INSTREAM FLOWS RESEARCH AND VALIDATION METHODOLOGY FRAMEWORK AND BRAZOS ESTUARY CHARACTERIZATION

Brazos River and Associated Bay and Estuary System

FINAL REPORT

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PURSUANT TO SENATE BILL 1 AS APPROVED BY THE 83RD 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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EXECUTIVE SUMMARY

The Natural Flow Paradigm describes fluvial communities as dependent upon the dynamic character of stream flows. Characteristics of stream flow differ across precipitation, water source, stream order, geomorphology, and other gradients but are similar by having a base flow punctuated by flows less than base (i.e., subsistence) and greater than base (i.e., high-flow pulses). Dynamic characters of stream flow can be 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 from a representative USGS stream gage site, ideally with a historical record sufficient to capture accurate seasonal central tendencies in dynamic characters. Magnitude and duration of flow tiers, when naturally occurring, can be protected by regulatory control, resulting in an environmental flow standard. When water withdrawals are regulated, flow tiers pass through a river reach, presumably maintaining the dynamic character of stream flow and a sound ecological environment. Water volumes in excess of flow tiers are presumably available for diversion, storage, or other uses. With dynamic characters of stream flow defined and protected among multiple river reaches, hypotheses about fluvial community dependencies on dynamic character of stream flows (i.e., Natural Flow Paradigm) can be developed and tested with replication across reaches and basins. Simultaneously, hypothesis testing in a context of an environmental flow standard provides a framework to predict and subsequently test community-flow relationships and to validate or refine environmental flow standards based on evidence.

This study was conducted in order to fill knowledge gaps about ecological linkages between instream flows and components of the natural environment in order to help inform management decisions for aquatic systems in the lower Brazos River (BRA).This research was performed in the context of Senate Bill 3 (SB 3) BBEST/BBASC recommendations and Texas Commission on Environmental Quality (TCEQ) Environmental Flow Standards for BRA and the Guadalupe, San Antonio, Mission, and Aransas Rivers and Mission, Copano, Aransas, and San Antonio Bays Basin and Bay Area (GSA). Purposes were to develop hypotheses about community-flow relationships via an Expert Workshop and subsequent preliminary field investigations, to prioritize and select hypotheses for subsequent testing via a second Expert Workshop, and to test predicted abiotic and biotic responses to flow recommendations and standards during a one-year period of field observations. Instream abiotic and biotic responses to flow tiers (i.e., subsistence flows, base flows, and 4/season [4-per-season], 3/season, 2/season, 1/season, and 1/year pulses) were tested at multiple stream and river sites within the BRA and GSA drainages (hereafter referred to as the aquatic component), multiple riparian zones within the BRA and GSA drainages (riparian component), multiple sites within the Brazos estuary (Brazos estuary component),and multiple GSA floodplain lakes (hereafter referred to as the floodplain lakes component).

The aquatic component quantified physical characteristics of riffle and shallow run instream habitats, macroinvertebrate communities within riffles, fish communities within riffle and run habitats, and egg release of fluvial fishes. A summary of findings includes that predicted abiotic and biotic responses to flow tiers were largely not supported among BBEST/BBASC and TCEQ flow tiers (i.e., base, 2/season, 1/season, and 1/year) for physical characteristics of riffle and shallow run instream habitats, macroinvertebrate communities within riffles, and fish communities within riffle and run habitats. Estimated egg release of fluvial fishes was inconclusive because of low sample size. However, a companion study suggested that flow pulses as low as 2/season were beneficial to the recruitment of fluvial fishes based on estimated time of egg release.

The riparian component quantified seedling and sapling distribution and survival and mature tree distributions of three common riparian trees along cross sections of the riparian zone. A summary of findings includes that seedlings were distributed and survived in the riparian zone at several sites during moderate flow pulses, sapling distribution and survival was inconclusive, and mature tree distributions often failed to receive at least 80% inundation of the riparian zone given current TCEQ standards, a necessary linkage for long-term persistence and recruitment. The across basin assessment confirmed that TCEQ environmental flow standards that did not have the benefit of site-specific, comprehensive instream flow studies are insufficient (in most cases) to meet inundation of at least 80% of the existing riparian zone species on a seasonal or annual basis. If maintenance of the existing riparian zones is a BBASC focus, the addition of higher flows with a 1/spring and 1/fall periodicity is recommended.

The Brazos estuary component described water quality and nekton community patterns and quantified estuary salinity regime, nutrients, suspended solids, and utilization by estuarine-dependent nekton. We found predictable responses of water quality and fish communities to freshwater inflow in the lower Brazos, with greater flow pulses corresponding to lower salinity levels, higher concentrations of suspended solids, chlorophyll-α, and nitrate-nitrite, and lower proportions of estuarine-dependent nekton The Brazos estuary conformed to predictions by transitioning to lower salinity levels and a higher proportion of freshwater nekton communities after 1/year pulse events. Additionally, maximum concentrations of suspended solids, chlorophyll-α, and nitrate-nitrate were observed after 1/season pulse events. Relationship between freshwater inflow and other variables were weak and inconclusive due to low samples sizes.

The floodplain lakes component estimated discharge magnitude resulting in floodplain lake connectivity and quantified fish community structure of floodplain habitats within the GSA. Although a floodplains lakes assessment was not included as part of the BRA study, GSA results are summarized in this report because they confirm ecological relationships documented in the literature for the Brazos basin, and the project team recommends expanding this component to the Brazos basin for future applied research and long-term monitoring. A summary of findings includes that floodplain lakes provide habitat for a unique community of lower Guadalupe River and San Antonio River fishes, in particular lentic fishes (e.g., Gizzard Shad and sunfishes) that are typically rare in mainstem rivers, and fishes in floodplain lakes add to the overall diversity of fishes within the lower reaches of both river. Three of the floodplain lakes were connected at base flows (i.e., protected by TCEQ standard flow tiers), and three lakes were connected by moderate-magnitude high-flow pulses themselves protected by TCEQ standard flow tiers (and consequently by BBEST/BBASC recommendations). However, one floodplain lake was not estimated to be connected by current TCEQ standards.

Among aquatic, riparian, and floodplain lakes components, we detected ecological value from base flow to 3/season through 1/year high-flow events. TCEQ environmental flow standards beyond subsistence and base flow for most of the BRA and GSA sites only included frequent, low-magnitude flow pulses. These pulses were included to maintain a dynamic ecological condition based predominantly on historical hydrology. However, this report, with the full set of qualifiers discussed within, suggests that frequent, low-magnitude pulses may not meet the definition of a dynamic ecological condition. . Study results suggest that higher flow pulses (e.g., 1/year) are likely necessary to maintain existing riparian communities during the spring and fall, and perhaps even higher pulses may be necessary to maintain biotic integrity of riverine communities.

Validation of the TCEQ environmental flow standards and BBEST/BBASC recommendations is currently in the beginning stages and can be refined to allow for additional replications and response variables to improve the validation methodology. Herein, we provide recommendations for a methodological approach with which to prioritize future validation efforts, several possible applied research projects to improve our understanding of the community-flow relationships, and ideas on how to integrate traditional biomonitoring protocols into monitoring long-term changes in aquatic and riparian communities given changes in water quantity.

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 eleven 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 for 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, limitations in establishing ecological linkages between flow levels and biological components (i.e., 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 improving scientific understanding of key relationships between BRA flow levels and regional 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 data gaps, the TWDB commissioned two similar environmental flows validation projects with funds designated by the Texas Legislature to be used in support of SB 3 activities. While one of these projects concerned the BRA while the other dealt with the Guadalupe, San Antonio, Mission, and Aransas Rivers and Mission, Copano, Aransas, and San Antonio Bays Basin and Bay Area (GSA), each of these projects shared the same goals of: (1) adding to the available dataset on flow-ecology relationships in these regions and (2) helping to inform the development of validation methodology which could potentially be used in the future for evaluating established flow standards.

Because the BRA and GSA basin environmental flows validation projects shared not only the same goals and objectives, but many of the same researchers, as well, aspects of each project were at times performed in concert with one another. One such useful combination was the joint

GSA/Brazos project workshop held in July 2014, which brought together environmental flow experts and biologists from throughout Texas. The experts' input was invaluable in helping the project teams target and scale research efforts by selecting meaningful hypotheses for field testing. The project teams then refined these hypotheses by conducting field observations during the summer and fall of 2014. A second joint workshop was held on October 27th, 2014, at which point the final hypotheses were selected. 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 (4/season), 3/season, 2/season, 1/season, and 1-per-year pulses), and (3) the length of time required to conduct field research (each project's deadline was in August 2015). Please note that while the focus of this report will be on the BRA project, references to and results from the GSA region are used in this report to support findings, further develop discussions, and guide future recommendations.

In 2014, following the initial selection and testing of hypotheses, the project teams submitted an interim report to the TWDB outlining the project decision process and included the scope of work for the remainder of the study (BIO-WEST, 2014). Content from the 2014 interim report found to give useful context is presented once more in this report. This report first provides an overview of the early decisions made for the BRA environmental flows validation project followed by a detailed description of the scientific investigations conducted within the BRA region as part of this project. The report closes with two integration sections, each with an eye towards future application. The first of these sections is a multidisciplinary evaluation dealing largely with ways in which this study's findings may be used to help inform and refine validation methodologies, to the eventual end of establishing a sound scientific approach for evaluating whether adopted environmental flow standards are protective of a sound ecological environment in the Brazos basin and bay area. This section goes on to offer preliminary guidance to the BRA BBASC regarding ways in which the application of these methodologies might be either partially or fully validated or used to suggest potential refinements of existing TCEQ flow standards at select BRA basin sites. The final section concerns recommendations for future applied research or long-term monitoring for BRA BBASC consideration.

1.1 Hypothesis development and indicator selection

Several key aquatic and riparian processes and characteristics were researched and discussed in detail during the first joint Expert Workshop held on July 8, 2014. A wide range of possible hypotheses were formulated and discussed, with the key factor being the predicted response of each process/characteristic in relation to stream flow. Workshop discussions focused on both community dynamics and determination of indicator species (e.g., fluvial specialists, individual riparian plants, etc.) in order to evaluate variables that could be tested to best determine short-term ecological responses to stream flows.

Upon development and discussion of an extensive list of hypotheses for testing, the following list of potential instream processes/characteristics were discussed and considered as parameters/variables for testing:

1. Instream habitat
    a. Hydromorphic units

        i. Runs, riffles, pools, backwaters
    b. Hydraulic
        i. Depth, velocity, shear stress
    c. Physical
        i. Substrate, instream cover, woody debris, aquatic vegetation
    d. Chemical
        i. Water quality – standard parameters (i.e., temperature, dissolved oxygen, pH, conductivity)
2. Aquatic biology
    a. Fish, macroinvertebrate, mussels
        i. Community assemblage
        ii. Fluvial specialists
        iii. Indexes (e.g., native versus nonnative species, IBI, EPT, condition)
    b. Fish diet
        i. Gut contents
    c. Larval fish responses
    d. Fish recruitment
        i. Aging using otoliths, scales
            1. Small, short-lived fluvial fish
            2. Large riverine fish
    e. Mussel, *Rangia* spp. recruitment
        i. Aging using shell rings
3. Riparian habitat
    a. Community mapping
    b. Distribution, germination, survival, recruitment
        i. Seedlings, saplings, mature trees
    c. Riparian maintenance
        i. Tree ring analyses
    d. Lateral connectivity
        i. Seedlings, saplings, mature trees
4. Floodplain connectivity
    a. Water level, water quality, habitat, biology
5. Sediment transport
    a. Total suspended solids, turbidity, bedload
6. Water chemistry
    a. Nutrients, contaminants, pharmaceuticals

The July 8th workshop attendees discussed the pros and cons of the indicators and/or parameters listed above. When considering hypotheses/variables/indicators, the workshop attendees also evaluated whether they might require additional resources, might not be amenable to the short time-frame of this effort, or if significant work on the subject had already been conducted by resource agencies or other researchers.

Following the first expert panel workshop, each respective project team was given from July through October 2014 to conduct preliminary testing of possible monitoring protocols and sampling techniques. On October 27, 2014, upon completion of this pilot period, participants were reconvened for a second expert panel workshop, which had the objective of using the existing scientific literature, the panel members' combined professional expertise, and the project teams' preliminary data to streamline the number of hypotheses to be tested, maximizing the value of parameters tested and indicators used, and refining experimental methodologies, if necessary. These steps were proposed in order to determine the most promising validation approach to be tested in the following year. At this workshop, the project teams reported their preliminary results, and the panel discussed study questions, site selection, sampling protocols and procedures, and lessons learned. There were discussions on true replication, temporal scales, random subsampling of fish for condition evaluation, and macroinvertebrate indicators, among other topics.

Based on workshop discussions, some variables and hypotheses which had been proposed were eliminated from consideration, while others were modified and retained. Workshop attendees removed mussels from consideration for the project due to the limited life history information available at the time. As had been noted in the first workshop, the participants acknowledged that there are a number of ongoing mussel investigations regarding habitat utilization in relation to flow dynamics taking place outside of this project, which would be valuable to help guide this project in the future. The hypotheses related to the linkage between flow pulses and macroinvertebrate reproduction was abandoned because of the apparent complexity and high level of effort anticipated to be necessary in order quantify a response. In the end, discussions from the second expert workshop were extremely valuable in assisting each project team with recommendations for the following year's sampling efforts, which are described in this report.

1.2 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 Brazos River during low flow conditions, large-river-type fishes, such as smalleye shiners, sharpnose shiners, silverband shiners, and chubs, are replaced with tributary/generalist type fishes, such as red shiners, bullhead minnows, and centrarchids (generalization is based on historical analyses [Runyan, 2007], but also on ecology of other similar prairie streams). 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). Increases in generalist fishes within mainstem rivers conform to the Native Invader Concept (Scott and Helfman, 2001), which states that the first indication of environmental degradation is increases in native, generalists taxa (i.e., native invaders) and can be easily applied to the Biological Gradient Concept (Davies and Jackson, 2006), which describes initial resistance followed by rapid changes in fish community structure (i.e., native generalist fishes replacing native specialist fishes) with increases anthropogenic alterations.

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 with a priori predictions. The hypotheses selected each concerned variables that were controlled by environmental flow standards, able to be tested with independent observations, and could be tested within project time.

Study objectives and predictions

Aquatic assessment objectives were to:

1. describe spatial and temporal trends in abiotic characters of riffle habitats;
2. quantify relative abundances, densities, and habitat associations of macroinvertebrates and fishes in riffle habitats;
3. assess patterns in condition factors, hepatic-somatic indices, and gut fullness of riffle fishes;
4. describe spatial and temporal trends in abiotic characters of run habitats;
5. quantify relative abundances, densities, and habitat associations of fishes in run habitats;
6. test for differences in abiotic and biotic responses among flow tiers (BBEST), basin, and season (differences in abiotic and biotic responses among basin and seasonal effects are of lesser interest than differences among tiers; however, relationships among response variables and tier might depend on basin and seasonal effects, and therefore be necessary to test concurrently); and,
7. collect juvenile specimens of fluvial specialists (chub [*Macrhybopsis* spp.]) during various intervals throughout the year in order to estimate ages and dates of hatching via analysis of otolith growth rings.

Silt and other fine sediments are removed through scouring action associated with higher flow pulses, which decrease the embeddedness of substrates and increase the amounts of coarser substrates (e.g., gravel and cobble) in riffle and run habitats (De Sutter et al., 2001). Mobilization of substrates increases current velocity and depth of riffle and run habitats (Jowett and Richardson, 1989), though dependent upon stream gradient (Coleman, 1986).

For abiotic factors, we predicted that:

1. flow tiers will be inversely related to amount of silt substrates in riffle and run habitats and directly related to amount of larger substrates (i.e., sand, gravel, cobble, boulder, and bedrock) in riffle and run habitats,
2. flow tiers will be inversely related to substrate embeddedness and percent vegetation in riffle and run habitats, and
3. flow tiers will be directly related to current velocity and depth of riffle and run habitats.

Relative abundances by densities and percent occurrences of riffle-specialist and fluvial-specialist macroinvertebrates and fishes are greater following flow pulses because of these specialists' abilities to seek refuge and minimize downstream displacement (Harrell, 1978; Meffe and Minkley, 1987; Extence et al., 1999; Dodds et al., 2004). Correspondingly, relative abundances and percent occurrences of slack-water specialists will be less following flow pulses. In addition, flow pulses are related to increases in nutrient pulses, thus increasing food sources for fishes (Brittain and Eikeland, 1988; Gibbins et al., 2007). Based on prior research findings on minnow species classified as fluvial specialists that reproduce by broadcast spawning of pelagic eggs during high-flow pulses, we hypothesized that related minnow species in the Brazos and San Antonio rivers likewise classified as fluvial specialists would show a positive relationship between number of successful recruits and high-flow pulses in these rivers. Many of the fluvial-specialist minnow species in these two rivers have already declined in abundance, but the shoal chub, *Macrhybopsis hyostoma*, in the Brazos River and the burrhead chub, *Macrhybopsis marconis*, in the San Antonio River can still be found in low to moderate numbers in certain habitats during certain periods.

For biotic factors, we predicted that:

1. flow tiers will be directly related to relative abundances of swift-water and moderately swift-water aquatic insects (defined in Section 2.1) and inversely related to relative abundances of slack-water aquatic insects in riffle habitats;
2. flow tiers will be directly related to relative abundances of riffle fishes and fluvial fishes and inversely related to slack-water fishes in riffle habitats;
3. flow tiers will be inversely related to fish species richness in riffle habitats;
4. flow tiers will be directly related to percent occurrences of riffle fishes and fluvial fishes, and inversely related to percent occurrences of slack-water fishes in riffle habitats;
5. flow tiers will be directly related to condition factor, hepatic-somatic index, and gut fullness of selected riffle and fluvial specialists in riffle habitats;
6. flow tiers will be directly related to relative abundances of swift-water and fluvial fishes and inversely related to slack-water fishes in run habitats;
7. flow tiers will be inversely related to fish species richness in run habitats;
8. flow tiers will be directly related to percent occurrences of swift-water and fluvial fishes and inversely related to slack-water fishes in run habitats; and
9. abundance of surviving chub (*Macrhybopsis* spp.) juveniles would be greater when river flow was increasing and high during hatching (high-flow hypothesis for recruitment of fluvial specialists).

To further explore biotic effects related to flow tiers, we also tested density response of macroinvertebrates and fishes (overall and by specialty) among flow tiers, response of selected fish families (Cyprinidae, Percidae, Centrarchidae), response of selected fish habitat guilds (benthic and top-water), and response of species of conservation concern.

1.3 Riparian

The environmental flow requirements for recruitment and persistence of bottomland hardwood species within riparian corridors in Texas are not well understood. Two key problems in identifying the flow needs of riparian trees are the physical and hydrological complexity of this transitional zone in the landscape and the differing germination and growth requirements of the diverse group of taxa that occur in it. Research in riparian areas has identified several factors that influence recruitment, including species and dispersion of trees at the site, seed production and dispersal (Clark et al., 1998; Houle and Payette, 1990), and establishment limitations (Houle and Payette, 1990; Houle, 1992; Shibata and Nakashizuka, 1995; Clark et al., 1998; Hampe, 2004).

Establishment limitation may be the strongest filter on recruitment for many taxa. Using a random permanent plot survey method, Liang and Seagle (2002) found that two microhabitat factors (soil moisture and leaf litter) were correlated with seedling spatial distributions, suggesting that microhabitat variability promotes seedling diversity. Battaglia and Sharitz (2006) developed logistic regressions to determine the probability of occurrence of bottomland hardwood species based on canopy openness and distance to water table.

Soil moisture is another important environmental variable for seed germination and seedling survival; too much water may not allow air to reach the plant roots, and too little will desiccate the plant. The hydrology of the riparian zone influences microhabitat conditions of germination sites such as soil moisture, nutrients, aeration, sedimentation, erosion, and disturbance. Riparian bottomland hardwood forests are characterized by high water tables and seasonal and periodic flooding from river pulse flows. The duration and level of flood inundation from these pulse flows are therefore likely to play important roles in determining the seedling recruitment and growth of trees in riparian areas.

Study objectives and predictions

Several key riparian processes/characteristics are given below, grouped by general life stage. The responses of these processes were considered in relation to stream flow:

1. seedling distribution/germination;
2. seedling survival;
3. sapling survival; and
4. mature tree survival/maintenance and distribution.

The study focused on riparian indicator species, rather than riparian community as a whole, in order to best determine short-term responses to stream flows. A set of key indicator species previously developed for the San Antonio River by Duke (2011) was used for this study. These species include: Black willow (*Salix nigra*), Box elder (*Acer negundo*), and Green ash *(Fraxinus pennsylvanica*). These three species were selected as representatives of a healthy, functioning riparian zone because they are broadly distributed across the GSA basin and its tributaries and are tightly connected to stream channel processes (primarily stream flow).

Several characteristics of these species make them valuable indicators of riparian health in a forest. Seedlings of these species are either tolerant of flooding or require considerable flooding to germinate. Black willows generally tend to drop seeds from April to July, which must then germinate immediately. Green ash and box elder generally tend to drop seeds in late fall and winter, but do not germinate until the next spring. Once germinated, all three indicator species then require periodic wetting in order to survive and thrive (Stromberg, 1998). Small flow pulses facilitate resiliency to larger floods in young members of these species (Middleton, 2002). Lack of streamside soil moisture not only threatens seedlings (Smith et.al., 1998) but also allows for encroachment by upland plants (Myers, 1989). Willows have been shown to be particularly sensitive to long-term flow alterations and susceptible to takeover by invasive species in areas of altered stream flows (Williams and Cooper, 2005).

Although seed germination is critically dependent on flood pulsing (Junk and Piedade, 1997), as plants mature they become both less dependent on frequent pulses and more tolerant of severe flow fluctuations. Seedling dispersal, establishment, and survival are key life stages to ensuring that riparian forest replacement is maintained.

Hypotheses were developed using the above major parameters for consideration, BBEST recommendations (Brazos BBEST, 2012), results from a recently-conducted intensive riparian study at two sites along the San Antonio River (M. Fontenot/Bio West, pers. comm.), TIFP recommendations (TIFP, 2011), and general riparian flow-ecology hypotheses developed by Duke and Davis (2014). The flow-ecology hypotheses were developed by the Southeast Aquatic Resources Partnership (SARP) and intended as a holistic suite of relationships that demonstrate ecological responses to alterations of the natural flow regimes. They form a scientific basis for setting ecological limits of hydrologic alteration for streams and rivers in the southeast, including Texas. Their purpose is to inform data synthesis and to design field studies to improve flow-ecology relationships and the science supporting instream flow standards in the region, and consequently work well as a foundation for hypothesis development.

Prior to the October 2014 expert panel workshop, a set of proposed woody riparian hypotheses were developed; these were refined following the workshop and field testing and are described below and in Table 1.

Mature woody riparian species

Rationale: Falling water tables caused by increased duration of extreme low-flow events and lack of flow pulses results in loss of plant vigor, increased mortality rates, and stand loss. But the recommended flows are adequate for maintaining current mature riparian tree distributions against falling water tables. Accordingly, a key assumption is that the standing mature riparian tree distributions at a given site are representative of historical adequate flows at that site.

Biotic Predictions:

1. Seasonal flows will correlate directly with riparian zone mature tree distribution.
2. TCEQ flow tiers will provide adequate coverage of existing riparian stands.

Woody riparian seedlings

Rationale: Seedling establishment and survival require multiple high-flow pulses, which distribute seeds and contribute to soil moisture in the shallow unsaturated zone, throughout the growing season.

Biotic Predictions:

1. For indicator species, seedling count and distribution will relate directly to frequency and magnitude of seasonal high-flow pulses.
2. If TCEQ flow tiers occur, seedling counts and distribution will correlate positively with them.
3. If TCEQ flow tiers do not occur, seedling counts and distribution will correlate with actual flows, if adequate (verifying whether flows do influence seedling dispersal and survival).

Woody riparian saplings

Rationale: Sapling survival along channel slopes requires multiple high-flow pulses (which provide soil moisture in the shallow unsaturated zone) throughout the growing season.

Biotic Predictions:

1. For indicator species, sapling count and distribution will relate directly to frequency and magnitude of high-flow pulses.
2. If TCEQ flow tiers occur, sapling counts and distribution will correlate positively with them.
3. If TCEQ flow tiers do not occur, sapling counts and distribution will correlate with actual flows, if adequate (verifying whether flows do influence sapling dispersal and survival). Nullification of this hypothesis would indicate that saplings have already begun to develop root systems deeply enough connected to soil water zones to protect them from within-year seasonal fluctuations.

Woody riparian community

Rationale: High-flow pulses both recharge groundwater availability to mature trees and scour/remove invasive/non-riparian species along the active channel and riparian zone.

Biotic Predictions:

1. Riparian relative abundance will correlate directly with flows. This is a hypothesis with limited confirmation within the one year study. However, establishment of the relative abundance, pre-study and post-study for each of the age classes will provide a baseline for follow-up studies. Once relative abundance is calculated, long-term monitoring of variation will allow managers to scale up the short-term processes and hypotheses to overall riparian health and functioning.
2. Age distributions of riparian populations reflect historic flow regimes, and can be used to detect the effect of major anomalies in flow.

1.4 Brazos Estuary

Estuaries can be classified based on multiple criteria including salinity regime, tidal influence, freshwater inflow, geomorphology, origin, and circulation/stratification (Savenije, 2005; Day et al., 2013). The Brazos River estuary is unique in that it is one of the few "riverine" estuaries along the Texas coast (Orlando, 1993; Savenije, 2005; Engle et al., 2007). Depending on freshwater inflow, depth and tidal regime riverine type estuaries can experience wide lateral (upstream to downstream) and vertical changes in salinity. For example, during low freshwater inflow upstream density currents coupled with flood tides can extend marine water far upstream (Orlando 1993).

A widely accepted conceptual model that describes the relationship between freshwater inflow and resulting geomorphological, physio-chemical and biological attributes was first proposed by Alber (2002).

Table 1. Summary of riparian hypothesis testing. The Y/N column was used to determine whether the hypothesis was supported/disproven.

Group	Hypothesis	Y/N	Pros	Cons	Usefulness
Mature tree distribution	Distribution of mature trees reflects seasonal flow standards				
	Seasonal flow standards are adequate to maintain distribution of mature trees				
Seedling distribution and survival	Seedling distribution correlates with seasonal flow standards				
	If flows observed are less than the flow standards, seedling distribution correlates with actual flows				
	Seedling survival across seasons correlates with flows received				
Sapling distribution and survival	Distribution of saplings correlates with seasonal flow standards				
	If flows observed are less than the flow standards, sapling distribution correlates with actual flows				
	Sapling survival across seasons correlates with flows received				
Riparian community	Riparian species show high relative abundance				
	Community age distribution reflects observed major flow anomalies				

This model describes the array of ecosystem services provided by freshwater inflow (Figure 1). Similar to the natural flow paradigm and river continuum concept for rivers, the proposed model states that the discharge of freshwater under natural conditions creates an optimal salinity gradient for the assemblage of organisms that have evolved for the range of conditions that occur within an estuary (Vannote, 1980; Poff et al., 1997; Alber, 2002). In addition, under these natural fluctuations other ecosystem services including delivery of delta forming sediments and nutrients that support primary producers are delivered to the estuary (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). This stratification and formation of a stable pycnocline limits vertical mixing and the formation of hypoxic or anoxic conditions along tidally influenced river bottoms (Kuo et al., 1991).

Hypoxia in Gulf coast estuaries has been linked with: (1) seasonal temperature increases which drive high oxygen demand, (2) neap-spring tidal cycles, 3) salinity and/or temperature stratification which limits vertical mixing, 4) eutrophication and 5) diurnal cycling of dissolved oxygen (DO) (Engle et al., 1999). This increased 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 DO was closely related to the vertical salinity gradient (ΔS). Hypoxia seldom occurred when ΔS (over 2.5 m) was <2 psu and occurred almost all the time when ΔS was >8 psu in the absence of extreme events like hurricanes (Park et al., 2007).

As with many estuarine systems, a significant amount of primary production in the Brazos River and nearshore Gulf of Mexico is driven by the import of upstream nutrients and detritus including high levels of particulate organic matter (POM) (Day et al., 2013). This nutrient and organic loading support both phytoplankton and benthic and planktonic heterotrophic protozoan which are fed upon by larger immigrating juvenile estuarine organisms into the Brazos River (Day et al., 2013).

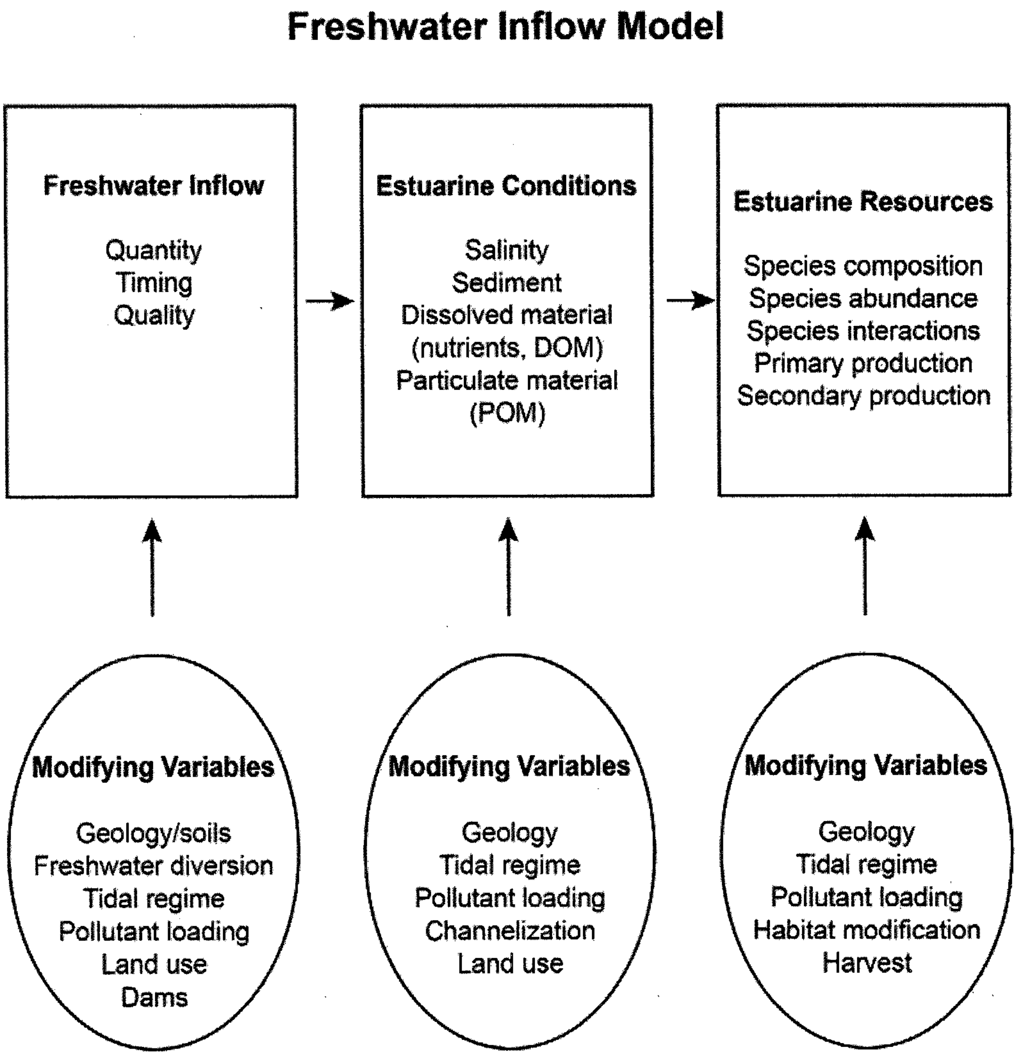


Figure 1. Schematic diagram of the effects of freshwater inflow on estuaries. Modified after Alber (2002).

Water flowing through a riverine estuaries, including the lower Brazos River, exhibit short residence times and high turnover rates (Engle et al., 2007). The productivity of riverine estuaries are dependent upon maintaining natural hydrographic variation since the majority of nutrient input is dependent on upstream sources (Orlando et al., 1993; Engle et al., 2007). Part of this natural variability includes large high-flow pulses that are important for maintaining the river delta geomorphology (Orlando et al., 1993; Gibeaut et al., 2000). The current Brazos River delta is an arcuate, wave-dominated delta that protrudes two kilometers 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 will be displaced downstream into the Gulf of Mexico and either return as salinity increases or die. Immobile benthic organisms however, will not be able to persist and high mortality is the likely outcome. 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, salinity in the Brazos River and other riverine estuaries will increase significantly and extend along the bottom far upstream (Orlando et al., 1993). During these periods estuarine and marine organisms will 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 stratification, 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).

Study objectives and predictions

Estuary assessment objectives included were:

1. to use new and historical data collected on the tidal portion of the lower Brazos River by
    a. characterizing flow regime and tidal dynamics,
    b. assessing water quality and nutrient patterns,
    c. describing the salinity regime of the Brazos estuary,
    d. characterizing nekton community composition, and
    e. assessing use by estuarine dependent species, and
2. to test predicted relationships between salinity, nutrients and proportions of estuarine species against flow tier and discharge.

We predicted that:

1. flow tiers and discharge would be inversely related to salinity levels in the Brazos River estuary,
2. flow tiers and discharge would be inversely related to pycnocline lateral extent and stability in the Brazos River estuary,
3. flow tiers and discharge would be directly related to nutrient and suspended solid levels in the Brazos River estuary, and
4. flow tiers and discharge would be inversely related to the occurrence of estuarine dependent species in the Brazos River estuary.

2 Materials and methods

2.1 Aquatic

Within the BRA and GSA regions, fourteen SB 3 GSA and Brazos gage locations were selected for the aquatic assessment. Sites were selected to represent tributaries and main stem reaches. Six of the fourteen sites sampled were from the Brazos River basin: four tributaries (11-Leon River at Gatesville, 12-Lampasas River near Kempner, 13-Little River at Little River and 17-Navasota River near Easterly) and two main stem sites (18-Brazos River at Hempstead and 20-Rosharon). Numbers correspond to site descriptions in BRA BBEST report (Figure 2). Eight of the fourteen sites sampled were within the GSA basins: three tributaries (Medina River at Bandera, San Marcos River at Luling, Cibolo Creek near Falls City) and four mainstem sites (San Antonio River at Falls City and Goliad and Guadalupe River at Gonzales and Cuero) (Figure 3; taken from BBEST Report).

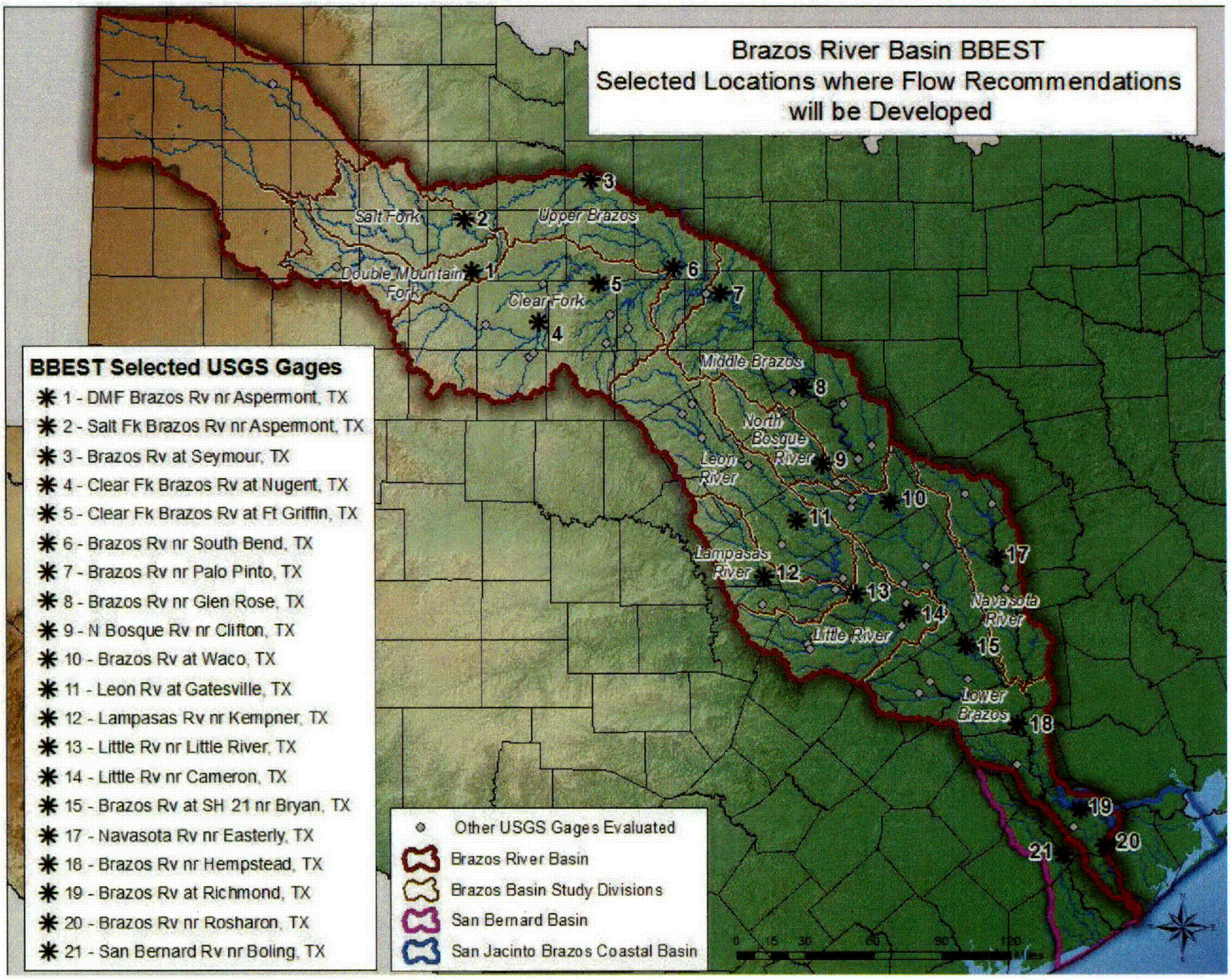


Figure 2. **Reference map of locations within the BRA (taken from BRA BBEST report). Specific sites used in this study are reported in the text.**

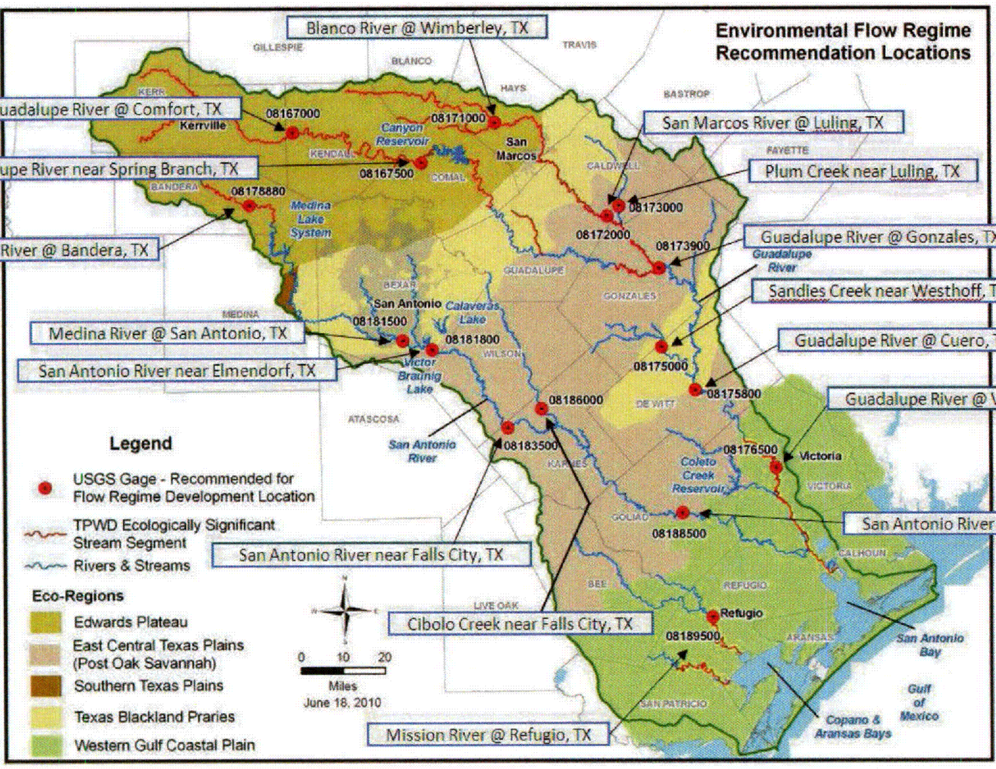


Figure 3. **Reference map of locations within the GSA (taken from GSA BBEST report). Specific sites used in this study are reported in the text.**

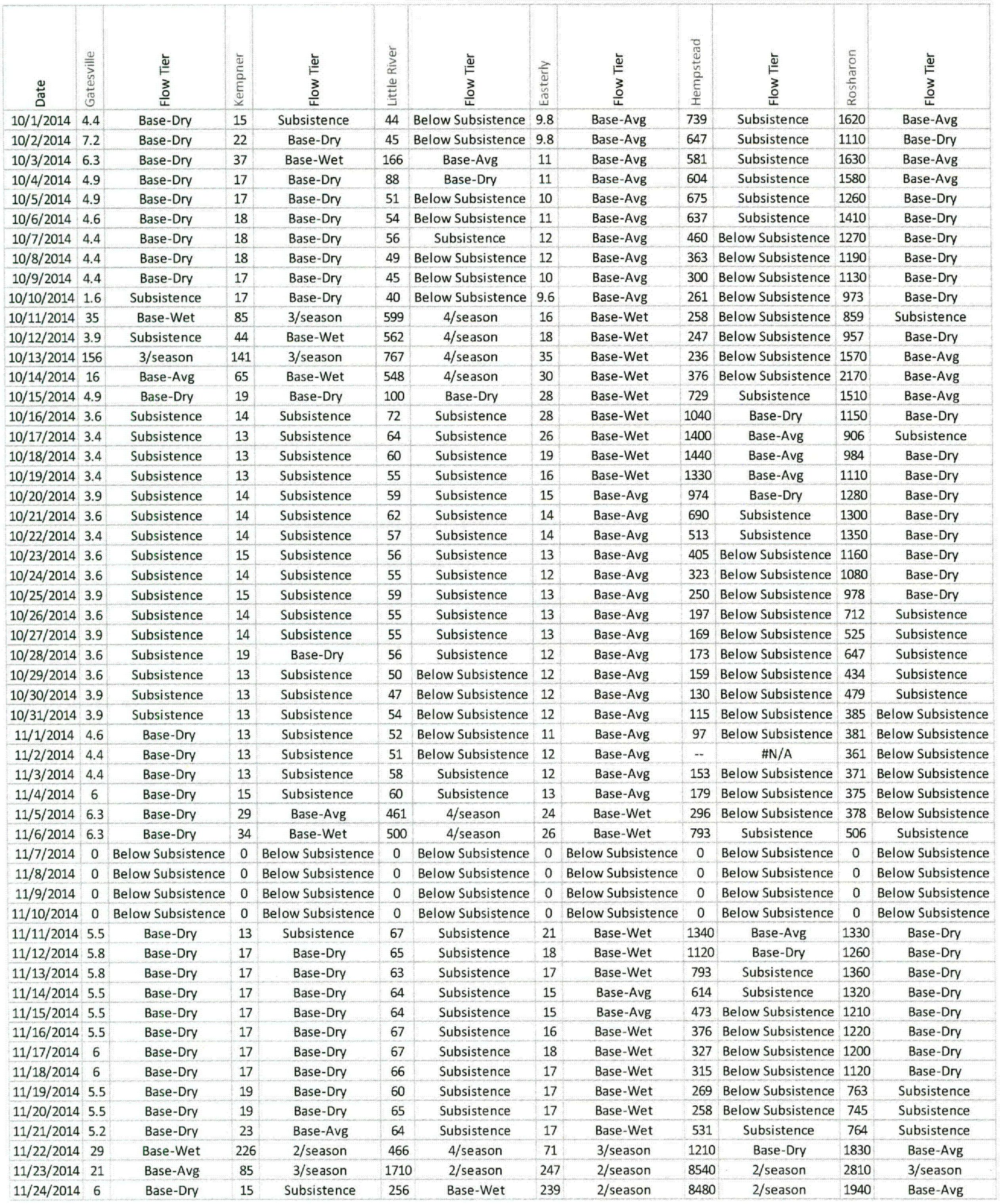
During each season (designated by BBEST recommendations), flows were monitored daily using USGS gaging stations at or near each site. Peak flow (cfs) of the day determined the classification of the peak flow event as 1 of 7 flow tiers [subsistence, base, 4-per-season, 3-per-season, 2-per-season, 1-per-season, and 1 per year high-flow pulses; assigned ordinal numbers 1 (subsistence) through 7 (1 per year high-flow pulse), respectively]. To automate the monitoring of daily peak flows and corresponding flow tier, we developed a program, using Excel that communicated with USGS stations each time the program was opened (Figure 4). Latest daily peak flows and flow tiers were updated and displayed on the spreadsheet, allowing us to simultaneously monitor flows and tiers among 14 sites. Sites with subsistence and base tiers were visited seasonally or between 10 and 15 days of continuously maintaining that tier. Sites with flow pulses were visited up to 15 days following the event but with the condition that flows returned to base tier. Therefore, visits and abiotic and biotic samples were taken at subsistence or base flow conditions and not during a high-flow event preventing a dilution effect.

For each site visit, one riffle and one or more shallow runs were selected, except at main stem Brazos River sites (i.e., Hempstead and Rosharon), which lacked riffle habitats. Among riffle habitats, three subsections of the riffle were designated (approximately 30 m^2) to capture variability within each riffle habitat (i.e., 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 (Figure 5). The electrofished area was inspected for any stunned fish on the benthos. 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 (Figure 6). 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*, *Macrohybopsis*, Percidae, juvenile Ictaluridae) were collected, euthanized with MS-222, and fixed in 10% formalin for laboratory quantification of gut fullness, condition, and hepatic-somatic index. Among run habitats, downstream seining (common or bag seine, depending on water depths) was used to quantify fish occurrence and abundance (Figure 7, Figure 8). Within the main stem Brazos River, seine hauls were taken from point-sand bar habitats. Fish and habitats were quantified identical to those described for riffle habitats, except Hess Samples were not taken and embeddedness was not recorded.

In the laboratory, benthic samples were rinsed using a 250 µm sieve, sorted to order, and enumerated. Fishes taken from riffles were weighed and measured to calculate Fulton Condition Factor (Anderson and Neumann, 1996). For hepatic-somatic index and gut fullness, fish were dissected by exposing the viscera with a longitudial cut from isthmus to posterior of urogental vent. The entire gut tract (from esophugus to anus) and other organs were removed from the abdominal cavity. With the use of a dissecting scope, stomachs were removed and seperated from the remaing gut tract at the pyloric sphincter muscle. Liver was removed from Percidae only and weighed. Gut fullness (i.e., proportion of stomach filled by contents) were independently assessed by two observers, assigning a number from 0 (empty) to 10 (full) in increments of 1. Descrepency in number assignment between independent observers required a third observer to assign a number.

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. Taxa richness was calculated by counting the number of unique species among the three subsections or multiple runs. 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. Consequently, 227 riffle subsections were reduced to 63 riffles, and 145 runs were reduced to 74 runs. Abiotic and biotic variables of experimental units were used in subsequent analyses.



Date	Gatesville	Flow Tier	Kempner	Flow Tier	Little River	Flow Tier	Easterly	Flow Tier	Hempstead	Flow Tier	Rosharon	Flow Tier
10/1/2014	4.4	Base-Dry	15	Subsistence	44	Below Subsistence	9.8	Base-Avg	739	Subsistence	1620	Base-Avg
10/2/2014	7.2	Base-Dry	22	Base-Dry	45	Below Subsistence	9.8	Base-Avg	647	Subsistence	1110	Base-Dry
10/3/2014	6.3	Base-Dry	37	Base-Wet	166	Base-Avg	11	Base-Avg	581	Subsistence	1630	Base-Avg
10/4/2014	4.9	Base-Dry	17	Base-Dry	88	Base-Dry	11	Base-Avg	604	Subsistence	1580	Base-Avg
10/5/2014	4.9	Base-Dry	17	Base-Dry	51	Below Subsistence	10	Base-Avg	675	Subsistence	1260	Base-Dry
10/6/2014	4.6	Base-Dry	18	Base-Dry	54	Below Subsistence	11	Base-Avg	637	Subsistence	1410	Base-Dry
10/7/2014	4.4	Base-Dry	18	Base-Dry	56	Subsistence	12	Base-Avg	460	Below Subsistence	1270	Base-Dry
10/8/2014	4.4	Base-Dry	18	Base-Dry	49	Below Subsistence	12	Base-Avg	363	Below Subsistence	1190	Base-Dry
10/9/2014	4.4	Base-Dry	17	Base-Dry	45	Below Subsistence	10	Base-Avg	300	Below Subsistence	1130	Base-Dry
10/10/2014	1.6	Subsistence	17	Base-Dry	40	Below Subsistence	9.6	Base-Avg	261	Below Subsistence	973	Base-Dry
10/11/2014	35	Base-Wet	85	3/season	599	4/season	16	Base-Wet	258	Below Subsistence	859	Subsistence
10/12/2014	3.9	Subsistence	44	Base-Wet	562	4/season	18	Base-Wet	247	Below Subsistence	957	Base-Dry
10/13/2014	156	3/season	141	3/season	767	4/season	35	Base-Wet	236	Below Subsistence	1570	Base-Avg
10/14/2014	16	Base-Avg	65	Base-Wet	548	4/season	30	Base-Wet	376	Below Subsistence	2170	Base-Avg
10/15/2014	4.9	Base-Dry	19	Base-Dry	100	Base-Dry	28	Base-Wet	729	Subsistence	1510	Base-Avg
10/16/2014	3.6	Subsistence	14	Subsistence	72	Subsistence	28	Base-Wet	1040	Base-Dry	1150	Base-Dry
10/17/2014	3.4	Subsistence	13	Subsistence	64	Subsistence	26	Base-Wet	1400	Base-Avg	906	Subsistence
10/18/2014	3.4	Subsistence	13	Subsistence	60	Subsistence	19	Base-Wet	1440	Base-Avg	984	Base-Dry
10/19/2014	3.4	Subsistence	13	Subsistence	55	Subsistence	16	Base-Wet	1330	Base-Avg	1110	Base-Dry
10/20/2014	3.9	Subsistence	14	Subsistence	59	Subsistence	15	Base-Avg	974	Base-Dry	1280	Base-Dry
10/21/2014	3.6	Subsistence	14	Subsistence	62	Subsistence	14	Base-Avg	690	Subsistence	1300	Base-Dry
10/22/2014	3.4	Subsistence	14	Subsistence	57	Subsistence	14	Base-Avg	513	Subsistence	1350	Base-Dry
10/23/2014	3.6	Subsistence	15	Subsistence	56	Subsistence	13	Base-Avg	405	Below Subsistence	1160	Base-Dry
10/24/2014	3.6	Subsistence	14	Subsistence	55	Subsistence	12	Base-Avg	323	Below Subsistence	1080	Base-Dry
10/25/2014	3.9	Subsistence	15	Subsistence	59	Subsistence	13	Base-Avg	250	Below Subsistence	978	Base-Dry
10/26/2014	3.6	Subsistence	14	Subsistence	55	Subsistence	13	Base-Avg	197	Below Subsistence	712	Subsistence
10/27/2014	3.9	Subsistence	14	Subsistence	55	Subsistence	13	Base-Avg	169	Below Subsistence	525	Subsistence
10/28/2014	3.6	Subsistence	19	Base-Dry	56	Subsistence	12	Base-Avg	173	Below Subsistence	647	Subsistence
10/29/2014	3.6	Subsistence	13	Subsistence	50	Below Subsistence	12	Base-Avg	159	Below Subsistence	434	Subsistence
10/30/2014	3.9	Subsistence	13	Subsistence	47	Below Subsistence	12	Base-Avg	130	Below Subsistence	479	Subsistence
10/31/2014	3.9	Subsistence	13	Subsistence	54	Below Subsistence	12	Base-Avg	115	Below Subsistence	385	Below Subsistence
11/1/2014	4.6	Base-Dry	13	Subsistence	52	Below Subsistence	11	Base-Avg	97	Below Subsistence	381	Below Subsistence
11/2/2014	4.4	Base-Dry	13	Subsistence	51	Below Subsistence	12	Base-Avg	--	#N/A	361	Below Subsistence
11/3/2014	4.4	Base-Dry	13	Subsistence	58	Subsistence	12	Base-Avg	153	Below Subsistence	371	Below Subsistence
11/4/2014	6	Base-Dry	15	Subsistence	60	Subsistence	13	Base-Avg	179	Below Subsistence	375	Below Subsistence
11/5/2014	6.3	Base-Dry	29	Base-Avg	461	4/season	24	Base-Wet	296	Below Subsistence	378	Below Subsistence
11/6/2014	6.3	Base-Dry	34	Base-Wet	500	4/season	26	Base-Wet	793	Subsistence	506	Subsistence
11/7/2014	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence
11/8/2014	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence
11/9/2014	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence
11/10/2014	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence	0	Below Subsistence
11/11/2014	5.5	Base-Dry	13	Subsistence	67	Subsistence	21	Base-Wet	1340	Base-Avg	1330	Base-Dry
11/12/2014	5.8	Base-Dry	17	Base-Dry	65	Subsistence	18	Base-Wet	1120	Base-Dry	1260	Base-Dry
11/13/2014	5.8	Base-Dry	17	Base-Dry	63	Subsistence	17	Base-Wet	793	Subsistence	1360	Base-Dry
11/14/2014	5.5	Base-Dry	17	Base-Dry	64	Subsistence	15	Base-Avg	614	Subsistence	1320	Base-Dry
11/15/2014	5.5	Base-Dry	17	Base-Dry	64	Subsistence	15	Base-Avg	473	Below Subsistence	1210	Base-Dry
11/16/2014	5.5	Base-Dry	17	Base-Dry	67	Subsistence	16	Base-Wet	376	Below Subsistence	1220	Base-Dry
11/17/2014	6	Base-Dry	17	Base-Dry	67	Subsistence	18	Base-Wet	327	Below Subsistence	1200	Base-Dry
11/18/2014	6	Base-Dry	17	Base-Dry	66	Subsistence	17	Base-Wet	315	Below Subsistence	1120	Base-Dry
11/19/2014	5.5	Base-Dry	19	Base-Dry	60	Subsistence	17	Base-Wet	269	Below Subsistence	763	Subsistence
11/20/2014	5.5	Base-Dry	19	Base-Dry	65	Subsistence	17	Base-Wet	258	Below Subsistence	745	Subsistence
11/21/2014	5.2	Base-Dry	23	Base-Avg	64	Subsistence	17	Base-Wet	531	Subsistence	764	Subsistence
11/22/2014	29	Base-Wet	226	2/season	466	4/season	71	3/season	1210	Base-Dry	1830	Base-Avg
11/23/2014	21	Base-Avg	85	3/season	1710	2/season	247	2/season	8540	2/season	2810	3/season
11/24/2014	6	Base-Dry	15	Subsistence	256	Base-Wet	239	2/season	8480	2/season	1940	Base-Avg

Figure 4. **Screenshot of Excel program illustrating tracking of daily stream flows and tiers among USGS stations located near sampling sites. Program code enabled the spreadsheet to communicate with USGS stations to obtain peak flow per station, each time the file was opened.**

Spatial (among sites) and temporal (among seasons) patterns in riffle and run abiotic factors were assessed with Principal Component analyses (PCA). PCA is an indirect gradient analysis used to reduced dimensionality of large datasets by the use of linear combinations. Sites and seasons were coded as dummy variables, embeddedness as ordinal data (1 – 4), and the remaining variables were treated as continuous variables. Spatial and temporal patterns in riffle and run biotic (macroinvertebrate and fish total N and densities) and their abiotic relationships were assessed with Canonical Correspondence analyses (CCA). CCA is a direct gradient analysis where an ordination of one multivariate matrix is constrained by a multiple linear regression on variables in a second matrix (McCune and Grace, 2002)

Figure 5. **Electroshocking one section of a riffle at Cibolo Creek near Falls City.**

Among riffle habitats, macroinvertebrates were grouped along a gradient of swift to slack-water specialists following the methodologies of Extence et al., (1999). Orders not annotated in the publication were assigned a category from habitat associations found in the available literature. Categories were swift-water insects, moderately swift-water insects, and slack-water insects. Categories were summed across densities to calculate each category per riffle. Likewise, Ephemeroptera-Plecoptera-Tricoptera (EPT) index was calculated for each riffle by summing densities. Relative abundances were calculated for each category (i.e., swift-water insects, moderately swift-water insects, slack-water insects, and EPT) by summing densities within a category, dividing by all insect densities, and multiplying by 100. Similarly, fishes were grouped along a gradient of swift 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 density within the category, divided by fish densities, and multiplying by 100. In addition, percent occurrences (number of species within a category, divided by the number of all species, multiplied by 100) were calculated for riffle fishes, fluvial fishes, slack-water fishes, Cyprinidae, Percidae, Ictaluridae, benthic fishes, top-water fishes (*Gambusia* and *Fundulus*), and species of conservation concern (SOC; listed by Texas Parks and Wildlife Department [TPWD]).

Figure 6. **Hess sample collection and abiotic parameters readings following electroshocking of the riffle sections on the San Antonio River near Goliad.**

Among run habitats, density, relative abundance, and percent occurrences were calculated for each run by the same methodology and similar categories (swift-water fishes, fluvial fishes, slack-water fishes, Cyprinidae, Centrarchidae, top-water fishes, and TPWD SOC). Consequently, two abiotic data sets (one for riffles and one for runs) and three biotic data sets (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., percent silt substrate, embeddedness, macroinvertebrate densities, swift-water fish relative abundances, percent occurrence of Cyprinidae) and tier (up to seven levels), drainage (BRA or GSA), and season (3 seasons in BRA were converted to a 4 seasons scale, while the GSA has 4 seasons). Replication was deemed adequate if treatment level had at least five replicates. Treatment levels with < 5 replicates were deleted prior to analyses. For each three-factor analysis, full model (three treatments and all two way and three way interactions terms) was tested first. If no interactions were detected ($\alpha = 0.05$ here and throughout), then a reduced model was tested with interactions terms dropped. Reduced model was reported in table only if a treatment effect was detected. Post hoc tests were conducted with Fisher's LSD test. If interactions were detected, then models were reduced accordingly (e.g., basin x tier effect; tier effects tested by drainage). Visualizations of response variables by tier are provided in appendices along with plots of response variables by peak flow.

Figure 7. A shallow run seine haul above the sampled riffle area on the Little River near Little River.

Figure 8. A shallow run bag seine haul on the mainstem Brazos River near Rosharon.

Daily growth increment (circuli) formation in otoliths of young-of-the-year cyprinids in the Brazos River have been validated as a reliable means to estimate hatch dates (Durham and Wilde 2008a). Specimens used in the otolith analysis were collected during aquatic component sampling described above. Total length (mm) and standard length (mm) were recorded for each *Macrhybopsis* spp. specimen prior to otolith examination. Procedures for otolith preparation and daily growth estimation generally followed those of Campana (1992) and Secor et al., (1992). Asteriscus otoliths, the largest otoliths in Cyprinidae (Secor et al., 1992), were removed using a dissecting microscope with two polarizing filters, one mounted between the light source and the otolith, and one mounted between the objective lens and otolith. After removal, otoliths were fixed to a glass slide using thermoplastic cement that had been heated on a hotplate. Before reading, a drop of immersion oil was placed on the otolith, and daily growth rings were counted using a compound light microscope at 40x magnification. Counts of daily growth rings on each otolith were made independently by two readers. Age estimates from the two readers that were within 10% were accepted as valid and retained for analysis. The daily age estimate was recorded as the mean of the two estimates (Durham and Wilde 2006, 2009). Otoliths, for which counts could not be reconciled within 10%, were excluded from further analysis. The number of usable *Macrhybopsis* spp. otoliths was 11 (0 excluded). To determine hatch dates from age estimates, 1 day was added to the final daily growth ring count. This was based on Bottrell et al.'s (1964) determination that eggs of Speckled Chub [*Macrhybopsis aestivalis*] hatch within 28 hours of spawning.

For the Brazos River sampling locations, daily stream flows were classified as subsistence, base, flow pulse, or overbanking flows using indicators of hydrologic alteration parameters for flow separation developed by the BRA BBEST (Table 3.3 in BRA BBEST 2012) for the nearest USGS gage. For the San Antonio River, daily stream flows were classified according to discharge levels categorized in the environmental flow regime recommendations for that basin (Table 6.1-13 and 6.1-15 in GSA BBEST 2011).

2.2 Riparian

Because both BRA BBEST recommendations and TCEQ flow standards were specific to study reaches, each of which possessed a number of unique characteristics, we opted not pool the site riparian data into a composite one-basin recommendation, or to run statistical analyses similar to those performed in the Aquatic assessment of this report. Instead, hypothesis testing was performed for each individual reach. Overall, within-basin recommendations were inferred from general response patterns observed at the study reaches and, when possible, from between-basin responses.

Six sites were chosen in the BRA basin from the recommended BRA BBEST (2012) USGS-monitored reaches (Figure 9 and Table 2). Criteria for site selection included: (1) that established riparian forests must be present, (2) that at least two of the three indicator species must be present, and (3) that the sites must not have any major tributaries between the USGS gage and study site. Three of the selected sites were located on the main stem Brazos River, and three on its tributaries (Leon River, Little River, and Navasota River). Early into the study, the Navasota site was lost because of land use changes that removed much of the study area's riparian growth.

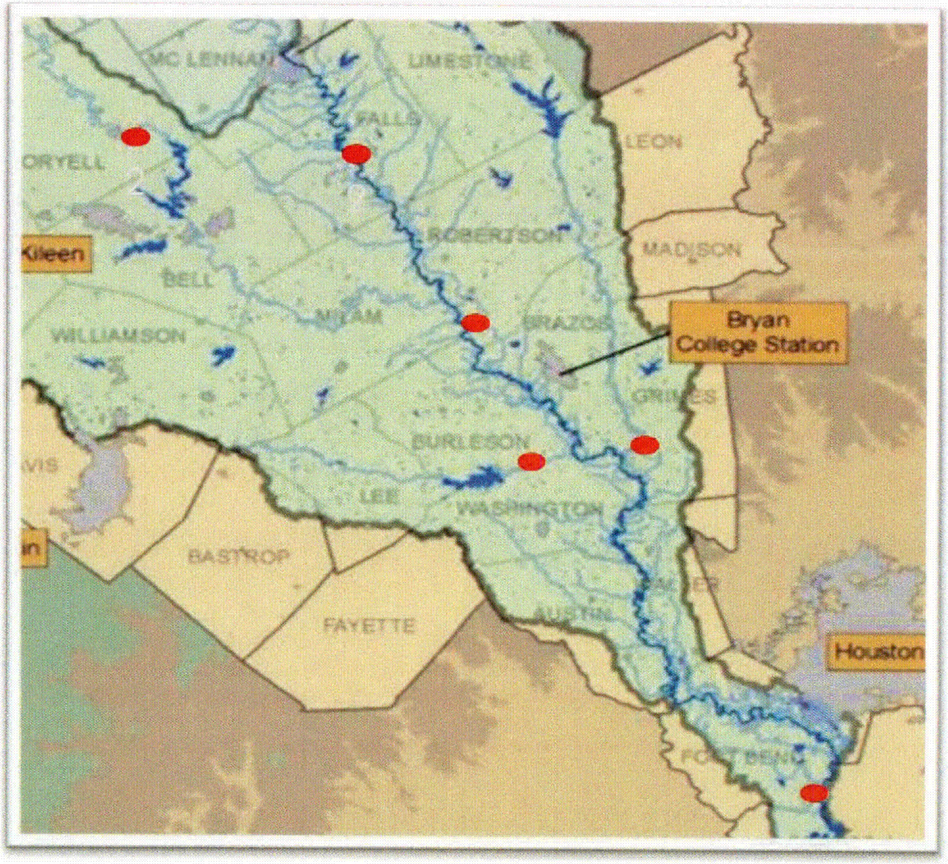


Figure 9. **Location of the six original sites (red dots) selected for the study. Credit: TWDB (modified).**

For each site, three transects were semi-permanently placed perpendicular to the river, beginning at water's edge. Transect lengths covered the extent of mature indicator species plus 2 meters. Study protocol stated that if seedling dispersal extended beyond the mature trees' distribution at any time in the study, transects would be adjusted accordingly; however, at no time did this occur for any sites. Labeled ½" rebar posts were placed at two meter intervals along each transect and GPS recordings taken. 2X2m quadrats were placed at the corner of each, with the rebar representing the upstream lowest point of the 2X2m plot. Sampling was done from the upstream side of the transect line to prevent trampling of species. Elevation above the stream was recorded along the transect lines and channel slope/stream bank profiles were generated (Figure 10). One representative profile per site was chosen for tree data comparisons.

To monitor flow inundation into the site, an Onset (2012) stream level logger was submerged (in sediment-resistant housing) in the stream within one to two meters of the stream bank, and depth of water at time of installation was recorded (Figure 11). Pressure recordings occurred at one-hour intervals, and were used to calculate water level depths. To monitor site-specific rainfall an Onset (2011) electronic rain gage was installed nearby in an open canopy area and recorded rainfall events in 0.01-inch increments. Four sampling events were conducted from summer 2014 to spring 2015: August 2014, October 2014, January 2015, and April 2015 (though only a few sites were accessible at this time because of flooding).

Table 2. **BBEST-recommended USGS gages selected for study.**

Gage Number	Site	Gage location
8116650	Brazos Bend	Brazos River near Rosharon
8108700	Hearne	Brazos River near Bryan
8100500	Leon	Leon River near Gatesville
8106500	Little River	Little River near Cameron
8096500	Marlin	Brazos River at Waco
8110500	Navasota	Navasota River near Easterly

Flow frequency was measured categorically as the number of flow tiers given in TCEQ flow standards and BBEST 1/year-recommended flow events of specified magnitude within the seasons defined in the TCEQ standards. Typically, rather than compare all individual base flows, an average of all base flows was used. Measured site inundation stream flows were used both to determine direct water levels at the site and to calibrate recorded flow to USGS gages. The nearest USGS river gage to each site was used for long-term, historical flows as calibrated by on-site measurements. First, stream logger data was compared against corresponding USGS data, to determine corresponding flow events based on flow event timing and peak heights. Differences in peak height at USGS gage and study reach were then used to calibrate USGS flows to study reach elevations when datasets required stream flow measurements prior to logger installation (long-term flows) or when missing data. This method ultimately provided only limited success, as during the study event very little flow was recorded until the heavy spring flows. With additional time, a better correlation (and better potential statistical analyses) of the two flows would be much more accurate and useful for this methodology.

Total number of seedlings, saplings and mature trees for each indicator species in each 2X2 transect plot were counted, and spatial coverages recorded during each sampling event except January 2015 (the deciduous trees were dormant). Age classes (life stages) were grouped into seedling, sapling and mature. Trees between 1 and 5cm DBH were classified as saplings, and seedlings as <1cm DBH or shorter than 1m; all other trees were classed as mature (Figure 12). Tree coring of a total of ten mature trees (of indicator species) was done at each site to establish general growth factors (relationship between number of tree rings and DBH). The growth factors were used, in conjunction with a growth factor developed by Duke (2011) for the San Antonio and Brazos River riparian trees, to establish estimated age distributions of mature trees. The two datasets were combined to generate a growth factor (Table 3) for basin-wide estimated age of mature trees given their DBH. Additionally, 10-15 saplings from several sites were sampled to determine a growth factor for saplings, and used in age classing saplings in the study.

Figure 10. Crewmembers take elevation at stream transect.

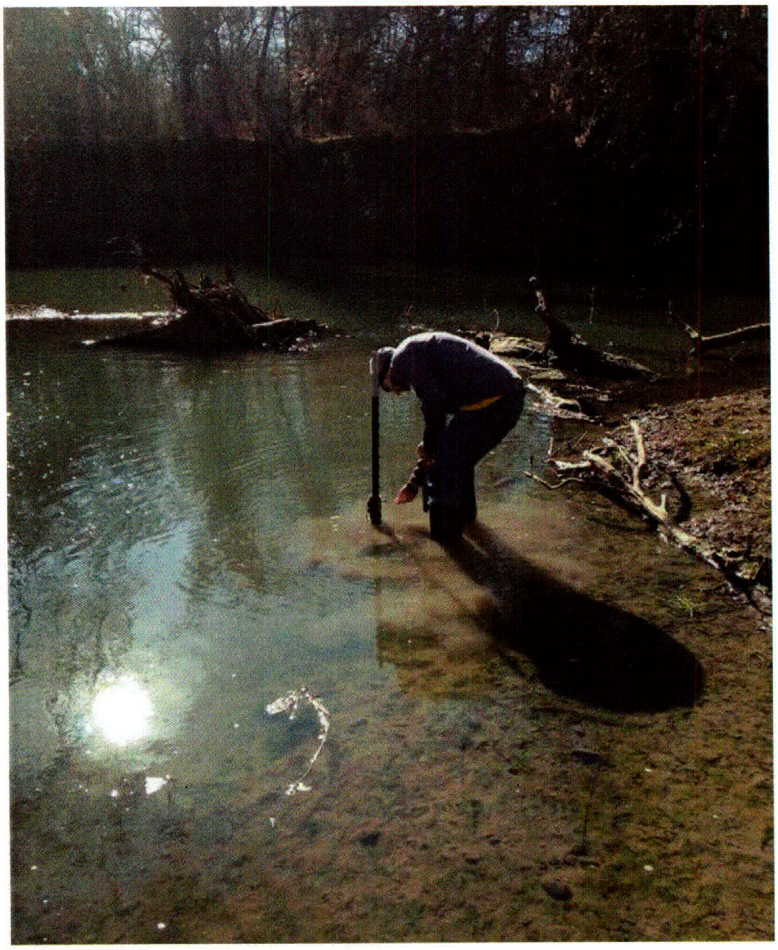


Figure 11. Crewmember installs a stream level logger.

Figure 12. **Crewmember collects tree core samples in the field.**

A comparison of TCEQ flow standards and the 1/year-recommended BBEST flows to mature riparian spatial distributions was made for each site to determine if recommended flows are adequate for maintenance of existing riparian stands (with the assumption that 'maintenance' of stands includes not only mature tree needs, but provision for seed dispersal and survival through all age classes). For each flow, percent coverage of each indicator species' mature stands was determined. For analysis of whether inundation of a species occurred, 80% or more was considered as a "yes" or supported hypothesis; below this was deemed a "no" or not supported. This percentage does not reflect an actual recommendation by the study authors. It was chosen as a way of simplifying the characterization. This 80% "rule" was selected because of a number of factors: (1) it is a relatively conservative coverage that given its slightly lower than 100% coverage would capture more near-magnitude flows than would the 100% coverage flow (more slightly less-than-target flows vs. less full-target flows; (2) most flow pulses don't hit the target precisely (e.g., a target/standard flow of 1000 cfs is met by an actual flow of 1250 cfs), therefore a "met" flow is often above the standard/required flow pulse, actually inundating further up the bank than the standard flow would indicate; and (3) capillary action in the stream bank often results in a shifting upward of flow pulse waters that wet channel slopes/floodplains - meeting the needs of plants whose roots extend downward toward saturated soils. Whether or not this 80% rule, or some other designator, should be used by riparian/stream managers can only be determined by those managers. All data presented includes all inundation levels (not just the 80%) so that managers can use their professional judgment in what levels are deemed appropriate.

An analysis of met vs. not-met flows (measured as inundation into the site) was performed for each site, grouped by TCEQ seasons and flow magnitude. Because not all flows occurred during the study duration (and not all flows provided coverage for the indicator species), a comparison of actual flows to seedling and sapling spatial coverage was also made. Rain gage information was used to determine if anomalous seedling/sapling distributions to streamflow might be better explained by local rainfall than streamflow. Changes to site seedling, sapling, and mature counts through seasons were calculated to determine if streamflow had an effect on survival and/or recruitment. Relative abundance of all tree species was limited to the first sampling, and could not be compared to final study results because of the severe flooding. Tree age classes for each species were graphed to better visualize age distribution and make predictions about future replacement.

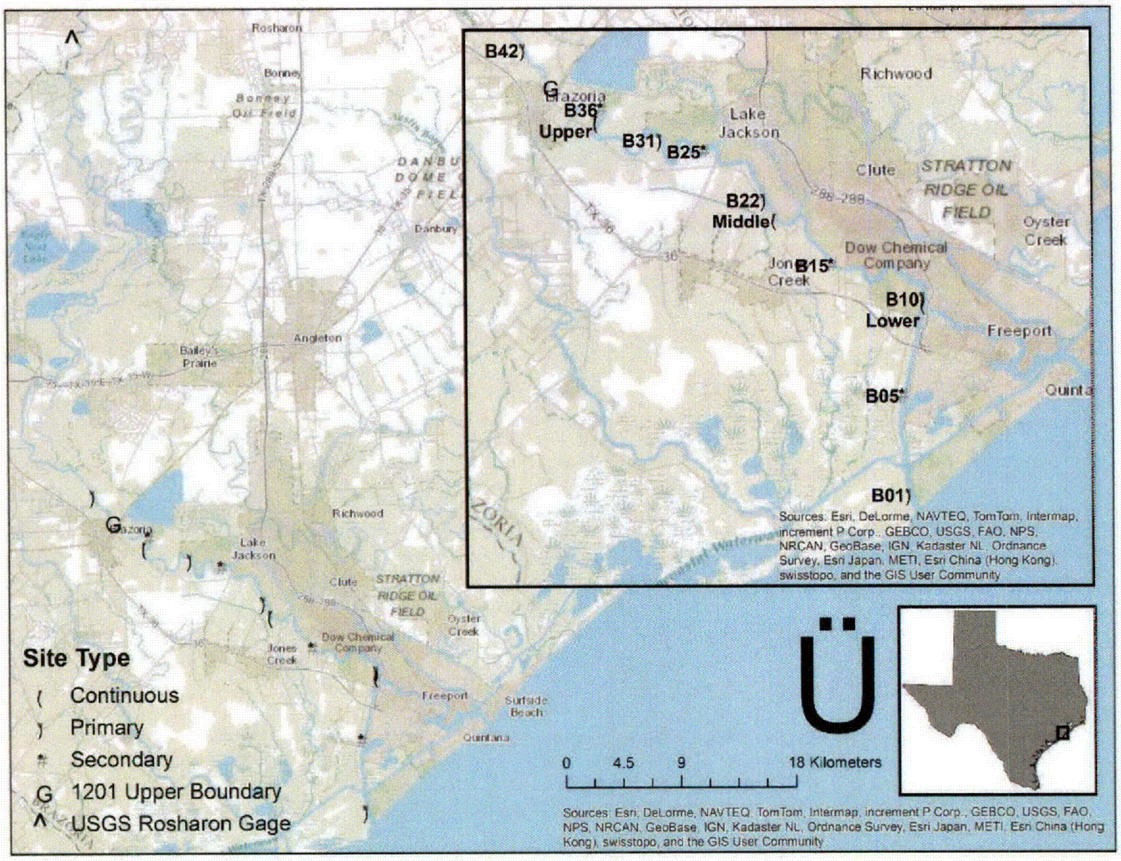
Table 3. **Growth factors for estimating mature tree ages.**

Species	Average number of rings per year	Number observed
Black Willow	0.900	46
Box Elder	0.318	41
Green Ash	0.277	19

2.3 Brazos Estuary

The tidal, or "lower" portion (TCEQ segment 1201) of the Brazos River is classified as the first 177 km from its confluence with the Gulf of Mexico in Freeport, Texas to a point about 100 meters 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). On average, the lower Brazos River exhibits oligohaline 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 water body 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 of the sampling area (42 rkm upstream; 7.23 m) (Miller, 2014).

During November 2014 to May 2015, a total of eight sampling events of the lower Brazos River were conducted at multiple monitoring sites (Figure 13). This included five primary monitoring sites at approximately 1, 10, 22, 31, and 42 river kilometers (rkm) upstream from the mouth (sites B01, B10, B22, B31, and B42, respectively; Table 4). Several of these sites corresponded with locations of a previous survey of the lower Brazos River conducted in 2012 (Miller, 2014). Each primary site was sampled for water quality, nutrients, nekton, and zooplankton during every sampling event. 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 every sampling event. Collection of data was conducted over a two day period during each sampling event as described below. In addition, continuous monitoring sites were established at 10, 21, and 35 rkm upstream of the mouth.



Sources: Esri, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community

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Figure 13. **Site map of the lower Brazos River depicting the locations of continuous, primary and secondary sampling sites as well as the USGS gage in Rosharon and the upper boundary of the 1201 tidal segment of the Brazos River.**

Hydrology and water quality

In order to assess instream flow recommendations of the lower Brazos River, hourly stream flow data was downloaded from USGS gage #08116650 in Rosharon, Texas for the duration of the study. Sampling events were divided into winter and spring seasons and classified by flow tier according to recommended environmental flow standards (BRA BBEST, 2012). Events were assigned a flow tier status ranging from subsistence flow to a one-per-season (1/season) high-flow pulse (1 – 8). Actual tide data was downloaded from NOAA tide station #8772447 at the USCG station in Freeport, TX to assess tidal influence.

Vertical profiles of water temperature (°C), salinity (psu), dissolved oxygen (mg/L), pH, and turbidity (NTU) were recorded at the thalweg of each primary and secondary sampling site using a YSI 600XLM multiprobe sonde (YSI Inc.; Yellow Springs, OH). Prior to and post 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 (0.3 m), 25% of total depth, 50% of total depth, 75% of total depth, and bottom (0.3 m above the bottom substrate) was recorded while conducting water quality profiles. Additionally, total depth was recorded at each site, and Secchi depth was recorded at all primary sites.

Table 4. **Sampling sites including distance from Gulf of Mexico (r-km), GPS coordinates and type of data collected at each site on the lower Brazos River from Nov. 2014 – May 2015; WQ: water quality, HOBO: stationary temperature, conductivity and dissolved oxygen sondes, ES: electroshocking, BT: beam trawl, OT: otter trawl and ZP: zooplankton.**

Site	Distance from Gulf (r-km)	Latitude	Longitude	Hydrology			Nekton and Zooplankton				Historical Sampling
				WQ	Nutrients	HOBO	ES	BT	OT	ZP	
B01	1	28.88368	-95.38227	X	X		X*	X	X	X	Miller (2014)
B05	5	28.92592	-95.38534	X							
Lower	10	28.96457	-95.37428			X					
B10	10	28.96682	-95.37464	X	X		X	X	X	X	Miller (2014)
B15	15	28.98117	-95.41979	X							
Middle	21	29.00054	-95.44773			X					
B22	22	29.00908	-95.45314	X	X		X	X	X	X	Miller (2014)
B25	25	29.02987	-95.48269	X							
B31	31	29.03473	-95.50422	X	X		X	X			
B34	34	29.03582	-95.53136						X**	X**	
Upper	35	29.04218	-95.53557			X					
B36	36	29.04785	-95.53343	X							
B42	42	29.07288	-95.57167	X	X		X	X	X	X	Miller (2014)

*Electroshocking conducted at B01 when conductivities were <18,000 μS/cm

**Otter trawl and zooplankton for B31 conducted at B34 due to snags at original location

Surface water grab samples were collected at primary sites during each sampling event. These samples were submitted to Eastex Environmental (Houston, TX). Nitrate and nitrite nitrogen (Nitrate+Nitrite; mg/L) were analyzed using EPA method SM 4500-NO_3 E and F. Total Kjehldahl nitrogen (TKN; mg/L) was analyzed using EPA methods SM4500 SM 4500-N_{org} B or C and SM 4500-NH_3 B. Total phosphorous (Total P; mg/L) was analyzed using EPA method SM 4500-PE. Total suspended solids (TSS; mg/L) were analyzed using methods SM 2540 D. Additional grab samples were collected for determination of relative chlorophyll-α concentration and were measured at the EIH laboratory using an Aquaflour® Handheld Fluorometer (Turner Designs, 2013). The relative concentration (RFU µg/L) of chlorophyll-α, was estimated using raw (in-vivo) water samples and reported in relative fluorescent units of equivalent chlorophyll-α (RFU µg/L). This represents a semi-quantitative estimate of the chlorophyll-α content for rapid analysis of water samples and serves as an index of primary production. Despite its semi-quantitative nature, in vivo fluorescence data provides valuable information on the spatial and temporal distribution of chlorophyll concentrations.

Continuous monitoring sites were equipped with temperature and conductivity U26-001 HOBO data loggers (Onset Computer Corporation; Bourne, MA). Data loggers were downloaded monthly and checked for battery life, fouling, and damage. Conductivity values were converted to salinity via the practical salinity scale (PSS-78) algorithm (Lewis and Perkin, 1978) available in HOBOware (ver. 3.7.2).

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. A two-factor analysis of variance (ANOVA) was used to test for differences ($\alpha = 0.05$) in depth integrated (surface, middle and bottom) salinity and dissolved oxygen concentrations as well as nutrients (N-NO_{2+3}, TKN, Total P), RFU chlorophyll-α and TSS between flow tiers and sites. If no interactions were detected, but 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. If interactions were detected, a reduced model was tested by assessing site differences within each flow tier. Fisher's LSD was used post-hoc to assess pairwise differences among tiers and sites when statistically significant.

Interpolated salinity contours for the entire river reach were created using Sigma Plot (ver. 11.2) by plotting percent total depth of vertical profile salinity measurements by site (river kilometer). 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 graphed against the hydrograph and tide data to visually assess the relationship of freshwater inflow and tides on salinity regime.

Results of visual and statistical analyses conducted on water quality and hydrological variables were compared against the current environmental flow hypotheses and conditions predictions by conceptual and best fit linear regression models in regards to critical functions (nursery habitat, salinity regime, nutrients) provided by various components of the flow regime. Regression models were used to describe potential relationships between river inflow and the response of salinity, water quality, and primary production as measured by chlorophyll-α (RFU). Regression models considered included linear, quadratic, and cubic functions that utilize discharge or ranked discharge TCEQ flow tiers as independent variables and water quality variables (vertically integrated salinity, chlorophyll-α (RFU), nitrate-nitrite nitrogen, TKN, TSS and total phosphorus).

Nekton

Nekton were collected using a combination of trawling and electrofishing and were identified to the lowest possible taxonomic level and counted. Nekton includes mobile finfish and invertebrates such as shrimp, 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 later identification and enumeration. Laboratory identification was conducted using taxonomic keys and recorded using common and scientific names from most current nomenclature used by the American Fisheries Society (Hoese and Moore, 1998; Turgeon et al., 1998; Cairns et al., 2003; Hubbs et al., 2008; Merryman et al., 2012; Page et al., 2013). 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 TPWD (Texas Parks and Wildlife) Scientific Collection Permit #SPR-0504-383.

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) deployed for 5-minutes in triplicate. 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 location. Shoreline nekton were collected at all primary sites using a modified 6.4 mm mesh Renfro beam trawl (Sea-Gear Corporation; Melbourne, FL; Renfro 1963). Triplicate hauls were pulled parallel to shore for approximately 15.2 m on one bank per site (alternating sides at each site). Larger nekton were collected using a boat mounted 9.0 GPP electrofishing unit (Smith-Root; Vancouver, WA) for a total of 20 minutes shock time per site. Electrofishing was conducted at sites B10, B22, B31, and B42 during each sampling event and opportunistically at site B01 depending on surface conductivity.

Total number (N), species richness (S), diversity (H') and evenness (J') were calculated for each sampling event across all sites and methods (Magurran, 2004). Species were classified into life history salinity preference groups of freshwater, estuarine or saltwater based on official AFS listings and/or published literature (Nelson, 1992; Hoese and Moore, 1998; Kells and Carpenter, 2011). Species classified as estuarine were those that regularly utilize estuaries to fulfill at least one portion of their life cycle. Analysis of variance was used to test for differences in percent occurrence of estuarine species between flow tiers and sites. Fisher's LSD was used post-hoc to assess pairwise differences among tiers and sites. Percent occurrence of estuarine species were graphed by flow tier and discharge to assess nekton response to flow. Regression models were used to describe potential relationships between river inflow and the response of estuarine nekton. Regression models that were considered included linear, quadratic and cubic functions that utilize discharge or ranked discharge TCEQ flow tiers as independent variables and percent occurrence of estuarine species as the dependent variable.

Spatial and flow tier mediated effects on nekton community composition were analyzed using PRIMER 6 statistical package (Clarke and Warwick, 2001). Prior to analysis nekton abundance data were transformed (log+1). A Bray-Curtis resemblance matrix was constructed on the transformed data to measure similarity between site/event community composition. Subsequently, classification and ordination of the communities were conducted using cluster analysis and non-metric multi-dimensional scaling using the default program settings (exception: 50 restarts, minimum stress = 0.001). One-way ANOSIM was conducted to test for significant ($\alpha = 0.05$) differences in species assemblages by flow tier and site. Sampling methods were combined for analysis of current data (i.e., OT, BT and ES), but it must be noted that electroshocking at B01 was only conducted when conductivity was less than 18,000 µS/cm (Event 3, 4 and 8). When grouping current and historical nekton data (Miller, 2014), otter trawl and beam trawl catch were only used for data analysis.

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 0.2 meter diameter wide, 0.6 meter long plankton net 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 possessed a 6.4 mm bar nylon netting. The species composition should be very similar. Since this data was compared using rank transformed data the effect due to gear differences should be trivial.

Water quality (salinity data from vertical profiles to assess relationships to flow tier and discharge) and nekton data (otter trawl and beam trawl data for MDS analysis) were incorporated to supplement the number of flow tiers sampled and account for additional flow tiers that did not exist during our 2014-15 field season (Table 5). Addition of the historical data resulted in the inclusion of flow tiers in the subsistence, three-per-season (3/season) and 1/season categories (note site B31 was not sampled by Miller 2014). Several historical events were excluded from nekton analysis due to prolonged time laps between samples from each event. For a complete summary of catch for 2012, see Miller (2014).

Table 5. Current and historical sampling events including season, USGS discharge (cfs) of the sampling event, flow tier classification, lag time from peak to sampling event and type of data collected at each site on the lower Brazos River from Nov. 2014 – May 2015 and Jan. – Dec. 2012; WQ: water quality, ES: electroshocking, BT: beam trawl, OT: otter trawl and ZP: zooplankton.

	Sampling Event						**Hydrology**		**Nekton and Zooplankton**			
	Date	**Event No.**	**Season**	**USGS Q**	**Flow Tier**	**Lag Time**	**WQ**	**Nutrients**	**ES**	**BT**	**OT**	**ZP**
Current	05/06/15	8	Spring	15,000	2ps	2	X	X	X	X	X	X
	04/28/15	7	Spring	27,700	2ps	9	X	X	X	X	X	X
	04/01/15	6	Spring	28,600	2ps	8	X	X	X	X	X	X
	02/18/15	5	Winter	2,070	Avg	—	X	X	X	X	X	X
	02/04/15	4	Winter	23,300	2ps	8	X	X	X	X	X	X
	01/06/15	3	Winter	4,690	4ps	14	X	X	X	X	X	X
	12/09/14	2	Winter	6,650	4ps	10	X	X	X	X	X	X
	11/11/14	1	Winter	856	Dry	—	X	X	X	X	X	X
Historical	12/13/12	20	Winter	350	Sub	—	X	—	—	X	X	—
	11/13/12	19	Winter	275	Sub	—	X	—	—	X	X	—
	10/16/12	18	Summer	920	Dry	—	X	—	—	X	X	—
	09/11/12	17	Summer	710	Dry	—	X	—	—	X	X	—
	08/14/12	16	Summer	475	Dry	—	X	—	—	X	X	—
	07/10/12	15	Summer	380	Sub	—	X	—	—	X	X	—
	06/12/12	14	Spring	304	Sub	—	X	—	—	X	X	—
	05/09/12	13	Spring	1,330	Avg	—	X	—	—	X	X	—
	04/11/12	12	Spring	47,500	1ps	16	X	—	—	X	X	—
	03/12/12	11	Spring	27,200	1ps	19	X	—	—	X	X	—
	02/14/12	10	Winter	22,500	2ps	6	X	—	—	X	X	—
	01/19/12	9	Winter	9,620	3ps	9	X	—	—	X	X	—

3 Results, discussion, and interdisciplinary assessment

3.1 Aquatics

Collection efforts yielded 63 riffle habitats and 74 run habitats, sampled between August 2014 and May 2015 and between subsistence flows to 1 per year high-flow pulse events. Nine insect orders and 51,460 macroinvertebrates were identified and enumerated, and 46 fish species and 21,452 fishes were identified and enumerated. Condition factors were calculated for 11 species and 435 individuals of fishes, gut fullness was calculated for 11 species and 332 individuals, and hepatic-somatic indices were calculated for seven species and 350 individuals.

Biota and habitat descriptions

Numbers of riffles sampled were 22 in the BRA drainage and 41 in the GSA for a total of 63 riffles. Riffles were sampled during or after Tiers 1 – 7 and among all four seasons (Table 6). PCA axes 1 and 2 explained 32% of the variation in habitat parameters. PC axis 1 explained 18% of the variation and described a water temperature and season gradient. Falls City (GSA) was associated (strong positive loading) with PC axis 1 (summer) because of restricted access and lack of winter and spring collections. PC axis 2 explained 14% and described a season, water quality, and substrate gradient. Kemper (BRA) and Falls City (GSA) were negatively associated with PC 2 because of higher conductivity at each site and because of greater amounts of bedrock (at Falls City only). Otherwise, riffle habitats were physically and chemical similar among remaining sites, as indicated by clustering and overlap of site means and standard deviations (Figure 14).

A total of 51,460 aquatic insects, representing 9 insect orders, was recorded among the 63 riffles (Table 7). Among all sites, Ephemeroptera was the most abundant insect order (39% of total N of macroinvertebrates) and exhibited the greatest density (38%), followed by Coleoptera (17% of N; 15% of density), Trichoptera (17%; 17%), and Diptera (14%; 15%).

A CCA model explained 47% of the variation ($F = 1.7$; $P < 0.01$) in total number of macroinvertebrates in riffles (Figure 15). Current velocity (CV), depth, and GSA basin were positively associated, and bedrock, conductivity, and boulder substrate were negatively associated with CCA axis 1. Winter season and sand substrates were positively associated, and summer season, water temperature, and pH were negatively associated with CCA axis 2. Along CCA axis 1, the macroinvertebrate group with the strongest positive association was Plecoptera, and the macroinvertebrate group with the strongest negative association was Odonata. Along CCA axis 2, the macroinvertebrate group with the strongest positive association was Diptera, and macroinvertebrate groups with the strongest negative association were Megaloptera, Hemiptera, and Lepidoptera.

Table 6. **Riffle habitat summary statistics taken overall (N = 14 sites) and by drainage from August 2014 – May 2015.**

	Overall					Brazos River Drainage					Guadalupe-San Antonio Drainages				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Riffle	63					22					41				
Area (m^2)	5,646	90	33.5	39	193	1,971	90	29.0	48	193	3,675	90	36.0	39	193
Tier (1 = subsistence; 7 = 1 per year)				1	7				1	7				1	7
Peak Flow (cfs)		1,372	2,740	4	15,600		1,214	3,299	4	15,600		1,452	2,427	8	9,570
Season															
Summer	18					6					12				
Fall	20					9					11				
Winter	16					5					11				
Spring	9					2					7				
Water Temperature (°C)		19.7	7.28	7.8	32.3		18.4	7.23	7.8	31.2		20.3	7.33	10.2	32.3
Dissolved Oxygen (mg/l)		9.3	2.26	6.0	15.9		9.6	2.45	6.6	15.2		9.1	2.16	6.0	15.9
Specific Conductance (µS/cm)		712	373.6	248	1,881		746	559.7	248	1881		671	217.1	498	1219
pH		7.9	0.39	6.9	8.8		7.7	0.43	7.0	8.8		7.9	0.36	6.9	8.6
Current Velocity (m/s)		0.61	0.256	0.00	1.27		0.50	0.238	0.12	0.88		0.67	0.244	0.00	1.27
Depth (m)		0.26	0.375	0.06	0.64		0.19	0.292	0.06	0.48		0.29	0.402	0.09	0.64
Vegetation (%)		15.3	20.80	0	80		23.1	25.73	0.0	70.0		11.2	16.75	0.0	80.0
Substrate															
Silt (%)		1.8	5.42	0	26.7		3	7.5	0	27		1	3.7	0	23
Sand (%)		13.1	11.13	0	46.7		19	12.8	0	47		10	9.3	0	33
Gravel (%)		44.8	20.25	0	80.0		47	16.2	20	75		43	22.2	0	80
Cobble (%)		31.3	26.74	0	90.0		18	17.9	0	55		40	27.5	0	90
Boulder (%)		2.6	7.70	0	50.0		2	6.7	0	25		2	8.2	0	50
Bedrock (%)		5.5	16.27	0	83.3		9	19.0	0	62		4	14.5	0	83
Embeddedness (0 = low; 1 = high)		0.2	0.29	0	1.0		0	0.3	0	1		0	0.3	0	1

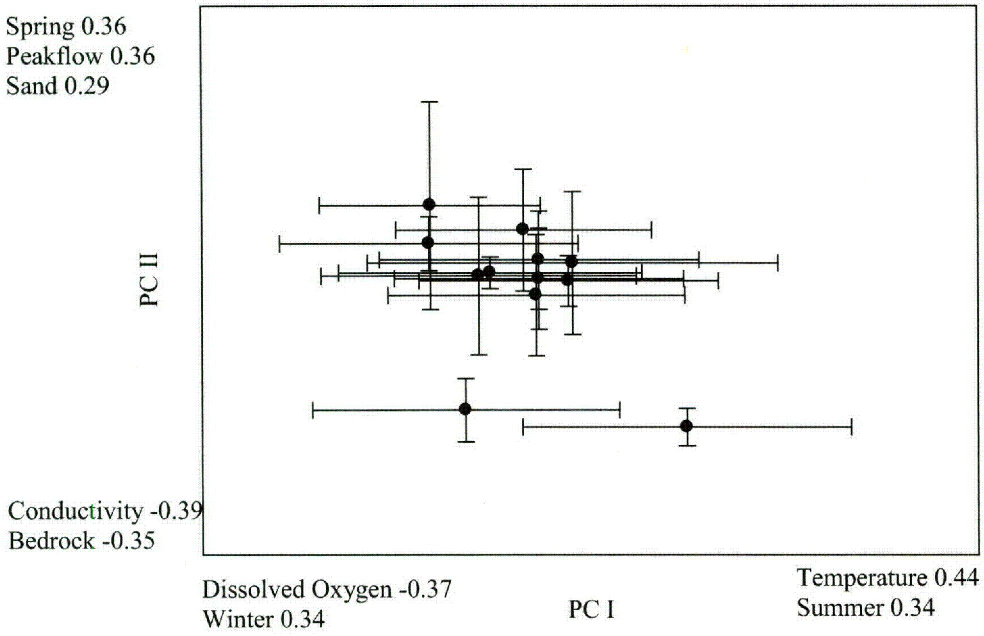


Figure 14. A Principal Component analyses (PCA) analysis of the association of riffle habitats for sites on the Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) by season, substrate and water quality parameters for from August 2014 – May 2015.

Table 7. Total number, mean density and flow association of macroinvertebrates taken among all sites from riffle habitats within the Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) from August 2014 – May 2015.

Species	Symbol	Flow association	Basin	Total N	Percent	Mean Density	Percent
Coleoptera	Col	Moderate	GSA-BRA	12459	24.2	62.9934	24.2
Diptera	Dip	Slackwater	GSA-BRA	7338	14.3	39.2063	15.1
Ephemeroptera	Eph	Swiftwater	GSA-BRA	19872	38.6	99.4193	38.2
Hemiptera	Hem	Slackwater	GSA-BRA	540	1.0	2.6772	1.0
Lepidoptera	Lep	Slackwater	GSA-BRA	114	0.2	0.6071	0.2
Megaloptera	Meg	Slackwater	GSA-BRA	322	0.6	1.4511	0.6
Odonata	Odo	Slackwater	GSA-BRA	1375	2.7	6.8228	2.6
Plecopotera	Ple	Swiftwater	GSA-BRA	483	0.9	2.3836	0.9
Tricoptera	Tri	Moderate	GSA-BRA	8957	17.4	44.5225	17.1
Total				51460		260.0833	

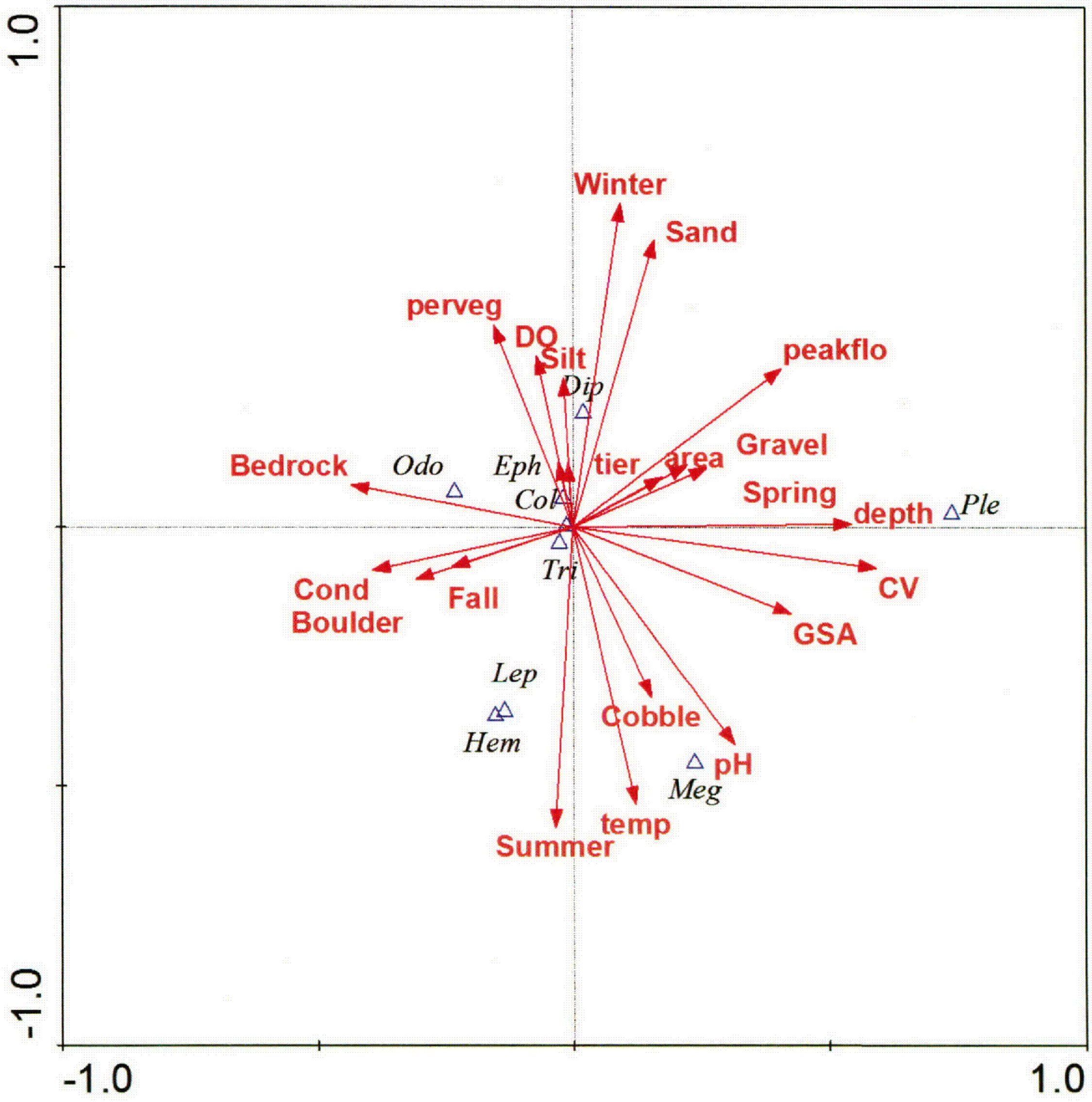


Figure 15. Canonical Correspondence analyses (CCA) of the total number of macroinvertebrates from riffle habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 7 for macroinvertebrate codes.

CCA model explained 53% of the variation (F = 2.3; P < 0.01) in density of macroinvertebrates in riffles (Figure 16). Winter season, sand substrates, and gravel substrates were positively related, and summer season and temperature were negatively associated with CCA axis 1. Cobble substrates were positively associated, and gravel substrates and water depth were negatively associated with CCA 2. Along CCA axis 1, the macroinvertebrate group with a strong positive association was Diptera, and macroinvertebrate group with a strong negative association was Megaloptera. Along CCA axis 2, macroinvertebrate groups with a strong positive association were Tricoptera and Hemiptera, and macroinvertebrate group with a strong negative association was Plecoptera.

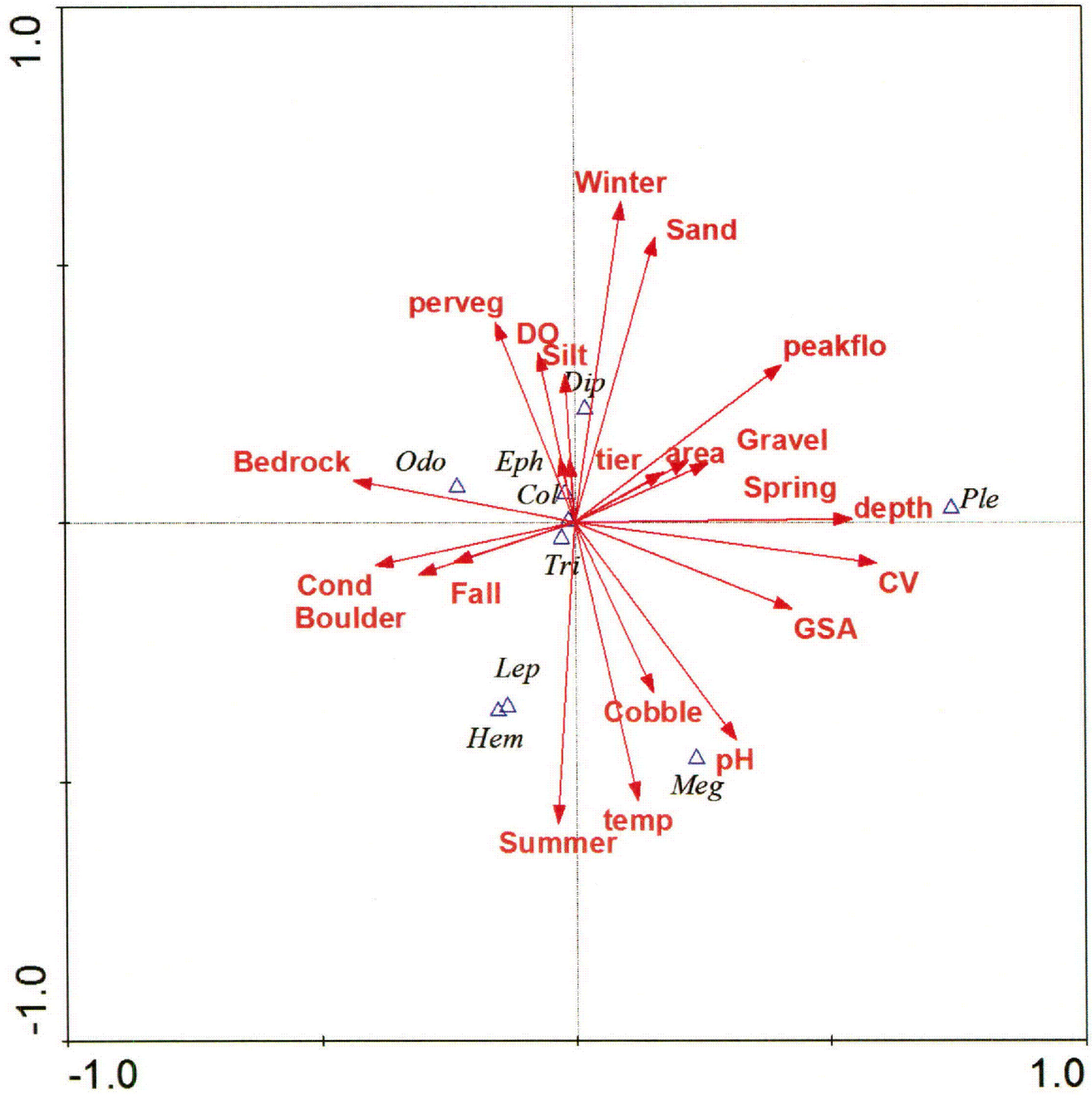


Figure 16. Canonical Correspondence analyses (CCA) of the density of macroinvertebrates from riffle habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 7 for macroinvertebrate codes.

A total of 6,612 fishes, representing 33 species of fishes, was recorded among the 63 riffles (Table 8). Among all sites, *Cyprinella lutrensis* was the most abundant (32% of total N of fishes) and with the greatest density (30% of total density of fishes), followed by *Cyprinella venusta* (17% of N; 15% of density), *Etheostoma spectabile* (16%; 17%), *Campostoma anomalum* (8%; 10%), and *Ictalurus punctatus* (6%; 5%).

Table 8. **Total number, mean density and flow association of riffle fishes taken among all sites from riffle habitats within the Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) from August 2014 – May 2015.**

Species	Symbol	Basin	Flow association	Total N	Percent	Mean Density	Percent
Anguilla rostrata	Ang ros	GSA	Slackwater	1	0.0	0.0003	0.0
Campostoma anomalum	Cam ano	GSA -BRA	Riffle	537	8.1	0.1408	9.8
Cyprinella lutrensis	Cyp lut	GSA -BRA	Fluvial	2,129	32.2	0.4309	30.1
Cyprinella venusta	Cyp ven	GSA -BRA	Fluvial	1,088	16.5	0.2086	14.6
Macrhybopsis marconis	Mac mar	GSA	Riffle	56	0.8	0.0088	0.6
Notropis amabilis	Not ama	GSA	Riffle	40	0.6	0.0160	1.1
Notropis buchanani	Not buc	GSA -BRA	Slackwater	22	0.3	0.0038	0.3
Notropis volucellus	Not vol	GSA -BRA	Fluvial	120	1.8	0.0330	2.3
Pimephales vigilax	Pim vig	GSA -BRA	Slackwater	282	4.3	0.0563	3.9
Moxostoma congestum	Mox con	GSA -BRA	Fluvial	5	0.1	0.0012	0.1
Astyanax mexicanus	Ast mex	GSA	Riffle	3	0.0	0.0008	0.1
Ictalurus punctatus	Ict pun	GSA -BRA	Riffle	390	5.9	0.0757	5.3
Noturus gyrinus	Not gyr	GSA -BRA	Slackwater	17	0.3	0.0036	0.3
Pylodictis olivaris	Pyl oli	GSA -BRA	Riffle	41	0.6	0.0123	0.9
Menidia beryllina	Men ber	GSA	Slackwater	1	0.0	0.0002	0.0
Fundulus notatus	Fun not	BRA	Slackwater	2	0.0	0.0003	0.0
Gambusia affinis	Gam aff	GSA -BRA	Slackwater	63	1.0	0.0154	1.1
Poecilia latipinna	Poe lat	GSA	Slackwater	4	0.1	0.0008	0.1
Lepomis auritus	Lep aur	GSA	Slackwater	2	0.0	0.0004	0.0
Lepomis cyanellus	Lep cya	GSA	Slackwater	1	0.0	0.0003	0.0
Lepomis macrochirus	Lep mac	GSA -BRA	Slackwater	9	0.1	0.0016	0.1
Lepomis megalotis	Lep meg	GSA -BRA	Slackwater	72	1.1	0.0153	1.1
Lepomis humilis	Lep hum	BRA	Slackwater	1	0.0	0.0002	0.0
Micropterus punctulatus	Mic pun	GSA	Slackwater	13	0.2	0.0038	0.3
Micropterus treculii	Mic tre	GSA -BRA	Fluvial	17	0.3	0.0042	0.3
Etheostoma gracile	Eth gra	GSA -BRA	Slackwater	14	0.2	0.0038	0.3
Etheostoma lepidum	Eth lep	GSA	Riffle	60	0.9	0.0157	1.1
Etheostoma spectabile	Eth spe	GSA -BRA	Riffle	1,046	15.8	0.2487	17.4
Percina apristis	Per apr	GSA	Riffle	75	1.1	0.0138	1.0
Percina carbonaria	Per car	GSA -BRA	Riffle	133	2.0	0.0304	2.1
Percian sciera	Per sci	BRA	Riffle	25	0.4	0.0058	0.4
Percina shumardi	Per shu	GSA -BRA	Riffle	285	4.3	0.0573	4.0
Herichthys cyanoguttatus	Her cya	GSA	Slackwater	58	0.9	0.0204	1.4
Total				6,612		1.4306	

CCA model explained 43% of the variation ($F = 5.3$; $P < 0.01$) in total number of fishes in riffles. Sand substrate, pH, peak stream flow, water depth, and spring season were positively associated, and summer season and cobble substrate was negatively associated with CCA axis 1 (Figure 17). BRA basin (as inferred from direction of GSA loading), sand substrate, and silt substrates were positively associated, and GSA basin, depth, and cobble substrate were negatively associated with CCA axis 2. Fishes ($N > 5$) with strong positive associations along CCA 1 were *Etheostoma gracile, Lepomis macrochirus, Notatus gyrinus, Notropis buchanani, Percina apristis*, and *Macrhybopsis marconis*. Fishes ($N > 5$) with strong negative associations along CCA 1 were *Etheostoma lepidum, Micropterus treculii*, and *Notropis amabilis*. Fishes (N

> 5) with strong positive associations along CCA 2 were *Etheostoma gracilis* and *Percina sciera*. Fishes (N > 5) with strong negative associations with CCA 2 were *Micropterus punctulatus*, *Macrhybopsis marconis*, *Percina shumardi*, and *Herichthys cyanoguttatus*.

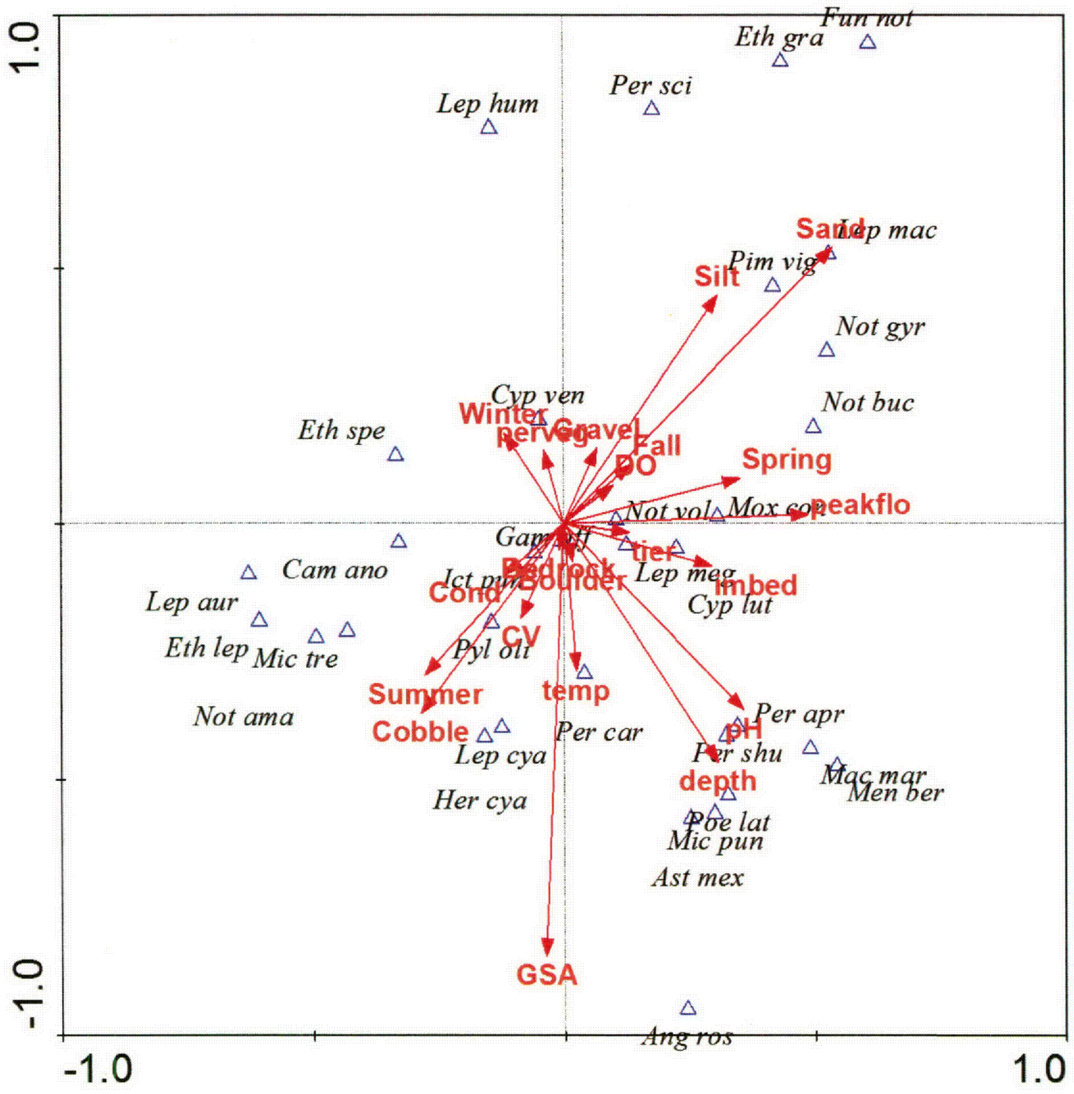


Figure 17. **Canonical Correspondence analyses (CCA) of the total number of fishes from riffle habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 8 for riffle fishes codes.**

CCA model explained 47% of the variation (F = 7.5; P < 0.01) in fish densities in riffles. GSA basin, current velocity (CV) were positively associated, and BRA basin, pH, and embeddedness were negatively associated with CCA axis 1 (Figure 18). BRA basin and silt substrate were positively associated, and GSA basin, current velocity, and depth were negatively associated with CCA axis 2. Fishes (N > 5) with strong positive associations along CCA 1 were *Notropis amabilis*, *Etheostoma lepidum*, *Etheostoma spectabile*, *Campostoma anomalum*, and *Micropterus treculii*. Fishes (N >5) with strong negative associations along CCA 1 were *Lepomis macrochirus*, *Pimephales vigilax*, *Notropis buchanani*, and *Cyprinella lutrensis*. Fishes (N > 5) with strong positive associations along CCA 2 were *Etheostoma gracilis*, *Percina sciera*, and *Noturus gyrinus*. Fishes (N > 5) with strong negative associations along CCA 2 were *Percina shumardi*, *Percina apristis*, and *Macrhybopsis marconis*.

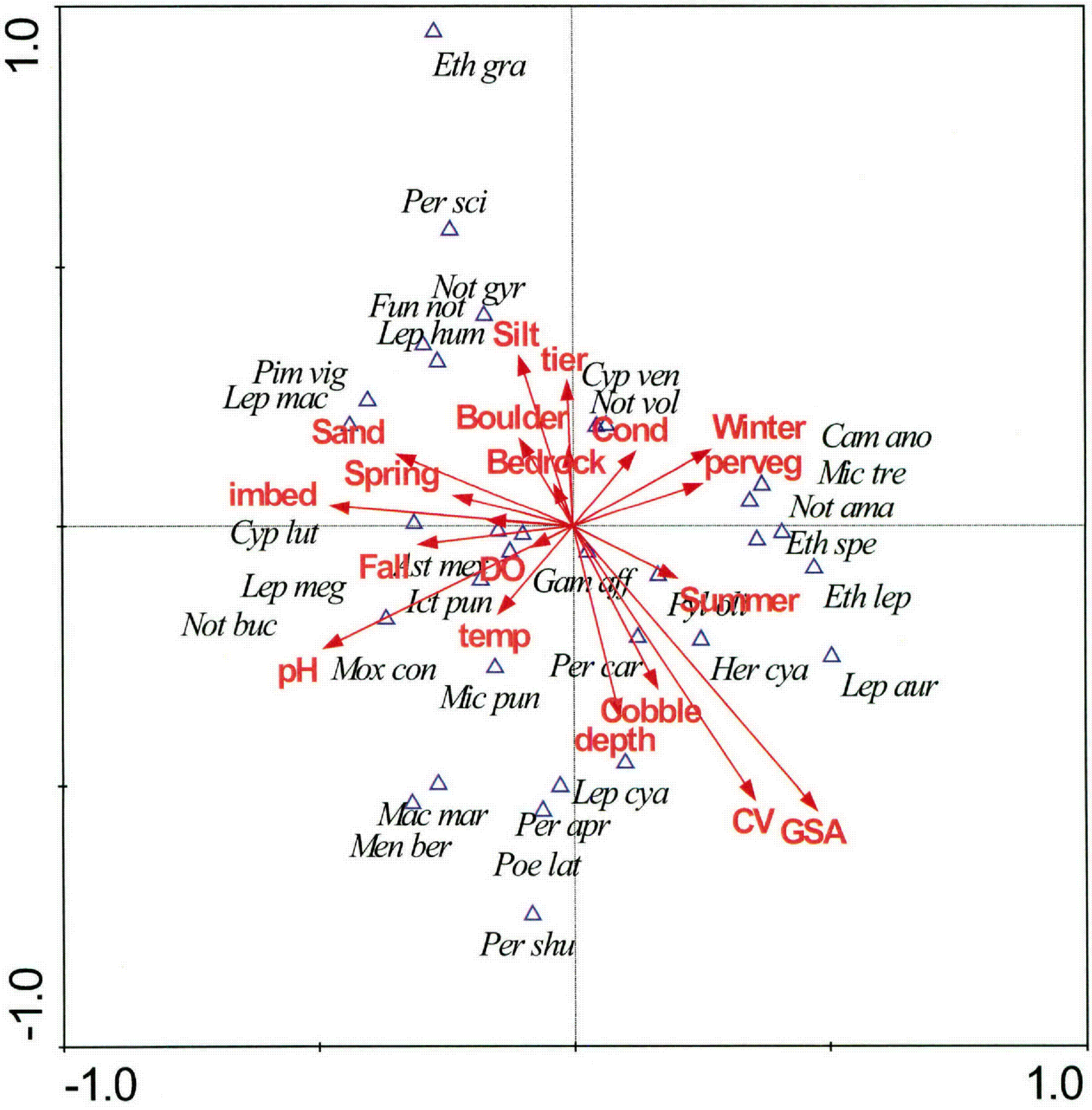


Figure 18. **Canonical Correspondence analyses (CCA) of the density of fishes from riffle habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 8 for riffle fishes codes.**

Condition factors were calculated for 11 species 435 individuals of fishes associated with riffles, hepatic-somatic indices were calculated for 7 species and 350 darters, and gut fullness was calculated for 11 species and 332 individuals of fishes associated with riffles (Table 9). Among all fishes, mean lengths (± 1 SD) ranged from 37 mm (± 4.6) in *Notropis buchanani* to 97 mm (±16.2) in *Percina carbonaria*. Condition factors (± 1 SD) ranged from 0.60 (0.07) in *Notropis* *buchanani* to 0.95 (.148) in *Percina shumardi*. Hepatic-somatic indices (± 1 SD) ranged from 1.2 (0.65) in *Etheostoma lepidum* to 3.1 (2.38) in *Etheostoma gracile*. Gut fullness (± 1 SD) ranged from 45% (43.1) in *Notropis volucellus* to 78% (28.2) in *Percina carbonaria*.

Table 9. **Mean length, weight, condition factor, hepatic-somatic index (HIS) and gut fullness of swift-water associated fishes collected from riffle habitats among all sites and seasons within the Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) from August 2014 – May 2015.**

		M. marconis	*N. amabilis*	*N. buchanani*	*N. volucellus*	*E. gracile*	*E. lepidum*	*E. spectabile*	*P. apristis*	*P. carbonaria*	*P. sciera*	*P. shumardi*
Basin		GSA	GSA	GSA-BRA	GSA-BRA	BRA	GSA	GSA-BRA	GSA	GSA-BRA	BRA	GSA
Total N		23	17	10	35	9	26	129	31	63	26	66
Length (mm)	Mean	56	46	37	45	48	39	43	68	97	75	57
	1 SD	6.7	6.1	4.6	9.2	4.1	5.5	6.7	12.2	16.2	12.3	7.1
Weight (g)	Mean	1.45	0.65	0.31	0.70	0.82	0.51	0.80	2.39	7.06	3.52	1.83
	1 SD	0.673	0.336	0.126	0.669	0.201	0.244	0.412	1.697	3.491	1.608	0.766
Condition Factor	Mean	0.79	0.65	0.60	0.68	0.72	0.83	0.92	0.67	0.72	0.75	0.95
	1 SD	0.078	0.113	0.069	0.062	0.066	0.167	0.127	0.117	0.095	0.115	0.148
Hepatic Index	Mean					3.1	1.2	2.2	1.4	1.6	2.3	2.4
	1 SD					2.38	0.65	1.95	0.97	0.91	1.60	1.88
Gut fullness (%)	N	16	8	8	14	9	22	111	22	48	26	48
	Mean	54	73	46	45	72	58	69	60	78	70	62
	1 SD	42.7	36.9	32.0	43.1	21.7	38.8	33.9	39.1	28.2	31.4	30.6

Numbers of runs sampled were 33 in the BRA drainage and 40 in the GSA for a total of 74 runs. Runs were sampled during or after Tiers 1 – 7 and among all four seasons (Table 10). PCA axes 1 and 2 explained 31% of the seasonal and habitat variation (Figure 19). PC axis 1 explained 16% and described a water quality, season, and peak stream flow gradient. Kempner (BRA) was negatively associated with PC axis 1, specifically runs with higher dissolved oxygen concentration and percent vegetation. PC axis 2 explained 15% and described primarily a seasonal gradient. Scatter plots of PCA 1 and 2 means and 1 SDs by site indicate clustering and overlap, which suggests similar of habitat parameters among sties, except for Kempner.

A total of 14,840 fishes, representing 37 species of fishes, were recorded among the 74 runs (Table 11). Among all sites, *Cyprinella lutrensis* was the most numerically abundant (45% of total N of fishes), followed by *Notropis amabilis* (21%) and *Notropis volucellus* (14%). *Notropis amabilis* had the greatest density (40%), followed by *Notropis volucellus* (22%) and *Cyprinella lutrensis* (22%).

Table 10. **Run habitat summary statistics taken overall (N = 14 sites) and by drainage from August 2014 – May 2015.**

	Overall					Brazos River Drainage					Guadalupe-San Antonio Drainages				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Run	73					33					40				
Area (m^2)	7,185	98	78.3	12	425	4,111	125	100.7	27	425	3,074	77	44.1	12	225
Tier (1 = subsistence; 7 = 1 per year)				1	7				1	7				1	7
Peak Flow (cfs)		2,233	5,375	4	40,600		3,137	7,495	4	40,600		1,488	2,447	21	9,570
Season															
Summer	19					8					11				
Fall	26					15					11				
Winter	18					7					11				
Spring	10					3					7				
Water Temperature (°C)		19.9	7.25	7.8	32.3		19.5	7.21	7.8	31.2		20.1	7.36	10.2	32.3
Dissolved Oxygen (mg/l)		9.4	2.15	6.0	15.9		9.7	2.15	6.6	15.2		9.2	2.16	6.0	15.9
Specific Conductance (µS/cm)		691	368.8	248	1,881		716	496.0	248	1881		671	219.9	498	1219
pH		7.8	0.42	6.9	8.8		7.7	0.46	6.9	8.8		7.9	0.37	6.9	8.6
Current Velocity (m/s)		0.28	0.171	0.01	0.63		0.26	0.176	0.01	0.58		0.29	0.168	0.01	0.63
Depth (m)		0.48	0.186	0.14	1.14		0.44	0.220	0.14	1.14		0.51	0.149	0.24	0.82
Vegetation (%)		7.4	19.81	0	95		12.9	23.80	0.0	95.0		2.8	14.58	0.0	90.0
Substrate															
Silt (%)		22	26.4	0	100		30	29.9	0	100		15	21.1	0	69
Sand (%)		30	34.4	0	100		36	37.6	0	100		25	31.1	0	100
Gravel (%)		26	22.2	0	75		22	19.6	0	75		29	23.8	0	70
Cobble (%)		10	19.3	0	80		1	2.2	0	10		17	23.7	0	80
Boulder (%)		3	12.0	0	95		5	17.1	0	95		2	4.6	0	20
Bedrock (%)		9	23.3	0	100		8	20.1	0	80		10	25.9	0	100

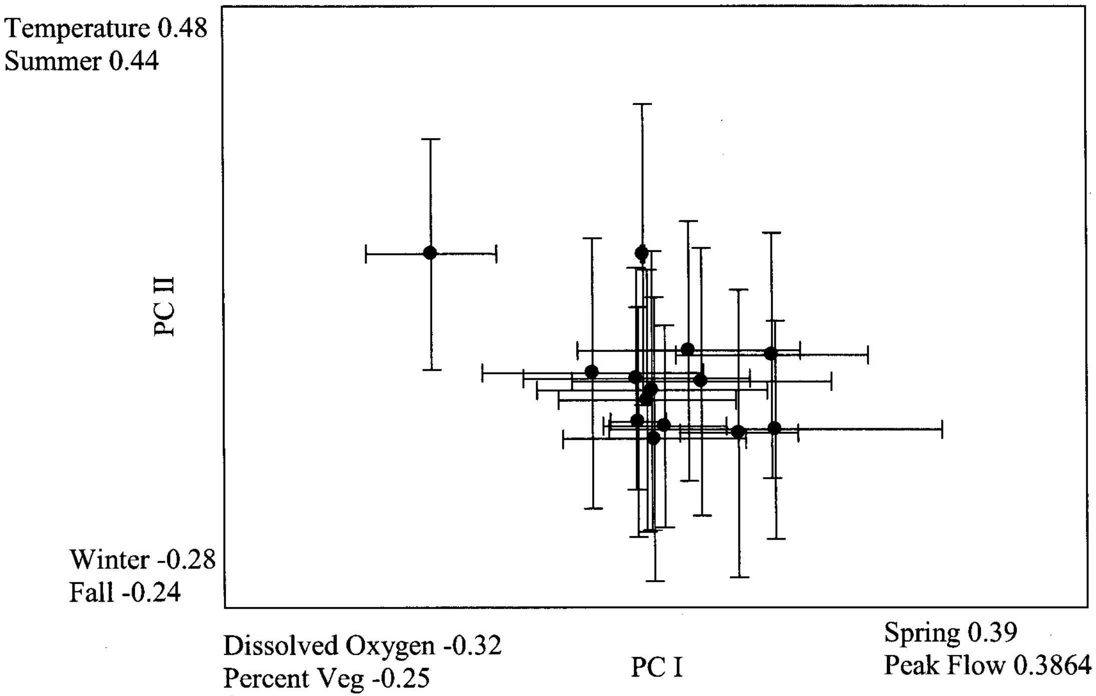


Figure 19. **A Principal Component analyses (PCA) analysis of the association of run habitats for sites on the Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) by season, substrate and water quality parameters for from August 2014 – May 2015.**

Table 11. Total number, mean density and flow association of fishes taken among all sites from run habitats within the Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) from August 2014 – May 2015.

Species	symbol	Basin	Flow association	Total N	Percent	Mean Density	Percent
Brevoortia patronus	Bre pat	BRA	Slackwater	54	0.4	0.0009	0.0
Dorosoma cepedianum	Dor cep	BRA	Slackwater	8	0.1	0.0007	0.0
Anchoa mitchilli	Anc mit	BRA	Slackwater	33	0.2	0.0011	0.0
Campostoma anomalum	Cam ano	GSA-BRA	Swiftwater	9	0.1	0.0021	0.1
Cyprinella lutrensis	Cyp lut	GSA-BRA	Fluvial	6,698	45.1	0.5813	21.5
Cyprinella venusta	Cyp ven	GSA-BRA	Fluvial	1,171	7.9	0.2422	9.0
Lythrurus fumeus	Lyt fum	BRA	Slackwater	43	0.3	0.0145	0.5
Macrhybopsis hyostoma	Mac hyo	BRA	Swiftwater	47	0.3	0.0010	0.0
Macrhybopsis marconis	Mac mar	GSA	Swiftwater	5	0.0	0.0014	0.1
Notropis amabilis	Not ama	GSA	Swiftwater	3,165	21.3	1.0662	39.5
Notropis buchanani	Not buc	GSA-BRA	Slackwater	356	2.4	0.0802	3.0
Notropis shumardi	Not shu	BRA	Swiftwater	12	0.1	0.0002	0.0
Notropis volucellus	Not vol	GSA-BRA	Fluvial	2,016	13.6	0.5876	21.8
Pimephales vigilax	Pim vig	GSA-BRA	Slackwater	707	4.8	0.0426	1.6
Moxostoma congestum	Mox con	GSA	Fluvial	6	0.0	0.0010	0.0
Ictalurus furcatus	Ict fur	BRA	Swiftwater	137	0.9	0.0039	0.1
Ictalurus punctatus	Ict pun	GSA-BRA	Swiftwater	39	0.3	0.0041	0.2
Noturus gyrinus	Not gyr	GSA	Slackwater	1	0.0	0.0003	0.0
Mugil cephalus	Mug cep	BRA	Slackwater	10	0.1	0.0005	0.0
Labidesthes sicculus	lab sic	BRA	Slackwater	5	0.0	0.0003	0.0
Menidia beryllina	Men ber	GSA	Slackwater	2	0.0	0.0012	0.0
Fundulus notatus	Fun not	BRA	Slackwater	16	0.1	0.0071	0.3
Gambusia affinis	Gam aff	GSA-BRA	Slackwater	172	1.2	0.0328	1.2
Lepomis auritus	Lep aur	BRA	Slackwater	8	0.1	0.0011	0.0
Lepomis macrochirus	Lep mac	GSA-BRA	Slackwater	16	0.1	0.0006	0.0
Lepomis megalotis	Lep meg	GSA-BRA	Slackwater	61	0.4	0.0113	0.4
Micropterus dolomieu	Mic dol	GSA	Fluvial	1	0.0	0.0003	0.0
Micropterus punctulatus	Mic pun	GSA-BRA	Slackwater	12	0.1	0.0033	0.1
Micropterus salmoides	Mic sal	GSA-BRA	Slackwater	4	0.0	0.0006	0.0
Micropterus treculii	Mic tre	GSA-BRA	Fluvial	4	0.0	0.0007	0.0
Pomoxis annularis	Pom ann	BRA	Slackwater	1	0.0	0.0000	0.0
Etheostoma chlorosoma	Eth chl	BRA	Slackwater	3	0.0	0.0006	0.0
Etheostoma gracile	Eth gra	BRA	Slackwater	9	0.1	0.0039	0.1
Etheostoma spectabile	Eth spe	GSA	Swiftwater	3	0.0	0.0006	0.0
Percina carbonaria	Per car	GSA	Swiftwater	1	0.0	0.0003	0.0
Percian sciera	Per sci	BRA	Swiftwater	4	0.0	0.0019	0.1
Herichthys cyanoguttatus	Her cya	GSA	Slackwater	1	0.0	0.0002	0.0
Total				14,840		2.6985	

CCA model explained 36% of the variation (F = 1.4; P = 0.01) in total number of fishes in runs. GSA basin and cobble substrates were positively associated, and BRA basin and sand substrates were negatively associated with CCA axis 1 (Figure 20). Percent vegetation was positively associated, and sand substrates and water depth were negatively associated with CCA axis 2. Fishes (N > 5) with strong positive associations along CCA 1 were *Notropis amabilis*, *Notropis volucellus*, *Noturus gyrinus*, and *Percina carbonaria*. Fishes (N>5) with strong negative associations along CCA 1 were *Macrhybopsis hyostoma*, *Notropis shumardi*, *Pimephales vigilax* and *Ictalurus furcatus*. Fishes (N > 5) with strong positive associations along CCA 2 were *Lythrurus fumeus*, *Campostoma anomalum*, and *Moxostoma congestum*. Fishes (N > 5) with strong negative associations with CCA 2 were *Noturus gyrinus*, *Percina carbonaria,* and *Macrhybopsis marconis*.

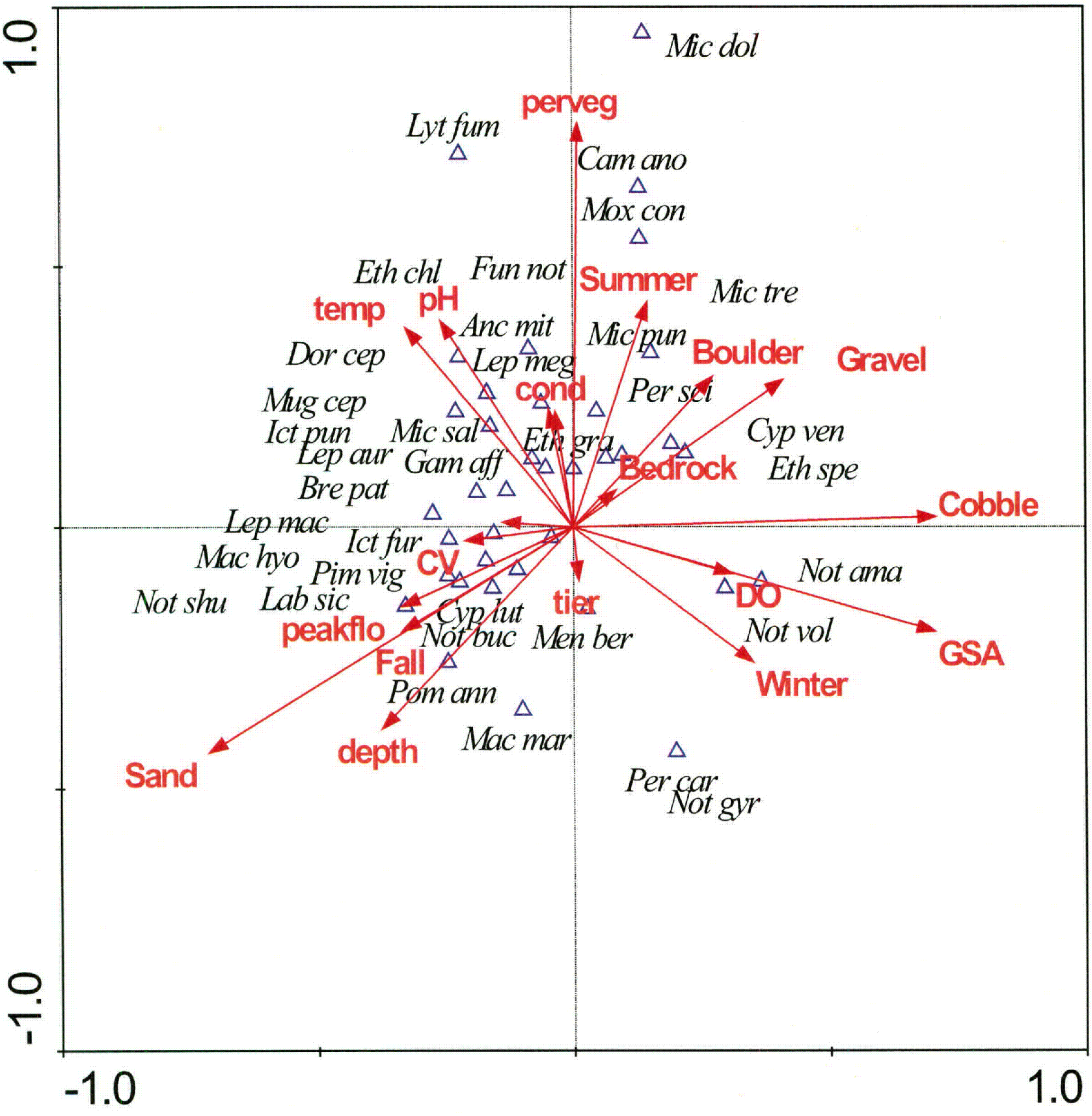


Figure 20. **Canonical Correspondence analyses (CCA) of the total number of fishes from run habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 11 for run fishes codes.**

CCA model explained 38% of the variation (F = 1.7; P < 0.01) in fish densities runs. Silt substrate, pH, and BRA basin were positively associated, and cobble substrates and GSA basin were negatively associated with CCA axis 1 (Figure 21). Percent vegetation and summer season were positively associated, and GSA basin were negatively associated with CCA axis 2. Fishes (N > 5) with strong positive associations along CCA 1 were *Ictalurus punctatus, Notropis* *shumardi* and *Macrhybopsis hyostoma*. Fishes (N >5) with an association along CCA 1 were *Notropis amabilis* and *Notropis volucellus*. Fishes (N > 5) with strong positive associations along CCA 2 were *Moxostoma congestum*, *Lythrurus fumeus*, and *Campostoma anomalum*.

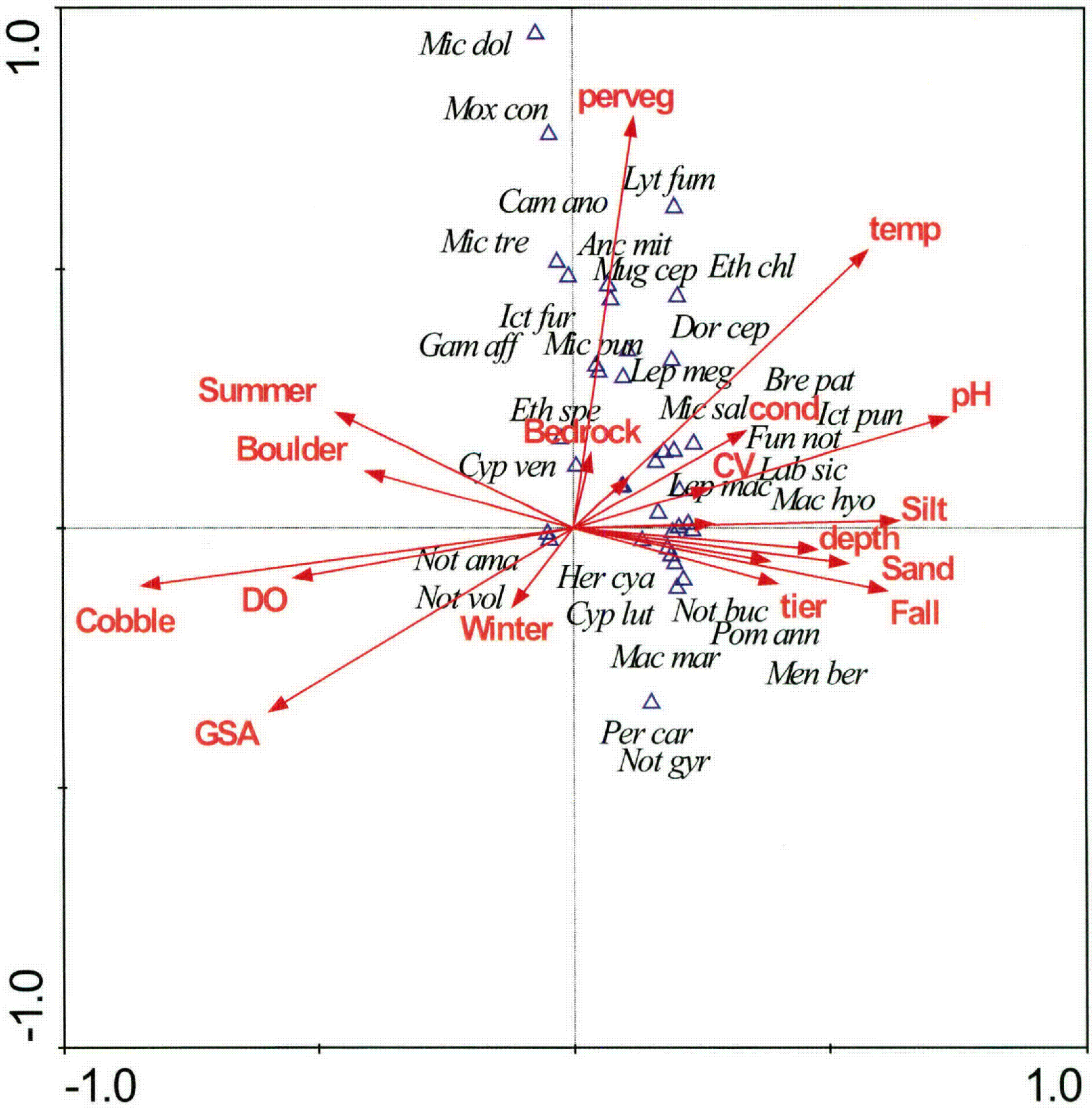


Figure 21. **Canonical Correspondence analyses (CCA) of the density of fishes from run habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 11 for run fishes codes.**

Flow tier analyses

Numbers of riffle and run habitats quantified by flow tier, basin, and season are provided in Table 12. Habitat descriptions by flow tier are provided in Appendices A (riffle) and B (run). Ten habitat hypotheses were tested for riffles among tiers, basins, and seasons (Table 13). Percentages of silt substrates, cobble substrates, and percent vegetation (in bold) differed among treatments. Cobble substrates differed by basin with GSA riffles consisting of more cobble substrates than BRA. For silt substrates and percent vegetation, interaction terms were significant for basin and tier. As such, tier treatment was tested separately for each basin. Silt substrates and percent vegetation did not differ (P > 0.05) among tier or season in the GSA. Likewise, silt substrates and percent vegetation did not differ among tier or season in the BRA, but tiers 6 and 7 were dropped from the analyses because each treatment only contained one replicate each.

Five aquatic insect community hypotheses were tested for riffles among tiers, basins, and seasons (Table 14; Appendix C). Percent relative abundance of densities differed for moderately swift insects and slack-water aquatic insects, but these differences were seasonal and not related to tiers.

Table 12. **Total number of riffles and runs sampled among basin, season and flow tiers within the Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) from August 2014 – May 2015.**

	Tier	1	2	3	4	5	6	7	
	Description	Subsistence	Base-average	4 per season	3 per season	2 per season	1 per season	1 per year	Totals
Riffles	N	3	30	2	2	9	12	5	63
	GSA	1	20	0	0	5	11	4	41
	BRA	2	10	2	2	4	1	1	22
	Summer	1	9	1	0	3	4	0	18
	Fall	1	9	0	2	5	2	1	20
	Winter	1	11	1	0	1	2	0	16
	Spring	0	1	0	0	0	4	4	9
Runs	N	4	36	2	4	10	13	5	74
	GSA	1	20	0	0	5	11	4	41
	BRA	3	16	2	4	5	2	1	33
	Summer	2	10	1	0	3	4	0	20
	Fall	1	12	0	4	6	2	1	26
	Winter	1	13	1	0	1	2	0	18
	Spring	0	1	0	0	0	5	4	10

Table 13. Degrees of freedom (N = numerator, D = denominator), F-statistics, and P-values for three factor analyses of variances related hypotheses testing of riffle abiotic response variables among tier, basin, and season treatment effects.

Dependent Variable	N	D	F-Statistic	P-value	Interactions		N	D	F-Statistic	P-value	Class variable	P-value	Class variable	P-value	Notes
Substrate (%)															
Silt	**19**	**36**	**3.28**	**0.001**	S	Basin x Tier					Tier by GSA	NS	Tier by BRA	NS	BRA Tiers 6 & 7 lacked replication
Sand	19	36	1.34	0.220	NS										
Gravel	19	36	1.08	0.405	NS										
Cobble	**19**	**36**	**2.10**	**0.027**	NS		7	48	4.28	0.001	Basin	<0.001			GSA > BRA
Boulder	19	36	0.79	0.704	NS										
Bedrock	19	36	0.90	0.588	NS										
Embeddedness	19	36	0.90	0.588	NS										
Vegetation (%)	**19**	**36**	**2.09**	**0.028**	S	Basin x Tier					Tier by GSA	NS	Tier by BRA	NS	BRA Tiers 6 & 7 lacked replication
Current Velocity (cm/s)	19	36	1.31	0.237	NS										
Depth (m)	19	36	1.33	0.227	NS		7	48	3.23	0.007	Basin	0.003			GSA > BRA

Interaction terms were tested and dropped from the model is not significant (NS). Only significant reduced models are shown. Under Notes, results of Fisher's LSD tests are provided for tier (1 – 7), basin (GSA, BRA), and season (Su = summer, F = fall, W = winter, Sp = spring). Different letters denote significant differences among treatment levels. Bold text represents significant response variables.

Table 14. Degrees of freedom (N = numerator, D = denominator), F-statistics, and P-values for three factor analyses of variances related hypotheses testing of riffle biotic response variables among tier, basin, and season treatment effects.

Response Variable		N	D	F-Statistic	P-value	Interactions	N	D	F-Statistic	P-value	Class variable	P-value	Class variable	P-value	Class variable	P-value	Notes
Aquatic Insects																	
Density	All insects	19	36	1.55	0.127	NS											
Relative abundance (%)	Swiftwater insects	19	36	1.29	0.250	NS											
	Moderately swift insects	19	36	1.59	0.114	NS	7	48	2.46	0.031	Season	0.009					Su a, F a, W b, Sp a
	Slack water insects	19	36	1.76	0.072	NS	7	48	4.42	0.001	Season	<0.001					Su b, F b, W a, Sp b
	EPT	19	36	1.77	0.069	NS											
Fishes																	
Density	All fishes	19	36	1.18	0.323	NS	7	48	2.82	0.015	Season	0.003					Su a, F b, W b, Sp b
	Riffle fishes	19	36	1.35	0.213	NS	7	48	2.48	0.029	Season	0.010					Su a, F b, W b, Sp b
	Fluvial fishes	19	36	1.25	0.274	NS											
	Slackwater fishes	19	36	1.18	0.326	NS											
Relative abundance (%)	Riffle fishes	19	36	1.44	0.168	NS											
	Fluvial fishes	19	36	1.75	0.074	NS											
	Slackwater fishes	19	36	2.21	0.020	NS	7	48	3.00	0.011	Tier	0.008					2bc 3c 6b 7a
Richness		19	36	5.06	<0.001	NS	7	48	10.06	<0.001	Season	<0.001					Su a, F b, W c, Sp b
Occurrence (%)	**Riffle fishes**	19	36	2.17	0.022	NS	7	48	3.99	0.002	Basin	0.002					GSA > BRA
	Fluvial fishes	19	36	1.11	0.379	NS	7	48	2.35	0.038	Tier	0.034					2ab 3a 6b 7ab
	Slackwater fishes	19	36	2.05	0.031	NS	7	48	3.98	0.002	Tier	0.001	Basin	0.005			2a 3b 6a 7a; BRA > GSA
	Cyprinidae	19	36	1.01	0.473	NS											
	Percidae	19	36	1.62	0.105	NS	7	48	4.48	0.001	Tier	0.034	Basin	0.004	Season	0.010	2a 3a 6ab 7b; GSA>BRA; Su b, F b, W a, Sp b
	Ictaluridae	19	36	1.23	0.289	NS											
	Benthic fishes	19	36	1.4	0.190	NS	7	48	2.75	0.018	Basin	0.013					GSA > BRA
	Gambusia, Fundulid	19	36	1.25	0.276	NS											
	SOC	19	36	3.41	<0.001	NS	7	48	3.83	0.002	Basin	<0.001					GSA > BRA
Condition Factor		19	28	1.27	0.28	NS											
Hepatic Somatic Index		19	28	1.66	0.11	NS											
Gut fullness		19	28	1.34	0.23	S											3-way interaction S; test by season, NS, lack of rep

Interaction terms were tested and dropped from the model is not significant (NS). Only significant reduced models are shown. Under Notes, results of Fisher's LSD tests are provided for tier (1 – 7), basin (GSA, BRA), and season (Su = summer, F = fall, W = winter, Sp = spring). Different letters denote significant differences among treatment levels. Tested hypothesis of all abiotic and biotic factors for riffle fishes and macroinvertebrates using a three factor analysis of variance among basin, season and flow tiers. Bold text represents significant response variables.

Seventeen fish community hypotheses were tested for riffles among tiers, basins, and seasons (Table 14; Appendix D - G). Fish density and riffle fish density differed among treatments with the removal of non-significant interaction terms; differences were related to seasonal effects with greater fish density and riffle fish density observed in the summer. Slack-water fish relative abundances by density differed among flow tiers; greater relative abundances were observed at Tier 7. Differences in percent occurrences of riffle fishes, fluvial fishes, slack-water fishes, Percidae, benthic fishes, and SOC were detected. Differences in percent occurrences of riffle fishes, benthic fishes, and SOC were attributed to basin effect, with greater percent occurrences of riffle fishes, benthic fishes, and SOC in the GSA than in BRA. The percent occurrence of fluvial fishes differed among tiers: while the percent occurrence of fluvial fishes was greater at Tier 5 than Tier 6, the percent occurrence at Tier 5 did not differ from that at Tiers 2 and 7. Slack-water fishes percent occurrences differed by tier and basin; percent occurrences at tiers 2, 6, and 7 were greater than Tier 5, and percent occurrences were greater in BRA than GSA. Percidae percent occurrences differed by tier, basin, and season; percent occurrences were greater at tiers 2 and 5 than Tier 7 with no differences detected among tiers 2, 5, and 6. Percidae percent occurrences were greater in GSA during the winter.

Three fish biology hypotheses were tested among tiers, basins, and seasons (Table 14; Appendix H). Condition factor and Hepatic-somatic index did not differ among tiers, basins, or season. For Gut Fullness, three-way interaction term was significant. Analyses of tier by season and basin lack sufficient replication to complete.

Nine habitat hypotheses were tested for runs among tiers, basins, and seasons (Table 15). Percentages of gravel substrates and cobble substrates differed among treatments. Gravel substrates differed by tier and season. Gravel substrates were greater at Tier 5 than tiers 1, 4, 6, and 7 and greater during the winter than in fall. Cobble substrates differed between basins with greater amounts in the GSA than BRA.

Fourteen fish community hypotheses were tested for runs among tiers, basins, and seasons (Table 16; Appendix I - L). Fluvial fishes' relative abundance, species richness, and Centrarchidae percent occurrences differed among tiers, basins, and seasons. Fluvial fishes' relative abundances differed among tiers with relative abundances greater at tiers 5 and 7 than tiers 2 and 6. Species richness of run fishes differed between basins with greater richness in BRA than GSA. Centrarchidae percent occurrences differed among seasons with greater percent occurrences in summer than in fall and winter.

Table 15. **Degrees of freedom (N = numerator, D = denominator), F-statistics, and P-values for three factor analyses of variances related hypotheses testing of run abiotic response variables among tier, basin, and season treatment effects.**

Dependent Variable	N	D	F-Statistic	P-value	Interactions	N	D	F-Statistic	P-value	Class variable	P-value	Class variable	P-value	Notes
Substrate (%)														
Silt	24	47	0.95	0.544	NS									
Sand	24	47	1.05	0.435	NS									
Gravel	24	47	1.39	0.167	NS	9	62	3.13	0.004	Tier	0.002	Season	0.027	1b 2ab 4b 5a 6b 7b; Su ab, F b, W a; Sp ab
Cobble	24	47	1.24	0.262	NS	9	62	2.83	0.007	Basin	<0.001			GSA > BRA
Boulder	24	47	0.51	0.960	NS									
Bedrock	24	47	0.90	0.596	NS									
Vegetation (%)	24	47	0.65	0.868	NS									
Current Velocity (cm/s)	24	47	0.8	0.716	NS									
Depth (m)	24	47	1.01	0.470	NS									

Interaction terms were tested and dropped from the model if not significant (NS). Only significant reduced models are shown. Under Notes, results of Fisher's LSD tests are provided for tier (1 – 7), basin (GSA, BRA), and season (Su = summer, F = fall, W = winter, Sp = spring). Different letters denote significant differences among treatment levels. Bold text represents significant response variables.

Table 16. **Degrees of freedom (N = numerator, D = denominator), F-statistics, and P-values for three factor analyses of variances related hypotheses testing of run biotic response variables among tier, basin, and season treatment effects.**

Dependent Variable		N	D	F-Statistic	P-value	Interactions	N	D	F-Statistic	P-value	Class variable	P-value	Class variable	P-value	Notes
Density	All fishes	24	47	0.34	0.997	NS									
	Swiftwater fishes	24	47	0.31	0.998	NS									
	Fluvial fishes	24	47	0.58	0.925	NS									
	Slackwater fishes	24	47	1.01	0.477	NS									
Relative abundance (%)	Swiftwater fishes	24	47	0.70	0.823	NS									
	Fluvial fishes	24	47	1.48	0.125	NS	9	62	2.18	0.036	Tier	0.015			1ab 2b 4a 5a 6b 7a
	Slackwater fishes	24	47	0.67	0.855	NS									
Richness		24	47	1.56	0.094	NS	9	62	3.87	<0.001	Basin	0.001			BRA > GSA
Occurrence (%)	Swiftwater fishes	24	47	0.70	0.824	NS									
	Fluvial fishes	24	47	1.13	0.349	NS									
	Slackwater fishes	24	47	0.69	0.840	NS									
	Cyprinidae	24	47	0.65	0.870	NS									
	Centrarchidae	24	47	1.46	0.133	NS	9	62	2.3	0.027	Season	0.002			Su a, F b, W b, Sp ab
	Gambusia, Fundulid	24	47	0.38	0.994	NS									
	SOC	24	47	0.84	0.673	NS									

Interaction terms were tested and dropped from the model is not significant (NS). Only significant reduced models are shown. Under Notes, results of Fisher's LSD tests are provided for tier (1 – 7), basin (GSA, BRA), and season (Su = summer, F = fall, W = winter, Sp = spring). Different letters denote significant differences among treatment levels. Bold text represents significant response variables.

Daily otolith aging

A total of 11 juvenile *Macrhybopsis* spp. were captured for use in the aging analysis. Shoal Chub *Macrhybopsis hyostoma* (n=8), from the Brazos River, made up the majority of the sample. Burrhead Chub *Macrhybopsis marconis* (n=3) were also collected from the San Antonio River. Shoal Chub were captured at two different locations on the lower Brazos River. Three individuals were captured near Hempstead and five individuals were captured near Rosharon. The Burrhead Chub sample was split between two locations on the San Antonio River. One individual was captured near Falls City and the other two were collected near Goliad. Mean length (SL, mm) and age (days) of Shoal Chub young-of-year for which otoliths were analyzed were 22.6 mm (range = 18.1-27.7 mm) and 44 days (range = 30-59 days), respectively. Burrhead Chub had a mean length of 20.3 mm (range = 13.9-28.1) and mean age of 40 days (range = 26 – 65). No general relationship between the flow regime and hatch date was apparent based on these very small samples for Shoal Chub or Burrhead Chub. In the Brazos River, one individual hatched during a pulse flow, two hatched during base flows, and five hatched during subsistence flows. Burrhead Chubs captured near Goliad both hatched during base flow conditions, and the Burrhead Chub specimen captured near Falls City hatched during subsistence flow conditions. These data are summarized in Table 17. Low sample sizes preclude the use of more powerful statistical analyses to determine relationships between hatch dates and the flow regime.

Table 17. **Summary of *Macrhybopsis spp.* otolith data. SL = standard length (mm), Age = estimated age of individual (days), Hatch date = back calculated estimated hatch date based on estimated age and date individual was sampled, Discharge = mean daily discharge (cfs), Rate of change = percent change from previous day's mean daily discharge.**

Species	River	Location	SL	Age	Hatch date	Discharge	Rate of change	Flow level
M. hyostoma	Brazos	Hempstead	18.1	30	9/3/2014	601	4	Base
M. hyostoma	Brazos	Hempstead	21.7	43	8/21/2014	214	-4	Subsistence
M. hyostoma	Brazos	Hempstead	22.4	45	8/19/2014	235	-19	Subsistence
M. hyostoma	Brazos	Rosharon	21.0	43	10/27/2014	151	-10	Subsistence
M. hyostoma	Brazos	Rosharon	21.8	34	11/4/2014	148	56	Subsistence
M. hyostoma	Brazos	Rosharon	23.1	46	7/6/2014	1730	-15	Base
M. hyostoma	Brazos	Rosharon	25.3	54	6/28/2014	3200	70	Pulse
M. hyostoma	Brazos	Rosharon	27.7	59	10/11/2014	242	-3	Subsistence
M. marconis	San Antonio	Falls City	28.1	65	9/10/2014	71	-10	Subsistence
M. marconis	San Antonio	Goliad	13.9	26	7/11/2014	146	5	Base
M. marconis	San Antonio	Goliad	19.0	29	7/8/2014	165	-6	Base

Interdisciplinary aquatic conclusions

Initial predictions about riffle and run habitat parameters, aquatic insect and fish community responses to flow tiers tested in this study, and fish biology were largely not supported. . Among the 58 hypotheses tested, tier effect was detected among six response variables (slack-water fish relative abundances in riffles, percent occurrences of fluvial fishes, slack-water fishes, and Percidae in riffles, gravel substrates in runs, and relative abundances of fluvial fishes in runs).

Among six response variables, two variables were linearly related to tier: relative abundances of slack-water fishes increased (expected to decrease) with increases in tiers (greatest percentage was at Tier 7), and percent occurrence of Percidae decreased (expected to increase) with increases in tiers (Tier 7 was lower than Tiers 2 and 5). Another variable, gravel substrates in runs, indicated a unimodal relationship with tier (Tier 5 was greater than Tiers 1, 4, 6, and 7 but did not differ from Tier 2), which was somewhat expected though lack of differences with Tier 2 suggests that effects were not different from base flow conditions). High-flow pulses were inconsistently related to the remaining three response variables: Tier 5 was greater than Tier 6, but Tier 5 was not different from Tier 7. Results of hypotheses tests are supported by descriptive analyses (PCA and CCA models), meaning that descriptive analyses, like hypotheses tests, did not indicate a strong association between tiers and habitat parameters and biota occurrence, abundances, and densities.

Very low sample sizes of juveniles of two *Macrhybopsis* species (combined N=11) from four locations on two different rivers made detecting relationships between stream flow and hatch dates virtually impossible. Limited results based on analysis of these few specimens cannot provide a reliable assessment of the influence of flow pulses on the recruitment of *Macrhybopsis* spp. Recently, Rodger (2015) estimated hatch dates of Shoal Chubs in the lower Brazos River using otoliths and found the greatest proportion of surviving young-of-year fish had hatched during pulse flows, and on days when discharge was increasing (Figure 22). Using otoliths to analyze hatch dates of young-of-year pelagic broadcast-spawning minnows allows for determination of quantitative estimates of discharge magnitude that promote recruitment in focal species. Based on a low sample size (n=68), Rodger concluded that Shoal Chub recruitment is greatest during flows categorized as the two-per-season flow pulse within the BRA BBEST environmental flows recommendations (Figure 23). Rodger's estimate was based on discharge data from the upstream USGS streamflow gage nearest to his survey site on the lower Brazos River. It is logical to assume that if high-flow pulses positively influence Shoal Chub recruitment, this occurs only to a certain threshold beyond which greater magnitude pulses result in lower recruitment.

Members of the *Macrhybopsis* genus belong to a unique reproductive guild of cyprinids known as pelagic broadcast-spawning minnows (Platania and Altenbach, 1998, Wilde and Durham, 2008, Perkin and Gido, 2011). Elevated gonadosomatic indices (GSI) throughout the reproductive season and oocytes in all stages of development provide concrete evidence that spawning occurs multiple times over an extended reproductive season for pelagic broadcast-spawning minnows (Durham and Wilde 2008b, 2014). Furthermore, based on short-term shifts in proportions of postovulatory follicles and reductions in female oocyte diameter and GSIs following flow pulses, species within this reproductive guild are known to undergo population-wide synchronized spawning events that are prompted by elevated discharge events (Durham and Wilde 2008b, 2014). Thus, flow pulses greatly increase the number of propagules released into the system, and there is recent evidence that recruitment success is also linked to high-flow pulses. To date, two studies have been completed on different segments of the Brazos River, and both document greater recruitment of pelagic broadcast-spawning minnows associated with intervals of higher discharge. In addition to Rodger's (2015) study, Durham and Wilde (2009) used otoliths to estimate hatch dates and found this relationship for Sharpnose Shiner *Notropis* *oxyrhynchus* and Smalleye Shiner *Notropis buccula*, two imperiled fluvial specialist species endemic to the Brazos River.

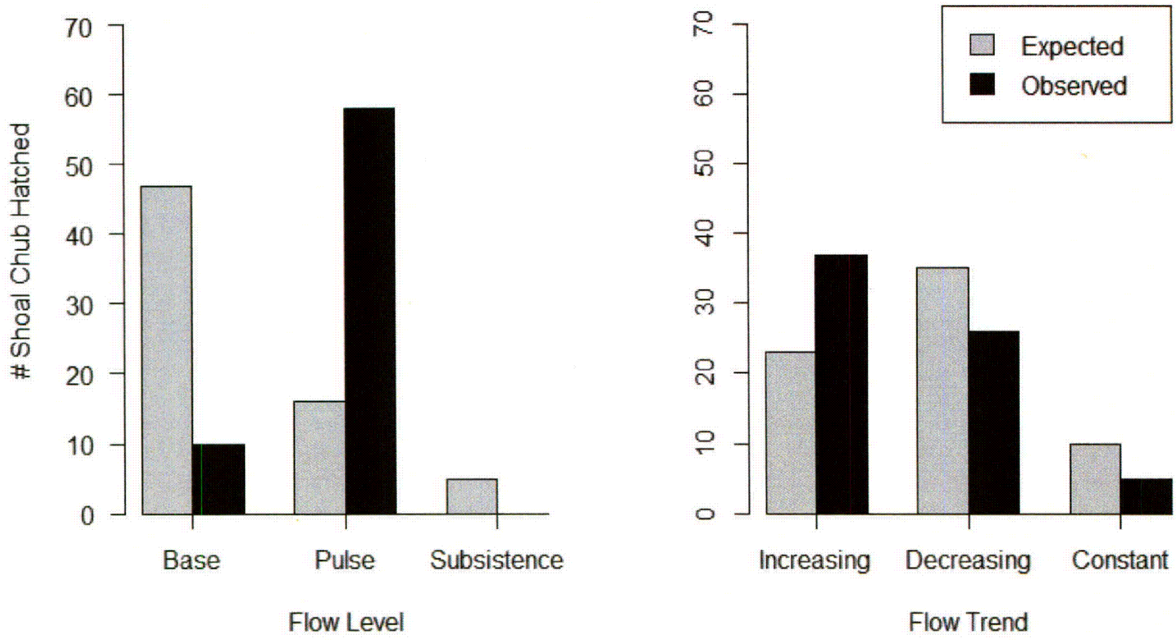


Figure 22. **$\chi2$ goodness-of-fit test results for Shoal Chub hatch dates in relation to hydrological categories. Results for both $\chi2$ goodness-of-fit tests analyzing flow levels and flow trends were significant ($\chi2 = 150.18$, df = 2, $P < 0.00001$) and ($\chi2 = 13.54$, df = 2, $P = 0.001$), respectively (adapted from Rodger 2015).**

To maintain a stable local population, pelagic broadcast-spawning cyprinids either need to take advantage of hydrologic conditions that reduce downstream transport of larvae, or else undergo upstream movements during the juvenile and/or adult stage to balance downstream drift of larvae, the latter being much more energetically expensive. Since flow pulses tend to be brief in prairie rivers (Hoagstrom and Turner, 2013), this explains the tendency for species in this reproductive guild to initiate spawning on the rising limb of a flow pulse (Medley et al., 2007), much like the pattern described by Rodger's (2015) study of Shoal Chub recruitment in the lower Brazos River. Spawning during short-lived flow pulses of moderate magnitude probably facilitates retention of drifting propagules in nearby nursery habitats following pulse subsidence (Medley et al., 2007; Widmer et al., 2012; Hoagstrom and Turner, 2013), which would reduce requirement for long upstream migrations by survivors to replace individuals displaced downstream. Based on a significant, non-linear, quadratic relationship between discharge magnitude and the number of Shoal Chubs recruits obtained by Rodger (2015)(Figure 24), our best current assessment is that flow pulses of moderate magnitude promote highest recruitment of Shoal Chubs in the lower Brazos River.

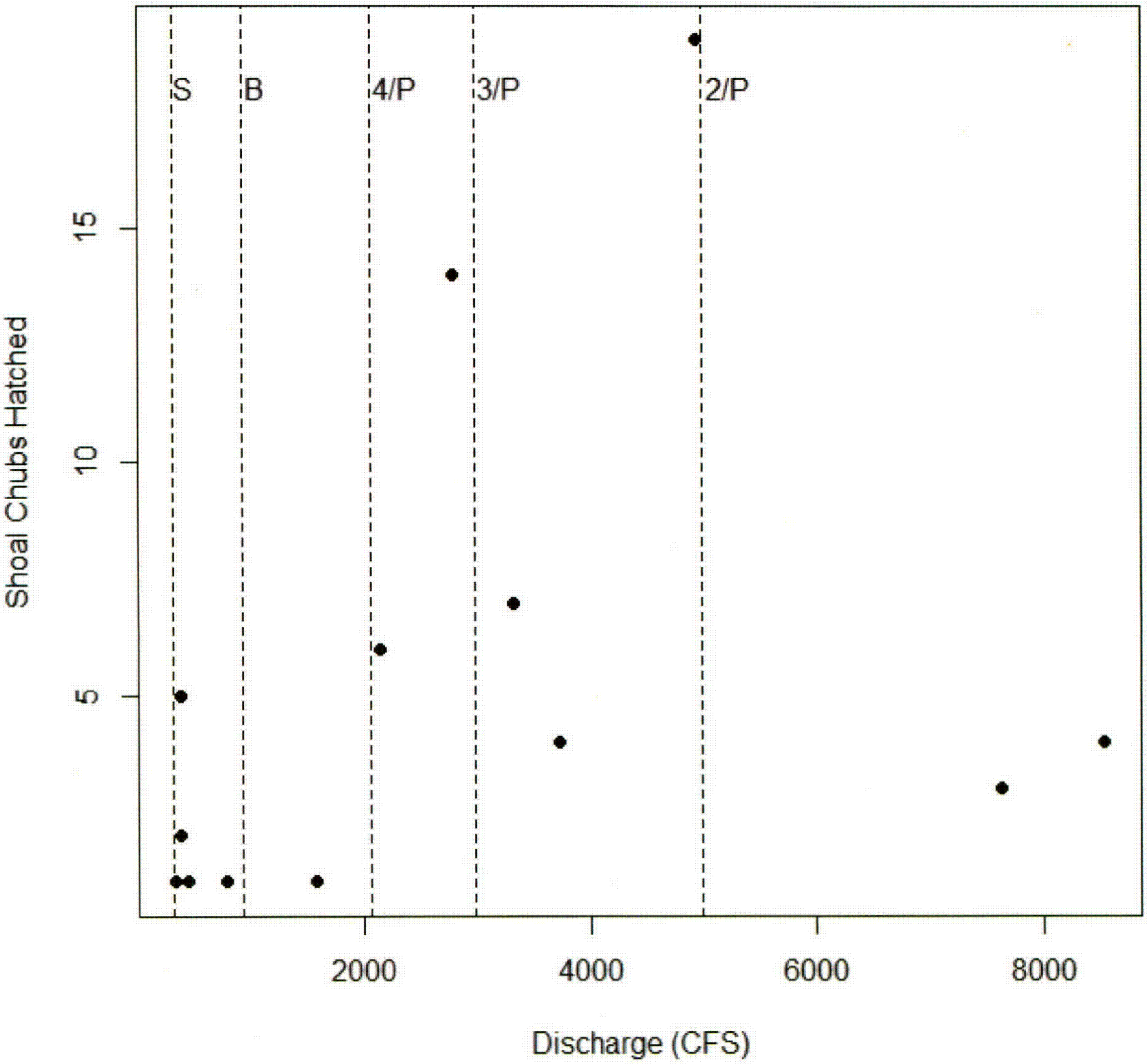


Figure 23. **Number of Shoal Chubs hatched and environmental flow standards. Environmental flow standards, for the summer period (June-October), based on USGS streamflow gauge 8108700 near Bryan, TX that represented the nearest upstream gauge from the field collection site. S = subsistence flow, B = base flow, 4/P = four-per-season flow pulse, 3/P = three-per-season flow pulse, 2/P = two-per-season flow pulse (adapted from Rodger 2015).**

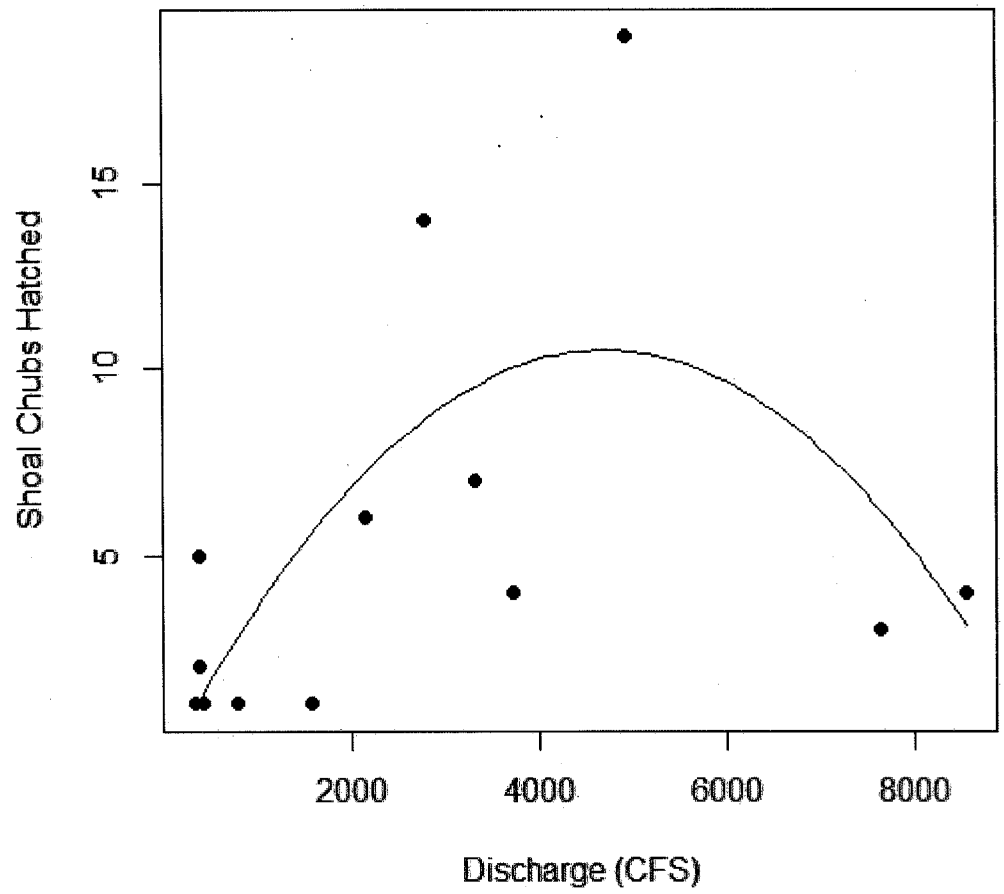


Figure 24. **Numbers of surviving Shoal Chubs hatched and discharge. Non-linear relationship was significant ($R^2 = 0.46$, $P = 0.048$). Equation for the regression line is $y = -5E\text{-}07x^2 + 0.0047x - 0.604$ (adapted from Rodger 2015).**

3.2 Riparian

Results and discussion of outcomes will be discussed by site rather than as individual hypotheses to better facilitate how all hypotheses/conditions combine and to determine overall riparian responses to flows. For both the BRA and GSA riparian assessments, a repeated theme that echoed throughout the results section was that TCEQ flow standards, in most cases, are not sufficient to meet 80% to 100% inundation of the existing mature tree distribution at these locations. Such flows are typically large, less frequent flows that may not happen every year. Additionally, trees have a lifespan greater than a single year, so not getting flows necessary to inundate 80% of the mature tree distribution each year would not necessarily cause a long-term reduction in riparian zone coverage. However, it is the repeated occurrence of such "no-inundation events" that would start to shrink the riparian community distribution. If maintenance of the existing riparian zones is the BBASC or TCEQ focus, then the protection of roughly the 1/year flow tiers (with an added component of timing) of these larger flows is essential. This ensures that when they do occur, the pulses are allowed to inundate.

Brazos Bend site

The Brazos Bend site (representing the USGS gage near Rosharon) represented the downstream-most site. The river here has deeply incised channel slopes that are often very steep-to-vertical, and little riparian zone forest remains along much of this stretch of river. The study site was within Brazos Bend State Park, located along a more gently sloping sand bar with an opposite cut bank. Green ash was not found at this site. Beyond the long running horizontal sand bar, the slope is 0.32 (meters rise/meters run) (Figure 25). While black willows are constrained within one vertical meter of the water's edge, box elder mature trees have a much greater spatial coverage, from 1m to almost 8m elevation. Not shown in the figure, but just beyond the limit of the riparian species is a run-off gully that runs parallel to the river uphill from the site. It may be influencing water availability to those box elders at highest elevations, and a very large dense stand of saplings was observed in the area.

At this site, all TCEQ flow tiers inundate some portion of the riparian species, all of which completely cover black willow (Figure 26). None of the TCEQ seasonal flows provide more than 60% coverage of box elder (Figure 27). Only the BBEST-recommended 1/year flow provides inundation to the full extent of box elder distribution.

Observed differences between site-specific measured flow and USGS measured flow can be explained by the channel morphology variation between the sites (Figure 28). Water level heights for individual flow events are not expected to be identical (though flow pulse timings should correlate) between the two because local stream reach characteristics (e.g., width of channel, steepness of slope, curvature of river, etc.) will drive distinct differences. Thus, one must be calibrated to the other.

TCEQ base flows were seen for all seasons (Table 18). In 2014 the Brazos Bend site experienced 2/spring, 3/summer, a 3/winter, and a 1/winter flows, and all TCEQ and BBEST-recommended flows occurred in spring 2015.

The effects of the drought extending through 2014, during which where many flows did not occur, were apparent (Table 18). The drought limited our ability to test seedling establishment and survival, and sapling survival to flows that did not occur. Therefore, we moved to the second propositions of the seedling and sapling hypotheses – comparing seeds and saplings to actual flows that did occur. The actual flows during the season more than covered the willow distribution (Figure 29). This would indicate two characteristics of note: One is that willows are resistant to full submersion of higher flows. This trait is well known and is confirmed with this study. The second is that both the willow seedlings and saplings are constrained to the near-water's edge even with the flows seen during the study. This could be an indicator that even with the higher flows, young willows were unable to thrive at those elevations. For example, even though a 1/spring event potentially allowed for seed dispersal at a higher elevation (with fewer subsequent flows), their distribution is limited exclusively to the 3/summer flow levels, as are saplings. Given that the black willow mature trees have the same spatial coverage as saplings, this could be the maximum extent of the willow's potential range regardless of flow fluctuations; however, the current year's flows resulted in seed dispersal at an even lower elevation than either – an effect of the lack of flow seen here.

Box elder seedlings and saplings are both distributed throughout the entire mature tree distribution (Figure 30), indicating that replacement of this stand is healthy. The seedling dispersal for this species correlates very closely with the 2014 spring flow of 8m, one of the additional flows that occurred early in the season. However, most seedlings fell within 4m (the height of the 2/spring flow) with just a few at the upper edges, so there was likely some attrition occurring beyond the 2/spring flows' inundation.

It is apparent that there was much recruitment from black willow seedling to sapling (32 new saplings recorded) from summer to fall (Table 19). And those seedlings that recruited to the next class were replaced (and 2 added) during this same time. This indicates that where the willows are distributed they are thriving and growing vigorously. So too are the box elders. As mentioned above there was an extremely dense stand of box elder saplings along the upper tiers of their distribution. Six of them recruited to mature class sizes from the summer to fall season, the sapling class size increased in numbers, and the seedling increase was prolific in October (likely the 2/summer event that reached 4.8m into the site during this species' late-season seed dispersal).

The presence of hackberry and chinaberry indicates some invasion of upland and non-natives, respectively, is occurring (Table 20). However, collectively box elders are 78% of the forest (saplings clearly dominate) and black willows are 20%. Clearly the riparian species are the overwhelmingly dominant species found in this healthy riparian zone. Because sites could not be resampled in summer 2015 no comparisons could be made to changes in annual relative abundance. This is true of all sites.

A typical (expected) distribution should include seedlings as the most abundant age class, followed by saplings and then older, mature trees, with large reductions seen with each class. This site's riparian community age distribution shows that seedling dispersal/survival is a fraction of what is expected when compared to sapling class size (Figure 31). Again, this is evidence of the negative impacts of the recent drought covering the past few years (Figure 32). Beyond saplings, the presence of older trees quickly drops to less than 10 for each age class, with a few lone sentinels several decades old. One year of study with this limited number of trees cannot answer why this is. It could be a normal age structure for theses riparian trees (disturbance removes older trees on a regular cycle here). Or it could be a past stressor. Subsequent studies can and definitely should explore this. Figure 32 shows that even though no anomalous flows seem present between 11 and 15 years, unfortunately the USGS gage data for several years prior to 1999 - the time frame for which an answer would lie - are missing from their dataset.

TCEQ flow standards appear to be relatively adequate for maintaining the existing mature tree distribution for the Brazos Bend site. They more than cover black willows but provide incomplete coverage for box elders. Even though only a few of the recommended flows actually occurred during the study timeframe, those flows that did occur appeared to have a positive influence on the willows. Willow seedlings and saplings correlated most closely only with base flow and the lowest flows; however, their spatial coverage was similar to their mature trees, which indicates that replacement is adequate for the existing stand, and only slightly lower than necessary this past year as indicated by seed dispersal. Maintenance includes much distribution, survival and recruitment over this short time period. Box elder seedling and saplings also fell within the full extent of the mature trees, and exhibited much dispersal, survival and recruitment. The extreme relative abundance of the two species in the zone (98% relative abundance) indicates that the Brazos Bend streamside forest is clearly functioning as a riparian zone, rather than an encroached-upon riparian zone or mixed forest.

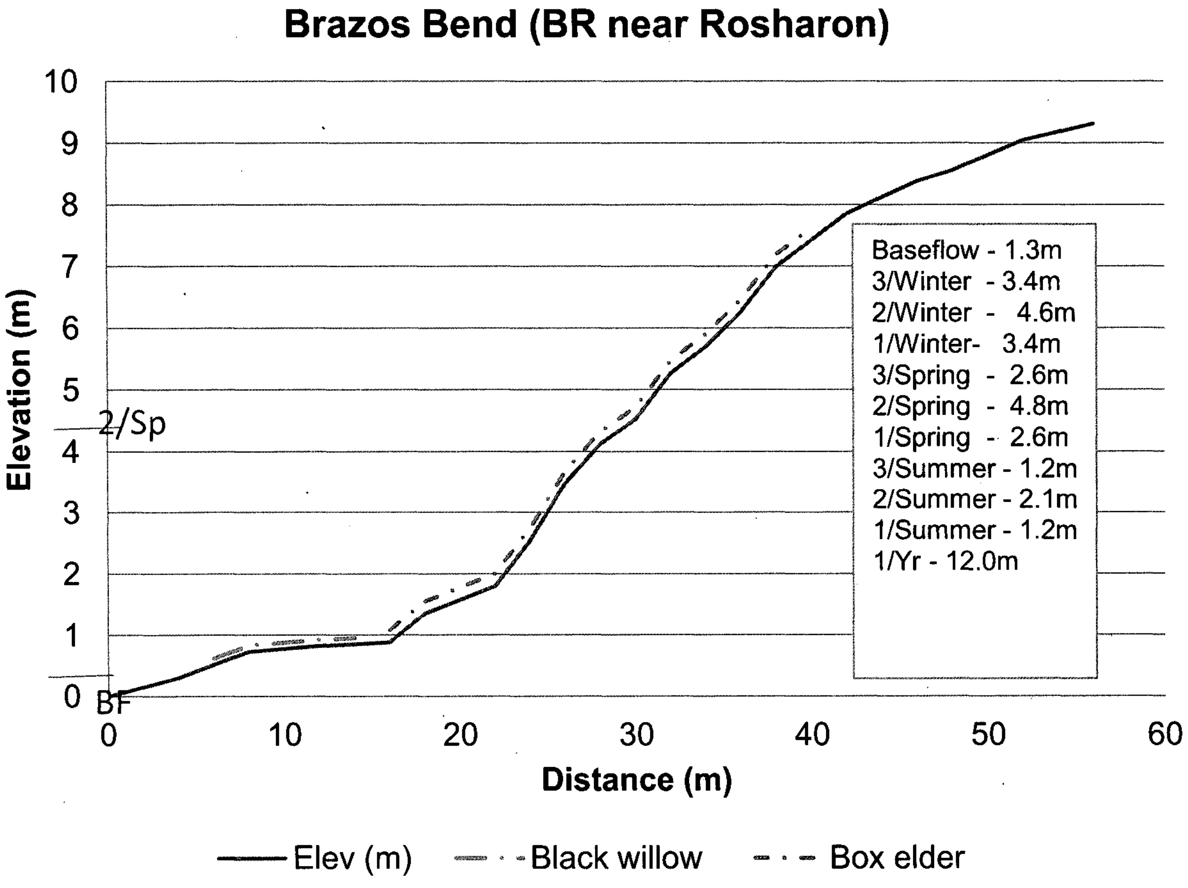


Figure 25. **Brazos Bend site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows estimated vertical inundation of the site at the given flow tiers. Flow elevation and select flows are shown on the y-axis.**

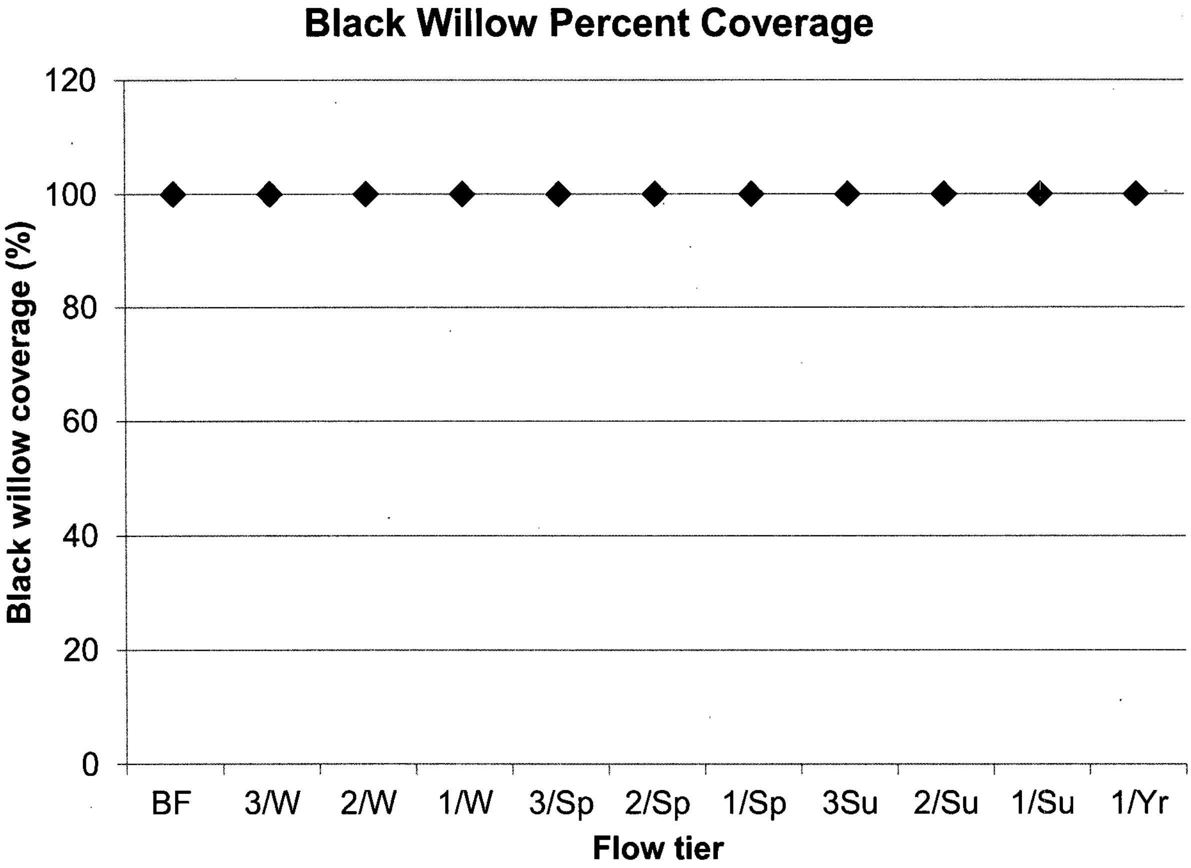


Figure 26. **Percent of mature black willow stand inundated by flow tier at the Brazos Bend site.**

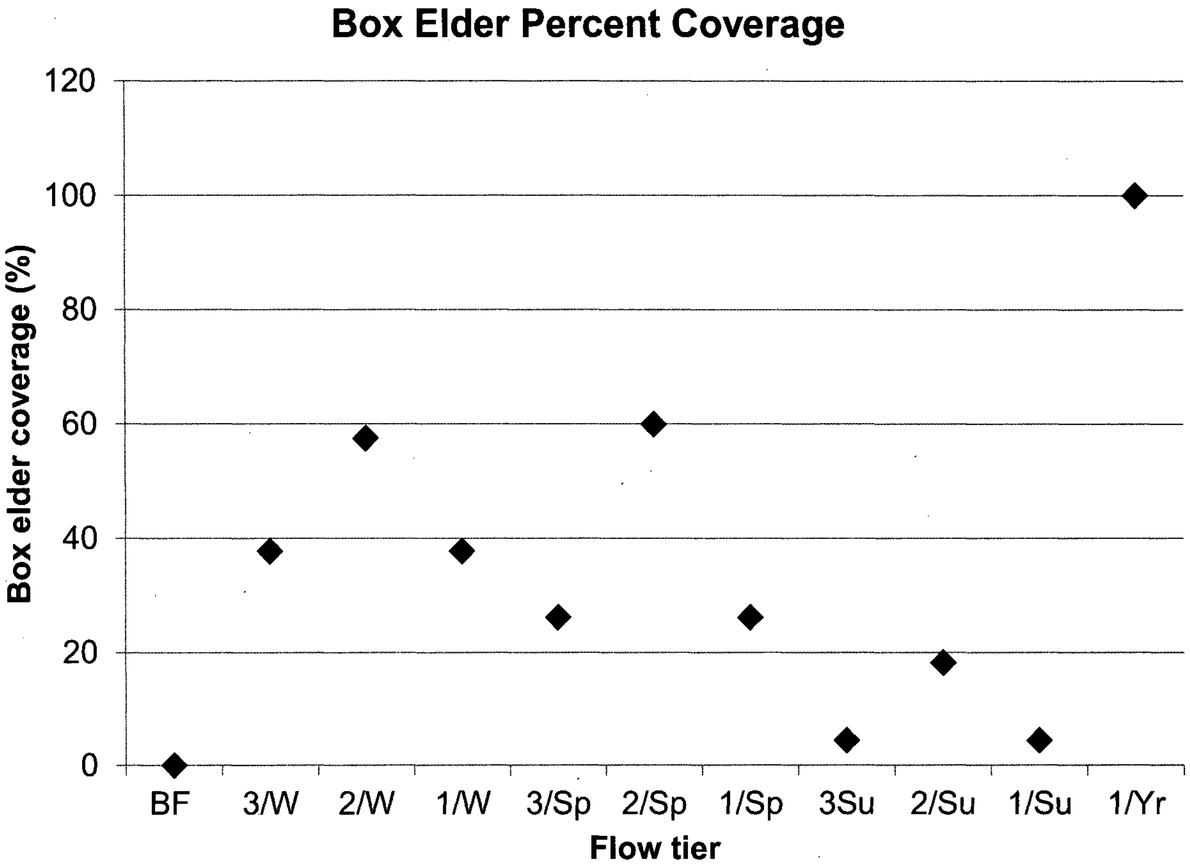


Figure 27. Percentage of mature box elder stand covered by flow tiers at the Brazos Bend site

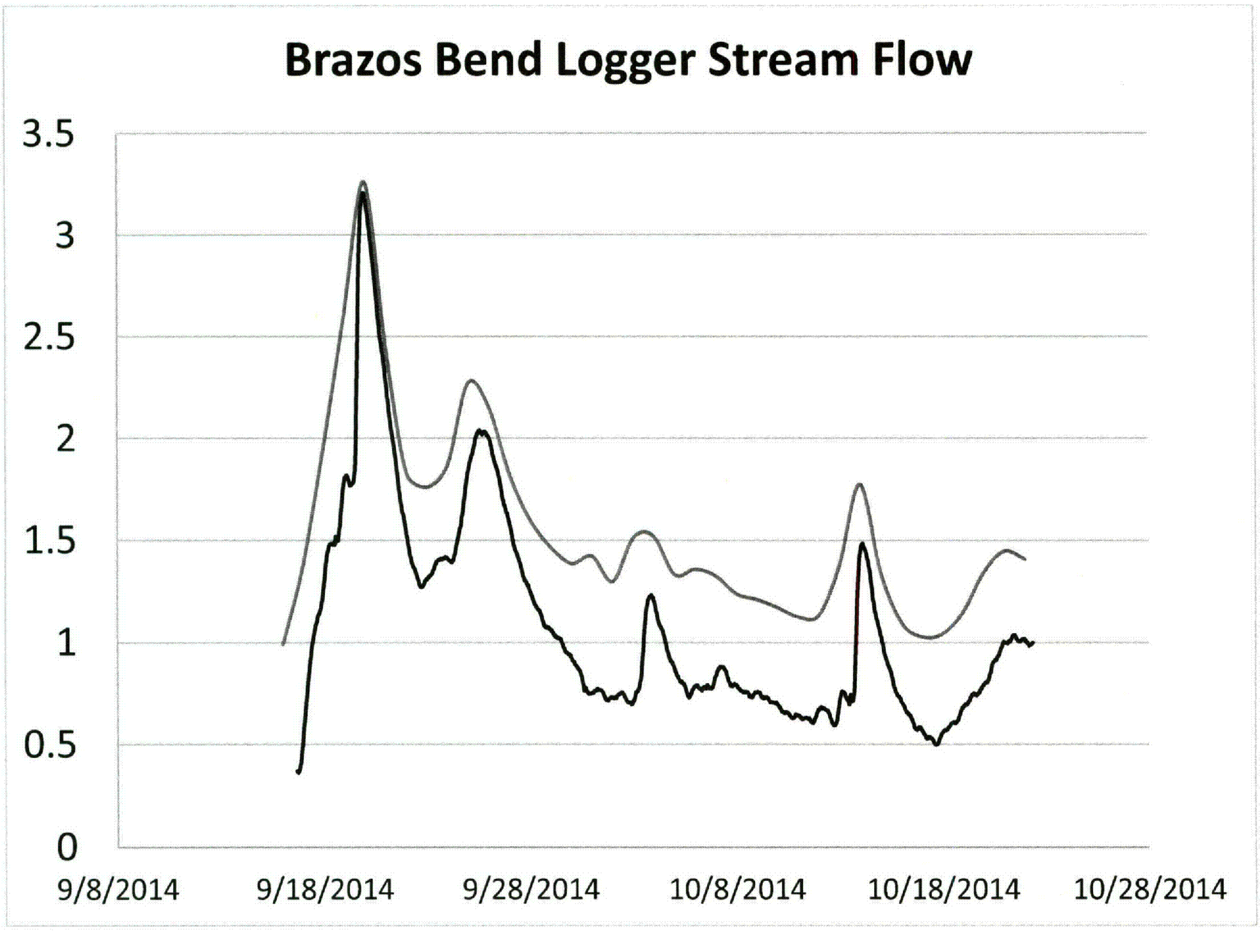


Figure 28. USGS flow (in red) in comparison to measured site water level heights (in blue) at the Brazos Bend site. Y-axis is elevation of water in the channel in meters.

Table 18. TCEQ- and BBEST-recommended flow tiers and their occurrences throughout the BBEST-designated seasons (shaded) at the Brazos Bend site. Y indicates flow occurred; dash indicates no flow occurred.

			2014 Spring	2014 Summer	2014 Winter	2015 Spring
Flow Tier	**CFS**	**Elevation (m)**	**Mar. - Jun.**	**Jul. - Oct.**	**Nov. - Feb.**	**Mar. - Jun.**
Baseflow	200	1.33	Y	Y	Y	Y
3/Winter	9090	3.35			-	
2/Winter	13600	4.64			-	
1/Winter	9090	3.35			Y	
3/Spring	6580	2.59	-			Y
2/Spring	14200	4.8	Y			Y
1/Spring	6580	2.59	-			Y
3/Summer	2490	1.17		Y		
2/Summer	4980	2.07		-		
1/Summer	2490	1.17		-		
1/Year	51000	11.96	-	-	-	Y

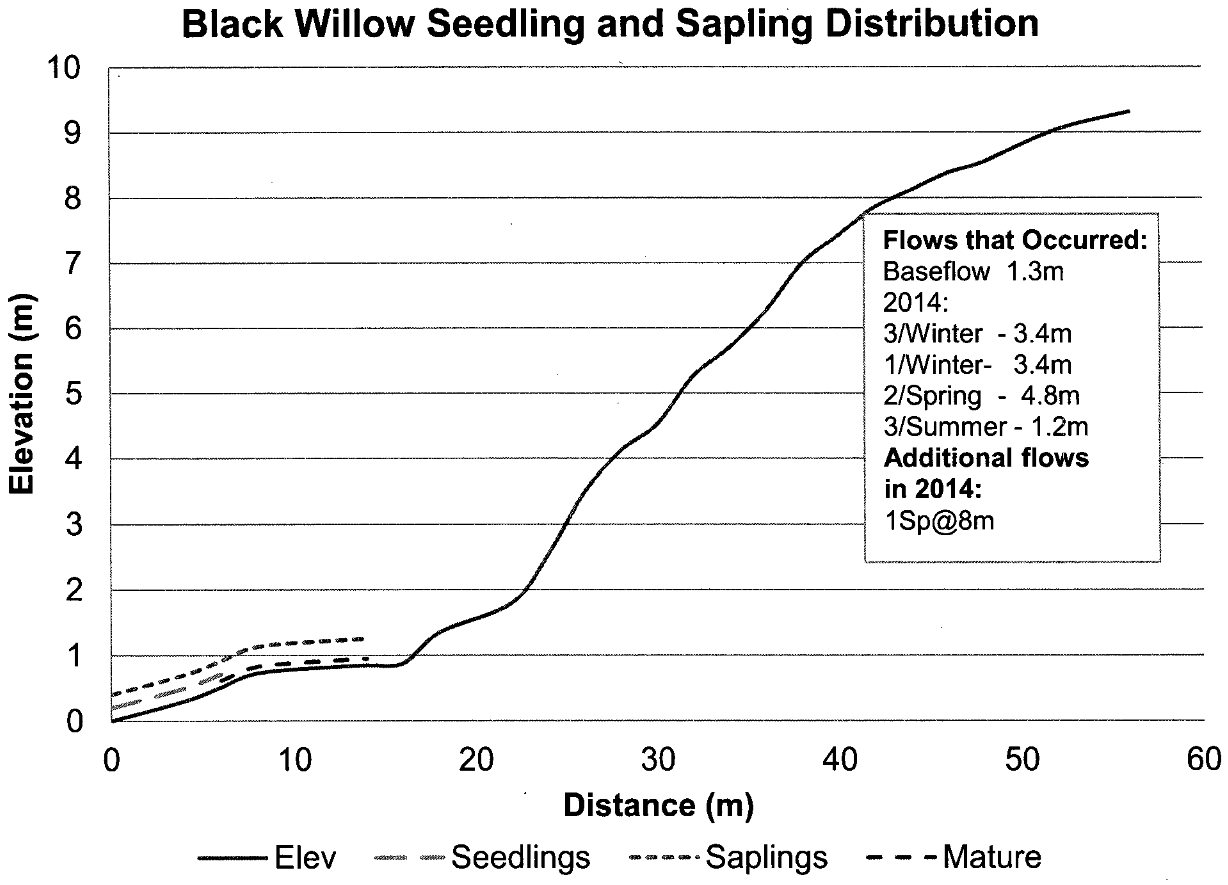


Figure 29. Black willow distributions at the Brazos Bend site. Inset box indicates which flow tiers actually occurred during the study. Additional flows are shown in the inset box.

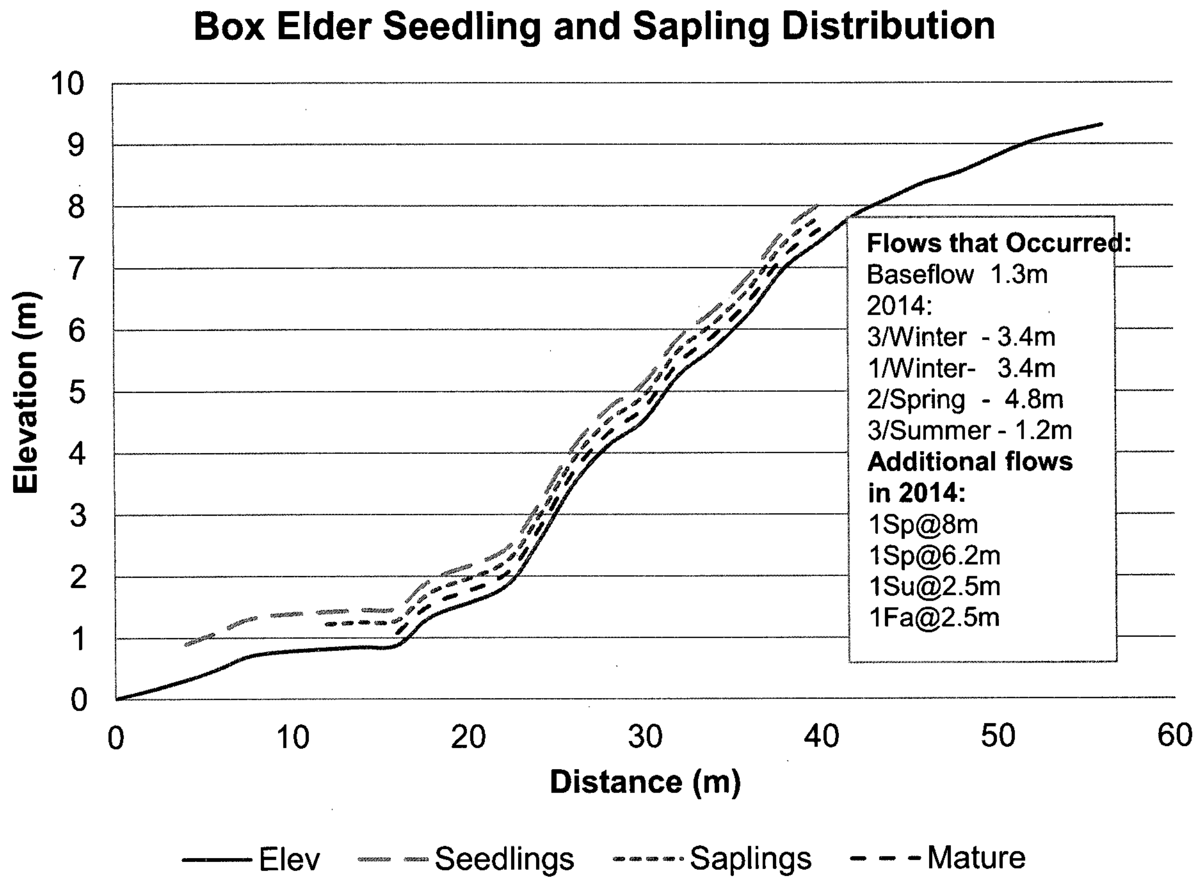


Figure 30. **Box elder distributions at the Brazos Bend site. Inset box indicates which flow tiers actually occurred during the study.**

Table 19. **Tree counts through time at the Brazos Bend site. Spring 2015 was not collected, as the site was almost entirely underwater (70%) in April 2015 and inaccessible.**

Species	Class	Summer 2014	Fall 2014
Black Willow	Mature	5	5
Black Willow	Sapling	60	92
Black Willow	Seedling	25	27
Box Elder	Mature	2	8
Box Elder	Sapling	333	340
Box Elder	Seedling	16	100

Table 20. **Relative abundances of woody species, grouped by tree type and age class, at the Brazos Bend site.**

Tree Species	Class	Relative abundance (%)
Black Willow	Mature	1.1
Black Willow	Sapling	13.3
Black Willow	Seedling	5.5
Box Elder	Mature	0.4
Box Elder	Sapling	73.8
Box Elder	Seedling	3.5
Chinaberry	Sapling	0.2
Cottonwood	Sapling	0.4
Dogwood	Sapling	0.9
Hackberry	Seedling	0.2
Pecan	Seedling	0.4
		100

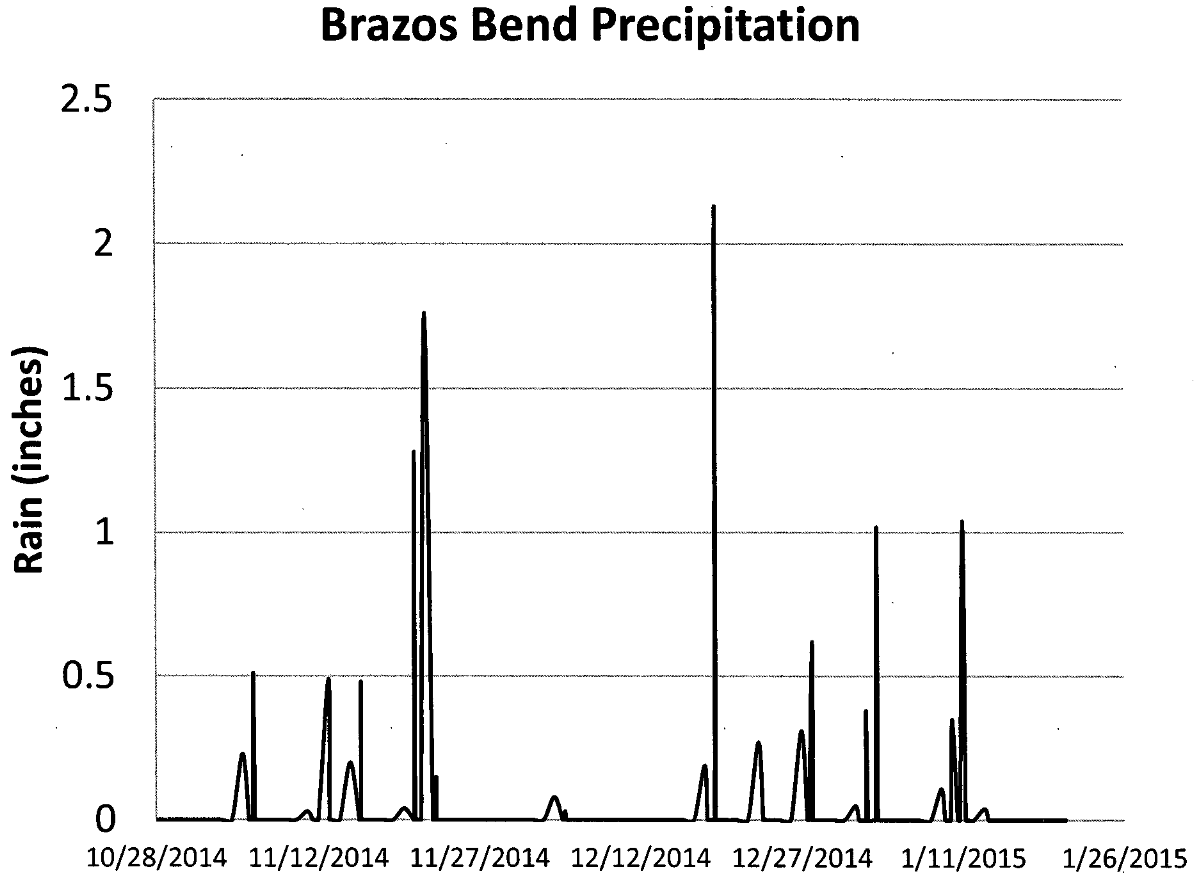


Figure 31. **Recorded Brazos Bend rainfall data in inches.**

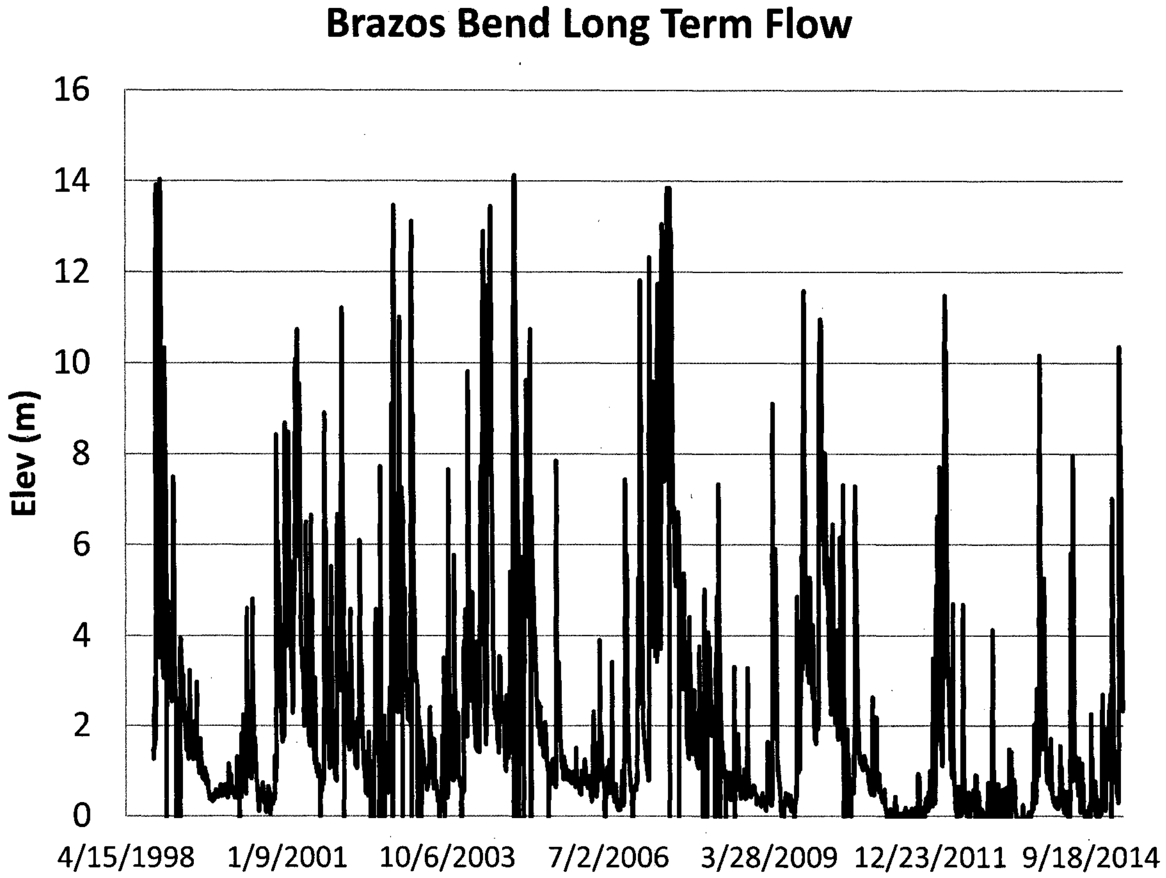


Figure 32. **Long term stream flow for the USGS gauge near Rosharon, adjusted for site elevation inundation. Graphed only as far back as data extend.**

Hearne site

The Hearne site (corresponding to the Brazos River USGS gage near Bryan) represented a mid-reach site on the Brazos River itself. The river here is incised, channel slopes are often steep with cut banks, and little riparian zone forest remains along much of the stretch. The site was located on private property (upland landscape is mostly agricultural), and was just upstream and converging with a gently sloping sand bar. Beyond the sand bar, the slope is 0.16 (rise/run) (Figure 33).

Box elders occupy the lowest tiers of the slope (between 3-5-4.5m), green ash the mid-slopes (3.5-5.5m), and black willows are distributed all the way to the upper bank edges. Not all TCEQ flow tiers are adequate for meeting the needs of all species: notably the 3/summer and 1/summer flows fell short of any tree species. Because black willow is the most widely dispersed, none of the within-year TCEQ flow tiers fully cover black willow mature stands beyond 55% (Figure 34). Only the 1/year BBEST-recommended flow covers this species fully. Box elder coverage shows a distinctive pattern (Figure 35) whereby all spring flows inundate 100% of their distribution as does a 2/summer. The 2/winter provides 80% inundation. Only the 1/year BBEST flows covers green ash (at 100% coverage) (Figure 36); all other flows inundate 50% or less of their distribution.

TCEQ base flows were maintained for all seasons (Table 21). In 2014 no flows other than a 1/winter occurred. All flows occurred in spring 2015 during the heavy rains. The effects of the drought extending through 2014 can clearly be seen (Table 21), where most flows did not occur. This limited our ability to test seedling establishment and survival, and sapling survival to those flows. Therefore, we moved to the second propositions of the seedling and sapling hypotheses – testing those actual flows that did occur. Black willow seedlings were dispersed only where base flow occurred (Figure 37) and were far removed from the mature distributions (they were much lower than mature); saplings covered both the seedling range and the upper reaches where mature are located. This pattern makes sense given that some of the saplings pre-date the drought at up to eight years old, and may reflect previous years' flows, whereas others represent more recent flow inundation – giving them a broad distribution.

Green ash seedling dispersal correlates generally with the one spring flow in 2014 that reach 4.4m (Figure 38). Sapling distribution was identical to mature, seedlings only covered the lower edge of that same distribution.

There were a number of rainfall events in September, October, and November that may have kept soil moisture sufficiently wet to keep the green ash and black willow seedlings going, especially given there were no summer flows, though the ultimate attrition by spring 2015 shows they weren't fully adequate (Figure 39). There is currently no method to determine how beneficial spring rains were with the dataset available.

This site had obvious signs of drought stress observed at the study's start. Several of the mature willow trees in the site were already dead, and from summer 2014 to spring 2015 two of the three in our transect plots were lost (Table 22). Though four willow saplings recruited from seedlings, by the next spring they had perished. Willow seedling survival dropped from almost 200 individuals in summer 2014 to slightly more than 50 by that fall. The early spring rains of 2015 did allow seed dispersal to bounce back to almost double the previous season. Box elders counts were low but mature trees survived through to spring. No new seed dispersal occurred (if any had occurred early in 2014 they had already been lost when the study began). Green ash mature trees also survived through the year, and it appears that most of the seedlings for this species were able to recruit into saplings by fall, but by spring 2015 had perished – with no new seed dispersal. The lack of flows in summer and fall 2014 obviously took their toll on the green ash saplings as they did on the willow seedlings. Collectively box elders are less than 2% of the forest, green ash are 5%, and black willows make up 80% (Table 23). Black willows are the dominant species found in this riparian zone, both in count and in spatial distribution. They were also the hardest hit in this past year's drought.

In comparisons of age class sizes, seedlings are the most prolific in the site, though as mentioned, they were hardest hit during the fall (Figure 40). Again, this is evidence of the negative impacts of the recent drought covering the past few years. Beyond saplings, the presence of older trees drops to less than 5 for each age class. One year of study cannot answer why this is. It could be a normal age structure for theses riparian trees (disturbance removes older trees on a regular cycle here); or it could be a past stressor. Subsequent studies could explore this. Even though no anomalous flows seem present between 11 and 15 years, unfortunately the USGS gage data for several years prior to 1999 - the time frame for which an answer would lie - are missing from their records (Figure 41).

TCEQ flow standards appear to be moderately adequate for maintenance of the existing mature tree distribution for the Hearne reach. Even though few of the TCEQ flows actually occurred over the study period, those that did occur appeared to have a positive influence on the willows. Willow seedlings were dispersed with spring 2014 flows and even some recruitment, but many perished (~95%) before the year's end with the lack of subsequent flows. Most notably, while willow sapling distributions span through the mature tree distribution, seedling dispersal was limited to the severely low elevations served only by base flow – indicating this season's replacement is inadequate for the existing stand's ranges. Green ash, too was limited to the elevation of one spring flow – short of its sapling and mature ranges. Box elder seedling dispersal was nonexistent (or all seedlings had perished before the study began). Age structure analysis indicates that a lack of streamflow pulses along the river have had noticeable impacts on seedling dispersal and future maintenance. The strong relative abundance of the riparian species in the zone indicates that the Hearne streamside forest is functioning as a riparian zone (at 87% relative abundance), rather than an encroached zone.

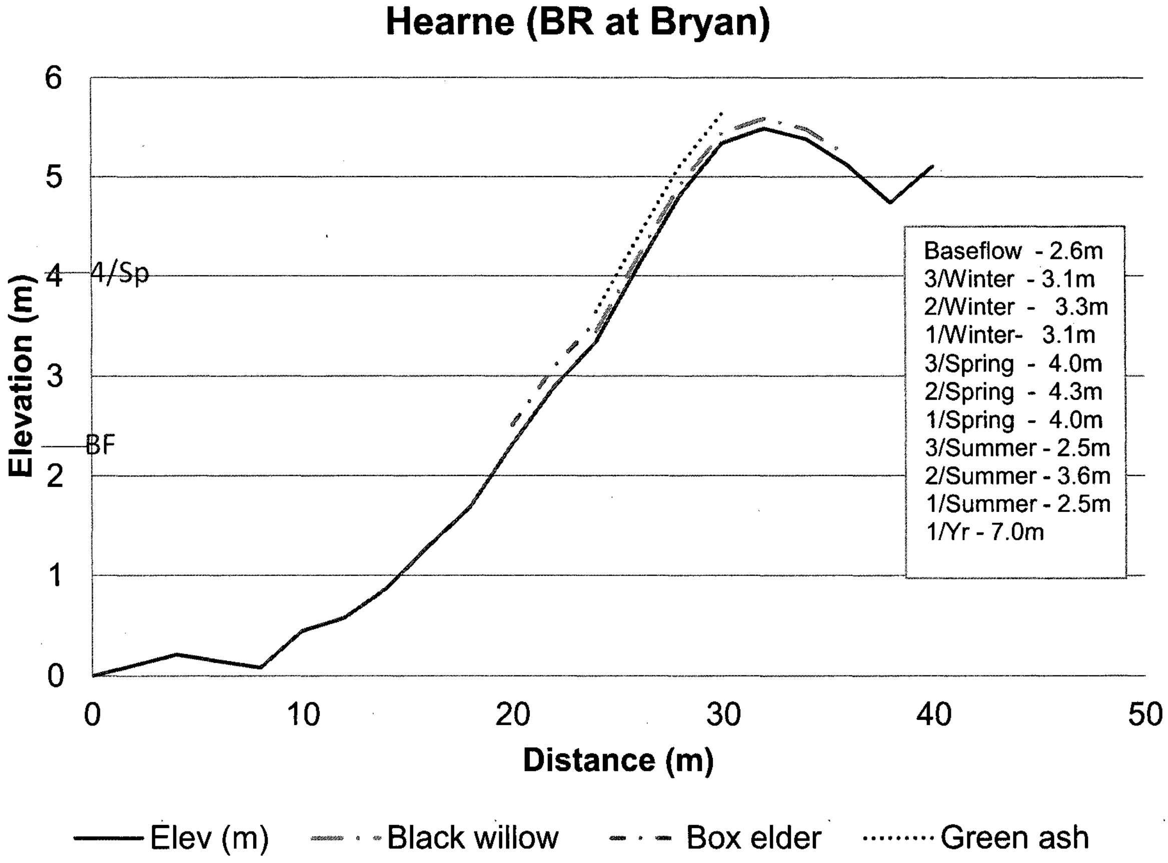


Figure 33. **Hearne site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows vertical inundation of flow tiers. Select flows are shown on the y-axis.**

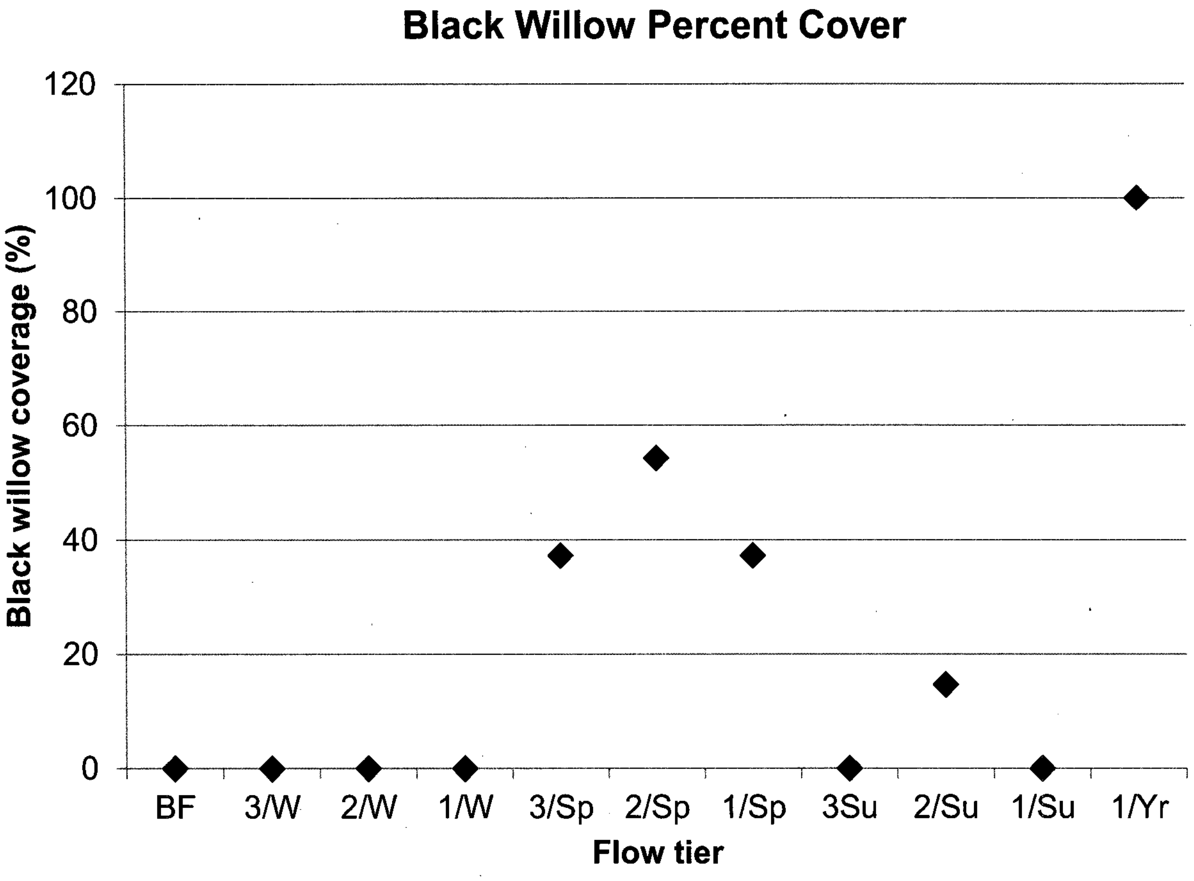


Figure 34. **Percentage of mature black willow stand covered by flow tiers at the Hearne site.**

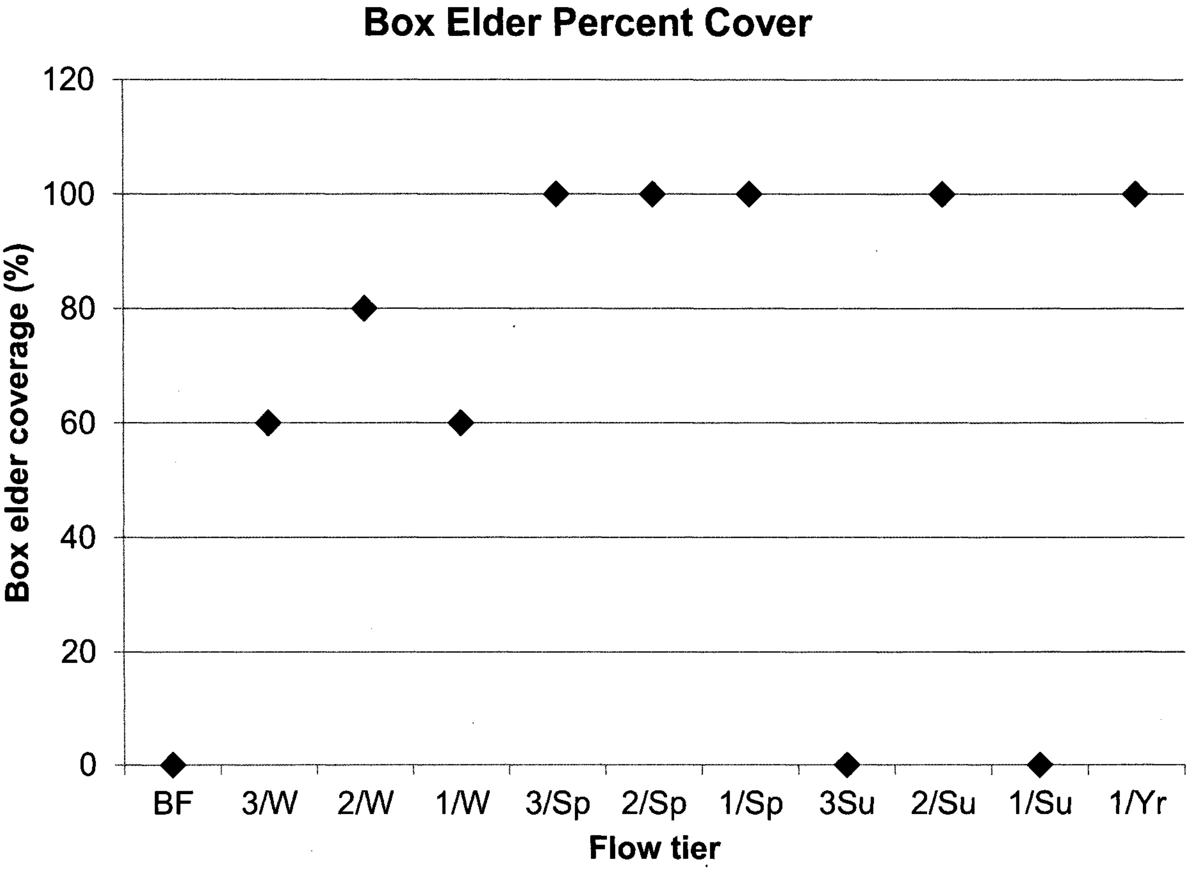


Figure 35. **Percentage of mature box elder stand covered by flow tiers at the Hearne site.**

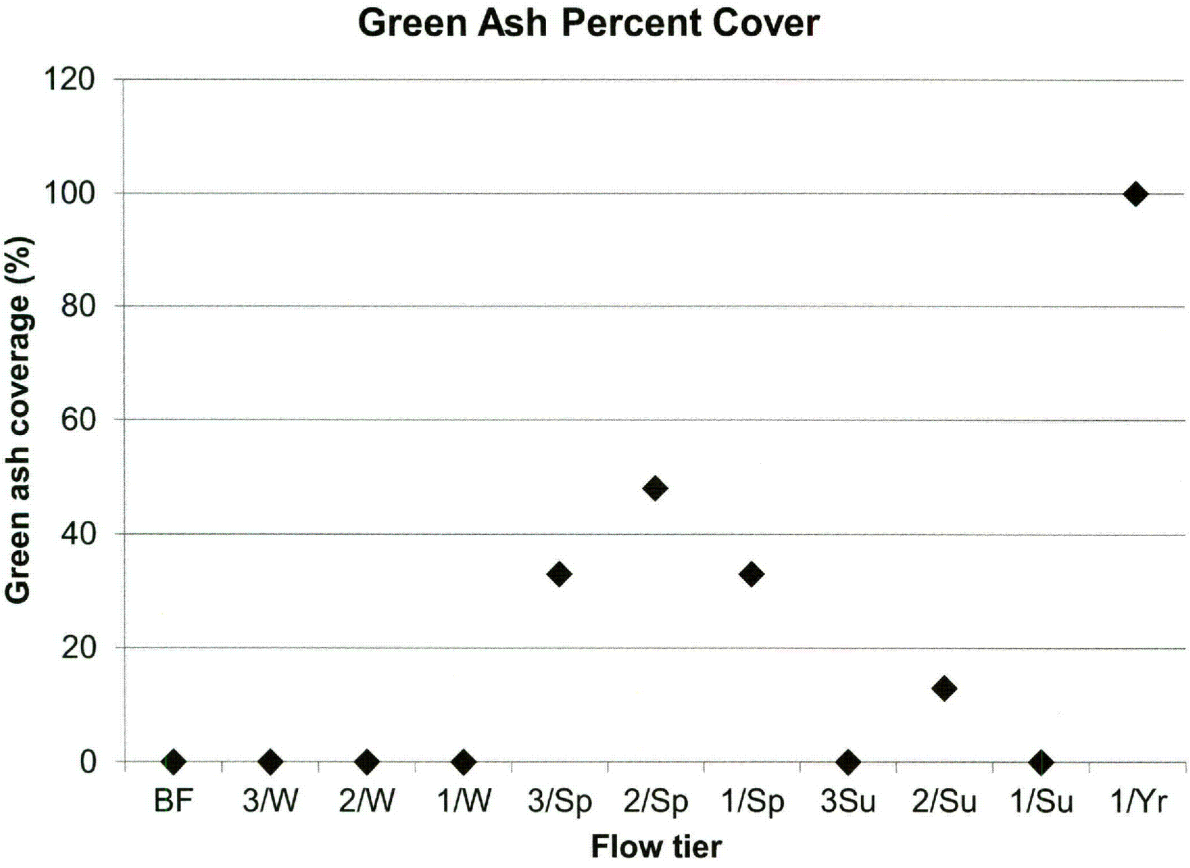


Figure 36. Percentage of mature green ash stand covered by flow tiers at the Hearne site.

Table 21. **TCEQ- and BBEST-recommended flow tiers and their occurrences throughout the BBEST-designated seasons at the Hearne site. Y indicates flow occurred; dash indicates no flow occurred.**

			2014 Spring	2014 Summer	2014 Winter	2015 Spring
Flow Tier	**CFS**	**Elevation (m)**	**Mar. - Jun.**	**Jul. - Oct.**	**Nov. - Feb.**	**Mar. - Jun.**
Baseflow	900	2.6	Y	Y	Y	Y
3/Winter	3230	3.1			-	
2/Winter	5570	3.3			-	
1/Winter	3230	3.1			Y	
3/Spring	6050	4	-			Y
2/Spring	10400	4.3	-			Y
1/Spring	6050	4	-			-
3/Summer	2060	2.5		-		
2/Summer	2990	3.6		-		
1/Summer	2060	2.5		-		
1/Year	49400	7	-	-	-	Y

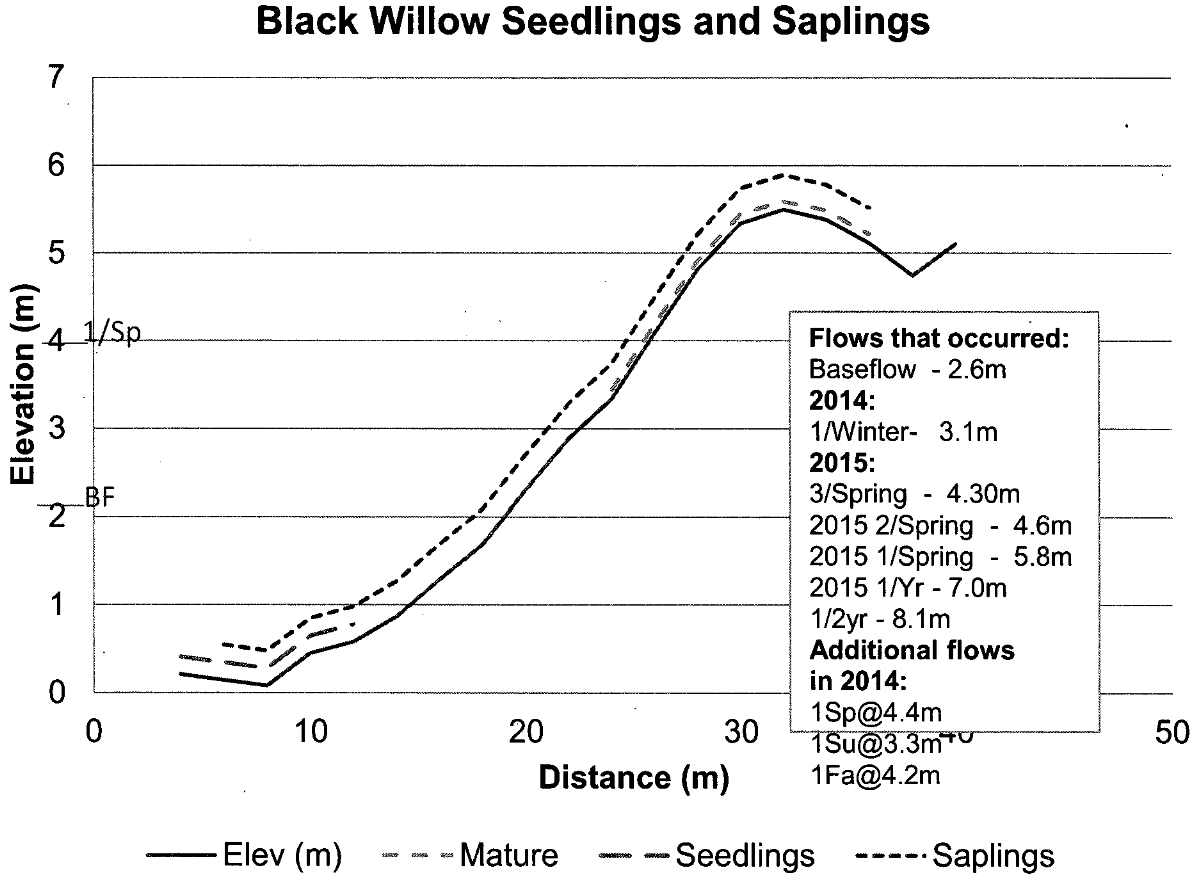


Figure 37. Black willow distributions at the Hearne site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred are shown in the inset box.

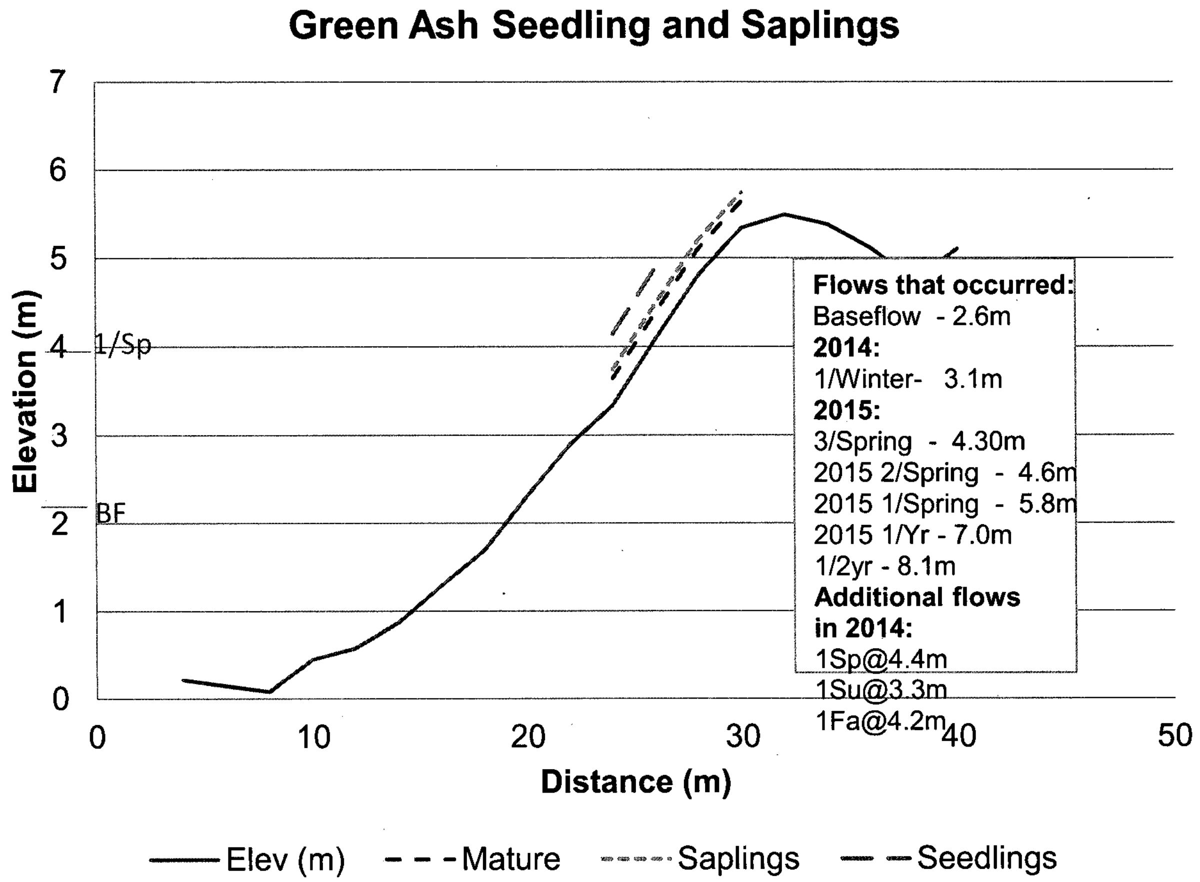


Figure 38. **Green ash distributions at the Hearne site. Inset box indicates which flow tiers actually occurred during the study.**

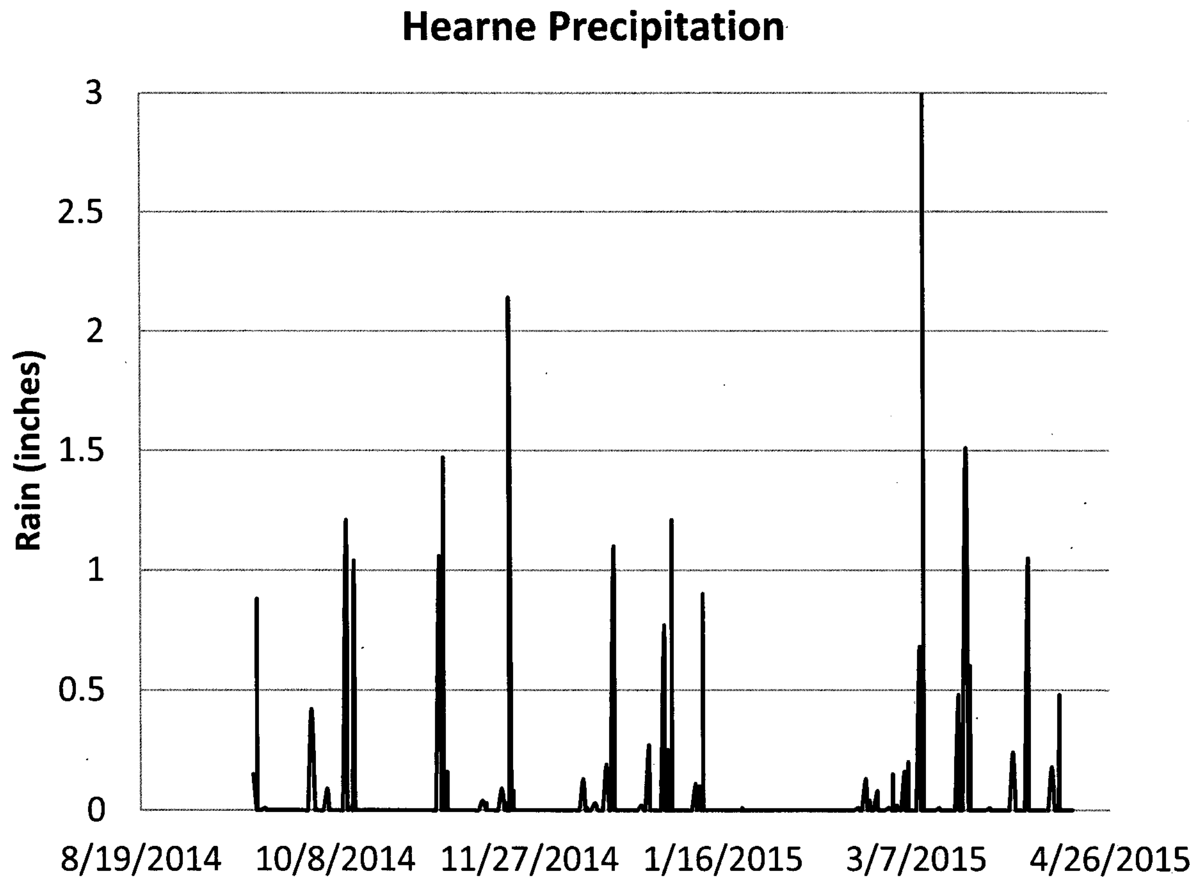


Figure 39. **Hearne local rainfall data in inches.**

Table 22. **Tree counts through time grouped by class at the Hearne site.**

Species	Class	Summer 2014	Fall 2014	Spring 2015
Black Willow	Mature	3	2	1
Black Willow	Sapling	8	12	0
Black Willow	Seedling	198	56	100
Box Elder	Mature	2	2	2
Box Elder	Sapling	1	0	1
Green Ash	Mature	3	3	3
Green Ash	Sapling	0	9	1
Green Ash	Seedling	10	1	1

Table 23. **Relative abundances of woody species at the Hearne site, grouped by tree type and age class.**

Tree Species	Class	Relative abundance (%)
American Elm	Sapling	0.4
American Elm	Seedling	0.8
Black Willow	Mature	1.1
Black Willow	Sapling	3.1
Black Willow	Seedling	75.6
Box Elder	Mature	0.8
Box Elder	Sapling	0.4
Cedar Elm	Seedling	0.4
Cottonwood	Sapling	0.4
Cottonwood	Seedling	5.0
Dogwood	Sapling	0.4
Elm	Seedling	1.1
Green Ash	Mature	1.1
Green Ash	Seedling	3.8
Hackberry	Mature	1.1
Hackberry	Sapling	0.4
Hackberry	Seedling	4.2
		100

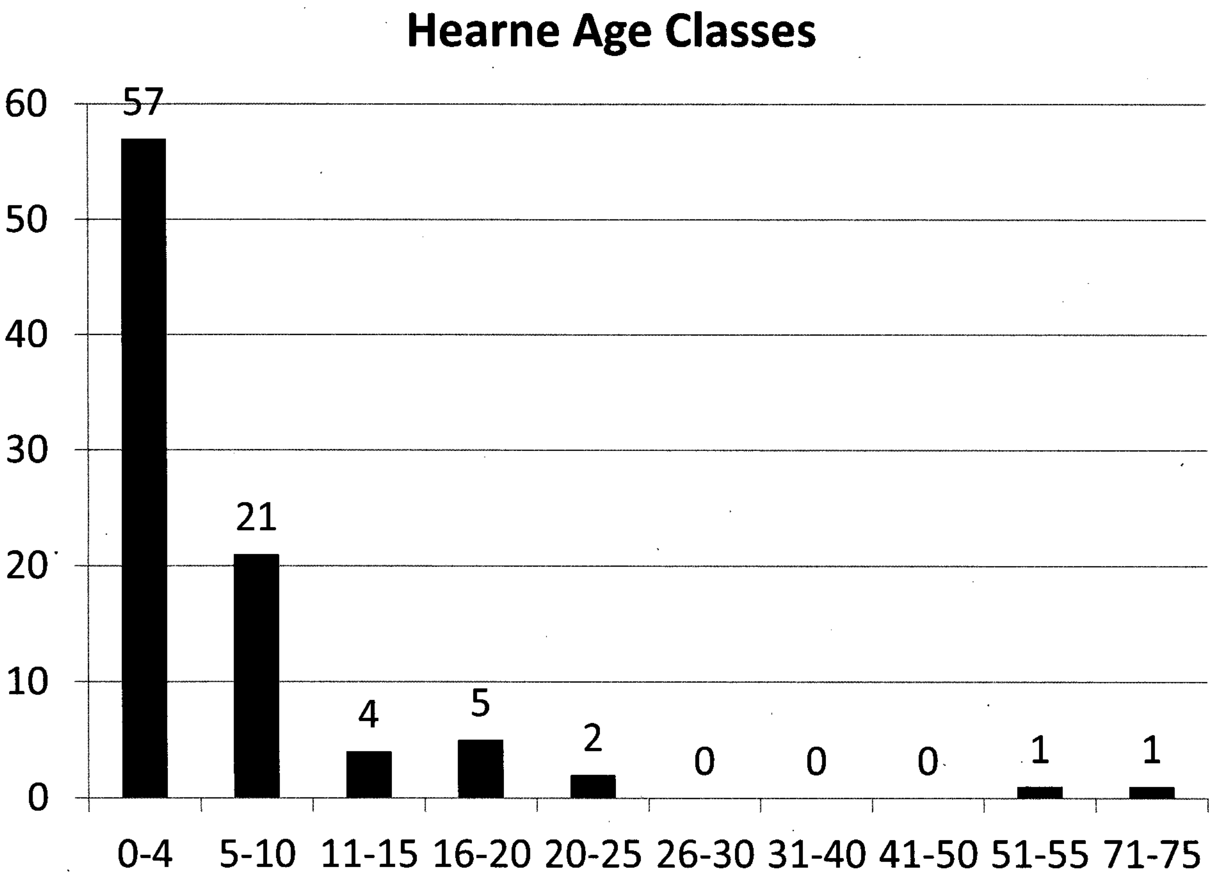


Figure 40. **Hearne trees grouped by age class.**

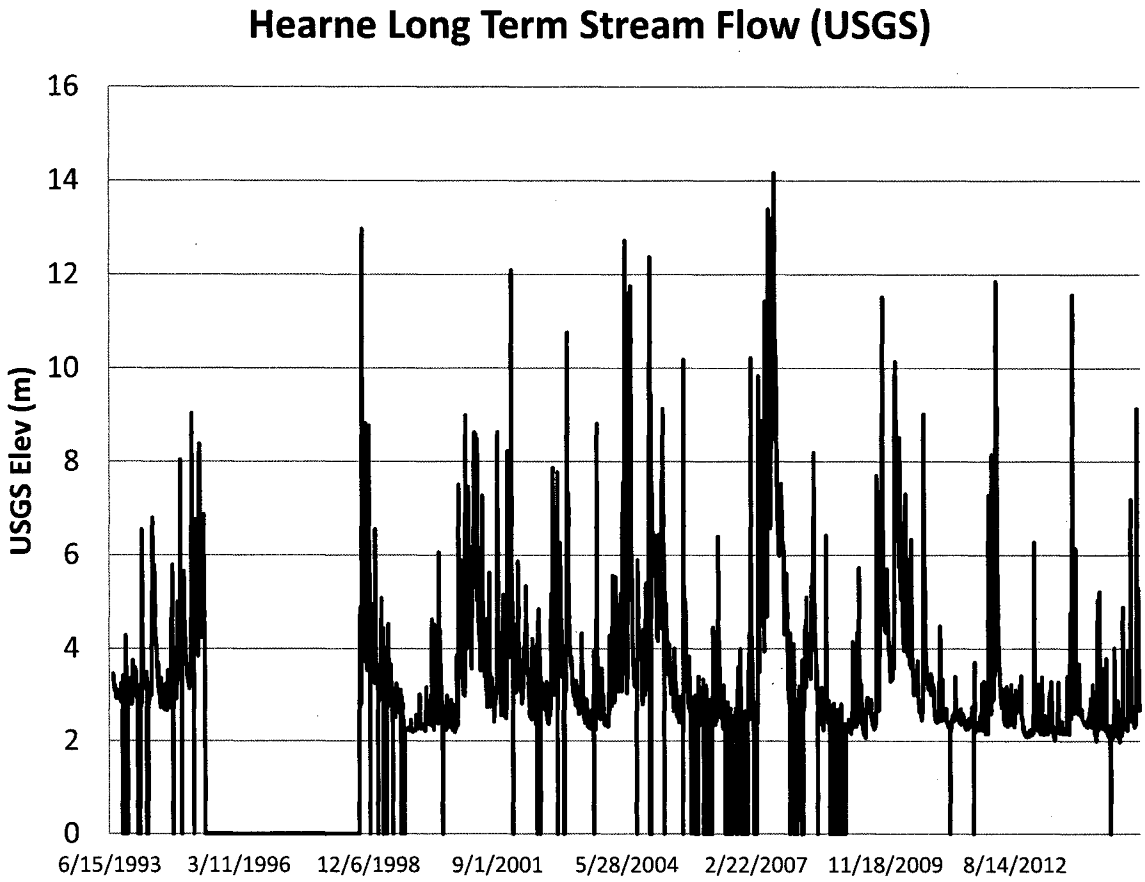


Figure 41. Long term stream flow for the USGS gauge near Bryan, adjusted for site elevation inundation.

Leon site

The Leon site (corresponding to the Leon River USGS gage near Gatesville) represented an upper tributary to the Brazos River site. This smaller river feeds into Belton Lake, and occasionally experiences backwater flooding from the reservoir during extremely high flooding. The study site was located on US Army Corps of Engineer property (the Horseshoe Bend Wildlife Management Area). The upland landscape is agricultural with lots of natural areas still intact. Though steep at water's edge, the floodplain is fairly horizontal just beyond the river channel.

The overall slope is 0.14 (rise/run), though the floodplain slope is 0.06 (Figure 42). Box elders occupy the edge of the river bank from 3.5 to 4.5 m, green ash are distributed from bank edge (3.5m elevation) to 35m distance (5m elevations); there were no black willows at this site. No TCEQ flow tiers inundate the existing range for any of the indicator species (Figure 42, Figure 43, and Figure 44). Table 24 shows the flow tiers (by season) and which ones occurred during the study period. TCEQ base flows were seen for most seasons, except winter. In 2014 all spring flows occurred, but no summer or winter flows did. With the heavy spring rains, all recommended flows occurred in spring 2015.

This site shows a very unique, distinct pattern not observed at other sites. The 3m downcut along the stream reach, coupled with low recommended flows in comparison, should result in a disconnection between the riparian zone and streamflow. However, box elder and green ash seedling and sapling distributions, (Figure 45 and Figure 46, respectively), indicate there are riparian species present. Evidence that the drought is impacting the site is that there was no box elder seed dispersal, despite the lack of mature trees. Even if the spring flows *had* resulted in seed dispersal they would only have dispersed about halfway up the downcut, shear side slope – and this area is too steep for them to have settled and germinated. Sampling of the saplings indicated they all range in the 5-7 yr. range. Conversations with park rangers revealed that flooding during that time frame resulted in the backup of Belton lake headwaters into the site for several weeks, which may be responsible for the stand of saplings (both box elder and green ash) of this age. Additionally, the saplings all cover the same spatial distribution as their mature counterparts.

Seed dispersal for green ash does not correlate with actual spring flows (Figure 46). These seeds may have been dispersed the previous fall and sprouted during 2014. A check into fall/winter 2013 flows showed there were a series of flows that did reach the upper banks of the stream and overbanked in the site; likely these seedlings are from that fall dispersal.
Unfortunately, there is no precipitation data for the Leon River site. Shortly after installation the rain gage stand was vandalized and the gage itself stolen. Whether there was sufficient rainfall to keep the seedlings in moist soil, or whether there is some other subsoil water availability (e.g., perched water table or seepage from headwater backups) cannot at this time be determined.

Through time the existing box elder saplings and mature trees appeared unaffected by the lack of flows, as each class held all members through spring 2015 (Table 25). One green ash mature tree perished during the study. From summer to fall, 13 green ash seedlings recruited to saplings while 14 at the upper elevations perished. From fall to the next spring another 6 recruited to saplings while many more perished (36). Interestingly, no new green ash seed dispersal was seen in spring 2015. As mentioned before, there was no box elder seed dispersal at the site.

Collectively box elders are less than 1% of the forest and green ash are 44% (Table 26). They were also the hardest hit in this past year's drought. What skewed the community abundances was the prolific cedar elm seed dispersal into the site. Obviously, there is potential encroachment into the site of upland species if periodic flows do not resume in the site.

An examination of the Leon River riparian community tree age classes gain is evidence of the negative impacts of the recent drought covering the past few years as seedling counts are considerably less than saplings (and went through further attrition over the next two seasons) (Figure 47). Beyond saplings, the presence of older trees drops to less than 5 for each age class. When considering Figure 48, one year of study cannot answer why this is. It could be a normal age structure for these riparian trees (disturbance removes older trees on a regular cycle here); or it could be a past stressor. Subsequent studies can and definitely should explore this. One method would be to increase specific sampling of mature trees to increase those class sizes.

TCEQ flow standards do not appear to be adequate to maintain the existing mature tree distribution for the Leon site. None of the flows provide any coverage to the riparian species present, and even the flows that did occur did not reach the riparian zone. However, the presence of riparian species indicates there must be some provision of requirements necessary to those species. Whether it was the previous year's flow coupled with saplings that have root depths accessing some below-ground source (maybe a perched water table), the mature and sapling classes are persisting. There was no box elder seed dispersal, but green ash did disperse seeds. While some of those went on to be recruited to the next class, all others perished and the following spring saw no seed germination of any species. This unique site seems to have other factors confounding its relation to stream flow and does not appear to be a good predictor site for the methodologies proposed here. Additionally, low relative abundance of riparian species in the zone (45% relative abundance) indicates that the Leon River streamside forest is at risk of being encroached upon by other species.

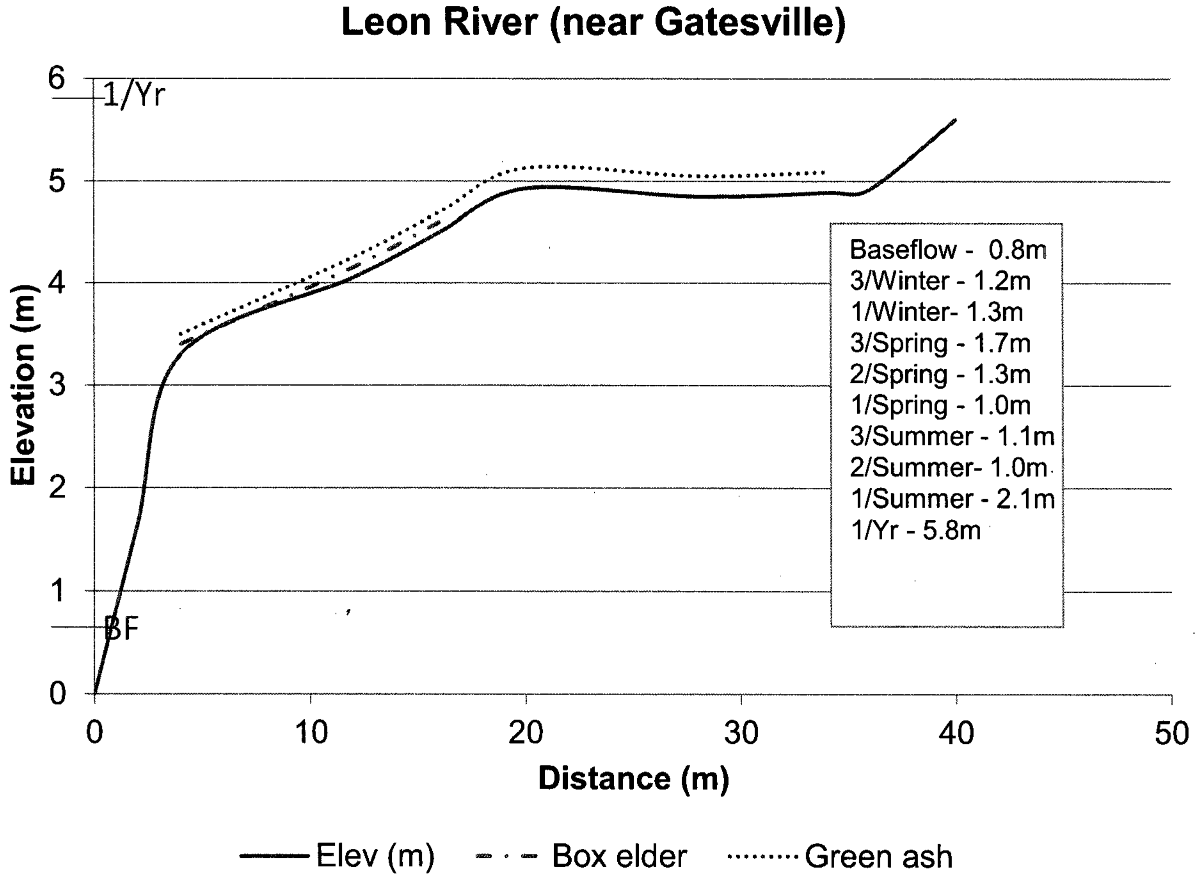


Figure 42. Leon site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows vertical inundation of flow tiers. Select flows are shown on the y-axis.

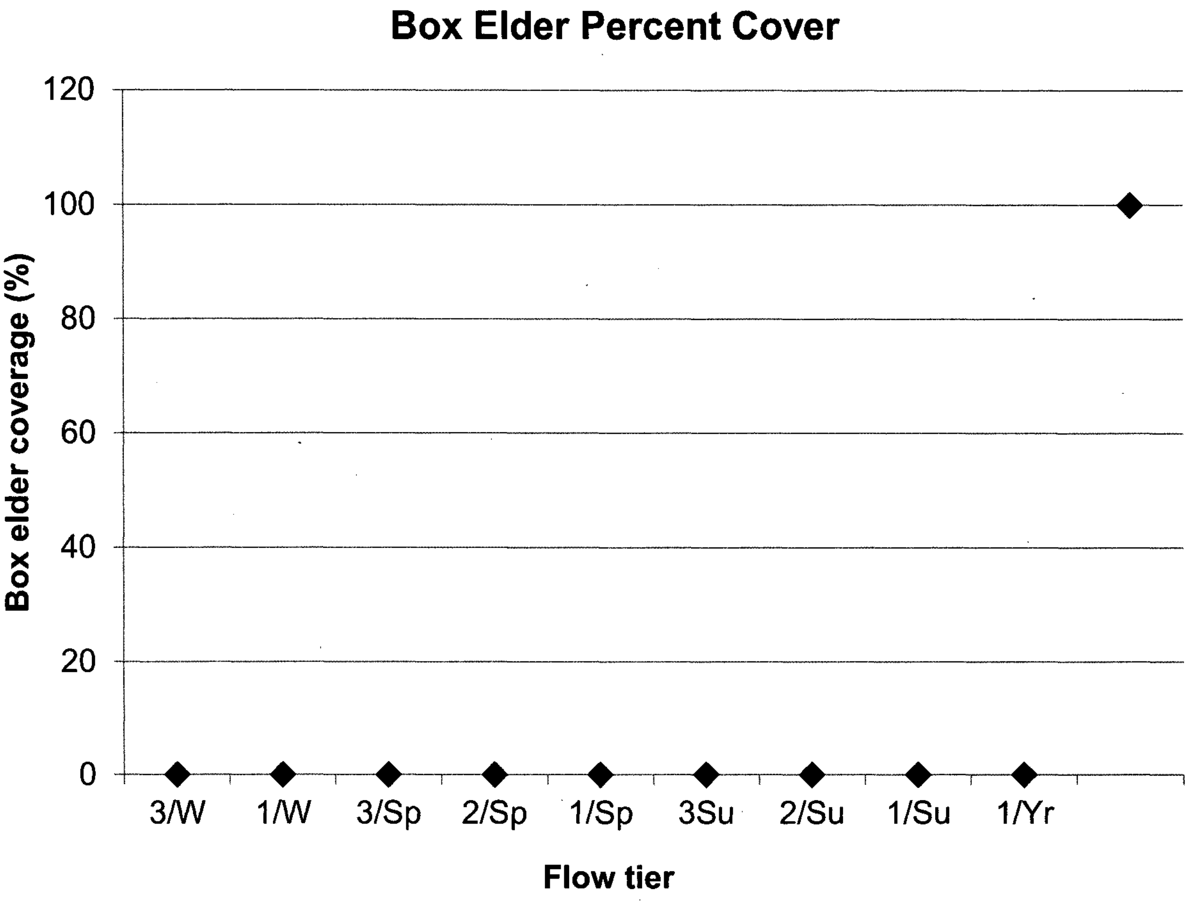


Figure 43. **Percentage of mature box elder stand covered by flow tiers at the Leon site.**

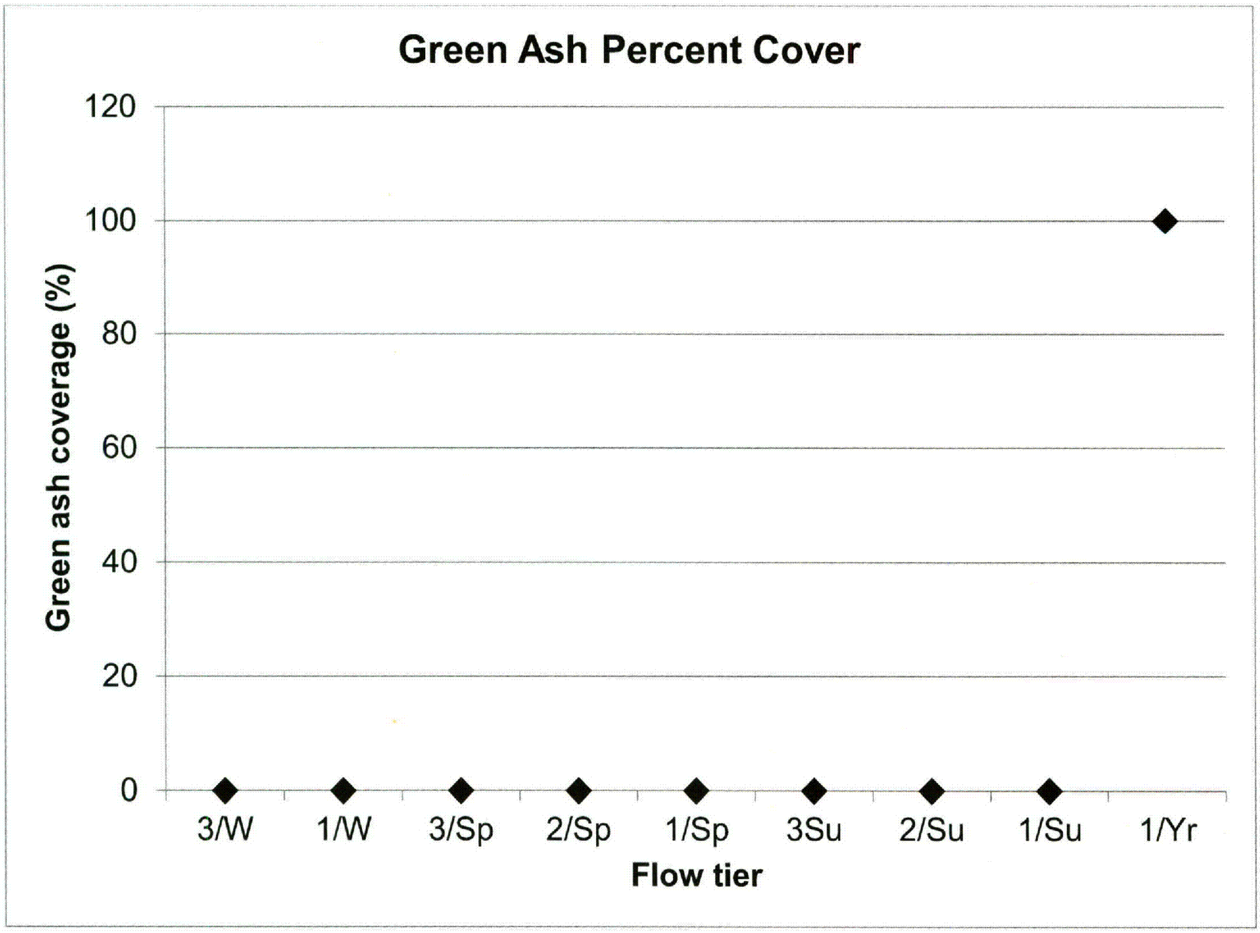


Figure 44. Percentage of mature green ash stand covered by flow tiers at the Leon site.

Table 24. **TCEQ- and BBEST-recommended flow tiers low tiers and their occurrences throughout the BBEST-designated seasons at the Leon site. Y indicates flow occurred; dash indicates no flow occurred.**

			2014 Spring	2014 Summer	2014 Winter	2015 Spring
Flow Tier	**CFS**	**Elevation (m)**	**Mar. - Jun.**	**Jul. - Oct.**	**Nov. - Feb.**	**Mar. - Jun.**
Baseflow	20	0.8	Y	Y	-	Y
3/Winter	100	1.2			-	
1/Winter	100	1.2			-	
3/Spring	340	1.3	Y			Y
2/Spring	630	1.7	Y			Y
1/Spring	340	1.3	Y			Y
3/Summer	58	1		-		
2/Summer	140	1.1		-		
1/Summer	58	1		-		
1/Year	5300	5.8	-	-	-	Y

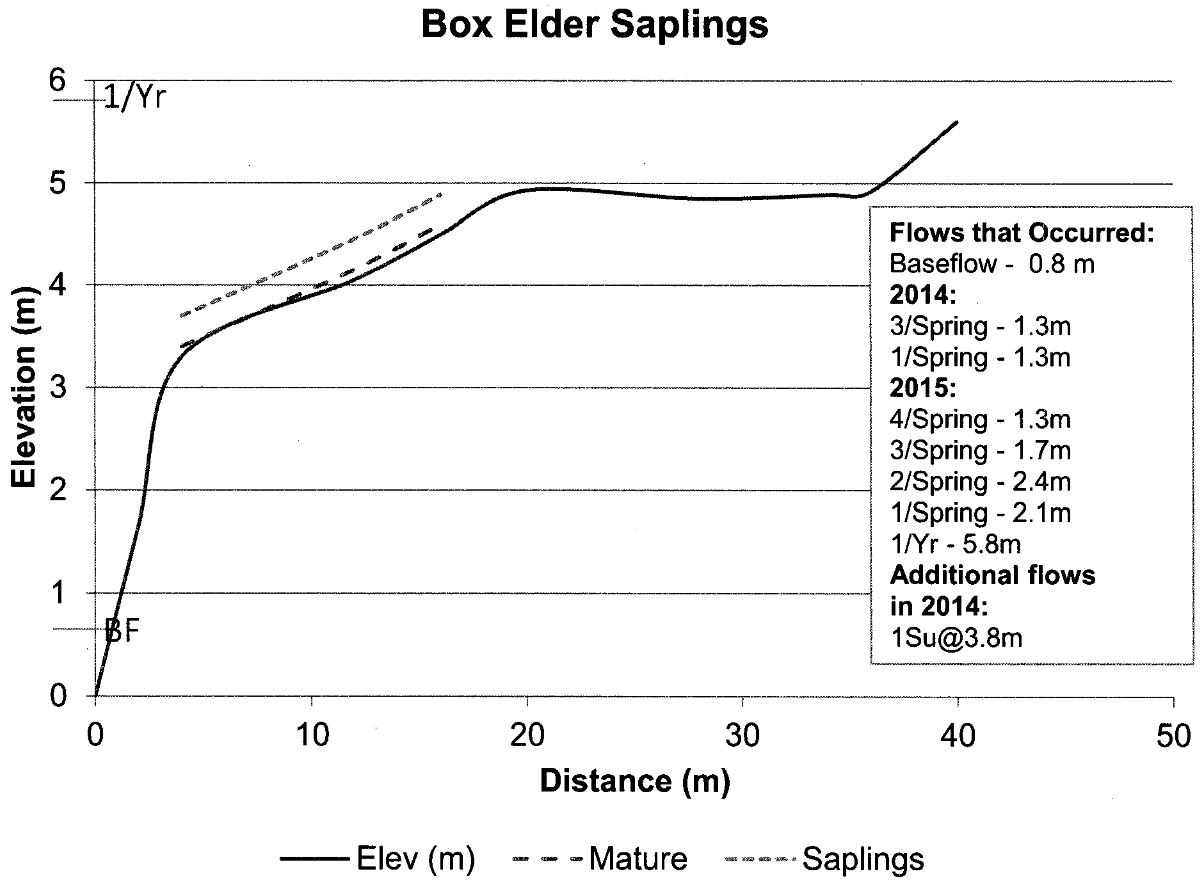


Figure 45. **Box Elder distributions at the Leon site. Inset box indicates both which flows actually occurred during the study as well as additional flows.**

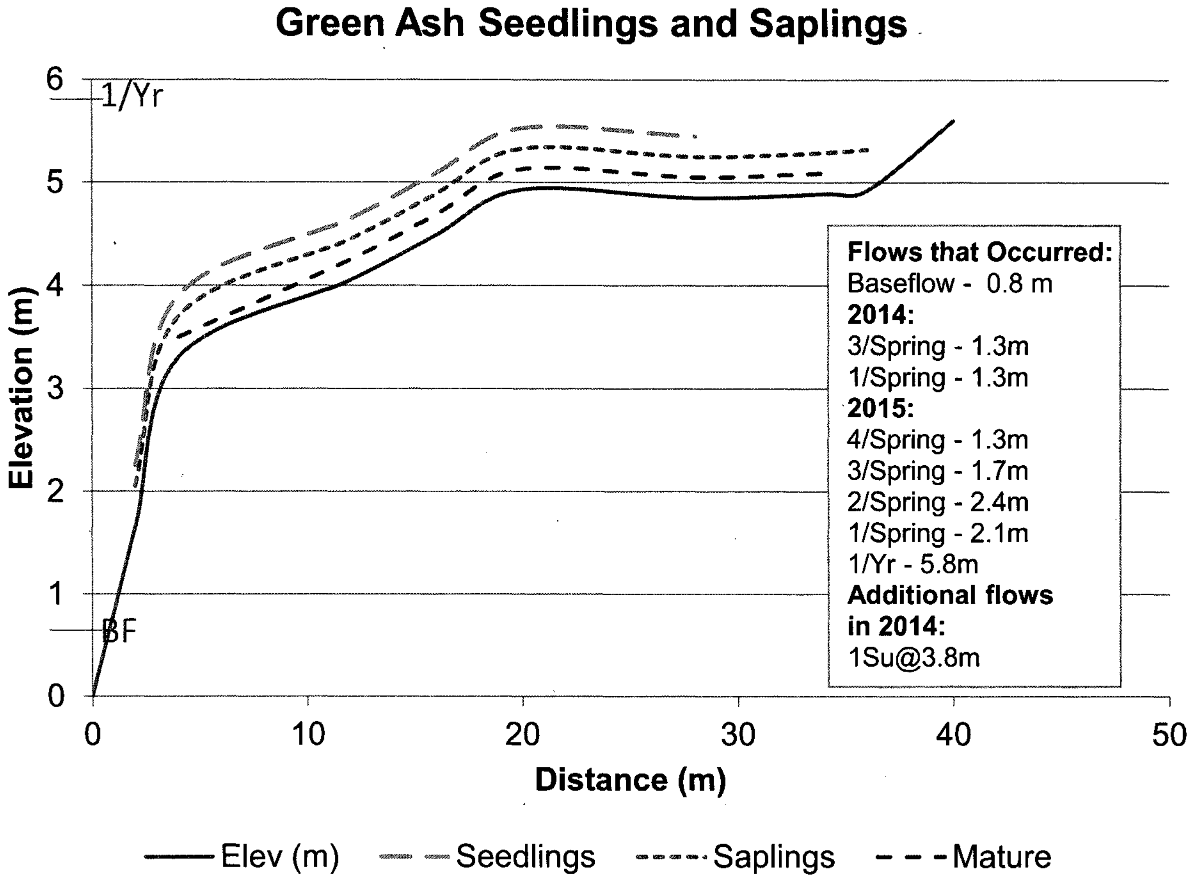


Figure 46. **Green ash distributions at the Leon site. Inset box indicates which flow tiers actually occurred during the study.**

Table 25. **Tree counts through time grouped by class at the Leon site.**

Species	Class	Summer 2014	Fall 2014	Spring 2015
Box Elder	Mature	2	2	2
Box Elder	Sapling	4	2	4
Green Ash	Mature	15	15	14
Green Ash	Seedling	69	42	0
Green Ash	Sapling	194	207	213

Table 26. **Relative abundances of woody species at the Leon site, grouped by tree type and age class.**

Tree Species	Class	Relative abundance (%)
American Elm	Seedling	0.3
Box Elder	Mature	0.3
Box Elder	Sapling	0.6
Cedar Elm	Mature	0.6
Cedar Elm	Seedling	50.3
Cedar Elm	Sapling	1.3
Desert Willow	Seedling	0.2
Desert Willow	Sapling	1.9
Desert Willow	Mature	0.3
Green Ash	Mature	2.4
Green Ash	Seedling	10.9
Green Ash	Sapling	30.7
Pecan	Mature	0.2
		100

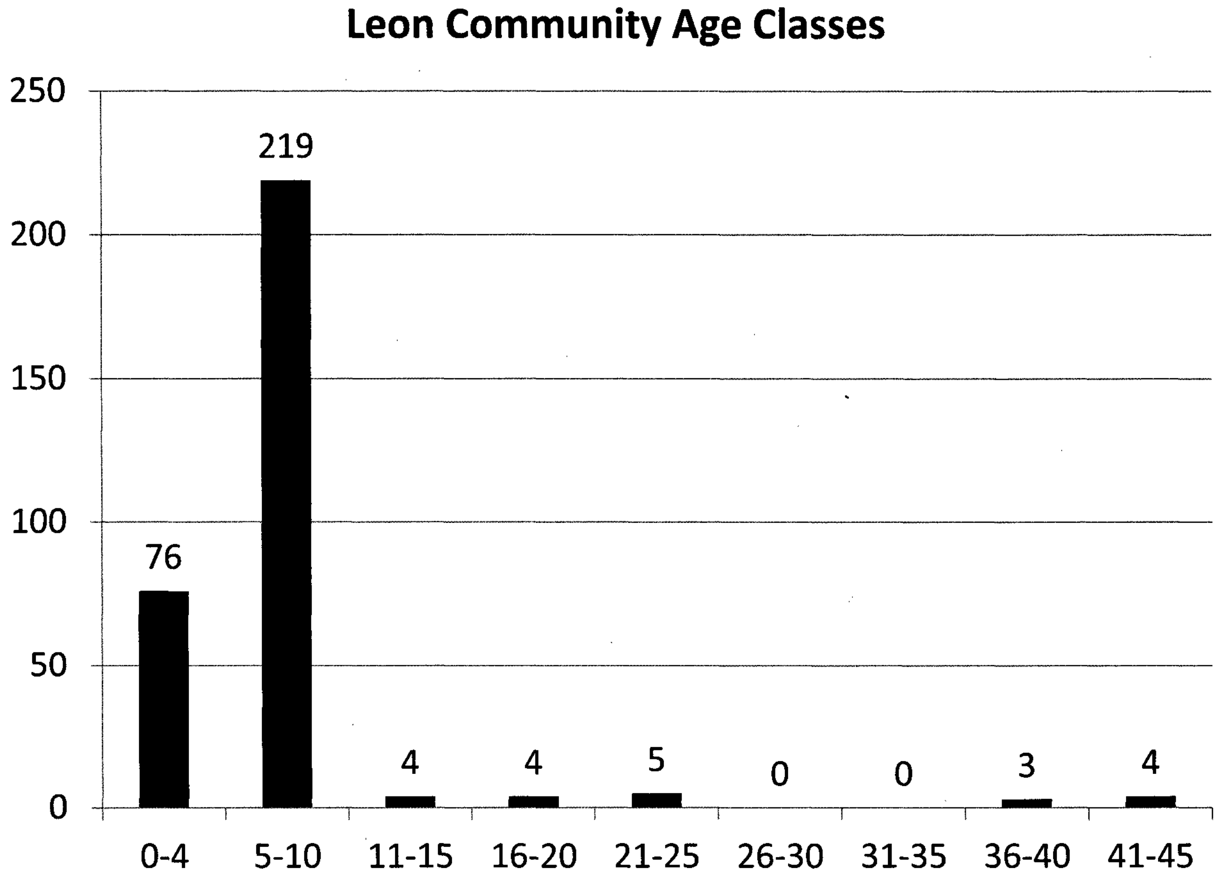


Figure 47. Leon trees grouped by age class. Columns represent the number of trees in each class.

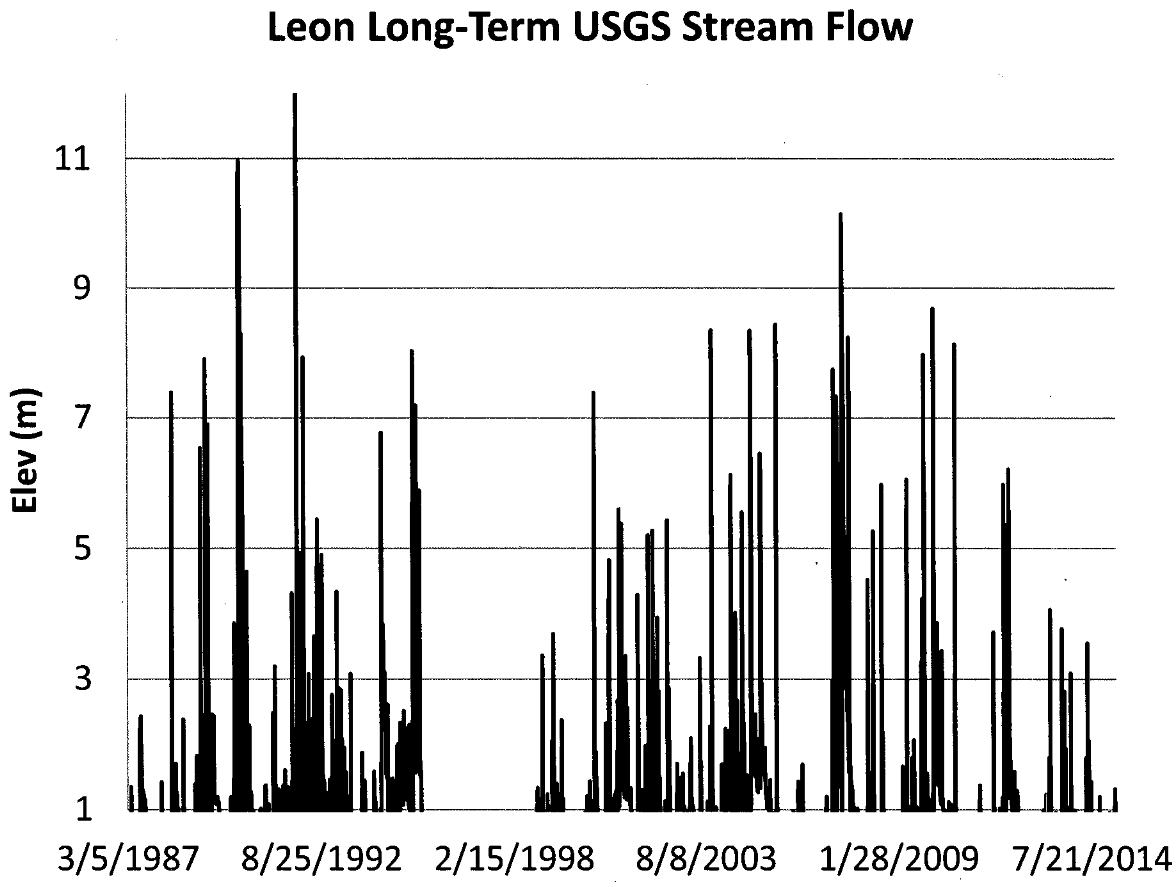


Figure 48. **Long-term stream flow for the USGS gauge near Gatesville, adjusted for site elevation inundation.**

Little River site

The Little River site (corresponding to the Little River USGS gage near Cameron) represented a mid-reach tributary to the Brazos River. The site was located on private property (upland landscape is mostly agricultural and fields are adjacent to the site) just downstream from a bend in the river. Between the riparian zone and the fields is a dense stand of chinaberry trees. Fortunately, they have not invaded into the lower reaches of the riparian zone to-date. The slope from river's edge to the top of a natural levee is 0.31 (rise/run), behind which is a low-lying floodplain (Figure 49). Box elders occupy from the lowest tiers of the slope (1.8m elevation) to the top of the natural levee (6.1m elevation), green ash and black willow distributions begin at a mid-slope bench about 4m above the water's edge, and green ash extend to 10m distance and 6m elevation. Black willows are constrained to this bench, but green ash are distributed across the low-lying floodplain. Not all TCEQ flow tiers are adequate for meeting the needs of all indicator species. All spring and one winter flow cover at least some portion of species, but summer flows do not reach any of the riparian species. Only the BBEST-recommended 1/year flow provides inundation to all three indicator species.

Because of the black willow distribution only at the top of the bench none of the TCEQ flows inundate their spatial distribution (Figure 50). Box elders, though partially inundated, have no flows beyond 40% of their distribution. (Figure 51) None of the TCEQ flows inundate any of the green ash distributions (Figure 52).

TCEQ base flows were seen for all seasons. In 2014 no spring flows occurred. The 1/summer as well as all winter flows did occur. All spring flows occurred in spring 2015 during the heavy rains (Table 27). The effects of the drought extending through 2014 can be seen (Table 27), where most flows did not occur. This limited our ability to test seedling establishment and survival, and sapling survival to those flows. Therefore, where necessary, we moved to the second propositions of the seedling and sapling hypotheses – testing actual flows that did occur. No black willow seedlings dispersed in the study plots. Box elder seedlings appear to have possibly been dispersed where the one highest spring flow inundation into the site occurred (5.m elevation), though their range is slightly higher at 6m (Figure 53), and all seeds fell well within both the mature and sapling ranges.

As with the box elders (Figure 54) green ash seedling dispersal also correlates (though a bit higher) with the one spring flow that occurred in 2014. Sapling distribution was identical to seedling distribution, but both fell short of the mature range. While there was some overlap, there has been a definite shift from mature to replacing age class distributions toward the stream (the zone is constricting toward the stream). Though some rain fell during the fall and winter, it was probably not sufficient to account for the complete lack of flows later in 2014 (Figure 55).

This site had signs of prolonged drought stress (Table 28). From fall 2014 to spring 2015 one of the two adult willows in our transect plots were lost, and no seedling dispersal was seen for this species (and a lack of saplings indicates it hasn't been occurring for some time here). Box elder seedlings faired pretty well. Four recruited to saplings from summer to fall and another 5 germinated in spring 2015. While many box elder saplings were still surviving in fall (though with obvious signs of distress observed in the field), by spring two of them had perished, the rest rebounded. Green ash mature trees also survived through the year. Seedlings for this species showed some recruitment into saplings by fall (7 of them), but an additional 25 perished during this same time. By spring 2015 another 9 were lost as well as 43 saplings. It appears that going into late fall with drought stress decreased fitness such that the following season reflected an increased loss, regardless of winter flows (or maybe because the flows further stressed the dormant trees with too much root moisture). The lack of flows in spring and summer seem to have taken a toll on the green ash saplings more so than the box elders.

Collectively box elders are 18% of the forest, green ash are 41%, and black willows make up approximately 1% (Table 29). This riparian zone is more diverse than others sampled, but has some encroachment from hackberry and other upland species. Saplings are the most prolific in the site (Figure 56). Again, the dearth of a comparable amount of new seedlings is evidence of the negative impacts of the recent drought the past few years, and indicates replacement is low this past year. Beyond saplings, the presence of older trees drops to less than 5 for each age class which prevents the detection of previous anomalous flows from the available data. Further sampling of mature trees may provide this information.

TCEQ flow standards appear to be mostly inadequate to maintain the existing mature tree distribution at the Little River site. Even though only a few of the recommended flows actually occurred over the study, those flows that did occur (whether recommended or otherwise) appeared to have a positive influence on the box elders and green ash. Lack of flows had a detriment on survival. Age structure analysis further supports that a lack of streamflow pulses along the river have had noticeable impacts on seedling dispersal and future maintenance. The relative abundance of the riparian species in the zone indicates that the Little River streamside forest is functioning as a riparian zone (60% relative abundance), rather than an encroached zone.

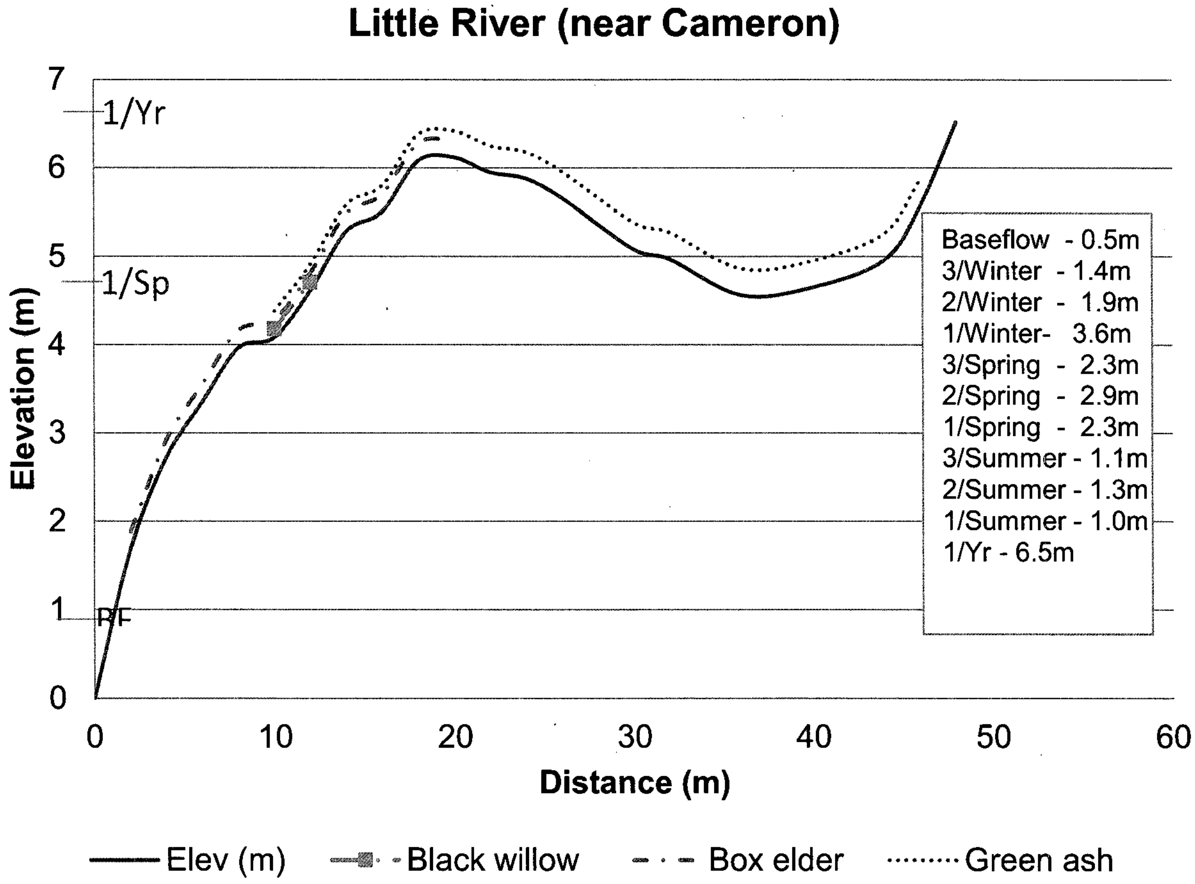


Figure 49. **Little River site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows vertical inundation of flow tiers. Select flows are shown on the y-axis.**

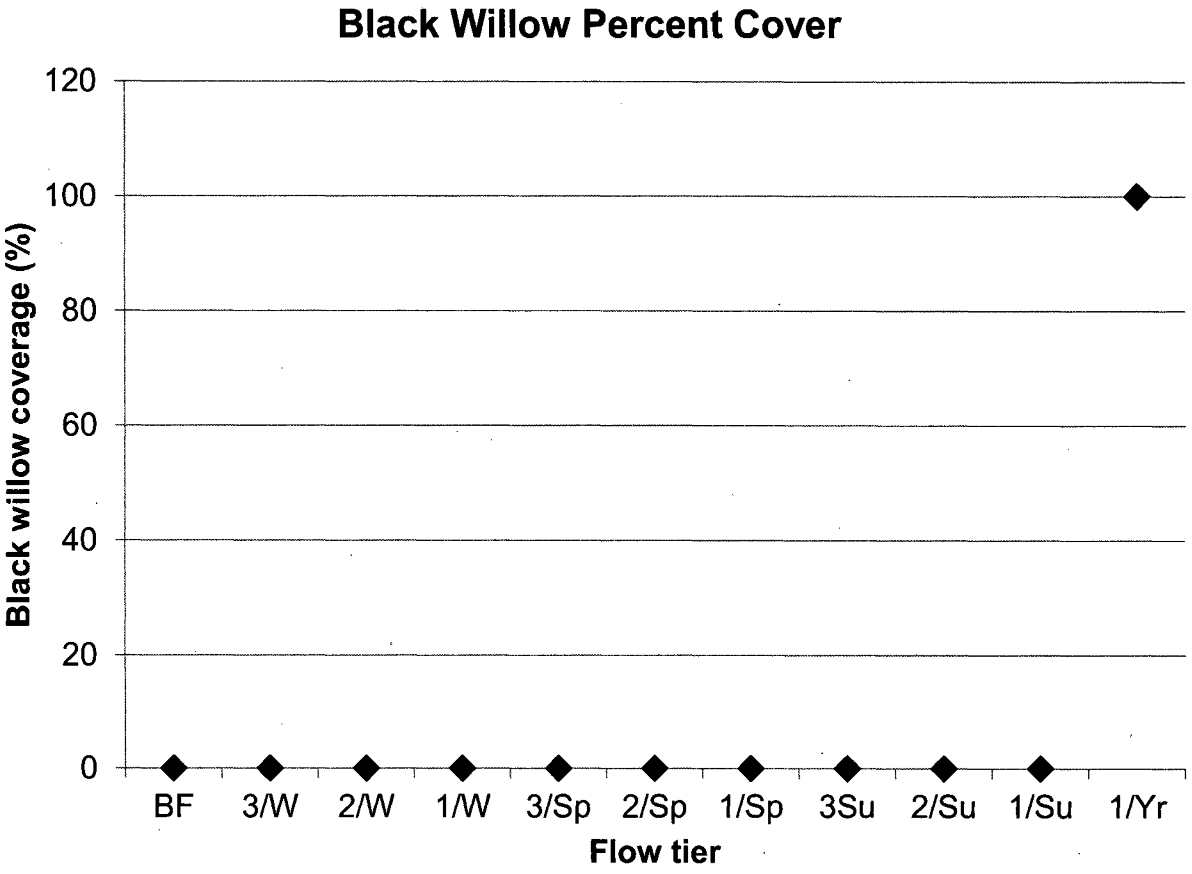


Figure 50. **Percentage of mature black willow stand covered by flow tiers at the Little River site.**

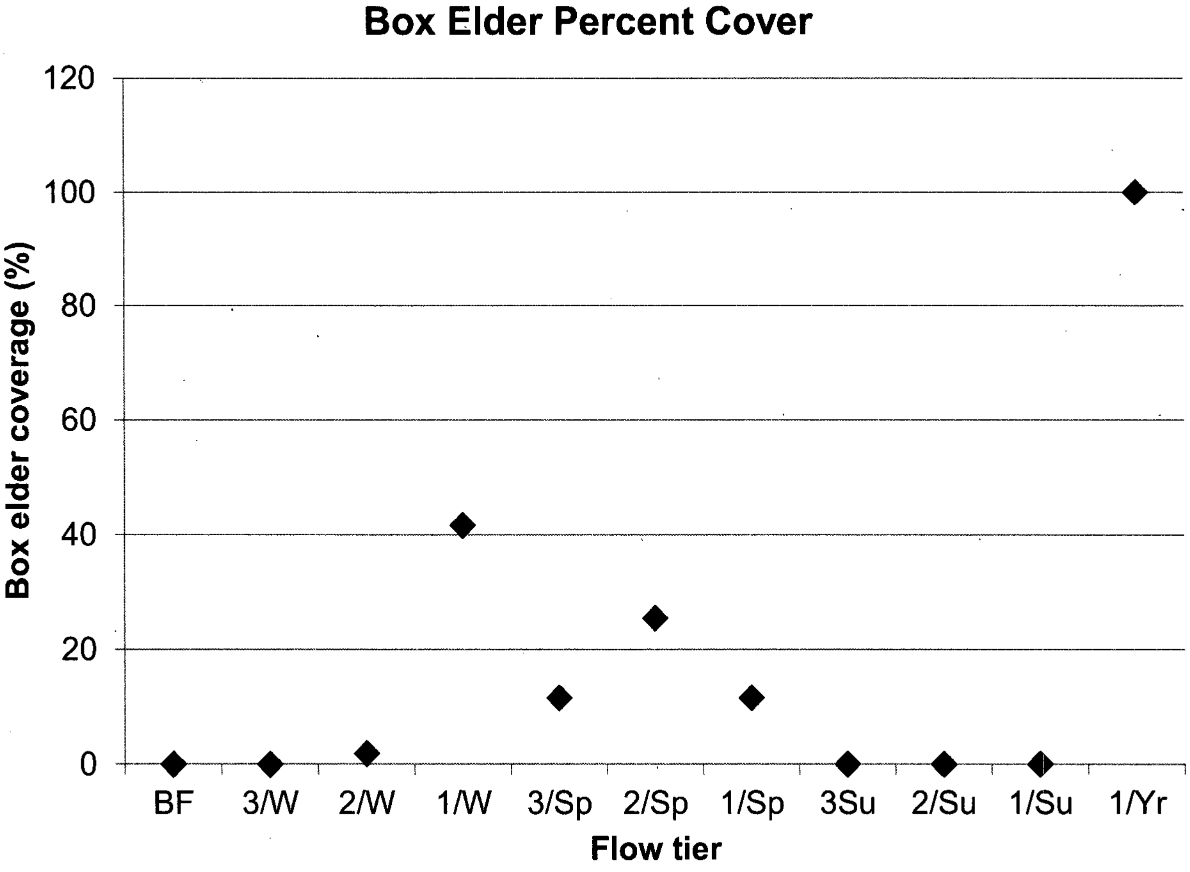


Figure 51. **Percentage of mature box elder stand covered by flow tiers at the Little River site.**

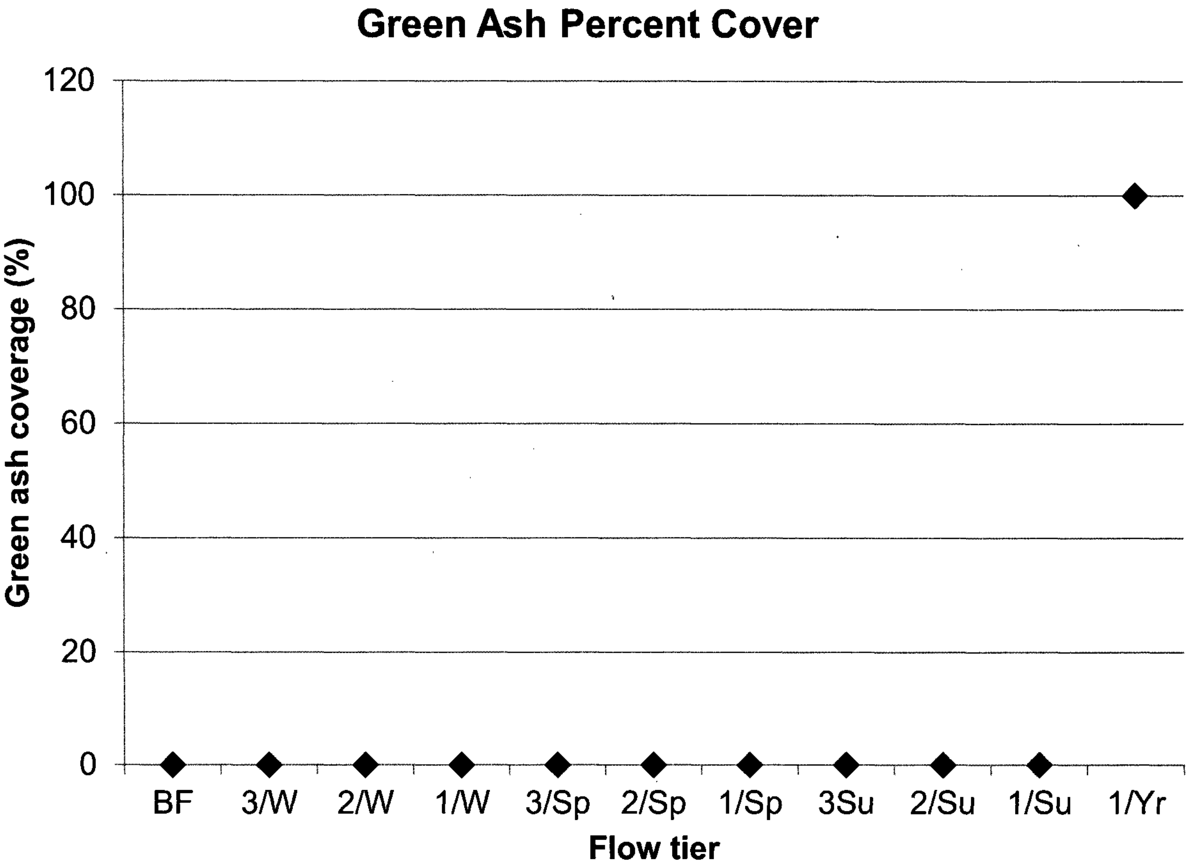


Figure 52. **Percentage of mature green ash stand covered by flow tiers at the Little River site.**

Table 27. **TCEQ- and BBEST-recommended flow tiers and their occurrences throughout the BBEST-designated seasons at the Little River site. Y indicates flow occurred; dash indicates no flow occurred.**

			2014 Spring	2014 Summer	2014 Winter	2015 Spring
Flow Tier	**CFS**	**Elevation (m)**	**Mar. - Jun.**	**Jul. - Oct.**	**Nov. - Feb.**	**Mar. - Jun.**
Baseflow	200	0.5	Y	Y	Y	Y
3/Winter	1080	1.4			Y	
2/Winter	2140	1.9			Y	
1/Winter	6680	3.6			Y	
3/Spring	3200	2.3	-			
2/Spring	4790	2.9	-			
1/Spring	3200	2.3	-			
3/Summer	560	1		-		
2/Summer	990	1.3		-		
1/Summer	560	1		Y		
1/Year	19700	6.5	-	-	-	Y

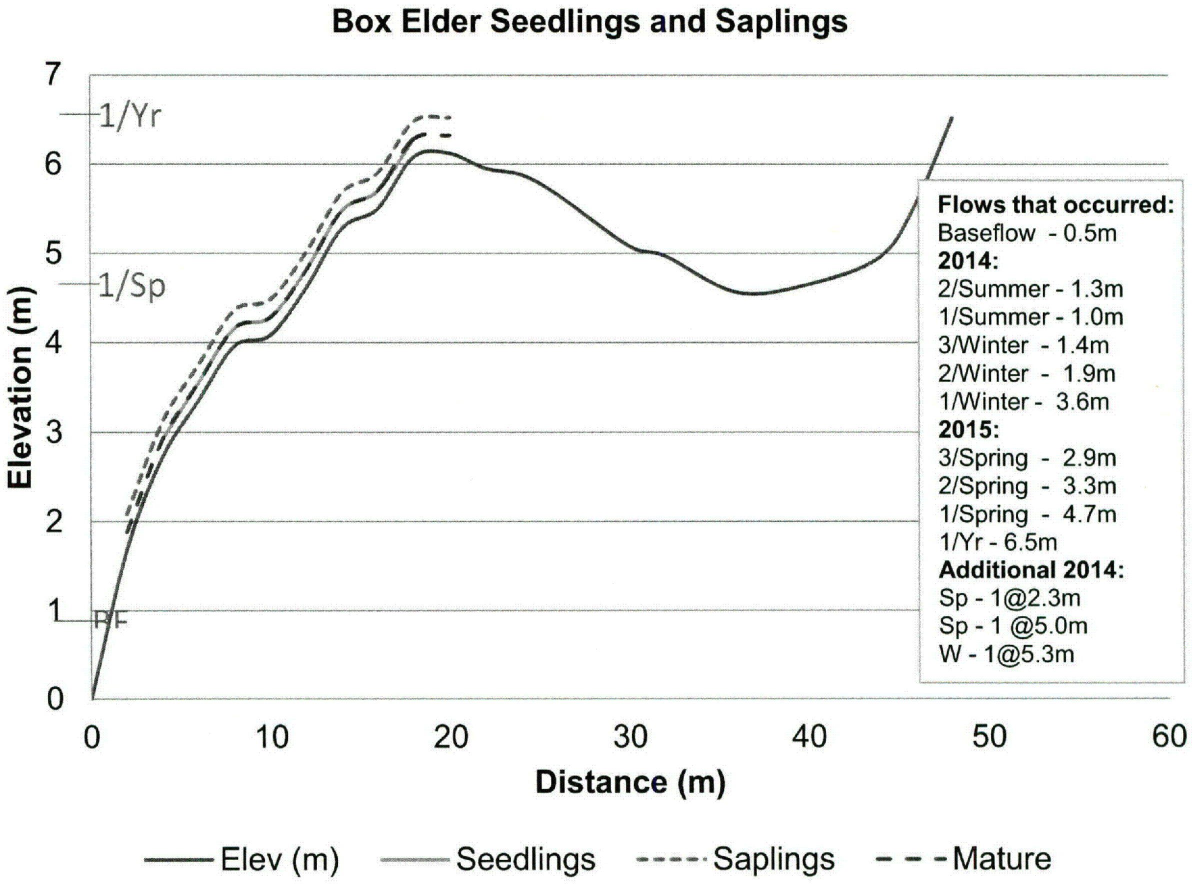


Figure 53. **Box elder distributions at the Little River site. Inset box indicates which flow tiers actually occurred during the study. Additional flows are shown in the inset box.**

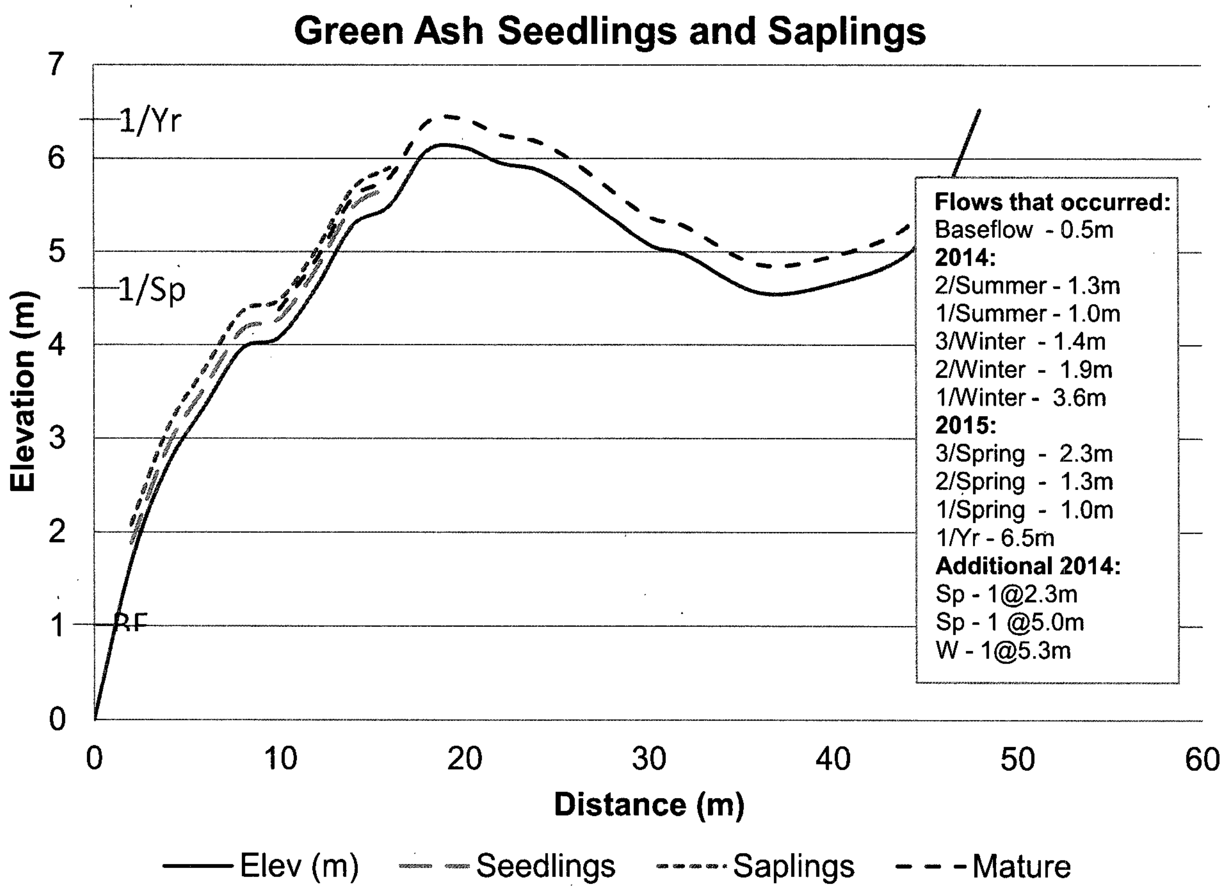


Figure 54. Green ash distributions at the Little River site. Inset box indicates which flow tiers actually occurred during the study.

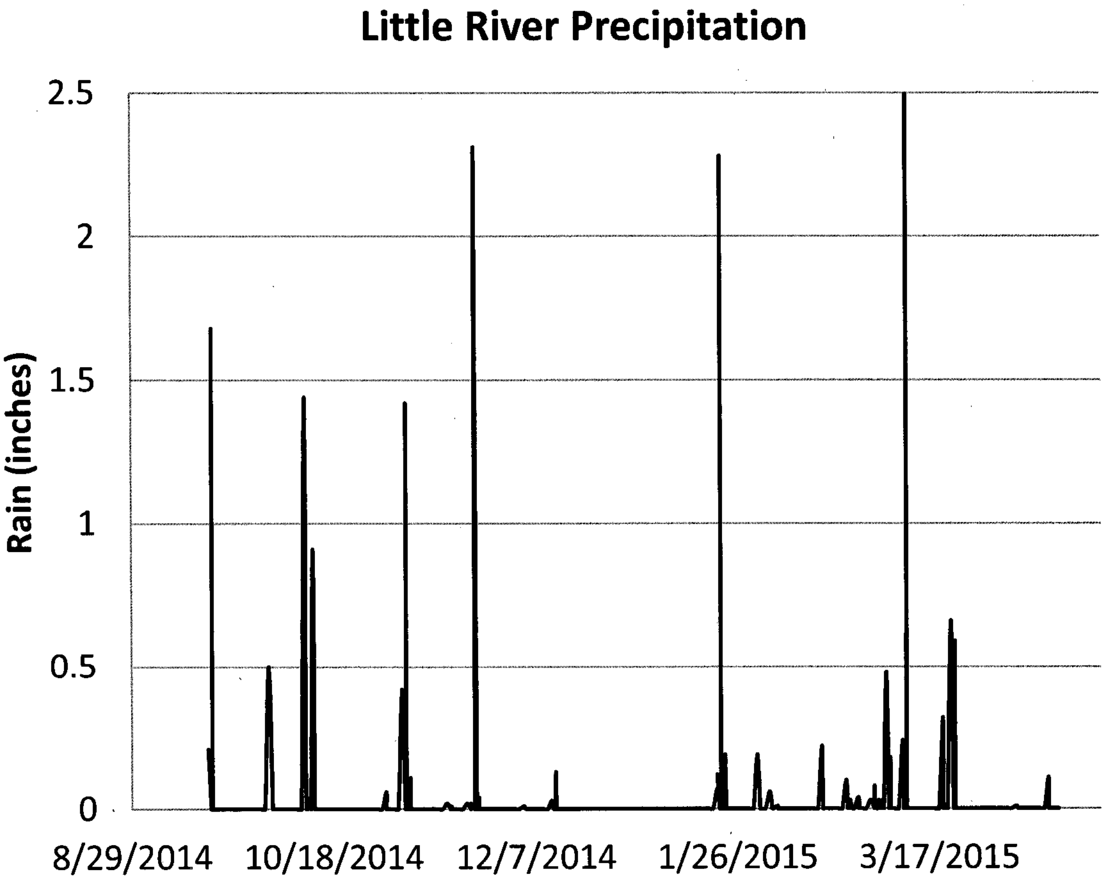


Figure 55. **Little River local rainfall data in inches.**

Table 28. **Tree counts through time grouped by class at the Little River site.**

Species	Class	Summer 2014	Fall 2014	Spring 2015
Black Willow	Mature	2	2	1
Box Elder	Sapling	48	52	50
Box Elder	Seedling	13	9	14
Green Ash	Mature	7	7	7
Green Ash	Sapling	77	84	41
Green Ash	Seedling	58	26	17

Table 29. **Relative abundances of woody species at the Little River site, grouped by tree type and age class.**

Tree Species	Class	Relative abundance (%)
American Elm	Mature	0.6
American Elm	Seedling	0.9
American Elm	Sapling	1.5
Black Willow	Mature	0.6
Box Elder	Seedling	3.8
Box Elder	Sapling	13.7
Cedar Elm	Sapling	6.1
Cedar Elm	Sapling	8.5
Cottonwood	Mature	0.3
Elm	Seedling	3.2
Elm	Sapling	4.1
Green Ash	Mature	2.0
Green Ash	Seedling	16.9
Green Ash	Sapling	22.4
Hackberry	Mature	0.3
Hackberry	Sapling	3.5
Hackberry	Seedling	11.1
Pecan	Seedling	0.3
Pecan	Mature	0.3
		100

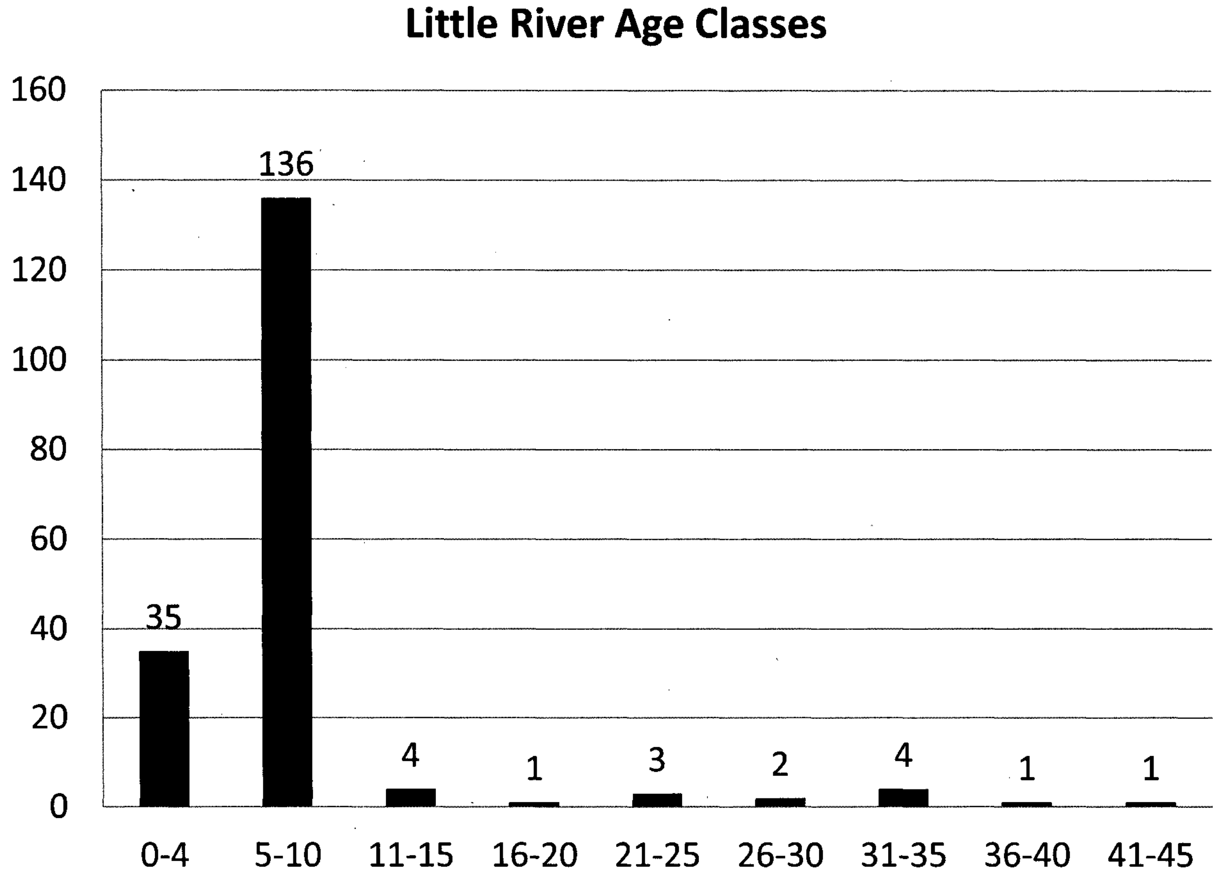


Figure 56. **Little River trees grouped by age class.**

Marlin site

The Marlin site (corresponding to the Brazos River USGS gage near Waco) represented an upper-most reach of the Brazos River. The site was located on private property (upland landscape is mostly agricultural and cattle range). The riparian forest is well developed and little-disturbed. The slope from river's edge to the top of the furthest natural levee is 0.19 (rise/run), and includes a 3.5 near-vertical rise at the stream's edge (Figure 57). None of the mature species occupy the shear channel drop to the stream. Black willows begin just after that and extend to 15m distance, roughly 3.5m above the stream. The box elder range begins shortly beyond the black willows and extends to the highest elevation, 5.6m. Green ash are limited and only one tree was found, right at 4.5m elevation. Most TCEQ flow tiers are inadequate for inundation of the indicator tree species throughout their existing range; only the 2/spring provides any coverage.

For black willows the 2/spring inundates 100% of the distribution, but no other flows provide any coverage (Figure 58) other than the BBEST-recommended 1/year flow. This same trend is seen for box elders (Figure 59) and green ash (Figure 60) except that the 2/s no longer inundates it. TCEQ base flows were seen for all seasons (Table 30). In 2014 no other flows occurred. The only flows to occur were all spring flows during the heavy spring 2015 rains. The effects of the drought extending through 2014 can clearly be seen, where most flows did not occur. This limited our ability to test seedling establishment and survival and sapling survival to those flows.

Therefore, where necessary, we moved to the second propositions of the seedling and sapling hypotheses – analyzing actual flows that did occur. An analysis of seedling dispersal showed no black willow seedlings dispersed or saplings present. No box elder seedlings were dispersed during 2014 (Figure 61), though box elder saplings fell well within the mature ranges.

One green ash seedling was found right at the top of the bank slope, along with one sapling - and both fell short of the mature range (a single tree) (Figure 62). Likely green ash will disappear from this site in the future unless seedling dispersal greatly increases.
Though some rain fell during the fall and winter, it was probably not sufficient to account for the complete lack of flows in 2014, though it may have benefited the standing box elder saplings to a small extent (Figure 63).

This site had signs of drought stress. Though the mature trees all survived the year, there was virtually no seedling dispersal (a single green ash was found in the study area), and only sparse saplings were observed (Table 31). Those saplings found were aged between 4 and 7 years, so probably had root depths sufficient to increase their resiliency to a lack of flows. These data support that either no new seedling dispersal has occurred in a number of years – or that all new individuals have perished. Collectively box elders are 20% of the forest, green ash are 8%, and black willows make up 31% (Table 32). The hackberry and American elm, both upland species, appear to be taking advantage of the lack of flows. Saplings are the most prolific age class in the site, though their numbers are much less than expected for a healthy, maintaining riparian zone (Figure 64). Again, the dearth of new seedlings is evidence of the negative impacts of the recent drought the past few years, and indicates replacement is low this past year and for several previous years.

Though some of the trees are very long lived, beyond saplings the presence of older trees drops to less than 5 for each age class, which prevents the detection of previous anomalous flows from the available data. Further sampling of mature trees may provide this information. The longevity implies that trees may develop to a certain maturity such that they become more impervious to flow reductions on a localized temporal scale.

This site plainly showed signs of prolonged drought stress. Though the mature trees all survived the year, there was virtually no seedling dispersal (a single green ash was the sole seedling found), and only sparse saplings were observed. Age structure analysis further supports that a lack of streamflow pulses along the river have had noticeable impacts on seedling dispersal and future maintenance. With continued low-flow conditions this site appears in danger of a lack of replacement of woody riparian vegetation, especially given that riparian species only represent only 59% of the current diversity.

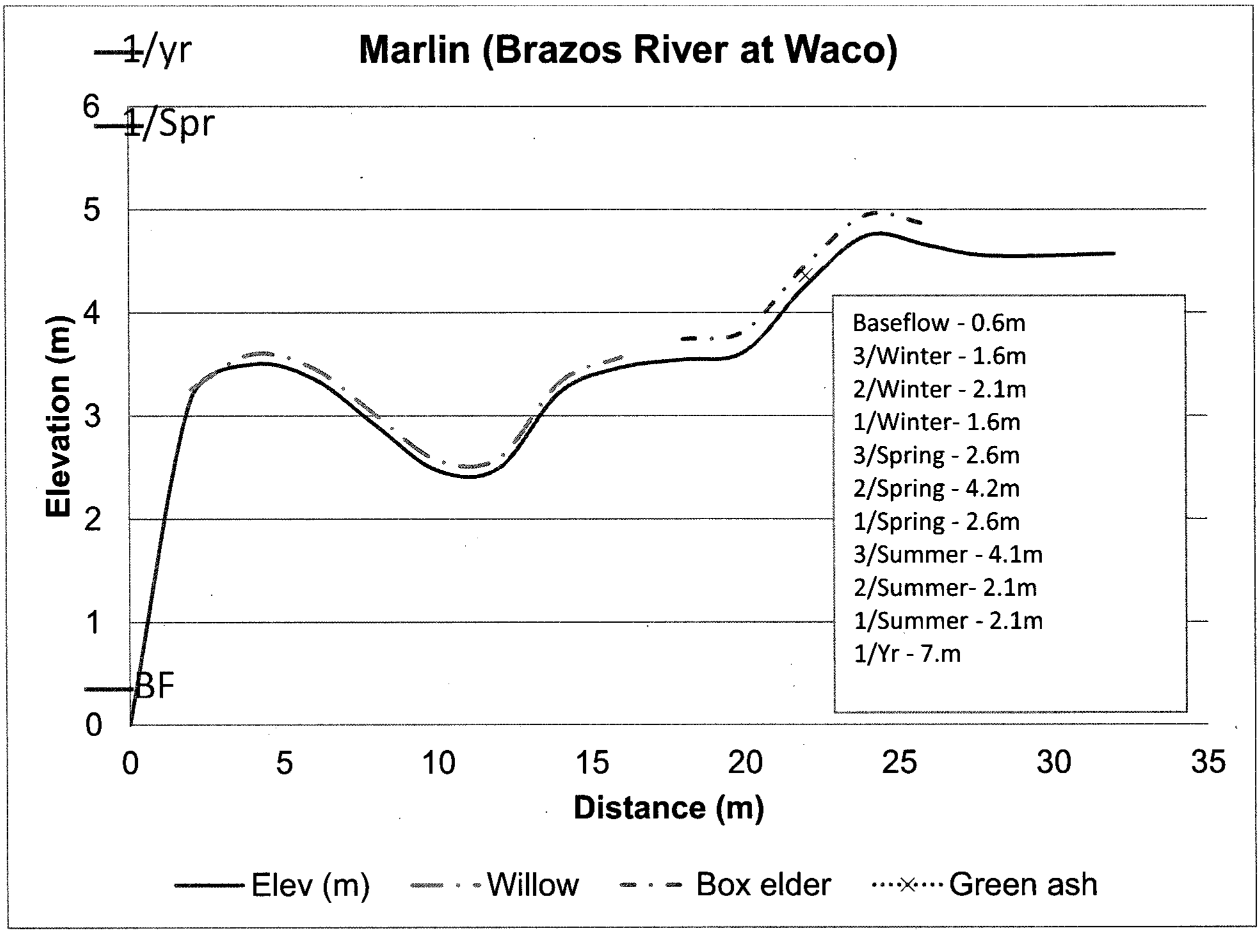


Figure 57. **Marlin site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows vertical inundation of flow tiers. Select flows are shown on the y-axis.**

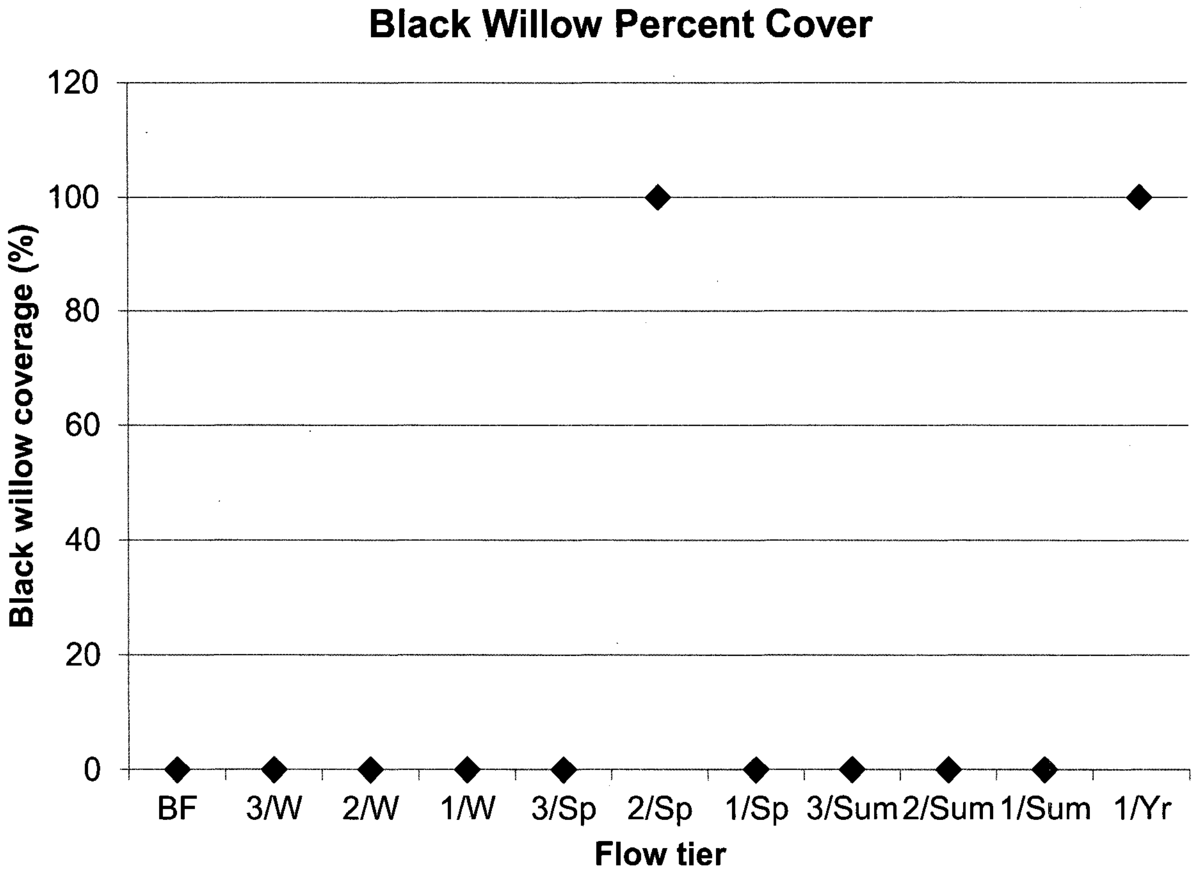


Figure 58. **Percentage of mature black willow stand covered by flow tiers at the Marlin site.**

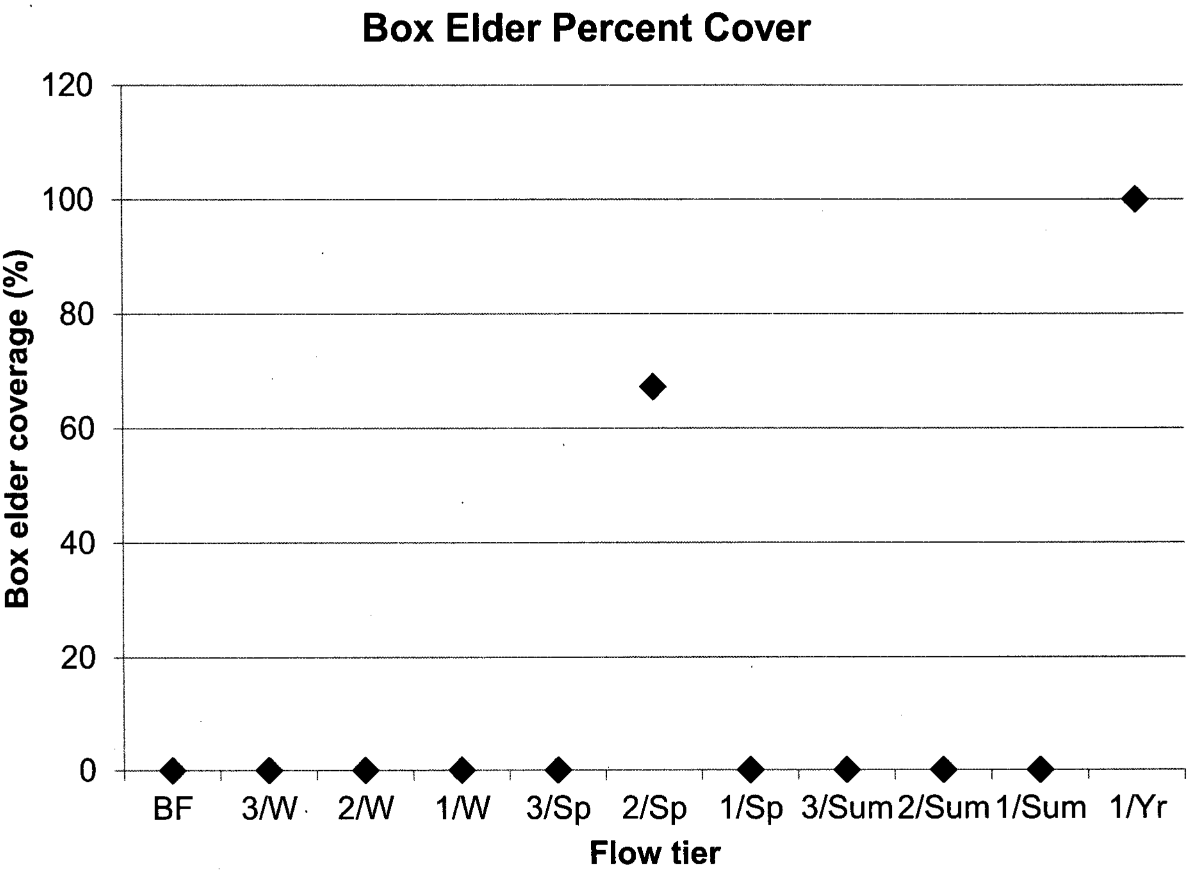


Figure 59. **Percentage of mature box elder stand covered by flow tiers at the Marlin site.**

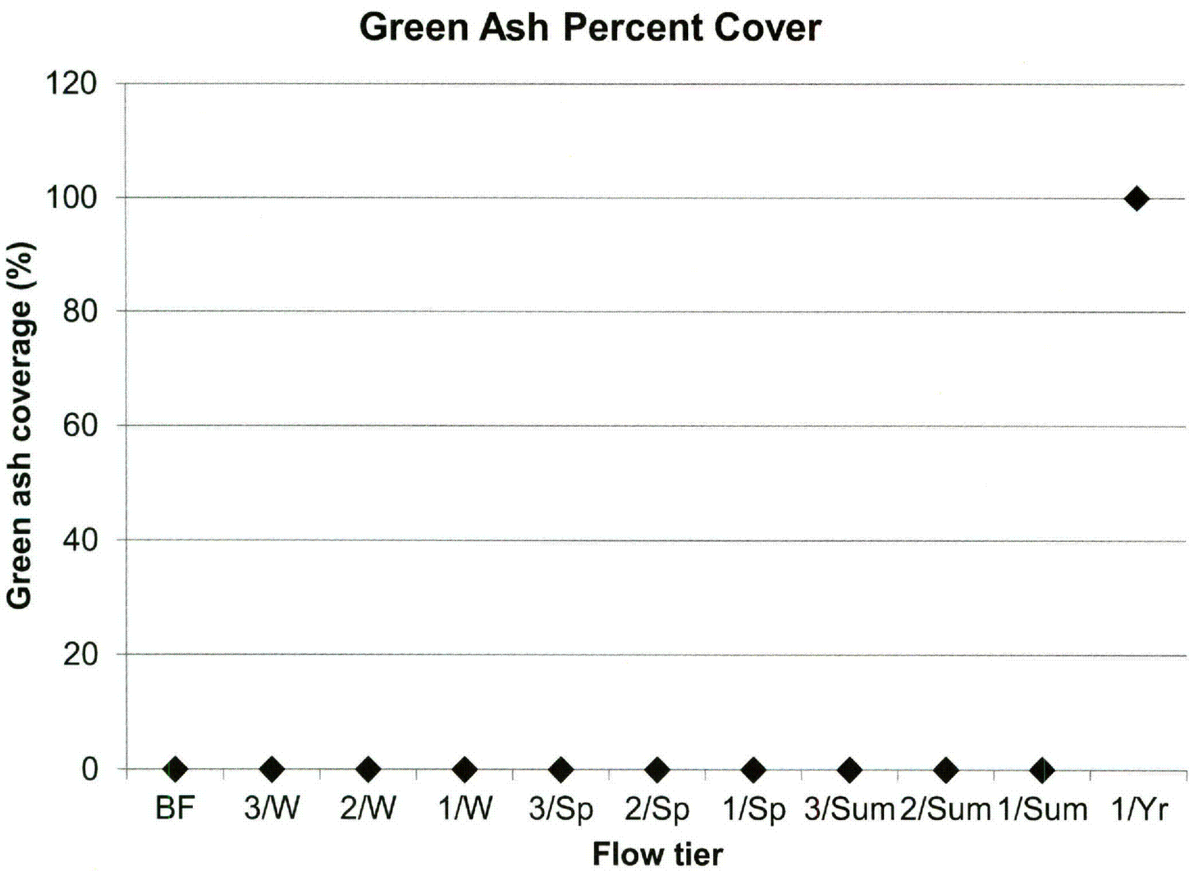


Figure 60. **Percentage of mature green ash stand covered by flow tiers at the Marlin site.**

Table 30. **TCEQ- and BBEST-recommended flow tiers and their occurrences throughout the BBEST-designated seasons at the Marlin site. Y indicates flow occurred; dash indicates no flow occurred.**

			2014 Spring	2014 Summer	2014 Winter	2015 Spring
Flow Tier	**CFS**	**Elevation (m)**	**Mar. - Jun.**	**Jul. - Oct.**	**Nov. - Dec.**	**Jan. - Apr.**
Baseflow	250	0.6	Y	Y	Y	Y
3/Winter	2320	1.6			-	
2/Winter	4180	2.1			-	
1/Winter	2320	1.6			-	
3/Spring	5330	2.6	-			Y
2/Spring	13600	4.2	-			Y
1/Spring	5350	2.6	-			Y
3/Summer	1980	1.4		-		
2/Summer	4160	2.1		-		
1/Summer	1980	2.1		-		
1/Year	30800	7.0	-	-	-	Y

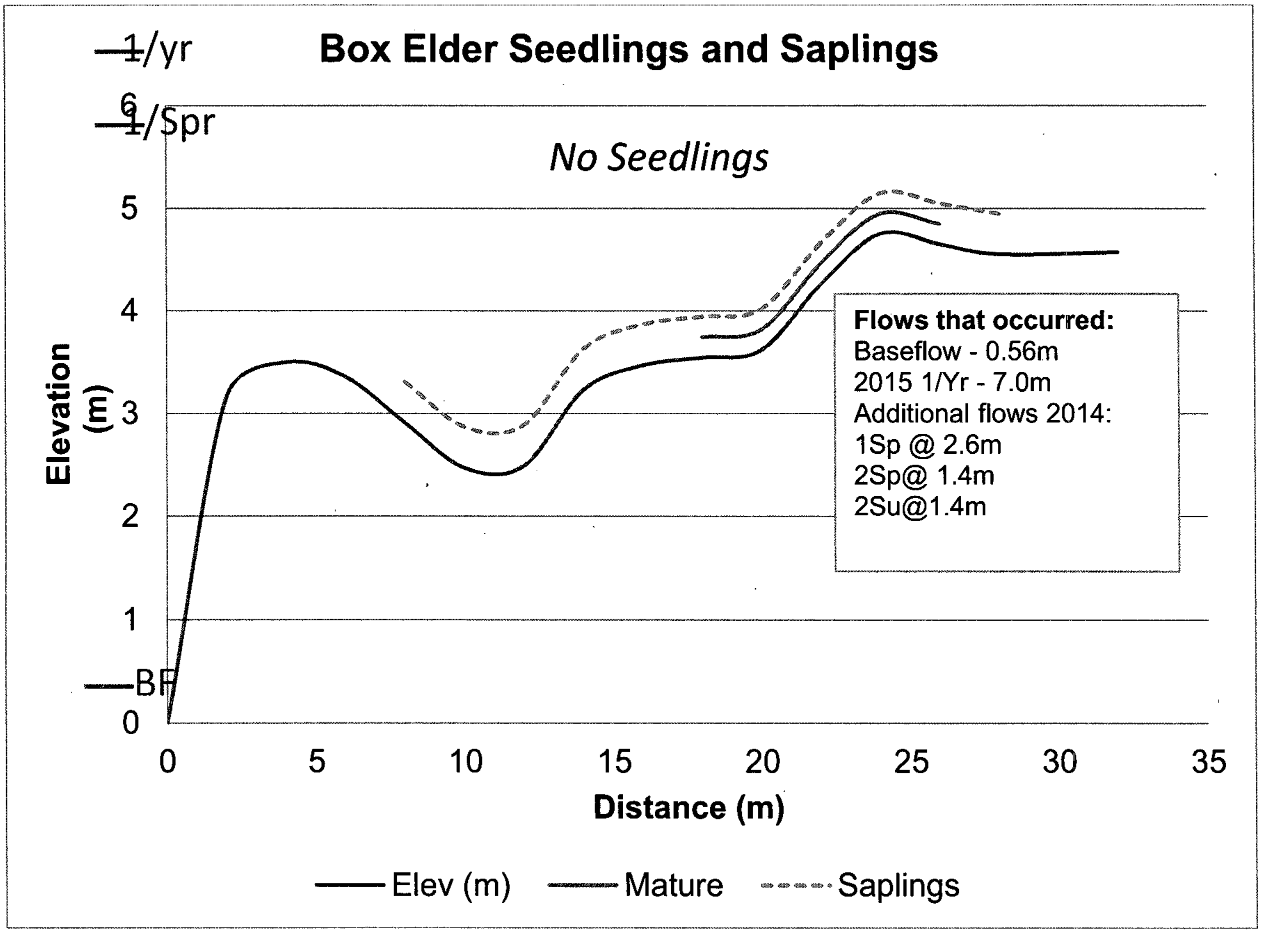


Figure 61. **Box elder distributions at the Marlin site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations criteria) are shown in the inset box.**

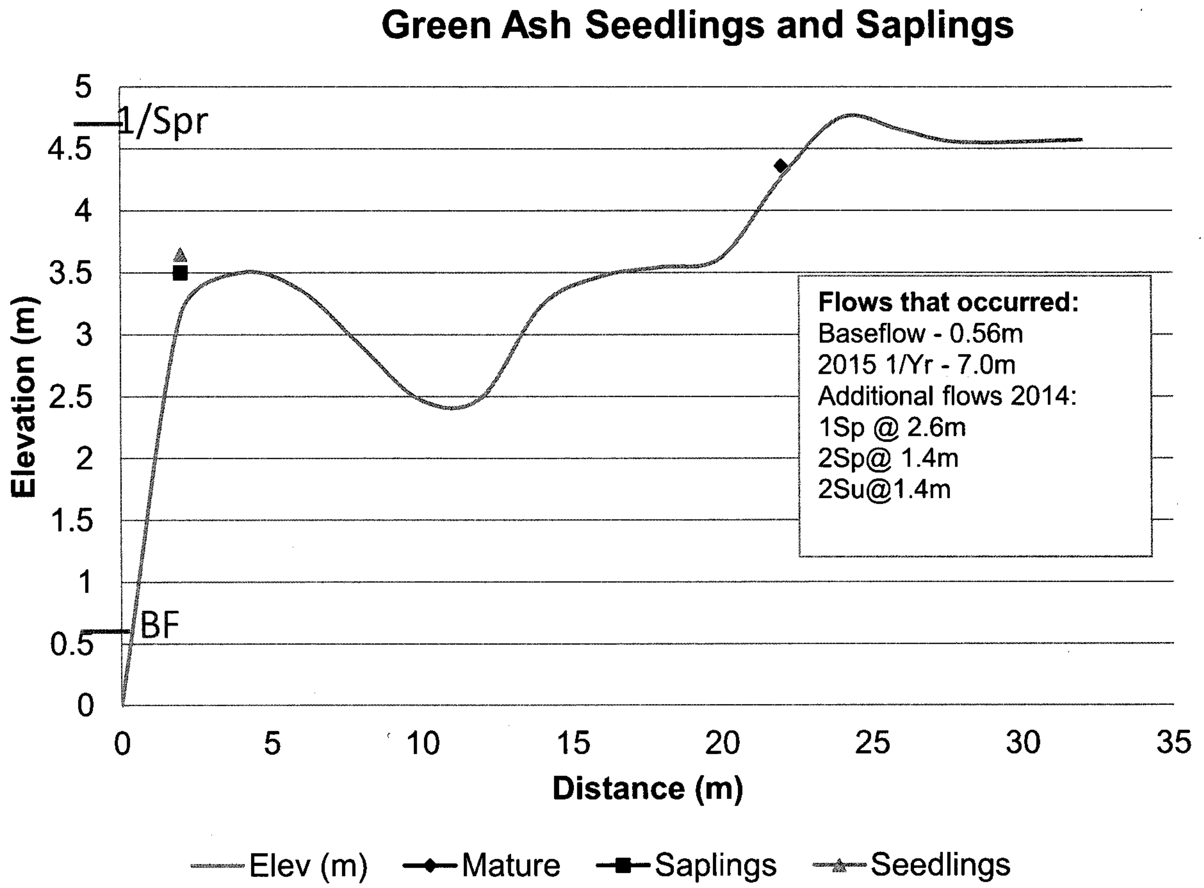


Figure 62. Green ash distributions at the Marlin site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.

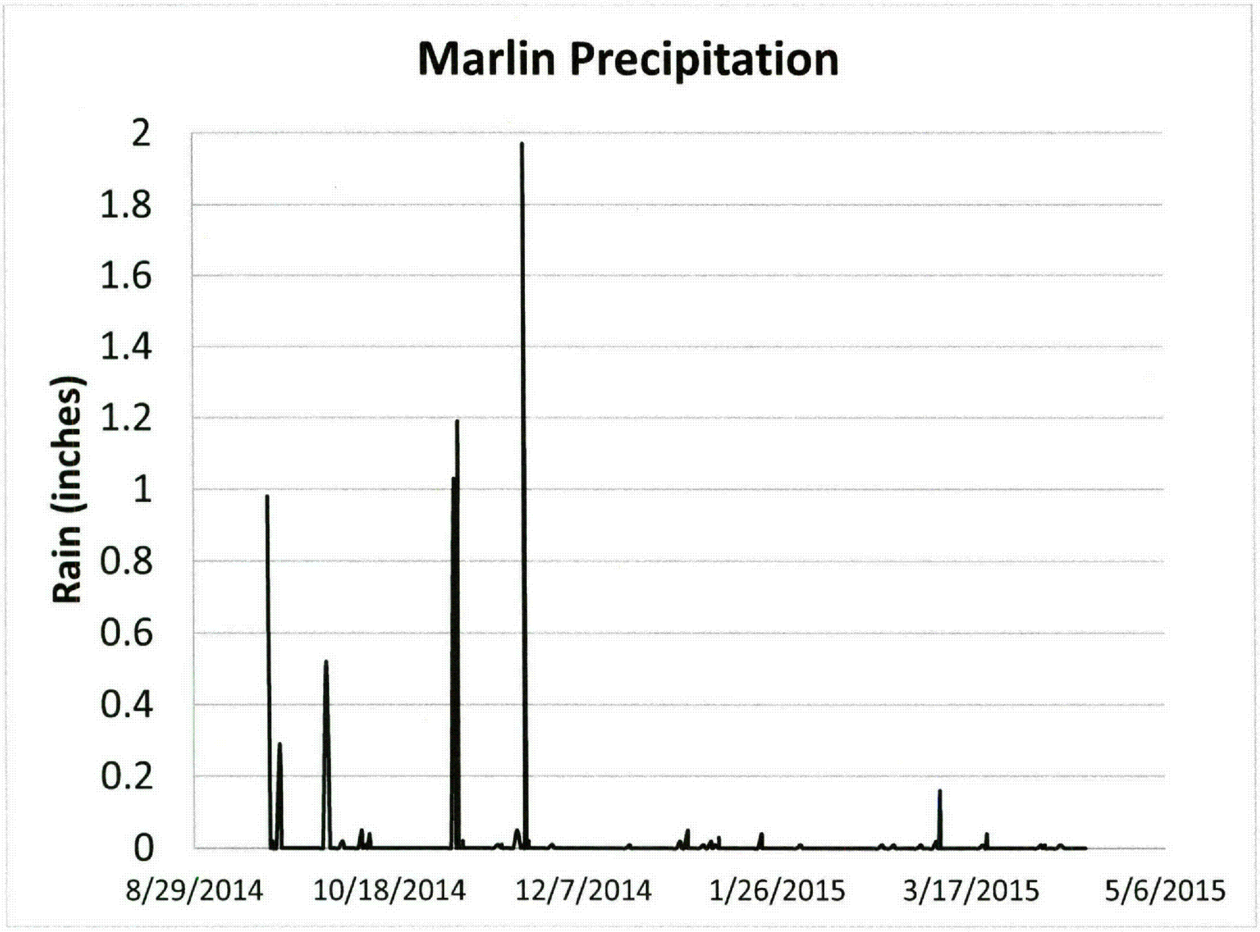


Figure 63. **Marlin local rainfall data in inches.**

Table 31. **Tree counts through time grouped by class at the Marlin site.**

Species	Class	Summer 2014	Fall 2014	Spring 2015
Black Willow	Mature	16	16	16
Box Elder	Mature	2	2	2
Box Elder	Sapling	8	8	8
Green Ash	Mature	1	1	1
Green Ash	Sapling	2	2	2
Green Ash	Seedling	1	1	1

Table 32. **Relative abundances of woody species, grouped by tree type and age class at the Marlin site.**

Tree Species	Class	Count	Relative abundance (%)
American Elm	Mature	1	2.0
American Elm	Sapling	6	11.8
Black Willow	Mature	16	31.4
Box Elder	Mature	2	3.9
Box Elder	Sapling	8	15.7
Cedar Elm	Sapling	1	2.0
Green Ash	Seedling	1	2.0
Green Ash	Mature	1	2.0
Green Ash	Sapling	2	3.9
Hackberry	Seedling	12	23.5
Mulberry	Mature	1	2.0
			100

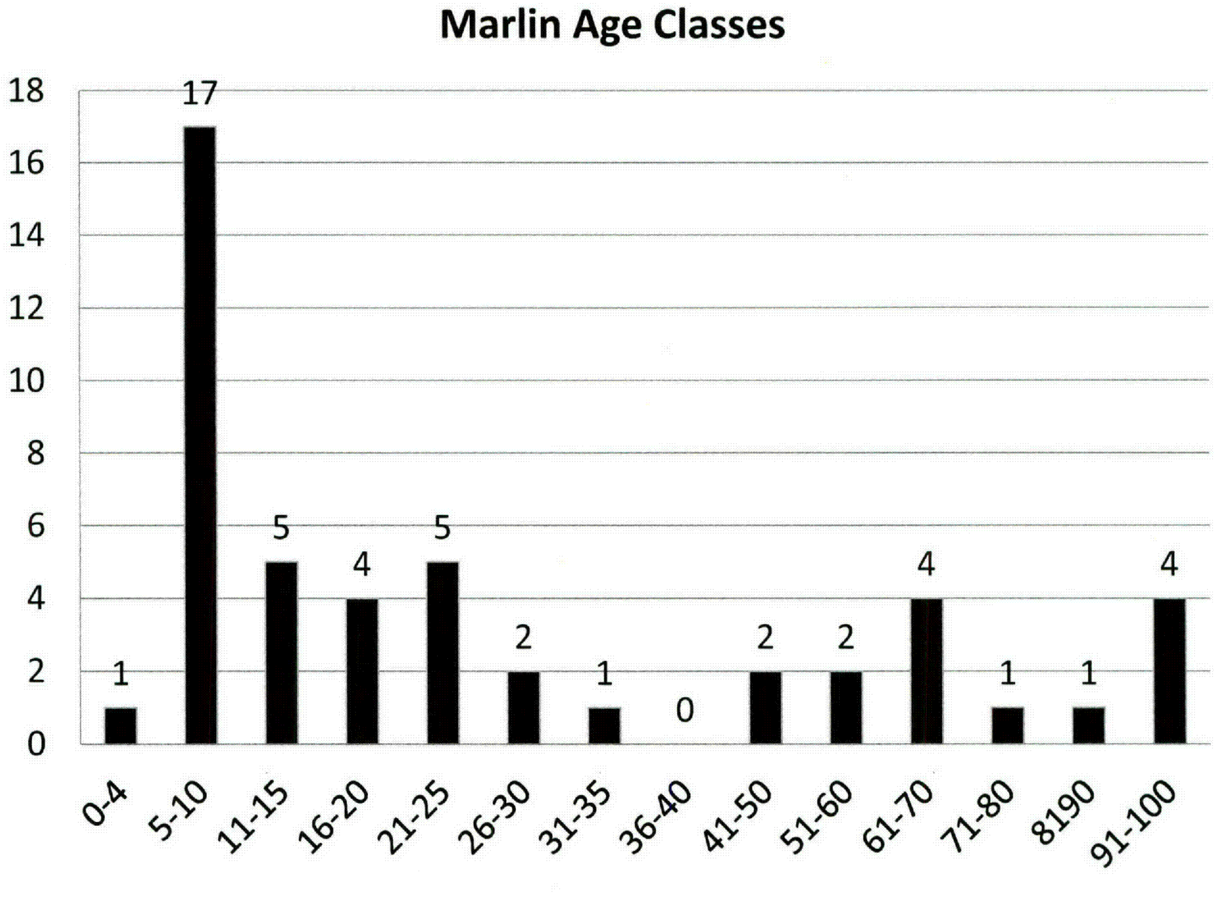


Figure 64. **Marlin trees grouped by age class.**

Basin-wide conclusions

When considering all flow tiers across the Brazos basin, base flow only inundates the range of one of the indicator species (Table 33).Conversely, the 1/year BBEST recommended flow covers all riparian indicator species at all sites. TCEQ flow tiers in the spring are generally below species' ranges – only 2 of 13 species' distributions were inundated at 80% or more with the 1/spring flow. Given that this is the season of seed dispersal and/or germination for all three indicator species, it should be concerning that this flow provided so little coverage. Only 1 of 13 species were covered at 80% or more by 1/summer flows. This too may be a critical flow for the seedling life stage; however, the 9-month study from later summer to spring did not allow for testing of this season. Winter, however, (which in reality covers the fall seed drop and more accurately reflects fall conditions) showed only 1 of 13 species receiving 80% or more inundation. Box elder and green ash depend on the late summer flows/early winter flows for their fall seed dispersals, and a 1/winter flow that serves them at 80% of their range is recommended. All three species' seedlings would also be maintained with this coverage. Please note that this study does not infer that other flows (winter and lower magnitude pulses) are not important to stream ecological function; but rather that they seem not to be related to riparian functioning only, or are at least not detectable with these methodologies.

Table 33. **Basin-wide summary of the total numbers of riparian species covered by TCEQ- and BBEST-recommended flows, total numbers of uppermost species covered, and the number of flows that occurred in 2014.**

TCEQ flow standards	Number of all species covered* by flow	Number of species at the highest elevation covered* by flow	Number that occurred in 2014
Baseflow	1/13	0/5	5/5
3/Winter	1/13	0/5	2/5
2/Winter	2/13	0/5	3/5
1/Winter	1/13	0/5	2/5
3/Spring	2/13	0/5	3/5
2/Spring	4/13	0/5	1/5
1/Spring	2/13	0/5	1/5
3/Summer	1/13	0/5	1/5
2/Summer	2/13	0/5	1/5
1/Summer	1/13	0/5	0/5
1/Year	13/13	5/5	0/5

* Flows inundated 80% or more the species range.

When only the highest-elevation species at each site (Table 33, Column 3) are considered, none of the uppermost species are covered by TCEQ flow tiers. The criterion of highest-elevation species is shown as a way of simplifying future management. If all species are considered, the recommended flows appear to provide more coverage. However, if only the uppermost are managed for (which by their very location automatically result in coverage for all others) then the recommended flow discrepancies to actual species locations becomes more apparent, and more simply managed. The lack of flows during the whole of 2014 really underscores the distressing conditions these riparian zones were shown to be under (Table 33, Column 4).

3.3 Brazos Estuary

Baseline characterization

Eight sampling events occurred in the lower Brazos River estuary between November 2014 and May 2015 encompassing four flow tiers: dry (n = 1), average (n = 1), four-per-season (4/season; n = 2) and two-per-season (2/season; n = 4) (Figure 65; Table 5). All three spring sampling events followed 2/season high-flow pulses. Twelve sampling events occurred in the lower Brazos River estuary between January and December 2012 encompassing six flow tiers: subsistence (n = 4), dry (n = 3), average (n = 1), 3/season (n = 1), 2/season (n = 1) and 1/season (n = 2). When current and historical data were combined, all flow tiers were accounted for except for wet base flow. Compilation and use of historical data facilitated assessment of the entire flow regime since 2012 was a relatively dry year in comparison to the current study which occurred under extremely wet conditions.

Hydrology and water quality

Overall trends in surface and/or bottom measurements of water temperature, salinity, dissolved oxygen (DO), pH, turbidity and Secchi disk are presented in Appendix M. Total depth at the thalweg increased with higher flows as expected (Appendix M). At all sites, surface and bottom water temperatures were highest during dry conditions (Appendix M). Surface and bottom pH readings at all sites remained relatively stable, although the lowest values were recorded during 4/season flow conditions (bottom) and the highest values were recorded during average flow conditions (Appendix M). Across all flow tiers, Secchi disk transparency generally declined as flow tiers increased from dry to 2/season conditions (Appendix M). Summary statistics for chlorophyll-α (RFU), TSS, nitrate and nitrite-N, total phosphorus are presented in Table 34. In comparison to other variables, total suspended solids (TSS) exhibited the widest variation in individual measurements. Specific trends for these variables including salinity and dissolved oxygen are discussed below.

Salinity exhibited significant differences between sites, flow tiers and exhibited significant interactions between sites and flow tier categories (Table 35). Subsequent ANOVA and multiple comparison of sites within flow tiers indicated that the number of sites within a group of similar salinity, and the number of site groupings exhibiting different salinities generally declined and increased respectively as flow tiers increased, that is river discharge increased (Table 35). This suggests greater heterogeneity in salinity regime during lower freshwater inflow regimes.

The upstream extent of the salinity wedge was influenced by the amount of freshwater inflow. The salinity wedge was located approximately 31-42 rkm upstream of the Gulf during dry base flows, approximately 22-36 rkm upstream during average and 4/season events, and approximately 0-10 rkm upstream during 2/season events (Figure 66). 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 (i.e., all 4/season flow events do not exhibit a similar response).

These broad-scale patterns in salinity gradients are primarily dependent on the timing of the sampling event and the magnitude and duration of the flow pulse.

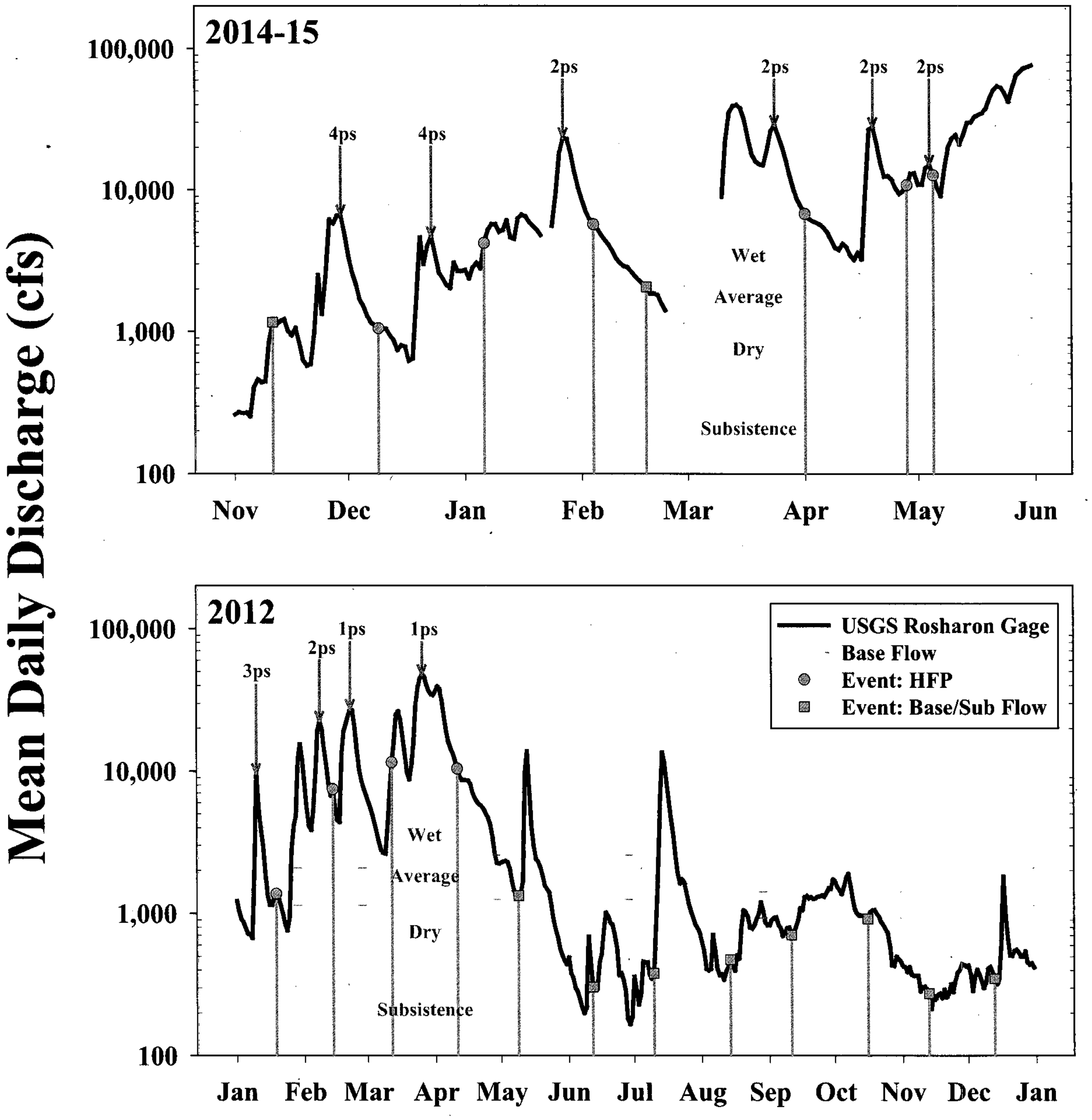


Figure 65. Current and historical hydrograph of mean daily discharge (cfs) from the USGS gage station near Rosharon, TX (USGS 08116650) on the lower Brazos River from 11/01/2014 – 05/31/2015 and 01/01/2012 – 12/31/2012.

Table 34. **Mean ± 1 standard error (SE), range, and number of samples (N) for relative fluorescent units for chlorophyll-a (RFU), total suspended solids (TSS), nitrate + nitrite nitrogen (Nitrate+Nitrite), total Kjeldahl nitrogen (TKN), and total phosphorus (Total P) values documented at primary sites during each flow tier sampled from November 2014-May 2015 in the Brazos River Tidal. All parameters reported in mg/L except chlorophyll-a (µg/L).**

Nutrient	Flow Tier	Mean ± SE	Range	N
RFU	Dry	10.57 ± 0.422	6.37-13.46	5
	Avg	5.85 ± 0.477	3.69-8.28	5
	4ps	12.70 ± 2.789	4.88-20.14	10
	2ps	5.97 ± 0.485	4.61-7.41	20
TSS	Dry	182.3 ± 24.44	24.5-454	5
	Avg	73.8 ± 14.14	14.8-122	5
	4ps	37.8 ± 2.09	32.3-43.5	10
	2ps	15.6 ± 5.12	9.4-36	20
Nitrate+Nitrite	Dry	1.18 ± 0.092	0.64-1.96	5
	Avg	1.22 ± 0.043	0.97-1.37	5
	4ps	0.95 ± 0.099	0.62-1.24	10
	2ps	0.39 ± 0.071	0.16-0.56	20
TKN	Dry	1.70 ± 0.172	0.6-3.7	5
	Avg	0.86 ± 0.159	0.1-1.7	5
	4ps	1.62 ± 0.453	0.5-2.6	10
	2ps	1.44 ± 0.296	0.7-2.5	20
Total P	Dry	0.59 ± 0.098	0.12-1.9	5
	Avg	0.32 ± 0.036	0.15-0.48	5
	4ps	0.19 ± 0.023	0.13-0.27	10
	2ps	0.30 ± 0.125	0.06-0.78	20

Table 35. **Results of two-way analysis of variance for water quality, nutrient and nekton variables across tiers and sites showing the degrees of freedom, test statistic, p-value, significance of the interaction term and subsequent post-hoc tests based on the interaction term. Comments include similarities across flow tiers and sites within flow tiers.**

Dependent Variable	Df_n	Df_d	F-Statistic	P-value	Interaction	Variable	Df_n	Df_d	F-Statistic	P-value	Comments
Water Quality											
Salinity	24	180	2.205	0.002	S	2ps-site	8	99	9.548	<0.001	(B01-B10) (B15-B42)
						4ps-site	8	45	3.783	0.002	(B01-B26) (B15-B31) (B26-B42)
						Avg-site	8	18	3.327	0.016	(B01-B22) (B15-B42)
						Dry-site	8	18	5.095	0.002	(B01-B22) (B15,B22,B31) (B22-B36) (B26-B42)
DO	24	180	0.801	0.732	NS	Tier	3	212	46.070	<0.001	All tiers different
Nutrients											
RFU	12	20	3.286	0.009	S	2ps-site	4	15	1.382	0.287	
						4ps-site	4	5	3.566	0.098	
						Avg-site					Not enough replicates
						Dry-site					Not enough replicates
TSS	12	20	0.163	0.999	NS	Tier	3	36	9.131	<0.001	(2ps) (4ps, Avg, Dry)
Nitrate-Nitrite	12	20	0.293	0.983	NS	Tier	3	36	9.265	<0.001	(2ps, 4ps, Avg) (Dry)
TKN	12	20	0.768	0.675	NS	Tier	3	36	3.036	0.042	(2ps, Avg, Dry) (4ps, Avg, Dry)
Total Phosphorous	12	20	0.575	0.837	NS	Tier	3	36	2.926	0.047	(2ps) (4ps, Avg, Dry)
Nekton											
Estuarine Species	12	20	4.045	0.003	S	2ps-site	4	15	64.850	<0.001	All sites different
						4ps-site	4	5	11.550	0.010	(B01-B22) (B22-B31) (B31-B42)
						Avg-site					Not enough replicates
						Dry-site					Not enough replicates

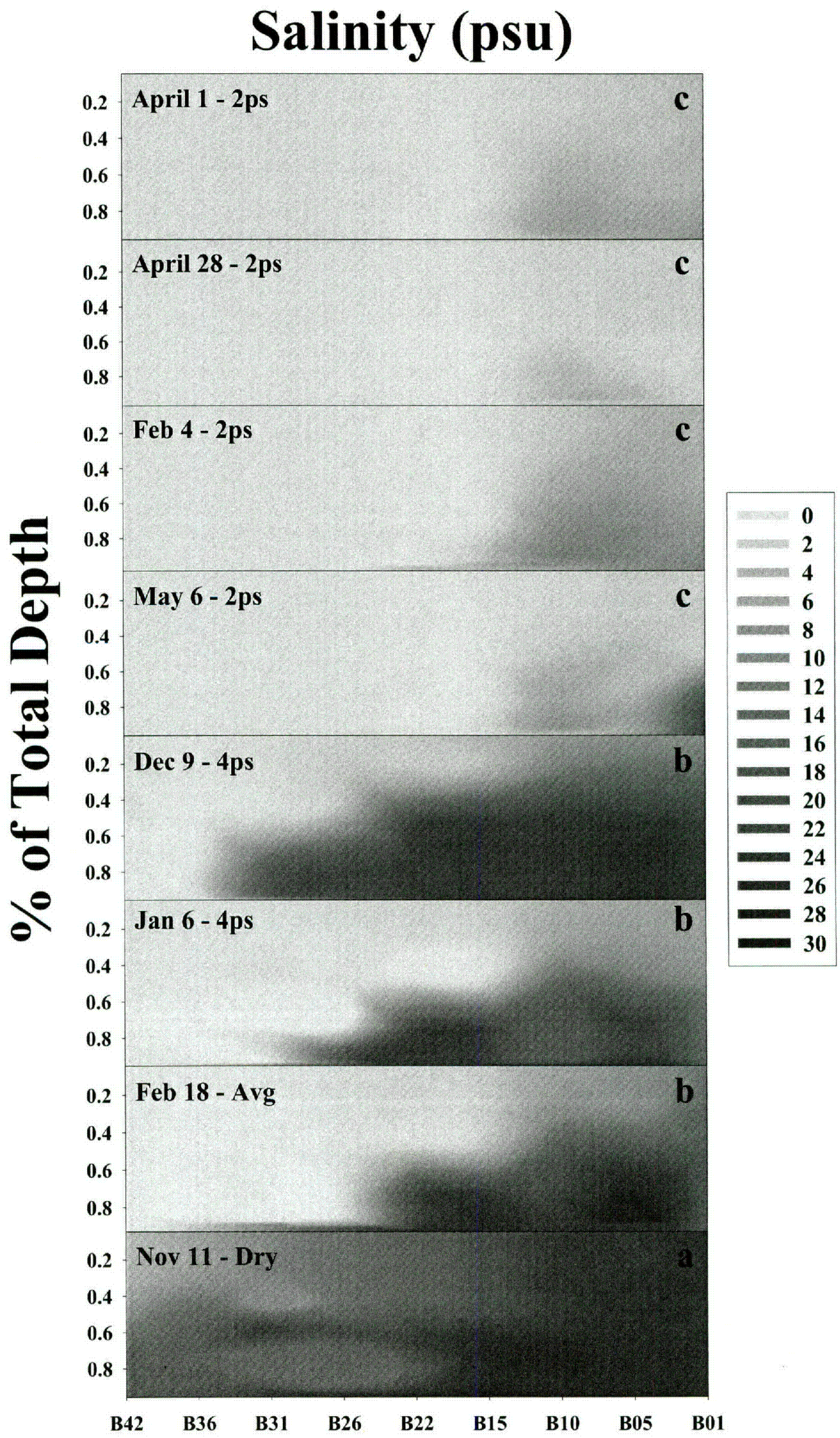


Figure 66. Salinity (psu) profiles organized by flow tier for all sampling events from B42 to B01 on the lower Brazos River from Nov 2014 – May 2015.

Continuous monitoring of salinity in the upper, middle and lower reach resulted in similar trends with salinity vertical profiles that were lowest upstream and highest downstream near the Gulf (Figure 67). Prior to and during high-flow pulse events, salinity decreased upstream to downstream. After high-flow pulse events, salinity increased downstream to 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). Salinity levels responded predictably to high-flow pulse events along the sampling reach (Figure 68). During dry base flow, tidal cycles (±1.0 ft) were greatest causing salinity fluctuations of ~5.9 ppt in the upper reach, ~3.3 ppt in the middle reach and ~3.9 ppt in the lower reach (Figure 68). During 2/season events, tidal cycles (±0.4 ft) were smallest causing salinity fluctuations of 0.0 ppt in the upper reach, 0.0 ppt in the middle reach and ~0.7 ppt in the lower reach. Tidal influence depended upon location within the hydrograph and was most evident on the tapered end of the salinity wedge.

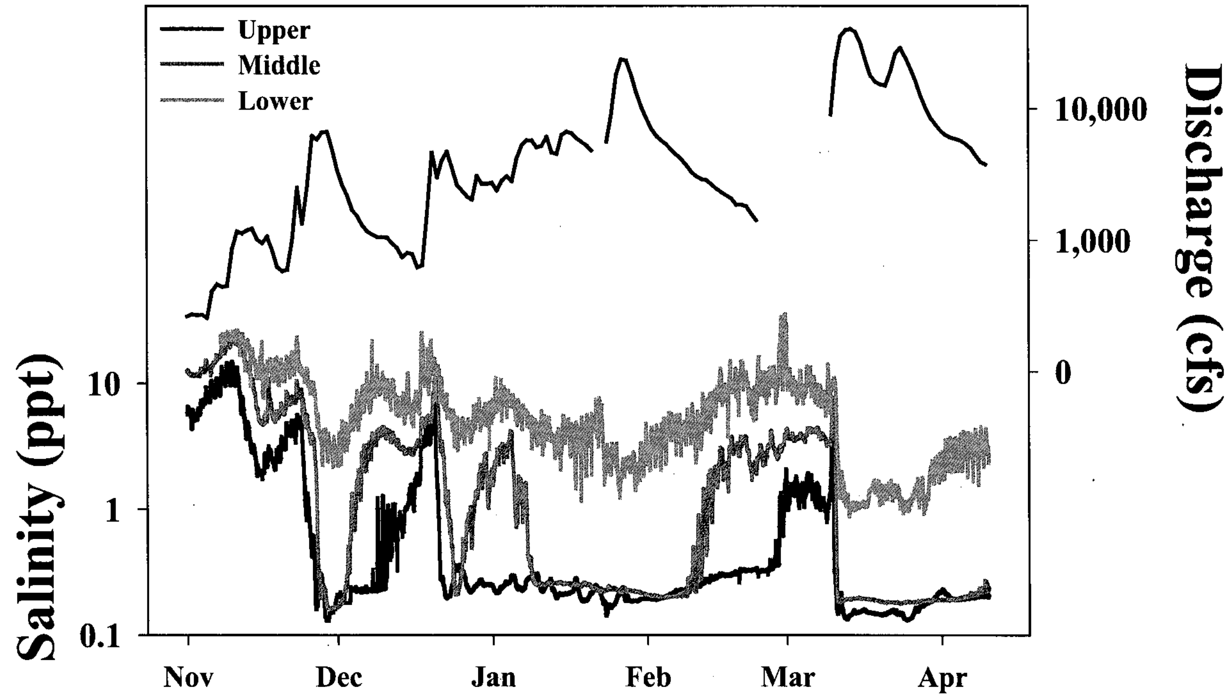


Figure 67. **Continuous salinity (ppt) monitoring and discharge (cfs) on the upper, middle and lower reach of the lower Brazos River from Nov 2014 – Apr 2015.**

Dissolved oxygen values from all depths, differed across all four flow tiers but did not exhibit significant differences between sites (Appendix M and Table 35). Examination of plots of individual dissolved oxygen measurements obtained at each sites and event by depth illustrates the interaction between flow regime and the vertical distribution of this variables (Figure 69). During 2/season events, DO was relatively mixed throughout the entire estuary and water column (Figure 69). During 4/season and average flow tiers, average DO was fairly homogenous across sites at the surface and became increasingly stratified at the upper and lower sites. During dry base flow DO exhibited the lowest values recorded in upper estuary. Depressed dissolved oxygen conditions usually occurred at the same location along the bottom near the extent of the leading edge of the salinity (Figure 66, Figure 69). The lowest DO levels observed during the study occurred during dry base flow conditions but never approached hypoxic (2 mg/L) conditions.

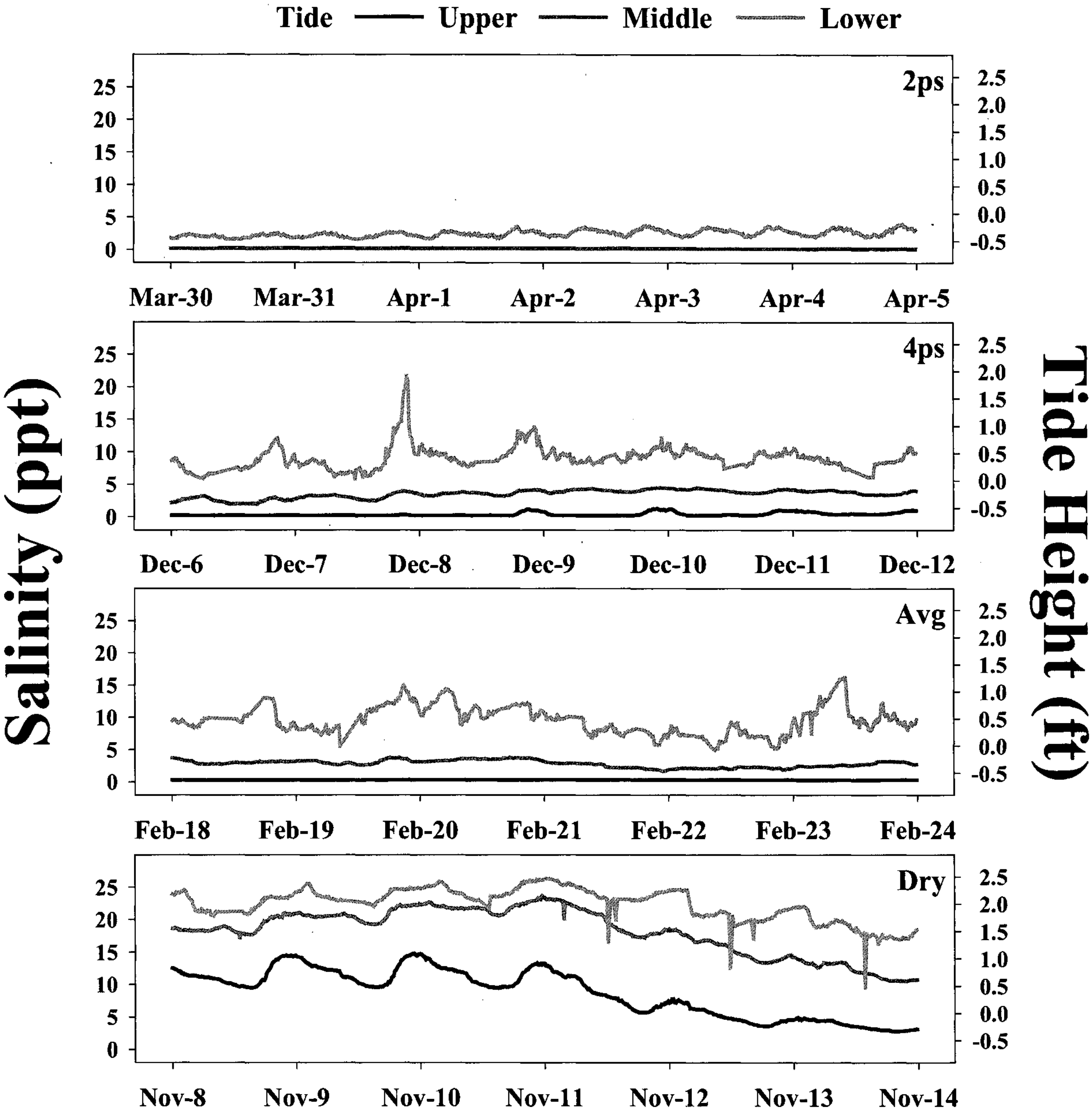


Figure 68. **Continuous salinity (ppt) monitoring and tide height (ft) classified by flow tier on the upper, middle and lower reach of the lower Brazos River from Nov 2014 – Apr 2015**

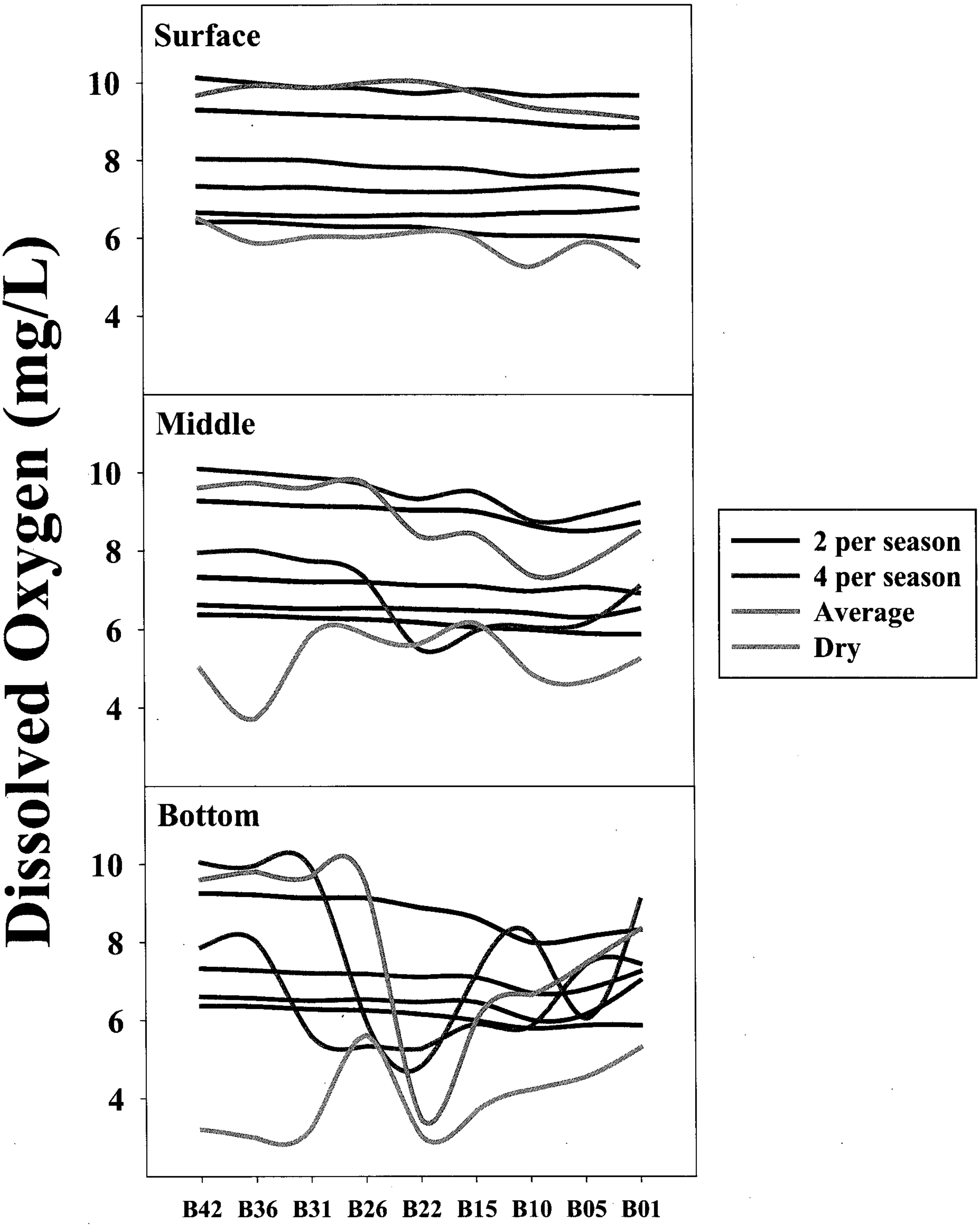


Figure 69. Dissolved oxygen (mg/L) profiles classified by flow tier at the surface, middle and bottom of the river for all sampling events from B42 to B01 on the lower Brazos River from Nov 2014 – May 2015.

We did not detect any statistically significant differences in chlorophyll-α RFU during the study period between sites or flow tiers (Estuary Tables 3, Table 35). However, the highest individual values occurred during average flow conditions (Table 34 and Figure 70). Significant differences in TSS were observed between flow tiers but not sites (Table 34, Table 35). Total suspended solids (TSS) were highest during 2/season events than all other events (Table 35, Figure 70). Although statistically insignificant, TSS levels during 4/season also exhibited individual elevated TSS values (Figure 70).

Nitrate+Nitrite-N levels measured during dry events were significantly lower than during all other tiers (Table 34, Table 35, and Figure 70). Although statistically insignificant nitrate-nitrite-N did exhibit a gradual increasing trend in individual measurements with increasing flow tiers (Figure 70). We did not detect any difference in this variable between sites (Figure 70). Total Kjeldahl nitrogen (TKN) concentrations exhibited statistically significant differences between flow tier groups with the lowest values being recorded during 4/season pulse events (Table 34, Table 35, and Figure 70). We did not detect any difference in TKN between sites. Highest total phosphorus concentrations were encountered during 2/season pulse events (Table 34, Table 35, and Figure 70). We did not detect any difference in total phosphorus between sites, although the highest individual measurements were recorded at site B22.

Nekton

A total of 21,024 individuals were collected throughout the current study with an overall species richness of 79 (Appendix N). Across all sites, *Micropogonias undulatus* was most abundant (N = 8,194, RA = 39%) followed by *Brevoortia patronus* (5,463, 26%), *Mugil cephalus* (2,812, 13%), *Anchoa mitchilli* (1,138, 5.4%) and *Ictalurus furcatus* (883, 4.2%). Total catch per sampling event was highest during event 3 (winter; N = 5,472), 8 (spring; 3,371) and 5 (winter; 3,240) likely due to winter spawning and recruitment into the estuary of *M. undulatus* (3 and 5) and delayed winter spawning of *B. patronus* (8) resulting in higher catch of juveniles and young-of-year during these sampling events. Lowest species abundance occurred during event 6 (1,027), 2 (1,550) and 4 (1,563) which all occurred following fairly high-magnitude pulses and likely resulted in a wash out effect.

Nekton catch was high for all sample methods (total catch by method: BT = 4,276, ES = 8,702, OT = 8,046). The three gear types used in the study effectively targeted diverse guilds of nekton including post-larval and newly recruited fishes and small invertebrates in the beam trawl, large transient fishes with the electroshocking boat, and demersal nekton with the otter trawl. The majority (65%) of species caught across all methods were classified as estuarine dependent (status = ES). Twenty-five percent were classified as fresh water species and only 3% were considered marine species.

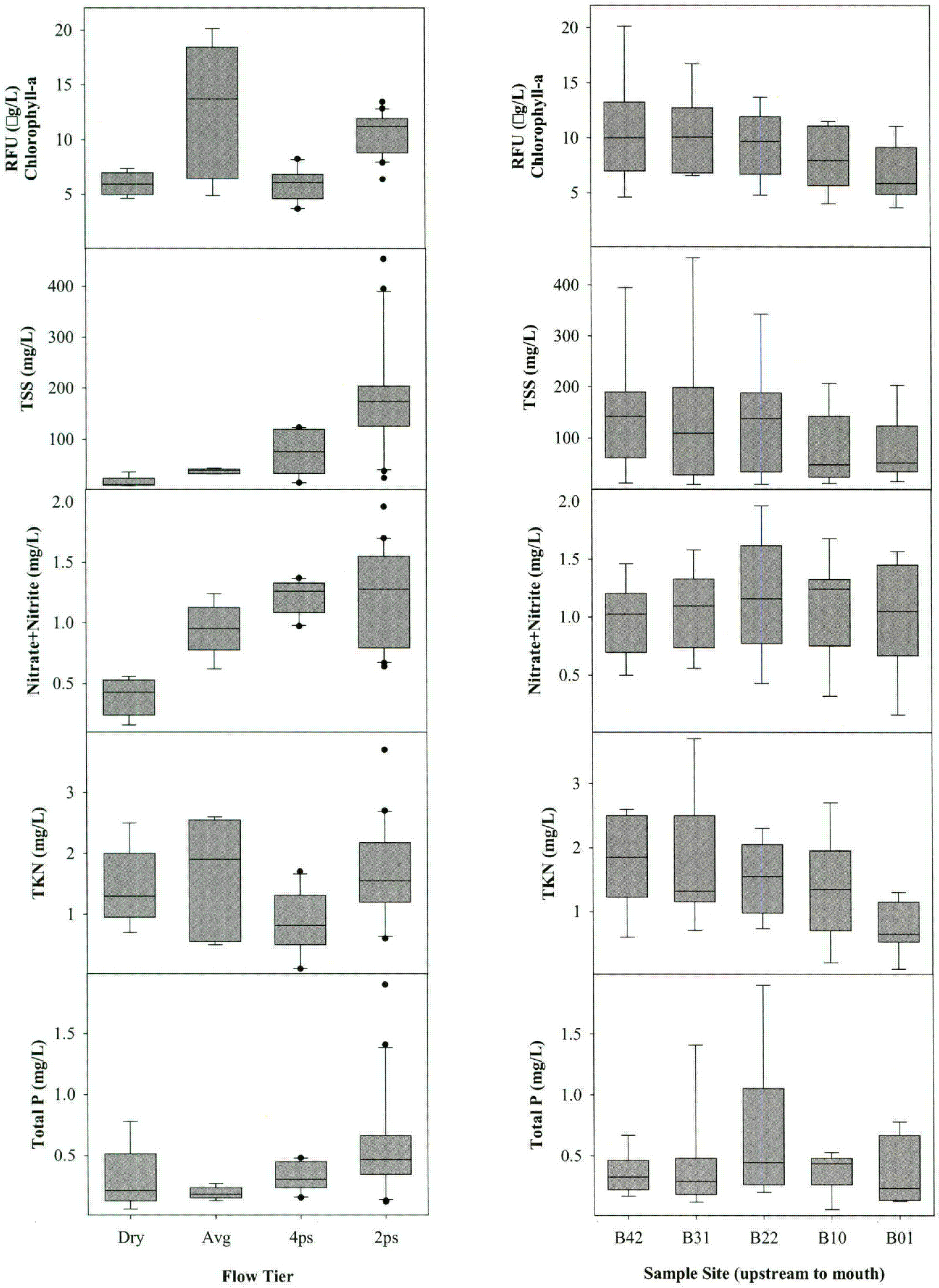


Figure 70. RFU (µg/L) chlorophyll-a, TSS (mg/L) Nitrate-Nitrite (mg/L), TKN (mg/L) and Total P (mg/L) grouped by flow tier and site for all sampling events from B42 to B01 on the lower Brazos River from Nov 2014 – May 2015.

The proportion of freshwater species was likely inflated due to the high amount of freshwater inflow encountered throughout the study period (Figure 71). Overall trends in the proportion of estuarine species indicate the proportion of estuarine-dependent species increases from upstream to downstream but also varied between flow tiers. Proportion of estuarine species differed by flow tier and site and exhibited significant interactions between these two factors (Table 35 and Figure 71). The proportion of estuarine species did not differ between sites during high-flow 2/season pulse events. During average base flow conditions, sites grouped according to lateral gradient with the lower 3 sites (B01-B22) usually grouping together and the upper two site (B31-B42) forming a third group (Table 35). However, site B22 and B31 also grouped together depending on the individual flow event. Average base flow tends to represent transitions to or from a high-flow pulse resulting in a wide range of community response depending on location within the hydrograph.

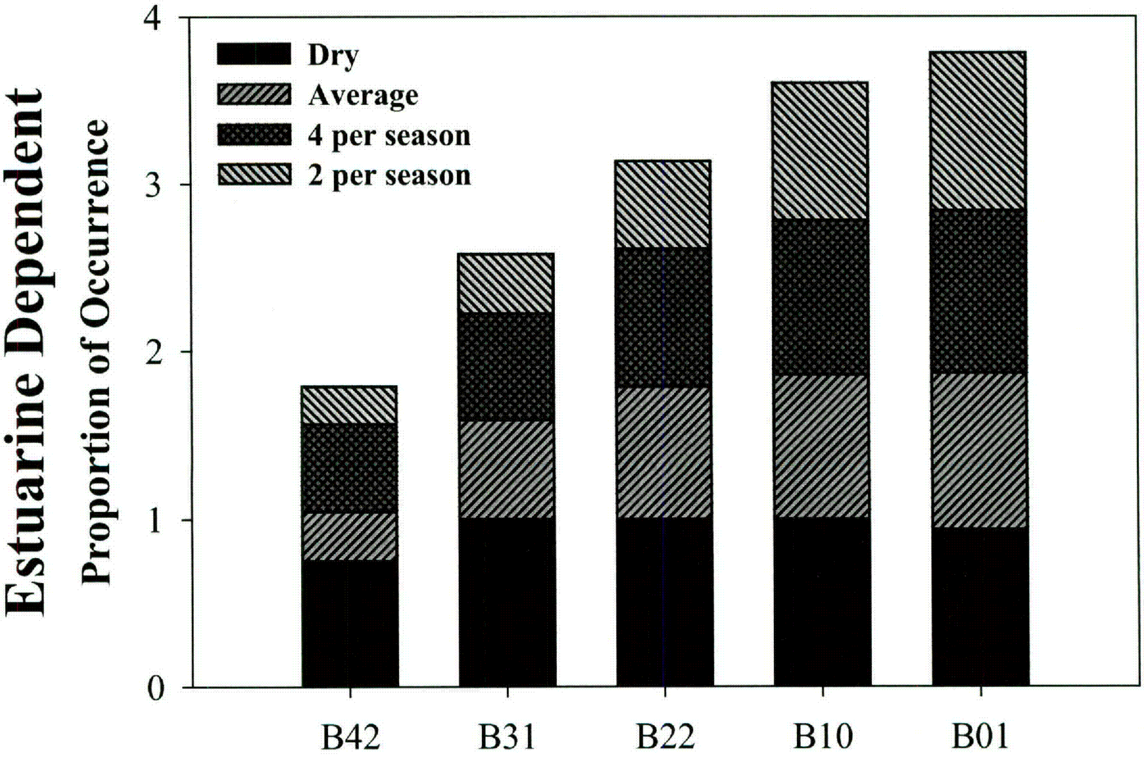


Figure 71. **Mean proportion of occurrence of estuarine dependent organisms classified by flow tier for all sampling events from B42 to B01 on the lower Brazos River from Nov 2014 – May 2015. Proportion of one means that 100% of the assemblage consisted of estuarine dependent nekton.**

During the current study, nekton exhibited community structure along a longitudinal gradient with lower estuary sites separating from upper estuary sites using a 33% similarity resemblance (Figure 72). The exception to this spatial grouping was site B22 which during this wetter than normal study period appears to be precisely mid-estuary. Additionally, NMDS plot for nekton abundance showed clear trends for flow tier and site location with both factors clustering in opposing linear gradients (Figure 73). The dry base flow event (tier 2) was significantly different from all other flow tiers (global R = 0.216; $p \leq 0.01$). All sites were significantly different except for sites B22 and B31 and B31 and B42 (global R = 0.351; $p \leq 0.01$). When the nekton MDS plots were overlaid with surface and bottom salinities, salinity thresholds were evident by depth (Figure 74). The dry base flow event (tier 2) was the only event where surface salinity >3 psu was sustained across all sites, however, most average (tier 3) and 4/season pulse events (tier 5) had sustained bottom salinity >3 psu. Overall, assemblages sampled during 2/season pulse events (tier 7) had lower surface and bottom salinities compared the rest of the flow tiers with salinity generally increasing from the upper estuary to lower estuary.

Nekton assemblages showed similar trends to the current data alone and differed by flow tier (global R = 0.263; $p \leq 0.01$) and site (global R = 0.235; $p \leq 0.01$) when historical data were combined with the current study (Figure 75). Clustering of nekton assemblages both spatially and by flow tier became more evident with the inclusion of additional extreme flow tiers (i.e., sub = 1, dry = 2, 2/season = 7 and 1/season = 8). Assemblages sampled under higher flows (7-8) tended to exhibit a greater spatial gradient between B42 and B01; however, nekton communities sampled on the low end of the hydrograph (1-2) tended to look more similar across the estuary. This is likely due to the influence of the salt wedge extending beyond B42 under low flow conditions. Several outlier collections were been identified that require further investigation including B42-flow tier 1 in the lower left corner of the MDS plot which is not following the typical trend as well as all flow tier 8 assemblages (event 11) which seem to tightly cluster spatially. This could be due to gear inefficiencies if sampling occurred under high-flow conditions that has not fully returned to base flow.

Figure 72. Cluster Analysis results for nekton abundance (log+1 transformed with Bray-Curtis resemblance) data from 2014-

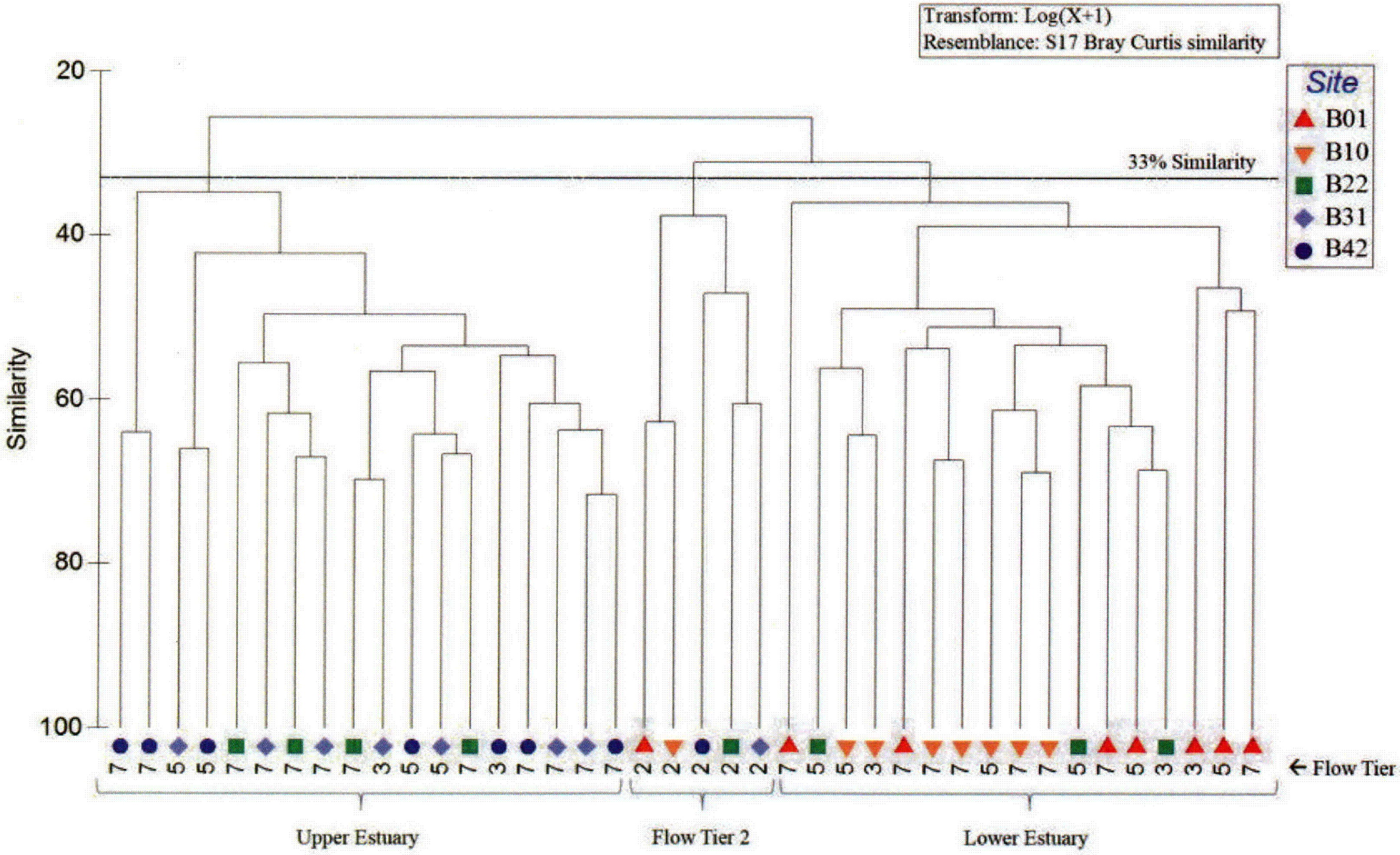


Figure 73. NMDS plot for nekton abundance (log+1 transformed with Bray-Curtis resemblance) data from 2014-15, all sampling methods combined. Points are labeled by TCEQ Flow Tier Category (2=dry base flow, 3=average wet flow, 5=four per season, and 7=two per season events). Relationships by Flow Tier (from top right to bottom left) and Site location (from bottom right to top left) are shown with general trend lines.

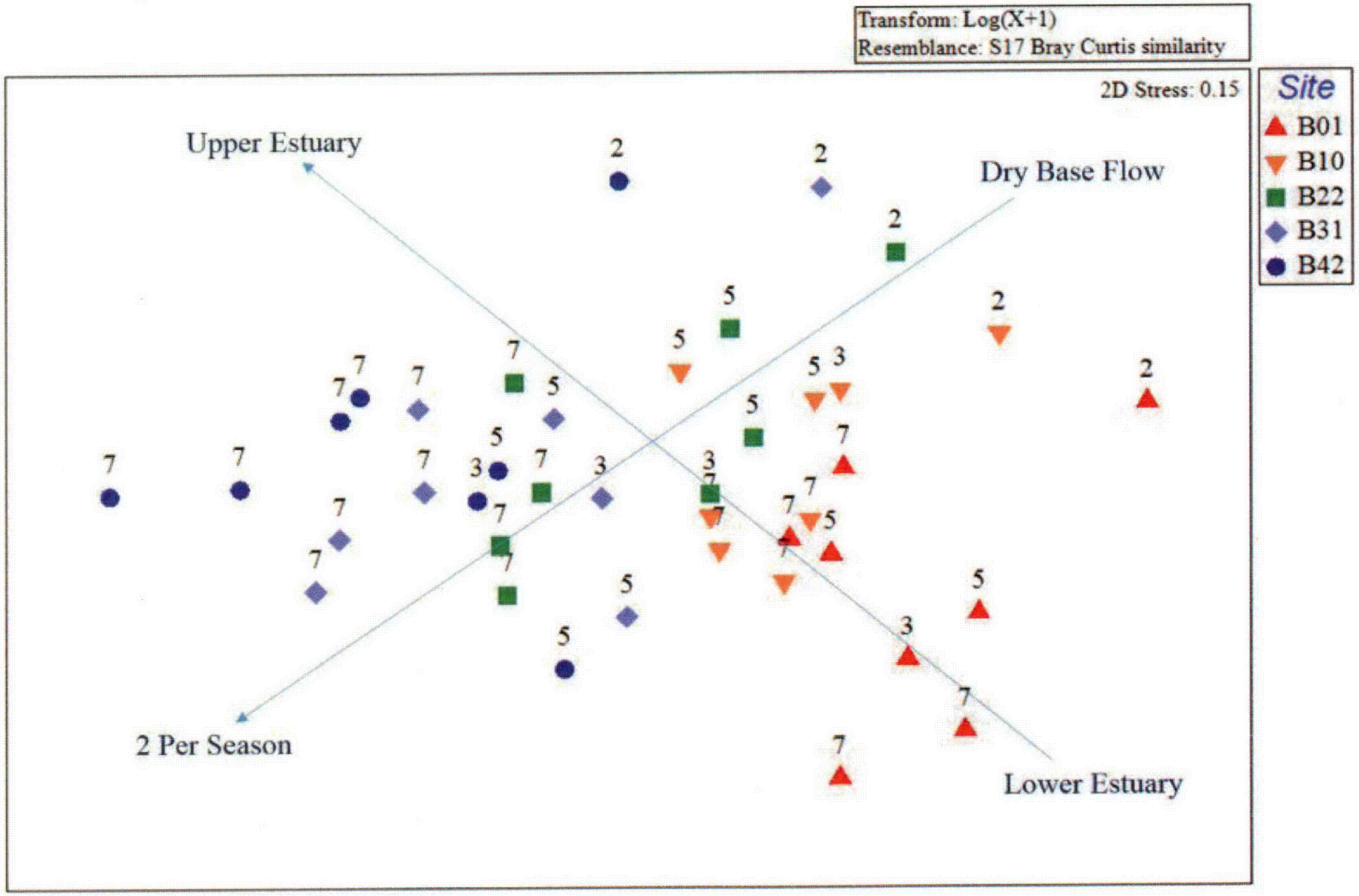


Figure 74. NMDS plots for nekton abundance (log+1 transformed with Bray-Curtis resemblance) data from 2014-2015. All sampling methods combined overlaid with 2D bubble using both surface (top two figures) and bottom (bottom two figures) salinity (psu). Points are labeled by Flow Tier Category (2=dry base flow, 3=average wet flow, 5=four per season, and 7=two per season events) (left two figures) and site numbers (right two figures).

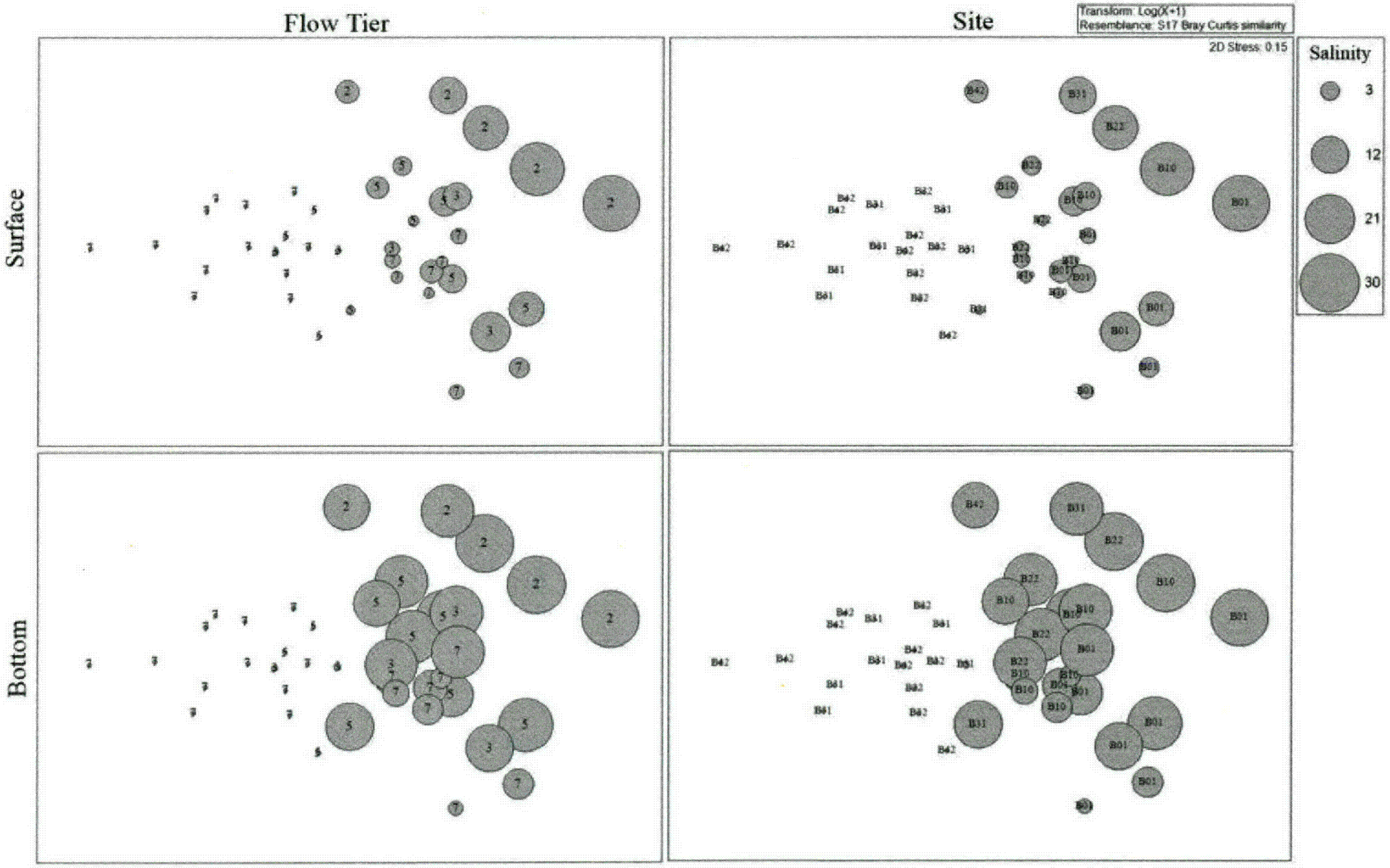
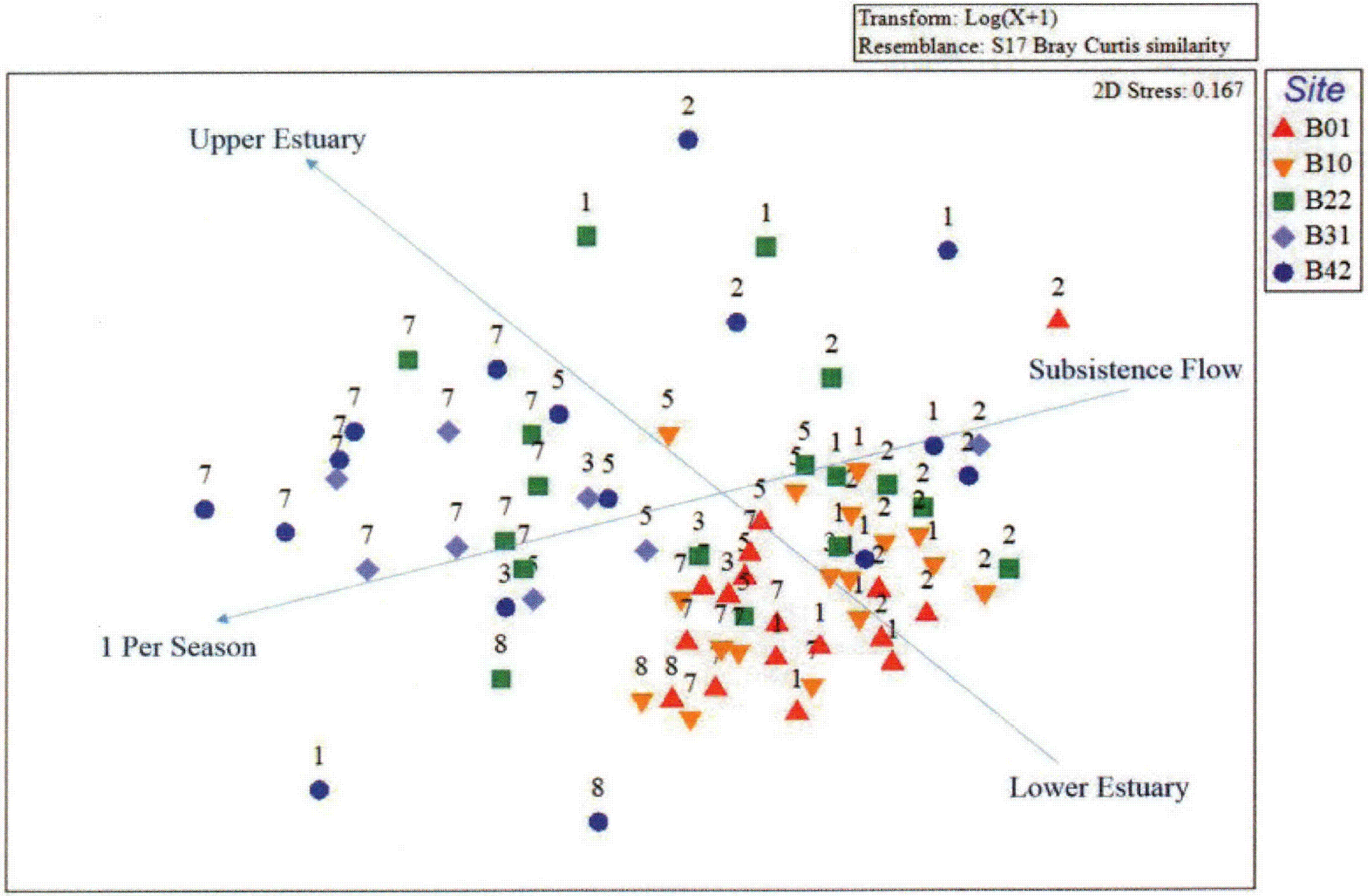


Figure 75. NMDS plot for nekton abundance (log+1 transformed with Bray-Curtis resemblance) from 2012 and 2014-15 using combined otter trawl and beam trawl data. Relationships by Flow Tier (from top right to bottom left) and Site location (from bottom right to top left) are shown with general trend lines. Points are labeled by TCEQ Flow Tier Category (1=Subsistence flow 2=dry base flow, 3=average wet flow, 5=four per season, 7=two per season events, and 8=one per season).



4 Multidisciplinary evaluation

For intensive biological data collection 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. In most basins, including the BRA, standards for the majority of sites were developed based on historical hydrology, existing biological and water quality data, and professional judgment. In certain cases (i.e., lower San Antonio River and Cibolo Creek in the GSA basin) extensive data were available from recent, comprehensive instream flow studies. Even in those instances, professional judgment influenced final BBEST and BBASC recommendations. Additionally, 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.

This section provides a summary of key ecological components that have been described so far. In order to inform the SB 3 process, components are then evaluated collectively, methodology development is described, and some potential application scenarios specific to the BRA basin are provided. It is acknowledged that this represents the first step in the development of validation methodologies with the ultimate goal of having a scientifically defensible approach for testing TCEQ environmental flow standards in the future.

4.1 Description of validation process

Aquatic

Biotic and abiotic responses, as measured in this study, were not detected among flow tiers and therefore, could not validate the predicted ecological values of high-flow pulses at the levels tested. Insufficient collections at subsistence flows (N = 4) prevented inferences into the ecological values of base flows over subsistence flows or information on the adequacies of subsistence flow standards. Collections at base flow (N = 36) and following several tiers of high pulse flows (N = 34) were sufficient, although some high-flow pulse tiers had low replication (i.e., Tiers 3 and 4).

Failure to detect differences in most of the initial predictions could be attributed to low number of replicates given the amount of variation observed in the response variables (i.e., lack of statistical power). Given that basin and season effects were rarely detected in aquatic insect and fish community structure, replication of riffle and run habitats can be made independent of basin and season, which provides greater opportunities to gather larger numbers of replicates.

Alternative to lack of statistical power, failure to detect differences in most of the initial predictions could be an accurate reflection of habitat and community responses to the defined tiers. Flow tiers and, more specifically, flow magnitudes observed and quantified in this study were not sufficient to elicit a habitat or community response. The following 2015 post-flood collection supports this finding.

Intensive and extensive precipitation and subsequent flooding occurred in May and early June 2015 at most of our sites. For the purposes of this study, we categorized pulse events broadly: "1/season", "2/season", "1/year", and so on, denote pulses of such magnitudes as typically occur a few times during each time period, while "large flood" denotes intense, infrequent flooding events. By the end of June 2015, the GSA Comfort site was nearest to base flow conditions among all sites, though flows at Comfort were still elevated at a magnitude considered a 2/season event. Current velocities within riffle habitats were too high to sample efficiently, but run habitats were suitable for seining. Comparing flow tiers taken only at the Comfort site, percentages of slack-water fishes were 13% at base flow, 0% at 1/season and 0% at large flood, percentages of fluvial fishes were 29% at base flow, 44% at 1/season, and 4.5% at large flood, percent of swift fishes were 58% at base flow, 55% at 1/season, and 95% at large flood. Responses of the fish community at Comfort after the large flood were consistent with theory that flow pulses help to maintain communities by displacing less lotic-adapted species.

Collectively, responses of macroinvertebrate and fish community structure (i.e., relative abundances of slack-water to swift-water specialists) were not detected at low magnitude flow pulses (4/season to 1/year), and therefore, cannot validate the ecological benefits of recommended high-flow pulses. However, response of fish community structure following a large flood is consistent with stream theory but with suggestion of refinement: only higher flow pulses (>1/year) might be sufficient to elicit a community response.

Independent of the findings, the validation approach used herein demonstrated that flow recommendations and standards can be tested with a priori hypotheses and with replication. Failure to detect differences with statistical tests is analogous to a "hung jury". Benefits or the lack thereof are unknown at this point. As such, we can reuse and refine the approach by continuing to test the same hypotheses to understand sources of large variation, especially within stream communities at base flow conditions, and to test additional hypotheses. Macroinvertebrate and fish community structure (% occurrence by density) in runs and riffles can be monitored into the future across sites to supply greater understanding on how communities respond to subsistence and high-flow conditions. Gut fullness and health (i.e., hepatic-somatic index, Fulton condition) can be measure over longer temporal scales to assess benefits of flow pulses to fish fitness.

A summary of the daily otolith investigation requires more of a literature based description as limited samples were collected during this study. Additionally, the limited sample size in the GSA basin resulted in no specific recommendations for this component in that basin. However, the description for the Brazos River is included below to show an example of a direct ecological linkage to flow.

Based on literature, to maintain a stable local population, pelagic broadcast-spawning cyprinids either need to spawn during intermediate-magnitude flow pulses that limit downstream transport of larvae, or else undergo upstream movements during the juvenile and/or adult stage to balance downstream drift of larvae, the latter being much more energetically expensive (Medley et al., 2007). Since flow pulses tend to be brief in prairie rivers (Hoagstrom and Turner, 2013), this explains the tendency for species in this reproductive guild to initiate spawning on the rising limb of a flow pulse (Medley et al., 2007), much like the pattern described by Rodger's (2015) study of Shoal Chub recruitment in the lower Brazos River. Spawning during short-lived flow pulses of moderate magnitude probably facilitates retention of drifting propagules in nearby nursery habitats following pulse subsidence (Medley et al., 2007; Widmer et al., 2012; Hoagstrom and Turner, 2013), which would reduce requirement for long upstream migrations by survivors to replace individuals displaced downstream. Based on a significant, non-linear, quadratic relationship between discharge magnitude and the number of Shoal Chubs recruits obtained by Rodger (2015), our best current assessment is that flow pulses of moderate magnitude promote highest recruitment of Shoal Chubs in the lower Brazos River.

Riparian

Within the Brazos basin, only 17 of 130 mature riparian distributions were inundated at 80% or more by TCEQ flow tiers (Table 36). Although the TCEQ flow standards do not include 1/year flows, the BBEST-recommended flows provided inundation in 13 of 13 tests. For most species at most sites, there is an apparent lack of correlation between distribution and TCEQ flow standards. When individual sites were combined, ten of 11 tests of TCEQ flows vs. seedling distribution were supported (i.e., the observed seedling distribution could be explained by one or more known TCEQ flow tiers), while 1 of 11 was inconclusive (primarily because so few flows occurred). Eleven of 11 tests of actual flow vs. seedling distribution were supported (i.e., all seedling distributions could be linked to at least one known flow). Tests of seedling survival across seasons in response to actual flows showed that 7 of 9 were supported, 2 of 9 were inconclusive. Testing of the sapling distribution in response to TCEQ flows resulted in 10 of 10 inconclusive - again so few flows occurred in 2014 that several could not be verified/disproved. Testing of sapling distributions in response to actual flows showed that 6 of 10 were supported, 3 of 10 were not supported, and 1 of 10 was inconclusive. This outcome suggests that saplings are developing greater tolerance to flow variation, likely as shoots are taller (above flood waters) and root systems are able to capture deeper water sources. When survival of saplings across seasons in response to flows was tested, 5 of 10 were supported, and 5 of 10 were not supported. This is even further evidence that the sapling life stage is less dependent of individual/within-year flows, and is an expected characteristic of the sapling stage (Middleton, 2002).

In conclusion, most of the TCEQ flow standards in the BRA did not provide for coverage of 80% or more of riparian species' distributions. SARA (2015) describes that the lower San Antonio River and Cibolo Creek locations had comprehensive instream flow studies that included a riparian component in the analysis, which was subsequently recommended by the GSA BBASC and adopted by TCEQ into the flow standards. Therefore, the TCEQ flow standards at those locations inherently meet the needs of the riparian communities. This study suggests that spring and fall are critical times particularly for the seedling stage. Without these seasonal flows not only is seed dispersal lessened/lost, but seedling germination and survival are also impacted. Although winter flows were not shown to be related to the seedling stage, they have been shown by others to be ecologically important in elevating groundwater to within tree rooting zones (Stromberg, 2001) – providing a benefit to the mature class stage, particularly in spring when trees begin leafing out. The importance of flow pulse events in the summer season is unclear. There were few linkages that could be made directly to summer flows. While summer flows have the potential to provide soil wetting for more mature age classes, newly germinated seedlings may actually face greater mortality with high/prolonged summer flows (Middleton, 2000). This area needs further attention, as this study (beginning in late summer and ending early spring) did not allow for examination of actual responses the summer season.

Table 36. **Summary of basin-wide riparian hypothesis-testing results.**

Group	Hypothesis	Hypothesis testing results	Comments
Mature tree distribution	Mature riparian distributions reflect seasonal TCEQ flow standards (and BBEST 1/year recommendation)	17/130 TCEQ flow standards (and 13/13 BBEST 1/year recommendation) tested in this basin inundate 80% or more of their species' ranges	There is an apparent lack of correlation between distribution of species and TCEQ flow standards for most species at most sites; in general, the standards fall well below riparian distributions.
Seedling distribution and survival	Seedling riparian distributions correlate with TCEQ seasonal flows	10/11 tests were Inconclusive, 1/11 failed to support	Many flows did not occur. Consequently, there were no conclusive results to compare seedling distribution with.
	Seedling riparian distributions correlate with actual flows	11/11 supported	For flows that did occur (TCEQ, BBEST-recommended, plus others recorded), seedlings correlated very closely with flow pulse inundation. For some sites, a lack of flow correlated with absence of seedling dispersal. This, too, is seen as a support for the importance of flow pulses in determining seed dispersal.
	Seedling survival through seasons correlate with flows received	7/9 supported, 2/9 inconclusive	Later flows observed to provide coverage; or a lack of coverage correlating with loss of seedlings
Sapling distribution and survival	Sapling distributions correlate with TCEQ/BBEST-recommended seasonal flows	10/10 inconclusive	Many flows did not occur. Consequently, there were no conclusive results to compare sapling distribution with.
	Sapling distributions correlate with actual flows	6/10 supported; 3/10 failed to support; 1/10 inconclusive	Suggests that saplings are less dependent on seasonal flows, as their distributions often reflected flow effects from several years prior, or appeared independent of any known current flows. The one inconclusive had too few saplings to determine a relationship.
	Sapling survival through seasons correlate with flows received	5/10 supported; 5/10 failed to support	Suggests that sapling life stage is less dependent on individual seasonal flows.
Riparian community	Riparian species show high relative abundance	4/5 supported; 1 failed to support	Overall average = 69. %. Range = 45 - 90%
	Age distributions detect the effect of major anomalies in flow	Seedlings: 5/5 supported; Mature trees: 5/5 inconclusive	Low seedling counts tended to strongly reflect 2014 drought conditions. Unfortunately, there were too few older trees to draw conclusions about past flows. A study that intensely samples mature trees (outside of a transect plot design) would better address this.

Many of the sites showed evidence of replacement only in the near-stream reaches because of low-flow conditions in 2014. This is a good example of what the future holds if flows are managed at 2014 levels. Droughts are a cyclic occurrence but human diversion is not. Even though the plants do show some resiliency against a lack of flows - otherwise die-backs could have been more severe, 2014 gave us an excellent view of how a lack of flows affects riparian reproduction and survival.

In order to provide riparian maintenance at the current riparian spatial distributions the existing TCEQ flow standards (spring and fall) would need adjustment. Otherwise, if future flow magnitudes are removed, the riparian zone width may face constriction in almost all cases. Management decisions should consider carefully the potential ecological loss of this important ecotone. Based on the spatial distribution of species across the basins, general flow needs for each reach can be determined, and are given here as a reference (Table 37). Even though the BBEST 1/year recommended flows provided adequate inundation of most species, this flow too is lacking in that it has no particular timing associated with it. In light of this study not only is magnitude important, but so too is timing. At a minimum, it is recommended that a 1/spring and 1/fall event be implemented for those reaches currently lacking these flow magnitudes and possibly a 1/summer, as well, though future research extending across the full growing season is necessary to verify the benefit of this pulse (Table 37).

Table 37. **General flow needs for each reach based on the distribution of currently present riparian species in the GSA and BRA basins.**

Site	Highest Elevation Indicator Species	Distribution (meters)	Elevation (m) 100%	Elevation (m) 80%	CFS 100%	CFS 80%
Blanco	Box Elder	6-40	5.7	5.3	27800	24100
Goliad	Green Ash	3-10	4.2	4.1	3334	3171
Gonzales	Box Elder/Green Ash	18-20	6.2	6.2	6058	6000
Guadalupe	Box Elder	14-24	5.6	5.1	18300	15700
Medina	Box Elder	0-60	3.8	3.0	1227	583
Victoria	Box Elder/Green Ash	4-90	5.3	4.4	6630	4743
Brazos Bend	Box Elder	15-40	7.4	6.1	20903	15359
Hearne	Black Willow	24-32	5.5	5.1	11598	9471
Leon	Green Ash	4-34	4.9	4.6	4080	3794
Little River	Green Ash	10-46	6	5.6	13584	12482
Marlin	Box Elder	18-26	4.75	4.5	16067	15174

This study showed a difference in how life stages were affected, with seedlings appearing to be most detrimentally affected by a general lack of flows. This is as expected, as saplings seemed to have some resiliency to lack of flows (though not complete immunity). Again, this supports previous studies of resilience by life stages. Mature trees were more resilient, though some were lost during the study (likely due to the prolonged lack of flows at some sites) and observations were made that many more mature trees had recently perished. The tree coring study did not provide a large enough sample size for long-term flow comparisons; however, more intensive sampling in the reaches may supplement the current work. This indicates that the seedling class is the best indicator for within-year riparian responses to flows, mature trees are better indicators of long-term flow responses, and saplings are useful for indicating flow responses over the past 5 to 10 years.

Seedling dispersal/germination is an excellent methodology for short-term frequent monitoring as well as a tool for providing a "snap shot" view of a current riparian's health/status. In most cases the flows received either in spring or the previous fall dictated a season's seedling distribution. Survival through the season was more difficult to track – many other variables affect survival (e.g., herbivory, trampling, rainfall, etc.) and the relationship to flow is more difficult to detect, except in the cases of severe lack of flows. But the very strong ecological linkage between flow inundation and seedling distributions makes for an excellent indicator of seasonal flows' effects on the early life stage. The increased resiliency of saplings is a characteristic that gives a little longer-term view of riparian functioning. Aging of saplings in addition to measuring their distributions gives a glimpse into recent, though not immediate, flow effects.

The strong resiliency in mature trees results in less connectivity to direct/individual flows. Instead their ecological linkage value lies in providing a long-term glimpse into riparian health and maintenance at the scale of decades. Age classes in this study did not provide enough data to draw strong conclusions about specific past flow events. However, more intensive sampling in these reaches would provide a more comprehensive age class structure that when used over time may provide valuable information of the long-term maintenance and functioning of the forest. And finally, now that an initial relative abundance has been calculated for each reach, it offers a baseline for future comparisons. This provides an ecological linkage to future flows in that a reduction of high-flow pulses may result in less riparian species and more encroachment by upland species, and vice versa.

Seasonal categories were adjusted for across-basin comparisons between the BRA and GSA basins, since the Brazos basin's winter flow more directly correlates with the GSA fall category (and hence was incorporated into that season). An accounting of the across-basins analyses of flow inundations for mature tree distributions is presented in Table 38. The across basin assessment further confirms what was observed in the BRA that TCEQ flow standards (that did not have the benefit of site-specific, comprehensive instream flow studies) are insufficient (in most cases) to meet inundation of at least 80% of the existing riparian zone species on a seasonal or annual basis. If maintenance of the existing riparian zones is a focus of the BBASC or TCEQ, protection of flows such as the BBEST recommended yearly flows with an added timing component should be considered.

Table 38. **Basin-wide riparian coverage by standard flows. Very few species' distributions are being inundated by current TCEQ flow standards.**

Flow Tiers**	Total number of all species covered* by flow	Total number of species at the highest elevation covered* by flow
Baseflow	2/27	0/11
2/Winter	1/14	0/6
1/Winter	1/14	0/6
3/Spring	2/13	0/5
2/Spring	4/27	0/11
1/Spring	5/25	1/10
3/Summer	1/13	0/5
2/Summer	3/27	0/11
1/Summer	2/27	0/11
3/Fall	1/13	0/5
2/Fall	3/27	0/11
1/Fall	4/27	0/11
1/Year	25/27	9/11

* Inundation of 80% or more of the species' distribution.

** Brazos winter was included in the fall category in order to compare across basins.

Brazos Estuary

The Brazos estuary validation assessment evaluated the relationship of the USGS Rosharon gage and estuarine flow regime. 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 including cubic and quadratic functions to evaluate relationships between streamflow and flow tiers estimated from the Rosharon gage and data collected in the lower river (0-42 km). Data collected by Miller (2014) was used to partially supplement data collected during this study.

Due to the unique nature of the Brazos River estuary and the paucity of previous biological data from the lower river the recommended environmental freshwater inflow standard including flow tiers and points of compliance (gage site) was by default based on the instream flow standard recommended for the Rosharon gage (BRA BBEST, 2012). It was assumed the freshwater inflows needs of the estuary should theoretically benefit from the same recommended instream environmental flow regime including the tiers and flow frequencies (BRA BBEST, 2012.) The Rosharon gage is located in Fort Bend County, in USGS Hydrologic Unit 12070104 at latitude 29.349444 N, and longitude 95.582222 W or approximately 89 km upstream of the mouth of the river (USGS web site waterdata.usgs.gov/nwis/nwismap). The drainage area at the gage is 117,428 km^2 and the contributing drainage area is 92,652 km^2. The TCEQ defines the lower Brazos River tidal segment (1201) as extending from 100 yd. upstream of the SH 332 crossing in Brazoria County downstream to the mouth or approximately 38 km. As noted earlier, data collected within the estuarine zone was confined to the lower 42 km of the river which encompasses the tidal segment.

The TWDB provides published estimates of the combined annual freshwater inflow discharge into an estuary (Schoenbaechler et al., 2011). This estimate is calculated as the sum of the gaged discharge plus modeled runoff from the ungaged portion of the watershed below this point after adjusting for diversions and return flows. For a typical estuary, the freshwater inflow balance is calculated as:

= Combined Inflow + Precipitation onto the estuary – Evaporation from the estuary.

The TWDB however, reasons that since the Brazos River drains directly into the Gulf of Mexico there is no bay surface area from which to estimate precipitation or evaporation. Thus, the freshwater inflow balance is calculated as the combined freshwater inflow of the Brazos River including gaged flows as measured at Rosharon and ungaged estimates below this point with adjustments for permitted discharges and diversions. There are a total of 7 permitted discharges and 16 diversions located below the Rosharon gage (Schoenbaechler et al., 2011). Based on the provided illustration of the drainage area (see Figure 1, HUC unit 12002 in Schoenbaechler et al., 2011) and the definition provided in the accompanying text it actually appears that the upper boundary of estuary for the purposes of freshwater inflow estimation was actually defined as the Gulf of Mexico and not the tidally influenced portion of the Brazos River. As noted above, the TCEQ has formally defined the tidal portion of the Brazos River. In general for the purposes of water quality protection, the TCEQ define tidal waters as "descriptive of coastal waters that are subject to the ebb and flow of tides. For purposes of standards applicability, tidal waters are considered to be saltwater. Classified tidal waters include all bays and estuaries with a segment number that begins with 24xx, all streams with the word tidal in the segment name, and the Gulf of Mexico" (State of Texas, 2014a).

The TCEQ also provides definitions for river basin waters which include tidal segments, coastal basin waters which include tidal streams not associated with major rivers, bay waters and Gulf waters (State of Texas, 2014a). Furthermore the TCEQ provides definitions of "saltwater" based primarily on the observable rise and fall of the tide but also in the absence of tidal information waterbodies containing 2 ppt salinity. It should also be noted that, during summer low flows, observable daily rise and fall of the river water consistent with a tidal signature have been observed at the Rosharon gage.

Therefore the extent of 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 (Orlando, 1993; Savenije, 2005; Engle et al., 2007). We adopted a definition that, lacking a recognizable bay system, the tidal segment of the Brazos River (segment 1201) 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 also attempted to measure actual stream flow at near the upstream portion of the Brazos River tidal zone at river km 42 to assess the relationship between streamflow measured at the Rosharon gage and estimated discharges measured at the upper end of the tidally influenced portion of the river (estuarine zone).

To evaluate the potential strength of the relationships of discharge measured at the Rosharon gage and water quality and biological variables we pooled the limited data from both winter and spring periods as defined in flow standards. Based on study results we detected statistically significant relationships between discharge (cfs) measured at the Rosharon gage and resulting flow tier levels and salinity, chlorophyll- α, TSS, $N\text{-}NO_{2+3}$, TSS, TKN and TP (Figure 76 and Figure 77). The highest r^2 values were observed when quadratic or cubic linear models were fitted to discharge and ranked flow tier values versus salinity, chlorophyll-α, TSS, and $N\text{-}NO_{2+3}$. Although significant, these linear models suggested only a weak positive relationship ($r^2 < 0.5$) between discharge and/or flow tiers and the measured variables. In addition, overall dissolved oxygen levels varied between flow tiers (Table 35). In addition, weak but significant negative relationship was detected between proportion of estuarine dependent species and stream discharge at the Rosharon gage and resulting flow tiers (Figure 78). This suggests that as stream flow increases we would expect a decline in estuarine species. Discharge levels also influenced the spatial distribution (between sites) of salinity, chlorophyll-α, and estuarine nekton (Table 35). However, significant interactions between sites and flow tier and discharge were detected indicating the response of these variables did not vary in a consistent pattern between sites across all tiers. However, salinity levels were highest in the lower river along with the proportion of estuarine species (Table 35, Figure 78).

The patterns in salinity, TSS, $NO_{2+3\text{-N}}\text{-N}$, total P, and estuarine nekton appeared to conform to previously predicted relationships between these variables and freshwater inflow. However, there was a large amount of variation in values within flow tiers. We believe that this reflects the amount of variability in flow within the different tiers, which in some cases was confirmed by the better fit of models, based on actual discharge values versus flow tiers. This should be expected since flow tiers collapse the variability of multiple discharge levels into a single classification variable (flow tier). Further research is needed to evaluate the relationship and statistical properties observed between actual flow values and flow tiers and the dependent variables. In addition, we did exhaustively explore varying 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.

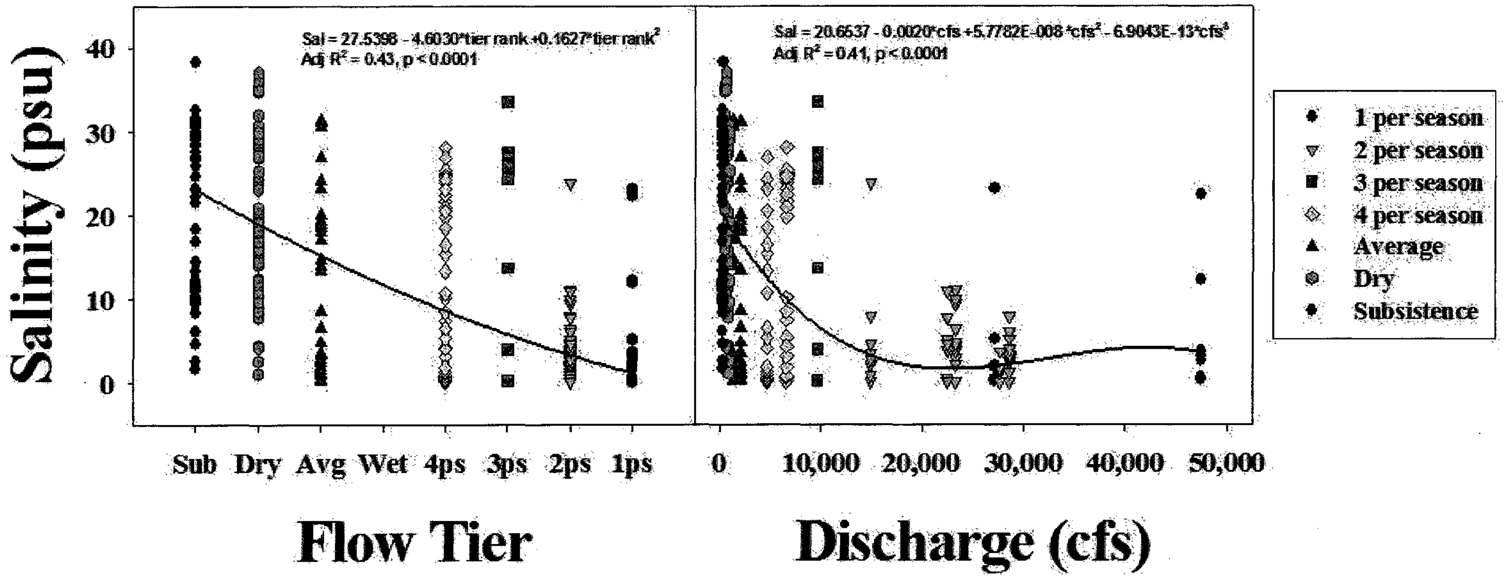
Another confounding factor that limits interpretation of data collected during this study is the lack of an entire annual period of data. Since the study did not span the entire year, we were unable to evaluate the influence of freshwater inflow during the summer (July-October) and a portion of the spring (June) season. It is important to note that, given the 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. It has been well documented that estuarine nekton exhibit significant seasonal variation in abundance and composition (Day et al., 2013;

Nelson 1992). This variation is driven primarily by the migration of sensitive juvenile stages (Able and Fahay 2010, Nelson 1992). For example, data collected during this study cannot be used to evaluate potential effects on summer nekton assemblages, which markedly differ from winter and early spring species. 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.

Another major obstacle that prevented us from surveying during May and June was the massive floods that occurred in late May through June that resulted in stream flows at the Rosharon gage exceeding 30,000 cfs during most of that time period. Based on previous experience from past studies on the Brazos River, we have established a threshold of 10,000 cfs for safety and logistical sample collection effectiveness. As of July 27, 2015, we have been unable to retrieve our depth/stage level due to high waters. Since late spring 2015 was dominated by heavy precipitation and resulting floods, this eliminated the possibility of sampling during a large proportion of the spring season.

In summary, we were able to use discharge data collected at Rosharon to initiate development of predictive models that relate environmental conditions in the estuarine zone to flow tier recommendations but not complete the task. Recommendations for future applied research and long-term monitoring of the estuary are provided in Section 5 to assist in completion of this charge.

Figure 76. Salinity (psu) values measured at the surface, middle and bottom of the river and classified by flow tier rank (TR) and discharge (cfs) for all sampling events from B42 to B01 on the lower Brazos River from Nov. 2014 – May 2015 and Jan. – Dec. 2012. The best fit linear model results and plot provided. The best fit linear model results and plots are provided.



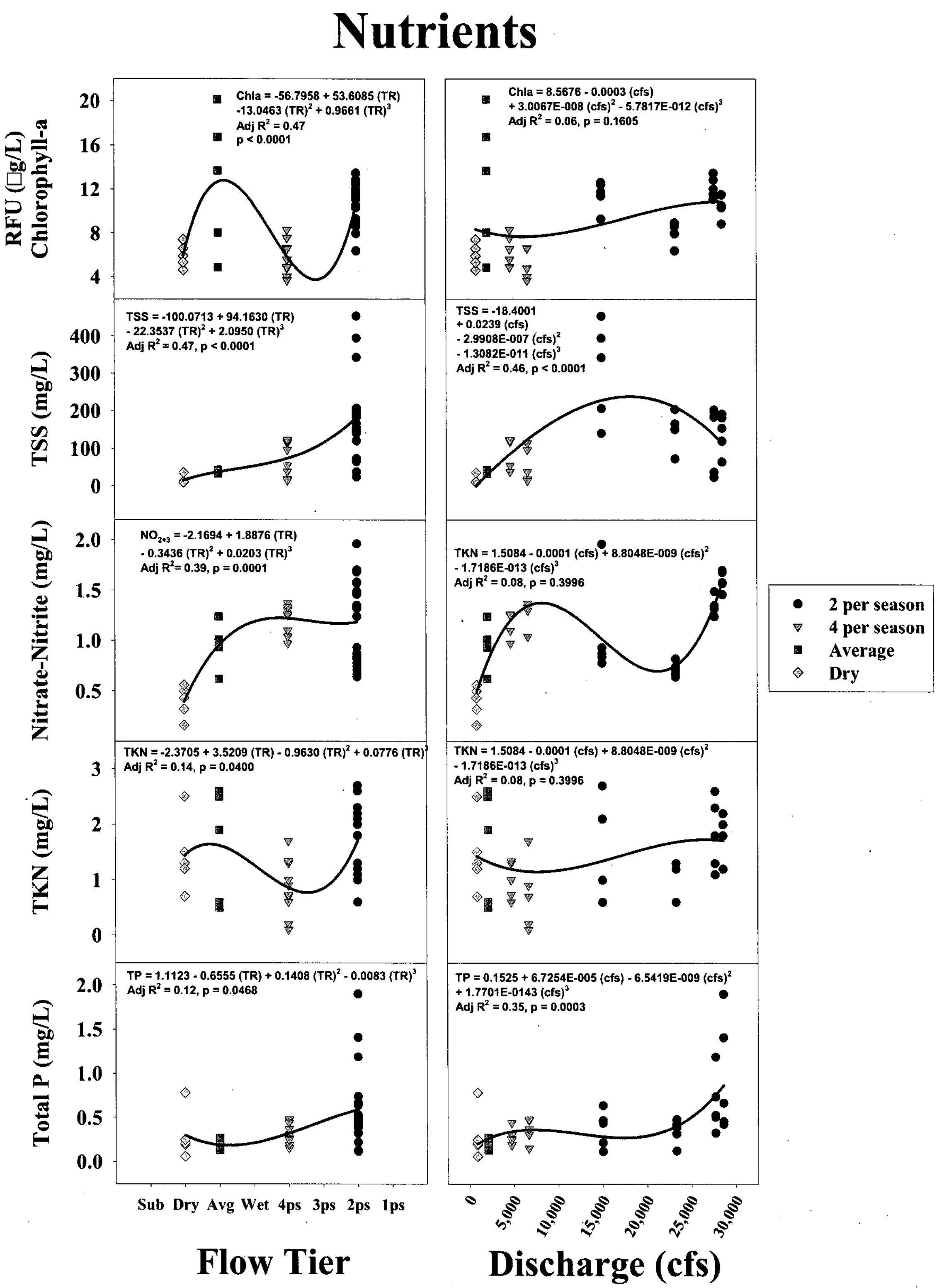
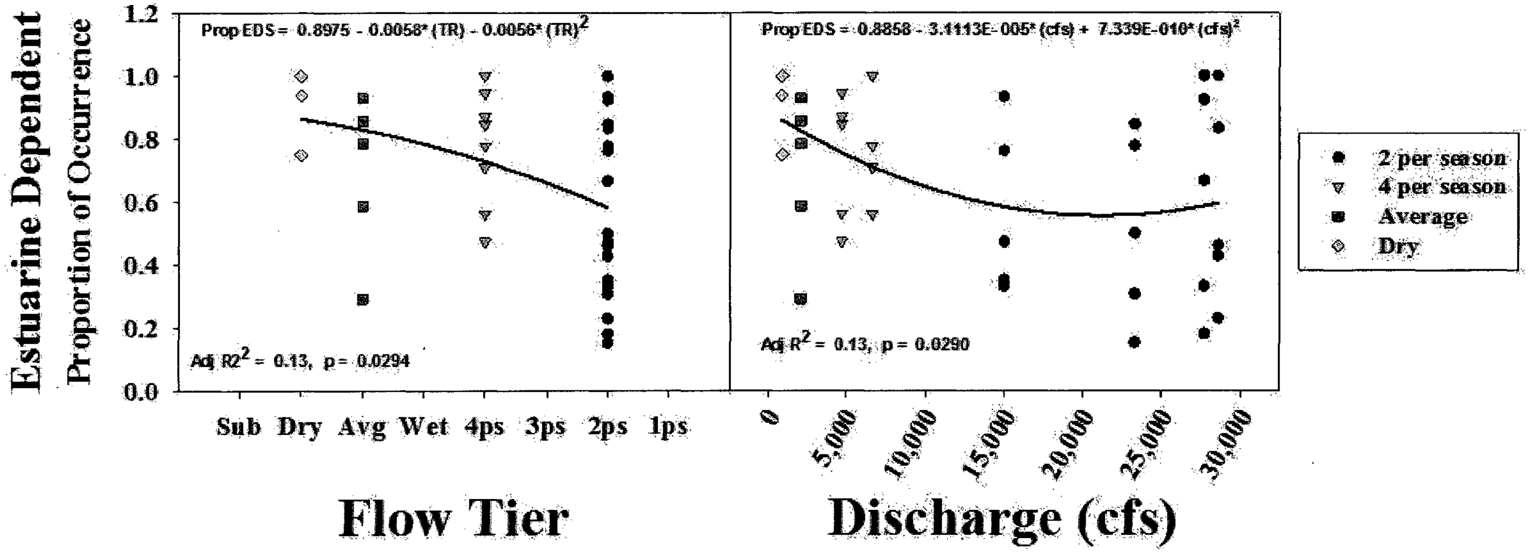


Figure 77. RFU (µg/L) chlorophyll-a, TSS (mg/L) Nitrate-Nitrite (mg/L), TKN (mg/L) and Total P (mg/L) classified by flow tier (TR) and discharge (cfs) for all sampling events from B42 to B01 on the lower Brazos River from Nov 2014 – May 2015.The best fit linear model results and plots are provided.

Figure 78. **Proportion of occurrence of estuarine dependent species classified by flow tier rank (TR) and discharge (cfs) for all sampling events from B42 to B01 on the lower Brazos River from Nov 2014 – May 2015. The best fit linear model results and plots are provided.**



4.2 Description of instream validation process development

Application of a validation methodology can occur at two different scales, each of which can provide useful information to environmental flow managers. The first is to test the TCEQ flow standards on a basin-wide scale to see if, in general, the standards meet ecological needs. The second application then could be conducted on a site-by-site basis in service of future water projects proposals in specific river reaches. Using the current protocol, whether or not a proposed water project is considered satisfactory for protection of environmental flows hinges solely on its ability to meet the TCEQ environmental flow standards. However, even though TCEQ does not currently require that permit applicants conduct site-specific studies, it is likely that BBASC members or other interested parties may consider conducting site-specific studies in an effort to inform the next round of environmental flow standard revisions. Currently, there is no standard method for collecting or assessing such information; at present this relies only on the professional judgment of the BBASC and the TCEQ. Consequently, a future application of such a validation methodology could be to standardize the assessment process for future projects. A standard methodology might be useful in the event of internal controversy amongst the BBASC. An agreed-upon methodology would also provide the TCEQ with a simplified and science-based tool for making the final decision of whether a proposed project passes or fails.

Building on the ecological components tested during this study, the following is proposed as the foundation of this methodology. We recognize that this is a first step in development of such a methodology, and therefore, a series of expert panel workshops to further refine and test this methodology is also proposed.

To answer the question, "Is the TCEQ flow standard at this site sufficient to maintain a sound ecological environment as defined by the BBASC?", a tiered approach is proposed. This tiered approach is proposed to start with the most direct ecological linkages and works through a checklist of ecological components. However, for specific SB 3 applications, each tier first starts with a question that can only be answered by the BBASC in the context of the balance between environmental and human needs. As previously mentioned, the validation approach can be conducted basin-wide or specific to individual sites. The example presented below describes an individual site evaluation.

Tier I Site Evaluation: Floodplain Connectivity

A. Does the study reach have oxbows and important backwaters or floodplain features that benefit from connectivity to the main river channel and if so, what is the BBASC goal for maintaining this ecological component?
B. If yes, and a goal is established, then proceed with the flood plain evaluation (D)
C. If no, then proceed to TIER II.
D. Floodplain evaluation is simply whether the existing TCEQ flow standards meet the connectivity requirements (water surface elevation) of important floodplain features with a reasonable frequency. This would require a field study (if elevation is not known) to determine the water surface elevation needed to connect study reach floodplain features. This would be followed by an examination of the fish community (existing information if possible or new collections if needed) for the seasonal need and review if the

timing/frequency of pulses are deemed appropriate. If flow amount or seasonal timing are deemed insufficient, then consider addition of this pulse and timing to standards.

Tier II Site Evaluation: Riparian Assessment

E. Does the study reach have important riparian habitat and if so, what is the BBASC goal for maintaining the existing (or some other) distribution of riparian species?
F. If yes and a goal is established, then proceed with the riparian evaluation (H).
G. If no, proceed to TIER III.
H. Riparian evaluation would consist of the establishment of "representative" field transects perpendicular to the stream throughout the riparian corridor within the downstream study reach. The evaluation would include the 3 indicator species described in this report along with the seedlings and mature trees life stages. Following the site visit, one would simply evaluate whether the TCEQ flow standards meet some level of inundation (goal established by the BBASC) necessary for watering and dispersal of these indicator species and life stages.

Tier III Site Evaluation: Aquatic Assessment

I. Does the study reach have important aquatic resources (endangered or threatened species, recreational or commercial fisheries, unique instream habitats, etc.) and if so, what is the BBASC goal for maintaining the current assemblage and community composition?
J. If yes, and a goal is established, then proceed with the aquatic evaluation (L)
K. If no, then your tiered evaluation is over.
L. Based on the results of this study, it is not possible to outline a defined aquatic evaluation at this time as only a few of the aquatic components tested had significant statistical relationships with flow. As such, additional data collection focused on the aquatic components that had trends but not statistical significance is recommended. Upon relationship development, it is anticipated that the aquatic evaluation would consist of a one-day field sampling effort to assess aquatic parameters (to be determined) within a representative study reach related to the relevant SB 3 gage. Following the site visit, one would simply evaluate whether the TCEQ flow standards meet the established goal for the aquatic component.

The above framework is a work in progress, and development should continue to be refined with additional data collection, proposed expert workshops, agency, BBEST and BBASC input, etc. Ultimately, when completed, the BBASC and TCEQ would have a specific, yet simplified methodology (approved upfront by each) that may require a day or two per site for field investigations, followed by desktop analysis specific to a proposed project. The analysis would include a comparison of the site-specific data to the basin-wide information on that ecological component in order to make an informed decision as to whether the flow standard is sufficient or needs potential adjustment.

The approach outlined above was used in the following section to provide examples of potential BBASC application. Being that the approach is not complete the following section is only included to provide the underlying thought process for such an assessment.

4.3 Potential application of results

Using the proposed tiered approach outlined in Section 4.2, two different sites within the Brazos basin were evaluated using data from this study. The first example involves an evaluation of the Brazos River at Rosharon. For this example, it was assumed that floodplain connectivity was deemed extremely important in the lower Brazos River and a BBASC goal was set to maintain this ecological component but not at the risk of flooding personal property. Of course, per methodology, these decisions would need to be made by the Brazos BBASC. Unlike the concurrent GSA assessment, this study did not assess floodplain connectivity, but published literature does exist for several floodplain features in the Brazos basin as described in the Brazos BBEST (2012) report. Although it is impossible to assess Tier I for this location using data only from this study; to carry forward the hypothetical example, let's assume that only flood stage levels would connect floodplain features at this location. Please be clear that this is a made up assumption just to carry the example forward, as we acknowledge that there are floodplain features in the Brazos basin that achieve lateral connection at fairly modest flow pulses as well. As our hypothetical oxbow would require overbanking flows to connect, it was discarded from consideration and the assessment progressed to Tier II.

The next step in this hypothetical example would be to answer the Tier II riparian question. For this example, the answer was that riparian habitat in the Rosharon study reach is very important but it is not vital to maintain everything that is currently there. This lead us to recommend assessing the TCEQ standards based on the amount of water necessary to inundate the riparian indicator species up to 70%, of their current distribution, rather than the recommended 80% in this report. In doing so, we acknowledged that such flows may cause shrinkage of the existing riparian community to some extent, especially if not addressed by an inter-year requirement. The TCEQ standards for Rosharon were then evaluated relative to the riparian needs for seedlings and mature trees at this location. An examination of the data from Section 3 shows that the existing TCEQ flow standards at Rosharon meet the requirements (both in volume and timing) for the riparian indicator species present and life stages evaluated. Thus, the TCEQ flow standard for the lower Brazos River at Rosharon passes the Tier II test for this hypothetical example.

Being that Tier III is not yet established, it is impossible to incorporate it in to this exercise. However, assuming the results from the aquatic assessment of this study are supported over time and that frequent, yet smaller seasonal pulses are not critical to the aquatic component of the in channel environment, then the following discussion could be held. In this example Tier I and Tier II needs were met by existing TCEQ flow standards with spring and fall prescribed events. Tier III hypothetically showed no ecological relationships. In this example, the BBASC may consider eliminating some of the frequency of those lower flow pulses because no ecological linkage had been established. Again, this section is only provided to stimulate discussion. We also reiterate that Tier III data collection is incomplete at this time, and that other considerations such as sediment transport and channel maintenance are not currently included in this proposed tiered approach.

To provide a second example, an evaluation of the Leon River site at Gatesville was conducted. For this example, no hypothetical answers to the initial questions posed to the BBASC are provided. Tier I is straightforward as there are no floodplain features as defined so no Tier I evaluation is conducted. In the Tier II riparian assessment at this site, the evaluation gets interesting in that none of the TCEQ flow standards during average conditions meet the requirements to achieve any riparian zone inundation. Therefore, should the answer to the Tier II question on riparian importance be extremely valuable, a BBASC discussion would need to occur regarding the potential increase in the volume of water assigned in the existing TCEQ flow standard or inclusion of an inter-year requirement with a higher volume to meet those environmental needs. In this example, the same hypothetical discussion could be held for Tier III as presented in the last example. Although, spring and fall flow standards may need to be increased to meet riparian needs, the frequency of smaller seasonal pulses might possibly be reduced. Again, these are just examples of how the BBASC could use this methodology for evaluation of existing TCEQ flow standards.

At present, Tier I (literature-based evaluations) and Tier II desktop evaluations could be conducted by the Brazos BBASC at each of the sites that were evaluated during this study because the field work for Tier II has already been conducted. However, the first question for each Tier must be also answered *a priori* by the BBASC. The proposed Tier III validation methodology is currently incomplete due to the lack of quantifiable aquatic responses to flow tiers tested during this study, so it cannot be evaluated at this time. Additional data is needed (as described in Section 5) before aquatic responses or lack thereof can be formally considered in such an evaluation. A site-by-site evaluation of each of the study sites is not presented in this report, but, as noted, could be conducted for Tier I and Tier II should the BBASC feel this is a useful exercise. Ultimately, while one would not want to make formal validation judgments based on preliminary information, this prospective approach, coupled with the preliminary indications offered by the aquatic assessment, does suggest that adjustments to the TCEQ standards (possibly in both directions) may be in order, depending on the specific sites and applicable flow standards.

Based on this study and our professional judgment, it is likely that adjustments for consideration may involve:

- increases or decreases in volumes needed in spring and fall pulses for either floodplain connectivity or maintenance of the existing riparian communities;
- adjustment in timing of seasonal pulses in conjunction with volume to meet the ecological needs of a certain ecological components (i.e., consideration of adding in the BBEST 1/per year event (at some sites) which is not in the standards but put it in with a seasonal component rather just an annual requirement);
- inclusion of an inter-year riparian pulse requirement; and
- a reduction in the frequency of some seasonal pulses if no ecological linkages become evident.

During the expert panel workshops proposed, other ecological components for testing or inclusion in the validation methodology may surface, possibly resulting in the eventual inclusion of additional Tiers for evaluation. Two such considerations that received considerable discussion by the project teams in the course of these studies are (1) the temporal needs of flows for riparian zones and (2) the incorporation of some type of sediment transport/channel maintenance component into the tiered structure. The first involves scientifically justifying the frequency needed for riparian inundation. If an indicator species lives for 20 years, there may be interest in better understanding how many years it requires inundation throughout its distribution in order to maintain its distribution over time. While it is possible to make educated guesses toward this end (e.g., strict yearly inundation is likely not required), we simply do not have the answer to this question yet. Additionally, we currently lack evidence to support the stance that allowing flows on a generally infrequent, less-than yearly basis, would suffice for maintaining this ecological linkage. The second consideration involves sediment transport and channel maintenance, which we acknowledge are critical components to maintaining the existing ecological community. Current literature suggests a large portion of channel forming occurs during major events which are beyond the scope of TCEQ flow standards. However, literature also suggests a dual mode of sediment transport, with some level of lower flows moving a significant amount of material through the system. In our professional judgment, it is these lower pulses that need further attention. For instance, although the ecological linkage to flow from the aquatics didn't materialize (so far) for these lower pulse events (in this study), maybe these events are controlling the habitat necessary for these species and over time (not instantly) changes in community structure for fish and/or macroinvertebrates would start to occur. That point highlights the importance of further applied research and the establishment of long-term monitoring at select locations which are the topics of the next section.

5 Recommendations for future applied research or long-term monitoring

This study has been a great first step at addressing real questions and concerns raised during the SB 3 process. However, we acknowledge that more work needs to be done to get to a usable endpoint for the BBASC and TCEQ. This section describes recommendations for additional focused research as well as the establishment of select locations for long-term monitoring. It is important to first clarify the difference between applied research and long-term monitoring upfront. Focused applied research (as conducted in this study) is needed 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. Long-term monitoring is to track ecological condition over time. However, to be informative to the SB 3 process, this long-term monitoring needs to be set up in a way to "validate" the short-term answers over time. Time may be in intervals of 5, 10, or 20 years, etc. It is also important to acknowledge upfront that any long-term monitoring and further research are subject to availability of funding.

Each component addressed in this study needs some combination of focus applied research and long-term monitoring moving forward, but each with a different balance. An initial overview of that balance is provided in the next paragraph followed by recommended applied research and long-term monitoring consideration per ecological components in the following sections. It is also recommended that a floodplain feature assessment be incorporated into the Brazos basin studies where published literature is not sufficient to answer the connectivity question.

The aquatics component needs to be heavy on applied research with a few reference sites to start long-term monitoring. The applied research would again focus on documenting baseline conditions and sampling after flow pulses over the course of the study. As aquatic components are quite dynamic, it is recommended that long-term monitoring occur at least annually in the spring, with an additional trip considered during hot summertime temperatures. We recommended that riparian applied research focus on opportunistic conditions (i.e., 2015 flooding) and to evaluate important BBASC sites not covered in this study. It is also recommended that a few representative sites be selected to track riparian conditions over time. Select Brazos River sites from this study are highlighted as potential long-term sites because of the initiated sampling record. If resources are limited, riparian long-term monitoring could be done at a longer temporal interval, say every other year, or every five years. Applied research for oxbows is recommended but only for those that the BBASC specifically might have an interest in that have not been studied to date. Long-term monitoring of select floodplain features on an annual or even every other year sampling to assess over time will be invaluable in determining if the TCEQ flow standards maintain the ecological function anticipated in the floodplain feature. Finally, it is recommended that continued focused applied research in the Brazos estuary be continued to inform the validation comparison to river flows at the Brazos River Rosharon site.

Aquatic

Focused applied research for the aquatic component will build off the extensive work conducted in 2014/2015. Further refinement of the experimental design is recommended. Represented flow tiers are proportionate to the specific magnitude at each site, which allows replication among flow tiers. Yet, a major question still remains. Do these magnitudes influence and affect stream community structure similarly along a longitudinal gradient? Lowland sites on the main stem (i.e., Hempstead and Rosharon; Cuero and Goliad) versus upper main stem or tributaries (e.g., Little River and Leon River; Comfort and Bandera) should be sampled with greater frequency and longer observation periods. This approach will provide greater understanding on how flow magnitudes influence stream communities within a lower gradient reaches (lowland sites) and higher gradient reaches (upstream sites) and the validity of combining low and high gradient reaches to achieve adequate replication.

Assignment of macroinvertebrates to a flow category is also in need of refinement. Macroinvertebrate orders were assigned to flow categories based on available literature, but information is obtainable to assign flow categories for families and genera of macroinvertebrates in the BRA and GSA drainages. Assignment at the families and genera to a flow category will improve the resolution to detect biotic responses to flow tiers, if differences exist.

Flow duration is another component of the standards and BBEST/BBASC recommendations and in need of applied research assessment. Based on preliminary calculations, durations were not met for any of the flow pulses observed during this study, except for the large flood that occurred in May. Future work would include abiotic and biotic responses to specific flow tiers but with duration met or not.

Additional applied research studies could be conducted to assess the mechanistic relationships between flow pulses (or subsistence flows) and community structure. Physical displacement of slack-water species downstream and nutrient pulses necessary for macroinvertebrates and fishes following high-flow pulses are supported with literature but additional projects, both observational and manipulative, can further refine the causal relationships between flow tiers and aquatic communities.

Biomonitoring will be necessary for two reasons: (1) aquatic community responses to a specific flow tier was variable, per our one year's worth of data; additional collections (and, consequently, a larger number of replicates and greater statistical power) will help to control the variability for the flow tiers quantified to date, and (2) sample size of most flow tiers (e.g., subsistence, 4/season, 3/season) were insufficient. Given that more samples at a site would help control variability, we suggest reducing the total number of sites surveyed but increase frequency of collections. Increased sampling frequency at few sites could also provide the resolution necessary to assess the mechanistic relationship between flow tiers and aquatic community responses. In addition, other habitat types (i.e., deep pools, deep runs, and backwater habitats) could be monitored at a site to help elucidate macroinvertebrate and fish movement patterns following a flow pulse (e.g., fish displaced from riffle but only moved a short distance downstream into a flow refuge habitat). Another major component for long-term monitoring is to create and refine an Index of Biological Integrity (IBI) specifically for instream flows. Our current assessment of flows is categorized into slack-water, fluvial, and swift-water or riffle associated macroinvertebrates and fishes. Creating a specialized instream flow IBI would allow us to assess streams that have environmental flow standards to determine the "health" of stream as surface water withdrawals becomes more prevalent. Developing and testing an IBI "Water Quantity" approach would enable a simplified biomonitoring technique, which could be executed by river authorities and TCEQ in the same way IBI Water Quality approach is used today.

Riparian

The methodology developed here for testing life stage responses to flow pulses would work well as a focused applied research study. By taking a quick survey of the riparian width, and count/spatial distribution of the three age classes (seedling, sapling, mature) of riparian indicator species a river manager can discern much about the health and status of the riparian zone, from the immediate/recent flow pulsing to longer term water inundation into the site. It also serves well in long-term monitoring, as a comparison of any given site using these techniques to the flow standards will allow a quick analysis of projected riparian persistence and guide managers in long-term management.

It is recommended that one or a few select sites be chosen for continued monitoring so that the methodology can be further validated and refined. On the Brazos basin, the Hearne, Little River and Brazos Bend sites would be excellent candidates for continued monitoring. Several additional sites from this study could then be scheduled in every 2 to 5 years for follow-up monitoring.

One limitation of this study was the extremely truncated time period, compounded with severe flooding that prevented much of the spring data from being collected. Because flows were so excessively low in 2014, it made correlations of on-site logger flows to USGS flows less reliable (there weren't large flows to calibrate with). To improve upon this, and better ensure that estimated inundation elevations are truly reflective of actual inundations, a longer study (with greater diversity in natural pulses) is highly recommended. This would also lend much more credence to information on flow coverages. Additionally, because the study time period did not span across summer seasons, little could be said about this season, and the flows within. Future studies would do well to incorporate this critical stage.

Following the spring 2015 floods, this would be an excellent time to begin a re-establishment study post-disturbance. Floods are the major disturbance regime for riparian zones, and

May/June 2015 provided an excellent example of a large-scale disturbance. Such a study might ask: "How does this large-scale disturbance affect diversity, and what are the successional stages? Do invasive species have greater advantage in establishment? What is the general time scale for recovery in this system?", and other such questions. A host of ecological linkage questions could potentially be addressed in such a study. Although all sites were affected, on the Brazos basin, the Marlin site experienced extensive rearrangement of river morphology, and a new sandbar was placed in the previously steeply sloped zone.

Another future effort that may eventually provide insight into flood pulses would be to study duration of inundation. For example, willow species are not only dependent on flow pulses, but also susceptible to desiccation from too-rapidly declining water levels. When regulated rivers draw flood pulses down too quickly, survival of first year seedlings rapidly decline. (Stella et al., 2010). A limitation of this current study was that only flow pulse frequency/magnitudes were tested, not regression times. Future studies may incorporate this.

Floodplain connectivity

Although connection of floodplain features provides support for high-flow pulses, exact connection discharge magnitudes should not be interpreted as static pulse flow goals given the assumptions of the analysis. For the purposes of a Brazos basin analysis, it would likely be assumed that connection of these habitats is static, and does not change through time. In reality, erosional and depositional processes occurring during each high-flow pulse event potentially modify the control point of each floodplain lake by scouring or depositing sediments. This is particularly true for large flood events that move the most sediment and have the greatest influence on channel migration. As oxbows and floodplain features age, they typically become more isolated and farther from the active river channel. However, occasionally the river meanders back to reconnect ancient floodplain features. The dynamic nature of these processes result in a continually changing floodplain environment within lowland river systems. Maintaining such a dynamic and active channel that interacts with floodplain habitats should be the goal.

Data from floodplain areas within the Brazos basin could certainly strengthen the analysis started in the GSA basin (SARA 2015). That analysis was based on data collection at seven of the 24 potential GSA sites identified from a desktop review. Additionally, repeat sampling data from a select few sites could be even more beneficial than data from additional sites. Therefore, the project team recommends a two-component long-term floodplain monitoring plan within the Brazos basin that focuses on: (1) intense seasonal biomonitoring (focused applied research) at a select few sites to evaluate specific community responses to connection events, and (2) long-term monitoring of additional sites to ensure active floodplain habitats remain combined , as detailed below:

Component 1 – Focused Applied Research.

1. Frequency: Seasonally for 2-3 years.
2. Location: 2-3 select floodplain lakes within the basin.
3. Data Collected: Seasonal and post-pulse biological collections.

Component 2 – Long-term habitat persistence evaluations.

1. Frequency: Once every five years.
2. Location: 5-10 random floodplain features. Sites will not necessarily be consistent.
3. Data Collected: Connection discharge/frequency, and fish community data.

Brazos Estuary

Best use of the estuary models described above would involve conducting future sampling to assess conditions within the lower estuary across all seasons and flow tiers; therefore, increasing the number of samples used to populate these predicted models. Once abiotic and biotic responses are more fully understood, environmental flow recommendations can then 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. Future research should focus on several aspects 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. Additional water quality monitoring and data collection is needed to evaluate and better define the response of salinity and vertical density stratification to varying discharge throughout the lower tidal portion of the Brazos River. The upstream extent, latitudinal gradient, and vertical change in salinity associated with the pycnocline affects multiple water quality and biological attributes including the probability of hypoxia and formation of barriers to movement of juvenile estuarine organisms. Future assessment and monitoring should employ both intensive surveys and the deployment of data sondes at multiple depths to characterize the dynamics of the lower estuarine vertical pycnocline in response to varying flow. Additional water quality data is also needed for the months not sampled during this study including a focus on nutrients, TSS, and chlorophyll-α and other algal pigments. Since the discharge of the Brazos River directly affects the nearshore Gulf of Mexico additional consideration should be given to evaluating fluctuations in water quality variables near the mouth of the river and nearshore Gulf of Mexico. Hydrological and water quality characterization should incorporate sampling for suspended solids which is needed to evaluate sediment transport and maintenance of the Brazos River delta. One of the major functions of freshwater inflow is the maintenance of the delta at the mouth of the river, which would otherwise erode away.

Additional biological monitoring similar to what was deployed during this study should be continued for at least 1 and ideally 2 years to capture and describe the complete annual cycle of biological communities that utilize the lower rivers and their respective response to varying flow regimes and the adopted flow tiers. This biological monitoring should include both nekton, zooplankton/ichthyoplankton, and algal pigments. Sampling should be conducted at the same frequency used in this study but also include intensive surveys to evaluate the influence of lunar tides over a 24-48 hour period during subsistence through moderate flow tier conditions. Furthermore stable isotope analysis and age growth analysis should be conducted to determine the relative importance and potential contribution of upstream nutrients versus marine sources to estuarine organisms including the influence of flow regime on the recruitment, survival, growth and production of nekton in the lower Brazos River estuary.

Expert panel workshops

As previously discussed, we recommend a series of expert panel workshops be conducted with the next round of legislative funding. The ultimate goal of the workshops will be to refine and finalize a validation methodology and engage scientists and stakeholders throughout the development process. We envision a series of three individual workshops over the first year of funding. The first workshop would be conducted soon after the formal award of a contract with the intent of discussing this report, introducing the validation methodology, and soliciting feedback on other considerations for inclusion in focus applied research and long-term monitoring. For example, participants may feel the methodology would benefit from other physical or biological components such as channel maintenance or freshwater mussel evaluations, for example. Discussion and incorporation of ideas aimed at strengthening the scientific validity of the validation approach as well as gaging and establishing BBASC support will be important during this early phase. Approximately 6 months in to the next round of data collection, we propose a second expert panel workshop aimed at further development of the tiered validation methodology. Following this workshop, a brief memorandum will be generated and circulated amongst participants for them to continue formulating ideas during the data collection phase. A third and final workshop is recommended approximately 1 year in to the process to finalize the validation methodology. Following this workshop, a formal memorandum would be prepared that documents the methodology. This documentation will be submitted to the Brazos BBASC and TCEQ for discussion and consideration for possible adoption.

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APPENDICES

Appendix A: Riffle habitat summary statistics taken by flow tiers (1-7) from August 2014 – May 2015.

	Tier 1					Tier 2				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Riffle										
Area (m^2)	248	83	13	70	97	2,692	90	36	39	193
Tier (1 = subsistence; 7 = 1 per year)										
Peak Flow (cfs)		102	164	6	292		223	274	4	937
Season										
Summer	1					9				
Fall	1					9				
Winter	1					11				
Spring	0					1				
Water Temperature (°C)		17.6	12.5	7.8	31.7		17.9	8.1	7.8	32.3
Dissolved Oxygen (mg/l)		9.9	1.0	8.9	10.8		10.4	2.3	6.0	15.9
Specific Conductance (µS/cm)		556	22	535	578		705	422	248	1881
pH		7.86	0.37	7.59	8.28		7.90	0.44	6.90	8.84
Current Velocity (m/s)		0.47	0.33	0.27	0.86		0.63	0.26	0.12	1.27
Depth (m)		0.13	0.21	0.08	0.23		0.23	0.33	0.08	0.46
Vegetation (%)		0	0	0	0		16	20	0	80
Substrate										
Silt (%)		0.56	0.96	0.00	1.67		1.86	4.72	0.00	20.00
Sand (%)		13.61	3.76	10.00	17.50		13.95	12.67	0.00	46.67
Gravel (%)		44.72	6.47	37.50	50.00		46.42	19.82	8.33	80.00
Cobble (%)		40.00	5.00	35.00	45.00		30.79	28.49	0.00	90.00
Boulder (%)		0.56	0.96	0.00	1.67		1.31	4.61	0.00	25.00
Bedrock (%)		0.00	0.00	0.00	0.00		3.81	12.70	0.00	61.67
Embeddedness (0 = low; 1 = high)		0.17	0.29	0.00	0.50		0.19	0.30	0.00	1.00

			Tier 3					Tier 4		
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Riffle										
Area (m^2)	147	73	11	66	81	221	110	24	93	127
Tier (1 = subsistence; 7 = 1 per year)										
Peak Flow (cfs)		1,259	977	568	1,950		149	11	141	156
Season										
Summer	1					0				
Fall	0					2				
Winter	1					0				
Spring	0					0				
Water Temperature (°C)		25.1	7.4	19.9	30.3		20.8	0.5	20.4	21.2
Dissolved Oxygen (mg/l)		6.9	0.1	6.8	7.0		7.5	0.7	7.0	7.9
Specific Conductance (µS/cm)		491	128	400	582		902	572	497	1306
pH		7.72	0.22	7.56	7.87		7.70	0.14	7.60	7.80
Current Velocity (m/s)		0.80	0.01	0.79	0.81		0.33	0.09	0.26	0.39
Depth (m)		0.29	0.40	0.21	0.37		0.12	0.20	0.06	0.18
Vegetation (%)		33	47	0	67		15	21	0	30
Substrate										
Silt (%)		0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00
Sand (%)		20.83	1.18	20.00	21.67		6.67	4.71	3.33	10.00
Gravel (%)		55.00	28.28	35.00	75.00		50.83	1.18	50.00	51.67
Cobble (%)		24.17	27.11	5.00	43.33		24.17	22.39	8.33	40.00
Boulder (%)		0.00	0.00	0.00	0.00		8.33	11.79	0.00	16.67
Bedrock (%)		0.00	0.00	0.00	0.00		10.00	14.14	0.00	20.00
Embeddedness (0 = low; 1 = high)		0.17	0.24	0.00	0.33		0.00	0.00	0.00	0.00

			Tier 5					Tier 6		
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Riffle										
Area (m^2)	885	98	37	71	193	1,012	84	39	44	189
Tier (1 = subsistence; 7 = 1 per year)										
Peak Flow (cfs)		997	882	226	2,410		2,042	2,529	193	9,570
Season										
Summer	3					4				
Fall	5					2				
Winter	1					2				
Spring	0					4				
Water Temperature (°C)		20.5	5.9	10.8	29.5		22.5	5.7	12.7	30.2
Dissolved Oxygen (mg/l)		9.3	2.6	6.6	15.2		7.8	1.2	6.1	9.8
Specific Conductance (µS/cm)		788	479	498	1810		718	253	429	1219
pH		7.68	0.40	7.00	8.15		7.95	0.32	7.35	8.34
Current Velocity (m/s)		0.70	0.28	0.22	1.10		0.55	0.24	0.00	0.95
Depth (m)		0.33	0.48	0.15	0.64		0.28	0.38	0.15	0.50
Vegetation (%)		18	25	0	70		12	16	0	43
Substrate										
Silt (%)		0.63	1.27	0.00	3.33		1.94	6.74	0.00	23.33
Sand (%)		12.69	9.30	0.00	30.00		7.74	9.52	0.00	31.67
Gravel (%)		52.56	24.60	10.00	76.67		32.92	15.06	6.67	60.00
Cobble (%)		23.10	26.98	1.00	72.50		48.55	23.33	3.33	78.33
Boulder (%)		1.78	4.97	0.00	15.00		5.44	14.33	0.00	50.00
Bedrock (%)		9.24	20.73	0.00	61.67		3.33	11.55	0.00	40.00
Embeddedness (0 = low; 1 = high)		0.24	0.34	0.00	1.00		0.22	0.33	0.00	1.00

			Tier 7		
	N	Mean	SD	Min	Max
Riffle					
Area (m^2)	440	88	15	76	109
Tier (1 = subsistence; 7 = 1 per year)					
Peak Flow (cfs)		8,354	4,685	3,220	15,600
Season					
Summer	0				
Fall	1				
Winter	0				
Spring	4				
Water Temperature (°C)		22.1	4.2	14.9	25.0
Dissolved Oxygen (mg/l)		7.6	0.9	6.9	9.1
Specific Conductance (µS/cm)		695	277	352	1053
pH		7.70	0.37	7.28	8.19
Current Velocity (m/s)		0.58	0.18	0.36	0.79
Depth (m)		0.33	0.44	0.21	0.50
Vegetation (%)		13	30	0	67
Substrate					
Silt (%)		5.33	11.93	0.00	26.67
Sand (%)		19.00	11.64	0.00	30.00
Gravel (%)		38.33	27.44	0.00	70.00
Cobble (%)		16.67	22.61	0.00	53.33
Boulder (%)		4.00	7.23	0.00	16.67
Bedrock (%)		16.67	37.27	0.00	83.33
Embeddedness (0 = low; 1 = high)		0.20	0.18	0.00	0.33

Appendix B: Run habitat summary statistics taken by flow tiers (1-7) from August 2014 – May 2015.

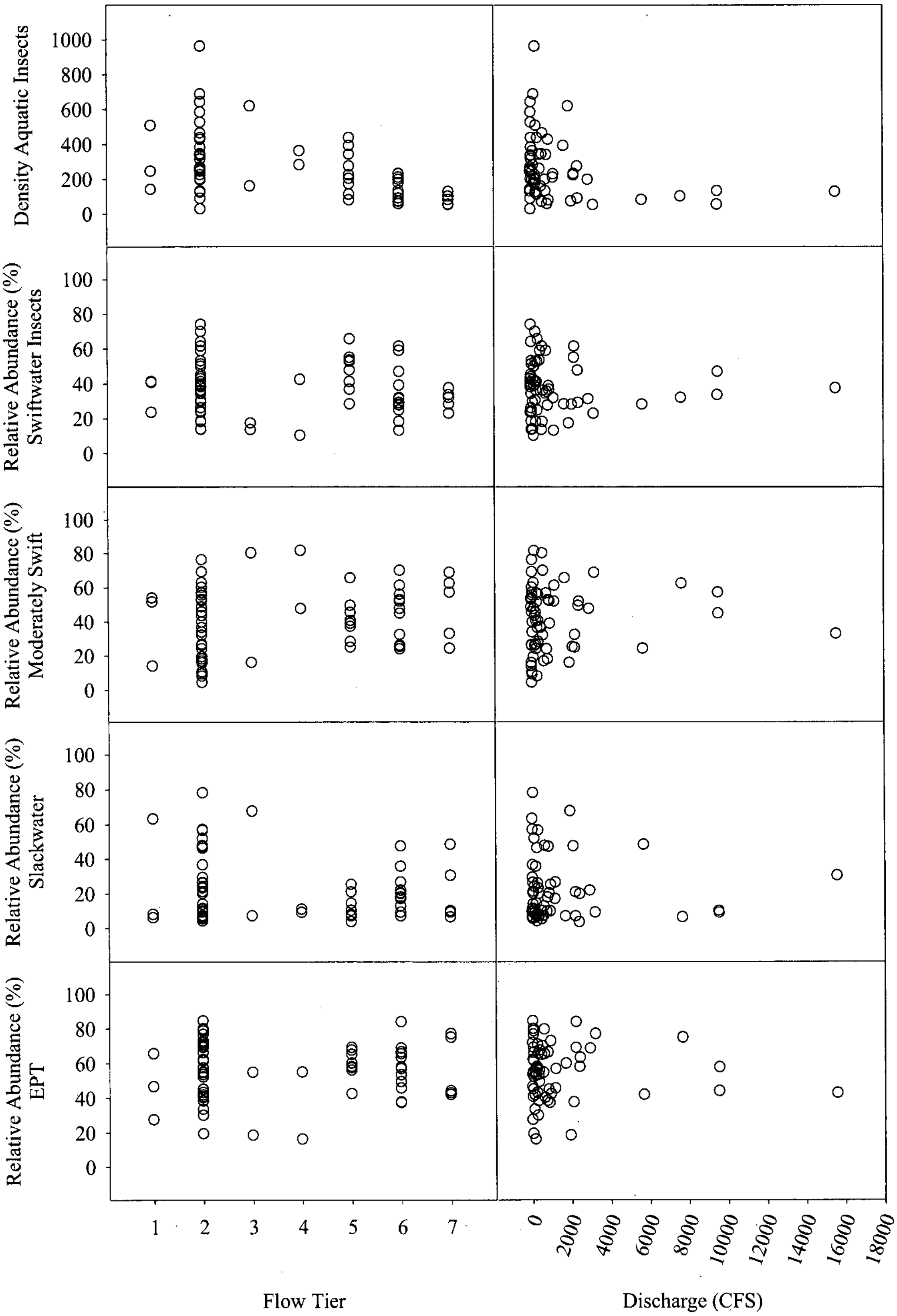
	Tier 1					Tier 2				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Run										
Area (m^2)	323	81	46	22	132	3,388	94	77	3	416
Peak Flow (cfs)		217	267	6	563		702	1,496	4	7,090
Season										
Summer	2					10				
Fall	1					12				
Winter	1					13				
Spring	0					1				
Total	4					36				
Water Temperature (°C)		20.7	11.9	7.8	31.7		17.3	7.8	7.8	32.3
Dissolved Oxygen (mg/l)		9.8	0.8	8.9	10.8		10.8	3.5	6.0	27.6
Specific Conductance (µS/cm)		675	237	535	1030		654	411	26	1881
pH		7.92	0.32	7.59	8.28		7.81	0.50	6.90	8.84
Current Velocity (m/s)		0.19	0.16	0.05	0.34		0.29	0.19	0.01	0.63
Depth (m)		0.33	0.12	0.24	0.50		0.46	0.19	0.14	0.89
Vegetation (%)		1	1	0	3		9	24	0	95
Substrate										
Silt (%)		26.67	22.24	0.00	45.00		21.89	27.03	0.00	90.00
Sand (%)		48.25	21.42	33.00	80.00		25.45	30.42	0.00	100.00
Gravel (%)		15.33	12.55	3.33	33.00		29.00	21.88	0.00	70.00
Cobble (%)		9.75	16.21	0.00	34.00		10.95	19.33	0.00	80.00
Boulder (%)		0.00	0.00	0.00	0.00		4.15	16.27	0.00	95.00
Bedrock (%)		0.00	0.00	0.00	0.00		7.18	21.63	0.00	92.00

	Tier 3					Tier 4				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Run										
Area (m^2)	147	73	11	66	81	747	187	114	96	336
Peak Flow (cfs)		1,259	977	568	1,950		3,097	3,967	141	8,540
Season										
Summer	1					0				
Fall	0					4				
Winter	1					0				
Spring	0					0				
Total	2					4				
Water Temperature (°C)		25.1	7.4	19.9	30.3		21.6	4.8	16.6	28.1
Dissolved Oxygen (mg/l)		6.9	0.1	6.8	7.0		10.1	2.4	7.9	13.1
Specific Conductance (μS/cm)		491	128	400	582		792	555	450	1619
pH		7.72	0.22	7.56	7.87		7.47	0.33	7.02	7.80
Current Velocity (m/s)		0.50	0.09	0.44	0.57		0.18	0.10	0.04	0.25
Depth (m)		0.81	0.46	0.49	1.14		0.39	0.23	0.14	0.70
Vegetation (%)		0	0	0	0		12	22	0	45
Substrate										
Silt (%)		58.75	15.91	47.50	70.00		15.00	21.21	0.00	45.00
Sand (%)		22.50	10.61	15.00	30.00		60.31	48.48	1.25	100.00
Gravel (%)		18.75	26.52	0.00	37.50		11.25	15.34	0.00	32.50
Cobble (%)		0.00	0.00	0.00	0.00		1.38	1.60	0.00	3.00
Boulder (%)		0.00	0.00	0.00	0.00		4.75	9.50	0.00	19.00
Bedrock (%)		0.00	0.00	0.00	0.00		12.19	24.38	0.00	48.75

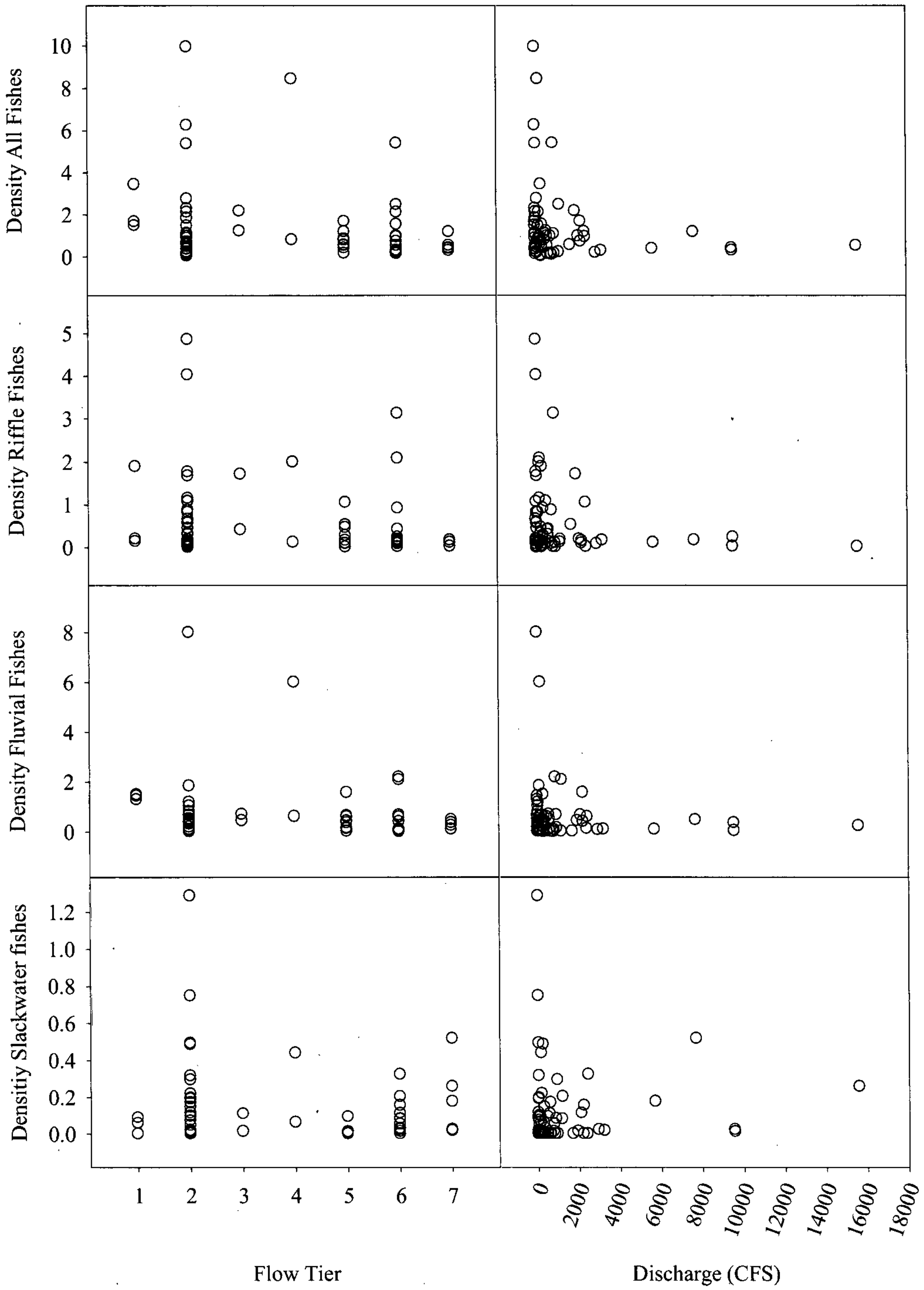
	Tier 5					Tier 6				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Run										
Area (m^2)	1,069	107	116	12	425	958	74	44	18	163
Peak Flow (cfs)		1,510	1,821	226	6,120		5,008	10,965	193	40,600
Season										
Summer	3					4				
Fall	6					2				
Winter	1					2				
Spring	0					5				
Total	10					13				
Water Temperature (°C)		21.3	6.1	10.8	29.5		22.5	5.5	12.7	30.2
Dissolved Oxygen (mg/l)		9.1	2.5	6.6	15.2		7.8	1.1	6.1	9.8
Specific Conductance (µS/cm)		752	465	434	1810		699	251	429	1219
pH		7.67	0.37	7.00	8.15		7.95	0.33	7.25	8.34
Current Velocity (m/s)		0.29	0.15	0.09	0.55		0.25	0.15	0.01	0.47
Depth (m)		0.39	0.10	0.25	0.51		0.53	0.14	0.36	0.75
Vegetation (%)		11	19	0	45		0	0	0	0
Substrate										
Silt (%)		17.66	19.80	0.00	55.00		13.40	20.61	0.00	69.17
Sand (%)		14.57	24.12	0.00	80.00		38.65	44.29	0.00	100.00
Gravel (%)		41.10	23.07	10.00	75.00		17.05	19.33	0.00	60.00
Cobble (%)		13.09	24.37	0.00	75.00		16.94	25.47	0.00	66.67
Boulder (%)		2.10	5.97	0.00	19.00		2.28	5.64	0.00	20.00
Bedrock (%)		9.01	18.39	0.00	48.75		11.67	26.65	0.00	95.00

	Tier 7				
	N	Mean	SD	Min	Max
Run					
Area (m^2)	424	85	35	50	131
Peak Flow (cfs)		8,354	4,685	3,220	15,600
Season					
Summer	0				
Fall	1				
Winter	0				
Spring	4				
Total	5				
Water Temperature (°C)		22.1	4.2	14.9	25.0
Dissolved Oxygen (mg/l)		7.6	0.9	6.9	9.1
Specific Conductance (µS/cm)		695	277	352	1053
pH		7.70	0.37	7.28	8.19
Current Velocity (m/s)		0.26	0.18	0.13	0.56
Depth (m)		0.60	0.10	0.51	0.78
Vegetation (%)		10	22	0	50
Substrate					
Silt (%)		36.00	44.64	0.00	100.00
Sand (%)		31.00	41.89	0.00	100.00
Gravel (%)		12.00	19.56	0.00	45.00
Cobble (%)		0.00	0.00	0.00	0.00
Boulder (%)		1.00	2.24	0.00	5.00
Bedrock (%)		20.00	44.72	0.00	100.00

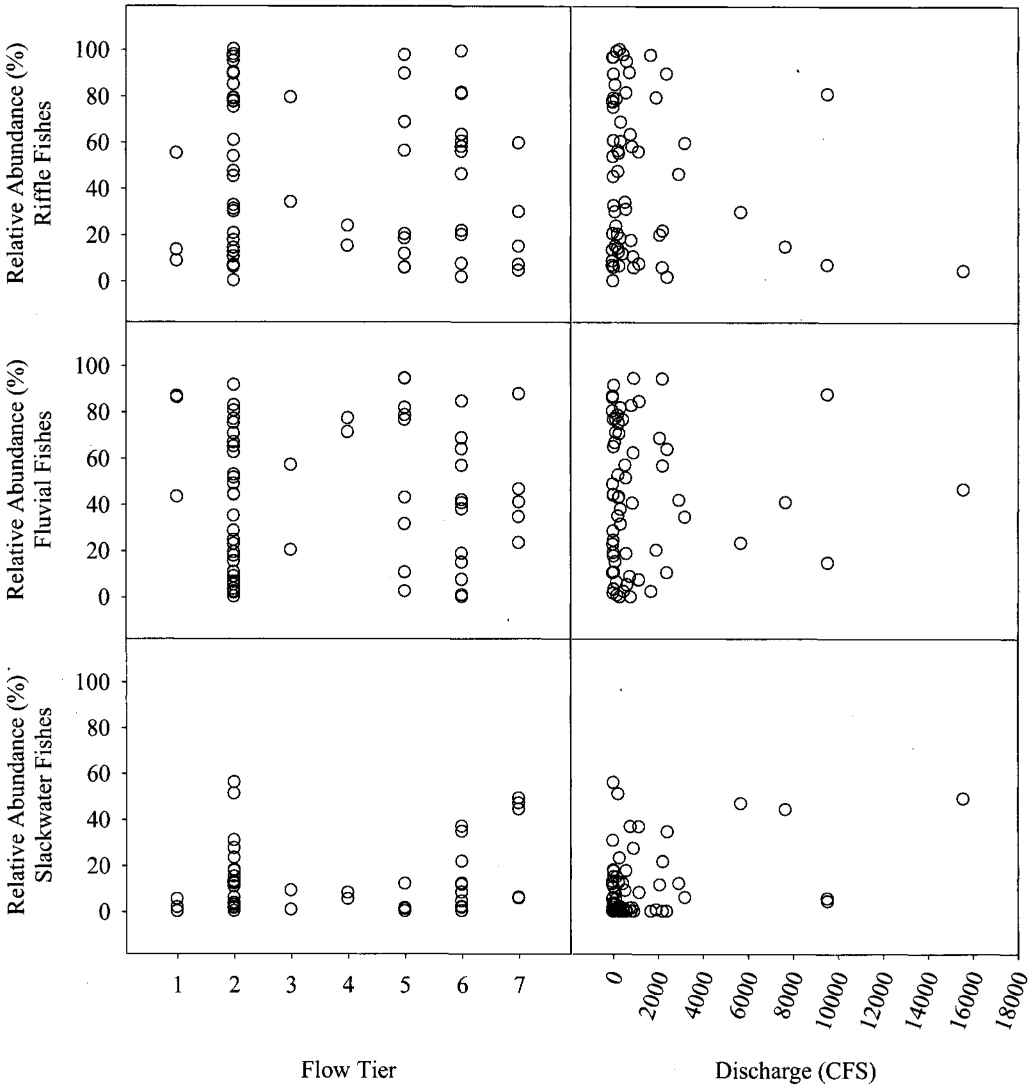
Appendix C: Density overall and relative abundances of swiftwater, moderately swift and slackwater macroinvertebrates plotted among flow tiers and discharge (CFS) from August 2014 – May 2015.



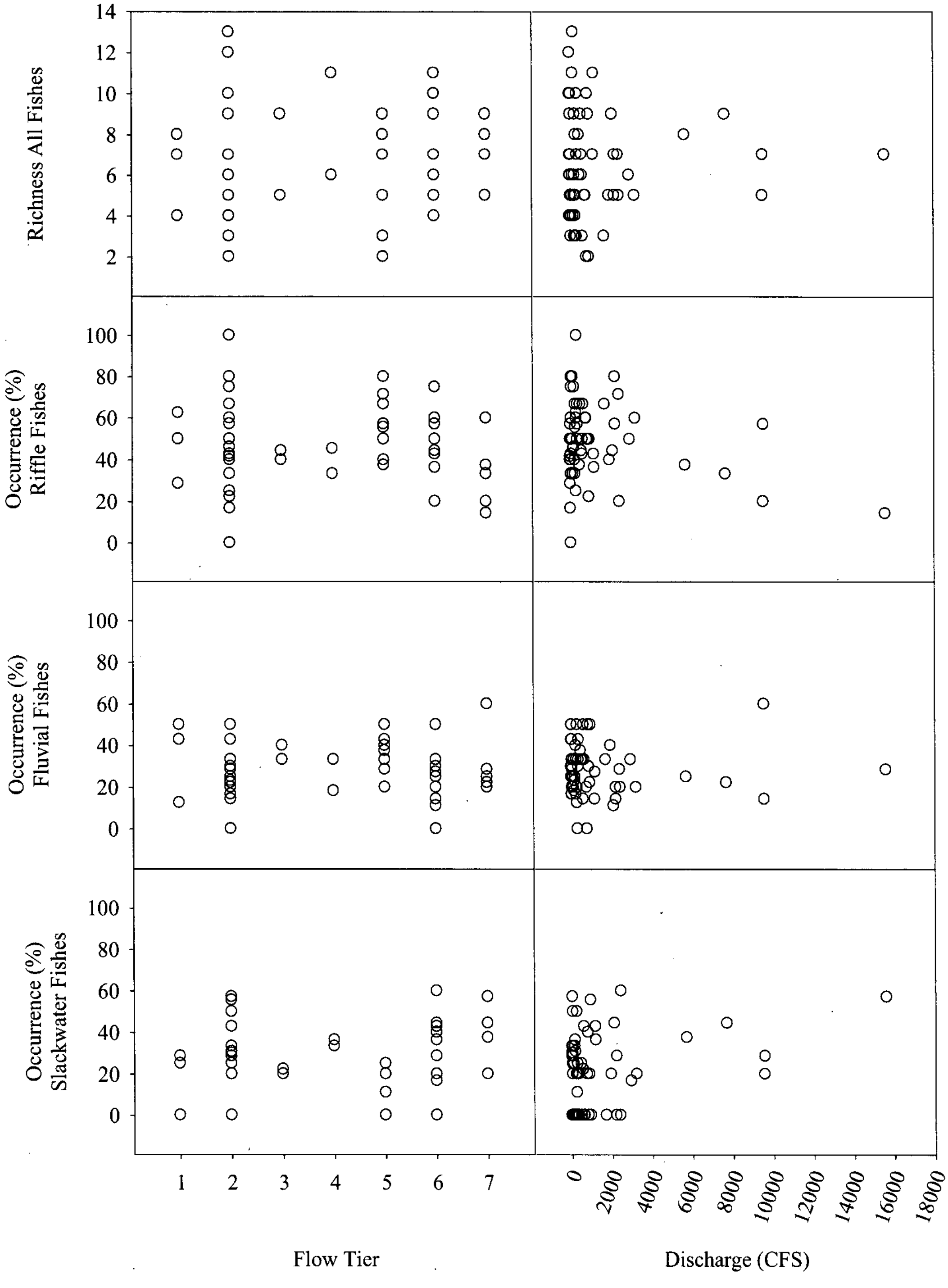
Appendix D: Densities overall and for riffle, fluvial and slackwater fishes plotted among flow tiers and discharge (CFS) from August 2014 – May 2015.



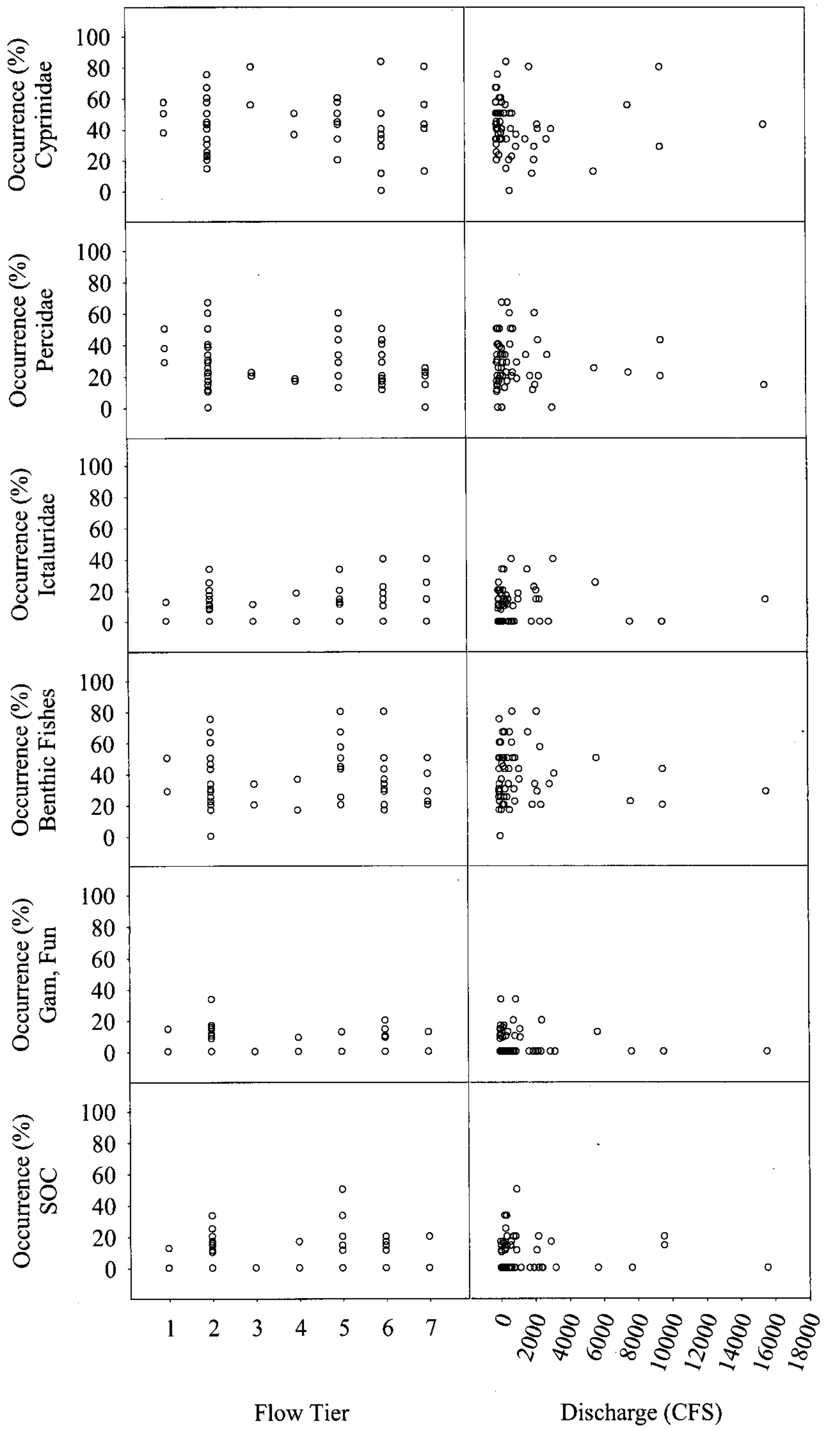
Appendix E: Relative abundances of riffle, fluvial and slackwater fishes plotted among flow tiers and discharge (CFS) from August 2014 – May 2015.



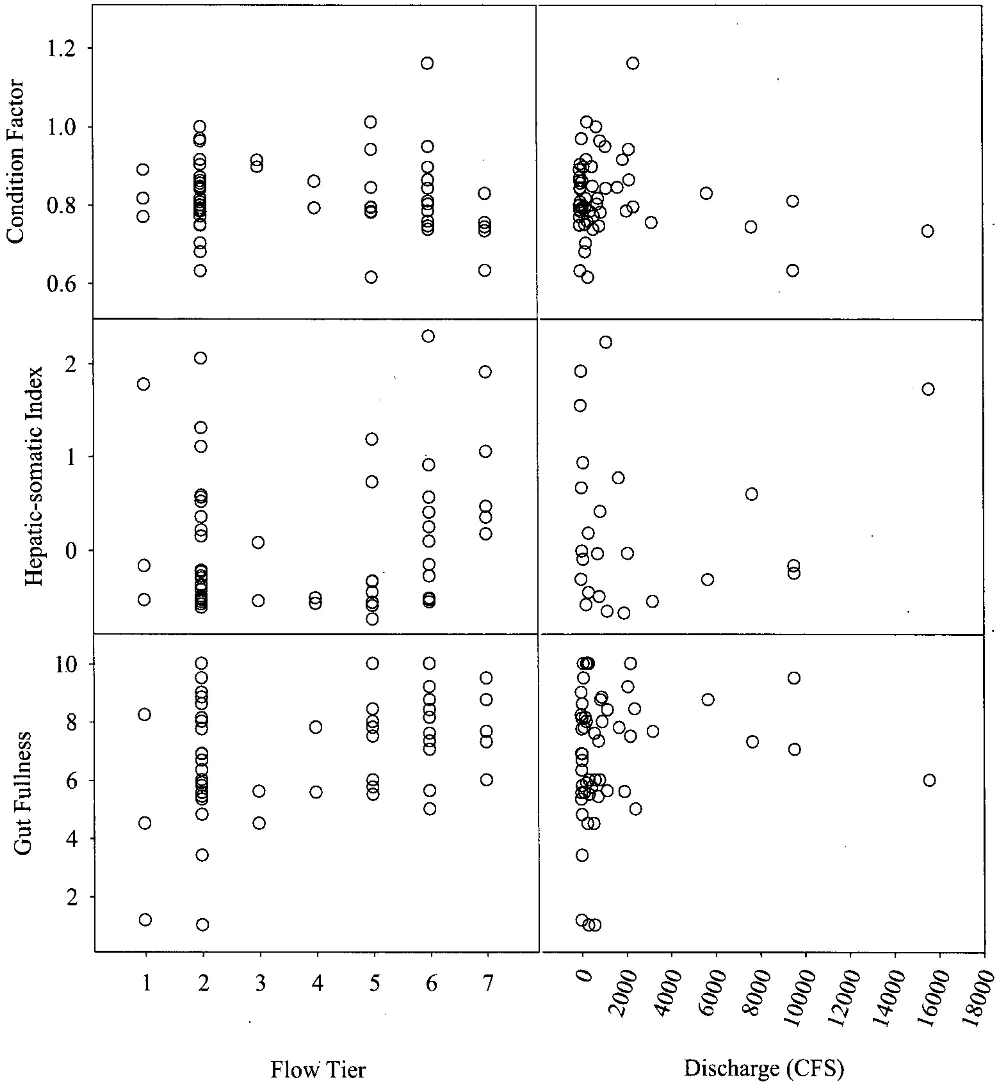
Appendix F: Richness and occurrence for riffle, fluvial and slackwater fishes plotted among flow tiers and discharge (CFS) from August 2014 – May 2015.



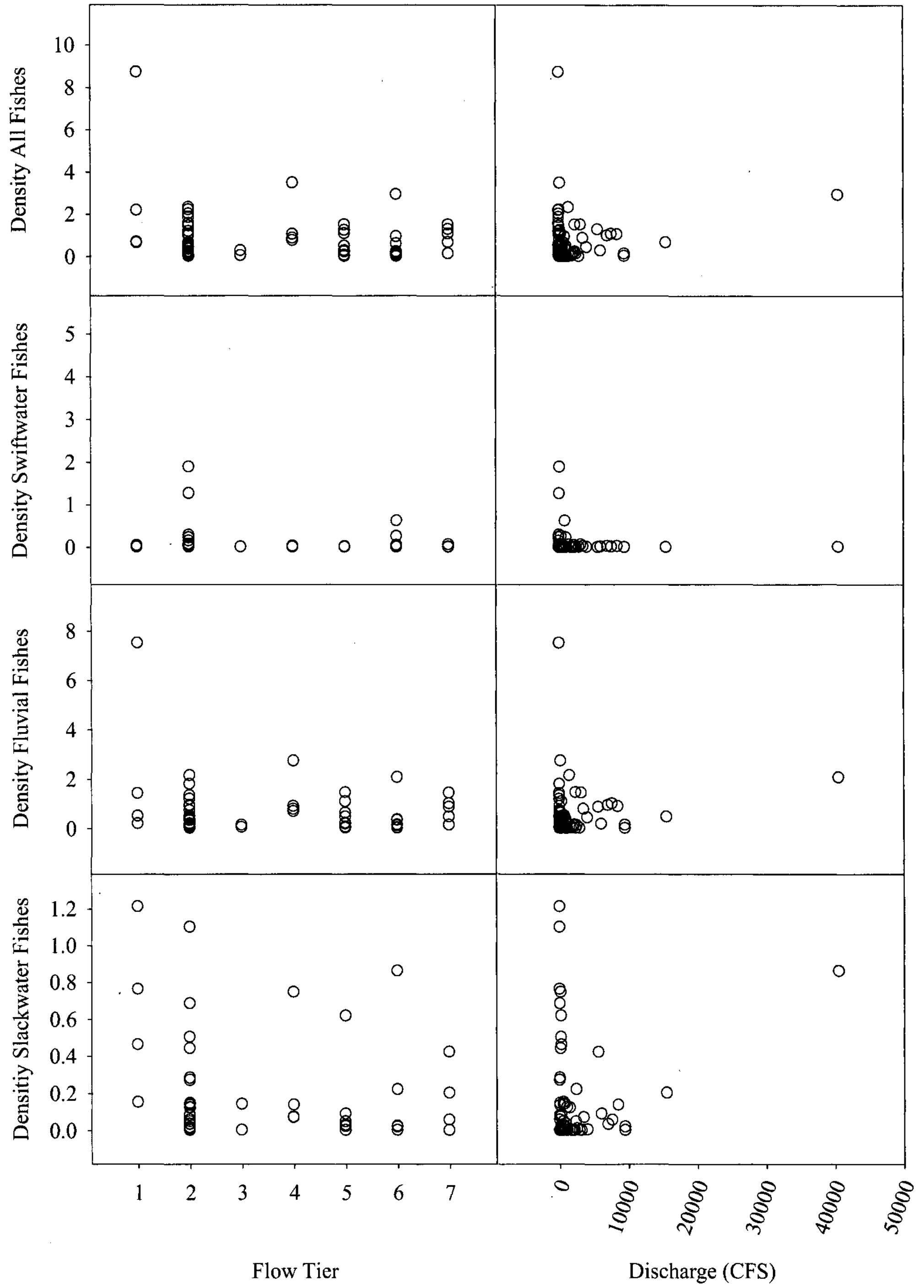
Appendix G: Occurrence for Cyprinidae, Percidae, Ictaluridae, benthic fishes, Gambusia and Fundulidae and species of concern plotted among flow tiers and discharge (CFS) for riffle species from August 2014 – May 2015.



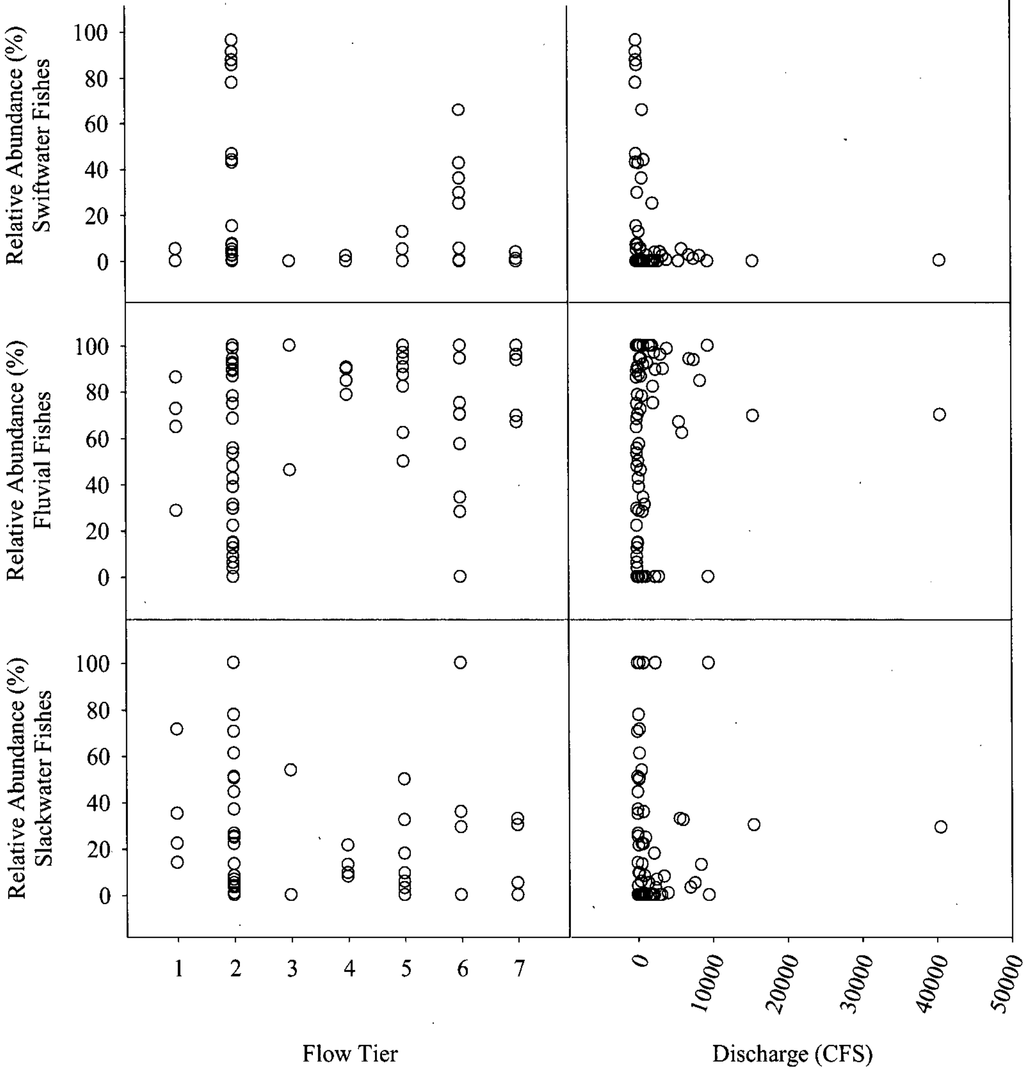
Appendix H: Condition factor, hepatic-somatic index (HIS) and gut fullness plotted among flow tiers and discharge (CFS) for riffle species from August 2014 – May 2015.



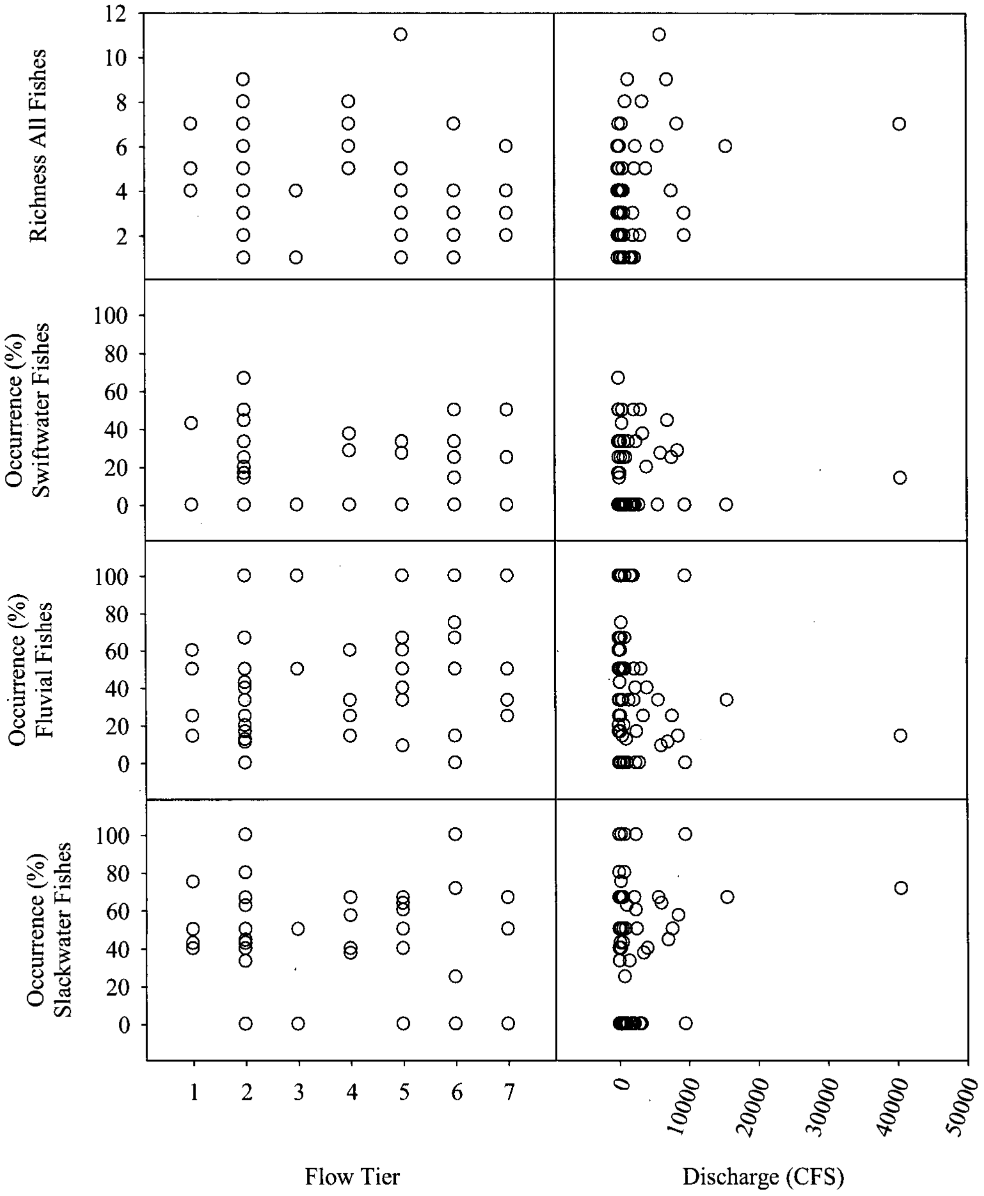
Appendix I: Densities overall and for swiftwater, fluvial and slackwater fishes plotted among flow tiers and discharge (CFS) for run species from August 2014 – May 2015.



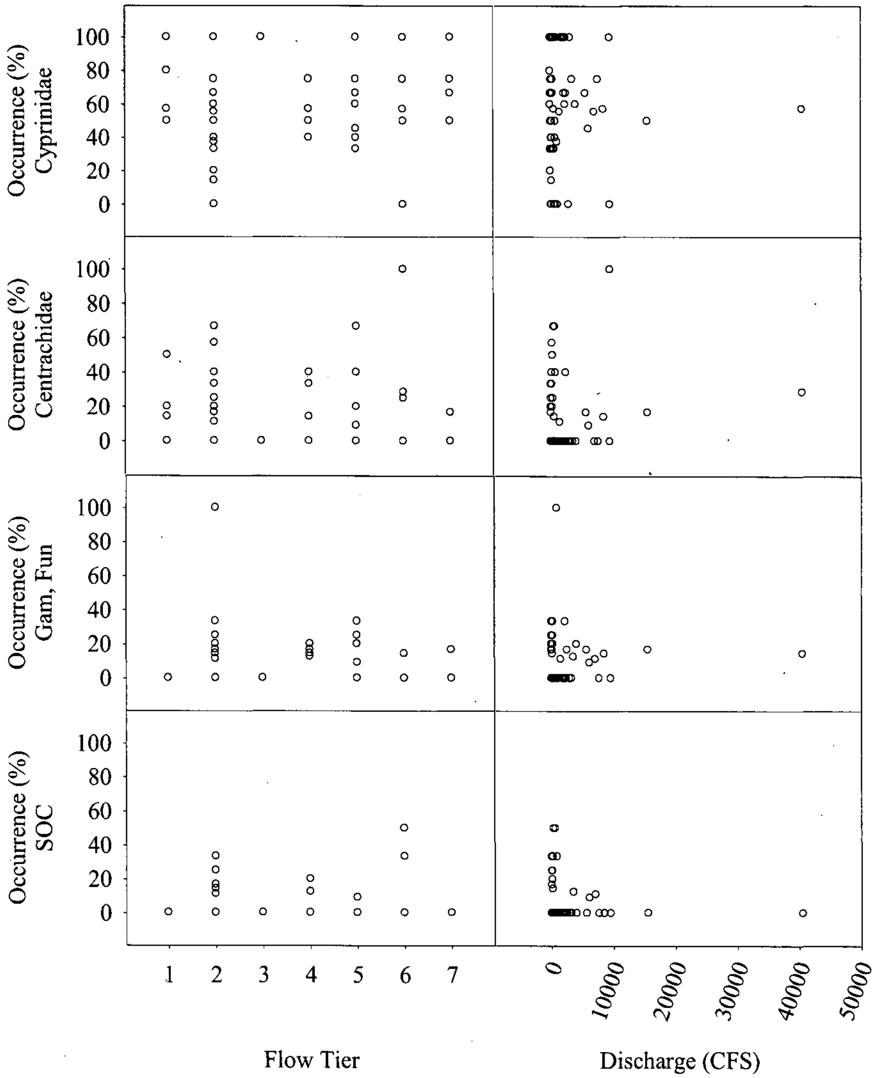
Appendix J: Relative abundances for swiftwater, fluvial and slackwater fishes plotted among flow tiers and discharge (CFS) for run species from August 2014 – May 2015.



Appendix K: Richness and occurrence for swiftwater, fluvial and slackwater fishes plotted among flow tiers and discharge (CFS) for run species from August 2014 – May2015.



Appendix L: Occurrence for Cyprinidae, Centrarchidae, Gambusia and Fundulidae and species of concern plotted among flow tiers and discharge (CFS) for run species from August 2014 – May 2015.



Appendix M. Mean ± 1 standard error (SE), range, and number of samples (N) for hydrological variables by site for each flow tier (Dry, Avg, 4ps, 2ps) sampled from November 2014-May 2015.

		Surface			Bottom		
		Mean ± SE	Range	N	Mean ± SE	Range	N
Total Depth (m)	Dry	5.13 ± 0.408	3.27-7.01	9			
	Avg	5.46 ± 0.417	3.55-7.11	9			
	4ps	5.78 ± 0.250	4.09-7.19	18			
	2ps	6.08 ± 0.245	3.04-8.34	36			
Water Temp (°C)	Dry	22.98 ± 0.169	22.26-23.97	9	23.86 ± 0.298	22.10-24.98	9
	Avg	15.62 ± 0.140	15.20-16.53	9	15.34 ± 0.467	13.33-18.02	9
	4ps	14.01 ± 0.734	10.02-18.03	18	15.37 ± 0.802	9.97-19.93	18
	2ps	20.96 ± 0.767	12.77-24.88	36	20.86 ± 0.760	12.79-24.76	36
Salinity (psu)	Dry	16.80 ± 2.659	4.49-27.60	9	24.43 ± 1.748	15.64-29.08	9
	Avg	3.85 ± 1.545	0.24-13.31	9	14.09 ± 4.390	0.24-31.09	9
	4ps	3.08 ± 0.775	0.15-10.12	18	16.61 ± 2.563	0.15-28.06	18
	2ps	0.86 ± 0.199	0.14-4.44	36	2.77 ± 0.817	0.14-23.78	36
D.O. (mg/L)	Dry	5.89 ± 0.133	5.26-6.51	9	3.98 ± 0.332	2.98-5.61	9
	Avg	9.67 ± 0.116	9.09-10.04	9	7.84 ± 0.722	3.46-9.81	9
	4ps	8.83 ± 0.245	7.59-10.13	18	7.23 ± 0.407	4.84-10.05	18
	2ps	7.30 ± 0.186	5.94-9.31	36	7.12 ± 0.178	5.80-9.26	36
pH	Dry	7.80 ± 0.027	7.69-7.90	9	7.64 ± 0.060	7.38-7.91	9
	Avg	7.99 ± 0.032	7.85-8.16	9	7.82 ± 0.071	7.40-8.03	9
	4ps	7.66 ± 0.035	7.37-7.84	18	7.62 ± 0.049	7.33-8.04	18
	2ps	7.62 ± 0.018	7.15-7.76	36	7.61 ± 0.022	7.09-8.01	36
Turbidity (NTU)	Dry*						
	Avg	18.2 ± 2.56	7.7-28.3	9	17.5 ± 3.89	2.2-31.0	9
	4ps	67.9 ± 10.50	1.8-119.3	18	128.8 ± 63.91	3.0-1196.5	18
	2ps	177.4 ± 13.45	58.0-356.8	36	223.8 ± 22.67	23.8-494.8	36
Secchi (m)	Dry	0.57 ± 0.077	0.34-0.73	5			
	Avg	0.21 ± 0.027	0.15-0.27	5			
	4ps	0.16 ± 0.039	0.05-0.38	10			
	2ps	0.09 ± 0.011	0.03-0.21	19**			

*Turbidity values for dry sampling event omitted due to equipment malfunction.

**One secchi reading not recorded at site B31 during 2ps sampling event.

Appendix N. Total N, species richness (S), diversity (H'), evenness (J') and habitat status (F = freshwater, ES = estuarine and S = saltwater) of nekton for sampling events across all sites on the lower Brazos River from Nov 2014 – May 2015.

					Winter				Spring		
			Dry	4ps	4ps	2ps	Avg	2ps	2ps	2ps	
Common Name	**Scientific Name**	**Status**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
Arthropoda: Shrimp											
Arrow Shrimp	*Tozeuma carolinense*	ES			2						**2**
Bigclaw River Shrimp	*Macrobrachium carcinus*	F					1				**1**
Brown Shrimp	*Farfantepenaeus aztecus*	ES	5	1	1				33	18	**58**
Daggerblade Grass Shrimp	*Palaemonetes pugio*	ES	21	3	26	2	5	25	6	20	**108**
Marsh Grass Shrimp	*Palaemonetes vulgaris*	ES	1			11	11		4	4	**31**
Ohio River Shrimp	*Macrobrachium ohione*	F			1	8	2	43	85	324	**463**
Pink Shrimp	*Farfantepenaeus duorarum*	ES	4								**4**
Roughneck Shrimp	*Rimapenaeus simlis*	S	1		1						**2**
Sergestid Shrimp	*Acetes americanus*	ES	97				25				**122**
White Shrimp	*Litopenaeus setiferus*	ES	259	35	27	4	7		30	5	**367**
Arthropoda: Crab											
Blue Crab	*Callinectes sapidus*	ES		42	9	7	34	8	5	8	**113**
Estuarine Mud Crab	*Rhithropanopeus harrisii*	ES					1			1	**2**
Lesser Blue Crab	*Callinectes similis*	ES	10								**10**
Mollusca: Squid											
Atlantic Brief Squid	*Lolliguncula brevis*	ES	3								**3**
Chordata: Fish											
Alligator Gar	*Atractosteus spatula*	F		1			1	2			**4**
Atlantic Bumper	*Chloroscombrus chrysurus*	ES	2								**2**
Atlantic Croaker	*Micropogonias undulatus*	ES	90	758	4,218	663	2,153	195	60	57	**8,194**
Atlantic Needlefish	*Strongylura marina*	ES							1	1	**2**

					Winter				Spring		
			Dry	4ps	4ps	2ps	Avg	2ps	2ps	2ps	
Common Name	**Scientific Name**	**Status**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
Atlantic Spadefish	***Chaetodipterus faber***	**ES**	1	1							**2**
Banded Drum	***Larimus fasciatus***	**ES**	6								**6**
Bay Anchovy	***Anchoa mitchilli***	**ES**	1,012	10	8	4	19	1	1	83	**1,138**
Bay Whiff	***Citharichthys spilopterus***	**ES**	2	1	4		4		3		**14**
Bighead Searobin	***Prionotus tribulus***	**ES**		1			1				**2**
Bigmouth Sleeper	***Gobiomorus dormitor***	**ES**			1						**1**
Black Bullhead	***Ameiurus melas***	**F**					1				**1**
Black Crappie	***Poxomis nigromaculatus***	**F**			2		1				**3**
Black Drum	***Pogonias cromis***	**ES**		3						2	**5**
Blackcheek Tonguefish	***Symphurus plagiusa***	**ES**	1		7						**8**
Blue Catfish	***Ictalurus furcatus***	**F**	6	121	65	229	282	97	49	34	**883**
Bluegill	***Lepomis machrochirus***	**F**		3	4	3		3	4	8	**25**
Bonefish	***Albula vulpes***	**S**					1			1	**2**
Bullhead Minnow	***Pimephales vigilax***	**F**				4	2	24	19	36	**85**
Channel Catfish	***Ictalurus punctatus***	**F**			1	27	31	7	7	6	**79**
Common Snook	***Centropomus undecimalis***	**ES**			1						**1**
Darter Goby	***Ctenogobius boleosoma***	**ES**	2	13	16	26	16	12	10	11	**106**
Flagfin Mojara	***Eucinostomus melanopterus***	**ES**	5	12	118						**135**
Freshwater Drum	***Aplodinotus grunniens***	**F**				2	1				**3**
Freshwater Goby	***Ctenogobius shufeldti***	**ES**							1	2	**3**
Gafftopsail Catfish	***Bagre marinus***	**ES**	4		1		4				**9**
Gizzard Shad	***Dorosoma cepedianum***	**F**		36	87	49	10	23	2	7	**214**
Gray Snapper	***Lutjanus griseus***	**ES**	2	1	6						**9**
Green Sunfish	***Lepomis cyanellus***	**F**						1		1	**2**
Gulf Menhaden	***Brevoortia patronus***	**ES**		10	132	132	115	225	2,297	2,552	**5,463**
Hardhead Catfish	***Ariopsis felis***	**ES**	10	10	2		4	9		1	**36**
Highfin Goby	***Gobionellus oceanicus***	**ES**		1			1				**2**

					Winter				Spring		
			Dry	4ps	4ps	2ps	Avg	2ps	2ps	2ps	
Common Name	**Scientific Name**	**Status**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
Hogchoker	***Trinectes maculatus***	**ES**		2	2	2		2		3	**11**
Inland Silverside	***Menidia beryllina***	**ES**		2	2	1		1	1	5	**12**
Lake Chubsucker	***Erimyzon sucetta***	**F**				1	1				**2**
Lined Sole	***Achirus lineatus***	**ES**		1	4						**5**
Longear Sunfish	***Lepomis megalotis***	**F**			3					1	**4**
Longnose Gar	***Lepisosteus osseus***	**F**						1	1	2	**4**
Lookdown	***Selene vomer***	**ES**	1								**1**
Naked Goby	***Gobiosoma bosc***	**ES**		1							**1**
Pinfish	***Lagodon rhomboides***	**ES**		5	1	1				1	**8**
Red Drum	***Sciaenops ocellatus***	**ES**	2		1	3	2			3	**11**
Red Shiner	***Cyprinella lutrensis***	**F**		1	2	1			2	6	**12**
Redear Sunfish	***Lepomis microlophus***	**F**				1					**1**
River Carpsucker	***Carpoides carpio***	**F**				1					**1**
Sailfin Molly	***Poecilia latipinna***	**ES**								1	**1**
Sand Seatrout	***Cynoscion arenarius***	**ES**	10	3	1		1			3	**18**
Sheepshead	***Archosargus probatocephalus***	**ES**	3	23	8	3	14		4	24	**79**
Sheepshead Minnow	***Cyprinodon variegatus***	**ES**						1			**1**
Silver Perch	***Bairdiella chrysoura***	**ES**	5	2	3	3	1	5		2	**21**
Skilletfish	***Gobiesox strumosus***	**ES**			1						**1**
Smallmouth Buffalo	***Ictiobus bubalus***	**F**		5		3	2				**10**
Southern Flounder	***Paralichthys lethostigma***	**ES**			4	3		1	9	5	**22**
Speckled Worm Eel	***Myrophis punctatus***	**ES**		1							**1**
Spot	***Leiostomus xanthurus***	**ES**	10	2	6	2	5		5	2	**32**
Spotfin Mojarra	***Eucinostomus argenteus***	**ES**		13							**13**
Spotted Gar	***Lepisosteus oculatus***	**F**		2	4	7	3	2	3	4	**25**
Spotted Seatrout	***Cynoscion nebulosus***	**ES**	3								**3**
Star Drum	***Stellifer lanceolatus***	**ES**		7	2	1	20	73			**103**

					Winter				Spring		
			Dry	4ps	4ps	2ps	Avg	2ps	2ps	2ps	
Common Name	**Scientific Name**	**Status**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
Striped Mullet	***Mugil cephalus***	**ES**	504	405	671	355	454	259	67	97	**2,812**
Threadfin Shad	***Dorosoma petenense***	**F**		10	2	2	2	3	3		**22**
Warmouth	***Lepomis gulosis***	**F**		1	8	2	1	3		1	**16**
Western Mosquitofish	***Gambusia affinis***	**F**	1				1		2	29	**33**
White Bass	***Morone chrysops***	**F**			1			1			**2**
White Crappie	***Pomoxis annularis***	**F**							2		**2**
Yellowfin Mojarra	***Gerres cinereus***	**ES**	2	1	6						**9**
	Total N:		**2,085**	**1,550**	**5,472**	**1,563**	**3,240**	**1,027**	**2,716**	**3,371**	**21,024**
	Richness (S):		**32**	**39**	**44**	**33**	**39**	**27**	**29**	**39**	**79**
	Diversity (H'):		**1.57**	**1.70**	**0.96**	**1.75**	**1.28**	**2.13**	**0.82**	**1.12**	**1.90**
	Evenness (J'):		**0.45**	**0.46**	**0.25**	**0.50**	**0.35**	**0.65**	**0.24**	**0.31**	**0.44**