INSTREAM FLOWS RESEARCH AND VALIDATION METHODOLOGY FRAMEWORK

Guadalupe, San Antonio, Mission, and Aransas Rivers and Mission, Copano, Aransas, and San Antonio Bays Basin

FINAL REPORT

Prepared for
Texas Water Development Board

Prepared by
San Antonio River Authority
Dr. Timothy Bonner (Texas State University)
Dr. Jacquelyn Duke (Baylor University)
BIO-WEST, Inc.

September 24, 2015

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 being 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 environmental flow standards provides a framework with which 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 Guadalupe, San Antonio, Mission, and Aransas Rivers and Mission, Copano, Aransas, and San Antonio Bays Basin and Bay Area (GSA). 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 the GSA and lower Brazos River basin (BRA). 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 GSA and BRA drainages (hereafter referred to as the aquatic component), multiple riparian zones within the GSA and BRA drainages (hereafter referred to as riparian 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. Summary of findings include 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. 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 results 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. An across-basin assessment confirmed that TCEQ environmental flow standards set without the benefit of site-specific, comprehensive instream flow studies are in most cases insufficient 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 floodplain lakes component estimated discharge magnitude resulting in floodplain lake connectivity, and quantified fish community structure of floodplain habitats within the GSA. Summary of findings include 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. Connection would be met at BBEST recommended overbank flows, but it is unclear at this time if water levels within this particular floodplain lake are dependent upon connectivity to the river or are influenced more by runoff from localized precipitation.

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 GSA and BRA 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 conditions (i.e., dynamic character) required to maintain sound ecological environments as defined in GSA and BRA BBEST reports. 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 Guadalupe, San Antonio, Mission, and Aransas Rivers and Mission, Copano, Aransas, and San Antonio Bays Basin and Bay Area (GSA), the regional stakeholder committee (GSA BBASC) and the regional expert science team (GSA BBEST) were formed in 2010. After numerous meetings and extensive data compilation and analysis, the GSA BBEST submitted their environmental flow recommendations report to the GSA BBASC in March 2011. Following a series of GSA BBASC meetings and balancing discussions, the approved stakeholder recommendations report was submitted to the Texas Commission for Environmental Quality (TCEQ) and the Environmental Flows Advisory Group (EFAG) in August 2011. Following a public comment period, the TCEQ then adopted environmental flow standards for the GSA, effective August 30, 2012.

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 arose as a major source of uncertainty in setting environmental flow standards for the GSA 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 GSA 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 to reduce the unwanted uncertainty that these data gaps introduced to the GSA environmental flow standards, primarily by improving scientific understanding of key relationships between GSA flow levels and regional ecology, emerged as a major point of emphasis following TCEQ rule development. This issue was acknowledged by the Environmental Flows Science Advisory Committee (SAC), the GSA BBASC, and the Texas Water Development Board (TWDB).

Seeking to address these needs, 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 GSA basin and the other the Brazos River basin (BRA), they 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 a methodology with potential future use in evaluating established flow standards. Because the GSA and Brazos 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 GSA project, references to and results from the Brazos basin 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 interim reports to TWDB outlining the project decision process and planned scope of work for the remainder (BIO-WEST, 2014). Some 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 GSA environmental flows validation project, followed by a detailed description of the scientific investigations conducted within the GSA basin 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 primarily with ways in which this study may be used to help inform and refine validation methodologies, to the eventual end of establishing a sound scientific approach for evaluating TCEQ environmental flow standards. This section goes on to offer preliminary guidance to the GSA 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 GSA basin sites. The final section concerns recommendations for future applied research or long-term monitoring for GSA 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 workgroups' 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, now 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 lower Guadalupe River, habitat generalist fishes dominate the fish community, whereas regionally endemic fishes and those with fluvial-adapted spawning strategies decrease during periods of reduced flood frequencies (Perkin and Bonner, 2011). In the Brazos River during low flow conditions, large-river-type fishes, such as smalleye shiners, sharpnose shiners, silverband shiners, and 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). 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 (Hoagstrom, 2014; Hoagstrom et al., 2015; Wilde and Durham, 2008)., 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 (GSA BBEST, 2011), 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 result in loss of plant vigor, increased mortality rates, and stand loss. 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.

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				

1.4 Floodplains

Occasional connections to off-channel floodplain habitats such as floodplain lakes and oxbows are important for maintaining diversity within large lowland river systems. These habitats have been shown to harbor unique floodplain specialists, which are rare in the main stem, and also provide highly productive recruitment zones, which supplement populations of many lentic-adapted species occurring in the main stem. Previous work in the Brazos River basin has documented the community composition of lower-basin oxbows, their connection frequencies, and their importance in source-sink dynamics relative to the main stem (Winemiller et al., 2000; Zeug et al., 2005). However, little information is available on floodplain/oxbow habitats within the lower Guadalupe and San Antonio River basins.

Study objectives and predictions

The objective of the floodplain analysis was to collect data on fish community composition in floodplain habitats of the lower Guadalupe and San Antonio Rivers, determine connection discharge and frequency for these habitats, and examine the relationship between community dynamics and floodplain connection in the context of pulse flow recommendations.

Biotic predictions:

1. Fish species richness is expected to be enhanced in floodplain habitats within the Guadalupe and San Antonio river basins as frequency of connection to the river increases.

Under stable hydrologic conditions, floodplain habitats eventually become dominated by slack-water specialists, such as centrarchids, that proliferate under the lentic conditions. In contrast, swift-water specialists are more abundant in the lotic environment in the river's main channel. Periodic connection of these two habitats allows for biotic exchange, thus increasing diversity in both systems as species intermingle. Therefore, as frequency of connection increases, species richness within floodplain habitats is also expected to increase.

2 Methods and materials

2.1 Aquatics

Fourteen GSA and Brazos gage locations were selected for the aquatic assessment. Sites were selected to represent tributaries and mainstem reaches. 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 1; taken from GSA BBEST 2011). 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 mainstem sites (18-Brazos River at Hempstead and 20-Rosharon). Numbers correspond to site descriptions in BRA BBEST report (Figure 2).

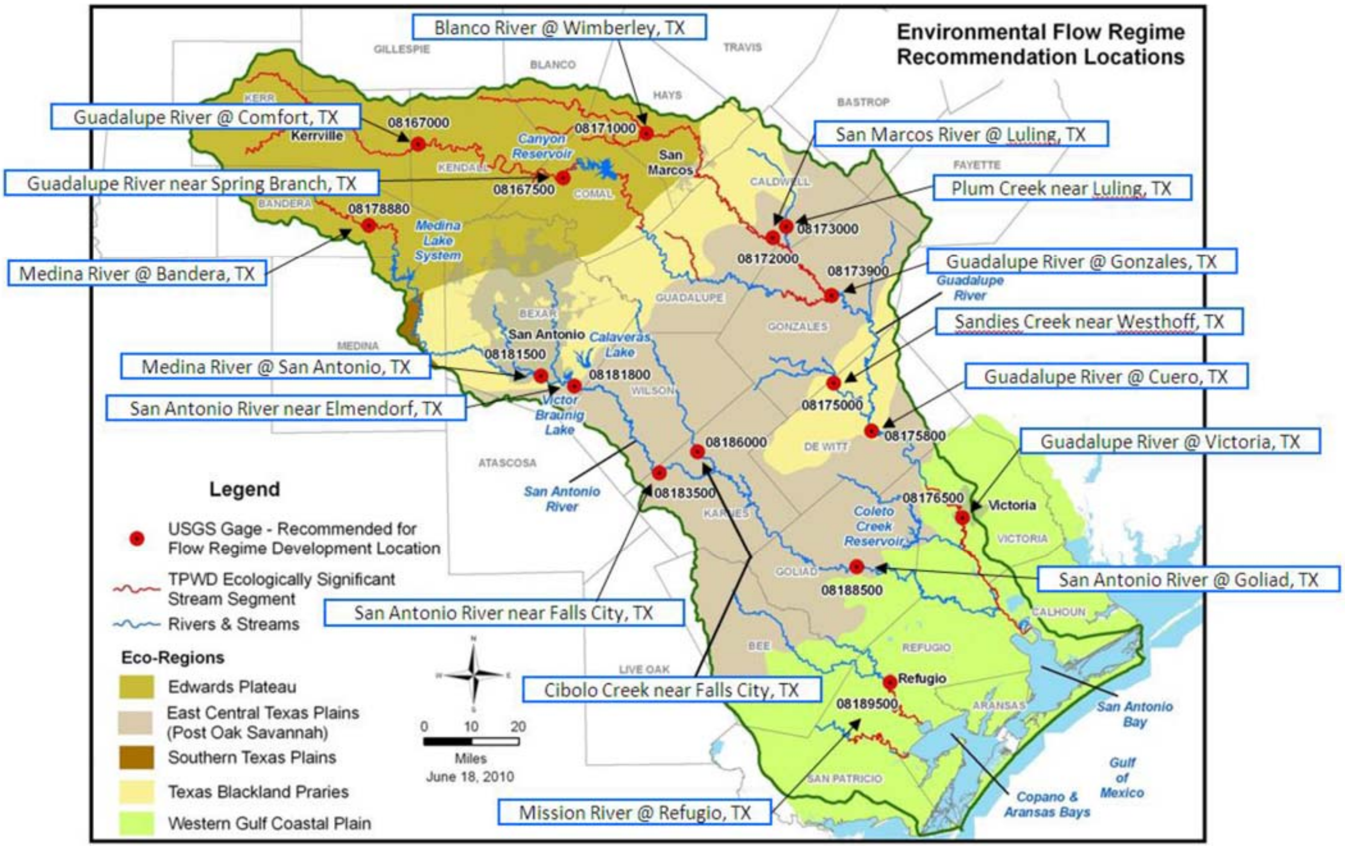


Figure 1. **Reference map of locations within the GSA (taken from GSA BBEST 2011). Specific sites used in this study are reported in the prose.**

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 3). 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.

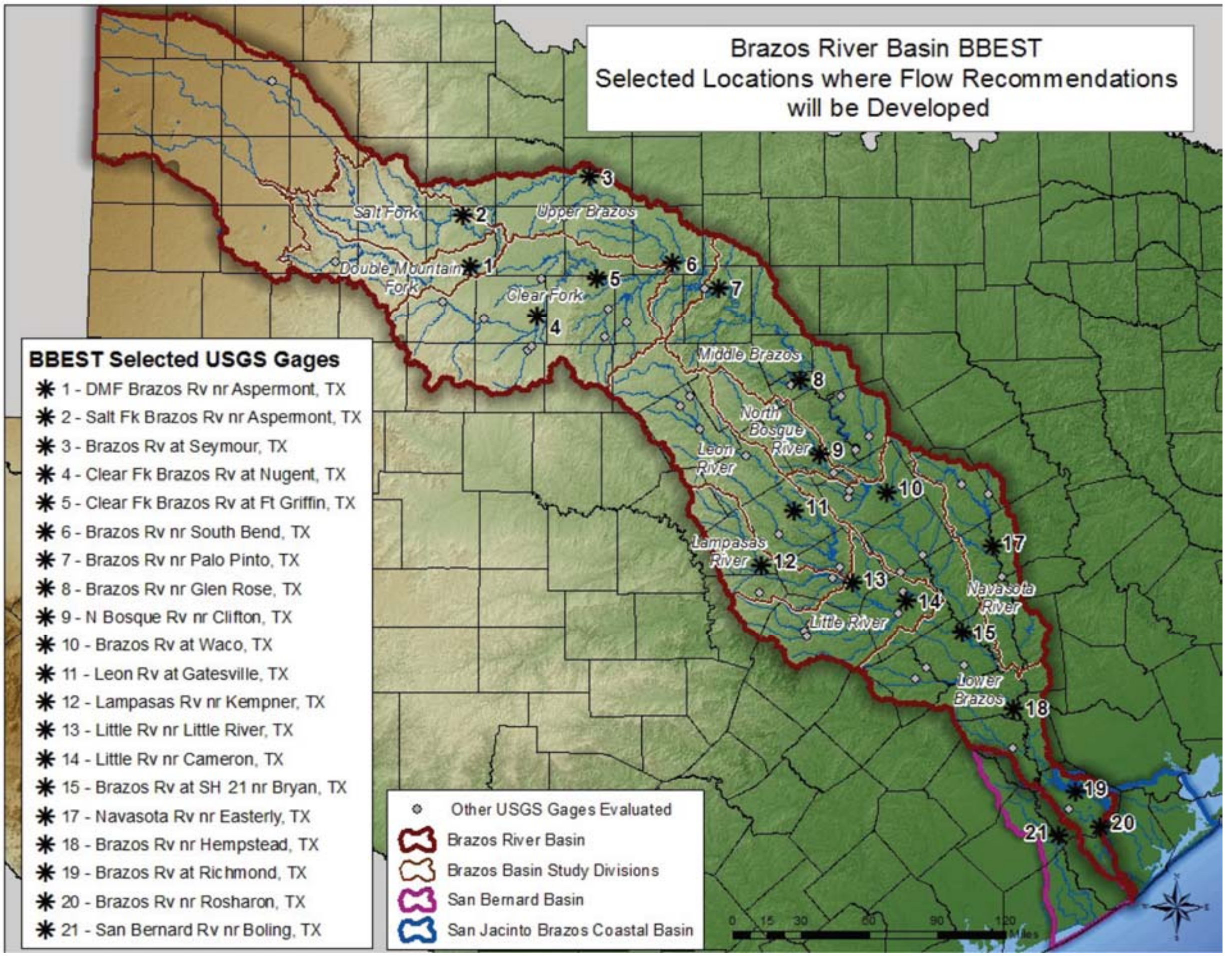


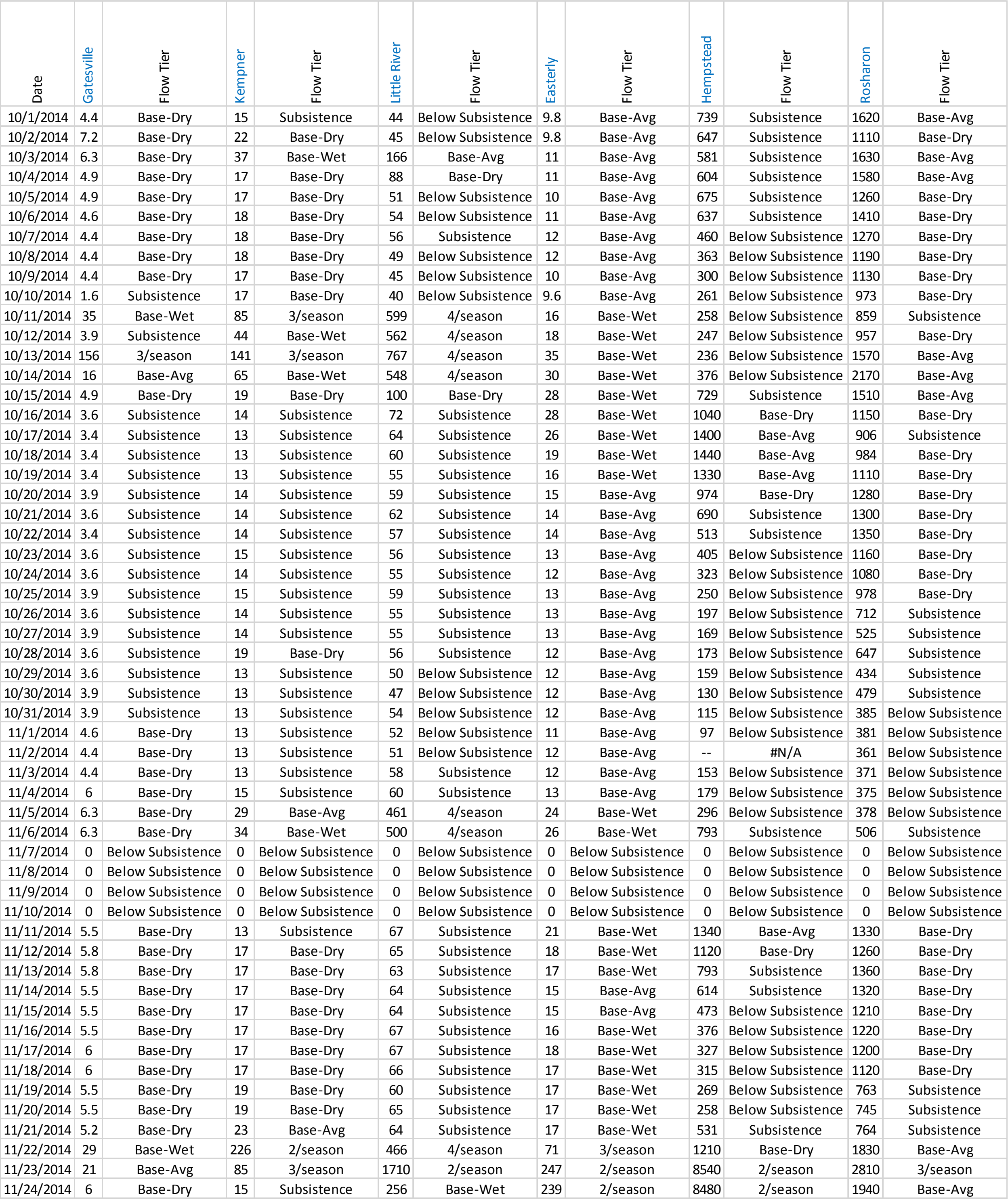
Figure 2. **Reference map of locations within the BRA (taken from BRA BBEST report). Specific sites used in this study are reported in the prose.**

For each site visit, one riffle and one or more shallow runs were selected, except at mainstem 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 (e.g, near shore vs. middle, swifter vs. slacker current velocities, shallower vs. deeper water) and sampled with a barge-mounted or backpack electrofisher. A blocking seine was placed at the downstream end of the subsection with the electrofisher positioned upstream, and the electrofisher was swept side-to-side within the width of seine and moved downstream until coming in contact with the seine (Figure 4). 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 5). Hess sample contents were preserved in 70% ethanol for subsequent identification in the laboratory. Length, width, standard water quality parameters (water temperature, specific conductance, dissolved oxygen, pH), percent substrate composition, substrate embeddedness (scored 1 = <25% embeddedness to 4 = 100% embeddedness), and percent vegetation were recorded once per riffle subsection. Water depth and current velocity were recorded from three locations within each subsection. At the riffle or from a nearby riffle, up to five individuals of riffle or fluvial specialist species (i.e., *Notropis*, *Macrhybopsis*, Percidae, and juvenile Ictaluridae) were collected, euthanized with MS-222, and fixed in 10% formalin for laboratory quantification of gut fullness, condition, and hepatic-somatic index. Among run habitats, downstream seining (common or bag seine, depending on water depths) was used to quantify fish occurrence and abundance (Figure 6, Figure 7). Within the mainstem 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. Therefore 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.

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).



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 3. **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.**

Figure 4. **Electroshocking one sections of a riffle selected at Cibolo Creek near Falls City.**

Figure 5. **Hess sample collection and abiotic parameters readings following electroshocking of the riffle sections on the San Antonio River near Goliad.**

Figure 6. A shallow run seine haul above the sampled riffle area on the Little River near Little River.

Figure 7. A shallow run bag seine haul on the mainstem Brazos River near Rosharon.

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]).

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 (GSA or BRA), and season (4 seasons in GSA, 3 seasons in BRA were converted to a 4 seasons scale). 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). Visualization of response variables by tier are provided in appendices along with plots of response variables by peak flow.

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; Durham and Wilde, 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 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). 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 Brazos River Basin and Bay Expert Science Team (Table 3.3 in Brazos BBES,T 2012) for the nearest USGS gage.

2.2 Riparian

Because both BBEST recommendations and TCEQ flow standards were specific to study reaches, it was not practical to combine site data into one basin recommendation or run statistical analyses as was performed with the aquatics assessment given the unique characteristics of each site. 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 GSA basin from the recommended BBEST (GSA BBEST, 2011) USGS-monitored reaches (Figure 8 and Table 2). Criteria for site selection included: (1) that established riparian forests be present, (2) that at least two of the three indicator species be present, and (3) that the site must not have any major tributaries between it and the USGS gage. One site was on the mainstem San Antonio River, three were on the mainstem Guadalupe River, and one each was from each river's tributaries (Medina River and Blanco, respectively). Table 2 references the site names, which will be used throughout this report. The Gonzales site was considered a reference site, as monitoring of it began prior to this study. It was used as a general model for study methodologies and it provided a longer term dataset for analysis.

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 9). 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 10). 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.

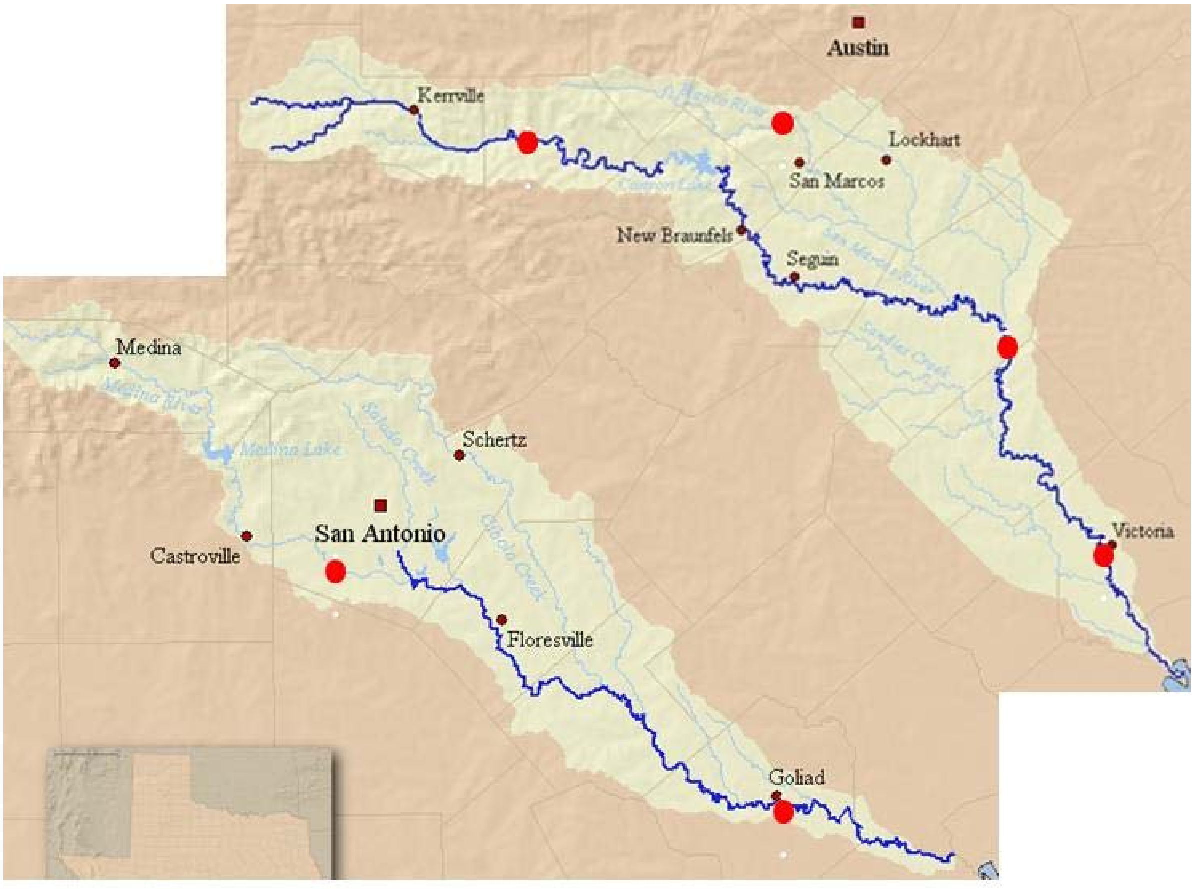


Figure 8. **Location of the six sites (red dots) selected for the study. Credit: TX Climate News (modified).**

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

Gage Number	Site	Gage Location
8171000	Blanco	Blanco River at Wimberley
8188500	Goliad	San Antonio River at Goliad
8167500	Guadalupe	Guadalupe River near Spring Branch
8173900	Gonzales	Guadalupe River at Gonzales
8181500	Medina	Medina River at San Antonio
8176500	Victoria	Guadalupe River at Victoria

Four sampling events were conducted from summer 2014 to spring 2015: August 2014, October 2014, January 2015, and April 2015 (though only select sites were accessible at this time because of flooding).

Flow frequency was measured categorically as the number of flow tiers given in the 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 baseflow tiers 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 11). 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 9. **Crew members take elevation at stream transect.**

Figure 10. **Crew member installs a stream level logger.**

A comparison of TCEQ flow tiers 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 more a useful "rule of thumb" and was selected because of a number of factors: (1) 80% 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/flow tier of 1000 cfs is met by an actual flow of 1250 cfs), therefore a "met" flow is often above the standard/required flow tier pulse, actually inundating further up the bank than the flow tier 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.

Figure 11. Crew member collects tree core samples in the field.

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 tier magnitudes. 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 stream flow might be better explained by local rainfall than stream flow.

Changes to site seedling, sapling, and mature counts through seasons were calculated to determine if stream flow 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 use in estimating age of mature riparian tree species.

Species	Average number of rings per year	Number observed
Black Willow	1.108	26
Box Elder	0.267	39
Green Ash	0.292	20

2.3 Floodplains

During the aforementioned workshops considerable discussion was held on whether TCEQ flow standards connect floodplain features to the river at the appropriate frequency needed for dependent aquatic species. It was concluded that with the considerable work already completed in the Brazos basin, resources related to this indicator would be only applied on the lower Guadalupe and San Antonio Rivers. Therefore, whereas the Aquatic component (Section 2.1) focuses on all sites within both the GSA and Brazos basins and the Riparian component (Section 2.2) focuses on individual GSA sites throughout the basin, the floodplain assessment focuses only on lower basin GSA sites.

To locate potential study sites, a desktop analysis using Google Earth imagery was conducted. This initial desktop analysis led to identification of 18 potential floodplain lake sites within the lower Guadalupe River basin and 6 potential sites within the lower San Antonio River basin. A subset of the 10 most promising sites (6 on lower Guadalupe, 4 on lower San Antonio) was then visited for further evaluation. After visiting, a few of these sites were determined to be inappropriate due to lack of water, distance from river, or access issues. As a result, data were collected at 5 floodplain lakes within the lower Guadalupe River basin and 2 floodplain lakes within the lower San Antonio River basin during March 30 – April 3, 2015 (Figure 12).

Gonzales1 is located on the Guadalupe River approximately 11 river miles downstream of the Hwy. 183 Bridge in Gonzales. Cuero1 is located approximately 1.5 miles downstream of the Hwy. 72 crossing on the Guadalupe River in Cuero. Data on this oxbow was also collected by Hudson (2010), who named it the “Cuero ’98 oxbow”, since it was evidently formed during a large flood event in 1998. Cuero2 is a large oxbow located approximately four miles downstream of Cuero1, and approximately 2.5 miles downstream of the Hwy. 183 Bridge over the Guadalupe River in Cuero. Victoria1 is located several miles downstream of Victoria, near Linn Lake, approximately 24 miles upstream from the mouth of the Guadalupe River. Victoria2 is located just upstream of the Invista plant near Bloomington, Texas, approximately 32 miles upstream from the mouth of the Guadalupe. LSAR1 is located on the lower San Antonio River approximately 7.2 miles upstream from the Hwy. 77 Bridge near McFaddin. LSAR2 is located on the lower San Antonio River approximately 19.4 miles upstream from the Hwy. 77 Bridge. To estimate the discharge level which results in surface water connectivity between floodplain features and the main channel of the river, on-site topography data and water surface elevation (WSE) data were collected at each site during March 30 – April 3, 2015. These data were then tied to corresponding data on water surface elevation and flow rate at nearby USGS gage locations using methods similar to Osting et al. (2004). The "control point" elevation was estimated from on-the-ground surveys and represented the water surface elevation, which would result in surface connection of each floodplain lake to the main channel of the river. The slope relationship between water surface elevation near each control point and the nearest upstream and downstream WSE data (from USGS gages and/or other study sites) was estimated. This relationship (assumed to be linear) was then used to estimate a flow rate at the gage, which would result in connection of each floodplain lake. Once these connection discharges were established, they were evaluated against the hydrologic record from the gage to estimate connection frequencies for each floodplain lake.

It is recognized that water surface elevation slope in river systems is not truly linear, but instead typically changes in a stepwise fashion, being steeper in riffle areas and flatter in pools. However, given the relatively flat lowland nature of these two systems and the distances over which slope was estimated, a linear function was deemed appropriate. Estimated slopes ranged from 0.88 – 1.79 feet/mile and R^2 values for slope equations ranged from 0.96 -0.99. It should also be noted that these estimates assume a constant water surface elevation slope for all flow rates. No adjustments were made to account for slope changes with changes in flow. Although detailed hydraulic flood-flow modeling can account for such changes, such modeling was beyond the scope of this study.

Fish communities within six of the seven floodplain lake sites were sampled with seines and/or boat or backpack electrofishing during March 30 – April 3, 2015. All fish were identified to species, measured to the nearest millimeter total length, enumerated, and released, except for voucher specimens. Voucher specimens were fixed in 10% formalin and brought back to the laboratory for identification and enumeration. A second fish sampling event scheduled for late May or early June 2015 had to be cancelled due to flooding and dangerous flow levels.

For floodplain lakes with sufficient fish community data (Cuero2, Victoria1, Victoria2, and Gonzales1), comparisons were made between floodplain lake fish communities and mainstem Guadalupe River fish communities. Recently collected Texas Instream Flow Program (TIFP) baseline data for the Guadalupe River, along with recent BIO-WEST collections from the Guadalupe River, were used to represent mainstem Guadalupe River fish communities. Each fish species was categorized into one of three basic habitat utilization categories based on available life history information and previous experience – Riverine, Floodplain, or Generalist. The proportion of riverine species was then compared among sites and riverine vs. floodplain habitats. The proportion of riverine species was incorporated into the analysis by treating the count of individuals of riverine species/sample (successes) and the count of individuals of non-riverine species/sample (failures) as a binomial response in a generalized linear model with quasibinomial errors to account for overdispersion in the data. This effectively weights the observations to limit the influence of extreme observations. Analysis of deviance was used to perform model selection and test for interaction of site and habitat. Species richness was also compared across all floodplain lake fish sampling events and was analyzed in the context of estimated connection discharge to examine potential patterns.

Although data from only one fish sampling event is available for six of the seven sites, one of the Guadalupe basin floodplain lakes (Gonzales1) included in this study was a site already being sampled per a Guadalupe-Blanco River Authority (GBRA) sponsored instream flow study. Two years of seasonal fish collection data were available from this particular site, and were incorporated into this analysis to examine temporal trends in fish community dynamics relative to connection events. To analyze the effect of connection events on fish community dynamics, the proportion of riverine species in each Gonzales1 sample was compared between events following connections and events not associated with connections using the same quasibinomial generalized linear model approach described above.

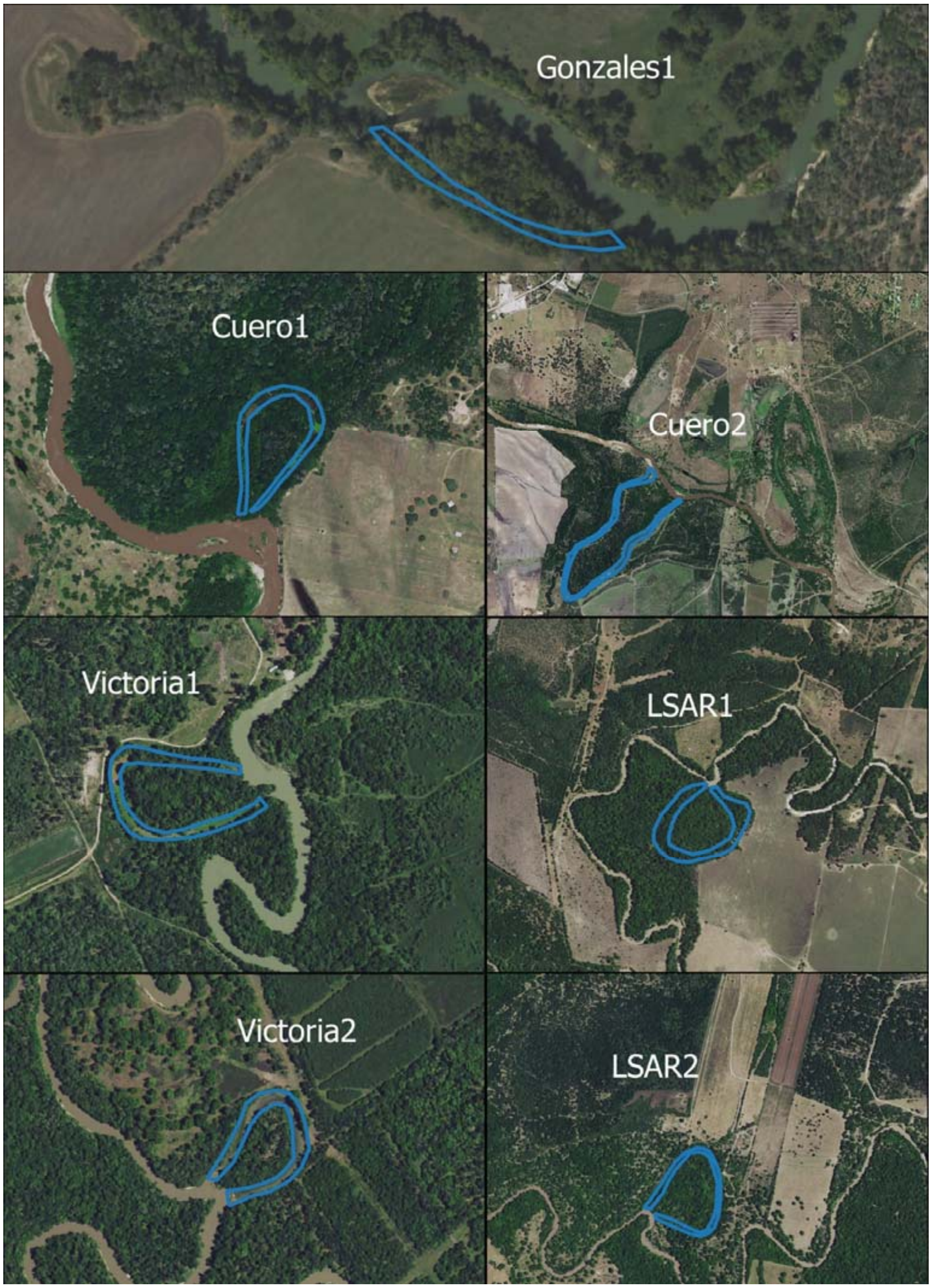


Figure 12. **Floodplain lake study sites.**

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 4). 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. Falls City (GSA) and Kemper (BRA) 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 13).

A total of 51,460 aquatic insects, representing 9 insect orders, was recorded among the 63 riffles (Table 5). Among all sites, Ephemeroptera was the most abundant insect order (39% of total N of macroinvertebrates) and exhibited with 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 14). 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 the macroinvertebrate groups with strong negative associations were Megaloptera, Hemiptera, and Lepidoptera.

Table 4. **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

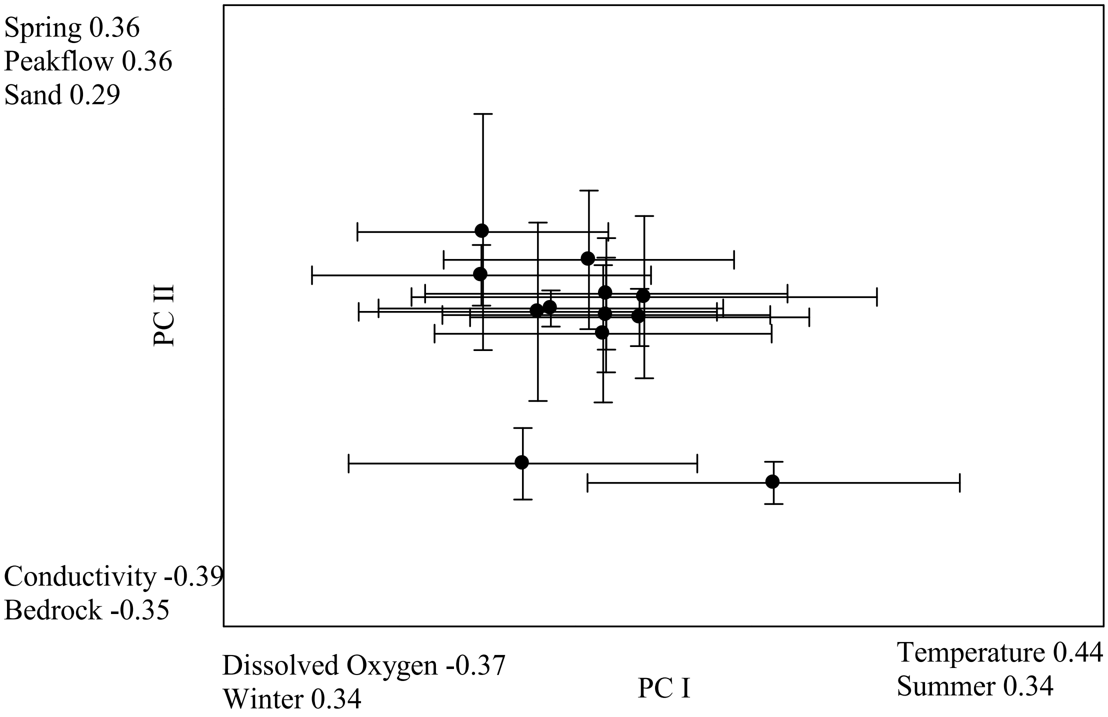


Figure 13. A Principal Component analyses (PCA) analysis of the association of riffle habitats for sites on the Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) by season, substrate and water quality parameters for from August 2014 – May 2015.

Table 5. 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	

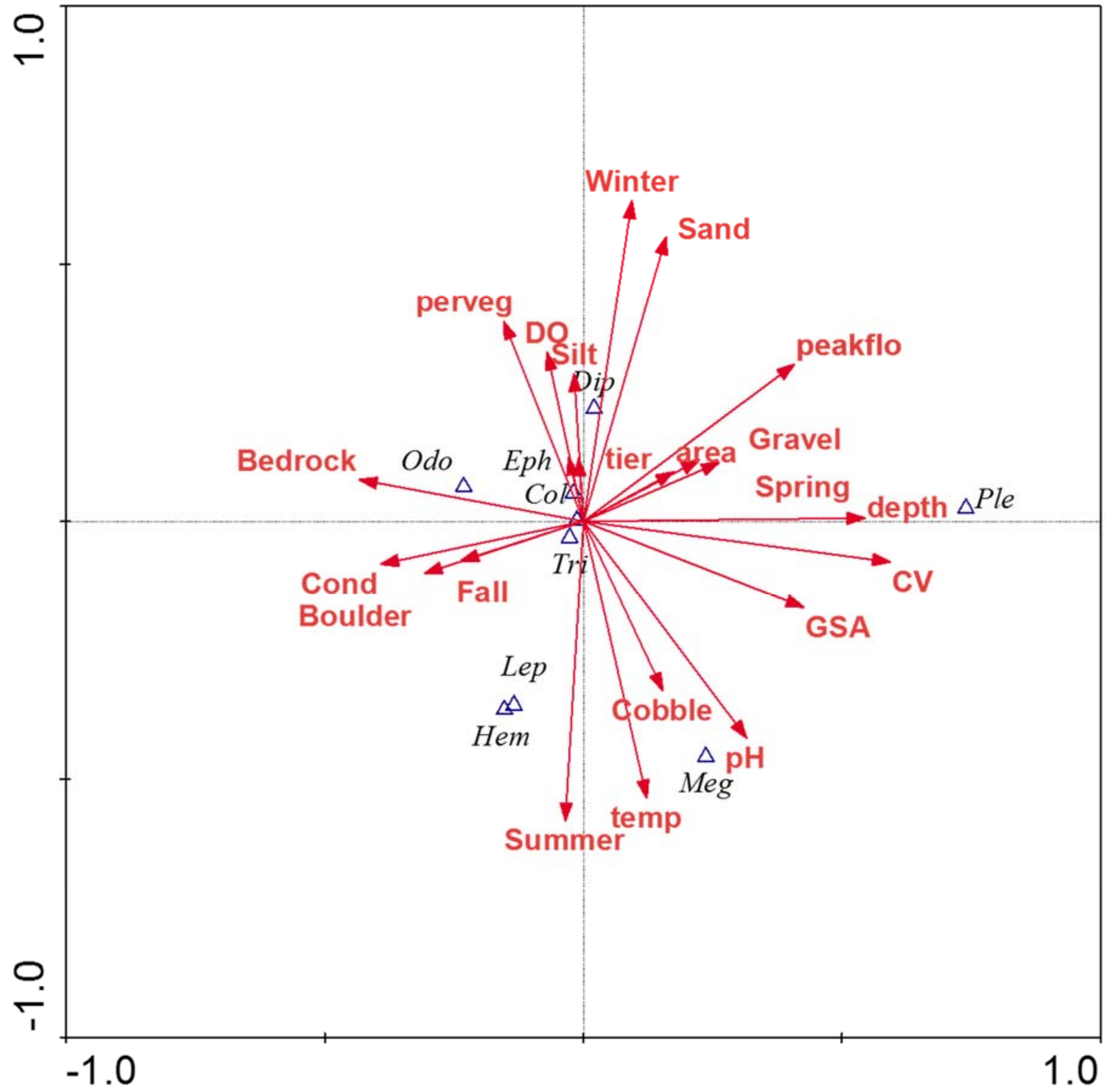


Figure 14. **Canonical Correspondence analyses (CCA) of the total number of macroinvertebrates from riffle habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 5 for macroinvertebrate codes.**

A CCA model explained 53% of the variation (F = 2.3; P < 0.01) in density of macroinvertebrates in riffles (Figure 15). 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 the strongest positive association was Diptera, and macroinvertebrate group with the strongest negative association was Megaloptera. Along CCA axis 2, macroinvertebrate groups with strong positive associations were Tricoptera and Hemiptera, and a macroinvertebrate group with strong negative association was Plecoptera.

A total of 6,612 fishes, representing 33 species of fishes, were recorded among the 63 riffles (Table 6). Among all sites, *Cyprinella lutrensis* was the most abundant (32% of total N of fishes) and had 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%).

A 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 were negatively associated with CCA axis 1 (Figure 16). 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*.

A CCA model explained 47% of the variation (F = 7.5; P < 0.01) in fish densities in riffles. GSA basin, and current velocity (CV) were positively associated, and BRA basin, pH, and embeddedness were negatively associated with CCA axis 1 (Figure 17). 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*.

Condition factors were calculated for 11 species and 435 individual 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 individual fishes associated with riffles (Table 7). 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*.

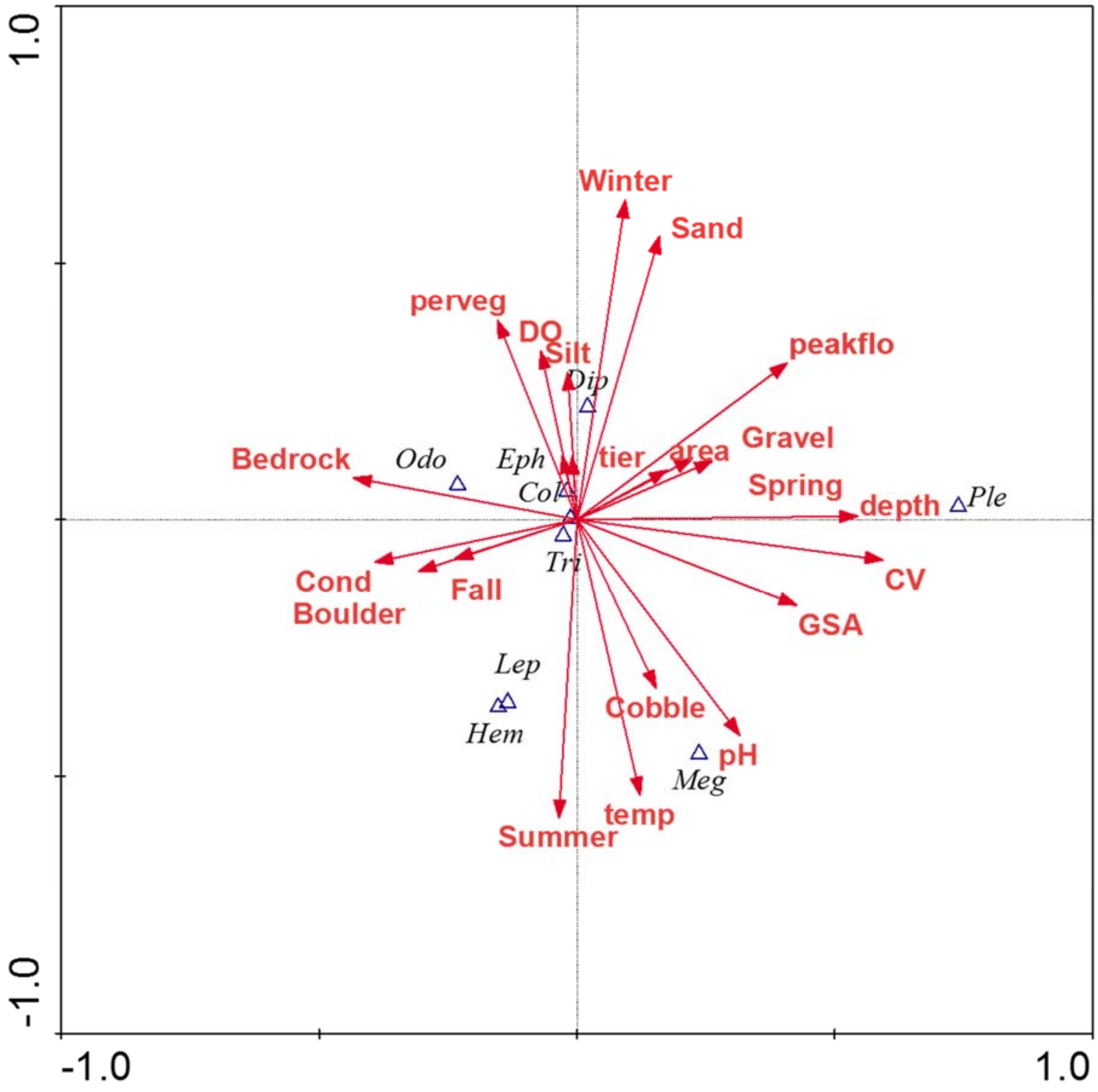


Figure 15. **Canonical Correspondence analyses (CCA) of the density of macroinvertebrates from riffle habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 5 for macroinvertebrate codes.**

Table 6. **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	

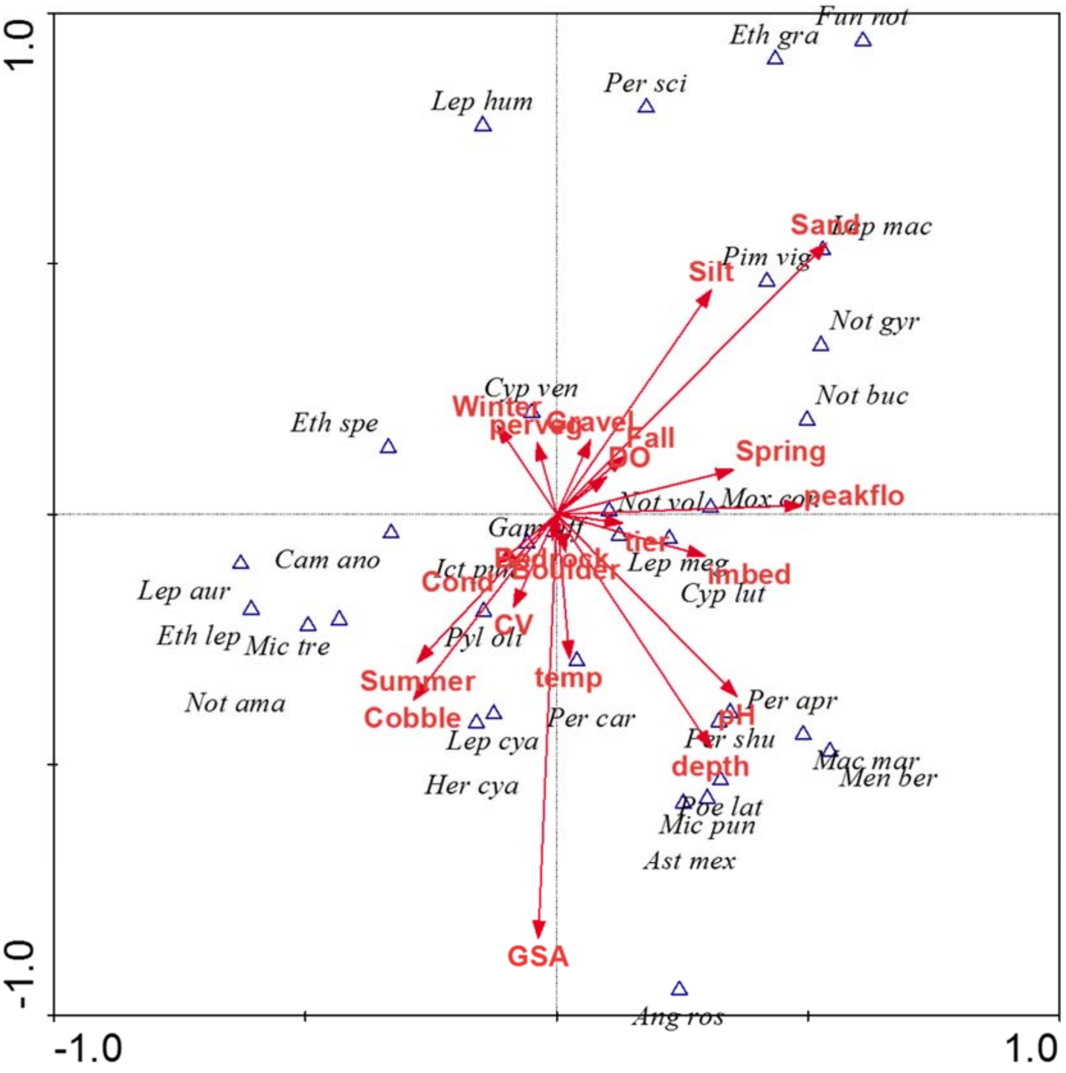


Figure 16. **Canonical Correspondence analyses (CCA) of the total number of fishes from riffle habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 6 for riffle fishes codes.**

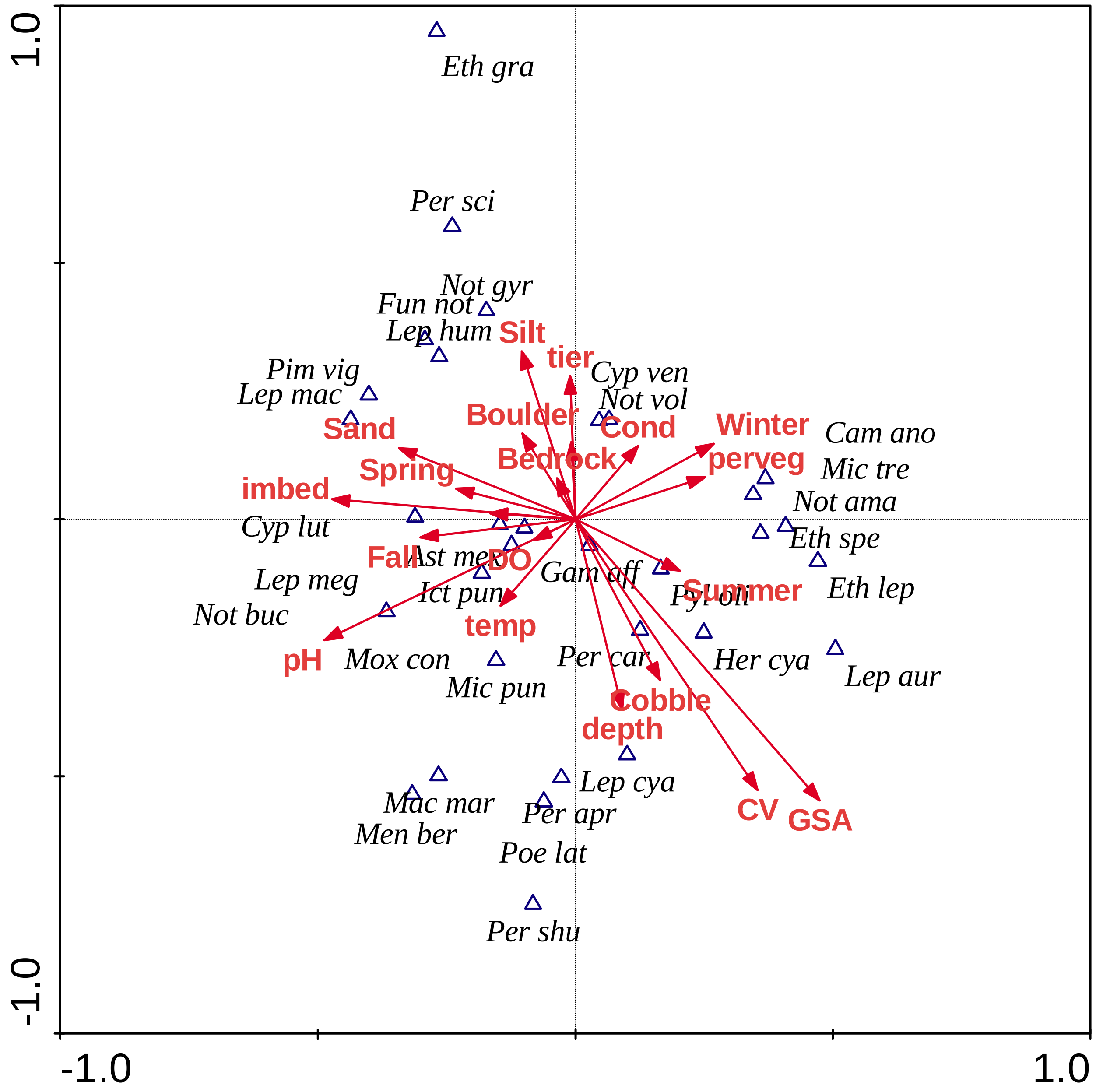


Figure 17. **Canonical Correspondence analyses (CCA) of the density of fishes from riffle habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 6 for riffle fishes codes.**

		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

Table 7. **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.**

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 8). PCA axes 1 and 2 explained 31% of the seasonal and habitat variation (Figure 18). PC axis 1 explained 16% of the variation 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% of the variation and described primarily a seasonal gradient. Scatter plots of PCA 1 and 2 means (+/- 1 SD) by site indicate clustering and overlap, which suggests similarity of habitat parameters among sites, except for Kempner.

A total of 14,840 fishes, representing 37 species of fishes, was recorded among the 74 runs (Table 9). 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%).

A 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 19). 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*.

A CCA model explained 38% of the variation (F = 1.7; P < 0.01) in fish densities within runs. Silt substrate, pH, and BRA basin were positively associated, and cobble substrates and GSA basin were negatively associated with CCA axis 1 (Figure 20). Percent vegetation and summer season were positively associated, and GSA basin was 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*.

	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

Table 8. **Run habitat summary statistics taken overall (N = 14 sites) and by drainage from August 2014 – May 2015.**

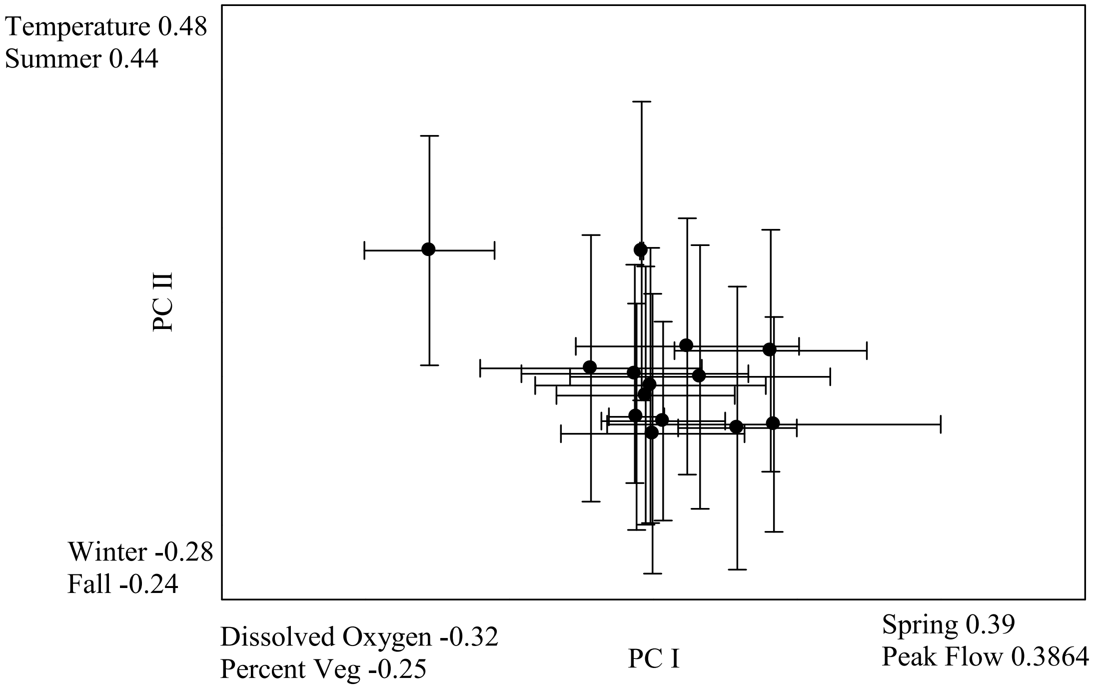


Figure 18. **A Principal Component analyses (PCA) analysis of the association of run habitats for sites on the Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) by season, substrate and water quality parameters for from August 2014 – May 2015.**

Table 9. **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	

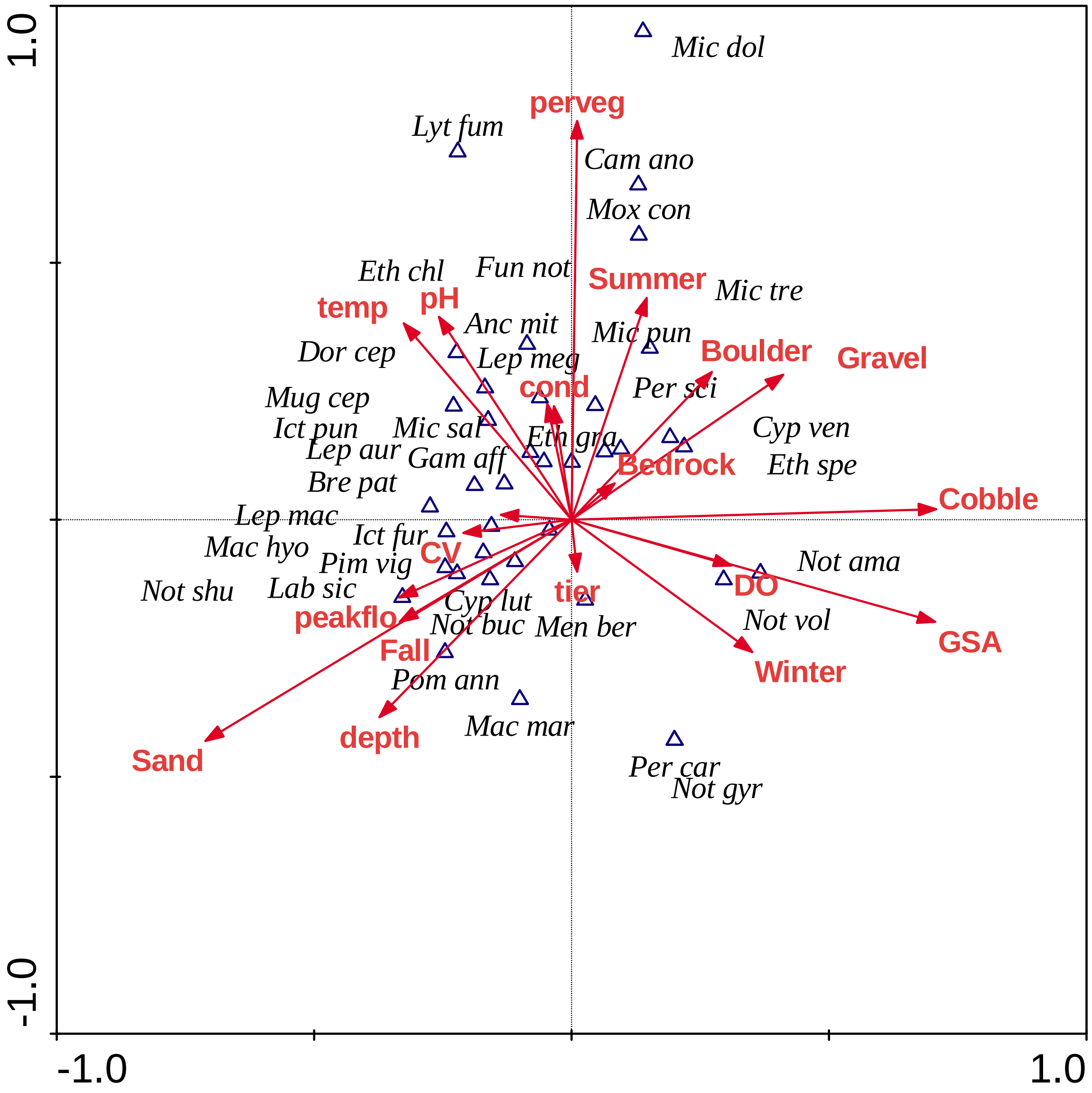


Figure 19. **Canonical Correspondence analyses (CCA) of the total number of fishes from run habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 9 for run fishes codes.**

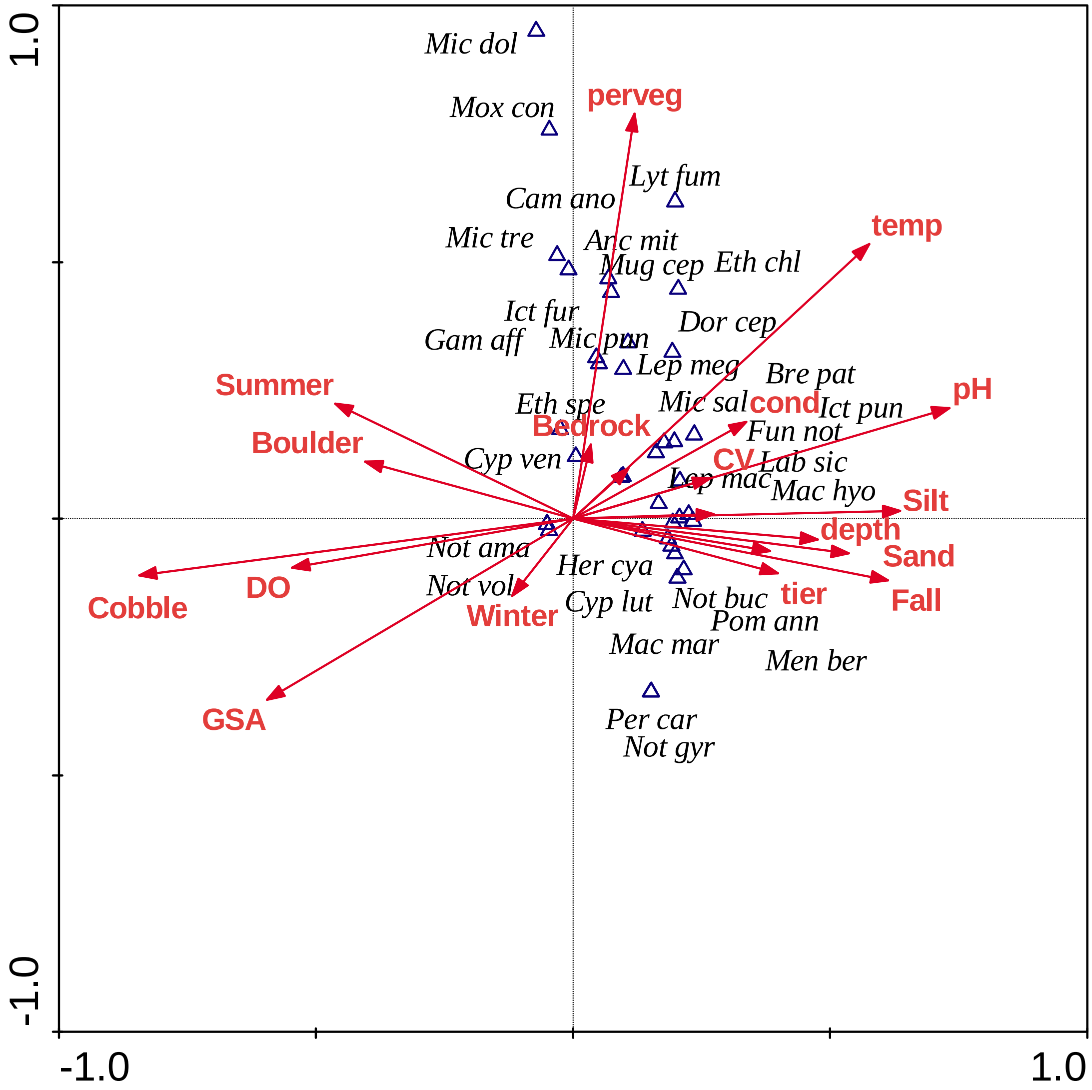


Figure 20. **Canonical Correspondence analyses (CCA) of the density of fishes from run habitats for Guadalupe-San Antonio Rivers (GSA) and Brazos River (BRA) associated among site, season, and abiotic factors from August 2014 – May 2015. See Table 9 for run fishes codes.**

Flow tier analyses

Numbers of riffle and run habitats quantified by flow tier, basin, and season are provided in Table 10. Habitat descriptions by flow tier are provided in Appendices A (riffle) and B (run).

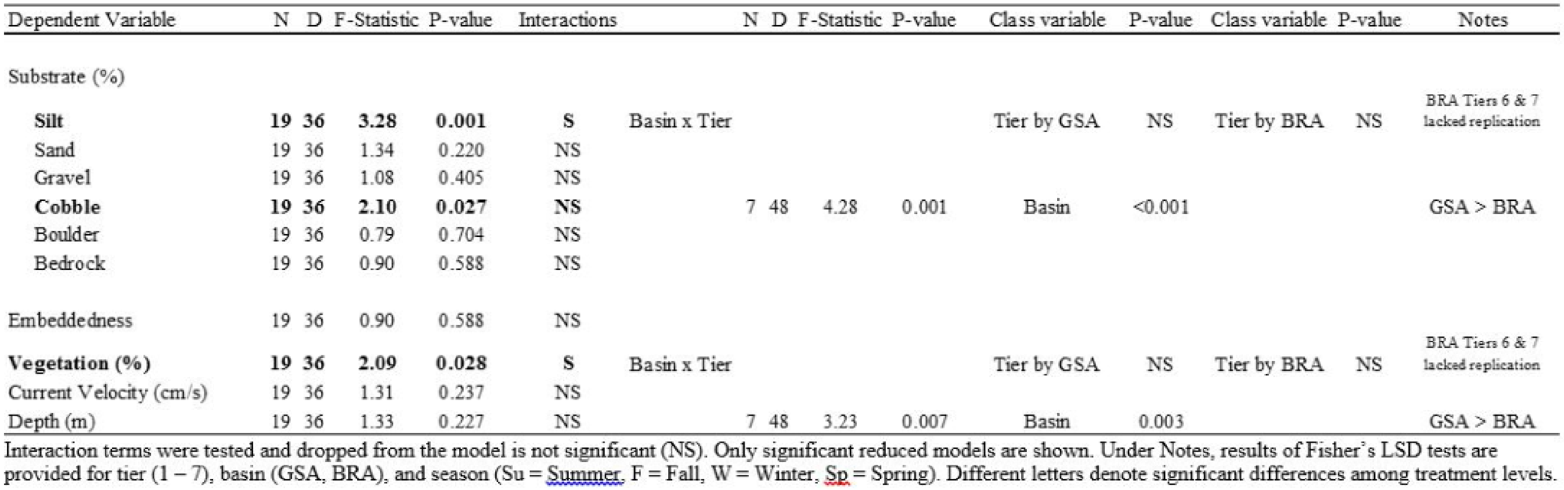
Ten habitat hypotheses were tested for riffles among tiers, basins, and seasons (Table 11). Percentages of silt substrates, cobble substrates, and percent vegetation (in bold) differed among treatments. Cobble substrates differed by basin with GSA riffles consisting of more cobble substrates than BRA. 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 12; 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 10. 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 Description	1 Subsistence	2 Base-average	3 4 per season	4 3 per season	5 2 per season	6 1 per season	7 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 11. **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.

Table 12. 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 5c 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 5a 6b 7ab
	Slackwater fishes	19	36	2.05	0.031	NS	7	48	3.98	0.002	Tier	0.001	Basin	0.005			2a 5b 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 5a 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.

Seventeen fish community hypotheses were tested for riffles among tiers, basins, and seasons (Table 12; 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 12; 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.

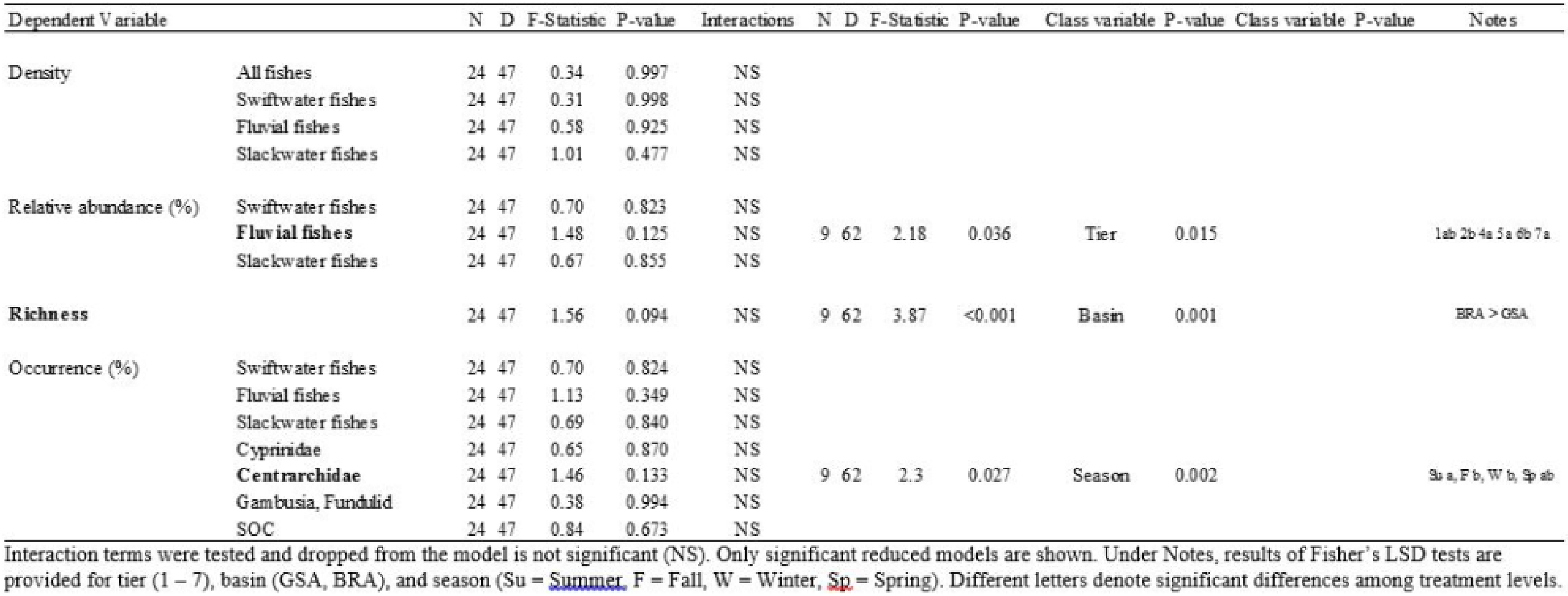
Nine habitat hypotheses were tested for runs among tiers, basins, and seasons (Table 13). Percentages of gravel substrates and cobble substrates differed among treatments. Gravel substrates differed by tier and season. Gravel substrates were greater at Tier 5 than tiers 1, 4, 6, and 7 and greater during 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 14; 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 13. **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									

Table 14. **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.

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 15. Low sample sizes preclude the use of more powerful statistical analyses to determine relationships between hatch dates and the flow regime.

Table 15. 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 unimodally 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, 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 21). 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 Brazos BBEST environmental flows recommendations (Figure 22). Rodger's estimate was based on discharge data from the upstream USGS stream flow 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; Durham and Wilde, 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; Durham and Wilde, 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.

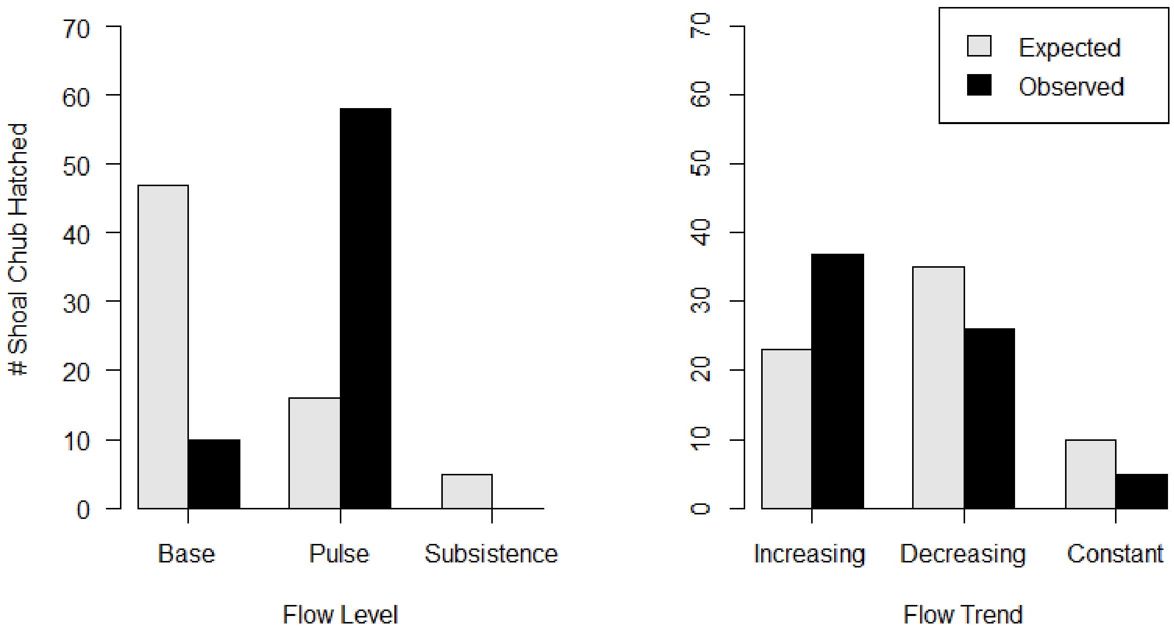


Figure 21. **χ2 goodness-of-fit test results for Shoal Chub hatch dates in relation to hydrological categories. Results for both χ2 goodness-of-fit tests analyzing flow levels and flow trends were significant (χ2 = 150.18, df = 2, P < 0.00001) and (χ2 = 13.54, df = 2, P = 0.001), respectively (adapted 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 (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, Figure 23), our best current assessment is that flow pulses of moderate magnitude promote highest recruitment of Shoal Chubs in the lower Brazos River.

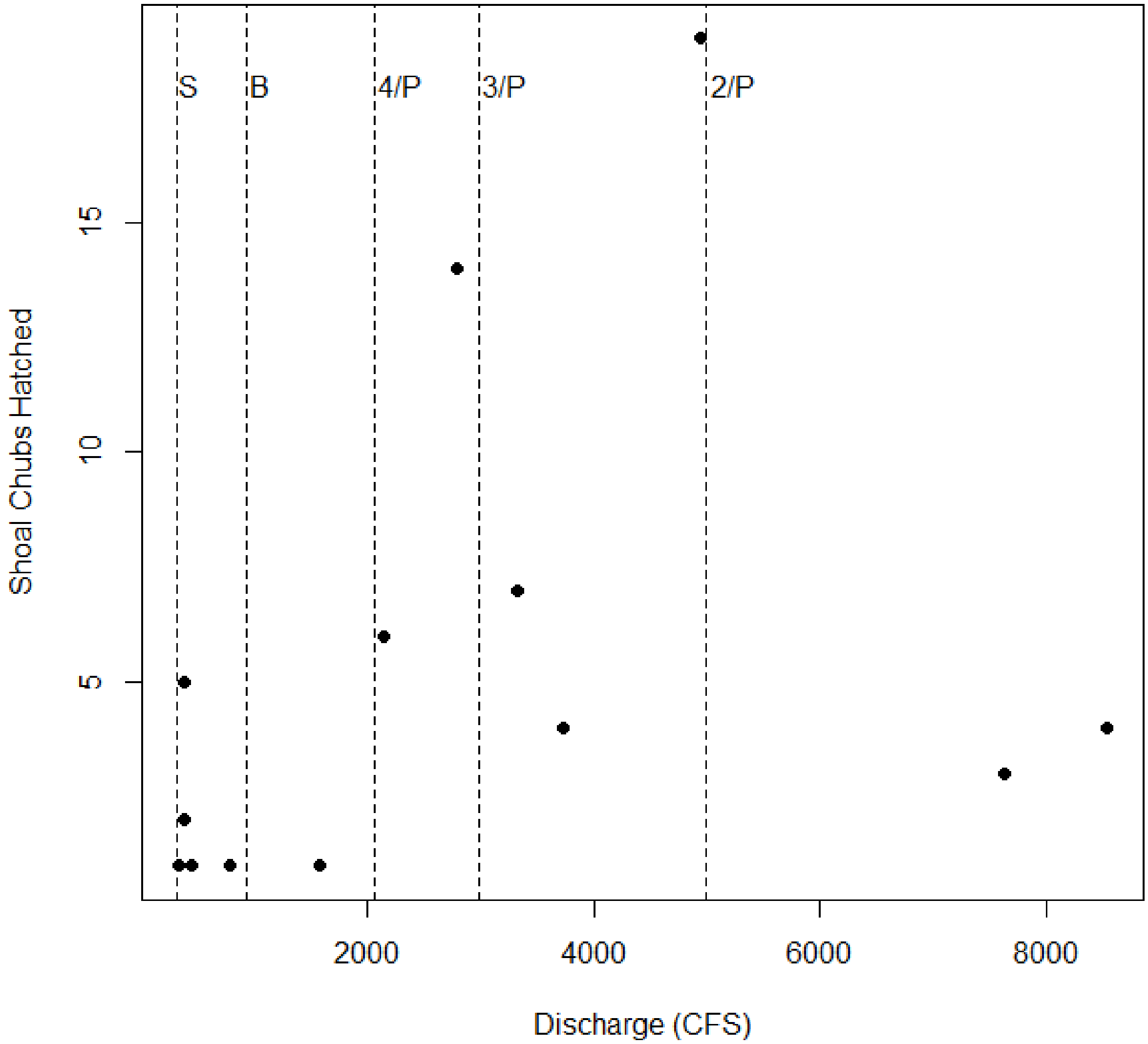


Figure 22. **Number of Shoal Chubs hatched and environmental flow standards. Environmental flow standards, for the summer period (June-October), based on USGS streamflow gauge 8108700 near Bryan, TX that represented the nearest upstream gauge from the field collection site. S = subsistence flow, B = 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).**

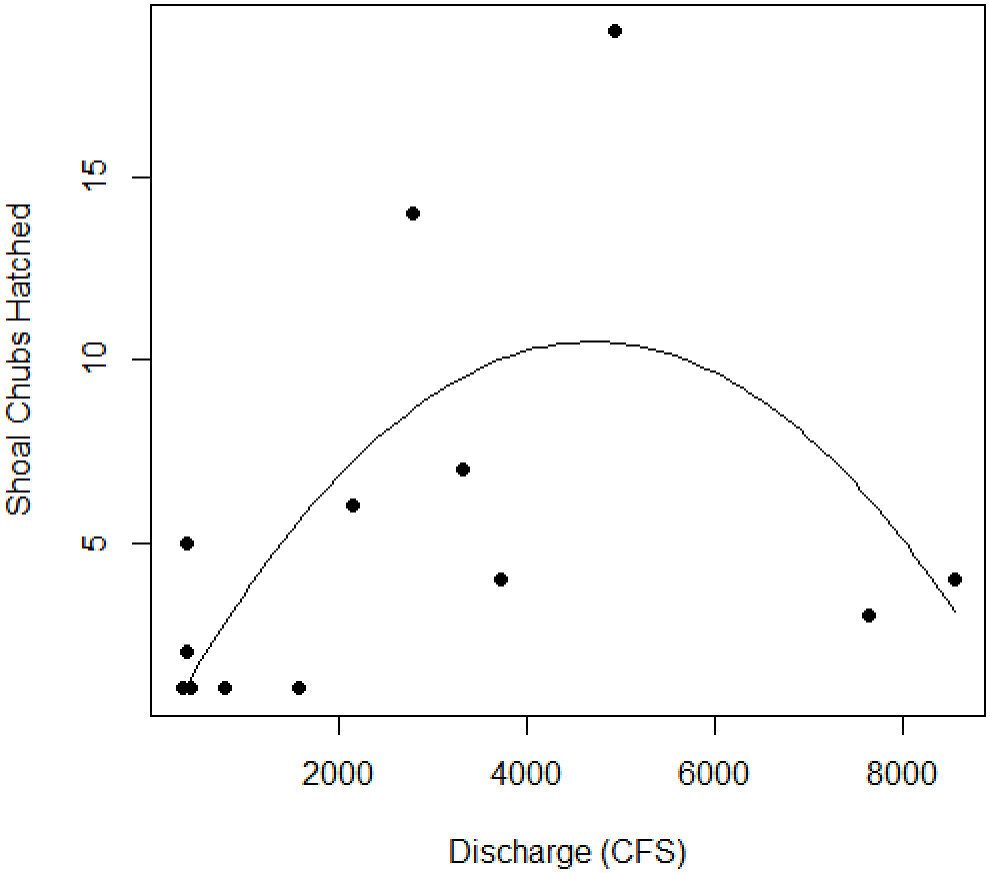


Figure 23. **Numbers of surviving Shoal Chubs hatched and discharge. Non-linear relationship was significant ($R^2 = 0.46$, $P = 0.048$). Equation for the regression line is $y = -5E\text{-}07x^2 + 0.0047x - 0.604$ (adapted from Rodger 2015).**

3.2 Riparian

Results and discussion of outcomes will be discussed by site rather than individual hypotheses to better facilitate how all hypotheses/conditions combine to determine overall riparian responses to flows. For both the GSA and Brazos 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 the occasional absence of 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 zone extent is desired, then protection of roughly the 1/year flow tiers (with an added component of timing) is essential.

Blanco site

The Blanco site (corresponding to the Blanco River USGS Gage at Wimberley) represented a headwater tributary to the Guadalupe River. The study site was located on private property (upland landscape is a mix of rural and housing zones). The river at this reach is mostly exposed bedrock with a shallow, wide channel. The slope from river's edge to the uppermost extent is 0.15 (meters rise/meters run). Beyond the sharp, almost 3m rise in elevation at stream's edge, the slope is a more gently sloping 0.07(Figure 24). Black willows occupy the lowest tiers of the slope, very near the vertical drop and completely within predicted baseflow. Green ash and elder distributions begin at just above baseflow and extend to 28m and almost 40m across the floodplain, respectively. Their vertical ranges are from about 4 to 6m. All recommended flow tiers provide coverage to some species, although coverage of box elder and green ash are considerably less than black willows (Figure 25, Figure 26, and Figure 27).

No flows provide 100% coverage for box elder or green ash. For box elders the 1/year is the only within-year flow that provides 80% of coverage. All other flows cover only 20% to 50% of the box elder range. Green ash showed a similar pattern, except the 1/year covers almost 100% and all others cover 40% to 62% of the mature trees. Recommended base flows occurred during all seasons (Table 16). In 2014 no recommended flow pulses other than the 2/winter occurred. All recommended spring flow tiers occurred in spring 2015 during the heavy rains. The effects of the drought extending through 2014 was clearly seen, as 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.

Black willow seedlings were dispersed where base flows occurred (Figure 28) and fell well within both the mature and sapling ranges. This appears to be the long-term limit of black willow distribution along this reach. Box elder seedlings (Figure 29) dispersed just above baseflow at the very lowest edges of the mature trees. Even though no spring/summer recommended flows occurred, the seedlings' distribution follows the spatial coverage provided by a single spring flow of 4.5m inundation, followed by summer and winter flows at the same general elevation. This seedling distribution is well below the saplings' range, which falls within the mature ranges. No green ash seedling dispersal occurred, and the sapling distributions fell within the spring and summer flows that did occur (Figure 30). While there was some overlap of saplings to mature ranges, there has been a definite shift from mature to replacing age classes toward the stream, and the lack of seedlings altogether indicates this trend is magnifying. Several rain events occurred during the fall and winter, however, it is not possible to determine how beneficial they were to the existing vegetation (Figure 31).

This site had signs of severe drought stress (Table 17. There were very few seeds dispersed by black willow and box elder, and none by green ash. Those seedlings and saplings that were present in summer were able to survive until the next spring (sustained by the few flows that did occur), and the site saw an increase of three new saplings in spring 2015. Obviously the lack of recommended flow pulses is limiting stand replacement in this site – both spatially and in individual numbers. Collectively box elders are 15.3% of the forest, green ash are 5.1%, and black willows make up 1.7% (Table 18). This riparian zone is a diverse community, but also has more encroachment from hackberry and other upland species, which dominate in abundance over riparian species.

Saplings are the most prolific in the site at a paltry 7 individuals (Figure 32). Again, the dearth of new seedlings is evidence for the negative impacts of the recent drought the past few years, and indicates replacement was severely stunted 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 only moderately adequate to maintain the existing riparian distribution for the reach - black willow mature distributions are covered completely by baseflow inundation but the two other species are not fully wetted with any flow tiers except the BBEST-recommended 1/year flow. Even though few of the flows actually occurred over the study time period, flows that did occur had a positive influence on box elders and green ash seedling distribution; lack of flows had a detrimental effect on dispersal and seedling/sapling distribution, but not on the survival of the handful of plants in the area. The relative abundance of the riparian species in the zone (20% relative abundance) indicates that this Blanco streamside forest is functioning less as a riparian zone and more as a mixed forest.

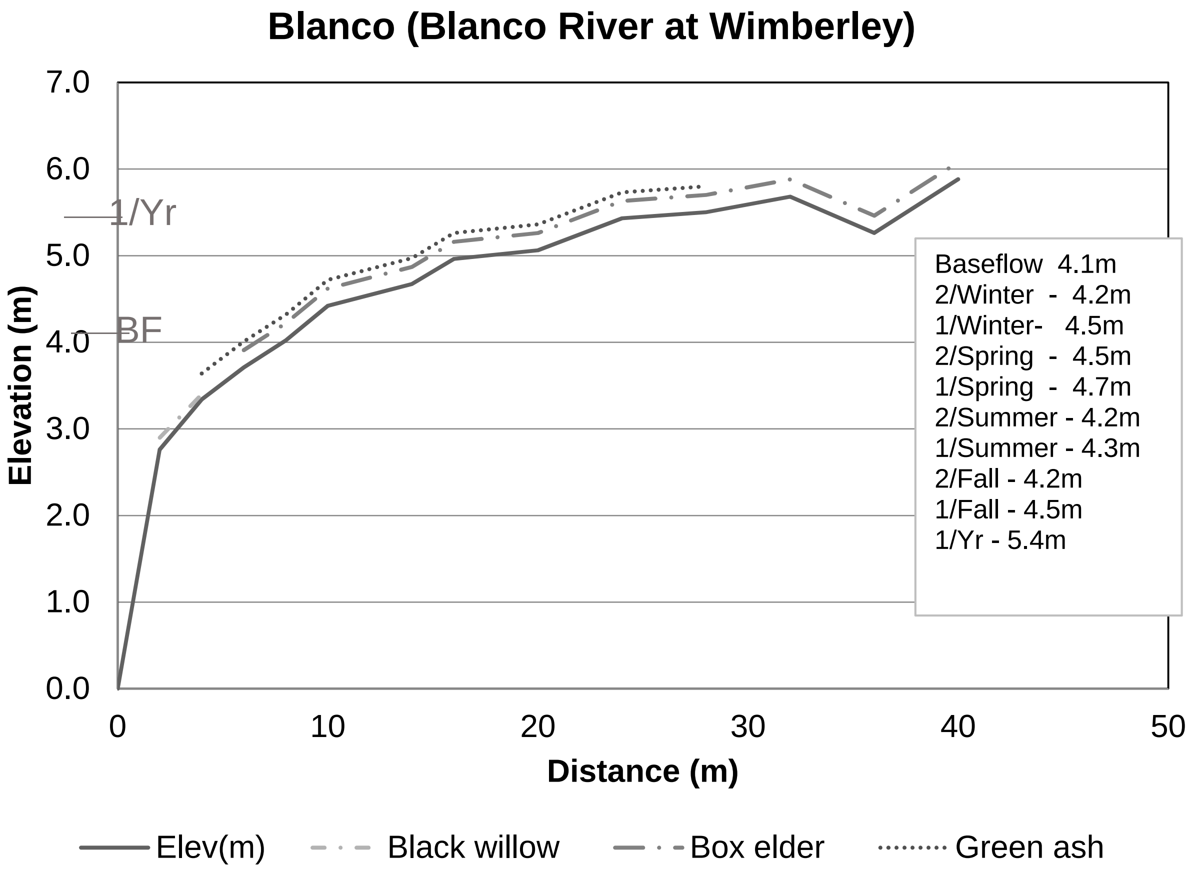


Figure 24. **Blanco site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows estimated vertical inundation of the site at the given flow tiers. Flow elevation and select flows are shown on the y-axis.**

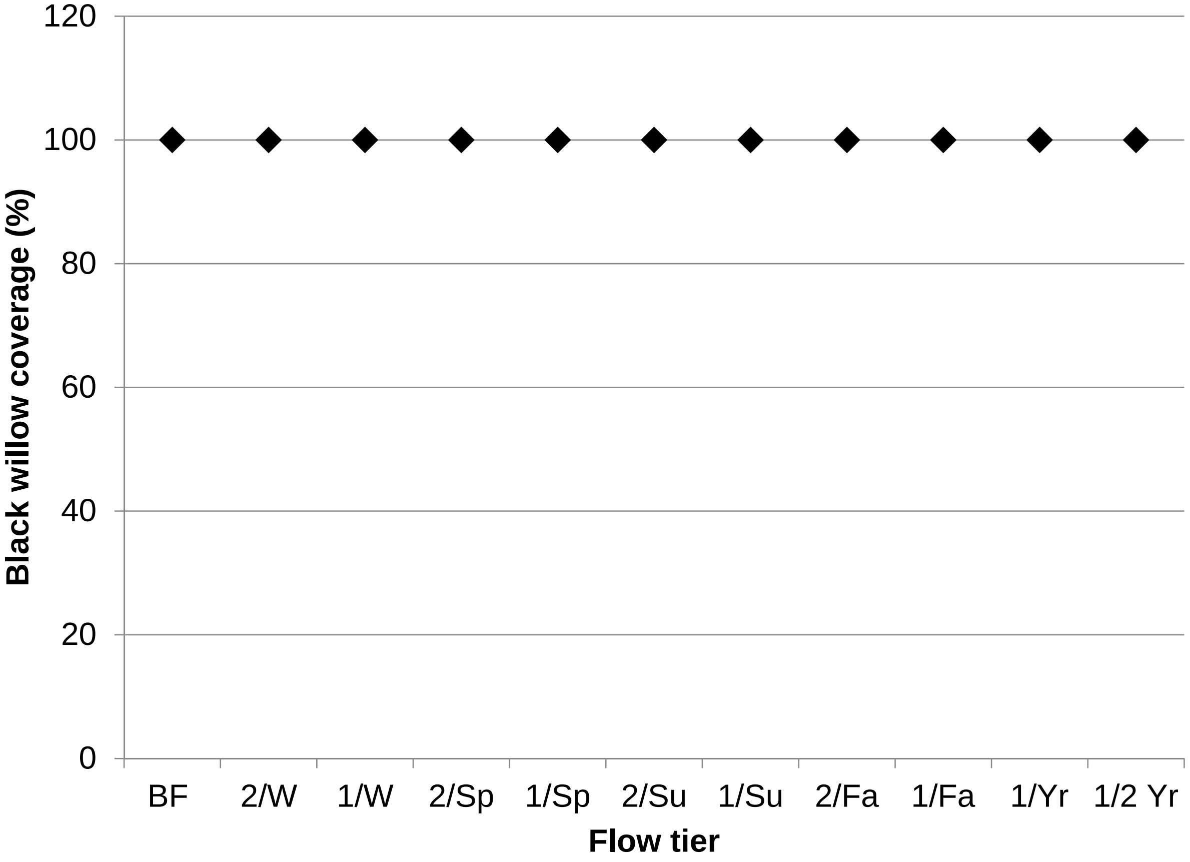


Figure 25. **Percentage of mature black willow stand at the Blanco site covered by flow tiers.**

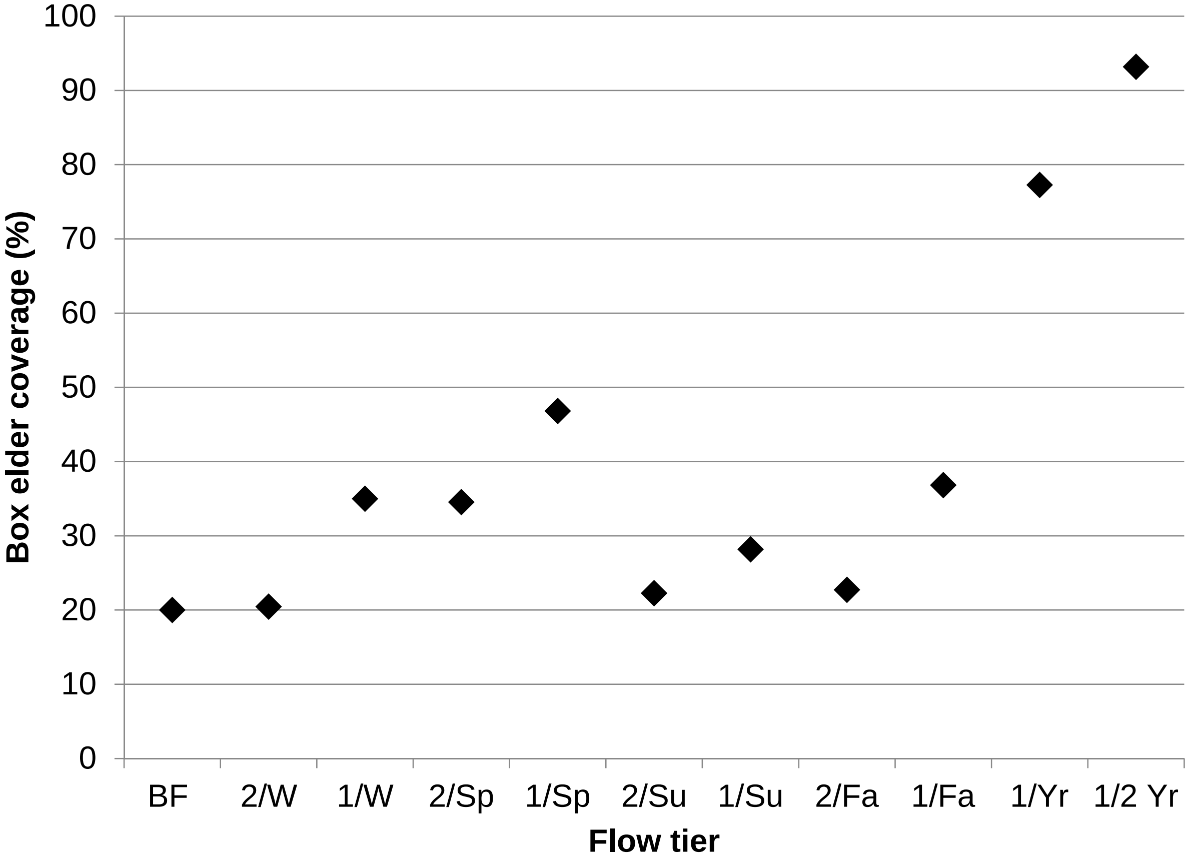


Figure 26. **Percentage of mature box elder stand at the Blanco site covered by flow tiers.**

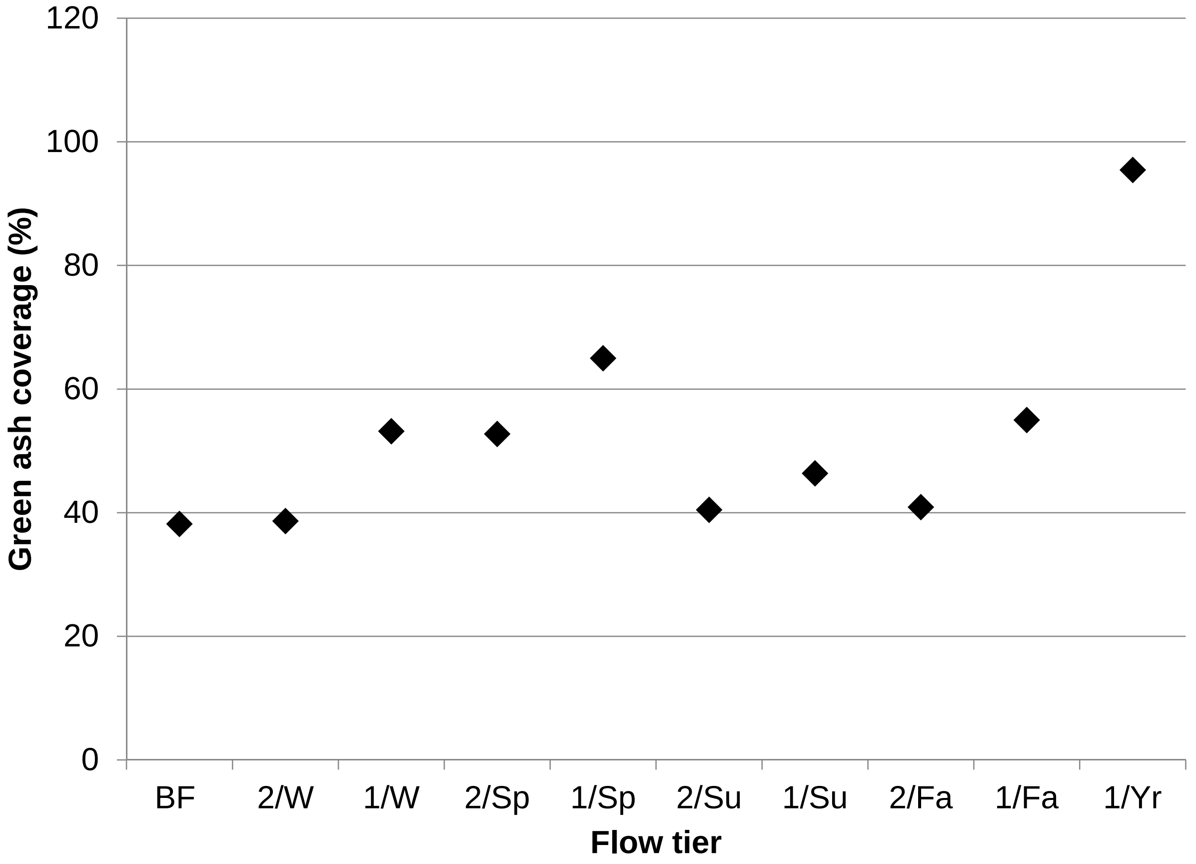


Figure 27. **Percentage of mature green ash stand at the Blanco site covered by flow tiers.**

Table 16. **Flow tiers (TCEQ flow standards and one BBEST 1/year recommendation) and their occurrences throughout the BBEST-designated seasons at the Blanco site. Y indicates flow occurred; dash indicates no flow occurred.**

		2014 Spring	2014 Summer	2014 Fall	2014 Winter	2015 Spring
Flow Tier	**CFS**	**Apr.-Jun.**	**Jul.-Sep.**	**Oct. - Dec.**	**Jan. - Mar.**	**Apr. - Jun.**
Baseflow	40	Y	N	Y	Y	Y
2/Winter	54				Y	
1/Winter	380				-	
2/Spring	360	-				Y
1/Spring	960	-				Y
2/Summer	74		-			
1/Summer	190		-			
2/Fall	82			-		
1/Fall	440			-		
1/Year	2820	-	-	-	-	Y
1/2 Years	4640	-	-	-	-	Y
1/5 Years	8310	-	-	-	-	Y

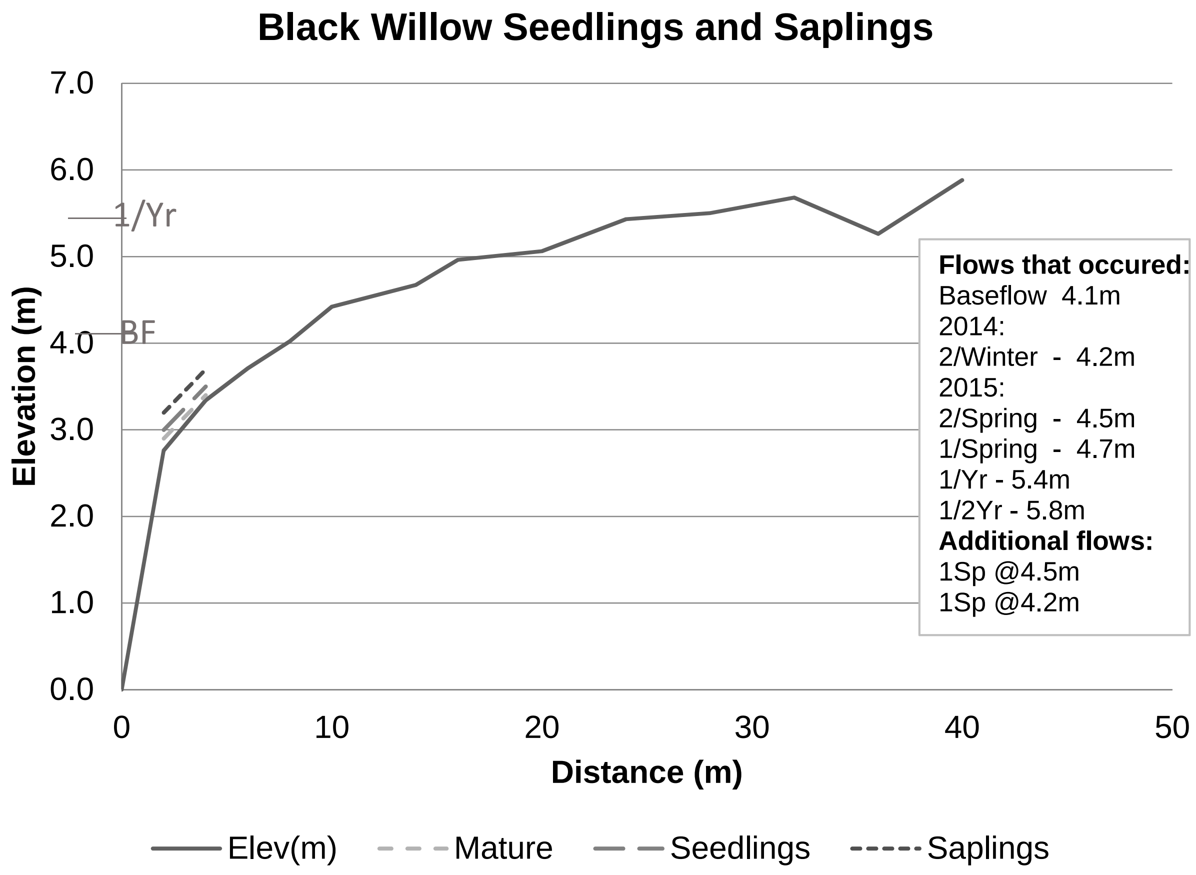


Figure 28. **Black willow distributions at the Blanco site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

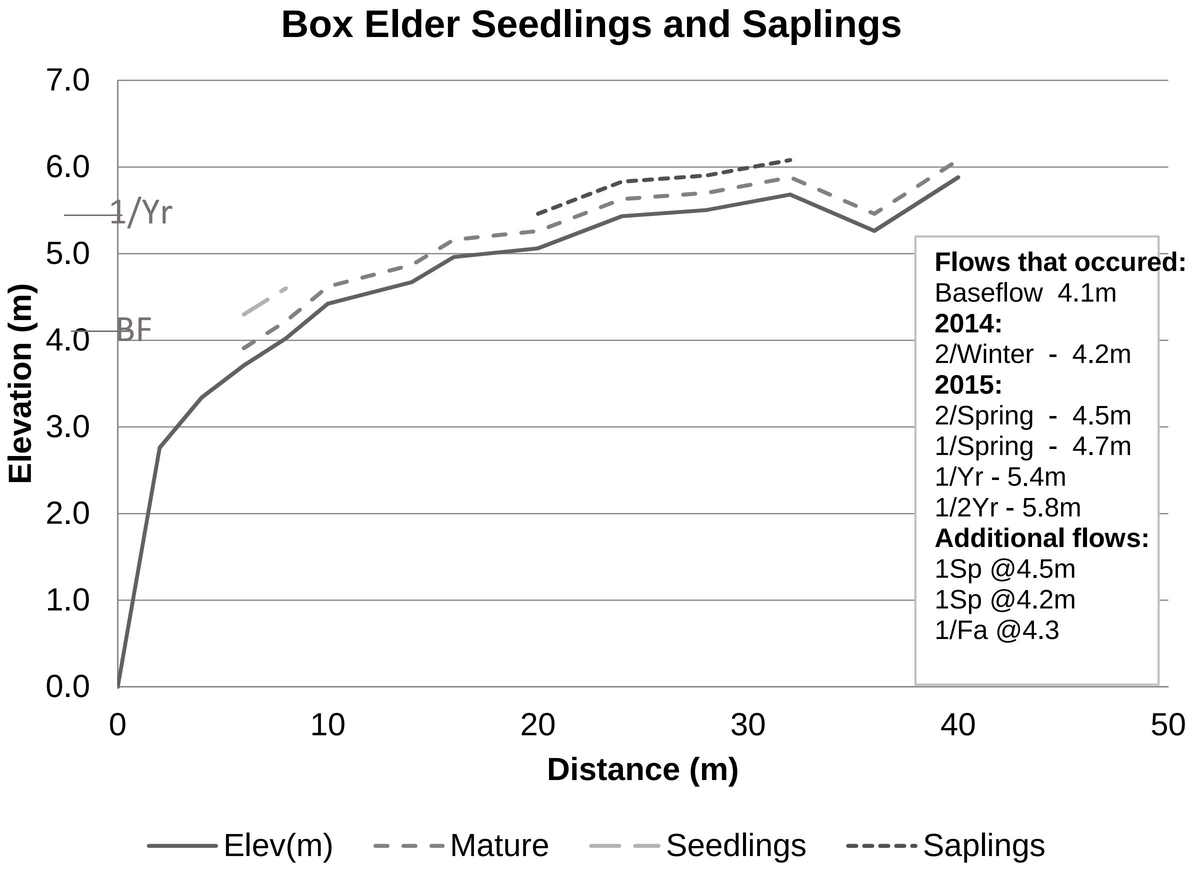


Figure 29. **Box elder distributions at the Blanco site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

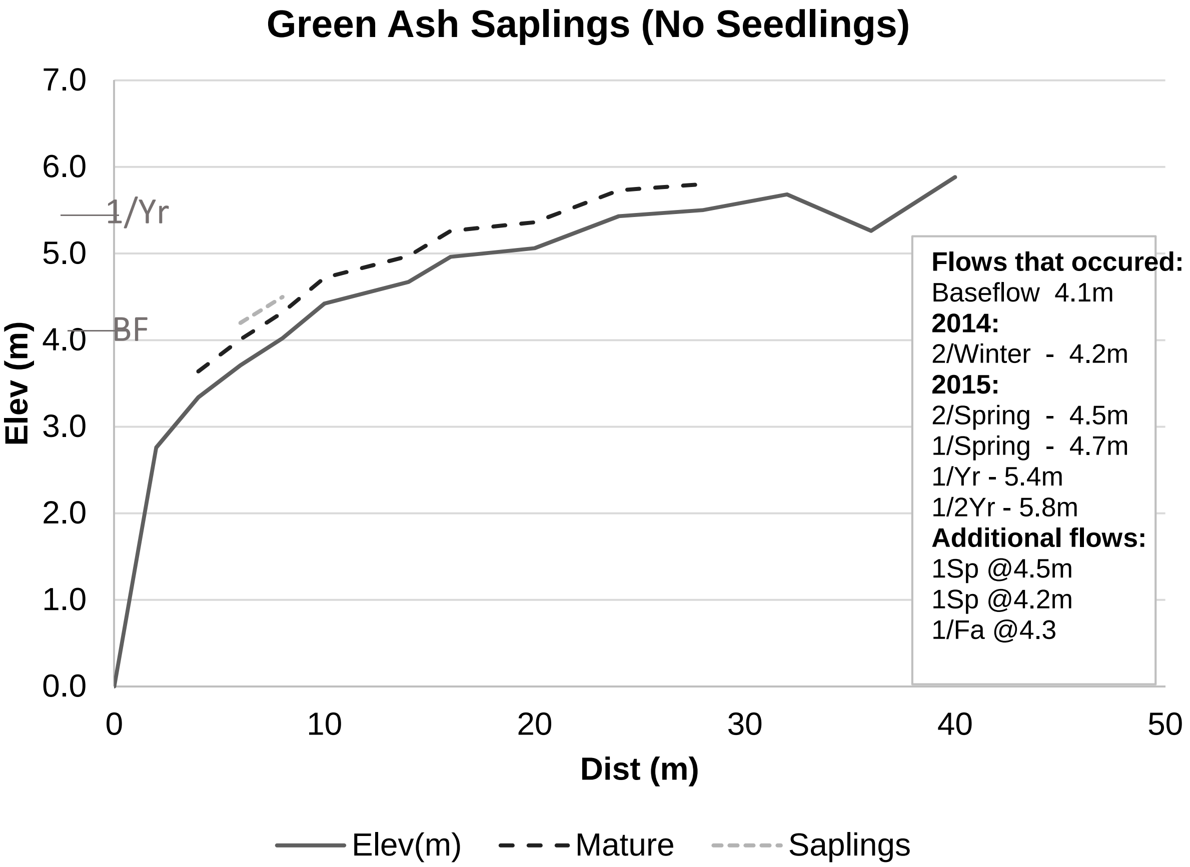


Figure 30. Green ash distributions at the Blanco site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.

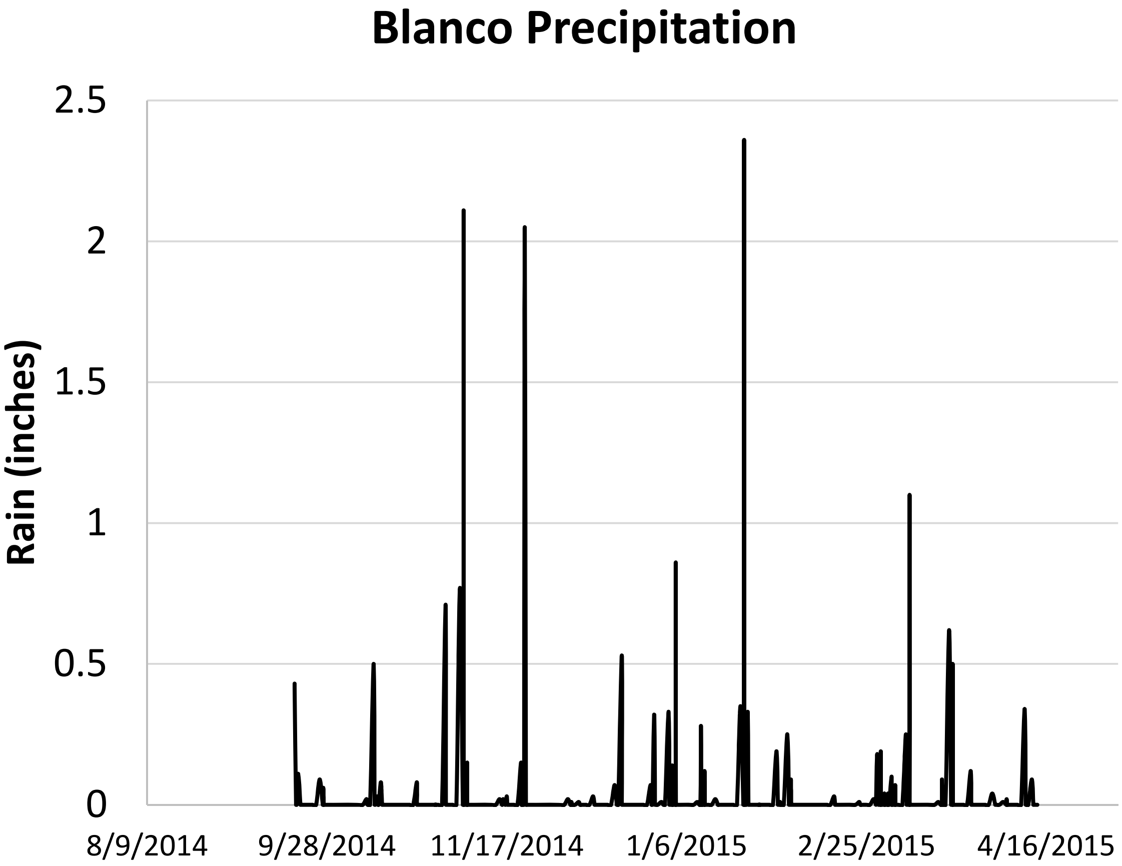


Figure 31. **Blanco site local rainfall data in inches.**

Table 17. **Blanco site tree counts through time grouped by class.**

Species	Class	Summer 2014	Fall 2014	Spring 2015
Black Willow	Sapling	1	1	1
Black Willow	Seedling	0	1	3
Box Elder	Mature	4	4	4
Box Elder	Sapling	5	5	5
Box Elder	Seedling	0	0	1
Green Ash	Mature	2	2	2
Green Ash	Sapling	1	1	1

Table 18. **Relative abundances of woody species at the Blanco site, grouped by tree type and age class.**

Tree Species	Class	Relative abundance (%)
Bald Cypress	Seedling	3.4
Black Willow	Seedling	0
Black Willow	Sapling	1.7
Box Elder	Seedling	0
Box Elder	Mature	6.8
Box Elder	Sapling	8.5
Buttonbush	Seedling	1.7
Buttonbush	Mature	3.4
Buttonbush	Sapling	3.4
Cedar Elm	Mature	1.7
Cedar Elm	Sapling	3.4
Cedar Elm	Seedling	10.2
Green Ash	Sapling	1.7
Green Ash	Mature	3.4
Hackberry	Seedling	16.9
Juniper	Seedling	0
Juniper	Mature	1.7
Juniper	Sapling	3.4
Live Oak	Seedling	5.1
Pecan	Mature	1.7
Pecan	Seedling	1.7
Pecan	Sapling	3.4
Sycamore	Mature	1.7
Sycamore	Sapling	5.1
Yaupon Holly	Sapling	5.1
Yaupon Holly	Seedling	5.1
		100

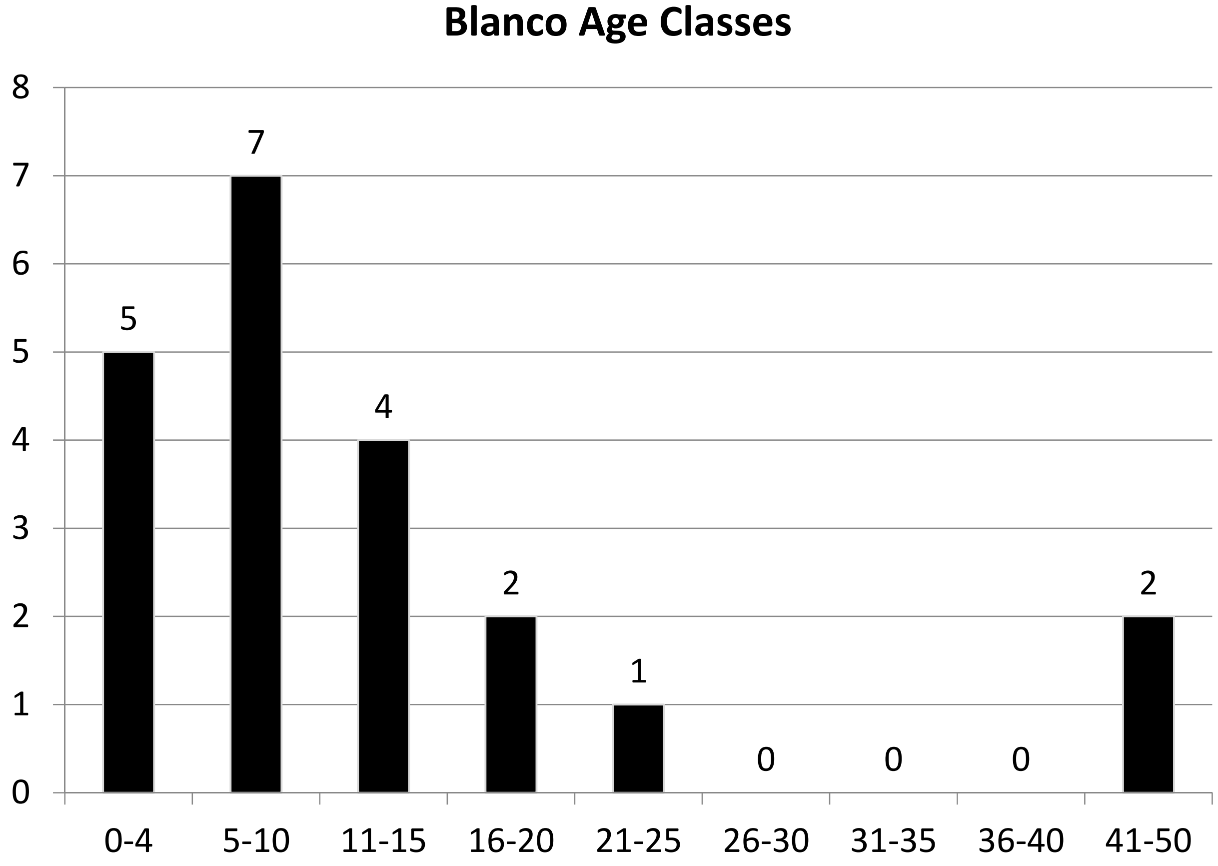


Figure 32. **Guadalupe site riparian community grouped by tree age classes; values are based on summer 2014 sampling.**

Goliad site

The Goliad site (corresponding to the San Antonio River USGS Gage at Goliad) represented a mid to lower reach of the San Antonio River. The study site was located within Goliad State Park. The river at this reach is more deeply incised with steep banks. Within the study site, banks are not sheer cliffs, but extremely steep near water's edge. Even though there are a number of foot paths through this site, the riparian forest is still largely intact and well preserved (though it is surrounded by areas of highly manicured landscape). The slope from river's edge to the uppermost extent is a steep 0.40 (meters rise/meters run; Figure 33). Beyond that the floodplain flattens out to horizontal.

Black willows occupy the lowest tiers of the slope, from within 1m of water's edge up to 4m elevation and 8m distance. Green ash distributions begin at the same location but extend up to almost 5m elevation and 10m distance. All but baseflow and the 2/fall flow tier provide coverage to some species, although very few provide 80% or more coverage.

Several flow tiers inundate black willow mature trees each year at 80% or more (Figure 34): TCEQ 3/spring, 2/Feb. – Mar., 2/Jul. – Nov., and the 1/year BBEST-recommended flows. Those same flows for green ash provide 100% inundation as well (Figure 35). Additionally, other than the 2/summer and the two large flow pulses for Feb. – Apr. and Jul. – Nov. most TCEQ flow standard pulses occurred (Table 19). All recommended spring flows occurred in spring 2015 during the heavy rains.

In addition to most of the recommended flows, there were three additional high-flow pulses observed during the study (Figure 36, Figure 37, and Figure 38) Black willow saplings were distributed from just above base flows (Figure 36) to almost 5m height - well within and just beyond the mature ranges. This appears to be the long-term spatial limit of black willow distribution at this site regardless of additional flow pulse heights. There was no black willow seedling dispersal during 2014.

Even though there were no mature box elders in the study plots, some were observed in the larger forest, and there were seedlings (Figure 37) of this species dispersed just above baseflow to about 4.5m elevation, correlating directing with the 1/spring event. This seedling distribution falls below the saplings' range, which extends up to the 1/year flow recommendation (which did occur in 2014). It appears timing of seedling dispersal likely occurred during the 1/spring event that did occur. The green ash saplings shown (Figure 38) (there were no seedlings dispersed) show a distribution directly comparable to their mature counterparts. This also corresponds to the 1/spring event that occurred and shows they are providing maximum spatial replacement for this stand.. Because of flooding along this reach the site was inaccessible during the spring count window, and so the counts only extend through fall (Table 20). The one mature willow that perished was lost during one of the late summer/early fall flow pulses, as it was near the stream's edge on a steep, highly erodible slope. Sapling count for this species increased between summer and fall by 3 individuals. Box elder saplings saw a reduction of 4 plants (probably the same scouring event) while 2 new seedlings emerged. Green ash saplings fared well, and two new seedlings of this species emerged by fall.

Collectively box elders are 22% of the forest, green ash are 41%, and black willows make up 20% (Table 21). This riparian zone is a diverse community, but is still highly dominated by riparian woody species, which make up 83% of the forest. Saplings are the most prolific in the site with 69 individuals (Figure 39). The near-total lack of new seedlings could be evidence for the negative impacts of the recent drought the past few years, and indicates replacement was severely stunted this past year. Beyond saplings, the presence of older trees drops to 5 or less 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.

Several TCEQ flow tiers provide full inundation of the riparian species at this reach, in particular those larger flow pulses implemented with intended riparian linkages that were generated during a site-specific, comprehensive instream flow study at this location. Many of the flows actually occurred over the study period, and those flows that did occur appeared to have a positive influence on dispersal, survival and even late season seed germination, while at the same removing other individuals. Seedling distributions closely correlated with actual flows during 2014, showing the importance of those flows in distributing the seedlings. Age structure analysis indicates that a lack of stream flow pulses along the river have had noticeable impacts on seedling dispersal and future maintenance. The dominance of the riparian species (83% relative abundance) in the zone indicates that this Goliad streamside forest is functioning well as a riparian zone though with reduced seed dispersal; prolonged lack of stream flow would likely further threaten or prevent replacement of all three species. This site probably represents the closest example of a riparian zone having received recommended high-flow pulses (rather than drought conditions) in comparison to the other sites because it did see multiple flows throughout the 2014 season. Even though the seedling counts were much lower than expected based on previous monitoring in this area (that could be prolonged drought impact), the fluctuations in age classes (and particularly the seed dispersal) seems to be reflecting later season flow inputs that both scour some of the previous woody vegetation, and allow for germination later in the year. In short, the turnover reflects both the effects of lack of flow, and high flow, throughout one growing season.

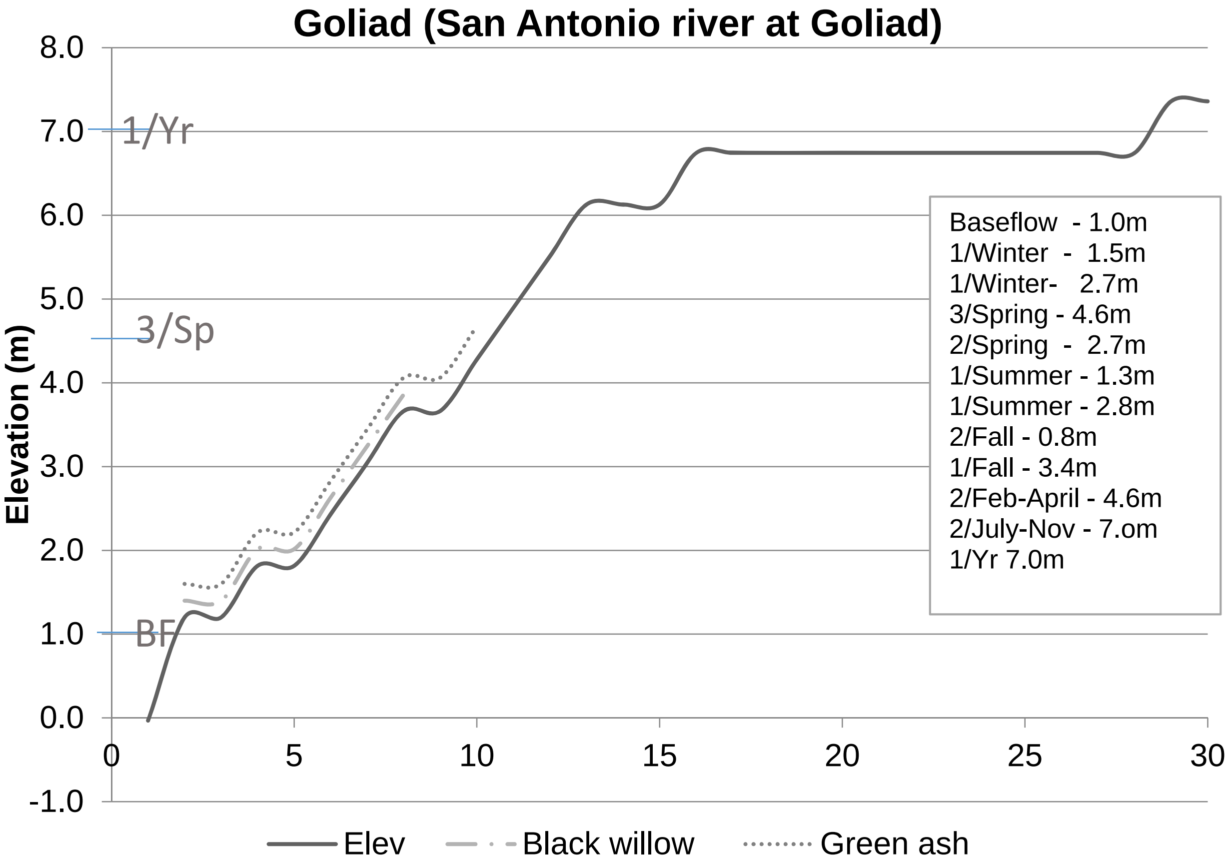


Figure 33. **Goliad site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows vertical inundation of flow tiers. Select flows are shown on the y-axis.**

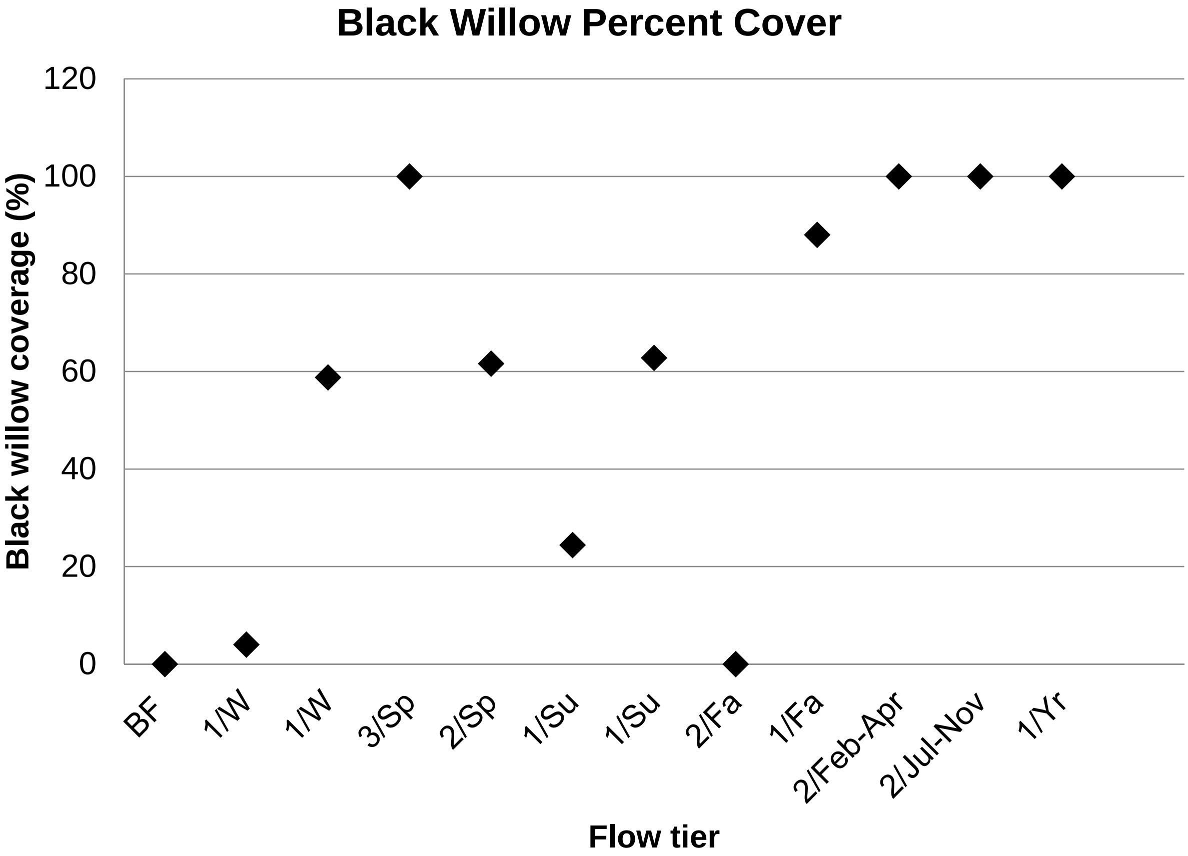


Figure 34. **Percentage of mature black willow stand at the Goliad site covered by flow tiers.**

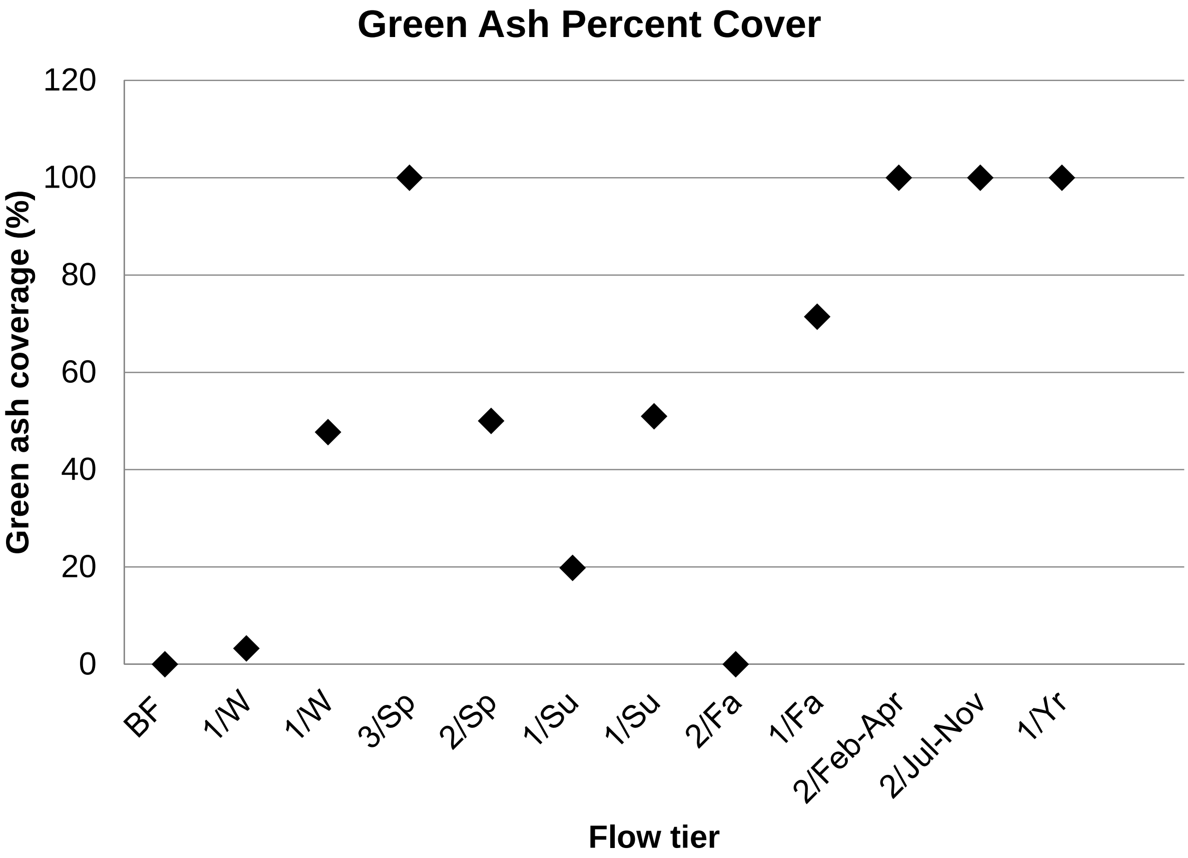


Figure 35. Percentage of mature box elder stand at the Goliad site covered by flow tiers.

Table 19. Flow tiers (TCEQ flow standards and one BBEST 1/year recommendation) and their occurrences throughout the BBEST-designated seasons at the Goliad site. Y indicates flow occurred; dash indicates no flow occurred.

		2014 Spring	**2014 Summer**	**2014 Fall**	**2014 Winter**	**2015 Spring**
Flow Tier	**CFS**	**Apr.-Jun.**	**Jul.-Sep.**	**Oct. - Dec.**	**Jan. - Mar.**	**Apr. - Jun.**
Baseflow	240	Y	Y	Y	Y	Y
1/Winter	570				Y	
1/Winter	1520				Y	
3/Spring	4000	Y				Y
2/Spring	1570	Y				Y
1/Spring	N/A					
1/Summer	390		-			
1/Summer	1640		Y			
2/Fall	190			Y		
1/Fall	2320			Y		
2/ Feb. - Apr.	4000	-				Y
2/Jul. - Nov.	8000		-			
1/Year	7680	Y	-	-	-	Y

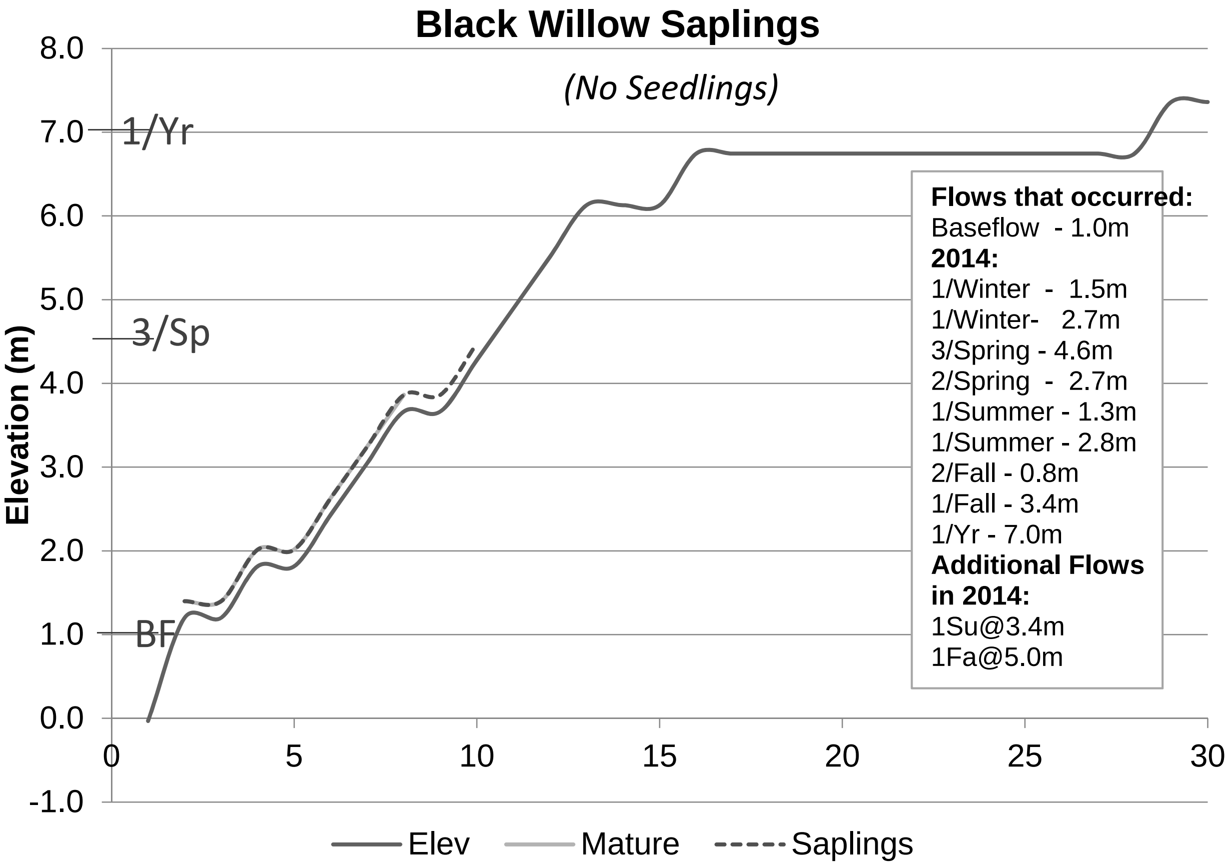


Figure 36. **Black willow distributions at the Goliad site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

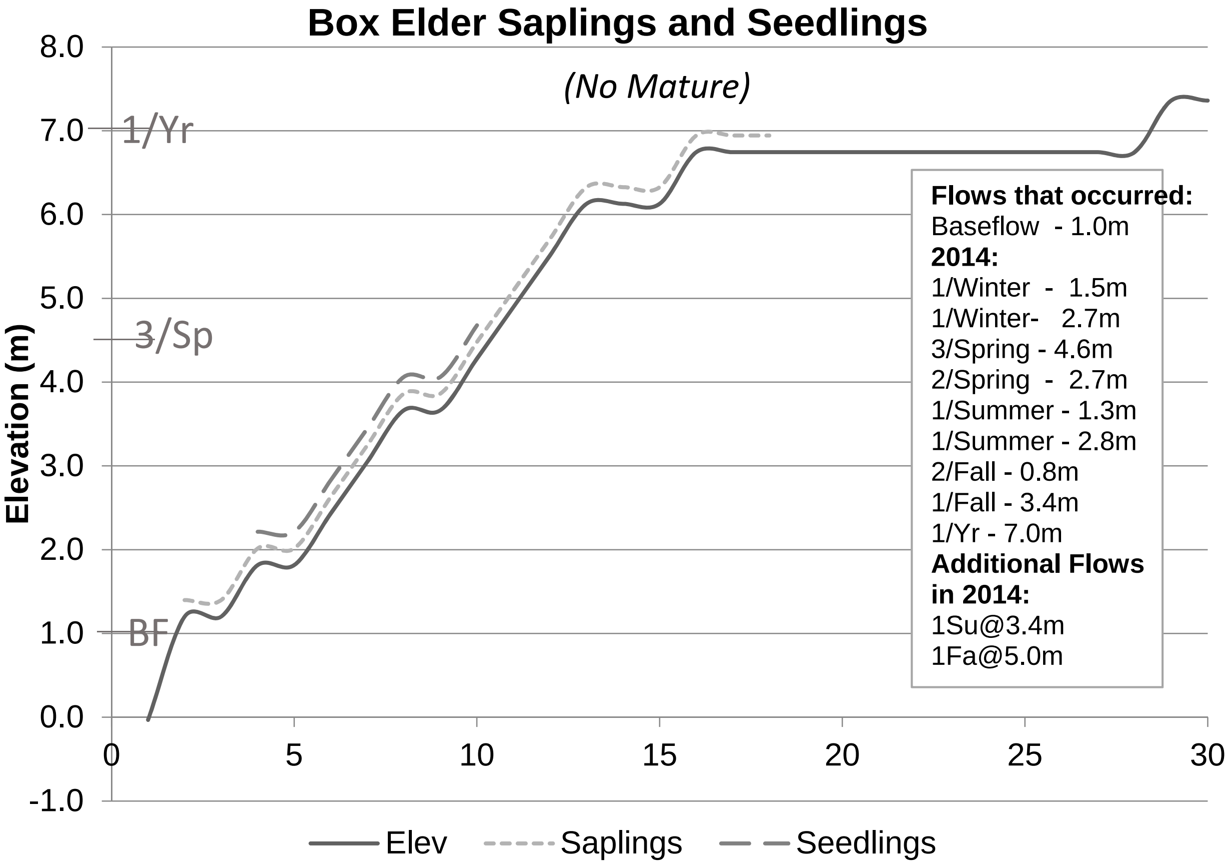


Figure 37. **Box elder distributions at the Goliad site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

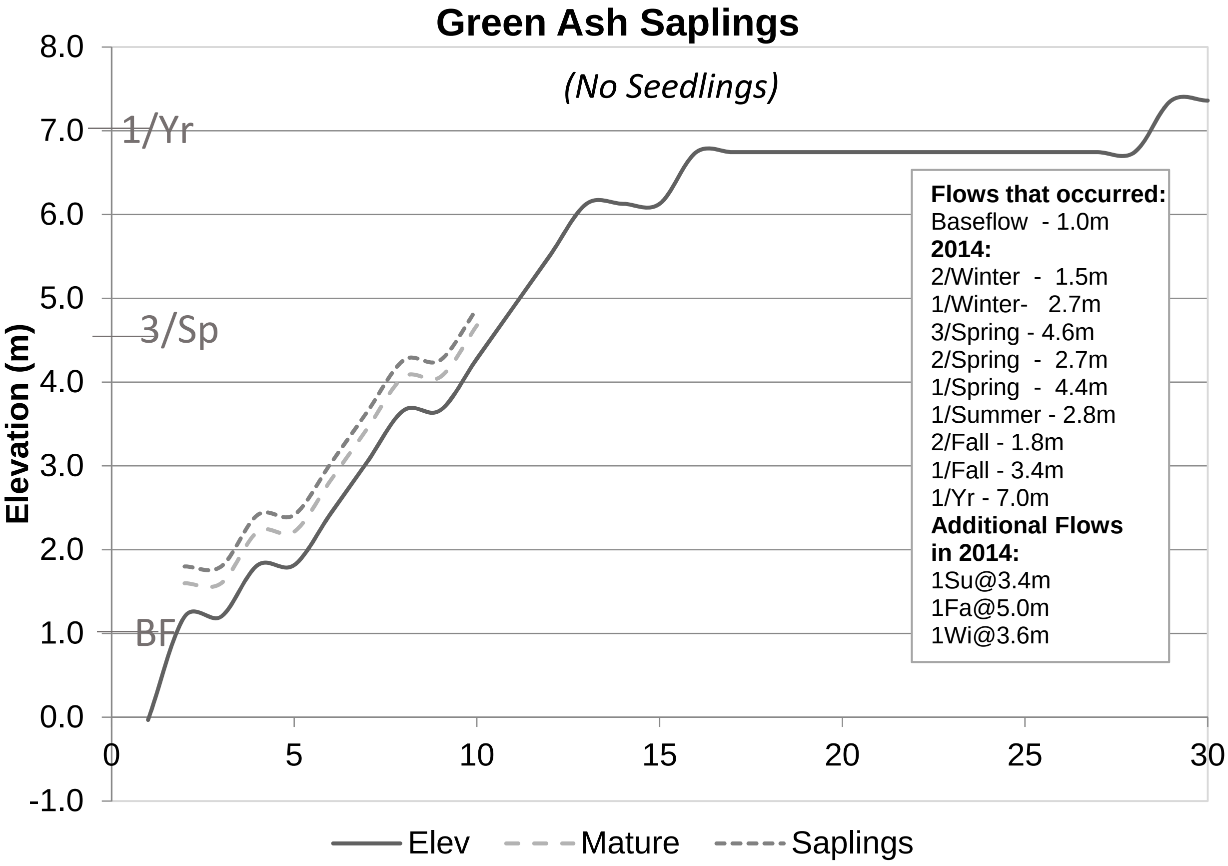


Figure 38. **Green ash distributions at the Goliad site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

Table 20. **Goliad site tree counts through time grouped by class.**

Species	Class	Summer 2014	Fall 2014
Black Willow	Mature	9	8
Black Willow	Sapling	13	16
Box Elder	Sapling	12	8
Box Elder	Seedling	12	10
Green Ash	Mature	4	4
Green Ash	Sapling	39	39
Green Ash	Seedling	0	2

Table 21. **Relative abundances of woody species at the Goliad site, grouped by tree type and age class.**

Tree Species	Class	Relative abundance (%)
Black Willow	Mature	8.2
Black Willow	Sapling	11.8
Box Elder	Sapling	10.9
Box Elder	Seedling	10.9
Cedar Elm	Seedling	0.9
Dogwood	Sapling	0.9
Elm	Sapling	0.9
Elm	Seedling	1.8
Green Ash	Mature	3.6
Green Ash	Sapling	35.5
Green Ash	Seedling	0
Hackberry	Seedling	8.2
Pecan	Seedling	5.5
Sycamore	Sapling	0.9
		100

Figure 39. **Goliad site riparian community grouped by tree age classes; values are based on summer 2014 sampling.**

Gonzales site

The Gonzales site (corresponding to the Guadalupe River USGS Gage at Gonzales) represented a mid-reach Guadalupe River site. The study site was located on private property (upland landscape is mostly agriculture and grazing pastures). The river at this reach is mostly exposed bedrock, cobble and gravel with a shallow, wide channel. The Gonzales site represents a longer term study than do the other sites. It has been monitored since September 2013 as part of the aforementioned GBRA sponsored study and was used as a model for the other site methodologies.

This site had a longer dataset of stream flow, and therefore, is a good example of calibration between the on-site and USGS flows (Figure 40). As stated in the methods section, as long as flow pulses are comparable, adjustments/calibrations can be made to either dataset to reflect the actual inundation of USGS flow pulses into the study site when considering flows prior to start of the study (or where data are missing as in the late fall 2013 stream logger data).

The slope from river's edge to the uppermost extent is 0.15 (meters rise/meters run). Beyond a steep, almost 6m rise in elevation, the slope levels to 0.05 (Figure 41). Interestingly, the ranges for the two species found here (box elder and green ash) overlap completely, are extremely truncated, and are confined to a height of 6m and distance of 18-20 meters. None of the TCEQ tiered flows for this reach inundate to this level, except the BBEST-recommended 1/year at 6.2m (Figure 42). In comparison, the percent coverage of box elder and green ash saplings from March 2015 (Figure 43), with ranges including lower tiers of the channel slopes had slightly more inundation than their mature counterparts. The 1/year covers a little less than 100% because their distribution also extends further up the slope.

Average base flows were seen for all seasons (Table 22). In 2014 no other flow pulses than the 2/winter occurred. All recommended spring flows occurred in spring 2015 during the heavy rains. The effects of the drought extending through 2014 can clearly be seen (Table 22, Table 23), 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. Box elder seedlings (Figure 44) dispersed just above 2m elevation and 2m distance up to 6.3m elevation and 34m distance, both above and below the mature trees' ranges. Even though no spring/summer recommended flows occurred, the seedlings' distribution follows the spatial coverage provided by two large flow pulses of 6.2m and 8.2m inundation in fall 2013. However, there were no subsequent flows at this level during 2014 presumably necessary to ensure survival.

Green ash seedling dispersal occurred up to an elevation of 7.2m, and sapling distributions fell within a comparable range (Figure 45). Both of these age classes were well above and below the mature stands. As with the box elders, the likely flow pulse(s) that deposited the seedlings were the fall 2013 8.2m and/or 6.2m flows. Several rain events occurred throughout the season, however, it is not possible to determine exactly how beneficial they were to the existing vegetation (Figure 46).

All box elder classes saw an increase from September 2013 to March 2015 (Riparian Tables 19 and 20). Green ash mature and saplings increased as well, but seedlings had a considerable die-off. Box elder seedlings lost 5 members and had 5 recruited to saplings from September 2013 to the next spring. Through the 2014 growing season another one was recruited and 5 new germinated. Two died by October, but another 58 were added early in 2015. Saplings of this species remained fairly healthy (only one perished over the study period and one was recruited to the mature class). Green ash seedlings saw a recruitment of 10 members to sapling and a loss of 20 members from September 2013 to the next spring. During the 2014 growing season several more were added and recruited up, but between fall 2014 and spring 2015 18 perished. At the same time that seedling loss was occurring by 2014 year's end, sapling and mature loss also occurred. It appears that the late 2013 high-flow pulses (6.2m and 8.2m) jumpstarted the green ash's reproduction and growth, but by the end of the very dry 2014 year, the stands were showing the stress of a lack of further flow pulses.

Collectively box elders were 7.8% of the forest and green ash are 7.5% for a combined total of 15.3% in Sept 2013 (Table 24). By March 2015 they were: box elder – 16.3% and green ash – 10.4%; for a combined total of 26.7%. This riparian zone is a diverse community, but recently it has seen a lot of encroachment from hackberry, and also is dominated more by dogwood than any other species. Dogwood is considered a riparian-functioning species, but the three indicator species do not dominate the forest. If the trend of increase in their relative abundance continues as it did between 2013 and 2015, the riparian indicator species may reach dominance in the future.

Saplings well outnumber seedlings in age classes (Figure 47). This shows that even though seedling dispersal is occurring at this site, it is not at the levels expected. As previous data have shown, this is likely a drought response, which is commonly seen in trees. 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.

For the indicator species, TCEQ flow standards fall below what would be necessary to maintain the existing riparian spatial range at this site. Even though few of the flows actually occurred over the study, flows that did occur appeared to have a positive influence on the box elders and green ash seedlings, whose distributions in 2014 directly correlated with a fall 2013 flow event. A lack of higher flows in 2014 had a negative effect on dispersal and seedling/sapling distribution, especially green ash seedling survival.

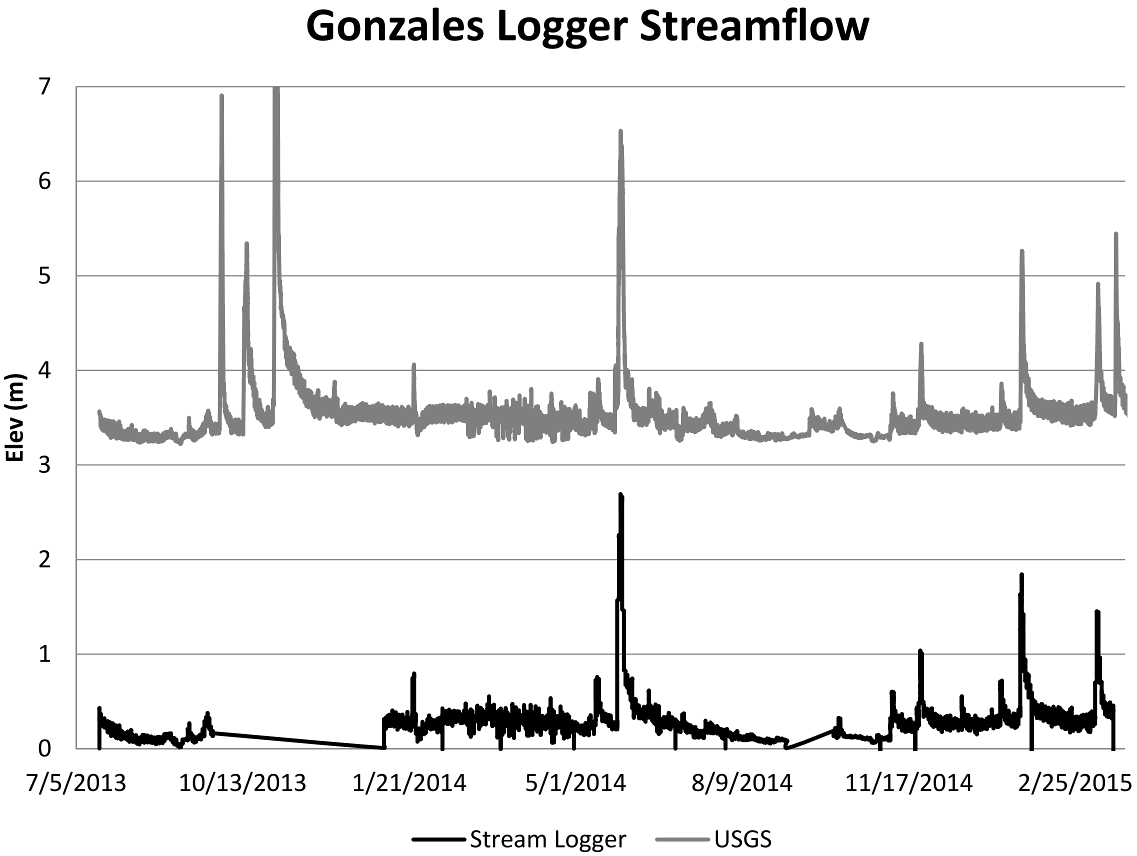


Figure 40. USGS flows in comparison to the Gonzales site stream level logger.

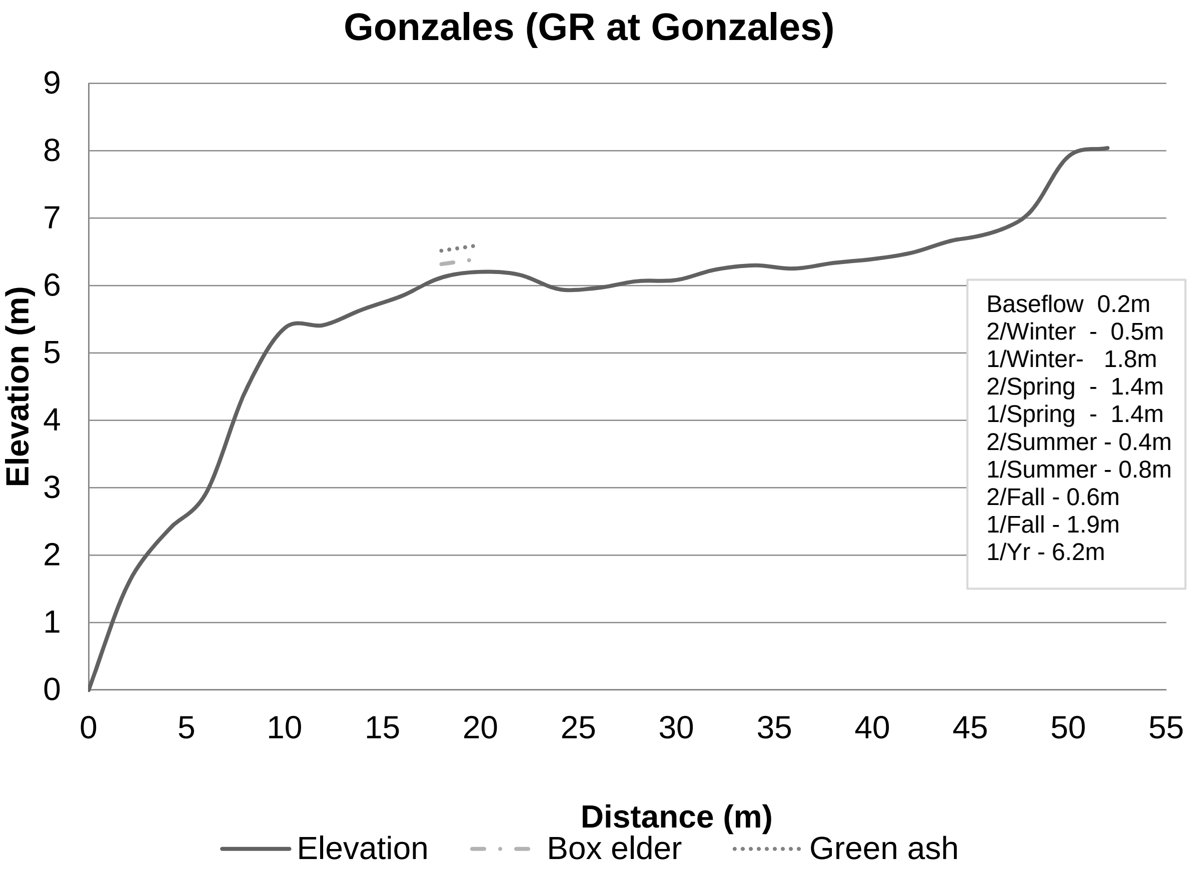


Figure 41. **Gonzales site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows vertical inundation of flow tiers.**

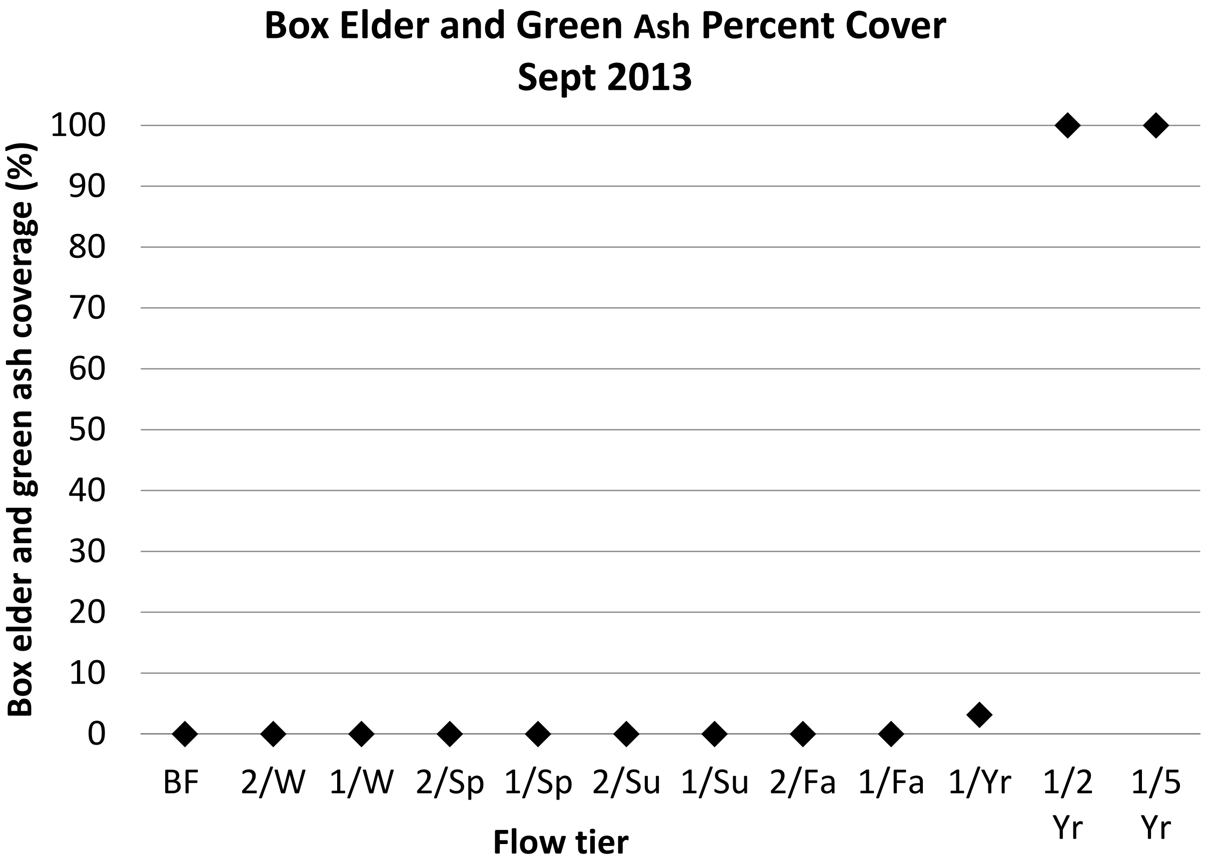


Figure 42. Percentage of mature box elder and green ash at the Gonzales site covered by flow tiers; values are based on sampling conducted in September 2013.

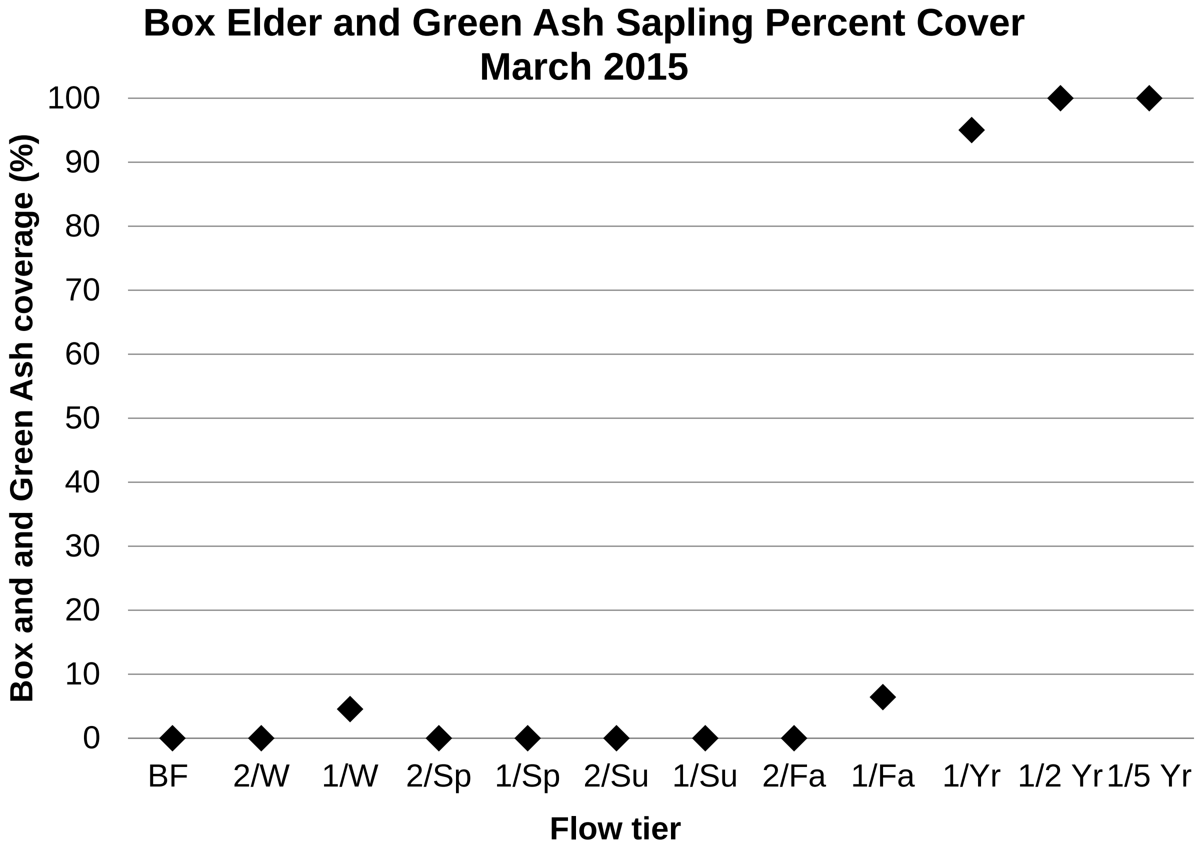


Figure 43. **2015 distribution of box elder and green ash sapling percent coverages at the Gonzales site by BBEST flow tiers; values are based on sampling conducted in March 2015.**

Table 22. **Flow tiers (TCEQ flow standards and one BBEST 1/year recommendation) and their occurrences throughout the BBEST-designated seasons at the Gonzales site. Y indicates flow occurred; dash indicates no flow occurred.**

		2014 Spring	2014 Summer	2014 Fall	2014 Winter	2015 Spring
Flow Tier	**CFS**	**Apr.- Jun.**	**Jul.- Sep.**	**Oct. - Dec.**	**Jan. - Mar.**	**Apr. - Jun.**
Baseflow	510	Y	Y	Y	Y	Y
2/Winter	1150				Y	
1/Winter	4140				-	
2/Spring	3250	-				Y
1/Spring	6590	-				Y
2/Summer	950		-			
1/Summer	1760		-			
2/Fall	1410			-		
1/Fall	4330			-		
1/Year	14300	-	-	-	-	Y
1/2 Years	24400	-	-	-	-	-
1/5 Years	36700	-	-	-	-	-

Table 23. **Gonzales site species counts through time grouped by class.**

Species	Class	Sep. 2013	Apr. 2014	Aug. 2014	Oct. 2014	Mar. 2015
Box Elder	Mature	1	1	2	2	2
Box Elder	Sapling	36	14	41	41	40
Box Elder	Seedling	17	8	12	10	68
Green Ash	Mature	1	1	2	2	1
Green Ash	Sapling	36	46	53	52	49
Green Ash	Seedling	53	23	37	38	20

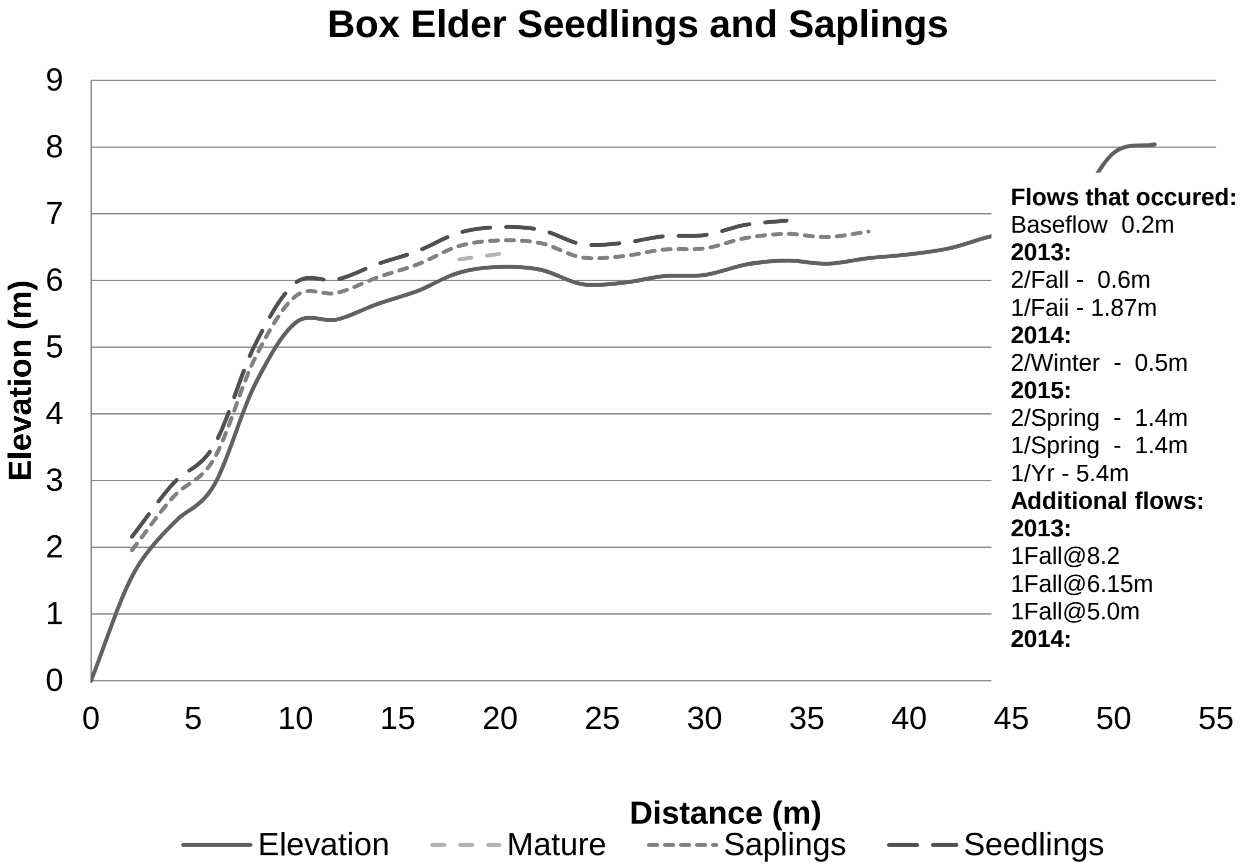


Figure 44. **Box elder distributions at the Gonzales site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

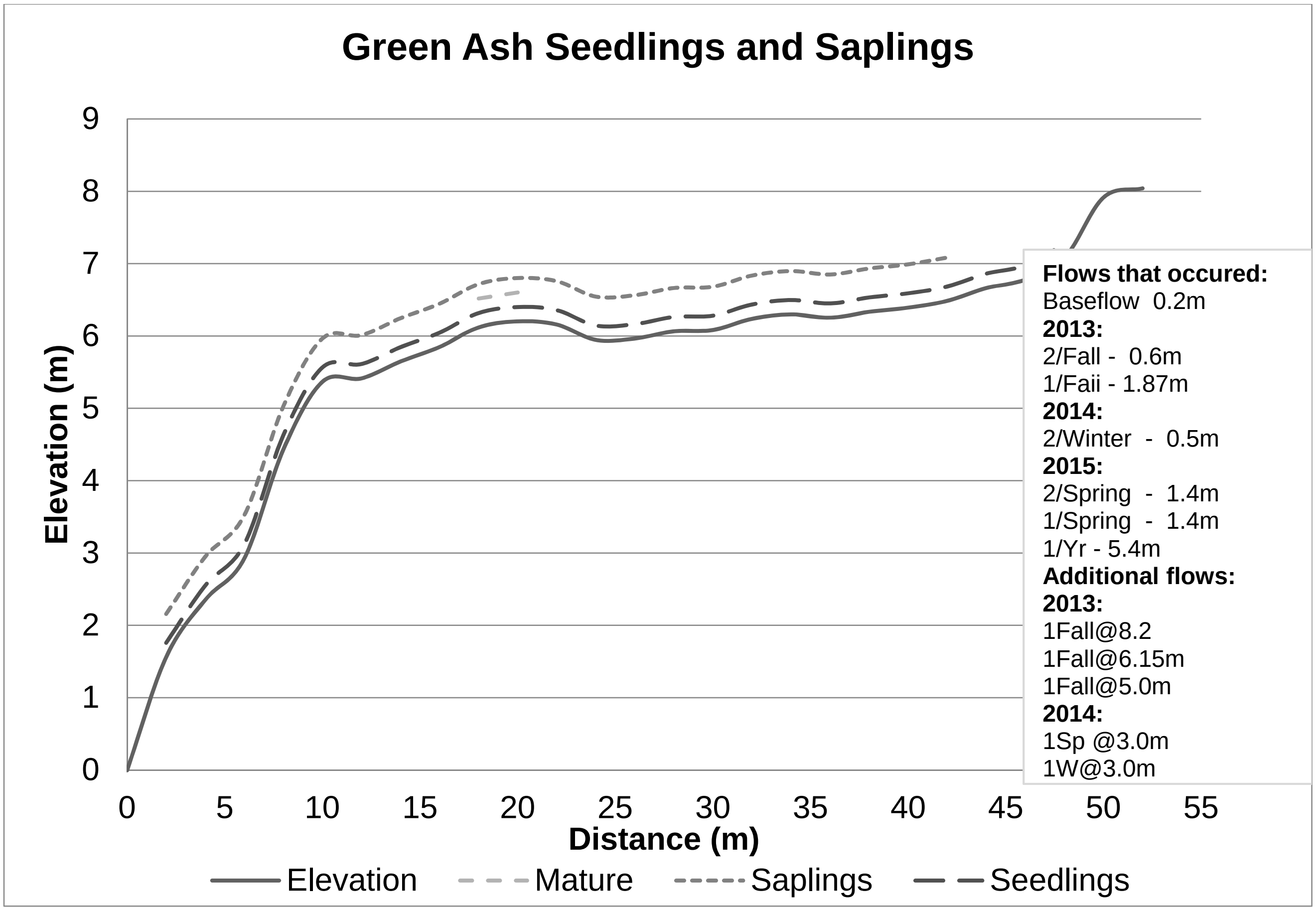


Figure 45. Green ash distributions at the Gonzales site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.

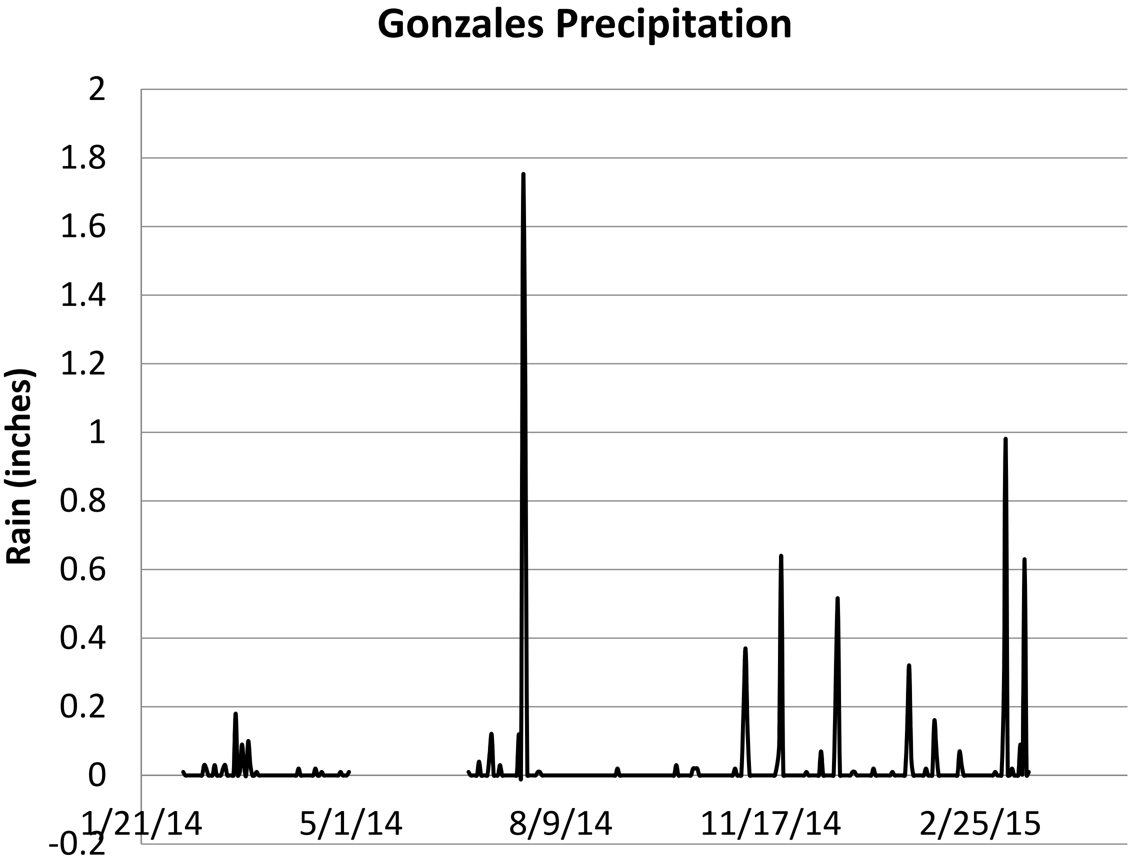


Figure 46. **Gonzales site local rainfall data in inches.**

Table 24. **Relative abundances of woody species at the Gonzales site, grouped by tree type and age class, and changes to abundances shown through time.**

Tree Species	Class	September 2013 Relative Abundance (%)	March 2015 Relative Abundance (%)	Change
American Elm	Mature	0.2	0.1	0.0
American Elm	Sapling	0.9	0.9	0.0
American Elm	Seedling	0.4	0.3	0.1
Anacua	Sapling	0.4	0.3	0.1
Anacua	Seedling	0.2	0	0.2
Box Elder	Mature	0.2	0.3	0.1
Box Elder	Sapling	6.7	5.9	0.8
Box Elder	Seedling	3.2	10.1	6.9
Cedar Elm	Mature	0.4	0.3	0.1
Cedar Elm	Sapling	8.2	8.6	0.4
Cedar Elm	Seedling	7.7	6.2	1.4
Cottonwood	Seedling	0.4	0	0.4
Dogwood	Mature	13.7	12.8	0.9
Dogwood	Seedling	3.7	3.7	0.0
Green Ash	Mature	0.2	0.1	0.0
Green Ash	Sapling	6.7	7.3	0.5
Green Ash	Seedling	9.9	3	7.0
Gum Bumelia	Sapling	0	0.3	0.3
Gum Bumelia	Seedling	0.7	2.1	1.3
Hackberry	Mature	0.4	0.4	0.1
Hackberry	Sapling	2.4	0	2.4
Hackberry	Seedling	23.8	23.5	0.3
Pecan	Mature	0.4	0.3	0.1
Pecan	Sapling	0.2	0.3	0.1
Pecan	Seedling	4.5	0	4.5
Slippery Elm	Sapling	0	0.3	0.3
Slippery Elm	Seedling	3.6	7.6	4.0
Sycamore	Mature	0.2	0.1	0.0
Sycamore	Sapling	0.2	0.1	0.0
Sycamore	Seedling	0	0.1	0.1
Western Soapberry	Mature	6	0.6	5.4

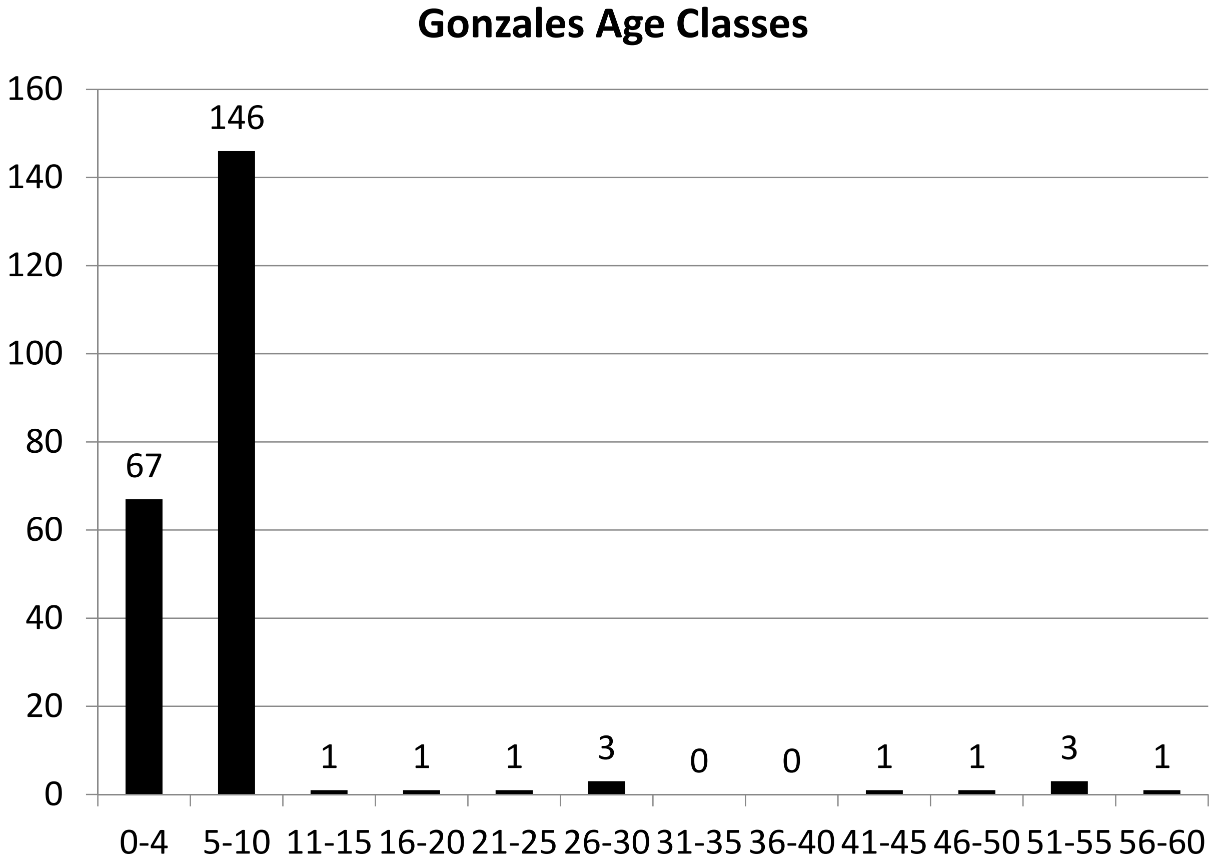


Figure 47. **Gonzales site riparian community grouped by tree age classes; values are based on summer 2014 sampling.**

Guadalupe site

The Guadalupe site (corresponding to the Guadalupe River USGS Gage near Spring Branch) represented an upstream reach of the Guadalupe River. The study site was located within the Guadalupe State Park (the upland landscape is mostly natural and protected). The river at this reach is wide and shallow with silt overlaying bedrock outcrops. Even though there is a footpath that traverses the upper limits of the zone, the riparian forest is intact and well preserved.
The slope from river's edge to the uppermost extent is 0.22 (meters rise/meters run) (Figure 48). Black willows occupy the lowest tiers of the slope, including a shallow bank exposed by prolonged low flows, and up to just less than 3m height. Box elder distributions extend from 13 to 23m distance and 3.5 to 5.5m elevation. All recommended flows provide some coverage to some species, although coverage of box elder is considerably less than black willows. None of the within-year BBEST recommended flows cover box elder mature trees (Figure 49). Only the 1/two-year BBEST-recommended flow fully covers their range. Black willow coverage (Figure 50) shows that even though all flows have some overlap with the species, only the 2/spring and 1/year flows cover more than 80% of this indicator species.

Recommended base flows were seen for all seasons except summer (Table 25). In 2014 the only TCEQ flow pulses to occur were the 2/spring, 1/spring, and 1/winter. All recommended spring flows occurred in spring 2015 during heavy rains. The effects of the drought extending through 2014 can clearly be seen (Table 25), 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. Box elder saplings (Figure 51) ranged from the exposed stream-edge bench to the bottom edge of the mature trees. This indicates that both baseflow and stream flow pulses have been lacking for some time – severe enough for saplings to be dispersed below normal distribution and time enough for them to have established for several years on the exposed bench.

Black willow saplings (Figure 52) ranged from the exposed stream-edge bench to the full range of the mature trees. This distribution correlates both generally with the recommended 2/spring event (2.5m) as well as (and even closer with) the additional 2.8m event in spring 2014. Black willow saplings were only located along the exposed bench, indicating (as did the box elder data) that this stream has been experiencing prolonged low flows and infrequent pulses.

Those seedlings and saplings that were present in summer were able to survive until the next spring, and box elder saplings saw recruitment of one individual to the mature class by spring 2015 (Table 26). Even though there was no seed dispersal by box elders in 2014, spring 2015 saw 3 new seedlings added. Although this riparian zone appears stressed because of a previous lack of flow pulses, the adequate spring flows seemed to revive the species somewhat, as so many of the seedlings and saplings that were present were able to survive the summer and winter lack of flow pulses.

Collectively box elders are 22% of the forest and black willows make up 38.2% (Table 27). This riparian zone is a diverse community that is still dominated by riparian indicator species. With only 2 seedlings present in fall, this is strong evidence for the negative impacts of the recent drought over the past few years, and indicates replacement was severely stunted this past year (Figure 53). Additionally, only 17 saplings were found in the study plots - also a very much lower than expected number for this age class. 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 only moderately adequate to maintain the existing riparian zone extent at this location. Even though few of the recommended flows actually occurred over the study period, flows that did occur had a positive influence on the species. Black willow seedlings were distributed at the level of inundation of the 2/spring event and an additional flow in spring 2014. Lack of flows had a negative effect on dispersal and seedling/sapling distribution (no box elder seeds were distributed/germinated in 2014, but with spring flows they were), but not on the survival of the handful of established plants in the area. The dominance of the riparian species in the zone indicates that this Guadalupe streamside forest is functioning as a riparian community (60% relative abundance), but with so few individuals present, a prolonged lack of stream flow could severely limit replacement of the species in their historic distributions.

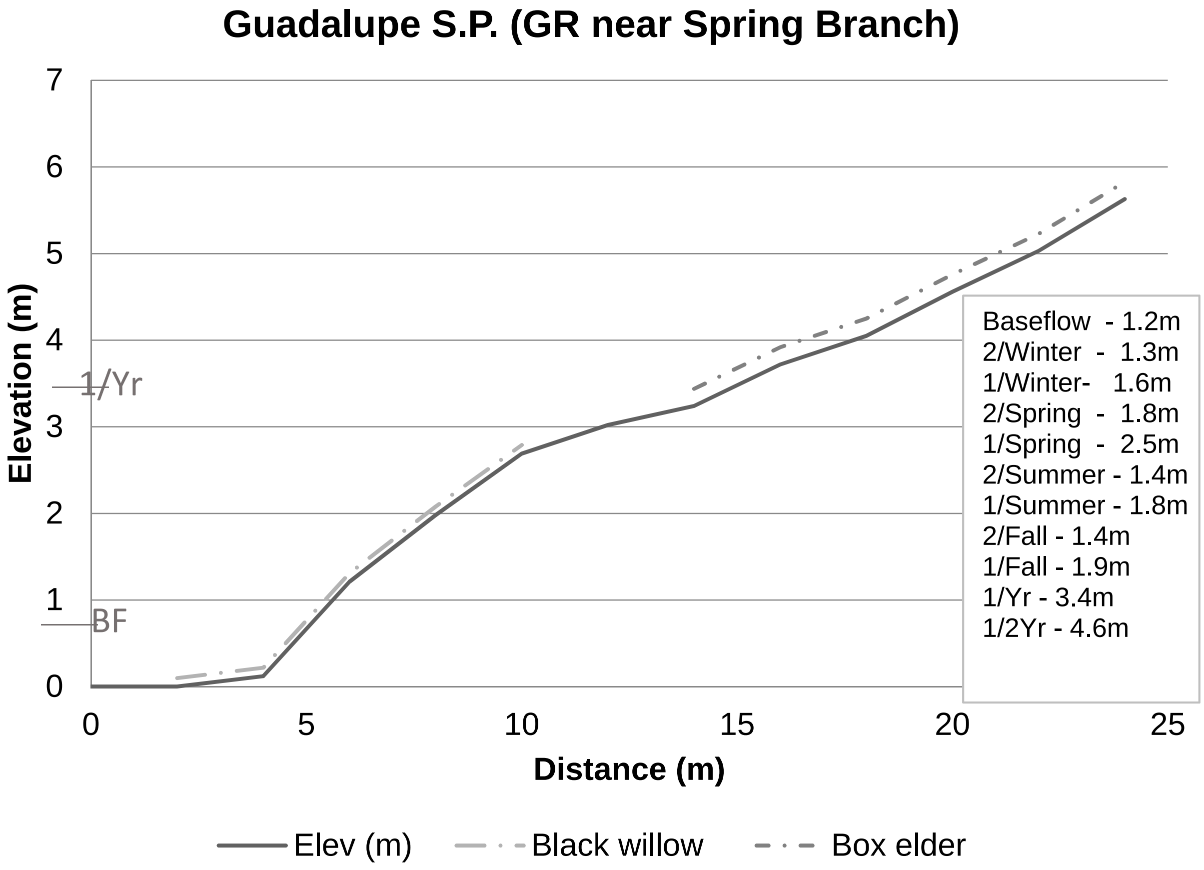


Figure 48. **Guadalupe site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows vertical inundation of flow tiers. Select flows are shown on the y-axis.**

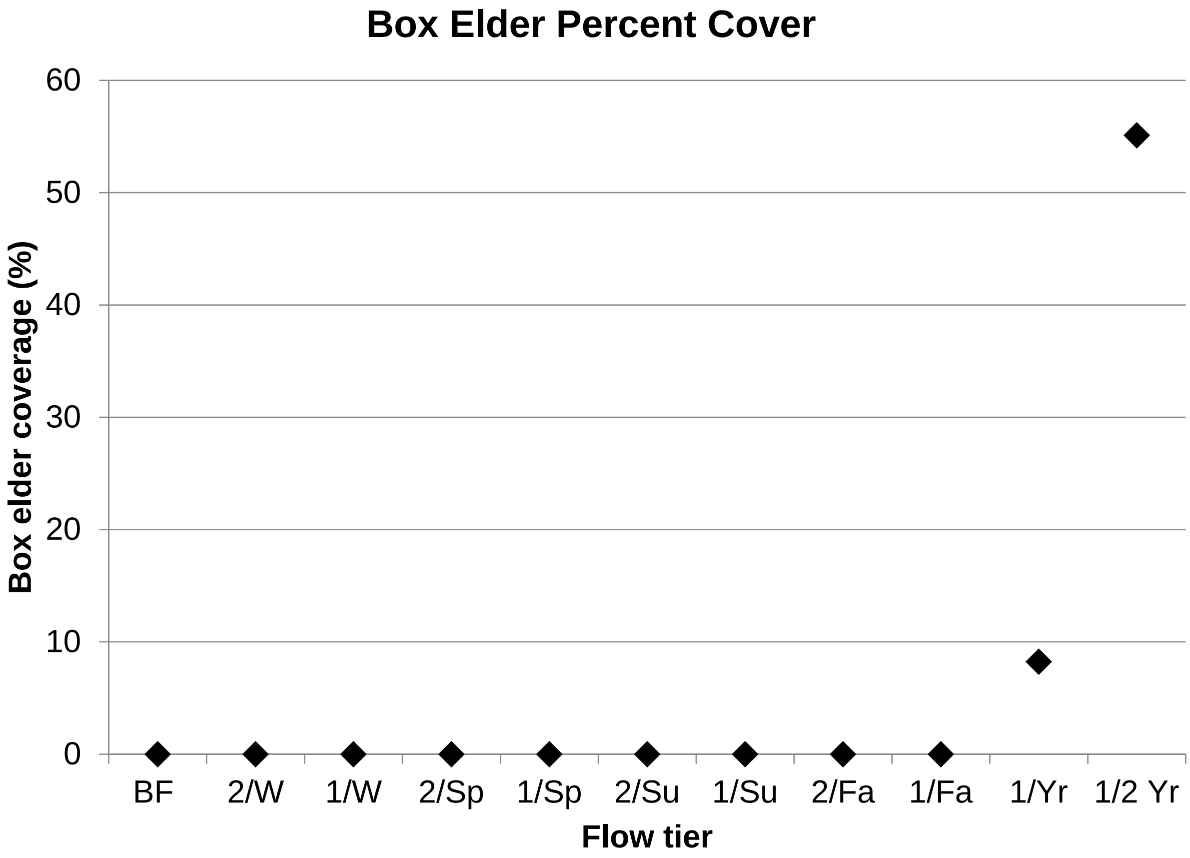


Figure 49. **Percentage of mature box elder stand at the Guadalupe site covered by flow tiers.**

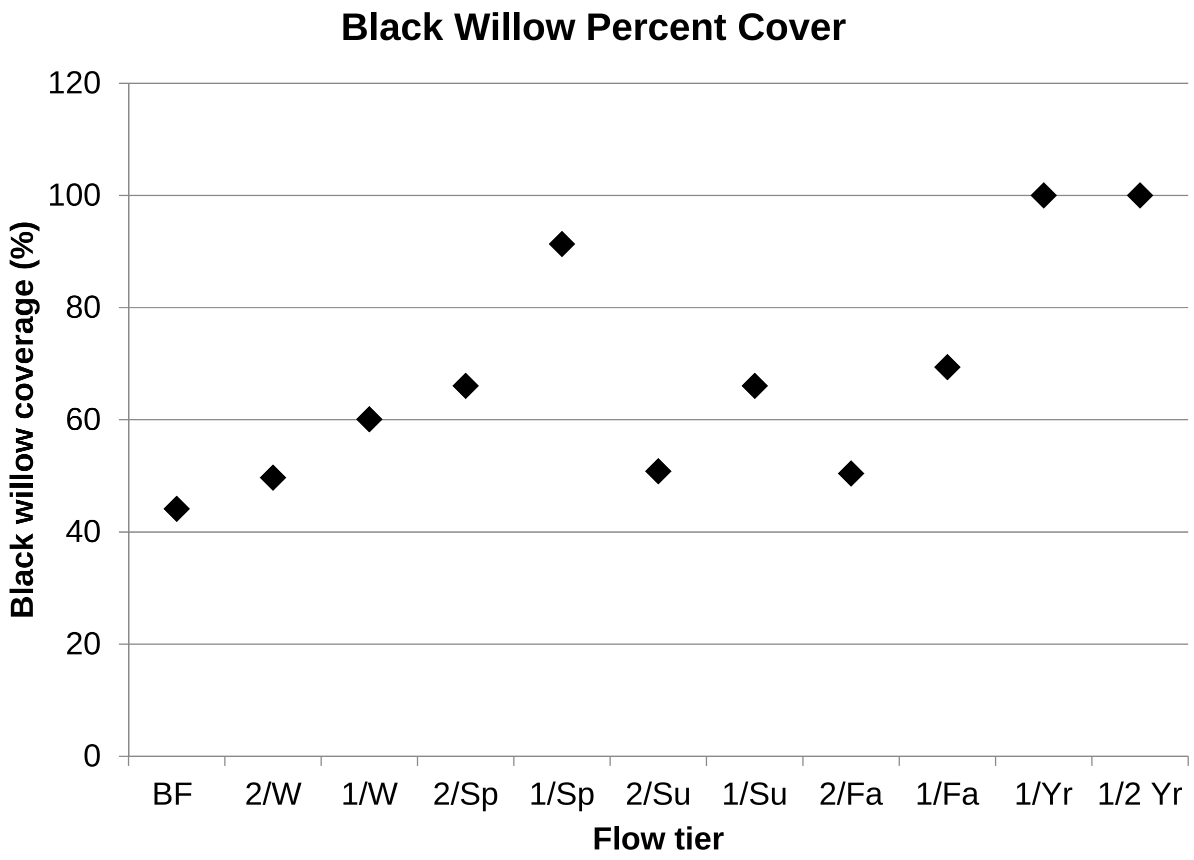


Figure 50. **Percentage of mature black willow stand at the Guadalupe site covered by flow tiers.**

Table 25. **Guadalupe site flow tiers and their occurrences throughout the BBEST-designated seasons. Y indicates flow occurred; dash indicates no flow occurred.**

		2014 Spring	**2014 Summer**	**2014 Fall**	**2014 Winter**	**2015 Spring**
Flow Standard	**CFS**	**Apr.-Jun.**	**Jul.-Sep.**	**Oct. - Dec.**	**Jan. - Mar.**	**Apr. - Jun.**
Baseflow	100	Y	-	Y	Y	Y
2/Winter	210				-	
1/Winter	570				Y	
2/Spring	870	Y				Y
1/Spring	2310	Y				Y
2/Summer	240		-			

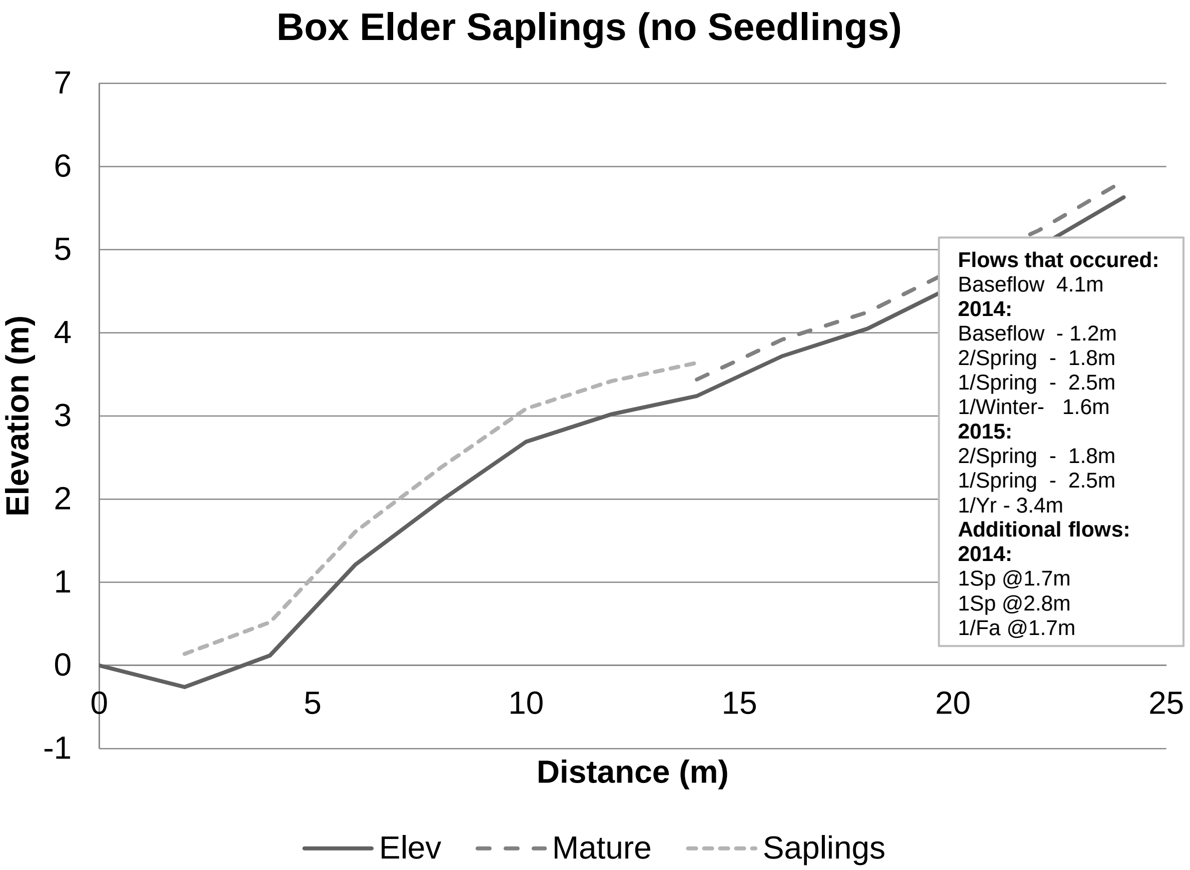


Figure 51. Box elder distributions at the Guadalupe site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.

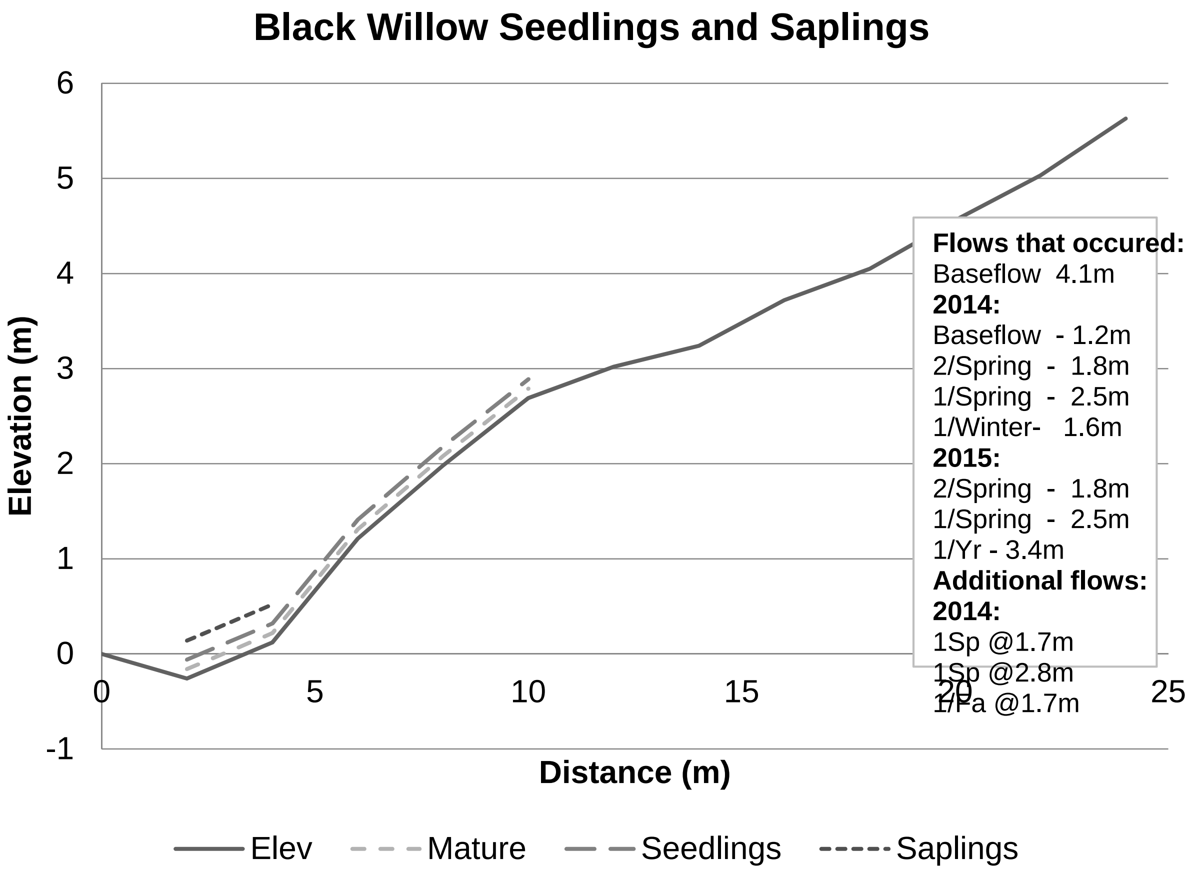


Figure 52. **Black willow distributions at the Guadalupe site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

Table 26. **Guadalupe site tree counts through time grouped by class.**

Species	Class	Summer 2014	Fall 2014	Spring 2015
Black Willow	Sapling	2	1	2
Black Willow	Seedling	30	30	30
Box elder	Mature	6	6	7
Box elder	Sapling	12	11	11
Box Elder	Seedling	0	0	3

Table 27. **Relative abundances of woody species at the Guadalupe site, grouped by tree type and age class.**

Tree Species	Class	Relative abundance (%)
Baldcypress	Sapling	1.2
Baldcypress	Seedling	2.4
Black Willow	Sapling	2.4
Black Willow	Seedling	36.6
Box Elder	Seedling	0
Box elder	Mature	7.3
Box elder	Sapling	14.6
Cedar Elm	Sapling	2.4
Cedar Elm	Seedling	2.4
Hackberry	Mature	3.7
Hackberry	Sapling	7.3
Pecan	Seedling	2.4
Pecan	Sapling	6.1
Sycamore	Sapling	1.2
Sycamore	Seedling	2.4
Water Elm	Seedling	1.2
Water Elm	Sapling	6.1
		100

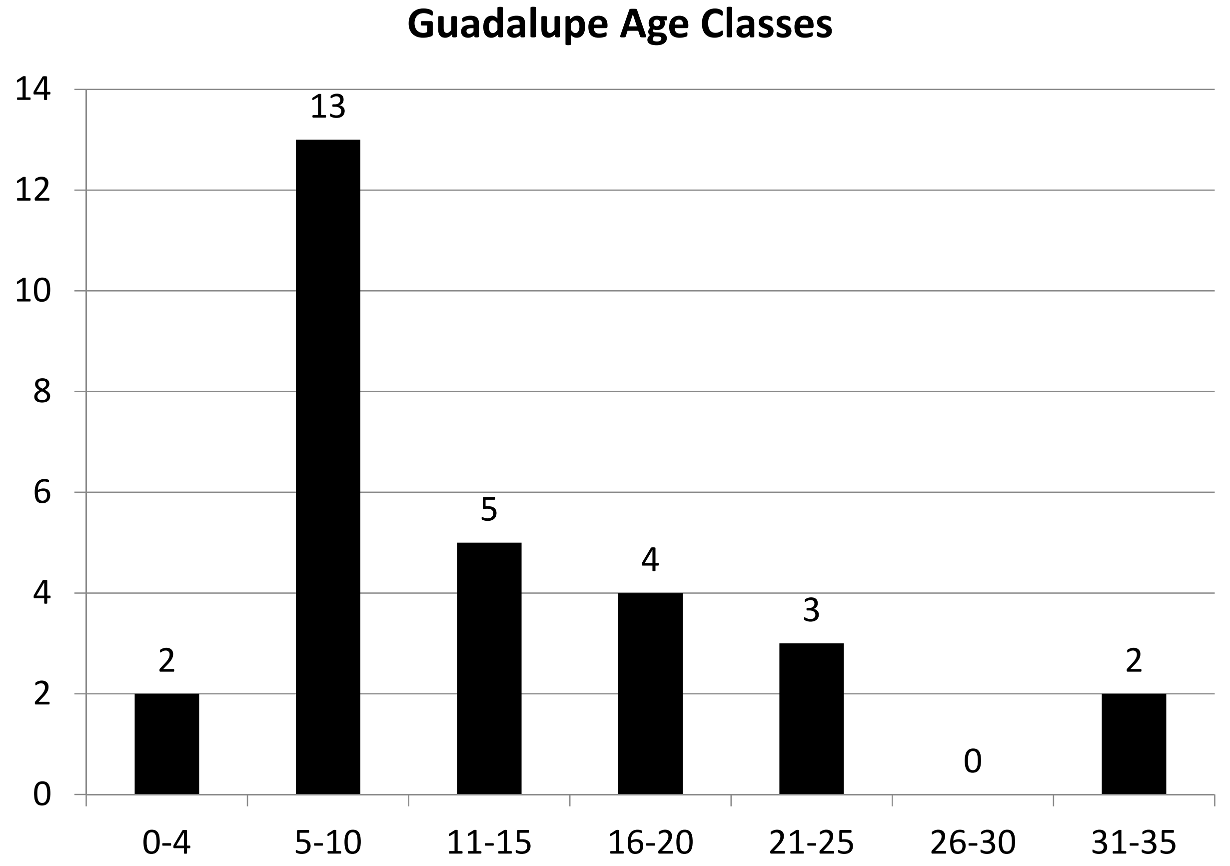


Figure 53. **Guadalupe site riparian community grouped by tree age classes; values are based on summer 2014 sampling.**

Medina site

The Medina site (corresponding to the Medina River USGS Gage at San Antonio) represented a headwater tributary to the San Antonio River. The study site was located on private property (upland landscape is mostly natural and actively protected as a wildlife preserve). This river is upstream of the partially constructed Applewhite Dam, and experiences lowland flooding with large, prolonged rains.

The slope from river's edge to the uppermost extent is 0.06 (meters rise/meters run); however, within the first 12m there is a 3m rise in elevation – beyond that the slope is 0.01 (Figure 54). Box elders occupy virtually the entire channel slope and floodplain – from water's edge to the uppermost reaches. Green ash are distributed from 20m to 50m distance - all at the 3.5-4m elevation. No flows except the BBEST-recommended 1/year provide coverage to the green ash. All flows provide some coverage to the box elders because of their broad distribution. There were no mature black willows at this site.

For box elders (Figure 55) the 1/year and 1/spring are the only within-year flows that provides 80% of coverage or more, though most others provide between 60-80% coverage. Green ash

(Figure 56), with their small location up on the floodplain, are only covered by the 1/year and beyond.

Baseflows were seen for all seasons (Table 28). In 2014 a number of TCEQ flow pulses occurred. Early in spring 2014 the BBEST-recommended 1/year occurred, and again in spring 2015. The only flow not occurring was the 2/summer in 2014. All recommended spring flows occurred in spring 2015 during the heavy rains.

Box elder seedlings (Figure 57) dispersed just above baseflow, about a meter above the very lowest edges of the mature trees. Their range continued up across the 3.6m floodplain to a distance of about 50m, just short of the mature range. This correlates directly with the 2014 spring flow that reached 5.4m elevation into the site (completely covering the floodplain). Sapling distribution covered most of the shear channel slope as well as the floodplain out to almost 70m distance.

Green ash seedling dispersal occurred from just above baseflow to along the upper floodplain at the 3.6m elevation, again directly in correlation with the 1/year flow in spring 2014 (Figure 58). Saplings were distributed along that same floodplain in the same distribution as the mature trees. It appears that even though seedlings are distributed along the channel slope, the long-term persistence of green ash (under average flow conditions rather than the recent drought) is well above that slope - where they survive better on the floodplains.

Several rain events occurred during the fall and winter, however, it is not possible to determine just how beneficial they were to the existing vegetation (Figure 59).

Because of flooding, this site was inaccessible during the spring sampling period. Therefore only 2014 data are available (Table 29). Even though there were no mature or sapling black willow present, one seedling was found in the study plots. Box elder mature and sapling classes maintained from summer to fall, while the seedling class saw the loss of one member. All green ash classes maintained their numbers. This shows that survival was robust, likely supplemented by the fall 2014 flow that provided soil wetting in the plant’s range. However, there was also no comparable summer flow, so their resiliency kept them going despite the lack of flows.

Collectively box elders are 10.8% of the forest, green ash are 3%, and black willows make up 0.3%, with a combined total of 14% (Table 30). This riparian zone is a diverse community, but shows encroachment from hackberry (seedlings = 51% of relative abundance) and other upland species, which dominate in abundance over riparian species. It is predicted that the spring flooding would be adequate to remove these upland encroachers and greatly increase riparian dominance. Follow up studies would verify if this is the case.

Saplings are the most prolific in the site at a paltry 25 individuals (Figure 60). The lack of many new seedlings (8 total) is evidence for the negative impacts of the recent drought the past few years, and indicates replacement was severely stunted this past year. Beyond saplings, the presence of older trees drops to less than 10 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 only moderately adequate to maintain the existing riparian distribution for the reach. Several recommended flows occurred over the study period, though most of them did not reach the riparian species. Because a BBEST-recommended 1/year did occur in spring there was corresponding seed dispersal/germination in the site and all but one box elder seedling survived through fall. Whether this is because of both a 2013 and 2014 fall flow that wetted their range, or despite the lack of summer flows into the site cannot be determined. Age structure analysis indicates that a lack of stream flow pulses along the river have had noticeable impacts on seedling dispersal and future maintenance. The lack of dominance of the riparian species in the zone (14% relative abundance) indicates that this Medina streamside forest is functioning less as a riparian zone than as a mixed forest, and a prolonged lack of stream flow would likely further threaten or prevent replacement of all three species. Because the dominant species/class is the hackberry/seedling, several robust flow pulses (as were seen in spring 2015) would likely remedy this encroachment.

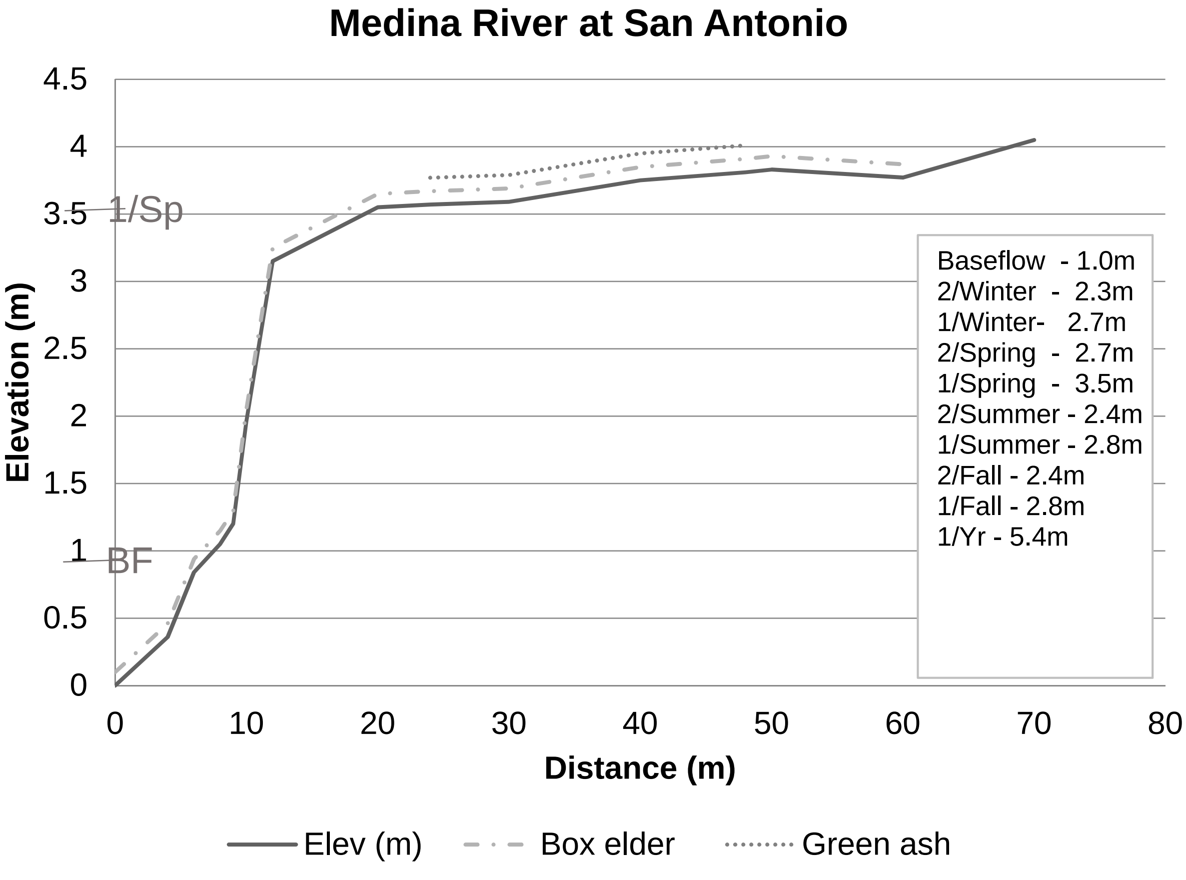


Figure 54. **Medina site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows vertical inundation of flow tiers. Select flows are shown on the y-axis.**

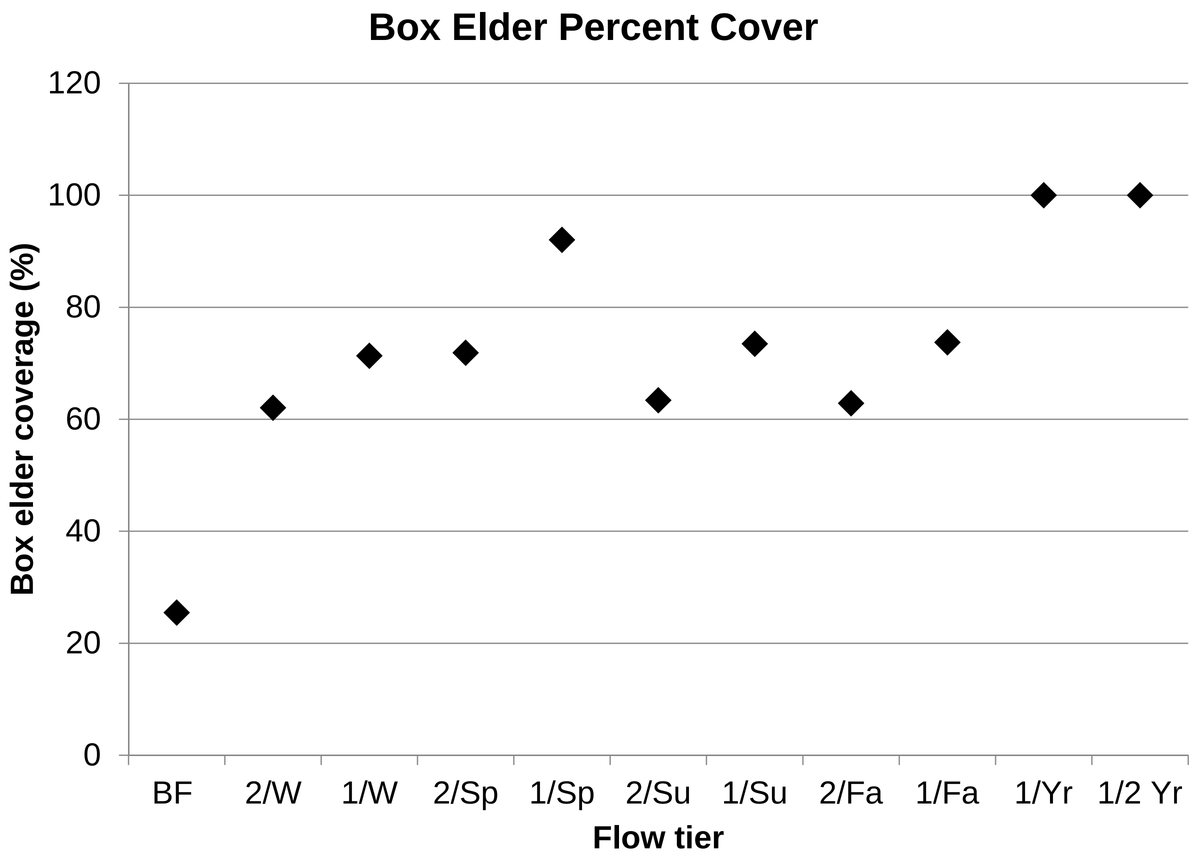


Figure 55. **Percentage of mature box elder stand at the Medina site covered by flow tiers.**

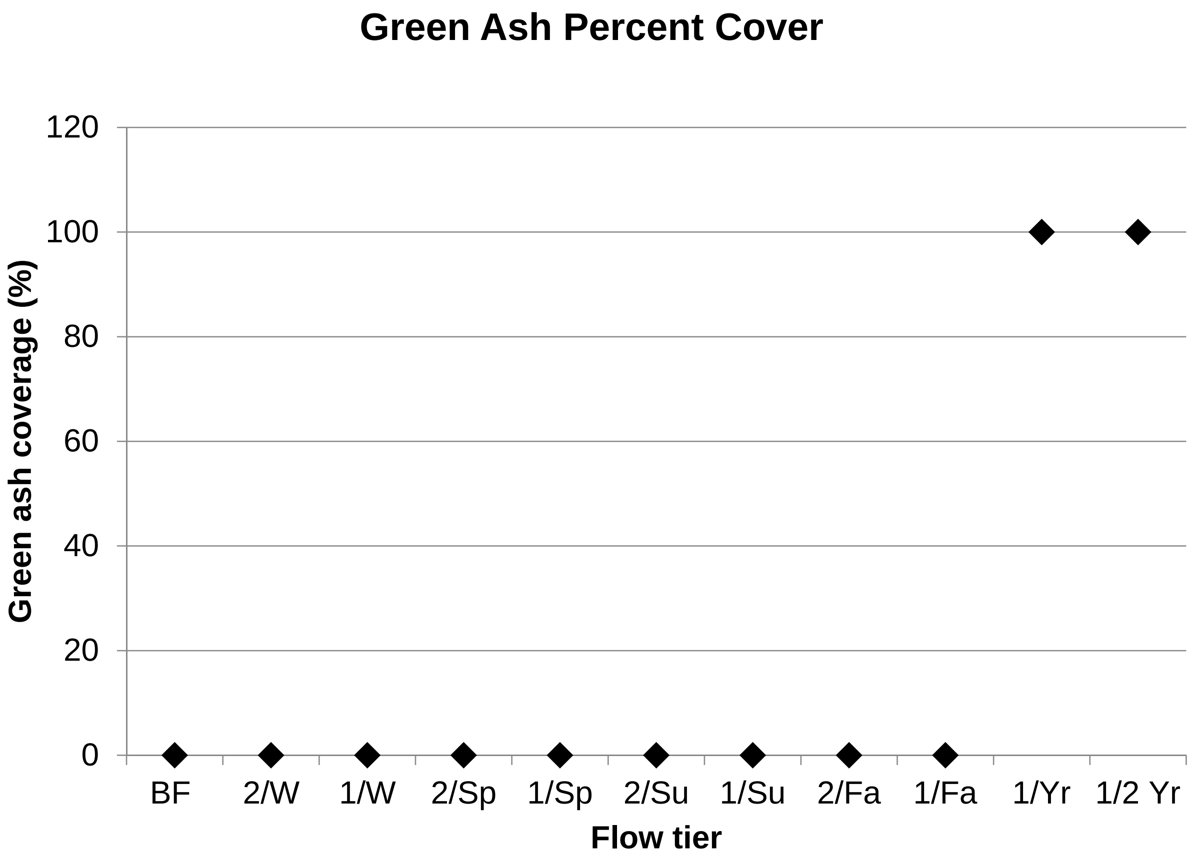


Figure 56. **Percentage of mature green ash at the Medina site stand covered by flow tiers.**

Table 28. **Flow tiers and their occurrences throughout the BBEST-designated seasons at the Medina site. Y indicates flow occurred; dash indicates no flow occurred.**

		2014 Spring	**2014 Summer**	**2014 Fall**	**2014 Winter**	**2015 Spring**
Flow Standard	**CFS**	**Apr.- Jun.**	**Jul.- Sep.**	**Oct. - Dec.**	**Jan. - Mar.**	**Apr. - Jun.**
Baseflow	57	Y	Y	Y	Y	Y
2/Winter	120				Y	
1/Winter	350				Y	
2/Spring	380	Y				Y
1/Spring	1000	Y				Y
2/Summer	140		-			
1/Summer	440		Y			
2/Fall	130			Y		
1/Fall	450			Y		
1/Year	2920	Y	-	-	-	Y
1/2 Years	6020	-	-	-	-	Y

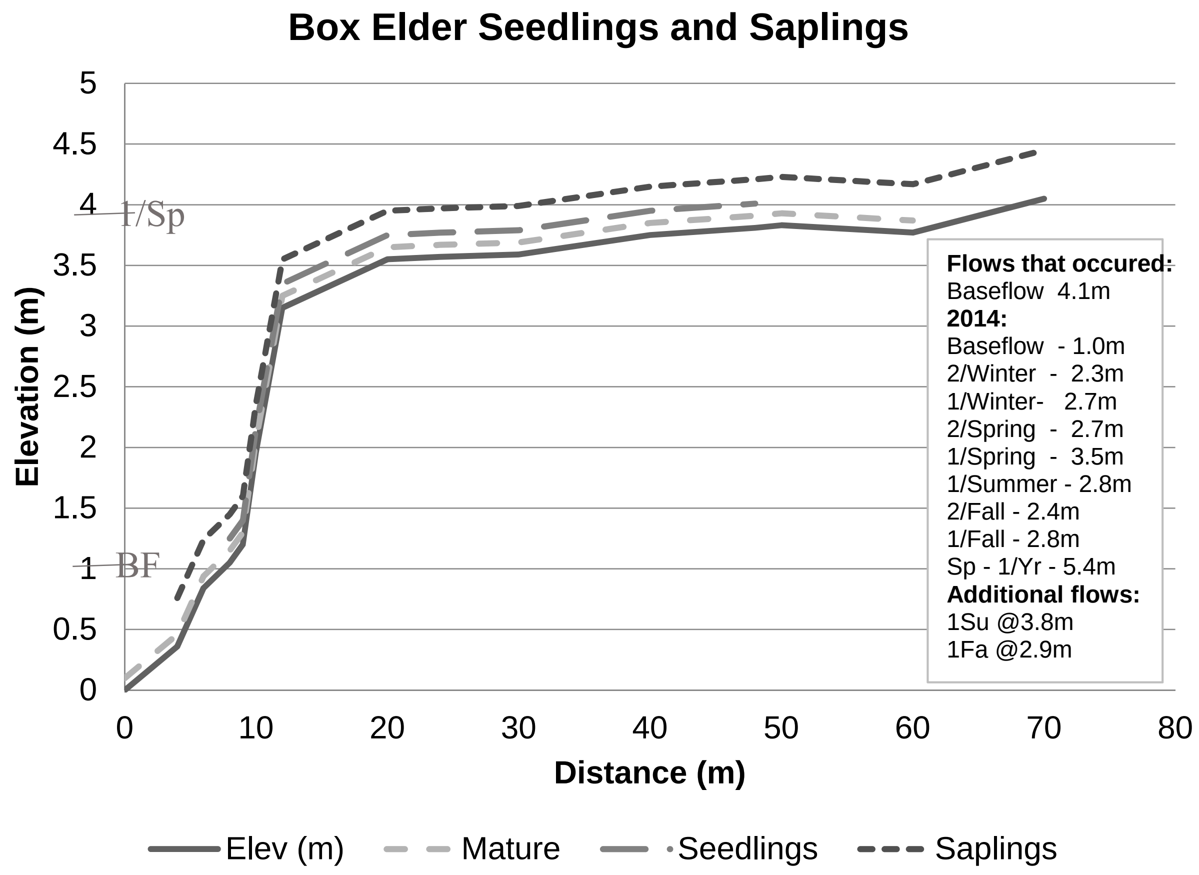


Figure 57. **Box elder distributions at the Medina site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

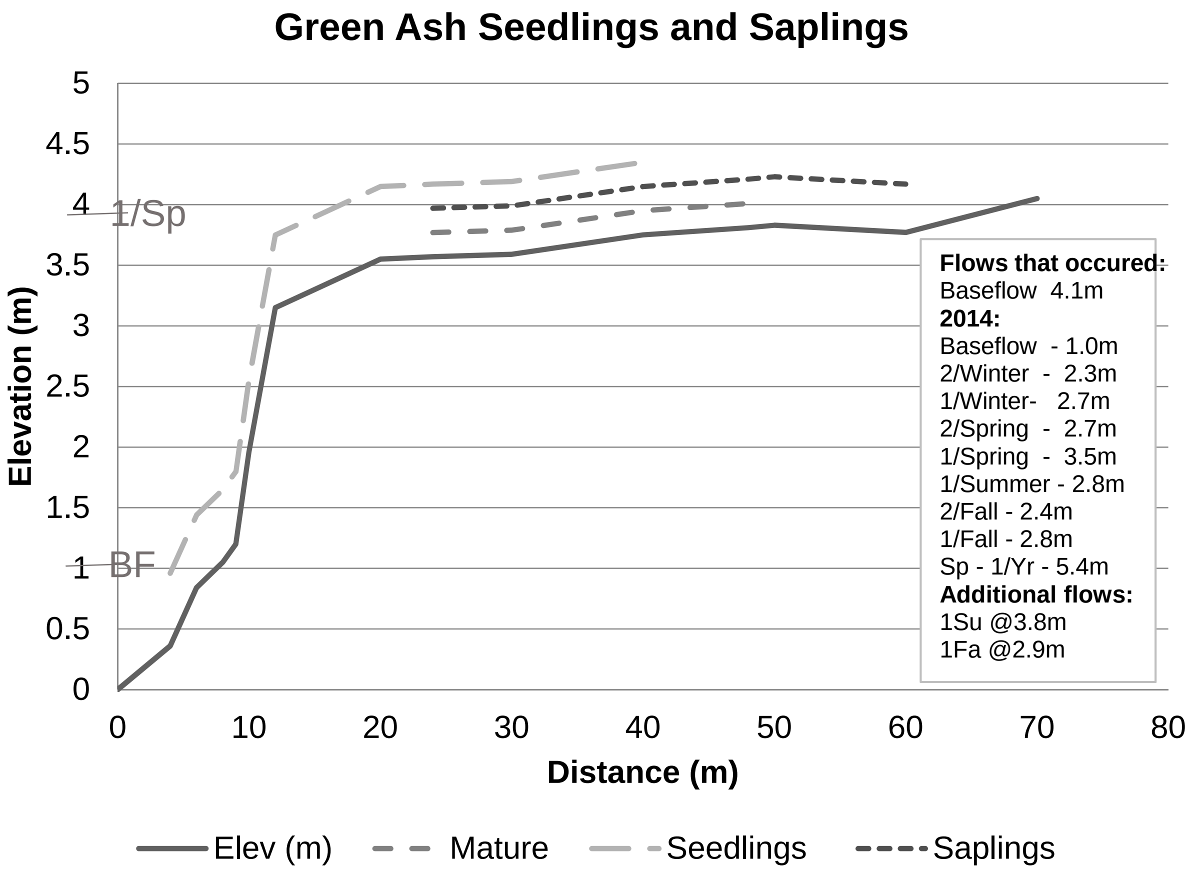


Figure 58. **Green ash distributions at the Medina site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

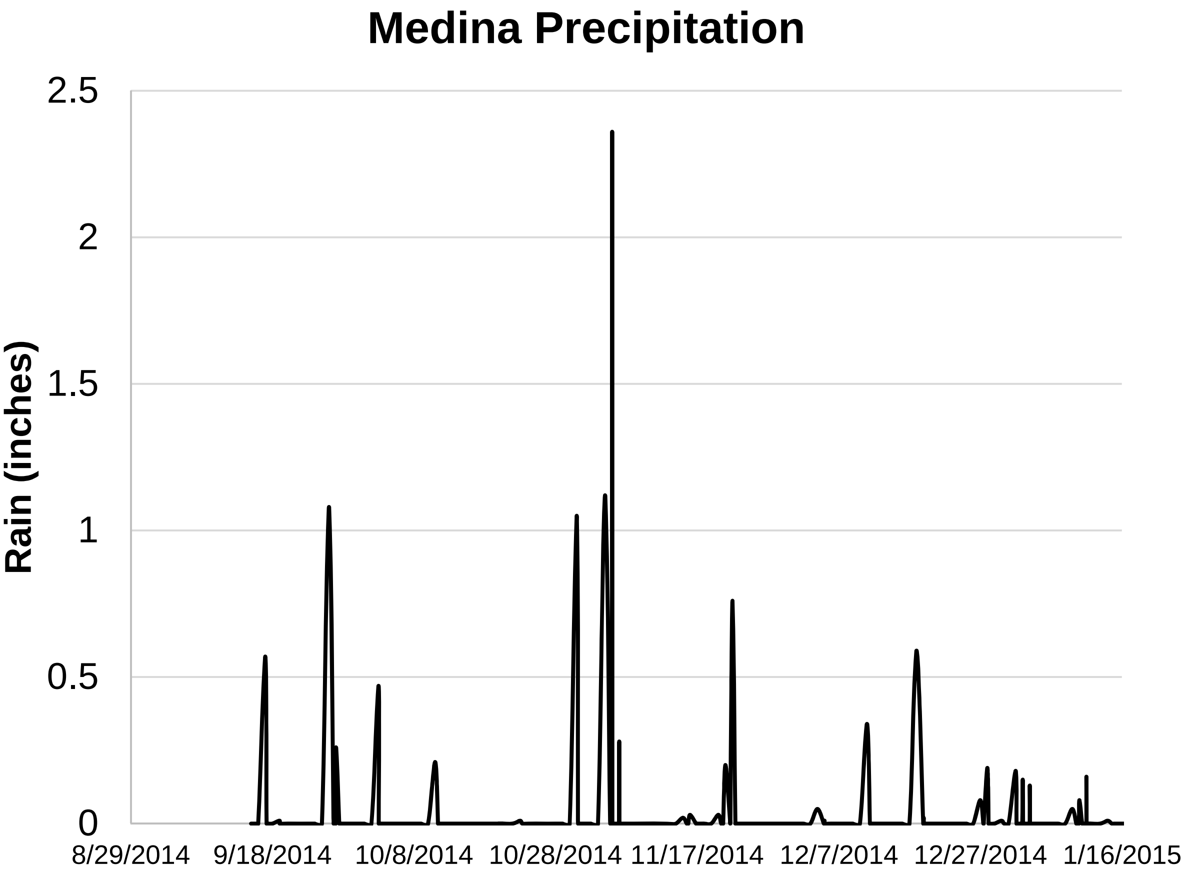


Figure 59. **Medina study site local rainfall data in inches.**

Table 29. **Tree counts through at the Medina site, time grouped by class.**

Species	Class	Summer 2014	Fall 2014
Black Willow	Seedling	1	1
Box Elder	Mature	16	16
Box Elder	Sapling	23	23
Box Elder	Seedling	7	6
Green Ash	Mature	10	10
Green Ash	Sapling	2	2
Green Ash	Seedling	2	2

Table 30. Relative abundances of woody species at the Medina site, grouped by tree type and age class.

Tree Species	Class	Relative abundance (%)
Baldcypress	Seedling	0.3
Black Willow	Seedling	0.3
Box Elder	Seedling	1.8
Box Elder	Mature	3
Box Elder	Sapling	5.8
Cedar Elm	Sapling	2.3
Cedar Elm	Seedling	5
Chinaberry	Sapling	0.3
Chinaberry	Seedling	2.5
Elm	Sapling	1
Elm	Seedling	16.3
Green Ash	Sapling	0.5
Green Ash	Seedling	0.5
Green Ash	Mature	2
Hackberry	Mature	1.8
Hackberry	Sapling	2.5
Hackberry	Seedling	51.5
Pecan	Seedling	0.5
Soapberry	Seedling	0.3
Sycamore	Seedling	0.8
Walnut	Seedling	0.5
Yaupon Holly	Sapling	1
		100

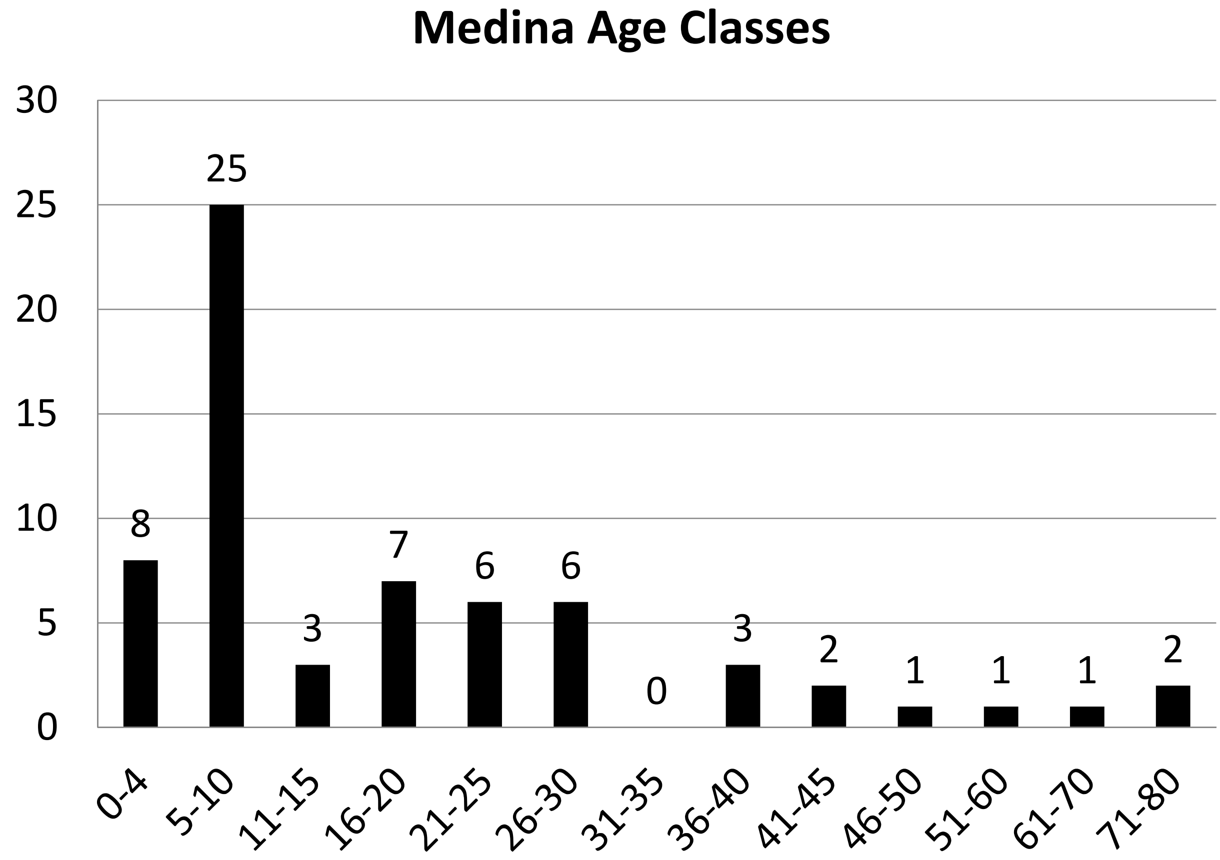


Figure 60. **Medina site riparian community grouped by tree age classes; values are based on summer 2014 sampling.**

Victoria site

The Victoria site (corresponding to the Guadalupe River USGS Gage at Victoria) represented a lower reach of the Guadalupe River. The study site was located on private property (upland landscape is mostly rural). The river at this site is more deeply incised with steep banks. The study site was located along an inner bend of the river, with less shear banks, but still steep. The slope from river's edge to the uppermost extent is 0.06 (meters rise/meters run). Because there is a land depression between 40 and 70m, considering only the first rise, the slope is 0.13 (Figure 61). This mid-slope depression allowed for a much broader riparian zone than would be expected otherwise, as the low draw has the potential to hold and seep water to more distantly located plants. Evidence that this does happen was a dense stand of green ash located up to 80m from the stream. All Black willows occupy the lowest tiers of the slope, within 5m of water's edge to 48m, covering the natural levee and its back side. Box elders run from the lower edge of black willows, across the levee and depression to a distance of 90m. Green ash distributions begin at almost 3m height and range from 15 to 90m distance. All flow tiers given in the TCEQ standards provide coverage to the indicator species, although coverage of box elder and green ash are considerably less than black willows.

Only the 1/fall flow inundates black willow at 100%, all other flows are below 80% (Figure 62). No within-year flows inundated box elder above 50%; however, the 1/year BBEST-recommended flow provided 100% coverage of their distribution (Figure 63). No within-year flows inundated green ash above 15%; however, the 1/year BBEST-recommended flow provided 100% coverage of their distribution (Figure 64). TCEQ base flows were seen for all seasons except summer 2014 (Table 31). In 2014 no recommended flow pulses other than the winter flows occurred. All recommended spring flows occurred in spring 2015 during the heavy rains. The effects of the drought extending through 2014 can clearly be seen (Table 31), as 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. Black willow seedlings were dispersed (Figure 65) within and just above baseflow. This appears to be the long-term limit of black willow distribution along this reach. There were no other flows until that winter, and apparently this is the event that allowed for dispersal as their distribution correlates with it. Saplings extended up to 4m, but still fell short of mature distributions. This species is undergoing a shrinking of its spatial range.

Box elder seedlings (Figure 66) dispersed from 3-4.5m elevations, or 20-78m distances. This range is still completely contained within the mature trees, which extend both closer to and more distant from the stream, but much greater in elevation that 2014 flows would explain. Because box elder seedlings drop in fall, a check of the USGS gage flow was made and verified that in November 2013 a flow that inundated the site up to 7m did occur. Sapling ranges very much reflect mature ranges for this site. Green ash seedlings and saplings (Figure 67) were distributed beyond the levee, from the back side of the depression and up the next slope at an elevation of 3-5.3m and distance of 60-80+m. Again, the fall 2013 7m inundation may explain how seeds were deposited so distant to the stream, but it doesn’t explain why neither the seedlings nor saplings are surviving any closer than 60m. No additional flows inundated here so scouring loss does not explain the pattern. During the fall sampling evidence of hog wallowing was present throughout the forest along the banks; possibly this activity is removing near-stream plants over time when flows wet the soil.

Because of high flows in spring 2015 this site could not be accessed during that season; data coverage is for 2014 only (Table 32). Three black willow seedlings were recruited to the sapling class. One sapling was recruited to mature. All others maintained. Four box elder seedlings perished. One green ash sapling perished, as did five seedlings. These losses are minimal, but given the low counts to begin with they have a greater impact on long-term riparian abundance than they otherwise would. It is curious that survival was as high as it was considering the lack of flows in 2014; however, the low-lying depression could provide the answer – these areas often retain standing water that more slowly seeps into surrounding soils, reducing the effects of river drawdown on seedlings and saplings. Collectively, box elders are 19.6% of the forest, green ash are 24.6%, and black willows make up 18.8%, with a combined total of 63% (Table 33). This riparian zone shows invasion by Chinese tallow but otherwise is dominated by riparian species.

Saplings are the most prolific age classes in the site, with 110 individuals (Figure 68). Again, the lack of new seedlings at the level expected is evidence for the negative impacts of the recent drought the past few years, and indicates replacement was stunted this past year. Beyond saplings, the presence of older trees drops to less than 10 for each age class, preventing the detection of previous anomalous flows from the available data. Further sampling of mature trees may provide this information.

TCEQ flow tiers did not provide inundation of much of the riparian zone. Even though few of the recommended flows actually occurred over the study, flows that did occur had a positive influence on the box elders and green ash. For the box elders and green ash, it appears the fall 2013 large flow pulse allowed for seed dispersal across the full extent of the riparian zone, while black willow were distributed within the baseflow inundation only. A lack of flows had no effect on dispersal and seedling/sapling survival for any species. The presence of a fairly deep and broad land depression in the zone likely allowed for water retention and supplementation to the nearby groundwater. Given that the seedlings and saplings near this spot had such high survival despite a lack of flows shows this is a very real possibility. Age structure analysis indicates that a lack of frequent stream flow pulses along the river have had noticeable impacts on seedling dispersal and future maintenance. The dominance of the riparian species in the zone (63% relative abundance) indicates that the Victoria streamside forest is functioning as a riparian zone despite the presence of a relatively abundant invasive species.

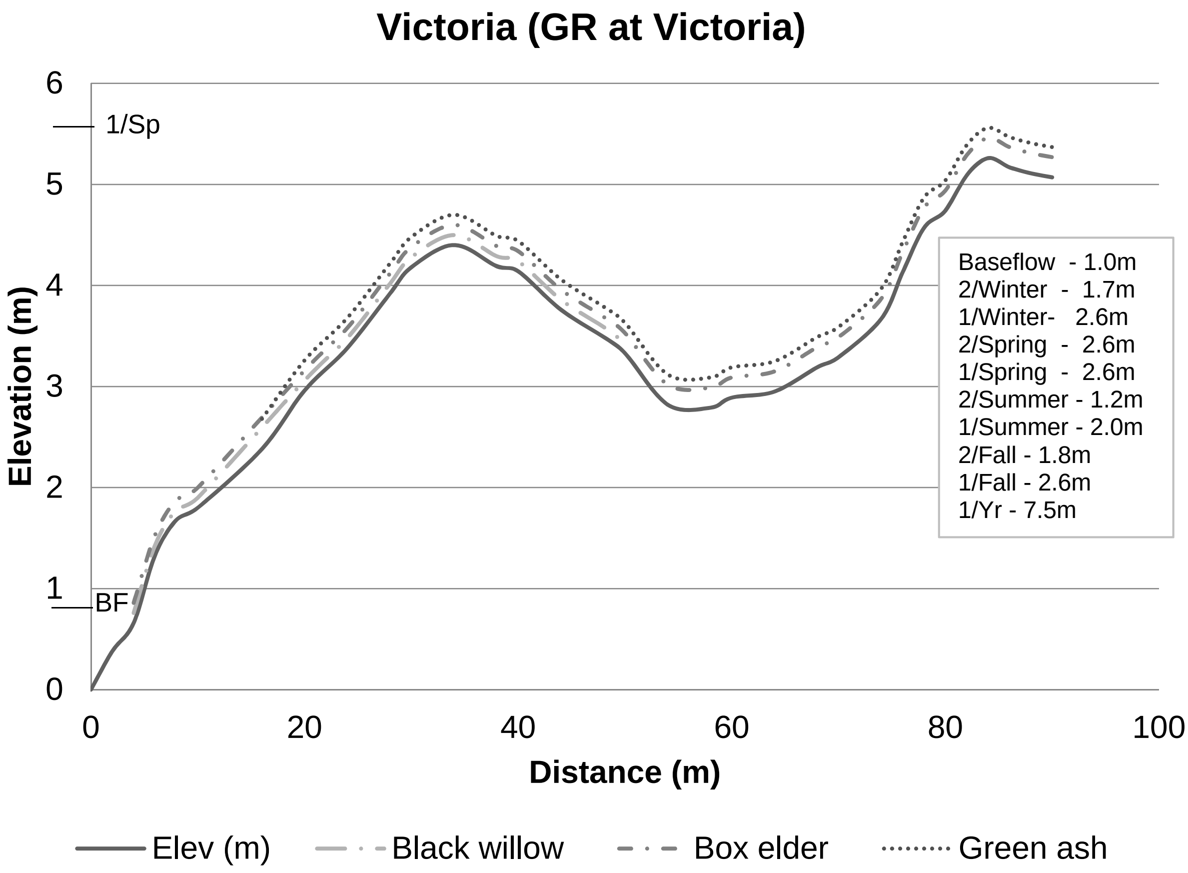


Figure 61. **Victoria site profile. Elevation is height above water's edge. Spatial distributions of mature indicator species are shown along the site profile. The box inset shows vertical inundation of flow tiers. Select flows are shown on the y-axis.**

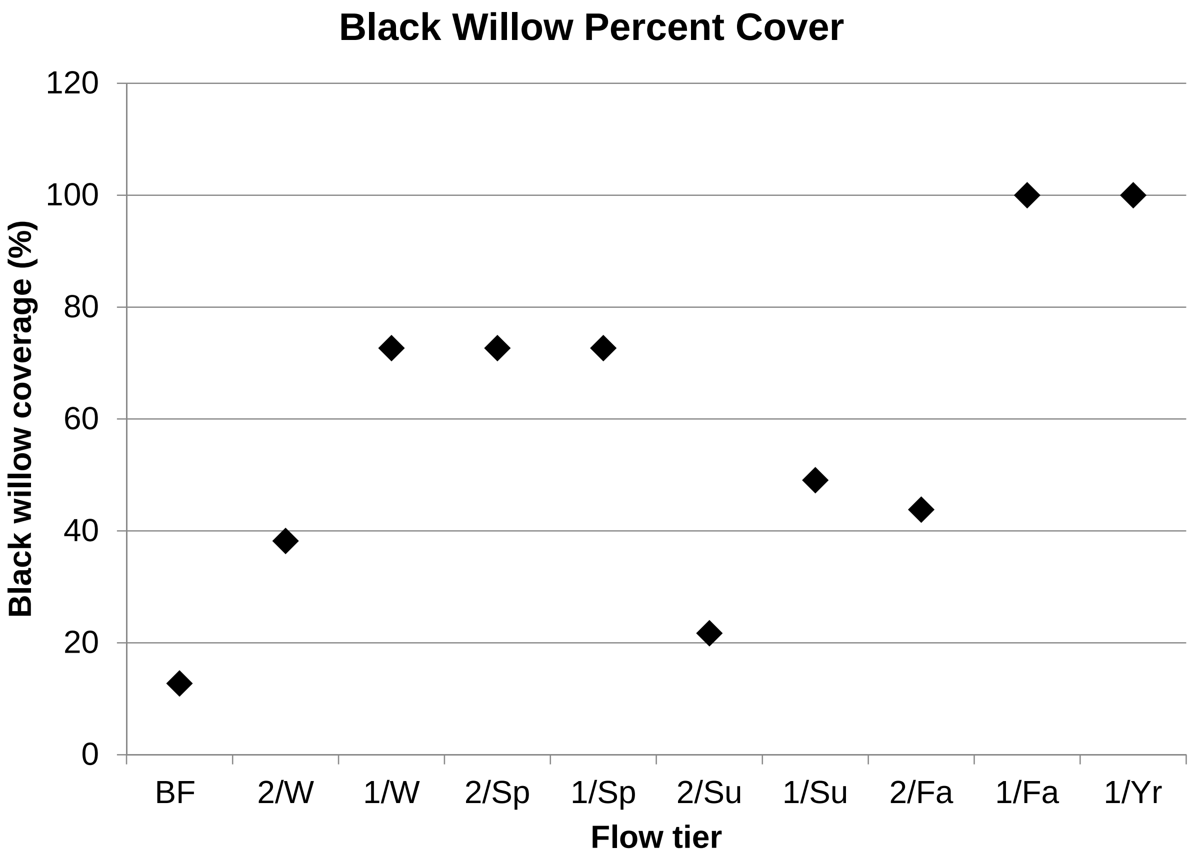


Figure 62. **Percentage of mature black willow stand at the Victoria site covered by flow tiers.**

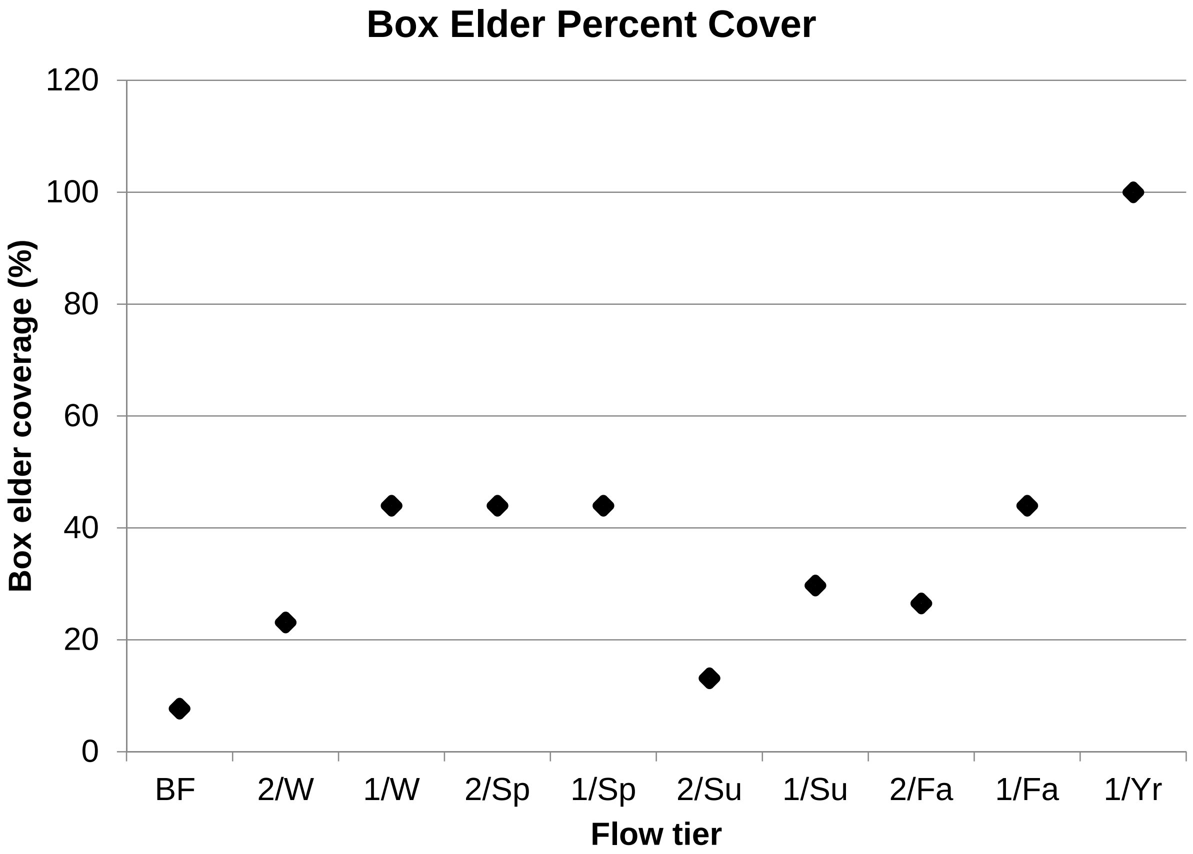


Figure 63. **Percentage of mature box elder stand at the Victoria site covered by flow tiers.**

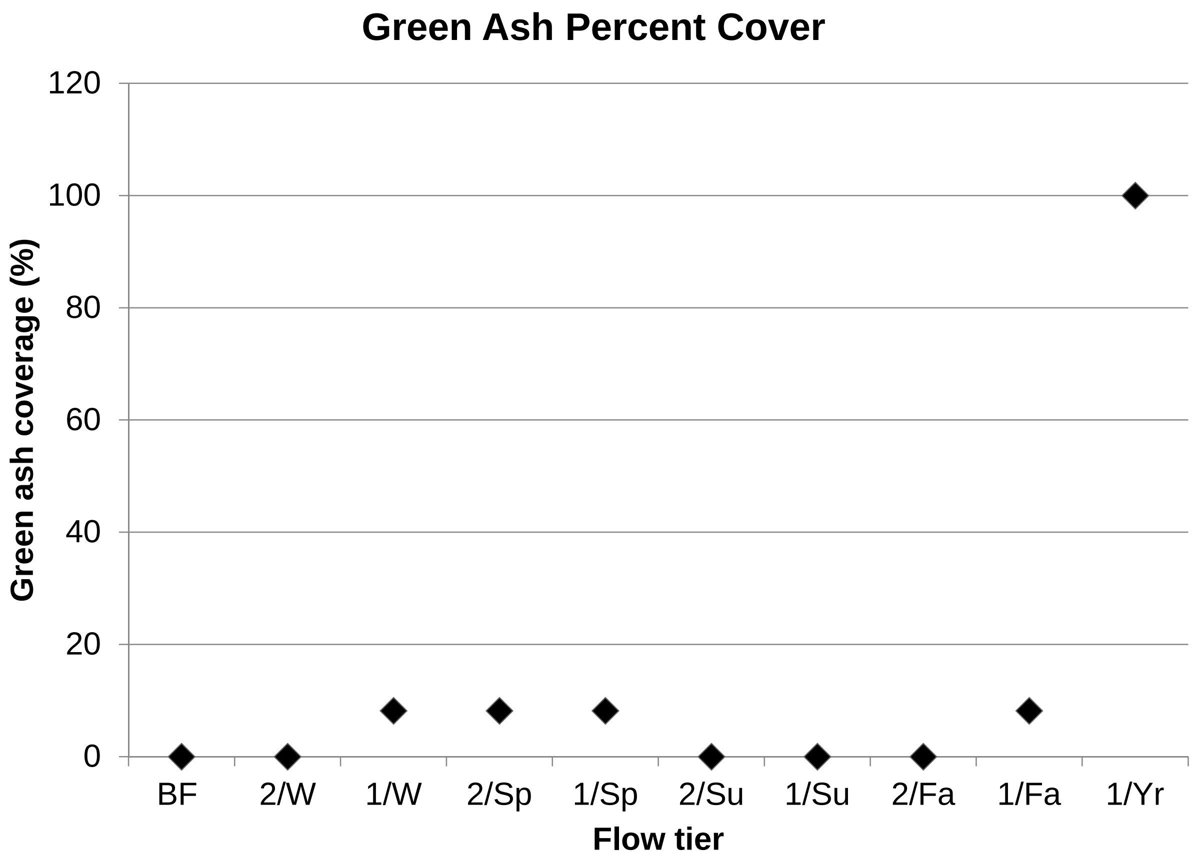


Figure 64. Percentage of mature green ash stand at the Victoria site covered by flow tiers.

Table 31. **Flow tiers and their occurrences throughout the BBEST-designated seasons (shaded) at the Victoria site. Y indicates flow occurred; dash indicates no flow occurred.**

		2014 Spring	2014 Summer	2014 Fall	2014 Winter	2015 Spring
Flow Standard	**CFS**	**Apr.- Jun.**	**Jul.- Sep.**	**Oct. - Dec.**	**Jan. - Mar.**	**Apr. - Jun.**
Baseflow	710	Y	-	Y	Y	Y
2/Winter	1690				Y	
1/Winter	4620				Y	
2/Spring	3300	-				Y
1/Spring	9020	-				Y
2/Summer	1040		-			
1/Summer	2060		-			
2/Fall	1880			-		
1/Fall	5370			-		
1/Year	16700	-	-	-	-	Y
1/2 Years	25500	-	-	-	-	Y
1/5 Years	48000	-	-	-	-	Y

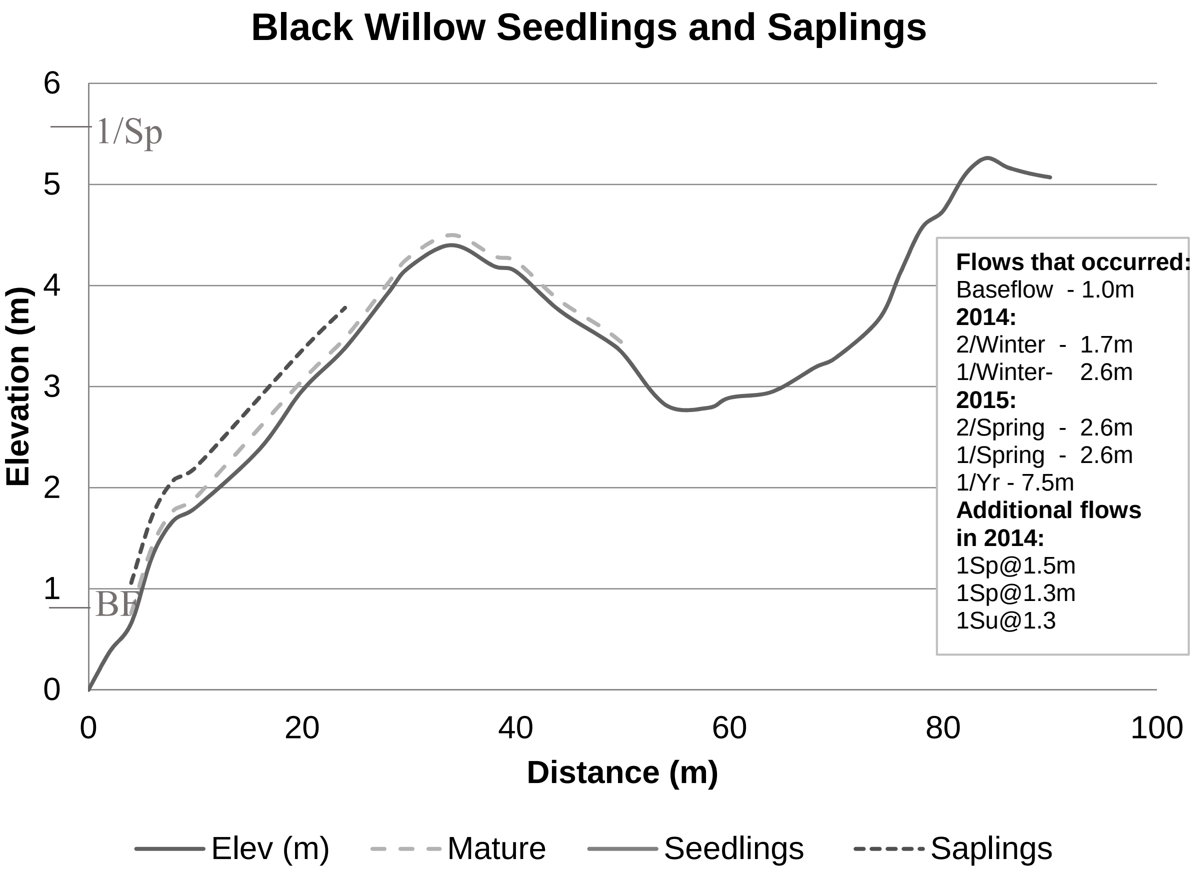


Figure 65. **Black willow distributions at the Victoria site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

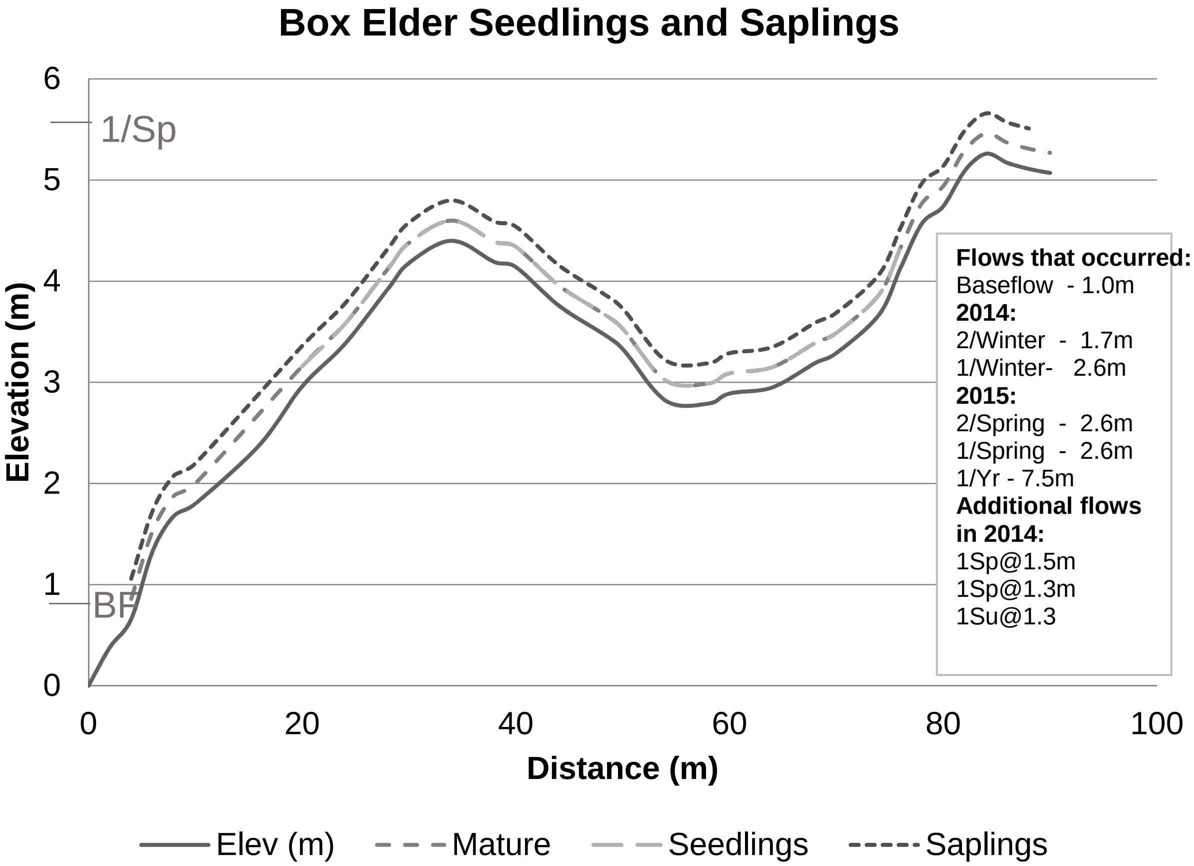


Figure 66. **Box elder distributions at the Victoria site. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

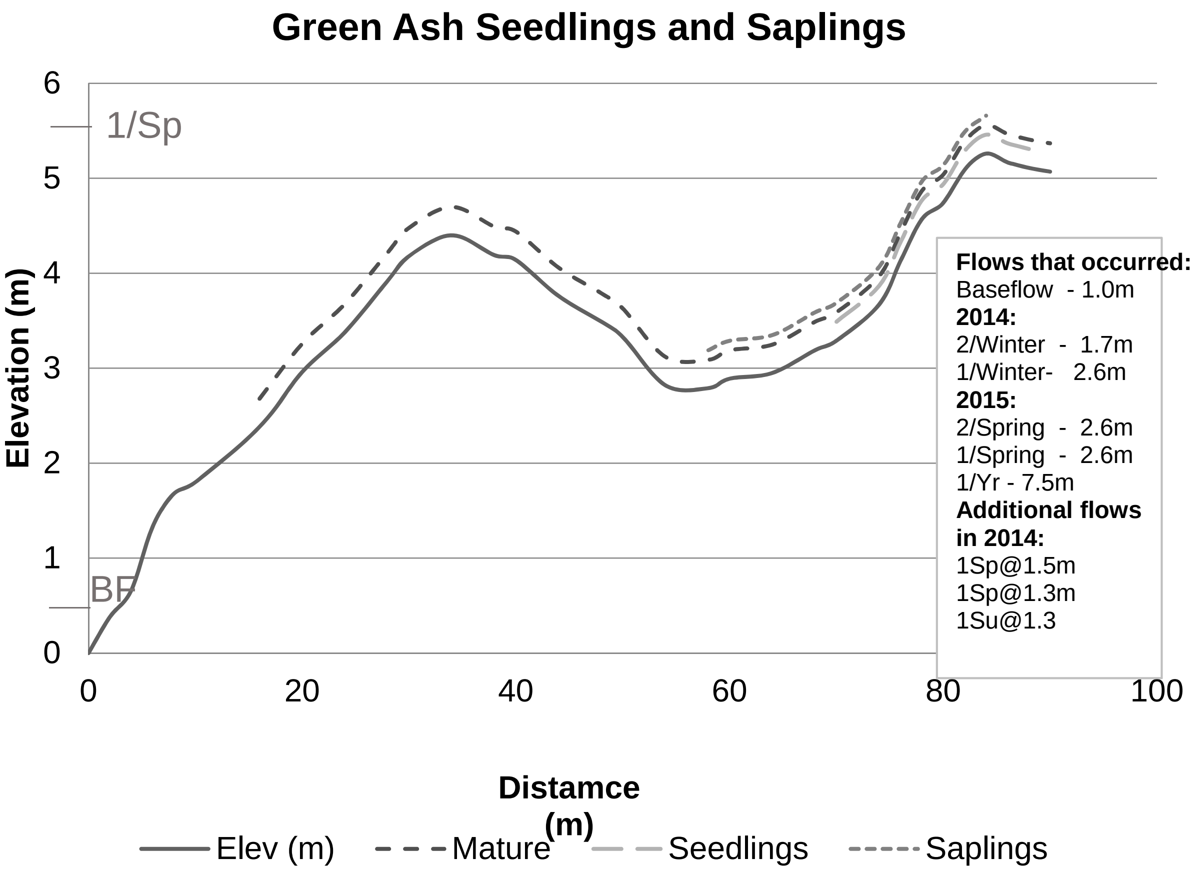


Figure 67. **Green ash distributions. Inset box indicates which flow tiers actually occurred during the study. Additional flows that occurred (but did not meet recommendations) are shown in the inset box.**

Table 32. **Tree counts through time grouped by class at the Victoria site.**

Species	Class	Summer 2014	Fall 2014
Black Willow	Mature	30	31
Black Willow	Sapling	20	22
Black Willow	Seedling	5	2
Box Elder	Sapling	39	39
Box Elder	Seedling	18	14
Green Ash	Mature	1	1
Green Ash	Sapling	43	42
Green Ash	Seedling	28	23

Table 33. **Relative abundances of woody riparian species, grouped by tree type and age class at the Victoria site.**

Tree Species	Class	Relative abundance (%)
Black Willow	Sapling	6.8
Black Willow	Mature	10.3
Black Willow	Seedling	1.7
Box Elder	Seedling	6.2
Box Elder	Sapling	13.4
Cedar Elm	Sapling	0.7
Chinese Tallow	Mature	2.7
Chinese Tallow	Seedling	3.8
Chinese Tallow	Sapling	8.6
Dogwood	Sapling	0.7
Green Ash	Mature	0.3
Green Ash	Seedling	9.6
Green Ash	Sapling	14.7
Hackberry	Seedling	3.1
Pecan	Seedling	1
Sycamore	Seedling	0.7
Sycamore	Mature	3.1
Sycamore	Sapling	12.7
		100

Figure 68. **Victoria site riparian community grouped by tree age classes; values are based on summer 2014 sampling.**

Table 34. **Basin-wide summary of the total number of species covered by flow standards, total numbers of uppermost species covered, and the number of flows that occurred in 2014.**

Flow Tier	Number of all species covered[*] by flow	Number of species at the highest elevation covered* by flow	Number that occurred in 2014
Baseflow	1/14	0/6	6/6
2/Winter	1/14	0/6	5/6
1/Winter	1/14	0/6	4/6
3/Spring[**]	2/2	1/1	1/1
2/Spring	0/14	0/6	2/6
1/Spring	3/12	1/5	3/6
2/Summer	1/14	0/6	3/6
1/Summer	1/14	0/6	0/6
2/Fall	1/14	0/6	2/6
1/Fall	3/14	0/6	2/6
2/Feb. - Apr.[**]	2/2	1/1	0/1
2/Jul. - Nov.[**]	2/2	1/1	0/1
1/Year	12/14	4/6	2/6

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

[**] Goliad large flow pulses.

Table 35. Connection discharges and associated hydrologic statistics for each floodplain lake study site.

Site	Corresponding USGS Gauge	Period of Record (Mean Daily Discharge)	Estimated Connection Discharge (cfs)	Connection Discharge Percent Exceedance	Number of Connections During Period of Record	Frequency of Connection Events
Gonzales1	Guadalupe River at Gonzales (#08173900)	1996 - 2015 (6,832 days)	2,822	13	97	5.2 / year
Cuero1	Guadalupe River at Cuero (#08175800)	1964 - 2015 (18,794 days)	1,710	27	341	6.6 / year
Cuero2	Guadalupe River at Cuero (#08175800)	1964 - 2015 (18,794 days)	207	97	Connected at baseflow	Connected at baseflow
Victoria1	Guadalupe River at Victoria (#08176500)	1934 - 2015 (29,443 days)	290	92	Connected at baseflow	Connected at baseflow
Victoria2	Guadalupe River at Victoria (#08176500)	1934 - 2015 (29,443 days)	144	97	Connected at baseflow	Connected at baseflow
LSAR1	San Antonio River at Goliad (#08188500)	1924 - 2015 (29,570 days)	2,740	4	328	4.0 / year
LSAR2	San Antonio River at Goliad (#08188500)	1924 - 2015 (29,570 days)	>10,000	1	64	0.8 / year

Basin-wide conclusions

When considering all flow tiers across the basin, baseflow only inundates the range of one of the indicator species (Table 34) whereas the 1/year BBEST-recommended flow inundates 80% or more of 12 of 14 species' distributions at all sites combined. Spring flows are generally below species' ranges – only 3 of 12 species' distributions were inundated at 80% or more by the 1/spring flow. Goliad had no 1/spring flows, but did experience a 3/spring flow event that provided 100% inundation of the site riparian species. Goliad also experienced two additional large pulses; these pulses do not align with the general seasonal categories designated across the basin and so are not included in Table 35, but are noteworthy in that they did provide varied seasonal coverage of 100% of species' distributions at Goliad. Given that spring is the season of seed dispersal and/or germination for all three species, it is noteworthy that this flow tier provided so little coverage (excluding the Goliad site) across the basin. Only 1 of 14 species was covered at 80% or more by 1/summer flows. This too may be a critical flow for the seedling life stage; however, this 9-month study from late summer to spring did not allow for testing of this season. Only 3 of 14 species received 80% or more coverage from 1/winter flows, though this is not seen as particularly detrimental to direct productivity, as these deciduous species are not transpiring/photosynthesizing during this season. Fall, however, also showed only 3 of 14 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/fall flow that serves them at 80% of their range is recommended. All three species' seedlings would also be maintained with this coverage. 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 at least not detectable with these methodologies.

When only the highest-elevation species at each site (Table 34, Column 3) are considered, only one of the uppermost species are covered by TCEQ flows (not including Goliad's unique large flow pulses). 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 additional large flows assigned to Goliad are an exception to this trend. Clearly, they provide adequate inundation specifically to the full riparian distribution. Given that TCEQ flow tiers across the other sites are already often below species' ranges, the lack of flows during 2014 really underscores the distressing conditions these riparian zones were shown to be under (Table 34, Column 4). This also places priority emphasis on the need to study the inter year requirements for inundation flows necessary to maintain an existing mature riparian distribution at the SB 3 sites.

3.3 Floodplains

Estimates of connection discharge ranged from 144 cfs at Victoria2 to over 10,000 cfs at LSAR2 (Table 35). Generally, connection discharge estimates fell into one of three categories. Cuero2, Victoria1, and Victoria2 represent relatively recently formed floodplain features which are still connected to the main stem of the river under typical base flow conditions (connection discharges ranged from 144 -290 cfs) and function as large backwater habitats. Gonzales1, Cuero1, and LSAR1 all had connection discharges typical of moderate pulse events (range: 1,710 – 2,822 cfs) with estimated connection frequencies ranging from 4-7 events per year. Lastly, LSAR2 had an estimated connection discharge of over 10,000 cfs and a frequency of less than one connection per year. Given poor GPS accuracies at LSAR2, only a rather rough estimate of greater than 10,000 cfs is provided. Additional surveying is needed at this location to refine connection estimates.

A total of 1,099 individual fishes representing 11 families and 26 species were collected during fish sampling at six of the floodplain lake study sites in March and April 2015 (Table 36). Numerically abundant species included Western Mosquitofish *Gambusia affinis* (25% of all fish collected), Red Shiner *Cyprinella lutrensis* (18%), Bluegill *Lepomis macrochirus* (16%), Orangespotted Sunfish *Lepomis humilis* (10%), and Gizzard Shad *Dorosoma cepedianum* (7%). At Gonzales1, previously collected fish community data from eight seasonal fish sampling events are available. This dataset includes 6,519 individual fishes representing 11 families and 32 species (Table 37). Numerically abundant species include Western Mosquitofish (48%), Red Shiner (11%), Gizzard Shad (8%), Mimic Shiner *Notropis volucellus* (8%), and Bluegill (4%). Although rare (<1% relative abundance), other notable species captured from this site include Slough Darter *Etheostoma gracile*, Bluntnose Darter *Etheostoma chlorosoma*, and Pugnose Minnow *Opsopoeodus emiliae*.

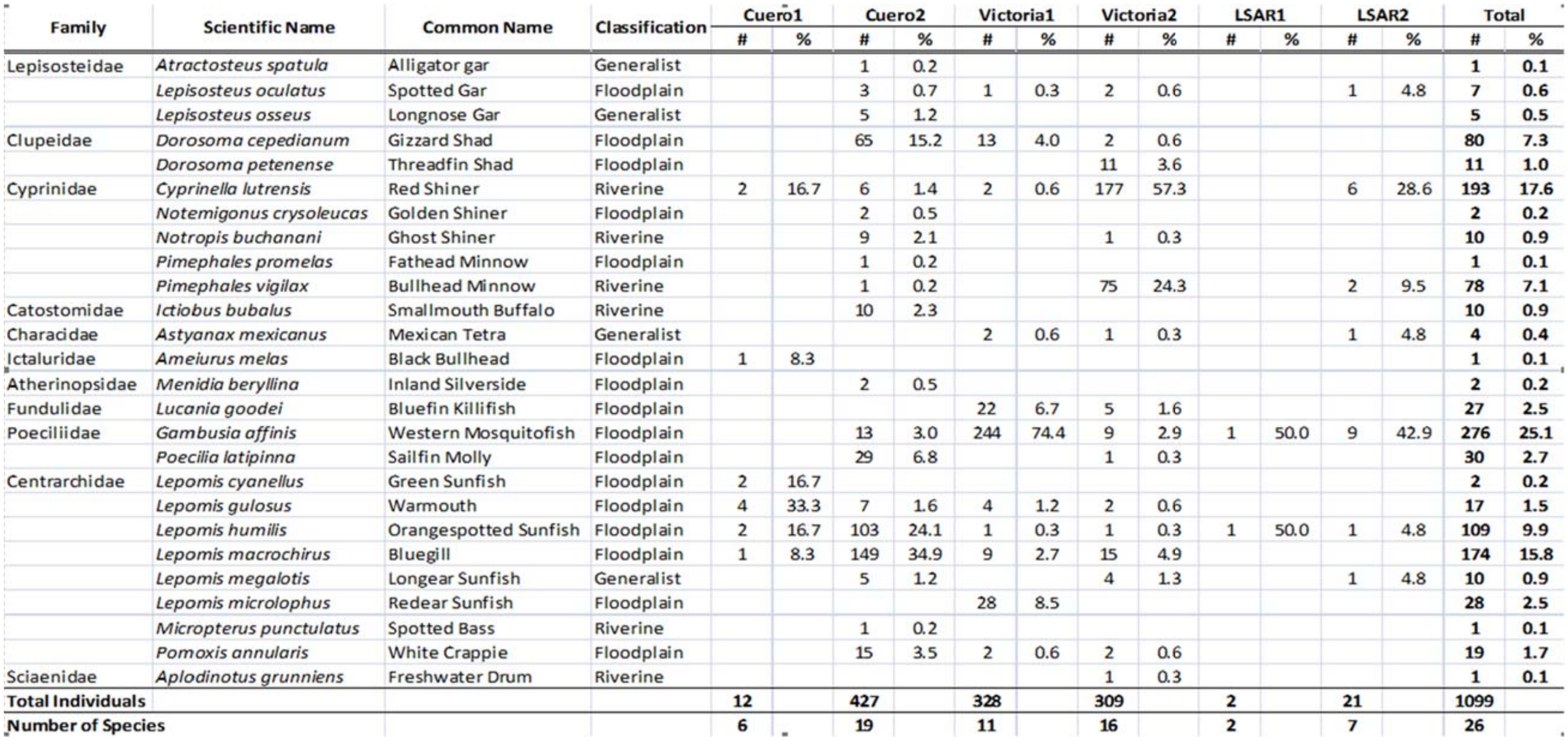
After inclusion of previously collected TIFP and BIO-WEST data, 19 individual river collections and 11 individual floodplain collections were available for analysis. The proportion of riverine and non-riverine species varied widely across sites (Figure 69). Results of the quasibinomial generalized linear model analyzing the proportion of riverine and non-riverine species demonstrate a significant difference between floodplain lake and mainstem Guadalupe River habitats ($p<0.001$). The effect of site ($p=0.62$), and the interaction term between site and habitat ($p=0.77$) were not significant. Based on this model, river habitats were on average 85% riverine species, while floodplain habitats were 23% riverine species on average.

Species richness documented during each floodplain feature sampling event ranged from 2 at LSAR1 to 23 at Gonzales1, and varied widely in relation to estimated connection discharge (Figure 70). Excluding Gonzales1, the three floodplain features which are connected at base flow levels exhibited higher species richness than the three which are disconnected at base flows. This is not surprising since these habitats have a constant potential for species exchange with the main stem. However, repeat sampling data from Gonzales 1 show how variable species richness can be between sampling events at a given site, and suggest that additional fish community data is needed from the other sites to strengthen this analysis.

To examine when connections occurred at Gonzales1, and thus link fish community data to hydrologic data, the percent of riverine species in each collection was overlaid on the hydrograph along with the estimated connection discharge (Figure 71). Based on this analysis, it appears that Gonzales1 was fully connected to the mainstem Guadalupe River four times over the study period. The first time was a brief connection on May 26-27, 2013. During this event, the river reached a maximum instantaneous discharge of 3,070 cfs (only slightly higher than the 2,822 cfs estimated for connection) and was estimated to maintain connectivity for less than 12 hours. A fish sampling event occurred immediately following this connection event on May 28, 2013. The other three connections all occurred between September 29, 2013 and November 5, 2013. During this time, three large pulses passed, with the last pulse exhibiting a maximum discharge of over 26,000 cfs and maintaining connectivity for approximately five days. A fish sampling event was conducted approximately one month before this series of pulses on September 3, 2013, and another event was conducted approximately one month afterwards on December 10, 2013.

The percent of riverine species captured from Gonzales1 showed a marked increase after the series of large pulses in fall 2013. However, no such effect was evident following the smaller-magnitude short-duration connection event in May 2013. Results of the quasibinomial generalized linear model confirm that samples following the large connection event had a significantly higher proportion of riverine species than other samples (p=0.001). This model predicts an increase of 62.5% riverine species after connection. A similar model analyzing days since connection was not significant (p=0.11), possibly a result of insufficient temporal data. It is also interesting to note that species richness declined after the series of connection events in fall 2013. The two sampling events prior to connection exhibited species richness values of 17 and 19, whereas the two events following the connection had species richness values of 12 and 9.

Table 36. Fishes collected from six floodplain lake study sites on the lower Guadalupe and San Antonio Rivers in March and April 2015.



Family	Scientific Name	Common Name	Classification	Cuero1		Cuero2		Victoria1		Victoria2		LSAR1		LSAR2		Total	
				#	%	#	%	#	%	#	%	#	%	#	%	#	%
Lepisosteidae	*Atractosteus spatula*	Alligator gar	Generalist			1	0.2									**1**	**0.1**
	Lepisosteus oculatus	Spotted Gar	Floodplain			3	0.7	1	0.3	2	0.6			1	4.8	**7**	**0.6**
	Lepisosteus osseus	Longnose Gar	Generalist			5	1.2									**5**	**0.5**
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	Floodplain			65	15.2	13	4.0	2	0.6					**80**	**7.3**
	Dorosoma petenense	Threadfin Shad	Floodplain							11	3.6					**11**	**1.0**
Cyprinidae	*Cyprinella lutrensis*	Red Shiner	Riverine	2	16.7	6	1.4	2	0.6	177	57.3			6	28.6	**193**	**17.6**
	Notemigonus crysoleucas	Golden Shiner	Floodplain			2	0.5									**2**	**0.2**
	Notropis buchanani	Ghost Shiner	Riverine			9	2.1			1	0.3					**10**	**0.9**
	Pimephales promelas	Fathead Minnow	Floodplain			1	0.2									**1**	**0.1**
	Pimephales vigilax	Bullhead Minnow	Riverine			1	0.2			75	24.3			2	9.5	**78**	**7.1**
Catostomidae	*Ictiobus bubalus*	Smallmouth Buffalo	Riverine			10	2.3									**10**	**0.9**
Characidae	*Astyanax mexicanus*	Mexican Tetra	Generalist					2	0.6	1	0.3			1	4.8	**4**	**0.4**
Ictaluridae	*Ameiurus melas*	Black Bullhead	Floodplain	1	8.3											**1**	**0.1**
Atherinopsidae	*Menidia beryllina*	Inland Silverside	Floodplain			2	0.5									**2**	**0.2**
Fundulidae	*Lucania goodei*	Bluefin Killifish	Floodplain					22	6.7	5	1.6					**27**	**2.5**
Poeciliidae	*Gambusia affinis*	Western Mosquitofish	Floodplain			13	3.0	244	74.4	9	2.9	1	50.0	9	42.9	**276**	**25.1**
	Poecilia latipinna	Sailfin Molly	Floodplain			29	6.8			1	0.3					**30**	**2.7**
Centrarchidae	*Lepomis cyanellus*	Green Sunfish	Floodplain	2	16.7											**2**	**0.2**
	Lepomis gulosus	Warmouth	Floodplain	4	33.3	7	1.6	4	1.2	2	0.6					**17**	**1.5**
	Lepomis humilis	Orangespotted Sunfish	Floodplain	2	16.7	103	24.1	1	0.3	1	0.3	1	50.0	1	4.8	**109**	**9.9**
	Lepomis macrochirus	Bluegill	Floodplain	1	8.3	149	34.9	9	2.7	15	4.9					**174**	**15.8**
	Lepomis megalotis	Longear Sunfish	Generalist			5	1.2			4	1.3			1	4.8	**10**	**0.9**
	Lepomis microlophus	Redear Sunfish	Floodplain					28	8.5							**28**	**2.5**
	Micropterus punctulatus	Spotted Bass	Riverine			1	0.2									**1**	**0.1**
	Pomoxis annularis	White Crappie	Floodplain			15	3.5	2	0.6	2	0.6					**19**	**1.7**
Sciaenidae	*Aplodinotus grunniens*	Freshwater Drum	Riverine							1	0.3					**1**	**0.1**
Total Individuals				**12**		**427**		**328**		**309**		**2**		**21**		**1099**	
Number of Species				**6**		**19**		**11**		**16**		**2**		**7**		**26**	

Table 37. Fishes collected from the Gonzales1 site during eight seasonal fish sampling events.

Family	Scientific Name	Common Name	Classification	Summer 2012		Fall 2012		Winter 2013		Spring 2013		Summer 2013		Fall 2013		Winter 2014		Spring 2014		Total	
				#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Lepisosteidae	*Lepisosteus oculatus*	Spotted Gar	Floodplain							2	0.5							2	0.1	**4**	**0.1**
	Lepisosteus osseus	Longnose Gar	Generalist	1	0.1					3	0.7									**4**	**0.1**
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	Floodplain	54	4.2			1	1.3	189	45.2							280	20.6	**524**	**8.0**
	Dorosoma petenense	Threadfin Shad	Floodplain	18	1.4							2	0.1							**20**	**0.3**
Cyprinidae	*Cyprinella lutrensis*	Red Shiner	Riverine	6	0.5	68	24.9	5	6.7	52	12.4	2	0.1	77	32.6	464	33.6	60	4.4	**734**	**11.3**
	Notemigonus crysoleucas	Golden Shiner	Floodplain	20	1.5			6	8.0			8	0.5					6	0.4	**40**	**0.6**
	Notropis buchanani	Ghost Shiner	Riverine	9	0.7			1	1.3											**10**	**0.2**
	Notropis volucellus	Mimic Shiner	Riverine	41	3.2					2	0.5	1	0.1	1	0.4	476	34.4			**521**	**8.0**
	Opsopoeodus emiliae	Pugnose Minnow	Floodplain	15	1.2															**15**	**0.2**
	Pimephales vigilax	Bullhead Minnow	Riverine	39	3.0	7	2.6	7	9.3	5	1.2	5	0.3	33	14.0	67	4.8	2	0.1	**165**	**2.5**
Catostomidae	*Carpiodes carpio*	River Carpsucker	Riverine	1	0.1													7	0.5	**8**	**0.1**
	Ictiobus bubalus	Smallmouth Buffalo	Riverine					1	1.3	6	1.4							2	0.1	**9**	**0.1**
	Moxostoma congestum	Gray Redhorse	Riverine					1	1.3									21	1.5	**22**	**0.3**
Ictaluridae	*Ameiurus melas*	Black Bullhead	Floodplain	3	0.2	11	4.0			9	2.2	13	0.9					2	0.1	**38**	**0.6**
Mugilidae	*Mugil cephalus*	Striped Mullet	Generalist							1	0.2									**1**	**0.0**
Atherinopsidae	*Menidia beryllina*	Inland Silverside	Floodplain					1	1.3			4	0.3	5	2.1					**10**	**0.2**
Poeciliidae	*Gambusia affinis*	Western Mosquitofish	Floodplain	915	70.8	143	52.4	2	2.7	79	18.9	1118	75.3	38	16.1	174	12.6	655	48.2	**3124**	**47.9**
	Poecilia formosa	Amazon Molly	Generalist							1	0.2									**1**	**0.0**
	Poecilia latipinna	Sailfin Molly	Floodplain	1	0.1			1	1.3			50	3.4					8	0.6	**60**	**0.9**
Centrarchidae	*Lepomis cyanellus*	Green Sunfish	Floodplain	11	0.9	2	0.7	2	2.7	5	1.2	12	0.8							**32**	**0.5**
	Lepomis gulosus	Warmouth	Floodplain	11	0.9			1	1.3	2	0.5	70	4.7	1	0.4	2	0.1	2	0.1	**89**	**1.4**
	Lepomis humilis	Orangespotted Sunfish	Floodplain	13	1.0	12	4.4	1	1.3			7	0.5			102	7.4	100	7.4	**235**	**3.6**
	Lepomis macrochirus	Bluegill	Floodplain	19	1.5	9	3.3	17	22.7	17	4.1	56	3.8	30	12.7	71	5.1	57	4.2	**276**	**4.2**
	Lepomis megalotis	Longear Sunfish	Generalist	24	1.9	19	7.0	20	26.7	41	9.8	103	6.9	21	8.9	19	1.4	24	1.8	**271**	**4.2**
	Lepomis microlophus	Redear Sunfish	Floodplain							1	0.2	1	0.1	3	1.3					**5**	**0.1**
	Lepomis sp.	sunfish	Generalist	36	2.8							10	0.7	21	8.9			63	4.6	**130**	**2.0**
	Micropterus punctulatus	Spotted Bass	Riverine	4	0.3			1	1.3	3	0.7									**8**	**0.1**
	Micropterus salmoides	Largemouth Bass	Floodplain	3	0.2							9	0.6	2	0.8			32	2.4	**46**	**0.7**
	Pomoxis annularis	White Crappie	Floodplain	44	3.4			5	6.7			1	0.1							**50**	**0.8**
Percidae	*Etheostoma chlorosoma*	Bluntnose Darter	Floodplain	1	0.1			2	2.7					3	1.3	7	0.5	21	1.5	**34**	**0.5**
	Etheostoma gracile	Slough Darter	Floodplain									1	0.1					14	1.0	**15**	**0.2**
	Percina apristis	Guadalupe Darter	Riverine											1	0.4					**1**	**0.0**
Cichlidae	*Herichthys cyanoguttatus*	Rio Grande Cichlid	Generalist	3	0.2	2	0.7					12	0.8							**17**	**0.3**
Total Individuals				**1292**		**273**		**75**		**418**		**1485**		**236**		**1382**		**1358**		**6519**	
Number of Species				**23**		**9**		**18**		**17**		**19**		**12**		**9**		**18**		**32**	

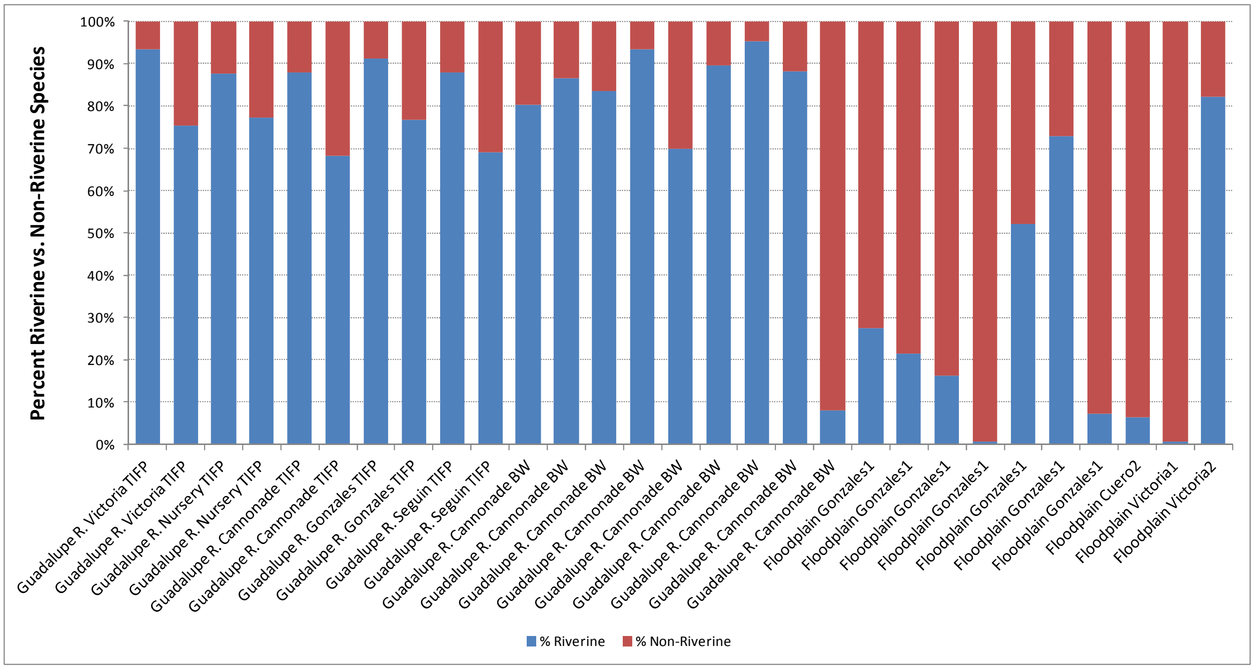


Figure 69. **Percentages of riverine and non-riverine species from 19 river and 11 floodplain fish collections within the Guadalupe River basin.**

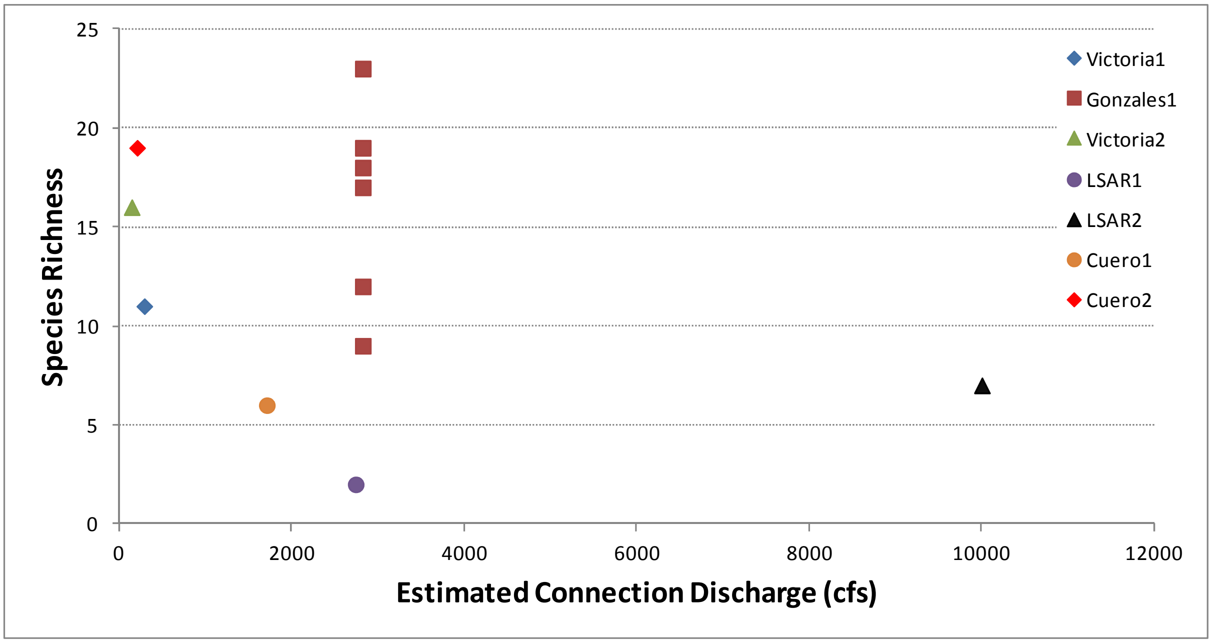


Figure 70. **Scatterplot of species richness versus estimated connection discharge for each floodplain lake fish sampling event.**

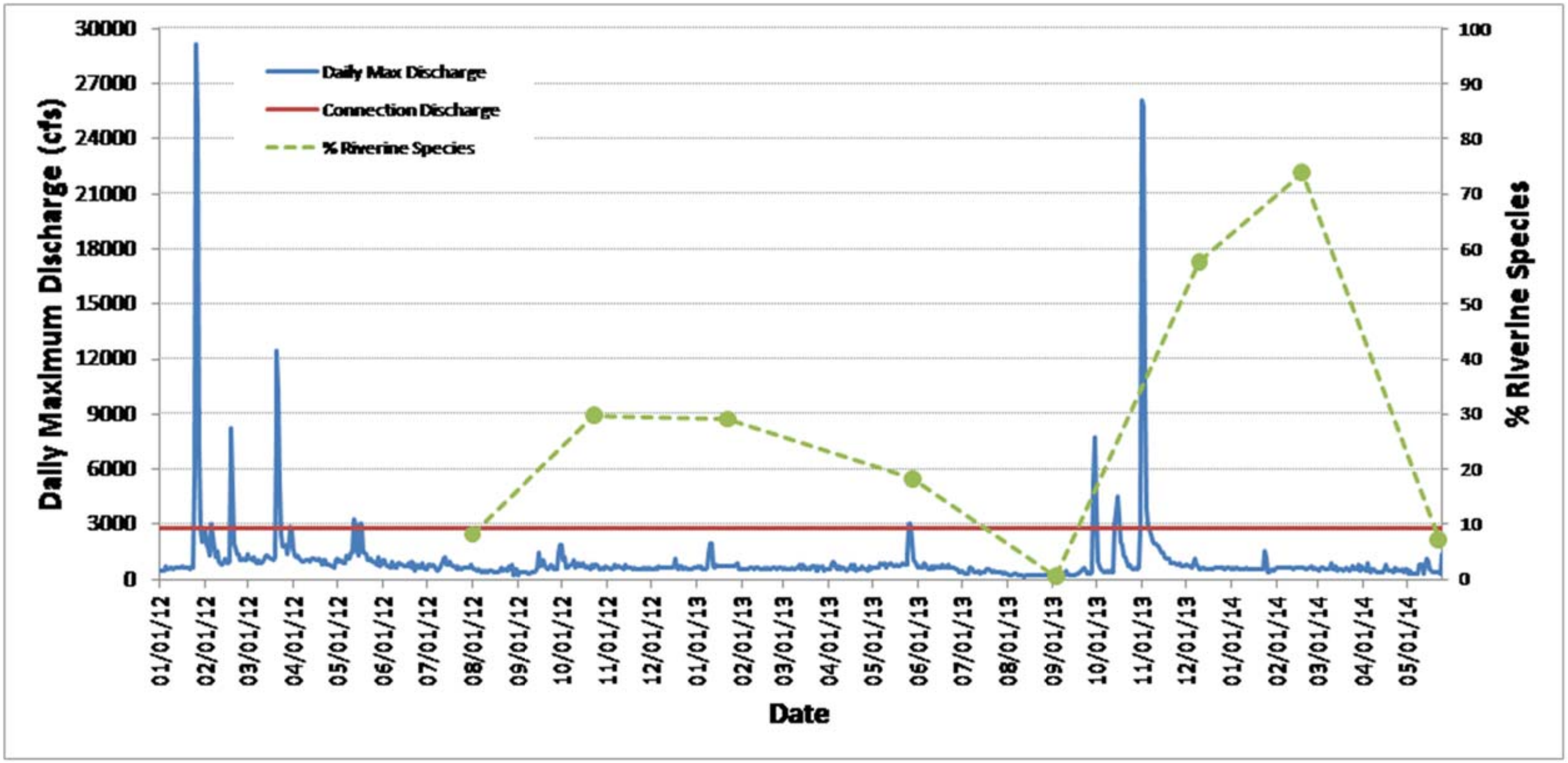


Figure 71. Daily maximum discharge (blue line) from the USGS gauge on the Guadalupe River at Gonzales (#08173900), estimated connection discharge for the floodplain lake (red line), and percent riverine species in each fish collection (green line).

Discussion/Conclusions

These data confirm that floodplain features within the lower Guadalupe River basin harbor a unique community of fishes significantly different from that found in the mainstem Guadalupe River. Although additional data collection is needed, these habitats are less common within the lower San Antonio River basin, and the ones sampled generally contained fewer species. Common species such as Western Mosquitofish, Red Shiner, Bluegill, and Gizzard Shad are the most numerically abundant fishes in these habitats. However, more importantly, these areas provide quality habitat for less common species including Bluntnose Darter, Slough Darter, and Pugnose Minnow. Floodplain lakes also serve as important habitat and recruitment zones for many Centrarchids, which are rare in the main stem including White Crappie and Orangespotted Sunfish. By providing habitat for these less common species, floodplain lakes are an important habitat component for maintaining basin-level biodiversity.

Occasional connection of floodplain lakes to the main stem of the river is crucial to prevent desiccation and allow biotic exchange. Estimates of connection discharge varied widely, ranging from 144 cfs to over 10,000 cfs. Several of the floodplain lakes maintained connection with the main stem of the river even under base flow conditions. These sites tended to be the most speciose, although species richness can fluctuate drastically in these habitats based on recent hydrological conditions. Gonzales1 tended to have a high species richness relative to other floodplain lakes of similar connection discharges. Cool water temperatures observed during sampling suggest that this site may have a groundwater connection that perhaps prevents desiccation even during long periods without connection.

Temporal analysis of fish community data from Gonzales1 shows a distinct change in species composition following a series of large pulses resulting in connectivity for several days. The increase in proportion of riverine species and decrease in overall species richness following this event was mainly due to an influx of riverine cyprinids (Red Shiner, Bullhead Minnow, Mimic Shiner), and a concomitant decrease in the occurrence and abundance of floodplain species such as Western Mosquitofish, Sailfin Molly, Green Sunfish, and Black Bullhead. This trend was maintained for a period of approximately three months from November 2013 through February 2014. However, by May 2014, floodplain species again dominated the assemblage. This reversal in composition was mainly due to a decrease in the number of riverine cyprinids, and an increase in the number of lentic spawning species such as Gizzard Shad, Western Mosquitofish, and various Centrarchids. With the onset of spring and resulting warmer water temperatures from February to May, mortality of riverine cyprinids likely increased as metabolism of predators increased, while spawning and recruitment of many lentic-spawning floodplain species was also occurring. Due to these biotic processes, the reversal from a riverine dominated assemblage to a floodplain dominated assemblage after a connection event may be expected to occur faster during the warmer months of March – October than during the colder non-reproductive season of November through February. However, more data is needed from additional connection events in varying seasons to investigate this hypothesis.

It is interesting to note that no substantial change in the proportion of riverine/floodplain species was noted after the brief connection event on May 26-27, 2013. Observations made on the following day confirm that a connection did occur. However, this connection may not have been of sufficient magnitude (i.e., it was likely a shallow connection) or duration (estimated to last

≈12 hours) to allow for substantial biotic exchange. Additional data on floodplain lake connectivity may help inform appropriate pulse duration recommendations.

Temporal analysis from Gonzales1 also shows a decline in species richness following the series of connection events in fall 2013. This is due to disappearance of several less-abundant floodplain species, as riverine species dominated these samples. Although this results in short-term reductions in species richness, these periodic connection events are necessary to maintain populations of riverine species in these typically lentic habitats, and thus preserve diversity within these habitats over the long-term.

Estimated connection discharge at each floodplain lake was examined in the context of both BBEST and BBASC pulse flow recommendations as well as TCEQ flow standards for each appropriate GSA gage location. Since three floodplain lakes were connected at base flow levels (Cuero2, Victoria1, Victoria2), they were not included in this analysis.

An estimated flow of 2,822 cfs must pass the USGS gage at Gonzales to fully connect Gonzales1. Estimated connection frequency for this habitat was 5.2 connections/year. BBEST and BBASC flow recommendations and TCEQ flow standards all include pulse flow recommendations adequate to meet this magnitude at least twice during the spring season and at least once during both winter and fall seasons. Flows of this magnitude are rare during the summer, and excluding BBEST/BBASC overbank flows, no high-flow pulse recommendations were made by any group to reach this magnitude during the summer season (July – September). Connection of this habitat once during the winter, twice during the spring, and once during the fall is likely sufficient for preventing desiccation of this unique floodplain habitat.

An estimated discharge of 1,710 cfs must pass the USGS gage on the Guadalupe River at Cuero to connect Cuero1. Estimated connection frequency for this floodplain lake was 6.6 connections/year. BBEST recommendations, BBASC recommendations, and TCEQ flow standards all contain the same high-flow pulse requirements at this location. This magnitude of pulse is protected once during the winter, twice during the spring, once during the summer, and twice during the fall. This connection regime is likely sufficient for preventing desiccation of this floodplain habitat.

An estimated discharge of 2,740 cfs must pass the USGS gage on the lower San Antonio River at Goliad to connect LSAR1. Estimated connection frequency for this habitat was 4.0 connections/year. Although pulse recommendations/standards varied among the three groups, TCEQ flow standards protect pulses of sufficient magnitude to connect this floodplain lake twice during February thru April, three times during April thru June, and twice from June to September. Should flows of sufficient magnitude occur, this connection regime is likely adequate to prevent desiccation of this habitat.

Lastly, an estimated discharge of over 10,000 cfs must pass the USGS gage on the San Antonio River at Goliad to connect LSAR2. Although large overbank flows of this magnitude were included in BBEST and BBASC recommendations, such flow magnitudes are absent from TCEQ standards. Flows within this magnitude typically occur during large flood events. Historically, this magnitude of event has occurred less than once per year. Occasional desiccation of this habitat would be expected at such rare connection frequencies. However, given that this floodplain lake maintained water and fish at the time of sampling, and a connection event of this magnitude had not recently occurred, it may receive enough runoff from the surrounding watershed to maintain wetted habitat. More data is needed from this particular location to further evaluate the contributing hydrology.

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 GSA, 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) extensive data were available from recent, comprehensive instream flow studies. Even in those instances, professional judgment still 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 GSA 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

Aquatics

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).

The 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. Perhaps 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 baseflow, 0% at 1/season and 0% at large flood, percentages of fluvial fishes were 29% at baseflow, 44% at 1/season, and 4.5% at large flood, percent of swift fishes were 58% at baseflow, 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 (or a subset of the 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 measured 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 this basin. However, the description for the Brazos River is included below to show an example of a direct ecological linkage to flow that may show promise to investigate further in the GSA basin.

Based on literature, 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 (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 GSA basin, only 15 of 124 sampled mature riparian distributions were inundated at 80% or more by TCEQ flow tiers (Table 38). Even though TCEQ has no standard 1/year flow, BBEST-recommended 1/year flows provided inundation in 12 of 14 tests. This study documented that there is a lack of correlation for most species at most sites between distribution of indicator species and TCEQ flow standards. When individual sites are combined, 8 of 14 tests of TCEQ flow tiers vs. seedling distribution were supported (seedling flow reflected a known TCEQ flow), while 6 of 14 were inconclusive (mainly because so few flows occurred). Thirteen of 13 tests of actual flow vs. seedling distribution were supported (all seedling distributions could be linked to at least one known flow). Tests on the survival of seedlings through seasons in response to actual flows showed that 6 of 13 were supported, 2 of 13 were inconclusive and 5 of 13 were not supported. Testing of the sapling distribution in response to TCEQ flow tiers resulted in 5 of 14 supported and 9 of 14 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 9 of 15 were supported, 6 of 15 were not supported. 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 sapling survival through the seasons in response to flow was tested 2 of 14 were supported, 10 of 14 were not supported and 2 of 14 were inconclusive. 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 tiers at the sites evaluated for this study did not provide for coverage of 80% or more of riparian species' distributions. It has already been described 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 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 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

Table 38. Summary of basin-wide riparian hypothesis-testing results.

Group	Hypothesis	Hypothesis testing results	Comments
Mature tree distribution	Distribution of mature trees reflects seasonal TCEQ flow standards (and BBEST 1/year recommendations)	15/127 TCEQ flow standards (and 12/14 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 flow standards	8/14 tests were supported, 6/14 were inconclusive	Several flows did not occur; for those: no conclusive results to compare seedling distributions with.
	Seedling riparian distributions correlate with actual flows	13/13 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 was correlated with no seedling dispersal. This too is seen as a support for flow pulses (or lack of) determining seed dispersal.
	Seedling survival across seasons correlates with flows received	6/13 supported; 2/13 inconclusive; 5/13 not supported	Later flows observed to provide coverage a little more than 50% of times; others were inconclusive or survived despite a lack of flow. Some appeared to have alternate water sources.
Sapling distribution and survival	Distribution of saplings correlates with seasonal flow standards	5/14 supported; 9/14 inconclusive	Many flows did not occur. For those there were no conclusive results to compare sapling distribution with. Others showed correlations.
	If flows observed are less than the flow standards, sapling distribution correlates with actual flows	9/15 supported; 6/15 not supported	Evidence that saplings are becoming less dependent on seasonal flows, as their distributions often reflected several years' prior flows or appeared independent of any known current flows.
	Sapling survival across seasons correlates with flows received	2/14 supported; 10/14 not supported; 2/14 inconclusive	Even more evidence that the sapling life stage is less dependent on individual seasonal flows.
Riparian community	Riparian species show high relative abundance	3/6 supported; 3/6 not supported	Overall average = 45%. Range = 14-83%
	Community age distribution reflects observed major flow anomalies	Seedlings: 6/6 supported; Mature trees: 6/6 inconclusive	Low seedling counts tended to reflect strongly the drought conditions in 2014. 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.

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 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 across the summer season.

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 at sites not including the lower San Antonio River or Cibolo Creek, the existing TCEQ flow standards (spring and fall tiers) would need adjustment. Otherwise, if future flow magnitudes are removed, the riparian zone width will likely face constriction in most 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 39). 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. It is recommended that the BBASC consider the value of the riparian zones throughout the basin on a site-by-site basis. At sites deemed high value, the consideration of setting a goal of maintaining the historical riparian distribution would be the next discussion. Following that discussion and potential setting of that goal, the BBASC would need to consider a 1/spring and 1/fall event in accordance with Table 39 be implemented for those reaches currently lacking these flow magnitudes in order to maintain historical riparian distributions. Another BBASC consideration may be the addition of a 1/summer event as well, though future research extending across the full growing season is necessary to verify the benefit of this pulse.
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 life stages. Mature trees were more resilient, though some were lost during the study (likely the prolonged lack of flows) and observations were made that many more mature trees had recently perished. The tree coring didn’t currently 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 and germination are an excellent target for short-term, frequent monitoring, and are useful tool for providing a “snapshot” view of riparian health and 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 will 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 methodology to monitor 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.

Table 39. 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

Seasonal categories were adjusted for across-basin comparisons between the Brazos 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 40. The across basin assessment further confirms what was observed in the GSA 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 warrant consideration.

Table 40. **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.

Floodplains

Estimated connection discharges for three of the floodplain features examined in this study (Cuero2, Victoria1, Victoria2) show that they remain connected to the river at base flow conditions. These are relatively newly formed oxbows which have only developed a separation from the river at one end, and essentially serve as large backwater lakes. Species richness was relatively high in these habitats. Although pulse flow events were no doubt important in establishing these complex habitats, maintenance of aquatic habitat in these areas is not directly tied to any specific pulse flow magnitude.

Three other floodplain features (Gonzales1, Cuero1, and LSAR1) exhibited connection discharges typical of moderate seasonal pulse events (1,710 – 2,822 cfs), and one older floodplain feature (LSAR2) had an estimated connection discharge typical of a large flood event (>10,000 cfs). Since these habitats are not connected to the river at base flow levels, their maintenance is assumed to be dependent upon pulse flow events. Table 41 Section 4 – FP1 evaluates pulse flow recommendations from TCEQ flow standards at the nearest upstream USGS gage location to each floodplain feature. Pulse flow recommendations labeled with a “Y” at a particular site/gage combination met the estimated connection discharge, whereas those marked with an “N” did not. Not all pulse flow recommendations would be expected to meet a given connection discharge. However, considering recommended frequencies, if the appropriate seasonal flows occur, recommendations generally meet annual connection frequencies similar to those experienced historically for these particular habitats (Table 42). The one exception to this trend is LSAR2 which had an estimated connection discharge greater than 10,000 cfs.

Table 41. TCEQ environmental flow standard pulse recommendations compared to connection discharges at four floodplain lakes in the lower GSA basin. Pulse events with a “Y” had a magnitude greater than the estimated connection discharge, whereas those with an “N” did not. Dashes represent recommendations which were not applicable at a particular gage.

Floodplain Lake			**Gonzales1**	**Cuero1**	**LSAR1**	**LSAR2**
Connection Discharge (cfs)			2,822	1,710	2,740	>10,000
USGS Gage			Guadalupe River at Gonzales	Guadalupe River at Cuero	San Antonio River at Goliad	San Antonio River at Goliad
Seasonal Pulses	Winter	2/season	N	N	-	-
		1/season	Y	Y	N	N
	Spring	2/season	Y	Y	N	N
		1/season	Y	Y	-	-
	Summer	2/season	N	N	-	-
		1/season	N	Y	N	N
	Fall	2/season	N	Y	-	-
		1/season	Y	Y	N	N
Large Pulses	Apr. - Jun.	3/period	-	-	Y	N
	Feb. - Apr.	2/period	-	-	Y	N
	Jul. - Nov.	2/period	-	-	Y	N

Table 42. **Connection discharges for four floodplain lakes within the GSA basin, their historical connection frequencies, and the number of annual connection events protected by TCEQ standards (if all flow standards occur).**

Floodplain Lake	Connection Discharge (cfs)	USGS Gage	Number of annual connection events protected by TCEQ flow standards	Historical connection frequency (connections/year)
Gonzales1	2,822	Guadalupe River at Gonzales	5	5.2
Cuero1	1,710	Guadalupe River at Cuero	8	6.6
LSAR1	2,740	San Antonio River at Goliad	7	4
LSAR2	>10,000	San Antonio River at Goliad	0	0.8

4.2 Description of 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 application is to test the TCEQ flow standards on a basin-wide scale to see if, in general, the standards are meeting ecological needs. The second application could be employed on a site-by-site basis, should future water projects be proposed in specific river reaches. Current TCEQ protocol suggests that should a future water project be able to meet TCEQ environmental flow standards, no further ecological assessment would be necessary. However, even though TCEQ does not currently require site-specific studies to be conducted to address this, it is likely that BBASC members or other interested parties may consider conducting specific studies in an effect to inform the next round of environmental flow standards revisions. However, there is currently no standard method for collecting or assessing that information rather than professional judgment by the BBASC and ultimately the TCEQ. Therefore, a future use of a validation methodology could be to standardize the assessment process for future projects. An agreed upon methodology upfront will provide TCEQ with a simplified and scientifically based tool for making the final decision of pass or fail.

Building on the ecological components tested during this study, the following is proposed as the foundation of this methodology. It is recognized 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 at this location as defined by the BBASC?” a tiered approach is proposed that starts with the most direct ecological linkages and works through a checklist of ecological components. However, for specific SB 3 application, 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, then 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 complete.
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.

It is acknowledged that 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

As a hypothetical example, the proposed tiered approach outlined in Section 4.2 was used to evaluate two different sites within the GSA basin using data from this study. The first example involves an evaluation of the San Antonio River at Goliad. For this example, it was assumed that floodplain connectivity was deemed extremely important in the lower San Antonio 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 GSA BBASC. These hypothetical answers allow for the Tier I evaluation of the TCEQ flow standards, since the field data for this site is already collected. As noted in Section 3, for one of the two floodplain features studied at this location, flows adequate to provide the connectivity and seasonal timing required for maintenance of the floodplain aquatic community are currently provided by the TCEQ flow standards. However, the other feature at this location is an ancient oxbow that requires overbanking flows, so it is discarded from consideration. Thus, in this example, the TCEQ flow standard for the lower San Antonio River at Goliad passes the Tier I test.

The next step in this hypothetical example would be to answer the Tier II riparian question. In this example, our answer was yes, deciding that though riparian habitat in the Goliad study reach is very important, it is not vital to maintain in its entirety. This led 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% - 100% in this report. In doing so, we acknowledge 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 flow standards for Goliad were then evaluated relative to the riparian needs for seedlings and mature trees. An examination of the data from Section 3 shows that the existing TCEQ flow standards at Goliad 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 San Antonio River at Goliad passes the Tier II test.

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 Blanco River at Wimberley was conducted. For this example, no hypothetical answers to the initial tier questions posed to the BBASC are provided. Addressing Tier I is straightforward, as the site possesses no floodplain features as defined, so no evaluation is necessary. However, addressing Tier II (the riparian assessment) is more interesting. For instance, when looking at one of the riparian indicator species, the black willow, TCEQ flow standards inundate 100% of the existing mature tree distribution for this species in both spring and fall. However, for box elder, a different riparian indicator species, TCEQ standard flows only inundate approximately 45% and 35% of the existing mature tree distribution in spring and fall, respectively. A similar result is noted for green ash, for which approximately 65% and 55% of the existing mature tree distribution is inundated by the respective spring and fall pulses when comparing against the existing TCEQ flow standards. Therefore, if the answer to the Tier II question on riparian importance is considered valuable, those evaluating the adequacy of the flow standards might discuss whether the potential increase in the volume of water assigned in the existing TCEQ flow standard or inclusion of a inter year requirement with a higher volume to meet those environmental needs is warranted. In this example, the same hypothetical discussion could be held for Tier III as presented in the last example. Although, spring and fall flow tiers 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 and Tier II desktop evaluations could be conducted by the GSA BBASC at each of the sites that were evaluated during this study because the field work has already been conducted. The only missing piece is that the first question for each Tier must be 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. As described in Section 5, additional data is needed before aquatic responses, or the 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:

1) increases or decreases in volumes needed in spring and fall pulses for either floodplain connectivity or maintenance of the existing riparian communities;
2) adjustment in timing of seasonal pulses in conjunction with volume to meet the ecological needs of certain ecological components (i.e., consideration of adding in the BBEST 1/per year event which is not in the standards (at some sites) but put it in with a seasonal component rather just an annual requirement);
3) inclusion of an inter year riparian pulse requirement; and
4) 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 an important and much-needed first step toward addressing important questions and concerns raised during the SB 3 process. However, it is acknowledged that more work needs to be done to get to a workable endpoint for the BBASC and TCEQ. This section describes recommendations for additional focused research as well as the establishment of a few targeted locations for long-term monitoring. It is important to first clarify the difference between applied research and long-term monitoring. 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.

Each component addressed in this study needs some combination of focused 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.

The aquatics component needs to emphasize 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. It is recommended that riparian applied research focus on opportunistic conditions (i.e., 2015 flooding) and evaluation of 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. The lower San Antonio River at Goliad and lower Guadalupe River at Gonzales are proposed as potential long-term sites because of their extended sampling record to date. If resources are limited, riparian long-term monitoring could be done at a longer temporal interval, say every other year, or every five years. Finally, it is recommended that applied research for oxbows be limited to those that the BBASC specifically might have an interest in that have not been studied to date. However, long-term monitoring of select floodplain features is recommended on an annual or even every other year sampling to assess over time if the TCEQ flow standards maintain the ecological function anticipated in the floodplain feature. The floodplain feature long-term monitoring applies only to the lower Guadalupe and San Antonio Rivers.

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 of the ways in which flow magnitudes influence stream communities within both lower-gradient reaches (lowland sites) and higher-gradient reaches (upstream sites). This approach should also help inform future research planning with regard 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 from TCEQ and TPWD 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. We focused on magnitude, but duration could also be evaluated. Future work could include abiotic and biotic responses to specific flow tiers with duration either 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 are chosen for continued monitoring so that the methodology can be further validated and refined. On the GSA basin, the lower Guadalupe River at Gonzales has the longest continuous record, and would be an excellent site to continue. Additionally, the lower San Antonio River at Goliad would also be an excellent site 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, as there were fewer flows available with which to calibrate equipment. 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 coverage. 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 GSA basin, the Blanco River site in particular presents an excellent opportunity, as the entire riparian zone was wiped completely away, and the lower Guadalupe River at Victoria site was covered over by several inches of sediment.

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

Although connection of the above 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 this analysis, it was 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. For the purposes of this analysis, it was assumed that the floodplain features examined above provide an adequate representation of those currently occurring in the system, and that they are similar in connectivity and function to those historically found in the system.

Additional data from other similar floodplain areas within the GSA basin could certainly strengthen this evaluation. This analysis was based on data collection at seven of the 24 potential 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. Seasonal data such as that available from Gonzales1 provide data useful in understanding the effects of these connection events on floodplain lake communities (and mainstem river communities) under different hydrologic scenarios. Therefore, the project team recommends a two-component long-term floodplain monitoring plan within the GSA that focuses on: (1) intense seasonal biomonitoring (i.e., 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.

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

Component 2 – Long-term habitat persistence evaluations.

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

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 GSA BBASC and TCEQ for discussion and consideration for possible adoption.

6 Acknowledgements

The SARA project team would like to thank the numerous property owners throughout the GSA basin that made this study possible. Stakeholders such as these allow for protection of natural resources important for people and natural systems. We also thank the TWDB for managing this contract and for their support and guidance during this accelerated process. We sincerely thank all participants of the two expert panel workshops for their thought-provoking comments and valuable suggestions. Acknowledgments would not be complete without recognizing the resource agency scientists and GSA BBEST and BBASC members who provided considerable time, guidance, and sharing of expertise both in front of and behind the scenes during this exciting project. Finally, a heartfelt thank you to all volunteers that supported field efforts to allow for data collection efforts above and beyond the scope of work.

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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.

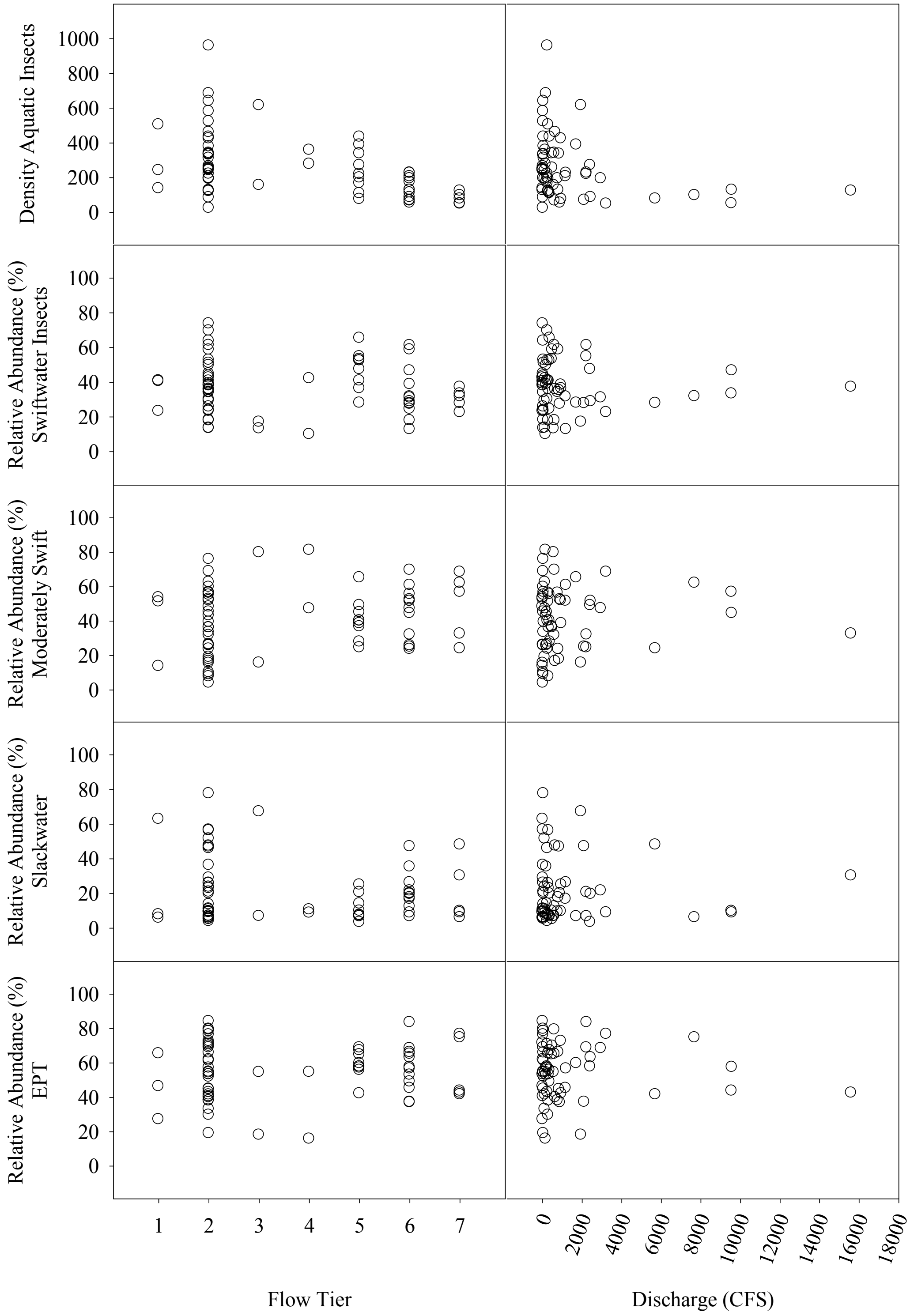
	Tier 1					Tier 2				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Run										
Area (m^2)	323	81	46	22	132	3,388	94	77	3	416
Peak Flow (cfs)		217	267	6	563		702	1,496	4	7,090
Season										
Summer	2					10				
Fall	1					12				
Winter	1					13				
Spring	0					1				
Total	4					36				
Water Temperature (°C)		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

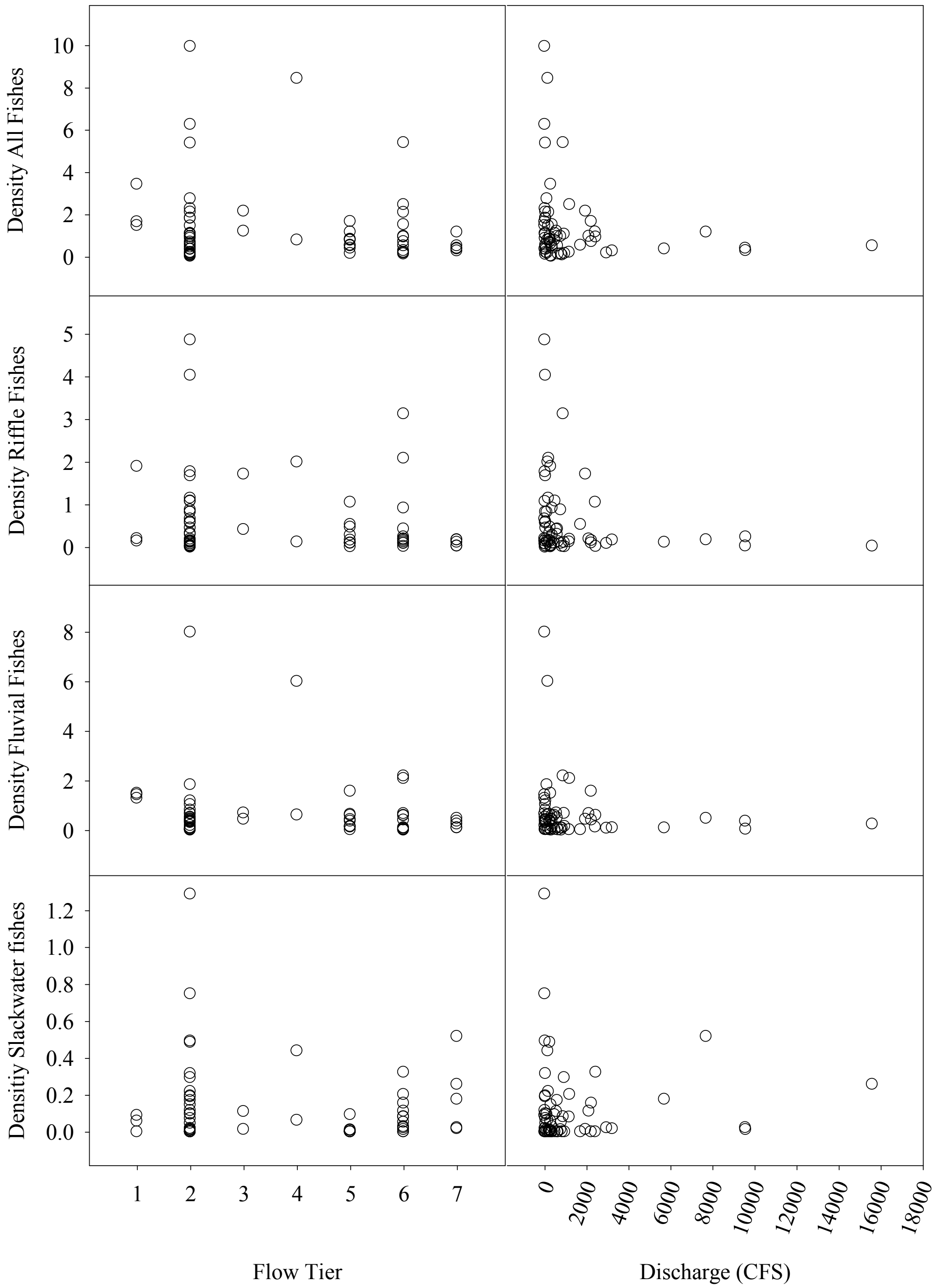
	Tier 5					Tier 6				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Run										
Area (m^2)	1,069	107	116	12	425	958	74	44	18	163
Peak Flow (cfs)		1,510	1,821	226	6,120		5,008	10,965	193	40,600
Season										
Summer	3					4				
Fall	6					2				
Winter	1					2				
Spring	0					5				
Total	10					13				
Water 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

	N	Mean	Tier 7 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

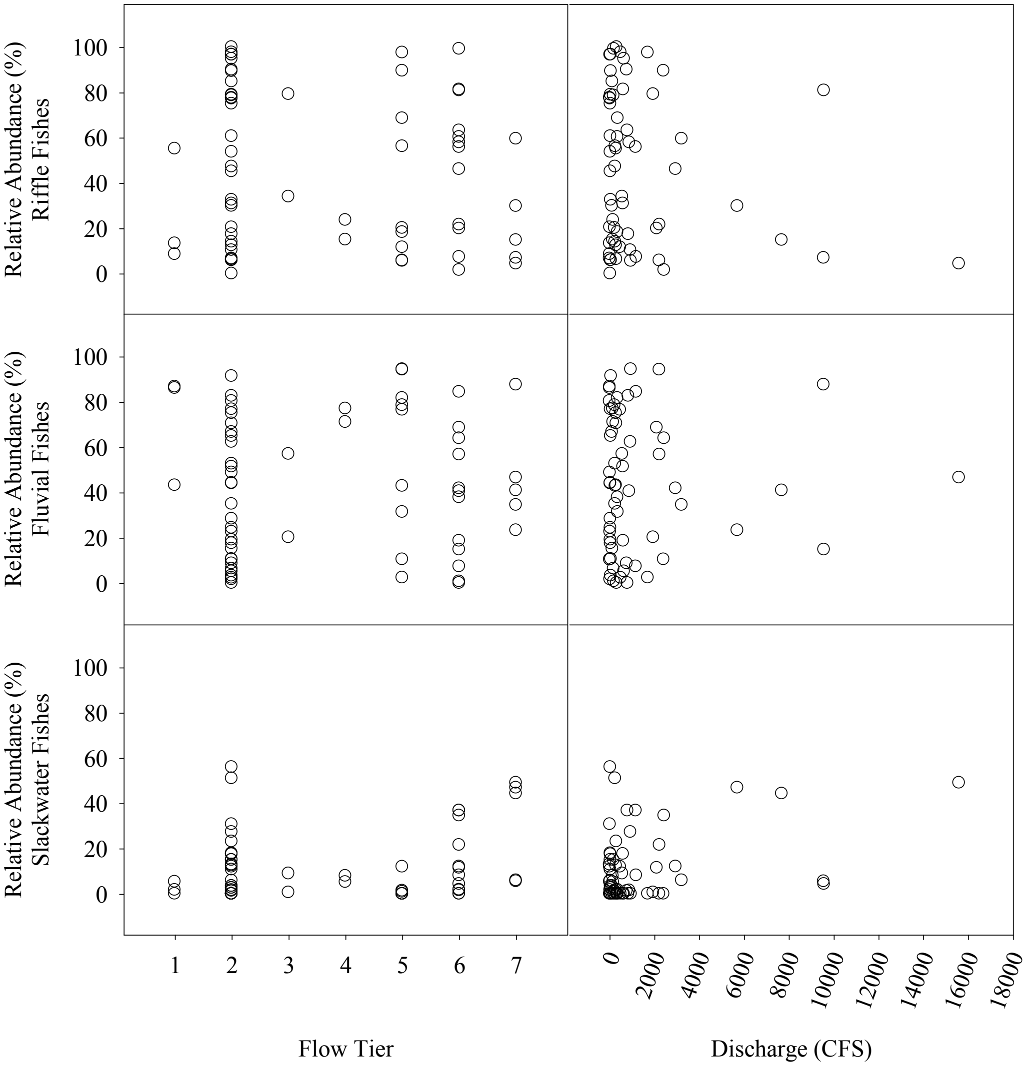
Appendix C: Density overall and relative abundances of swiftwater, moderately swift and slackwater macroinvertebrates plotted among flow tiers and discharge (CFS) from August 2014 – May 2015.



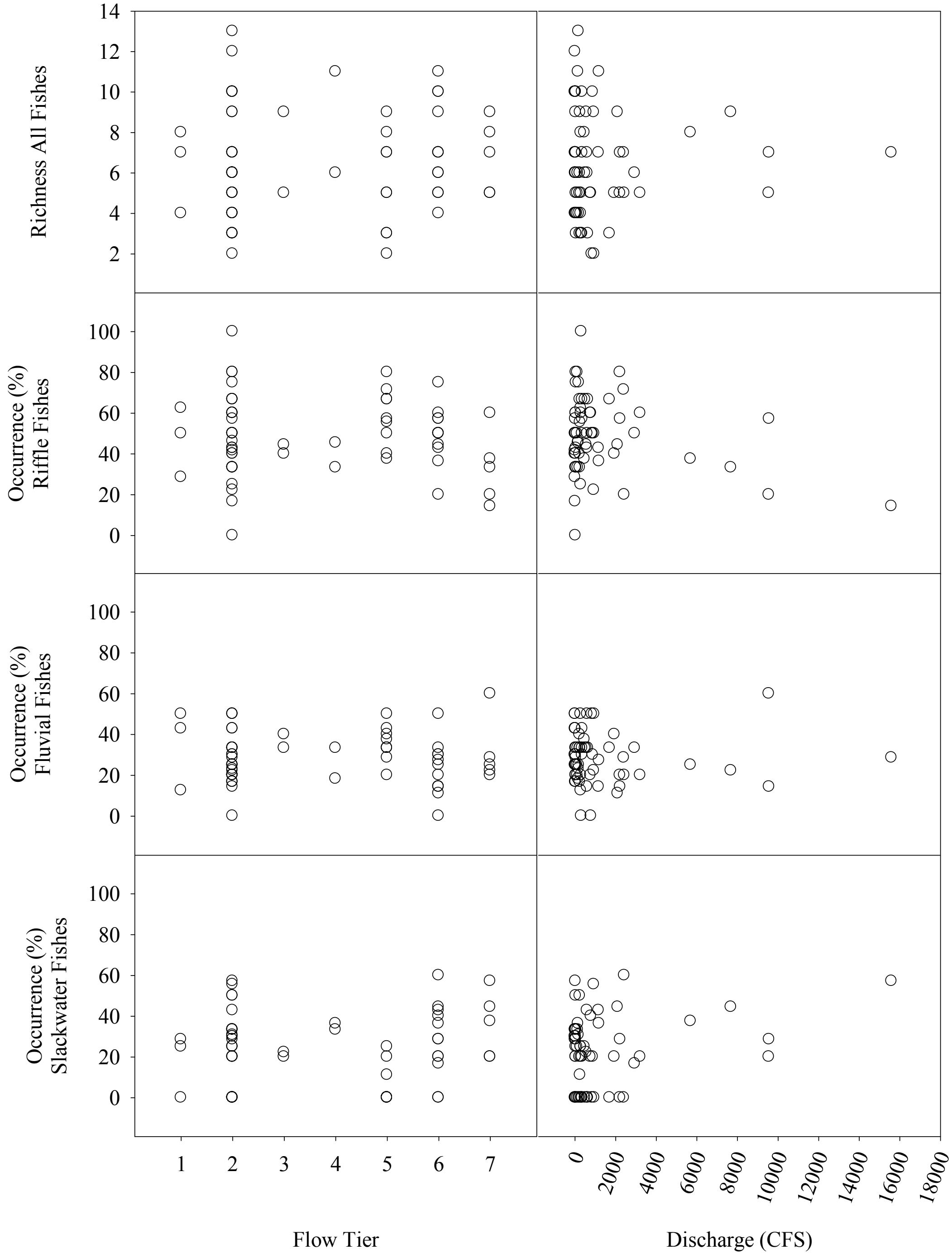
Appendix D: Densities overall and for riffle, fluvial and slackwater fishes plotted among flow tiers and discharge (CFS) from August 2014 – May 2015.



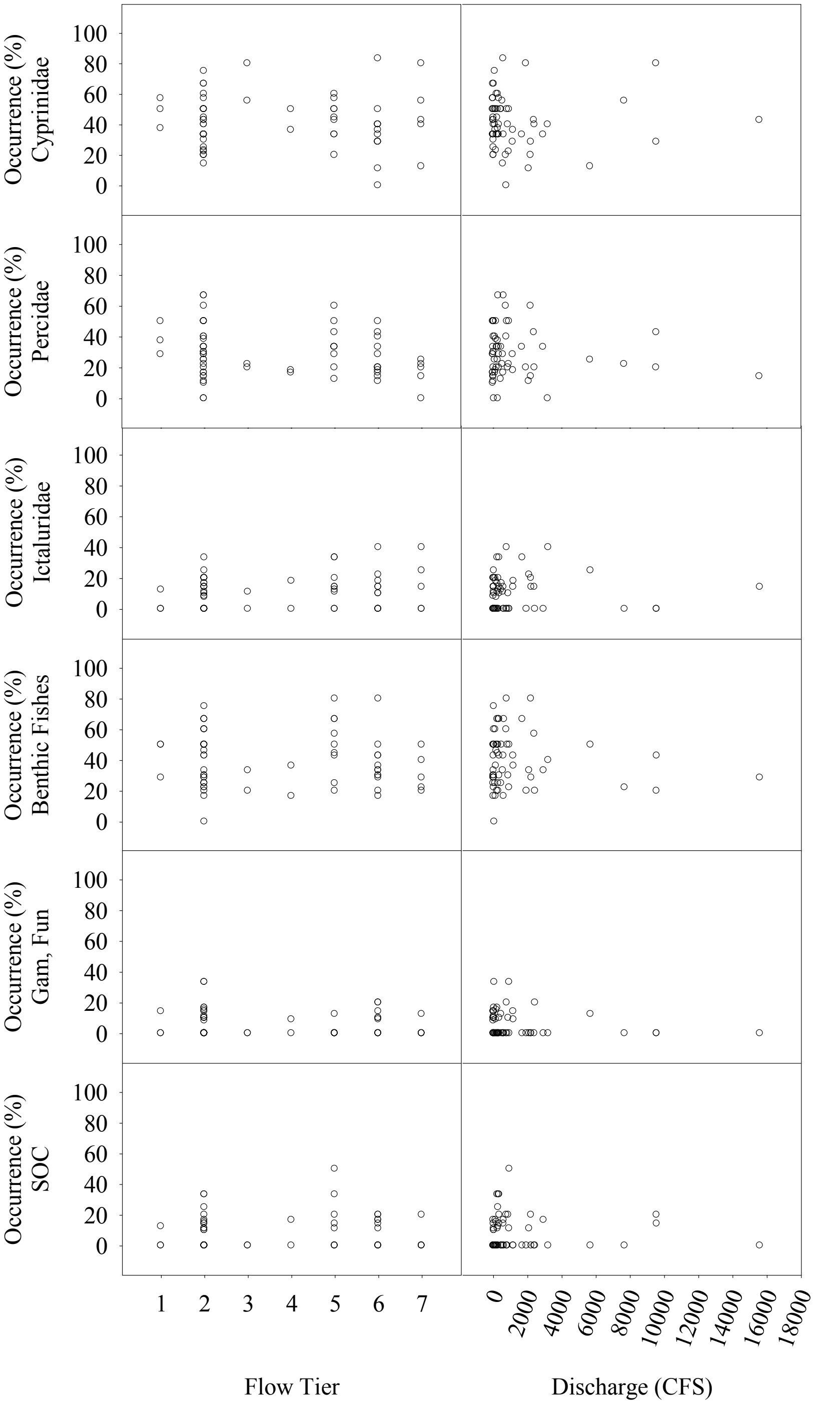
Appendix E: Relative abundances of riffle, fluvial and slackwater fishes plotted among flow tiers and discharge (CFS) from August 2014 – May 2015.



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.



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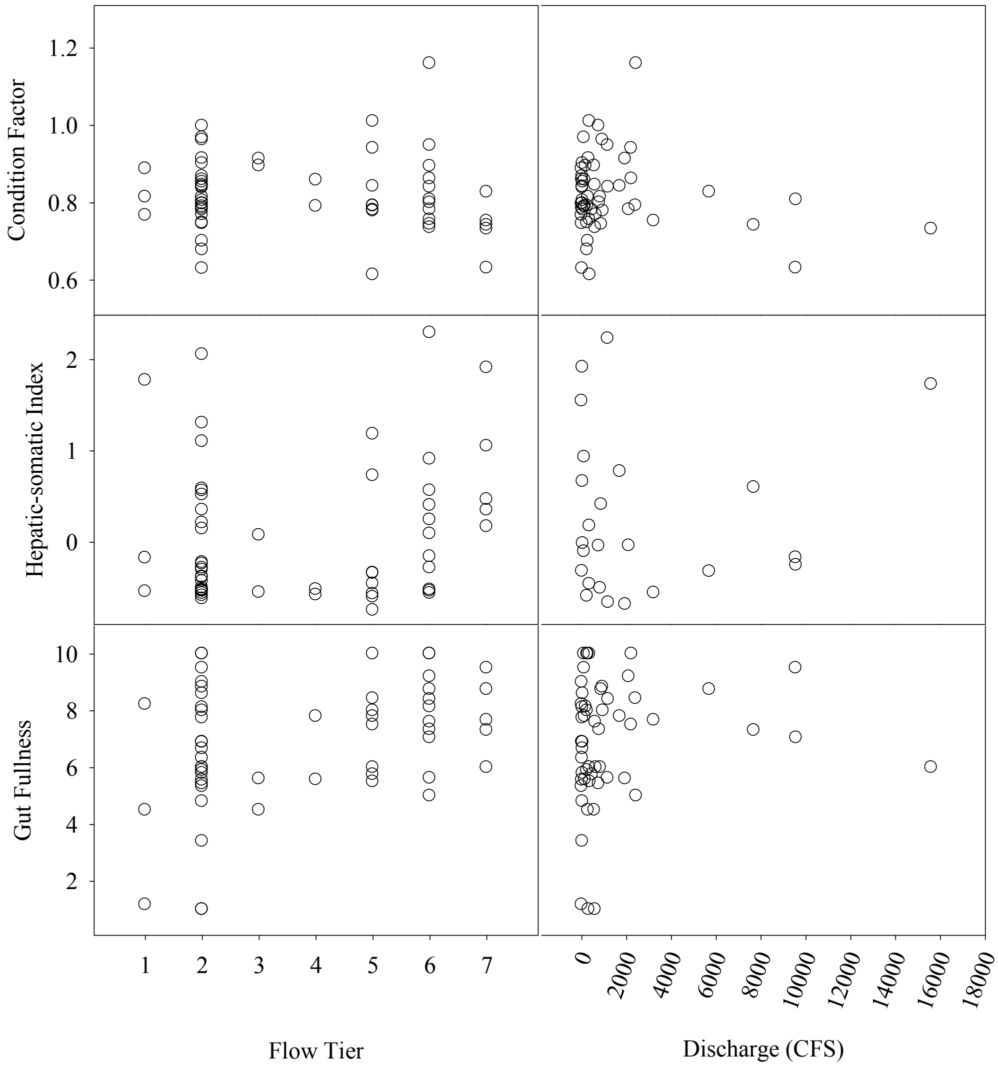
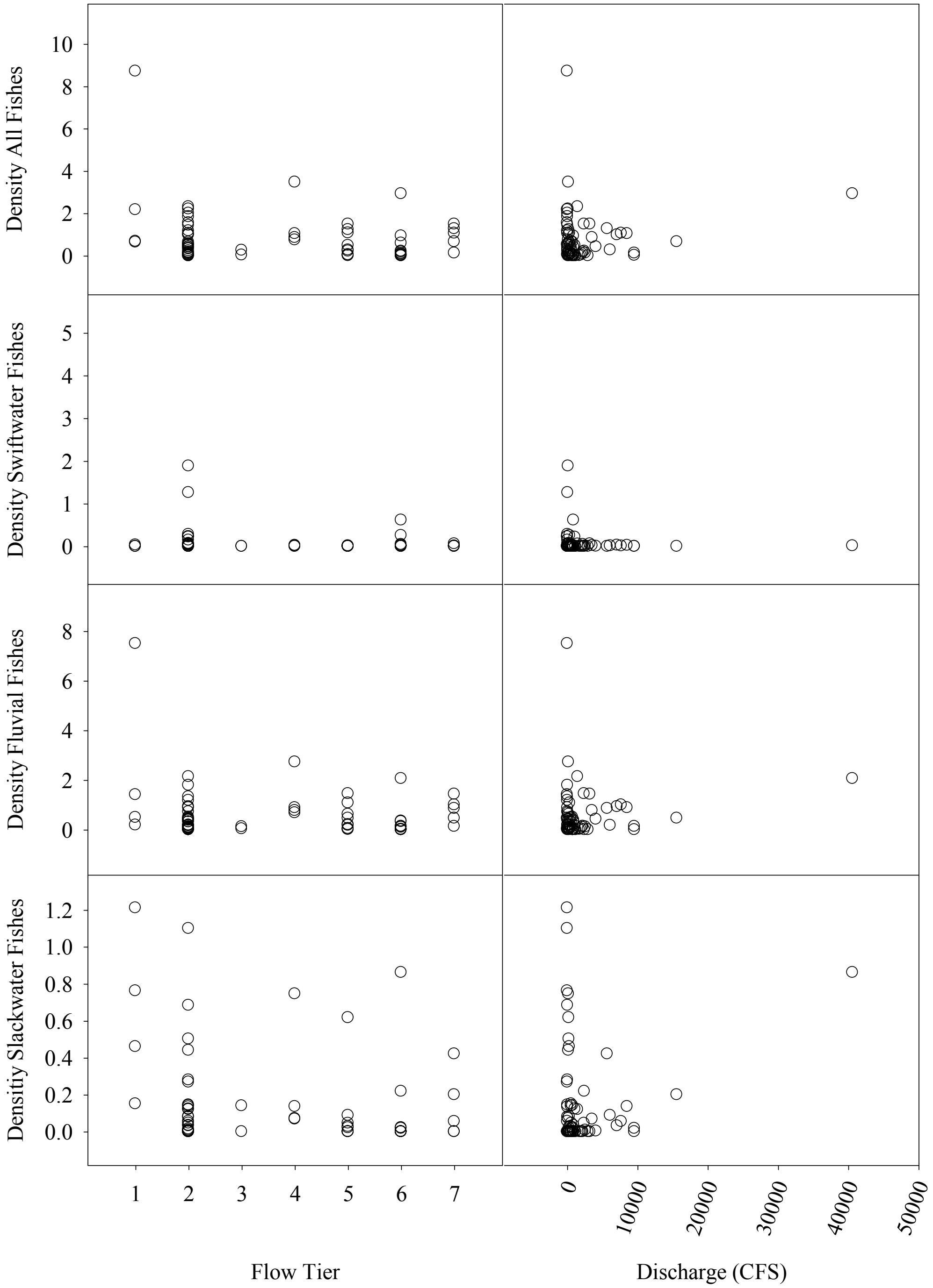
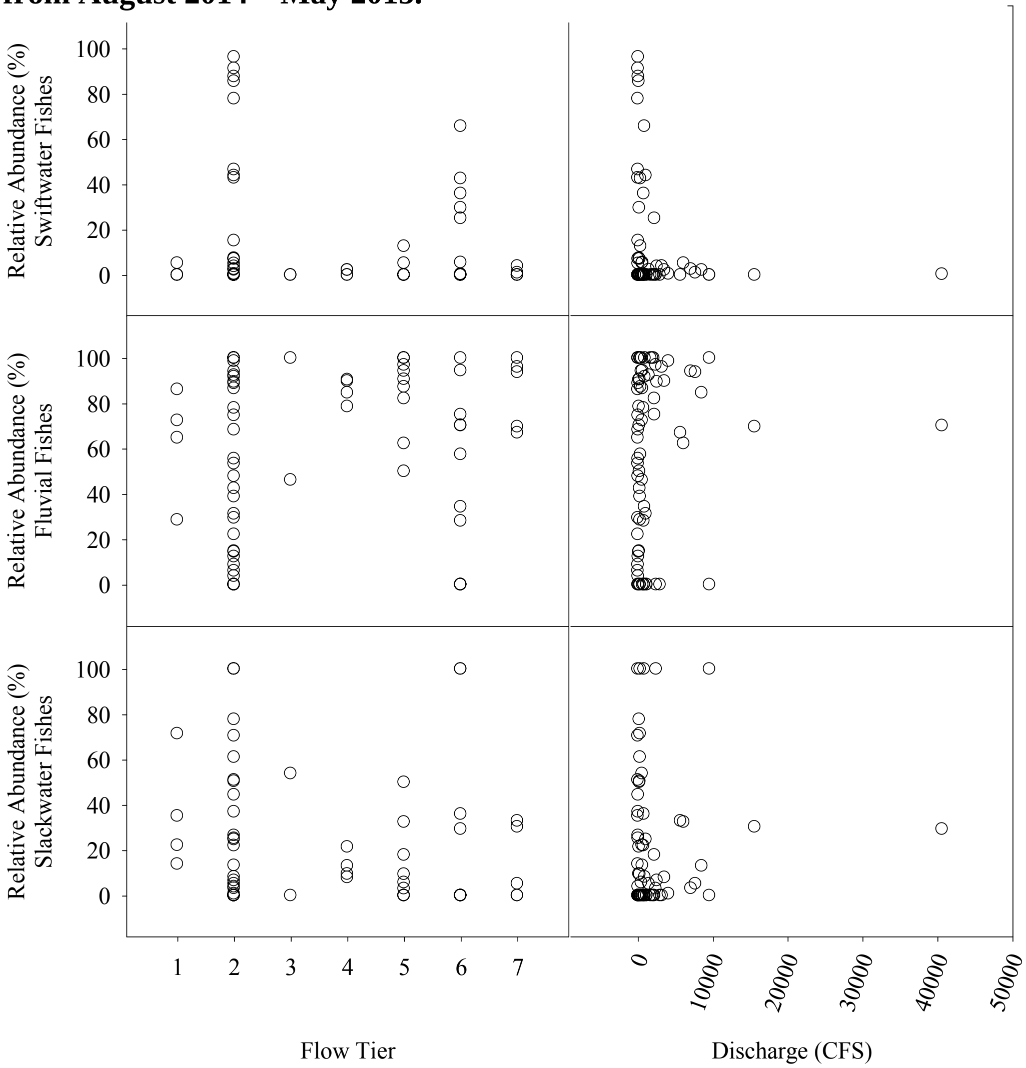
Appendix H: Condition factor, hepatic-somatic index (HIS) and gut fullness plotted among flow tiers and discharge (CFS) for riffle species from August 2014 – May 2015.



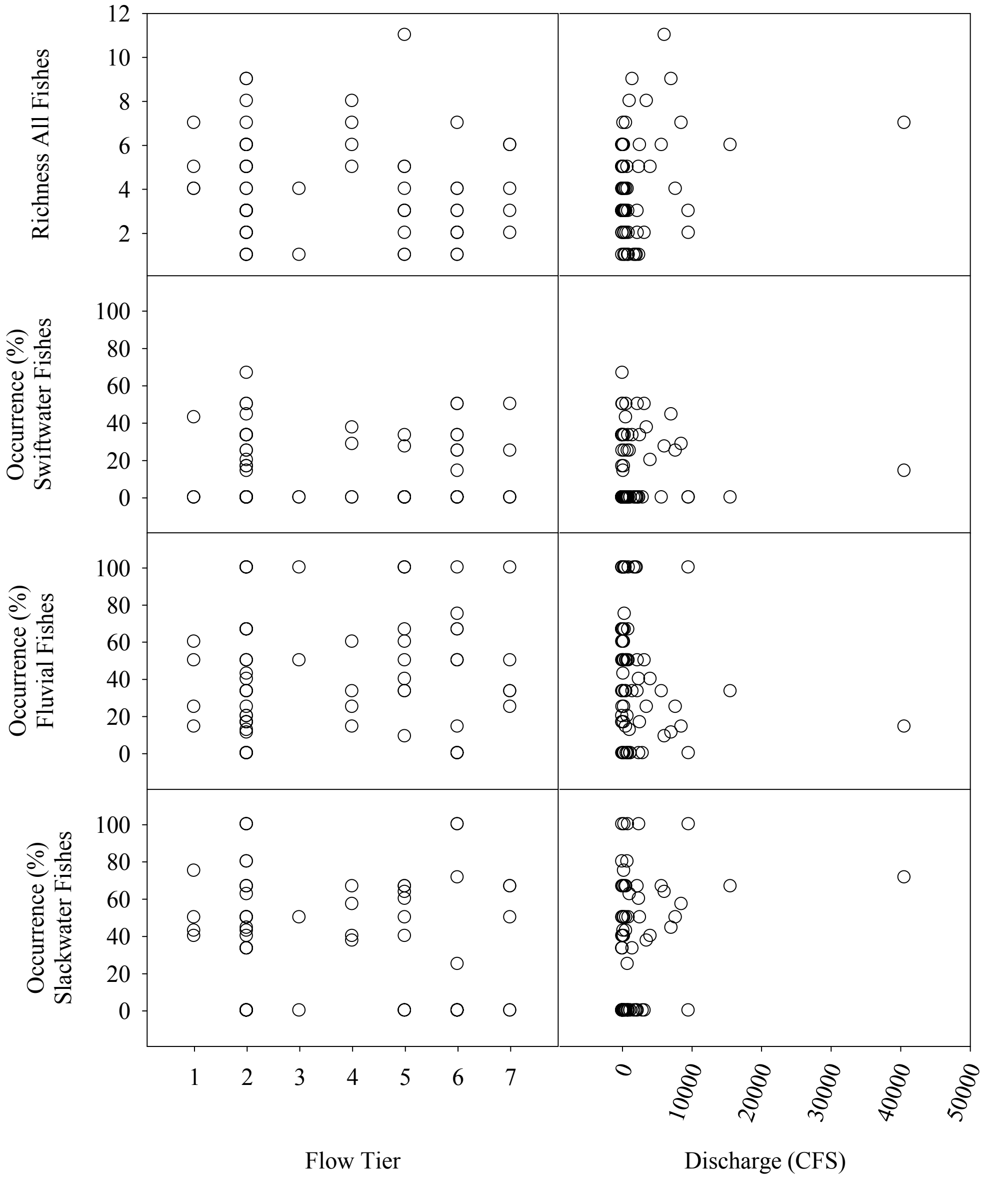
Appendix I: Densities overall and for swiftwater, fluvial and slackwater fishes plotted among flow tiers and discharge (CFS) for run species from August 2014 – May 2015.



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.