levels). This site's biological community does not reflect an expected riparian distribution, which again is being influenced by the shallow point bar and recent scouring. This would be an excellent candidate site to monitor riparian re-establishment and community succession through time.

The discharge estimated to inundate all of Level 1 is more than approximately 35,500 cfs (Table 13). Level 2 inundation would require approximately 42,500 cfs, and Level 3 would require approximately 43,500 cfs to fully inundate. Table 14 shows that all TCEQ flow standards fall well short of these approximate inundation levels.

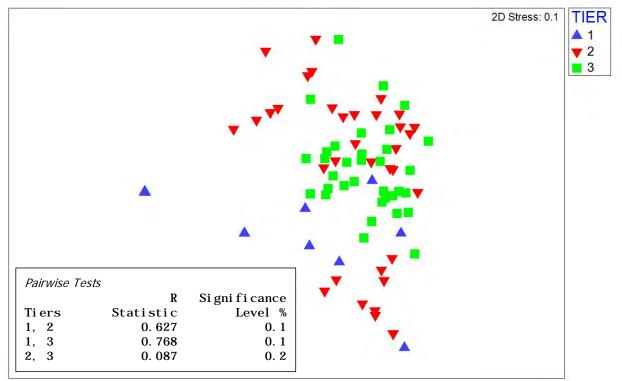


Figure 30. An nMDS analysis of Brazos Bend levels' WI class differences. The inset box shows ANOSIM results; p=.1%.

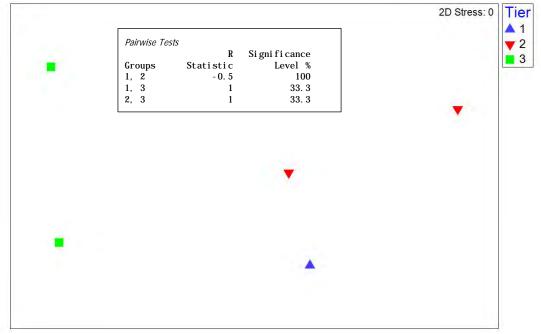


Figure 31. An nMDS analysis of Brazos Bend levels' WI class differences. The inset box shows ANOSIM results; significance=.1%.

Table 13.Stream discharge estimated to inundate Riparian site level based on USGS gage rating
curves.

Riparian Site	Strata	Estimated Inundation Flow Rate (cfs)
	1	35,500
Brazos Bend State Park	2	42,500
	3	43,500
	1	700
Hearne	2	5,000
	3	8,500

Table 14.TCEQ flow standards for selected sites in the Brazos Basin. Source: TCEQ 2014.

Gauge Location	Study Site	Season / Time Period	Subsistence (cfs)	Hydrologic Condition	Base (cfs)	Dry Pulse (cfs)	Average Pulse (cfs)	Wet Pulse (cfs)
Rosharon	Brazos Bend	Winter	430	Dry	1,140	9,090	9,090	13,600
		Winter	430	Avg	2,090	9,090	9,090	13,600
		Winter	430	Wet	4,700	9,090	9,090	13,600
		Spring	430	Dry	1,250	6,580	6,580	14,200
		Spring	430	Avg	2,570	6,580	6,580	14,200
		Spring	430	Wet	4,740	6,580	6,580	14,200
		Summer	430	Dry	930	2,490	2,490	4,980
		Summer	430	Avg	1,420	2,490	2,490	4,980
		Summer	430	Wet	2,630	2,490	2,490	4,980
Bryan	Hearne	Winter	300	Dry	540	3,230	3,320	5,570
		Winter	300	Avg	860	3,230	3,320	5,570
		Winter	300	Wet	1760	3,230	3,320	5,570
		Spring	300	Dry	710	6,050	6,050	10,400
		Spring	300	Avg	1260	6,050	6,050	10,400
		Spring	300	Wet	2460	6,050	6,050	10,400
		Summer	300	Dry	630	2,060	2,060	2,990
		Summer	300	Avg	920	2,060	2,060	2,990
		Summer	300	Wet	1470	2,060	2,060	2,990

3.2.2 Hearne Site

Data at this location were collected in May 2017 as a spring sampling event. Level 1 was a flat, mostly bare point bar (Figure 32). Due to high water levels from June 2015 to November 2016, this level was completely inundated for prolonged periods of time, which led to removal of established vegetation. Level 2 was a little higher in elevation and located on a sandy bank. Portions of Level 2 showed evidence of recent and prolonged inundation, but pioneer species including cocklebur, ragweed, and Johnson grass were prevalent. The wooded portions of Level 2 were dominated by large, mature trees including box elder and black willow, with a shrubby understory of roughleaf dogwood and woody vines. Level 3 was located near the same elevation as Level 2 but consisted of a dense, woody community dominated by hackberry and dogwood species. Giant cane was prevalent, forming dense colonies in both Level 2 and Level 3.

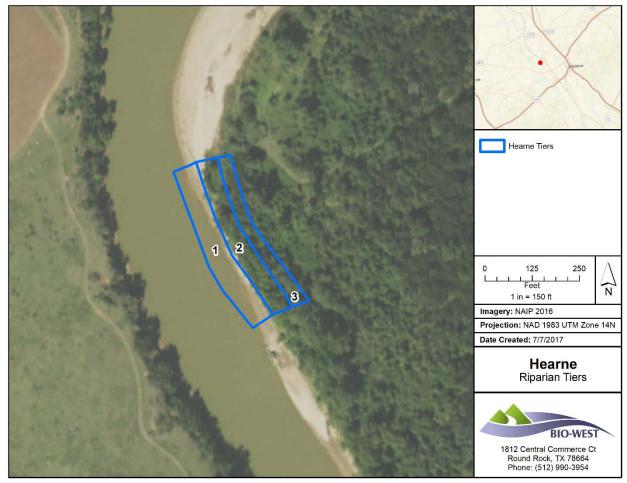


Figure 32.

Overview of Hearne Site showing the three level boundaries (in blue). At the time of the aerial photo, the river had risen to cover the exposed sand bar.

A representative profile (Figure 33) shows the slope from river's edge to the uppermost extent has a site steepness factor of 0.04 (see Table 11). Table 15 shows the overall community species' and mature trees' abundances. Cedar sedge, gamma grass, switch grass, and horseweed, collectively comprised 55% of the community. Because of the large presences of these grasses and forbs, the most abundant woody riparian species was box elder at 4% and black willow was a sparse 0.3% of the community. Hackberry was the most prevalent woody species, at 47% abundance, which also explains why FACU species show this same dominance in the site. Box elder was second-most prevalent at 18%, and the total FACW abundance was 28%.

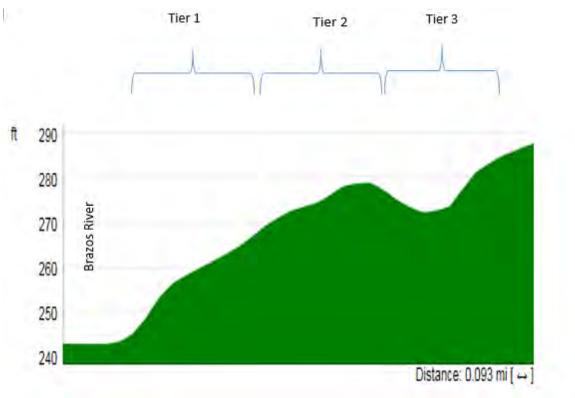


Figure 33. Hearne Site profile showing general level locations.

Table 15.	Hearne community and matur	e tree abundances.
Table 13.	meanine community and matur	e ti ce abunuances.

Plots	
Species	% of Total
Cedar sedge	22.1
Gamma grass	11.3
Switchgrass	11.3
Horseweed	10.1
Water pepper	8.4
Roughleaf dogwood	6.0
Hackberry	4.4
Box elder	4.1
Inland seaoats	3.6
Giant cutgrass	3.4
Swamp sweetscent	2.3
Hellers rosettegrass	2.1
Oxalis sp.	2.0
Coralberry	1.6
Indian grass	1.1
Slippery elm	0.9
Creepingburclover	0.9
Snailseed	0.7
Green ash	0.6
Shade betony	0.4
Black willow	0.3
Cottonwood	0.3
Soapberry	0.3
Agarita	0.3
Beakedcornsalad	0.3
Horse briar	0.3
Maxamillion sunflower	0.3
American elm	0.2
Goldencrown grass	0.2
Mexican hat	0.2
Gum bumelia	0.1
Pecan	0.1
Trumpetcreeper	0.1
N=1126	

Mature Trees	
Species	% of Total
Hackberry	47.4
Box elder	18.4
Slippery elm	7.9
American elm	6.6
Green ash	6.6
Cottonwood	3.9
Roughleaf dogwood	3.9
Blackwillow	2.6
Pecan	2.6
N=76	
FACU	47.4
FACW	27.6
FAC	25.0
UPL	0.0
OBL	0.0
Invasive	0.0

An nMDS ordination plot of Hearne's levels shows a progression of moderate dissimilarities with increasingly higher and distant levels, supported by the ANOSIM R values (Figure 34). The major contributors to similarity indicate that cocklebur is prevalent throughout the site as is hackberry (see Appendix F, Table 3). Very similar to Brazos Bend, Level 1 of this site experienced considerable scouring and vegetation removal in 2015 and 2016. Many species present in Levels 2 and 3 are missing or virtually nonexistent in Level 1 (see Appendix F, Table 4). Some box elder was found in Level 1 but most were located in Level 2.

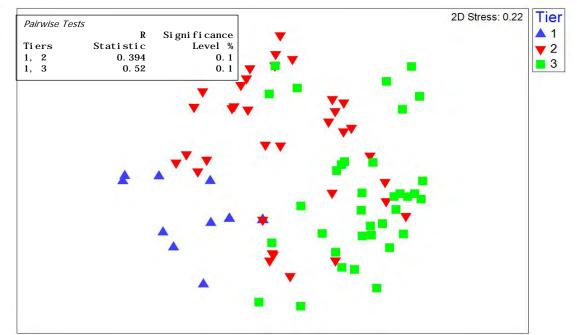


Figure 34. An nMDS analysis of the Hearne Site levels' community differences. The inset box shows ANOSIM results; p=.1% (Levels 2,3 stats not shown).

Grouping species by WI classes does little to refine community assemblages, though it does indicate there are a number of distinctly unique plots in all levels (Figure 35). These results expose one of the drawbacks to using a randomized method for sampling in riparian zones. There were so few FACW and OBL species randomly sampled, that they are lacking from statistical analyses of those assemblages (see Appendix F, Table 5). Additionally, the overwhelmingly high number of understory grasses obscures the variability of the lower-numbered species. This is a distinct disadvantage when using randomized vs. riparian-targeted sampling techniques. Despite this limitation, there are other, obvious explanations for the low dissimilarities. The FACU species pervaded all levels, though they showed increasing abundance values from Level 1 to Level 3. The FACW and OBL species' sampled counts were so low that they are completely absent from similarity and dissimilarity rankings at this site.

There are apparent differences among the mature trees in the two levels (Figure 36), but the sample size was too low to produce significant ANOSIM results. Level 1 is lacking from the ordination plot because it had no sampled mature trees; Level 2 was mostly slippery elm and American elm, while Level 3 consisted largely of cottonwood, box elder, green ash, and hackberry. This would indicate that at this point bar location, the established riparian species are further up the bank and rely on periodic flow pulses that scour the bar and bring water up into the upper reaches of the zone.

Overall community assemblages at this site showed much overlap between levels and significant encroachment into all levels by non-riparian-associated species. The mature-tree sampling lacked FACW and OBL classes, which 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 high abundance of these herbaceous/understory species also made distinguishing level community assemblages difficult.

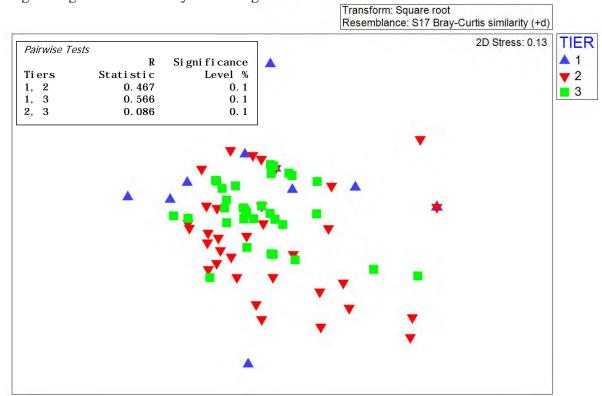


Figure 35. An nMDS analysis of the Hearne Site levels' WI class differences. The inset box shows ANOSIM results; p=.1%.

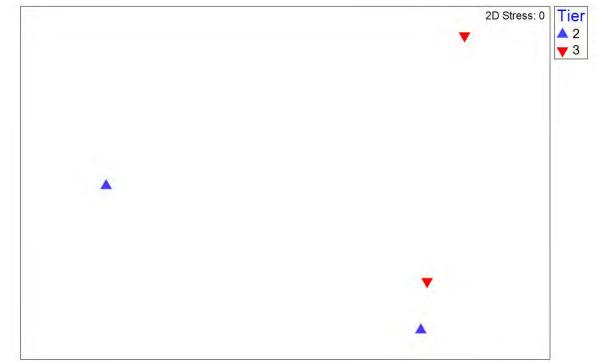


Figure 36. An nMDS analysis of the Hearne Site levels' mature-tree differences.

The discharge estimated to inundate all of Level 1 is approximately 700 cfs (Table 13). Level 2's inundation requires approximately 5,000 cfs, while Level 3 would require roughly 8,500 cfs to fully inundate. Table 14 shows that the TCEQ spring wet pulses do meet these elevations, though all other flow pulses fall short. This finding is in opposition from the Round One study (Bonner et.al 2015), which found that no TCEQ flow pulses reached full coverage of the riparian zone. This may indicate that either much stream channel geomorphology and biotic community alteration occurred in this site with the 2015 and 2016 floods, or it may indicate that the two differing methods of channel and stream elevations gave different estimated values.

3.2.3 Community and Basin Assessments

One of the important questions this study aimed to explore was the homogeneity of sites within the basin, or the 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 Brazos basin is provided in Appendix F.

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 evaluating riparian habitats within the GSA, Brazos, and Col/Lav basins is provided in Appendix F.

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 following section.

3.2.4 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' short-comings 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 samples 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 seaoats, 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.

3.2.5 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 necessary in order to make sound conclusions.

This study confirmed that with the field and statistical techniques employed, community assemblages could be well-characterized. 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. 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.

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 showed:

- 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 Round One outcomes: that in order to provide continued conservation and maintenance of the current riparian spatial distributions at many sites (excluding the Goliad site) the existing TCEQ flow standards (spring and fall) may need

adjustment based on existing information and future research. Without seasonal flows along the Guadalupe and San Antonio Rivers, the already-impacted riparian zones will likely face further longitudinal and perpendicular constriction in most cases. Management decisions must consider carefully the potential ecological loss of this important ecotone.

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.

3.3 Brazos Estuary

3.3.1 Hydrology Meteorology and Flow Tiers

Data collected from historical and study periods spanned a range of hydrological conditions (Table 17 and Figure 37). Quick visual examination of the hydrograph shows that in general the amount and distribution of freshwater inflow differed during each study period. Overall flows were comparable in 2012 and 2017, but they were lower compared to levels in 2015. The sustained high May peak flows made it impossible to sample the river during the May 2015 time period. The distribution of high-flow peaks also varied between study periods. During 2012, peak flows occurred more frequently during winter and spring months (Figure 37). In contrast, peak flows occurred in late spring and early summer during 2015. Smaller peak flows have been more evenly distrusted during the 2016–2017 study period. The distribution of sampling effort also differed between 2012 and the other two study periods. During 2014–2015 and 2016–2017 sampling effort was primarily limited to winter and spring months. Although the Brazos River was not sampled during late 2015 through 2016, extremely high peak flows occurred in December 2015 and even higher peak flows resulting in extensive flooding occurred during early June 2016. This pattern is easily visualized in the monthly average discharge hydrograph (Figure 38). Based on visual examination of stream banks and the Brazos River delta, the large June 2016 flood altered the channel and delta geomorphology and generated significant sediment loading to the estuary and nearshore Gulf of Mexico that will influence aquatic and wetland habitat.

Miller (2014) collected water quality and nekton data at four sites in the lower Brazos River once a month during January to December 2012 (Table 2 and 5, and Figure G1). During 2012, the highest (>20,000 cfs) daily average flows were observed during the winter and spring, with highest peaks occurring in March and April (Figure G1). This data was combined with information collected during the current study to expand our scope and include more flow tiers. Each collection was classified by season and flow tier using the methodology outlined in State of Texas (2014b) (Table 5 and Appendix G). Surveys conducted during 2012 were classified into a total of six flow tiers including: winter dry base (N=2); spring dry base (N=1), spring dry 1ps (N=2), spring dry subsistence (N=1); summer dry subsistence (N=4); and winter dry subsistence (N=2) (Tables 5 and 17).

During the first phase of our project we collected nekton during eight sampling events during the months of November 2014 through May 2015 (Table 17 and Figure G2). During the majority of the study period, the daily average flow at the Rosharon gage seldom exceeded 10,000 cfs

(Figure G2). Smaller peak flows were observed during January, March, and April. Field sampling had to be terminated during most of the month of May extending through July due to an extreme flood that started around May 15, 2015. Daily discharge rates continued to rise to a maximum discharge of 67,500 cfs on June 4, 2015 (Appendix H). A total of four flow tiers include winter average subsistence (N=3), winter average base (N=2), and spring average 3 ps (N=3) (Table 5 and 17).

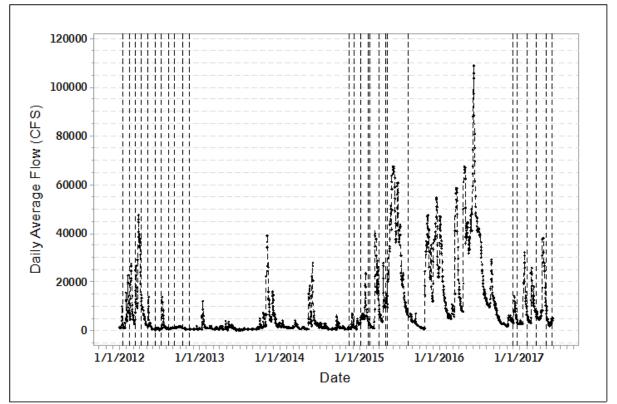
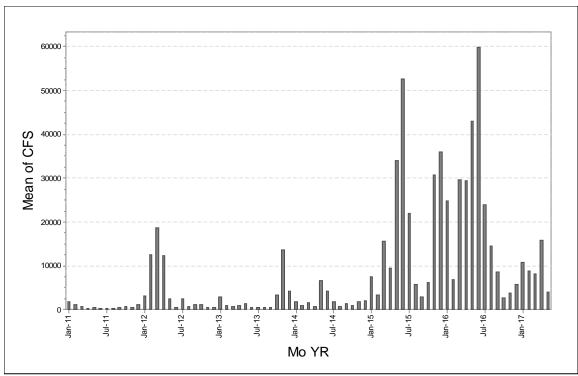


Figure 37. Daily average discharge measured at the USGS gage station near Rosharon, TX (USGS 08116650) during January 1, 2012, to May 31, 2017. Vertical lines denote sampling dates from this study and past investigations (Miller 2014; Bonner et al. 2015).



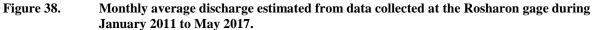


Table 16.Summary of flow tiers sampled during this and other historical surveys cited and analyzed
in this report.

Group	Season ^a	Hydrologic Conditions	Flow Tier	Ν
1	Spring	Average	3ps	3
2	Spring	Dry	Base	1
3	Spring	Dry	1ps	2
4	Spring	Dry	Subsistence	1
5	Spring	Wet	Base	2
6	Spring	Wet	Subsistence	1
7	Summer	Dry	Subsistence	4
8	Summer	Wet	2ps	1
9	Winter	Average	Base	2
10	Winter	Average	Subsistence	3
11	Winter	Dry	Subsistence	2
12	Winter	Dry	Base	2
13	Winter	Wet	Base	1
14	Winter	Wet	Subsistence	2

^a "Season" refers to hydrologic condition and flow tiers defined in Environmental Flow Standards Brazos River (State of Texas 2014b).

The field sampling for the first phase of the project in 2015 officially ended in May. However, EIH conducted one additional limited survey in August 2015 as part of an effort to retrieve instrumentation (e.g., depth gage) that had been deployed prior to the high-flow pulse conditions.

The gage had been left in May prior to the onset of one of the largest peak flows on the Brazos River that started in early May and extended to early August 2015. This event occurred during a summer wet 2-per-season flow tier (Table 5). During this survey, field water quality and nekton collections were completed.

During the current phase of field sampling a total of six additional surveys were conducted in the lower Brazos River estuary between December 2016 and May 2017 (Figure G3). Four distinct pulse flows were observed during the study period—in December, January, February, and April. The highest (37,900 cfs) peak flows were observed during April 17, 2017. During this time, we collected data during two seasons and two flow tiers including winter wet subsistence (N=2), winter wet base (N=1); spring wet base (N=2), spring wet subsistence (N=1) (Table 5). The use of data from all three study periods (2012, 2014–2015, 2016–2017) allowed us to sample 13 distinct combinations of season, hydrologic conditions and flow tiers.

The daily and monthly precipitation patterns in the study area as measured at the Plantation Lakes gage are illustrated in Figures E4 and E5. Local precipitation generally concurs with overall freshwater inflow patterns discussed earlier. In addition, local rainfall also indicates that 2012 was a generally dry year in the lower basin. Based on previous water budget studies, direct local rainfall contribution to the lower Brazos River has not been defined (Schoenbaechler et al. 2011). This is primarily due to a lack of a formally recognized "open bay area surface" that is used to estimate direct precipitation input to the receiving water body surface. Furthermore, local and regional ungaged freshwater inflow that would capture runoff from rain events in the lower basin are considered to be minimal compared to gaged inflows as measured at the Rosharon gage. During 1977–2009, gaged inflow from the Brazos River accounted for approximately 93 percent of the combined inflow into the lower river (Schoenbaechler et al. 2011).

Hourly and daily averaged water surface levels measured at the Freeport tide gage are displayed in Figures E6 and E7. The Freeport gage is not located within the main channel of the Brazos River but instead several miles west within the ICWW near the town of Surfside. Therefore, it would probably only respond to large river discharges. We also calculated the deviation in successive hourly water surface level measurements (Δ ft/hr). Examination of tidal data for the Brazos River provides evidence to support the hypothesis that the influence of astronomical tides is overwhelmed by meteorological events such as strong cold fronts that occurred during the study period and high flow events. During the winter months of our study water levels would often drop rapidly during passage of cold weather fronts or "blue northers" (Ward and Montague 1996). The lowest water levels were consistently observed during the months of December-February when cold weather fronts are common (Figure G7). The passage of cold weather fronts possessing strong north winds would effectively increase the outgoing stream velocity as coastal waterbodies drained. This reinforces the export and movement of freshwater into the lower portions of the estuary. This would also expose extensive stretches of the shoreline. This is potentially a significant bottleneck in the life history of any small juvenile fish living along the river shoreline. These fish would essentially be exposed to additional predation risk as water receded from brushy and vegetated areas. The interaction between river discharge, astronomical tides and weather can be seen in Figures E8 and E9. Water level fluctuations were more variable and more influenced by daily average discharges above 400 cfs. In addition, extremely low and declining water levels were encountered each winter when strong high pressure storms

descended from the north. Higher water levels were observed in the spring and fall month when strong southerly winds occur preceding northerly cold fronts.

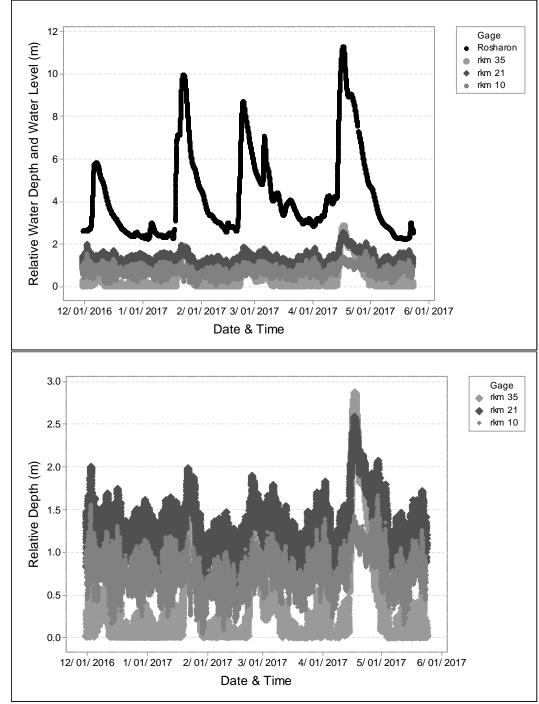
During each sampling period in 2014-2017 we also monitored water levels and flow in the lower river with a Sontek River Surveyor to determine if there were any significant differences between inflow levels measured at the most upstream site (B42) located at river kilometer 42 and the Rosharon gage. Previously in 2015 we found good agreement between our pressure transducer gage located at rkm 21 and the recorded water levels at the Rosharon gage (Figure G8). Due to the greater width of the lower downstream portions of the river the water levels would only rise approximately three meters in comparison to concurrent river stage rises of more than 14 meters at the Rosharon gage during the same period. Our estimates of river discharge also correlated well with the estimated discharges recorded at Rosharon (Figure G9). Greater deviation between our measurements and estimated flows at Rosharon occurred at lower (<8,000 cfs) discharge levels. Data collected during the 2016-17 study period provided additional information on how the lower river responds to flood pulses recorded at the Rosharon gage (Figure 39). These data generally support observations made in 2015 at river kilometer 21. These data indicate that depending on the magnitude and duration, high flow pulses recorded at Rosharon ranging up to 11 meters over base flows had diminished to 2.8, 2.6 and 1.4 meters at river kilometer 35, 21 and 10 respectively as they moved downstream.

The salinity regime within the lower Brazos River was strongly influenced by the amount of freshwater inflow. Salinity levels generally oscillated out of phase with fluctuations in freshwater inflow. Generally, as water levels increased due to higher flows the salinity would rapidly decline due in part to dilution and hydraulic forcing of the salt wedge downstream. The change in salinity could be very quick and rapid especially in the lower river below river kilometer 10 (Figure 40). During these periods of rapid rises in river stage, surface salinity measured at river kilometer 10 could increase or decline by as much as 25 psu within a few days. In contrast, less deviation was observed at river kilometer sites 21 and 35 (Figure 40). During these same periods, wide fluctuations in dissolved oxygen were also observed (Figure 41). These fluctuations were less predictable. In some cases, it appears that dissolved oxygen would decline during base flow periods perhaps due to less mechanical mixing or the establishment of a stable halocline that reduced vertical mixing. In addition, oxygen production due to suspended plankton would vary with other factors including the amount of suspended solids that when elevated would increase shading and reduced photosynthesis.

3.3.2 Water Quality

Summary statistics in surface and/or bottom measurements of total depth, water temperature, salinity, DO, pH, turbidity, Secchi disk transparency, chlorophyll-a, TSS, nitrate and nitrite nitrogen, total phosphorus are presented in Table 18 and Appendix I. Combined nitrate+nitrite-N levels exhibited a significant difference in concentration between tiers (Appendix J, see Model 3). During wet events nitrate-nitrite levels were significantly lower than during all other tiers (Appendix J Model 3). We did not detect any difference in this variable between sites. Total Kjeldahl nitrogen (TKN) concentrations exhibited statistically significant differences between

flow tier groups with the lowest values being recorded during wet base spring and winter events (Appendix J, Model 6). We did not detect any difference in TKN between sites.





Comparison of river stage at Rosharon gage and relative water depth measured by InSitu pressure transducers deployed at river kilometer 10, 21, and 35.

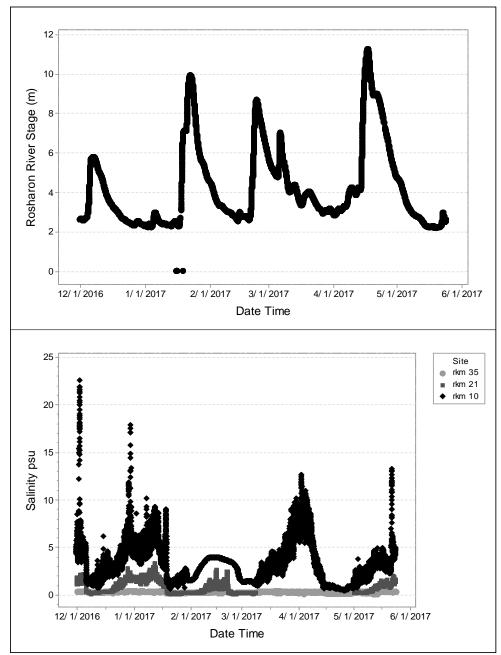


Figure 40. River stage measured at Rosharon versus surface salinity measured with continuous monitoring temperature and conductivity meters (HOBO model) during December 2016 to May 24, 2017.

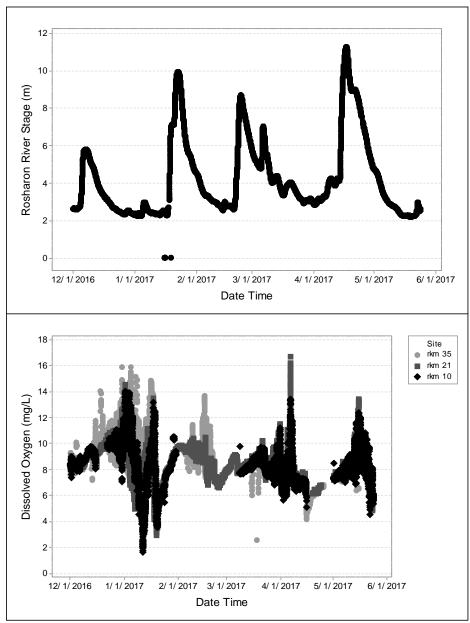


Figure 41. River stage measured at Rosharon versus dissolved oxygen measured with continuous monitoring temperature and conductivity meters (HOBO model) during December 2016 to May 24, 2017.

Table 17.Summary statistics for nitrate + nitrite nitrogen NO2+3 (mg/L-N), total Kjeldahl nitrogen
TKN (mg/L-N), total phosphorus (mg/L-P), total suspended solids TSS (mg/L), and
Chlorophyll-a Chl-a (ppb) measured over multiple flow tiers.

		Total					
Variable	Flow Tier	Count	Ν	Mean	SE Mean	Minimum	Maximum
NO ₂₊₃ mg/L	Avg-3ps S	15	15	1.3407	0.0910	0.7800	1.9600
	Avg-Base W	10	10	0.9420	0.0815	0.6400	1.2600
	Avg-Sub W	15	15	0.873	0.106	0.160	1.370
	Wet-3ps Su	10	10	0.3710	0.0232	0.2600	0.4700
	Wet-Base S	5	5	0.8700	0.0110	0.8300	0.8900
	Wet-Base W	5	5	0.9200	0.0249	0.8500	0.9900
	Wet-Sub W	10	10	0.964	0.132	0.230	1.610
TKN mg/L	Avg-3ps S	15	15	1.893	0.203	0.600	3.700
	Avg-Base W	10	10	1.0570	0.0958	0.6000	1.3400
	Avg-Sub W	15	15	1.267	0.213	0.200	2.600
	Wet-3ps Su	10	10	1.220	0.190	0.300	2.000
	Wet-Base S	5	5	0.980	0.220	0.600	1.800
	Wet-Base W	5	5	0.660	0.169	0.300	1.300
	Wet-Sub W	10	10	1.570	0.226	0.800	2.900
TP mg/L	Avg-3ps S	15	15	0.671	0.123	0.120	1.900
	Avg-Base W	10	10	0.3178	0.0361	0.1280	0.4800
	Avg-Sub W	15	15	0.2817	0.0470	0.0600	0.7800
	Wet-3ps Su	10	10	0.1340	0.0229	0.0300	0.2700
	Wet-Base S	5	5	0.22200	0.00860	0.20000	0.25000
	Wet-Base W	5	5	0.660	0.173	0.290	1.150
	Wet-Sub W	10	10	0.3660	0.0483	0.2100	0.7600
TSS mg/L	Avg-3ps S	15	15	193.4	31.7	24.5	454.0
	Avg-Base W	10	10	120.0	16.4	38.5	204.0
	Avg-Sub W	15	15	36.69	7.94	9.40	114.00
	Wet-3ps Su	10	10	33.89	6.39	11.20	66.50
	Wet-Base S	5	5	88.2	15.0	53.0	125.0
	Wet-Base W	5	5	279.9	56.6	83.5	392.0
	Wet-Sub W	10	10	54.18	6.29	18.40	85.00
Chl-a ppb	Avg-3ps S	15	0	*	*	*	*
	Avg-Base W	10	0	*	*	*	*
	Avg-Sub W	15	0	*	*	*	*
	Wet-3ps Su	10	0	*	*	*	*
	Wet-Base S	5	5	11.460	0.175	10.800	11.800
	Wet-Base W	5	5	3.0200	0.0200	3.0000	3.1000
	Wet-Sub W	10	10	13.19	2.21	5.30	24.80

Highest total phosphorus concentrations were observed during 2ps events (Appendices G-H). We did not detect any difference in total phosphorus between sites, although the lowest average reported values usually occurred during wet-3ps summer pulses (Appendix J, Model 9).

In comparison to other variables, total suspended solids (TSS) exhibited the widest variation in individual measurements (Appendix I). The lowest TSS levels generally occurred during low flow dry tiers, although low values were also reported during wet-3ps-summer tiers (Appendix J, Models 10-12). The average TSS concentrations within the upper river sites (rkm 31-42) were also higher than the lower river site (rkm 1) (Appendix J, model 12). During the study period, we found that chlorophyll-a generally declined with increasing flow (Appendix I and Appendix J, Models 13-14). However, we were not able to detect any difference in chlorophyll-a between flow tiers or river kilometer distance (Appendix I and Appendix J, Model 15).

At all sites, surface and bottom water temperatures exhibited normal seasonal fluctuations (Figure 42, Appendix I). Water temperature varied seasonally but exhibited only slight differences in levels due to depth (Figure 42 and Figure G12). Water temperature near the bottom of the river was only slightly different from the surface readings. This lack of a strong thermocline is probably due to the heavy suspended solids that shades the majority of the water column and the dynamic nature of the river that under all but low flows insures some physical mixing that leads to more thermal homogeneity (Figure G13). Slightly cooler temperatures were generally observed during higher flows, although there may be some bias associated with more high flow events usually occurring in cooler months. However, some of the lowest water temperatures reported was observed during base flow periods (Figure G14).

The surface and bottom pH readings at all sites remained relatively stable throughout the study period and are not discussed further (Appendix I). Across all flow tiers, Secchi disk transparency generally declined and turbidity (NTU) increased as flow tiers increased from subsistence to 2p-3p conditions (Appendix I). This pattern is similar to the previously reported patterns in TSS.

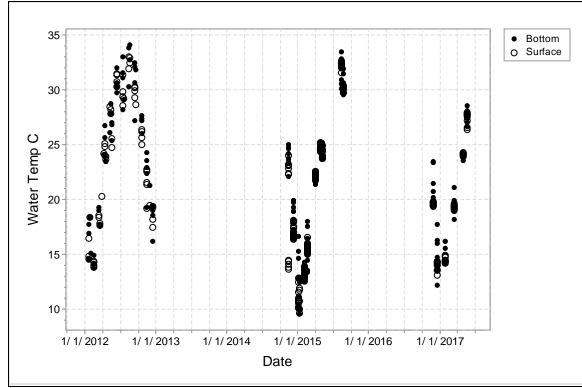


Figure 42. Water temperatures measured in surface and bottom waters at all sites during the study period.

Surface and bottom salinity exhibited significant fluctuation during the various studies including major differences between sites, flow tiers and significant interactions between sites and flow tier categories (Figures 43, and E15-E17). However, it appeared that bottom salinity was more variable and responded more rapidly to changes in river flow (Figures E15-E16). We therefore focused our modeling efforts primarily on bottom salinity only. Regression and ANOVA models and multiple comparisons of sites and flow tiers indicated that bottom salinity generally declined as discharge increased (Appendix J, models 16 and 17). In addition, the bottom salinity generally increased the closer the observation was to the river mouth. However, the regression models indicated there was significant interaction between the influence of daily average discharge and river kilometer on bottom salinity. Sites rkm 1-15 almost consistently displayed higher bottom salinities than sites rkm 31-42 (Appendix J, Model 18; Figure G15). The highest bottom salinities overall generally occurred during spring and winter dry subsistence flows (Figure G17). These patterns in bottom salinity suggest greater site heterogeneity in the salinity regime during low freshwater inflow regimes. This heterogeneity in the physicochemical environment can lead to the creation of multiple habitat niches and potentially cause organisms to migrate to more favorable salinity concentrations.

The existence of a halocline (salt wedge, salinity wedge) was clearly visible in our salinity measurements and calculated Δ (surface-bottom) salinity measurements obtained from vertical profile deployments (Figure 44, Figures E18-E19). The greatest differences between surface and bottom readings (large - Δ salinity) occurred at sites rkm 1-22 (Figure G18). The highest salinity readings were measured by Miller (2014) in 2012 when salinities approached 40 psu within the lower portion of the study area. Minimal Δ salinity was consistently observed during the Avg-

3ps S flow tier which indicates high flow pulses almost always reduced the magnitude of the salt wedge throughout the lower Brazos River (Figure G19).

Ultimately the upstream extent of the salinity wedge was influenced by the amount of freshwater inflow and the physical upstream extent of the sampling location. Areas located far upstream are less likely to experience salt intrusion except during drought periods and/or tropical storm events that can transport considerable amounts of salt water upstream during to storm surge and prevailing southerly winds. Although the salinity in the lower river was higher than the upper sites as expected, it also exhibited considerably more vertical variability, especially at sites rkm 5 and 15 (Figure 40 and E18). However, site rkm 26 also exhibited wide variation in Δ salinity, including sometimes exhibiting and inverse vertical gradient (Figure G18). These data illustrate the more dynamic environment found in the lower river that is influenced more by wind driven water movement, tides and varying freshwater inflow.

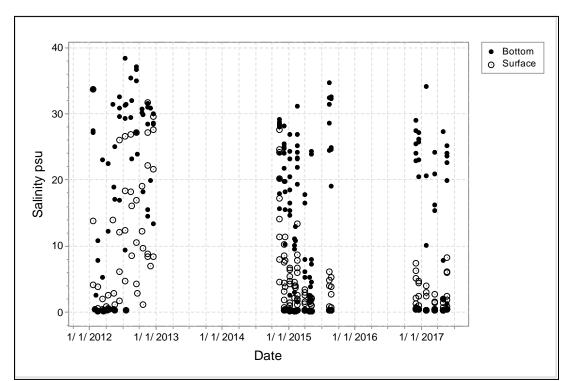


Figure 43. Salinity measured in surface and bottom waters at all sites during the study period.

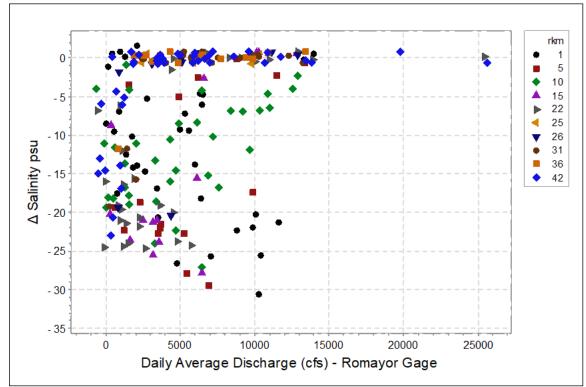


Figure 44.Delta (surface-bottom) salinity values calculated based on the difference between surface
and bottom salinity measurements made at each site and river kilometer (rkm) versus
various river discharges recorded at the Rosharon gage.

Based on examination of surface, bottom, and delta salinity values, the upstream extent of the salinity wedge was on average found approximately 25-42 rkm upstream of the Gulf during subsistence flows. The upstream extent of the salinity wedge along the sampling reach was influenced by the size of the inflow event and timing within the hydrograph (Figure 40). Bottom salinities seldom fell below 10-20 psu when average daily flows declined below 3,000 cfs (Figure G16). The broad-scale patterns in observed salinity gradients are heavily dependent on the timing of the sampling event and the magnitude and duration of the flow pulse. The highest bottom and surface salinities were observed most frequently during wet-subsistence winter and dry-subsistence winter and spring tiers (Figure G19).

Continuous and instantaneous monitoring of salinity in the upper, middle and lower reach of the Brazos River also documented salinity concentrations that were generally lowest upstream and highest downstream near the Gulf (Figure G13). However, prior to and during high flow pulse events, a decline in salinity was first observed upstream which then progressed downstream. After high flow pulse events, salinity increased first downstream which progressed upstream. Flow thresholds existed at which the salinity wedge did not return to the upper reach (2-3,000 cfs) and the middle reach (3-4,000 cfs) (Figure 26). When flows remained below these levels a very strong (Δ -25 ppt) salinity wedge could occur up to 15 rkm upstream (Figure 44). As stated previously the presence of any density layer (pycnocline) caused either salinity (halocline), temperature (thermocline) gradients or both can influence the amount of dissolved oxygen present within the parcel of water entrained within the deeper higher density water mass. In many cases there were distinct differences in surface and bottom dissolved oxygen

measurements (Figure G20). This may be due to poor mixing caused by a stable halocline. Surface dissolved oxygen measurements were in general higher than bottom readings and seldom fell below 4-6 mg/L (Figure G22). As previously noted the influence of the halocline was more evident in the lower river where the difference between surface and bottom salinities, i.e. delta (Δ) was greatest indicating there was a very stable salinity wedge (Figure G18). Lower bottom dissolved oxygen levels were observed in the lower and middle portions (rkm 5 to 22) of the river where the halocline was more stable (large - Δ salinity) (Figures E15 and E21). The variation between surface and bottom dissolved oxygen readings was dependent on the daily average discharge levels (Figure G22). At flows below 2,000 cfs the difference between surface and bottom readings falling below 4.0 and sometimes even 2.0 mg/L. In contrast hypoxia (< 2.0 mg/L) was never observed at flows above 10,000 cfs (Figure G22).

Dissolved oxygen values from all depths, differed across flow tiers and only exhibited significant differences between sites depending on the depth of measurement (Appendix J, Models 22-25 and Figure G23). As described earlier the variation in surface dissolved oxygen was generally less than bottom measurements and usually exhibited a higher concentration (Figure G21). The results of regression and ANOVA models supports this hypothesis since we failed to find any statistically significant differences in surface dissolved oxygen across measured discharges or sites (Appendix J, Model 22). We did however detect a significant difference in surface dissolved oxygen between flow tiers (Appendix J, Model 23). Tukeys multiple comparison test however failed to clearly define flow tier groups that followed any recognizable pattern in surface dissolved oxygen associated with flow intensity. Fluctuations in dissolved oxygen appeared to be more correlated with seasonality. For example, the higher average surface dissolved oxygen occurred during the winter flow tiers. This is consistent with the known solubility of dissolved oxygen which has a higher solubility in colder water. We also constructed regression and ANOVA models of bottom dissolved oxygen versus average daily discharge, site (rkm) and flow tiers (Appendix J, models 24-25). We failed to detect any significant relationship using the linear regression model between average daily discharge, river kilometer and bottom dissolved oxygen. However, we did detect significant differences in bottom dissolved oxygen and flow tier and river kilometer using the two-way crossed ANOVA model. Similar to surface dissolved oxygen readings we found that the higher dissolved oxygen readings were found to occur during winter flow tiers (Appendix J, model 25). However, the Tukeys multiple comparison tests failed to generate clearly separable groups of flow tiers based on bottom dissolved oxygen.

3.3.3 Nekton

We combined the nekton data collected from this study with historical information to increase our ability to detect and evaluate the response of these organisms to changing hydrology and water quality. A total of 8,967 individuals representing 49 different taxa were captured by beam trawl and otter trawl during 2016-17. The composition of the entire species assemblage collected during 2016-17 is provided in Appendix L. A total of 43,813 organisms with an overall diversity of 121 taxa was collected during 2012, 2014-15, and 2016-17 (Table 19). Based on the combined catch of otter trawls and beams trawls, the Atlantic croaker, *Micropogonias undulatus*, was most abundant (N = 15,677, RA = 35.7%) followed by the Gulf

Menhaden, *Brevoortia patronus* (7,339, 17.6%). Overall estuarine and marine nekton numerically represented 92.0% (otter trawl) and 97.4% (beam trawl) of the total catch. Only 13 out of the 77 taxa (17%) collected with the beam trawl were considered freshwater species. Similarly, only 13 out of the 85 taxa (18%) collected with the otter trawl were considered freshwater species. Some taxa such as Sand Seatrout, *Cynoscion arenarius* which were relatively common in the demersal river nekton were absent from shoreline habitats and beam trawl collections.

We observed a statistically significant relationship in total nekton catch in otter trawls between sites and discharge (Figure G42 and G43, Appendix J, Model 26). However, the model explained little of the variation in total catch ($r^2 = 3.47\%$). We found a statistically significant difference in total nekton abundance in bottom trawls between sites and flow tiers (Figure G44 and Appendix J, Model 27). Site rkm 1 and rkm 42 exhibited statistically different catch rates with the lowest rate occurring at the site rkm 42 and the highest at the site rkm 1. The highest total catch per bottom trawl tow generally occurred at the lower site rkm 1 when salinity exceeded 20 psu and dissolved oxygen was above 6 mg/L (Figure 45). This is supported by the regression model that indicated a significant positive effect overall between increasing salinity and dissolved oxygen and increasing total trawl catch (Appendix J, models 28 and 29). Once again however the strength of this relationship was very weak ($r^2 < 3$). In summary, these models and observed data suggest that there is a weak yet discernible trend of increasing numbers of nekton with increasing salinity and dissolved oxygen coupled with a slight increase in numbers downstream during lower flows.

We did not generate detailed graphics to evaluate the number of estuarine and marine nekton collected, since this group composed over 92% of the total catch. However, careful examination of statistical models showed that in fact they did behave similarly to total catch in respect to their response to average daily discharge, river kilometer, flow tier, salinity and dissolved oxygen (Appendix J, Models 30-33).

The number of nekton taxa collected by otter trawls appeared to decline with increasing average daily discharge and distance upstream from the mouth of the river (Figure G45 and G46). This observed trend is supported by the regression model that indicated a weak inverse relationship between flow and river kilometer versus number of taxa (Appendix J, Model 34, $r^2 = 33\%$). The ANOVA model also indicated significant differences in number of nekton taxa collected by otter trawls (Appendix J, Model 35). It was difficult to detect any pattern in number of taxa related to flow tiers (Figure G47). Number of taxa was most variable during dry-subsistence flow conditions in the spring season. However, the Tukeys multiple range tests indicated that sites rkm 1 and 10 exhibited a higher average number of taxa collected with otter trawls than rkm 22, 31 and 42. These patterns in number of nekton taxa between sites, discharge levels and flow tiers are also supported by the regression model that indicated a significant but weak positive relationship between salinity and total number of trawl captured nekton taxa (Figure 46 and Appendix J, Model 36, $r^2 = 20.3$). We failed to detect any relationship between bottom dissolved oxygen and number of nekton taxa captured in otter trawls (Figure 46 and Appendix J, Model 36, $r^2 = 20.3$).

The patterns observed for number of estuarine taxa collected by otter trawl were very similar to those exhibited by the number of taxa (Figures 47-49 and Figures G48; Appendix J, Models 38-41) and therefore is not discussed in great detail. Based on close examination of the regression model it appears that highest number of estuarine taxa can be expected in the lower river under lower flow rates. Evaluation of ANOVA table output and Tukey's test results suggest that the number of nekton taxa exhibits a strong gradient related to river kilometer with three groups identified. One difference that was observed was the significant relationship between number of estuarine nekton taxa and dissolved oxygen (Figure 47 and Appendix J, Model 41). This relationship was however very weak ($r^2 = 2.0\%$). Similar to trawl captured nekton we observed gradients in the number of shoreline nekton (total catch) collected with the beam trawl that mimics patterns exhibited in our trawl landings. Although detailed graphics are not provided we have provided tabular output from the statistical models that are discussed below.

Catch rates of shoreline nekton collected with the beam trawl increased during periods of lower flow and were generally highest at the downstream sites, although the model failed to explain much of the variation in total shoreline nekton abundance ($r^2 = 2.5\%$) (Appendix J, Model 42). Based on the two-way ANOVA model we observed significant differences in the abundance of shoreline nekton with the highest catches occurring at the downstream site (rkm 1) (Appendix J, Model 43). Although significant, salinity and dissolved oxygen exhibited only a very week ($r^2 < 6.7$) relationship with the number of shoreline nekton taxa (Appendix J, Models 44-45).

As expected the number of estuarine shoreline nekton exhibited very similar spatial and temporal patterns in abundance and relationships with discharge, river kilometer, flow tier, salinity, and dissolved oxygen when compared to overall shoreline nekton catches (Appendix J, Models 46-49). This is not too surprising since the vast majority (97.4%) of specimens collected from the shoreline nekton community in the lower Brazos were classified as estuarine or marine taxa.

The number of shoreline nekton taxa exhibited trends similar to nekton catch rates, with the highest number of taxa occurring lower in the river (Appendix J, Models 50-53). This spatial trend was however weak ($r^2 = 5.9\%$). However, we failed to detect any significant difference in number of shoreline taxa versus average daily flow (Model 50). We did observe significant differences in the number of shoreline taxa between flow tiers and river kilometer (Appendix J, Model 51). The Tukeys multiple comparison test did not provide a clear interpretable pattern of flow tiers (dry-wet). The lowest average number of shoreline taxa generally occurred at the upstream site (rkm 42). The number of shoreline estuarine nekton taxa exhibited a significant but very weak relationship ($r^2 < 1$) with salinity and dissolved oxygen levels (Models 52-53). This suggests that salinity and dissolved oxygen may not have varied sufficiently over the range of number of shoreline nekton taxa values observed. As expected, the number of shoreline estuarine nekton taxa displayed similar spatial and temporal trends exhibited by the number of shoreline nekton taxa being classified as marine or estuarine.

Gear	Таха	Number	Percent		
Otter Trawl	Atlantic Croaker	14,926	46.5		
	Micropogonias undulatus				
	Bay Anchovy	6,585	20.5		
	Anchoa mitchilli				
	Brown shrimp	2,254	7.0		
	Farfantepenaeus aztecus				
	Blue Catfish	1,457	4.5		
	Ictalurus furcatus				
	White shrimp	1,272	4.0		
	Litopeneaus setiferus				
	Star Drum	1,195	3.7		
	Stellifer lanceolatus				
	Ohio Shrimp	830	2.6		
	Macrobrachium ohione				
	Sand Seatrout	734	2.3		
	Cynoscion arenarius				
	Gulf Menhaden	407	1.3		
	Brevoortia patronus				
	Subtotal Number	32,081			
	Subtotal Number of Taxa	96			
Beam Trawl	Gulf Menhaden	6,932	59.1		
	Brevoortia patronus				
	Bay Anchovy	1,231	10.5		
	Anchoa mitchilli				
	White shrimp	780	6.5		
	Litopenaeus setiferus				
	Atlantic croaker	751	6.4		
	Micropogonias undulatus				
	Clown Goby	183	1.6		
	Microgobius gulosus				
	Daggerblade grass shrimp	181	1.5		
	Palaemonetes pugio				
	Ohio shrimp	174	1.5		
	Macrobrachium ohione				
	Subtotal Number	11,732			
	Subtotal Number of Taxa	76			
Grand To	otal (both gears)	43,813			
	f Taxa (both gears)	121			

Table 18.Summary statistics for nekton collected during 2012 (Miller 2014), 2014-15 and 2016-17 with
otter trawls and shoreline beam trawls.

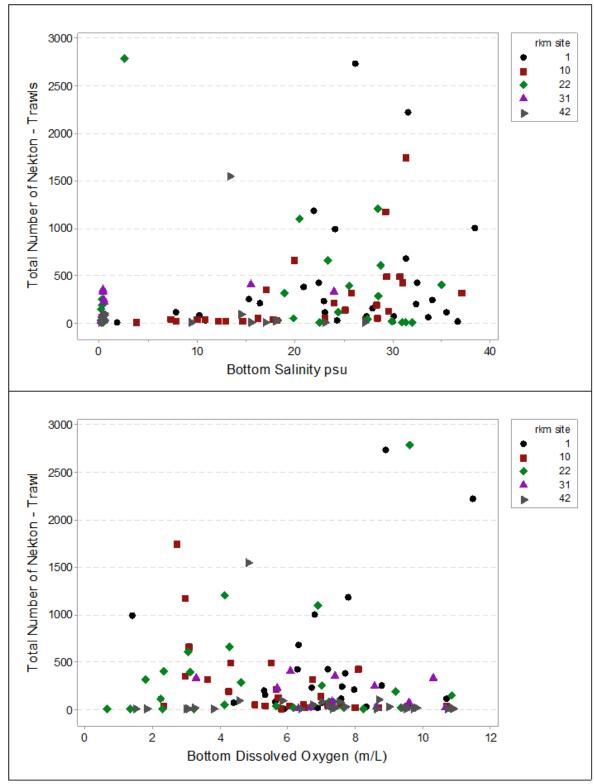


Figure 45. The total number of nekton (all replicates combined) collected with trawls at each site and date versus bottom salinity and dissolved oxygen.

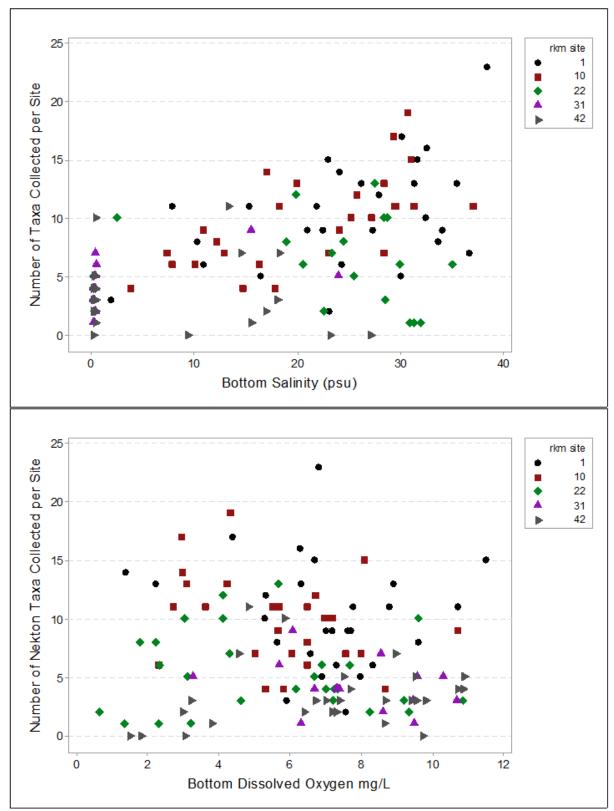


Figure 46. The total number of nekton taxa (all replicates combined) collected with trawls at each site and date versus bottom salinity and dissolved oxygen.

94

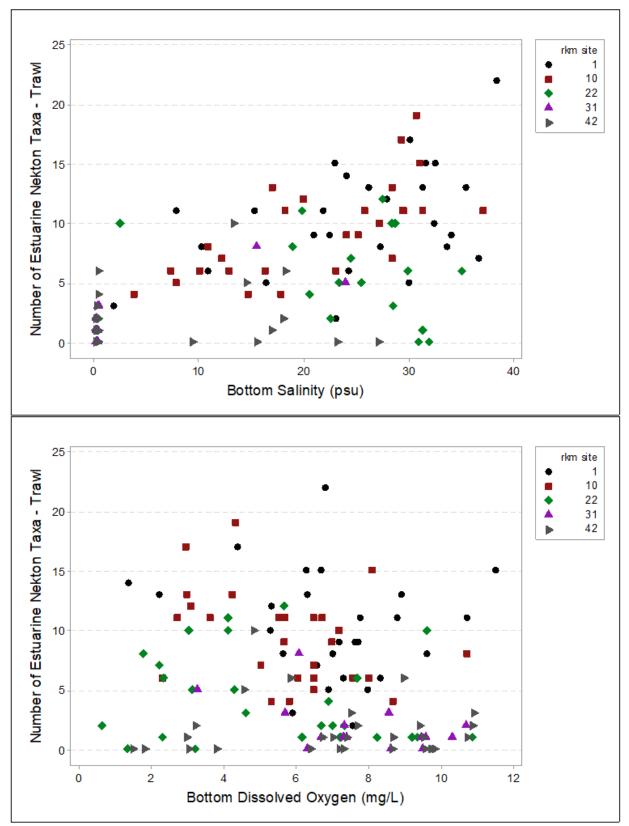


Figure 47. The total number of estuarine nekton taxa (all replicates combined) collected with trawls at each site and date versus bottom salinity and dissolved oxygen.

95

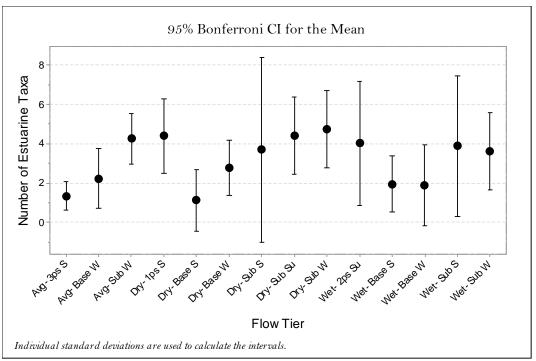


Figure 48. Confidence interval plot for the number of estuarine and marine nekton taxa collected with otter trawls during 2012 through 2017 per flow tier.

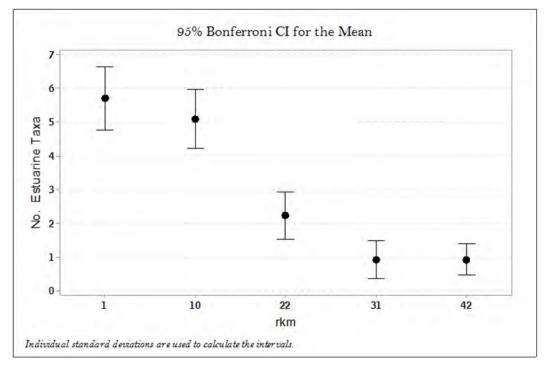


Figure 49. Confidence interval plot for number of estuarine and marine nekton taxa collected with otter trawls at each site during 2012 through 2017.

We combined data from 2012, 2014-2015 and this current phase (2016-17) in order to increase our ability to discern patterns in community composition that may be due to the varying hydrology including relatively dry conditions (2012) and wetter conditions (2014-2015). We analyzed our shoreline beam trawl nekton data separately from our demersal otter trawl nekton data due to the dissimilar habit and sampling bias associated with each gear type. Prior to classification and ordination, we generated total catch per species estimates based on the sum of catches from each replicate tow. This was done to reduce the number of sampling units and ease the interpretation of sample analysis output.

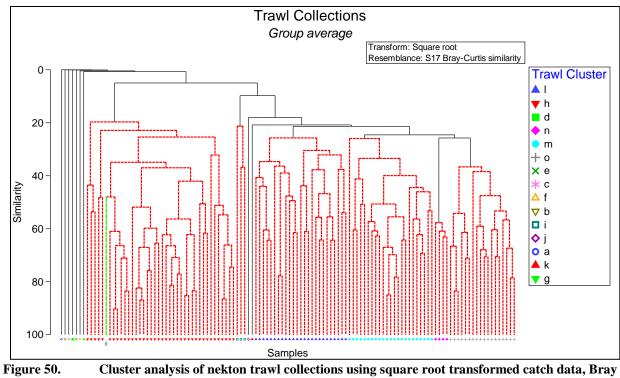
The cluster dendrogram generated from the cluster analysis of otter trawl collections generated 15 groups based on the similarity of species composition (Figure 50). When we examined the classification produced based on the nekton community similarity of trawl collections we found that there were some groups that consisted of only one collection (singletons). We also conducted an ordination of the community data using nMDS in order to better describe the describe patterns in the nekton community structure collected by otter trawl. The dendrogram also showed that the 15 smaller groups appeared to segregate into two large group clusters. This was also exhibited by the nMDS plot that showed a high number of sites exhibiting very short multidimensional distance in terms of similarity and therefore appearing to be grouped close together (Figure 51). This could be an artifact of the excessive number of zeros in the data matrix which sometimes generates groups of collections containing few individuals from a few taxa and many zero counts. However, the collections made in 2012 at site rkm 42 appeared to be very different from the majority of other collections. These collections were mainly from summer months during a relatively dry period compared to later years. These "outlier" collections were mainly obtained during March, July, August and September 2012. The common trait they possessed was the fact they were almost all composed of zero catches. We are still examining the patterns of classification to determine if there is some other common trait that might assist us in identifying potential key habitat needs for each species. In the future, we may reduce the number of traits (species) to only include numerically dominant or common taxa that could be used in subsequent classifications and ordinations. This would reduce the influence of many zero counts.

The cluster analysis and nMDS conducted on the beam trawl data generated even more (19) groups based on the species similarity of the different collections (Figures G49 and G50). Once again, the "outlier" collections were obtained in 2012. However, this time the majority of the 2012 sites were collected from spring months. They also all had very low or non-existent (zero) catches. Even though the patterns generated from the community composition data were difficult to interpret, it is clear that varying salinity and the upstream extent of the salt wedge has a profound influence on the species composition of the lower river.

We conducted an analysis of similarity test (ANOSIM) to determine whether sites that belong to different flow tiers or physical location (e.g. rkm) would exhibit significant differences in community similarity based on the Bray-Curtis similarity matrix. The test was run on the previously generated resemblance matrix created during cluster analysis. The results of these tests are listed in Tables 20-23. The way the table is interpreted is that low sig. % levels (\leq 5%) are similar to low alpha values (e.g. < 0.05) indicating there is a low probability of the observed differences in patterns in species composition within each paired collection occurring by chance.

The ANOSIM results did provide evidence to suggest that the physical location differences between site river kilometer 1 and 42 as well as the hydrological differences associated with wet and dry tiers were associated with observed differences in the associated nekton communities. For example, the most significant difference between the community compositions of beam trawl collections was generally observed between peaks and other categories or subsistence and other categories (Table 20). This suggests that hydrology is a major driver in predicting community composition in beam trawl collections. This is partially supported by observed significant differences in community composition occurred between the lower (rkm 1-10) and upper (rkm 31 and 42) sites. These sites also experienced distinct differences in salinity regime that were primarily caused by changing hydrology.

The ANOSIM results generated for otter trawl collections also provided evidence of differences in community composition associated with different flow regimes (Table 22). The most significant differences in community composition between tiers were observed between collections obtained during subsistence flows versus other tiers, and/or between collections obtained during peak flows and other tiers. The ANOSIM results for comparisons of collections from different sites suggest that the greatest difference in community composition occurred between the lower river (rkm 1-10), and upper river (22-42) sites (Table 23). The differences observed between the beam trawl and otter trawl comparisons most likely reflect the greater variation in physical attributes (e.g. bottom salinity) in the bottom of the river which influenced the demersal nekton more so than the less variable shallow salinity which shoreline nekton were exposed to.



Cluster analysis of nekton trawl collections using square root transformed catch data, Bray Curtis similarity and group averaging. A total of 15 groups were defined by the SIMPROF algorithm in PRIMER software.

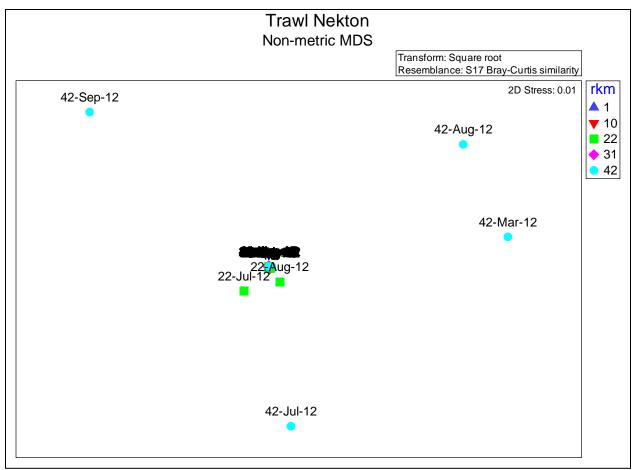


Figure 51. Non-metric dimensional scaling (nMDS) plot of trawl catch. The majority of sites are located in the dark centroid.

Table 19.Results of ANOSIM analysis used to determine statistical significance of differences in
community composition of nekton collected with the beam trawl within each flow tier. Low
significant values indicate the two sites exhibit different species compositions. Shaded
comparisons are considered significant.

Beam Trawl - Flow Tier Pair Wise Comparisons - ANOSIM										
Flow Tier	Flow Tier	Sig %	Flow Tier	Flow Tier	Sig %					
Dry-Base W	Avg-3ps S	0.1	Dry-Sub Su	Avg-3ps S	0.1					
Dry-1ps S	Avg-3ps S	0.1	Dry-Sub Su	Wet-Base S	0.1					
Dry-1ps S	Avg-Sub W	0.3	Dry-Sub W	Avg-3ps S	0.1					
Dry-Base S	Avg-3ps S	0.4	Avg-Sub W	Avg-3ps S	0.1					
Dry-Base S	Dry-Sub W	1.2	Avg-3ps S	Wet-Sub W	0.1					
Dry-Base S	Avg-Base W	1.2	Avg-3ps S	Wet-Base W	0.1					
Dry-Base S	Avg-Sub W	2.1	Avg-3ps S	Wet-Base S	0.1					
Dry-Base W	Avg-Sub W	3.0	Avg-3ps S	Wet-Sub S	0.2					
Dry-Base S	Wet-Sub W	3.7	Dry-Sub S	Avg-3ps S	0.4					
Dry-1ps S	Dry-Sub W	5.9	Avg-Base W	Wet-Sub W	0.4					
Dry-Base S	Dry-Sub Su	6.1	Avg-Base W	Wet-Base W	0.4					
Dry-Base W	Dry-Sub W	6.2	Avg-Base W	Wet-Base S	0.8					
Dry-Base W	Avg-Base W	7.4	Dry-Sub S	Avg-Sub W	1.0					
Dry-Sub S	Dry-Sub Su	8.0	Dry-Sub S	Dry-Sub W	1.2					
Dry-1ps S	Avg-Base W	10.4	Dry-Sub S	Avg-Base W	1.2					
Dry-Base W	Wet-Sub W	12.3	Dry-Sub W	Avg-Base W	1.2					
Dry-1ps S	Wet-Sub W	14.8	Dry-Sub W	Wet-Base W	1.2					
Dry-Base W	Dry-Sub Su	16.0	Dry-Sub W	Wet-Sub S	1.2					
Dry-1ps S	Wet-Base S	20.0	Avg-Base W	Avg-3ps S	1.3					
Dry-1ps S	Wet-Base W	25.0	Avg-Sub W	Wet-Sub W	1.8					
Dry-1ps S	Dry-Sub Su	29.8	Dry-Sub Su	Wet-Base W	1.9					
Dry-1ps S	Wet-Sub S	30.6	Avg-Base W	Wet-Sub S	2.1					
Dry-Base W	Wet-Base S	38.3	Dry-Sub W	Wet-Sub W	2.5					
Dry-Base S	Wet-Base S	51.9	Dry-Sub S	Wet-Sub W	3.7					
Dry-Base W	Dry-1ps S	54.4	Wet-Sub W	Wet-Base S	4.5					
Dry-Base W	Dry-Sub S	55.6	Avg-Sub W	Wet-Base S	4.9					
Dry-Base W	Wet-Base W	55.6	Avg-Sub W	Wet-Sub S	5.5					
Dry-Base W	Wet-Sub S	55.6	Wet-Sub W	Wet-Sub S	7.4					
Dry-1ps S	Dry-Sub S	66.7	Wet-Sub W	Wet-Base W	8.6					
Dry-Base W	Dry-Base S	77.8	Dry-Sub W	Wet-Base S	9.9					
Dry-Base S	Dry-1ps S	88.9	Dry-Sub W	Avg-Sub W	10.7					
Dry-Sub Su	Avg-Sub W	24.5	Dry-Sub S	Wet-Base S	11.1					
Dry-Sub Su	Wet-Sub W	26.1	Dry-Sub Su	Avg-Base W	15.7					
Wet-Base S	Wet-Sub S	45.7	Avg-Sub W	Wet-Base W	18.0					
Dry-Sub Su	Dry-Sub W	46.0	Dry-Sub Su	Wet-Sub S	22.2					
Wet-Base W	Wet-Base S	77.0	Avg-Sub W	Avg-Base W	90.9					

Table 20.Results of ANOSIM analysis used to determine statistical significance of differences in
community composition of nekton collected with the beam trawl within each river kilometer
site. Low significant values indicate the two sites exhibit different species compositions.
Shaded comparisons are considered significant.

rkm	rkm	Sig %
1	10	5.3
1	22	34.9
1	42	2.3
1	31	3.7
10	22	17.9
10	42	5.2
10	31	1.3
22	42	66.2
22	31	17.4
42	31	49.8

	comparisons are				
			w Tier Pair wise tests - A		C'= 0/
Flow Tier Dry-1ps S	Flow Tier Avg-3ps S	Sig %	Flow Tier Dry-Sub Su	Flow Tier	Sig %
Dry-Base W	Avg-3ps S	0.30	Avg-Sub W	Avg-3ps S Avg-3ps S	0.10
Dry-Base W	Dry-Sub W			Avg-3ps S	0.20
Dry-Sub S	Avg-3ps S	1.17	Avg-3ps S	Wet-2ps Su	0.40
Dry-Base S	Wet-Sub W	1.23	Avg-3ps S	Wet-Sub W	0.40
Dry-Sub S	Wet-Sub W	1.23	Avg-Sub W	Wet-Base S	1.10
Dry-Sub S	Avg-Sub W	2.34	Wet-Sub W	Wet-Base S	1.23
Dry-Base W	Wet-Sub W	2.47	Dry-Sub Su	Wet-Base S	1.30
Dry-Base S	Avg-Sub W	3.13	Dry-Sub W	Wet-Sub W	1.48
Dry-Base W	Dry-Sub Su	6.00	Avg-3ps S	Wet-Sub S	1.50
Dry-1ps S	Wet-Sub W	7.41	Dry-Sub Su	Avg-Base W	1.60
Dry-Sub S	Avg-Base W	7.41	Dry-Sub W	Wet-Base S	2.22
Dry-Base S	Dry-Sub W	8.33	Avg-Base W	Wet-Sub W	2.47
Dry-Base S	Dry-Sub Su	8.80	Avg-Base W	Avg-3ps S	2.60
Dry-1ps S	Dry-Sub W	13.70	Dry-Sub W	Avg-Base W	2.96
Dry-Base W	Avg-Sub W	14.70	Dry-Sub Su	Avg-Sub W	3.50
Dry-Base S	Avg-3ps S	15.63	Avg-3ps S	Wet-Base W	4.40
Dry-Base W	Wet-Sub S	18.52	Dry-Sub W	Avg-Sub W	5.70
Dry-1ps S	Avg-Sub W	21.30	Avg-Base W	Wet-Sub S	6.17
Dry-Base W	Wet-Base S	22.22	Avg-Sub W	Wet-Base W	6.90
Dry-1ps S	Wet-Base S	27.16	Avg-3ps S	Wet-Base S	7.00
Dry-1ps S	Dry-Sub Su	27.80	Avg-Sub W	Wet-Sub S	7.40
Dry-1ps S	Avg-Base W	28.40	Wet-Sub W	Wet-Base W	8.64
Dry-Sub S	Dry-Sub W	31.48	Avg-Sub W	Avg-Base W	9.50
Dry-Base W	Dry-1ps S	39.51	Dry-Sub W	Wet-Sub S	10.19
Dry-Sub S	Dry-Sub Su	43.20	Wet-Base S	Wet-Sub S	16.87
Dry-Base S	Avg-Base W	44.44	Wet-2ps Su	Wet-Base S	20.99
Dry-Base W	Wet-2ps Su	45.68	Dry-Sub Su	Wet-Sub W	21.20
Dry-Base S	Dry-1ps S	53.09	Dry-Sub W	Wet-Base W	22.22
Dry-Base W	Dry-Sub S	55.56	Avg-Base W	Wet-Base S	25.93
Dry-1ps S	Dry-Sub S	61.73	Dry-Sub W	Wet-2ps Su	34.26
Dry-1ps S	Wet-2ps Su	61.73	Avg-Base W	Wet-2ps Su	39.51
Dry-1ps S	Wet-Sub S	61.73	Wet-Sub W	Wet-Sub S	39.51
Dry-Sub Su	Dry-Sub W	65.80	Dry-Sub Su	Wet-Base W	45.76
Dry-Base S	Wet-Base S	74.07	Dry-Sub Su	Wet-Sub S	47.20
Dry-Base W	Avg-Base W	77.78	Avg-Sub W	Wet-Sub W	51.10
Dry-Sub S	Wet-Base S	81.48	Avg-Base W	Wet-Base W	52.67
Dry-Base W	Dry-Base S	85.19	Wet-2ps Su	Wet-Sub W	60.49
Dry-Base W	Wet-Base W	88.89	Dry-Sub Su	Wet-2ps Su	71.20
Dry-1ps S	Wet-Base W	93.83	Wet-Base W	Wet-Base S	79.01
			Avg-Sub W	Wet-2ps Su	84.20

Table 21.Results of ANOSIM analysis used to determine statistical significance of differences in
community composition of nekton collected with the otter trawl within each flow tier. Low
significant values indicate the two sites exhibit different species compositions. Shaded
comparisons are considered significant.

Table 22.Results of ANOSIM analysis used to determine statistical significance of differences in
community composition of nekton collected with the otter trawl within each river kilometer
site. Low significant values indicate the two sites exhibit different species compositions.
Shaded comparisons are considered significant.

River Kilomete	River Kilometer Pair wise Test				
1	10	2			
1	22	0.6			
1	42	0.1			
1	31	0.1			
10	22	2.3			
10	42	0.4			
10	31	0.1			
22	42	26.5			
22	31	2			
42	31	3.8			

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 Instream Ecological Components

4.1.1 Aquatics

The flow tier analysis completed across the GSA and Brazos basins for both fishes and macroinvertebrates revealed 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- and post-flood evaluation using the historical dataset 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 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 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.

4.1.3 Floodplains

As previously discussed, there were no floodplain connectivity studies conducted during either round of sampling in the Brazos basin. 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 24).

Table 23.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	\checkmark	\checkmark								
Riparian Community			\checkmark							
Floodplain Connectivity			\checkmark							

The Round Two effort expanded our understanding of ecological responses (statistical differences in relative abundance or diversity caused by flow) of main-stem fish and macroinvertebrates 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 may be 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.

4.2 Brazos Estuary

During this study we were able to achieve the primary objectives of the Brazos estuary study including

- a. Characterization of the flow regime and tidal dynamics,
- b. Description of the response of the salinity regime to varying flow throughout the tidal portion of the river,
- c. Assessment of the response of water quality variables to varying flow,
- d. Characterization of the nekton community composition, diversity, and abundance
- e. Began development of potential models that predict the relationship between discharge, flow tiers, seasonality, salinity, nutrients and nekton composition including estuarine species within the lower tidal portion of the Brazos River.

Salinity and water levels values throughout the lower Brazos River estuary exhibited a significant inverse relationship with river discharge and associated flow tier (Appendix J). Those relationships that were tested frequently exhibited a fairly weak response ($r^2 < 0.7$). However, the general trends in discharge, river kilometer and depth versus salinity and dissolved oxygen were characterized and confirmed. They also conform to the conceptual model of an estuarine system (Alber 2002). Furthermore, we were able to describe the response of the nekton community to varying river discharge in more complete detail for the first time. The emerging pattern that was documented is, how unlike other estuaries, the Brazos estuarine zone can rapidly change due to high freshwater turnover. During the higher flow events, we found that the salinity and related physical characteristics can change rapidly, often within a day, depending on the amount and duration of freshwater. The Brazos River estuary is a dynamic ecosystem dominated by species with a wide tolerance or ability to adapt or behaviorally respond to a wide range of flow regimes and salinity. Due to the wide fluctuation in salinity possible in the lower estuary it is not unusual to find strictly marine species during one visit and later capture freshwater catfish.

Since initiation of this project and including past studies we have observed a wide range of salinity values ranging from subsistence flows to massive floods. In addition, the interaction of tides on transport of larval fish and the presence of small juvenile fish in the shoreline zone of the river is still not fully understood given the high discharge volumes that are possible. Somehow many of these juvenile species of estuarine (e.g. Brown Shrimp, Atlantic Croaker) and freshwater (*Macrobrachium ohione*) survive in the highly variable hydrology of the lower Brazos River. In addition, we did not exhaustively explore various linear (e.g. quadratic, cubic) or nonlinear models that might better describe the relationship of discharge and multiple response variables. Additional exploration of these models is needed upon collection of

sufficient data to support them. Serious consideration should be given to multimetric predictors using multiple variables and/or the use of time lagged variables that capture the influence of past events that may influence the variability in numbers and types of organisms. Additional work is needed during the summer months to better characterize the hydrology, water quality and use of the Brazos River by immature fish and shellfish. The increased frequency of hypoxia during low discharge years (e.g. 2012) can potentially have serious negative impacts on the survival of future cohorts of fish and shellfish if they are prevented from immigrating into the system or are exposed to stressful conditions. Based on our analysis of the hydrology of the lower river during low flows the halocline or salt wedge would be able to extend past the upper most site and expand the estuarine portion of the Brazos River further inland.

During this phase of the study we observed relatively weak ($r^2 < 0.7$) but significant relationships between key nutrients to flow tier and/or river discharge and river kilometer (Appendix J). Total suspended solids appeared to have the strongest positive relationship with river discharge. Nutrient concentrations exhibited weak positive relationships ($r^2 < 0.12$) between flow tier and discharge. Nutrient levels might not have fully conformed to the conceptual model predictions due to the fact that we had a low sample size (14 events during 2014-17). This can problematic in the case of sampling transient properties that are highly dependent on the timing of and shape of the hydrograph. As stated in our earlier report the timing of sampling along the hydrograph can strongly influence the levels of expected suspended and dissolved substances including whether nutrients are being measured during the ascending or receding arm of the hydrograph (Hudson 2003; Brandes et al. 2009). The timing between storm events also influences the availability of fine-grained sediment from the watershed, such that an initial runoff flow following relatively dry conditions contains greater suspended and dissolved solids than subsequent flows of similar magnitude. In addition, certain nutrients like phosphorus binds strongly to clay particles under aerobic conditions (Day et al. 2013; Anderson 2007; Bianchi 2007). However, under subsequent anaerobic conditions that exist in buried sediments in the summer, phosphorus is liberated back into soluble forms that are available to support estuarine phytoplankton populations. Nutrient pulses such as nitrogen, phosphorous and chlorophyll-a can benefit the estuary by supporting primary producers in the downstream estuary and adjacent Gulf of Mexico (Anderson 2007; Bianchi 2007; Olsen et al.2011; Gillson 2011; Livingston 1997).

The number of estuarine dependent organisms exhibited a statistically significant but moderate inverse relationship ($r^2 < 0.45$) with increasing discharge. These weak relationships were most likely due to several factors including 1) incomplete sampling of the river during the entire year, 2) lack of monitoring of adjacent waterways that may serve as refugium for nekton during high flows. For example, we currently do not have a clear understanding of the role of the intracoastal waterway (ICWW) and nearshore Gulf of Mexico for species that may not be able to tolerate prolonged high or low flow conditions and discharge. For example, several well studies species that are known to exhibit a strong response to freshwater inflow (e.g. Spotted Seatrout, Pinfish) were not encountered in high numbers during their seasonal peak periods during our study period. Additionally, each sampling event provides only a snap-shot at rather short temporal scale of the current physicochemical and biological conditions.

We feel that additional work is needed to evaluate short term variation within flow tiers caused by tidal fluctuation and diel (day vs. night) fluctuations in water quality, hydrology and

biological communities. The types of species found in the estuary during a sampling event (i.e., specified flow tier) is also probably more dependent in part on the previous long-term conditions that existed in the estuary rather than conditions that exist only during the day of collection. Past studies by Purtlebaugh and Allen (2010) on the Suwanee River, Florida using 9 years of monitoring data have reported positive relationships between river discharge and the relative abundance of age 1 Spotted Seatrout, Sand Seatrout, and Red Drum, and negative relationships between Pinfish and river discharge. The incorporation of multiple years of monitoring data reflecting various flow tiers and hydrographs is necessary to fully characterize the response of nekton to varying freshwater inflow in this highly variable and dynamic estuary. Furthermore, we feel that it is necessary to monitor the nearshore Gulf of Mexico for potential feedback mechanisms such as larval transport, temporary displacement of marine fish during floods, and transport of nutrients.

One of the primary objectives of this study was to use new and historical data collected on the tidal portion of the lower Brazos River to develop and test predicted relationships between salinity, sediments, nutrients, and proportions of estuarine species against flow tier and discharge. To accomplish this, we compared these variables using graphical methods and preliminary linear models to evaluate relationships between streamflow and flow tiers estimated from the Rosharon gage and data collected in the lower river (0-42 km). We supplemented our data with data previously collected in 2012 by Miller (2014). One of the important accomplishments of the project team during the last several years is the detailed documentation of the hydrological behavior of the lower river in relation to measured stage and discharge at the upstream Rosharon gage. This was done using several approaches including 1) installation of an expanded in-situ monitoring network consisting of water level, temperature, salinity, and dissolved oxygen meters, 2) extensive use of the ADCP meters to estimate flow in the lower river while adjusting for flood tide effects and 3) sampling of shoreline habitats to document how these areas serve as habitat during the critical early life of estuarine nekton.

The Brazos River "estuary" has not been consistently defined either by hydrological, geomorphological, or biological criteria. This is likely a result of the fact that unlike most other Texas estuaries the Brazos River estuary is more properly defined as a riverine estuary possessing both a short hydrological residency period and deltaic mouth which extends into the Gulf of Mexico and is formed by the deposition of river sediment (Orlando1993, Savenije 2005, Engle et al. 2007). The definition we continue to use as a reasonable definition of the Brazos River estuary is the tidal segment of the Brazos River (segment 1201), which is a reasonable description of the estuarine zone of the watershed.

The flows at Rosharon gage are therefore intended to serve as an "index" of the flow regime in the lower estuary as measured at the beginning of the tidal segment at river kilometer 38 (51 km downstream) or the mouth of the estuary (89 km downstream). We were able to successfully measure actual stream flow at near the upstream portion of the Brazos River tidal zone at river km 42. We were able to assess the relationship between streamflow measured at the Rosharon gage and estimated discharges at the upper end of the tidally influenced portion of the river (estuarine zone). We found that the Rosharon gage was a good conservative predictor of water delivery to the estuary.

The patterns in salinity, TSS, N-NO₂₊₃, total P, and estuarine nekton observed during this phase of the study appeared to agree with previously defined relationships between these variables and freshwater inflow. However, there was a large amount of variation in values within flow tiers. Future redefinition of flow tiers may be necessary to reflect this variability. Further research is needed to evaluate the relationship and statistical properties observed between actual flow values and flow tiers and the dependent variables. We did not exhaustively explore all possible linear or nonlinear models that might better describe the relationship of discharge and multiple response variables. Additional exploration of these models is needed upon collection of sufficient data to support them. Serious consideration of multimetric predictors using multiple variables or the use of time lag variables that captures the influence of past events that may influence the viability of organisms (e.g. inclusion of not just current flow data but past data (e.g. 30-day average flow) that reflects the full impacts of the hydrological regime on target species and community composition is needed.

Another confounding factor that limits interpretation of data collected during this study is the similar to before, a lack of an entire annual period of data. Since the study did not span the entire year we were unable to evaluate the influence of freshwater inflow during the summer (July-October) and a portion of the spring (June) season. It is important to note that given the documented seasonality of estuarine organisms, this represents a major limitation in using this data for evaluating the effect of the existing freshwater inflow standard for the estuary. Estuarine nekton exhibit significant seasonal variation in abundance and composition (Nelson 1992). This variation is driven primarily by the migration of sensitive juvenile stages (Nelson 1992). For example, data collected during this study cannot be used to evaluate potential effects on summer nekton assemblages, which is very different form winter and early spring communities. Due to the fact that the summer season was not sampled, it is critical that a future study be conducted to address this data gap. During summer months when flows are normally low and the weather is hot there is also a higher risk of hypoxia. We were fortunate to have some limited summer data from an earlier study in 2012 (Miller 2014). However, the hydrological conditions were much different during that year compared to 2014-2017.

Prior to our study we generated several hypotheses regarding the influence of high discharge rates. These are listed below along with our conclusions regarding these hypotheses.

- 1. Salinity levels in the Brazos River estuary would decline rapidly. This was observed and confirmed starting at the upstream sites and progressing downstream. Generally sustained (> 3 days) flows above 5-10,000 cfs induced the greatest decline in salinity.
- 2. The lateral extent and vertical stability of the pycnocline would decline. This was also observed concurrent with the general decline in salinity.
- 3. Nutrient and suspended solid levels would increase. This prediction was confirmed for total phosphorus and TSS.
- 4. The occurrence and density of estuarine dependent species would decline. This was confirmed for upper sites located above 30 rkm.
- 5. Under moderately high flows, vertical mixing and reaeration would increase, leading to higher abundances of nekton in trawl samples. This prediction was not totally confirmed. Although vertical mixing occurred, i.e. less salinity stratification, the number of nekton did not always increase.

5 Recommendations for Future Applied Research or Long-term Monitoring

The second phase of this study has contributed to the understanding of ecological responses to flow, 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 linkages 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.

Focused applied research

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

- *Post-flood aquatic community shift dynamics.* An evaluation of post-flood fish and macroinvertebrate shifts would focus on the sites that exhibited discernible changes during the first two rounds of study. Aquatic applied research would 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.* 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 per flow tier.
- **Brazos estuary.** Future applied research should focus on several aspects (water quality, sediment transport, and biological communities) of validating and if appropriate refining relationships between adopted flow tiers and the response of water quality and biological variables that define the estuarine ecological health. Studies should be extended to encompass the entire year including missed seasons not sampled during this study in order to more accurately assess the response of water quality, biological resources and other ecological services associated with freshwater inflow.

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

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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 elevation anticipated in the unique floodplain features.

Estuarine long-term water quality and biological monitoring similar to what was deployed during this study should be maintained on a monthly to quarterly basis for a period of 3 to 5 years at the same locations with a focus on collection additional data during summer months to capture and describe the complete annual cycle of biological communities that utilize the lower river and their respective response to varying flow regimes and the adopted flow tiers.

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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)
 - o 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

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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

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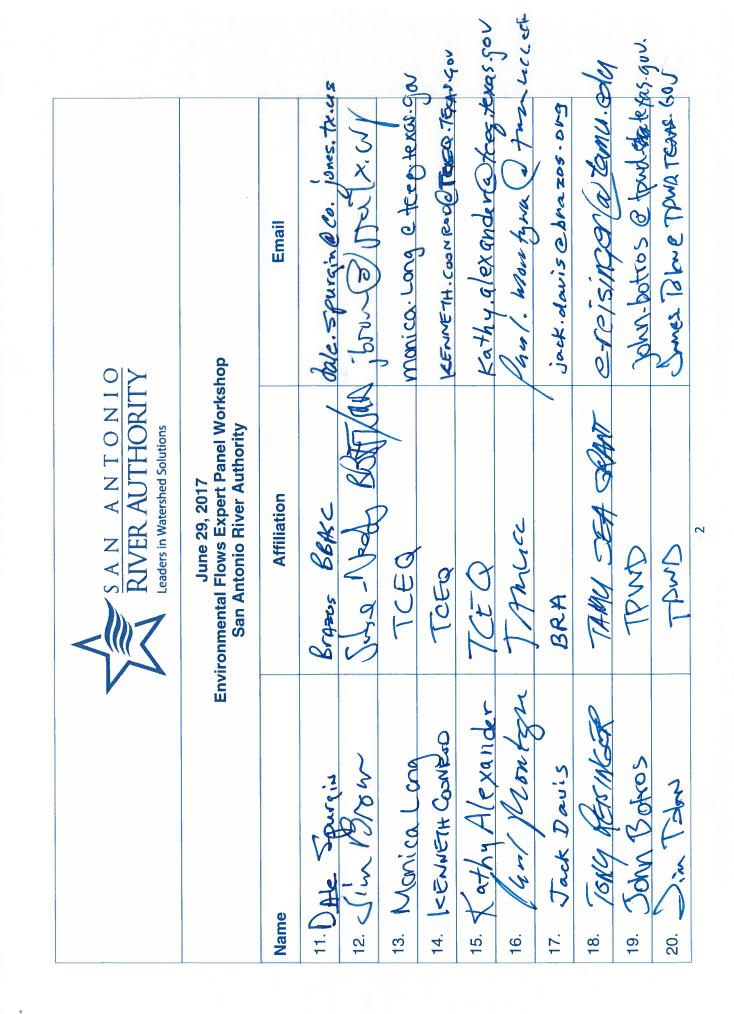
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GSA / BRAZOS / COLORADO ENVIRONMENTAL FLOWS VALIDATION PROJECT 2017 WORKSHOP AGENDA June 29, 2017

10:00 to 10:15	Welcome and Introductions – SARA
10:15 to 10:30	 Introduction - Oborny 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 Sites and Methods Results and Conclusions Paths forward
11:00 to 11:30	 FLOODPLAIN - Littrell Sites and Methods Results and Conclusions Paths forward
11:30 to 12:00	 RIPARIAN – Duke Sites and Methods Results and Conclusions Paths forward
12:00 to 1:00	Lunch – on site
1:00 to 1:30	 AQUATIC – Bonner Sites and Methods Results and Conclusions Paths forward
1:30 to 1:45	 Instream Flow Validation Tool – Oborny 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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Appendix B. Flow (CFS) on Day of **Subsample per Site**

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Appendix C. Hydrographs from Brazos Basin Study Sites

Leon River - Gatesville USGS 08100500

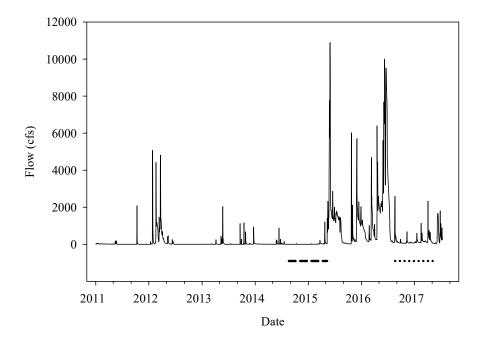


Figure C1. Hydrograph of instantaneous streamflow from the USGS gage on the Leon River near Gatesville (USGS #08100500) during January 2011 - July 2017. Lampasas River – Kempner USGS 08103800

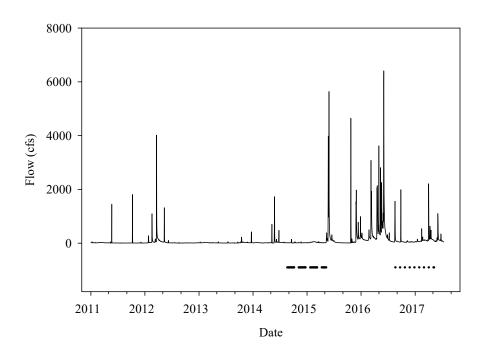


Figure C2. Hydrograph of instantaneous streamflow from the USGS gage on the Lampasas River near Kempner (USGS#08103800) during January 2011 - July 2017.

Little River – Little River USGS 08104500

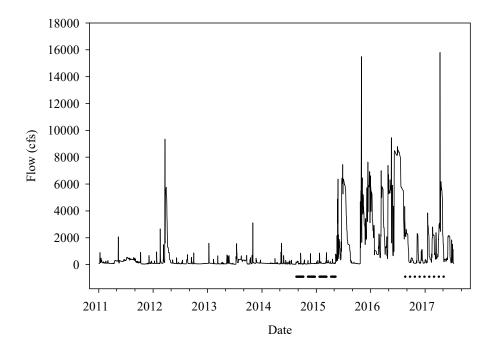


Figure C3. Hydrograph of instantaneous streamflow from the USGS gage on the Little River near Little River (USGS #08104500) during January 2011 - July 2017.

Navasota River - Easterly USGS 08110500

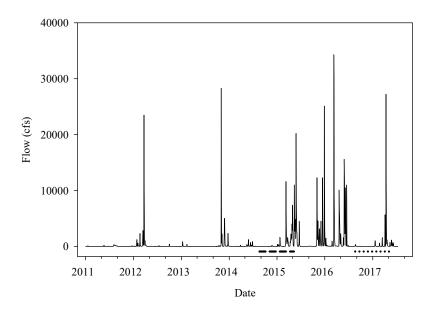


Figure C4. Hydrograph of instantaneous streamflow from the USGS gage on the Navasota River at Easterly (USGS #08110500) during January 2011 - July 2017.

Brazos River - Hempstead USGS 08111500

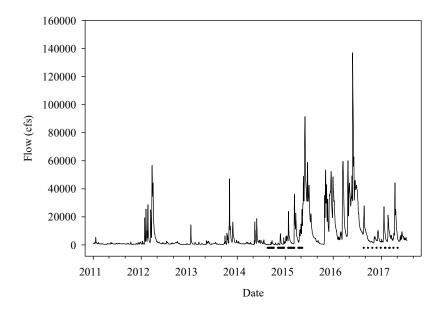


Figure C5. Hydrograph of instantaneous streamflow from the USGS gage on the Brazos River at Hempstead (USGS #08111500) during January 2011 - July 2017.

Brazos River - Rosharon USGS 08116650

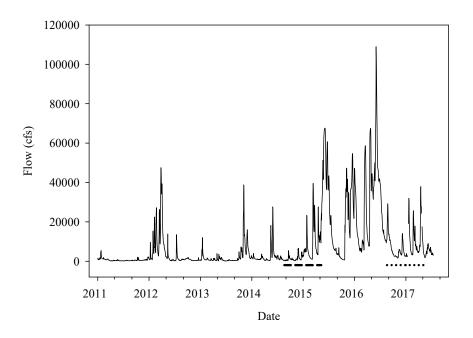


Figure C6. Hydrograph of instantaneous streamflow from the USGS gage on the Brazos River near Rosharon (USGS #08116650) during January 2011 - July 2017.

Appendix D. Aquatic Habitat Data Summarized by HMU

	Ν	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 D1. Description of riffle habitats taken from GSA, Brazos, and Colorado basins.

	Ν	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
рН				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 D2. Description of run habitats taken from GSA, Brazos, and Colorado basins.

	Ν	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 D3. Description of pool habitats taken from GSA, Brazos, and Colorado basins.

	Ν	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

Table D4. Description of backwater habitats taken from GSA, Brazos, and Colorado basins.

Appendix E. Relative Abundance of Macroinvertebrates Summarized by Basin

	Medina	Cibolo Creek	Guadalupe			San Antoni	0	San Marcos
Order	Bandera	Cestohowa	Comfort	Cuero	Gonzales	Falls City	Goliad	Luling
Ephemeroptera	37.62	40.50	27.65	35.47	41.91	25.70	39.25	55.77
Tricoptera	22.02	28.41	35.21	12.71	18.16	27.65	9.11	4.75
Diptera	10.33	13.33	9.91	20.29	24.30	28.49	19.22	8.37
Coleoptera	19.33	12.36	22.48	26.37	10.37	15.08	9.11	25.83
Odonata	8.15	3.85	2.13	0.72	0.38	1.40	1.33	1.25
Plecoptera	0.89	0.98	0.38	3.17	3.60	0.00	5.14	1.10
Hemiptera	0.37	0.00	1.63	0.08	0.38	0.00	0.13	1.16
Megaloptera	0.96	0.36	0.39	1.01	0.55	0.84	0.51	1.67
Lepidoptera	0.32	0.20	0.21	0.18	0.36	0.84	0.00	0.11
EPT	60.53	69.89	63.24	51.35	63.67	53.35	53.50	61.62
Richness	9	8	9	9	9	7	8	9
Total N	3,737	5,617	10,646	3,879	5,300	358	2,329	10,124

Table E1. Relative abundances of macroinvertebrates taken from GSA from 2014 through 2017.

	Lampasas	Leon	Little River	Navasota	Brazos	
Order	Kempner	Gatesville	LRA	Easterly	Rosharon	Hempstead
Ephemeroptera	54.88	20.95	31.50	34.82	47.21	48.29
Tricoptera	15.77	45.24	13.66	10.80	27.49	14.36
Diptera	8.37	18.21	36.85	38.72	12.65	21.45
Coleoptera	14.38	13.63	13.47	12.65	8.14	3.35
Odonata	3.46	0.89	0.87	2.09	2.02	5.74
Plecoptera	0.23	0.12	3.45	0.32	1.69	0.96
Hemiptera	2.09	0.65	0.07	0.39	0.40	0.00
Megaloptera	0.08	0.02	0.13	0.16	0.40	5.86
Lepidoptera	0.74	0.30	0.01	0.04	0.00	0.00
EPT	70.88	66.31	48.61	45.94	76.39	63.61
Richness	9	9	9	9	8	7
Total N	16,635	12,556	13,805	7,989	248	209

Table E2. Relative abundances of macroinvertebrates taken from Brazos River from 2014 through 2017.

	San Saba	Colorado	Onion Creek	Lavaca	Navidad
Order	San Saba	Bend	Driftwood	Edna	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

Table E3. Relative abundances of macroinvertebrates taken from Colorado River from 2016 through 2017.

Appendix F. Additional Riparian Data and Analyses

Table 1. SIMPER dissimilarity analysis for pairwise tests between Brazos Bend tier community assemblages.

<i>Tiers 1 & 2</i> Average dissimilarity = 93.93								
Tier 1	Tier 2							
Species Av. Abund	Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %			
Cockleburr 1.04	2.87	30.84	0.85	32.83	32.83			
Black willow 0.16	2.16	27.96	0.94	29.77	62.60			
Creepburclover 0.00	0.57	5.60	0.43	5.96	68.56			
Bermuda grass 0.18	0.58	5.38	0.58	5.73	74.29			
Tiers 1 & 3								
Average dissimilarity = 99.51	l							
Tier 1	Tier 3							
Species Av. Abund	Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %			
Box el der 0.00	2.50	27.30	1.76	27.43	27.43			
Sycamore 0.00	2.43	22.72	1.26	22.84	50.27			
Pepper vine 0.00	0.60	6.85	0.56	6.89	57.15			
Cockleburr 1.04	0.03	6.55	0.49	6.58	63.74			
Black willow 0.16	0.52	5.97	0.36	6.00	69.74			
Trumpet creeper 0.00	0.45	5.12	0.55	5.15	74.88			
Tiers 2 & 3								
Average dissimilarity = 89.56								
Tier 2	Tier 3							
Species Av. Abund	Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %			
Cockleburr 2.87	0.03	15.81	0.76	17.65	17.65			
Box el der 0.42	2.50	13.36	1.52	14.92	32.56			
Black willow 2.16	0.52	12.97	1.15	14.48	47.04			
Sycamore 0.30	2.43	12.87	1.13	14.37	61.41			
Pepper vine 0.42	0.60	4.48	0.76	5.01	66. 42			
Creepi nburcl over0. 57	0.11	3.40	0.52	3.80	70.21			

<i>Tiers 1 & 2</i> Average dissir	milarity = 91.17								
Speci es FACW FAC	Ti er 1 Av. Abund 0. 16 1. 06	Ti er 2 Av. Abund 2. 66 3. 51	Av. Diss 37. 12 36. 73	Di ss/SD 1. 19 1. 09	Contri b% 40. 71 40. 28	Cum. % 40. 71 81. 00			
<i>Tiers 1 & 3</i> Average dissir	<i>Tiers 1 & 3</i> Average dissimilarity = 88.33								
Speci es FACW FAC	Ti er 1 Av. Abund 0. 16 1. 06	Ti er 3 Av. Abund 3. 02 3. 14	Av. Diss 36. 29 34. 49	Di ss/SD 1. 99 1. 71	Contri b% 41. 08 39. 04	Cum. % 41. 08 80. 13			
<i>Tiers 2 & 3</i> Average dissir	milarity = 48.91								
Speci es FAC FACW FACU	Ti er 2 Av. Abund 3. 51 2. 66 0. 61	Ti er 3 Av. Abund 3. 14 3. 02 1. 31	Av. Di ss 20. 19 12. 56 8. 94	Di ss/SD 1. 25 1. 33 1. 05	Contri b% 41. 27 25. 68 18. 27	Cum. % 41. 27 66. 95 85. 22			

Table 2. SIMPER dissimilarity analysis for pairwise tests between Brazos Bend tiers' WI classes.

Table 3. SIMPER similarity analysis for Hearne tiers.

<i>Tier 1</i> Average similarity: 4	1.67							
Species Av. Cockleburr	Abund 0. 43	Av. Si m 3. 82	Sim/SD 0.29	Contri b% 81. 83	Cum. % 81. 83			
<i>Tier 2</i> Average similarity: 22.36								
Species Av.	Abund	Av. Sim	Si m/SD	Contri b%	Cum. %			
Trumpetcreeper	0.83	8.23	0.67	36.80	36.80			
Box elder	0.45	4.28	0.32	19.14	55.93			
Pepper vine	0.46	3.03	0.38	13.55	69.48			
Gi ant ragweed	0.50	2.20	0.31	9.82	79.30			
<i>Tier 3</i> Average similarity: 27.25								
Species Av.	Abund	Av. Si m	Si m/SD	Contri b%	Cum. %			
Roughleaf dogwo	od0. 82	12.98	0.86	47.63	47.63			
Hackberry	0.59	5.55	0.55	20.36	67.99			
Inl and seaoats	0.56	3.61	0.41	13.26	81.25			

Table 4. SIMPER dissimilarity analysis for pairwise tests between Hearne tier community assemblages

<i>Tiers 1 & 2</i> Average dissimilarity = 97.	74				
Tier 1	Tier 2				
Species Av. Abun	d Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Trumpetcreeper 0.0	0 0.83	15.42	1.04	15.78	15.78
Box elder 0.03		14.76	0.56	15.10	30.88
Pepper vine 0.1	7 0.46	9.79	0.77	10.02	40.90
Giantragweed 0.00		8.29	0.63	8.48	49.38
Inlandseaoats 0.0	0.40	7.78	0.62	7.96	57.34
cockleburr 0.43	3 0.16	7.76	0.59	7.94	65.28
Green ash 0.03	3 0.14	4.16	0.40	4.26	69.54
Johnson grass 0.0	0 0.25	3.84	0.44	3.93	73.47
<i>Tiers 1 & 3</i> Average dissimilarity = 99.	40				
Tier 1	Tier 3				
Species Av. Abun	d Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Roughl eaf dogwood0.00	0 0.82	19.34	1.02	19.45	19.45
Hackberry 0.0	0 0.59	12.10	0.72	12.18	31.63
Inlandseaoats 0.0	0 0.56	10.18	0.70	10.24	41.87
Wildrye 0.0	0 0.42	6.43	0.51	6.47	48.34
Trumpetcreeper 0.0	0 0.27	5.70	0.50	5.73	54.07
Cockleburr 0.43	3 0.00	5.45	0.50	5.48	59.55
Horse briar 0.0	7 0.24	5.26	0.52	5.30	64.85
Box elder 0.03	3 0.11	4.13	0.27	4.16	69.01
Poison ivy 0.00	0.20	3.97	0.42	3.99	73.00
<i>Tiers 2 & 3</i> Average dissimilarity = 85.	85				
Tier 2	Tier 3				
Species Av. Abune	d Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Rleaf dogwood 0.13	8 0.82	10.12	1.10	11.79	11.79
Trumpetcreeper 0.8	3 0.27	9.23	1.10	10.76	22.55
Inland seaoats 0.4	0 0.56	7.88	0.90	9.17	31.72
Hackberry 0.14	4 0.59	6.88	0.89	8.01	39.73
Box el der 0.4	5 0.11	6.83	0.68	7.95	47.69
Giantragweed 0.5	0.17	5.96	0.71	6.95	54.63
Pepper vine 0.4	6 0.10	5.66	0.76	6.60	61.23
Wildrye 0.0	0 0.42	4.21	0.53	4.90	66.13
Horse briar 0.0	6 0.24	3.34	0.52	3.90	70.03

Table 5. SIMPER dissimilarity analysis for pairwise tests between Hearne tiers' WI classes.

<i>Tiers 1 & 2</i> Average dissim	nilarity = 88.36							
Speci es FAC FACU	Tier 1 Av. Abund 1. 08 0. 25	Ti er 2 Av. Abund 2. 27 1. 49	Av. Di ss 42. 13 24. 23	Diss/SD 1.46 1.13	Contri b% 47. 68 27. 42	Cum. % 47. 68 75. 11		
<i>Tiers 1 & 3</i> Average dissimilarity = 86.65								
Speci es FAC FACU	Tier 1 Av. Abund 1. 08 0. 25	Ti er 3 Av. Abund 2. 79 1. 20	Av. Di ss 55. 39 23. 42	Di ss/SD 1. 92 1. 10	Contri b% 63. 92 27. 02	Cum. % 63. 92 90. 95		
<i>Tiers 2 & 3</i> Average dissim	<i>Tiers 2 & 3</i> Average dissimilarity = 48.91							
Speci es FAC FACU	Tier 2 Av. Abund 2. 27 1. 49	Ti er 3 Av. Abund 2. 79 1. 20	Av. Di ss 21. 65 16. 19	Di ss/SD 1. 19 1. 27	Cont ri b% 44. 27 33. 09	Cum. % 44. 27 77. 37		

Community-Wide Assessment

One of the important questions this study aimed to explore was the homogeneity of sites across 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. This section will discuss results of that focus, with the multi-basin section to follow. Figure 7 shows the 3-D ordination plot of the Brazos Basin's two sites and tiers, and indicates there were dissimilarities between the overall communities. The ANOSIM stats show those differences are moderate, and there exists commonalities between the two sites. When plotted by tier (Figure 8) those differences are lessened as each tier level across the basin has similarities with all other tiers. There does exist a progression, as would be expected, of heterogeneity between the tier levels from lowest to highest. Tiers 1 and Tiers 3 were most distinct; Tiers 2 overlapped with each other. This is verified with ANOSIM statistics in the figure. A SIMPER test (Table 6) shows that both sites' major similarities arise from the presence of box elder and black willow. Therefore, even though herbaceous plants may proliferate, these two ripariansentinel species are seen as commonalities. A SIMPER test (not shown) of the dissimilarities between these sites shows that the same general species were present in both, but with variation in their abundances.

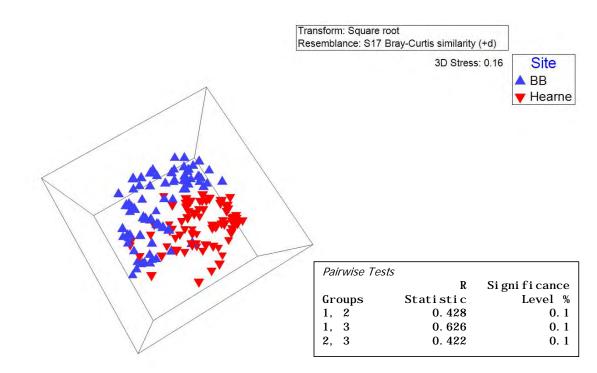


Figure 7. nMDS 3-D analysis of the BRAZOS Basin's community assemblage differences across sites and tiers. Inset box is of ANOSIM results; p=.1%.

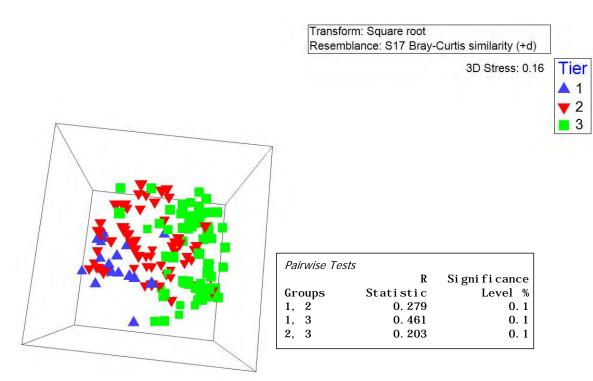


Figure 8. nMDS 3-D analysis of the BRAZOS Basin's community assemblage differences across comparable tiers. Inset box is of ANOSIM results; p=.1%.

Table 6. SIMPER similarity analysis the BRAZOS Basin's community assemblages.

<i>SIMPER Examines Site group</i> <i>(across all Tier group Group BB</i> Average similarity: 2	os)						
Species Av.	Abund	Av. Si m	Si m/SD	Contri b%	Cum. %		
Box elder	0.98	8. 81	0.68	30. 82	30.82		
Black willow	0.94	5.89	0.46	20.62	51.44		
Sycamore	0.91	5.14	0.50	17.98	69.42		
cockl eburr	1.31	4.85	0.30	16.98	86.40		
<i>Group Hearne</i> Average similarity: 1	8.73						
Speci es	A	Av. Abund	Av. Sim	Si m/SD	Contri b%	Cum. %	
Rleaf dogwood		0.41	5.07	0.45	27.06	27.06	
Trumpcreeper		0.53	3.52	0.40	18.79	45.85	
Inland seaoats		0.46	2.13	0.30	11.39	57.24	
Hackberry		0.29	1.88	0.33	10.04	67.28	
Box elder		0.25	1.69	0.20	9.01	76.29	
<i>Groups BB & Hearn</i> Average dissimilarity							
Gro	up BB	Group Hea	rne				
	Abund	Av. Ab		Av. Di ss	Di ss/SD	Contri b%	Cum. %
cockl eburr	1.31	0	. 39	19.46	0.63	20.81	20.81
Box elder	0.98	0	. 25	9.69	0.92	10.36	31.17
Black willow	0.94	0	. 03	8.97	0.63	9.59	40.76
Sycamore	0.91	0	. 00	7.07	0.63	7.56	48.33
Pepper vine	0.34	0	. 32	5.18	0.61	5.54	53.87
Trumpcreeper	0.15	0	. 53	5.17	0.71	5.53	59.40
Inland seaoats	0.05	0	. 46	3. 92	0.56	4.20	63.60
Rleaf dogwood	0.17	0	. 41	3.47	0.63	3.71	67.31
Bermudagrass	0.29	0	. 02	2.81	0.42	3.00	70.31

When grouped by WI classes and plotted by site and tier, as seen in Figure 9, the two basin sites showed low statistical dissimilarity because of variation across all tiers. The SIMPER test for similarity (Table 7) underscores the lack of riparian (and most species) from Tier 1, which again was dominated by facultative herbaceous grasses and forbs following the recent flooding disturbance. Tiers 2 and 3 both had a mixture of FAC and FACW species as their dominant contributors to similarity, and lacked FACU. This underscores why there exists so much overlap in the community compositions of these two sites.

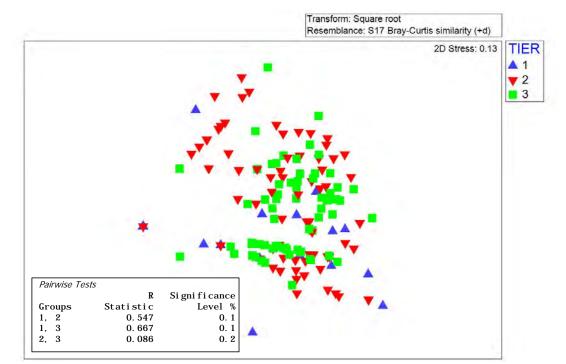


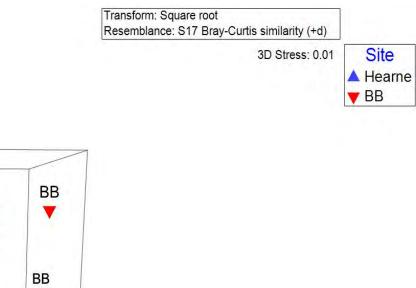
Figure 9. nMDS analysis of the BRAZOS Basin's WI community assemblage differences across comparable tiers. Inset box is of ANOSIM results; p=.1%.

Table 7. SIMPER similarity analysis the BRAZOS Basin's WI community assemblages across comparable tiers. Inset box shows ANOSIM results; p=.1%

<i>Examines TIEF (across all Site Group 1</i> Average simila	e groups)								
Speci es	Av. Abund	Av. Sim	Si m/SD	Contri b%	Cum. %				
FAC	1.07	5.53	0.34	90.42	90.42				
Group 2 Average similarity: 44.90									
Speci es	Av. Abund	Av. Sim	Si m/SD	Contri b%	Cum. %				
FAC	2.89	22.47	1.01	50.04	50.04				
FACW	1.71	14.77	0.74	32.90	82.93				
Group 3 Average similarity: 63.17									
Speci es	Av. Abund	Av. Si m	Si m/SD	Contri b%	Cum. %				
FAC	2.96	37.37	1.72	59.17	59.17				
FACW	1.58	15.56	0.91	24.64	83.81				

The canopy trees for the Brazos Basin were 3-D plotted (Figure 10) and the results reveal that in comparison to within-site plots, there exists greater dissimilarity between the two communities. When plotted by tier (Figure 11), Hearne Tiers 2 and 3 had variation that caused their plots to

overlap (Hearne had too few Tier 1 sampled mature trees to display, and the ANOSIM reflects that). Brazos Bend's Tier 2 and Tier 3 plots showed distinctive dissimilarities, while the Tier 1 plot had a direct overlap with one of the Tier 2 plots. Table 8 shows the major contributors to dissimilarity for those tiers. Three prevalent species (black willow, slippery elm, and American elm) are all present in Tier 2 but lacking from Tier 1's (very sparsely inhabited) ranks. Box elder did inhabit Tier 1 but was in lower abundance than in Tier 2 or 3. Hackberry and cottonwood, also missing in Tier 1 were found in Tiers 2 and 3; sycamore was found in Tier 3.



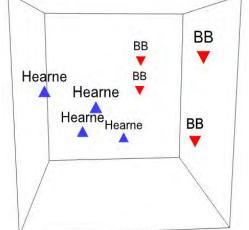


Figure 10. nMDS 3-D plot of the BRAZOS Basin's mature tree dissimilarities across sites.

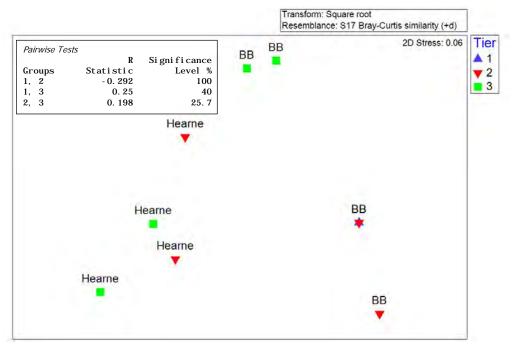


Figure 11. nMDS analysis of the BRAZOS Basin's mature tree community differences across sites and tiers. Inset box shows ANOSIM results; p=.1%

Table 8. SIMPER dissimilarity analysis for pairwise tests between the BRAZOS Basin's mature trees across comparable tiers (labeled as groups).

,	Groups 1 & 2 Average dissimilarity = 64.17									
Species blackwillow boxelder slippery el americanelm	1.00 m 0.00	Group 2 Av. Abund 0. 71 1. 29 0. 71 0. 60	Av. Di ss 18. 10 16. 79 7. 66 6. 65	Di ss/SD 0. 65 0. 82 0. 86 0. 81	Contrib% 28.20 26.16 11.93 10.36	Cum. % 28. 20 54. 36 66. 30 76. 65				
Groups 1 & 3 Average dissimilarity = 85.74										
Species boxelder hackberry cottonwood sycamore	Group 1 Av. Abund 1. 00 0. 00 0. 00 0. 00	Group 3 Av. Abund 3. 12 1. 95 1. 22 0. 99	Av. Di ss 24. 39 17. 80 10. 41 9. 25	Di ss/SD 1. 28 0. 83 1. 28 0. 86	Contrib% 28.44 20.76 12.15 10.79	Cum. % 28. 44 49. 20 61. 35 72. 14				
<i>Groups 2 & 3</i> Average dissim										
Species boxelder hackberry cottonwood sycamore blackwillow	Group 2 Av. Abund 1. 29 0. 50 0. 00 0. 00 0. 71	Group 3 Av. Abund 3. 12 1. 95 1. 22 0. 99 0. 25	Av. Di ss 19. 80 14. 10 8. 32 7. 28 5. 09	Di ss/SD 1. 22 0. 93 1. 31 0. 91 0. 98	Contrib% 26.07 18.56 10.96 9.58 6.70	Cum. % 26. 07 44. 64 55. 59 65. 18 71. 88				

Overall, the communities of the two Brazos sites showed similarities in that they both represented riparian zones located along reaches with well developed sand bars that had been

recently scoured by large flooding disturbances. However, there do exist differences between other abiotic variables than can be tested.

A PCA plot of the Brazos Basin sites (Figure 12) shows that Brazos Bend with its deeply incised channel slopes is strongly influenced by steepness/elevation differences, while Hearne is correlated strongly with channel width and dominant soil type. At the Hearne site the stream is shallow and wide-spread, having a width of over 70m (**Error! Reference source not found.**), considerably wider than the 50m width of Brazos Bend much further downstream. These features apparently are driving some of the variation seen between the two sites. Figure 13 displays the tiers within each site, and shows a very distinct progression of those effects with increasing tier number, such that Tiers 3 of both sites are considerably affected by their distance and elevation above the stream. The ANOSIM result (in the figure) displays very strong values, underscoring this strong pattern.

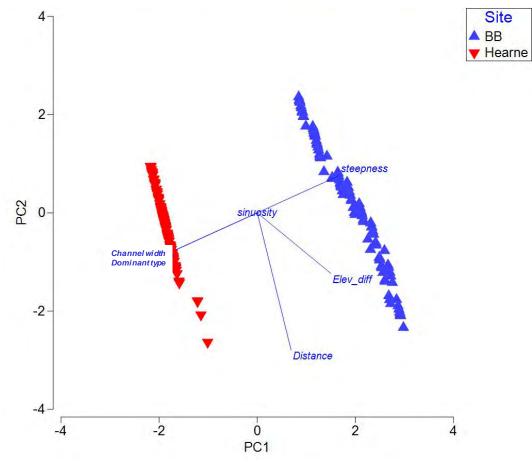


Figure 12. Principal component analysis (PCA) of the community assemblages for the Brazos Basin associated among site, tiers, and abiotic factors.

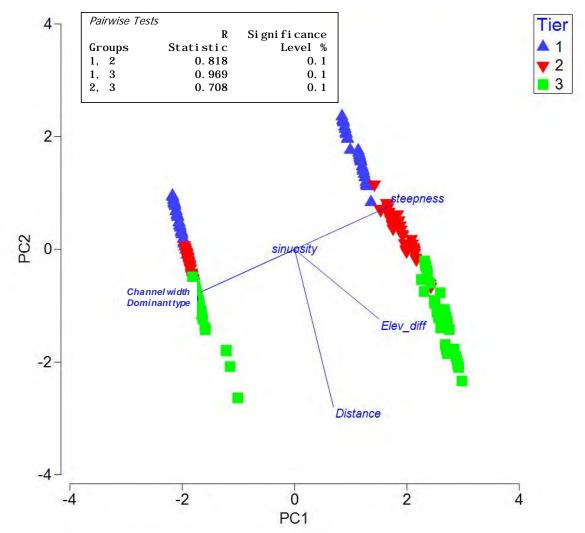


Figure 13. Principal component analysis (PCA) of the community assemblages for the Brazos Basin associated among sites, tiers, and abiotic factors. Inset box shows ANOSIM results; p=.1%.

A PCA of mature trees for the Brazos Basin (Figure 14) duplicates results seen in the general community assemblages. What is driving variation in the site's overall communities is also acting on each site's canopy layer. When overlain with site tiers (Figure 15) Brazos Bend shows that Tier 1 and Tier 3 are more closely influenced by distance than Tier 2. This could be an effect of so few trees in Tier 1; with only a single plot and few mature trees, the sampling error was very large. Hearne had too few trees to analyze, thus only two tiers are shown; but as with previous analyses in the community assemblages, Tier 3 is most strongly influenced by distance to stream.

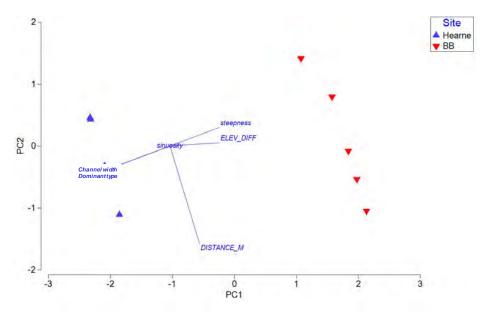


Figure 14. Principal component analysis (PCA) of mature trees for the Brazos Basin associated among site and abiotic factors.

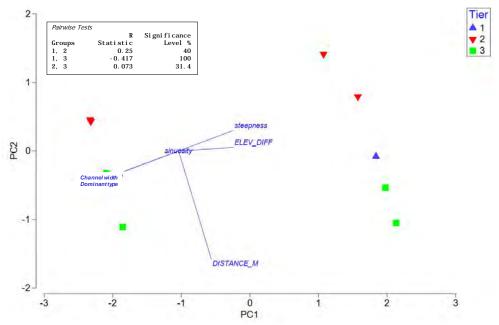


Figure 15. Principal component analysis (PCA) of the mature tree assemblages for the Brazos Basin associated among site, tiers, and abiotic factors. Inset box shows the ANOSIM statistic for differences; p=.4%.

Across Basin Assessment

One of the important 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 16 shows an nMDS 3-D ordination plot of the community assemblages for all three basins – GSA, Colorado-Lavaca, and Brazos. There are noticeable dissimilarities between them, 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 17) those dissimilarities dissolve as shown in the figure and verified by the ANOSIM results. An examination of the major contributing species to dissimilarity between basins sheds light on the overall community assemblages.

Table 9 (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. Cedar elm, which contributes 6% to the dissimilarity, is the second-ranked species and the only canopy species to be located in Colorado-Lavaca but 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 10 (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 a matter of abundance differences.

Table 11 (comparing Brazos and GSA) shows 16 species contributed 72% of the dissimilarity between the two basins. These two basins 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 variation in their abundances that created dissimilarity.

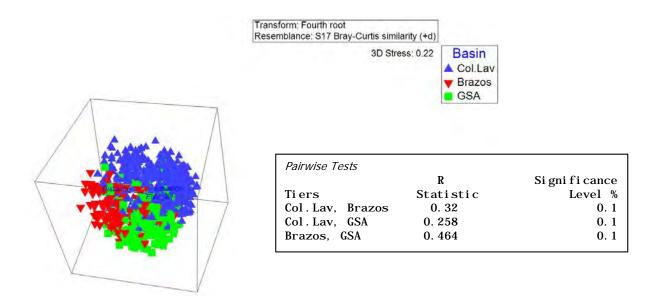


Figure 16. nMDS 3-D analysis of the community assemblage dissimilarities across the GSA, Brazos, and Colorado-Lavaca basins. The inset box shows the ANOSIM statistic for differences; p=.1%.

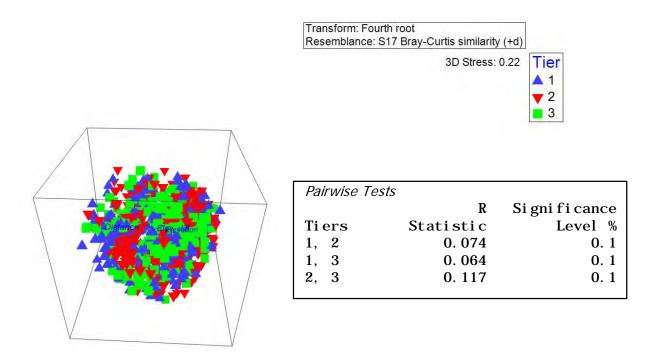


Figure 17. nMDS 3-D analysis of the community assemblage differences across tiers from all three basins. The inset box shows the ANOSIM statistic for differences; p=.1%.

Table 9. SIMPER dissimilarity analysis for pairwise tests between Colorado-Lavaca and Brazos Basins' community assemblages.

Col.Lav & GSA Average dissimilarity = 92.35

Co	ol . Lav	GSA				
Species Av.	Abund	Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Giantragweed	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
Sea0ats	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
stickywilly	0.01	0.26	2.01	0.43	2.17	59.40
purpl el eatherfl ower	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 10.SIMPER dissimilarity analysis for pairwise tests between Colorado-Lavaca and GSA basins'community assemblages.

Col.Lav & Brazos Average dissimilarity = 97.64

	Col . Lav	Brazos				
Speci es	Av. Abund	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
Cockl eburr	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 dogwoo	d 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
Sea0ats	0.28	0.00	3.15	0.40	3.22	41.44
Trumpetcreeper	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
Carol i nasedge	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
Gol deneye	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
Gi ant 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 11.
 SIMPER dissimilarity analysis for pairwise tests between Brazos and GSA basins' community assemblages.

Brazos & GSA

Average dissimilarity = 03.81

Average dissimilarity	= 93.81					
	Brazos	GSA				
Speci es	Av. Abund	Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Giantragweed	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
Inl and seaoats	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
Cockl eburr	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 creepe	r 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 dogwoo	od 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 very little dissimilarities to investigate, for either overall community assemblages (shown on the right in Figure 18) or grouped by tiers (on the left in the figure). Based on these results and low ANOSIM R statistics (not shown), no further analyses were performed on this grouping. A comparison (verified by both nMDS and ANOSIM) of the mature canopy across basins (Figure 19) 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). Grouped by tier (Figure 20), these differences diminish (as did the overall community assemblages above).

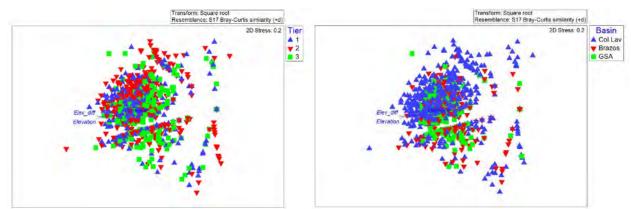


Figure 18. nMDS analysis of the community assemblage differences across all three basins' WI classes. On the left the WI classes are grouped by tier, on the right are the overall community assemblages.

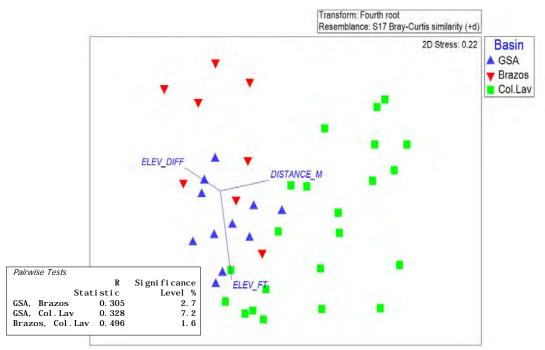


Figure 19. nMDS analysis of the GSA Basin's mature tree differences across all sites. The inset box shows the ANOSIM results; p=.1%.

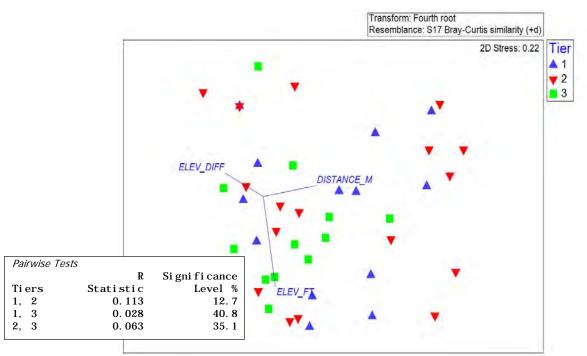


Figure 20. nMDS analysis of the GSA Basin's mature tree differences across comparable tiers. Inset box shows the ANOSIM results; p=.1%.

An examination of the dissimilarity between basins (Table 12) 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 many 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 of their ability to be community assemblage indicators, as these are species known to be present across many ecosystems in Texas. However, this may warrant further investigation to narrow how prevalently they exist in various riparian sites. Black willow and slippery elm were missing from Colorado-Lavaca but present in the Brazos Basin. However, again this does not justify the species as community assemblage indicators as these are wellknown riparian inhabitants across Texas.

Table 12. SIMPER dissimilarity analysis for pairwise tests for all basins' mature trees across sites.

<i>GSA & Brazos</i> Average dissimil							
Sugai an	GSA	Brazos	Art Dian		Control h%	C	
Speci es	Av. Abund	Av. Abund	Av. Di ss	Diss/SD	Contrib%	Cum. %	
Hackberry	1.11	0.59	11.11	1.08	15.58	15.58	
Pecan Boxel der	1.15 0.71	0.35 1.19	9.45 9.14	1.42 1.08	13. 25 12. 81	28.83 41.64	
	0. 99		9.14 8.30	0.99			
Green ash		0.38			11.65	53.29	
Sycamore	0.45	0.31	5.26	0.76	7.38	60. 67 67 89	
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 dissimil							
Average dissimil	GSA	Col . Lav					
Speci es	Av. Abund	Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %	
Hackberry	1. 11	0. 71	AV. DI SS 7. 10	1. 33	9.49	9. 49	
Green ash	0.99	0. 71	6.94	1. 33	9.49 9.28	9.49 18.78	
Pecan	1. 15	0. 58	6. 45	1. 12	9.28 8.63	27.40	
Cedar elm	0.18	0. 58	6. 38	1.42	8. 53	27.40 35.94	
Boxel der	0. 18	0.85	6. 38 5. 80	1.15	8.53 7.75	35.94 43.69	
	0. 71	0. 18	5.80 4.51	0. 92	6.03		
Americanelm	0. 31	0.47		0. 92	6. 03 5. 92	49.72	
Yaupon	0.00	0. 64	4.43 3.70	0.85	5.92 4.95	55.63	
Ashej uni per						60.58	
Sycamore	0.45	0.28	3.45	0.82	4.62	65.20	
Red mulberry		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.L.	av						
Average dissimil	arity = 90.27						
	Brazos		Col . Lav				
Speci es	Av. Abund		Av. Abund	Av. Di ss	Di ss/SD	Contri b%	Cum. %
Boxel der	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
Ashej uni per	0.00		0.44	5.09	0.62	5.64	54.57
Bl ackwill ow	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			0.00	3.49	0.73	3.87	71.16
-rr-j orn						·	

The community dissimilarities between sites and basins, although moderately low, warranted an attempt at examination of the biotic community-to-environmental variables. Table 13 shows the PCA statistics for the community assemblages in all three basins associated among basin, site, and abiotic factors. Figure 21 is a visual representation of that PCA and 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 13.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 ir	n the linear co	mbinations of var	iables making u	p PC's)	
Variable	PC1	PC2	PC3	PC4	PC 5
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_ty	/pe 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

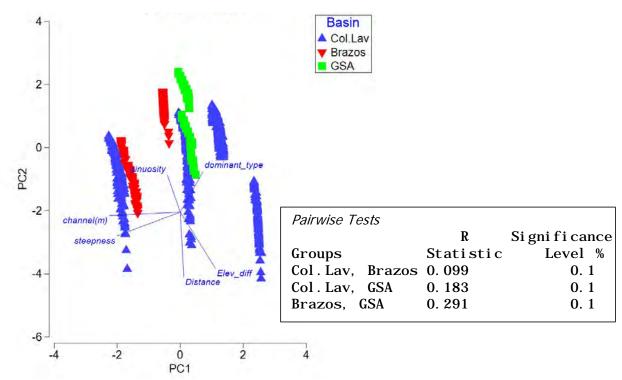


Figure 21. Principal component analysis (PCA) of the community assemblages for the GSA, Brazos, and Colorado-Lavaca Basins associated among basin and abiotic factors. Inset box shows the ANOSIM results; p=.1%.

When associated among individual site and environmental factors (Figure 22), 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.

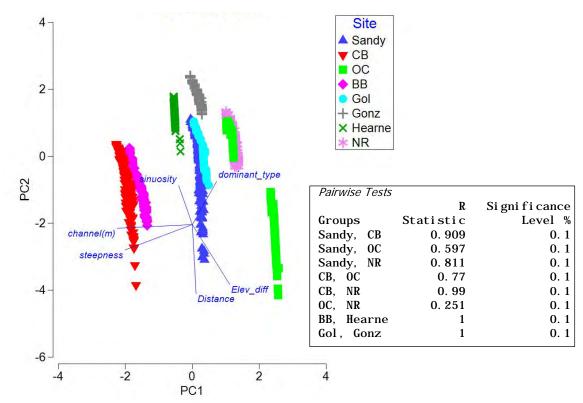


Figure 22. Principal component analysis (PCA) of the community assemblages for the GSA, Brazos, and Colorado-Lavaca Basins associated among site and abiotic factors. Inset box shows the ANOSIM results; p=.1%.

When associated by tier (Figure 23) 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.

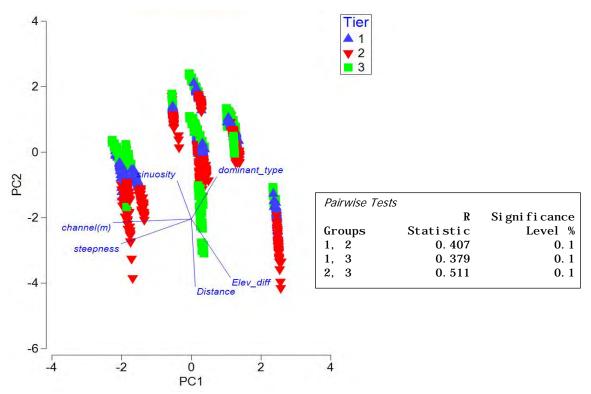


Figure 23. Principal component analysis (PCA) of the community assemblages for the GSA, Brazos, and Colorado-Lavaca Basins associated among site, tiers, and abiotic factors. Inset box shows the ANOSIM results; p=.1%.

The mature trees' correlations to abiotic variables across the three basins are shown in Table 14, Figure 24, and Figure 25. Figure 24 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 25 groups the trees by site, which adds detail to the findings. For example, the division in Brazos Basin sites' influences can now been 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 14.Principal component analysis (PCA) of the community assemblages for the GSA, Brazos,and Colorado-Lavaca Basins associated among basin, site and abiotic factors.

<i>Eigenvectors</i> (Coefficients in	the linear co	mbinations of var	iables making u	p PC's)	
Variable	PC1	PC2	PC3	PC4	PC 5
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_ty	pe 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

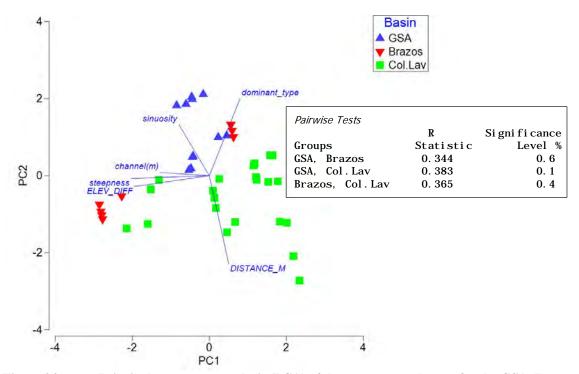


Figure 24. Principal component analysis (PCA) of the mature tree dataset for the GSA, Brazos, and Colorado-Lavaca Basins associated among basin and abiotic factors. The inset box shows the ANOSIM results; p=.1%.

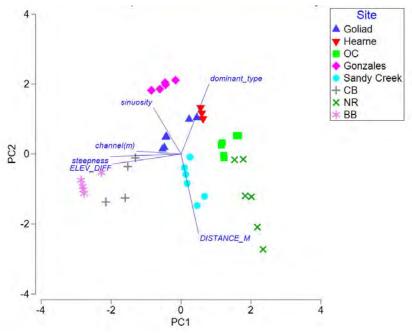


Figure 25. Principal component analysis (PCA) of the mature tree datasets for the GSA, Brazos and Colorado-Lavaca Basins associated among site and abiotic factors.

Table 15 is a summary of abiotic variables' influences on each site. The top half of the table displays within basin correlations; the bottom half displays all 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 acrossbasin 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 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 15.Summary of abiotic influences both within each basin (above) and across each basin (below).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 easilydistinguishable 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 G. Additional Brazos Estuary Data and Analyses

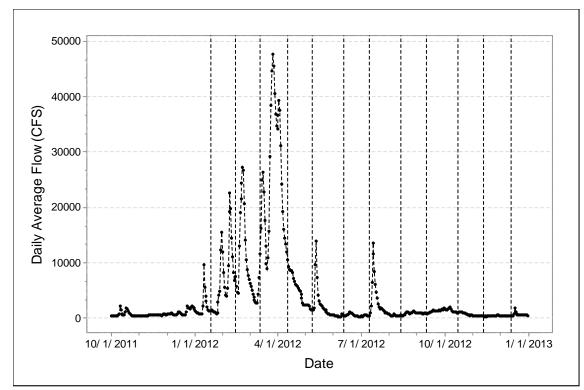


Figure G-1. Historical hydrograph of mean daily discharge (cfs) measured at the USGS gage station near Rosharon, TX (USGS 08116650) on the Brazos River from 10/01/2011 – 12/31/2012. Dashed vertical lines denote dates when nekton sampling was conducted by Miller (2014).

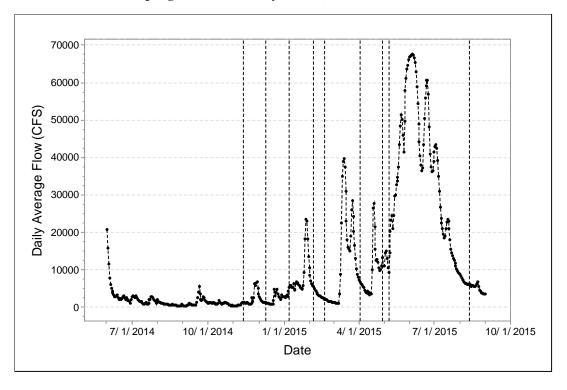


Figure G-2. Historical hydrograph of mean daily discharge (cfs) measured at the USGS gage station near Rosharon, TX (USGS 08116650) on the Brazos River from 6/1/2014 – 8/31/2015. Dashed vertical lines denote dates when nekton sampling was conducted by Bonner et al. (2015).

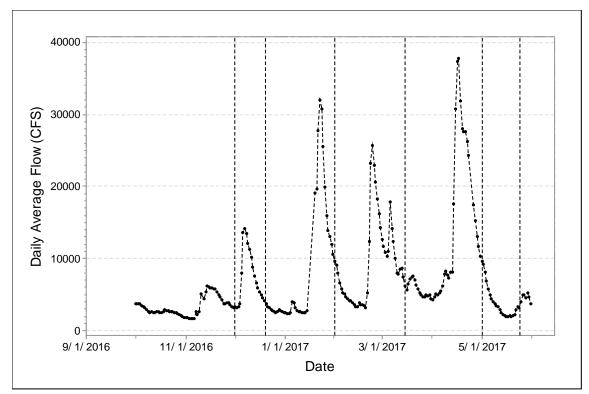


Figure G-3. Hydrograph of mean daily discharge (cfs) measured at the USGS gage station near Rosharon, TX (USGS 08116650) on the Brazos River during this study from 10/1/2016 – 5/31/2017. Dashed vertical lines denote dates when nekton sampling was conducted.

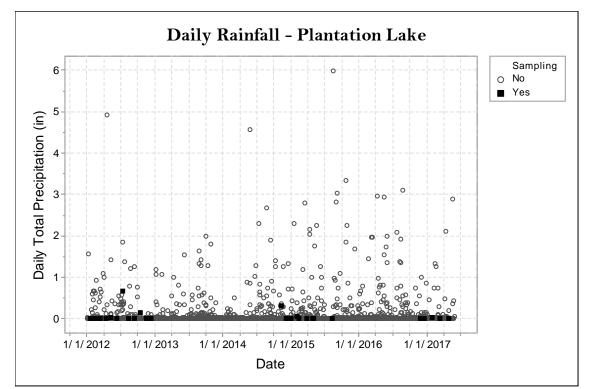


Figure G-4. Daily precipitation measured at the Plantation Lake gage site near Lake Jackson in the lower Brazos River watershed. Sampling groups identifies dates when nekton and water quality were monitored.

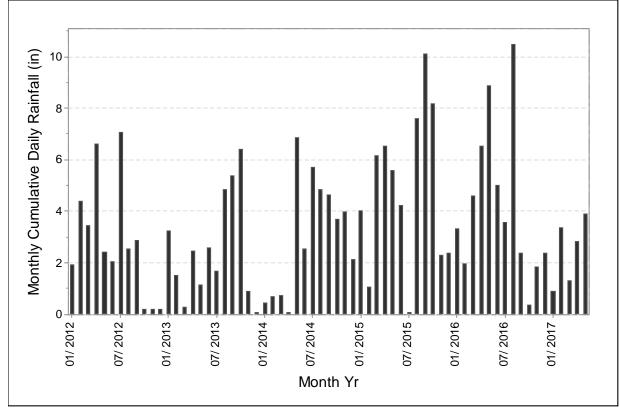


Figure G-5. Monthly cumulative daily rainfall measured at the Plantation Lake rain gage.

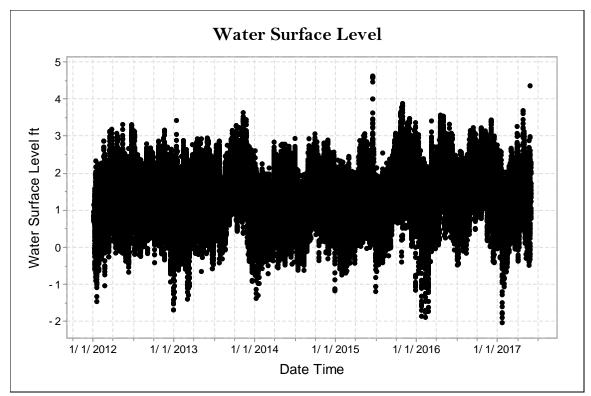


Figure G-6. Hourly water surface elevations recorded at the Freeport NOAA tide gage during January 1, 2012 to Mary 31, 2017.

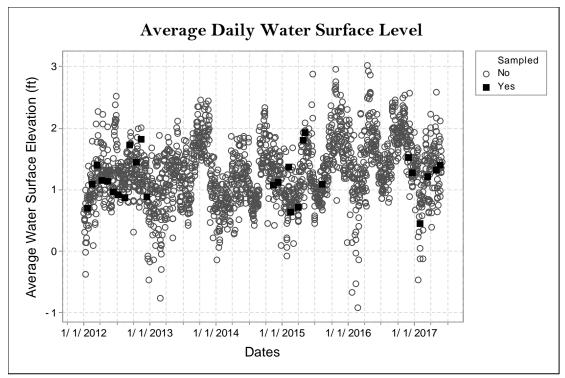


Figure G-7. Average daily water surface elevations based on hourly data recorded at the Freeport NOAA tide gage during January 1, 2012 to Mary 31, 2017. Dates when nekton and water quality were monitored are depicted by black squares.

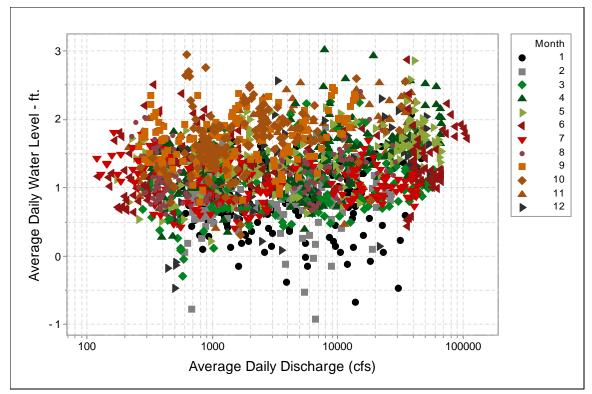


Figure G-8. Average daily water levels measured at the Freeport NOAA tide gage versus daily average discharge measured at the USGS Rosharon gage from January 2012 to May 31, 2017 by month.

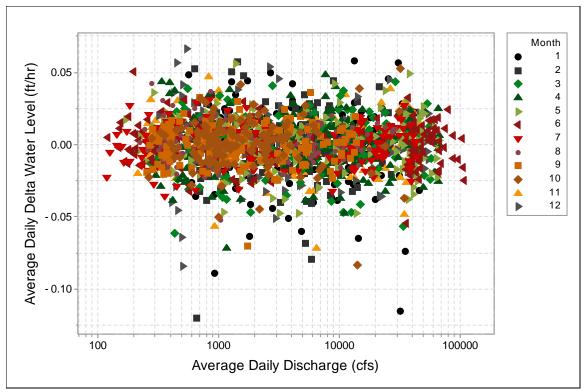


Figure G-9. Average daily water level fluctuation (Δ ft/hr) based on hourly readings obtained from the Freeport NOAA tide gage versus daily average discharge measured at the USGS Rosharon gage from January 2012 to May 31, 2017 by month.

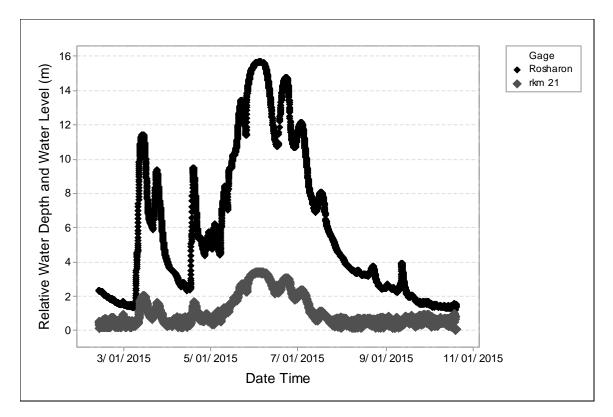


Figure G-10. Comparison of river stage measured at the Rosharon gage and relative water depth measured with the In-Situ pressure transducer deployed at river kilometer 21 during 2015.

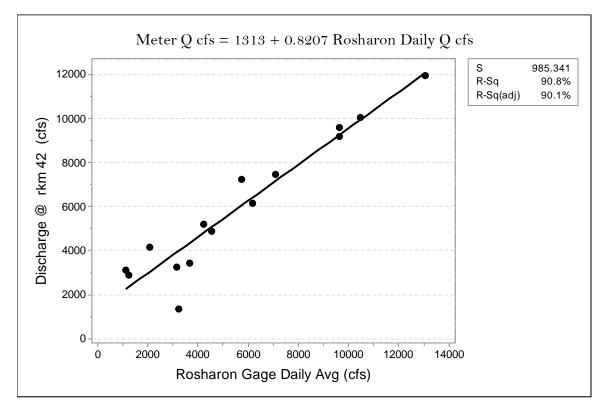


Figure G-11. Comparison of instantaneous discharge measured at river kilometer 42 and daily average flows recorded at the Rosharon Gage.

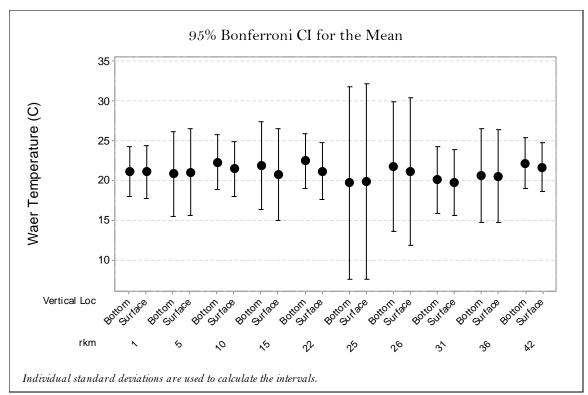


Figure G-12. Confidence intervals (95%) plot of surface (S) and bottom (B) water temperature for each site (river kilometer) monitored during the study period.

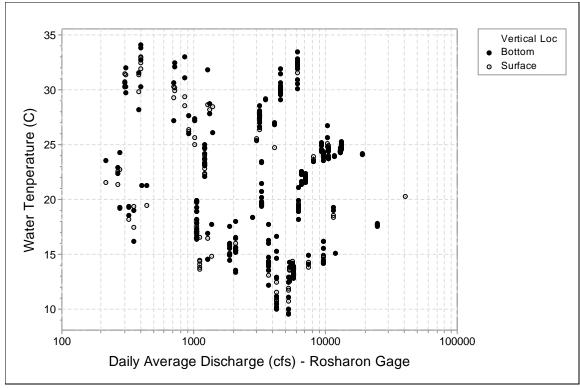


Figure G-13. Surface and bottom water temperatures at all sites versus daily average flow. X-axis is log10 scale.

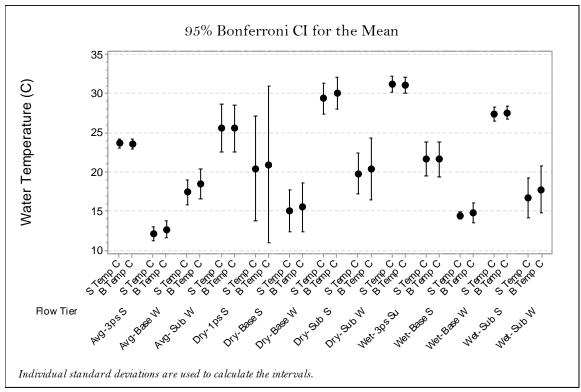


Figure G-14. Confidence interval (95%) plot of surface (S) and bottom (B) water temperature during each flow tier during the study period including past research (2012, 2014-207).

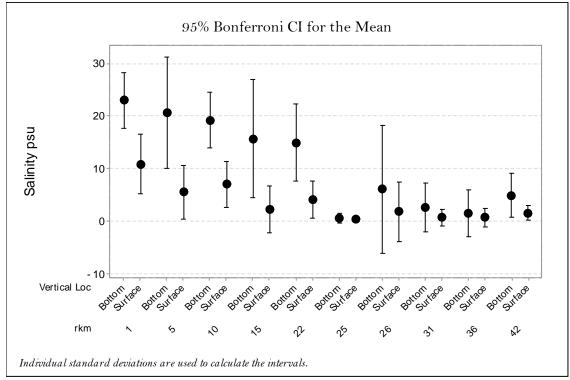


Figure G-15. Confidence interval (95%) plot of surface (S) and bottom (B) salinity for each site (river kilometer) monitored during the study period.

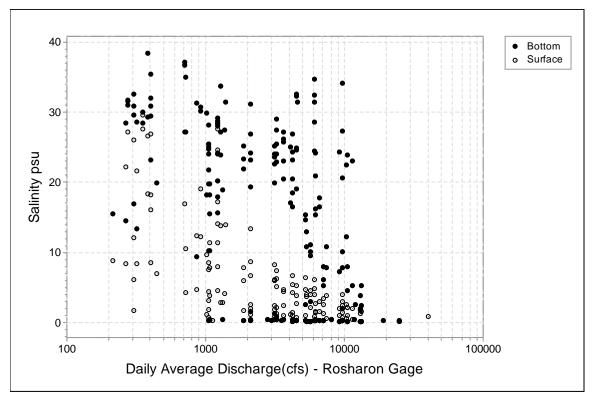


Figure G-16. Surface and bottom water salinity levels measured at all sites versus daily average flow. X-axis is depicted on a log_{10} scale.

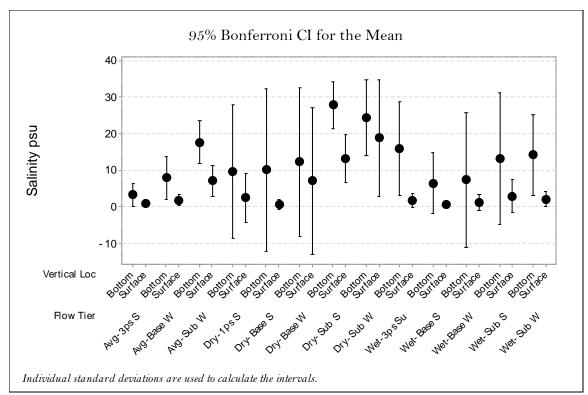


Figure G-17. Confidence interval (95%) plot of surface (S) and bottom (B) salinity for each flow tier during the study period including past research (2012, 2014-207).

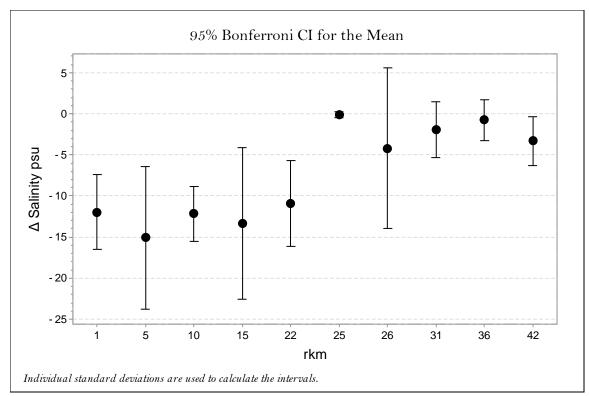


Figure G-18. Confidence interval (95%) plot of delta (surface - bottom salinity) for each site (river kilometer) monitored during the study period.

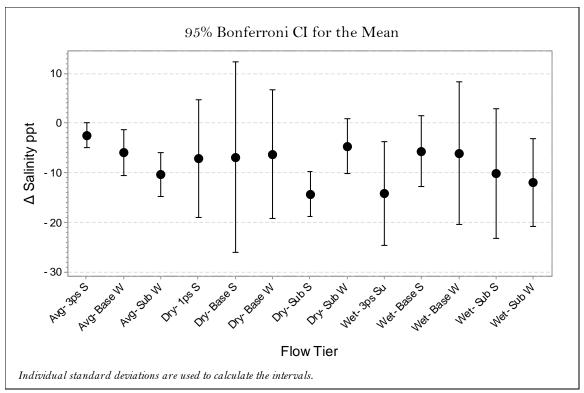


Figure G-19. Confidence interval of delta (surface-bottom) salinity values for each flow tier during the study period including past research (2012, 2014-207).

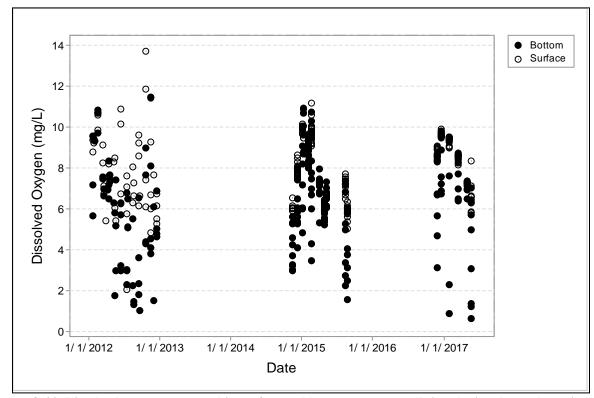


Figure G-20. Dissolved oxygen measured in surface and bottom waters at all sites during the study period.

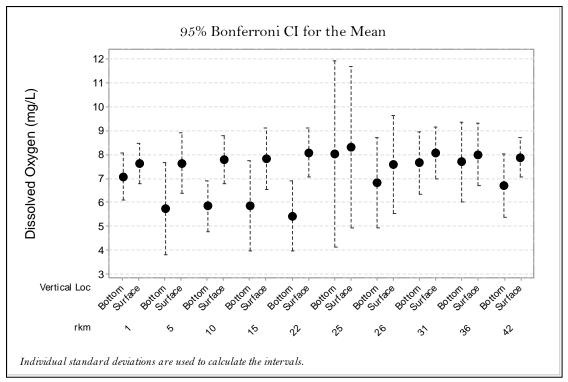


Figure G-21. Confidence interval (95%) plot of surface (S) and bottom (B) dissolved oxygen for each site (river kilometer) monitored during the study period.

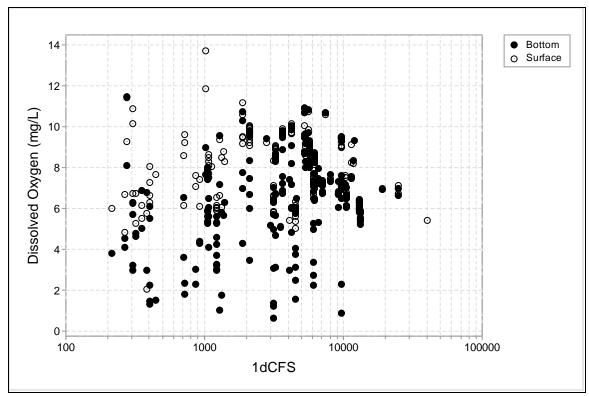


Figure G-22. Surface and bottom water dissolved oxygen levels measured at all sites versus daily average flow. X-axis is log₁₀ scale.

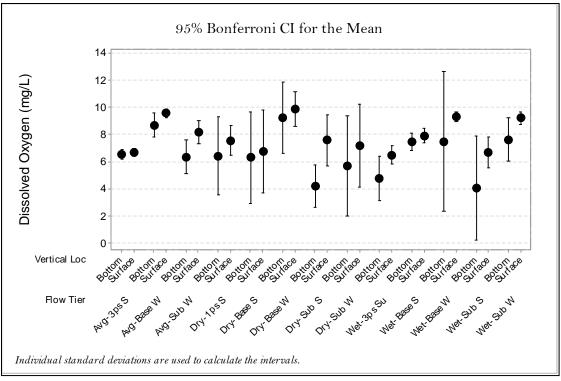


Figure G-23. Confidence interval (95%) plot of surface (S) and bottom (B) dissolved oxygen during each flow tier during the study period including past research (2012, 2014-207).

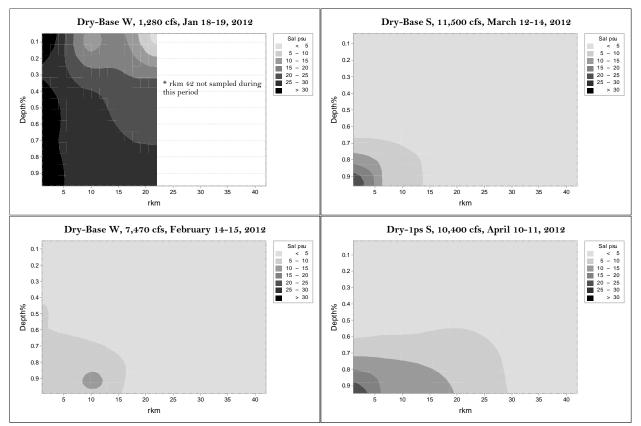


Figure G-24. Contour plot of salinity versus river kilometer during January 18-19, February 14-15, March 12-14, and April 10-11, 2012 at a daily average discharge of 1280, 7,470, 11,500 and 10,400 cfs measured respectively at the Rosharon gage under a dry base winter flows (Jan and Feb), dry base spring flows (March) and dry 1 peak per season high flow pulse (April) flow regime tiers.

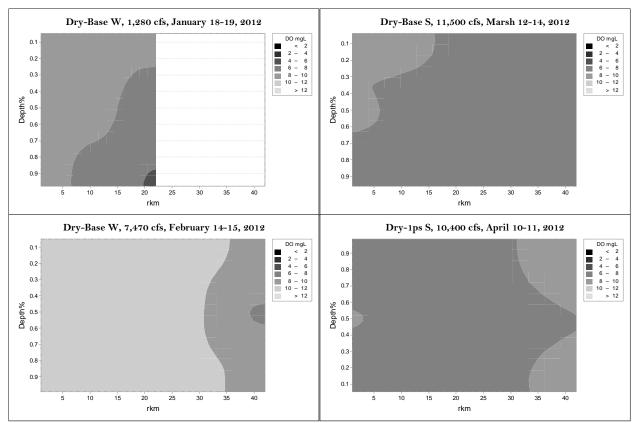


Figure G-25. Contour plot of dissolved oxygen (mg/L) versus river kilometer during January 18-19, February 14-15, March 12-14, and April 10-11, 2012 at a daily average discharge of 1280, 7,470, 11,500 and 10,400 cfs measured respectively at the Rosharon gage under a dry base winter flows (Jan and Feb), dry base spring flows (March) and dry 1 peak per season high flow pulse (April) flow regime tiers.

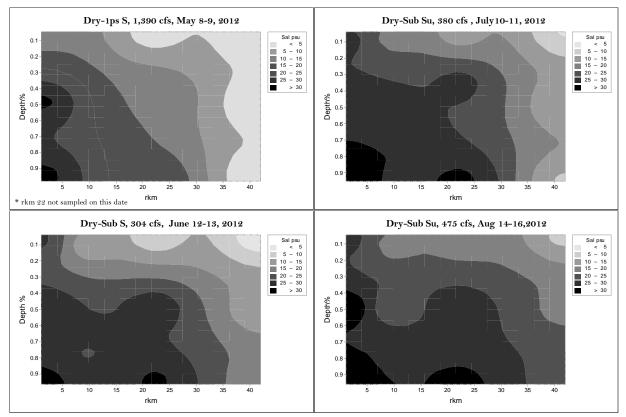


Figure G-26. Contour plot of salinity versus river kilometer during May 8-9, June 12-13, July10-11, and August 14-16, 2012 at a daily average discharge of 1,390, 304, 380, and 475 cfs measured respectively at the Rosharon gage under a dry 1 peak per season (May), dry subsistence spring (June) and dry subsistence summer (July and August) flow regime tiers.

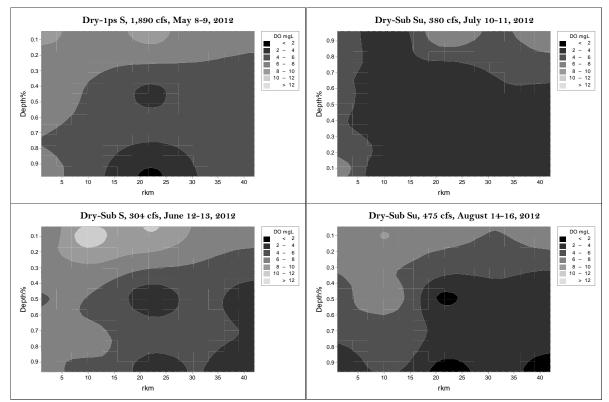


Figure G-27. Contour plot of dissolved oxygen (mg/L) versus river kilometer during May 8-9, June 12-13, July10-11, and August 14-16, 2012 at a daily average discharge of 1,390, 304, 380, and 475 cfs measured respectively at the Rosharon gage under a dry 1 peak per season (May), dry subsistence spring (June) and dry subsistence summer (July and August) flow regime tiers.

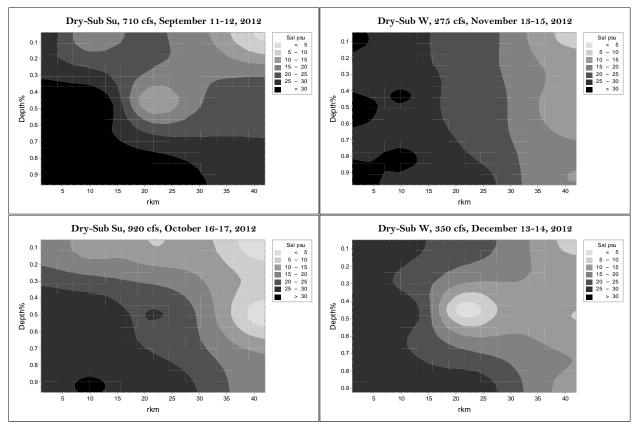


Figure G-28. Contour plot of salinity versus river kilometer during September 11-12, October 15-17, November 13-15, and December 13-14, 2012 at a daily average discharge of 710, 920, 275, and 350 cfs measured respectively at the Rosharon gage under a dry subsistence summer (September and October) and dry subsistence winter (November and December) flow regime tiers.

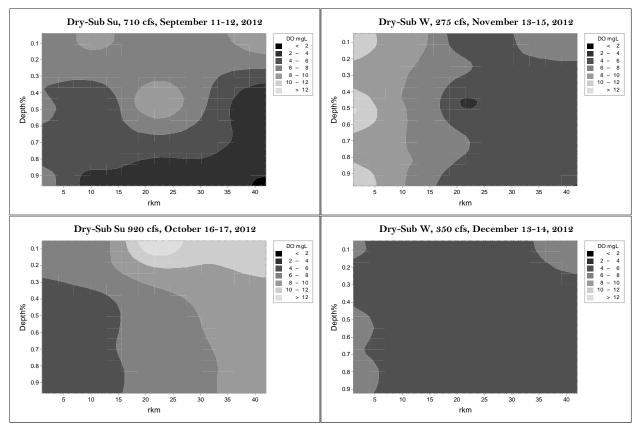


Figure G-29. Contour plot of dissolved oxygen (mg/L) versus river kilometer during September 11-12, October 15-17, November 13-15, and December 13-14, 2012 at a daily average discharge of 710, 920, 275, and 350 cfs measured respectively at the Rosharon gage under a dry subsistence summer (September and October) and dry subsistence winter (November and December) flow regime tiers.

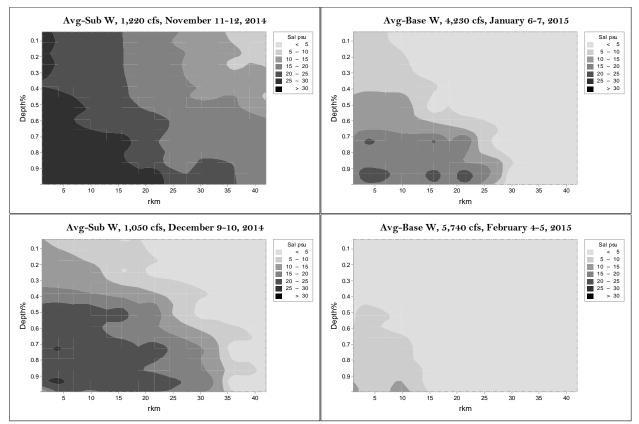


Figure G-30. Contour plot of salinity versus river kilometer during November 11-12 and December 9-10, 2014, and January 6-7 and February 4-5, 2015 at a daily average discharge of 1,220, 1,050, 4,230, and 5,740 cfs measured respectively at the Rosharon gage under a average subsistence winter (November and December) and average base winter (January and February) flow regime tiers.

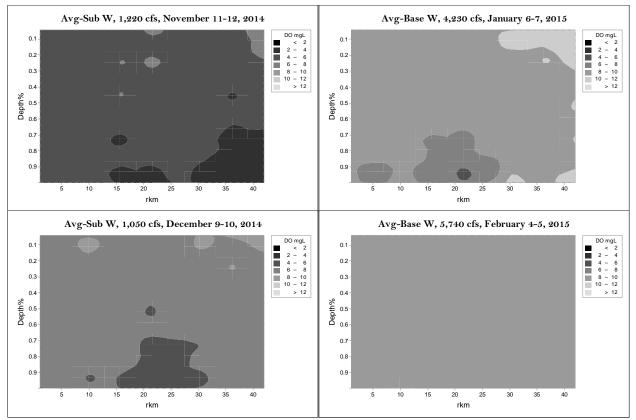


Figure G-31. Contour plot of dissolved oxygen (mg/L) versus river kilometer during November 11-12 and December 9-10, 2014, and January 6-7 and February 4-5, 2015 at a daily average discharge of 1,220, 1,050, 4,230, and 5,740 cfs measured respectively at the Rosharon gage under a average subsistence winter (November and December) and average base winter (January and February) flow regime tiers.

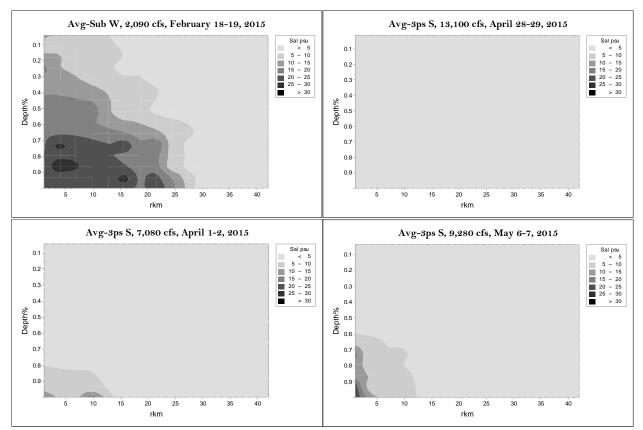


Figure G-32. Contour plot of salinity versus river kilometer during February 18-19, April 1-2, April 28-29 and May 6-7, 2015 at a daily average discharge of 2,090, 7,080, 13,100, and 9,280 cfs measured respectively at the Rosharon gage under a average subsistence winter (February) and average 3 pulse per season spring (April and May) flow regime tiers.

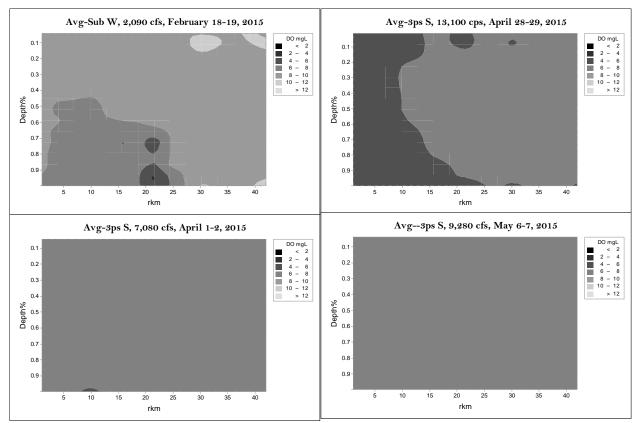


Figure G-33. Contour plot of dissolved oxygen (mg/L) versus river kilometer during February 18-19, April 1-2, April 28-29 and May 6-7, 2015 at a daily average discharge of 2,090, 7,080, 13,100, and 9,280 cfs measured respectively at the Rosharon gage under a average subsistence winter (February) and average 3 pulse per season spring (April and May) flow regime tiers.

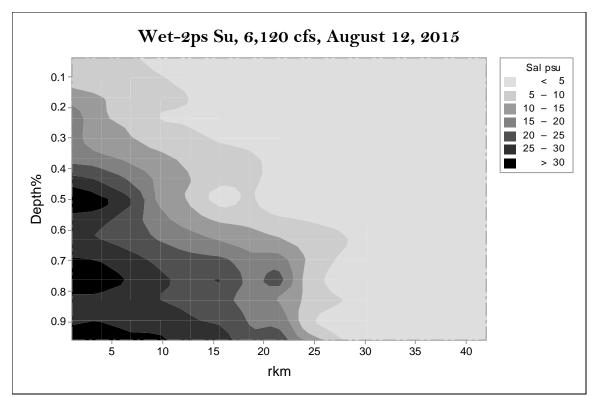


Figure G-34 Contour plot of salinity versus river kilometer during August 12, 2015 at a daily average discharge of 6,120 cfs measured at the Rosharon gage under a wet 2 pulses per season summer flow regime tier.

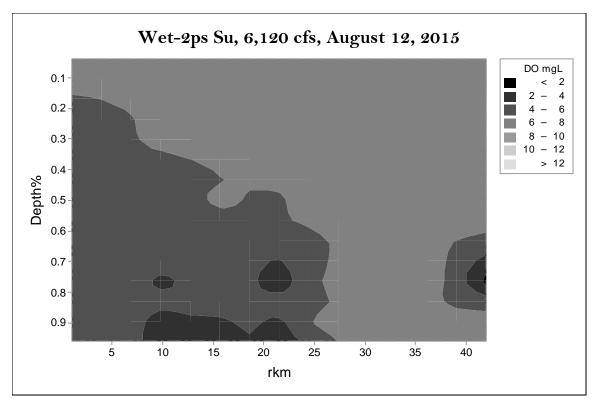


Figure G-35 Contour plot of dissolved oxygen (mg/L) versus river kilometer during August 12, 2015 at a daily average discharge of 6,120 cfs measured at the Rosharon gage under a wet 2 pulses per season summer flow regime tier.

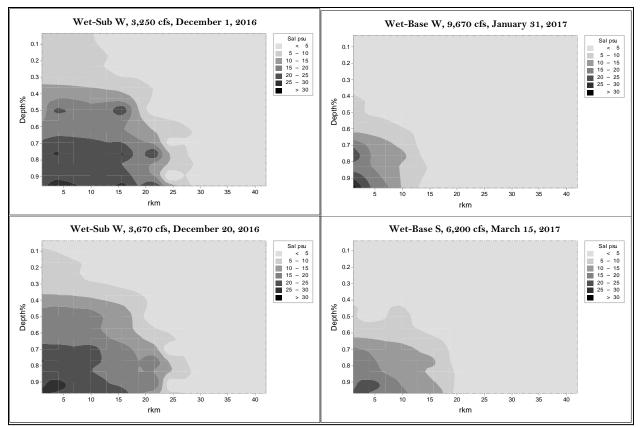


Figure G-36. Contour plot of salinity versus river kilometer during December 1 and 20, 2016, and January 31 and March 15, 2017 at a daily average discharge of 3,250, 3,670, 9,670, and 6,200 cfs measured respectively at the Rosharon gage under a wet subsistence winter (December 1 and 20) and wet base winter (January) and wet base spring (March) flow regime tiers.

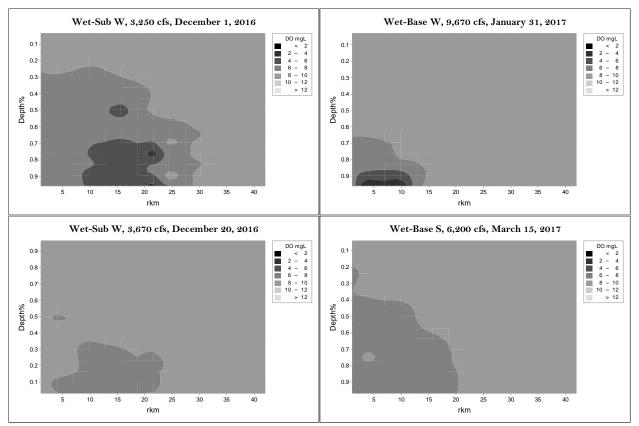


Figure G-37. Contour plot of dissolved oxygen versus river kilometer during December 1 and 20, 2016, and January 31 and March 15, 2017 at a daily average discharge of 3,250, 3,670, 9,670, and 6,200 cfs measured respectively at the Rosharon gage under a wet subsistence winter (December 1 and 20) and wet base winter (January) and wet base spring (March) flow regime tiers.

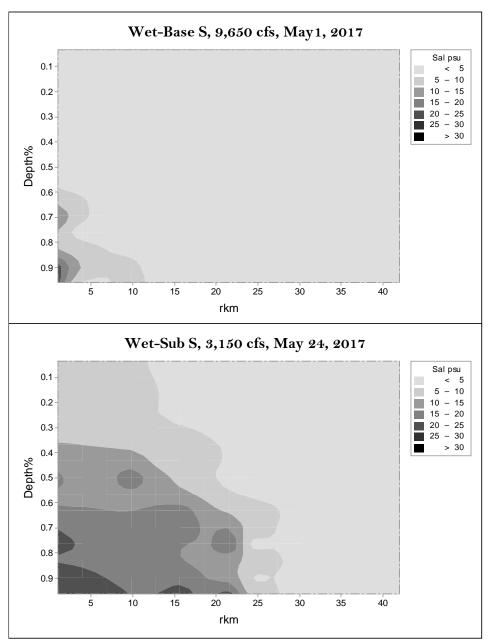


Figure G-38. Contour plot of salinity versus river kilometer during May 1 and 24, 2017 at a daily average discharge of 9,650 and 3,150 cfs measured respectively at the Rosharon gage under a wet base spring (May 1) and wet subsistence spring (May 24) flow regime tiers.

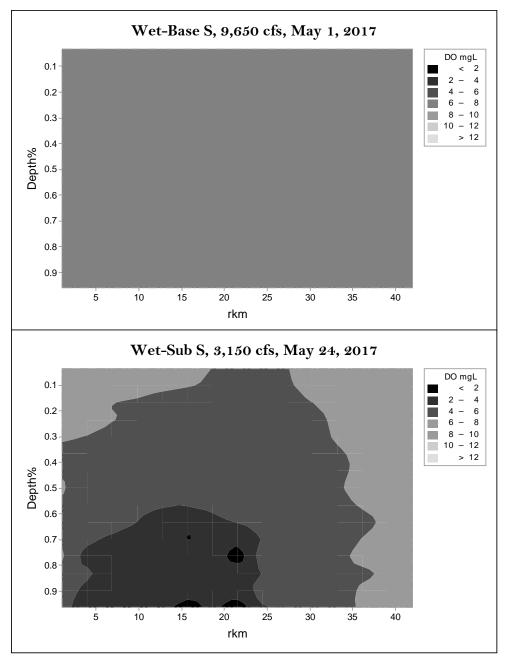


Figure G-39. Contour plot of dissolved oxygen (mg/L) versus river kilometer during May 1 and 24, 2017 at a daily average discharge of 9,650 and 3,150 cfs measured respectively at the Rosharon gage under a wet base spring (May 1) and wet subsistence spring (May 24) flow regime tiers.

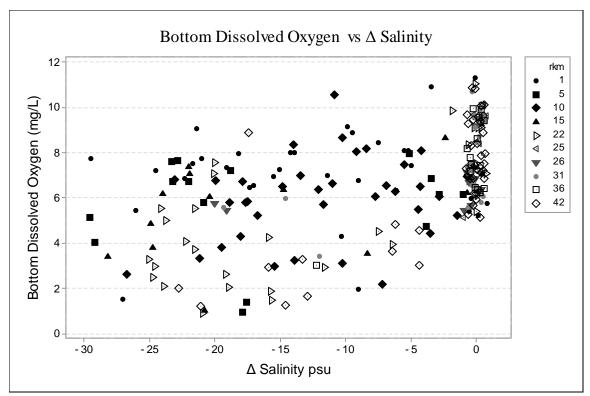


Figure G-40. Bottom dissolved oxygen versus delta (surface – bottom) salinity at each site (river kilometer) during the study period.

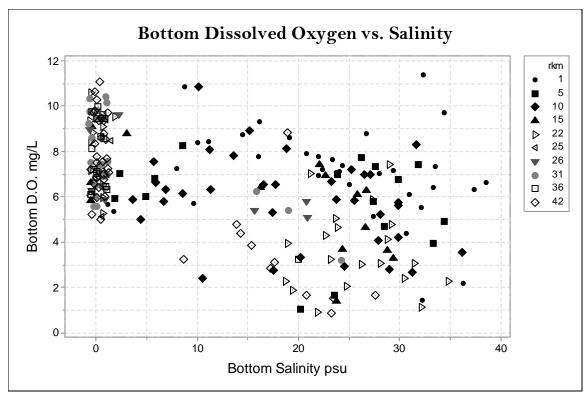


Figure G-41. Bottom dissolved oxygen versus bottom salinity at each site (river kilometer) during the study period.

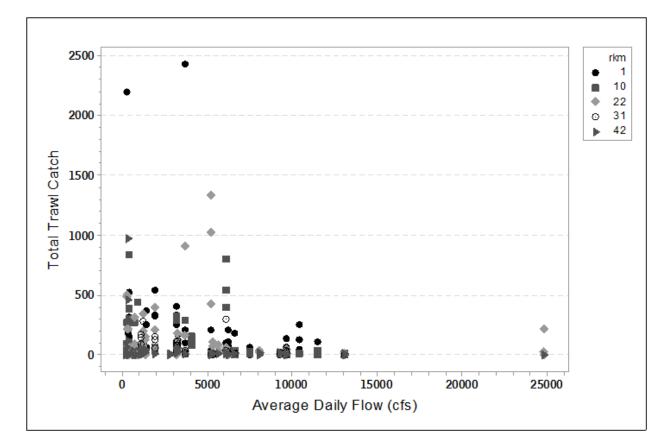


Figure G-42. The total number of nekton collected with otter trawls at each site versus average daily discharge (cfs) measured at the Rosharon gage.

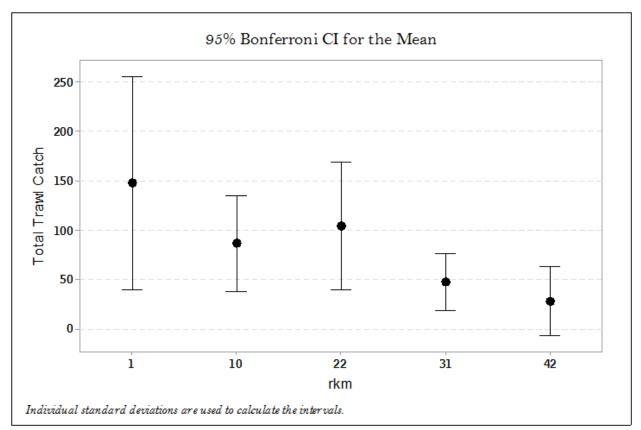


Figure G-43. Confidence interval plot for total number of nekton collected with otter trawls at each site during 2012 through 2017.

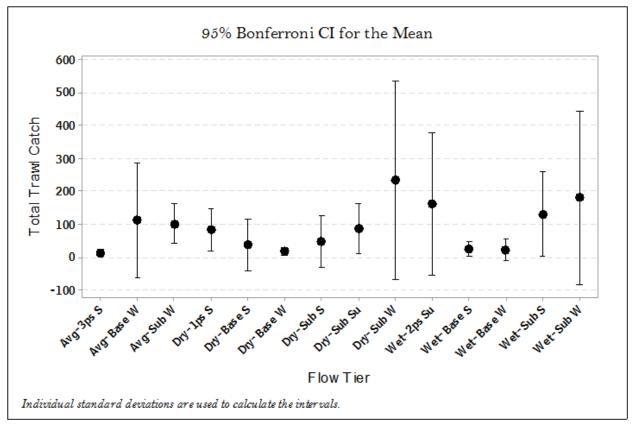


Figure G-44. Confidence interval plot for total number of nekton collected with otter trawls within each flow tier during 2012 through 2017.

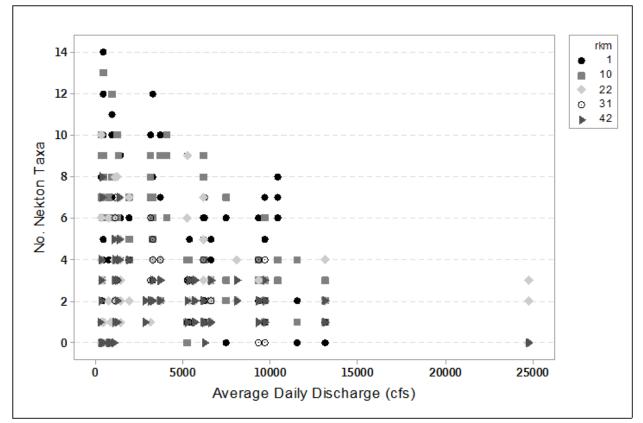


Figure G-45. The number of nekton taxa collected with otter trawls at each site versus average daily discharge (cfs) measured at the Rosharon gage.

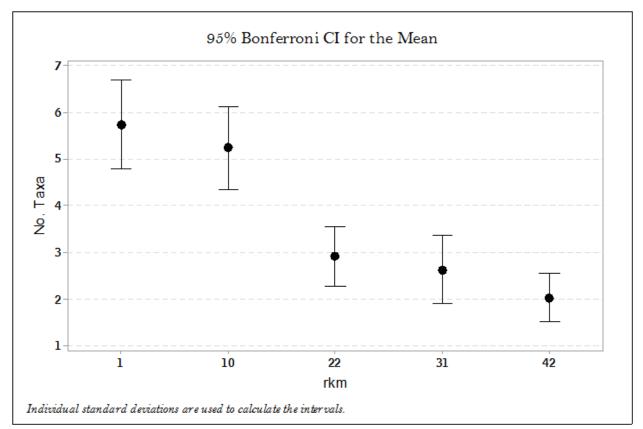


Figure G-46. Confidence interval plot for number of nekton taxa collected with otter trawls at each site during 2012 through 2017.

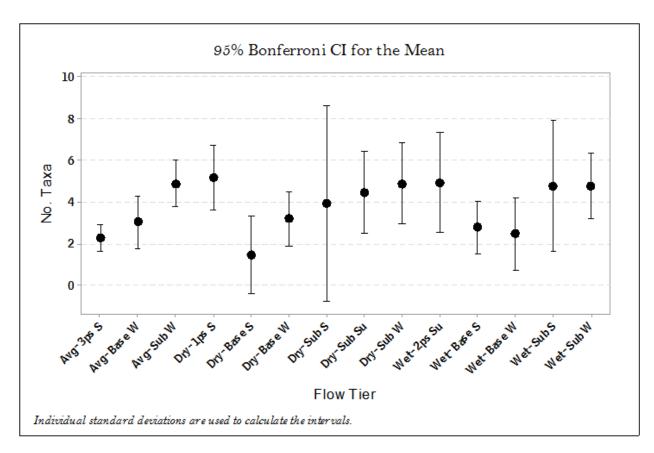


Figure G-47. Confidence interval plot for the number of estuarine nekton taxa collected per bottom trawl tow during 2012 through 2017 per flow tier.

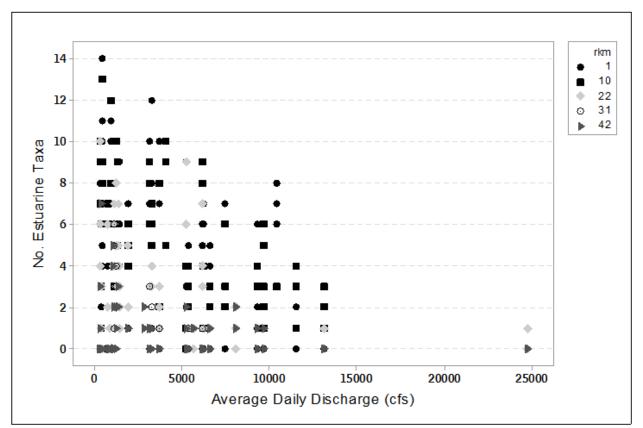


Figure G-48. The number of estuarine and marine nekton taxa collected with otter trawls at each site versus average daily discharge (cfs) measured at the Rosharon gage.

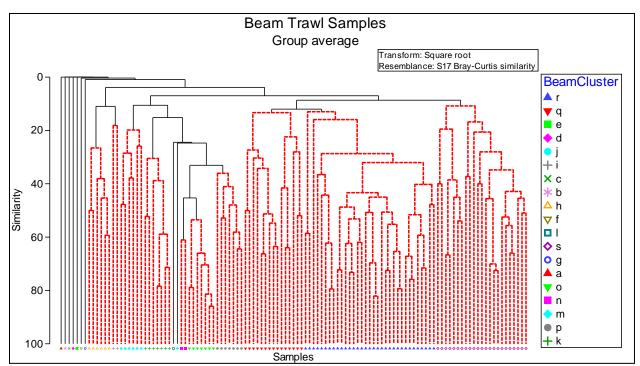


Figure G-49. Cluster analysis of shoreline nekton beam trawl collections using square root transformed catch data, Bray Curtis similarity and group averaging. Groups defined by the SIMPROF algorithm in PRIMER software. A total of 19 groups were identified based on similar community composition.

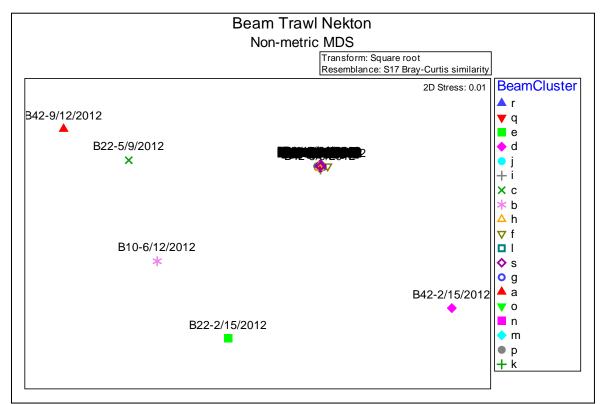
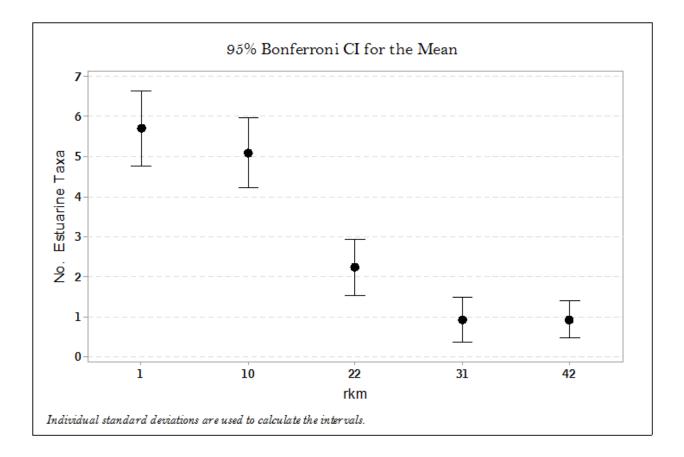


Figure G-50. Non-metric dimensional scaling (nMDS) plot of beam trawl catch. The majority of sites are located in the dark centroid.



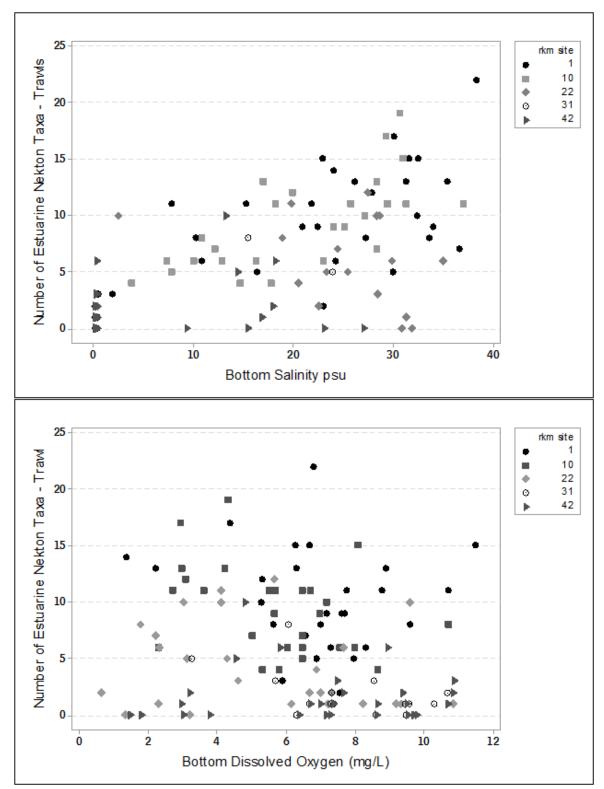


Figure G-53. The number of estuarine and marine nekton taxa (all replicates combined) collected with trawls at each site and date versus bottom salinity and dissolved oxygen.

Appendix H. Hydrologic Conditions Calculations Worksheet for the Brazos Estuary

Worksheet used to calculate hydrologic condition in the Brazos River estuary. Regional Palmer Method used to determine season, estimate PHDI, and calculate hydrological condition is described in Environmental Flow Standards for the Brazos River Brazos River Environmental Flow - Rosharon (Figure 30 TAC 298.470(c) (State of Texas 2014b). Hydrologic Drought Index data obtained from https://waterdatafortexas.org/drought/phdi/monthly?time=2011-09.

	Last Mo	N	orth Ce	ntral]	East Te		Edv	vards P	lateau	So	outh Ce	entral	U	pper C		PHDI Index =	Hydrologic
Season	Prev Season			(1) Wt X			(2) WtX			(3) WtX			(4) WtX			(5) WtX	Cell:	Condition
	riev beason	Wt	PHDI	PHDI	Wt	PHDI	PHDI	1+2+3+4+5	contantion									
Winter	11-Oct	0.619	-5.99	-3.70781	0.147	-6.86	-1.00842	0.057	-6.39	-0.36423	0.132	-6.21	-0.81972	0.045	-5.7	-0.2565	-6.15668	Dry
Winter	11-Oct	0.619	-5.99	-3.70781	0.147	-6.86	-1.00842	0.057	-6.39	-0.36423	0.132	-6.21	-0.81972	0.045	-5.7	-0.2565	-6.15668	Dry
Spring	12-Feb	0.619	-2.03	-1.25657	0.147	-4.36	-0.64092	0.057	-3.75	-0.21375	0.132	-4.37	-0.57684	0.045	-4.72	-0.2124	-2.90048	Dry
Spring	12-Feb	0.619	-2.03	-1.25657	0.147	-4.36	-0.64092	0.057	-3.75	-0.21375	0.132	-4.37	-0.57684	0.045	-4.72	-0.2124	-2.90048	Dry
Spring	12-Feb	0.619	-2.03	-1.25657	0.147	-4.36	-0.64092	0.057	-3.75	-0.21375	0.132	-4.37	-0.57684	0.045	-4.72	-0.2124	-2.90048	Dry
Spring	12-Feb	0.619	-2.03	-1.25657	0.147	-4.36	-0.64092	0.057	-3.75	-0.21375	0.132	-4.37	-0.57684	0.045	-4.72	-0.2124	-2.90048	Dry
Summer	12-Jun	0.619	-2.23	-1.38037	0.147	-3.19	-0.46893	0.057	-2.03	-0.11571	0.132	-2.67	-0.35244	0.045	-2.61	-0.11745	-2.4349	Dry
Summer	12-Jun	0.619	-2.23	-1.38037	0.147	-3.19	-0.46893	0.057	-2.03	-0.11571	0.132	-2.67	-0.35244	0.045	-2.61	-0.11745	-2.4349	Dry
Summer	12-Jun	0.619	-2.23	-1.38037	0.147	-3.19	-0.46893	0.057	-2.03	-0.11571	0.132	-2.67	-0.35244	0.045	-2.61	-0.11745	-2.4349	Dry
Summer	12-Jun	0.619	-2.23	-1.38037	0.147	-3.19	-0.46893	0.057	-2.03	-0.11571	0.132	-2.67	-0.35244	0.045	-2.61	-0.11745	-2.4349	Dry
Winter	12-Oct	0.619	-2.21	-1.36799	0.147	-1.59	-0.23373	0.057	-1.91	-0.10887	0.132	-2.41	-0.31812	0.045	-1.62	-0.0729	-2.10161	Dry
Winter	12-Oct	0.619	-2.21	-1.36799	0.147	-1.59	-0.23373	0.057	-1.91	-0.10887	0.132	-2.41	-0.31812	0.045	-1.62	-0.0729	-2.10161	Dry
Winter	14-Oct	0.619	-1.58	-0.97802	0.147	1.04	0.15288	0.057	-2.29	-0.13053	0.132	-2.93	-0.38676	0.045	-1.32	-0.0594	-1.40183	Average
Winter	14-Oct	0.619	-1.58	-0.97802	0.147	1.04	0.15288	0.057	-2.29	-0.13053	0.132	-2.93	-0.38676	0.045	-1.32	-0.0594	-1.40183	Average
Winter	14-Oct	0.619	-1.58	-0.97802	0.147	1.04	0.15288	0.057	-2.29	-0.13053	0.132	-2.93	-0.38676	0.045	-1.32	-0.0594	-1.40183	Average
Winter	14-Oct	0.619	-1.58	-0.97802	0.147	1.04	0.15288	0.057	-2.29	-0.13053	0.132	-2.93	-0.38676	0.045	-1.32	-0.0594	-1.40183	Average
Winter	14-Oct	0.619	-1.58	-0.97802	0.147	1.04	0.15288	0.057	-2.29	-0.13053	0.132	-2.93	-0.38676	0.045	-1.32	-0.0594	-1.40183	Average
Spring	15-Feb	0.619	-0.95	-0.58805	0.147	1.3	0.1911	0.057	-1.25	-0.07125	0.132	-1.47	-0.19404	0.045	0.58	0.0261	-0.63614	Average
Spring	15-Feb	0.619	-0.95	-0.58805	0.147	1.3	0.1911	0.057	-1.25	-0.07125	0.132	-1.47	-0.19404	0.045	0.58	0.0261	-0.63614	Average
Spring	15-Feb	0.619	-0.95	-0.58805	0.147	1.3	0.1911	0.057	-1.25	-0.07125	0.132	-1.47	-0.19404	0.045	0.58	0.0261	-0.63614	Average
Summer	15-Jun	0.619	4.07	2.51933	0.147	4.13	0.60711	0.057	3.22	0.18354	0.132	4.32	0.57024	0.045	3.38	0.1521	4.03232	Wet
Winter	16-Oct	0.619	4.99	3.08881	0.147	3.13	0.46011	0.057	4.5	0.2565	0.132	3.26	0.43032	0.045	3.4	0.153	4.38874	Wet
Winter	16-Oct	0.619	4.99	3.08881	0.147	3.13	0.46011	0.057	4.5	0.2565	0.132	3.26	0.43032	0.045	3.4	0.153	4.38874	Wet
Winter	16-Oct	0.619	4.99	3.08881	0.147	3.13	0.46011	0.057	4.5	0.2565	0.132	3.26	0.43032	0.045	3.4	0.153	4.38874	Wet
Spring	17-Feb	0.619	2.66	1.64654	0.147	0.77	0.11319	0.057	2.96	0.16872	0.132	1.55	0.2046	0.045	1.06	0.0477	2.18075	Wet
Spring	17-Feb	0.619	2.66	1.64654	0.147	0.77	0.11319	0.057	2.96	0.16872	0.132	1.55	0.2046	0.045	1.06	0.0477	2.18075	Wet
Spring	17-Feb	0.619	2.66	1.64654	0.147	0.77	0.11319	0.057	2.96	0.16872	0.132	1.55	0.2046	0.045	1.06	0.0477	2.18075	Wet

Appendix I. Summary Statistics of Brazos Estuary Water Quality Variables

Summary statistics for all water quality variables evaluated based on data from this study (2014-2017) and historical data (2012). Flow tiers = Dry, Avg, Wet; # ps = number of peaks season criterion met, base = base flow, sub = subsistence; Season: W = winter, S = spring, Su = summer. Data on total depth is presented separately at the end of the appendix.

Variable	Vertical Loc	Total Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	43	0	*	*	*	*
	Surface	43	14	0.0979	0.0143	0.0280	0.2060
W Temp C	Bottom	43	43	23.542	0.193	21.380	25.230
L	Surface	43	43	23.661	0.169	21.700	25.210
Sal psu	Bottom	43	43	3.261	0.940	0.130	24.250
-	Surface	43	43	0.753	0.141	0.130	3.340
DO mgL	Bottom	43	43	6.536	0.101	5.210	7.960
-	Surface	43	43	6.6770	0.0874	5.4800	7.4800
рН	Bottom	43	43	7.6519	0.0177	7.5100	8.0300
-	Surface	43	43	7.6433	0.0102	7.5300	7.8400
NTU	Bottom	43	43	237.8	21.0	28.0	524.8
	Surface	43	43	177.0	12.2	62.1	356.8
Chl-a ppb	Bottom	43	0	*	*	*	*
	Surface	43	0	*	*	*	*
NO23 mgL	Bottom	43	0	*	*	*	*
	Surface	43	15	1.3407	0.0910	0.7800	1.9600
TKN mgL	Bottom	43	0	*	*	*	*
	Surface	43	15	1.893	0.203	0.600	3.700
TP mgL	Bottom	43	0	*	*	*	*
	Surface	43	15	0.671	0.123	0.120	1.900
TSS mgL	Bottom	43	0	*	*	*	*
	Surface	43	15	193.4	31.7	24.5	454.0

1) Flow Tier = Avg-3ps S

2) Flow Tier = Avg-Base W

	Vertical	Total					
Variable	Loc	Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	28	0	*	*	*	*
	Surface	28	10	0.0992	0.0254	0.0400	0.3100
W Temp C	Bottom	28	28	12.679	0.315	9.520	16.600
	Surface	28	28	12.080	0.253	9.550	13.840
Sal psu	Bottom	28	28	7.89	1.70	0.14	26.88
	Surface	28	28	1.816	0.418	0.140	6.560
DO mgL	Bottom	28	28	8.681	0.258	4.840	10.880
	Surface	28	28	9.538	0.102	8.580	10.950
рН	Bottom	28	28	7.6075	0.0332	7.0900	7.9100
	Surface	28	28	7.6357	0.0277	7.1500	7.8100
NTU	Bottom	28	28	167.5	44.8	7.0	1196.5
	Surface	28	28	107.52	8.53	27.60	181.90
Chl-a ppb	Bottom	28	0	*	*	*	*
	Surface	28	0	*	*	*	*
NO23 mgL	Bottom	28	0	*	*	*	*
	Surface	28	10	0.9420	0.0815	0.6400	1.2600
TKN mgL	Bottom	28	0	*	*	*	*
	Surface	28	10	1.0570	0.0958	0.6000	1.3400
TP mgL	Bottom	28	0	*	*	*	*
	Surface	28	10	0.3178	0.0361	0.1280	0.4800
TSS mgL	Bottom	28	0	*	*	*	*
	Surface	28	10	120.0	16.4	38.5	204.0

3) Flow Tier = Avg-Sub W

Variable	Vertical Loc	Total Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	37	0		se mean	*	Maximum
Secont m	Surface	42	15	0.3205	0.0564	0.0580	0.7300
W Temp C	Bottom	37	37	18.507	0.574	13.330	24.980
	Surface	42	42	17.433	0.479	13.580	23.970
Sal psu	Bottom	37	37	17.57	1.77	0.17	31.09
	Surface	42	37	7.06	1.25	0.17	27.60
DO mgL	Bottom	37	37	6.348	0.362	2.980	10.710
	Surface	42	37	8.152	0.260	5.260	11.180
рн	Bottom	37	37	7.6370	0.0550	6.7300	8.1800
	Surface	42	37	7.8038	0.0376	7.3700	8.2100
NTU	Bottom	37	27	35.89	8.16	0.80	154.40
	Surface	42	26	30.63	6.53	1.80	119.30
Chl-a ppb	Bottom Surface	37 42	0 0	*	*	*	*
NO23 mgL	Bottom	37	0	*	*	*	*
	Surface	42	15	0.873	0.106	0.160	1.370
TKN mgL	Bottom	37	0	*	*	*	*
	Surface	42	14	1.343	0.214	0.200	2.600
TP mgL	Bottom	37	0	*	*	*	*
	Surface	42	15	0.2817	0.0470	0.0600	0.7800
TSS mgL	Bottom	37	0	*	*	*	*
	Surface	42	15	36.69	7.94	9.40	114.00

4) Flow Tier = Dry-1ps S

	Vertical	Total					
Variable	Loc	Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	9	3	0.3600	0.0732	0.2580	0.5020
	Surface	9	9	0.1592	0.0556	0.0130	0.5020
W Temp C	Bottom	9	9	25.557	0.648	23.470	28.720
	Surface	9	9	25.569	0.676	23.640	28.500
Sal psu	Bottom	9	9	9.60	4.01	0.27	31.31
	Surface	9	9	2.38	1.48	0.27	13.89
DO mgL	Bottom	9	9	6.417	0.633	1.760	8.320
	Surface	9	9	7.546	0.247	6.040	8.470
рН	Bottom	9	9	7.7289	0.0705	7.2800	8.0200
	Surface	9	9	7.8411	0.0540	7.6200	8.1400
NTU	Bottom	9	0	*	*	*	*
	Surface	9	9	183	100	9	800
Chl-a ppb	Bottom	9	0	*	*	*	*
	Surface	9	0	*	*	*	*
			~				
NO23 mgL	Bottom	9	0	*	*	*	*
	Surface	9	0	*	*	*	*
TTAL	Dathan	0	0	*	*	*	*
TKN mgL	Bottom	9	0	*	*	*	*
	Surface	9	0	^	^	^	^
D mart	Bottom	9	0	*	*	*	*
TP mgL	Surface	9	0	*	*	*	*
	SULLACE	9	U	~	~	^	^
TSS mgL	Bottom	9	0	*	*	*	*
TOO IIIGT	Surface	9	0	*	*	*	*
	Surrace	9	U				

5) Flow Tier = Dry-Base S

	Vertical	Total					
Variable	Loc	Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	8	0	*	*	*	*
	Surface	7	7	0.04357	0.00566	0.02200	0.06800
W Temp C	Bottom	8	7	21.80	1.65	17.48	26.99
W remp c	Surface	7	7	20.42	1.27	17.64	25.57
Sal psu	Bottom	8	7	10.08	4.24	0.15	24.92
Sai þsu	Surface	7	7	0.666	0.260	0.150	1.970
DO wet	Dathan	0	7	C 207	0 640		
DO mgL	Bottom Surface	8 7	7	6.307 6.743	0.640 0.581	2.950 5.170	7.560 9.140
рH	Bottom	8	7	7.7114	0.0777	7.5300	8.0400
	Surface	7	7	7.7057	0.0400	7.6000	7.8600
NTU	Bottom	8	0	*	*	*	*
	Surface	7	6	683	271	61	1560
Chl-a ppb	Bottom	8	0	*	*	*	*
11	Surface	7	0	*	*	*	*
NO23 mgL	Bottom	8	0	*	*	*	*
NO20 MgH	Surface	7	0	*	*	*	*
TKN mgL	Bottom	8	0	*	*	*	*
IKN MGL	Surface	° 7	0	*	*	*	*
		_	_				
TP mgL	Bottom Surface	8 7	0	*	*	*	*
	SULLACE	/	U	^	^	~	~
TSS mgL	Bottom	8	0	*	*	*	*
	Surface	7	0	*	*	*	*

6) Flow Tier = Dry-Base W

	Vertical	Total					
Variable	Loc	Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	9	0	*	*	*	*
	Surface	8	8	0.1433	0.0600	0.0200	0.5280
W Temp C	Bottom	9	9	15.479	0.570	13.710	18.330
	Surface	8	8	15.061	0.547	13.820	18.340
Sal psu	Bottom	9	9	12.20	4.51	0.10	33.63
our pou	Surface	8	8	7.02	4.13	0.10	33.64
	burrace	0	0	1.02	1.10	0.10	33.01
DO mgL	Bottom	9	9	9.229	0.581	5.650	10.850
	Surface	8	8	9.846	0.265	8.790	10.740
	Dattam	0	9	7.7789	0.0656	7.4600	8.0000
рH	Bottom	9 8					
	Surface	8	8	7.9375	0.0343	7.7900	8.1100
NTU	Bottom	9	0	*	*	*	*
	Surface	8	8	293	133	6	931
Chl-a ppb	Bottom	9	0	*	*	*	*
	Surface	8	0	*	*	*	*
NO23 mgL	Bottom	9	0	*	*	*	*
	Surface	8	0	*	*	*	*
TKN mgL	Bottom	9	0	*	*	*	*
IIII IIIJE	Surface	8	0	*	*	*	*
	Surrace	0	0				
TP mgL	Bottom	9	0	*	*	*	*
	Surface	8	0	*	*	*	*
TSS mgL	Bottom	9	0	*	*	*	*
TOO IIIGTI	Surface	8	0	*	*	*	*
	SULLACE	0	U	~	~	~	~

7) Flow Tier = Dry-Sub S

Variable	Vertical Loc	Total Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	24	14	0.4212	0.0367	0.0270	0.5800
	Surface	23	23	0.3801	0.0271	0.0270	0.5800
W Temp C	Bottom	24	24	30.045	0.557	22.870	34.100
	Surface	23	23	29.421	0.562	22.510	33.040
Sal psu	Bottom	24	24	27.75	1.82	0.22	38.33
	Surface	23	23	13.16	1.84	0.22	27.05
DO mgL	Bottom	24	24	4.161	0.446	1.000	8.960
	Surface	23	23	7.562	0.526	2.030	13.710
2 11	Bottom	24	2.4	7.4408	0.0775	6.6100	7.9900
рH	Surface	24	24 23	7.9661	0.0681	6.9100	7.9900 8.5400
	Surlace	23	23	1.9001	0.0681	6.9100	8.5400
NTU	Bottom	24	0	*	*	*	*
	Surface	23	23	9.17	1.96	3.98	51.27
Chl-a ppb	Bottom	24	0	*	*	*	*
	Surface	23	0	*	*	*	*
NO23 mgL	Bottom	24	0	*	*	*	*
	Surface	23	0	*	*	*	*
			0		*	*	
TKN mgL	Bottom	24	0	*	*	*	*
	Surface	23	0	*	*	*	*
TP mgL	Bottom	24	0	*	*	*	*
тт шды	Surface	23	0	*	*	*	*
	Surrace	20	U				
TSS mgL	Bottom	24	0	*	*	*	*
-	Surface	23	0	*	*	*	*

8) Flow Tier = Dry-Sub W

Variable	Vertical Loc	Total Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	10	10	0.4801	0.0670	0.1500	0.9280
	Surface	9	9	0.4801	0.0749	0.1500	0.9280
W Temp C	Bottom	10	10	20.506	0.786	16.190	24.270
	Surface	9	9	19.818	0.567	17.400	22.680
Sal psu	Bottom	10	10	24.32	2.41	13.26	31.57
	Surface	9	9	18.85	3.52	6.91	31.60
DO mgL	Bottom	10	10	5.679	0.855	1.510	11.480
	Surface	9	9	7.182	0.669	5.260	11.420
рН	Bottom	10	10	7.6360	0.0638	7.2100	7.8700
	Surface	9	9	7.7911	0.0295	7.6900	7.9300
NTU	Bottom	10	1	6.5800	*	6.5800	6.5800
	Surface	9	9	9.78	2.48	4.91	28.97
Chl-a ppb	Bottom Surface	10 9	0 0	*	*	*	*
NO23 mgL	Bottom Surface	10 9	0 0	*	*	*	*
TKN mgL	Bottom Surface	10 9	0 0	*	*	*	*
TP mgL	Bottom Surface	10 9	0 0	*	*	*	*
TSS mgL	Bottom Surface	10 9	0 0	*	*	*	*

9) Flow Tier = Wet-3ps Su

Variable	Vertical Loc	Total Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom Surface	18 18	0 10	* 0.1625	* 0.0190	* 0.1000	*
W Temp C	Bottom	18	18	31.122	0.283	29.600	33.450
	Surface	18	18	31.208	0.268	29.710	32.690
Sal psu	Bottom	18	18	15.92	3.50	0.26	34.65
	Surface	18	18	1.694	0.486	0.260	6.010
DO mgL	Bottom	18	18	4.753	0.446	1.560	7.370
	Surface	18	18	6.468	0.187	5.030	7.720
рH	Bottom	18	18	7.7461	0.0480	7.3200	8.0700
	Surface	18	18	7.8989	0.0364	7.6500	8.1600
NTU	Bottom	18	18	51.76	7.41	12.60	126.00
	Surface	18	18	45.42	4.16	14.70	70.40
Chl-a ppb	Bottom Surface	18 18	0 0	*	*	*	*
NO23 mgL	Bottom	18	0	*	*	*	*
	Surface	18	10	0.3710	0.0232	0.2600	0.4700
TKN mgL	Bottom	18	0	*	*	*	*
	Surface	18	10	1.220	0.190	0.300	2.000
TP mgL	Bottom	18	0	*	*	*	*
	Surface	18	10	0.1340	0.0229	0.0300	0.2700
TSS mgL	Bottom	18	0	*	*	*	*
	Surface	18	10	33.89	6.39	11.20	66.50

10) Flow Tier = Wet-Base S

Variable	Vertical Loc	Total Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	18	18	0.1010	0.0102	0.0430	0.1720
	Surface	18	18	0.1010	0.0102	0.0430	0.1720
W Temp C	Bottom	18	18	21.612	0.593	18.120	24.330
	Surface	18	18	21.694	0.583	18.990	24.260
Sal psu	Bottom	18	18	6.46	2.27	0.22	27.27
	Surface	18	18	0.701	0.171	0.220	2.590
DO mgL	Bottom	18	18	7.433	0.179	6.370	8.620
	Surface	18	18	7.908	0.150	7.250	8.720
рН	Bottom	18	18	7.8122	0.0228	7.6100	7.9500
	Surface	18	18	7.8772	0.0163	7.7800	8.0100
NTU	Bottom	18	18	154.4	26.6	18.6	315.5
	Surface	18	18	111.7	19.4	32.8	268.9
Chl-a ppb	Bottom	18	0	*	*	*	*
	Surface	18	5	11.460	0.175	10.800	11.800
NO23 mgL	Bottom	18	0	*	*	*	*
	Surface	18	5	0.8700	0.0110	0.8300	0.8900
TKN mgL	Bottom	18	0	*	*	*	*
	Surface	18	5	0.980	0.220	0.600	1.800
TP mgL	Bottom	18	0	*	*	*	*
	Surface	18	5	0.22200	0.00860	0.20000	0.25000
TSS mgL	Bottom	18	0	*	*	*	*
	Surface	18	5	88.2	15.0	53.0	125.0

11) Flow Tier = Wet-Base W

Variable	Vertical Loc	Total Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom Surface	9 9	9 9	0.04622 0.04622		0.02000 0.02000	0.09400 0.09400
W Temp C	Bottom Surface	9 9	9 9		0.294 0.0900	14.120 14.170	16.160 14.890
Sal psu	Bottom	9	9	7.32	4.07	0.22	34.01
	Surface	9	9	1.182	0.494	0.220	3.930
DO mgL	Bottom Surface	9 9	9 9	7.47 9.3022		0.86 8.9700	
PН	Bottom Surface	9 9	9 9	7.9000 7.8967		7.6500 7.7900	
NTU	Bottom	9	9	192.8	45.2	-1.7	333.2
	Surface	9	9	194.6	24.2	67.2	253.2
Chl-a ppb	Bottom Surface	9 9	0 5		* 0.0200		* 3.1000
NO23 mgL	Bottom	9	0	*	*	*	*
	Surface	9	5	0.9200	0.0249	0.8500	0.9900
TKN mgL	Bottom	9	0	*	*	*	*
	Surface	9	5	0.660	0.169	0.300	1.300
TP mgL	Bottom	9	0	*	*	*	*
	Surface	9	5	0.660	0.173	0.290	1.150
TSS mgL	Bottom	9	0	*	*	*	*
	Surface	9	5	279.9	56.6	83.5	392.0

12) Flow Tier = Wet-Sub S

Variable	Vertical Loc	Total Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	9	9	0.1914	0.0270	0.1020	0.3010
	Surface	9	9	0.1914	0.0270	0.1020	0.3010
W Temp C	Bottom	9	9	27.567	0.181	26.680	28.570
	Surface	9	9	27.420	0.191	26.410	27.920
Sal psu	Bottom	9	9	13.05	3.97	0.34	25.14
	Surface	9	9	2.884	0.997	0.340	8.130
DO mgL	Bottom	9	9	4.071	0.842	0.630	7.000
	Surface	9	9	6.642	0.250	5.790	8.320
рН	Bottom	9	9	7.4978	0.0958	7.1100	7.8000
	Surface	9	9	7.7644	0.0354	7.6500	7.9600
NTU	Bottom	9	9	31.77	6.68	5.10	53.60
	Surface	9	9	30.21	4.22	14.20	45.10
Chl-a ppb	Bottom Surface	9 9	0 0	*	*	*	*
NO23 mgL	Bottom Surface	9 9	0 0	*	*	*	*
TKN mgL	Bottom Surface	9 9	0 0	*	*	*	*
TP mgL	Bottom Surface	9 9	0 0	*	*	*	*
TSS mgL	Bottom Surface	9 9	0 0	*	*	*	*

13) Flow Tier = Wet-Sub W

Variable	Vertical Loc	Total Count	N	Mean	SE Mean	Minimum	Maximum
Secchi m	Bottom	18	18	0.1590	0.0141	0.1040	0.3180
	Surface	18	18	0.1590	0.0141	0.1040	0.3180
W Temp C	Bottom	18	18	17.786	0.827	12.130	23.440
	Surface	18	18	16.745	0.693	13.090	19.790
Sal psu	Bottom	18	18	14.14	3.01	0.34	29.00
	Surface	18	18	2.084	0.554	0.340	7.310
DO mgL	Bottom	18	18	7.597	0.434	3.100	9.790
	Surface	18	18	9.194	0.123	8.300	9.890
рН	Bottom	18	18	7.9128	0.0356	7.5300	8.0800
	Surface	18	18	8.0706	0.0206	7.8900	8.1900
NTU	Bottom	18	17	49.2	10.5	1.1	177.2
	Surface	18	18	35.14	4.04	3.30	60.20
Chl-a ppb	Bottom	18	0	*	*	*	*
	Surface	18	10	13.19	2.21	5.30	24.80
NO23 mgL	Bottom	18	0	*	*	*	*
	Surface	18	10	0.964	0.132	0.230	1.610
TKN mgL	Bottom	18	0	*	*	*	*
	Surface	18	10	1.570	0.226	0.800	2.900
TP mgL	Bottom	18	0	*	*	*	*
	Surface	18	10	0.3660	0.0483	0.2100	0.7600
TSS mgL	Bottom	18	0	*	*	*	*
	Surface	18	10	54.18	6.29	18.40	85.00

14) All Flow Tiers: Total Depth Total

		TOLAL					
Variable	Flow Tier	Count	Ν	Mean	SE Mean	Minimum	Maximum
T Depth m	Avg-3ps S	86	27	6.166	0.297	3.037	8.339
	Avg-Base W	56	18	5.803	0.266	3.535	7.385
	Avg-Sub W	79	27	5.451	0.228	3.271	7.167
	Dry-1ps S	18	9	6.802	0.495	4.457	9.450
	Dry-Base S	15	7	6.045	0.568	4.432	8.102
	Dry-Base W	17	9	5.347	0.500	3.585	7.142
	Dry-Sub S	47	24	5.987	0.300	3.300	7.556
	Dry-Sub W	19	10	5.640	0.375	4.309	7.030
	Wet-3ps Su	36	18	6.733	0.300	3.889	8.136
	Wet-Base S	36	18	6.748	0.293	4.620	8.820
	Wet-Base W	18	9	6.316	0.488	4.323	8.896
	Wet-Sub S	18	9	6.360	0.490	4.301	8.430
	Wet-Sub W	36	18	6.252	0.384	3.042	9.047

Appendix J. Linear Models (ANOVA and Regression) Used to Evaluate the Spatial Response of Various Water Quality and Biological Indices to Flow Tiers and River Discharge

Linear models (ANOVA and regression) used to evaluate the spatial response of various water quality and biological indices to flow tiers and river discharge. All models were constructed and run with the Minitab statistical software package.

Model 1: Regression Analysis: NO₂₊₃ – N mg/L versus daily average flow and river kilometer

Analysis of Variance Source DF Adj SS Adj MS F-Value P-Value Regression 3 0.9262 0.30874 1.83 0.150 0.074 CFS 1 0.5552 0.55519 3.29 rkm10.06460.06455CFS*rkm10.07270.07270Error6611.12170.16851Total6912.0479 0.38 0.538 0.38 0.514 Model Summary S R-sq R-sq(adj) R-sq(pred) 0.410501 7.69% 3.49% 0.00% Coefficients Coef SE Coef T-Value P-Value VIF Term COEI SE COEI I VALLE 0.662 0.166 3.99 0.000 Constant 1dCFS0.0000460.000025rkm0.004000.00646 1.82 0.074 3.11 0.62 0.538 3.69 0.00400 0.00646 1dCFS*rkm -0.000001 0.000001 -0.66 0.514 5.80 Regression Equation NO23 mgL = 0.662 + 0.000046 1dCFS + 0.00400 rkm - 0.000001 1dCFS*rkm Fits and Diagnostics for Unusual Observations Std Obs NO23 mgL Fit Resid Resid 1.490 1.259 0.231 0.65 31 Х 1.240 1.076 0.164 0.46 35 Х 38 1.960 1.083 0.877 2.18 R R Large residual X Unusual X

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value Regression 1 0.8514 0.85143 5.17 0.026 1dCFS 1 0.8514 0.85143 5.17 0.026 Error 68 11.1965 0.16465 0.000 Lack-of-Fit 13 8.1447 0.62652 11.29 0.000 Pure Error 55 3.0518 0.05549 10.05549 10.010
Model Summary
SR-sqR-sq(adj)R-sq(pred)0.4057767.07%5.70%2.18%
Coefficients
Term Coef SE Coef T-Value P-Value VIF Constant 0.7468 0.0931 8.02 0.000 CFS 0.000032 0.000014 2.27 0.026 1.00
Regression Equation
NO ₂₃ mgL = 0.7468 + 0.000032 CFS
Fits and Diagnostics for Unusual Observations
Std Obs NO23 mgL Fit Resid Resid 38 1.9600 1.0851 0.8749 2.20 R
R Large residual

Model 2: Regression Analysis: NO₂₊₃ –N mg/L versus average daily discharge at Rosharon gage only.

Model 3: ANOVA General Linear Model: NO₂₊₃ –N mg/L versus rkm and flow tier categories.

Factor coding (-1, 0, +1)Factor Information Levels Values Factor Type 5 1, 10, 22, 31, 42 rkm Fixed 7 Avg-3ps S, Avg-Base W, Avg-Sub W, Wet-3ps Su, Wet-Base S, Flow Tier Fixed Wet-Base W, Wet-Sub W Analysis of Variance Source DF Adj SS Adj MS F-Value P-Value 4 0.1514 0.03786 0.27 0.895 rkm 6 5.7349 0.95581 6.85 Flow Tier 0.000 0.34 rkm*Flow Tier 24 1.1478 0.04782 0.996 Error 35 4.8872 0.13963 Total 69 12.0479 Model Summary R-sq(adj) R-sq(pred) S R-sq 0.373677 59.44% 20.03% Coefficients Term Coef SE Coef T-Value P-Value VTF Constant 0.8972 0.0487 18.41 0.000 rkm -0.0731 0.0975 -0.75 0.458 1.90 1 0.0975 0.52 0.608 1.90 10 0.0504 0.0975 22 0.0566 0.58 0.565 1.90 31 0.0076 0.0975 0.08 0.939 1.90 Flow Tier 4.67 0.40 Avg-3ps S 0.4435 0.0950 0.000 1.59 Avg-Base W 0.045 0.111 0.689 1.77 0.0950 0.798 1.59 -0.0245 -0.26 Avg-Sub W -0.526 -4.74 0.000 1.77 Wet-3ps Su 0.111 0.149 0.857 2.34 -0.027 -0.18 Wet-Base S Wet-Base W 0.023 0.149 0.15 0.880 2.34 rkm*Flow Tier coefficients not presented Grouping Information Using the Tukey Method and 95% Confidence Flow Tier Mean Grouping Ν Avg-3ps S 15 1.34067 A Wet-Sub W 10 0.96400 A В Avg-Base W 10 0.94200 A В Wet-Base W 5 0.92000 A В Avg-Sub W 15 0.87267 В Wet-Base S 5 0.87000 Α B C Wet-3ps Su 10 0.37100 С Means that do not share a letter are significantly different.

Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value Regression 2 4.1511 2.0755 4.27 0.018 1dCFS 1 0.6178 0.6178 1.27 0.264 rkm 1 3.6110 3.6110 7.43 0.008 Error 66 32.0934 0.4863 7.43 0.008
Model Summary
S R-sq R-sq(adj) R-sq(pred) 0.697326 11.45% 8.77% 2.76%
Coefficients
TermCoefSE CoefT-ValueP-ValueVIFConstant0.8630.2084.160.0001dCFS0.0000280.0000251.130.2641.00rkm0.015810.005802.730.0081.00
Regression Equation
TKN mg/L = 0.863 + 0.000028 cfs + 0.01581 rkm
Fits and Diagnostics for Unusual Observations
Std Obs TKN mgL Fit Resid 37 2.700 1.313 1.387 2.04 R 39 3.700 1.645 2.055 3.02 R 58 2.900 1.313 1.587 2.30 R
R Large residual

Model 4: Regression analysis: TKN mg/L versus average daily discharge at Rosharon (cfs) and distance from the river mouth (rkm).

Model 5. Regression Analysis: TKN mg/L versus Average Daily discharge at Rosharon (cfs).

Analysis of Variance

Regression ldCFS Error Lack-of-Fit Pure Error		0.5400 0.5400 0.5329 1.0679	1.01 1.01	0.318 0.318
Model Summary				
S R-sq 0.730002 1.49%	q R-sq(adj) % 0.02%	R-sq(pred) 0.00%		
Coefficients				
Term C Constant 1. 1dCFS 0.000	.213 0.171	1 7.10		
Regression Equa	ation			
TKN mg/L = 1.21	13 + 0.000026	cfs		
Fits and Diagno	ostics for Uni	usual Obser	vations	
Obs TKN mgL 39 3.700 1 58 2.900 1 60 2.800 1 R Large residu	1.486 2.214 1.308 1.592 1.308 1.492	3.10 R 2.20 R		
it harge restuu	A.4.1			

Model 6. ANOVA General Linear Model: TKN mg/L versus river kilometer (rkm) and flow tier.

Factor coding (-1, 0, +1) Factor Information Factor Type Levels Values rkm Fixed 5 1, 10, 22, 31, 42 Flow Tier Fixed 7 Avg-3ps S, Avg-Base W, Avg-Sub W, Wet-3ps Su, Wet-Base S, Wet-Base W, Wet-Sub W

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
rkm	4	2.610	0.6525	1.44	0.241
Flow Tier	6	9.048	1.5081	3.33	0.011
rkm*Flow Tier	24	8.183	0.3410	0.75	0.763
Error	34	15.383	0.4524		
Total	68	36.244			

Model Summary

S R-sq R-sq(adj) R-sq(pred) 0.672643 57.56% 15.11% *

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1.2391	0.0881	14.07	0.000	
rkm					
1	-0.158	0.178	-0.89	0.381	1.89
10	-0.270	0.176	-1.54	0.133	1.91
22	0.034	0.176	0.20	0.846	1.91
31	0.045	0.176	0.25	0.801	1.91
Flow Tier					
Avg-3ps S	0.654	0.171	3.82	0.001	1.60
Avg-Base W	-0.182	0.200	-0.91	0.369	1.77
Avg-Sub W	0.054	0.177	0.31	0.762	1.65
Wet-3ps Su	-0.019	0.200	-0.10	0.925	1.77
Wet-Base S	-0.259	0.269	-0.96	0.342	2.34
Wet-Base W	-0.579	0.269	-2.15	0.039	2.34
* rkm*flow tier	coeffici	ents not	presented		

Grouping Information Using the Tukey Method and 95% Confidence

Flow Tier	Ν	Mean	Grouping
Avg-3ps S	15	1.89333	A
Wet-Sub W	10	1.57000	A B
Avg-Sub W	14	1.32329	A B
Wet-3ps Su	10	1.22000	A B
Avg-Base W	10	1.05700	В
Wet-Base S	5	0.98000	A B
Wet-Base W	5	0.66000	В

Means that do not share a letter are significantly different.

Model 7. Regression Analysis: Total phosphorus (TP) mg/L versus daily average discharge at Rosharon (cfs) and river kilometer (rkm).

Analysis o	f Variance	2					
rkm Error	2 0.91 1 0.90 1 0.01	1978 0.45 0650 0.90 1319 0.01 3707 0.09	989 650 319	4.90 0 9.66 0	.010 .003		
Model Summ	Model Summary						
S R-sq R-sq(adj) R-sq(pred) 0.306328 12.76% 10.16% 6.49%							
Coefficients							
Constant CFS		0.0881	2.01 3.1		3 1.00		
Regression	Equation						

TP mgL = $0.1775 + 0.000033 \, 1dCFS + 0.00094 \, rkm$

Model 8. Regression Analysis: TP mg/L versus daily average discharge measured at the Rosharon gage.

Analysis of Variance
 Source
 DF
 Adj SS
 Adj MS
 F-Value
 P-Value

 Regression
 1
 0.9066
 0.90659
 9.79
 0.003

 1dCFS
 1
 0.9066
 0.90659
 9.79
 0.003

 Error
 68
 6.3003
 0.09265
 0.003
 Lack-of-Fit 13 2.6429 0.20330 3.06 0.002 Pure Error 55 3.6574 0.06650 Total 69 7.2069 Model Summary R-sq R-sq(adj) R-sq(pred) S 0.304386 12.58% 11.29% 7.70% Coefficients Coef SE Coef T-Value P-Value VIF Term Constant0.19750.06982.830.0061dCFS0.0000330.0000113.130.0031.00 Regression Equation TP mgL = 0.1975 + 0.000033 1dCFSFits and Diagnostics for Unusual Observations Std Obs TP mgL Fit Resid Resid
 28
 1.9000
 0.4329
 1.4671
 4.86
 R

 29
 1.4100
 0.4329
 0.9771
 3.24
 R

 63
 1.1500
 0.5191
 0.6309
 2.11
 R
 R Large residual

Model 9. ANOVA General Linear Model: Total Phosphorus (TP) mg/L versus river kilometer (rkm) and flow tier.

Method Factor coding (-1, 0, +1)Factor Information Factor Туре Levels Values 5 1, 10, 22, 31, 42 rkm Fixed 7 Avg-3ps S, Avg-Base W, Avg-Sub W, Wet-3ps Su, Wet-Base S, Flow Tier Fixed Wet-Base W, Wet-Sub W Analysis of Variance Source DF Adj SS Adj MS F-Value P-Value 4 0.5100 0.12751 1.82 rkm 0.136 Flow Tier 6 2.5737 0.42895 6.14 0.000 59 4.1231 0.06988 Error Lack-of-Fit 24 1.6338 0.06807 0.96 0.537 Pure Error 35 2.4894 0.07112 Total 69 7.2069 Model Summary R-sq R-sq(adj) R-sq(pred) S 0.264355 42.79% 33.09% 21.68% Coefficients Term Coef SE Coef T-Value P-Value VTF 0.3789 0.0345 10.99 0.000 Constant rkm -0.0766 0.0632 -1.21 0.230 1.60 1 -0.56 -0.0353 0.0632 0.578 1.60 10 2.58 0.0632 22 0.1632 0.012 1.60 31 0.0004 0.0632 0.01 0.995 1.60 Flow Tier Avg-3ps S 0.2918 0.0672 4.34 0.000 1.59 Avg-Base W -0.0611 0.0786 -0.78 0.440 1.77 0.0672 0.154 1.59 Avg-Sub W -0.0972 -1.45 0.003 1.77 Wet-3ps Su -0.2449 0.0786 -3.12 0.143 2.34 Wet-Base S -0.157 0.106 -1.48 Wet-Base W 0.281 0.106 2.66 0.010 2.34 Grouping Information Using the Tukey Method and 95% Confidence Flow Tier Ν Mean Grouping Avg-3ps S 15 0.670667 A 5 0.660000 A Wet-Base W В Wet-Sub W 10 0.366000 A B C Avg-Base W 10 0.317800 B C Avg-Sub W 15 0.281733 в С Wet-Base S 5 0.222000 B C Wet-3ps Su 10 0.134000 С Means that do not share a letter are significantly different.

Model 10. Regression analysis of total suspended solids (TSS) mgL versus daily average discharge measured at the Rosharon gage and river kilometer (rkm).

Analysis of Variance Source DF Adj SS Adj MS F-Value P-Value Regression 2 345472 172736 29.04 0.000 1dCFS 1 282320 282320 47.46 0.000 rkm 1 63041 63041 10.60 0.002 Error 67 398578 5949 Total 69 744050 Model Summary S R-sq R-sq(adj) R-sq(pred) 77.1293 46.43% 44.83% 39.65% Coefficients Term Coef SE Coef T-Value P-Value VIF Constant -42.3 22.2 -1.91 0.061 1dCFS 0.01856 0.00269 6.89 0.000 1.00 rkm 2.058 0.632 3.26 0.002 1.00 Regression Equation

TSS mgL = $-42.3 + 0.01856 \ 1dCFS + 2.058 \ rkm$

Analysis of Va	rian	се							
Error Lack-of-Fit Pure Error	1 68 13 55	282431 282431 461619 256790 204829	282431 282431 6789 19753	41.60	0.000 0.000				
Total	69	/44050							
Model Summary									
S R-s 82.3924 37.96	-	-sq(adj) 37.05%							
Coefficients									
Term C Constant				e P-Value 7 0.946					
1dCFS 0.01856 0.00288 6.45 0.000 1.00									
Regression Equation									
TSS mgL = 1.3	TSS mgL = $1.3 + 0.01856 \ 1dCFS$								

Model 11. Regression analysis of total suspended solids (TSS) mg/L versus daily average discharge measured at the Rosharon gage.

Model 12. ANOVA General Linear Model: total suspended solids (TSS) versus river kilometer (rkm) and flow tier.

```
Method
Factor coding (-1, 0, +1)
Factor Information
Factor
        Type Levels Values
               Fixed
rkm
Flow Tier Fixed
Wet-Base
                    W, Wet-Sub W
Analysis of Variance
Source
            DF Adj SS Adj MS F-Value P-Value
 rkm
             4 78092 19523 3.57 0.015
             6 420325
                                    0.000
 Flow Tier
                      70054 12.82
rkm*Flow Tier 24 65225
Error 35 191330
                      2718
                             0.50 0.962
                       5467
Total
             69 744050
Model Summary
       R-sq R-sq(adj) R-sq(pred)
    S
73.9362 74.29%
             49.31%
```

Tukey Pairwise Comparisons: Response = TSS mgL, Term = rkm

Grouping Information Using the Tukey Method and 95% Confidence rkm N Mean Grouping 14 151.464 A 42 14 150.040 A 14 128.240 A 31 22 В 87.083 A 10 14 В 59.067 1 14 В

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Response = TSS mgL, Term = Flow Tier

Grouping Information Using the Tukey Method and 95% Confidence Ν Mean Grouping Flow Tier 5 279.900 A Wet-Base W Avg-3ps S 15 193.447 A В Avg-Base W 10 119.950 B C 88.200 вC Wet-Base S 5 Wet-Sub W 10 54.180 С Avg-Sub W 15 36.687 С Wet-3ps Su 10 33.890 С

Means that do not share a letter are significantly different.

Model 13. Regression analysis of Chlorophyll-a (ug/L) versus daily average discharge measured at the Rosharon gage and river kilometer (rkm).

Analysis of Variance

Rogrossic	on 2 1 1 17	449.21 95.25 353.96 346.06	224.60 95.25 353.96	F-Value 11.03 4.68 17.39	0 001						
Model Summary											
S 4.51179	S R-sq R-sq(adj) R-sq(pred) 4.51179 56.49% 51.37% 40.39%										
Coefficie	ents										
Constant	10	5.42	2.87	T-Value 5.72 2.16 -4.17	0.000						
Regressio	on Equat	tion									
Chl-a ppb = 16.42 + 0.1497 rkm - 0.001646 CFS											
Fits and	Fits and Diagnostics for Unusual Observations										
		Fit	Resid H 9.09	Std Resid 2.15 R							
R Large	residua	al									

Model 14. Regression Analysis: Chlorophyll-a (ug/L) versus daily average discharge at Rosharon gage.

Analysis of Variance

DF	Adj SS	Adj MS	F-Value	P-Value
1	354.0	353.96	14.44	0.001
1	354.0	353.96	14.44	0.001
18	441.3	24.52		
2	173.4	86.68	5.18	0.018
16	267.9	16.75		
19	795.3			
	1 1 18 2 16	1 354.0 1 354.0 18 441.3 2 173.4 16 267.9	1 354.0 353.96 1 354.0 353.96 18 441.3 24.52 2 173.4 86.68 16 267.9 16.75	1 354.0 353.96 14.44 1 354.0 353.96 14.44 18 441.3 24.52 2 173.4 86.68 5.18 16 267.9 16.75

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.95147	44.51%	41.43%	33.07%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	19.59	2.70	7.24	0.000	
1dCFS	-0.001646	0.000433	-3.80	0.001	1.00

Regression Equation

Chl-a ppb = 19.59 - 0.001646 1dCFS

Model 15. ANOVA General Linear Model: chlorophyll-a (ug/L) versus river kilometer (rkm) and flow tier.

Method

Factor coding (-1, 0, +1)Rows unused 50 Factor Information Type Levels Values Factor FactorTypeLevelsvaluesrkmFixed51, 10, 22, 31, 42Flow TierFixed3Wet-Base S, Wet-Base W, Wet-Sub W Analysis of Variance Source DF Adj SS Adj MS F-Value P-Value rkm451.4012.850.310.858Flow Tier2355.10177.554.340.081rkm*Flow Tier8112.7114.090.340.913rror5204.6040.9210.92 rkm 5 204. 19 795.27 Error Total Model Summary S R-sq R-sq(adj) R-sq(pred) 6.39680 74.27% 2.24%

Model 16. Regression analysis of bottom salinity (psu) versus daily average discharge measured at the Rosharon gage and river kilometer (rkm).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	21871.3	10935.7	171.93	0.000
1dCFS	1	9253.3	9253.3	145.48	0.000
rkm	1	11815.0	11815.0	185.76	0.000
Error	232	14756.4	63.6		
Lack-of-Fit	231	14756.4	63.9	*	*
Pure Error	1	0.0	0.0		
Total	234	36627.7			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7.97528	59.71%	59.37%	58.10%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	30.88	1.12	27.58	0.000	
CFS	-0.001483	0.000123	-12.06	0.000	1.00
rkm	-0.4975	0.0365	-13.63	0.000	1.00

Regression Equation

Bot Sal psu = 30.88 - 0.001483 1CFS - 0.4975 rkm

Model 17. Regression analysis of bottom salinity (psu) versus daily average discharge measured at the Rosharon gage.

Innary	515 01 14	I Lunce								
CFS Error Lac Pur	ssion	1 1 233 53 180	10056 10056 26571 8965 17606	10056.3 10056.3 114.0 169.2	1.73	0.000 0.000				
Model	Summary									
10.67	S R-s 90 27.46	q R-sq % 2	[(adj) 7.14%	R-sq(pre 26.3	ed) 11%					
Coeff	icients									
Const	ant	20.69	1.1	12 18	lue P-Val .54 0.0 .39 0.0	00				
CFS	-0.0	01343	0.00010	54 -9	.39 0.0	00 1.00				
Regre	ssion Equ	ation								
Bot S	al psu =	20.69 -	0.0015	545 ldCFS	5					
Fits	Fits and Diagnostics for Unusual Observations									
	Bot Sal			Std						
Obs 11	psu 0 15	-17 61	Resid 17 76	Resid 1.75	Х					
12	0.16	-17.61	17.77	1.75	Х					
14	0.27	-8.81	9.08	0.87	Х					
	34.65	11.24	23.41	2.20	R					
	34.01									
224	27.27	5.79	21.48	2.02	R					

Analysis of Variance

Model 18. ANOVA General Linear Model: bottom salinity versus river kilometer (rkm) and flow tier.

```
Factor coding (-1, 0, +1)
Rows unused
             6
Factor Information
Factor
         Type Levels Values
         Fixed 10 1, 5, 10, 15, 22, 25, 26, 31, 36, 42
rkm
Flow Tier Fixed
                   13 Avg-3ps S, Avg-Base W, Avg-Sub W, Dry-1ps S, Dry-Base S,
Dry-Base
                        W, Dry-Sub S, Dry-Sub W, Wet-3ps Su, Wet-Base S, Wet-Base W,
                        Wet-Sub S, Wet-Sub W
Analysis of Variance
                         Adj MS F-Value P-Value
             DF Adj SS
Source
              9 13583 1509.28 31.84
                                          0.000
 rkm
 Flow Tier
             12 11695 974.57 20.56
                                          0.000
            213 10095 47.39
Error
 Lack-of-Fit 70 4616 65.94 1.72 0.003
 Pure Error 143 5479 38.31
           234 36628
Total
Model Summary
Regression Equation
Bot Sal psu = 11.895 + 10.65 rkm_1 + 10.63 rkm_5 + 6.94 rkm_10 + 5.71 rkm_15
+ 2.45 rkm_22
             - 9.30 rkm 25 - 3.96 rkm 26 - 6.31 rkm 31 - 8.47 rkm 36 - 8.33 rkm 42
             - 9.51 Flow Tier Avg-3ps S - 5.06 Flow Tier Avg-Base W
+ 4.63 Flow Tier Avg-Sub
             W - 2.27 Flow Tier Dry-1ps S - 6.00 Flow Tier Dry-Base S
             - 1.40 Flow Tier Dry-Base W + 13.77 Flow Tier Dry-Sub S
             + 11.23 Flow Tier Dry-Sub W + 2.99 Flow Tier Wet-3ps Su
             - 5.87 Flow Tier Wet-Base S - 5.02 Flow Tier Wet-Base W
             + 0.71 Flow Tier Wet-Sub S + 1.80 Flow Tier Wet-Sub W
Tukey Pairwise Comparisons: Response = Bot Sal psu, Term = rkm
Grouping Information Using the Tukey Method and 95% Confidence
rkm N
          Mean Grouping
1
    35 22.5435 A
5
    16 22.5235 A
```

10	35	18.8375	А	В		
15	16	17.6053	A	В		
22	35	14.3460		В	С	
26	10	7.9321			С	D
31	24	5.5846				D
42	42	3.5610				D
36	16	3.4260				D
25	6	2.5941				D

Means that do not share a letter are significantly different.

Flow Tier	Ν	Mean		G	rou	pin	g	
Dry-Sub S	23	25.6633	A					
Dry-Sub W	9	23.1218	A	В				
Avg-Sub W	37	16.5285		В	С			
Wet-3ps Su	18	14.8899		В	С	D		
Wet-Sub W	18	13.6969		В	С	D	Е	
Wet-Sub S	9	12.6096		В	С	D	Е	
Dry-Base W	8	10.4909			С	D	Е	F
Dry-1ps S	9	9.6231			С	D	Е	F
Wet-Base W	9	6.8774				D	Е	F
Avg-Base W	28	6.8395					Е	F
Wet-Base S	18	6.0235					Е	F
Dry-Base S	6	5.8926				D	Е	F
Avg-3ps S	43	2.3828						F

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Model 19. Regression analysis of Δ salinity psu versus daily average discharge measured at the Rosharon gage and river kilometer.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	6261.5	3130.76	51.27	0.000
CFS	1	1984.9	1984.89	32.51	0.000
rkm	1	4058.2	4058.20	66.46	0.000
Error	232	14166.3	61.06		
Lack-of-Fit	231	14166.3	61.33	*	*
Pure Error	1	0.0	0.00		
Total	234	20427.8			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7.81419	30.65%	30.05%	28.84%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-17.99	1.10	-16.40	0.000	
CFS	0.000687	0.000120	5.70	0.000	1.00
rkm	0.2916	0.0358	8.15	0.000	1.00

Regression Equation

 Δ Sal ppt = -17.99 + 0.000687 1dCFS + 0.2916 rkm

Model 20. Regression analysis of Δ salinity psu versus daily average discharge measured at the Rosharon gage.

Analysis of Va	arianc	e					
Source	DF	Adj SS	Adj MS	F-Value	P-Value		
Regression					0.000		
1dCFS				28.17	0.000		
Error Lack-of-Fit					0 1 0 1		
Pure Error				1.20	0.191		
Total			/4.05				
Model Summary							
S R-s 8.84402 10.79	-		R-sq(pre 9.5				
Coefficients							
Term							
Constant -12							
CFS 0.000723 0.000136 5.31 0.000 1.00							
Regression Equation							
Δ Sal ppt = -12.015 + 0.000723 CFS							

Model 21. ANOVA General linear model of Δ salinity versus river kilometer (rkm) and flow tier.

```
The following terms cannot be estimated and were removed:
  Flow Tier*rkm
Method
Factor coding (-1, 0, +1)
Rows unused 6
Factor Information
         Type Levels Values
Factor
Flow Tier Fixed 13 Avg-3ps S, Avg-Base W, Avg-Sub W, Dry-1ps S, Dry-Base S,
Dry-Base
                        W, Dry-Sub S, Dry-Sub W, Wet-3ps Su, Wet-Base S, Wet-Base W,
                        Wet-Sub S, Wet-Sub W
                    10 1, 5, 10, 15, 22, 25, 26, 31, 36, 42
rkm
          Fixed
Analysis of Variance
Source
              DF Adj SS Adj MS F-Value P-Value
 Flow Tier
              12 3645 303.76 5.92 0.000
 rkm 9 5055 5
rror 213 10927 51.30
              9 5639 626.55 12.21
                                           0.000
Error
 Lack-of-Fit 70 6001 85.73 2.49 0.000
 Pure Error 143
                    4926 34.45
             234
                  20428
Total
Model Summary
     S
        R-sq R-sq(adj) R-sq(pred)
7.16253 46.51%
                41.23%
                             34.59%
Regression Equation
\triangle Sal ppt = -7.326 + 5.60 Flow Tier Avg-3ps S + 2.17 Flow Tier Avg-Base W
           - 2.28 Flow Tier Avg-Sub W + 0.71 Flow Tier_Dry-1ps S
+ 2.06 Flow Tier Dry-Base S
           + 3.22 Flow Tier_Dry-Base W - 5.31 Flow Tier_Dry-Sub S
+ 3.35 Flow Tier Dry-Sub W
           - 5.99 Flow Tier_Wet-3ps Su + 1.88 Flow Tier_Wet-Base S
+ 1.51 Flow Tier Wet-Base
           W - 2.52 Flow Tier Wet-Sub S - 4.41 Flow Tier Wet-Sub W - 4.71 rkm 1
- 7.52 rkm 5
           - 4.98 rkm 10 - 5.81 rkm 15 - 3.41 rkm 22 + 8.22 rkm 25 + 2.87 rkm 26
           + 4.60 rkm 31 + 6.79 rkm 36 + 3.94 rkm 42
```

Method							
Rows unused 5							
Analysis of Va							
	DF Adj						
Regression							
rkm	1 2.	087 2.08	731	0.81	0.368		
ldCFS	1 8.	433 8.43	268	3.29	0.071		
rkm*1dCFS	1 0.	195 0.19	454	0.08	0.783		
Error	232 594.	516 2.56	257				
Lack-of-Fit				58.92	0.018		
Pure Error	1 0.	001 0.00	125				
Total							
Model Summary S R-sq 1.60080 4.40%	R-sq(adj 3.17						
Coefficients							
Term							
Constant							
rkm	0.0094	0.0104	0.90	0.368	2.04		
1dCFS -0.	000060 0.	000033	-1.81	0.071	2.33		
rkm*1dCFS -0.	000000 0.	000001	-0.28	0.783	3.34		
Regression Equa DO mgL = 8.036		rkm - 0.0	00060 1	dCFS - 0	.000000	rkm*1dCFS	

Model 22. Regression analysis of surface dissolved oxygen (mg/L) versus daily average discharge measured at the Rosharon gage and river kilometer.

Model 23. ANOVA General linear model of surface dissolved oxygen (mg/L) versus river kilometer (rkm) and flow tier.

The following terms cannot be estimated and were removed: Flow Tier*rkm Method Factor coding (-1, 0, +1)Rows unused 5 Factor Information Type Levels Values Factor Flow Tier Fixed 13 Avg-3ps S, Avg-Base W, Avg-Sub W, Dry-1ps S, Dry-Base S, Dry-Base W, Dry-Sub S, Dry-Sub W, Wet-3ps Su, Wet-Base S, Wet-Base W, Wet-Sub S, Wet-Sub W rkm Fixed 10 1, 5, 10, 15, 22, 25, 26, 31, 36, 42 Analysis of Variance Source DF Seq SS Contribution Adj SS Adj MS F-Value P-Value

 12
 288.506
 46.39%
 286.671
 23.8892
 15.62

 0.000 Flow Tier rkm 9 0.101 0.6818 1.5292 0.99% 6.136 0.45 0.909 52.62% 327.247 Error Lack-of-Fit 70 40.390 40.390 0.5770 0.29 1.000 6.49% Pure Error 144 286.857 46.13% 286.857 1.9921 235 621.889 100.00% Total Model Summary S R-sq R-sq(adj) PRESS R-sq(pred) 1.23660 47.38% 42.21% 390.891 37.14% Grouping Information Using the Tukey Method and 95% Confidence Flow Tier N Mean Grouping Dry-Base W 8 9.82660 A Avg-Base W 28 9.51323 A 9.29886 A B Wet-Base W 9 Wet-Sub W 18 9.19053 A B 37 8.13182 Avg-Sub W ВC Wet-Base S 18 7.90442 в С Dry-Sub S 23 7.52569 C D Dry-1ps S 9 7.49688 в с р Dry-Sub W 9 7.16243 C D Dry-Base S 7 6.75078 C D Avg-3ps S 43 6.64773 D Wet-Sub S 9 6.63886 C D Wet-3ps Su 18 6.46356 D Means that do not share a letter are significantly different.

		-					
Analysis of Va							
Source							
Regression							
		0.67					
1dCFS	1	5.55	5.5453	1.10	0.296		
rkm*1dCFS	1	3.20	3.1976	0.63	0.427		
Error	235	1187.07	5.0514				
Lack-of-Fit	234	1187.07	5.0730	2818.31	0.015		
Pure Error	1	0.00	0.0018				
Total	238	1254.78					
Model Summary							
S R-sq	R-s	q(adj) R-	sq(pred)				
2.24753 5.40%		4.19%	1.98%				
Coefficients							
Term	Coef	SE Coef	T-Value	e P-Value	e VIF		
Constant	5.850	0.413	14.16	5 0.000)		
rkm 0	.0057	0.0157	0.36	5 0.715	5 2.40		
1dCFS 0.0	00067	0.000064	1.05	5 0.296	5 3.44		
rkm*1dCFS 0.0	00002	0.000002	0.80	0.427	7 4.97		
Regression Equ	ation						
DO mgL = 5.850	+ 0.	0057 rkm +	0.000067	7 1dCFS +	0.000002	rkm*1dCFS	
2							

Model 24. Regression analysis of bottom dissolved oxygen (mg/L) versus daily average discharge measured at the Rosharon gage and river kilometer.

Model 25. ANOVA General linear model of bottom dissolved oxygen (mg/L) versus river kilometer (rkm) and flow tier.

The following terms cannot be estimated and were removed: Flow Tier*rkm Method Factor coding (-1, 0, +1)Rows unused 1 Factor Information Type Levels Values Factor Flow Tier Fixed 13 Avg-3ps S, Avg-Base W, Avg-Sub W, Dry-1ps S, Dry-Base S, Dry-Base W, Dry-Sub S, Dry-Sub W, Wet-3ps Su, Wet-Base S, Wet-Base W, Wet-Sub S, Wet-Sub W rkm Fixed 10 1, 5, 10, 15, 22, 25, 26, 31, 36, 42 Analysis of Variance Source DF Seq SS Contribution Adj SS Adj MS F-Value P-Value Flow Tier 12 493.1 39.30% 475.3 39.605 13.77 0.000 rkm 9 137.5 10.96% 137.5 15.280 5.31 0.000 Error 217 624.2 49.74% 624.2 2.876 251.1 3.587 Lack-of-Fit 70 20.01% 1.41 0.041 251.1 373.1 Pure Error 147 373.1 2.538 29.73% 238 1254.8 Total 100.00% Model Summary S R-sq R-sq(adj) PRESS R-sq(pred) 1.69601 50.26% 45.44% 769.067 38.71% Grouping Information Using the Tukey Method and 95% Confidence Flow Tier N Mean Grouping 9 9.40046 A Dry-Base W Avg-Base W 28 8.77699 A B Wet-Sub W 18 7.61552 A B С Wet-Base W 9 7.48829 A B С Wet-Base S 18 7.45107 A B C Avg-3ps S 43 6.61162 С Dry-1ps S C D E 9 6.50141 Avg-Sub W C D 37 6.44934 Dry-Base S 7 6.44318 BCDE Dry-Sub W 10 5.66481 CDE Wet-3ps Su 18 4.87332 DE Dry-Sub S 24 4.37700 Ε Wet-Sub S 9 4.08941 Ε Grouping Information Using the Tukey Method and 95% Confidence rkm N Mean Grouping 6 7.67543 A B C D 25 16 7.52280 A B 24 7.36459 A 36 31 39 7.11560 A B C 1 42 6.88264 A B C 42 10 6.76022 A B C D 26 35 5.86828 вср 10 15 16 5.70092 A B C D 5 16 5.58155 C D 22 35 5.48369 D Means that do not share a letter are significantly different.

Model 26. Regression analysis of the total number of nekton captured per bottom tow versus river discharge measured at Rosharon and river kilometer (rkm).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	3	1005423	335141	6.74	0.000
CFS	1	323081	323081	6.50	0.011
rkm	1	495455	495455	9.97	0.002
CFS*rkm	1	83805	83805	1.69	0.195
Error	366	18186506	49690		
Lack-of-Fit	120	8858670	73822	1.95	0.000
Pure Error	246	9327836	37918		
Total	369	19191929			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
222.912	5.24%	4.46%	3.47%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	194.2	29.1	6.67	0.000	
CFS	-0.01210	0.00474	-2.55	0.011	3.42
rkm	-3.57	1.13	-3.16	0.002	2.10
CFS*rkm	0.000225	0.000173	1.30	0.195	4.69

Regression Equation

Total = 194.2 - 0.01210 CFS - 3.57 rkm + 0.000225 CFS*rkm

Model 27. General linear model of total number of nekton collected with bottom trawls versus river kilometer (rkm) and flow tier.

The following terms cannot be estimated and were removed: Flow Tier*rkm Method Factor coding (-1, 0, +1)Factor Information Levels Values Factor Type 14 Avg-3ps S, Avg-Base W, Avg-Sub W, Dry-1ps S, Dry-Base S, Flow Tier Fixed Dry-Base W, Dry-Sub S, Dry-Sub Su, Dry-Sub W, Wet-2ps Su, Wet-Base S, Wet-Base W, Wet-Sub S, Wet-Sub W rkm Fixed 5 1, 10, 22, 31, 42 Analysis of Variance DF Seq SS Contribution Adj SS Adj MS F-Value P-Value Source 1569616 8.18% 1591656 122435 2.55 Flow Tier 13 0.002 4 699010 3.64% 699010 174753 3.63 0.006 rkm 352 16923303 88.18% 16923303 Error 48078 3129239 68027 16.30% 1.51 0.024 Lack-of-Fit 46 3129239 Pure Error 306 13794064 71.87% 13794064 45079 369 19191929 100.00% Total Model Summary S R-sq R-sq(adj) PRESS R-sq(pred) 219.266 11.82% 7.56% 18617936 2.99% Grouping Information Using the Tukey Method and 95% Confidence Flow Tier Ν Mean Grouping 25 226.509 A Dry-Sub W 30 180.167 A Wet-Sub W В Wet-2ps Su 15 160.600 A В 15 129.667 A Wet-Sub S В Avg-Base W 30 111.033 A B 97.032 A Avq-Sub W 42 В Dry-Sub Su 48 76.422 A В Dry-1ps S 24 72.880 A В Dry-Sub S 12 36.547 A В Dry-Base S 11 32.102 A В Wet-Base S 30 23.267 В Wet-Base W 15 19.733 A В Avg-3ps S 45 10.889 В Dry-Base W 28 2.913 В Grouping Information Using the Tukey Method and 95% Confidence Mean Grouping rkm Ν 81 149.828 A 1 22 84 108.273 A В 10 81 87.776 A В 31 44 47.790 A В 42 80 27.677 В Means that do not share a letter are significantly different.

Model 28. Regression analysis of the total number of nekton captured per bottom tow versus bottom salinity.

Analysis of Va	riance					
Source Regression Sal psu Error Lack-of-Fit Pure Error Total	1 74 368 1844 101 906 267 937	9256 749 9256 749 2673 50 3025 89 9648 35	256 14. 256 14. 116 733 2.	95 95	0.000 0.000	
Model Summary						
S R-sq R-sq(adj) R-sq(pred) 223.866 3.90% 3.64% 2.84%						
Coefficients						
Term Coe Constant 33. Sal psu 3.49	7 18.0	1.87	0.062			
Regression Equation						
Total = 33.7 +	3.499 Sal	psu				

Analysis of Va	riance					
Source Regression DO mgL Error Lack-of-Fit Pure Error Total	1 1 368 191 112 95 256 96	1222 1 90707 52 69006 85 21701 37	.222 0 .222 0 .149 .5438 2	.02 .02	0.878	
Model Summary						
S R-sq 228.361 0.01%	R-sq(ad 0.0					
Coefficients						
Term Coef Constant 81.8 DO mgL 0.76	34.4	2.38	0.018			
Regression Equ	ation					
Total = 81.8 +	0.76 DO 1	mgL				

Model 29. Regression analysis of the total number of nekton captured per bottom tow versus bottom dissolved oxygen.

Model 30. Regression analysis of the total number of estuarine nekton captured per bottom tow versus bottom river kilometer and average daily discharge measured at the Rosharon gage.

Analysis of Variance							
Source	DF	Adj SS	Adj MS	F-Value	P-Value		
Regression	3	1159383	386461	r varue 7.77	0.000		
rkm	1	608182	608182	12.23	0.001		
CFS	1	340647	340647	6.85	0.009		
rkm*CFS	1	94969	94969	1.91	0.168		
Error	366	18202009	49732				
Lack-of-Fit	120	8938667	74489	1.98	0.000		
Pure Error	246	9263342	37656				
Total	369	19361392					

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
223.007	5.99%	5.22%	4.23%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	195.5	29.1	6.71	0.000	
rkm	-3.95	1.13	-3.50	0.001	2.10
CFS	-0.01242	0.00475	-2.62	0.009	3.42
rkm*CFS	0.000239	0.000173	1.38	0.168	4.69

Regression Equation

Tot Est Nek = 195.5 - 3.95 rkm - 0.01242 CFS + 0.000239 rkm*CFS

The following terms cannot be estimated and were removed: Flow Tier*rkm Method Factor coding (-1, 0, +1)Factor Information Levels Values Factor Type 14 Avg-3ps S, Avg-Base W, Avg-Sub W, Dry-1ps S, Dry-Base S, Flow Tier Fixed Dry-Base W, Dry-Sub S, Dry-Sub Su, Dry-Sub W, Wet-2ps Su, Wet-Base S, Wet-Base W, Wet-Sub S, Wet-Sub W rkm Fixed 5 1, 10, 22, 31, 42 Analysis of Variance DF Seq SS Contribution Adj SS Adj MS F-Value P-Value Source 1477199 7.63% 1487294 114407 Flow Tier 13 2.37 0.005 4 860465 4.44% 860465 215116 4.45 0.002 rkm 87.93% 17023728 352 17023728 Error 48363 3103069 67458 16.03% 1.48 0.029 Lack-of-Fit 46 3103069 Pure Error 306 13920660 71.90% 13920660 45492 369 19361392 100.00% Total Model Summary S R-sq R-sq(adj) PRESS R-sq(pred) 219.916 12.07% 7.83% 18726167 3.28% Grouping Information Using the Tukey Method and 95% Confidence Flow Tier Ν Mean Grouping 25 221.181 A Dry-Sub W Wet-Sub W 30 166.067 А В Wet-2ps Su 15 136.000 A В Wet-Sub S 15 110.267 A В Avg-Base W 30 103.233 A В Avq-Sub W 86.436 A 42 B Dry-Sub Su 48 71.040 A В Dry-1ps S 24 61.770 A В Dry-Sub S 12 30.853 A В Dry-Base S 11 24.309 A В Wet-Base W 15 18.667 A В 17.033 В Wet-Base S 30 45 7.400 В Avg-3ps S Dry-Base W 28 -7.589 В Grouping Information Using the Tukey Method and 95% Confidence rkm Ν Mean Grouping 81 148.928 A 1 84 100.361 A 22 R 10 81 86.633 A В 42 80 20.715 В 31 44 17.174 В Means that do not share a letter are significantly different.

Model 31. General linear model of total number of estuarine nekton collected with bottom trawls versus river kilometer (rkm) and flow tier.

Model 32. Regression analysis of the total number of estuarine nekton captured per bottom tow versus bottom salinity.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	1009879	1009879	20.25	0.000
Sal psu	1	1009879	1009879	20.25	0.000
Error	368	18351513	49868		
Lack-of-Fit	101	9074584	89847	2.59	0.000
Pure Error	267	9276929	34745		
Total	369	19361392			

Model Summary

S R-sq R-sq(adj) R-sq(pred) 223.312 5.22% 4.96% 4.17%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	18.5	17.9	1.03	0.302	
Sal psu	4.062	0.903	4.50	0.000	1.00

Regression Equation

Tot Est Nek = 18.5 + 4.062 Sal psu

Analysis of Va	riance					
Source Regression DO mgL Error Lack-of-Fit Pure Error Total	1 368 1935 112 980 256 955	2428 2 2428 2 2428 2 8964 52 1917 87 7047 37	428 0. 428 0. 606 517 2.	05 0.3	830 830	
Model Summary						
S R-sq 229.360 0.01%	R-sq(adj 0.00					
Coefficients						
Term Coe Constant 87. DO mgL -1.0	1 34.5	2.52	0.012			
Regression Equation						
Tot Est Nek =	87.1 - 1.0	7 DO mgL				

Model 33. Regression analysis of the total number of estuarine nekton captured per bottom tow versus bottom dissolved oxygen.

Model 34. Regression analysis of the number of nekton taxa captured per bottom tow versus river kilometer (rkm) and annual daily discharge at the Rosharon gage.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	3	1095.80	365.267	62.77	0.000
rkm	1	581.86	581.856	99.99	0.000
CFS	1	225.03	225.032	38.67	0.000
rkm*CFS	1	56.35	56.346	9.68	0.002
Error	366	2129.78	5.819		
Lack-of-Fit	120	1393.78	11.615	3.88	0.000
Pure Error	246	736.00	2.992		
Total	369	3225.58			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.41227	33.97%	33.43%	32.65%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.239	0.315	22.99	0.000	
rkm	-0.1223	0.0122	-10.00	0.000	2.10
CFS	-0.000319	0.000051	-6.22	0.000	3.42
rkm*CFS	0.000006	0.000002	3.11	0.002	4.69

Regression Equation

No. Taxa = 7.239 - 0.1223 rkm - 0.000319 CFS + 0.000006 rkm*CFS

The following terms cannot be estimated and were removed: Flow Tier*rkm Method Factor coding (-1, 0, +1)Factor Information Factor Levels Values Type 14 Avg-3ps S, Avg-Base W, Avg-Sub W, Dry-1ps S, Dry-Base S, Flow Tier Fixed Dry-Base W, Dry-Sub S, Dry-Sub Su, Dry-Sub W, Wet-2ps Su, Wet-Base S, Wet-Base W, Wet-Sub S, Wet-Sub W rkm Fixed 5 1, 10, 22, 31, 42 Analysis of Variance DF Seq SS Contribution Adj SS Adj MS F-Value P-Value Source Flow Tier 13 457.3 14.18% 423.5 32.575 5.88 0.000 rkm 4 818.2 25.36% 818.2 204.539 36.92 0.000 60.46% 1950.1 352 1950.1 5.540 Error 2.76 0.000 Lack-of-Fit 46 572.3 17.74% 572.3 12.442 Pure Error 306 1377.8 42.72% 1377.8 4.503 369 3225.6 100.00% Total Model Summary S R-sq R-sq(adj) PRESS R-sq(pred) 2.35375 39.54% 36.62% 2143.63 33.54% Grouping Information Using the Tukey Method and 95% Confidence Flow Tier N Mean Grouping Wet-2ps Su 15 4.93333 A В Dry-1ps S 24 4.92788 A В Wet-Sub W 30 4.76667 А В Avg-Sub W 42 4.75324 A Wet-Sub S 15 4.73333 Α В 25 4.71894 A Dry-Sub W В Dry-Sub Su 48 4.19872 A В 12 3.67788 A Dry-Sub S B C Avg-Base W 30 3.03333 A в С Dry-Base W 28 2.99012 A ВC Wet-Base S 30 2.76667 B C Wet-Base W 15 2.46667 A B C С Avg-3ps S 45 2.24444 Dry-Base S 11 1.37390 С Means that do not share a letter are significantly different. Grouping Information Using the Tukey Method and 95% Confidence rkm Ν Mean Grouping 81 5.66296 1 А 10 81 5.17674 Α 84 2.87381 22 В 31 44 2.72952 В 42 80 1.98024 В Means that do not share a letter are significantly different.

Model 35. ANOVA general linear model of the number of nekton taxa captured per bottom tow versus river kilometer (rkm) and flow tier.

Model 36. Regression analysis of the number of nekton taxa captured per bottom tow versus bottom salinity.

Analysis of Va	rianc	e				
Source Regression Sal psu Error Lack-of-Fit Pure Error Total	1 368 101 267	683.0 683.0 2542.6 1758.0 784.6	683.028 683.028 6.909 17.406 2.939	98.86 98.86 5.92	0.000 0.000	
Total 369 3225.6 Model Summary S R-sq R-sq(adj) R-sq(pred) 2.62852 21.18% 20.96% 20.28%						
Coefficients Term Co Constant 2.2 Sal psu 0.10	24	0.211	10.53	0.000		
Regression Equation No. Taxa = 2.224 + 0.1056 Sal psu						

Analysis of Va	rianc	e				
Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Regression	1	23.86	23.856	2.74	0.099	
DO mgL	1	23.86	23.856	2.74	0.099	
Error						
Lack-of-Fit	112	2313.17	20.653	5.95	0.000	
Pure Error	256	888.56	3.471			
Total	369	3225.58				
Model Summary S R-sq R-sq(adj) R-sq(pred) 2.94963 0.74% 0.47% 0.00%						
Coefficients						
Term C	oef	SE Coef	T-Value	P-Value	VIF	
Constant 4.	514	0.444	10.17	0.000		
DO mgL -0.1	061	0.0641	-1.66	0.099	1.00	
Regression Equation No. Taxa = 4.514 - 0.1061 DO mgL						

Model 37. Regression analysis of the number of nekton taxa captured per bottom tow versus bottom dissolved oxygen.

Model 38. Regression analysis of the number of estuarine nekton taxa captured per bottom tow versus river kilometer (rkm) and annual daily discharge at the Rosharon gage.

Analysis of Variance						
Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Regression	3	1793.27	597.758	106.20	0.000	
rkm	1	948.33	948.327	168.48	0.000	
CFS	1	277.39	277.394	49.28	0.000	
rkm*CFS	1	63.22	63.224	11.23	0.001	
Error	366	2060.10	5.629			
Lack-of-Fit	120	1406.94	11.724	4.42	0.000	
Pure Error	246	653.17	2.655			
Total	369	3853.38				

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.37249	46.54%	46.10%	45.43%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.423	0.310	23.97	0.000	
rkm	-0.1562	0.0120	-12.98	0.000	2.10
CFS	-0.000354	0.000050	-7.02	0.000	3.42
rkm*CFS	0.00006	0.000002	3.35	0.001	4.69

Regression Equation

No Est Spp = 7.423 - 0.1562 rkm - 0.000354 CFS + 0.000006 rkm*CFS

Model 39. ANOVA general linear model of the number of estuarine nekton taxa captured per bottom tow versus river kilometer (rkm) and flow tier.

The following terms cannot be estimated and were removed: Flow Tier*rkm Method Factor coding (-1, 0, +1)Factor Information Factor Type Levels Values Flow Tier Fixed 14 Avg-3ps S, Avg-Base W, Avg-Sub W, Dry-1ps S, Dry-Base S, Dry-Base W, Dry-Sub S, Dry-Sub Su, Dry-Sub W, Wet-2ps Su, Wet-Base S, Wet-Base W, Wet-Sub S, Wet-Sub W rkm Fixed 5 1, 10, 22, 31, 42 Analysis of Variance Adj MS F-Value P-Value Source DF Seq SS Contribution Adj SS 546.014.17%456.535.1161432.737.18%1432.7358.164 Flow Tier 13 35.116 6.59 0.000 4 1432.7 67.25 0.000 rkm 48.65% 1874.7 352 1874.7 Error 5.326 13.61% 524.6 11.404 524.6 2.58 0.000 Lack-of-Fit 46 Pure Error 306 1350.1 35.04% 1350.1 4.412 369 3853.4 Total 100.00% Model Summary S R-sq R-sq(adj) PRESS R-sq(pred) 2.30777 51.35% 49.00% 2062.11 46.49% Grouping Information Using the Tukey Method and 95% Confidence Flow Tier Ν Mean Grouping Dry-Sub W 25 4.37254 A Avg-Sub W 42 4.09489 A 4.00000 A B C Wet-2ps Su 15 Dry-Sub Su 48 3.92523 A B Dry-1ps S 24 3.92523 A B C Wet-Sub S 15 3.86667 A B C D Wet-Sub W 30 3.60000 A B C Dry-Sub S 12 3.21690 A B C D E Dry-Base W 28 2.35495 A B C D E Avg-Base W 30 2.20000 BCDE Wet-Base S 30 1.93333 C D E Wet-Base W 15 1.86667 A B C D E Avg-3ps S 45 1.31111 E Dry-Base S 11 0.84048 DΕ rkm Ν Mean Grouping 81 5.60733 1 Α 81 5.02109 A 10 84 2.17307 22 В 31 44 1.16579 B C 42 80 0.85701 С Means that do not share a letter are significantly different.

Model 40. Regression analysis of the number of estuarine nekton taxa captured per bottom tow versus bottom salinity.

Analysis of Variance								
Regression Sal psu	1 368 101 267	1402.1 1402.1 2451.2 1790.4 660.8	1402.14 1402.14 6.66 17.73 2.47	210.50 7.16	0.000 0.000			
Model Summary								
S R-sq R-sq(adj) R-sq(pred) 2.58088 36.39% 36.21% 35.67%								
Coefficients								
Term Co Constant 0.8 Sal psu 0.15	98	0.207	4.33	0.000				
F								
Regression Equation								
No Est Spp = $0.898 + 0.1514$ Sal psu								

Model 41. Regression analysis of the number of estuarine nekton taxa captured per tow by bottom trawl versus bottom dissolved oxygen.

Analysis of Variance

Source Regression DO mgL Error Lack-of-Fit Pure Error Total	1 368 112 256	122.3 122.3 3731.1 2896.9 834.2	122.271 122.271 10.139 25.865 3.259	12.06 12.06	0.001 0.001			
Model Summary								
S R-sq R-sq(adj) R-sq(pred) 3.18416 3.17% 2.91% 2.06% Coefficients								
Term Constant 4. DO mgL -0.24	754	0.479	9.92	0.000				
Regression Equation								
No Est Spp = 4.754 - 0.2401 DO mgL								

Model 42. Regression analysis of the number of shoreline nekton captured with the shallow beam trawl versus river discharge measured at Rosharon and river kilometer (rkm).

Analysis of Variance: Beam Trawl							
Source	DF	Adj SS	Adj MS	F-Value	P-Value		
Regression	3	-	-	3.97			
			175941	4.59	0.033		
rkm	1	175941 422850	422850	11.04	0.001		
CFS*rkm				4.39			
		13218955					
Lack-of-Fit	113	10182209	90108	6.88	0.000		
Pure Error							
Total	348	13675411					
Model Summary							
S R-sq	R-s	q(adj) R-	sq(pred)				
195.744 3.34%		2.50%	0.20%				
Coefficients							
Term							
Constant 1							
CFS -0.0							
rkm -							
CFS*rkm 0.00	0316	0.000151	2.09	0.037	4.72		
Regression Equation							
Grand Total = 112.4 - 0.00898 CFS - 3.33 rkm + 0.000316 CFS*rkm							

Model 43. ANOVA general linear model of the number of shoreline nekton captured with the beam trawl versus river kilometer (rkm) and flow tier.

The following terms cannot be estimated and were removed: Flow Tier*rkm Method Factor coding (-1, 0, +1)Factor Information Factor Type Levels Values 13 Avg-3ps S, Avg-Base S, Avg-Sub W, Dry-1ps S, Dry-Base S, Flow Tier Fixed Dry-Base W, Dry-Sub S, Dry-Sub Su, Dry-Sub W, Wet-Base S, Wet-Base W, Wet-Sub S, Wet-Sub W rkm Fixed 5 1, 10, 22, 31, 42 Analysis of Variance Adj SS Adj MS F-Value P-Value Seq SS Contribution Source DF Flow Tier 12 1863756 13.63% 1903799 158650 4.67 0.000 544909 136227 rkm 4 544909 3.98% 4.01 0.003 Error 332 11266746 82.39% 11266746 33936 7036411 Lack-of-Fit 42 Pure Error 290 51.45% 7036411 167534 11.48 0.000 4230335 30.93% 4230335 14587 348 13675411 Total 100.00% Model Summary S R-sq R-sq(adj) PRESS R-sq(pred) 13.64% 12658361 184.217 17.61% 7.44% Grouping Information Using the Tukey Method and 95% Confidence Flow Tier Ν Mean Grouping Dry-Sub W 22 311.711 A Dry-Base S 12 89.106 В Dry-Base W 24 55.648 В 23.981 Dry-1ps S 24 B Wet-Base W 15 16.467 В 13.460 Dry-Sub Su 48 В Avg-Base S 30 7.067 В 5.767 Wet-Base S 30 В Avg-Sub W 45 5.533 В Avg-3ps S 45 5.467 В Wet-Sub W 27 3.992 В Wet-Sub S 15 1.267 В Dry-Sub S 12 1.190 В * Means that do not share a letter are significantly different. Grouping Information Using the Tukey Method and 95% Confidence Mean Grouping rkm N 76 115.139 A 1 40.014 A 31 42 В 10 78 20.555 В 22 75 18.180 В 42 78 14.055 В

Means that do not share a letter are significantly different.

Model 44. Regression analysis of the number of shoreline nekton captured with the shallow beam trawl versus salinity.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	894560	894560	24.29	0.000
Sal psu	1	894560	894560	24.29	0.000
Error	347	12780850	36832		
Lack-of-Fit	93	9733735	104664	8.72	0.000
Pure Error	254	3047115	11997		
Total	348	13675411			

Model Summary

S R-sq R-sq(adj) R-sq(pred) 191.918 6.54% 6.27% 1.30%

Coefficients

 Term
 Coef
 SE Coef
 T-Value
 P-Value
 VIF

 Constant
 -2.9
 12.7
 -0.23
 0.816

 Sal psu
 5.99
 1.21
 4.93
 0.000
 1.00

Regression Equation

Grand Total = -2.9 + 5.99 Sal psu

Model 45. Regression analysis of the number of shoreline nekton captured with the shallow beam trawl versus dissolved oxygen.

Analysis of Va	rianc	e							
Source	DF	Adj	SS	Adj M	S I	F-Value	e P-V	alue	
Regression	1	3738	42	37384	2	9.75	5 0	.002	
		3738				9.75	5 0	.002	
Error									
Lack-of-Fit	99	98574	27	9957	0	7.1	7 0	.000	
Pure Error	248	34441	42	1388	8				
Total	348	136754	11						
Model Summary									
S R-sq 195.788 2.73%									
Coefficients									
Term Coef SE Coef T-Value P-Value VIF Constant -115.5 48.9 -2.36 0.019									
DO mgL 18.	52	5.93		3.12		0.002	1.00		
Regression Equation									
Grand Total = $-115.5 + 18.52$ DO mgL									

Model 46. Regression analysis of the total number of shoreline estuarine nekton collected with the shallow water beam trawl versus river discharge measured at Rosharon and river kilometer (rkm).

Analysis o	f Varianc	e				
		Adj SS				
Regression	3	468097	156032	4.07	0.007	
		172978		4.51		
rkm	1	428010	428010	11.17	0.001	
CFS*rkm	1	160108	160108	4.18	0.042	
Error	345	13219220	38317			
Lack-of-	Fit 113	10183139	90116	6.89	0.000	
Pure Err	or 232	3036081	13087			
Total	348	13687317				
Model Summ S 1 195.746 3	R-sq R-s	q(adj) R-				
199.740 9	. 72 0	2.000	0.200			
Coefficien	ts					
Term	Coef	SE Coef	T-Value	P-Value	VIF	
Constant	112.4	26.0	4.32	0.000		
CFS						
rkm						
CFS*rkm						
Regression	-					
Total Est (Catch = 1	12.4 - 0.0	0891 CFS	- 3.35 rl	cm + 0.000	309 CFS*rkm

Model 47. ANOVA general linear model of the total number of shoreline estuarine nekton captured with the beam trawl versus river kilometer (rkm) and flow tier.

The following terms cannot be estimated and were removed: Flow Tier*rkm Method Factor coding (-1, 0, +1)Factor Information Factor Type Levels Values Flow Tier Fixed 13 Avg-3ps S, Avg-Base S, Avg-Sub W, Dry-1ps S, Dry-Base S, Dry-Base W, Dry-Sub S, Dry-Sub Su, Dry-Sub W, Wet-Base S, Wet-Base W, Wet-Sub S, Wet-Sub W 5 1, 10, 22, 31, 42 rkm Fixed Analysis of Variance Source DF Seq SS Contribution Adj SS Adj MS F-Value P-Value 12 1876540 13.71% 1915480 159623 4.71 0.000 Flow Tier rkm 4 554651 4.05% 554651 138663 4.09 0.003

 rror
 332
 11256126

 Lack-of-Fit
 42
 7026664

 Pure Error
 290
 4229463

 otal
 348
 13687317

 82.24%
 11256126
 33904

 51.34%
 7026664
 167302

 30.90%
 4229463
 14584
 Error 11.47 0.000 Total 100.00% Model Summary R-sq R-sq(adj) PRESS R-sq(pred) S 184.130 17.76% 13.80% 12646022 7.61% Grouping Information Using the Tukey Method and 95% Confidence Flow Tier Ν Mean Grouping 22 311.706 A Dry-Sub W Dry-Base S 12 89.052 В Dry-Base W 24 55.010 В Dry-1ps S 24 23.302 В 13.406 Dry-Sub Su 48 В 15 Wet-Base W 9.067 В 6.233 Avg-Base S 30 В 5.200 Avg-Sub W 45 В Wet-Base S 30 4.800 В Avg-3ps S 45 3.867 В Wet-Sub W 27 3.649 В Dry-Sub S 12 1.135 В Wet-Sub S 15 1.133 В *Means that do not share a letter are significantly different. Grouping Information Using the Tukey Method and 95% Confidence rkm N Mean Grouping 76 114.721 A 1 31 42 38.789 A В 20.289 10 78 В 75 17.756 22 В 78 11.353 42 В Means that do not share a letter are significantly different.

Model 48. Regression analysis of the total number of shoreline estuarine nekton collected with the shallow water beam trawl versus salinity.

Analysis of Variance

Source Regression Sal psu Error Lack-of-Fit Pure Error Total	1 91 1 91 347 1277 93 972 254 304	.2987 912 .2987 912 74330 36 29439 104 14892 11	987 24 987 24 814 618 8	1.80 1.80	0.000 0.000	
Model Summary						
S R-sq 191.869 6.67%	R-sq(adj 6.40					
Coefficients						
Term Coef Constant -4.2 Sal psu 6.05	12.7		0.743			
Regression Equation						
Total Est Catch = $-4.2 + 6.05$ Sal psu						

Analysis of Va	rianc	е				
Source	DF	Adj S	S Adj M	IS F-Valu	e P-Valu	e
Regression	1	36818	36 36818	6 9.5	9 0.00	2
DO mgL	1	36818	36 36818	6 9.5	9 0.00	2
Error						
Lack-of-Fit					9 0.00	0
Pure Error				3		
Total	348	1368731	.7			
Model Summary S R-sq 195.917 2.69%	R-s					
Coefficients						
Term Co	ef S	E Coef	T-Value	P-Value	VIF	
Constant -115						
DO mgL 18.						
Regression Equation Total Est Catch = -115.2 + 18.38 DO mgL						

Model 49. Regression analysis of the total number of shoreline estuarine nekton collected with the shallow water beam trawl versus dissolved oxygen.

Model 50. Regression analysis of the number of shoreline nekton taxa captured by the shoreline beam trawl versus river discharge measured at Rosharon and river kilometer (rkm).

Analysis of Variance								
Source	DF	Adj SS	Adj MS	F-Value	P-Value			
Regression	3	81.71	27.2375	8.35	0.000			
CFS	1	7.01	7.0051	2.15	0.144			
rkm	1	29.93	29.9340	9.18	0.003			
CFS*rkm	1	0.08	0.0817	0.03	0.874			
Error	345	1124.87	3.2605					
Lack-of-Fit	113	723.53	6.4029	3.70	0.000			
Pure Error	232	401.33	1.7299					
Total	348	1206.58						

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.80568	6.77%	5.96%	4.60%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.582	0.240	10.76	0.000	
CFS	0.000057	0.000039	1.47	0.144	3.49
rkm	-0.02802	0.00925	-3.03	0.003	2.06
CFS*rkm	0.00000	0.000001	0.16	0.874	4.72

Regression Equation

No Taxa = 2.582 + 0.000057 CFS - 0.02802 rkm + 0.000000 CFS*rkm

Model 51. ANOVA general linear model of the number of shoreline nekton taxa collected per tow with the beam trawl versus river kilometer (rkm) and flow tier.

The following terms cannot be estimated and were removed: Flow Tier*rkm Method Factor coding (-1, 0, +1)Factor Information Type Levels Values Factor Flow Tier Fixed 13 Avg-3ps S, Avg-Base S, Avg-Sub W, Dry-1ps S, Dry-Base S, Dry-Base W, Dry-Sub S, Dry-Sub Su, Dry-Sub W, Wet-Base S, Wet-Base W, Wet-Sub S, Wet-Sub W rkm Fixed 5 1, 10, 22, 31, 42 Analysis of Variance DF Seq SS Contribution Adj SS Adj MS F-Value P-Value Source Flow Tier 12 251.64 20.86% 252.14 21.012 7.91 0.000 72.99 6.05% 72.99 18.247 6.87 0.000 rkm 4 332 881.95 73.09% 881.95 2.656 Error Lack-of-Fit 42 218.59 18.12% 218.59 5.204 2.28 0.000 663.36 Pure Error 290 54.98% 663.36 2.287 348 1206.58 Total 100.00% Model Summary S R-sq R-sq(adj) PRESS R-sq(pred) 1.62987 26.91% 23.38% 978.938 18.87% Grouping Information Using the Tukey Method and 95% Confidence Flow Tier Ν Mean Grouping Dry-Base S 12 3.81987 A Avg-Base S 30 3.46667 A Dry-1ps S 24 3.31987 A Dry-Sub W 22 3.18049 A Avg-Sub W 45 2.75556 A B Avg-3ps S 45 2.68889 A B C Wet-Base W 15 2.26667 A B C D Dry-Base W 24 2.02820 A B C D C D Dry-Sub Su 48 1.59070 Wet-Base S 30 1.36667 D Wet-Sub W 27 1.24376 D Dry-Sub S 12 1.06987 B C D Wet-Sub S 15 1.00000 D Grouping Information Using the Tukey Method and 95% Confidence rkm Ν Mean Grouping 76 2.93906 A 1 75 2.48596 A 22 В 42 2.23822 A B C 31 10 78 2.19989 B C 42 78 1.59733 С * Means that do not share a letter are significantly different.

Analysis of Va	rianc	е				
Source	DF	Adj S	S Adj MS	F-Value	P-Value	
Regression				0.63		
-				0.63	0.429	
			0 3.471			
Lack-of-Fit					0.000	
Pure Error						
Total	348	1206.5	8			
Model Summary S R-sq R-sq(adj) R-sq(pred) 1.86303 0.18% 0.00% 0.00%						
1.00303 0.10%		0.00%	0.0	0.5		
Coefficients						
Term Co Constant 2.2						
Sal psu 0.00	93	0.0118	0.79	0.429	1.00	
Regression Equation						
No Taxa = 2.252 + 0.0093 Sal psu						

Model 52. Regression analysis of the number of shoreline nekton taxa captured with the shallow beam trawl versus salinity.

Analysis of Var	riance						
Source Regression DO mgL Error Lack-of-Fit Pure Error Total	1 0.2 347 1206.4 99 731.0 248 475.3	10 0.0963 10 0.0963 48 3.4768 09 7.3847 39 1.9168	9 0.03 9 0.03 9 3.85	0.868			
Model Summary S R-sq 1.86464 0.01%	R-sq(adj) 0.00%						
Coefficients							
Term Coef SE Coef T-Value P-Value VIF Constant 2.234 0.466 4.80 0.000 DO mgL 0.0094 0.0565 0.17 0.868 1.00							
Regression Equation							
No Taxa = 2.234 + 0.0094 DO mgL							

Model 53. Regression analysis of the number of shoreline nekton taxa captured with the shallow beam trawl versus dissolved oxygen.

Model 54. Regression analysis of the number of estuarine taxa captured with shoreline beam trawls versus river discharge measured at Rosharon and river kilometer (rkm).

Analysis of Variance

Source Regression	DF 2	Adj SS 141.04	Adj MS 70.518	F-Value 23.16	P-Value 0.000
CFS	1	9.76	9.759	3.21	0.074
rkm	1	134.84	134.841	44.29	0.000
Error	346	1053.48	3.045		
Lack-of-Fit	114	696.81	6.112	3.98	0.000
Pure Error	232	356.67	1.537		
Total	348	1194.52			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.74492	11.81%	11.30%	10.27%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.708	0.181	14.95	0.000	
CFS	0.000036	0.000020	1.79	0.074	1.00
rkm	-0.04155	0.00624	-6.65	0.000	1.00

Regression Equation

No Est Spp = 2.708 + 0.000036 CFS - 0.04155 rkm

Model 55. ANOVA general linear model of the number of shoreline estuarine taxa collected per tow with the beam trawl versus river kilometer (rkm) and flow tier.

The following terms cannot be estimated and was removed: Flow Tier*rkm Method Factor coding (-1, 0, +1)Factor Information Type Levels Values Factor 13 Avg-3ps S, Avg-Base S, Avg-Sub W, Dry-1ps S, Dry-Base S, Flow Tier Fixed Dry-Base W, Dry-Sub S, Dry-Sub Su, Dry-Sub W, Wet-Base S, Wet-Base W, Wet-Sub S, Wet-Sub W rkm Fixed 5 1, 10, 22, 31, 42 Analysis of Variance DF Seq SS Contribution Adj SS Adj MS F-Value P-Value Source Flow Tier 12 242.5 20.30% 235.9 19.658 8.12 0.000 rkm 148.0 804.0 148.0 37.002 4 12.39% 15.28 0.000 rror 332 804.0 Lack-of-Fit 42 194.4 Error 67.31% 804.0 2.422 16.27% 194.4 4.628 2.20 0.000 Pure Error 290 609.6 51.04% 609.6 2.102 348 1194.5 100.00% Total Model Summary PRESS R-sq(pred) S R-sq R-sq(adj) 1.55619 32.69% 29.45% 891.687 25.35% Grouping Information Using the Tukey Method and 95% Confidence Flow Tier Ν Mean Grouping Dry-Base S 12 3.73326 A Dry-Sub W 22 3.10676 A B Avg-Base S 30 3.03333 A B Dry-1ps S 24 3.02493 A B Avg-Sub W 45 2.55556 A B C Avg-3ps S 45 1.86667 B C D Dry-Base W 24 1.81660 B C D Wet-Base W 15 1.73333 A B C D Dry-Sub Su 48 1.50410 C D Wet-Sub W 27 1.00193 D Wet-Base S 30 1.00000 D Dry-Sub S 12 0.98326 Wet-Sub S 15 0.86667 0.98326 C D D Grouping Information Using the Tukey Method and 95% Confidence Ν Mean Grouping rkm 76 2.89259 A 1 22 75 2.36816 A В 10 78 2.16229 В 31 42 1.61714 B C 42 78 1.04690 С Means that do not share a letter are significantly different.

Analysis of Variance								
Source Regression Sal psu Error Lack-of-Fit Pure Error Total	1 21. 1 21. 347 1172. 93 723. 254 449.	80 21.799 80 21.799 72 3.380 58 7.780 13 1.768	6.45 6.45 4.40	0.012 0.012				
Model Summary S R-sq 1.83837 1.82%	R-sq(adj) 1.54%							
Coefficients								
Term Coe Constant 1.8 Sal psu 0.02	57 0.121	15.30	0.000					
Regression Equa	ation							
No Est Spp = 1	.857 + 0.02	96 Sal psu						

Model 56. Regression analysis of the number of shoreline estuarine nekton taxa collected with the beam trawl versus surface salinity.

Model 57. Regression analysis of the number of shoreline estuarine nekton taxa collected with the shallow water beam trawl versus surface dissolved oxygen.

Analysis of Va	rianc	е				
Source Regression DO mgL	1 1	1 0.20 1 0.20		2033 2033	0.06 0.06	0.808
Error Lack-of-Fit Pure Error Total	99 248	753.6 440.6	7 7. 4 1.	6129	4.28	0.000
Model Summary						
S R-sq 1.85521 0.02%						
Coefficients						
Term Coe Constant 1.92						
DO mgL 0.01						
Regression Equa	ation					
No Est Spp = 1	.927	+ 0.013	7 DO	mgL		

Appendix K. Results of ANOSIM Analysis of Beam and Otter Trawl Nekton Collections

Beam Trawl -					
Flow Tier	Flow Tier	Sig %	Flow Tier	Flow Tier	Sig %
Dry-Base W	Avg-3ps S	0.1	Dry-Sub Su	Avg-3ps S	0.1
Dry-1ps S	Avg-3ps S	0.1	Dry-Sub Su	Wet-Base S	0.1
Dry-1ps S	Avg-Sub W	0.3	Dry-Sub W	Avg-3ps S	0.1
Dry-Base S	Avg-3ps S	0.4	Avg-Sub W	Avg-3ps S	0.1
Dry-Base S	Dry-Sub W	1.2	Avg-3ps S	Wet-Sub W	0.1
Dry-Base S	Avg-Base W	1.2	Avg-3ps S	Wet-Base W	0.1
Dry-Base S	Avg-Sub W	2.1	Avg-3ps S	Wet-Base S	0.1
Dry-Base W	Avg-Sub W	3.0	Avg-3ps S	Wet-Sub S	0.2
Dry-Base S	Wet-Sub W	3.7	Dry-Sub S	Avg-3ps S	0.4
Dry-1ps S	Dry-Sub W	5.9	Avg-Base W	Wet-Sub W	0.4
Dry-Base S	Dry-Sub Su	6.1	Avg-Base W	Wet-Base W	0.4
Dry-Base W	Dry-Sub W	6.2	Avg-Base W	Wet-Base S	0.8
Dry-Base W	Avg-Base W	7.4	Dry-Sub S	Avg-Sub W	1.0
Dry-Sub S	Dry-Sub Su	8.0	Dry-Sub S	Dry-Sub W	1.2
Dry-1ps S	Avg-Base W	10.4	Dry-Sub S	Avg-Base W	1.2
Dry-Base W	Wet-Sub W	12.3	Dry-Sub W	Avg-Base W	1.2
Dry-1ps S	Wet-Sub W	14.8	Dry-Sub W	Wet-Base W	1.2
Dry-Base W	Dry-Sub Su	16.0	Dry-Sub W	Wet-Sub S	1.2
Dry-1ps S	Wet-Base S	20.0	Avg-Base W	Avg-3ps S	1.3
Dry-1ps S	Wet-Base W	25.0	Avg-Sub W	Wet-Sub W	1.8
Dry-1ps S	Dry-Sub Su	29.8	Dry-Sub Su	Wet-Base W	1.9
Dry-1ps S	Wet-Sub S	30.6	Avg-Base W	Wet-Sub S	2.1
Dry-Base W	Wet-Base S	38.3	Dry-Sub W	Wet-Sub W	2.5
Dry-Base S	Wet-Base S	51.9	Dry-Sub S	Wet-Sub W	3.7
Dry-Base W	Dry-1ps S	54.4	Wet-Sub W	Wet-Base S	4.5
Dry-Base W	Dry-Sub S	55.6	Avg-Sub W	Wet-Base S	4.9
Dry-Base W	Wet-Base W	55.6	Avg-Sub W	Wet-Sub S	5.5
Dry-Base W	Wet-Sub S	55.6	Wet-Sub W	Wet-Sub S	7.4
Dry-1ps S	Dry-Sub S	66.7	Wet-Sub W	Wet-Base W	8.6
Dry-Base W	Dry-Base S	77.8	Dry-Sub W	Wet-Base S	9.9
Dry-Base S	Dry-1ps S	88.9	Dry-Sub W	Avg-Sub W	10.7
Dry-Sub Su	Avg-Sub W	24.5	Dry-Sub S	Wet-Base S	11.1
Dry-Sub Su	Wet-Sub W	26.1	Dry-Sub Su	Avg-Base W	15.7
Wet-Base S	Wet-Sub S	45.7	Avg-Sub W	Wet-Base W	18.0
Dry-Sub Su	Dry-Sub W	46.0	Dry-Sub Su	Wet-Sub S	22.2
Wet-Base W	Wet-Base S	77.0	Avg-Sub W	Avg-Base W	90.9

Table K-1. Results of ANOSIM analysis used to determine statistical significance of differences in community composition of nekton collected with the beam trawl within each flow tier. Low significant values indicate the two sites exhibit different species compositions. Shaded comparisons are considered significant.

Table K-2. Results of ANOSIM analysis used to determine statistical significance of differences in community composition of nekton collected with the beam trawl within each river kilometer site. Low significant values indicate the two sites exhibit different species compositions. Shaded comparisons are considered significant.

rkm	rkm	Sig %
1	10	5.3
1	22	34.9
1	42	2.3
1	31	3.7
10	22	17.9
10	42	5.2
10	31	1.3
22	42	66.2
22	31	17.4
42	31	49.8

Table K-3. Results of ANOSIM analysis used to determine statistical significance of differences in community composition of nekton collected with the otter trawl within each flow tier. Low significant values indicate the two sites exhibit different species compositions. Shaded comparisons are considered significant.

Otter Trawl Flow	Tier Pair wise tests - A	NOSIM			
Flow Tier	Flow Tier	Sig %	Flow Tier	Flow Tier	Sig %
Dry-1ps S	Avg-3ps S	0.10	Dry-Sub Su	Avg-3ps S	0.10
Dry-Base W Dry-Base W	Avg-3ps S Dry-Sub W	0.30	Avg-Sub W Dry-Sub W	Avg-3ps S Avg-3ps S	0.10
Dry-Sub S	Avg-3ps S	1.17	Avg-3ps S	Wet-2ps Su	0.40
Dry-Base S	Wet-Sub W	1.23	Avg-3ps S	Wet-Sub W	0.40
Dry-Sub S	Wet-Sub W	1.23	Avg-Sub W	Wet-Base S	1.10
Dry-Sub S	Avg-Sub W	2.34	Wet-Sub W	Wet-Base S	1.23
Dry-Base W	Wet-Sub W	2.47	Dry-Sub Su	Wet-Base S	1.30
Dry-Base S	Avg-Sub W	3.13	Dry-Sub W	Wet-Sub W	1.48
Dry-Base W	Dry-Sub Su	6.00	Avg-3ps S	Wet-Sub S	1.50
Dry-1ps S	Wet-Sub W	7.41	Dry-Sub Su	Avg-Base W	1.60
Dry-Sub S	Avg-Base W	7.41	Dry-Sub W	Wet-Base S	2.22
Dry-Base S	Dry-Sub W	8.33	Avg-Base W	Wet-Sub W	2.47
Dry-Base S	Dry-Sub Su	8.80	Avg-Base W	Avg-3ps S	2.60
Dry-1ps S	Dry-Sub W	13.70	Dry-Sub W	Avg-Base W	2.96
Dry-Base W	Avg-Sub W	14.70	Dry-Sub Su	Avg-Sub W	3.50
Dry-Base S	Avg-3ps S	15.63	Avg-3ps S	Wet-Base W	4.40
Dry-Base W	Wet-Sub S	18.52	Dry-Sub W	Avg-Sub W	5.70
Dry-1ps S	Avg-Sub W	21.30	Avg-Base W	Wet-Sub S	6.17
Dry-Base W	Wet-Base S	22.22	Avg-Sub W	Wet-Base W	6.90
Dry-1ps S	Wet-Base S	27.16	Avg-3ps S	Wet-Base S	7.00
Dry-1ps S	Dry-Sub Su	27.80	Avg-Sub W	Wet-Sub S	7.40
Dry-1ps S	Avg-Base W	28.40	Wet-Sub W	Wet-Base W	8.64
Dry-Sub S	Dry-Sub W	31.48	Avg-Sub W	Avg-Base W	9.50
Dry-Base W	Dry-1ps S	39.51	Dry-Sub W	Wet-Sub S	10.19
Dry-Sub S	Dry-Sub Su	43.20	Wet-Base S	Wet-Sub S	16.87
Dry-Base S	Avg-Base W	44.44	Wet-2ps Su	Wet-Base S	20.99
Dry-Base W	Wet-2ps Su	45.68	Dry-Sub Su	Wet-Sub W	21.20
Dry-Base S	Dry-1ps S	53.09	Dry-Sub W	Wet-Base W	22.22
Dry-Base W	Dry-Sub S	55.56	Avg-Base W	Wet-Base S	25.93
Dry-1ps S	Dry-Sub S	61.73	Dry-Sub W	Wet-2ps Su	34.26
Dry-1ps S	Wet-2ps Su	61.73	Avg-Base W	Wet-2ps Su	39.51
Dry-1ps S	Wet-Sub S	61.73	Wet-Sub W	Wet-Sub S	39.51
Dry-Sub Su	Dry-Sub W	65.80	Dry-Sub Su	Wet-Base W	45.76
Dry-Base S	Wet-Base S	74.07	Dry-Sub Su	Wet-Sub S	47.20
Dry-Base W	Avg-Base W	77.78	Avg-Sub W	Wet-Sub W	51.10
Dry-Sub S	Wet-Base S	81.48	Avg-Base W	Wet-Base W	52.67
Dry-Base W	Dry-Base S	85.19	Wet-2ps Su	Wet-Sub W	60.49
Dry-Base W	Wet-Base W	88.89	Dry-Sub Su	Wet-2ps Su	71.20
Dry-1ps S	Wet-Base W	93.83	Wet-Base W	Wet-Base S	79.01
			Avg-Sub W	Wet-2ps Su	84.20

Table K-4. Results of ANOSIM analysis used to determine statistical significance of differences in community composition of nekton collected with the otter trawl within each river kilometer site. Low significant values indicate the two sites exhibit different species compositions. Shaded comparisons are considered significant.

River Kilometer Pair wise Test		Sig. Level %
1	10	2
1	22	0.6
1	42	0.1
1	31	0.1
10	22	2.3
10	42	0.4
10	31	0.1
22	42	26.5
22	31	2
42	31	3.8

Appendix L. Total Number of Organisms Captured by Taxa, Total Number of Organisms Captured, Total Number of Organisms Captured Adjusted for Number of Flow Tiers (#/n), Total Number of Organisms Classified as Estuarine or Marine Captured, Total Number of Organisms Classified as Estuarine or Marine Captured Adjusted for Number of Flow Tiers (#/n), Total Number of Taxa Captured and Total Number of Taxa Classified as Estuarine or Marine Captured during 2016 and 2017

Table L1. Total number of organisms captured by taxa, total number of organisms captured, total number of organisms captured adjusted for number of flow tiers (#/n), total number of organisms classified as estuarine or marine captured, total number of organisms classified as estuarine or marine captured, total number of taxa captured adjusted for number of flow tiers (#/n), total number of taxa captured and total number of taxa classified as estuarine or marine captured during 2016 and 2017. Status refers to salinity preference assigned (FW – freshwater, E – estuarine, M – marine transient). Number in parentheses is frequency of sampling of selected flow tier.

		Beam T	rawl			Otter Trawl				
Common and Scientific Name	Status	Wet Base S (2)	Wet Base W (1)	Wet Sub S (1)	Wet Sub W (2)	Wet Base S (2)	Wet Base W (1)	Wet Sub S (1)	Wet Sub W (2)	
Shrimp										
Ohio Shrimp Macrobrachium ohione	FW	21	97	1	1	0	0	0	0	
brown shrimp Farfantepenaeus aztecus	Е	2	0	2	1	0	0	0	1	
daggerblade grass shrimp Palaemonetes pugio	Е	24	9	3	11	6	0	0	3	
marsh grass shrimp Palaemonetes vulgaris	Е	0	0	0	0	0	0	1	0	
white shrimp Litopenaeus setiferus	Е	2	10	0	46	15	1	11	140	
Crabs										
spider crabs Superfamily Majoidea	Е	0	0	0	0	0	0	0	1	
portly spider crab Libinia emarginata	Е	0	0	0	0	0	0	0	1	
blue crab Callinectes sapidus	Е	2	3	6	1	0	0	0	0	
lesser blue crab Callinectes similis	Е	0	2	0	0	0	0	0	0	
Finfish										
Ladyfish Elops saurus	Е	0	0	0	0	0	0	0	3	
Striped Anchovy Anchoa hepsetus	Е	0	0	0	0	2	3	459	86	
Bay Anchovy Anchoa mitchilli	Е	0	1	3	0	1	0	0	9	
Herrings Family Clupeidae	E	0	0	0	0	0	0	1	0	
Skipjack Herring Alosa chrysochloris	Е	0	5	0	0	0	0	0	0	
Gulf Menhaden Brevoortia patronus	Е	29	0	0	19	12	5	11	23	
Gizzard Shad Dorosoma cepedianum	FW	0	0	0	0	0	0	0	5	
Threadfin Shad Dorosoma petenense	FW	0	1	0	0	0	0	0	4	
minnows Family Cyprinidae	FW	1	1	0	0	0	0	0	1	
Ribbon Shiner Lythurus fumeus	FW	6	7	0	0	0	0	0	0	

		Beam T	rawl			Otter Trawl			
Common and Scientific Name	Status	Wet Base S (2)	Wet Base W (1)	Wet Sub S (1)	Wet Sub W (2)	Wet Base S (2)	Wet Base W (1)	Wet Sub S (1)	Wet Sub W (2)
Shoal Chub Macrhybopsis hyostoma	FW	1	0	0	0	135	3	249	258
armored catfishes <i>Pterygoplichthys</i> spp.	FW	0	0	1	0	0	0	0	0
Hardhead Catfish Ariopsis felis	Е	0	0	0	0	8	4	1	2
Gafftopsail Catfish Bagre marinus	Е	0	0	0	0	26	1	7	8
Blue Catfish	FW	0	0	0	0	51	11	35	147
Ictalurus furcatus Channel Catfish	FW	0	0	0	0	0	1	2	4
Ictalurus punctatus Striped Mullet	Е	80	5	1	0	0	0	0	4
Mugil cephalus Inland Silverside	E	0	0	2	1	0	0	0	0
Menidia beryllina Sheepshead Minnow	Е	0	0	0	0	1	0	0	0
Cyprinodon variegatus Western Mosquitofish Gambusia affinis	FW^1	0	6	0	2	0	0	0	0
Warmouth Lepomis gulosus	FW	0	0	0	0	0	0	5	0
Atlantic Bumper Chloroscombrus chrysurus	Е	0	0	0	0	0	0	1	0
Flagfin Mojarra Eucinostomus melanopterus	Е	0	0	0	0	38	0	941	1
Sheepshead Archosargus probatocephalus	Е	0	0	0	3	27	16	35	12
Atlantic Threadfin Polydactylus octonemus	М	0	0	0	0	0	0	1	0
drums and croakers Family Sciaenidae	Е	0	0	0	0	0	0	1	0
Freshwater Drum Aplodinotus grunniens	FW	0	0	0	0	0	0	0	0
Silver Perch Bairdiella chrysoura	Е	0	0	0	0	26	1	7	8
Spot Leiostomus xanthurus	Е	0	0	0	0	0	1	2	3
Atlantic Croaker Micropogonias undulatus	Е	0	0	0	0	88	179	90	4304
Black Drum Pogonias cromis	Е	0	0	0	0	1	6	0	14
Star Drum Stellifer lanceolatus	Е	0	0	0	0	272	64	27	306
gobies Family Gobiidae	Е	0	0	0	0	0	0	0	3
Darter Goby Ctenogobius boleosoma	Е	1	0	0	0	0	0	0	0

		Beam T	rawl			Otter Trawl			
Common and Scientific Name	Status	Wet Base S (2)	Wet Base W (1)	Wet Sub S (1)	Wet Sub W (2)	Wet Base S (2)	Wet Base W (1)	Wet Sub S (1)	Wet Sub W (2)
Freshwater Goby Ctenogobius shufeldti	Е	0	0	0	0	9	0	59	12
Highfin Goby Gobionellus oceanicus	Е	0	0	0	0	1	0	0	0
Naked Goby Gobiosoma bosc	Е	0	6	0	0	0	0	0	0
Clown Goby Microgobius gulosus	Е	4	93	0	85	0	0	0	0
Atlantic Spadefish Chaetodipterus faber	М	0	0	0	0	0	0	1	0
flounder Paralichthys spp.	Е	0	2	0	0	0	0	1	0
Southern Flounder Paralichthys lethostigma	Е	0	0	0	0	0	0	1	0
Hogchoker Trinectes maculatus	Е	0	0	0	0	1	0	0	4
Southern Puffer Sphoeroides nephelus	М	0	0	0	0	0	0	1	0
Total		173	248	19	170	720	296	1950	5367
Weighted Total		87	248	19	85	360	296	1950	2684
Number of Taxa		12	15	8	10	19	14	25	28
Total Estuarine Taxa		51	122	15	60	24	4	471	244
Weighted Total Estuarine T	axa	26	122	15	30	12	4	471	122
Number of Estuarine/Marin	e Taxa	5	6	5	5	4	2	3	8

¹Western Mosquitofish is sometimes classified as an estuarine species. It is often found in very low salinity portions of estuaries. However, it has a wide distribution in freshwater environments.

Appendix M. 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 are provided in Blue. All Brazos Estuary responses are provided at the conclusion of this document.

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 reminder 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 proceeded 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 flowecology 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 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 \geq 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.

All Brazos Estuary comment responses provided at the conclusion of this appendix.

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.

All Brazos Estuary comment responses provided at the conclusion of this appendix.

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.

All Brazos Estuary comment responses provided at the conclusion of this appendix.

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 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. oxyrhynchus 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. oxyrhynchus 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 homogenously 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 flow regime 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 preregulation/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 it 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 (<u>https://link.springer.com/referenceworkentry/10.1007%2F978-1-4020-4399-4_13</u>), "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, not-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.

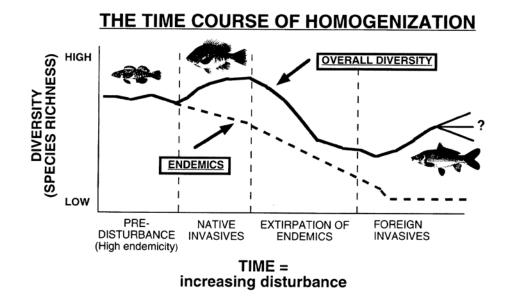
Testing occurs and, based on results, the narrative is supported, and can be revised and (hopefully) becomes more accurate, or the narrative is discarded.

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 intraannual patterns? We think so, therefore part of our narrative.

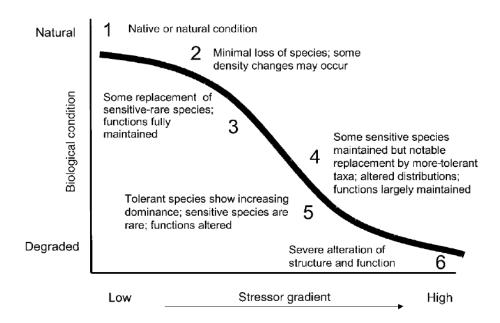
In contrast to our narrative, what are the other narratives that explain long-term changes in fish communities? What are the testable predictions? How can these be tested in the context of existing standards and recommendations?

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.

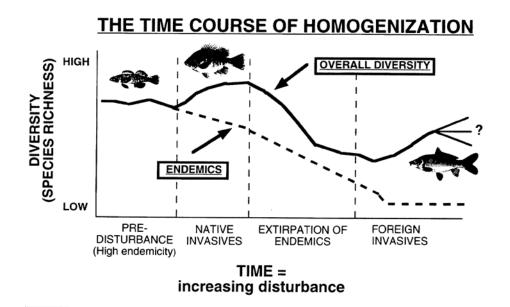
In an unregulated river (left side of x-axis, flows pulse through a system. For a species type or guild of species, densities and relative abundances before a flow pulse (base condition, assuming this is what is meant by "low flows") at "pre-disturbance" can be less than, equal to, or greater than the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



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 longlived, 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 <u>not</u> 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.

All Brazos Estuary comment responses provided at the conclusion of this appendix.

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. 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 5year 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 "preflood" 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 flowrelated 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-perseason (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.

7 5. 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 addition 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-bysite 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. Pleas 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 entire riparian distribution and not 80% of the 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 TCEO 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 <u>more</u> than a 25% reduction rather than no <u>less</u> 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 <u>before</u> "implementation" of SB3 standards are not really any different than flows of a similar magnitude occurring <u>after</u> "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.

3. 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.

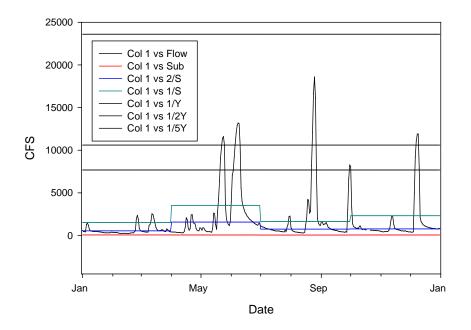
Release of this information will be forthcoming in future publications.

4. 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.
- 6. 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 since 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 highflow 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 SB3compliant 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 TCEO 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 connectingmagnitude 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 'postproject / 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.

All Brazos Estuary comment responses provided at the conclusion of this appendix.

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.

All Brazos Estuary comment responses provided at the conclusion of this appendix.

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.

RESPONSE TO TWDB/BBASC REVIEW COMMENTS AND ASSOCIATED FINAL REPORT ADDENDUM – ESTUARINE COMPONENT INSTREAM FLOWS RESEARCH AND VALIDATION METHODOLOGY FRAMEWORK 2016-2017

Response to TWDB/BBASC Review Comments and Associated Final Report Addendum – Estuarine Component Instream Flows Research and Validation Methodology Framework 2016-2017 Contract number 1600012009 Prepared by Dr. George Guillen

The following text and associated addendum report are provided for the Brazos River estuarine component of the Instream Flow Research and Validations Methodology Framework and Brazos Estuary. Review Comments and Action Items are listed and numbered according to the original number order in **black.**

Comment 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.

Reply to Comment 10. Page 4.

There are three specific comments and conclusions embedded in item 10. We have parsed them below and will address each comment and conclusion.

10.1) The Brazos estuarine research suffers from the *same basic limitation* as the aquatic research in this report.

10.2) 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.

10.3) 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.

Reply to Comment 10.1. Page 4.

In regards to comment 10.1, the author of the estuarine section respectfully and strongly disagrees with the characterization of the estuarine research suffering from "*the same basic limitation as the aquatic research*". Setting aside the criticisms of other sections of the report, we remind the reviewers about the different primary objectives of the individual components (freshwater, riparian, estuary) of these initial studies. Therefore any limitations in regards to the estuarine component of this study must be viewed from the point of view of the unique characteristics of the individual study components. In regards to the estuarine component these

Estuary-2

characteristics include the lack of 1) long term historical data, and 2) the lack of data from similar model systems that can be used to inform and augment data within the target system. The justification for this point of view is provided below in combination with comments regarding comment 10.1.

Reply to Comment 10.2. Page 4.

The author concurs with reviewers comment 10.2 "this data represents valuable information that will be used in the future to evaluate the ecological responses to flow variation". This was above all else the major intent and objective of these initial studies since comprehensive spatial and temporal biological and water quality data on the Brazos River estuary was largely lacking.

The need to conduct baseline characterization studies in the Brazos estuary was clearly documented in the 2012 Brazos River Basin and Bay Expert Science Team Environmental Flow Regime Recommendations Report. The Brazos River estuary unlike many other Texas estuaries lacks the 35+ years of historical standardized fisheries independent CPUE data (e.g. TPWD bag seine, trawl, gill seines, oyster dredge etc). This is due to several factors including distance from field stations, the lack of known oyster reefs, the bottom topography of the river and the inability to deploy bag seines or gillnets safely and effectively in a riverine environment. In summary the unique river topography and hydrology inhibits the use of standardized TPWD sampling gear with the exception of bottom trawls.

A similar deficiency in comprehensive historical water quality monitoring exists within the estuarine portion of the river. Currently there is only <u>one</u> routine water quality monitoring site in the tidal portion of the Brazos River (TCEQ site 11843) located at rkm 9.3 (river mile 5.8). Historical and ongoing water quality monitoring at this site consists of surface and depth interval sampling of water temperature, dissolved oxygen, pH, and standard conductance/salinity at monthly to quarterly intervals since 1969. In addition, concentrations of surface measurements of TSS and nutrients (TKN, nitrate-nitrites and ammonia nitrogen, total and orthophosphates) have been monitored at the same intervals since 1972.

As described above unlike other estuaries the Brazos River discharges directly into the Gulf of Mexico instead of an estuarine lagoon or bay, and possesses a "riverine estuarine zone" that extends at least 42 km (26.1 miles) upstream from the mouth. This effectively decreases the residence time of water in the lower estuarine zone and ultimately results in the discharge of large amounts of dissolved and suspended constituents directly into the Gulf of Mexico. These nutrients and sediments are considered highly important for sustaining the river delta and the productivity of offshore biotic communities (Rodriquez et al. 2000; Fraticelli 2006). Currently there is virtually no information on the nearshore nekton community response, primary productivity, the dynamics of delta and wetland formation of the Brazos River, and connectivity between the estuarine portion of the river and offshore habitats in regards to nekton life cycle.

The lack of any reliable historical data on the biological communities of the Brazos River under varying flow conditions, and the unique geomorphology and hydrology of the estuarine zone represents a serious limitation on being able to *immediately* develop, validate and/or develop alternative flow regime recommendations (Brazos BBEST 2012). With the exception of the Rio

Grande (more arid) and perhaps Mississippi River (highly modified and much larger) there is no other comparable system to model to generate projections as to the expected response of estuarine biota to varying Brazos River flows. As a result of these limitations the BBEST had no option other than to recommended adoption of the upstream instream flow standards, as defined at the Rosharon USGS gage, which is located approximately 90.9 km (56.5 miles) upstream from the mouth at the Gulf of Mexico as the default estuary inflow standards (Brazos BBEST 2012). This is the basis for the current estuarine inflow standard (TCEQ 2014).

Prior to formal adoption of the TCEQ freshwater inflow standards the Brazos BBASC in consultation with the BBEST recognized the need to validate and if necessary modify these inflow standards after conducting both characterization and *longterm monitoring* of the estuarine zone as described in their *Work Plan for Adaptive Management* (Brazos BBASC 2013). Specifically item 3.1.6 Priority 2 of the Work Plan states "A *long term study will be commissioned to monitor salinity, nutrient transport, sediment transport and deposition, and associated estuarine health in order to detect any negative effects as upstream projects are implemented over the next few decades*". In order to determine if there are effects however, we must first collect baseline data. The Work Plan further described the specifics of these needed studies in section 3.1.6 Priority 2 (Brazos BBASC 2013). They state that:

"Due to the lack of paired long-term biological, water quality, and hydrological data, fish assemblage studies should be initiated within the Brazos and San Bernard Rivers estuaries and compared to water quality and hydrological data".

They concluded that "*The estimated costs for these studies are \$3M over 5 years*" (Brazos BBASC 2013). The actual budget allocated for estuarine studies for the 2 year period (both projects) was approximately \$145K.

The primary goal of the estuarine component of the Brazos study these first two rounds (2 years) was to first provide an initial characterization of the lower estuarine zone of the study area and then to start developing predictive relationships between response variables (e.g. fish, water quality) and inflow. The two objectives of the estuarine component of this study were clearly described in the first report entitled *Instream Flows Research and Validation Methodology Framework and Brazos Estuary Characterization 2015* and are listed below:

Objective 1. To use new and historical data collected on the tidal portion of the lower Brazos River to:

- a. characterize flow regime and tidal dynamics
- b. assess water quality and nutrient patterns
- c. describe the salinity regime of the Brazos estuary
- d. characterize the nekton community composition, and
- e. asses use by estuarine dependent species, and

Objective 2. To test predicted relationships between salinity, nutrients, and proportions of estuarine species against flow tier and discharge.

For phase 2 of the Brazos Project the Texas Water Development Board provided additional details and guidance on the estuarine component of the study. In the TWDB Request for Qualifications No. 580-16-RFQ0011 2016, the agency delineated in section 2.2 Scope of Work described their request for a contractor to conduct studies including item 4: *perform studies and collect field data to determine the relationship between flows in the Brazos River and fish and nekton response in the Brazos River Estuary (the tidally influenced section of the river) as well as assess responses with respect to he adopted environmental flow standards.*

In proposal submitted to the TWDB entitled *BIO-WEST*, *Inc. Project Team Technical Approach* 2016, the project team clearly stated:

Analysis of Brazos estuary data collected during the original study was also influenced by a truncated period of data collection. Additional data on water quality, nutrients, and nekton will be collected at up to five previously established primary monitoring sites from summer 2013 to summer 12017 to provide one complete annual cycle of estuarine conditions. Combined with the previously data collected, this will provide additional replication to examine flow ecology linkages within the Brazos estuary.

On page 11 of this current draft of this report we clearly outlined the objectives of this report in regards to the estuarine component. These objectives are relisted below.

The primary objectives of the Brazos estuary study were listed as follows:

- 1. To use relevant historical and new data collected within the tidal portion of the lower Brazos River to:
- a. characterize flow regime and tidal dynamics,
- b. describe the response of salinity regime to varying flow,
- c. assess water quality and nutrient patterns, and
- d. characterize nekton abundance, diversity, and community composition.
- 2. To investigate and begin development of potential models that predict the relationship between discharge, flow tiers, seasonality, salinity, nutrients and nekton composition including estuarine species within the lower tidal portion of the Brazos River.

It was hypothesized that at higher flow tiers and discharge:

- 1. Salinity levels in the Brazos River estuary would decline rapidly;
- 2. The lateral extent and vertical stability of the pycnocline would decline;
- 3. Nutrient and suspended solid levels would increase;
- 4. The occurrence and density of estuarine dependent species would decline; and
- 5. Under moderately high flows, vertical mixing and reaeration would increase, leading
- to higher abundances of nekton in trawl samples.

Sections of the report that specifically address each objective are listed below.

a. characterize flow regime and tidal dynamics,

See section 3.3.1 Pages 75-79. Detailed information on flow conditions and tidal regime are described during the study.

b. describe the response of salinity regime to varying flow,

See section 3.3.2. Pages 78-85. Detailed information on the response of salinity both vertically (depth) and horizontally (river mile) is provided for various flow regimes using both horizontally intense point measurements during sampling dates and continuous monitoring data. The continuous monitoring sites located at the upper, mid and downstream portion of the survey area provides information on the temporal response of depth, salinity and dissolved oxygen during the entire hydrographic record including high flow and flood conditions are render the river unsafe for sampling. This approach provides the most comprehensive method of describing the influence of freshwater inflow along the entire tidally influenced (estuarine) portion of the lower Brazos River based on current resource limitations and logistical constraints.

c. assess water quality and nutrient patterns

See section 3.3.2. Pages 78-85. Detailed data on the response of surface and bottom water temperature, dissolved oxygen, and surface nutrients and TSS are provided by river mile is provided for various flow regimes using both horizontally intense point measurements during sampling dates. Furthermore data on the horizontal and vertical distribution of water temperature, dissolved oxygen, pH, and turbidity is provided. This approach provides the most comprehensive method of describing the influence of freshwater inflow along the entire tidally influenced (estuarine) portion of the lower Brazos River based on current resource limitations and logistical constraints.

d. characterize nekton abundance, diversity, and community composition.

Refer to section 3.3.2. Pages 86-99 and Appendix H. Detailed information on nekton communities inhabiting the mid-channel areas using otter trawls and shoreline habitats using beam trawls is presented. In addition, the author provided historical data collected by Dr. Guillen's graduate student who conducted a single year's (2012) trawl survey at most of the study sites surveyed during this investigation. This data was critical for augmenting the limited data that could be collected during the short period of time allotted for each phase of this project. For example samples were not collected during the summer "critical period" during either phase 1 or 2 of the studies when river flow is typically lower, water temperature is higher, and hypoxia is more commonly encountered based on the limited historical data. This was due to the contract period deadlines which precluded summer field work and/or the late spring floods during both study periods that prevented additional sampling safely.

Reply to Comment 10.3. Page 4.

The author partially disagrees with comment 3 "these descriptive data do not allow us to make decisions about the need for flows of specific magnitudes, frequencies and durations".

The author would remind the reviewer that due to lack of any biological or water quality response data the existing state flow standard for the estuary that was recommended by the Brazos BBEST and ultimately adopted by the TCEQ is based solely on the instream flow standard adopted for the Rosharon gage, located 90.9 km (56.5 miles) upstream from the mouth of the river and the upper boundary of the tidal extent (TCEQ segment 1201) at river mile 24.3 (Brazos BBEST 2012; TCEQ 2014b).¹ Due to the lack of comprehensive site specific water quality and biological data this adopted standard was recommended using the *Hydrology-Based Approach* (SAC 2009).

As stated earlier the limited studies that we conducted, that included only limited summer conditions and flow tier data based on historical data, provides the baseline data and beginning of a framework to either confirm the appropriateness of the existing standards or refinement in the future. The data provided in the initial phase of this study (Bonner et al. 2015) along with the current work performed during the data collected during this study in 2016-17 provides the basis for future assessments using a variety of potential methods that were presented and described in the report. For example, data was presented specifically and models developed to describe how water quality and fauna (freshwater, estuarine and marine species) in the tidal portion of the river responded to various flow tiers and daily average discharge. Combined (historical 2012; 2014-15; 2016-17) data sets were used to generate estimates of CPUE in critical areas within the estuarine zone including shoreline and main channel benthic zones. Specific questions that were addressed include how salinity and dissolved oxygen (including hypoxia events) respond to varying flow tiers and daily average discharge. These areas were defined both in terms of spatial and vertical extent. Graphical and modeling output describing the response to all measured variables and discharge or flow tiers are provided in Appendix F - H.

Comment 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

¹ TCEQ defines the boundary as a point 100 meters upstream of SH 332 but extending 25 miles downstream to mouth (2002 Texas Water Quality Inventory).

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.

Reply to Comment 11.

There are three major statements within comment 11 presented by the reviewers. They are addressed separately.

Comment 11.1

"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".

Reply to Comment 11.1

We concur. See previous reply to Comment 10.2. Page 4.The data from this study serves as the basis for future studies, review, validation, and/or development of estuarine flow standards, after completion of additional data collection that will address the following data gaps.

- 1. Lack of summer data
- 2. Nekton size distribution and growth estimates across flow regime conditions
- 3. Suspended solids sampling lower river, delta, and nearshore GOM
- 4. Nutrient and primary production sampling lower river and nearshore GOM

Comment 11.2

"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".

Reply to Comment 11.2

We cannot provide any guidance or an answer to the hypothetical question regarding "evaluating the relative benefit of freshwater inflow for estuarine species versus instream flows for freshwater fishes" for multiple reasons. Also the author apologizes if the reviewer has been misled to conclude that this scenario was being evaluated or presented. Although intriguing the authors have not yet considered this comparative analysis since at this time no "conflicting" estuarine inflow scenario has been presented. Furthermore, the existing default adopted standard for estuarine inflow is the instream flow standard adopted for Romayor gage (TCEQ 2014b). However, if the future if there was a perceived tradeoff it would be not only involve biological natural resource question but ultimately other services and functions and will involve a collective "value judgment" since the SB3 legislation does not discuss or provide guidance on how to deal with issues involving "competing" water allocation for *environmental* instream versus freshwater inflow needs.

However, the language of SB3 clearly states the an "Environmental flow regime means a schedule of flow quantities that reflects seasonal and yearly fluctuation that typically would vary geographically, by specific location in a watershed, and that are shown to be adequate to support a *sound ecological environment to maintain the productivity, extent, and persistence of key aquatic habitats* in a along the affected water bodies" (State of Texas 2007).. The Science Advisory Committee provides additional guidance on defining a "sound ecological environment" (SAC 2006). The SAC defined a sound ecological environment as one that:

- 1. Sustains the *full complement of native species* in perpetuity
- 2. Sustains key habitat features required by these species
- 3. Retains key features of the natural flow regime required by these species to complete their life cycles, and
- 4. Sustains key ecosystem processes and services, such as elemental cycling and the productivity of important plan and animal populations.

Clearly definition bullet 1 speaks directly to the need for documenting and defining the "normal range" of native species, including marine, estuarine and freshwater species (e.g. blue catfish) that inhabit the tidally influenced estuarine zone of the Brazos River. Since there is essentially no historical data this task must be accomplished first before proceeding to additional tasks.

Key habitat features sustained by the freshwater inflows in the Brazos River include the river delta which includes wetlands and acceptable water quality (e.g. dissolved oxygen levels > hypoxia, salinity) that sustains the various life stages of nekton that utilize the tidal portion of the river (Brazos BBEST 2012). The current study through the combined analysis of historical data and collection of new data provides evidence that documents which combination of seasonal flow regimes and currently adopted flow tiers is most likely able to produce hypoxic conditions in the lower river Further focused studies are also needed to document how freshwater inflow influences delta formation and maintenance. This will require additional collection of suspended sediment data and careful examination of the response of delta geomorphology which was beyond the scope of this current study.

Defining what key features of the natural flow regime required by biota to complete their life cycles is an ongoing process which is complicated by the unique geomorphology of the Brazos River estuary and the relationship to the Gulf of Mexico. Many of the other adopted inflow standards were developed for open lagoon type estuaries using monthly time steps or focusing on specific non-nekton resources such as oysters, Rangia, or seagrasses. In contrast, there is a lack of these resources in the Brazos estuary and instead there exists a direct link between the Brazos River and the resources of the nearshore Gulf of Mexico. State natural resources utilize the lower river estuarine zone and the adjacent nearshore GOM which extends out 9 nm. This includes commercially and recreationally important nekton such as Atlantic Croaker, Spotted Seatrout, Red Drum, Striped Mullet, Gulf Menhaden, Blue Crab and Penaeid shrimp. The lower river supports a blue crab fishery and the nearshore GOM a shrimp fishery.

Many important nekton resources utilize estuaries as nursery habitat for immature stages but complete their life history in the nearshore GOM. The exact influence of Brazos River on these

resources is largely unknown but is probably very large since the Brazos River is ranked 2nd in terms of average annual discharge in terms of Texas Rivers (Solis and Powell 1999). Therefore additional consideration of the direct affects of the freshwater inflow on the Gulf of Mexico fauna needs to be evaluated. Additional sampling of the GOM may be required since only limited environmental and fisheries data is available for the area adjacent to the Brazos River mouth.

We are not attempting to suggest that stenohaline marine conditions are necessary. More importantly, the mixture of freshwater and marine species that reflects the normal range of variability observed in the tidal portion of the Brazos River reflects one measure of a sound ecological environment.

Comment 11.3. 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.

Reply to Comment 11.3

We disagree with the criticism that *no* attempt was made to evaluate the numerical criteria that currently exists for estuarine organisms. Although primary goal of this estuarine portion of the study was to characterize the hydrology, water quality and biota we specifically constructed multiple linear models and graphical representations depicting how salinity, water quality and biota responded to varying daily average discharge and adopted flow tiers at Rosharon. The reader is encouraged to carefully review the tables and figures within the text and appendix G. In appendix G, discharge, water quality and nekton biological indices were tested between flow tiers x location (e.g. Model 35 ANOVA of number of nekton taxa/tow). Due to the low sample number for some categories (e.g. n = 5) some of these statistical comparisons have low power. Additional sampling within many tiers will increase the statistical power and ability to evaluate the sensitivity of various flow tiers on biological and water quality response variables.

Comment 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.

Reply to Comment 12

We concur and support the need for future targeted or expanded projects that will attempt to provide better estimates of sediment and nutrient loading and in relation to delta formation and

primary production. Furthermore, future studies should be extended to include evaluation of nutrient sediment dynamics in the lower rivers and into the GOM. This has been added in the text of the manuscript as a recommendation for future studies and monitoring.

Comment 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.

Reply to Comment 61.

You are correct only continuous (15 minute recording interval), daily average, and monthly average data were downloaded and utilized. Daily average values were used to estimate monthly values for more recent months (post October 2016) when required. Top of the hour (e.g. 8:00, 9:00, 10:00 etc) values or those closest to top of the hour values were extracted to facilitate direct comparison with hourly extracted data generated from automated water quality monitors that record at intervals that differ from the USGS flow discharge data. Since the intent was to illustrate potential co-variation between variables at the sub-day recording interval it was necessary to make these comparisons. However, it was not necessary to examine sub-hourly variation. To be clear we did not download any compiled "statistics" (e.g. average May flows) from the USGS site if I understand your question. This has been clarified in the methods.

Brazos Estuary Specific Draft Final Report Comments (Brazos Report only). Comments renumbered from original comments.

Comment E1.

Section 3.3: This is a very lengthy section that presents a great deal of environmental and biological data. However, it does not provide evaluation or recommendations for environmental flow standards, or a clear roadmap for how these survey data could be used to make evaluations and recommendations. The appendix material is dominated by data associated with the estuarine component of the project. This is useful information for estuarine ecologists. The findings should more clearly convey the major spatial and temporal patterns, perhaps integrating the various abiotic and biotic components that were surveyed. Please rewrite this section to offer a clear outline of the pros and cons (as done in the riparian section) regarding the likely capability for study design elements to evaluate environmental flow standards.

Reply to Comment E1.

The authors feel that it is not necessary to rewrite the entire estuarine section to mimic the structure illustrated in the riparian section. This is based on the fact that the objectives, hypothesis questions and existing state of knowledge for each component (estuarine, riparian, inland) are different. The primary objective of the estuarine section was to characterize the biota

and physicochemical features of the estuary and begin to develop predictive models that relate these variables to river discharge as measured at Rosharon gage. In regards to characterization the study focused on defining characteristics that describe a sound ecological environment in the Brazos River including:

- 1. the *full complement of native nekton species* in perpetuity
- 2. key habitat features required by these species
- 3. key features of the natural flow regime required by these species to complete their life cycles, and
- 4. Sustains key ecosystem processes and services, such as elemental cycling and the productivity of important plan and animal populations.

Finally, additional verbiage has been added to the summary and recommendation portion of the manuscript dealing with the estuarine component.

Comment E2.

Section 3.3.1: This section does a good job meeting the first primary objective (p.11) of describing and characterizing relevant physical, chemical, and biological data collected in the estuary. There were many models developed to begin to address the second primary objective. These model outputs, presented in Appendix H will be difficult to interpret and use to answer the questions on p. 11. Many of the regression models perform poorly. They could be significantly improved if models, other than simple linear regression with no time lags, were investigated. Please comment on the need/potential to investigate other models.

Reply to Comment E2.

The author agrees. Several models will be evaluated if the study continues. This includes both linear (e.g. linear, quadratic, cubic) and non-linear models if needed that may produce do a better job of describing the relationship of variables. A linear model with a quadratic term is particularly useful for describing the distribution of variables that exhibit a unimodal response to various levels of discharge. This has been added as a suggestion for future analyses in the recommendations section of the report.

Comment E3.

Please include graphics that depict the flow-salinity relationships. This would help stakeholders understand how sensitive salinity levels are to flow. Please consider developing similar figures to aid in addressing the other questions.

Reply to Comment E3.

These salinity versus discharge relationships are illustrated in Figures 40; 43-44; Appendix E. Fig. 13-17; Figure 22-37. Furthermore, linear model 16 relating river kilometer and stream discharge to bottom salinity is provided. Similar relationships are depicted for water temperature and dissolved oxygen either in combination with salinity or separately within the appendix.

Comment E4.

Section 3.3.3, Figures 45, 46, and 47, pages 91 through 93: The figures would be clearer if there were a separate figure for each river kilometer. If it is important to show the relationship among river kilometers on the same plot, color should be used as well as shape to differentiate among river kilometers. Please consider employing other means to display this data.

Reply to Comment E4.

We concur. Color figures are provided and will replace the original figures.

Comment E5.

Section 3.3.3: Reviewers noted that the study design and analysis strategy is inappropriate to evaluate environmental flows. As one might expect, more fish and shrimp are caught when flows are lower and there is less water volume (habitat) for the organisms to occupy -- i.e., their perunit-area densities are higher. This does not imply that higher flows with expanded habitat, nutrient delivery, sediment movement, etc. are not a critical requirement for the maintenance of freshwater and estuarine fish and invertebrate populations (as well as vegetation, birds, reptiles, etc.). Please respond to or acknowledge this limitation of the study approach.

Reply to Comment E5.

We disagree with the assessment of nekton density versus flow. The primary method of determining the density of bottom nekton was the bottom trawl catch per unit effort (CPUE). The primary method of determining the density of shoreline nekton was the beam trawl CPUE. The bottom trawl only samples demersal organisms that utilize the bottom depths which are constant over a wide range of flows. In the lower river that is tidally influence the bottom "surface area" is fairly constant irrespective of flow even during the lowest drought conditions. In contrast the shoreline zone of the Brazos River through most of the flows sampled is largely devoid of vegetation consisting of steep muddy clay banks. The shoreline consists of steep eroded banks or consists of extensive mud banks containing large amounts of deposited silt and clay. Consequently the amount of cover consisting of vegetation varies little over the range of discharges that were sampled during the studied. At higher flows water would inundate woody riparian vegetation. However, these flows usually exceeded 10,000 cfs when conditions became unsafe for boat navigation and operation and deployment of gear. We therefore conclude that under the range of conditions sampled the amount and type of bottom and shoreline habitat available to nekton would not vary considerably and neither would trawl and beam trawl efficiency.

Also, we did not mean to imply that the higher flows with "expanded habitat", nutrient delivery, sediment movement, etc. were NOT a critical requirement for the maintenance of freshwater and estuarine fish and invertebrate populations. Perhaps the reviewers thought we were implying this by our documentation of higher catches of marine organisms at lower flows. This is true but it does not take into account the other "services" provided by freshwater inflow including short and

long term loading of sediments and nutrients. In addition, the estuary is not defined as one point in the estuary but rather the entire continuum extending from the upper tidal limit to the mouth of the river. This includes the oligohaline nekton such as blue catfish and river prawn. Finally, we did document a higher likelihood of hypoxia during warm seasons and lower flows approaching hypoxic conditions. Since we did not sample the lower river during critical summer months (July-August) it is difficult to spatially and temporally delineate the extent of critical (e.g. salinity stratified induced hypoxia) which would reduce the amount of bottom habitat during lower flows.

Comment E6.

Section 3.3.3: In reference to this sentence:

"Based on close examination of the regression model it appears that highest number of estuarine taxa can be expected in the lower river under lower flow rates."

Please explain how this information will allow us to evaluate the environmental flow tiers – involving magnitudes, frequencies, and duration.

Reply to Comment E6.

Recall that due to the lack of any historical data on the biota of the tidal portion of the river, the default standard for the Brazos estuary was assumed to be identical to the instream standards at the Rosharon gage (TCEQ 2014b). We are therefore attempting to determine or validate whether these adopted standards (flow tiers, frequency, seasonality etc) to determine whether they are actually related to any measure or indicator of *a sound ecological environment* for the Brazos estuary. Such indicators may include 1) a full complement of native species in perpetuity, 2) key habitat features required by these species, 3) key features of natural flow regime required by these species to complete their life cycle and 4) the presence of key ecosystem processes and services, such as elemental cycling and productivity of important plan and animal populations are such measures (SAC 2006).

By itself the information collected during this study provides data on how oligohaline freshwater, estuarine and marine organisms respond to changes in seasonal river discharge, and indirectly to resulting salinity and dissolved oxygen in the lower river. These initial analyses represent the first step toward evaluating how populations of nekton respond to varying river discharge. During this phase only simple linear models were tested. However, we plan to construct more complex linear models (e.g. quadratic, cubic, multiple independent variables, lag time factors) as sufficient new data is accumulated during varying flow conditions and tiers across all seasons (e.g. summer months). We plan to utilize quadratic linear models which are frequently better at modeling response variables that exhibit modal responses over a range of independent variables (e.g. discharge).

The more complex models at times provide a better fit of the relationship of many water quality variables (e.g. suspended solids) that may exhibit dilution effects (lower concentrations at higher

flows) after initial mobilization of sediments. Similar phenomena are often observed during individual storm hydrographs. The variable does not exhibit the same concentration of a dissolved or suspended substance during the ascending and declining arm of the hydrograph. This phenomenon is called hysteresis and is often observed in hydrology when studying related variables like discharge, gage height, concentrations of dissolved and suspended substances. Hysteresis is more formally described as the time –based dependence of a system's output on present and past inputs. The dependence arises because the history affects the value of an internal state. To predict its future outputs, either its internal state or its history must be known.

The construction of more complex models if often limited by the range of conditions surveyed. The range of conditions surveyed during the study period was limited and therefore limits the modeling options. This situation is improving, although there are gaps in seasonal coverage, i.e. summer months. Once constructed these quantitative models will be compared using various performance metrics including R^2 , p-values, AICc, etc.

We did compare flow tiers (see Appendix G – flow tiers summary; Appendix H Linear Models) to determine, based on this preliminary data, whether there were any differences in selected water quality and biological indicators across distance (river kilometer) and flow tiers. As an example, we found significant differences in total phosphorus (TP) between flow tiers but not river kilometer (Appendix H Model 9). However, there was broad overlap in the various tier groups making it difficult to discern any patterns attributable to flow, season, and antecedent conditions (Wet, Dry etc). This is probably due to the low sample size (e.g.. wet base summer n = 5) for some categories, but could also be due to the fact there is no real difference between the flow tiers. If this were found to be true and total phosphorus was the only indicator of important then we could argue that the existing flow tiers don't appear to be related to any important process in the estuary related to total phosphorus. This might suggest the need to develop or explore new alternative tiers, consider fewer broader tiers, or define the tiers differently using different time periods (e.g. monthly targets).

In regards to biological metrics, we observed significant differences in number of taxa between flow tiers and river kilometer (Appendix H Model 35). Based on examination of subsequent multiple range tests there appeared to be very broad overlap in number of taxa over the range of flow tiers examined. A lower number of taxa were generally observed during dry, average, and subsistence base flow conditions in contrast to higher number of taxa being observed during dry, wet and average subsistence flow tiers. However, there was a stronger gradient that existed between downstream and upstream sites sampled in the estuarine zone. The highest average number of taxa was generally captured at the lower (< 10 km) sites.

So in conclusion we are beginning to evaluate how these various metrics (salinity, dissolved oxygen, nutrients, TSS, number of taxa and indicator taxa) responds to various flow tiers. Based on the limited data presented some of the results for selected metrics were inconclusive or exhibited low R^2 values meaning they do not explain most of the variability in the data. This may be due to low sample size and lack of sufficient seasonal sampling.

If this project continues we will continue to characterize the response (levels) of these variables at various flow tiers. Once characterized (high, low, average values of the indicator) we can

begin to document the actual benefits of each tier or if needed a reconstructed flow tier the better describes the relationship of various flow regimes and environmental indicators. While conducting these studies and developing predictive model we may find that other flow regimes (magnitude, frequencies, and duration) may provide a better statistical model and description of the relationship of these indicators and flow regime. This is our ultimate goal, but since we are starting with no historical data it will require multiple years including summer collections to obtain sufficient data to construct some of the more complex models. It is important to note that the construction of multivariate models will require concurrent paired measurements of many variables.

Comment E7.

Section 3.3.3: Please state whether it is good or bad to have more estuarine taxa distributed further upstream during low flow conditions. Perhaps freshwater taxa should be afforded more river habitat, given that estuarine taxa are much more widely distributed and abundant overall.

Reply to Comment E7.

Good/bad are values judgments that imply a preference for one assemblage (e.g. freshwater) over another. The question should be rephrased to ask whether the observed species reflects the "normal assemblage" of nekton found within the estuarine zone starting 42 km upstream of the mouth. For example, the upper portions of the estuarine zone generally support blue catfish. If water was diverted or a long term drought occurred, the upper zone would probably not support blue catfish. Since blue catfish are considered part of the normal assemblage of estuarine species in the upper estuary we might conclude that the use of the estuary was impaired. The trading of marine habitat for more freshwater or low saline species habitat is more of a social question. However, again referring to the SAC (2006) guidance on what constitutes a sound ecological environment for the Brazos estuary we can only refer back to the definition of various indicators including a full complement of native species in perpetuity. Therefore, the loss of any species of this assemblage that includes more marine species in the lower river and more oligohaline freshwater species in the upper (e.g. 42 km) could suggest some type of impairment. Currently we are trying to establish a baseline measure of the range of "normal" conditions for water quality and biota so we can determine if there is any detectable response that is sensitive to changes in hydrology and current flow tiers.

Comment E8.

Section 3.3.3: Much of this text may be too technical to be appreciated by BBASC members and many others. Please restate results and interpretations in a more straightforward manner.

Reply to Comment E8.

Table 19 includes common names in addition to presenting the data using scientific names.

Comment E9.

Section 3.3.3: Zero-catch data should be deleted from the multivariate community analysis. Please include rarely caught taxa. These results will be very skewed (biased) by these outlier surveys and taxa.

Reply to Comment E9.

We respectfully disagree. All taxa including rarely caught taxa were included in our multivariate analysis. However, this is a very difficult question. Zero can be real (organism not there) or not (failed to capture due to avoidance, selectivity). To reduce this tendency we used average values per site/collection. If we did delete zero catches we could severely bias the analysis by inflating the catch rates with only positive catches or completely eliminating an entire collection from consideration. If the study continues we will explore other approaches including presence/absence analysis and truncating the data using an apriori rule based on prevalence.

Comment E10.

Section 3.3.3: Reviewers commented that it would be more informative to select just one site located in the dynamic, transition zone between freshwater faunal dominance and estuarine faunal dominance -- and then study that location intensively to understand how flow variation affects ecological process that influence both freshwater and estuarine fishes. The descriptive work done to date is valuable to determine the best location to do the more intensive research. It was necessary to frame the spatial and temporal variation of salinity in the lower Brazos. Also, important would be research on nutrient dynamics, sediment dynamics, and aquatic primary productivity. These things influence coastal habitats outside the mouth of the Brazos River. Please respond.

Reply to Comment E10.

We agree and have included in our recommendation the need for future research in nutrient and sediment dynamics in the lower river and its influence on delta formation and primary productivity in the lower river and nearshore GOM. However, we do believe that it is important to measure response variables across the estuarine portion of the Brazos River since the flow standard applies to the entire segment and it is necessary to capture the extent of various gradients in salinity, oxygen and other variables and their influence on aquatic organisms.

Comment E11.

Section 4.4: Please clearly state study conclusions in this section. There was no Brazos Estuary recommendation by the BBEST because there was not much data upon which to make one. Rather, than trying to validate a non-recommendation from the BBEST based on HEFR flows at Rosharon, this section should propose how, based on the data that has been collected, a true riverine estuary flow recommendations could be developed.

Reply to Comment E11.

Agree –Verbiage was inserted into the estuary recommendations section regarding new and continued approaches to assessing the relationship of indicators and freshwater inflow in the future.

Comment E12.

Section 4.4: The following statement is too vague:

"To fully understand these interactions the use of other population assessment methods are probably needed to understand the interaction of tidal currents, and river discharge on the transport of larval fish and use of "nursery habitat" is needed."

Please state clearly how the recently completed study can provide guidance for a more focused research study in the future.

Reply to Comment E12.

See previous response to Comment E11 and text modifications in Section 4.4.

Comment E13.

Section 4.4: Reviewers commented that saltwater intrusion would impact the riparian plant community. The aquatic and marine faunas will ebb and flow with changes in discharge and salinity, but the plants cannot migrate. Please comment on this additional aspect of saltwater intrusion and (if necessary) the need for additional study.

Reply to Comment E13.

Salinity is a major factor structuring intertidal plant communities. The intertidal portion of the Brazos River lacks extensive shoreline areas that support emergent and submerged vegetation due in part to the dynamic nature of the river and steep banks. Therefore there is only limited vegetation along the stream banks. However, rapid assessment of dominant plant communities (e..g S. alterniflora vs. Juncus etc) will provide a quick method to assess the long-term trends in salinity in addition to automated monitoring instrumentation. The inclusion of this monitoring method would certainly enhance the ability of future aquatic studies to detect changes in salinity regime. A statement recommending this approach has been inserted in the recommendations section.

Comment E14.

Section 4.4: Yes, more research is needed regarding nutrients in the estuarine reach, but not only concentrations of dissolved inorganic nutrients, but more importantly nutrient cycling rates under different flow conditions.

Reply to Comment E14.

We concur. There are currently new studies underway in tidal rivers that examine the cycling of N and P within different areas of the intertidal portion of tidal rivers (e.g. head of tide, head of halocline etc). Preliminary data indicate some degree of internal cycling of nutrients between sediment and water column.

Comment E15.

Section 4.4: Please comment as to why surveys were not conducted during the summer period.

Reply to Comment E15.

The funding period did not extend across the summer period sufficiently to allow sampling during the months of July-September.

Comment E16.

Section 4.4: This section lacked evaluations for environmental flow standards offered, and there was not even a clear statement of the specific studies needed to make such recommendations. Please discuss methodologies that could be used to make specific recommendations about flows needed to support key ecological functions in the estuarine segment or coastal wetlands just outside the river mouth.

Reply to Comment E16.

Information regarding objectives are now listed in 4.4 and the recommendations section to describe multiple methods and approaches for assessing the response of nekton and other important processes including delta formation, nutrient transport and movement of larger nekton in response to freshwater inflow.

SUGGESTED CHANGES

Specific Draft Final Report Comments

Comment 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.

Reply to Comment 2.

The author concurs that additional samples spanning a range of flow conditions and seasons would increase the sensitivity of existing and future models in their ability (power) to detect changes in water quality, salinity, and biota in response to varying flow conditions and help validate and/or refine the existing environmental flow standard. Furthermore the continuous

time series extending 2-4 years would maximize the ability to tract impacts on cohorts using a combination of tools including size frequency analysis and mark recapture methodology. Additional sampling conducted at monthly intervals at greater spatial resolution would also improve the discriminatory ability to detect both spatial and temporal responses in biota and water quality. Finally, comparison of periodicity of relative density versus comparable data collected by similar gear in adjacent estuaries would provide some measure of similarity of responses of nekton to large scale differences in recruitment not induced by freshwater inflow.

Comment 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.

Reply to Comment 2.

Yes, the goals were listed on page 11 of the manuscript and have been inserted in the Section 4.4 at the end and revisited to indicate whether the objectives were achieved and hypotheses tested.

Figures and Tables Comments:

Comment 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.

Reply to Comment 2.

Changes made.

Comment 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.

Reply to Comment 7.

Changed to color.

Comment 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.

Reply to Comment 12.

The cluster symbols cannot be made larger. It is really not important to view the labels since it is just used to illustrate the groupings.

Replacement Figures and Tables.

Comment E4.

Section 3.3.3, Replaced Figures 45, 46, and 47 in color, pages 91 through 93

Literature Cited Additions

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