TWDB Contract No. 1800012317
Final Report
Received: March 28, 2022

Final Report: Environmental Flow Regime Assessment and Development of a Monitoring Framework

Texas Water Development Board Contract No. 1800012317

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March 17, 2022

Pursuant to Senate Bill 1 as approved by the 86th 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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Glossary

allochory	dispersal of seeds by animals, wind, water or another external agent or force
annuli	annually formed rings visible in a cross section of a fish otolith, mussel shell or tree core that is associated with reduced growth during winter or other stressful periods
asterisci	the smallest otoliths located in the semicircular canals located on each side of the skull
daily growth ring	rings visible in a cross section of a fish otolith that mark the separation of daily growth intervals
FRAT	Flow regime analysis tool that uses disaggregated monthly flows from the WAM to produce daily estimates based on a daily flow from an appropriate reference gage
geomorphology	the study of landform, herein with respect to fluvial systems under the influence of erosion and deposition of sediments under the influence of water flow
HEFR model	Hydrology-based environmental flow regime model used to derive tiered flow components of various frequencies, magnitudes, durations, and timing for evaluations leading to proposal of environmental flow standards
hydrochory	dissemination of seeds by water
IHA model	Indicators of hydrologic alteration model used to derive flow components from records of daily flows
lapilli	otoliths located in the semicircular canals located on each side of the skull
otolith	mineral deposit in the vestibular system of the fish skull that is involved in the sense of balance and linear acceleration and that can be used for age estimation
rate-based approach	analyses aimed at modeling the dynamics of a variable or system indicator
riparian	referring to areas along the margins of rivers and streams
state-based approach	analyses based on tracking variation in the states of variables or indicators of system status over time or space

umbo	the highest part of each valve of the mussel shell, which usually contains the beak, the oldest point of the valve
WAM	Water availability model uses naturalized flows and water management assumptions to simulate flows on a monthly time step

List of Acronyms and Abbreviations

acft	acre feet
AIC	Akaike's information criterion
ANCOVA	analysis of covariance
BBASC	Basin and Bay Area Stakeholder Committee
BBEST	Basin and Bay Expert Science Team
BIC	Bayesian information criterion
cfs	cubic feet per second
ft^3/y	cubic feet per year
FRAT	flow regime analysis tool
GAM	generalized additive model
GSA	Guadalupe and San Antonio
HEFR	hydrology-based environmental flow regimes model
IHA	indices of hydrologic alteration model
LCRA	Lower Colorado River Authority
mm	millimeters
NFP	natural flow paradigm
NOAA	National Oceanographic and Atmospheric Administration
OCR	off-channel reservoir
OTC	oxytetracycline
RFQ	request for qualifications
RI	recruitment index
SB3	Senate Bill 3
TCEQ	Texas Commission on Environmental Quality
TIFP	Texas Instream Flow Program
TPWD	Texas Parks and Wildlife Department
TWDB	Texas Water Development Board
USGS	United States Geological Survey
VIF	variance inflation factor
WAM	water availability model
WMP	water management plan

WMS	water management strategy
WRAP	water rights analysis package

Executive Summary

To enhance understanding of flow-ecology relationships for future validation of environmental flow standards, the TWDB issued RFQ No. 580-18-RFQ0067, *Environmental Flow Regime Assessment and Development of a Monitoring Framework*. Texas A&M AgriLife Research was contracted, and technical collaborators from Texas State University, University of North Texas, Trungale Engineering, and Freese and Nichols, Inc. were subcontracted to assist under TWDB Contract No. 1800012317. The project developed approaches for retrospective analysis of monitoring data as well as assessment of responses of key indicator taxa (riparian trees, fish, mussels) to flow-regime components.

Preliminary analysis of habitat occupancy in relation to flow regime components was performed for fishes in the Brazos River using Senate Bill 3 (SB3)-funded project data provided by the TWDB. Dynamic occupancy modelling revealed little evidence that community-wide persistence and colonization were related to antecedent flow conditions. However, model results were significant for several fish species (e.g., *Cyprinella lutrensis, Gambusia affinis*), and these merit further research. Species colonization and persistence were analyzed, but analyses were compromised by surveys done multiple times within a single year instead of doing surveys once per year across many years. For long-term monitoring, fish sampling should be done during September and October, the period with highest levels of species persistence and detection.

Field surveys and lab studies were conducted to determine ages of tree, fish and mussel species to estimate recruitment and growth in response to flow regime components. Study sites were selected along lower reaches of the Brazos, Colorado and Guadalupe rivers. Six riparian species were chosen for this study, representing fast maturing species (*Acer negundo, Populus deltoides*, *Salix nigra)* and slow maturing species (*Fraxinus pennsylvanica, Platanus occidentalis, Taxodium distichum*). For most species in the three rivers, the probability of germination was positively correlated with spring and summer average flows and smaller flow pulses. Precipitation and flows from late winter to early summer generally had the most influence on growth, and species-specific responses were detected between rivers. This framework showed good potential for assessing environmental flows; however, some of the species did not consistently produce distinct rings for dendrochronological analysis.

Smallmouth Buffalo (*Ictiobus bubalus*) were surveyed at sites in the Brazos, Colorado, and Guadalupe rivers to investigate the relationship between river flows and interannual recruitment success of a fish with a periodic life history strategy (high fecundity, seasonal spawning, episodic recruitment). Otoliths were used to estimate fish age and year of hatching. Population age structure was used to infer effects of flow variation on recruitment. After controlling for mortality using recruitment index (RI) values, interannual variation in recruitment was modeled using multiple components of the flow regime quantified as Indicators of Hydrologic Alteration (IHA) variables based on daily discharge data from USGS gaging stations. Duration of high flow pulses during the spawning season was determined to be significantly associated with successful recruitment in this species.

A rate-based method was developed for assessing effects of flow variation on recruitment of a short-lived fish, Shoal Chub (*Macrhybopsis hyostoma*), surveyed in the Brazos River.

Otoliths were extracted from specimens and used to determine age in days. Relationships between the number of fish hatched and flow regime components on a given date were analyzed with generalized additive models (GAMs). Greater recruitment followed pulses of relatively low magnitude and slow rates of change. Analysis using all flow variables dating back to 1900 revealed strong agreement between Brazos BBEST environmental flow targets and recruitment. Use of the model for hindcasting indicated that Shoal Chub recruitment in the lower Brazos River has declined over the past half-century.

Methods were developed to estimate freshwater mussel age and growth for retrospective analysis of recruitment in relation to flow history. Mussels (*Amblema plicata* and *Lampsilis teres*) were collected from sites in the Colorado, Brazos, and Guadalupe river basins in fall 2019 and summer/fall 2020. Mussel shells were thin sectioned for age determination. Historical daily streamflow data were obtained for nearby gage stations and analyzed with IHA software. Multiple regression revealed four IHA predictors that explained 48% of the variation in *A. plicata* recruitment in the Guadalupe River. A similar amount of variation in *A.plicata* recruitment was explained by the base flow index, rise rate, and date of the 1-day maximum in the Brazos River. Recruitment of *A. plicata* in the Colorado River tended to be lower when flow duration was high. Mussels appear to be sensitive to extreme flow conditions, and negative associations were found between recruitment of *A. plicata* and measures of low as well as high flow. The same analysis could not be conducted for *L. teres* due to a lack of data. Determining the age of mussels is challenging, mussels tend to have patchy spatial distributions, and collecting mussels for aging might contribute to population declines. Thus, it remains uncertain if mussels are a suitable indicator for analysis of flow-ecology relationships.

Past, current, and hypothetical future water management scenarios were simulated for use in projecting recruitment dynamics of indicator species. Hydrological analysis for sites on the Brazos, Colorado and Guadalupe rivers suggested that the potential for future alterations is limited. As a demonstration, the flow-ecology relationship modeled for a riparian tree (*Acer negundo*) was applied at a site in the Brazos River to predict changes in probabilities of germination based on the number of days flows meet or exceed high flow pulse thresholds. The analysis revealed how the number of pulse days per season in simulated flow scenarios influenced tree germination. In the future, flow-recruitment relationships of other riparian tree species, Smallmouth Buffalo, Shoal Chub, mussels, and perhaps other species could be used to project recruitment dynamics under alternative flow scenarios. The approach we developed for simulating flow regime scenarios might be improved with further development of the daily Water Availability Models.

Understanding the mechanisms that link ecological processes with flow variability are best addressed with *rate-based* approaches that generate testable predictions for ecological responses of indicator species to flow alteration. *State-based* approaches are suitable for long-term monitoring of indicators of community or ecosystem status.

Prologue

The passage of Senate Bill 3 (80th Texas Legislature, 2007) amended Section 11.0235 of the Texas Water Code to establish a stakeholder-driven process for identifying and quantifying environmental flow regimes needed *to maintain a sound ecological environment* in Texas rivers and bays. Environmental flow regimes, which were recommended by Basin and Bay Expert Science Teams (BBEST) and Basin and Bay Area Stakeholder Committees (BBASC), served as the basis for environmental flow standards adopted by the Texas Commission on Environmental Quality (TCEQ) between 2011 and 2014 in seven major river basins and five estuarine/bay systems. The SB3 environmental flows process created an adaptive management process (Texas Water Code §11.02362(p)) by which each BBASC could develop a work plan to guide their periodic review and refinement of the environmental flow regime analyses, recommendations, standards, and strategies within their basin-bay area. Implementation of the adaptive management process through funding of priority work plans resulted a series of studies aimed at validating or potentially revising the initial environmental flow standards established by the TCEQ.

In response to the need to explore additional approaches for understanding flow-ecology relationships and research for future validation of environmental flow standards, the TWDB issued RFQ No. 580-18-RFQ0067, *Environmental Flow Regime Assessment and Development of a Monitoring Framework*. The agency contracted Texas A&M AgriLife Research to address the tasks outlined in that RFQ, and technical collaborators from Texas State University, University of North Texas, Trungale Engineering, and Freese and Nichols, Inc. were subcontracted to assist Texas A&M AgriLife Research under TWDB Contract No. 1800012317. An initial task was to obtain and review datasets from previous studies associated with SB3 as well as other relevant investigations. Datasets were accessed, including SB3-funded projects conducted on the Guadalupe, Colorado, and Brazos basins. Most of these datasets lacked sufficient data and/or were collected during time intervals, locations or using methods that rendered them unsuitable for robust statistical analysis of responses to flow variation, which was a second project objective. A preliminary analysis of habitat occupancy in relation to flow regime components was performed for fishes in the Brazos River. A major objective of this project was to develop and demonstrate methods for retrospective analysis of how ecological indicators respond to short-term and long-term variation in the flow regime. Field and lab studies were conducted to survey and determine ages of tree, fish and mussel species for estimation of recruitment levels and their relationships to flow regime components. Another project objective was to evaluate requirements for monitoring programs. Long-term monitoring allows for tracking of indicators of ecosystem status (i.e., levels of soundness) in relation to flow regime changes. An additional objective was to develop an approach for projecting how ecological indicators should respond to future flow scenarios. Approaches, findings, and recommendations for further environmental flows studies were presented during a series of workshops conducted for stakeholders and technicians. The components of this multifaceted project are reported here as separate chapters, each presenting background, methods, results, discussion, and literature cited.

The project component reported in Chapter 2 analyzed fish survey datasets collected during previous SB3 research and provided by the TWDB to assess whether rate-based processes or detection limitations could be identified. The first objective was to test the hypothesis that increases in flow prior to sampling altered fish assemblage composition. The second objective was to determine whether and to what extent imperfect detection (i.e., fish are not collected when they occur at a site) occurred within the existing dataset. The third objective was to determine the most effective season for sampling stream fish assemblages so that samples might be collected when persistence (i.e., continuous occurrence of fish at sampling sites) is highest, colonization (i.e., arrival of new species to a site) is lowest, and detection probability is highest. To address these objectives, a hierarchical dynamic multi-species occupancy model was fit to the existing SB3 dataset provided by the TWDB. Dynamic occupancy modelling revealed little evidence that community-wide persistence and colonization were related to antecedent flow conditions based on the sites and fishes included in the existing dataset. However, modelling revealed evidence that persistence and detection varied by fish species and sampling sites, and the model results provide candidate fish species and locations that might be the focus of future research pertaining to flow-ecology relationships. Furthermore, the model highlighted that fish sampling during September and October is most appropriate because this period had the highest levels of persistence and detection of fishes at sampling sites, and this same timeframe is widely used in flow-ecology research elsewhere. Though repeated states assessments of flow-ecology relationships are useful for uncovering patterns, understanding the mechanisms that link ecological processes with flow variability are best addressed with rate-based approaches that test specific mechanisms. This research tested for two such mechanisms (persistence and colonization) but analyses were to some degree compromised by time steps that were intra-annual instead of the more widely employed annual time step (e.g., fish surveyed once per year across many years). The research is helpful for guiding future study designs to address the need for a long-term dataset for Texas river basins that could ultimately be used to inform environmental flows and water resource management.

With increased regulation of rivers for human use, an improved understanding of the needs of the riparian ecosystem is necessary to develop management practices that sustain these ecosystems. The primary objective of the study component reported in Chapter 3 was to develop a reliable, efficient, and economical method to assess the riparian condition by relating germination and growth to flow histories. Study sites were selected along the lower reaches of the Brazos River, Colorado River and Guadalupe River in Texas. Six target riparian species were chosen for this study, representing fast maturing species (*Acer negundo, Populus deltoides*, *Salix nigra)* and slow maturing species (*Fraxinus pennsylvanica, Platanus occidentalis, Taxodium distichum*). Germination years and growth indices were derived from tree cores collected from across the three rivers. The germination response to seasonal average flows and flow pulses was analyzed using binary logistic regression. Relationships between growth and precipitation and flows across various periods were analyzed using simple linear regression. Results showed that probability of germination was positively correlated with spring and summer average flows, along with smaller flow pulses, for most species along the three rivers. For growth, precipitation had a stronger effect than flows. The period of late winter to early summer for both precipitation and flows was the most influential on growth, though in some instances these same conditions decreased germination and growth on different rivers. The results also suggest that there are detectable differences in species-specific responses across rivers with different flow regimes. The framework presented in this study is the first of its kind to assess the effects of flows on germination and growth using tree rings and has promise for more widespread use on rivers in Texas and elsewhere. Further studies on the tree species examined here, including more extensive sampling, would be beneficial in expanding our understanding of riparian tree growth and germination processes to improve management.

Chapter 4 presents methods, findings and interpretations from a subproject that investigated the relationship between instream flows and Smallmouth Buffalo (*Ictiobus bubalus*) recruitment in rivers of the Gulf Coastal Plain of Texas. Smallmouth Buffalo is a good model species to investigate the relationship between river flows and variable interannual recruitment success of periodic life-history strategist fish species. Smallmouth Buffalo were collected from the Brazos, Colorado, and Guadalupe rivers, and otoliths were extracted from individuals in the field and sectioned and photographed in the lab. Photographs of sectioned otoliths were used to estimate age and thus the year in which the individual was spawned by counting back from the time of capture. Population age structure (i.e., a 'state' or condition at a point in time) was used to infer effects of flow variation on a rates-based process (i.e., recruitment). After controlling for mortality using RI values, interannual variation in recruitment was modeled using multiple components of the flow regime quantified as Indicators of Hydrologic Alteration (IHA) variables based on daily discharge data from USGS gaging stations in each river system. Model selection followed a two-tier approach, first fitting models using only flow attributes associated with the spawning season then adding additional informative parameters from the pre-spawn and post-spawn periods. The primary finding from model selection was that duration of high flow pulses during the spawning season is a critical component of the flow regime associated with successful Smallmouth Buffalo recruitment. These findings have implications for river management and conservation of ecological integrity, in particular populations of species with periodic life-history strategies.

Chapter 5 develops and tests a rate-based method for assessing the effects of flow variation on recruitment of a short-lived fish. Recruitment, defined here as *the increase in populations size as progeny grow and survive*, was measured for Shoal Chub (*Macrhybopsis hyostoma*) from the Brazos River using fish collected from the Farm to Market Road 485 crossing west of Hearne, Texas, and streamflow data from the State Highway 21 crossing near Bryan, Texas. A total of 254 Shoal Chub collected during summers of 2019 and 2020 was preserved in ethanol and returned to the laboratory where lapilli otoliths were removed and used to estimate age in days. Relationships between the number of fish hatched on a calendar day and flow regime components on that same day including discharge magnitude, duration, and timing were explored with generalized additive models (GAMs). Results from GAMs revealed greater recruitment at discharge magnitudes between Brazos Basin and Bay Expert Science Team (BBEST) thresholds for subsistence (i.e., 300 cubic feet per second, cfs) and 3 pulses per summer (i.e., 2,990 cfs), within 10 days (but continuing at a lower level for up to 50 days) after flows descended past the BBEST threshold for 2 pulses per summer (i.e., 5,000 cfs), within 10 days after flows descended past the BBEST threshold for base flow (i.e., 920 cfs), and at slow but negative rates of change that occurred as flows declined following pulses. These results collectively illustrate that Shoal Chub recruitment is linked to a dynamic flow regime characterized by increasing and then decreasing flows through time. When a multivariate GAM including all flow variables was fit to the time series of flows dating back to 1900, the estimated number of total recruits during May, June, July, and August was variable but averaged 280 during 1900-1960, declined sharply during 1961-1975, and then began to level off during 1976-2020. Example summer hydrographs from five recent (i.e., post-1980) years with high estimated recruitment (i.e., 2000, 2001, 2002, 2008, 2014) showed strong agreement between the BBEST's recommended flow targets and observed flows, while five recent years with low estimated recruitment (i.e., 1984, 1996, 2007, 2011, 2013) where characterized by flows regimes that did not attain the BBEST's flow targets or, in the case of 2007, maintained elevated flows throughout the duration of the summer. This study highlights the application of daily growth increments to establish flow-ecology relationships that are useful for managing environmental flows and provides focal hypotheses that should be evaluated during future research. These hypotheses include: (1) Shoal Chub recruitment is greatest when spawning occurs on the descending limb of the hydrograph; (2) the window of time for recruitment becomes increasingly narrow as flows decline towards base flow; and (3) the long-term trajectory of shoal chub recruitment in the lower Brazos River has declined over the past half-century.

Chapter 6 reports methods, findings, and recommendations from a study of freshwater mussel age and growth for retrospective analysis of recruitment in relation to flow history. Mussels (*Amblema plicata* and *Lampsilis teres*) were collected from sites in the Colorado (2 sites), Brazos (3 sites), and Guadalupe (3 sites) river basins in the fall 2019 and summer/fall 2020. Sites were surveyed for mussels using a two-phase sampling design that first includes a qualitative timed search to identify whether the target species is present at a site, followed by a quantitative approach. A total of 446 mussels from 3 rivers (302 *A. plicata*, 144 *L. teres*) were used for analysis. Most of the mussels were collected in the Guadalupe River (199 mussels, all *A. plicata*), 155 mussels collected from the Colorado River (80 *A. plicata*, 75 *L. teres*), and 92 mussels from the Brazos River basin (23 *A. plicata,* 69 *L. teres*). Mussel shells were thin-sectioned, and two observers counted annuli under stereomicroscope. To correlate annual recruitment rates of mussels to antecedent hydrologic conditions, historical daily streamflow data were obtained for nearby gage stations and analyzed with the Indicators of Hydrologic Alteration (IHA) software. Recruitment was calculated as the proportion of collected mussels in the basin with age 0 in a particular year. Multiple regression followed by a stepwise AIC model selection process was used for each river basin to examine which IHA parameters best explained the variation in mussel recruitment. Following model selection, four IHA predictors (1-day and 7-day minimums, Julian date of 1-day minimum, high pulse count) explained 48% of the variation in *A. plicata* recruitment in the Guadalupe River. A similar amount of variation (49%) in *A. plicata* recruitment was explained by the base flow index, rise rate, and the Julian date of the 1-day maximum in the Brazos River. Recruitment of *A. plicata* in the Colorado River was negatively associated with increased high flow duration and increased low pulse counts. Mussels are known to be sensitive to extreme flow conditions, and negative associations we found between recruitment of *A. plicata* and measures of low flow (low pulse counts, Day 1 minimum) as well as high flow (high flow durations, high pulse counts, flow variability rise rate). The same analysis could not be conducted for *L. teres* due to a lack of data. The positive correlation with Julian date of extreme flow events (Julian date of each annual 1-day minimum and maximum) suggests that timing may also play an important role or could be co-incidental. It remains to be determined if mussels are a suitable indicator for rates-based retrospective analysis of flow-ecology relationships. Determining the age of mussels is challenging because annual growth lines can be difficult to define, and this can vary between species. Also, mussels tend to have patchy spatial distributions, and collecting and euthanizing mussels for aging might contribute to further declines for some species. Despite limited sample sizes, this study did find statistically significant relationship between recruitment and several flow regime components, and it is suggested that further research is warranted to determine if mussels are a useful indicator.

Chapter 7 presents an approach with preliminary findings from simulation of alternative flow regime scenarios, which then were used to project recruitment dynamics of an indicator species (Box Elder, a riparian tree) based on flow-ecology relationship derived in Chapter 3. Daily flow time series were developed for conditions representative of past, current, and hypothetical future water management scenarios. The hydrological analysis was applied at one site in each of the Brazos, Colorado and Guadalupe river basins and suggests that while some of the flow regime components identified by the BBESTs have been altered from there more natural conditions, the potential for increased future alterations is limited, both because there is often little water available for appropriation beyond that which has already been appropriated and because the TCEQ standards apply constraints on additional diversions to ensure that diversions for new permits are limited when flows approach the levels identified by the BBESTs. The results of this analysis could be used to inform stakeholder and policy makers as to where and when environmental flows may be of concern and potentially focus future studies to evaluate the significance of lower attainment frequencies of meeting environmental flow standards. The flow-ecology relationship developed for Box Elder were applied at one site (Brazos River at Bryan) to predict potential changes in probabilities of germination based on the number of days flows meet or exceed high flow pulse thresholds. This type of analysis provides a means for validation of specific flow standard components and may suggest refinements for water management strategies. This analysis may also be useful in suggesting strategies to support a sound ecological environment, especially for flow components that may not be specifically incorporated into the adopted standards.

Chapter 8 summarizes some of the main lessons learned from the various project studies and offers recommendations for research and monitoring. The significant findings and recommendations are organized according to three research areas. The first area is research aimed at evaluating flow-ecology relationships for indicator taxa. Suitable indicators are aquatic and riparian species that are directly affected by changes in flow regime components and that can be collected in sufficient numbers to achieve reliable numerical analysis. In Texas, several aquatic species that are highly sensitive to flow variation are currently considered threatened or endangered, and these are not good candidates as environmental flow indicators. Our pilot studies to demonstrate the usefulness of the flow-ecology relationship in fishes focused on species with recruitment that is sensitive to flow variation and are widely distributed and common within appropriate habitats. Our indicator fish species that is a fluvial specialist with a fast life history (i.e., short-lived, recruitment must occur annually to maintain population) was the Shoal Chub (*Macrhybopsis hyostoma*). Our indicator fish species that is flow-dependent with slow life history (i.e., long-lived, recruitment is episodic with high inter-annual variation often resulting in populations with dominant cohorts) was the Smallmouth Buffalo (*Ictiobus bubalus*), and we also summarized findings from recent studies of an even longer-lived, flow-dependent species, the Alligator Gar (*Atractosteus spatula*).

Among other flow components that were analyzed, flow pulse fall rate was found to be particularly influential for recruitment of the Shoal Chub. Additional research on the relationship between fall rate and fish recruitment is necessary to explore the mechanistic relationship between flow variability and fish recruitment. Smallmouth Buffalo recruitment was significantly associated with flow pulses during the spawning period, but the specific mechanisms driving recruitment success are undocumented. Converting discharge data to spawning habitat availability and/or availability and persistence of nursery habitat would be an important refinement of this approach beyond characterizing the flow regime alone.

Mussels are known to be sensitive to extreme flow conditions, both droughts and floods, and we selected two relatively common mussel species as flow-ecology indicators, *Amblema plicata* and *Lampsilis teres*. We found negative associations between *A. plicata* recruitment and various measures of high and low flow tiers. We could not carry out the same analysis for *L. teres* due to a lack of sufficient data. There are several challenges that need to be considered when evaluating mussels as environmental flow indicators. Determining age of mussels can be challenging, because annual growth lines can be difficult to define and often varies between species. Another issue to consider is that many freshwater mussels tend to be patchily distributed and some populations are declining.

The riparian tree species selected as environmental flow indicators included fast maturing species (*Salix nigra, Acer negundo*, and *Populus deltoides*) and slow maturing species (*Platanus occidentalis, Fraxinus pennsylvanica*, and *Taxodium distichum*). Considering the species response and the lab work necessary to identify the rings, the easiest and most reliable species for this type of study were *F. pennsylvanica*, *P. deltoides*, and *P. occidentalis*. The riparian tree analysis identified a strong correlation between number of pulse days per season and likelihood of germination for most of the indicator species and sites. Some of these tree species did not consistently produce distinct rings for dendrochronological analysis. Thus, more work on methods for better identifying tree rings or knowledge of the wood anatomy for these species is needed to obtain more precise results for germination dates, but especially growth trends. Our study also suggests that larger sample sizes are needed for binary logistic regression.

The second broad research area was simulation of flow scenarios and projecting recruitment outcomes. Further research is needed to fill in knowledge gaps and assess whether proactive strategies may be needed to provide flows to maintain a sound ecological environment. The approach we developed for simulating daily flows for alternative flow regime scenarios might be improved with further development of the daily WAMs (Water Availability Models). Draft daily WAM model have been developed for the Brazos, Colorado, and Guadalupe basins. These models were considered for use in the current study; however, they are somewhat out of date and lacking some modifications to water management in the three basins. The riparian tree analysis revealed how the number of pulse days per season in simulated flow scenarios influenced tree germination. In the future, the flow-recruitment relationships of other riparian tree species, Smallmouth Buffalo, Shoal Chub, mussels, and perhaps Alligator Gar and other species could be used to project recruitment dynamics under alternative flow scenarios.

The third research area is long-term monitoring of ecosystem indicators. Systematic long-term monitoring has been identified as a priority for SB3 studies. Long-term monitoring allows tracking of indicators of the status (i.e., degree of *soundness*) of the ecosystem within a river segment. Useful indicators for the environmental flow program must be highly responsive to variation in flow. Indicators can be physical features of the ecosystem, such as aspects of water quality and habitat (e.g., dissolved oxygen concentration, temperature). Other indicators of ecosystem status are aquatic and riparian species, functional groups of species, such as habitat guilds and life history strategies. Our project was strongly focused on biological indicators, with a particular emphasis on taxa that are known to be dependent upon flow pulses of varying magnitude, duration, frequency, and timing. We advocate use of a *state-based approach* for environmental flows monitoring programs in Texas. Standardized methods for monitoring are required to track the state of ecosystem indicators over time, as well as to analyze statistically the responses of key indicator taxa to antecedent flow regimes.

With respect to monitoring programs to detect long-term trends in ecosystem status, a major challenge is establishment of suitable indicator criteria for assessing a sound ecological environment, which is the ultimate goal of environmental flows mandated by SB3. We suggest several criteria for selection of indicators. Though repeated states assessments of flow-ecology relationships are useful for uncovering patterns, understanding the mechanisms that link ecological processes with flow variability are best addressed with a *rate-based approach* that tests specific mechanisms. Rate-based approaches that focus on ecological responses that can be linked (directly or indirectly) to demographic processes provide two notable advantages to abundance- or density-based methodologies. Rate-based approaches provide mechanism-based inference into ecological responses, and second, rate-based approaches generate testable, temporally specific predictions for ecological responses to flow variability and alteration. The choice of approach for generating flow-ecology relationships will influence the type of hydrologic metrics chosen for use as predictor variables. States approaches generally rely on summary metrics that characterize flow conditions over long time series, whereas rate approaches often analyze multiple flow regime components over shorter time intervals.

Chapter 1. Background for Studies Completed for TWDB Contract No. 1800012317

The passage of Senate Bill 3 (80th Texas Legislature, 2007) amended Section 11.0235 of the Texas Water Code to establish a stakeholder-driven process for identifying and quantifying environmental flow regimes needed *to maintain a sound ecological environment* in Texas rivers and bays. Environmental flow regimes, which were recommended by Basin and Bay Expert Science Teams (BBEST) and Basin and Bay Area Stakeholder Committees (BBASC), served as the basis for environmental flow standards adopted by the Texas Commission on Environmental Quality (TCEQ) between 2011 and 2014 in seven major river basins and five estuarine/bay systems. The SB3 environmental flows process created an adaptive management process (Texas Water Code §11.02362(p)) by which each BBASC could develop a work plan to guide their periodic review and refinement of the environmental flow regime analyses, recommendations, standards, and strategies within their basin-bay area.

Implementation of the adaptive management process through funding of priority work plans resulted a series of studies aimed at validating or potentially revising the initial environmental flow standards established by the TCEQ. A recent review led by Texas State University (Hardy et al. 2021) under contract with the Texas Water Development Board (TWDB Contract 1900012284) found that most SB3-funded studies were focused one or more of the following issues: i) filling key data gaps identified by the BBESTs and BBASCs, ii) understanding freshwater inflows required to maintain the health of estuaries and bays, iii) refinement of indicator species responses to flow and/or salinity in rivers, bays and estuaries, and iv) model/tool development. Several studies considered strategies for achieving freshwater inflow standards, and a few studies attempted to validate select components of the adopted environmental flow standards, including studies focused primarily on pulse flows. Although, the instream flow validation studies identified issues related to some pulse flow elements of the adopted standards that might not meet long-term riparian community needs, the studies did not specifically recommend changes to the adopted flow standards. The Hardy et al. (2021) review found no evidence among SB3 studies conducted to date that specifically could be used validate adopted standards for instream flows or freshwater inflows for Texas rivers and bays.

In response to the need to explore additional approaches for understanding flow-ecology relationships and research for future validation of environmental flow standards, the TWDB issued RFQ No. 580-18-RFQ0067, *Environmental Flow Regime Assessment and Development of a Monitoring Framework*. The agency contracted Texas A&M AgriLife Research to address the tasks outlined in that RFQ, and technical collaborators from Texas State University, University of North Texas, Trungale Engineering, and Freese and Nichols, Inc. were subcontracted to assist Texas A&M AgriLife Research under TWDB Contract No. 1800012317. This final project report provides findings from subprojects that evaluated suitable ecological indicators and methods to quantify flow-ecology relationships. The project explored approaches for retrospective analysis of monitoring data (long-term data) for system indicators as well as retrospective analysis designed to assess responses (recruitment) of key indicator taxa (riparian trees, fish, mussels) to flow-regime components to facilitate inferences about cause and effect regarding ecological mechanisms. Approaches were developed to i) analyze existing SB3-related datasets, ii) collect and process new material and data for retrospective analyses with selected indicator species, iii) interpret findings relative to ecology-flow relationships in three Texas rivers (Brazos, Colorado, Guadalupe), and iv) demonstrate methodologies for evaluating environmental flow standards in accordance with the tasks described in TWDB Contract No. 1800012317. The studies emphasized flow-ecology relationships involving the magnitude, frequency, duration and timing of high flow pulses, because these tended to be the components of the environmental flow standards lacking direct evidence based on studies conducted within the river basins. The research projects were focused on instream and riparian flows and did not address flow-ecology relationships of coastal systems. A great deal of prior SB3-related research has focused on coastal ecology. Estuarine and bay ecosystems reveal high levels of natural environmental and biological variation driven by coastal currents, tides, freshwater inflows, wind, precipitation, temperature, sediment/nutrient delivery, and animal migration. Given that strong flow-ecology relationships have been documented for many aspects of fluvial ecosystems (stream and river channels and associated riparian habitats) (Poff et al. 1997, Arthington et al. 2018), the current contract requested studies focused on three rivers in central Texas, the Brazos, Colorado, and Guadalupe.

Task 1 – Compile existing relevant environmental flow study datasets

The first task was to obtain and review datasets from previous studies associated with SB3 as well as other relevant investigations conducted on the three rivers by universities, state agencies, and non-governmental organizations. Datasets deemed suitable for analysis were analyzed to reveal flow-ecology relationships. Findings from this analysis were intended to provide a foundation for recommending ways to improve monitoring protocols that can improve interpretation of flow-ecology relationships and evaluation of environmental flow standards. Multivariate analyses were conducted using existing population (species) and community (local species assemblage) data for fish to evaluate the strength of flow-ecology relationships based on time intervals of variable length. Datasets from most of the reviewed studies contained insufficient data for retrospective analysis of flow-ecology relationships, either because the survey methods were not sufficiently standardized and/or reliable, or because there were insufficient data from the same river reach for statistical analysis. The best available data were for fish surveys, and analysis of that dataset is presented in Chapter 2 of this report.

Task 2 – Propose indicators for use in analyses to reveal flow-ecology relationships and for use in long-term monitoring to track ecosystem status

Long-term monitoring allows for tracking of indicators of ecosystem status (i.e., levels of *soundness*) in relation to flow regime changes. These indicators can include aspects of habitat (e.g., aspects of water quality, bed geomorphology), species that are particularly responsive to flow variation, and functional groups of species (e.g., habitat guilds, life history strategies). Sources of existing data were accessed, including SB3-funded projects conducted on the Guadalupe, Colorado, and Brazos basins, along with data collected for SB2 studies in the Trinity, Brazos and Guadalupe basins. Additional published and unpublished data were evaluated for their suitability for long-term monitoring. Recommendations to refine methods and procedures used for evaluation of environmental flow standards are provided in Chapters 2, 7 and 8.

Task 3 – Develop and demonstrate methods for retrospective analysis of how ecological indicators respond to short-term and long-term variation in the flow regime

Retrospective flow analysis compares biological, physical or chemical indicators to flows that preceded the states of indicators in an ecologically relevant timeframe. Retrospective analysis improves our understanding of flow-ecology relationships and can help refine flow standards by focusing on biotic responses to specific flow regime components. These analyses are consistent with the "rates" approach discussed by Wheeler et al. (2017). In the rates approach, ecological response reflects temporal change. According to Wheeler et al. (2017), "*Because of their explicit or implicit links with demographic processes, rates approaches offer the potential for a more demographically mechanistic understanding of flow-ecology relationships*".

The most suitable indicators for retrospective flow analysis are aquatic and riparian species that are directly affected (either negatively or positively) by changes in flow regime components and that can be collected in sufficient numbers to achieve reliable numerical analysis. The studies reported in Chapters 3, 4, 5, and 6 targeted tree, fish, and mussel species that are known to be fluvial specialists (reproduction and recruitment are strongly dependent on hydrological dynamics of fluvial ecosystems). The objective was to contrast species with fast vs. slow life histories. To achieve population persistence, a species that is a fluvial specialist with a fast life history (i.e., early maturation, short-lived) must reproduce and achieve some amount of recruitment on an annual basis. In contrast, flow-dependent species with a slow life history (i.e., late maturation, long-lived) generally have recruitment that is episodic with high inter-annual variation often resulting in populations comprised of dominant age cohorts. The goal of the studies reported in Chapters 3, 4, 5 and 6 was to collect and age samples of flow-dependent tree, fish, and mussel species for retrospective analysis of recruitment levels in relation to flow variation. Attempts were made to collect robust samples of indicator species to contrast dynamics of fast vs. slow life histories. For short lived indicator species, the age of young individuals can be used to determine the date of hatching or germination and flow conditions before, during and after that date. For long-lived indicator species, age-structure data can be used to assess flow conditions associated with recruitment and development of strong year classes. Fish age and growth rates can be estimated from otoliths. The age distribution and growth rates of freshwater mussels can be estimated by thin sectioning of shells, and the age of trees can be estimated from growth rings in core samples.

Task 4 – Develop guidance for stakeholders on how project findings can be applied to future scenarios in which available water is fully permitted and utilized

Application of environmental flow standards involves a trade-off between leaving water in rivers and bays for the maintenance of a sound ecological environment vs. diversion for other beneficial uses. The project investigated methods that would assist stakeholders to evaluate these trade-offs by predicting ecological outcomes resulting from alternative instream flow regimes produced by different management strategies. Our focus for indicator taxa was recruitment, because without sufficient recruitment over time, populations do not persist in the system. The relative change in ecological conditions from the current flow conditions to a more fully appropriated future can support the development of strategies and identification of priorities both in terms of where and when the adverse impacts are likely to occur.

In Chapter 7, hydrologic time series at locations within the study area were developed for alternative water management scenarios for the selected river basins. Since flow ecology relationships operate on daily rather than monthly time steps, outputs from the WAMs were disaggregated from monthly to daily values using the Flow Regime Analysis Tool (FRAT) developed by the Texas Parks and Wildlife Department. The daily flow time series were used as an input for the flow-ecology relationships derived from Tasks 1-3 to predict ecological outcomes resulting from the alternative water management scenarios. Chapter 7 presents monthly and daily flow time series for select locations in the study area. Summary statistics provide frequencies, durations, timing, and variability of the flow magnitudes included in the flow standards included in the Hydrology-Based Environmental Flow Regime analyses conducted by the SB3 Expert Science Teams. Simulations were performed for riparian tree recruitment dynamics in relation to flow variation based on two alternative flow scenarios for a segment of the Brazos River. These preliminary findings are provided as a demonstration of how flow-ecology relationships can be useful for projecting outcomes from alternative flow regimes, including scenarios simulating impacts from past, present, and future water management and projects.

Task 5 – Evaluate methods for long-term monitoring of indicators of ecosystem status and further analysis of flow-ecology relationships

Each of the chapters in this report provides an assessment of data limitations with recommendations for future research involving field surveys. To be useful for statistical analysis of trends and flow-ecology relationships, such as recruitment in response to flow regimes and regime components, field surveys must be carefully designed in terms of spatial and temporal aspects as well as sampling gear and effort.

Task 6 – Organize and conduct workshops

An online workshop for stakeholders was organized for August 5, 2021, and were recorded. This stakeholder's workshop presented an overview of work completed under the contract, including research strategies, methods, findings, interpretations, and recommendations for future studies and their applications. Four online technical workshops were held on August 23-26, 2021, and recorded. These workshops were primarily for personnel from entities responsible for monitoring aquatic system health and providing input into environmental flows standards development. The workshops presented rationales and technical guidance for procedures developed and demonstrated by the project. These included protocols for survey design, sample collection, estimation of age and growth, hydrologic analysis and simulation, and data analyses that can be used to evaluate environmental flow standards. Draft instructional videos outlining the protocol were created for potential use in posting online guidance for interested parties. These draft instructional videos, workshop video/audio recordings and workshop Powerpoint files are included in appendices of the final project report.

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Chapter 2. Trend Analysis Using Data from Prior Environmental Flow Studies

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Background

Previous Senate Bill 3 (SB3) instream flows research focused on flow pulses and fish densities using a repeated states approach in which multiple snapshots of fish density were used to establish flow-ecology relationships. The pattern emerging from this research was a general change in the density of some, but not all, fish species following flow pulses (Bonner et al. 2017a, 2017b, San Antonio River Authority et al. 2017). However, the short temporal extent of previous studies (i.e., one year) and insufficient natural replication of flow magnitudes meant that testing the influence of specific flow thresholds was not possible. Furthermore, no mechanistic relationship between flow and fish density could be established using such an approach. For example, changes in fish density could be related to fish mortality (i.e., death) or displacement (i.e., emigration) or arrival (i.e., colonization) or recruitment (i.e., birth) of new individuals into the population during high flow events. An additional consideration is imperfect detection, or the phenomenon in which species that are present during a sample are not collected and therefore are erroneously considered absent from the sampling site. These example processes describe rate-based mechanisms that could be further explored using the existing data.

Here we analyze data provided by the Texas Water Development Board (TWDB) and collected during previous SB3 research to assess whether rate-based processes or detection limitations could be identified. The first objective was to test the hypothesis that increases in flow alter fish assemblage composition. This hypothesis is based on the notion that juvenile or small-bodied fishes are displaced during high flow pulses as reported from other river systems (e.g., Harvey 1987, Franssen et al. 2006), especially in close proximity to dams in regulated river systems (Pool and Olden 2015, Shea and Bettoli 2015). The second objective was to determine whether or not and to what extent imperfect detection occurred within the existing dataset. Imperfect detection can have strong implications for inference gained from ecological monitoring (e.g., Comte and Grenouillet 2013) and should be considered when designing long-term monitoring frameworks (Radinger et al. 2019). The third objective of this analysis was to determine the most effective season for sampling stream fish assemblages so that samples are collected when persistence (i.e., continues occurrence of fish at sampling sites) is highest, colonization (i.e., arrive of new species to a site) is lowest, and detection probability is highest. To address these objectives, a hierarchical dynamic multi-species occupancy model was fit to the existing SB3 dataset provided by the TWDB.

Study area

The study area included 15 sampling locations distributed across the Brazos, Colorado, and Guadalupe-San Antonio (GSA) basins (Table 2.1). These sampling locations were sampled as a part of previous Senate Bill 3 Instream Flow Process research and are reported in detail by Bonner et al. (2017a), Bonner et al. (2017b), and the San Antonio River Authority et al. (2017). Sampling locations were spatially distributed near United States Geological Survey (USGS) streamflow gages that were a part of earlier environmental flow recommendations suggested by expert science teams assembled for each basin (Colorado BBEST 2011, GSA BBEST 2011, Brazos BBEST 2012). The 15 sampling sites included six sampling sites in the Brazos Basin, three sampling sites in the Colorado Basin, and six sampling sites in the GSA Basin. Two sites with existing data from the Lavaca Basin were not included in this analysis because they occurred outside of the scope of this project (i.e., this project focused on only the Brazos, Colorado, and Guadalupe-San Antonio). Additionally, a single, unrepeated sample from the Guadalupe River at Cuero, Texas on March 31, 2017, was excluded from analysis.

Methods

Fish data collection. – Fish assemblage data were collected from sampling sites by previous researchers using a combination of barge-mounted or backpack electrofishing and a block seine. At each sampling location and date, sampling included at least one riffle, one pool, one backwater, and one or more shallow runs, depending on habitat availability (Bonner et al. 2017a). The block seine was placed at the downstream extent of the sampling unit and the electrofisher was passed side-to-side in a downstream direction towards the net. All stunned fishes were collected and identified to species. The resulting compiled data spreadsheet of fish abundances from sampling dates and locations was used for the analyses presented here.

Data processing. – All data across multiple visits to sites were considered for inclusion in the analysis. Data were retained for analysis if the sampling site had at least four sampling occasions. This resulted in the removal of the single sample on the Guadalupe River at Cuero, Texas. Fish species were retained for analysis if they occurred in at least 5% of all collections, resulting in 14 species being retained for analysis (Table 2.2). A hierarchical analysis design was used to model replicated geomorphic units sampled across repeated visits to sites. That is, each site was visited multiple times at approximately seasonal intervals, and during each visit multiple geomorphic units were sampled. To create a balanced data design, up to six replicated geomorphic unit samples from each site and date were retained for analysis. If more than 6 replicates were collected, then only the first six were retained. When less than six replicates were collected, “NA” was inserted for any replicates that were missing. For example, if only four replicates were collected, then “NA” values were inserted for fish occurrences for replicates five and six. Site visits were assigned to a monthly time step, corresponding to the month in which sampling occurred. The only exception was that the Easterly sampling site on the Navasota River was sampled twice during March of 2017 (March 3 and March 23), and the second sample was assigned to the month of April (which did not include a visit). Individual sites were not sampled every month, but across all sampling at least one data point was taken during every month starting in July 2016 and continuing through May 2017.

Model fitting. – A hierarchical dynamic multi-species occupancy model was fit to the fish assemblage data. This model matched the model previously used by Wheeler et al. (2018) to assess fish assemblage responses to flow variability and is consistent with models described by Peterson and Shea (2015) and MacKenzie et al. (2017). These hierarchical models include a state process model representing temporal changes in site occupancy (i.e., occurrence of a fish species) and an observational model that accounts for imperfect detection (i.e., fish are not always collected when they are present), and these two models are linked by a term representing the true presence or absence of a species at a site on a specific date. This modelling framework allows for assessing fish colonization (i.e., arriving after being absent during the previous sample), persistence (remaining at a site after being present during the previous sample), and detection probability as functions of specified covariates. Covariates included in the model were a fixed effect for flow magnitude adjusted for drainage area (i.e., magnitude divided by drainage area) from the previous month, a fixed effect for season, and random effects for fish species, sampling site, and monthly intervals between sampling events. Adjusting discharge magnitude by drainage area allowed for direct comparison of flow magnitudes among sampling sites (Nguyen et al. In Press) and allowed for testing the hypothesis that increases in flow magnitude affected fish persistence. The model was fit using a Bayesian framework and the software JAGS (Plummer 2003) accessed through R using the R2jags package (Su and Yajima 2015). The model used diffuse priors and was fit with 15,000 iterations, a burn-in period of 3,000 iterations, a thin rate of 6, and was based on three separate Monte Carlo Markov chains. Model output was interpreted based on estimated posterior distributions and 95% credible intervals for fixed and random effects. Model terms were considered non-zero, statistically significant, and therefore representing evidence of an ecological pattern if the credible intervals did not include zero.

Results

Dynamic occupancy modelling revealed little evidence that community-wide persistence and colonization were dynamic processes. The logit scale parameter estimate for community-wide persistence was 2.17 with 95% credible intervals that overlapped zero (-0.70 – 4.70), and the estimate for community-wide colonization was 0.86 with 95% credible intervals that overlapped zero (-2.16 – 3.75). The community-wide mean estimate for detection was -0.98 and the 95% credible intervals did not overlap zero (-1.99 – -0.06), suggesting average detection of fishes when they were present was less than perfect. The hypothesis that flow magnitude negatively affected persistence was not supported. The logit-scale parameter estimate for the effect of discharge magnitude during the previous month was 0.50 with 95% credible intervals that overlapped zero (-2.07 – 3.24). There was no evidence that the season during which sampling occurred affected detection. When compared to detection during summer, parameter estimates had credible intervals that overlapped zero for fall (estimate = 0.55, credible interval = -0.32 – 1.38), winter (0.36, -0.53 – 1.23), and spring (0.34, -0.52 – 1.18).

The model random effect for fish species revealed evidence that persistence and detection, but not colonization, varied by species. Positive persistence parameter estimates with 95% credible intervals that did not overlap zero occurred for Red Shiner (9.10, 1.60 – 20.41) and western mosquitofish (6.06, 0.15 – 16.68), while all other species had estimates that overlapped zero (Figure 2.1). Colonization parameter estimates included zero for all species included in the analysis, suggesting no evidence species-specific patterns in appearance at sites after not being present during earlier sampling events (Figure 2.1). Species-specific parameter estimates for detection were positive for two species and negative for two species. Positive parameter estimates occurred for Red Shiner (1.41, 0.87 – 1.99) and Blacktail Shiner (1.68, 1.11 – 2.27), suggesting these species were detected to a greater extent than other fishes in the analysis. Negative parameter estimates occurred for Western Mosquitofish (-0.71, -1.28 – -0.13) and Bluegill (-1.37, -2.24 – -0.56), suggesting these species were less likely to be detected given presence compared to other fishes included in the analysis.

The model random effect for site revealed evidence that persistence and detection, but not colonization, varied by sampling site. Estimates for persistence overlapped zero for all sites in the Brazos Basin (Figure 2.2). Two sites in the Colorado Basin had positive persistence estimates that did not overlap zero, including the San Saba River at San Saba, Texas (6.39, 0.16 – 17.55) and the Colorado River at Bend, Texas (6.68, 0.60 – 17.84). This suggests fish persistence at these sites was on-average greater than other sites. Sites with high persistence of species would represent good monitoring sites if routine assessment of the abundance of indicator species is of interest. The Guadalupe River at Gonzales had a negative parameter estimates that did not overlap zero (-5.28, -11.53 – -0.81), suggesting fish persistence at this site was on-average lower than other sites. Parameter estimates for colonization overlapped zero at all sampling sites (Figure 2.3). Parameter estimates for detection were negative for the Lampasas River at Kemper (-0.68, -1.19 – -0.23) and the Little River at Little River (-0.50, -1.01 – -0.03), but positive for the Brazos River at Rosharon (0.99, 0.35 – 1.68) and Hempstead (1.37, 0.63 – 2.20). This pattern suggests lower detection at tributary sites but higher detection at mainstem sites lower in the basin where variation in geomorphic units was less according to Bonner et al. (2017a). Detection was negative at the San Saba River at San Saba (-0.67, -1.15 – -0.22), otherwise detection parameter estimates overlapped zero for all other sampling locations (Figure 2.4).

The model random effect for sampling interval revealed evidence for an effect of interval on persistence but not colonization. The sampling interval between September and October had a parameter estimate of 5.96 and 95% credible intervals that did not include zero (0.14 – 17.32). This suggests study-wide persistence of fishes was more prevalent for this time period. All of the interval-based parameter estimates for colonization included zero (Figure 2.5). Finally, month-specific parameter estimates for detection revealed all months overlapped zero (Figure 2.6), meaning the timing of sampling had no notable influence on detection.

Discussion

Model results revealed no relationship between dynamic processes and discharge magnitude; nonetheless, they did reveal some important considerations for future monitoring. Although discharge magnitude during the month prior to the sampling event did not correlate with community-wide persistence, there was evidence that some fish species persisted to a greater extent compared to others. These fishes included Red Shiner, Western Mosquitofish, and to a lesser extent Bullhead Minnow and Ghost Shiner. Each of these species are small-bodied opportunistic life history strategists (Winemiller and Rose 1992) and represent excellent candidates for indicator taxa for use in studies of the effects of flow on ecological mechanisms. For example, Perkin et al. (2016) showed that opportunistic strategists were the most response group of fishes in the Sabine River system shortly after flows were regulated by impoundment construction. Fishes with less-than-average detection included Bluegill and Western Mosquitofish, suggesting these fishes are not always detected when they are present at a sampling site; whereas, Red Shiner and Blacktail Shiner had greater-than-average detection and therefore were more likely to be collected when present. These differences likely reflect differences in the availability of habitats used by these fishes. Red Shiner and Blacktail Shiner are generally dominant members of fish communities and occur in a variety of habitats, while Bluegill and Western Mosquitofish are generally more abundant in slow moving habitats such as pools or backwaters. If slow moving habitats are rare or temporally variable at sampling sites, then these two species, in particular, may be difficult to detect. For example, the two sampling sites with the highest on-average detection of fishes were both in the lower Brazos River where habitats were simpler (i.e., no riffles present) compared to other sites according to Bonner et al. (2017a). The analysis presented here also suggests that persistence of fishes was greatest during the transition between September and October, and detection was on-average highest (though still encompassed zero) during September. These results collectively suggest standardized sampling during the month of September might be most appropriate for assessing long-term patterns in stream fish communities within the focal study systems. This is the same sampling time (i.e., September and October) used in other studies where annual flow regimes are linked to annual changes in stream fish communities (e.g., Propst and Gido 2004; Kiernan et al. 2012).

The hypothesis that increases in discharge magnitude would influence fish community composition was not supported. This result might have occurred because the time step in the existing database included intra-annual sampling of sites within the same year. Such an approach creates a challenge when separating the effects of seasonality from flow responses. The life cycle of most fishes plays out on an annual timeline (e.g., defined spawning season followed by some recruitment period) and recruitment is likely not evident over such short time scales. Similarly, annual bottlenecks in survival, such as winter mortality, further confound seasonal sampling. For repeated states analysis to be useful for establishing flow-ecology relationships, the time step should be annual rather than seasonal. The strongest responses by fish communities to flow regime variability are typically detected at annual time scales rather than intra-annual time scales (e.g., Kiernan et al. 2012, Stewart-Koster et al. 2014). As an example, Nguyen et al. (In Press) recently used historical data from the upper Brazos, Canadian, and Red river basins of Texas to assess relationships between flow metrics and fish species occurrence. Similarly, the rates-based approach used here was based on the analysis conducted by Wheeler et al. (2018), a study that assessed colonization and persistence at inter-annual rather than intra-annual time steps. As the Instream Flows Process continues and new validation methods are sought, a chief consideration should be the establishment of annual surveys of fish assemblages, perhaps collected during September and October.

Previous research suggested that seasonal sampling was insufficient to establish links between flow variability and fish assemblage structure (San Antonio River Authority et al. 2017). The analysis conducted here supports this conclusion but also presents future direction for establishment of an annual monitoring program. Specifically, sampling during September and October is routinely used in flow-ecology research (e.g., Propst and Gido 2004, Kiernan et al. 2012) and the analysis presented here suggests added benefits of higher persistence and detection during this time period. The analysis presented also identified a number of candidate species for assessing flow-ecology relationships that have been used elsewhere, including Red Shiner (Stewart-Koster et al. 2014) and Blacktail Shiner (Perkin et al. 2016). Though repeated states assessments of flow-ecology relationships are useful for uncovering patterns, understanding the mechanisms that link ecological processes with flow variability are best addressed with rate-based approaches that test specific mechanisms. Wheeler et al. (2018) suggested that rate-based approaches that focus on ecological responses that can be linked (directly or indirectly) to demographic processes provide two notable advantages to abundance- or density-based methodologies. First, rate-based approaches provide mechanism-based inference into ecological responses, and second, rate-based approaches generate testable, temporally specific predictions for ecological responses to flow variability and alteration. Recruitment of new individuals into a population for specific taxa is an emerging methodology and is the focus of other sections of this report. However, if long-term, community wide data are generated in the coming years rate-based analyses like the one applied here could provide useful insight into the validity of flow standards developed for Texas rivers.

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Table 2.1. List of sampling locations including river, basin, and USGS streamflow gage included in analysis. A single sample on the Guadalupe River at Cuero Texas on March 31, 2017, was excluded from analysis.

Basin	River	Site	Dates sampled	USGS gage ID
Brazos	Brazos River	Hempstead	10/7/2016, 12/29/2016, 2/12/20174, 3/14/2017, 5/9/2017	08111500
Brazos	Brazos River	Rosharon	11/1/2016, 12/29/2016, 2/12/2017, 3/23/2017, 5/9/2017	08116650
Brazos	Leon River	Gatesville	9/19/2016, 12/28/2016, 2/3/2017, 3/13/2017, 5/8/2017	08100500
Brazos	Little River	Little River Academy	9/21/2016, 10/24/2016, 11/30/2016, 12/28/2016, 2/3/2017, 3/13/2017, 3/23/2017, 5/8/2017	08104500
Brazos	Navasota River	Easterly	9/11/2016, 10/7/2016, 12/29/2016, 2/3/2017, 3/3/2017, 3/23/2017, 5/9/2017	08110500
Brazos	San Saba River	San Saba	9/19/2016, 11/30/2016, 12/28/2016, 1/30/2017, 3/13/2017, 4/14/2017	08146000
Colorado	Colorado River	Bend	9/19/2016, 10/16/2016, 11/30/2016, 12/28/2016, 3/13/2017, 4/14/2017	08147000
Colorado	Lampasas River	Kempner	9/19/2016, 11/30/2016, 1/30/2017, 3/13/2017, 4/14/2017, 5/8/2017	08103800
Colorado	Onion Creek	Driftwood	12/28/2016, 3/6/2017, 4/7/2017, 4/20/2017	08158700
Guadalupe-San Antonio	Cibolo Creek	Cestohowa	11/17/2016, 12/30/2016, 3/31/2017	08186000
Guadalupe-San Antonio	Guadalupe River	Comfort	8/8/2016, 10/24/2016, 4/14/2017	08167000
Guadalupe-San Antonio	Guadalupe	Gonzales	11/17/2016, 3/31/2017, 4/20/2017, 5/16/2017	08173900
Guadalupe-San Antonio	Medina River	Bandera	7/20/2016, 10/24/2016, 4/14/2017	08178880
Guadalupe-San Antonio	San Antonio River	Goliad	3/31/2017, 4/20/2017, 5/16/2017	08188500
Guadalupe-San Antonio	San Marcos River	Luling	11/17/2016, 12/30/2016, 1/27/2017, 4/20/2017	08172000

Table 2.2. Fish species retained for analysis and the total number (%) of collections in which they occurred.

Common name	Species	Collections (%)	River basins
Red Shiner	*Cyprinella lutrensis*	214 (53%)	Brazos, Colorado, GSA
Blacktail Shiner	*Cyprinella venusta*	225 (55%)	Brazos, Colorado, GSA
Ghost Shiner	*Notropis buchanani*	25 (6%)	Brazos, GSA
Silverband Shiner	*Notropis shumardi*	24 (6%)	Brazos
Mimic Shiner	*Notropis volucellus*	49 (12%)	Brazos, Colorado, GSA
Bullhead Minnow	*Pimephales vigilax*	133 (33%)	Brazos, Colorado, GSA
Channel Catfish	*Ictalurus punctatus*	61 (15%)	Brazos, Colorado, GSA
Flathead Catfish	*Pylodictis olivaris*	25 (6%)	Brazos, Colorado, GSA
Western Mosquitofish	*Gambusia affinis*	77 (19%)	Brazos, Colorado, GSA
Bluegill	*Lepomis macrochirus*	21 (5%)	Brazos, Colorado, GSA
Longear Sunfish	*Lepomis megalotis*	69 (17%)	Brazos, Colorado, GSA
Orangethroat Darter	*Etheostoma spectabile*	45 (11%)	Brazos, Colorado, GSA
Texas Logperch	*Percina carbonaria*	30 (7%)	Brazos, Colorado, GSA
Dusky Darter	*Percian sciera*	21 (5%)	Brazos, Colorado

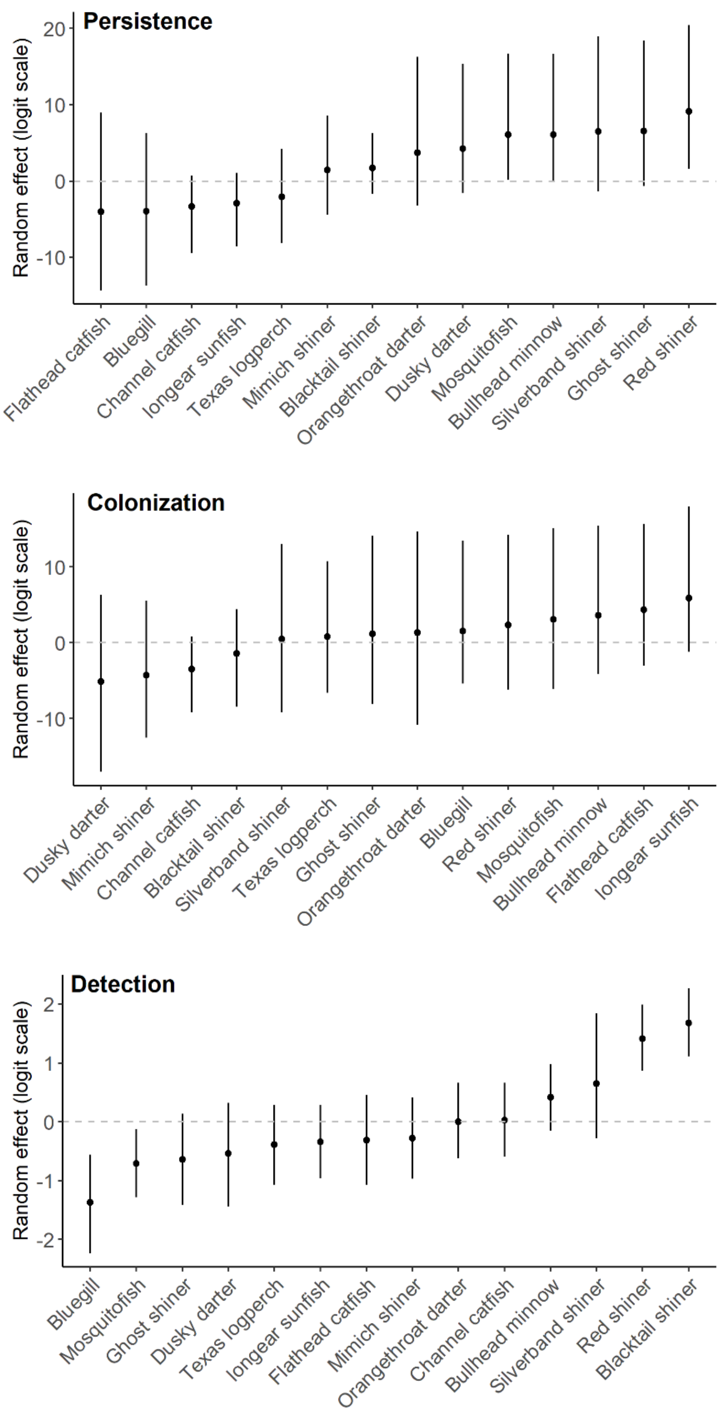

Figure 2.1. Parameter estimates for the random effect of fish species on persistence, colonization, and detection from a dynamic multi-species occupancy model. Values are on the logit scale, points represent mean variance by species level, and error bars represent 95% credible intervals. The horizontal dashed line represents zero. Parameters estimates with 95% credible intervals that overlap zero are interpreted as unrelated to occupancy.

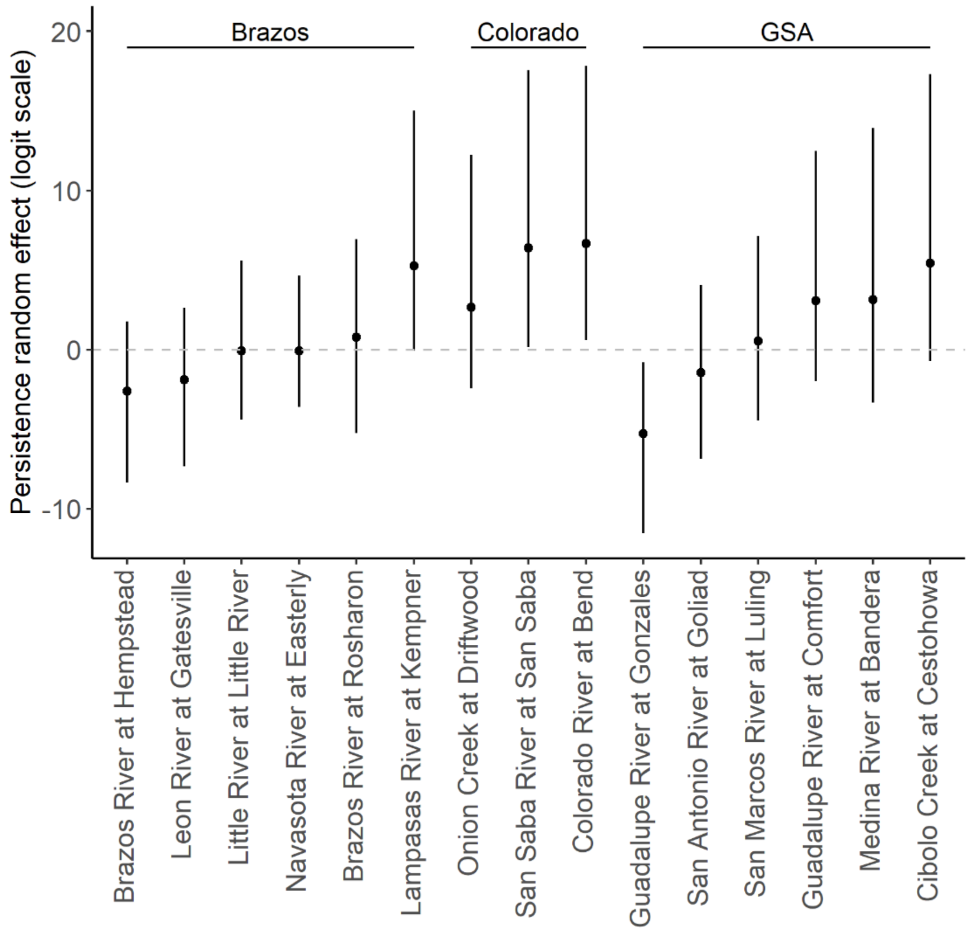

Figure 2.2. Parameter estimates for the random effect of site location on persistence from a dynamic multi-species occupancy model. Values are on the logit scale, points represent mean variance by sampling site level, and error bars represent 95% credible intervals. The horizontal dashed line represents zero. Parameters estimates with 95% credible intervals that overlap zero are interpreted as unrelated to persistence. Sites are grouped by the drainage basin in which they occur, including the Brazos, Colorado, and Guadalupe-San Antonio (GSA) river basins.

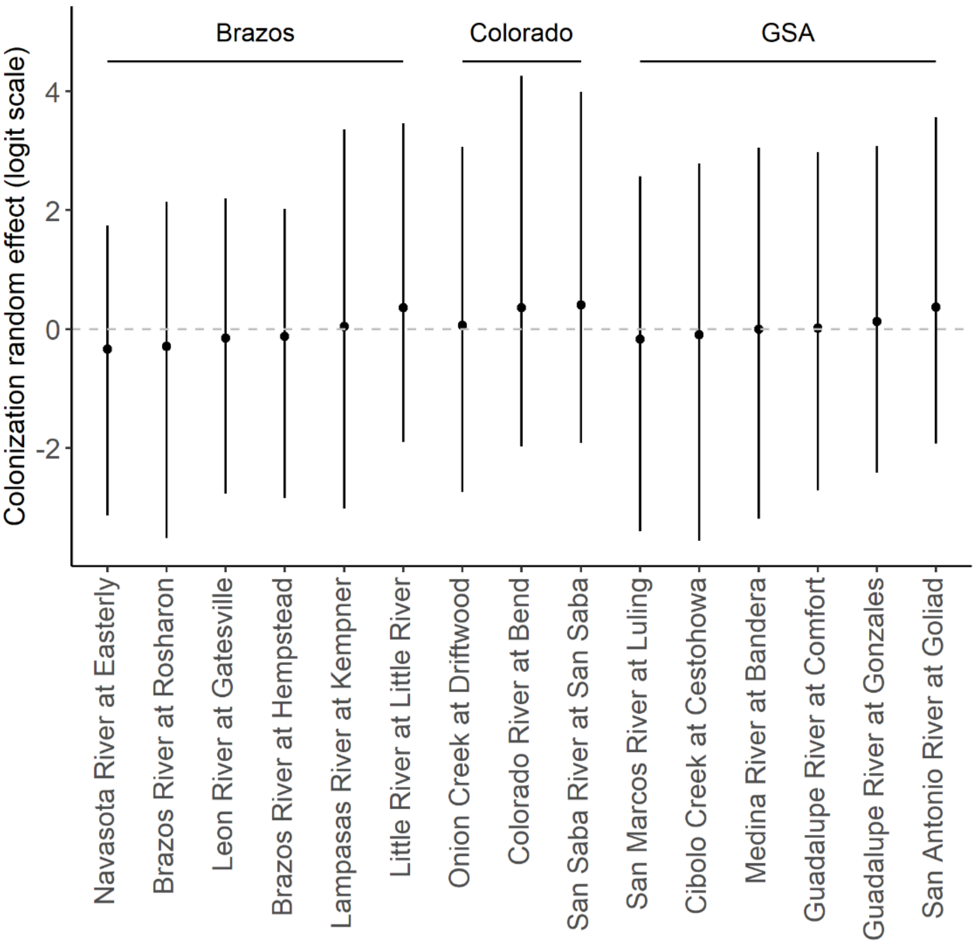

Figure 2.3. Parameter estimates for the random effect of site location on colonization from a dynamic multi-species occupancy model. Values are on the logit scale, points represent mean variance by sampling site level, and error bars represent 95% credible intervals. The horizontal dashed line represents zero. Parameters estimates with 95% credible intervals that overlap zero are interpreted as unrelated to colonization. Sites are grouped by the drainage basin in which they occur, including the Brazos, Colorado, and Guadalupe-San Antonio (GSA) river basins.

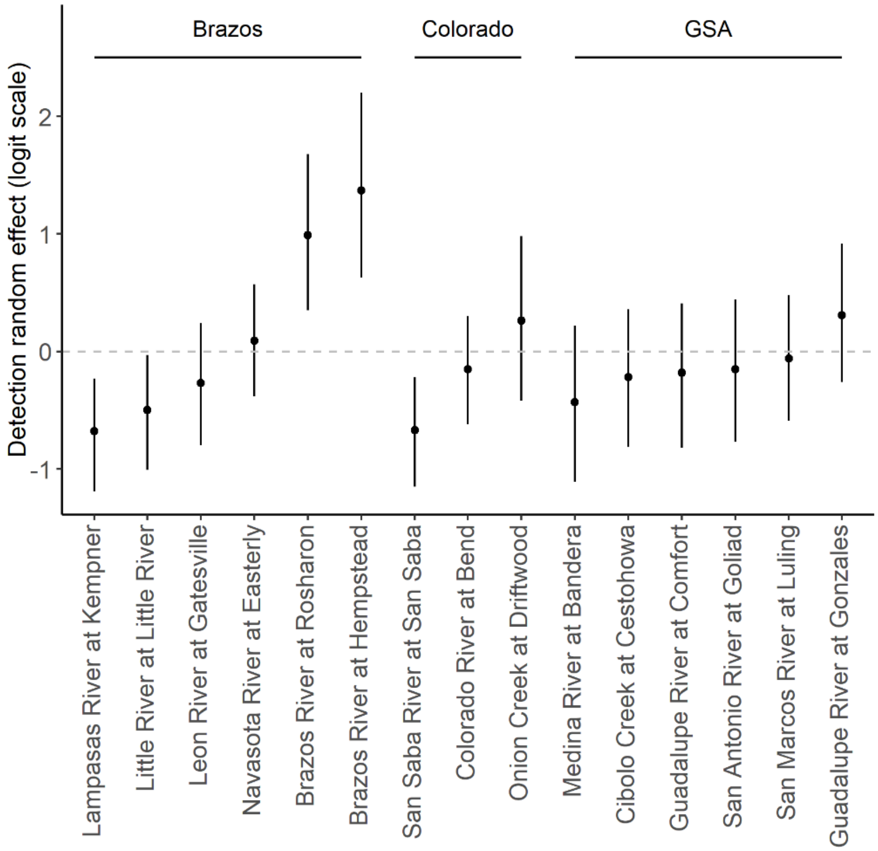

Figure 2.4. Parameter estimates for the random effect of site location on detection from a dynamic multi-species occupancy model. Values are on the logit scale, points represent mean variance by sampling site level, and error bars represent 95% credible intervals. The horizontal dashed line represents zero. Parameters estimates with 95% credible intervals that overlap zero are interpreted as unrelated to detection. Sites are grouped by the drainage basin in which they occur, including the Brazos, Colorado, and Guadalupe-San Antonio (GSA) river basins.

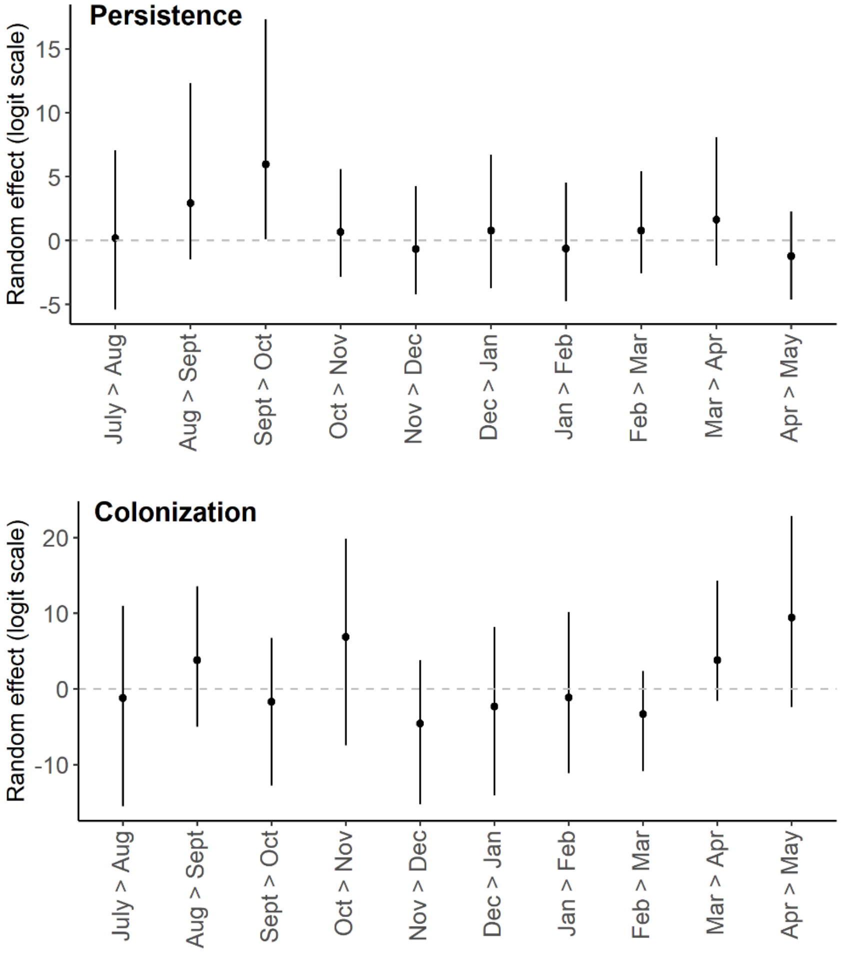

Figure 2.5. Parameter estimates for the random effect of monthly intervals between samples on persistence and colonization from a dynamic multi-species occupancy model. Values are on the logit scale, points represent mean variance by sampling interval, and error bars represent 95% credible intervals. The horizontal dashed line represents zero. Parameters estimates with 95% credible intervals that overlap zero are interpreted as unrelated to persistence or colonization. Intervals are shown as monthly time steps beginning when the first samples were collected (July 2016) and continuing until the final samples were collected (May 2017).

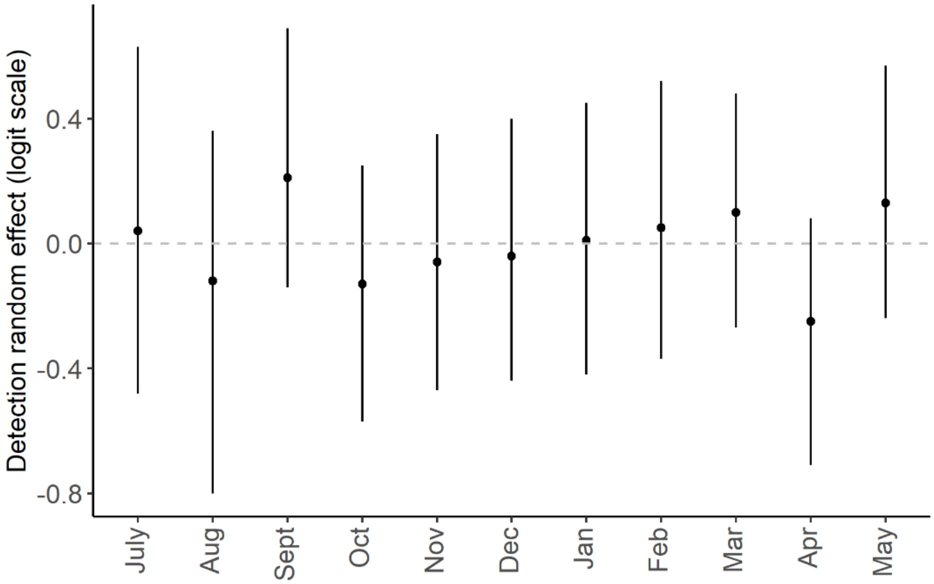

Figure 2.6. Parameter estimates for the random effect of sampling month on fish species detection from a dynamic multi-species occupancy model. Values are on the logit scale, points represent mean variance by month, and error bars represent 95% credible intervals. The horizontal dashed line represents zero. Parameters estimates with 95% credible intervals that overlap zero are interpreted as unrelated to detection. Time is shown as sampling month beginning when the first samples were collected (July 2016) and continuing until the final samples were collected (May 2017).

Chapter 3. Response of Indicator Taxa to Flow Variation – Riparian Trees[1]

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Background

Riparian ecosystems are transitional ecosystems between terrestrial and aquatic systems and are key components that maintain hydrologic function, promote biodiversity, and regulate geomorphic processes in the landscape (Gregory et al. 1991, Naiman et al. 1993). As riparian forest land has declined on average 36.6% since the 1970s, Texas has experienced some of the largest growth and losses, in its High Plains ecoregion and Southern Texas Plains respectively (Jones et al. 2010). As further losses of riparian ecosystems occurs, a myriad of consequences will continue, including degradation of water quality, increased levels of erosion, loss of flood regulations, pollution filtration, disruption of nutrient cycling, and loss of wildlife habitat (Sweeney et al. 2004).

Tree Establishment. – One of the most significant impacts on river systems is regulation from damming and municipal water usage and its impact on riparian tree establishment. The geomorphic processes of channel narrowing, meandering, and flood deposition are key determinants in the spatial and temporal distribution of establishment of riparian trees (Scott et al. 1996, Scott et al. 1997). These processes influence turnover of riparian bank conditions vital to seedling germination. Studies have documented that new dams initially cause an increase in areas able to be colonized by riparian species, though this increase is subsequently lost as vegetative succession progresses due to the lack of river meandering (Friedman et al. 1998, Dixon and Turner 2006). Areas occupied by riparian vegetation may or may not change post-dam; however, a decrease in area occupied by newly established riparian trees is likely (Benjankar et al. 2012).

Often regulations require seasonal pulses to maintain biota; however, timing of these events may not coordinate with crucial riparian seed dispersal and germination times. Burns and Honkala (1990) detail that “softwood” hardwoods, such as willows (*Salix spp.*) and cottonwoods (*Populus spp.*), utilize allochory and hydrochory as means for dispersal. These seeds remain viable for less than two weeks if not in a moist area, and require open, often freshly deposited sediment for germination. Other common riparian species, such as Box Elder (*Acer negundo*), possess longer dispersal times with seeds that have both longer viability as well as tolerance for variable initial conditions. Notable characteristics of these trees are their early age of maturation, often between 5 and 10 years of age, and short lifespans, averaging around 70 years. The American Sycamore (*Platanus occidentalis*), like the willows and cottonwoods, relies upon allochory and hydrochory to deliver seeds to fresh alluvial sites; however, *P. occidentalis* produces seeds for 50 to 250 years after finally reaching maturity around 25 years of age. Green Ash (*Fraxinus pennsylvanica*), like Sycamore, normally seeds at a later age, but also possess more persistent wind spread seeds that can be dormant for years in the seedbank waiting for moist, partially shaded conditions. Bald Cypress (*Taxodium distichum)* is one of the longest-lived riparian species averaging a 600-year lifespan, but capable of exceeding 1200 years. Their seed strategy relies on hydrochory and a 1-to-3-month period of exposure to wetness before being able to germinate. *T. distichum* seeds can lay dormant in the seedbank for many years until conditions for germination become favorable. In summation, both *F. pennsylvanica* and *T. distichum* share utilization of hydrochory and allochory, trends towards short period of seed viability, and needs moist conditions for germination, but differentiate on lifespans, age of maturation and mature age capacity for drier environments.

While mature trees have the capacity to withstand a variety of hydrologic conditions, most riparian species have seeds with short viability periods that require specific conditions to germinate. An example of this is found in willows, which once established, have the capacity to survive in a wide range of soil types and moisture conditions. To regenerate, though, germination is heavily dependent on alluvial deposits (Mcleod and Mcpherson 1973). Some riparian trees (primarily *Salicaceae spp.*) have demonstrated a synchronization with hydrology in which they drop or disperse their seeds within a few week(s) following the peak spring flows, thus maximizing either the window of transport or the availability of fresh alluvial surfaces. The timing of seed dispersal often correlates strongly with changes in temperature and precipitation rather than streamflow as an initiating factor (Stella et al. 2006, Sedlacek et al. 2015).

When these factors are desynchronized from natural hydrological regimes, the normal suite of co-occurring conditions are dissociated, thus preventing successful germination. An example of this is sensitivity to wet conditions, where willow and cottonwood seedling mortality corresponds with both the rate of drawdown as well as displaying species specific responses under experimental drawdowns (Stella et al. 2010). This illustrates the relevance of not only timing, duration, and intensity of discharge in pulses, but the stage-discharge relationship across the riverscape to ensure both germination and seedling success. Studies on Bald Cypress found that a long spanning

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[1] Chapter 3 presents material from – Trimble, A. 2021. Hydrologic drivers of riparian tree germination and growth along three Texas rivers. MSc Thesis, Texas A&M University, College Station, Texas.

seed window is linked closely to hydrologic regimes as a means of maximizing the range of areas (Schneider and Sharitz 1988). The trees relied upon the normally variable conditions, which covered some of the lowest and highest flows, to reach varying locations within the riparian area. When considering the denaturalization of river's flood events, the same process that promote germination and dispersal can become inhibitory processes preventing natural reforestation when out of sync (Doulatyari et al. 2014). A study on the Verde River found that minor changes in flood timing had no significant impact on changes in riparian tree germination. These findings support the capacity for a successful interaction between human needs in regulating waterways and the maintenance of riparian function and processes.

Tree Growth. – Obligate riparian tree species are likely more vulnerable to hydrologic denaturalization than facultative or upland species that occur in riparian zones. For example, a case study comparing black cottonwood (*Populus trichopta*), an obligate riparian tree, to Jeffrey pine (*Pinus jeffreyi*), a facultative riparian tree, found cottonwood growth was more closely correlated to the stream flows on the year samples were taken, whereas pine growth was more correlated to the prior years flow (Stromberg and Patten 1990). In that same study, prior year stream flows significantly correlated with the following year's growth for more upland trees, whereas current year stream flows more strongly correlated with wetland tree growth. Lagged growth could be an indicator that residual soil moisture is important for non-obligate trees, whereas immediate effects on growth from high flows during a particular year may indicate flood tolerance adaptations. Interestingly, Stromberg and Patten (1990) concluded that distance from stream or elevation on the bank had a significant effect on current year tree growth, with higher relevance for precipitation (rather than stream flow) farther from the stream. In another case of reduced flows from damming, riparian species, such as cottonwoods, may experience a collective reduced growth or sacrifice branches and roots for periods when water needs are not met from the reduced flows recharging stream levels and/or groundwater (Schook et al. 2016a).

While total discharge is identified as a crucial factor affecting riparian tree growth, the depth and accessibility to the water table is also an important determinant. The withholding and regulated release of water can cause fluctuations in the water table as the availability of water for groundwater recharge is altered (Tockner et al. 2000, Bejarano et al. 2018). In a study on phreatophyte riparian species, or those that develop deep root systems to access its water supply, obligate species show greater sensitivity to water table depth change as opposed to absolute water table depth than do facultative species (Shafroth et al. 2000). The other noted impact of highly variable water table fluctuation is the development of shallow mean root depths as compared to low variability water table fluctuation which promotes deeper root depth on average (Tron et al. 2014). While still dependent on plant rooting behavior and soil water retention, this response likely occurs as a mechanism to avoid anaerobic conditions presented by the prolonged inundation of roots. Riparian growth and development are also influenced by the elevation of a watershed. Variability in riparian trees grown within higher elevation watersheds is explained mostly by river discharge as deep roots may not have as consistent access to the water tables, whereas in lower elevation watersheds where proximity to river flow gives more consistent connection between deep roots and the closer water table and instead precipitation through surface interactions begins to contribute to growth as much as flows (Schook et al. 2016b). When considering the cascading effects of riparian presence, it is important to note that riparian establishment is not a one-way path of hydrologic and fluvial geomorphic impacts, but the vegetation itself can impact those processes in return (Doulatyari et al. 2014). The reduction of riparian area in one location facilitates altered flows downstream that perpetuate the loss of riparian areas.

Dendrochronology. – In understanding past influences on tree growth and tracking establishment, many researchers use dendrochronological approaches to assess the influences of natural regimes and disturbances. Dendrochronology, or tree ring analysis, utilizes tree rings and the variation in their widths to development indices for evaluating drivers of change in the environment (Stokes and Smiley 1968, Speer 2010). At the site level, tree ring chronologies measure the magnitude and frequency of reactions to environmental change. By developing chronologies for stands, a site's environmental history can be viewed, and annual patterns deduced. In a study in the Apalachicola River, Florida, hydrologic conditions often account for more of the variation found in tree growth than climatic conditions for riparian trees (Smith et al. 2013). It is important to consider that the many forms of disturbance have varying effects of the expression within the tree ring (Stoffel and Corona 2014).

Prior Flow Analyses. – Texas is a critical place to evaluate the effects of flows on riparian tree germination and growth because of interest in finding minimum flow requirements to maintain riparian health on Texas Rivers with ever-increasing human demands for water. In the 2018 Texas Instream Flow Program (TIFP) report, flows were assessed along the middle and lower reaches of the Brazos for inundation of riparian area. The recommended flow regimes in TIFP were to provide beneficiary flood frequency and duration for seeding and germinations periods determined from prior studies along the Brazos. This prior study does not fully address the necessary site conditions for seed germination, as it bases flood pulse levels on meeting the requirements for successful germination but not the development of newly exposed area for seeds to establish. The Bonner et al. (2017) report examined flow rates and species composition in riparian habitat the Brazos River, denoting dissimilarities between tree species composition in streamside, slope, and upper bank riparian zones that could be associated with differences in flow inundation patterns. They found that Texas Commission on Environmental Quality (TCEQ) flow standards were inconsistent in meeting the inundation needs of the riparian zone. However, they also noted that the riparian community did display an ecological response to the pulses and overbank flows, but not base and subsistence flows under the TIFP flow categorization. They attributed this discrepancy to potential shifts in the stream channels geomorphology and biotic community as responses of life stage changes in the riparian vegetation (Bonner et al. 2017).

In a study done on the San Antonio and Brazos rivers, riparian tree responses were analyzed to assess flows that were conducive to unsuppressed growth (Duke 2011). The study used tree cores and assessed ring widths to correlate with flows that were either so high or so low that growth was suppressed. The ideal annual total flows were found to be site dependent with the lowest minimum of 28.25 ft^3/y and the highest maximum of 70.6 ft^3/y on the San Antonio River, whereas the Brazos River had a lowest minimum of 63.6 ft^3/y and a highest maximum of 430.8 ft^3/y. That study highlights not only the expected species level differences in flow requirements, but also site level differences regarding flows that promote optimal growth. Other findings in that study concluded that along these rivers the regulated flows appear to facilitate the invasion of more upland or deeper-rooted plants that can tolerate reduced flows.

The riparian framework of the Colorado-Lavaca Bay and Basin Expert Science Team (BBEST) Environmental Flow Regimes Report (Colorado River Authority 2011) noted that there was a distinct lack of site-level information for riparian response to instream flows for the river systems in question. To make an assessment, they used vegetation community maps with modeled flow and stage levels under different return interval flood events to determine which size events best maintain the riparian community. Due to the lack of more in-depth riparian-flow analyses on this river, that study did not address whether the flows were resulting in diminishing riparian area. Rather, the BBEST 2011 report only assessed whether the current trajectory was sustainable, be it changing or stable. Similarly, in the 2015 San Antonio River Authority (San Antonio River Authority 2015) study on the Guadalupe River, riparian area persistence and recruitment was assessed by seedling, sapling, and mature tree counts of indicator species and evaluated whether the inundation needs were met or not. Their findings were that TCEQ flows were insufficient to reach 80-100% of the mature trees at their sampling sites. They also assessed the response of the trees by taking cores to develop general growth factors.

The current study further investigates the long-term impacts that can result from regulation of rivers and potential future shifts in riparian community age or species composition. Currently, few studies have examined the phenomena of delayed local extirpation of riparian species caused by the desynchronization of the riparian community and river hydrology (Vesipa et al. 2017). The aforementioned studies correlated growth to river flows and quantified recruitment by size class counts, but they neglected to assess recruitment under the same flows used to analyze growth. Also, these studies while showing relations of growth to flows, fall short in determining the magnitudes and the changes in flows that would affect riparian growth. This study examines these influences on riparian tree communities for segments of three Texas rivers (Brazos, Colorado, and Guadalupe).

Objectives. – The primary objective of this study was to develop a low-expense but effective method for the Texas Water Development Board to assess riparian conditions in relation to flow regimes. Specifically, this study relates riparian tree germination and growth to flood histories at locations in three Texas rivers. Our target species in this study represent common obligate wetland tree species that can reflect the overall condition the riparian habitat. Based on prior research, we tested the hypothesis that probability of germination would increase with floods of low size and decrease with floods of high tier, while being most beneficial in the Spring and Summer seasons. Our second hypothesis was that growth will be positively correlated with increases in floods and flows in the spring and summer seasons.

Methods

Study Area. – The study was conducted at six sites along the lower reaches of the Brazos, Colorado and Guadalupe Rivers in Texas (Figure 3.1). Sites were selected from active point bars within the vicinity of predetermined United States Geologic Survey (USGS) gaging stations. We selected point bars that display active deposition representative of fluvial succession, and active vegetative succession, where there is visible evidence of progressive growth and recruitment of tree growth. Permission from site landowners was granted prior to access properties from the riverside.

For the Brazos River sites, the Hearne Site (Figure 3.2) also is located on private property along the Brazos River in Caldwell, TX 44.5 km downstream of the USGS gage station 08098290. From the gage station to the sampling site, it is joined by Pond Creek, Little River, Threemile Creek, and Sixmile Creek. The site had a slightly steep but sloping bank to the water's edge. Once at the vegetated edge, the topography is primarily flat with recurring troughs. Willow Baccharis (*Baccharis salicina*) dominated much of the understory and clearings. The Bryan Site (Figure 3.3) also was located on private property along the Brazos River in Bryan, TX 6.1 km downstream from USGS gage station 08098450 near the confluence of the Little Brazos River. The site is relatively flat with slight undulations in topography away from the river. We observed 1-2ft tall *Salix nigra* and *P. deltoides* seedling/saplings commonly occurred throughout the forest floor and many clearings.

For the Colorado River Sites, Colorado-Bastrop Site (Figure 3.4) is located along the Colorado River in Bastrop, TX 1.14 km upstream of USGS gage station 08159200. The site is open for public access on Bastrop City property in Fisherman's Park. Colorado-La Grange Site (Figure 3.5) is located along the Colorado River near La Grange, TX 0.7 km upstream USGS gage station 08160400. This site used was bordered by pastureland, above and at the meander apex was deemed unviable due to absence of target species, so we sampled trees from the lower portion of the meander instead.

For the two adjacent Guadalupe Sites, Seguin upstream (Figure 3.6) and Seguin downstream (Figure 3.7), are located off FM 1117 bridge near Seguin, TX, where upstream site is 0.47 km upstream and downstream site 0.85 km downstream from USGS gage station 08169792. Both sites represent meanders with sandy soil almost entirely dominated by *P. occidentalis.* Beyond the sample boundary, both the sites are bordered by agricultural land, with evidence that cattle do come through the upstream site. This is noted as the presence of grazing cattle may have removed or prevented establishment of some target species, prior to sampling.

Tree Sample Data Collection. – The target riparian species in this study include Black Willow (*Salix nigra*), Box Elder (*Acer negundo*), Green Ash (*Fraxinus pennsylvanica*), Eastern Cottonwood (*Populus deltoides*), American Sycamore (*Platanus occidentalis*), and Southern Bald Cypress (*Taxodium distichum*). The species were primarily selected for their representation of fast maturing species (*S. nigra, A. negundo*, and *P. deltoides*) and slow maturing species (*P. occidentalis, F. pennsylvanica* and *T. distichum*). They also represent common species and indicators of quality of riparian habitat that cover a range of seedling dispersal strategies and germination requirements. The study area falls in the Atlantic Gulf Coast plain region of Texas where the indicator status of *S. nigra* and *T. distichum* are wetland obligate species, and *F. pennsylvanica, P. occidentalis, A. negundo* and *P. deltoides* are considered facultative wetland species.

For sites that exhibited progressive point bars, transects 50 m long with 10 m intervals were used. Transect endpoints were GPS assigned prior to site visits and then located on site with a GPS unit. At each 10-m interval, a 10-m transect was placed on alternating sides from the starting point. All viable trees within a 5-m width band were sampled. At the beginning and end of each 10-m transect, a laser range finder (mention the make and model) was used to measure elevation above the river.

Along rivers that did not form progressive point bars, sampling locations were at outer bends or meanders that exhibited staggered cohorts of trees along the bank. Generally, these cohorts appear to have been established in a narrow band of new deposits following a previous flow event. Due to the limited reach of the stand in these conditions at these sites all viable trees were sampled within 100 m x 30 m plot that ran adjacent to the river. At every 10 m within each vegetation band, a laser range finder was used to measure the elevation from the river.

All target species with a diameter at breast height (DBH) greater than 5 cm were cored at breast height (1.3 m or lower, pending trunk suitability) on the upstream side perpendicular to the river using a 5.15 mm increment borer (Haglöf, Sweden). Trees with a DBH less than 5 cm were cut at 50 cm height and cookies were extracted for age determination. If a target species exhibited multi-stemmed growth, the largest stem was used for sampling. Trees were cored to the pith or to the maximum depth of the borer. A minimum of 30 trees per transect were cored.

Cores were stored in paper straws and dried at 60^0 C until constant weight was recorded. Once dried, cores were mounted to a wooden mount with glue and sanded using progressively finer sandpaper ranging from 60 to 400 grit (Speer 2010). Cores that showed low or no ring visibility were removed. Annual growth ring increments were recorded by placing cores on a sliding-stage microscope and using MeasureJ2X software to measure tree ring widths to the nearest 0.001 mm. Incremental growth rings for each year were then cross-dated first using skeleton plots and verified using measured chronologies with the COFECHA software (Holmes 1983), after which problem cores were removed. For the cores that reached the pith a germination date was noted for use in germination trend analysis. For cores that did not reach the pith were not included in germination analysis data but were kept for use with the other cores in the growth analysis. The measurements of the increment cores were then combined by species for each site as well as combined into total for each river. These grouped chronologies were then standardized to master chronologies using the Regional Curve standardization using a 20-year spline in the dplyr R package (R Core Team 2021).

Hydrologic Data. – Available discharge and gage height data from the USGS gage stations was collected for the nearest stations for the time periods corresponding to the oldest trees sampled until the last dated tree ring (Table 3.1). If a gage station had periods of missing records, the nearest gage station where the hydrology was most similar was used to supplement the missing data. The TCEQ adopted flow rule and site level evaluations from Hydrology-Based Environmental Flow Regimes (HEFR) models that were used to determine threshold riverine conditions for average flow volumes and high flow pulse volumes and frequencies unique to each gage station (Table 3.1). These include levels that were within the 75th percentile of base flow, tier one pulses that occurred at twice per season return intervals, tier two pulses that are suggested to occur once per season return intervals, tier three pulses that are suggested to occur at once per year intervals, tier four pulses that are suggested to occur at once per two-year intervals, and tier five pulses that are suggested to occur at once per five-year intervals. From the data, seasonal average flows and tier pulses (number of days) were compiled for use as explanatory variables. NOAA precipitation data were acquired for each river as an additional explanatory variable for the statistical models.

Data Analysis. – A scatter plot and regression model of annual precipitation against annual average flow was conducted as a proxy to assess the accountancy of precipitation driving the rivers flows. This metric will also make note of rivers' flows being influenced by inputs of groundwater.

Tree germination was analyzed as the response variable in statistical models using univariate binary logistic regression with the logit function.

$$\text{Logit} = \beta_0 + \beta_1 X = \text{log odds (LO)} \quad (1)$$

where β_0, β_1, and X, are the intercept, logit defined coefficient of the independent variable, and the independent variable, respectively. The produced log odds (LO) were then converted to probabilities with the following function.

$$\frac{\exp(\mathrm{LO})}{1+\exp(\mathrm{LO})} * 100 = \text{Probability (\%)} \tag{2}$$

Probability curves were made using seasonal average flows (cfs) and high flow pulses (count) as independent variables in the model. Given the low sample size in this study and stochastic behavior for all possible germination events that occurred on these rivers, a threshold of p-value greater than 0.2 was used to distinguish notable trends on curves. However, we retain a standard of $p < 0.05$ for reporting significant trends.

The effects of hydroclimate on tree growth were analyzed using univariate linear regression. The regressions were conducted with river wide and species-specific annual ring-width indexes against monthly precipitation and mean monthly flow. To estimate the multi-month influence of season or cross seasonal periods in the growing season, we averaged monthly climate data over progressively longer periods from January to September.

Results

A total of 230 trees was assessed to determine germination dates with notable differences in species make-up unique to each river (Table 3.2). The most prevalent species was *A. negundo*, making up 34% of all trees sampled with its occurrences primarily restricted to the Brazos (42%) and Colorado Rivers (63%) and with lower presence on the Guadalupe River (3%). On the Brazos, *A. negundo* and *S. nigra* were the dominant species making up 42% and 39% of the samples collected, respectively. On the Colorado River, the remaining 37% comprised roughly equal proportions of *F. pennsylvanica*, *P. occidentalis*, and *S. nigra*. While *P. occidentalis* made up 100% and 95% of the total samples collected on the Guadalupe River and Seguin Site A, respectively, their presence at other sites was minimal. *F. pennsylvanica* and *P. deltoides* were found on both the Colorado and Brazos Rivers, though their numbers were on average 3 and 5 per site on average, respectively. The least encountered species, *T. distichum,* was encountered once within sampled areas, making up only 0.004% of all samples.

Although high correlations between annual precipitation (mm) and flows (m^3s^{-1}) were expected, the Colorado River despite having increased regulation through dams maintain a high correlation of annual precipitation to flows. Annual flow was most closely related to precipitation on the Guadalupe ($R^2 = 0.76$), whereas the Colorado and Brazos Rivers had R^2 values of 0.70 and 0.62 respectively (Figure 3.8).

On the Brazos River, spring and summer average flows were the highest during most years relative to the other seasons, with winter average flows occasionally reaching the similar levels (Figure 3.9). The highest germination counts on the Brazos River occurred in 2003 and 2008 which both comprised 12% of the germination. These periods did not overlap with high flow years, though 2008 did follow a year with the highest summer flows. *S. nigra* (SANI) experienced its highest germination in 2008, with 15% of its germination occurring in this year. *Acer negundo* (ACNE), *F. pennsylvanica* (FRPE), and *P. deltoides* (PODE) germinations were distributed over a wide range of flows, though only *F. pennsylvanica* had an occurrence during a high flow year.

From the logistic regression, germination events on the Brazos River were generally associated with increased spring and summer flows and exhibited negative to no correlation in fall and winter flows, respectively (Figure 3.10). As average spring flows increased, *A. negundo* (p= 0.08) and *P. deltoides* (p= 0.1) probability of germination increased from 5% to 93% and 14% to 90%, respectively. For increasing summer average flows, the overall species (p= 0.2), *A. negundo* (p= 0.04) and *F. pennsylvanica* (p= 0.2) germination probabilities increased from 38% to 100%, 4% to 99% and 9% to 85%, respectively. Fall and winter flows were not associated with germination probability; however, *P. deltoides* did exhibit a positive relationship to fall flows that did not achieve significance due to low observations.

For the relationships between germination and high flow pulses (HFPs) on the Brazos River, spring and summer HFPs that commonly accumulated much longer periods of flooding also exhibited mostly positive correlations with germination probabilities (Figure 3.11). *P. deltoides* displayed a significant positive response to spring 75th percentile pulses (p= 0.04) with probabilities increasing from 3% to 72% as flow durations ranged up to 92 days. *A. negundo* also showed a significant positive response to summer 75th percentile pulses (p= 0.04), summer tier one pulses (p= 0.04), and summer tier two pulses (p= 0.04) with probabilities increasing from 3% to 73%. Since the tier two pulses were the same duration as the smaller HFPs, it is difficult to assess ideal flood size for *A. negundo*, i.e., its germination may have responded solely to the tier two magnitude events that occurred in a single year.

On the Colorado River, summer flows generally were the highest amongst the season with winter occasionally surpassing them (Figure 2.12). Years with flows higher than 9,994 cfs occurred at both sites during the summers of 1987, 2002, and 2007, winter of 1992, and spring and summer 1997. While none of these years themselves had the highest germination, they were often succeeded by periods of high germination. The largest counts of germinations occurred in 1991, 1994 and 1996, each having 8% of total germination those years, the latter two of which occurred with 5 years of the largest flow recorded being the winter of 1992. *A. negundo* (ACNE), being most common, made up the largest germination events at both sites, often appearing soon after years of high spring or summer flows. The only instance of *P. occidentalis* (PLOC) germination occurred in 1979 which itself did not have nor was it preceded by any high or low flows. The remaining species were widely distributed yet tended to occur in years within a short period of high flow seasons.

For the germination responses to seasonal average flows on the Colorado River, there were few correlations (Figure 3.13). The probability of *A. negundo* germination increased with flows for all seasons, but this trend was only marginally significant for summer flows (p= 0.1). Due to *A. negundo* making up most of the sampled species, the overall germination reflects a similar relationship despite not having a notable correlation. No relations were found for other species likely due to low numbers of germination observations.

On the Colorado River, we found a general trend that flood pulses of varying sizes had a positive effect on germination (Figure 3.14). In contrast to the Brazos River where spring and summer events were important predictors of germination, we found that fall events appear to be critical for germination on the Colorado River. Germination of *P. deltoides* responded positively to fall events above the 75% percentile (p= 0.009) as well as fall tier one pulses (p= 0.08), increasing probability from 2% to 40% and 4% to 57%, respectively for flow durations up to 90 days. Despite the rarity of tier three and tier four events, *A. negundo* appears to germinate in response to those larger events lasting as much as 18 days in the summer (p= 0.07 and 0.08, respectively) with probabilities increasing from 28% to 79% and 28% to 86%, respectively, compared with smaller high flow pulses.

On the Guadalupe River summer flows were regularly the highest among seasons (Figure 3.15). The year 2007 had the highest flows in the record with summer flows reaching 5,014 cfs. During this year 10% of germination occurred with 3 counts of *P. occidentalis* and 1 of *S. nigra*. However, 2011 to 2014 was a drier than average period, yet contained 42% of the germination with 2013 containing 21% of total germination occurred, all of which was comprised by *P. occidentalis*. The only instance of *S. nigra* germination was in 2007 and the only instance of *A. negundo* was in 2011.

For the logistic regressions of flows to germination, most models suggest a declining probability of germination with increasing flows, but none of those trends were statistically significant (Figure 3.16). Note that the germination record on the Guadalupe only extends back to 2004, so limited data were available to test germination trends on this river. *P. occidentalis* was dominant species along this river and displayed no notable relationship to the seasonal flows. Relationships of *A. negundo* and *S. nigra* are of limited use due to their minimal number of observations.

HFPs on the Guadalupe River, often showed minimal influence on germination probabilities (Figure 3.17). However, two instances of winter tier one (p= 0.2) and tier two (p= 0.2) pulses lasting 71 and 29 days, respectively, had a weak negative effect on *P. occidentalis* germination, decreasing probabilities from 88% to 21% and 90% to 4%, respectively. Overall, target species did demonstrate changes as HFPS either increased in size or increased in duration but did not pass the threshold.

In the ring width development, annual growth was correlated with hydroclimate, but those effects differed from site-to-site, even within the same river (Figure 3.18). Trees at both the Brazos River sites reached peak growth in 1996 and exhibited a similar upward trend of growth from 2006 to 2009; however, the upstream Hearne site experienced a spike in growth from 2000 to 2003 unlike the Bryan site. As for the Colorado River, trees at both sites were similar in the minimal variation in ring width, despite earlier peaks in their chronologies. On the Guadalupe River, ring widths increased sharply in 2006 before dropping in 2007, which was an extreme wet year, and returned to a slow increase from 2008 to 2012, which encompasses several low flow years.

On the Brazos River, trees grew significantly more during years when spring to early summer precipitation was greater (Table 3.3). The multiple species growth was strongly correlated to April precipitation (R^2= 0.19, p= 0.03) where a 10 mm increase in precipitation resulted in a 0.035 increase in growth. For *A. negundo*, growth was most strongly and highly correlated to July precipitation (R^2= 0.3, p= 0.008) where a 10 mm increase resulted in a 0.04 increase in growth. May precipitation (R^2= 0.28, p= 0.03) was highly correlated to *F. pennsylvanica* growth, increasing growth by 0.05 per 10 mm increase in precipitation. The March to June period (R^2= 0.28, p= 0.03) and May-June period precipitation (R^2= 0.28, p= 0.03) were also highly correlated growth, but did not produce as strong of an increase in growth, only increasing growth by 0.03 and 0.04 per 10 mm increase in precipitation, respectively. Growth for *P. deltoides* was strongly correlated with April precipitation (R^2= 0.3, p= 0.008) with a 10 mm increase in precipitation increase growth by 0.04. *S. nigra* did not produce any significant regressions.

The flow results for the Brazos River resulted in significant regressions ranging from late winter to early summer (Table 3.4). There were no significant regressions for the multiple species assessment, *A. negundo*, *P. deltoides*, and *S. nigra*. While many combinations of months during the early growing season until mid-summer were related to growth of *F. pennsylvanica*, the months of February to May were most highly correlated with growth (R^2= 0.37, p= 0.01). During this period, growth is predicted to increase about 0.01 per 100 cfs increase in flows. Over the average flows for this period, growth was predicted to be 1.43, 32% higher than the average growth overall.

For the Colorado River, the precipitation regressions resulted in notable species differences and significant periods extending throughout the year (Table 3.5). For the multiple-species regressions, the growth had a low negative correlation with the May to June period precipitation (R^2= 0.073, p= 0.04) decreasing growth by 0.011 per 10 mm increase in precipitation. For *A. negundo*, *P. occidentalis, P. deltoides*, and *S. nigra* no significant correlations were found. For *F. pennsylvanica*, growth was found to have a very significant but low correlation to May precipitation (R^2= 0.13 p= 0.005), where a 10 mm increase in precipitation decreases growth by 0.03.

For the flow regression on the Colorado River, tree growth was most negatively impacted by increasing flows in the late winter and early-spring months with periods that span the entire year (Table 3.6). For the multiple species analyses, *A. negundo*, *F.*

pennsylvanica, *P. occidentalis*, and *P. deltoides* no significant regression was found. For *S. nigra*, growth was very significantly and strongly correlate with the March to April period (R^2= 0.25, p= 0.005) with a 100 cfs in average flows reducing growth by 0.011. Additional periods with significant correlations all started in late-winter to early-spring and spanned to progressive periods throughout the year.

For the Guadalupe River, growth responses varied even amongst tree species, but were most often affected by late-springs to early-winter (Table 3.7). There were no significant regressions from the all-species analyses. For *A. negundo*, growth was most highly correlated with March precipitation (R^2= 0.50, p= 0.03) which decreases growth by 0.01 per 10 mm increase in precipitation. For *P. occidentalis*, growth was highly correlated to July precipitation (R^2= 0.39, p= 0.01) with a 10 mm increase in precipitation increasing growth by 0.022. For *S. nigra*, was highly correlated to the March to May period (R^2= 0.39 p = 0.03) with growth increasing by 0.013 per 10 mm increase in precipitation.

For the flow interaction, there were no significant correlations found within the examined time period for any species on the Guadalupe River (data and results in Appendix 3D).

Discussion

Tree Germination. – This study found that across three Texas rivers, increased flows generally benefitted riparian tree germination. Higher flows during the spring and summer months were the most critical for riparian trees to germinate on these rivers (Figures 3.11, 3.14, 3.17). Although our results support the hypotheses that riparian-adapted tree species are more likely to germinate under higher flow conditions, it is possible that many germination events occurred under sub-optimal flow conditions, but those trees did not survive to maturity. Given each river has its own unique flow regime and bank characteristics, we found it difficult to generalize across the three rivers, though the high flow pulses for BBEST tiers 1 and 2 were generally the strongest correlations for recruitment. Although further study is needed to decipher more species-specific interactions, there was substantial evidence that higher flows in a given year and season were associated with higher probabilities of germination.

For trees along the Brazos River, average flows and lower-tier high flow pulses were the most common conditions found to increase germination (Figures 3.9, 3.10, 3.11). The fast-maturing species, *A. negundo* and *P. deltoides,* and slow maturing *F. pennsylvanica* were the most responsive, all of which would naturally rely on high flows to wet areas of the riverbank. These species' germination responded well to conditions that represented flows just above average. A study by Auble et al. (1997) that also included *Fraxinus spp.*, *Populus spp.* and *Salix spp.*, examined germination in a more urban setting and found that optimal germination at discharge rates around 530 cfs with these rates being more in the mid-ranges of experienced flows. Interestingly, despite large sample sizes in our study, we found little effect of flows on *S. nigra* germination, even though its life history is very similar to *P. deltoides* in terms of reproductive strategy and physiology (Burns and Honkala 1990). This may allude to additional factors influencing germination, such as light availability or interspecific competition. A noteworthy consideration comes from field observations as both sites near to where *S. nigra* was collected exhibited extensive steep and eroded banks giving the river a very channelized appearance. It is possible the tiered topography of the Brazos River restricts flows from reaching the vegetated bank. Additionally, this bank topography common to the Brazos River likely prevents crucial sediment from being deposited in areas accessible for long term colonization by riparian tree species.

Along the Colorado River, flows and pulses of spring and summer were found to positively impact probability of germination (Figures 3.12, 3.13, 3.14); however, responses were weaker compared to the Brazos River. Of the fast-maturing species, *A. negundo* had highest probability of germination at higher spring and summer seasonal average flows and low flood pulses since it readily germinate in shaded or sunny areas with higher moisture levels. Relatively high spring and summer flow levels are likely associated with good growing conditions, whereas prior winter precipitation and high winter flows may either move seeds into more favorable growing areas or prepare alluvial soils for spring germination. Surprisingly, *P. deltoides* germination was also associated with the lowest fall flood pulse tiers, which is contrary to its known germination process. This may have been an artifact of the trees sampled. When looking at slower maturing species on the Colorado River, *F. pennsylvanica* germination years showed only weak negative associations with most increased flows, likely pointing to a lower level of flood dependency or even a negative impact of high flows. Greater sampling of this species may be needed to detect more significant trends.

For the Guadalupe River sites, few relationships between flows and germination were detected in our study (Figures 3.16, 3.17). It is important to note that of the sites sampled, the Guadalupe site had overall lower flows and smaller and less frequent high flow pulses. Literature on the most common species sampled on the Guadalupe River, *P. occidentalis*, notes that while moist conditions on alluvial soil are necessary for *P. occidentalis* germination, high light availability is also crucial for seedling survival. There is potential that the rarely observed winter high flow pulse events on the Guadalupe River near the study site scour the riverbank, but timing and magnitude of the flood disturbance may have been insufficient to impact survival. From field observations, sites further down river, while not suitable for sampling, did show greater diversity of target species, suggesting that effects of these flows may have been more beneficial under a different bank topography.

Tree Growth. – Surprisingly, we did not find any generalizable trends for growth responses to precipitation or flows across all three rivers. In most cases late winter to early summer precipitation, and to a lesser extent winter to late spring flows, positively impacted growth (Tables 3.4, 3.6). We found precipitation was a stronger predictor for growth than flow (Tables 3.3, 3.5, 3.7), however, even these relationships were fairly weak. It is possible that riparian tree growth is influenced by regulated flow conditions on these rivers in ways that we could not capture in our study. Despite, the regressions showing the most regulated river having a high correlation of precipitation to flows its likely there is an interaction not captured by simple regression and it is this interaction that causes irregularities in growth. Other possibilities could be the presence of lagged effects of previous conditions, such as wet prior years, or that some response might be non-linear. A study by Keeland et al. (1997) that examined riparian trees in similar environments in different regions found that species response to flow regimes can vary among locations. Our study also found that, for individual species, growth in response to flow varied among locations.

For the fast-maturing species, late winter precipitation through spring and even to early summer were the best predictors of growth. *Acer negundo* displayed mixed results in its response to precipitation. On the Brazos River, *A. negundo* responded positively to July precipitation, but responded negatively to March precipitation on the Guadalupe River. This difference may be explained by the single odd years driving trends in the negative direction, given the low sample size for the Guadalupe River. Interestingly, *A. negundo*, showed no response to precipitation or flows on the Colorado River, to any period of precipitation. This suggest that along the Colorado River *A. negundo* and other species may be rooting to the water table and thereby buffering themselves from variation in precipitation or instances where channelization restricts flows. Notably, *A. negundo* has shown rapid mortality from inundation lasting over 85 days and likely would shows signs of stress for inundation periods that would approach this threshold (Friedman and Auble 1999). The lack of correlation might be explained by growth being negatively impacted by both high and low flows. *Populus deltoides* only showed a single response to April precipitation on the Brazos River, which suggests that it is most reliant upon mid-spring precipitation for growth. *Salix nigra* also displayed mixed results. On the Colorado River, precipitation during March to April negatively affected growth, whereas on the Guadalupe River, March to May precipitation had a positive effect on growth. The discrepancy between the two likely stems from differences in sample size, and further analysis with more samples and replication is needed for the Colorado River.

For the slow maturing species, the timing of precipitation in the early to mid-growing season (February to May) appeared to influence annual growth. *Fraxinus pennsylvanica*, had a positive response to May precipitation where on the Brazos River where its growth also responded well to flows from February to May. *Platanus occidentalis* responded well only to increased precipitation that occurred in July, the drier and hotter period of the year, and only showed a significant response on the Guadalupe River. That response was likely due to the large sample size on the Guadalupe River. Also, some survey sites had relatively flat topography that likely was associated a relatively high water table accessible to riparian trees. Sample sizes for *Platanus occidentalis* on other rivers were insufficient to evaluate responses.

Study Implications. – This study evaluated a rates-based approach to estimate the influence of flow variation on tree germination and growth. We found that the use of binary logistic regression allowed detection of the probability of germination across a range of flow conditions. However, some of the target species did not consistently produce ideal rings for dendrochronological analysis. More work on methods for identifying tree rings for these species is needed in order to get reliable estimates for germination dates and growth trends. Sample size was found to be a critical for binary logistic regression. For future research, we recommend at least 30 viable samples of a target species per site to develop better species-specific chronologies and germination data.

Among the species investigated, those most feasible and reliable for this type of study were *F. pennsylvanica*, *P. deltoides*, and *P. occidentalis*. Though samples from only one location were collected and ultimately could not be used, the literature supports the use of *T. distichum* for dendrochronological analyses because of its excellent ring production and known tendency to show strong correlations to hydroclimate. Smith et al. (2013) and Young et al. (1995) found that *T. distichum* annual growth correlates primarily with flows and precipitation experienced in the same year. In general, this species shows high correlations between growth and local hydroclimate as well as correlations between germination and hydrologic regime.

Acer negundo and *S. nigra* posed challenges for age and growth estimation. Although *A. negundo* had clear associations between germination and flows, it produces barely visible rings, whereas *S. nigra* germination was relatively unresponsive to flows and often had rot that rendered cores illegible or incomplete. *A. negundo* and *S. nigra* were very abundant at most locations, which suggests flows were sufficient to meet their needs for germination and growth. Despite its uncommon occurrence, *P. deltoides* is the best candidate for study of a fast-maturing species. Its cores yield highly legible rings. Further, *P. deltoides'* life history and indicator status is a combination of characteristics found in both *S. nigra* and *A. negundo*. Among slow-maturing species, *F. pennsylvanica* or *P. occidentalis* are suitable indicators, with the former found in greater abundance on the Brazos and Colorado rivers, the latter more frequently encountered on the Guadalupe River. Both *F. pennsylvanica* and *P. occidentalis* showed good growth rings and responses to flow.

Conclusions. – From this study the conventional knowledge that riparian trees benefit from increased precipitation and flows is partially supported. We found that ultimately, increased flows and low flood pulses in the spring and summer season best benefit germination, but that precipitation often was a stronger driver than flows for growth. Use of binary logistic regression for germination analysis shows promise as means to acquire detailed information on past hydrologic conditions and their effects. However, it is important to note that this method relies upon a large sample size of target species as well as having an extensive record of environmental conditions to predict germination following future events. Larger samples could facilitate use of ordinal logistic regression to achieve better estimates. Larger samples also would allow use of multiple regression to evaluate legacy effects of flows in prior years as well as other factors, such as hydropower pulsing.

Supplemental Analysis of Riparian Tree Germination and Survival on the Brazos River at Bryan using BBEST Standards

We evaluated flow metrics in a systematic way starting with annual flow averages and flood pulses that spanned all four seasons. The purpose of this supplementary analysis was to test whether the specific flow standards developed by Senate Bill 3 and the BBEST would meet requirements for riparian tree germination and subsequent survival. This analysis was done during the last months of the project, and therefore was conducted for only one site –the Brazos River at Bryan. The same methods as described above were applied.

General conclusions did not change substantially from what was reported from the prior analysis, but there were some notable findings (Figures 3.19 and 3.20). Results indicate that probability of germination was positively correlated with spring flow pulses in *A. negundo*, *P. deltoides*, and *S. nigra*, as well as summer flows in *S. nigra*. These findings were generally supportive of the current TCEQ standards for pulse flows in terms of the number of flow events, their magnitudes, and durations. We found a weak association between the number of winter flow pulses and germination of *S. nigra* for flow pulses reaching the 75th percentile flow standards (1,760 cfs). For *A. negundo* germination responses, only tier 3 and tier 4 events were statistically significant for the number of flow pulses. Notably, we predicted an 80% or higher probability of *A. negundo* germination with more than 4 pulse events at tier 3, but that same level of probability was reached with only 1-2 events at tier 4. However, *S. nigra* reached 80% or higher probability of germination with 1-2 events at tier 3. Furthermore, we found strong evidence that longer duration of spring flow pulses has a positive effect on germination of *A. negundo* and *P. deltoides*, and it takes shorter duration of flows at a higher tier to have the same effect. Since *T. distichum, P. occidentalis,* and *F. pennsylvanica* were absent or uncommon at the Bryan site on the Brazos River, and further analysis is needed to determine how environmental flow standards may affect germination and survival of those species.

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Table 3.19. List of primary USGS Gage Stations and their tiered flows from HEFR model calculations. Values reported are average discharge (cfs).

Brazos River USGS Gage Station 08098290				
	Spring	Summer	Fall	Winter
Baseflow 75th Percentile	1200	1236	1024	1236
High Flow Pulse 1	6003	2542	2507	3743
High Flow Pulse 2	12784	5191	4908	6992
High Flow Pulse 3	25603			
High Flow Pulse 4	33089			
High Flow Pulse 5	37892			
Brazos River USGS Gage Station 08108700				
	Spring	Summer	Fall	Winter
Baseflow 75th Percentile	2507	1554	1766	2119
High Flow Pulse 1	6286	NA	NA	6392
High Flow Pulse 2	22601	NA	12395	18399
High Flow Pulse 3	43790			
High Flow Pulse 4	57068			
High Flow Pulse 5	66850			
Colorado River USGS Gage Station 08159200				
	Spring	Summer	Fall	Winter
Baseflow 75th Percentile	1660	1907	1342	812
High Flow Pulse 1	3425	2507	2295	2931
High Flow Pulse 2	7133	3461	3814	4626
High Flow Pulse 3	15291			
High Flow Pulse 4	24473			
High Flow Pulse 5	31995			
Colorado River USGS Gage Station 08160400				
	Spring	Summer	Fall	Winter
Baseflow 75th Percentile	1554	1585	1306	840
High Flow Pulse 1	5332	2578	2648	3461
High Flow Pulse 2	9323	3743	4485	7239
High Flow Pulse 3	21083			
High Flow Pulse 4	29699			
High Flow Pulse 5	44673			
Guadalupe River USGS Gage Station 08169792				
	Spring	Summer	Fall	Winter
Baseflow 75th Percentile	530	530	565	530
High Flow Pulse 1	1271	883	812	812
High Flow Pulse 2	3249	4520	1907	1377
High Flow Pulse 3	6356			
High Flow Pulse 4	7451			
High Flow Pulse 5	9570			

Table 3.20. Total number of trees sampled by river, site, and species location.

River Sites	**Site Location**	**Trees Sampled**
Brazos River		
Bryan Site	7 km downstream of USGS Gage Station 08108700	Total (62) *A. negundo* (26) *F. pennsylvanica* (1) *P. deltoides* (12) *P. occidentalis* (1) *S. nigra* (22)
Hearne Site	46 km downstream of USGS Gage Station 08098290	Total (30) *A. negundo* (13) *F. pennsylvanica* (2) *P. deltoides* (1) *S. nigra* (14)
Colorado River		
Bastrop Site	1.14 km upstream from USGS Gage Station 08159200	Total: (30) *A. negundo* (17) *F. pennsylvanica* (2) *P. deltoides* (5) *S. nigra* (4)
La Grange Site	0.69 km away from USGS Gage Station 08160400	Total: (30) *A. negundo* (21) *F. pennsylvanica* (5) *P. occidentalis* (2) *S. nigra* (2)
Guadalupe River		
Seguin Site A	0.47 km upstream from USGS Gage Station 0816792	Total (37) *P. occidentalis* (37)
Seguin Site B	0.85 km downstream from USGS Gage Station 08169792	Total (41) *A. negundo* (2) *P. occidentalis* (37) *S. nigra* (1) *T. distichum* (1)

Table 3.21. Simple linear regression results of precipitation (mm) from the corresponding months (predictor variables) and annual ring-width index (mm) of the same year (response variable) on the Brazos River. Only statistically significant regressions are reported.

Species	Month Period	R^2	p-value
All Species			
	April	0.19	0.03
	August	0.16	0.04
ACNE			
	July	0.30	0.008
FRPE			
	May	0.28	0.03
	March–May	0.24	0.05
	March–June	0.28	0.03
	April–May	0.25	0.04
	April–June	0.27	0.03
	May–June	0.28	0.03
PODE			
	February	0.20	0.02
	April	0.27	0.008
	January–February	0.17	0.04
SANI			
	None		

Table 3.22. Simple linear regression results between average flows (cfs) from the corresponding months (predictor variables) and annual ring-width index (mm) of the same year (response variable) on the Brazos River. Only statistically significant regressions are reported.

Species	Month Period	R^2	p-value
All Species			
	None		
ACNE (5)			
	None		
FRPE (3)			
	March	0.24	0.04
	January–March	0.24	0.05
	January–April	0.26	0.04
	January–May	0.34	0.01
	January–June	0.32	0.02
	January–July	0.23	0.05
	February–March	0.25	0.04
	February–April	0.29	0.03
	February–May	0.37	0.01
	February–June	0.34	0.01
	February–July	0.24	0.05
	March–April	0.25	0.04
	March–May	0.29	0.02
	March–June	0.26	0.03
	April–May	0.24	0.05
PODE (5)			
	None		
SANI (9)			
	None		

Table 3.23. Colorado River results obtained from univariate linear regressions between precipitation from the corresponding months (predictor variables) and annual ring-width index of the same year (response variable). Only statistically significant regressions are reported.

Species	Month Period	R2	p-value
All Species			
	May	0.073	0.04
	May–June	0.11	0.01
ACNE			
	None		
FRPE			
	May	0.13	0.005
	May–June	0.082	0.03
PLOC			
	None		
PODE			
	None		
SANI			
	None		

Table 3.24. Colorado River results obtained from univariate linear regressions between average flow from the corresponding months (predictor variables) and annual ring-width index of the same year (response variable). Only statistically significant regressions are reported.

Species	Month Period	R^2	p-value
All Species			
	None		
ACNE			
	None		
FRPE			
	None		
PLOC			
	None		
PODE			
	None		
SANI			
	February	0.14	0.04
	March	0.23	0.007
	April	0.20	0.01
	January–February	0.13	0.05
	January–March	0.17	0.02
	January–April	0.19	0.02
	January–May	0.18	0.02
	January–June	0.18	0.02
	January–July	0.17	0.02
	January–August	0.17	0.02
	January–September	0.17	0.02
	February–March	0.18	0.02
	February–April	0.21	0.01
	February–May	0.18	0.02
	February–June	0.18	0.02
	February–July	0.17	0.02
	February–August	0.17	0.02
	February–September	0.17	0.02
	March–April	0.25	0.005
	March–May	0.17	0.02
	March–June	0.16	0.03
	March–July	0.13	0.05
	March–August	0.13	0.05
	March–September	0.13	0.05

Table 3.25. Guadalupe River results obtained from univariate linear regressions between precipitation from the corresponding months (predictor variables) and annual ring-width index of the same year (response variable). Only statistically significant regressions are reported.

Species	Month Period	R^2	p-value
All Species			
	None		
ACNE			
	March	0.50	0.03
	January–April	0.46	0.04
PLOC			
	July	0.39	0.01
	June–September	0.27	0.04
	July–August	0.33	0.02
	July–September	0.31	0.03
SANI			
	January–May	0.35	0.04
	March–May	0.38	0.03

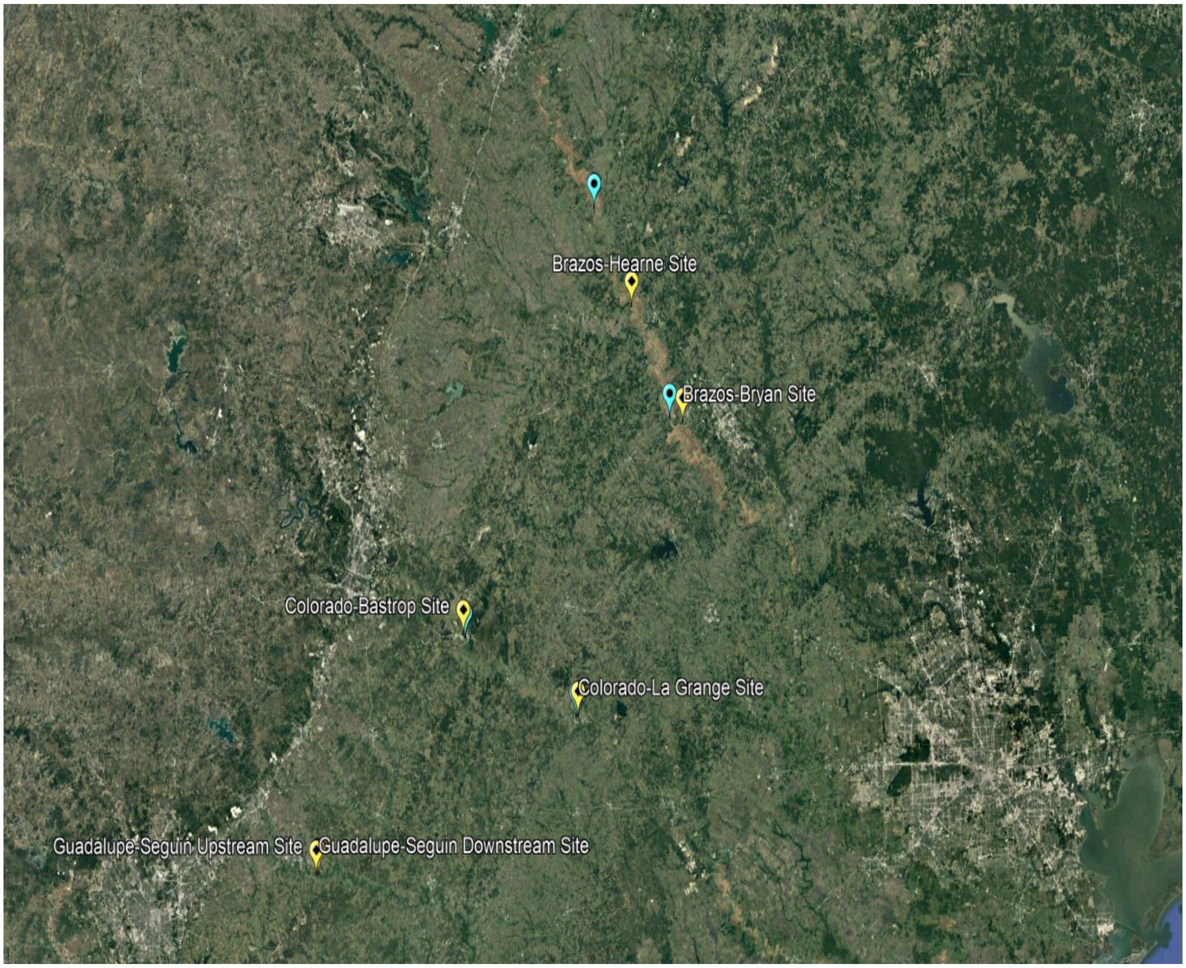

Figure 3.22. Site locations in yellow with name description and gage stations in blue.

Figure 3.23. Hearne, TX site on the Brazos River. The white outlined polygon represents the surveyed riparian area**.**

Figure 3.24. Bryan, TX site on Brazos River. The white outlined polygon represents the surveyed riparian area.

Figure 3.25. Bastrop, TX site on Colorado River. The white outlined polygon represents the surveyed riparian area.

Figure 3.26. La Grange, TX site on Guadalupe River. The white outlined polygon represents the surveyed riparian area.

Figure 3.27. Upstream Seguin, TX site on the Guadalupe River. The white outlined polygon represents the surveyed riparian area.

Figure 3.28. Downstream Seguin, TX site on Guadalupe River. The white outlined polygon represents the surveyed riparian area.

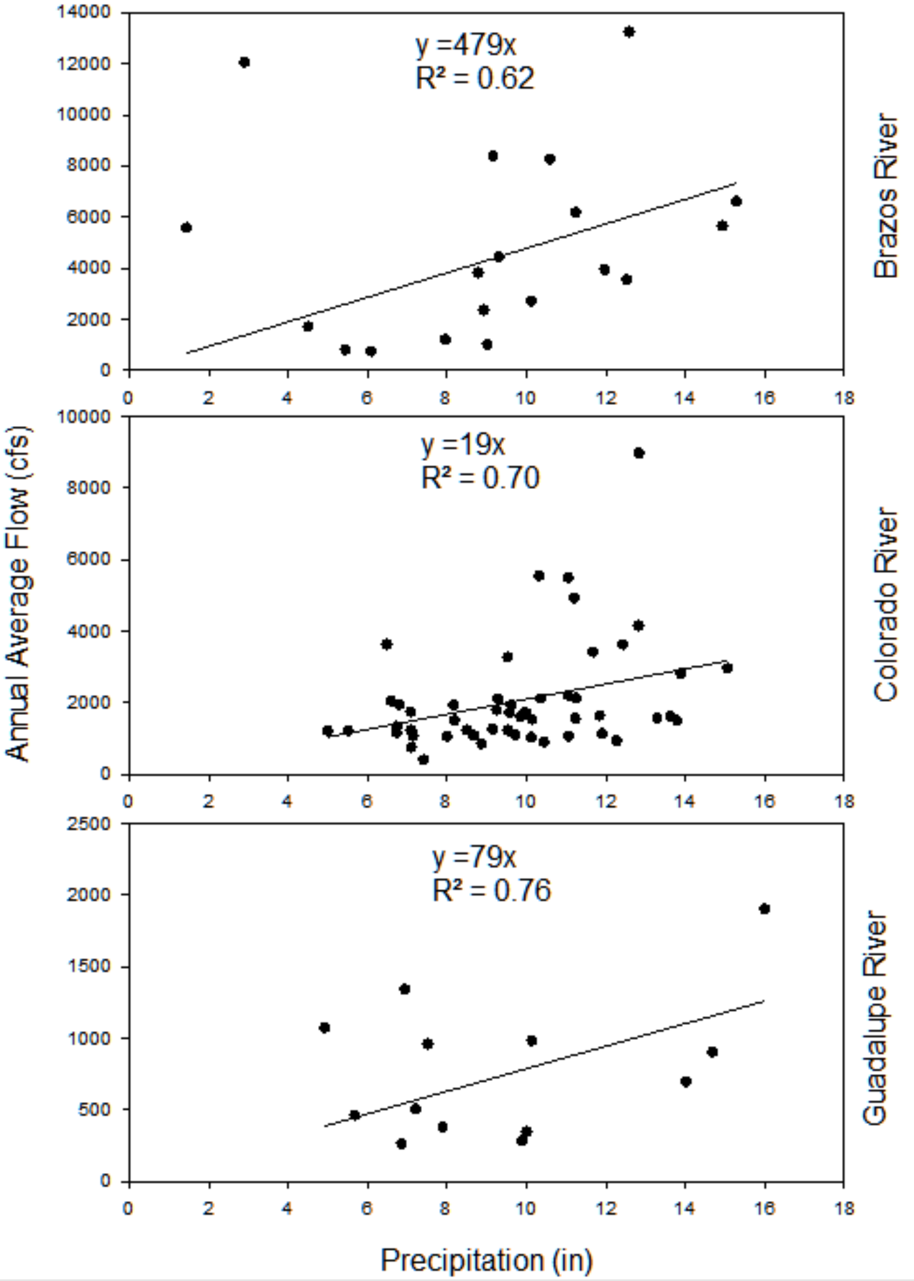

Figure 3.29. Relationship of precipitation (mm) and annual flows (m^3s^{-1}) on the Brazos River ($p < 0.0001$), Colorado River ($p < 0.0001$), and Guadalupe River ($p < 0.0001$).

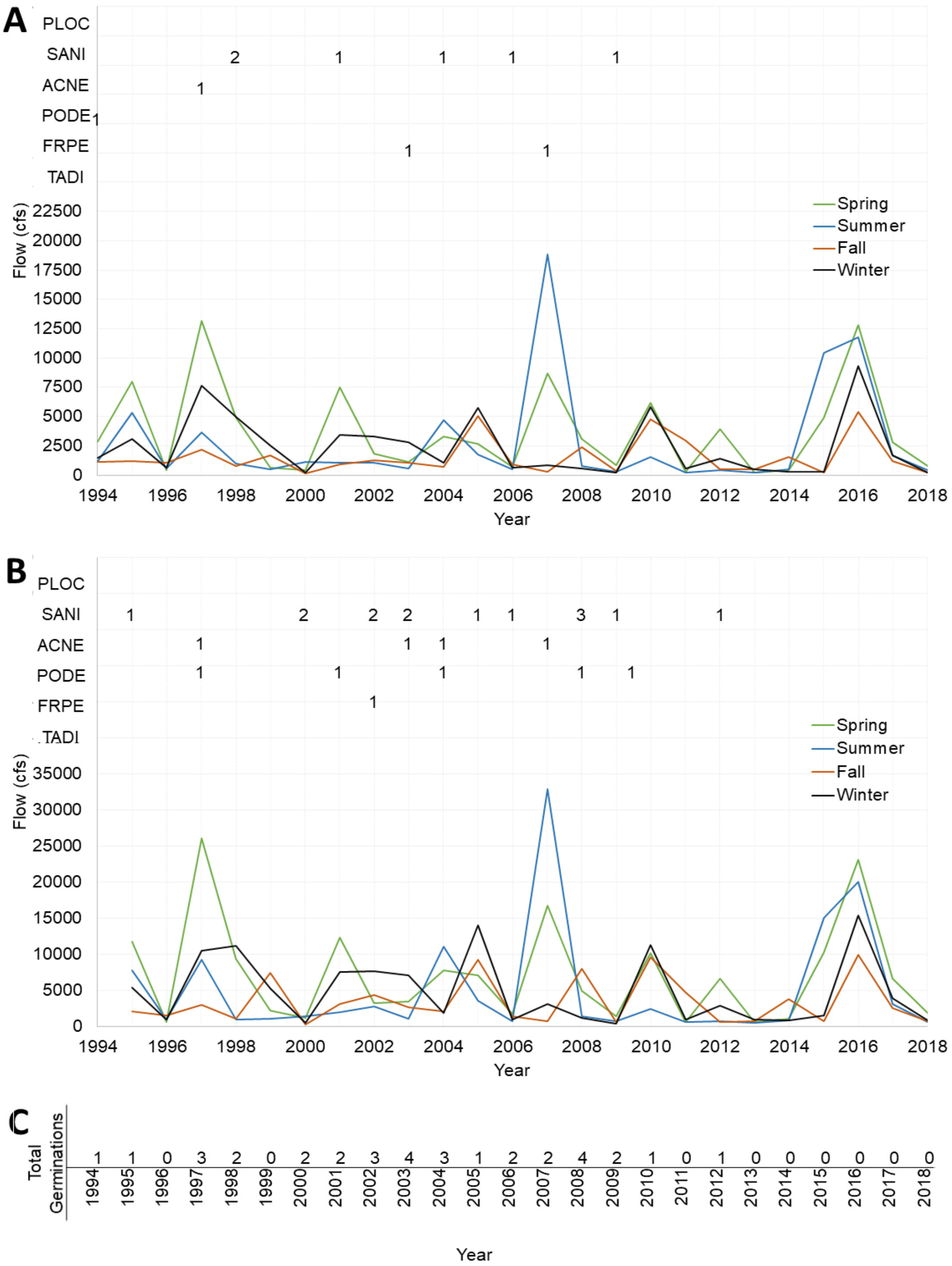

Figure 3.30. Seasonal flows and germination counts on the Brazos River at the Hearne, TX sites (A), Bryan, TX site (B) and total river consolidated germination counts by year (C). PLOC (*P. occidentalis*), SANI (*S. nigra*), ACNE (*A. negundo*), PODE (*P. deltoides*), FRPE (*F. pennsylvanica*), and TADI (*T. distichum)*.

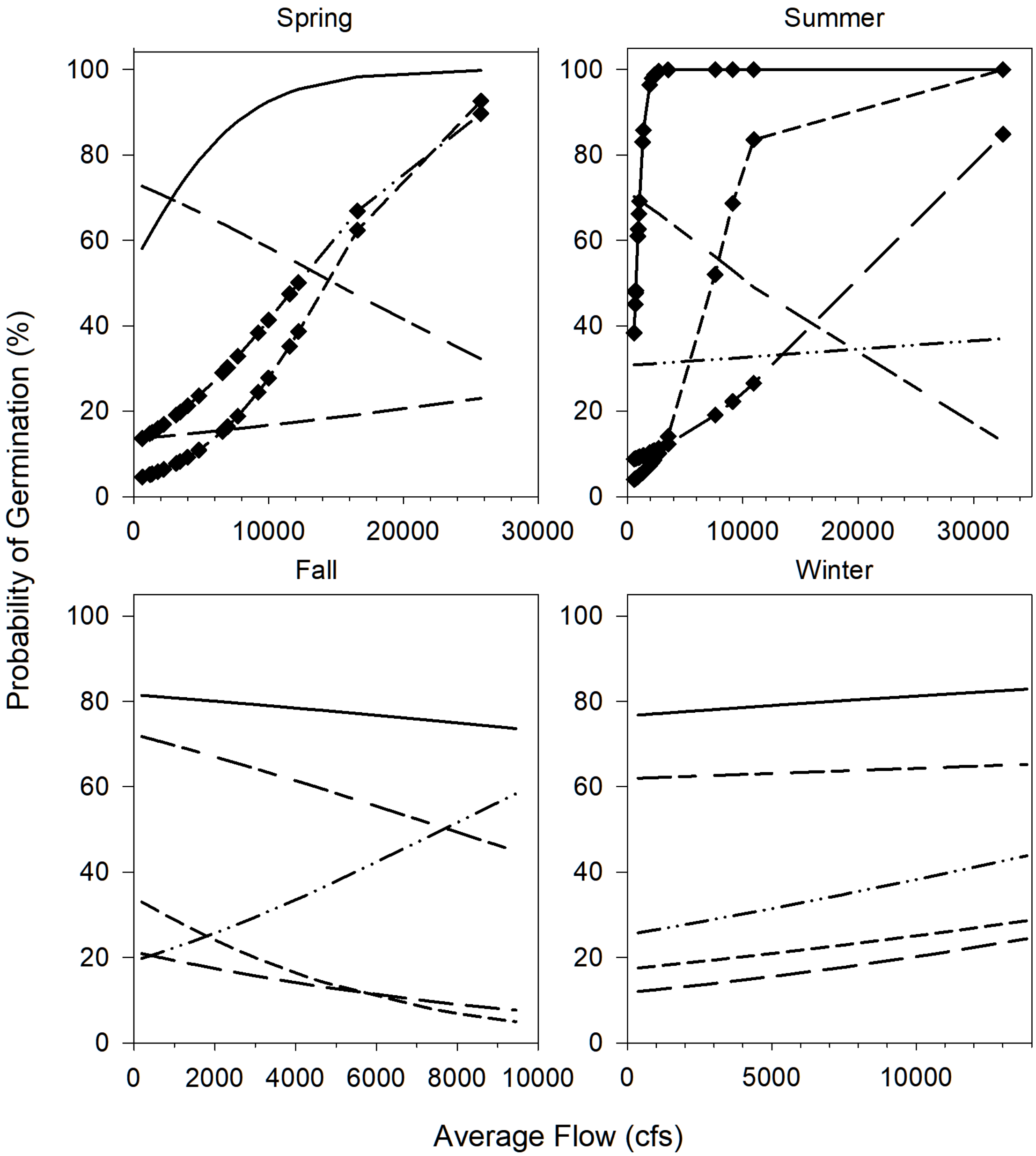

Figure 3.31. Brazos River average flow germination response. This figure displays the germination probabilities of the target species to the seasonal flows of the Brazos River. All species germination represented by (——), *Acer negundo* (– – – –), *Fraxinus pennsylvanica* (—— ——), *Populus deltoides* (— ·· — ·· —), and *Salix nigra* (— – — –). The ♦ denotes an interaction with a p-value < 0.2.

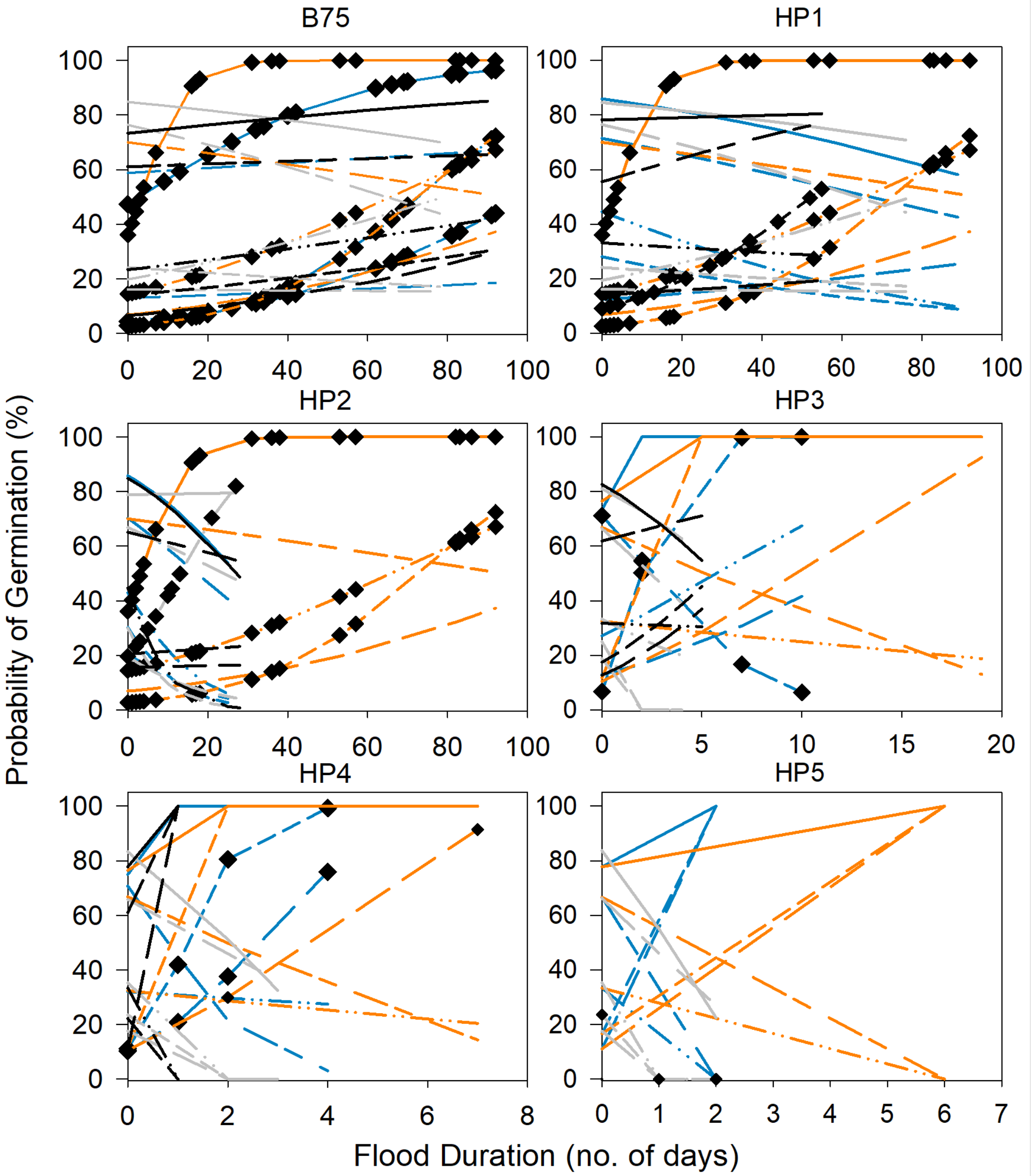

Figure 3.32. Brazos River high flow pulse germination response. This figure displays the germination probabilities of the target species to the HFPs of the Brazos River. All species germination represented by (——), *Acer negundo* (– – – –), *Fraxinus pennsylvanica* (—— ——), *Populus deltoides* (— ·· — ·· —), and *Salix nigra* (— – — –). The ♦ denotes an interaction with a p-value < 0.2. Seasons are in color coded as spring(blue), summer(orange), fall(gray) and winter(black).

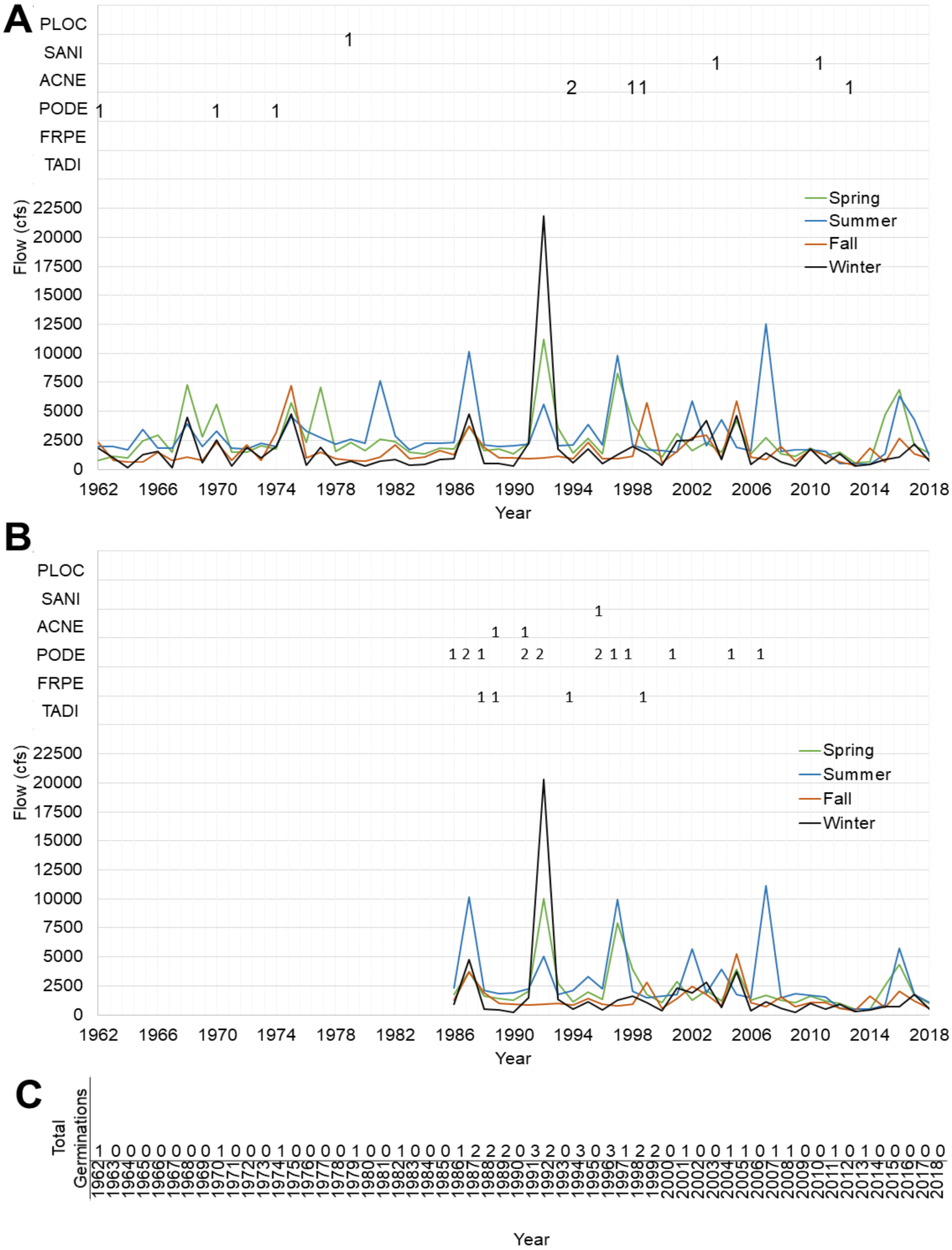

Figure 3.33. Seasonal flows and germination counts on the Colorado River at the Bastrop, TX sites (A), La Grange, TX site (B) and total river consolidated germination counts by year (C). PLOC (*P. occidentalis*), SANI (*S. nigra*), ACNE (*A. negundo*), PODE (*P. deltoides*), FRPE (*F. pennsylvanica*), and TADI (*T. distichum)*.

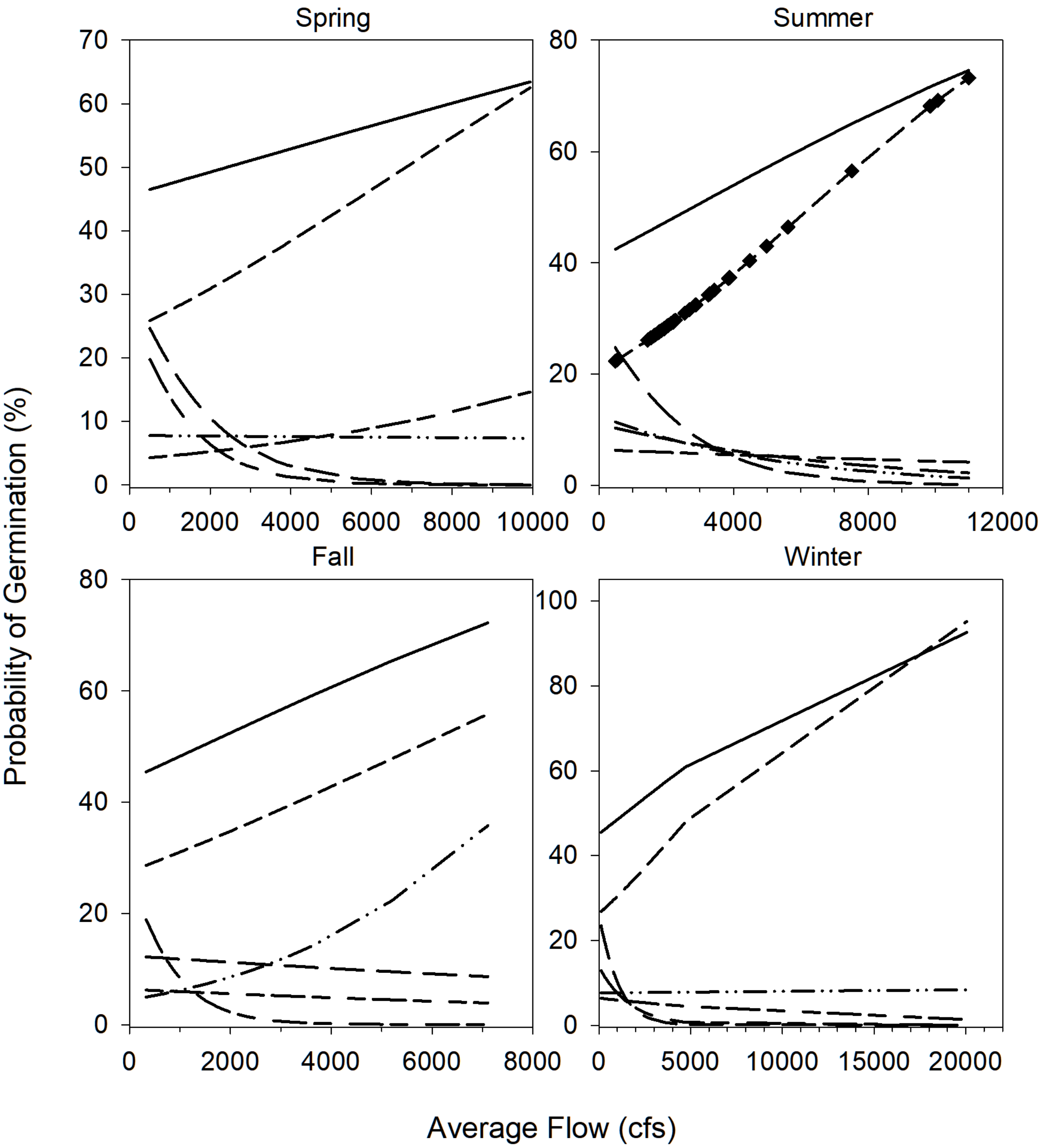

Figure 3.34. Colorado River average flow germination response. This figure displays the germination probabilities of the target species to the seasonal average flows of the Colorado River. All species germination represented by (——), *Acer negundo* (— — — —), *Fraxinus pennsylvanica* (—— ——), *Platanus occidentalis* (– — – – — –), *Populus deltoides* (— ·· — ·· —), and *Salix nigra* (— – — –). ♦ denotes an interaction with a p-value < 0.2.

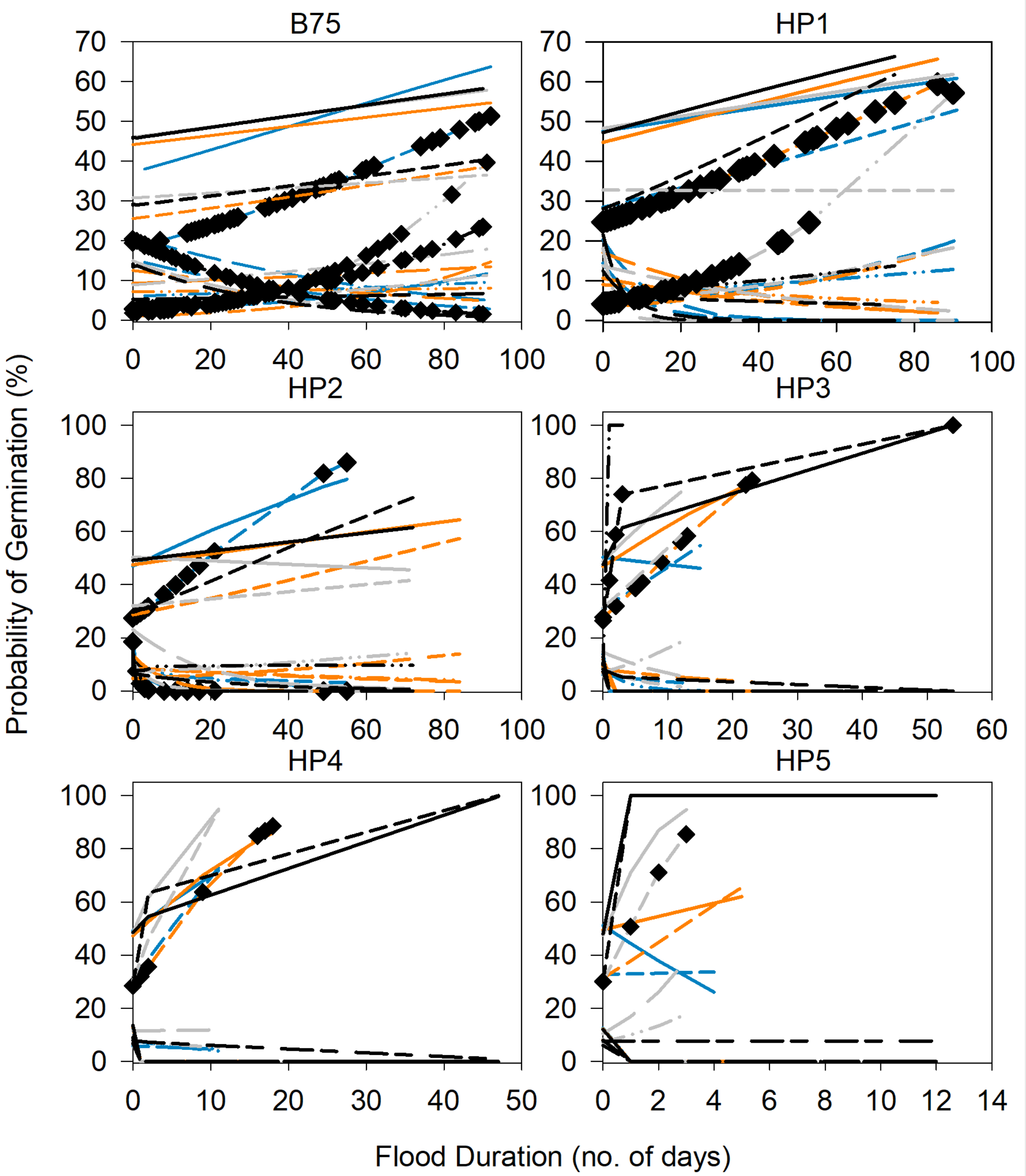

Figure 3.35. Colorado River high flow pulse germination response. This figure displays the germination probabilities of the target species to the seasonal HFPs of the Colorado River. All species germination represented by (———), *Acer negundo* (— — — —), *Fraxinus pennsylvanica* (—— ——), *Platanus occidentalis* (− — − − — −), *Populus deltoides* (— ·· — ·· —), and *Salix nigra* (— − — −). ♦ denotes an interaction with a p-value < 0.2. Seasons are in color coded as spring(blue), summer(orange), fall(gray) and winter(black).

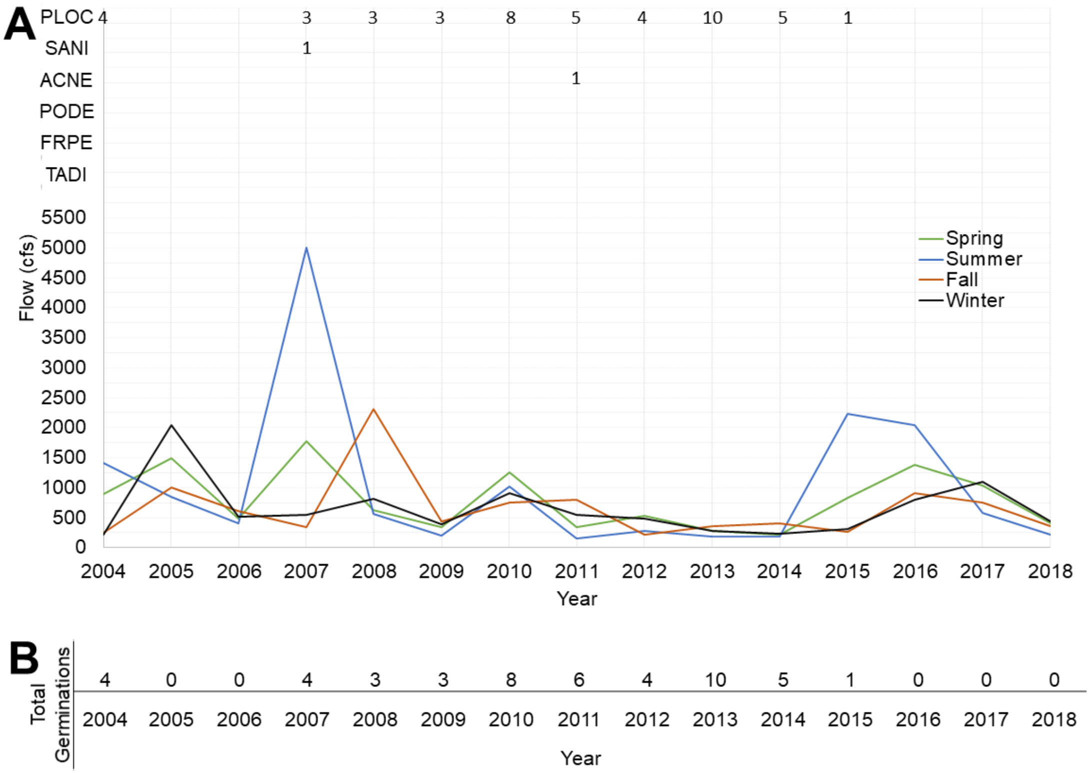

Figure 3.36. Seasonal flows and germination counts on the Guadalupe River at the Seguin, TX sites (A) and total river consolidated germination counts by year (B). PLOC (*P. occidentalis*), SANI (*S. nigra*), ACNE (*A. negundo*), PODE (*P. deltoides*), FRPE (*F. pennsylvanica*), and TADI (*T. distichum)*.

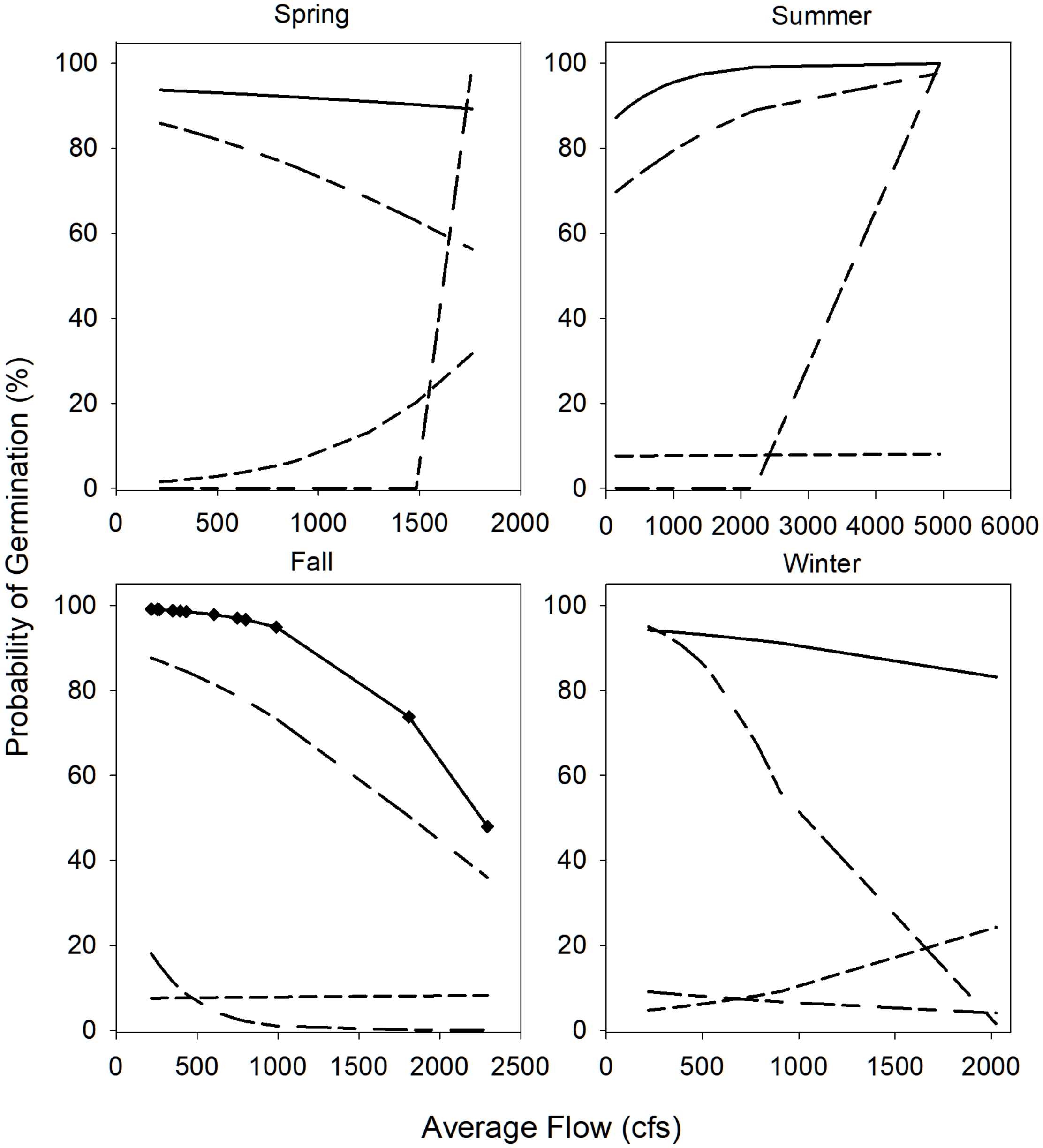

Figure 3.37. Guadalupe River average flow germination response. This figure displays the germination probabilities of the target species to the seasonal average flows of the Guadalupe River. All species germination represented by (——), *Acer negundo* (— — — —), *Platanus occidentalis* (– — – – — –),and *Salix nigra* (— – — –). ♦ denotes an interaction with a p-value < 0.2.

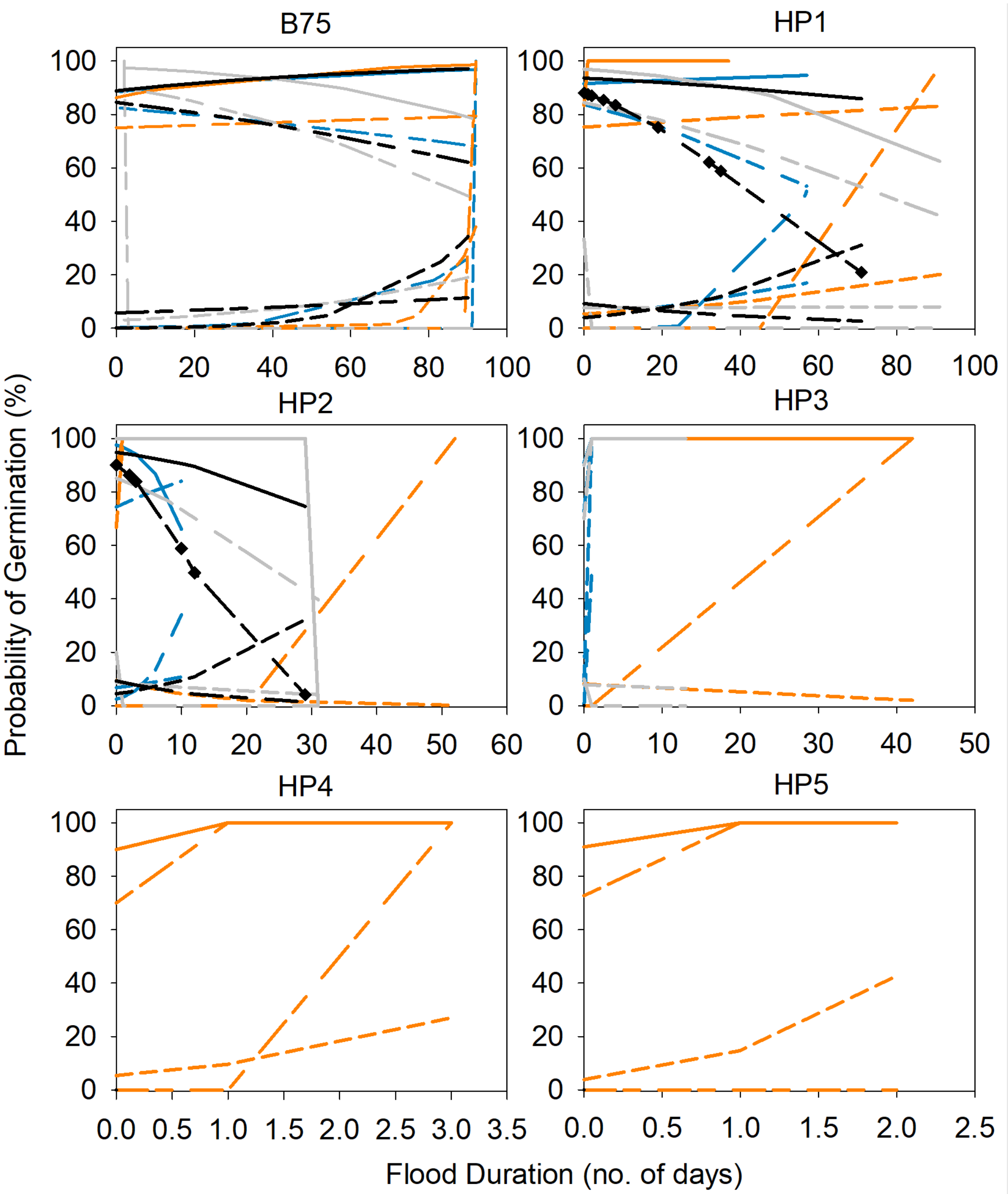

Figure 3.38. Guadalupe River high flow pulse germination response. This figure displays the germination probabilities of the target species to the seasonal HFPs of the Guadalupe River. All species germination represented by (——), *Acer negundo* (— — — —), *Platanus occidentalis* (− — − — —),and *Salix nigra* (— − — —). ♦ denotes an interaction with a p-value < 0.2. Seasons are in color coded as spring(blue), summer(orange), fall(gray) and winter(black).

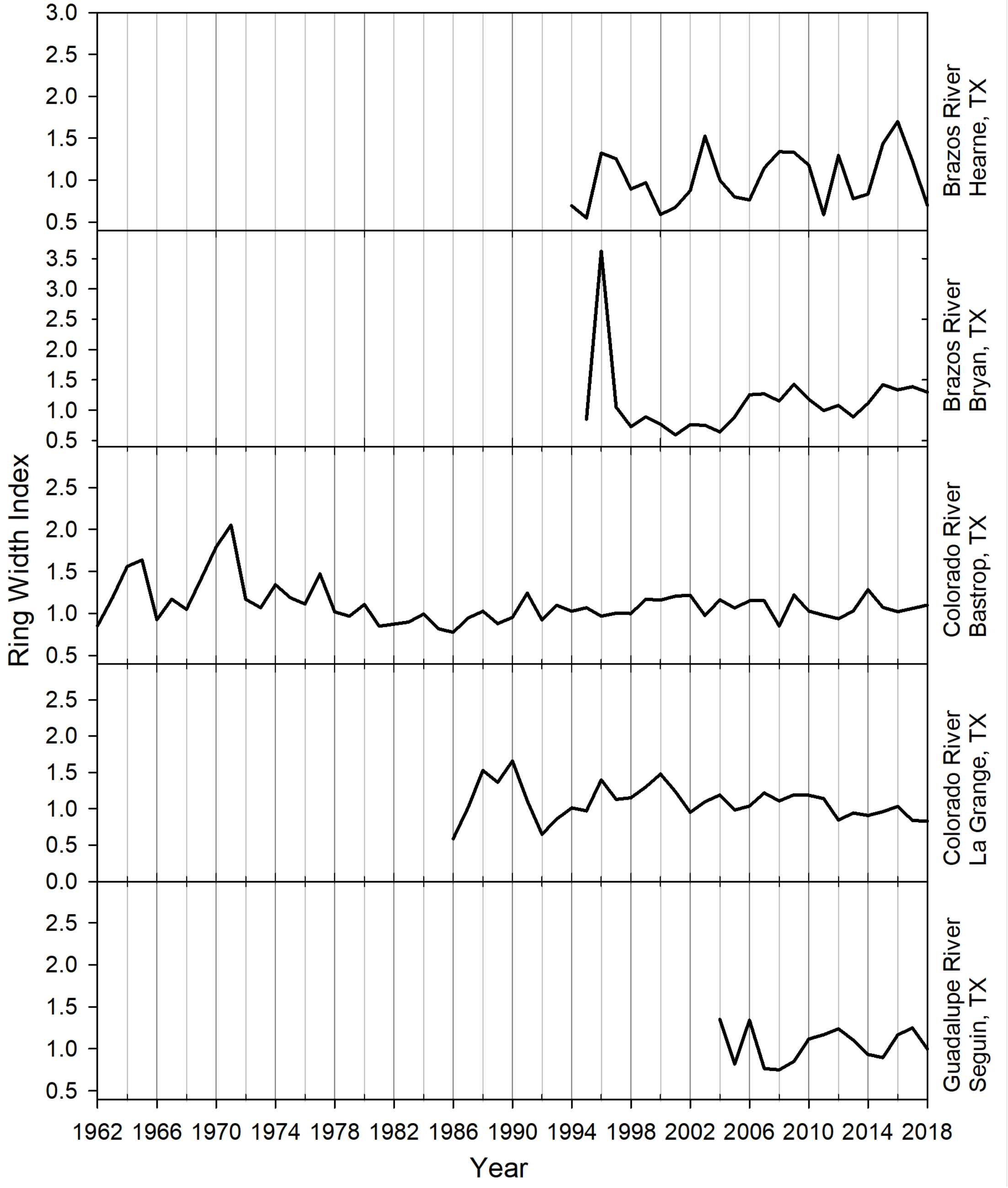

Figure 3.39. Standardized ring width indexes (RWIs) of all species for all sites across the three rivers.

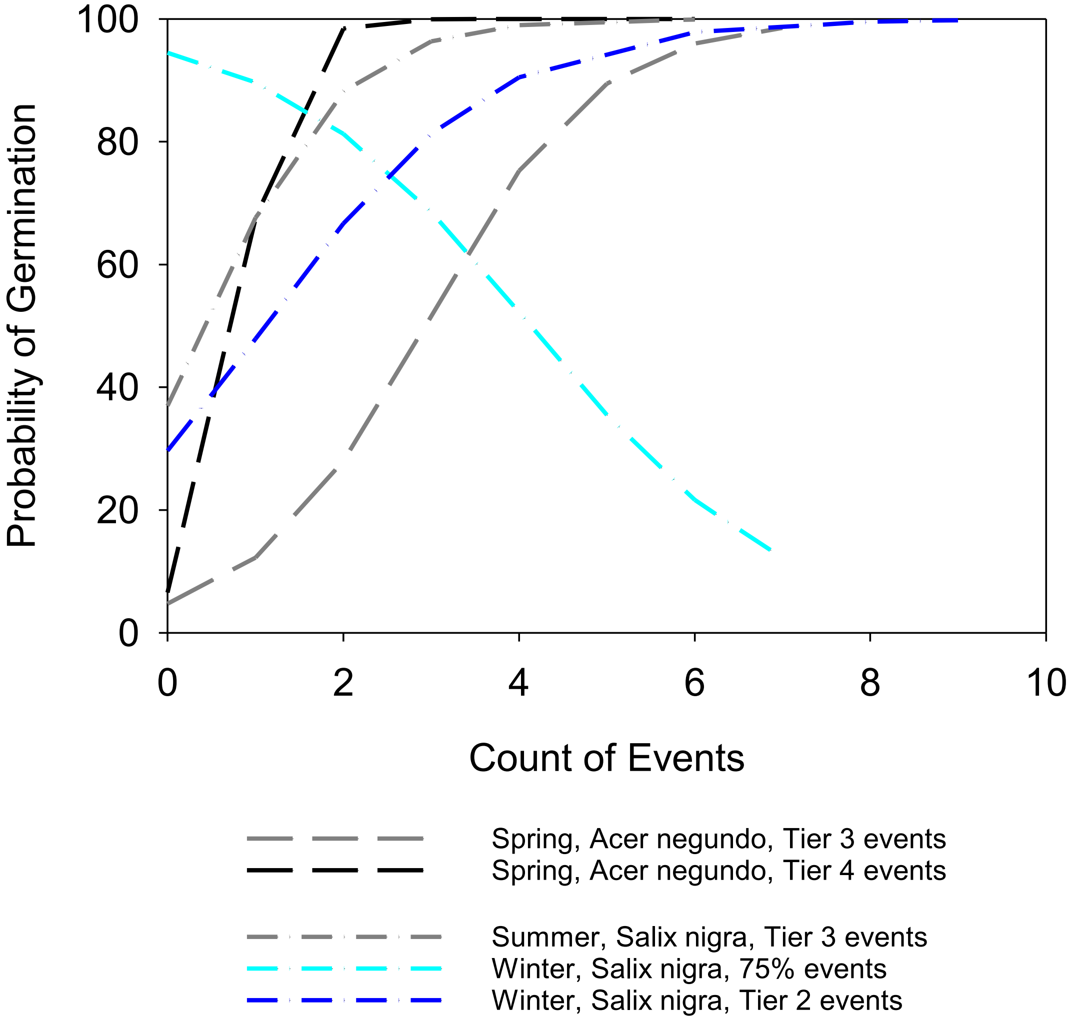

Figure 3.19. Probability of germination of *Acer negundo* (Box Elder) was statistically significant ($P < 0.05$) for tier 3 spring events (gray dashes) and tier 4 spring events (black dashes). Probability of germination of *Salix nigra* (Black Willow) was weakly significant ($P < 0.1$) for tier 3 summer events (gray dash-dot lines), winter 75% events (light blue dash-dot lines), and winter tier 2 events (blue dash-dot lines). Note the weak negative association found for *Salix nigra* in winter related to 75% events ($P < 0.1$).

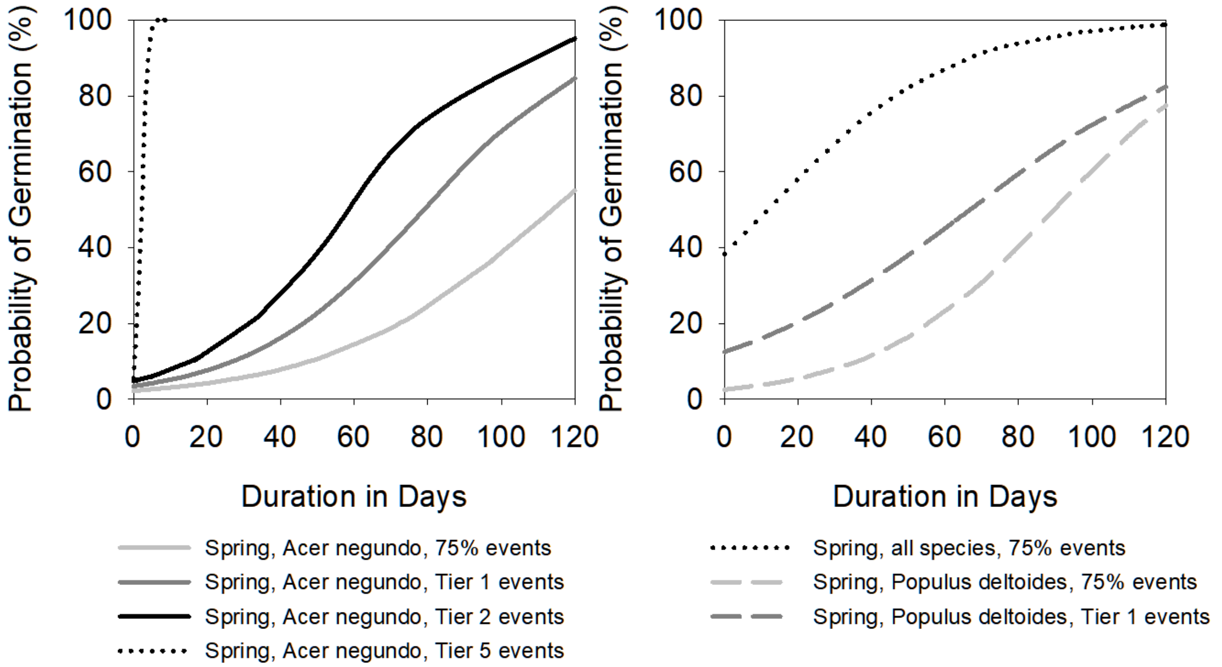

Figure 3.20. Probability of germination of *Acer negundo* progressively increased with increased duration of spring flows at 75th percentile ($p < 0.1$), tier 1 ($p < 0.05$), tier 2 ($p < 0.1$), and tier 5 ($p < 0.1$). Similarly, probability of germination of *Populus deltoides* (Cottonwood) progressively increased with increased duration of spring flows at 75th percentile ($p < 0.05$) and tier 1 ($p < 0.1$). A combination of all species found at the Bryan site also were positively associated with flow duration at the 75th percentile ($p < 0.1$).

Chapter 4. Response of Indicator Taxa to Flow Variation – Smallmouth Buffalo, with Comments on Alligator Gar[2]

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Background

Freshwater ecosystems are severely impacted by anthropogenic activities, particularly by flow regime alteration (Vorosmarty et al. 2010). Balancing human and environmental water needs is a priority for conservation and natural resource management, and environmental water needs are inextricably bound to the natural flow regime (Acreman et al. 2014, Bunn and Arthington 2002). Temporal change in instream flows, the basis of the natural flow regime, sustains ecosystem processes and maintains biodiversity (Poff and Zimmerman 2010). Identifying the key components of the natural flow regime needed to prevent the extirpation of species and to preserve ecosystem services is, therefore, critical to effective water management.

Temporal variation in environmental conditions has long been understood to play a critical role in maintaining species diversity and ecosystem processes (Hutchinson 1961). This insight led researchers to classify rivers based on flow regime and investigate the relationships between temporal flow variation and various population and community parameters (e.g., fisheries yield) (Poff and Ward 1989, Bayley 1991, Richter et al. 1996, Poff et al. 2017). The "natural flow regime paradigm" (Poff et al. 1997) synthesized relevant flow-ecology work, outlined how flow regimes shape ecosystem processes in and around the floodplain, and described how different forms of flow alteration impact the flow regime and ecosystem processes. It also outlined a model whereby river flow could be managed to preserve native species and the ecological integrity of lotic systems.

The natural flow regime paradigm is fundamentally based on five critical components of the flow regime: 1) magnitude, 2) frequency, 3) duration, 4) timing, and 5) rate of change of flow conditions (Richter et al. 1996). Magnitude refers to the amount of water passing through a system at a point in time. Frequency refers to how often a flow condition occurs, often in terms of probability of exceedance. Duration refers to the length of time a flow condition occurs. Timing has two components: the predictability of a flow condition over a set period of time and the approximate time within that period in which that flow condition occurs. Rate of change refers to how quickly flow conditions shift from one state to another. Richter et al. (1996) proposed a suite of indices called the Indicators of Hydrologic Alteration (IHA) that quantify ecologically relevant aspects of the flow regime, and which can be used to characterize variation in river flows and investigate flow-ecology relationships (Kim et al. 2010, Monk et al. 2011).

Flow-ecology research operates on a continuum from state-based to rate-based approaches (Wheeler et al. 2018). States-based approaches assess flow-ecology relationships of a system at a specific point in time. Rates-based approaches, on the other hand, look at a system over time using repeated measurements of that system. The factor of interest can be the same as in state-based investigations, but rates-based approaches can also investigate factors that fundamentally require a temporal component to have meaning (e.g., growth rate). Studies that are further towards the rates end of the continuum tend to provide a more accurate picture of a parameter and allow for more predictive power, but they are more expensive and time consuming to conduct. In contrast, the closer a study gets to a pure states-based approach, the less predictive power the study has, but execution is faster, easier, and less expensive. Flow ecology studies can effectively fuse the positive aspects of both sides of the states-rates continuum, i.e. high predictive power at low expense, by using a pure state to infer a rate-based process in relation to variation in annual flow regimes. For example, Robertson et al. (2018) used age structure data at a point in time, a state, to infer the rate of recruitment of Alligator Gar *Atractosteus spatula* in relation to variation in the flow regime and floodplain inundation over a period of nearly two decades. The combination of logistical practicality and high predictive power make this approach particularly appealing for natural resource management and conservation applications.

Similar to Robertson et al. (2018), this research used a pure state (i.e., population age structure at a point in time) to infer effects of flow variation on a rates-based process (i.e. recruitment). Specifically, we modeled flow-recruitment relationships of Smallmouth Buffalo *Ictiobus bubalus*, a periodic life-history fish species, in three Texas river basins using contemporary age-structure data and retrospective flow analyses. Age-structure data, which correspond to spawning year for all individuals collected, were generated for each basin using age estimates from counting annuli on otoliths. A recruitment index (RI) was made using this age-structure data by comparing observed age class strength to expected age class strength based on constant mortality and recruitment. Retrospective flow analyses were conducted using daily streamflow data from USGS gaging stations and IHA indices. Model selection procedures used a two-tiered approach that first identified attributes of the flow regime during the spawning season that were correlated with RI values, then added antecedent and post-spawn flow attributes that improved model fit.

Periodic life-history strategists are characterized by high fecundity, slow maturation, and low juvenile survivorship and recruitment success is often associated with occurrence of suitable conditions that are patchy in space and/or time, leading to ‘boom’ or ‘bust’ year class structure (Winemiller and Rose 1992, Winemiller 2005). Based on the limited previous reproductive life-history studies for Smallmouth Buffalo and broader knowledge of related taxa, we expect Smallmouth Buffalo to spawn in association with high-flow pulses and that recruitment success within a year is related to 1) sufficient flood magnitude to inundate critical spawning habitat, 2) sufficient flood duration to prevent eggs from desiccating with lowering waters, 3) coincident timing of the flood pulse with the spawning season, 4) appropriate rate of change such that

[2] Reeves, C. G. (2021). Flow-recruitment relationships of Smallmouth Buffalo (Ictiobus bubalus) in three Texas river basins. MS Thesis, University of North Texas, Denton, Texas.

weakly swimming larvae are not stranded by quickly receding waters, 5) slow rate of change of flow conditions antecedent to the spawning season such that spawning habitat is not destroyed, and 6) sufficient magnitude of base flows after the spawning season to allow young of year Smallmouth Buffalo to grow and avoid predators (Walker and Frank 1953, Jester 1971, 1973).

Methods

Smallmouth Buffalo were chosen as the focal species for this research for several reasons. First, as a species of 'least concern' they are abundant and common enough to enable large sample sizes for age-structure and flow-ecology analyses across replicate basins while not significantly impacting populations. Second, Smallmouth Buffalo are a suitable representative or indicator of periodic life-history strategist species in general. Many periodic life-history strategist species are species of conservation concern and are challenging to study when harvest is required to quantify age-structure because of limited population sizes and ethical concerns about impacting populations of such species. Finally, although broadly distributed and often numerically abundant components of aquatic ecosystems, Smallmouth Buffalo are surprisingly poorly studied in terms of their age and growth and relationships with flow regimes.

Study Systems. – The Texas Gulf Coastal Plain is a system with high temporal variability in flows, with base flows highest in spring into early summer and lowest base flows in late summer into early fall. The rivers in this region flow Northwest to Southeast, with the broader ecosystems around them shifting from dry and arid to wet and humid as the rivers approach the coast. The Brazos, Colorado, and Guadalupe rivers serve as replicate study systems for this research (Figure 4.1). Within the study area, these rivers are best described as temperate aseasonal/stochastic floodplain rivers, with flow regimes that are highly variable and based on weather patterns rather than seasonal snowmelt (Winemiller 2004). The region is also affected by anthropogenic flow alterations. All three rivers are extensively impounded higher in the drainages, and agricultural and municipal water withdrawals are common in our study area.

Fish Collection and Otolith Extraction. – A random sample of Smallmouth Buffalo were collected from each study system to harvest otoliths (lapilli and asterisci) to estimate population age structure. Fish were primarily collected using boat electrofishing, though a few individuals were also collected using experimental gill nets. All individuals were measured for total length (mm TL) and euthanized immediately after capture via cervical dislocation. Lapilli and asterisci were removed from the inner ear system of the Smallmouth Buffalo in the field and retained for further processing in the lab. Subsequently, sex and maturity were determined by examining the gonads, and samples of dorsal muscle and liver tissue were collected from each fish and stored frozen for use in other studies.

Otolith Processing and Age Estimation. – Asterisci and lapilli were marked across the nucleus with a graphite pencil and cast in epoxy (Alumilite Amazing Clear Cast, Galesburg, MI) blocks using a silicone mold. Embedded otoliths were sectioned using a Buehler Isomet low speed sectioning saw to a thickness of 0.5mm. If necessary, otolith sections were sanded with a fine grit sandpaper to remove scratches and make the sections slightly thinner. Prepared sections were photographed using a Leica stereomicroscope outfitted with a camera (Figure 4.2).

Three trained readers independently estimated age for each fish by counting annuli in photographs of each otolith. All aging was conducted ‘blind’, i.e. the reader had no additional information (e.g. size, sex, location of capture) other than a numerical identifier and date of collection (useful when assessing possible annuli formation at the edge of the otolith). Fish ages for subsequent analyses were assigned using the age estimates of Cole Reeves, with the age estimates from the other readers used to check for systematic bias and provide an approximation (since true age is unknown) of age assignment accuracy. A random subset of 50 otoliths was aged again by two readers to assess the precision of age estimates for each reader. Correlation was used to quantify repeatability of age estimates among readers as well as between repeat reads by individual readers. These analyses of accuracy and precision inform the degree of confidence in subsequent recruitment modeling that ultimately depends on the age structure data.

Recruitment Index. – A recruitment index (RI) was utilized to allow for comparability of year class strength over time by accounting for loss of individuals from populations due to mortality. The RI is calculated as the observed year class strength divided by the expected year class strength, both of which are calculated based on contemporary samples. Observed year class strength is simply the number of individuals in a particular river (i.e., Brazos, Colorado and Guadalupe) assigned to a particular year class based on age estimates. Expected year class strength accounts for mortality which was modeled using the updated Hoenig estimator developed by Then et al. (2015):

$$M = 4.899 t_m^{-0.916}$$

where M is natural mortality and t_m is maximum age. The values 4.899 and -0.916 are constants that were derived by averaging observed values from more than 200 fish stocks. With natural mortality estimated, we can quantify expected year class strength by using an observed number of individuals in the random sample of the population and the equation $N_x = N_{x-1} \times (1 - M)$ where N_x is the number of individuals expected to be assigned to a given year class and N_{x-1} is the number of individuals from the previous year class. N is adjusted such that the sum of expected individuals matches the sum of observed individuals in the random sample.

Quantifying Variation in Flow among Years and Retrospective Analysis. – Age estimates for all individuals were used to identify the year (and thus flow conditions) that each individual recruited to the population. Retrospective flow analyses were conducted using daily streamflow data from USGS gaging stations in each basin (Brazos River at Waco USGS 08096500, Colorado River at Bastrop USGS 08159200, Guadalupe River at Victoria USGS 08176500) for the period of record (oldest to youngest age class). Flow indices from IHA were quantified for the spawning season (March through July, determined based on previous studies and observations of gonads during field collections), and separately for the pre-spawn (January and February) and post-spawn (August through December) periods for each year. A reduced set of indices was selected by eliminating indices that were significantly correlated (Table 4.1), and the retained streamflow indices from IHA were used as predictor variables to model year-class strength for each basin independently. That is, basins served as replicate study systems with independent populations and flow regimes.

Multiple regression models for each basin were fitted using the selected IHA indices quantified for each year for the period 1985 to 2015. Year classes before 1985 were excluded because of greater aging uncertainty, strong leverage in the overall model, and because they were interspersed with too many zero recruitment years. Year classes after 2015 were excluded because of extremely low abundance, most likely because those younger (smaller) fish had not yet recruited to the sampling gear. Importantly, the 30-year period used for analyses includes sufficient variation in flow attributes within and among years to enable a robust assessment of flow-recruitment relationships.

Modeling procedures followed a two-tiered approach, starting with fitting a model using variables (i.e., IHA indices) characterizing stream flow conditions during the spawning season and then adding additional informative variables characterizing antecedent and post-spawn flow conditions. The first tier started with the 'beyond optimum' model (Zuur et al. 2009), which included all variables listed in Table 4.1 for the spawning season from March to July. Variance inflation factor (VIF) scores were used to remove redundant variables, in this case by eliminating variables with VIF scores > 5.0. When two factors had similar VIF scores, the factor that preserved the most information about the flow regime was retained. Rise_rate and Fall_rate, although useful indicators of rate of change in flow conditions, were unfortunately far too collinear with other variables to be included in any of the models. In addition, max_7day and Hi_pulseL also tended to be highly collinear with one another and Hi_pulseL was retained over max_7day because max_7day was slightly more likely to be collinear with other variables and because Hi_pulseL was considered to be of greater concern for water management. Model selection then proceeded by comparing all possible models using a combination of adjusted R^2, Akaike's Information Criterion (AIC), Bayesian Information Criterion (BIC), Mallow's C_p and model significance (assessed at $\alpha \leq 0.05$; Burnham and Anderson 2002). Better model fit is indicated by maximizing adjusted-R^2 while achieving lowest values for AIC and the more stringent BIC. Mallow's C_p indicates a 'good' model when the value is less than the number of parameters in the model, and with lower values indicating better model fit.

The second tier of modeling integrated informative IHA indices as variables characterizing flow conditions before and after the spawning season by using a step-wise function to add significant variables (i.e. explain a significant amount of additional variation considering the other variables already present in the model) to those present in the competing models (i.e. models with $\Delta AIC \leq 2.0$) from the first tier. Again, collinearity was assessed using VIF scores, redundant variables were excluded, and all possible models including significant pre-spawn and post-spawn variables were assessed. Final competing models were compared using adjusted R^2, AIC, BIC, Mallow's C_p and model significance. As a general note, individual variable significance was not considered a primary concern when comparing competing models because variable significance values are not independent of the other variables in the models and do not reflect contributions to model performance associated with collinearity or higher-order interactions among variables, which are prevalent in flow attributes even after removing variables with VIF > 5.0. Instead, model selection was focused on the model as a unit and based on model statistics as indicated above. Modeling was performed in R using the olsrr package (Hebbali 2020).

Results

Population Age Structure and Reruitment Index Values. – In total, 446 individuals were collected and aged, including 150 from the Brazos, 95 from the Colorado, and 201 from the Guadalupe. Age estimates ranged from 1 to 65 years. Individuals assigned to age classes between 1985 and 2015 accounted for 95.3%, 86.3% and 94.1% for the Brazos, Colorado and Guadalupe population samples, respectively. Age estimates appear to have sufficient accuracy and precision for robust retrospective flow-recruitment analyses, based on strong correlations between estimates from Reader 1 (Cole Reeves) used for analyses and blind reads from two additional trained readers ($R^2 = 0.9873$ and 0.9755; Figure 4.3) and strong correlations of age estimates from blind re-reads of a random subset of 50 otoliths ($R^2= 0.9879$ for Cole Reeves and $R^2= 0.9896$ for the other reader; Figure 4.4). After controlling for mortality, recruitment index values showed highly variable interannual recruitment in Smallmouth Buffalo in all three basins (Figures 4.5-4.7). The largest spread in recruitment index values for the 1985-2015 period was observed in the Guadalupe River (RI= 0.00 - 6.70) and the smallest range of recruitment index values was observed in the Brazos River (RI= 0.00 - 2.85).

Models of Recruitment Success. – Competing flow-recruitment models for all three basins, identified as those with $\Delta AIC \leq 2.0$ of the model with the lowest AIC value, consistently included the length of high flow pulses during the spawning season (Hi_pulseL) and the coefficient for that variable was almost always positive (Tables 4.2-4.7). When present, the number of high flow pulses (Hi_pulseN) during the spawning season consistently had a negative coefficient (Tables 4.3, 4.5 and 4.7). Mean monthly flows during the spawning season were not consistently included in competing models, but when present, coefficients for May and June mean flows were positive whereas coefficients for April and July mean flows were negative (Tables 4.2-4.7). The number (Lo_pulseN) and length (Lo_pulseL) of low flow pulses during the spawning season were also regularly included in competing models, but the sign of the coefficients differed among basins and when flow attributes from outside the spawning season were included. For example, the coefficients for Lo-pulseL changed from positive to negative for the Brazos River when Lo-pulseL during the post-spawn period was included (Table 4.3), although that result appears to be driven almost entirely by the recruitment index value associated with just one year. Other spawning season flow attributes included in some of the competing models for all three basins included the number of flow reversals (Reversals) and date of maximum flow (Date max), though the coefficients differed among basins (Tables 4.3, 4.5 and 4.7). For the Brazos and Colorado basins, including flow attributes from the post-spawn period improved model fit and substantially increased adjusted R^2 values (Tables 4.2 and 4.4). For the Brazos River, Lo_pulseL during the post-spawning season had positive coefficients (Table 4.3), whereas for the Colorado River, Date-max had negative coefficients (Table 4.5).

Based on the combined model selection criteria, one or two best-fit models were clearly evident for each basin (Tables 4.2, 4.4 and 4.6). For the Brazos River, none of the competing models including only flow attributes during the spawning season were significant; adding Lo_pulseL during the post-spawn period (as indicated above) resulted in significant models and substantially improved adjusted R^2 (Table 4.2). Of the competing models including Lo_pulseL during the post-spawn period, the best fit model (lowest AIC, BIC, and Mallow's C_p, and high adjusted R^2) was model F1 (Table 4.2). Model F1 included the spawning season flow attributes Hi_pulseL and Lo_pulseL along with Lo_pulseL during the post-spawn period; Hi_pulseL and

Lo_pulseL during the post-spawn period had positive coefficients whereas Lo_pulseL during the spawning season had a negative coefficient (Table 4.3). For the Colorado River, significant and informative flow-recruitment models were possible using only flow attributes during the spawning season, but including flow attributes from the post-spawn period increased the adjusted R^2 of the best-fit models (S1 and F3) from 0.45 to 0.68 and reduced AIC and BIC values (Table 4.4). Flow attributes included in Model S1 were Hi_pulseL, Hi_pulseN, Lo_pulseN, June mean, and July mean; Hi_pulseL, Lo_pulseN and June mean had positive coefficients (Table 4.5). Model F3 had those same variables except June mean and Hi_pulseN and included Date max during the post-spawn period (negative coefficient). Finally, few competing models were indentified for the Guadalupe River and the only significant models included only flow attributes during the spawning season (Table 4.6). Model S2 was the best-fit model with adjusted R^2= 0.84, lowest BIC, C_p, and p-value, and comparable AIC (Table 6). Flow attributes included in model S2 were Hi_pulseL, Lo_pulseL, April mean, Date max, and Reversals; only Hi_pulseL had a positive coefficient (Table 4.7).

Discussion

As expected, modeling results supported the importance of high flow pulses for successful recruitment of Smallmouth Buffalo. In particular, length of the high flow pulse during the spawning season was included in all competing models and had a positive coefficient in all cases with two exceptions (neither of which was the best fit model). It is also worth noting that when high pulse length during the spawning season was experimentally removed from models during model selection procedures, adjusted R^2 was greatly reduced (adjusted $R^2 < 0.10$) and models were non-significant. Thus, even though duration of the high flow pulse during the spawning season was only marginally significant in many cases, it was critical to developing significant models with high explanatory power (Tables 4.3, 4.5, 4.7). This dichotomy is likely due to correlations with other flow attributes (even when excluding variables based on VIF scores) and higher-order effects. For example, duration of high flow pulses during the spawning season was positively correlated with rise and fall rates, monthly mean and max flows, and max 3, 7, 30 and 90 day flows during the spawning season (typically > 0.80 for max 3, 7, 30 and 90 day flows and rise and fall rates, which were excluded from models due to high multicollinearity) and negatively correlated with reversals, and the number and duration of low flow pulses during the spawning season.

Previous studies indicated the importance of high flow events for spawning by Smallmouth Buffalo, and high flow pulses have been identified as important spawning cues for other Catostomid species, such as Razorback Sucker *Xyrauchen texanus* and Blue Sucker *Cycleptus elongatus* (Tyus and Karp 1990, Tournabene et al. 2020). For Smallmouth Buffalo, rising water levels is known to serve as a spawning cue in aquaculture settings (Jester 1973, Walker and Frank 1953) and inundated vegetation is generally considered the preferred spawning substrate of Smallmouth Buffalo (Jester 1973, Hoyt 1979, Wrenn 1968). Moreover, larval Smallmouth Buffalo swim very feebly for their first few days of life, and consequently a long duration and slowly receding flood pulse would allow larvae to develop and grow in the productive and relatively safe floodplain habitats and slack waters that lack the high energetic demands of swimming in fast current (Hoyt et al. 1979, Sabo and Kelso 1991, Pease et al. 2006, Hoagstrom and Turner 2015). Those conditions correspond with the ‘fundamental triad’ of processes – nutrient enrichment, food concentration, and retention-dispersal of food resources and fish larvae – supporting recruitment of fishes in river systems (Hoagstrom and Turner 2015, Humphries et al. 2020). According to the Riverscape Recruitment Synthesis Model (Humphries et al. 2020), recruitment of periodic life-history strategist species in particular is driven by floodplain inundation, which is associated with nutrient enrichment from release from soil nutrients and decomposing vegetation (among other sources), concentration of food resources for larval fish in floodplain habitats and into slack waters during flood recession, and dispersal of larvae onto floodplains and into slack waters during flood recession.

In contrast with high pulse duration, the number of high flow pulses during the spawning season and mean monthly flows for April-July were only occasionally included in competing models (Tables 4.3, 4.5, 4.7). When present, the number of high flow pulses and mean monthly flows for April and July always had negative coefficients even though those variables were positively correlated with high flow pulse duration. In contrast, mean monthly flows for May and June had positive coefficients when included in models. The negative coefficient for the number of high flow pulses may be due to diminishing returns in terms of the benefits of additional high flow pulses to spawning and recruitment. Although the life history of Smallmouth Buffalo is not well known (e.g., Love et al. 2019), they likely exhibit a single predominant spawning bout per year with release of all eggs in a single spawning event (Winemiller and Rose 1992). Thus, additional high flow pulses during the spawning season may contribute only marginally to additional recruitment if most individuals had already spawned during earlier high flow events. In addition, frequent flood pulses during the spawning season could be disruptive to development and survivorship of Smallmouth Buffalo larvae. For example, additional high flow pulses after a successful spawning event could force weakly swimming larvae into suboptimal habitats or strand larvae in drying floodplain habitats as floodwaters recede (e.g., Bolland et al. 2015) or otherwise disrupt the 'fundamental triad' of processes described above that are beneficial for recruitment (Humphries et al. 2020). Positive coefficients for mean monthly flows in May and June but negative coefficients for April and July may indicate that the ideal conditions for spawning and subsequent recruitment (e.g., abundant floodplain vegetation, high aquatic productivity and appropriate temperatures for larval development) occur in the middle of the spawning season or that the spawning season is shorter than we initially believed.

The results discussed above support the importance of high flow events of sufficient magnitude, duration and timing for Smallmouth Buffalo spawning and successful recruitment. Unfortunately, rate of change of flows during spawning season could not be directly assessed in our modeling due to strong correlations with other flow attributes, including the duration of high flow pulses during the spawning season. The final two expectations, slow rate of change during the pre-spawn period and sufficient baseflows during post-spawn period were not well-supported by our models. Overall, very few flow attributes from pre- and post-spawn periods were included in candidate models, and flow attributes from the pre-spawn period were never included in the best-fit models. In contrast, low pulse duration during the post-spawn period was included in the best-fit model for the Brazos River and date of maximum flow was included in the best model for the Colorado River. The inclusion of low pulse duration post-spawn for the Brazos River appears to be driven almost entirely by the recruitment index value associated with just one year. As such, interpretations for that relationship would likely be premature. It is worth noting that previous research on flow variability and fish recruitment dynamics on the Brazos River (Zeug and Winemiller 2007), based on trends in gonadosomatic index values, found that recruitment of other periodic species (Longnose Gar *Lepisosteus osseus*, Spotted Gar *Lepisosteus oculatus*, and Gizzard Shad *Dorosoma cepedianum*) was associated with long-term flow trends, in particular high flow pulses during spring (spawning season). That said, there was evidence that many species, including a couple periodic life-history strategist species, were able to recruit in off-channel habitats even during dry years when the floodplain was not inundated (Zeug and Winemiller 2008). The negative coefficient for date of max flow for the Colorado River may indicate that recruitment is inhibited when flow pulses do not occur until late in the post-spawn period. Such flow pulses may be important for providing access to alternative habitats, including allowing individuals in backwater floodplain habitats to recruit to the main river channel (e.g. Zeug and Winemiller 2008), as well as ameliorating water quality (e.g. temperature and dissolved oxygen) and providing a pulse of food resources (e.g. Górski et al. 2016).

Studies demonstrating differences in the functional composition (based on life-history strategies) or structure of fish assemblages among rivers with different hydrology or after impoundment or water withdrawal are relatively common and observed worldwide. For example, species richness and abundance of periodic species tend to be higher in rivers with high periodicity to their flows, in particular high flow pulses (e.g., Hoeinghaus et al. 2007, Tedesco et al. 2008, Mims and Olden 2012), and population sizes, persistence and distributions of periodic species are negatively impacted by flow homogenization due to impoundments (Olden et al 2006, Bice et al. 2013, McManamay and Frimpong 2015, Winemiller et al. 2016, Arantes et al. 2019). In contrast, few studies of flow-recruitment relationships use similar methodological approaches as used in this study, specifically retrospective flow analysis modeling year-class strength as a function of attributes of the flow regime. That said, two recent studies on Alligator Gar *Atractosteus spatula* in the Trinity River, Texas, used year-class strength data and retrospective flow analyses to identify factors affecting differential recruitment among years (Robertson et al. 2018, Hoeinghaus et al. unpublished manuscript). Alligator Gar is an extreme periodic life-history strategist that, similar to Smallmouth Buffalo, spawns over shallow flooded vegetation during high flow pulses and exhibits high variability in year class strength. Recruitment of Alligator Gar can be limited to a few 'good' years when conditions are optimal interspersed by several 'poor' years when conditions are not favorable for recruitment, with population storage in long-lived adults (>70 years) sustaining the population through time (Robertson et al. 2018, Hoeinghaus et al. unpublished manuscript). Similar to findings for Smallmouth Buffalo reported herein, those studies found that Alligator Gar recruitment was strongly affected by sufficient duration of high flow pulses during the spawning season. An important difference is that both studies coupled floodplain inundation models with the daily discharge data to relate year-class strength to interannual variation in flow regimes and availability of preferred spawning habitat. This is a subtle yet important difference because spawning habitat availability does not increase linearly with increasing discharge but rather is a function of river channel and floodplain geomorphology and vegetation structure (Robertson et al. 2018, Hoeinghaus et al. unpublished). Stage-specific floodplain inundation models may also be useful for identifying the availability and temporal persistence of productive floodplain and slack water habitats that are expected to contribute to recruitment of periodic species in river-floodplain systems (Humphries et al. 2020). Thus, converting discharge data to spawning habitat availability and/or availability and persistence of nursery habitat would be an important refinement of this approach beyond characterizing the flow regime alone.

Additional refinements could be made to validate or improve assumptions associated with age estimates and estimated background mortality used to calculate recruitment index values. Specifically, otolith annuli formation can be validated using oxytetracycline (OTC) marking (e.g., Bettinger and Crane 2011, Buckmeier and Smith 2020). OTC can be injected at multiple known time points and is retained in the otolith enabling the researcher to validate several key attributes of otolith growth. In particular, OTC marking would be valuable to confirm where (relative to the nucleus) and in what time of year the first annuli is laid down. Answering both of these questions would help ensure that age estimates, and therefore recruitment index values, are accurate. Incorporating multiple readers and blind re-reads to assess accuracy and precision in the age estimates for this study indicated that our results should be robust. That said, error associated with the identification of the first annuli can lead to systematic bias, and even a single year of error in age estimates can substantially affect results due to the high degree of interannual variability in flows in these systems. Similarly, extreme high (e.g., Young et al. 2010) or low flows (e.g., Archdeacon and Reale 2020) can affect mortality rates, and flow-dependent recruitment of predator species such as Alligator Gar could further induce a flow-dependent component of mortality and thus bias recruitment index values calculated based on an assumption of constant mortality. The assumption of constant mortality could be further evaluated using field or experimental methods, and sensitivity of results to changes in the background mortality rate could be assessed analytically.

Finally, it is worth reiterating the potential utility of Smallmouth Buffalo as an indicator species for periodic life-history strategist species in flow-ecology studies. Their wide geographic range, with large populations throughout the Mississippi River drainage and drainages in the Gulf of Mexico as far south as the Rio Grande, coupled with a lack of conservation concern, means that they can be studied in diverse systems and used as an indicator for diverse periodic species of conservation concern found in those same systems (e.g., Robertson et al. 2018, Acre et al. 2021). The utility of Smallmouth Buffalo to serve as an indicator species for other periodic species of conservation concern could be further improved by studies of the reproductive biology and life-history of Smallmouth Buffalo, as well as research into preferred spawning habitat (e.g., vegetation type, depth, temperature) and conditions maximizing growth and survivorship of young-of-the-year individuals.

The refinements mentioned above would further elevate an already highly promising approach that integrates state-based and rate-based approaches to take advantage of the best elements of each. In this study, retrospective flow analyses using contemporary age-structure data to model flow attributes affecting recruitment success among years demonstrated the importance of high flow pulse duration during the spawning season and other complementary flow attributes in determining recruitment success. In addition to elucidating hydrological drivers of recruitment success, the models can be used to predict population responses to flow management scenarios and environmental change, and thus should be of interest to natural resource managers.

Brief Summary of Research Using Retrospective Analysis of Alligator Gar Recruitment in Relation to Flows in the Trinity River, Texas

Alligator Gar *Atractosteus spatula* is an extreme periodic life-history strategist species that, similar to Smallmouth Buffalo, spawns over shallow flooded vegetation during high flow pulses and exhibits high inter-annual variation in year class strength. Recruitment of Alligator Gar can be limited to a few 'good' years when conditions are optimal, separated by several 'poor' years when conditions are not favorable for recruitment (Robertson et al. 2018, Hoeinghaus et al. unpublished manuscript). In effect, Alligator Gar populations persist due to what has been termed a 'storage effect' whereby long-lived adults (>70 years) survive good and bad years and undertake repeated bouts of reproduction that, over the long term, results in sufficient recruitment.

Recent studies on Alligator Gar in the Trinity River, Texas, used year-class data and retrospective flow analyses that included floodplain inundation modeling of spawning habitat availability to identify factors affecting recruitment among years (Robertson et al. 2018, Hoeinghaus et al. unpublished manuscript). Similar to the findings for Smallmouth Buffalo reported from this project, those studies found that Alligator Gar recruitment was strongly affected by factors associated with high flow pulses during the spawning season. Specifically, Robertson et al. (2018) found that years of past Alligator Gar recruitment success in the middle Trinity River were correlated with spawning habitat availability from May through July. PCA (principal components analysis) of hydrological variables and subsequent Spearman rank correlation indicated 30-day maximum spawning habitat availability, 90-day maximum spawning habitat availability, July maximum cumulative duration of high flow pulse, and mean spawning habitat availability in June were positively correlated with Alligator Gar recruitment success. The authors identified several additional variables that appeared to be highly correlated with the significant variables indicated above, yet by themselves were not statistically significant: maximum spawning habitat availability, 3-day maximum spawning habitat availability, 7-day maximum spawning habitat availability, July high flow pulse total duration, and July mean spawning habitat availability.

Hoeinghaus et al. (unpublished manuscript) adopted a similar approach for the lower Trinity River, but also included multiple levels for spawning habitat suitability (rather than just availability) and modeled recruitment based on recruitment index values as done in the Smallmouth Buffalo analyses for the present project. Similar to findings reported by Robertson et al. (2018), results from correlations with exceptional year classes in PCA indicate high mean and max flows of long duration, both early in the spawning season and especially later in the spawning season. Multiple models were run for the entire time series (i.e., 1950-2012) as well as a reduced time series (i.e., 1980-2012) and using recruitment index values as a continuous response variable (multiple linear regression) as well as exceptional year class as a categorial variable (logistic regression) to control for bias associated with increasing error of age estimation and increasing number of zeros in the dataset for older age classes. Using the entire time series in a logistic regression of exceptional age classes, with an adjustment of the oldest-aged individuals based on evidence from the PCA, resulted in a significant model with a single predictor variable: maximum 7-day spawning habitat availability. The same parameter was included in the reduced time series logistic regression model (1980-2012), but due in part to the limited number of exceptional year classes, this model exhibited complete separation and thus parameter estimates for forecasting were not available (which was one important reason for choosing Smallmouth Buffalo for the current project). Multiple regression models of recruitment index values for the 1980-2012 period indicated that recruitment of Alligator Gar in the lower Trinity River was positively associated with high monthly mean flows, especially later in the spawning season and particularly for July, and negatively affected by the number of pulse events.

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Table 4.26. Hydrologic parameters used to quantify variation in the flow regime based on daily data from USGS gages. Factors with an 'x' in the Pre-Spawn, Spawn, and Post-Spawn columns were quantified for those periods as indicated. Pre-spawn is January and February, spawn is March through July, and post-spawn is August through December; [month] indicates for each month, assigned to the appropriate season.

Variable (unit)	Definition	Variable abbreviations	Pre-Spawn	Spawn	Post-Spawn
Mean monthly flow (cfs)	Mean flow for each month	[month]_mean	x	x	x
7-day minimum flow (cfs)	7-day minimum flow	min_7day			x
7-day maximum flow (cfs)	7-day maximum flow	max_7day	x	x	x
Zero Days	Number of zero-flow days	n_Zero			x
Base flow index	7-day minimum flow/mean flow	Base_Flow			x
Date max	Julian date of maximum flow	Date_max	x	x	x
Low flow pulse number	Number of low flow pulse events	Lo_pulseN		x	x
Low flow pulse length (days)	Mean number of days per low flow pulse event	Lo_pulseL		x	x
High flow pulse number	Number of high flow pulse events	Hi_pulseN	x	x	x
High flow pulse length (days)	Mean number of days per high flow pulse event	Hi_pulseL	x	x	x
Rise rate (cfs)	Mean of all positive differences between consecutive daily values	Rise_rate	x	x	x
Fall rate (cfs)	Mean of all negative differences between consecutive daily values	Fall_rate	x	x	x
Reversals	Number of changes in flow to different environmental flow components	Reversals	x	x	x

Table 4.2. Summary statistics of the competing flow-recruitment models for the Brazos River. Models designated with an ‘S’ include only parameters from the spawning season, and models designated with an ‘F’ include parameters for the full year. Yellow highlight and bold font indicate the best-fit model primarily used in interpretations.

Model	R^2	Adj. R^2	AIC	ΔAIC	BIC	C_p	p-value	K
S1	0.29	0.17	40.28		0.21	1.01	0.13	2
S2	0.38	0.21	40.33	0.04	2.42	1.79	0.14	3
S3	0.42	0.18	41.37	1.09	5.66	3.24	0.21	4
S4	0.33	0.15	41.47	1.19	2.88	2.48	0.21	3
S5	0.33	0.14	41.57	1.28	2.92	2.54	0.21	3
S6	0.32	0.14	41.61	1.33	2.94	2.57	0.21	3
S7	0.41	0.17	41.61	1.33	5.70	3.38	0.22	4
S8	0.47	0.18	41.89	1.60	9.39	4.47	0.25	5
S9	0.38	0.14	42.19	1.90	5.81	3.70	0.26	4
S10	0.38	0.14	42.23	1.94	5.81	3.73	0.26	4
S11	0.29	0.10	42.28	1.99	3.20	3.01	0.27	3
S12	0.29	0.10	42.28	2.00	3.21	3.01	0.27	3
F1	**0.59**	**0.47**	**34.22**		**-2.34**	**0.49**	**0.02**	**3**
F2	0.61	0.46	35.16	0.93	1.44	1.98	0.03	4
F3	0.70	0.48	35.22	0.99	11.34	4.36	0.07	6
F4	0.65	0.46	35.57	1.34	5.82	3.28	0.05	5
F5	0.60	0.44	35.75	1.53	1.57	2.26	0.04	4
F6	0.59	0.43	36.06	1.84	1.64	2.41	0.05	4
F7	0.59	0.43	36.07	1.84	1.64	2.41	0.05	4
F8	0.59	0.42	36.20	1.98	1.67	2.48	0.05	4
F9	0.59	0.42	36.21	1.98	1.67	2.48	0.05	4

Table 4.3. Brazos River multiple regression models with coefficients of constituent IHA parameters included as independent variables. Variables with a p-value < 0.05 are in bold; variables with a p-value > 0.1 are in gray. Models designated with an 'S' include only parameters from the spawning season, and models designated with an 'F' include parameters for the full year. Yellow highlight indicates the best-fit model primarily used in interpretations. Model statistics are in Table 4.2.

	Spawning season flow attributes								Post-spawn
Model	May mean	July mean	Lo_pulseN	Lo_pulseL	Hi_pulseN	Hi_pulseL	Reversals	Date max	Lo_pulseL
S1				0.24		0.44			
S2				0.40	-0.41	0.66			
S3				0.36	-0.60	0.64	-0.29		
S4			0.22	0.28		0.53			
S5		-0.23		0.28		0.56			
S6				0.31		0.39		-0.19	
S7	0.21			0.45	-0.54	0.62			
S8	0.31			0.42	-0.83	0.59	-0.39		
S9		-0.10		0.40	-0.37	0.69			
S10				0.41	-0.37	0.62		-0.08	
S11	0.02			0.24		0.43			
S12				0.24		0.44	0.00		
F1				-0.14		0.39			**0.67**
F2	0.20			-0.16		0.29			**0.73**
F3	0.38			-0.02	-0.59	0.39	-0.35		**0.64**
F4	0.30			0.00	-0.32	0.42			**0.66**
F5				-0.04	-0.17	0.48			**0.62**
F6				-0.17		0.35	-0.08		**0.68**
F7				-0.12		0.38		-0.02	**0.66**
F8			-0.03	-0.15		0.38			**0.68**
F9			-0.03	-0.15		0.38			**0.68**

Table 4.4. Summary statistics of the competing flow-recruitment models for the Colorado River. Models designated with an ‘S’ include only parameters from the spawning season, and models designated with an ‘F’ include parameters for the full year. Yellow highlight and bold font indicate the best-fit model(s) primarily used in interpretations.

Model	R^2	Adj. R^2	AIC	ΔAIC	BIC	C_p	p-value	K
S1	**0.65**	**0.45**	**54.36**		**14.27**	**5.46**	**0.04**	**5**
S2	0.57	0.39	55.86	1.50	10.92	5.50	0.05	4
S3	0.62	0.41	55.90	1.55	14.14	6.31	0.06	5
S4	0.66	0.41	55.94	1.59	18.21	7.25	0.07	6
S5	0.65	0.41	56.02	1.66	18.18	7.29	0.08	6
S6	0.56	0.38	56.28	1.92	10.99	5.77	0.05	4
F1	0.82	0.70	44.17		6.92	7.07	0.00	6
F2	0.80	0.69	44.29	0.12	2.75	6.20	0.00	5
F3	**0.77**	**0.68**	**44.60**	**0.42**	**-0.46**	**5.59**	**0.00**	**4**
F4	0.82	0.66	46.04	1.87	10.96	9.00	0.01	7
F5	0.80	0.66	46.15	1.97	6.32	8.12	0.01	6

Table 4.5. Colorado River multiple regression models with coefficients of constituent IHA parameters included as independent variables. Variables with a p-value < 0.05 are in bold; variables with a p-value > 0.1 are in gray. Models designated with an ‘S’ include only parameters from the spawning season, and models designated with an ‘F’ include parameters for the full year. Yellow highlight indicates the best-fit model primarily used in interpretations. Model statistics are in Table 4.4.

	Spawning season flow attributes							Pre-spawn	Post-sp
Model	June mean	July mean	Lo_pulseN	Hi_pulseN	Hi_pulseL	Reversals	Date max	Fall_rate	Date n
S1	0.52	-0.43	**1.01**	-0.51	0.20				
S2	0.44		**0.93**		0.02	**0.61**			
S3	0.52		**1.00**	-0.37	-0.02	0.38			
S4	0.46	-0.50	**1.00**	-0.53	0.20		0.14		
S5	0.52	-0.35	**1.02**	-0.42	0.17	0.16			
S6	0.50		**0.95**	**-0.63**	-0.09				
F1	0.32	**-0.53**	**0.72**	-0.23	0.23				**-0.55**
F2	0.22	**-0.60**	**0.60**		0.27				**-0.63**
F3		**-0.56**	**0.56**		0.32				**-0.66**
F4	0.33	**-0.54**	**0.73**	-0.23	0.23		**-0.54**	0.38	
F5	0.23	**-0.61**	**0.62**		0.27			0.43	**-0.62**

Table 4.6. Summary statistics of the competing flow-recruitment models for the Guadalupe River. Models designated with an ‘S’ include only parameters from the spawning season, and models designated with an ‘F’ include parameters for the full year. Yellow highlight and bold font indicate the best-fit model primarily used in interpretations.

Model	R^2	Adj. R^2	AIC	ΔAIC	BIC	C_p	p-value	K
S1	0.94	0.83	2.87		-1.47	6.01	0.05	6
S2	0.93	0.84	3.23	0.35	-7.16	4.54	0.02	5
F1	0.95	0.75	4.84		8.46	8.00	0.18	8

Table 4.7. Guadalupe River multiple regression models with coefficients of constituent IHA parameters included as independent variables. Variables with a p-value < 0.05 are in bold; variables with a p-value > 0.1 are in gray. Models designated with an ‘S’ include only parameters from the spawning season, and models designated with an ‘F’ include parameters for the full year. Yellow highlight indicates the best-fit model primarily used in interpretations. Model statistics are in Table 4.6.

	Spawning season flow attributes							Pre-spawn
Model	April mean	Lo_pulseN	Lo_pulseL	Hi_pulseN	Hi_pulseL	Date max	Reversals	Date max
S1	-0.68	0.17	-0.3		0.12	-0.43	**-1.21**	
S2	**-0.86**		-0.36		0.25	-0.53	**-1.29**	
F1	-0.68	0.17	-0.31	-0.13	0.12	-0.44	-1.2	-0.02

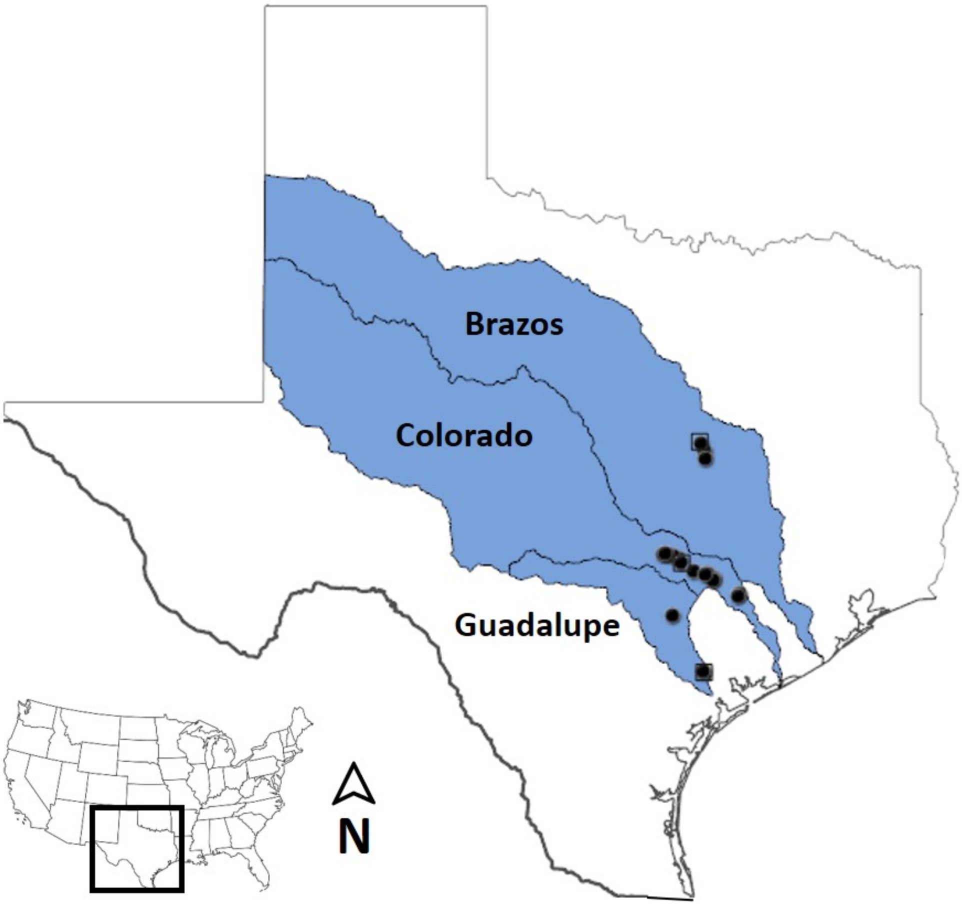

Figure 4.1. Map of Texas with Brazos, Colorado and Guadalupe river basins identified in blue. Sampling locations (black circles) and the USGS gaging station used for flow analyses (open squares: Brazos River at Waco USGS 08096500, Colorado River at Bastrop USGS 08159200, Guadalupe River at Victoria USGS 08176500) are identified for each basin. Inset identifies location of Texas within the continental United States.

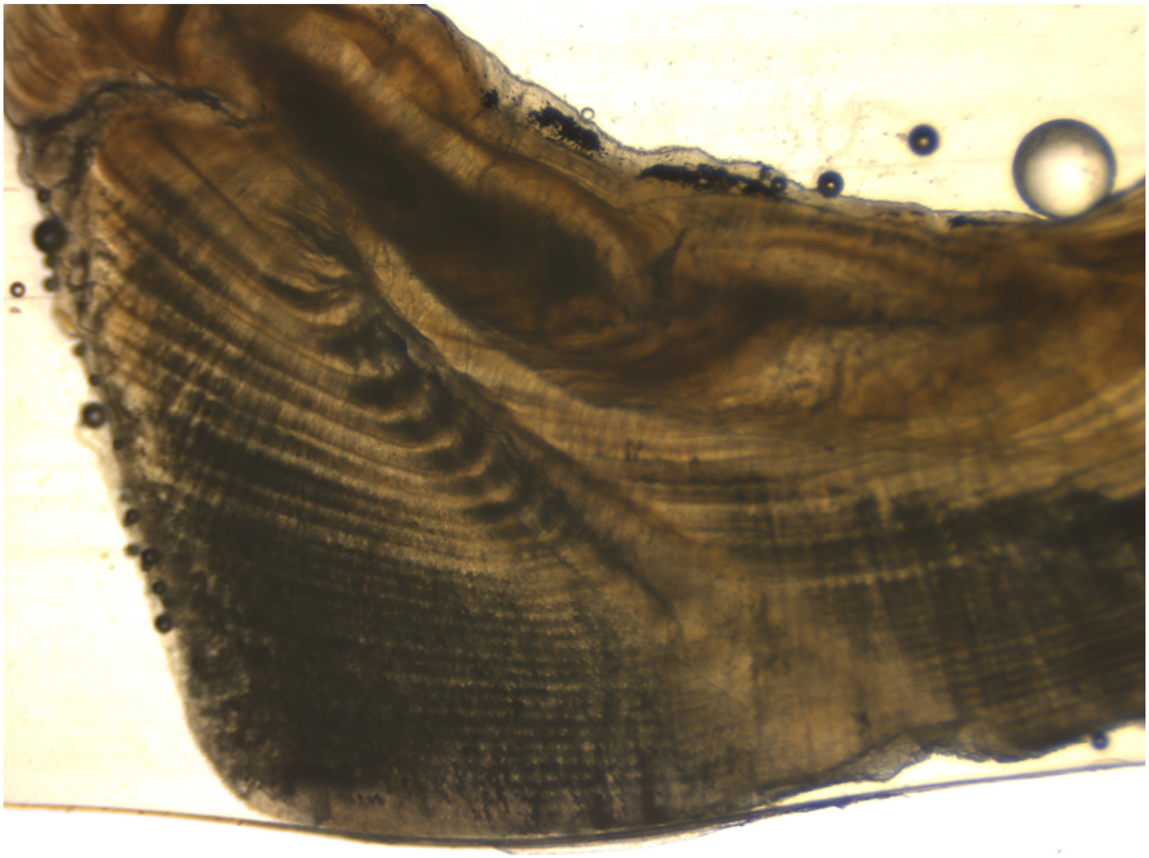

Figure 4.2. Sectioned lapillar otolith from a Smallmouth Buffalo.

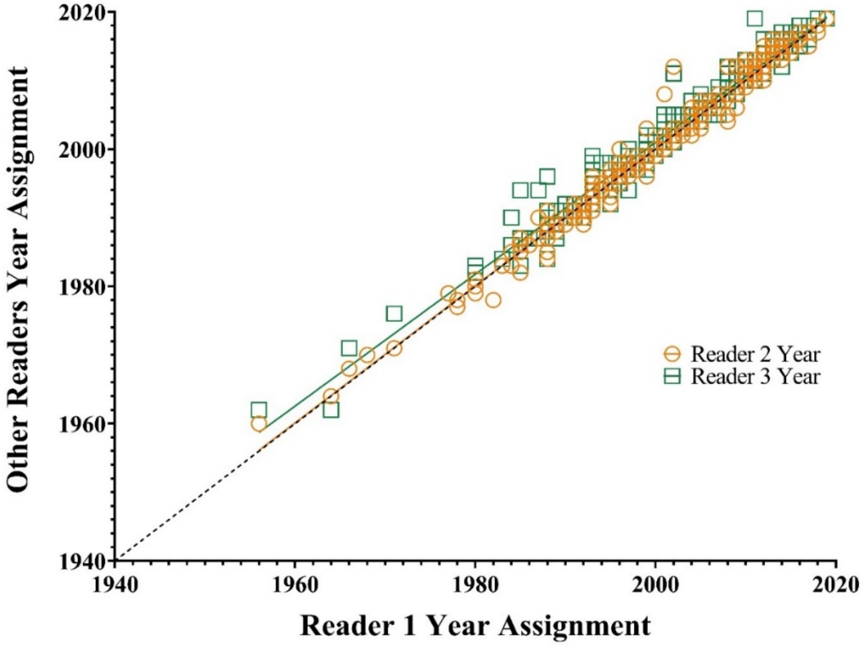

Figure 4.3. Correlation of year class assignments between Reader 1 (Cole Reeves) and Reader 2 ($R^2 = 0.987$) and Reader 3 ($R^2 = 0.976$). Age estimates by Reader 1 were used in all analyses. The dashed line is a 1:1 line.

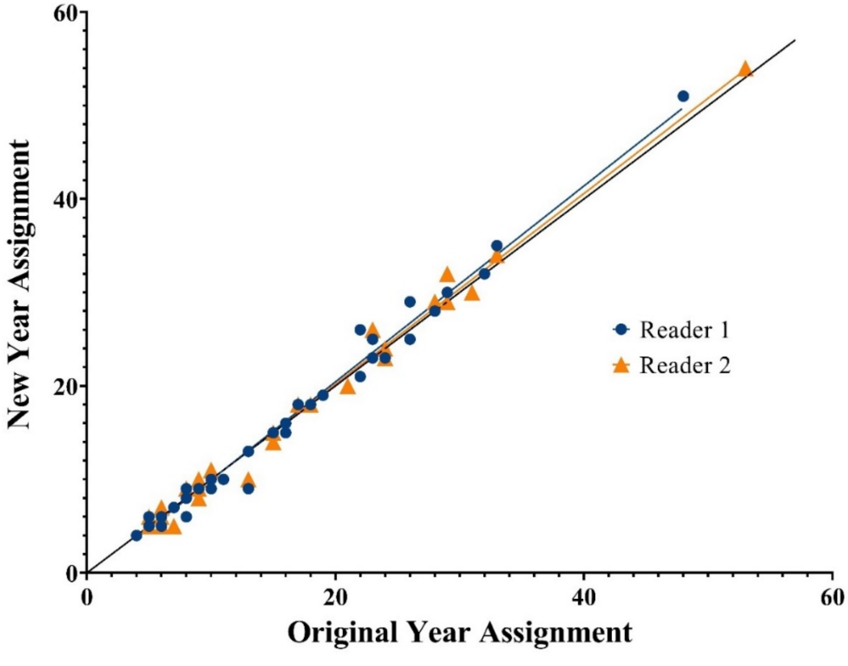

Figure 4.4. Correlation of original and repeat age estimates for 50 randomly selected otoliths for two independent readers (Reader 1: $R^2 = 0.988$; Reader 2: $R^2 = 0.990$). Original age estimates were used in all analyses. The black line is a 1:1 line.

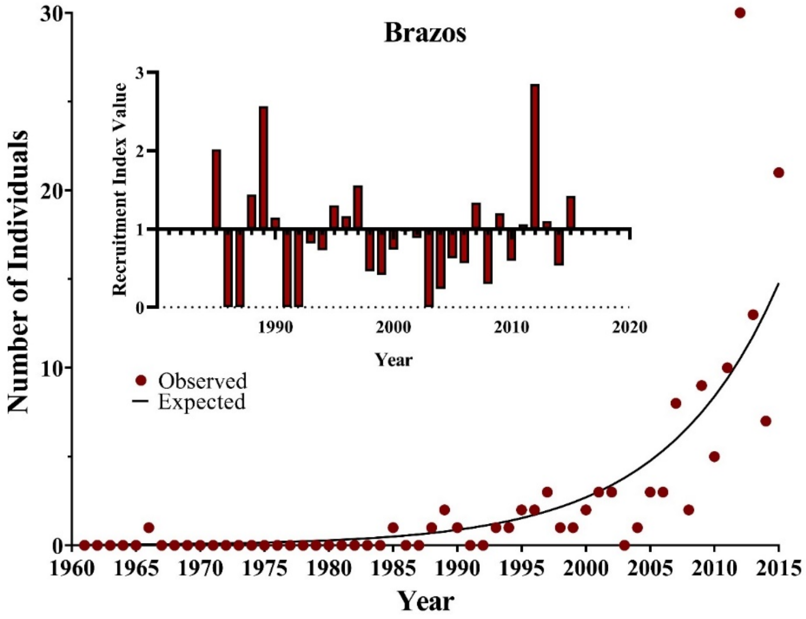

Figure 4.5. Observed (red circles) vs. expected (black line) number of Smallmouth Buffalo from the Brazos River assigned to each year class. The inset figure depicts Recruitment Index values for each year for the period 1985-2015.

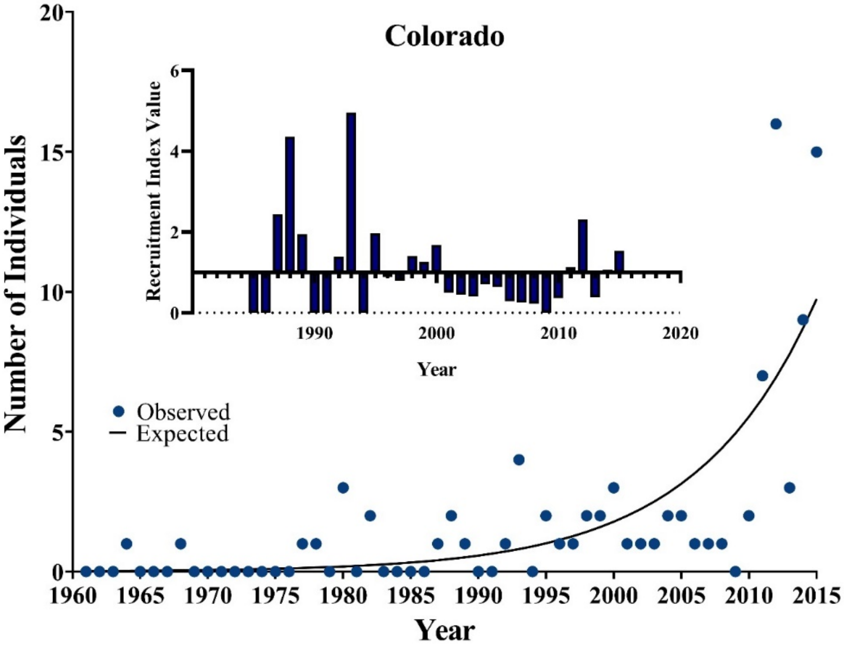

Figure 4.6. Observed (blue circles) vs. expected (black line) number of Smallmouth Buffalo from the Colorado River assigned to each year class. The inset figure depicts Recruitment Index values for each year for the period 1985-2015.

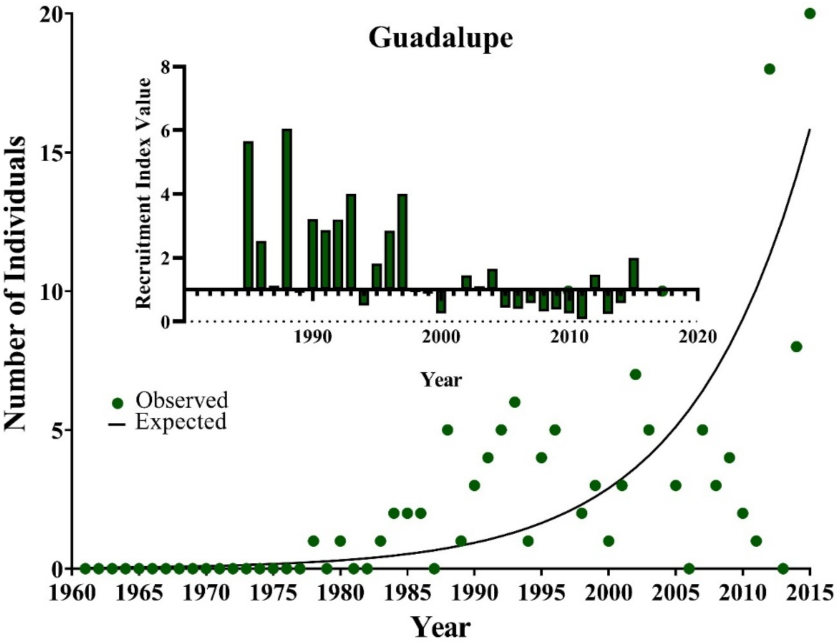

Figure 4.7. Observed (green circles) vs. expected (black line) number of Smallmouth Buffalo from the Guadalupe River assigned to each year class. The inset figure depicts Recruitment Index values for each year for the period 1985-2015.

Chapter 5. Response of Indicator Taxa to Flow Variation – Shoal Chub

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Background

Flows in rivers the world over are influenced by human activities such as impoundment construction and flow regime alteration (Lehner et al. 2011). A central concept in the conservation, management, and protection of riverine resources and biodiversity is the natural flow regime paradigm (NFR) developed by Poff et al. (1997). The NFR describes temporal dynamics (i.e., regimes) in riverine systems by quantifying five characteristics of hydrology, including magnitude (i.e., how much water is present), frequency (i.e., how often flows of a given magnitude occur), duration (i.e., how long flows of a given magnitude persist), timing (i.e., when flows of a given magnitude occur), and rate of change (i.e., how quickly flows transition among magnitudes). These characteristics can be used to assess departures from natural flow regimes caused by river modifications (e.g., reservoir construction) using a suite of metrics collectively known as indicators of hydrologic alteration (described in detail by Richter et al. 1996, Mathews and Richter 2007). Because of links between the NRP and ecological settings (e.g., Bunn and Arthington 2002), water resource management frequently seeks to establish links between flow metrics and ecological processes as a means of informing water decisions such as reservoir releases and water rights permitting. This process is commonly referred to as "environmental flows" (Arthington et al. 2018) and is often guided by the establishment of "flow-ecology relationships" (Davies et al. 2013) that describe the effects of flow modification on ecological conditions and processes (Tonkin et al. 2014). A recent synthesis of flow-ecology relationships points to a continuum of approaches based somewhere between "pure state" approaches that assess the instantaneous condition of a river ecosystem to "pure rate" approaches that assess dynamic processes within river ecosystems (Wheeler et al. 2018).

An emerging rate-based method for assessing flow-ecology relationships is the consideration of the effects of flow on recruitment of organisms. Recruitment – *defined as the increase in populations size as progeny grow and survive* – can be measured across riverine organisms (e.g., from fishes to riparian trees) using a number of methods. A commonly used method to measure recruitment is to ascertain the age of individuals and back-calculate when they were born (or hatched or germinated). For fishes, back-calculation of age is commonly performed on hard structures such as scales, fin rays, and otoliths (Maceina et al. 2007, Quist and Isermann 2017). Among these, otoliths are the most commonly used hard structure used to estimate age. Otoliths are calcium carbonate structures that occur in three pairs (asteriscus, lapillus, sagittal) in the heads of fishes, and grow as fish grow by accumulating new material around the edges of a core through a process known as accretion (Campana 1999). Accretion results in daily growth increments that can be used to age fishes at daily (in young or short-lived specimens) and annual time scales (Brothers et al. 1976). In the context of flow-ecology relationships, estimation of fish recruitment using otoliths is an emerging method for establishing linkages between flow and ecological rates (e.g., Rodger et al. 2016, Buckmeier et al. 2017).

Daily growth increments and the resulting developmental patterns on otoliths have received widespread attention among studies of Great Plains Cyprinidae fishes (Table 1). Studies routinely include flow-ecology investigations for "chubs", such as Shoal Chub (*Macrhybopsis hyostoma*; Rodger et al. 2016, Starks et al. 2016), Peppered Chub (*M. tetranema*; Durham and Wilde 2006), and Flathead Chub (*Platygobio gracilis*; Haworth and Bestgen 2016, 2017). Among these studies, emergent patterns include higher recruitment associated with high flow periods for Shoal Chub (Rodger et al. 2016) or higher recruitment associated with declining flow periods immediately following flow pulses for Shoal Chub and Flathead Chub (Durham and Wilde 2006; Haworth and Bestgen 2017). These patterns are evident despite the fact that these studies all varied slightly in their methods used to estimate hatch dates. Durham and Wilde (2006) extracted sagittal otoliths from Peppered Chub and Flathead Chub collected with seines; Rodger et al. (2016) extracted asteriscus otoliths from Shoal Chub collected with drift nets; Haworth and Bestgen (2016, 2017) extracted sagittal otoliths from Flathead Chub collected with both drift nets and seines. Comparisons suggest hatch dates estimated from drift net samples and seine samples produce similar results (Haworth and Bestgetn 2017). Furthermore, validation studies in which fish of known age were used to assess the reliability of otoliths for aging exist for lapillus otoliths (Durham and Wilde 2008a) and sagittal otoliths (Haworth and Bestgen 2016). Regardless of otolith choice, these studies confirmed daily increments after hatching. Therefore, daily growth increments of otoliths represent a reliable method for assessing age of young fish.

The purpose of this study was to use daily growth increments of Shoal Chub collected from the lower Brazos River to assess relationships between flow and Shoal Chub recruitment. Previous research on this same species in this same river system revealed a quadratic relationship between flow and recruitment and emphasized that additional research over a longer time period and broader range of flows was *"urgently needed"* (Rodger et al. 2016). Although discharge magnitude is commonly the focus of flow-ecology investigations (Poff and Zimmerman 2010), there is a need for greater inclusion of additional natural flow paradigm (NFP) characteristics in flow-ecology research (Nguyen et al. In Press). This work addresses this research need by including consideration of relationships between Shoal Chub recruitment and flow magnitude, frequency, rate of change, and duration. Flow magnitude was assessed by considering the relationship between the number of Chub hatched on a given day and the daily average discharge on that day (Rodger et al. 2016). Frequency of high flow events from the Brazos BBEST (2012) were considered simultaneously with duration measured as the number of days since flows fell below frequency thresholds, and the combined effects of these parameters were regressed against Chub recruitment to reveal the duration of recruitment windows following flows of specified frequency and magnitude. Rate of change was considered by regressing fall rate (i.e., the sequential decrease in discharge between two days during the declining limb of the hydrograph) and rise rate (i.e., the sequential increase in discharge between two days during the ascending limb of the hydrograph) against Shoal Chub recruitment. Together, these approaches fill a research need pertaining to extending flow-ecology relationships beyond only measures of discharge magnitude and address a need to understand flow requirements for an imperiled fish species.

Methods

Study Area. – Initial investigation of Shoal Chub flow-recruitment relationships included portions of the Brazos, Colorado, and Guadalupe river basins. However, high flows and difficulty collecting large numbers of specimens from the Colorado and Guadalupe rivers relegated the study to focus on the Brazos River at the Farm to Market Road 485 crossing west of Hearne, Texas in Milam County. At this location, age-0 Shoal Chub were easily encountered over a large gravel bed just upstream of the bridge crossing and just downstream of remnants of an old lock-and-dam structure. The sampling location (30° 51' 54.3"; 96° 41' 42.6") has a contributing drainage area of 56,332 km^2 and is surrounded by agricultural land uses including cultivated crops. The nearest U.S. Geological Survey stream gage included in the Senate Bill 3 validation process (Brazos BBEST 2012) is gage 08108700 located 46 km downstream at the State Highway 21 crossing near Bryan, Texas, and all analyses of flow are based on data obtained from this gage in cubic feet per second (cfs).

Field collections. – Seining collections were conducted on the Brazos River on August 30, September 2, September 8, September 21, September 26, and October 1 of 2019, and on June 19, August 18, August 23, and August 30 of 2020. Collections consisted of seining with a larval seine (3.6 m long, 1.8 m deep, 0.8 mm mesh) for one hour and retaining all Shoal Chub <40 mm total length (TL). The threshold of 40 mm TL was selected because this is likely the upper limit of age-0 individuals (Starks et al. 2016). Collected fish were euthanized in a lethal dose of MS-222 (80 mg/L), stored together by sampling date in 95% ethanol, and transported to the laboratory for later processing.

Laboratory methods. – In the laboratory, total length of all individuals was measured before removal of the lapillus otolith from both sides of the head. Once removed, individual otoliths were mounted on a microscope slide using thermoplastic cement with the macular hump facing upwards (Starks et al. 2016). The surface of each otolith was sanded using 6, 3, and 1 micrometer grit sandpaper until the macular hump was sanded down and an evenly polished surface was apparent. A drop of emersion oil was then added to the polished surface and the slide was placed underneath a compound microscope. Under 20x magnification, the polished surface of the otolith was brought into focus and then focus was continued downward until the aging plane of the otolith revealed daily growth increment rings. Rings were counted beginning at the nucleus and extending along the longest plane of the otolith to the edge of the otolith to estimate age in days. Data from 20 individuals processed in the previously described manner were combined with 68 Shoal Chub aged using the asteriscus otoliths by Rodger et al. (2016) to assess the relationship between age (days) and total length (mm).

Statistical analyses. – The relationship between age and total length was analyzed using linear regression. Differences in the linear relationships between age and length for data collected in this study versus data collected by Rodger et al. (2016) were tested using analysis of covariance (ANCOVA). The relationship between the number of Chub hatched on a given day and the discharge magnitude on that same day was quantified using a generalized additive model

(GAM). A GAM was selected because the relationship was non-linear and the response variable was count data, meaning a Poisson-based error distribution was more appropriate than a Gaussian-based error distribution (i.e., because count data cannot be negative). Prior to this analysis, discharge values for the day each Chub hatched were binned into 200-cfs bins so that fish hatched on days with very similar discharges were combined. The relationship between number of Chub hatched and flow (cfs) was explored based on data from this study only as well as data from this study plus data from Rodger et al. (2016). Next, the relationship between the number of Chub hatched and time since flows declined past Brazos BBEST (2012) flow thresholds was quantified using GAMs. These thresholds included the recommended magnitudes for two pulses per summer season under average flow conditions (5,000 cfs), three pulses per summer season under average flow conditions (2,990 cfs), four pulses per summer season under average flow conditions (2,060 cfs), and the threshold for base flow under average summer conditions (920 cfs). Finally, the relationship between rise rate and fall rate and the number of Chub hatched on a given date were analyzed with GAMs.

The additive effects of discharge magnitude, timing, and rate of change on Shoal Chub hatches with subsequent survival were analyzed using a GAM. Parameters considered for inclusion in the model were discharge magnitude on the day of hatch (cubic feet per second; cfs), rate of change in discharge from the day prior to hatch to the day of hatch (cfs/d), and the number of days that pasted between hatch date and when flows had previously declined past Brazos BBEST (2012) thresholds for base flow (920 cfs), four pulses per season (2,060 cfs), three pulses per season (2,990 cfs), and two pulses per season (5,000 cfs). Prior to fitting the model, multicollinearity among predictor variables was tested by computing pairwise Pearson correlation coefficients and any relationships with a value $>|0.70|$ were addressed by removing correlated variables. A GAM was then fit using the 'gam' function from the 'mgcv' package in R version 4.0.4 (Wood 2004). The 'gam' function allows for automatic variable selection and testing significance of smoothing parameters. Parameter selection followed the methods of Mara and Wood (2011) by setting the argument "select = T" in the R script. The resulting GAM was checked for model fit and used to predict the number of Chub hatched on each day during May 1 through August 31 for each year for which historical flow data were available at the Brazos River at Highway 21 crossing USGS gage, which was combined with the former Brazos River near Bryan, Texas gage (Brazos BBEST 2012). Estimated hatches for each day were summed across the entire summer season (May 1 to August 31) to generate an estimate of the number of Chub hatched and surviving during the summer. The total estimated fish that hatched and survived for each year was then regressed against time using a locally weighted regression to illustrate long-term patterns in estimated Shoal Chub recruitment. Summer flow regimes for the five most recent high recruitment years and the five most recent low recruitment years were plotted to illustrate differences in flow regime, particularly in meeting Brazos BBEST (2012) flow recommendations.

Results

A total of 254 age-0 Shoal Chub was collected from the lower Brazos River between 2019 (n = 38) and 2020 (n = 216; Table 2). The relationship between measured length and age estimated from otolith readings was consistent for fishes aged during this study (n = 20) and the study by Rodger et al. (2016; n = 68; Figure 5.1a). The ANCOVA analysis revealed no significant interaction effect between age and the categorical factor for study ($F_{1,84} = 0.114$, p=

0.74) when predicting total length. This result confirmed that the relationship between age and length was consistent among the two aging studies and data were therefore combined (Figure 5.1b). The linear regression model ($y = 6.7 + 0.56*x$) fit to the relationship between age and total length was significant ($p< 0.001$) and illustrated that age explained 97% of the variation in total length. Based on this strong relationship, this model was used to assign an estimated age to all remaining 234 Shoal Chub of known length collected from the Brazos River during 2019 and 2020 (Figure 5.2).

The temporal sequence of Shoal Chub recruitment revealed periods of increased recruitment following flow pulses (Figure 5.3). In 2019, there was a period of recruitment in late July and early August following elevated flows in the prior months of May and June. In 2020, bursts of recruitment were evident after smaller flow pulses (relative to those in 2019), though the number of fish that hatched and survived was greater in 2020. The relationship between daily discharge and the number of Chub that hatched and survived reveals a general decline in the number of surviving hatchlings as flow tiers increased, though the relationship was characterized by multiple "humps" that represented increases in recruitment near the thresholds for 4, 3, and 2 pulses per season (Figure 5.4a). The smoothing function for discharge in the GAM was significant ($p< 0.001$), and the model revealed that discharge explained 68% of variation in the number of Chub that hatched and survived. Though this result suggests that lower flow magnitudes equated to higher recruitment, subsistence flows did not occur during the period of study. Furthermore, there was a decline in recruitment across the three smallest discharge values that suggested unobserved flows below the threshold for subsistence were unlikely to result in a continued increase in recruitment. When data from Rodger et al. (2016) were included in the analysis, the general pattern did not change except that the second-highest increase in Chub recruitment occurred between the thresholds for 4 and 3 pulses per season (Figure 5.4b). The smoothing parameter for discharge magnitude was significant ($p< 0.001$) and discharge explained 48% of variation in the number of Chub that hatched and survived.

Analyses revealed that flow frequency and duration were correlated with Shoal Chub recruitment. The relationship between the number of Chub hatched and time since flows fell below Brazos BBEST flow thresholds revealed that larger pulses were associated with longer recruitment windows during the descending limb of the hydrograph. The GAM fit to the relationship between the number of Chub hatched/surviving and time since flows fell below the two pulses per season threshold illustrated that recruitment was greatest during 25-30 days and continued for up to 50 days after flows fell below the threshold (Figure 5.5). The smoothing parameter for time in the GAM was significant ($p< 0.001$) and showed that time explained 44% of variation in the number of Chub that hatched and survived. When the threshold for three pulses per season was analyzed, the smoothing parameter for time was again significant ($p< 0.001$), and time explained 41% of variation in the number of fish that hatched and survived. However, the largest number of hatches with survival occurred either while flows were above the three pulses per season threshold (i.e., zero days since decline past the threshold) or one day following decline past the threshold. The GAM model fit to the threshold for four pulses per season had a significant smoothing parameter for time ($p< 0.001$) and illustrated that time since decline past the threshold explained 90% of variation in the number of Chub that hatched and survived. This was driven by the large number of individuals that recruited prior to flows falling past the threshold and, to a lesser extent, during the first two days after flows declined past the threshold. The period for recruitment extended approximately 30 days past the thresholds for both three and four pulses per season (Figure 5.5). Finally, the GAM fit to the relationship between the number of Chub that hatched/survived and days since flow fell below the base flow threshold revealed a significant effect of time ($p < 0.001$) that explained 99% of variation in the number of Chub that hatched and survived. The high level of variation explained was associated with a large number of fish recruiting prior to flows declining past the base flow threshold and a narrow window of time (i.e., 12 days) over which recruitment continued as flows declined below the threshold for base flow.

Shoal Chub recruitment correlated with flow fall rate but not rise rate. Only six fish recruited at a rate of change of exactly zero, and these fish were excluded from the analyses of rate of change. The GAM fit to the relationship between the number of Chub that hatched and fall rate had a significant smoothing term for fall rate ($p < 0.001$) and revealed that fall rate explained 58% of variation in the number of Chub that hatched and survived (Figure 5.6). The highest number of hatches with subsequent survival occurred at slow fall rates (<-5 cfs/d) and remained low when fall rate exceeded -5 CFS/d. The GAM fit to the relationship between the number of Chub hatched/surviving and rise rate did not have a significant smoothing function for rise rate ($p = 0.215$), and rise rate consequently explained only 2% of variation in the number of Chub that hatched and survived.

Only one variable, time since flows declined past the threshold for 4 pulses per season, was removed prior to model fitting because of concern over multicollinearity (Table 4). All remaining variables were retained. The resulting GAM had an adjusted coefficient of determination (R^2) of 0.42 and explained 58.5% of deviance in Shoal Chub recruitment. Smoothing terms were significant for discharge magnitude ($p < 0.001$), time since flows declined past two pulses per season ($p < 0.001$), and time since flows declined past base flow ($p = 0.002$; Table 5). The smoothing term for rate of change was marginally significant ($p = 0.084$) and the smoothing term for time since flow declined past 3 pulses per season was not significant ($p = 0.887$). Based on individual smoothing terms in the model, shoal Chub hatches with survival declined as discharge magnitude on the day of hatch increased (Figure 5.7a), and the highest number of Chub hatched with survival occurred within 10 days of flows declining past the threshold for 2 pulses per season (Figure 5.7b). The number of surviving Chub hatchlings ranged 3-6 for up to a week after flows declined past the threshold for base flow, then decline rapidly (Figure 5.7c). Surviving Shoal Chub hatchlings were highest at positive or only slightly negative rates of change, but declined as fall rate became increasingly negative (Figure 5.7d).

Long-term annual projections (1900-2020) for the number of Chub hatched with subsequent survival during May through August showed a decline after approximately 1960 (Figure 5.8). The locally weighted regression model was essentially flat during 1900-1960 despite missing flow data during the early 1900s. After 1960, there was a precipitous decline in estimated recruitment until approximately 1975, when the rate of decline slowed but remained steady until 2020. The years with the lowest estimated recruitment all occurred after 1975. Examples of recent (i.e., post-1980) years with above average estimated recruitment included 2000, 2001, 2002, 2008, and 2014, while examples of recent years with much lower than average estimated recruitment included 1984, 1996, 2007, 2011, and 2013. Hydrographs illustrating summer flow regimes for these years revealed that higher than average recruitment years generally met or exceeded pulse recommendations made by the Brazos BBEST (2012), while years with very low estimated recruitment either did not attaint recommended pulses or, in the case of 2007, included sustained high flows for the entire summer (Figure 5.9).

Discussion

This study provides empirical evidence for linkages between stream flow regime and recruitment by Shoal Chub. Specifically, Shoal Chub recruitment was highest during flow reductions following pulses when fall rates reached near-zero and discharge magnitude was above or just below the threshold for base flow. The timing of recruitment was 30-50 days following higher pulses but became truncated to 12 days after flows declined below the base flow threshold. There was no evidence that Shoal Chub spawned during the ascending limb of the hydrograph or that spawning was synchronized within a narrow window of time. These findings represent the basis for more focused hypotheses regarding flow-ecology relationships for opportunistic life history strategists that have short life spans, mature early, and recolonize quickly (Winemiller and Rose 1992). Flow-ecology relationships or flow requirements are currently unquantified for a large number of imperiled fishes in the Great Plains (Worthington et al. 2018; but see Nguyen et al., In press) and this research provides new insight into flow requirements for one of the most broadly distributed members of the *Macrhybopsis* genus.

This study broadens the number of otolith-based age and recruitment studies for Great Plains minnows. Durham and Wilde (2005) reported strong linear relationships between age (independent variable) and size (dependent variable) for five species from the Canadian River, Texas, while Starks et al. (2016) reported similar linear relationships for three species from the Missouri River, Missouri (Table 3). The slopes of these regressions suggest these fishes grow 0.47-1.39 mm per day during their early life. The estimate for Shoal Chub growth based on the regression slope in this study fell within this range (i.e., 0.56). Regression-based growth estimates for Shoal Chub differed between the lower Brazos River (i.e., 0.56) and the Missouri River (i.e., 1.24) and indicated that Shoal Chub may grow twice as fast in the Missouri River. Aging larger individuals becomes increasingly difficult as daily growth increments begin to crowd near the edge of otoliths as fish growth slows during the transition into cooler seasons. This means that although linear models predict growth during the first summer of life, an asymptotic relationship likely emerges as fish live for longer periods of time. Estimates of age suggest *Macrhybopsis* spp. live up to 2.5 years (Wilde and Durham 2008; Perkin 2014). This means that estimating age of large individuals is not possible with these models, though fish of known length <40-50 mm total length can be measured in the field to estimate age and therefore hatch date. This opens new research opportunities in which standardized field monitoring in which the lengths of all age-0 fishes are measured can be used to assess recruitment. However, this might be challenging in lower portions of river basins where sustained high flows sometimes prevent seining collections, as was the case for this study.

The relationship between discharge magnitude and Shoal Chub recruitment quantified in this study differs from that quantified by Rodger et al. (2016). The shape of the relationship quantified by Rodger et al. (2016) was quadratic and was characterized by high recruitment at discharge magnitudes between the thresholds for two and three pulses per season (i.e., 5,000-2,990 cfs) but less recruitment occurred at flows of lesser or greater magnitudes. The relationship quantified as a part of this study was characterized by greater recruitment at discharge magnitudes between the thresholds for subsistence and base flow (i.e., 300-920 cfs) that followed flow pulses. These differences might be attributed to a number of factors. First, the collection method for fishes differed between the two studies, including drift nets by Rodger et al. (2016) and seining in this study. This difference resulted in clear segregation in the sizes of fish collected between the two studies (see Figure 5.1). However, Haworth and Bestgen (2016) showed that hatching date estimates for Flathead Chub were consistent between drift net and seining collections. Consequently, it is unlikely that collection method was the major driver of differences in the flow-recruitment relationships. Second, the two studies differed in the otoliths used to estimate the age of Chub, including asteriscus (Rodger et al. 2016) and lapillus (here). However, the ANCOVA analysis presented here showed that the relationship between age and total length did not differ between the two studies, and therefore differences in otoliths are unlikely the primary driver of differences in flow-recruitment relationships. A third difference between the two studies is the scale of flow conditions and time intervals over which fish were collected. Rodger et al. (2016) collected most of their fish (i.e., 67 of 68) on the same sampling date (i.e., October 25, 2013) following a large flow pulse that exceeded 8,000 cfs, whereas fish collected as a part of this study were collected on 10 occasions spanning exactly one year (August 30, 2019 to August 30, 2020). Consequently, the relationship reported by Rodger et al. (2016) represents recruitment variability for fish of various sizes that were hatched at various intervals before and after a large pulse (i.e., tier magnitude of 2 pulse per summer season; Brazos BBEST 2012), whereas the current study represents recruitment variability across multiple pulses of varying size. In doing so, the current study addressed the call Rodger et al. (2016) made regarding the needed for continued research of "*age and abundance data from multiple years encompassing a broad range of flow conditions.*" It is therefore possible that future, long-term studies encompassing even longer time spans and broader flow gradients will improve upon the flow-recruitment models even future. Such studies should be increasingly feasible in the future based on findings from earlier studies. The age-length key developed here might be used to convert field measurements of lengths to ages without the need to age large numbers of fish; however, this would need to be done with caution and involving environmental conditions that do not deviate greatly from those experienced by fish analyzed to develop the key.

The relationship between discharge magnitude and Shoal Chub recruitment quantified in this study is consistent with previous research on multiple Great Plains minnows. Durham and Wilde (2006) first showed relationships between discharge and fish recruitment that hinted at recruitment following flow pulses. The authors constructed multiple models in which different measures of discharge magnitude were used to predict the number of fish hatched and subsequently surviving to capture (i.e., recruitment). Results suggested that a model that included the presence of discharge as a predictor variable best fit the data, resulting in the conclusion that the presence of discharge, regardless of magnitude, was necessary for successful reproduction (Durham and Wilde 2006). Although unquantified by the original authors, the figure shown for the year 2001 in Durham and Wilde (2006) is suggestive of a pattern in which recruitment was highest at slow fall rates following flow pulses. This same pattern of recruitment during declining or near-stable base flows was reported for Flathead Chub in Fountain Creek, Colorado (Haworth and Bestgen 2017). Furthermore, Durham and Wilde (2009) showed compelling evidence, though unquantified, of higher recruitment during falling flows after flow pulses in the upper Brazos River. A clear future research direction might be to test hypotheses regarding mechanisms underpinning the relationship through which descending flows result in successful recruitment of pelagic-broadcast spawning fishes. Such research would advance conservation of pelagic-broadcasts spawning fishes in particular (Worthington et al. 2018) and endangered river fishes in general (Cooke et al. 2012).

Fall rate might be an important flow regime attribute for managing fishes such as Shoal Chub. The relationship between Shoal Chub recruitment and fall rate quantified in this study revealed that slow, but not zero, fall rates were correlated with higher recruitment. This could be a mechanism through which pelagic-broadcast spawning fishes synchronize spawning while effectively reducing the downstream transport of ova (Durham and Wilde 2008b). Previous authors have suggested spawning during the descending limb of the hydrograph promoted growth and survival while preventing long-distance downstream transport of offspring (Dudley and Platania 2007; Hoagstrom and Turner 2015; Haworth and Bestgen 2017). Interestingly, Costigan and Daniels (2012) found that increased fall rates were among the most substantial anthropogenic changes to Great Plains river flow regimes during the past half century. This could be a leading factor in the decline of endemic Great Plains fishes given that flow regime alteration is listed among the top causes of imperilment (Hoagstrom et al. 2011). Furthermore, Nguyen et al. (In press) found that fall rate, particularly a slow but non-zero rate, was an important flow regime characteristic for predicting the occurrence of Great Plains fishes, including Shoal Chub in the upper Brazos River. Additional research on the relationship between fall rate and fish recruitment is necessary to explore the mechanistic relationship between flow variability and fish recruitment.

Insight into flow-recruitment dynamics for fishes such as Shoal Chub could be used to direct flow management and promote conservation planning during periods of critical low flow. Based on the results of this study, flow pulses and the ensuing recession of flows are necessary for Shoal Chub recruitment, and higher flow pulses provide longer windows of time for recruitment during descending flows. Although lower discharges and slower fall rates promote enhanced recruitment, there appears to be a lower limit to magnitude during which recruitment occurs. For example, findings from this study suggest a narrow window of recruitment occurs after flows drop below the threshold for base flow. Extreme low flows that cause discharge to fall below base flow or subsistence flow for long periods of time could result in failed recruitment. This was recently the case for at two *Macrhybopsis* spp. in a Kansas riverscape during extreme drought (Perkin et al. 2019) and likely for Burrhead Chub (*M. marconis*) during historical drought extremes in Texas (Perkin et al. 2013). Flow-recruitment patterns for Shoal Chub provide insight into only a fraction of the necessary hydrographic requirements necessary to maintain “a sound ecological environment” as mandated by Senate Bill 3 and the Texas Instream Flow Program (Brazos BBEST 2012). This ultimately means that understating flow-recruitment dynamics for a broad range of organisms will ultimately be necessary for designing instream flow management regimes that sustain a broad range of biodiversity.

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Table 5.1. Studies using daily growth-increment formation to study age and flow-ecology relationships among North American Cyprinidae fishes.

Fish species	Location	Citations
Arkansas River shiner (*Notropis girardi*) Peppered chub (*Macrhybopsis tetranema*) Plains minnow (*Hybognathus placitus*) Flathead chub (*Platygobio gracilis*) Red shiner (*Cyprinella lutrensis*)	Canadian River, Texas	Durham and Wilde (2006)
Sharpnose shiner (*Nortopis oxyrhynchus*) Smalleye shiner (*Notropis buccula*) Plains minnow (*Hybognathus placitus*)	Upper Brazos River, Texas	Durham and Wilde (2008a) Durham and Wilde (2009)
Shoal chub (*Macrhybopsis hyostoma*)	Lower Brazos River, Texas	Rodger et al. (2016)
Sturgeon chub (*Macrhybopsis gelida*) Shoal chub (*Macrhybopsis hyostoma*) Sicklefin chub (*Macrhybopsis meeki*)	Lower Missouri River, Missouri	Starks et al. (2016)
Flathead chub (*Platygobio gracilis*)	Fountain Creek, Colorado	Haworth and Bestgen (2016) Haworth and Bestgen (2017)

Table 5.2. Number of Shoal Chub collected, range of total lengths, and range of ages during visits to the Farm to Market Road 485 crossing on the lower Brazos River during 2019 and 2020.

Date	Number collected	Length range (mm)	Age range (days)
8/30/2019	1	28	42
9/2/2019	1	27	40
9/8/2019	1	21	28
9/21/2019	7	32-39	49-63
9/26/2019	27	30-39	45-63
10/1/2019	1	33	51
6/19/2020	36	13-36	12-57
8/18/2020	68	21-39	28-63
8/23/2020	83	24-40	34-65
8/30/2020	29	28-39	42-63

Table 5.3. Linear regression models describing the relationship between estimated fish age (age) as the independent variable and total length (TL) as the dependent variable for multiple Great Plains cyprinids, including the location of the study, the linear regression model, model fit (coefficient of determination, R^2), and significance of the model (signif.).

Species	Location	Model	Fit	Signif.	Citation
Arkansas River shiner (*Notropis Girardi*)	Canadian River, Texas (2000)	TL=0.628(age)+0.52	0.74	<0.001	Durham and Wilde (2005)
Arkansas River shiner (*Notropis Girardi*)	Canadian River, Texas (2001)	TL=0.764(age)+0.085	0.63	<0.001	Durham and Wilde (2005)
Peppered chub (*Macrhybopsis tetranema*)	Canadian River, Texas (2000)	TL=0.876(age)-0.142	0.86	<0.001	Durham and Wilde (2005)
Peppered chub (*Macrhybopsis tetranema*)	Canadian River, Texas (2001)	TL=0.821(age)+0.016	0.67	<0.001	Durham and Wilde (2005)
Plains minnow (*Hybognathus placitus*)	Canadian River, Texas (2000)	TL=0.636(age)+0.669	0.71	<0.001	Durham and Wilde (2005)
Plains minnow (*Hybognathus placitus*)	Canadian River, Texas (2001)	TL=0.876(age)-0.077	0.75	<0.001	Durham and Wilde (2005)
Flathead chub (*Platygobio gracilis*)	Canadian River, Texas (2000)	TL=0.905(age)-0.191	0.86	<0.001	Durham and Wilde (2005)
Flathead chub (*Platygobio gracilis*)	Canadian River, Texas (2001)	TL=0.664(age)+0.354	0.61	<0.001	Durham and Wilde (2005)
Red shiner (*Cyprinella lutrensis*)	Canadian River, Texas (2000)	TL=0.672(age)+0.347	0.81	<0.001	Durham and Wilde (2005)
Red shiner (*Cyprinella lutrensis*)	Canadian River, Texas (2001)	TL=0.467(age)+0.994	0.46	<0.001	Durham and Wilde (2005)
Sturgeon chub (*Macrhybopsis gelida*)	Lower Missouri River, Missouri	TL=1.39(age)-7.45	0.77	<0.01	Starks et al. (2016)
Shoal chub (*M. hyostoma*)	Lower Missouri River, Missouri	TL=1.24(age)-20.88	0.56	<0.01	Starks et al. (2016)
Sicklefin chub (*M. meeki*)	Lower Missouri River, Missouri	TL=0.79(age)+5.21	0.82	<0.01	Starks et al. (2016)
Shoal chub (*M. hyostoma*)	Lower Brazos River, Texas	TL=0.56(age)+6.7	0.97	<0.001	This study

Table 5.4. Pairwise correlation coefficients for flow metrics included in the generalized additive model used to predict the number of Shoal Chub hatched and surviving during 2019 and 2020. Parameters were removed when the absolute value of correlation coefficient was >0.70. The parameter time since 4 pulses per season (4/S) was removed from the regression analysis to avoid multicollinearity

	Time since base	Time since 4/S	Time since 3/S	Time since 2/S	Magnitude	Rate of change
Time since base	1	0.79	0.62	0.22	-0.52	0.00
Time since 4/S	0.79	1	0.82	0.25	-0.62	0.00
Time since 3/S	0.62	0.82	1	0.36	-0.61	0.13
Time since 2/S	0.22	0.25	0.36	1	-0.49	0.08
Magnitude	-0.52	-0.62	-0.61	-0.49	1	0.14
Rate of change	0.00	0.00	0.13	0.08	0.14	1

Table 5.5. Results of parameter selection for the generalized additive model fit to the relationship between flow and number of Shoal Chub hatched and surviving in the lower Brazos River during 2019 and 2020. Flow parameters include discharge measured on the day of hatch (cubic feet per second, cfs), rate of change in cfs between the previous day and the day of hatch (cfs/d), and time in days since flow fell below the threshold for three pulses per season (i.e., 2,990 cfs), two pulses per season (i.e., 5,000 cfs), and base flow (i.e., 920 cfs) for an average summer season. The effective and reference degrees of freedom (DF), chi-square test statistic, and p-value are given for each smoothing parameter in the model.

Parameter	DF	Chi-Square	P-value
Discharge (cfs)	1,9	14.49	<0.001
Time since 3/S (days)	1,9	0.00	0.887
Time since 2/S (days)	7,9	34.53	<0.001
Time since base (days)	4,9	14.34	0.002
Rate of change (cfs/d)	1,9	2.26	0.084

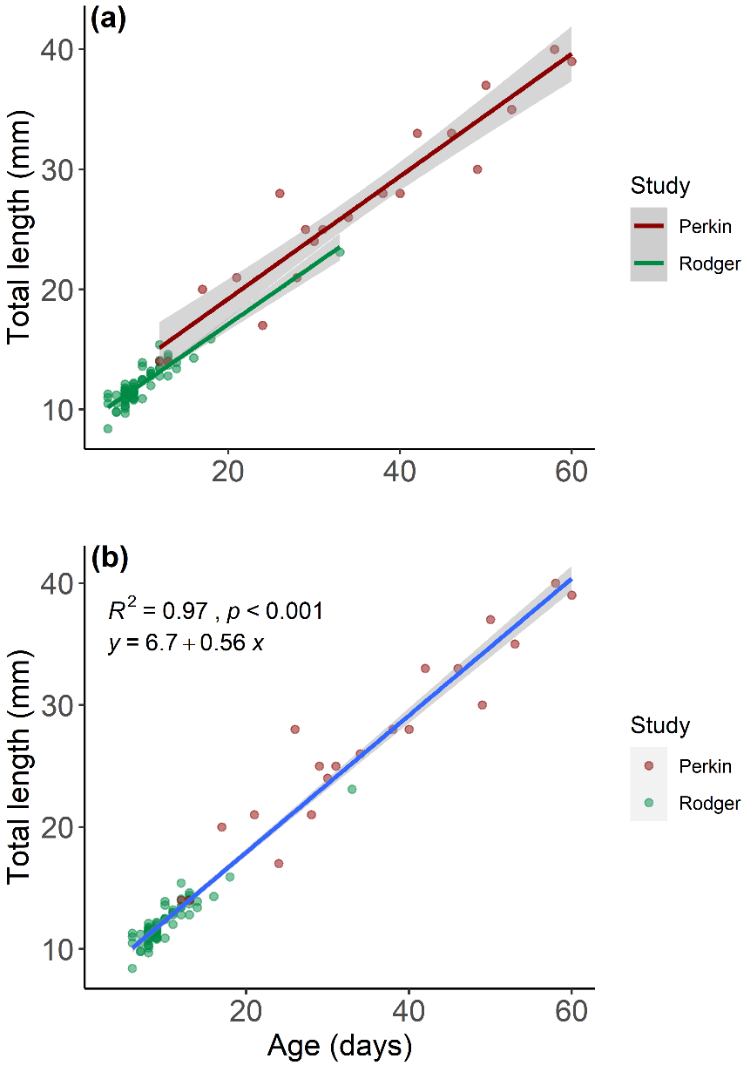

Figure 5.1. Relationship between age (days) and total length (mm) for Shoal Chub collected from the Brazos River during this study (Perkin; n = 20) and during a previous study by Rodger et al. (2016; n = 68). (a) Shows the relationship for each study independently, but analysis of covariance revealed no difference in the relationship between age and length between the two studies (see text), thus data were combined into a single regression model. (b) Shows the regression model for the combined data, including the regression equation used as an age-length key to estimate ages of shoal chub of known length.

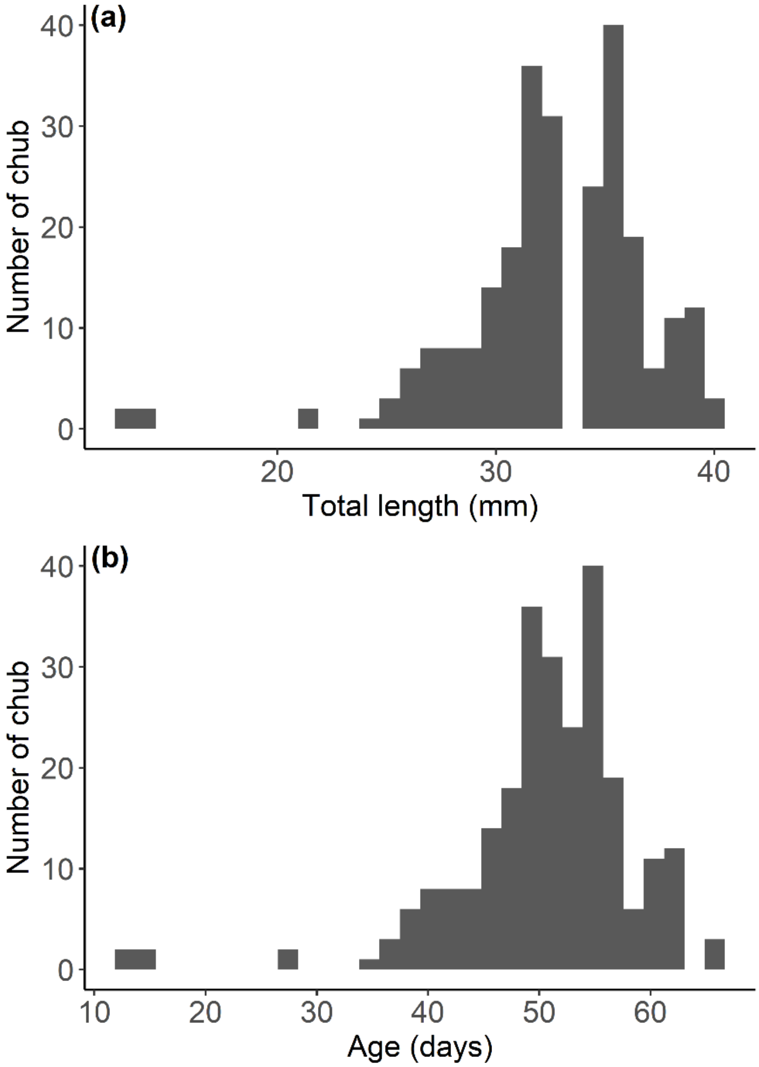

Figure 5.2. Histograms showing the distributions of (a) total length and (b) age for 254 Shoal Chub collected from the lower Brazos River during summers of 2019 and 2020.

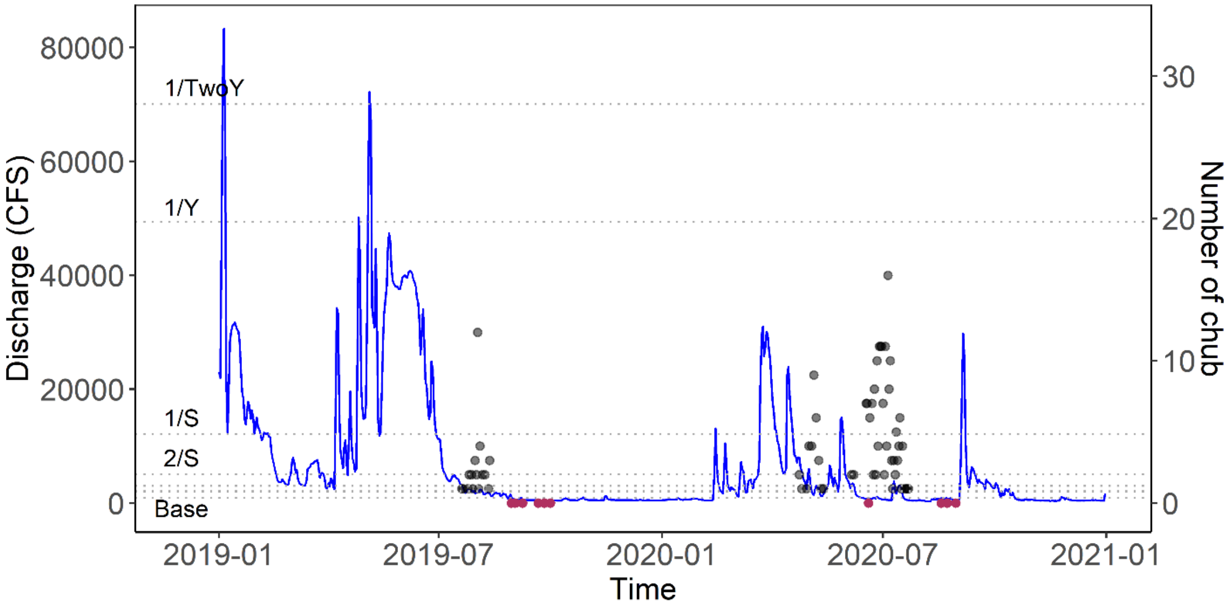

Figure 5.3. Hydrograph for the Brazos River at State Highway 21 near Bryan, Texas (blue line; left axis) illustrating flow tiers (dashed grey lines) developed by Brazos BBEST (2012), including one overbank pulse per two years (1/TwoY; 66,900 cubic feet per second, cfs), one overbank pulse per year (1/Y; 49,400 cfs), one high flow pulse per season (1/S; 12,100 cfs), two high flow pulses per season (2/S; 5,000 cfs), three high flow pulses per season (not labeled; 2,990 cfs), four high flow pulses per season (not labeled; 2,060 cfs) and base flow (Base; 920 cfs). The number of Shoal Chub recruited per day (grey points; right axis) are shown on the same time line but on a different axis (i.e., left and right axes scales differ). The dates of sampling are shown as red circles at the base of the graph. Sampling during 2019 was hindered by high flows early in the summer, while 2020 sampling was limited by high flow pulses as well as travel restrictions associated with the COVID-19 pandemic.

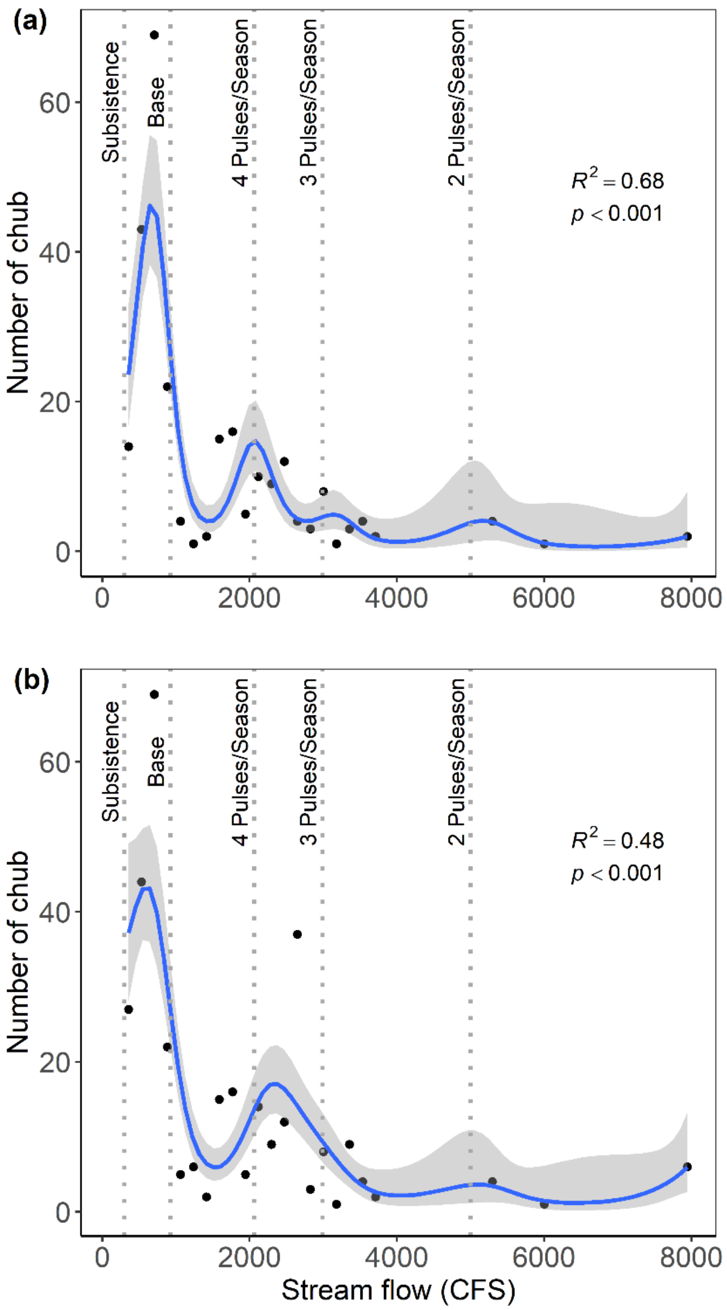

Figure 5.4. Relationship between the number of Shoal Chub recruited and the discharge (cfs) on the day of hatch for (a) data from this study only and (b) data from this study combined with data from Rodger et al. (2016). Thresholds are shown as vertical dashed lines for subsistence flow (300 cfs), base flow (920 cfs), four pulses per season (2,060 cfs), three pulses per season (2,990 cfs), and two pulses per season (5,000 cfs) based on values given in Brazos BBEST (2012). The blue lines represent generalized additive model fits, and the shaded areas are the 95% confidence intervals. The adjusted coefficient of determination (R^2) and test of significance (p-value) are given for each model.

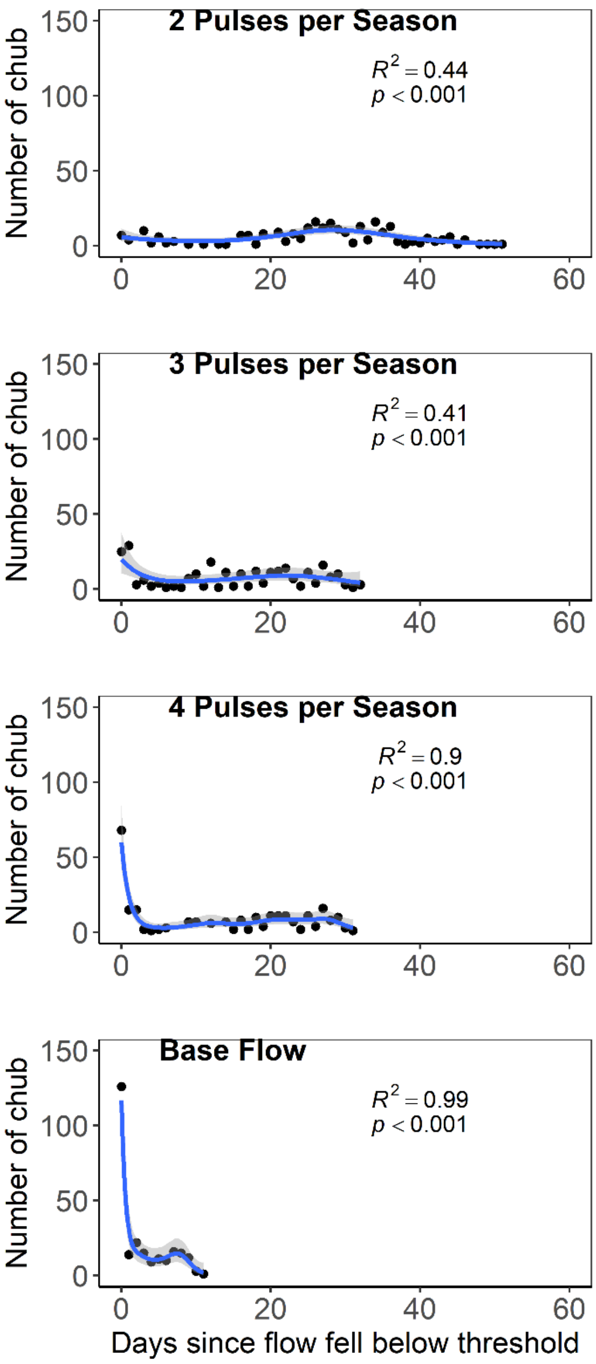

Figure 5.5. Relationship between the number of Shoal Chub recruited and the time (days) since flow declined below thresholds for two pulses per season, three pulses per season, four pulses per season, and base flow. Blue lines represent generalized additive model fits and shaded areas are 95% confidence intervals. The adjusted coefficient of determination (R^2) and test of significance (p-value) are given for each model.

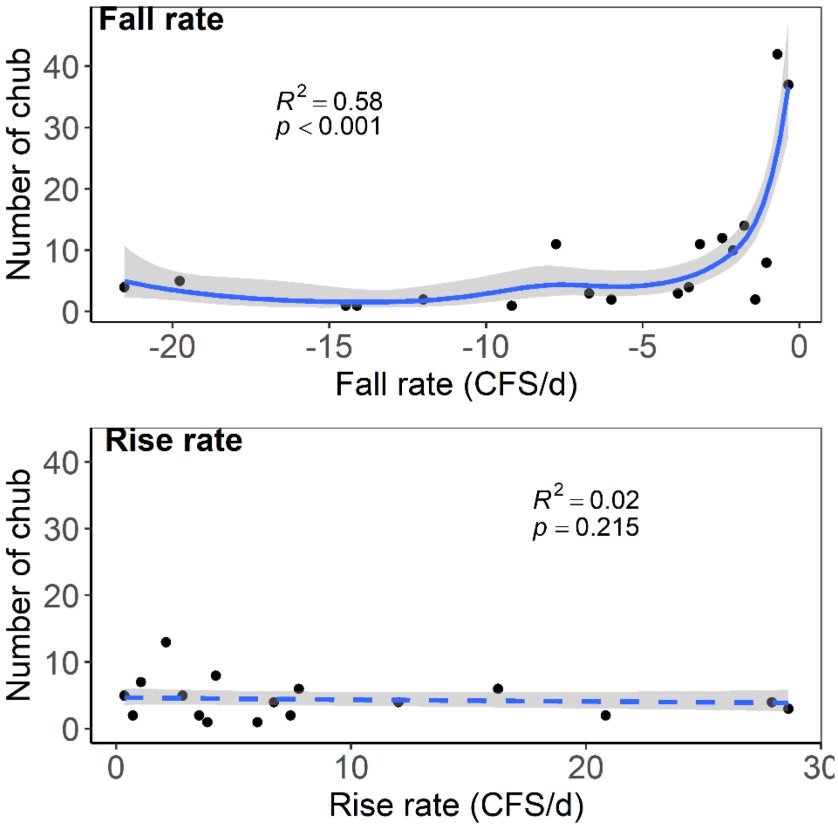

Figure 5.6. Relationship between the number of Shoal Chub recruited and rate of change in flow, including fall rate (i.e., descending limb; upper) and rise rate (i.e., ascending limb; lower). Blue lines represent generalized additive model fits and shaded areas are 95% confidence intervals. The adjusted coefficient of determination (R^2) and test of significance (p-value) are given for each model. The rise rate model was non-significant and is shown as a dashed line.

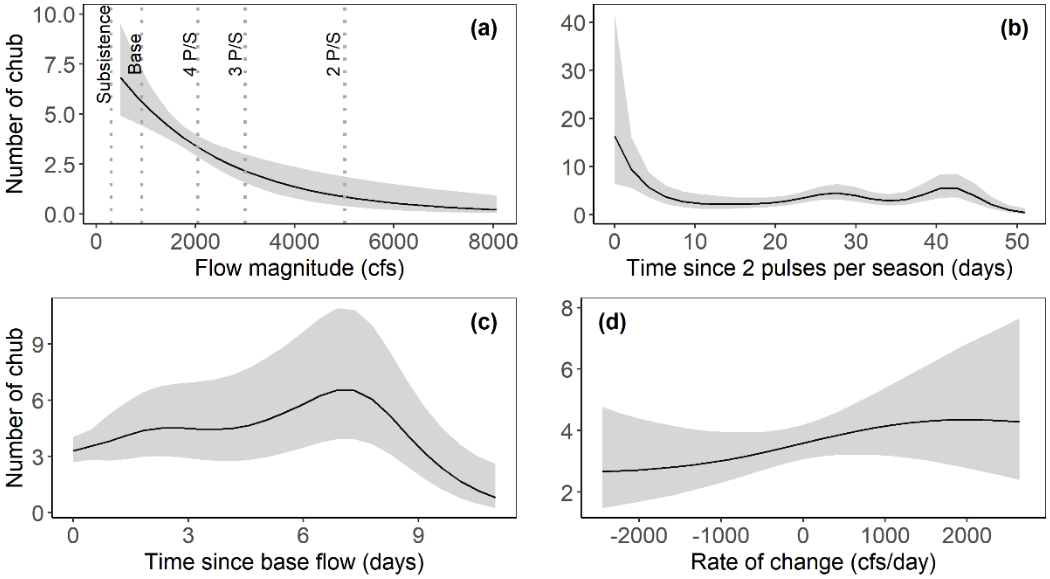

Figure 5.7. Smoothing functions included in a generalized additive model used to predict the number of Shoal Chub recruited based on (a) flow magnitude measured on the day of hatch (cubic feet per second; cfs), (b) time in days between hatch date and the day that flows descended past the threshold for 2 pulses per season (i.e., 5,000 cfs), (c) time in days between hatch date and the day that flows descended below the threshold for base flow (i.e., 920 cfs), and (d) daily rate of change in flow magnitude between the day of hatch and the day prior to hatch. Solid black lines represent fitted smoothing functions for relationships and grey shaded areas represent 95% confidence intervals. Thresholds from the Brazos BBEST (2012) are shown as grey dashed lines in (a), including subsistence flow (300 cfs), base flow (920 cfs), four pulses per season (4 P/S; 2,060 cfs), three pulses per season (3 P/S; 2,990), and two pulses per season (2 P/S; 5,000).

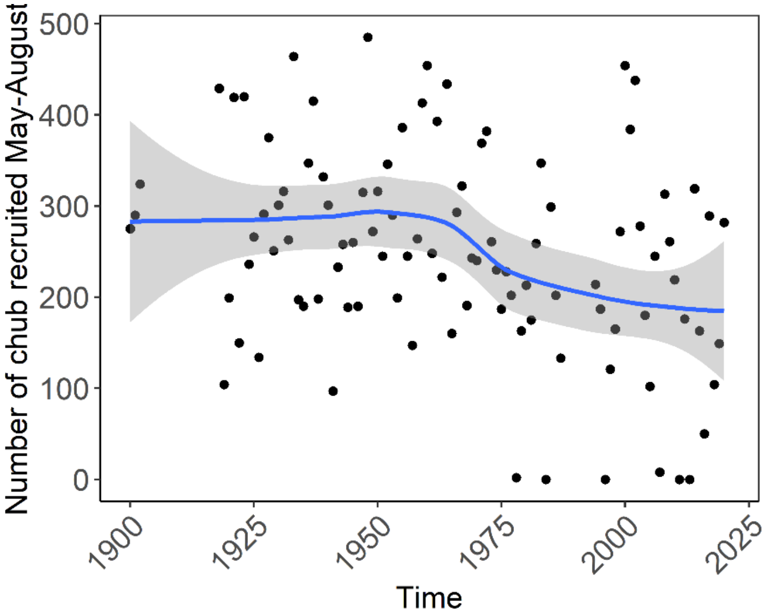

Figure 5.8. Estimated number of Shoal Chub recruited during May-August for years with historical flow data for the Brazos River near Bryan, Texas based on a generalized additive model fit. Daily streamflow data were used to estimate daily hatches and daily hatches were summed across all days in the months of May through August to develop a recruitment estimate for each year. The blue line and grey shaded area represent a locally weighted regression fit (line) and 95% confidence interval (area) used to summarize the long-term pattern.

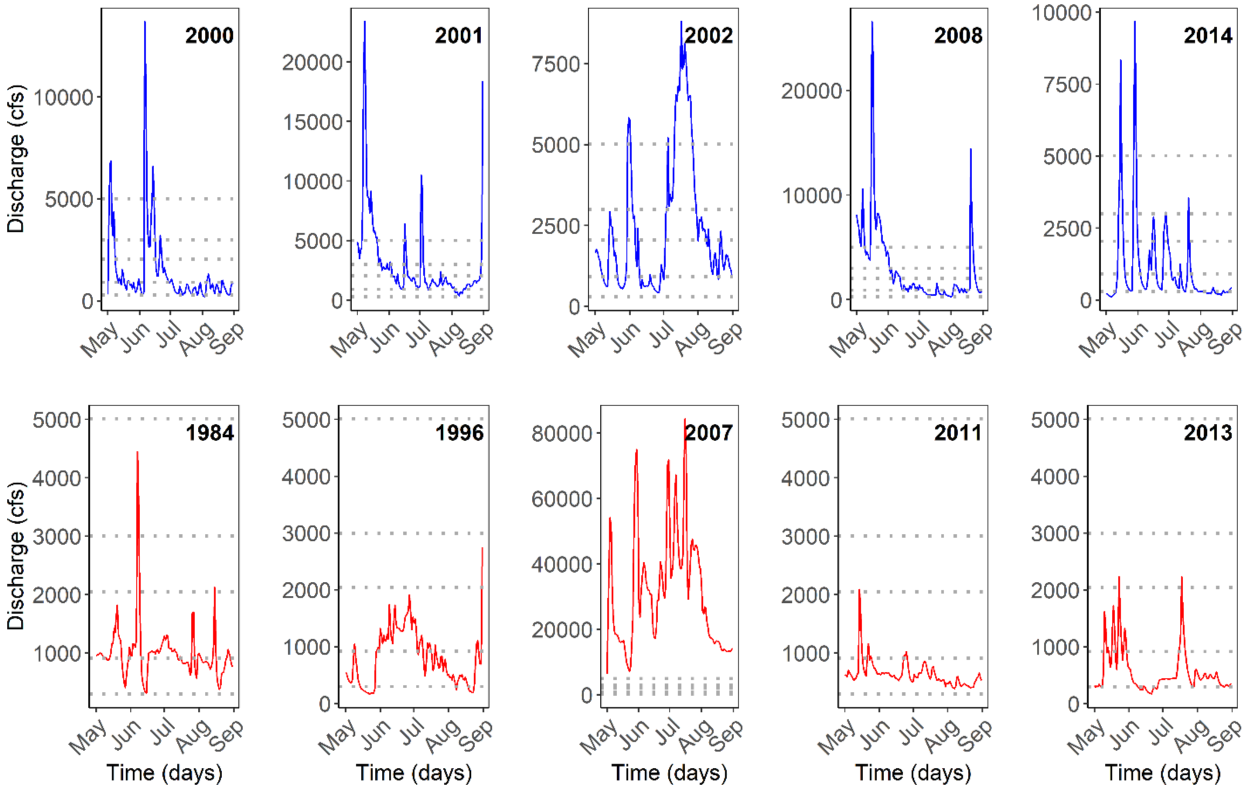

Figure 5.9. Example summer flow regimes for recent years (i.e., post-1980) with high estimated Shoal Chub recruitment (top row, blue lines) and years with low estimated shoal chub recruitment (bottom row, red lines). Each panel shows summertime (May 1 through August 31) mean daily discharge magnitude (cubic feet per second; cfs) in relation to Brazos BBest (2012) flow standards, including 2 pulses per summer (top dashed line; 5,000 cfs), 3 pulses per summer (second from top dashed line; 2,990 cfs), 4 pulses per summer (middle dashed line; 2,060 cfs), base flow (second from bottom dashed line; 920 cfs), and subsistence flow (bottom dashed line; 300 cfs).

Chapter 6. Response of Indicator Taxa to Flow Variation – Freshwater Mussels

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Background

Freshwater mussels provide crucial ecosystem services by filtering the water (biofiltration), nutrient recycling and storage, creating and structuring habitat (Vaughn 2018). North America has the highest diversity of freshwater mussels in the world with about 300 species, about 50 of those occurring in Texas (Haag 2012). However, they are also considered one of the most imperiled groups of organisms in North America (Lopes-Lima et al. 2018) and their decline leads to losses in ecosystem functions (Vaughn 2018). Understanding and mitigating threats that cause the declines are therefore important. Freshwater mussels are vulnerable to changes in flow conditions because they are rather sessile and cannot easily find refuge from adverse conditions. They therefore require suitable flow conditions that maintains their habitat, water quality and sustain food resources. Mussels also have a complex life cycle which includes a parasitic stage on host fish and require flow that facilitates the encounter with host fish during their reproductive season and successful settlement of juvenile mussels.

Flow modification can have various negative impacts on mussels. Frequent fluctuations in flow, such as those caused by dam releases, have led to declines and local extirpation of mussels (Vaughn and Taylor 1999; Allen et al. 2013). Newly settled juveniles may become dislodged during high flow and adults may be washed out their habitat during floods (e.g., Layzer and Madison 1995; Hastie et al 2001), which pose a major threat when mussels are dislodged and become stranded in degraded habitat (Karatayev et al. 2019). Low flow events caused by drought and/or excessive groundwater extraction also have detrimental effects on mussels (e.g., Galbraith et al. 2010; Sousa et al. 2018; Mitchell et al. 2019). Changes in the timing of low and high stream flows change temperature regimes and can affect mussel communities directly or indirectly via the absence of suitable host fish (Gido et al. 2010; Galbraith and Vaughn 2011).

Mussels are long-lived organisms; some species can live for several decades, and declines can occur undetected if the age structure of a population is not considered. Similar to trees their age can be determined by counting their annuli when the shell is thin sectioned (Haag and Commens-Carson 2008). Aging of mussels has been used to gain insights into their ecology

(Metcalfe-Smith and Green 1992; Rogers et al. 2001; Haag 2012; Daniel and Brown 2014), but to the best of our knowledge it has not been used to link recruitment to antecedent flow conditions, although a few studies have examined the relationship of growth to flow and other stream characteristics (Rypel et al. 2008, 2009, Dycus et al. 2015). The objective of this study was to determine whether aging of mussels could reveal relationships of antecedent flow conditions with recruitment and growth of mussels. Two common mussel species (*Lampsilis teres* and *Amblema plicata*) with different life history strategies were used. *Lampsilis teres* is considered an opportunistic species with shorter life span and high growth rates, whereas *A. plicata,* a mussel with an equilibrium life history strategy, is a longer-lived and slower growing species (Haag 2012).

Methods

Site Selection and Survey Design. – Mussels (*A. plicata* and *L. teres*) were collected from eight sites in the Colorado (2 sites), Brazos (3 sites), and Guadalupe (3 sites) River basins in the fall 2019 and summer/fall 2020 (Table 6.1, Figure 6.1). Mussel sites were chosen based on prior survey data that reported relatively high abundances for our study species. Historical mussel distribution data were collected from a variety of government reports (Randklev et al. 2014, Randklev 2016, Bonner et al. 2018) and from data previously collected by the Schwalb Stream Ecology Lab. Six additional sites (two from each study basin) were surveyed but the study species were either absent or were in very low abundances and thus not used for this study.

Sites were surveyed for mussels using a two-phase sampling design (Strayer and Smith 2003) that first includes a qualitative timed search to identify whether the target species is present at a site, followed by a quantitative approach (i.e., quadrat sampling). Each site was qualitatively searched for at least four person hours. If the study species was present and in relatively high abundance, then mussels were sampled using quadrats. Quantitative sampling (i.e., quadrat sampling) results in a more accurate size and age distribution, because timed searches are known to be biased towards larger individuals (Vaughn et al. 1995, Hornbach and Deneka 1996, Strayer and Smith 2003). A minimum of 18 quadrats (range: 18-105; Table 6.1) were randomly placed along the riverbank at each site. Additional quadrats were added until up to 100 mussels were sampled at each site. Mussels were searched within each quadrat by removing the top 10 cm of sediment and carefully examining it for mussels. While quantitative sampling is less biased towards larger mussel sizes than timed searches, juvenile mussels are easily missed, and a lack of very young mussels does not necessarily reflect a lack of recruitment but can be caused by sampling bias.

Mussel Aging. – Mussels were transported back to the Schwalb Stream Ecology Lab at Texas State University in insulated and aerated coolers filled with sediment and water from each site. Upon arriving at Texas State University, mussels were immediately put into freezers at -18° C for at least 48 hours. Once euthanized, the flesh was thawed and removed from the shells.

Mussel shells were thin sectioned using the Fisheries and Ocean Protocol for Ageing Mussel Shells. Thin sections were cut through the shell’s umbo to the longest exterior point using a diamond wafering blade and Buehler IsoMet 1000 precision saw (Buehler Ltd., Lake Bluff, Illinois). Thin sections were then sanded using four increasing sandpaper grits (course, medium, fine, and ultra-fine) and epoxied (General Purpose Epoxy Resin, East Coast Epoxy, USA) to a microscope slide. Two independent observers counted annuli under a Nikon SMZ800N stereomicroscope for each mussel. Annuli are lines that represent annual growth increments and originate at the umbo and are traceable to the margin of the shell (Haag and Commens-Carson 2008). In addition to annuli, mussels can form disturbance lines. If the annulus does not traverse from the origin of the umbo to the margin of the shell, it is considered a disturbance line. Disturbance lines can be caused by stressful environmental conditions or from handling (Haag and Commens-Carson 2008; Rypel et al. 2008). Disturbance lines are visually similar to annuli and can result in reduced growth of mussels and inaccurate age estimates (Haag and Commens-Carson 2008). Disturbance lines can be particularly challenging to identify in older individuals with tightly crowded annuli (Haag and Commens-Carson 2008).

Age estimates from the two independent observers were compared before data analysis. If there was a discrepancy between the two observers, the shell section was viewed together, and consensus annuli were identified. The age estimates used in the analyses were the total number of consensus annuli (see also Dycus et al. 2015). If a consensus could not be reached, the sample was excluded from the analysis. Specimens were also removed from the study if the slide were 1) cut improperly, 2) over sanded, or 3) broken and a replacement could not be made. A total of 54 out of 500 specimens were removed from the study.

Hydrological Data. – In order to correlate annual recruitment rates of mussels to antecedent hydrologic conditions within our study basins, historical daily streamflow data were gathered from the USGS website from nearby gage stations (Appendix Ch6) and analyzed with the Indicators of Hydrologic Alteration (IHA) software, version 7.1 (TNC, 2007). Non-parametric analyses were conducted in IHA to compute twenty ecologically relevant hydrologic parameters that describe the flow conditions for each study basin (Table 6.2; see Olden and Poff 2003 and TNC 2007, for detailed descriptions of IHA parameters). For each site, the earliest flow year used was the year prior to the recruitment year for the oldest mussel.

Data Analysis. – Recruitment was calculated as the proportion of collected mussels in the basin with age 0 in a particular year. Multiple regression followed by a stepwise AIC model selection process was used for each river basin to examine which IHA parameters best explained the variation in mussel recruitment (%). Prior to analysis, datasets were examined for multicollinearity based upon correlation matrices between predictor variables and variance inflation factor values. Highly correlated variables ($r > 0.75$ or $VIF > 10$) were removed prior to analysis. Three regression models were developed for *A. plicata* but too few data were available for *L. teres*, thus no models could be fit with *L. teres* and IHA data.

To examine the variability in annuli counts within observers, the observers independently counted annuli of 10-11 randomly chosen specimens three separate times. To be as unbiased as possible, specimens were randomized, and new IDs were given for each count. Variability within and between observers were examined by computing R^2-values for linear regressions between observers or blind repeated counts and by comparing how often a perfect match was found.

Results

After removing damaged and unaged samples, a total of 446 mussels from 3 rivers (302 *A. plicata*, 144 *L. teres*; Table 6.3) were used for analysis. Most of the mussels were collected in the Guadalupe River (199 mussels, all *A. plicata*), 155 mussels collected from the Colorado River (80 *A. plicata*, 75 *L. teres*), and 92 mussels from the Brazos River basin (23 *A. plicata,* 69 *L. teres*; Table 6.3). No *L. teres* were found at the Guadalupe River sites, except for one site which contained less than ten individuals.

The perceived age of the majority of *A. plicata* ranged roughly between 9 and 20 years and between 3 and 7 years for *L. teres* (Figure 6.2). Although several 3 to 4 years old *A. plicata* were encountered (6 to 7 individuals per year), only few 5 to 8 years old were found (1-2 ind. per year), indicating lower recruitment in the years 2011-2014 (most *A. plicata* were collected in 2019, thus recruitment of an 8-year-old mussel occurred in 2011). The highest proportion of *A. plicata* was found in the age groups 13 to 16 years old, whereas the highest proportion of *L. teres* was 3 to 5 years old (Figure 6.2).

Following model selection, four IHA predictors (1-day and 7-day minimums, Julian date of 1-day minimum, high pulse count) explained 48% of the variation in *A. plicata* recruitment in the Guadalupe River ($F_{(4,18)} = 5.997$; $P = 0.003$; Adj. $R^2 = 0.476$; Table 6.4). Day 1 minimums and high pulse count were negatively associated with mussel recruitment, whereas 7-day minimums and the Julian date of each annual 1-day minimum were positively associated with mussel recruitment. A similar amount of variation (49%) in *A.plicata* recruitment was explained by the base flow index, rise rate, and the Julian date of the 1-day maximum in the Brazos River ($F_{(3,15)} = 6.69$; $P = 0.004$; Adj. $R^2 = 0.49$; Table 6.4). Recruitment was negatively associated with base flow index and rise rate, and positively associated with the Julian date of each annual 1-day maximum. The least amount of variation in *A. plicata* recruitment was explained by IHA variables in the Colorado River ($F_{(2,23)} = 3.88$; $p= 0.04$; Adj. $R^2= 0.19$; Table 6.4). Recruitment of *A. plicata* in the Colorado River was negatively associated with increased high flow duration and increased low pulse counts (Table 6.4).

There was variation between and within observers in counting annuli (Fig. 6.3 and 6.4). A stronger correlation between observers was found for *A. plicata* ($R^2= 0.91$) compared to *L. teres.* (R^2 ranging between 0.58 and 0.84, Figure 6.3). The difference in R^2 could be at least partly driven by little variation between observers for younger *A. plicata* as the counted annuli of 80% of younger (≤ 4 years, n= 15) matched perfectly between observers. In addition, samples of *A. plicata* covered a larger age range. When only *A. plicata* 8-18 years were considered the R^2 (0.77) was more similar to those obtained for *L. teres.*

A perfect match between observers occurred more often for *L. teres* (on average 51 % of samples) compared to *A. plicata* (35% of samples, Table 6.5). The opposite was found for counts that were off by (+/-) 1 year, which was found in a larger proportion of samples (46%) of *A. plicata* compared to *L. teres* (32%). Differences by (+/-) 2 years and by 3 to 4 years occurred in a similar proportion of samples for both species (2 years difference: 13 vs. 11%, 3-4 years difference: 5 vs 6 % difference for *A. plicata* and *L. teres* respectively, Table 6.5). There was a small difference between positive (28%) and negative (37%) differences in annuli counts for *A. plicata*, whereas the difference was up to 45% for higher values of observer 1 compared to observer 3 vs. 13 % negative differences for *L. teres* (Figure 6.3).

There also was variation within observers. Correlation between repeated counts was high (R^2 ranging between 0.89 and 0.96, Figure 6.4) but only resulted in perfect match on average in 30% of cases (range: 10-45%) for *A. plicata.* A higher proportion of perfect matches was found for *L. teres* (70-80%), the species with the smaller age range.

Discussion

Mussels are known to be detrimentally affected by drought and several studies have shown that droughts can increase mortality, decrease mussel diversity, and change mussel community composition (Gagnon et al. 2004, Galbraith et al. 2010, Golladay et al. 2004, Haag and Warren 2008, Sousa et al. 2018). Drought does not only affect mussels by drying of their habitat, but also through secondary effects, such as higher temperatures and lower levels of dissolved oxygen (Haag and Warren 2008, Mitchell et al. 2019). Texas experienced an exceptional drought in 2011 (associated with a strong La Niña event in 2010-2011), and drought conditions persisted until a climatic reversal owing to a strong El Niño year in 2015-2016 brought high precipitation and intense flooding events. Studies in Texas found declines of mussels in Texas after the exceptional drought in 2011 in the upper Colorado River Basin (Mitchell et al. 2019) and the Neches River basin (Tarter 2019). Data from this study suggest that the drought period not only increased mortality as previously shown in a number of studies (see above), but likely also caused a decline in recruitment.

Mussels are known to be sensitive to extreme flow conditions, both droughts (see above) and floods (Hastie et al. 2001), which is in accordance with the negative associations we found between *A. plicata* recruitment and measures of high flow (high flow durations, high pulse counts, flow variability rise rate), and low flow (low pulse counts, Day 1 minimum). Equilibrium species may be especially impacted by disturbance events, and the mussel community may shift to more opportunistic or generalist species (Gagnon et al. 2004). Unfortunately, we could not carry out the same analysis for the opportunistic *L. teres* due to a lack of data. The positive correlation with Julian date of extreme flow events (Julian date of each annual 1-day minimum and maximum) suggests that timing may also play an important role or could be co-incidental such as the positive association with 7-day minimum.

Maximum age for *A. plicata* and *L. teres* in this study (26 and 9 years respectively) was lower compared to a study in southeastern Louisiana (39 and 14 years respectively, Daniel and Brown 2014). Compared to other studies summarized by Haag (2012) maximum age for *A. plicata* was within the range reported by other studies (18 to 54 years), but lower for *L. teres* in this study (9 vs. 13 years). In contrast, maximum size for *L. teres* was larger in this study (142 mm vs. 122 mm) and similar for *A. plicata* (123 vs. 122mm, Daniel and Brown 2014). These comparisons suggests that *L. teres* and to some degree *A. plicata* may have a shorter life span in Texas, but they may also be growing faster.

While aging of mussels has been useful in the past to better understand the ecology and life history strategies of mussels (Metcalfe-Smith and Green 1992, Rogers et al. 2001, Haag 2012, Daniel and Brown 2014), it may be difficult to use the technique to relate it to antecedent flow conditions, because the perceived age may easily vary from the actual age by one year or more. Other studies have also noted potential problem with age determination using internal annuli. For example, a study with *Elliptio complanata* and *Lampsilis radiata* found that actual annual changes in length determined by annual measurements of marked mussels were significantly lower than annual changes in length based on measurements of internal annuli and length-at-age predictions (Kesler and Downing 2003). However, the examination of growth can provide useful insights into factors affecting mussel populations (Dycus et al. 2015) and gathering useful growth data from mark-recapture studies will take decades or at least multiple years, whereas information of past growth rates can be examined via thin sectioning.

There are several challenges that need to be considered. Determining age of mussels can be somewhat subjective as annual growth lines can be difficult to define and this can vary between species. The appearance of the lines is dependent on how the slide specimen is prepared: 1) the cut of the mussel; 2) the amount of epoxy used (too little epoxy can cause the thin-section to “fleck” off the slide and obstruct vision; too much epoxy becomes difficult to sand down); 3) the adequacy to which the specimen is sanded down (both too little and too much sanding can cause the specimen to be opaque and lines hard to distinguish); and 4) disturbance lines can be difficult to distinguish from actual annuli. While there was a high correlation within observers and at least for *A. plicata* between counts of observers, a perfect match was only obtained in roughly 50% of the cases or less, which is a problem, when the analysis is geared towards determining recruitment and growth for specific years. Another issue to consider is that mussels are declining rapidly. For example, we initially surveyed six additional sites (two from each study basin), but the study species were either absent or were in very low abundances, although previous reports indicated relatively high abundances of these species. Collecting mussels for aging would contribute to further declines and alternative assessment strategies should be considered.

Whereas the detrimental impact of extreme low flow and rapid changes in flow on mussels is fairly well known, the impact of flooding is not well understood. High temperatures during extreme low flow impact mussels (e.g., Galbraith et al. 2010) and minimum flows should sustain a temperature regime that supports the growth and recruitment of mussels. Similarly, various studies have shown that mussels cannot respond quickly enough to rapid changes in flow (e.g., Mitchell et al. 2018). A better understanding of the impact of flooding will require an integration of field experiments and hydrodynamic modelling (Karatayev et al. 2019). In order to protect mussels from adverse impacts of flooding, the restoration and protection of continuous suitable habitat is needed (Karatayev et al. 2019). This will require a combined effort by several agencies that should be combined with an effort to establish a long-term monitoring program in Texas with mark-recapture studies that will allow to assess the impact of flow modification and other human impacts on population dynamics.

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Table 6.1. Summary of sampling dates and sampling effort (number of 0.25 m^2 quadrats sampled) for each site.

Site	**River**	**Date Sampled**	**Sample Effort**
Bellville	Brazos	8/28/2020	45
Navasota	Brazos	10/8/2019	55
Simonton	Brazos	8/24/2020	80
Altair	Colorado	10/3/2019	23
Bay City	Colorado	5/5/2020	105
Laker Wood	Guadalupe	10/15/2020	30
Gonzalez	Guadalupe	9/24/2019	18
Victoria	Guadalupe	9/4/2019	20

Table 6.2. Hydrologic parameters calculated using the Indicators of Hydrologic Alteration (IHA) software for each study basin. See TNC (2009) for a detailed description of each parameter.

IHA Parameter Group	Hydrologic Parameter
Magnitude and duration of annual extreme water conditions	Annual minima, 1-day mean
	Annual minima, 3-day mean
	Annual minima, 7-day mean
	Annual minima, 30-day mean
	Annual minima, 90-day mean
	Annual maxima, 1-day mean
	Annual maxima, 3-day mean
	Annual maxima, 7-day mean
	Annual maxima, 30-day mean
	Annual maxima, 90-day mean
	Base flow index
Timing of annual extreme water conditions	Julian Date of each annual 1-day maximum
	Julian Date of each annual 1-day minimum
Frequency and duration of high and low pulses	Number of low pulse count within each year
	Low pulse duration (days)
	Number of high pulse count within each year
	High pulse duration (days)
Rate and frequency of water condition changes	Rise rate
	Fall rate
	Number of hydrologic reversals

Table 6.3. Summary of length, age, sample size and sites of *A. plicata* and *L. teres* in the Guadalupe, Colorado, and Brazos River basins.

	Guadalupe	Colorado	Brazos
Amblema plicata			
Length (mm)	81.2 ±20.4 (range 15-115)	99.0 ±7.7 (range: 73-123)	87.3 ± 19.5 (range: 24-104)
Age (years)	13± 4 (range 2-23)	15 ± 4 (range 9-26)	12 ± 4 (range 1-18)
Sample size	199	80	23
Lampsilis teres			
Length (mm)		113.4 ±20.3 (range: 40-142)	89.6 ± 23.0 (range: 19-128)
Age (years)		5 ± 2 (range 1-9)	4 ± 2 (range 1-7)
Sample size		75	69

Table 6.4. Summary of results from regression analyses examining the relationship between hydrologic variables calculated in IHA and percent recruitment of *A. plicata* within three study basins.

River	**Coefficient**	**Estimate**	**SE**	**t**	**P**
Guadalupe	Intercept	-53.8	16.9	-3.18	0.005
	7-day minimum	9.2	2.1	4.38	0.0004
	Julian date of 1-day minimum	0.05	0.02	3.77	0.001
	1-day minimum	-5.3	1.7	-3.18	0.005
	High pulse count	-0.4	0.1	-2.55	0.02
Brazos	Intercept	13.9	5.5	2.54	0.02
	Base flow index	-16.4	5.4	-3.05	0.008
	Rise rate	-14.1	6.9	-2.03	0.06
	Julian date of 1-day maximum	0.01	0.009	1.33	0.02
Colorado	Intercept	8.7	1.9	4.51	< 0.0001
	Low pulse count	-0.5	0.21	-2.26	0.03
	High pulse duration	-0.7	0.03	-2.07	0.05

Table 6.5 Differences in annuli count between observers for *A. plicata* (2 observers) and *L. teres* (3 observers).

	Proportion of samples		
Difference in annuli count	*Amblema plicata*	*Lampsilis teres (average)*	*Lampsilis teres* (range)
4	1.0	1.5	0.00 - 2.2
3	1.6	3.0	0.8 – 4.4
2	3.9	9.7	4.7 -14.8
1	21.7	22.0	18.1 – 24.1
0	35.2	50.9	41.5 – 62.2
-1	24.7	9.6	8.0 – 12.6
-2	9.2	1.7	0.8 – 2.9
-3	2.3	1.0	0.0 – 2.2
-4	0.3	0.5	0.0 – 0.7

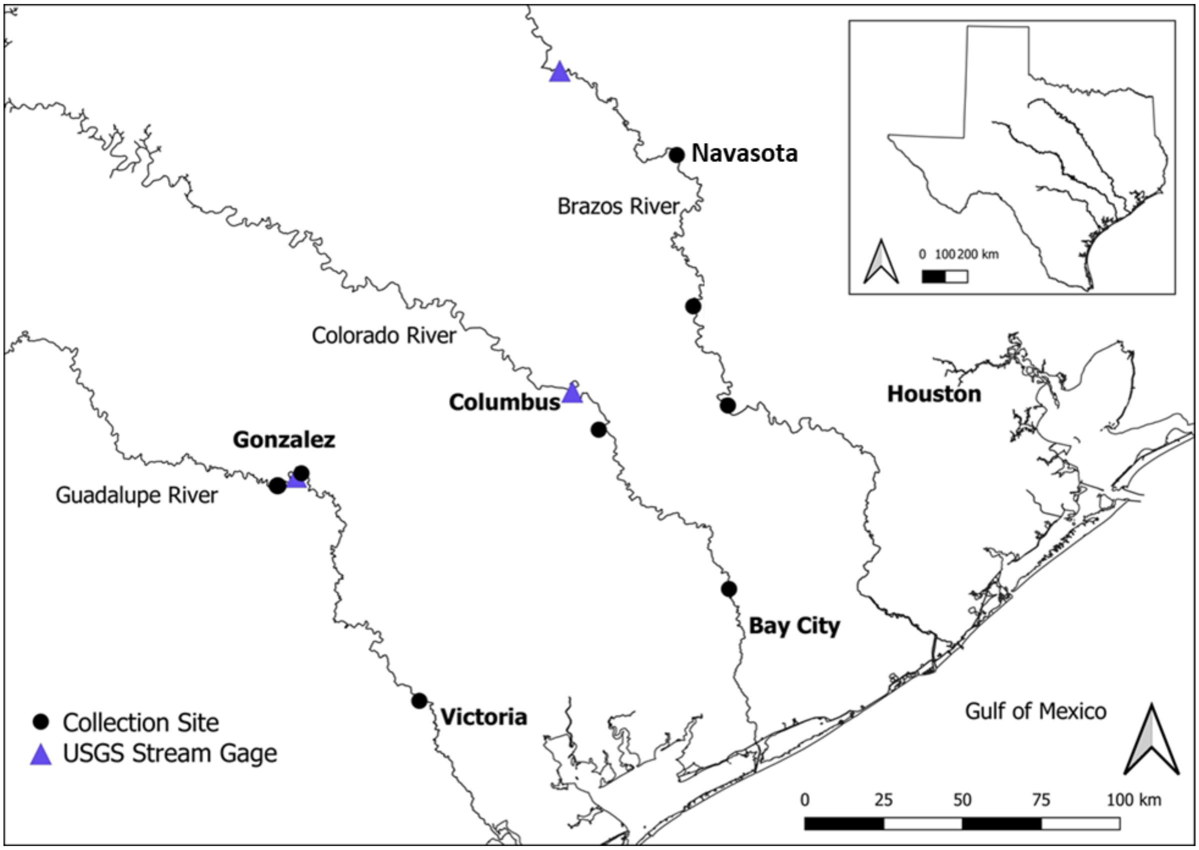

Figure 6.1. Locations of mussel collection sites and USGS stream gages within the Brazos, Colorado, and Guadalupe River basins.

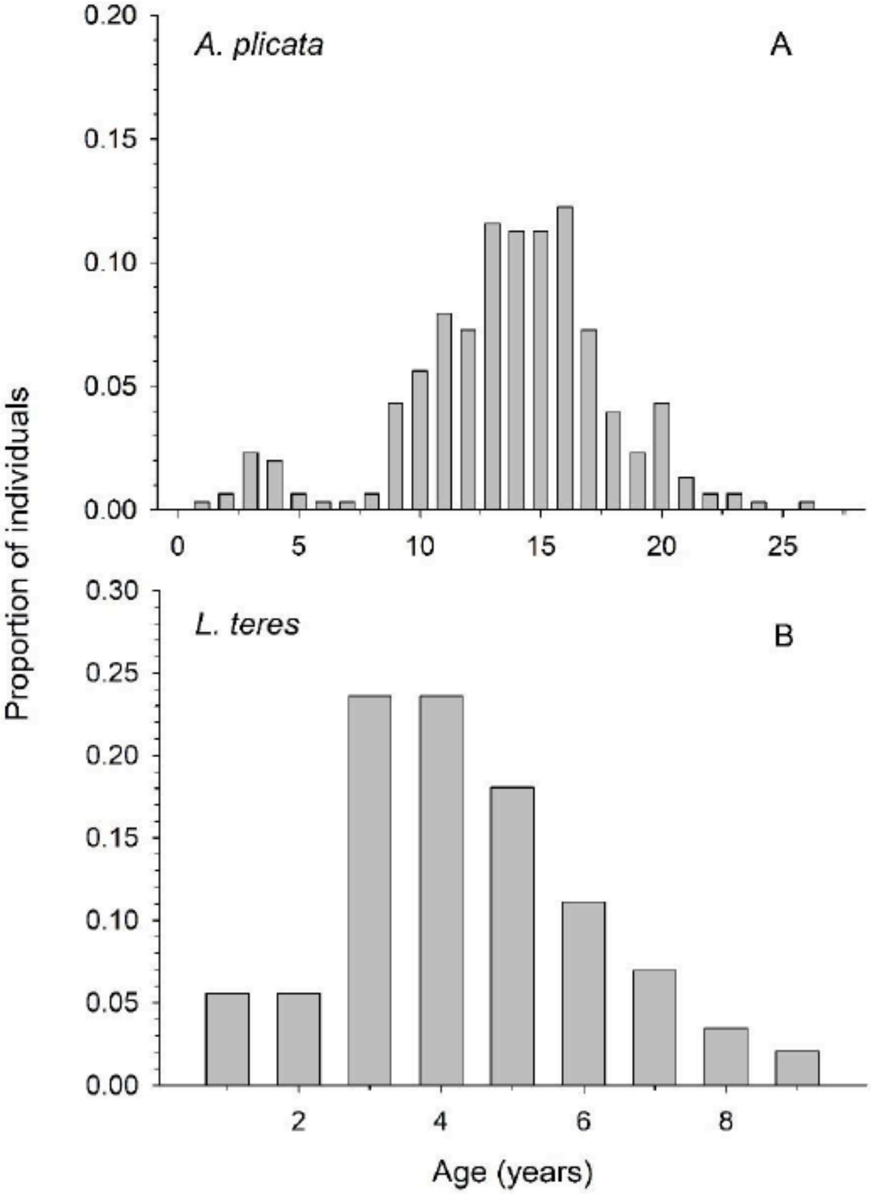

Figure 6.2. Age histogram for A) *A. plicata* (n = 302) and B) *L. teres* (n=144).

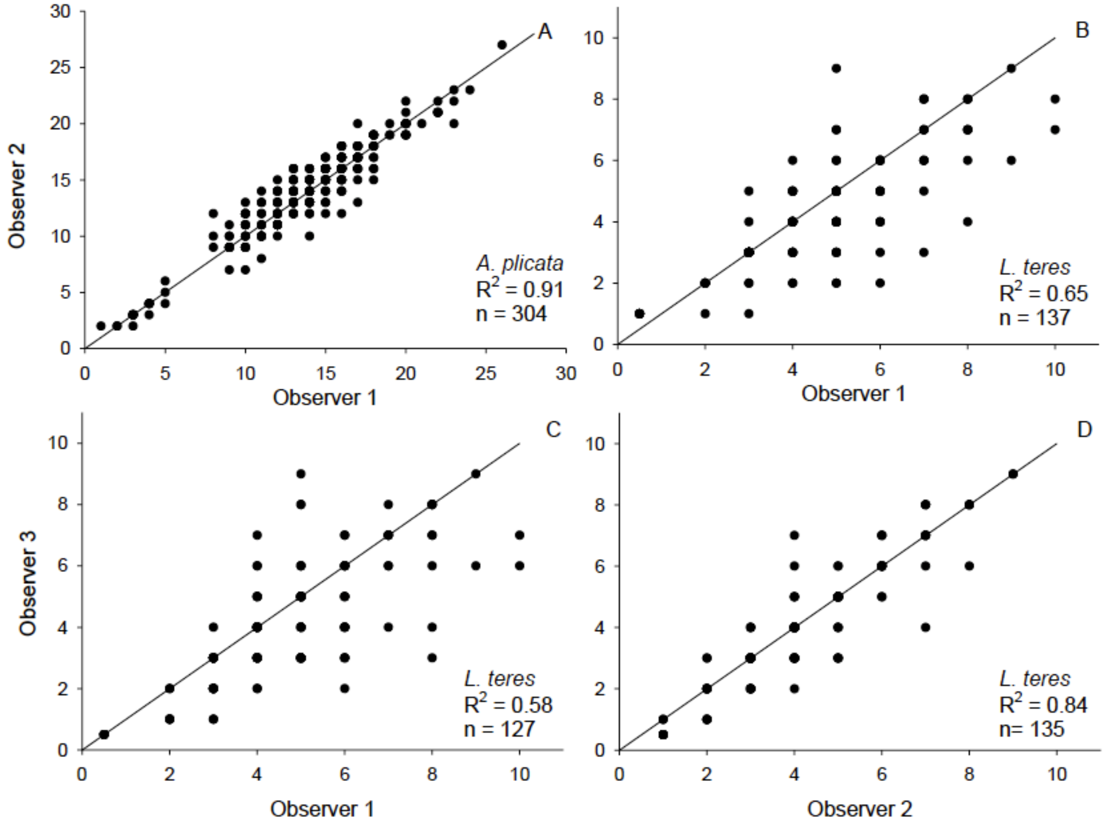

Figure 6.3. Variation between different observers for A) *A. plicata* and B-D) *L. teres*. The line represents the 1:1 line.

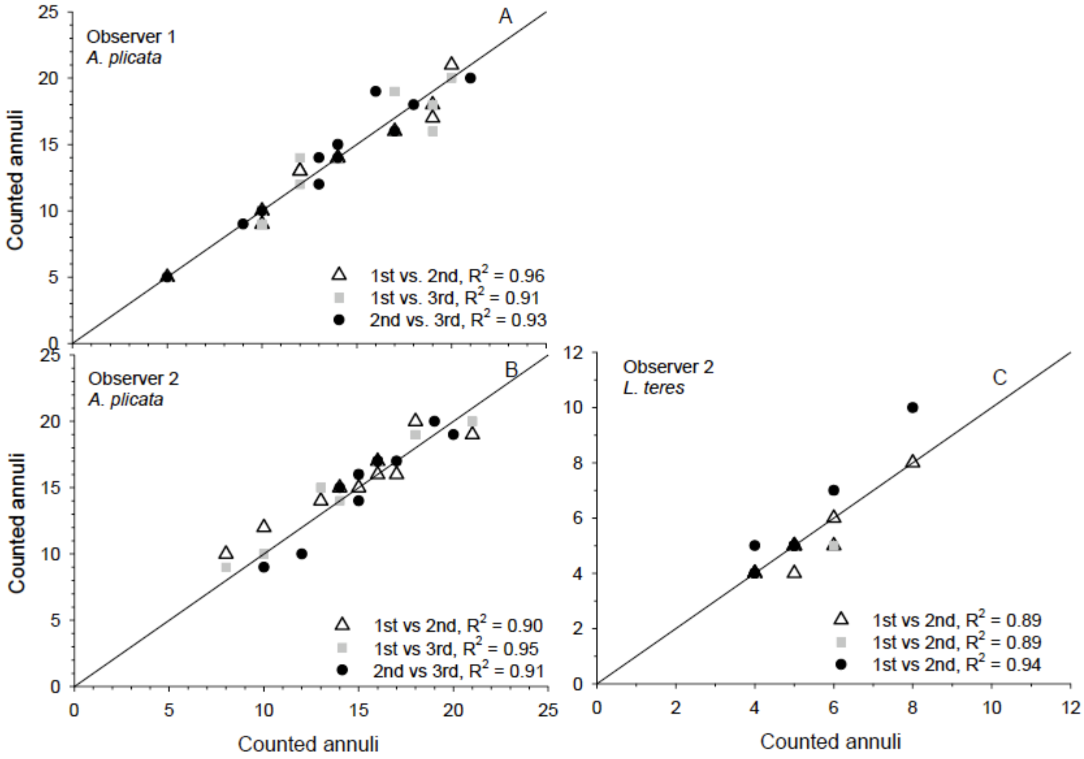

Figure 6.4. Variation within observers comparing repeated blind observations for A) observer 1 and B, C) observer 2 for *A. plicata* (A, B) and *L. teres* (C). The line represents the 1:1 line.

Chapter 7. Simulation of Flow Scenarios and Projection of Indicator Taxa Response

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Background

Application of environmental flow standards involves trade-offs between leaving water in rivers and bays for the maintenance of a sound ecological environment versus diversions for other beneficial uses. These trade-offs may be evaluated in part by predicting ecological outcomes resulting from alternative instream flow regimes produced by different management strategies. The relative change in ecological conditions from the current flow conditions to a more fully appropriated future can inform the development of strategies and identification of priorities both in terms of where and when the adverse impacts are likely to occur.

This study proposes two complementary approaches to assessing the potential impact of flow alteration on a sound ecological environment, both of which are dependent on the development of hydrologic time series of flows under natural or historic conditions and current or future conditions. All the SB3 BBESTs relied to varying degrees on the NFP in the development of flow targets. The following statement from the Brazos BBEST is consistent with the approach taken by each BBEST – *"… virtually all contemporary river ecologists accept the natural flow regime as a robust model for establishing environmental flows. In essence, this model proposes that key ecological processes (both physical and biotic) that sustain native species and their habitats and resources derive from flow variation that mimics, at least qualitatively, the natural pattern."*

A simple desktop approach to validating the flow targets and whether the application of TCEQ adopted standards to future water right permits or other water management strategies is to assess the degree to which flow targets mimic the key components of the natural flow regime. This approach, which has been applied by several BBASC groups (GSA BBASC 2011, Brazos BBASC 2012, Nueces BBASC 2012) and in workplan studies (San Antonio Bay Partnership et al 2015), quantifies the frequencies at which the flow targets at a given location meet or exceed the targets identified by the SB3 BBEST for the river basin in which that site is located. The second approach is based on the application of what was termed, during the SB3 process, as application of ecological overlays. Flow-ecology relationships described in other sections of this report are applied to the hydrologic time series to quantify ecological impacts.

Hydrologic time series at locations within the study area have been developed for alternative water management scenarios by simulating the Water Availability Model (WAM) for the selected river basins. The WAM uses naturalized flows and water management assumptions to simulate flows on a monthly time step for fully permitted scenarios.

Since flow ecology relationships operate on daily rather than monthly time steps, inputs and outputs from the WAMs were disaggregated from monthly to daily values using the Flow Regime Analysis Tool (FRAT) developed by the Texas Parks and Wildlife Department. The FRAT operates at a select location and uses disaggregated monthly flows from the WAM to produce daily estimates based on a daily flow from an appropriate reference gage. Subject to flow standards, FRAT also calculates water available for diversions, available yield from a water diversion or storage project, and the resulting flow downstream from project location.

In recent years, there has been extensive analysis of water management, availability and resulting instream flows in each of the basins in the study area. In 2018, the Brazos River Authority was granted a systems operation permit that included a water management plan and a daily accounting plan that estimates daily flows. The Lower Colorado River Authority regularly updates its water management plan and is currently initiating its next update. In the Guadalupe and San Antonio basins, there have been analyses for proposed off-channel reservoir projects and dedicated wastewater return flows. These system-wide operations and proposed water management plans are complex and not necessarily fully captured by the WAMs. However, the WAMs are the best tool available for generating flow time series. The framework proposed could be adopted to be applied to time series developed by other means.

The following sections describe the process to estimate daily flow time series for select locations in the study area including naturalized, historic/current (as represented by USGS gage flows), fully permitted and the daily flows that would result from the implementation of a hypothetical water management strategy (WMS) subject to diversion restrictions as mandated by the adopted TCEQ environmental flow standards. Calculations of attainment frequencies of the instream flow targets identified in the BBEST reports have been made for each of these alternative flow time series. Comparisons between these alternative time series can be used estimate the extent to which flows have already been altered from more natural conditions and predict potential future alterations as demands for water development continue. This in turn may inform policy recommendations and research priorities.

A main objective of the current study was to move beyond the application of the natural flow paradigm to develop flow-ecology relationships that could be applied to alternative flow scenarios in order to predict ecological response. Application of ecological overlays, as this was described during the development of the flow standards, was carried out by the BBESTs but in many cases site specific analysis was not available. Other sections of this report detail the development of these relationships (for mussels, Shoal Chub, Smallmouth Buffalo and riparian vegetation communities). Ecological outcomes are influenced by a myriad of biotic and abiotic factors and isolating responses to specific components of a flow regime is challenging. An ongoing systematic monitoring and adaptive management program may be required to further define research priorities and fill in knowledge gaps, however select findings from these ecological studies have been applied to the alternative flow regimes to demonstrate how these analyses can be used to validate or refine flow standards and support the development of strategies to maintain the ecological health of aquatic systems in Texas.

Methods

This study presents a framework for how flow-ecology relationships can be applied to alternative flow scenarios. To demonstrate this framework daily time series were developed for four alternative flow scenarios (naturalized, current/historical, fully permitted, and hypothetical water management strategy) for stream locations at Brazos River at Waco, Colorado River at Columbus and Guadalupe River at Victoria (the process described was subsequently repeated for additional sites to apply relationships from the ecological studies contained in this report).

As there are very many flow targets and these vary among sites, an effort was made to develop a consistent framework across the three sites chosen. So, for example, the time series set were developed for the period of record from 1940-1989. This period was selected because it is the longest period that is available for the Brazos, Colorado and GSA WAMs and avoids performing the analysis in the different basins with different periods of record that may include contrasting dry, average, and wet hydrologic conditions. Also, the BBEST target recommendations for the sites in the lower Colorado basin including the site at Columbus, were very different from other sites across the state. The flow targets and adopted standards in the lower Colorado are based primarily on a site-specific study (Bio-West 2008) rather than on the application of the HERF analysis, and thus have a very different format (monthly base flows and unique HFP recurrence frequencies) than almost all other SB3 analyses in the state. For simplicity, the monthly base flow targets were averaged to seasonal values in the attainment frequency analysis. The framework is flexible and could incorporate different periods of record or monthly quantification of attainment frequencies should that be needed.

Current (historical) flows are available from USGS as daily flows and therefore no processing was required to develop these data. Naturalized flows represent the flows that would have been at these locations in the absence of human alterations including reservoir development, streamflow diversions and wastewater returns. Monthly naturalized flows are available from the TCEQ WAM for the three basins. These were disaggregated to daily flows using daily flow patterns. The daily flow patterns for the Brazos River at Waco, and Colorado River at Columbus were acquired from a draft version of the daily WAM models (Wurbs 2019, 2013) which used daily flows from the U.S. Army Corps of Engineers (USACE) Daily Unregulated Flows. The draft daily WAM model for the Guadalupe-San Antonio WAM (Wurbs 2014) also contains daily naturalized flows, however in that model the daily flows were derived from the application of a SWAT model. A comparison the daily naturalized flows to daily gaged flow for Guadalupe River at Victoria suggests that the SWAT results do not closely track the observed daily pattern of flows, and therefore the daily flows developed for the FRAT analysis performed by the SB3 BBEST (GSA BBEST 2011) were used instead. The same disaggregation approach used for naturalized flows was used for the disaggregation of monthly flows produced by the fully permitted WAMs (Run3 – regulated flows). These fully permitted regulated daily flows were also used as inputs to the FRAT model to estimate flows that would occur as a result from implementation of a hypothetical water management strategy.

The FRAT model was used to estimate regulated flows assuming a hypothetical WMS were implemented subject to the rules contained TCEQ adopted flow standards. For the purposes of this study only, this hypothetical WMS assumes the development of a 100,000 acft capacity off-channel reservoir (OCR) with a 500 cfs maximum diversion at the measurement site and a target yield of 30,000 acft/yr. Based on the regulated flows and senior water right pass throughs estimated by the WAM, and the limitations on diversion defined by the implementation of the TCEQ adopted flow standards, the FRAT model determines, on a daily timestep, how much water can be diverted into the off-channel reservoir and how much water would remain in the stream. This remaining water represents the regulated flow for a hypothetical water management strategy. It is important to note that while this hypothetical scenario is intended to represent a reasonably realistic WMS based on consideration of real proposed WMS, this analysis should not be confused with these proposed projects, all of which are somewhat more complicated relying on existing water rights or complex conjunctive surface-ground water strategies. It is also worth noting that the hypothetical WMS in the study does not produce anywhere near the 30,000 acft desired firm yield. In each of the basins where the WMS is modeled, the OCR is empty much of the time and, as will be seen in the analysis of results, was very often unable to make diversions and thus does not significantly alter flows. The reason for this is that the mainstem rivers in these three basins are nearly, if not fully, appropriated. Therefore, even when there is regulated flow at the proposed discharge location, most of the time this water must be passed to meet the needs of downstream senior water rights holders. This combined with the rules contained within the FRAT model to limit diversions subject to the adopted standards means there is often little water available for diversion, and this is why the WMS OCR time series differs very little from the existing fully permitted simulation.

Although the FRAT model incorporates the TCEQ adopted standards, which include specific implementation rules, this report is focused on a quantification of how often the flows exceeded the targets included in the respective BBEST reports. The BBEST targets represent what, in the opinion of the bay and basin expert scientists, were important to maintain a sound ecological environment. The TCEQ standards considered other factors and ultimately adopted standards which were intended to balance the needs of the environment with other beneficial uses. Part of validation is to determine the degree to which the standards and implementation developed by TCEQ meet instream flow needs, and it is for this reason that the focus is on the BBEST target attainment rather than the values provided in the TCEQ standards.

For base flows, attainment frequencies are reported as the percentage of days for which flow met or exceeded the specified base flow magnitudes. For pulse flows, attainment is reported as the percentage of years in which the specific frequency of events (e.g., 4 per season, …, 1 per season, …, 1 per year, …., 1 per 5 years) are met. For example, the 4 per season pulse flow for the Brazos River at Waco is 2,430 cfs (Table 7.1). If this value is exceeded 4 or more times in a given year, then that year is counted as having met the target; if it is only met 3 times, it is not counted as having met the target for that year. Results for targets with frequencies less than 1 per year (e.g., 1 per 2 year, 1 per 5 year) are reported simply as annual frequencies, so whereas a 1 per year target might ideally occur in 100% of the years, a 1 per 2 year target would ideally be met 50% of the years (for the moment, ignoring the different ecological responses that would be expected from flow regimes that met the target every other year versus one that met the target for 10 years in a row followed by 10 years of failing to meet the target, both of which would equal a 50% attainment but would probably produce different ecological responses).

The analysis was initially performed based on meeting the magnitude targets only, magnitude and duration, magnitude and volume, and magnitude, duration and volume. The results of this analysis revealed that, at least for these 3 locations, whenever the duration was met the volume was also met, and thus there were no differences between the event counts for magnitude and duration and the event counts for magnitude, duration and volume. Including more than magnitude only in the calculation significantly decreased event counts and showed almost no differences between scenarios, and therefore only the analysis containing the magnitude only results are included in this report.

Flow ecology relationships derived from the riparian community and Shoal Chub analysis have been applied to the alternative flow scenarios to predict ecological outcomes in response to these flow time series. Emerging from this analysis is the idea that the rate of change of flow may be an important parameter, specifically with respect to Shoal Chub recruitment success. Since the FRAT method for disaggregating monthly flows to daily relies on the application of a daily flow pattern to monthly naturalized and regulated flows from the WAMs, this tool is not suitable for the calculation of rate of change statistics. Therefore, a newer draft version of the Brazos daily WAM was obtained from the TWDB. Several versions of this model include naturalized, current conditions, partial exercise of existing permits and full exercise of existing permits. A fifth scenario including the hypothetical ORC WMS was also developed. For the riparian analysis, these time series data were used to quantify the number of days in each season when the flow met or exceeded the HFPs identified by the BBESTs. The regression equations described in the riparian forest section of this report were then applied to predict germination success. The Shoal Chub analysis identified the rate of change of the descending limb of high flow pulses as a potentially important factor in determining recruitment success.

Results

Daily flow time series for each of the three sites are shown in Figures 7.1-7.3. Although it is impossible to visualize at a 50-year time scale, these flow magnitudes follow expected trends and are generally lower as water development increases. There are also times, at some locations, where naturalized flows are lower than flows reflecting various stages of water development during dry periods and higher than the other sets during wetter periods. This is consistent with the general homogenizing effect of reservoirs (Richter 2010) that capture high flows and release water for downstream needs during low flow periods. Quantification of the frequency at which the BBEST flow targets are met confirms this general pattern.

For reference, Tables 7.1 and 7.2 show the BBEST targets and TCEQ adopted flow standards from the Brazos River at Waco. Table 7.3 shows the attainment frequency results for the BBEST targets. The last column in this table notes the differences between the BBEST and TCEQ targets. At the Brazos River at Waco location, the TCEQ standards did not include HFPs above the 2 per season values. The smaller pulses were incorporated into the implementation approach where the BBEST 4 per season became targets for dry and average conditions, and the

3 per season targets became standards for wet conditions. The middle columns show attainment frequencies for the four sets of flow time series. Lower targets (subsistence and base dry) show increasing frequency of meeting the targets as diversions or other water management projects increase from naturalized to gaged, fully permitted and with the hypothetical OCR WMS. This pattern flips at the higher flow target magnitudes including the base average, base wet and high flow pulse levels. At these higher target flows, attainment frequencies decrease, with a few exceptions, under model assumptions which include increased water development.

Tables 7.4-7.6 provide the same information for the Colorado River at Columbus. As noted above, BBEST targets and TCEQ standards for the lower Colorado are monthly rather than seasonal values. The last column in this table notes that the original base flow targets and standards were converted from monthly to seasonal values. Similar to the situation for the Brazos River at Waco, the TCEQ standards for Colorado at Columbus did not include HFPs above the 2 per season values. These 2 per season pulses are, however, applicable for all hydrologic conditions. Attainment frequencies for this site show a decline in attainment from naturalized to fully permitted conditions. The inclusion of the OCR WMS does not impact any of the attainment frequencies (see discussion section below for explanation for this somewhat unexpected result).

Finally, Tables 7.7-7.9 provides the same information for the Guadalupe River at Victoria. As noted in the last column of Table 7.9, there were several modifications to the BBEST targets to create the TCEQ standards for this location. Most notably the three tiers of base flow targets were compressed into a single base flow standard for this location. Similar to the situation for the two sites discussed above, the TCEQ standards for Guadalupe at Victoria did not include the larger HFPs in this case above the 1 per season values. There were several other discrepancies between the BEBST targets (Table 7.7) and the TCEQ standards (Table 7.8), the explanation for which is not immediately apparent and beyond the scope of this study to address but may be worth additional review by the BBASC or TCEQ. Attainment frequencies for this site also follow a similar pattern to the sites discussed above, with a decline in attainment frequencies as water development increases.

Flow-ecology relationships for Box Elder were applied to the Brazos River at Bryan and the BBEST targets for this site are shown in Table 7.10. Figures 7.4-7.6 show the results of the application of flow ecology relationships between counts of days that met or exceeded the HFP target magnitudes and the probability of germination for Box Elder. Figure 7.4 shows the counts of days in the spring season which meet the 3 per season target magnitude under five alternative flow scenarios. Figure 7.5 shows the application of flow-ecology relationships to convert these counts into a probability of germination for each year. Figure 7.6 then presents these annual probabilities into exceedance frequencies. This figure shows that the probability of germination was greater than 30% in about 26% of years under natural conditions (NAT), about 18% of years under current conditions (CUR) with partial exercise of existing water rights (PART), and about 16% of years under fully permitted (FULL) and fully permitted plus and a hypothetical new off-channel reservoir (OCR).

Discussion

The methodology described in this study is intended to provide a rational framework for estimating flow alterations through time and develop hydrologic time series for estimations of past, present, and future conditions. The calculation of attainment frequencies under different water management scenarios has been applied by several SB1 regional planning groups based on monthly flows, and a move towards analysis based on daily flows provides a better basis upon which to consider environmental flows to which ecological response are more directly linked. These hydrologic time series can then be used to assess attainment frequencies of flow components identified by the BBEST that are supportive of a sound ecological environment and to which the results of studies which quantify flow-ecology relationships can be applied to predict ecological response to these alterations. The framework relies on a standard approach, incorporating tools developed by the TPWD and others during the SB3 planning process (FRAT) for disaggregating flows from monthly to daily values.

The analysis of attainment frequencies for environmental flow targets to maintain a sound ecological environment produced results that are generally consistent with expectations. As water development increases, the frequencies of flows meeting targets generally decreased between naturalized and fully appropriated flow scenarios. However, as demonstrated by the analysis of the Brazos at Waco, attainment frequencies for low flows (subsistence and base dry) have increased, likely due to releases for downstream diversions. Flows at the Colorado at Columbus show reductions in the frequency of meeting the subsistence attainment targets that, in some seasons, approach 90%. It is however important to acknowledge that flows in the lower Colorado are largely determined by the operations dictated by the LCRA WMP (water management plan), which contains specific rules related to operations of the Highland Lakes to provide for environmental flows in the lower Colorado. Although a primary purpose of the SB3 flow program was to develop standards to be used in the TCEQ permitting process, both the LCRA and BRA water management plans, which are themselves permitted water rights, have considered the SB3 standards as they have been developed, and it may be useful to consider WAM results developed specifically for these WMPs. The results for subsistence flow attainment frequencies for the Guadalupe at Victoria show very modest changes to attainment frequencies between naturalized and fully permitted scenarios, however simulated declines in attainment frequencies are greater at base dry levels. The fact that the TCEQ standards do not include the multiple tiers of base flow levels may result in greater diversion when the river is at base dry levels (allowing for diversions down to the subsistence flows at certain times when a tier base flow standard might otherwise limit these diversions).

As discussed below, the use of gage flows presents several challenges when comparing longer term attainment frequencies to modeled flows based on static water management scenarios. However, a review of the results for attainment frequencies based on gage flows does provide some insight into how much alteration has occurred relative to the natural conditions, and how much alteration may be expected to result from permitted, but as yet unperfected, water rights. A review of the results for the three sites included in this study does not readily produce general patterns for these comparisons. However, it can identify specific seasons and flow levels where declines in attainment frequencies have already occurred or can predict where these may occur if water rights are fully exercised. Further research is needed to fill in knowledge gaps and assess whether proactive strategies may be needed to provide flows to maintain a sound environment. Results produced here for subsistence and base flow components for Colorado at Columbus suggest that additional studies should examine base dry and base average conditions during winter. At that gage, attainment frequencies based on gage flow data are significantly lower than the WAM indicates would occur under more natural conditions. Also, it may be worth considering the potential future reduction in dry and average base flow in the fall season for the Guadalupe at Victoria where percent reductions in attainment frequencies are predicted to be near or above 20%.

Application of ecological overlays can be used to validate or suggest refinements to the adopted flow standards. The riparian vegetation analysis identified a strong correlation between number of pulse days per season and likelihood of germination. Figures 7.4-67. show the application of this relationship for the 3 pulses per spring season at the Brazos River at Bryan site. A second application of ecological overlays is to support the development of strategies to support a sound environment. The Shoal Chub analysis suggests that the rate of fall from a pulse event may influence recruitment success. While TCEQ adopted standards do not directly address rate of change, if this finding is true, it could be used to inform a reservoir management strategy to schedule a step-down flood pool release in a pattern more in keeping with a more natural pattern. Systematic long-term monitoring has been identified as a priority (Hardy et al. 2021). The Shoal Chub age-length analysis could be used to evaluate future sampling efforts to further refine or support this rate of change recruitment relationship.

The approach adopted here might be improved with further development of the daily WAMs. Draft daily WAM model have been developed for the Brazos, Colorado, and Guadalupe basins. These models were considered for use in the current study; however, they are somewhat out of date. The most recent version of the WRAP model (Water Rights Analysis Package) includes significant improvements to modeling environmental flow targets, which relate to ecological response on daily rather than monthly time steps, over earlier versions. The draft daily WAMs also do not include major modifications to water management in the three basins, specifically the most recent changes to the LCRA water management plan in the Colorado basin, the Brazos water management plan developed as part of the BRA systems operation permit, and several significant new or amended water rights permit in the Guadalupe basin. Each of these recent additions incorporate, to some extent, SB3 flow standards, but do so on a monthly time step. While updating the daily WAMs was investigated as part of the present study, this proved to be beyond the scope and resources available under the agency contract. During course of the present study, TWDB funded studies to create daily WAMs for the Brazos, Neches and Red River basins. The study funded by the TWDB (Anchor and Hoffpauir 2021) utilized already available daily WAMs for the Brazos, Trinity, and Neches river basins. Those WAMs include source model files that reflect no (naturalized), current, and fully authorized diversions and impoundments. To more accurately model current conditions (including system operations and actual diversion locations), Anchor and Hoffpauir (2021) developed source model files for the daily Brazos WAM based on source model files available from Wurbs (2019), TCEQ, and the Brazos Region G Regional Planning Group. This newer draft version of the Brazos daily WAM, including the new sources files for the current conditions, was used in this current study. Daily WAMs for the other basins could be used to developed more suitable hydrologic time series to which the flow ecology relationships developed in this study could be applied.

This study also relies on gage flow data to represent historic/current flow conditions. The use of gage flow data has both advantages and disadvantages relative to the use of model flows. The main advantage being that gage flow represents the actual observed flow to which the existing ecological community has responded. The disadvantage is that unlike the other three flow time series to which it is compared, the gage flows are impacted by non-static water management conditions. It would be useful to consider flow regimes representative of current conditions applied to historical flow patterns similar to the fully permitted simulations contained in the TCEQ run 3 model simulations. There have been current conditions WAM simulation created for each of the basins in Texas (TCEQ Run8) however many these models, including the ones for the three basins included in this study, appear to be well out of date and may no longer accurately reflect current conditions.

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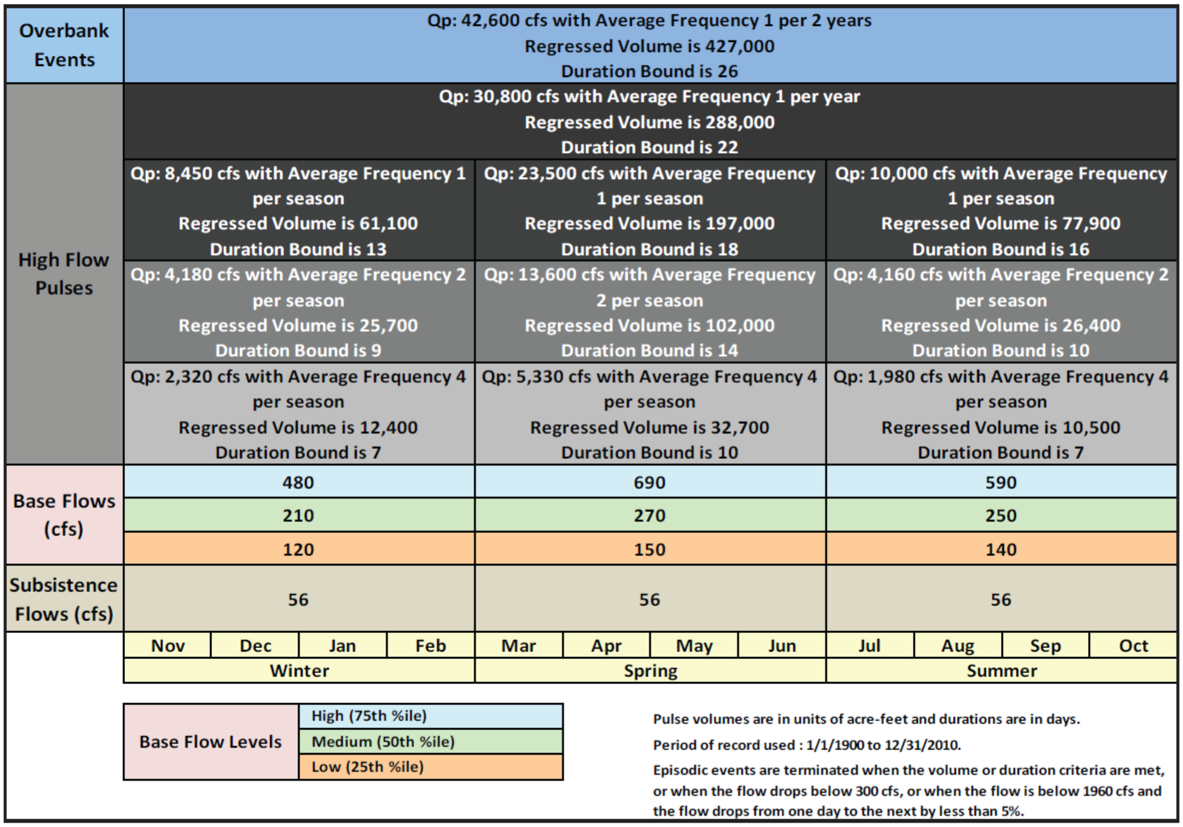
Table 7.27. BBEST Environmental Flows Report for Brazos River at Waco.



Overbank Events	Qp: 42,600 cfs with Average Frequency 1 per 2 years Regressed Volume is 427,000 Duration Bound is 26		
High Flow Pulses	Qp: 30,800 cfs with Average Frequency 1 per year Regressed Volume is 288,000 Duration Bound is 22		
	Qp: 8,450 cfs with Average Frequency 1 per season Regressed Volume is 61,100 Duration Bound is 13	Qp: 23,500 cfs with Average Frequency 1 per season Regressed Volume is 197,000 Duration Bound is 18	Qp: 10,000 cfs with Average Frequency 1 per season Regressed Volume is 77,900 Duration Bound is 16
	Qp: 4,180 cfs with Average Frequency 2 per season Regressed Volume is 25,700 Duration Bound is 9	Qp: 13,600 cfs with Average Frequency 2 per season Regressed Volume is 102,000 Duration Bound is 14	Qp: 4,160 cfs with Average Frequency 2 per season Regressed Volume is 26,400 Duration Bound is 10
	Qp: 2,320 cfs with Average Frequency 4 per season Regressed Volume is 12,400 Duration Bound is 7	Qp: 5,330 cfs with Average Frequency 4 per season Regressed Volume is 32,700 Duration Bound is 10	Qp: 1,980 cfs with Average Frequency 4 per season Regressed Volume is 10,500 Duration Bound is 7
Base Flows (cfs)	480	690	590
	210	270	250
	120	150	140
Subsistence Flows (cfs)	56	56	56
	Nov Dec Jan Feb	Mar Apr May Jun	Jul Aug Sep Oct
	Winter	Spring	Summer

Base Flow Levels	
	High (75th %ile)
	Medium (50th %ile)
	Low (25th %ile)

Pulse volumes are in units of acre-feet and durations are in days.

Period of record used : 1/1/1900 to 12/31/2010.

Episodic events are terminated when the volume or duration criteria are met, or when the flow drops below 300 cfs, or when the flow is below 1960 cfs and the flow drops from one day to the next by less than 5%.

Table 7.28. TCEQ Adopted Standards - 30 TAC §298.480(10) for Brazos River at Waco.

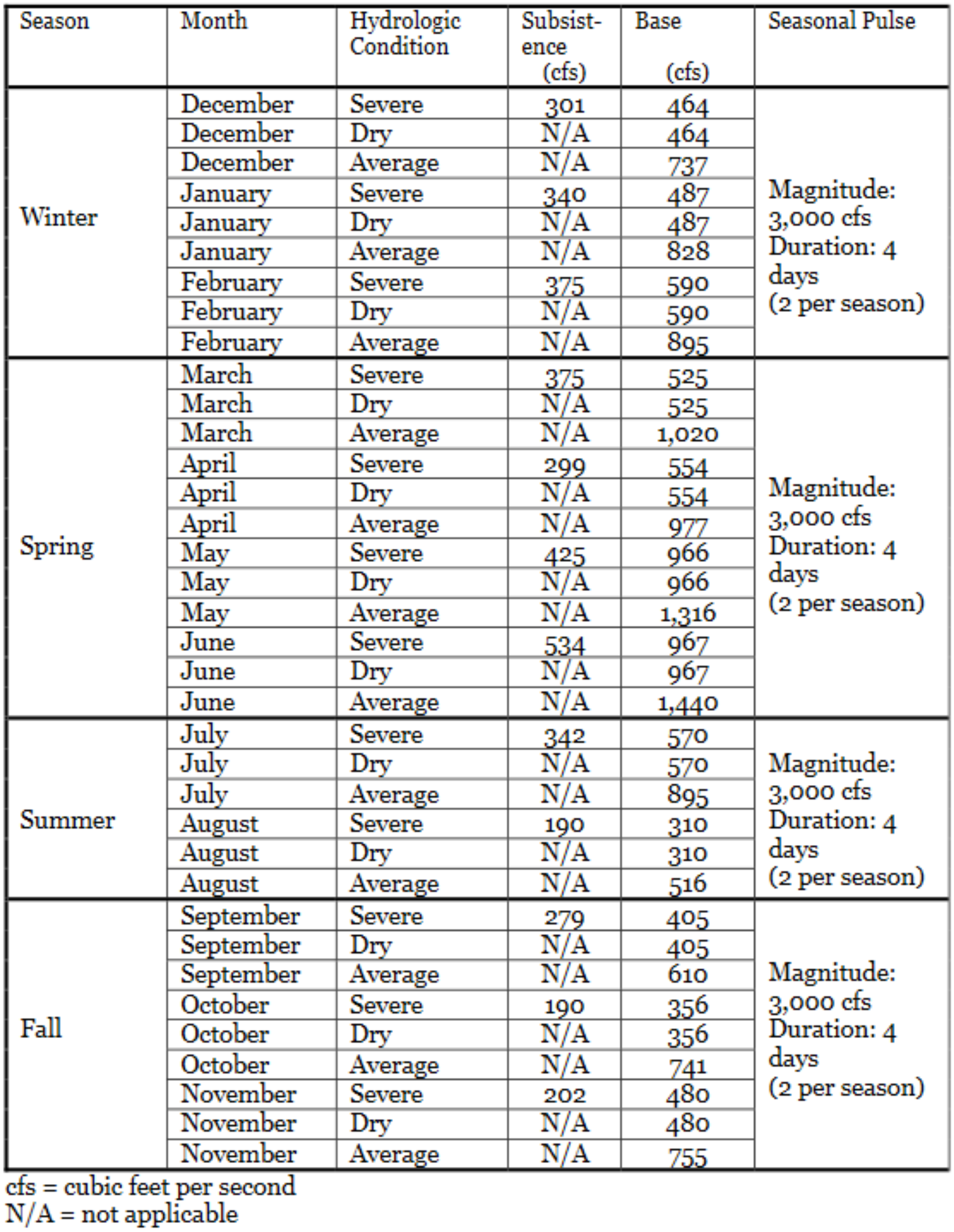
Season	**Subsistence**	**Hydrologic Condition**	**Base**	**Dry Condition Seasonal Pulse**	**Average Condition Seasonal Pulse**	**Wet Condition Seasonal Pulse**
Winter	56 cfs	Dry	120 cfs	1 per season Trigger: 2,320 cfs Volume: 12,400 af Duration: 7 days	3 per season Trigger: 2,320 cfs Volume: 12,400 af Duration: 7 days	2 per season Trigger: 4,180 cfs Volume: 25,700 af Duration: 9 days
		Average	210 cfs			
		Wet	480 cfs			
Spring	56 cfs	Dry	150 cfs	1 per season Trigger: 5,330 cfs Volume: 32,700 af Duration: 10 days	3 per season Trigger: 5,330 cfs Volume: 32,700 af Duration: 10 days	2 per season Trigger: 13,600 cfs Volume: 102,000 af Duration: 14 days
		Average	270 cfs			
		Wet	690 cfs			
Summer	56 cfs	Dry	140 cfs	1 per season Trigger: 1,980 cfs Volume: 10,500 af Duration: 7 days	3 per season Trigger: 1,980 cfs Volume: 10,500 af Duration: 7 days	2 per season Trigger: 4,160 cfs Volume: 26,400 af Duration: 10 days
		Average	250 cfs			
		Wet	590 cfs			

cfs = cubic feet per second
af = acre-feet
N/A = not applicable

Table 7.29. Attainment frequencies for Brazos River at Waco.

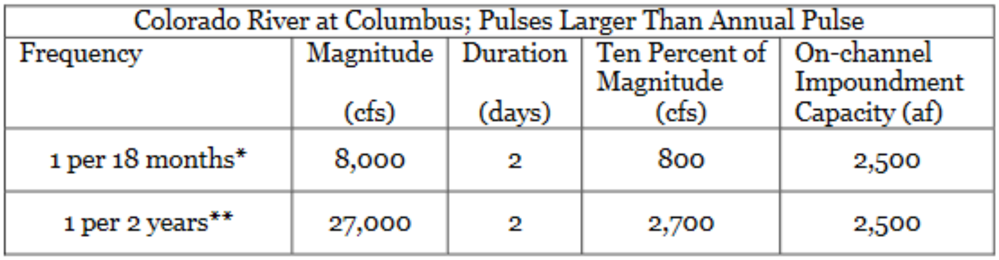
BBEST	Targets	Attainments (Base = % of days, HFP = % of years with events)				
Flow Tier	Magnitude (cfs)	Naturalized	Gaged	Fully Permited	WMS (OCR)	BBEST vs TCEQ
Subs_Win	56	95	95	97	97	same
Subs_Spr	56	98	99	97	97	same
Subs_Sum	56	92	97	97	97	same
Dry_Win	120	86	87	88	88	same
Dry_Spr	150	94	91	90	90	same
Dry_Sum	140	80	91	93	93	same
Avg_Win	210	73	77	77	77	same
Avg_Spr	270	87	84	81	81	same
Avg_Sum	250	67	82	86	86	same
Wet_Win	480	50	58	53	50	same
Wet_Spr	690	66	64	57	57	same
Wet_Sum	590	43	58	60	59	same
4ps_Win	2,320	40	44	34	32	1ps dry, 3ps avg
4ps_Spr	5,330	36	26	26	28	1ps dry, 3ps avg
4ps_Sum	1,980	38	56	70	70	1ps dry, 3ps avg
2ps_Win	4,180	44	40	28	28	2ps wet
2ps_Spr	13,600	42	50	28	28	2ps wet
2ps_Sum	4,160	56	36	60	60	2ps wet
1ps_Win	8,450	50	46	36	32	not part of TCEQ
1ps_Spr	23,500	50	40	38	38	not part of TCEQ
1ps_Sum	10,000	58	36	46	46	not part of TCEQ
1py_Ann	30,800	60	30	40	40	not part of TCEQ
1p2y_Ann	42,600	44	22	26	26	not part of TCEQ

Table 7.30. BBEST Environmental Flows Report for Colorado River at Columbus.



Season	Month	Hydrologic Condition	Subsistence (cfs)	Base (cfs)	Seasonal Pulse
Winter	December	Severe	301	464	Magnitude: 3,000 cfs Duration: 4 days (2 per season)
	December	Dry	N/A	464	
	December	Average	N/A	737	
	January	Severe	340	487	
	January	Dry	N/A	487	
	January	Average	N/A	828	
	February	Severe	375	590	
	February	Dry	N/A	590	
	February	Average	N/A	895	
Spring	March	Severe	375	525	Magnitude: 3,000 cfs Duration: 4 days (2 per season)
	March	Dry	N/A	525	
	March	Average	N/A	1,020	
	April	Severe	299	554	
	April	Dry	N/A	554	
	April	Average	N/A	977	
	May	Severe	425	966	
	May	Dry	N/A	966	
	May	Average	N/A	1,316	
	June	Severe	534	967	
	June	Dry	N/A	967	
	June	Average	N/A	1,440	
Summer	July	Severe	342	570	Magnitude: 3,000 cfs Duration: 4 days (2 per season)
	July	Dry	N/A	570	
	July	Average	N/A	895	
	August	Severe	190	310	
	August	Dry	N/A	310	
	August	Average	N/A	516	
Fall	September	Severe	279	405	Magnitude: 3,000 cfs Duration: 4 days (2 per season)
	September	Dry	N/A	405	
	September	Average	N/A	610	
	October	Severe	190	356	
	October	Dry	N/A	356	
	October	Average	N/A	741	
	November	Severe	202	480	
	November	Dry	N/A	480	
	November	Average	N/A	755	

cfs = cubic feet per second
N/A = not applicable



Colorado River at Columbus; Pulses Larger Than Annual Pulse				
Frequency	Magnitude (cfs)	Duration (days)	Ten Percent of Magnitude (cfs)	On-channel Impoundment Capacity (af)
1 per 18 months*	8,000	2	800	2,500
1 per 2 years**	27,000	2	2,700	2,500

Table 7.31. TCEQ Adopted Standards - 30 TAC §298.330(e)(13)(A) for Colorado River at Columbus.

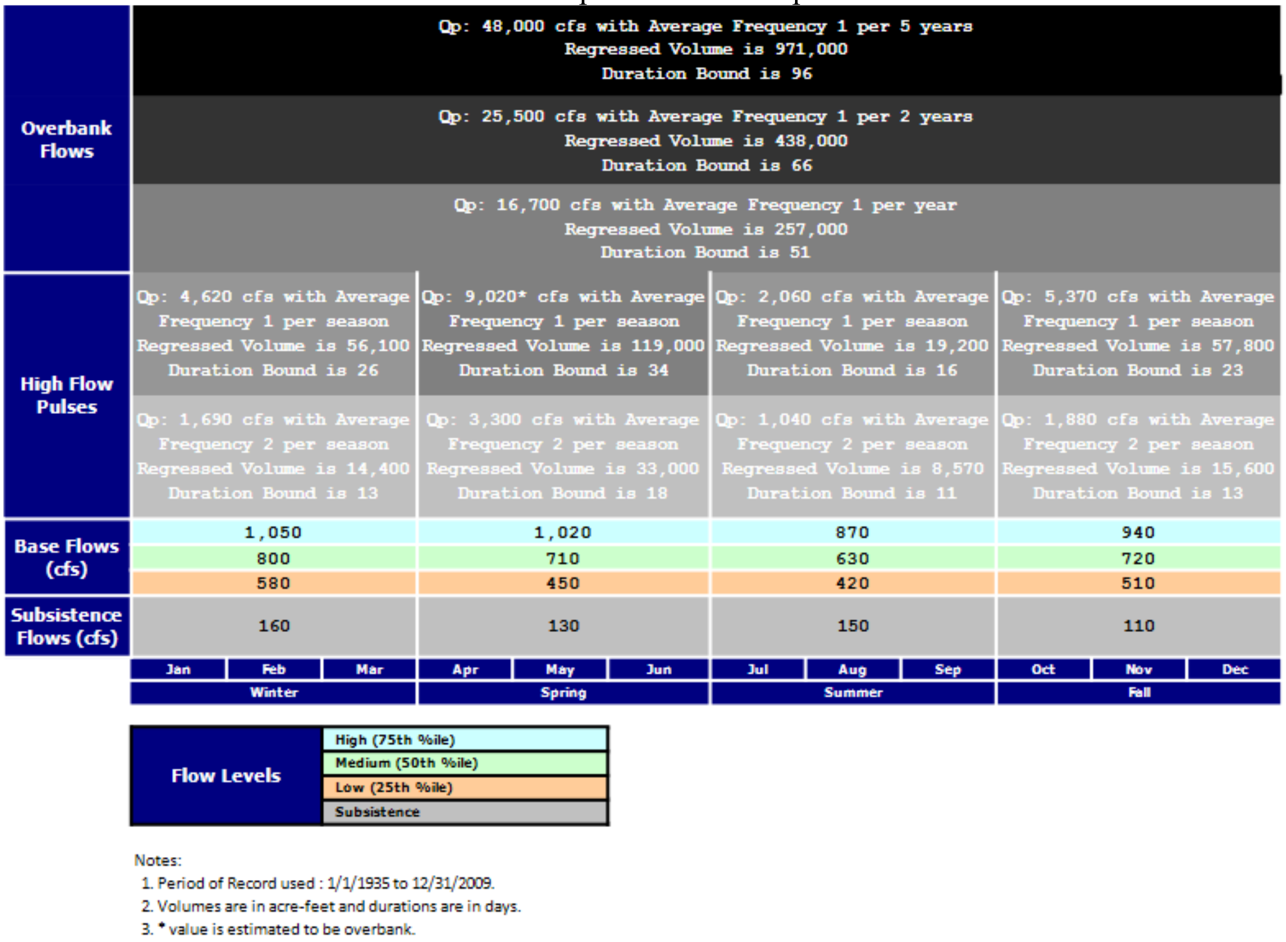
Season	**Month**	**Hydrologic Condition**	**Subsistence**	**Base**	**Seasonal Pulse (2 per season)**
Winter	December	Severe	301 cfs	464 cfs	Magnitude: 3,000 cfs Duration: 4 days
	December	Dry	N/A	464 cfs	
	December	Average	N/A	737 cfs	
	January	Severe	340 cfs	487 cfs	
	January	Dry	N/A	487 cfs	
	January	Average	N/A	828 cfs	
	February	Severe	375	590 cfs	
	February	Dry	N/A	590 cfs	
	February	Average	N/A	895 cfs	
Spring	March	Severe	375 cfs	525 cfs	Magnitude: 3,000 cfs Duration: 4 days
	March	Dry	N/A	525 cfs	
	March	Average	N/A	1,020 cfs	
	April	Severe	299 cfs	554 cfs	
	April	Dry	N/A	554 cfs	
	April	Average	N/A	977 cfs	
	May	Severe	425 cfs	966 cfs	
	May	Dry	N/A	966 cfs	
	May	Average	N/A	1,316 cfs	
	June	Severe	534 cfs	967 cfs	
	June	Dry	N/A	967 cfs	
	June	Average	N/A	1,440 cfs	
Summer	July	Severe	342 cfs	570 cfs	Magnitude: 3,000 cfs Duration: 4 days
	July	Dry	N/A	570 cfs	
	July	Average	N/A	895 cfs	
	August	Severe	190 cfs	310 cfs	
	August	Dry	N/A	310 cfs	
	August	Average	N/A	516 cfs	
Fall	September	Severe	279 cfs	405 cfs	Magnitude: 3,000 cfs Duration: 4 days
	September	Dry	N/A	405 cfs	
	September	Average	N/A	610 cfs	
	October	Severe	190 cfs	356 cfs	
	October	Dry	N/A	356 cfs	
	October	Average	N/A	741 cfs	
	November	Severe	202 cfs	480 cfs	
	November	Dry	N/A	480 cfs	
	November	Average	N/A	755 cfs	

cfs = cubic feet per second
N/A = not applicable

Table 7.32. Attainment frequencies for Colorado River at Columbus.

BBEST	Targets	Attainments (Base = % of days, HFP = % of years with events)				
Flow Tier	Magnitude (cfs)	Naturalized	Gaged	Fully Permited	WMS (OCR)	Note
Subs_Win	339	100	86	88	88	Monthly
Subs_Spr	408	100	95	91	91	Monthly
Subs_Sum	266	100	100	100	100	Monthly
Subs_Fall	224	100	98	96	96	Monthly
Dry_Win	514	93	73	67	67	Monthly
Dry_Spr	753	93	89	71	71	Monthly
Dry_Sum	440	98	100	97	97	Monthly
Dry_Fall	414	97	86	81	81	Monthly
Avg_Win	827	79	58	41	41	Monthly
Avg_Spr	1,239	79	77	52	51	Monthly
Avg_Sum	714	88	100	85	85	Monthly
Avg_Fall	710	84	74	56	56	Monthly
2ps_Win	3,000	58	56	40	40	Yes
2ps_Spr	3,000	86	84	90	90	Yes
2ps_Sum	3,000	34	30	22	22	Yes
2ps_Fall	3,000	64	60	54	54	Yes
1p18months	8,000	98	88	86	86	No
1p2y_Ann	27,000	70	44	30	30	No

Table 7.33. BBEST Environmental Flows Report for Guadalupe River at Victoria.



	Winter	Spring	Summer	Fall
Overbank Flows	Qp: 48,000 cfs with Average Frequency 1 per 5 years Regressed Volume is 971,000 Duration Bound is 96			
	Qp: 25,500 cfs with Average Frequency 1 per 2 years Regressed Volume is 438,000 Duration Bound is 66			
	Qp: 16,700 cfs with Average Frequency 1 per year Regressed Volume is 257,000 Duration Bound is 51			
High Flow Pulses	Qp: 4,620 cfs with Average Frequency 1 per season Regressed Volume is 56,100 Duration Bound is 26	Qp: 9,020* cfs with Average Frequency 1 per season Regressed Volume is 119,000 Duration Bound is 34	Qp: 2,060 cfs with Average Frequency 1 per season Regressed Volume is 19,200 Duration Bound is 16	Qp: 5,370 cfs with Average Frequency 1 per season Regressed Volume is 57,800 Duration Bound is 23
	Qp: 1,690 cfs with Average Frequency 2 per season Regressed Volume is 14,400 Duration Bound is 13	Qp: 3,300 cfs with Average Frequency 2 per season Regressed Volume is 33,000 Duration Bound is 18	Qp: 1,040 cfs with Average Frequency 2 per season Regressed Volume is 8,570 Duration Bound is 11	Qp: 1,880 cfs with Average Frequency 2 per season Regressed Volume is 15,600 Duration Bound is 13
Base Flows (cfs)	1,050	1,020	870	940
	800	710	630	720
	580	450	420	510
Subsistence Flows (cfs)	160	130	150	110
	Jan Feb Mar	Apr May Jun	Jul Aug Sep	Oct Nov Dec
	Winter	Spring	Summer	Fall

Flow Levels	
	High (75th %ile)
	Medium (50th %ile)
	Low (25th %ile)
	Subsistence

Notes:

1. Period of Record used : 1/1/1935 to 12/31/2009.
2. Volumes are in acre-feet and durations are in days.
3. * value is estimated to be overbank.

Table 7.34. TCEQ Adopted Standards - 30 TAC §298.380(c)(9) for Guadalupe River at Victoria.

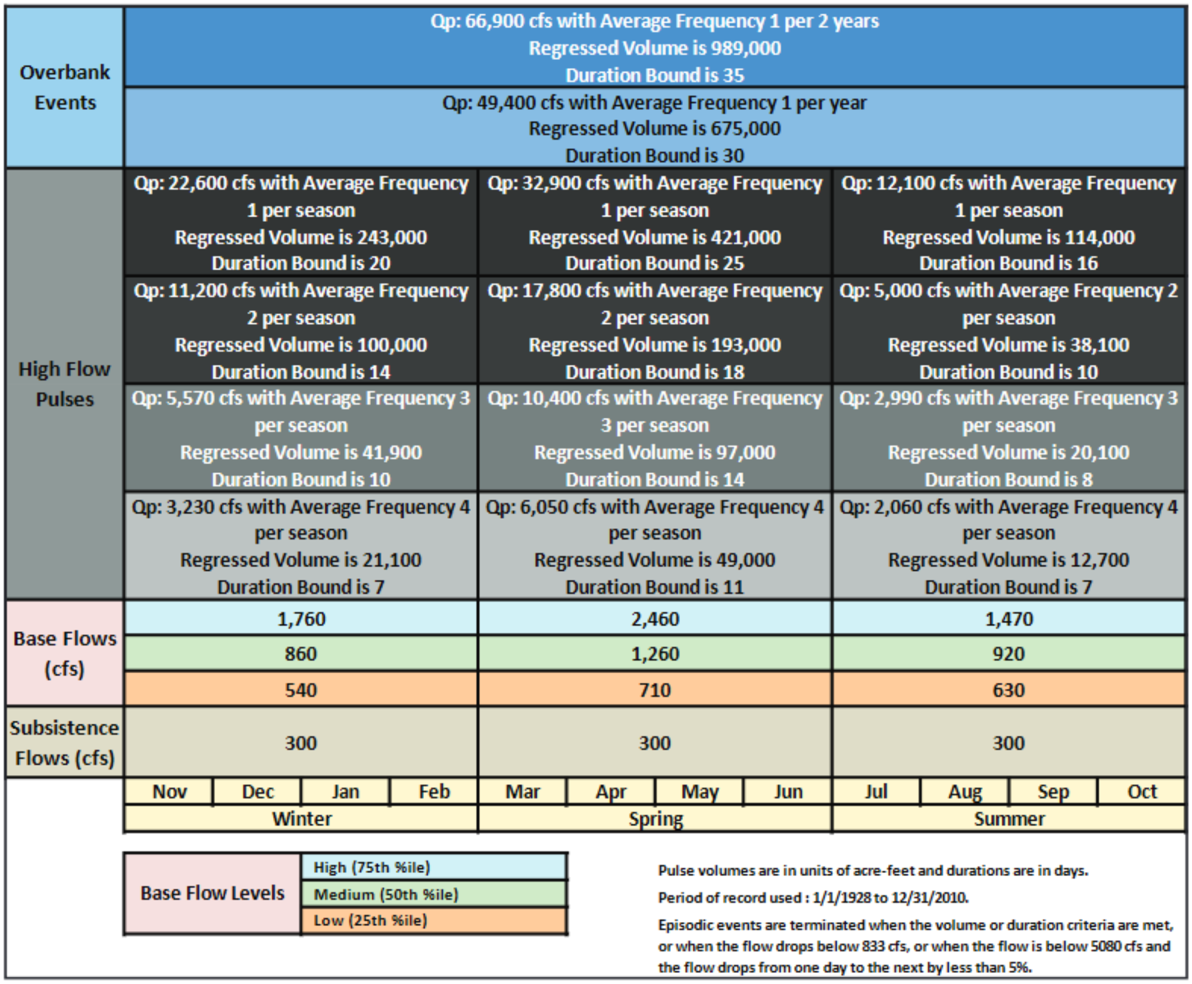
Season	Subsistence	Base	Small Seasonal Pulse (2 per season)	Large Seasonal Pulse (1 per season)
Winter	160 cfs	975 cfs	Trigger: 1,690 cfs Volume: 14,400 af Duration: 13 days	Trigger: 3,240 cfs Volume: 33,000 af Duration: 18 days
Spring	130 cfs	945 cfs	Trigger: 3,240 cfs Volume: 33,000 af Duration: 18 days	Trigger: 3,240 cfs Volume: 43,500 af Duration: 25 days
Summer	150 cfs	795 cfs	Trigger: 1,040 cfs Volume: 8,570 af Duration: 11 days	Trigger: 2,060 cfs Volume: 19,200 af Duration: 16 days
Fall	110 cfs	865 cfs	Trigger: 1,880 cfs Volume: 15,600 af Duration: 13 days	Trigger: 3,240 cfs Volume: 35,500 af Duration: 23 days

cfs = cubic feet per second
af = acre-feet

Table 7.35. Attainment frequencies for Guadalupe River at Victoria.

BBEST	Targets	Attainments (Base = % of days, HFP = % of years with events)				
Flow Tier	Magnitude (cfs)	Naturalized	Gaged	Fully Permited	WMS (OCR)	BBEST vs TCEQ
Subs_Win	160	100	99	98	98	Yes
Subs_Spr	130	100	98	98	98	Yes
Subs_Sum	150	99	92	93	93	Yes
Subs_Fall	110	99	97	97	97	Yes
Dry_Win	580	88	83	75	75	only 1 tier
Dry_Spr	450	93	86	81	81	only 1 tier
Dry_Sum	420	83	75	70	70	only 1 tier
Dry_Fall	510	86	82	75	75	only 1 tier
Avg_Win	800	74	67	60	60	only 1 tier
Avg_Spr	710	79	74	68	68	only 1 tier
Avg_Sum	630	71	63	52	52	only 1 tier
Avg_Fall	720	74	67	60	60	only 1 tier
Wet_Win	1,050	57	51	46	41	only 1 tier
Wet_Spr	1,020	65	60	54	50	only 1 tier
Wet_Sum	870	55	45	35	33	only 1 tier
Wet_Fall	940	60	48	44	40	only 1 tier
2ps_Win	1,690	52	48	40	42	Yes
2ps_Spr	3,300	62	58	52	48	Minor mod 3,240
2ps_Sum	1,040	66	68	52	50	Yes
2ps_Fall	1,880	56	56	54	54	Yes
1ps_Win	4,620	50	44	42	42	? - used 2ps Spr modified
1ps_Spr	9,020	62	58	58	56	? - used 2ps Spr modified
1ps_Sum	2,060	66	64	58	58	yes
1ps_Fall	5,370	50	50	48	48	? - used 2ps Spr modified
1py_Ann	16,700	46	44	42	42	no
1p2y_Ann	25,500	34	32	30	30	no
1p5y_Ann	48,000	20	16	16	16	no

Table 7.36. BBEST Environmental Flows Report for Brazos River at Bryan.



Overbank Events	Qp: 66,900 cfs with Average Frequency 1 per 2 years Regressed Volume is 989,000 Duration Bound is 35		
	Qp: 49,400 cfs with Average Frequency 1 per year Regressed Volume is 675,000 Duration Bound is 30		
High Flow Pulses	Qp: 22,600 cfs with Average Frequency 1 per season Regressed Volume is 243,000 Duration Bound is 20	Qp: 32,900 cfs with Average Frequency 1 per season Regressed Volume is 421,000 Duration Bound is 25	Qp: 12,100 cfs with Average Frequency 1 per season Regressed Volume is 114,000 Duration Bound is 16
	Qp: 11,200 cfs with Average Frequency 2 per season Regressed Volume is 100,000 Duration Bound is 14	Qp: 17,800 cfs with Average Frequency 2 per season Regressed Volume is 193,000 Duration Bound is 18	Qp: 5,000 cfs with Average Frequency 2 per season Regressed Volume is 38,100 Duration Bound is 10
	Qp: 5,570 cfs with Average Frequency 3 per season Regressed Volume is 41,900 Duration Bound is 10	Qp: 10,400 cfs with Average Frequency 3 per season Regressed Volume is 97,000 Duration Bound is 14	Qp: 2,990 cfs with Average Frequency 3 per season Regressed Volume is 20,100 Duration Bound is 8
	Qp: 3,230 cfs with Average Frequency 4 per season Regressed Volume is 21,100 Duration Bound is 7	Qp: 6,050 cfs with Average Frequency 4 per season Regressed Volume is 49,000 Duration Bound is 11	Qp: 2,060 cfs with Average Frequency 4 per season Regressed Volume is 12,700 Duration Bound is 7
Base Flows (cfs)	1,760	2,460	1,470
	860	1,260	920
	540	710	630
Subsistence Flows (cfs)	300	300	300
	Nov Dec Jan Feb	Mar Apr May Jun	Jul Aug Sep Oct
	Winter	Spring	Summer

Base Flow Levels	
	High (75th %ile)
	Medium (50th %ile)
	Low (25th %ile)

Pulse volumes are in units of acre-feet and durations are in days.

Period of record used : 1/1/1928 to 12/31/2010.

Episodic events are terminated when the volume or duration criteria are met, or when the flow drops below 833 cfs, or when the flow is below 5080 cfs and the flow drops from one day to the next by less than 5%.

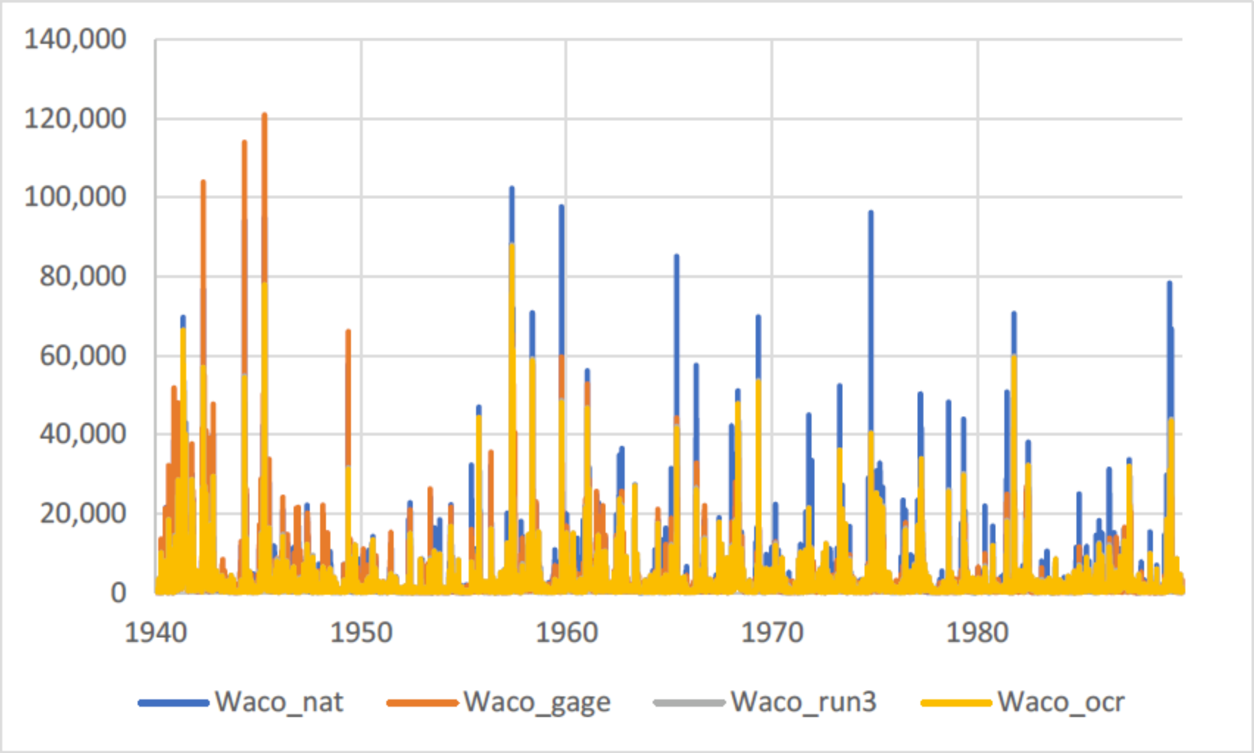


Figure 7.1. Brazos River at Waco daily time series.

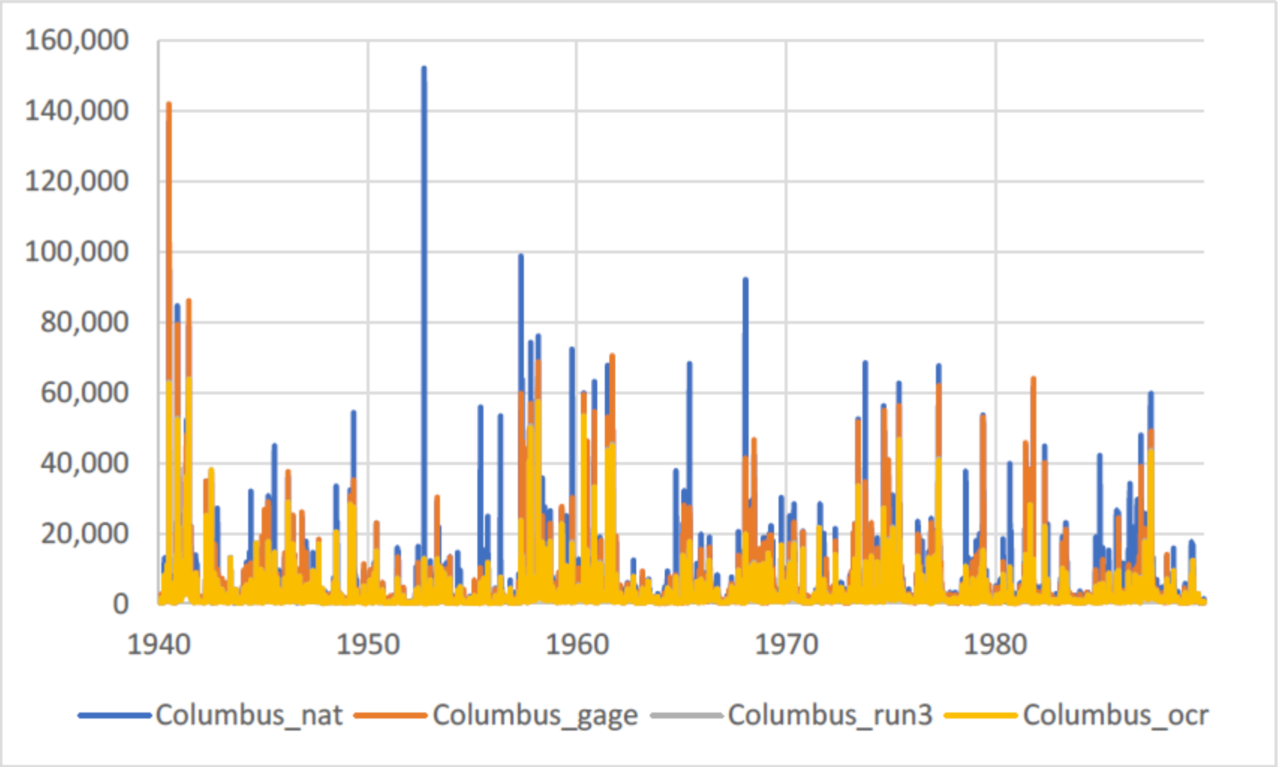


Figure 7.2. Colorado River at Columbus daily time series.

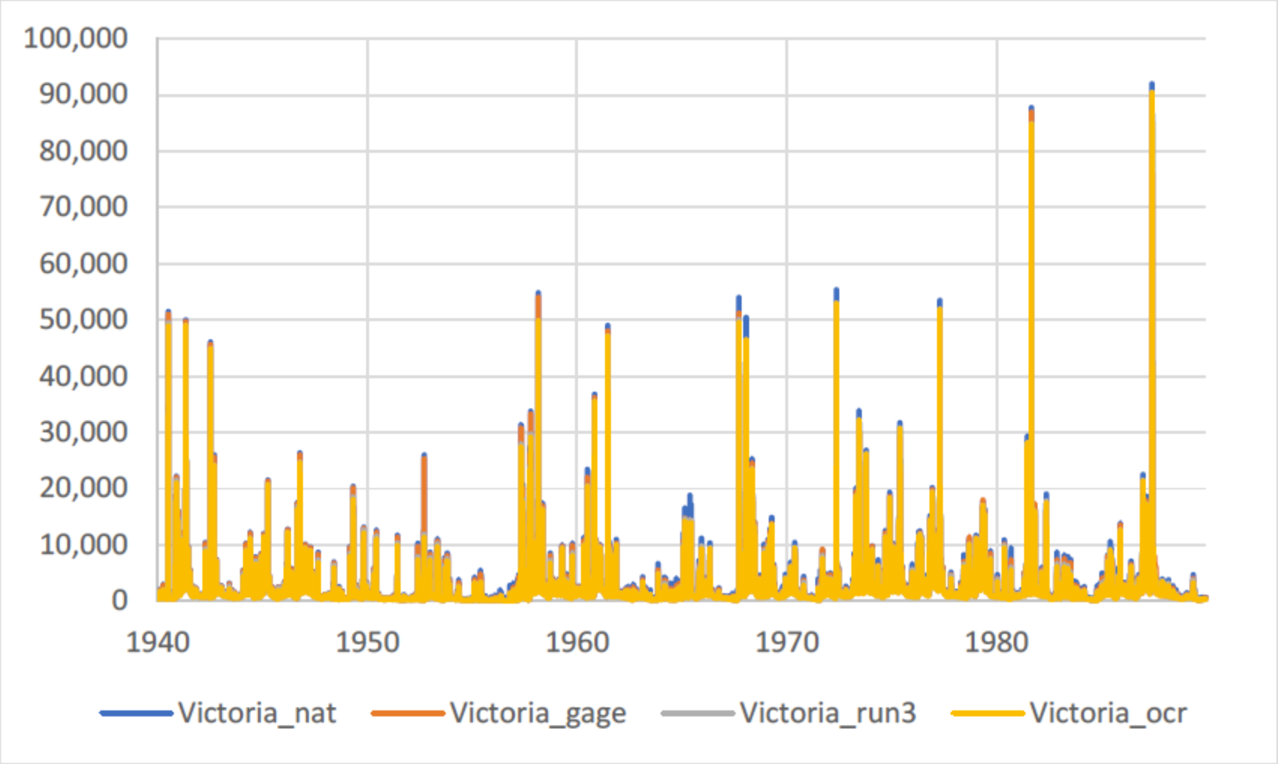


Figure 7.3. Guadalupe River at Victoria daily time series.

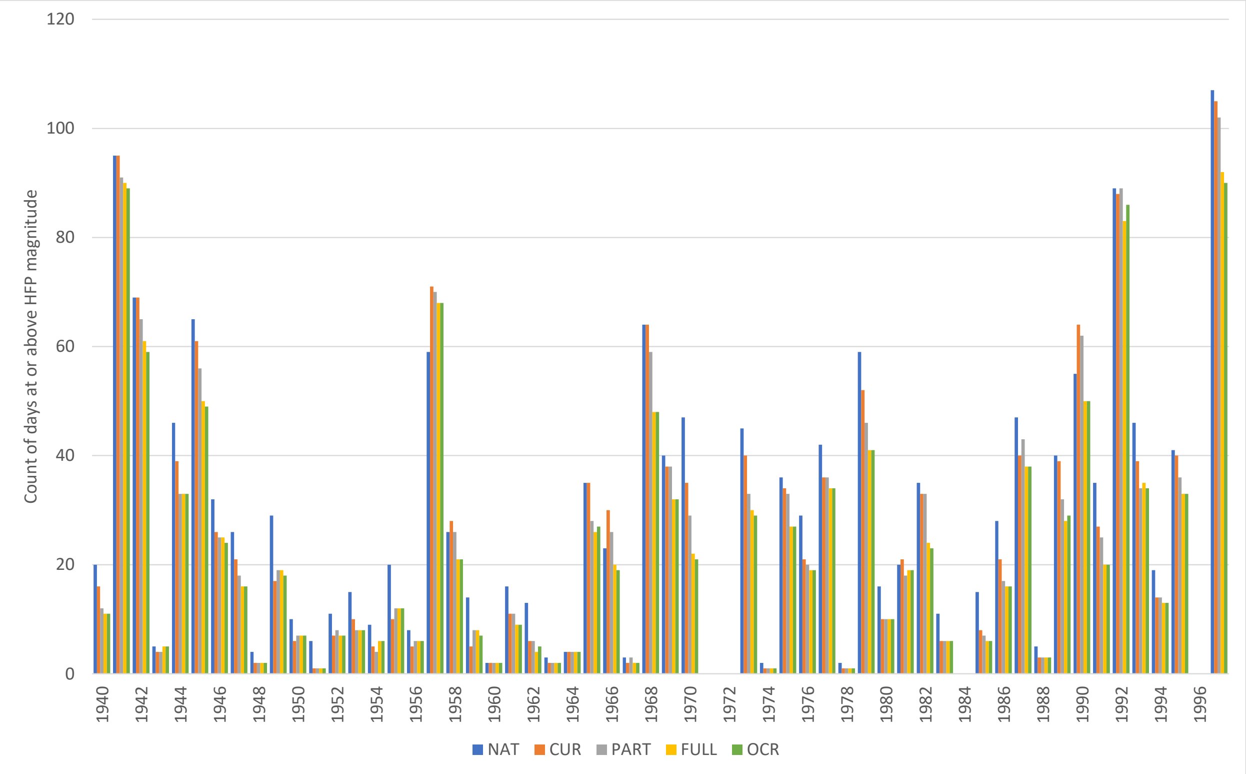


Figure 7.40. Count of number of days for which flows met or exceeded the 3-per-season spring high flow pulse magnitude at the Brazos River at Bryan.

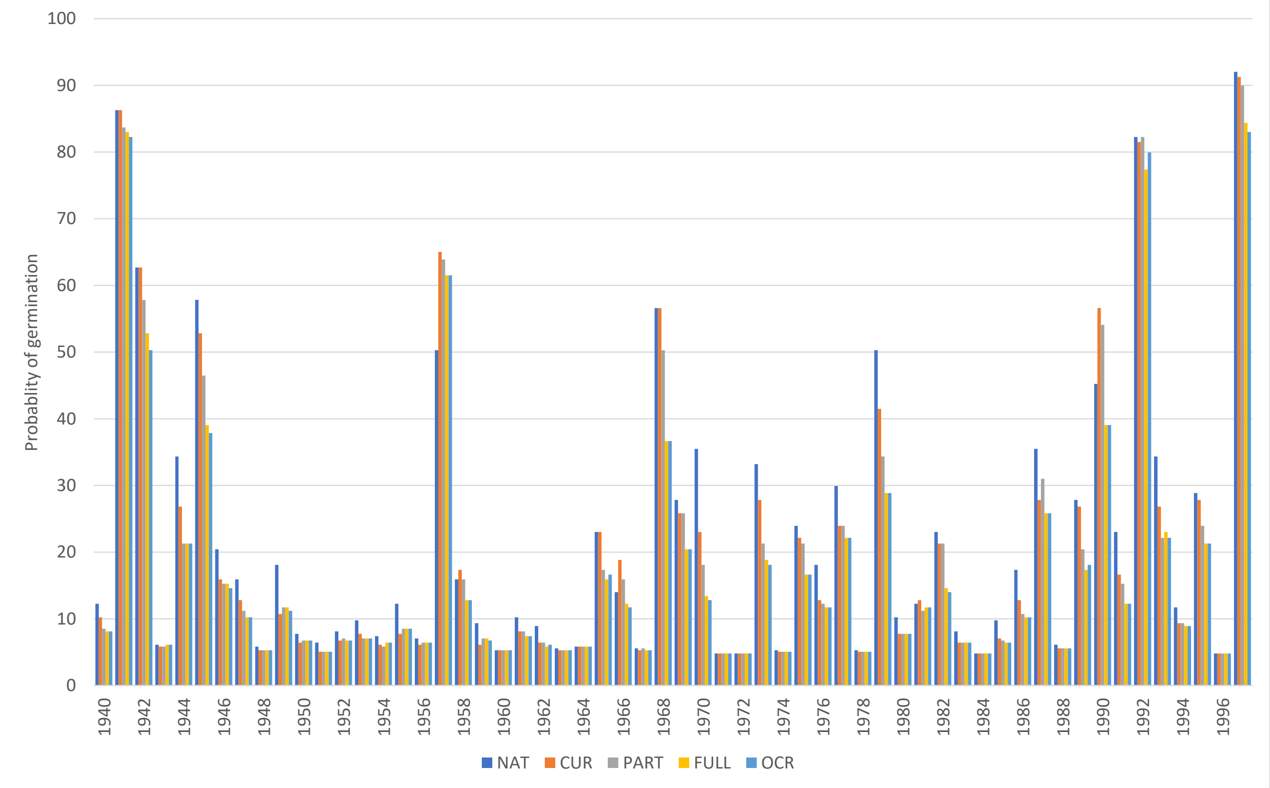


Figure 7.41. Probability of germination for Box Elder based on the flow ecology relationship to number of days meeting or exceeding the 3-per-season spring high flow pulse magnitude at the Brazos River at Bryan.

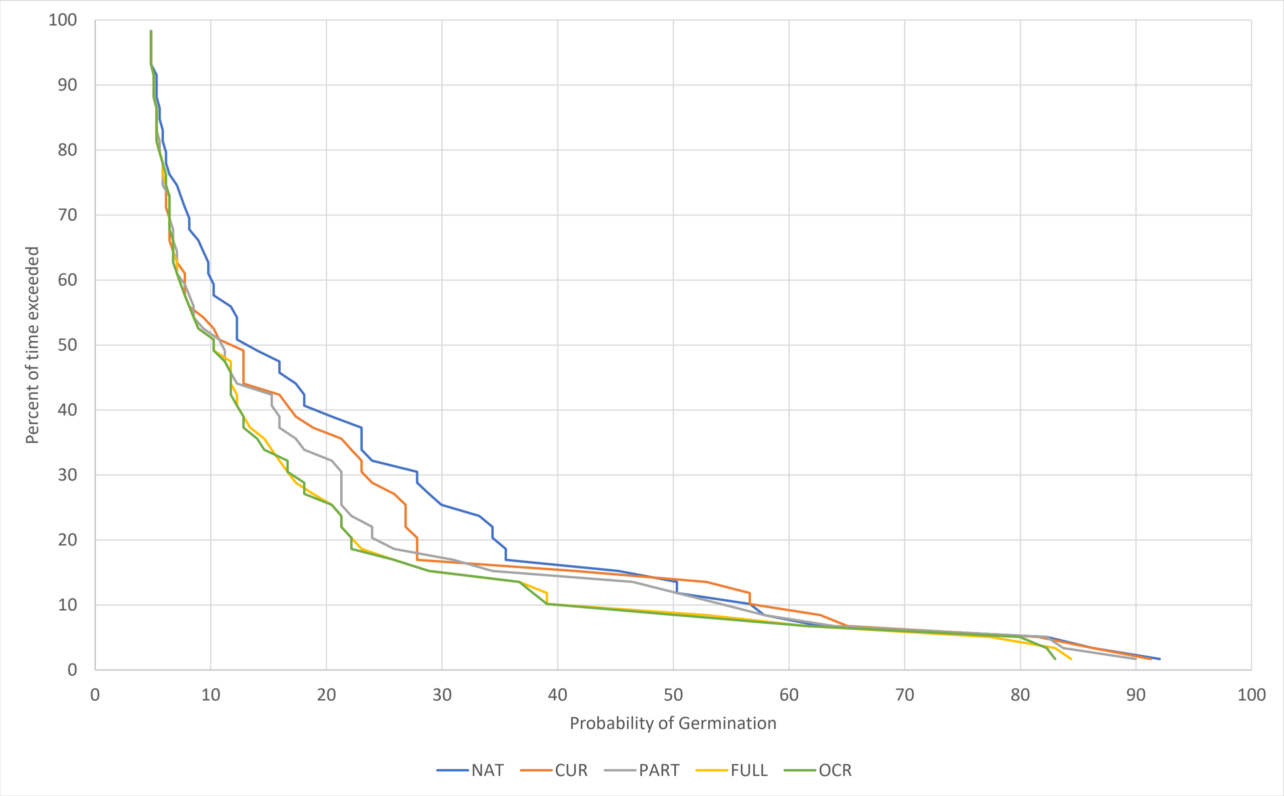


Figure 7.42. Exceedance plot of probability of germination for Box Elder based on the flow ecology relationship to number of days meeting or exceeding the 3-per-season spring high flow pulse magnitude at the Brazos River at Bryan.

Chapter 8. Lessons Learned and Recommendations for Research and Monitoring

The project and the multidisciplinary subprojects contained therein were contracted during February 21, 2019, and amended twice to provide later project termination dates that were requested due to unforeseen difficulties that were outside the control of our research team and the funding agency. During both 2019 and 2020, the study region experienced several periods of heavy precipitation that created high flow conditions in the three rivers that hindered access to study sites with unsafe conditions for fieldwork. Beginning in March 2020 and throughout the remainder of that year and the first half of 2021, the COVID-19 pandemic resulted in restrictions on travel and research activities for employees of the three universities. The travel restrictions placed particularly severe constraints on our teams' abilities to conduct field surveys. Despite these difficulties, our project team was able to obtain samples for several indicator taxa that were sufficient for statistical analysis. Obviously, larger sample sizes would improve statistical power of analyses and the reliability, precision, and accuracy of results. The approaches, methods, and findings presented in this report reveal great potential for future research to reveal mechanisms underlying flow-ecology relationships and long-term trends in Texas rivers. The ability to evaluate (validate) environmental flow standards for the state's rivers and streams will depend on robust data and analyses based on standardized methodologies.

Research to Evaluate Flow-Ecology Relationships for Indicator Taxa

Suitable indicators for retrospective flow analysis are aquatic and riparian species that are directly affected (either negatively or positively) by changes in flow regime components and that can be collected in sufficient numbers to achieve reliable numerical analysis. Indicator species need to be common in the ecosystem such that sufficient samples can be collected for statistical analyses and there is minimal risk of adversely impacting local populations. In Texas, several aquatic species that are highly sensitive to flow variation are currently considered threatened or endangered, and these are not good candidates as environmental flow indicators. Consequently, our pilot studies to demonstrate the usefulness of the flow-ecology relationship in fishes focused on species with recruitment that is sensitive to flow variation and are widely distributed and common within appropriate habitats. Our indicator fish species that is a fluvial specialist with fast life history (i.e., short-lived, recruitment must occur annually to maintain population) was the Shoal Chub (*Macrhybopsis hyostoma*). Our indicator fish species that is flow-dependent with slow life history (i.e., long-lived, recruitment is episodic with high inter-annual variation often resulting in populations with dominant cohorts) was the smallmouth Buffalo (*Ictiobus bubalus*), and we also summarized findings from recent studies of an even longer-lived, flow-dependent species, the Alligator Gar (*Atractosteus spatula*). Smallmouth Buffalo are widely distributed throughout Texas, and the species is common in rivers of intermediate to large size as well as floodplain lakes. Even though they are not currently considered threatened in Texas, Alligator Gar are not a good candidate for extensive research on age and growth, because this research requires euthanizing animals to extract otoliths. Alligator Gar are naturally long lived and have declined in distribution and abundance throughout much of its native range, and Texas rivers remain one of the species' last strongholds.

Our analysis indicated that flow pulse fall rate might be an important flow regime attribute for managing fishes like Shoal Chub. The relationship between shoal chub recruitment and fall rate quantified in this study revealed that slow (but > 0) fall rates were correlated with higher recruitment. This could be a mechanism through which pelagic-broadcast spawning fishes synchronize spawning while effectively reducing the downstream transport of ova. Additional research on the relationship between fall rate and fish recruitment is necessary to explore the mechanistic relationship between flow variability and fish recruitment.

For Smallmouth Buffalo, additional refinements could be made to validate or improve assumptions associated with age estimates and estimated background mortality used to calculate recruitment index values. Specifically, otolith annuli formation can be validated using oxytetracycline marking. Much like Smallmouth Buffalo, Alligator Gar in the Trinity River are reported to have recruitment that is strongly affected by duration of high flow pulses during the spawning season. Recent research on Alligator Gar coupled floodplain inundation models with daily discharge data to relate year-class strength to interannual variation in flow regimes and availability of preferred spawning habitat. Therefore, stage-specific floodplain inundation models may be useful for identifying the availability and temporal persistence of floodplain and slack water habitats that are expected to contribute to recruitment of periodic-type fishes in river-floodplain systems. Converting discharge data to spawning habitat availability and/or availability and persistence of nursery habitat would be an important refinement of this approach beyond characterizing the flow regime alone.

Mussels are known to be sensitive to extreme flow conditions, both droughts and floods, which is in accordance with the negative associations we found between *A. plicata* recruitment and various measures of high and low flow tiers. Unfortunately, we could not carry out the same analysis for the other mussel species, *L. teres*, due to a lack of data. There are several challenges that need to be considered when evaluating mussels as environmental flow indicators. Determining age of mussels can be challenging, because annual growth lines can be difficult to define and often varies between species. The appearance of the lines depends on how the slide specimen is prepared. While there was a high correlation within observers and, at least for *A. plicata,* between counts of observers, a perfect match was only obtained in roughly 50% of the cases or less, which is a problem, when the analysis is aimed at determining recruitment and growth for specific years. Another issue to consider is that many freshwater mussels are declining rapidly. For example, we initially surveyed six additional sites (two from each of the three study basins), but the target species were either absent or were in very low abundances, even though previous reports indicated relatively high abundances.

The riparian tree species selected as environmental flow indicators for our project were Black Willow (*Salix nigra*), Box Elder (*Acer negundo*), Green Ash (*Fraxinus pennsylvanica*), Eastern Cottonwood (*Populus deltoides*), American Sycamore (*Platanus occidentalis*), and Southern Bald Cypress (*Taxodium distichum*). These species were selected for their representation of fast maturing species (*S. nigra, A. negundo*, and *P. deltoides*) and slow maturing species (*P. occidentalis, F. pennsylvanica,* and *T. distichum*). They also represent common species and indicators of quality of riparian habitat that cover a range of seedling dispersal strategies and germination requirements.

Considering the species response and the lab work necessary to identify the rings, the easiest and most reliable species for this type of study were *F. pennsylvanica*, *P. deltoides*, and *P. occidentalis*. Though only one sample was collected during our study, literature supports the use of *T. distichum* for dendrochronological analyses because of its excellent ring production and known tendency to show strong correlations to hydroclimate. Some of these tree species did not consistently produce distinct rings for dendrochronological analysis. Thus, more work on methods for better identifying tree rings or knowledge of the wood anatomy for these species is needed to obtain more precise results for germination dates, but especially growth trends. Our study also suggests that larger sample sizes are needed for binary logistic regression. To develop better species-specific chronologies and germination data, we recommend at least 30 viable samples of a given target species per river segment.

Simulation of Flow Scenarios and Projecting Recruitment Outcomes

The use of gage flows presents several challenges when comparing longer term attainment frequencies to modeled flows based on static water management scenarios. Nonetheless, results for attainment frequencies based on gage flows does provide insight into how much alteration has occurred relative to the natural conditions, and how much alteration may be expected to result from permitted water rights. Although our Chapter 7 estimates did not produce general patterns for comparisons, one can identify specific seasons and flow levels where declines in attainment frequencies have already occurred or can predict where these may occur if water rights are fully exercised. Further research is needed to fill in knowledge gaps and assess whether proactive strategies may be needed to provide flows to maintain a sound ecological environment. Results produced for subsistence and base flow components for the Colorado River at Columbus suggest that additional studies should examine base dry and base average conditions during winter. At that gage, attainment frequencies based on gage flow data are significantly lower than would occur under more natural conditions based on the WAM analysis.

The approach adopted in Chapter 7 might be improved with further development of the daily WAMs. Draft daily WAM model have been developed for the Brazos, Colorado and Guadalupe basins. These models were considered for use in the current study; however, they are somewhat out of date. The most recent version of the WRAP model includes significant improvements to modeling environmental flow targets, which relate to ecological response on daily rather than monthly time steps. The draft daily WAMs also do not include major modifications to water management in the three basins

The riparian vegetation analysis identified a strong correlation between number of pulse days per season and likelihood of germination. In the future, the flow-recruitment relationships of other riparian tree species (Chapter 3), Smallmouth Buffalo (Chapter 4), Shoal Chub (Chapter 5), mussels (Chapter 6) and perhaps Alligator Gar and other species could be used to project recruitment dynamics under alternative flow scenarios.

Long-term Monitoring of Ecosystem Indicators

Systematic long-term monitoring has been identified as a priority for SB3 studies (Hardy et al. 2021). Long-term monitoring allows tracking of indicators of the status (i.e., degree of *soundness*) of the ecosystem within a river segment. Useful indicators for the environmental flow program must be highly responsive to variation in flow. Indicators can be physical features of the ecosystem, such as aspects of water quality and habitat (e.g., dissolved oxygen concentration, temperature). Other indicators of ecosystem status are aquatic and riparian species, functional groups of species, such as habitat guilds and life history strategies. Our project was strongly focused on biological indicators, with a particular emphasis on taxa that are known to be dependent upon flow pulses of varying magnitude, duration, frequency, and timing. Many stakeholders have requested biological justification for the various pulse tiers in the current environmental flow standards. The approaches explored during this project were formulated to address this challenge.

In Chapter 2 of this report, we applied a strategy for assessing flow-ecology relationships using a fish dataset compiled from SB3 studies conducted on the Brazos River. This approach essentially is equivalent to the "states" approach discussed in a recent review and synthesis of environmental flows science by Wheeler et al. (2017). According to Wheeler et al. (2017), *"Pure states approaches are found at one end of the continuum. In these approaches, the ecological response is a snapshot of a condition or property (i.e., a state) derived from a single measurement in time. … Common examples of ecological states in stream ecology include species richness, abundance, biomass, occupancy and indices used in bioassessments."* Wheeler et al. (2017) further explain the states approach may involve multiple measurements of an ecological response made over time (repeated states approach). We advocate use of this approach for environmental flows monitoring programs in Texas. Standardized methods for monitoring are required to track the state of ecosystem indicators over time, as well as to analyze statistically the responses of key indicator taxa to antecedent flow regimes.

All of the chapters in this report provide recommendations for appropriate survey and monitoring protocols for indicator taxa useful for evaluating environmental flow standards in river and stream segments throughout the state. Naturally, different taxa require different survey methods, but a key for any survey or monitoring effort is standard methodologies that are consistently applied over time within suitable habitats. With respect to monitoring programs to detect long-term trends in ecosystem status, a major challenge is establishment of suitable indicator criteria for assessing a sound ecological environment, which is the ultimate goal of environmental flows mandated by SB3. To advance this objective, and at a minimum, we suggest the following:

- Fish – Long-term changes in flow should not eliminate species or functional groups of fish. This implies that recruitment for short-lived (opportunistic type life history) indicator species must be sustained annually above thresholds that cause significant population decline. This implies that for longer-lived (periodic type life history) indicator species, recruitment must be high during years with favorable conditions as predicted by the models.

- Benthic macroinvertebrates – 80 percent of baseline species should always be present and key species should always be present within reference habitats (e.g., riffles, shallow runs).

- Mussels – Long-term changes in flow should not eliminate any mussel species. If robust flow-ecology relationships can be developed for mussels (i.e., with prospects for achieving similar levels of confidence as demonstrated here for the fish and tree models), then recruitment thresholds could be projected.

- Riparian vegetation – Long-term changes in flow should not eliminate wetland species. Again, this implies that for these late-maturing, long-lived (periodic-type) indicator species, recruitment must be high during years with favorable conditions as predicted by the models.

- Mesohabitats – No essential mesohabitats will be lost, with none reduced by >20 percent in area. Mesohabitats include riffles, runs, deep pools, and oxbow lakes. Extensive research on fish and macroinvertebrate mesohabitat availability in relation to flow has been conducted under SB2 and SB3.

- Water quality –TCEQ water quality standards and the requirements determined for sensitive indicator taxa should always be met.

The analysis presented in Chapter 2 identified some additional candidate species for assessing flow-ecology relationships, including Red Shiner (*Cyprinella lutrensis*) and Blacktail Shiner (*Cyprinella venusta*). Though repeated states assessments of flow-ecology relationships are useful for uncovering patterns, understanding the mechanisms that link ecological processes with flow variability are best addressed with rate-based approaches that test specific mechanisms. Rate-based approaches that focus on ecological responses that can be linked (directly or indirectly) to demographic processes provide two notable advantages to abundance- or density-based methodologies. Rate-based approaches provide mechanism-based inference into ecological responses, and also generate testable predictions about critical ecological responses (e.g., reproduction, recruitment, growth, survival) to flow variability and flow alteration.

In Chapter 2, model results yielded no relationship between fish densities community composition in habitat samples and flow magnitude, yet nonetheless revealed some important considerations for future monitoring. Although discharge magnitude during the month prior to the sampling event did not correlate with community-wide persistence, there was evidence that some fish species persisted to a greater extent compared to others. These fishes included Red Shiner, Western Mosquitofish, and to a lesser extent Bullhead Minnow and Ghost Shiner. That analysis also suggests that persistence of fishes was greatest during the transition between September and October, and detection tended to be higher during September. These results suggest standardized sampling during the month of September is optimal for assessing long-term patterns in fish communities within riverine habitats.

The choice of approach for generating flow-ecology relationships will influence the type of hydrologic metrics chosen for use as predictor variables (Wheeler et al. 2017). State-based approaches generally rely on summary metrics that characterize flow conditions over long time series, whereas rate-based approaches often analyze multiple flow regime components over shorter time intervals. The idea that greater discharge magnitude would influence fish community composition was not supported. This result might have occurred because the time step in the existing database included intra-annual sampling of sites within the same year. Such an approach creates a challenge when separating the effects of seasonality from flow responses. Annual bottlenecks in survival, such as winter mortality, confound seasonal sampling. Use of an annual rather than seasonal (intra-annual) time step is more effective for establishing flow-ecology relationships based on a state-based approach for analysis of long-term trends.

Literature Cited

Hardy, T., Winemiller, K., Buskey, E., Guillen, G., Trungale, J., Opdyke, D., Annear, T., Locke, A. & Estes, C. (2021). Statewide Synthesis of Environmental Flow Studies (2014-2017) Draft Report, Austin.

Wheeler, K., Wenger, S. J., & Freeman, M. C. (2018). States and rates: Complementary approaches to developing flow-ecology relationships. Freshwater Biology, 63(8), 906-916.

Appendix 1A: Summary of Responses to

TWDB Comments to Draft Report

Contract No. 1800012317

Texas A&M AgriLife Research Center

"ENVIRONMENTAL FLOW REGIME ASSESSMENT AND DEVELOPMENT OF A MONITORING FRAMEWORK"

Overall, the authors have made an exceptional effort to complete the work set out in the scope of work and to complete the contract tasks. Fundamentally, this work greatly increases the body of knowledge of environmental flow science and furthers the State's role in understanding the relationship of environmental flows to the flora and fauna of the state.

Required Changes

General Comments

1. Executive Summary – The Executive Summary, in principle, should be an extremely brief abstract overview of the findings of the report that should be limited to a maximum of two pages, but preferable in a single page. Because the report itself is a large body of work, this may necessitate a tiered summary approach. For example, a one-page Executive Summary must encapsulate the findings of all chapters taken together as a whole. These summary details should include the species that were found to respond favorably to environmental flow analysis and those that are not favorable for analysis. This is the single most important take away from the body of work. A second summary section (i.e. Prologue), should be created using the individual chapter summaries and background information that was formerly the Executive Summary.

These revisions were made – new Executive Summary was produced based on the main objectives and most salient findings (2 pp.), and remaining text was revised to produce a Prologue section.

2. Please check the final report for grammar, typographical, spacing, and spelling errors and ensure the final report adheres to the formatting guidelines for Texas Water Development Board reports. See "Helpful Contracting Documents at the following link: http://www.twdb.texas.gov/about/contract_admin/index.asp.

Entire document was checked for errors and formatting, and corrections were made.

3. Most of readers of this report will be familiar with the Imperial system of measurement, where things are measured in feet, inches, pounds, ounces, etc. Please use the Imperial system as the primary unit descriptors. The International system units can be include as a secondary unit of measurement, i.e., 100 cfs (2.83 cms).

All flow measurements were converted and reported in the Imperial units. For reporting units of measurement for growth and for precipitation, we retained use of millimeters. This was for two reasons. All published research in tree, mussel and fish growth reports these metric units. Many of our regression analyses used these units, and to convert to inches would require redoing analyses to derive new slopes and intercepts, etc. Correlations would not change of course.

4. Please be consistent with table and figure placement. Sometimes they are located in the text and others after the literature cited.

All tables and figures were placed in order at the end of chapters in the revised document.

5. Please reference tables and figures in the discussion when conclusions are made based on them.

Where appropriate, tables and figures are now cited in discussions. These revisions mostly applied to chapters 3 and 4.

Specific Draft Final Report Comments:

1. Executive Summary Page 1: “Datasets were accessed, including SB3-funded projects conducted on the Guadalupe, Colorado, and Brazos basins, and most datasets were insufficient or not suitable for statistical analysis of responses to flow variation, a second project objective.” This is a run-on sentence. Please reword more simply for the reader to understand.

Revision was made to provide clearer explanation.

2. Page 11: “This stakeholder’s workshop will present an overview of work completed under the contract, including research strategies, methods, findings, interpretations, and recommendations for future studies and their applications.” Please switch to past tense (“presented”) for the purposes of this report documenting things after the fact.

Revision was made with switch to past tense.

3. Page 13: Please correct “ Lead Invesgtigators” to “Lead Investigators.”

Correction was made.

4. Page 16: “This suggests fish persistence at these sites was on-average greater than other sites.” Please discuss if “on-average greater” makes it a good site to sample fish because they’ll always be there. Or does it make it a bad site to monitor fish because they will completely die off everywhere else before you see an affect here.

Revision was made to clarify the implication of the finding.

5. Chapter 2, Page 17: “This is not to say” – Although this is grammatically correct the use of the phrase “this is not to say” is convoluted and difficult for the reader, especially those that are non-native speakers. Please rephrase the sentence to be clearer to what you are trying to convey to the audience.

Sentence was revised.

6. Page 30: “Often regulations rule for seasonal pulses to maintain biota; however, timing of these events may not coordinate with crucial riparian seed dispersal and germination times. Burns and Honkala (1990) detail that “softwood” hardwoods, such as willows (Salix spp.) and cottonwoods (Populus spp.), utilize allochory and hydrochory as means for dispersal.” Please change “regulations rule” to “regulations require.”

Sentence was revised.

7. Please add a Glossary to the report that provides explanations of technical jargon for terms such as Annuli, asterisci, shell’s umbo, allochory and hydrochory, etc. Many readers will not be familiar with these very specific subject matter terms.

Glossary was created and added to docuemnt.

8. Page 32: Please note that TIFP denotes the Texas Instream Flow Program. DocuSign Envelope ID: AE0EA114-3D68-4798-A2C4-A427F156A31C Attachment 1, Page 3 of 4

Sentence was revised.

9. Page 32: “The Bonner et al. (2017) report on the Brazos River examined inundation flow rates and species composition, denoting dissimilarities between flow pulses sizes groups from riverside as well as proportion of wetland indicators in the mature trees.” The term “flow pulses sizes groups” is unknown and makes the meaning of this sentence unclear. Please clarify.

Sentence was revised.

10. Page 39: “The sampled cores were then measured to the nearest 0.001 mm on the Texas A&M University’s Department of Geography using the MeasureJ2X program linked to a sliding-stage microscope constructing core specific chronologies.” This sentence is awkwardly worded and difficult to understand. Please revise and clarify.

Sentence was revised.

11. Page 40: “The TCEQ House and Senate Bill 3 adopted flow rule and site level evaluations from Hydrology-Based Environmental Flow Regimes (HEFR) models that were used to determine threshold riverine conditions for average flow volumes and high flow pulse volumes and frequencies unique to each gage station (Table 3.1).” Please change “The TCEQ House and Senate Bill 3 adopted” to “The TCEQ adopted.”

Sentence was revised.

12. Page 40: “Using data acquired through NOAA precipitation(mm) for each river.” This sentence is awkwardly worded and how precipitation was used for each river is not clearly demonstrated. Please clarify.

Sentence was revised.

13. Page 66: “Some studies suggest that while not only do these rivers, especially in degraded or disturbed stages, undergo frequent topographical shifts, but as the systems recover the linkages and feedbacks that control for recruitment become poorly understood in part because of their transient nature (Hupp 1992, Bendix and Hupp 2000).” The meaning of this sentence is unclear. Please revise and clarify.

Sentence was revised.

14. Page 67: “However, it had a weaker negative positive effect on the Colorado River, which may be caused by the irregularities from a more regulated river.” This sentence is unclear. Please clarify what is meant by “a weaker negative positive effect.”

Sentence was revised.

15. Page 68: “In future research, we recommend requiring at least 30 viable samples of a target species per river to develop better species-specific chronologies and germination data.” Please clarify if site to site data on the same river can be combined. If not, should the recommendation be “30 viable samples of a target species per site” instead of “per river?”

Sentence was revised for clarification.

16. Page 150: “During the course of the present study, TWDB funded studies to create daily WAMs for the Brazos, Neches and Red River basins.” Please use the following DocuSign Envelope ID: AE0EA114-3D68-4798-A2C4-A427F156A31C Attachment 1, Page 4 of 4 text or something similar to provide additional description of the daily Brazos WAMs:

> *The study funded by the TWDB (Anchor and Hoffpauir 2021) utilized already available daily WAMs for the Brazos, Trinity, and Neches river basins. Those WAMs include source model files that reflect no (naturalized), current, and fully authorized diversions and impoundments. To more accurately model current conditions (including system operations and actual diversion locations), Anchor and Hoffpauir (2021) developed source model files for the daily Brazos WAM based on source model files available from Wurbs (2019), TCEQ, and the Brazos Region G Regional Planning Group.*

And include the following reference:

> *Anchor QEA, LLC, and Hoffpauir Consulting, PLLC, 2021. Evaluating the Attainment of Environmental Flow Standards. TWDB Contracted Report No. 2000012465*

Suggested text and reference were added.

Also, please confirm that the current conditions daily WAM for the Brazos developed by Anchor and Hoffpauir (2021) was used during this study.

Revision contains language that confirms.

SUGGESTED CHANGES

Specific Draft Final Report Comments:

1. Please consider numbering the tasks.

Numbers were added to tasks.

2. Page 1: "and most datasets were insufficient or not suitable for statistical analysis of responses to flow variation, a second project objective." It would be helpful to the reader of this report to provide a brief description of why datasets were not sufficient or suitable for statistical analysis of responses to flow.

Revised text clarifies the rationale.

2. Chapter 3 reads like a thesis. Any way to distill this down to the salient points would be appreciated.

Some nonessential text and citations/references were deleted from chapter 3. Text was revised throughout to conform better with language used in the other chapters of the report.

4. Figures 3.10, 3.11, 3.13, 3.14, 3.16, and 3.17 related to Average Flow Germination Response. For many species, it is difficult to identify which line they correspond to on the graphs. Please consider putting a legend on the graphs (rather than in the title of the figure).

It would be very difficult to revise these figures in this manner. We prefer to retain identification keys in the figure legends.

6. Table 3.8. Guadalupe River results obtained from univariate linear regressions between average flow from the corresponding months (predictor variables) and annual ring-width index of the same year (response variable). Only statistically significant regressions are reported. Please consider removing this table since there are no significant findings.

Table was removed from document.