SEASONAL ECOLOGICAL ASSESSMENT IN THE UPPER GUADALUPE ESTUARY

FINAL REPORT

Prepared for

Texas Water Development Board Contract #1800012267

Prepared by

Kyle T. Sullivan Casey R. Williams Bradley M. Littrell Edmund L. Oborny, Jr.

BIO-WEST, Inc. 1405 United Drive, Suite 111 San Marcos, Texas 78666

Pursuant to Senate Bill 1 as approved by the 85th 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.

TABLE OF CONTENTS

Letter of Transmittal
TABLE OF CONTENTS i
LIST OF FIGURES
LIST OF TABLES
EXECUTIVE SUMMARY 1
1.0 INTRODUCTION
2.0 METHODS
2.1 Study Area
2.2 Vegetation Community
2.3Faunal Community
2.4Data Analysis6
3.0 RESULTS
3.1 Vegetation Community
3.1.1 Community composition
3.1.2 Salinity tolerance
3.1.3 Biomass and decomposition
3.2 Faunal Community
3.2.1 Habitat and environmental conditions
3.2.2 Faunal abundance
3.2.3 Assemblage structure
3.2.4 Habitat associations
4.0 DISCUSSION
5.0 SUMMARY
6.0 LITERATURE CITED
APPENDIX A. SCOPE OF WORK
APPENDIX B. COMMENT RESPONSES TO TWDB REVIEW

LIST OF FIGURES

Figure 1. Study sites in the Guadalupe Delta.	4	4
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Figure 2.	Throw-trap	sampling at	Site 3	. 6
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LIST OF TABLES

Table 1.	Salinity (ppt) recorded at each site during vegetation sampling events in spring (May) and fall (October) 2019.	. 8
Table 2.	Percent dominance of plant species identified from sampling plots at three sites in the Guadalupe Delta during spring (May) and fall (October) 2019.	. 9
Table 3.	Average salinity values (ppt; ± standard error) measured at each site during faunal community sampling	11
Table 4.	Number of Families, taxa richness, counts (#), and relative abundance (%) of guilds among faunal groups at all sites. Species guilds include freshwater (F), estuarine-resident (R), and estuarine-dependent (D).	13
Table 5.	Counts (#), relative abundance (%), taxa richness, diversity (Shannon-diversity index[S-DI]), and species evenness (SE) of faunal communities among sites and seasons. Species guilds include freshwater (F), estuarine-resident (R), and estuarine-dependent (D).	14

EXECUTIVE SUMMARY

Passage of Senate Bill 3 (SB3) by the 80th Texas Legislature in 2007 established a process to develop and implement environmental flow standards for each of the major rivers and estuaries in Texas. This process resulted in establishment of the Guadalupe, San Antonio, Mission, and Aransas Rivers, and Mission, Copano, Aransas, and San Antonio Bays Basin and Bay Area Stakeholders Committee (GSA BBASC) that, working with an expert science team, was charged with developing environmental flow recommendations for the specified basin and bay area. Ultimately, the process led to adoption of environmental flow standards for this area by the Texas Commission on Environmental Quality (TCEQ), which became effective on August 30, 2012.

As part of the process, the GSA BBASC also submitted a Work Plan for Adaptive Management (Workplan) which identified data gaps and prioritized additional research tasks for validation and refinement of environmental flow recommendations and standards. The Workplan identified life cycle, habitat, and salinity studies for key bay and estuary faunal species as a Tier 1 high-priority task. It also called for additional studies on distribution and abundance of marsh vegetation in relation to salinity and elevation in the Guadalupe Delta. This study was conducted to supplement the available data on these priority research tasks. Specific objectives of the study were to initiate establishment of baseline conditions of marsh productivity for the upper Guadalupe Delta, evaluate the role of salinity and inundation relative to marsh vegetation community dynamics, and quantify aquatic organism species abundance and community composition within shallow habitats in relation to physical habitat and salinity.

To accomplish this, three sampling sites were established within the Guadalupe River Delta (the Delta) along a longitudinal gradient from near the sources of freshwater inflow to near the tip of the Delta in close proximity to open bay areas. To bracket the growing season, species composition, relative abundance, and biomass of the marsh vegetation community were quantified from multiple plots along fixed transects at each site in spring (May) and fall (October) 2019. To target seasons when key economically important faunal species are utilizing shallow estuarine areas, nekton sampling was conducted using throw-traps in summer (July) and fall (October) 2019. Fish, macrocrustaceans (shrimp and crabs), and mollusks were quantified from each throw-trap sample. Habitat (emergent and submergent vegetation composition and coverage) and water quality (e.g., salinity, water temperature) conditions were recorded to examine relationships between taxa occurrence/abundance and environmental variables. Descriptive and multivariate statistical techniques were utilized to examine spatiotemporal patterns in community composition, abundance, and habitat utilization.

A diverse community of wetland and marsh plants were documented, with a distinct longitudinal gradient in species composition apparent across sites, following a pattern in long-term salinity conditions. Site 1, near the main river inflow, was characterized by emergent freshwater marsh plants including alligatorweed, Beggar's ticks, and swamp smartweed. Submerged aquatic vegetation such as coontail and water stargrass were also common at Site 1. Site 2, near the middle of the Delta, exhibited the most diverse plant community and included a mixture of freshwater and salt-tolerant species. It was dominated by saltmarsh bulrush, Olney bulrush, and

wiregrass. Lastly, Site 3, near the tip of the Delta, was the least diverse site and was dominated by salt-tolerant taxa such as smooth cordgrass and common reed.

The resulting gradient in salinity and habitat conditions influenced the spatial distribution of the Delta fauna. Several freshwater taxa were only found at Site 1, including Bluegill, White Crappie, Channel Catfish, Tadpole Madtom, and Red Swamp Crayfish. Most estuarine-resident and estuarine-dependent taxa were more common at Sites 2 and 3. Sampling stations along the emergent marsh edge contained significantly higher abundance of organisms compared to those in more open-water environments.

Temporal variation in community composition and species abundance between sampling events were likely influenced by a variety of factors including well-established seasonality in reproductive and migratory patterns of many estuarine-dependent taxa as well as short-term changes to environmental conditions due to climate and freshwater inflow patterns observed during the study period.

Although this study provides important baseline data on the composition and abundance of Guadalupe Delta floral and faunal communities and their relationship to environmental variables, it represents a snapshot of conditions observed in 2019. Additional sampling under varying seasons and environmental conditions is necessary to provide a more thorough understanding of typical seasonal variation in taxa composition and abundance, and thus assess the influence of specific environmental variables such as freshwater inflow on this diverse and dynamic community.

1.0 INTRODUCTION

Estuarine ecosystems are particularly complex and dynamic due to the interaction between freshwater and marine communities (Methven et al. 2001; Elliot and Hemingway 2002; Akin et al. 2003). Floral and faunal composition of estuarine ecosystems consists of a combination of freshwater and euryhaline (adapted to a wide range of salinities) taxa, with community composition and abundance varying widely depending on a variety of factors including freshwater inflows, tidal influences, and predator/prey interactions, among other factors. Among these, freshwater inflows are recognized as a major influence on estuarine function (Alber 2002; Longley 1994; Quigg et al. 2009), influencing environmental parameters, such as salinity, organic matter, and nutrients that directly impact the ecological function and integrity of these systems (Copeland 1966; Alber 2002; Palmer et al. 2011; Montagna et al. 2013). Freshwater inflows fluctuate interannually and seasonally and the timing of inflows is important in structuring estuarine communities (Goberville et al. 2011). Therefore, variations in quantity and timing of freshwater inflow contributions can have both long-term and short-term effects on the organization of estuarine biota (Loneragan et al. 1989; Longley 1994).

Changes in freshwater inflow patterns to estuaries may affect salinity and marsh inundation patterns, which can play an important role in determining wetland vegetation community structure, vegetation productivity, and subsequent habitat utilization by other organisms. Natural climate patterns (e.g., drought) coupled with human utilization of water resources (e.g., storage, diversion) can alter hydrologic patterns of rivers (Steichen and Quigg 2018), thus influencing the timing and quantity of freshwater inflows into coastal estuarine systems (Longley 1994).

Senate Bill 3 (SB3), passed by the 80th Texas Legislature in 2007, established the need for developing and implementing environmental flow standards in Texas to maintain sound ecological environments in the state's rivers and estuaries (BBEST 2011). The implementation of SB3 revealed that major data gaps exist in the understanding of the role of freshwater inflows to bays and estuaries along the Texas Gulf Coast. Particularly, the lack of ecological data at the interface between river and bays (i.e., tidal/delta areas) is concerning due to the importance of these estuarine areas as nurseries for economically and ecologically important species (Longley 1994).

Developing an understanding of the relationship between freshwater inflows and biological productivity is an essential function in developing inflow recommendations for these understudied ecosystems (Alber 2002; Longley 1994; Quigg et al. 2009). This requires long-term datasets in order to characterize temporal differences in biotic communities based on the quantity, timing, and duration of freshwater inflows over extended time periods. The goal of this study was to initiate establishment of baseline conditions of marsh productivity for the upper Guadalupe Estuary. Establishing a baseline of the biota present in the system, along with typical seasonal and temporal variability in occurrence and abundance of taxa, will allow for development of more targeted freshwater inflow studies in coming years to develop and validate freshwater inflow recommendations. Specific objectives of this study included: 1.) evaluating the role of salinity and inundation relative to marsh vegetation community dynamics; and 2.) quantifying seasonal nekton abundance and community composition within shallow estuarine habitats in relation to physical habitat and salinity.

2.0 METHODS

2.1 Study Area

The Upper Guadalupe Estuary consists of a series of interconnected bays, bayous, and riverine systems located at the mouth of the Guadalupe River in Refugio and Calhoun Counties, Texas. This estuary represents the terminus of the Guadalupe-San Antonio River basin, receiving about 60% of its total freshwater inflow from the Guadalupe River drainage, with the remainder attributed to the San Antonio River and local drainages (Longley 1994; BBEST 2011). This study was conducted in the upper portions of this system (**Figure 1**) in marsh wetlands of the Guadalupe River Delta (from here on, the Delta). Within the Delta, river flow splits into multiple channels and exhibits a complex hydrology dependent on flow conditions. During base flows, the majority of inflows drain into Mission Lake via Traylor Cut and Guadalupe Bay via the Guadalupe River channel. Traylor Cut is a manmade channel that local authorities artificially trenched in 1935. This diversion of approximately two-thirds of the Guadalupe River freshwater discharge created additional wetlands habitat at the sub-delta that formed near its outlet into southwestern Mission Lake (Morton and Donaldson 1978). During flood events, there are greater contributions of inflow to Hynes Bay and the upper portion of San Antonio Bay (Longley 1994).



Figure 1. Study sites in the Guadalupe Delta.

Three sites were chosen to evaluate longitudinal trends in environmental conditions and biotic assemblages across the Delta. Site 1 was located near the mouth of the river's first outlet (Traylor Cut) at the western edge of Mission Lake. Due to its location at the mouth of Traylor Cut, Site 1 represents the "freshest" site in a gradient of fresh-to-salt. Site 2 was located approximately mid-way between the river mouth and the tip of the Delta, within a marsh lake that drains into Guadalupe Bay via Redfish Bayou. Site 3 occurred at the most southern point of the Delta in the outer perimeter of Lucas Lake, which is directly connected to the upper openwater portions of San Antonio Bay (**Figure 1**). Site 3 represented the "saltiest" site in a fresh-to-salt gradient.

2.2 Vegetation Community

For vegetation community sampling each site was visited twice, once in the spring (May) and once in the fall (October). During the first sampling trip in May, a transect was established perpendicular to the shoreline at each site. The beginning of each transect started at the water's edge, at the time, and continued 25 meters (m) inland from the shoreline. A steel marker was driven into the ground at the beginning of each transect for relocation purposes. Along each transect, one static plot (1 m²) was established for repeat temporal monitoring of the vegetation community and two randomly-selected plots (1 m²) were selected for biomass sampling. Random plot selection was conducted by using a random number generator to generate a starting location along the transect. From this location, a second random number was generated to select a distance from the transect line in a perpendicular direction, from 0-5 meters on either side.

For each temporal monitoring plot, species diversity, individual stem count, and percent cover estimates for dominant taxa were collected. Common and scientific names for wetland plants follow Stutzenbaker (1999). The plot was then photographed and marked for future revisits. In each of the randomized plots, the same data were collected, with the addition of standing biomass estimates. To estimate standing plant biomass, all plants within each plot were clipped at ground level, placed into a bag and weighed. For the first sampling event, one-half of the sample was returned and oven dried at 60°C to constant weight. The remaining one-half of the sample was placed into galvanized mesh bags and staked into place within the plot to decompose for collection during the fall (October) sample event. Upon the return visit in October, plant material remaining in the mesh bags was harvested and dried for dry weight measurements of remaining biomass. This allowed for an evaluation of decomposition rates at each site. Plant species diversity in the areas surrounding each transect was also recorded to help note the presence of species which may not have been captured in the transect plots.

Standard water quality parameters (temperature [°C], pH, dissolved oxygen [mg/L and percent saturation], specific conductance [μ S/cm], and salinity [ppt]) were measured with a HydroLab Model CDS5A water-quality sonde in the water column adjacent to each transect.

2.3 Faunal Community

Shallow water habitats were surveyed using a 1 m² throw-trap (**Figure 2**), which is effective at sampling macrocrustaceans (e.g., crabs, shrimp) and small-bodied fishes in shallow estuarine habitats (Jordan et al. 1997, MBHE 2007, Rozas and Minello 1997). To assess seasonal variation in faunal communities, sampling was conducted in July and October of 2019. Shallow non-

emergent vegetated bay bottom (SB) and emergent vegetated marsh edge (ME) habitats were sampled within three transects at each site during each event. Within each throw-trap sample, habitat measurements were taken. Temperature (°C), pH, dissolved oxygen (mg/L and percent saturation), specific conductance (μ S/cm), and salinity (ppt) were measured with a HydroLab Model CDS5A water-quality sonde. Additionally, emergent and submergent macrophyte composition and coverage (%) was estimated within each plot and the presence/absence of floating periphyton and vegetation was recorded. To collect organisms, a large dip net was then used within each throw-trap and swept along the length of the substratum a minimum of 10 times. All biota from each sample were then fixed in 10% formalin, brought back to the BIO-WEST laboratory, identified to a practical taxonomic level, and enumerated. All fishes were measured to the nearest millimeter.



Figure 2. Throw-trap sampling at Site 3.

2.4 Data Analysis

Vegetation community composition and percent dominance were calculated for each site. Once a species list was established, additional literature review was conducted to examine the salinity tolerance of the plant species observed and thus infer long-term patterns in typical salinity conditions at each site. Salinity tolerance values were based on data and information from Stutzenbaker (1999), Burdick and Konisky (2003), and USDA (2000).

Faunal taxa relative abundance (%), richness, diversity, and evenness were calculated for each site and between seasons. Diversity and evenness were calculated using the Shannon Index. Specific taxa were assigned to one of three guilds based on life history patterns and salinity tolerance. Guilds included freshwater, estuarine-resident (complete life cycle in estuaries; Day et al. 1989), and estuarine-dependent (reproduce offshore and occupy estuaries periodically as larvae/juveniles; Day et al. 1989). For this analysis, grass shrimp (*Paleon* sp.) were evaluated in aggregate due to the large numbers collected and difficulty in identifying to the species level.

For all statistical analyses, continuous environmental parameters and taxa abundances were log(x+1) transformed. Principal components analysis (PCA) was used to describe differences in environmental characteristics of each site and to identify any seasonal patterns in environmental variables. Species-habitat associations were evaluated using canonical correspondence analysis (CCA). Relative importance of environmental parameters, site, and season were tested using variance-partitioning techniques in CCA. Dissolved oxygen parameters were removed from the PCA and CCA due to strong correlations with time of day (LDO: r = 0.84; DO %saturation: r = 0.79) and specific conductance was removed due to its strong correlation with salinity (r = 0.99). Taxa that occurred at <5% of throw-trap samples were not included in the CCA. This resulted in exclusion of six of the seven taxa in the freshwater guild due to their low occurrence.

Differences in taxa abundance among sites, seasons, and habitat type (ME vs. SB) were tested using two-way analysis of variance with permutations (AOVP, $\alpha = 0.05$, 10,000 permutations). For this analysis, replicates were based on each throw-trap sample. To test for differences in faunal community structure between sites and seasons within sites, a one-way analysis of similarities (ANOSIM, $\alpha = 0.05$, 10,000 permutations) was used. Similarity percentages were also calculated to discriminate the contribution of individual taxa to overall dissimilarities among assemblages. Species that occurred at <5% of throw-trap samples were not included in the ANOSIM. All statistical analyses were performed in R 3.6 with the packages ImPerm (Wheeler and Torchiano 2016), stats (R-core package), and vegan (Oksanen 2017).

3.0 RESULTS

3.1 Vegetation Community

3.1.1 Community composition

All three sites were low-lying tidal marsh and showed signs of regular and fluctuating levels of inundation. Salinity measurements recorded during each sampling event documented tidal freshwater conditions at Site 1, moderate oligohaline conditions at Site 2, and mesohaline conditions at Site 3 (**Table 1**). In general, the marsh vegetation at Site 1 was dominated by broad leaved herbaceous species while the other two sites were dominated by graminoid species with less dominate herbaceous species. Species overlap between sites was minimal with only one species, swamp smartweed (*Polygonum hydropiperoides*), found within plots at more than one site.

Site	Spring (May)	Fall (Oct.)
1	0.33	0.39
2	2.92	9.13
3	1.95	14.78

Table 1. Salinity (ppt) recorded at each site during vegetation sampling events in spring (May) and fall (October) 2019.

Five species were documented in plots from Site 1, with the community composed entirely of herbaceous freshwater littoral and semiaquatic species (**Table 2**). Beggar's ticks (*Bidens laevis*) was by far the most dominant species at this location. It was found producing a monoculture with a few other species interspersed. During the spring (May) sampling, alligatorweed (*Alternanthera philoxeroides*), an invasive species, was the second most dominant species in sampling plots, followed by swamp smartweed. By fall (October), the dominance rank between these species shifted with swamp smartweed more abundant than alligatorweed. Several other nonnative and invasive species were present at Site 1 and captured in sampling plots. These included wild taro (*Colocasia esculenta*) and water hyacinth (*Eichhornia crassipes*). Although not dominant along the transect these species were quite common in the surrounding area.

Site 2, the oligohaline site, had the highest species diversity, with seven species identified from sampling plots (**Table 2**). The community was comprised mostly of graminoid species, rushes and grasses, as well as several herbaceous species. The graminoids saltmarsh bulrush (*Scirpus maritimus*) and smooth bulrush (*Schoenoplectus tabernaemontani*) were widespread throughout the community. Dominance between species was captured differently between spring (May) and fall (October) sampling, but this was an artifact of community heterogeneity rather than loss or expansion of individual species. With few exceptions, the plant species sampled in this site are considered facultative halophytes and have wide tolerances to salinity, but can also be found in freshwater habitats or require occasional freshwater inputs.

Site 3 was the least diverse site, with only three species documented from sampling plots (**Table 2**). This site was composed almost entirely of a homogenous stand of smooth cordgrass (*Spartina alterniflora*). The site was noted as having the largest variation in salinity between spring (May) and fall (October) sampling events, ranging from 1.95 to 14.78, respectively. Given the plant community present, low salinity observed at this site in the spring (May) sampling event likely represents an atypical situation caused by above average spring rainfall throughout the watershed. Smooth cordgrass is a common estuarine marsh plant that is considered a facultative halophyte. Therefore, although it can sustain itself in occasional low salinity waters (< 10 ppt), it is typically common in consistent brackish waters.

C:40	Common Nomo	Scientific Norre	Percent De	ominance
Sile	Common Name	Scientific Name	Spring	Fall
	Alligatorweed	Alternanthera philoxeroides	11	10
	Beggar's ticks	Bidens laevis	82	70
1	Swamp smartweed	Polygonum hydropiperoides	5	20
	Wild taro	Colocasia esculenta	<1	<1
	Water hyacinth	Eichhornia crassipes	n/a	<1
	Saltmarsh bulrush	Scirpus maritimus	30	40
	Wiregrass	Spartina patens	8	22
	Swamp smartweed	Polygonum hydropiperoides	1	7
2	Purple loosestrife	Lythrum lineare	12	8
	Water hyssop	Bacopa monnieri	n/a	11
	Smooth bulrush	Schoenoplectus tabernaemontani	27	n/a
	Olney bulrush	Scirpus americanus	20	10
	Smooth cordgrass	Spartina alterniflora	91	80
3	Common reed	Phragmites australis	9	20
	Sea myrtle	Baccharis halimifolia	<1	<1

Table 2. Percent dominance of plant species identified from sampling plots at three sites in the Guadalupe Delta during spring (May) and fall (October) 2019.

3.1.2 Salinity tolerance

To further explore salinity tolerance of the species observed, additional literature review was conducted to examine the range of salinity tolerance reported for each species. Reported ranges demonstrate that the plant community present at Site 1 is intolerant of salinity, whereas communities at Site 2 and 3 exhibited wide variation in salinity tolerance (**Figure 3**).

3.1.3 Biomass and decomposition

Estimates of standing plant biomass from spring (May) samples demonstrate that Site 3 had the highest mean biomass (811.40 grams [g]), followed by Site 1 (593.05 g). Mean biomass at Site 2 was lowest at 395.95 g. In fall (October), mean standing plant biomass was highest at Site 3 (459.30 g), intermediate at Site 2 (371.35 g), and lowest at Site 1 (260.65 g). Although this is a limited dataset, it suggests an increase in biomass along a freshwater to brackish water gradient, and a reduction in biomass from spring to fall at all sites in 2019. Additional data is needed to further explore trends in biomass between sites, seasons, and inflow conditions. Unfortunately, only one of the three leaf litter bags left on site was recovered, so no comparison can be made as to the decomposition rate of leaf litter between sites.



Figure 3. Reported salinity tolerance ranges for plant taxa observed at each site. Salinity tolerances are based on data and information from Stutzenbaker 1999, Burdick and Konisky 2003, and USDA 2000.

3.2 Faunal Community

3.2.1 Habitat and environmental conditions

Principal components axes I and II explained 39.1% of the variation in environmental parameters among 78 throw-trap samples. Axis I explained 26.0% of the variation and described a water quality and vegetation gradient. Strongest loadings for PC axis I were water hyacinth (0.46), salinity (-0.42), and pH (-0.42). Axis II explained 13.1% of the variation and represented a vegetation gradient. Strongest loadings were vine (*Mikania scandens* and *Vigna luteola*, in aggregate, 0.52), alligatorweed (0.46), and water stargrass (*Heteranthera dubia*, -0.45; Figure 4).

Among sites, mean sample scores from the PCA distinguished two different groups that varied in salinity concentrations and vegetation composition. The first group is represented by Site 1, with mean site scores positively associated with PC axis I and II in the summer (July), whereas scores were positively associated with PC axis I and negatively associated with PC axis II in the fall (October) (**Figure 4**).

The positive association with PC axis I can be attributed to lower mean salinity at Site 1 in the summer and fall (**Table 3**). Differences between mean sample scores for Site 1 between summer (July) and fall (October) can be attributed to differences in vegetation composition (**Figure 4**). The summer (July) mean sample score had a stronger positive association with PC axis I due to greater mean coverage of water hyacinth (34.2%). The fall (October) mean sample score had a

stronger negative association with PC axis II, which can be attributed to a shift in submergent vegetation coverage, with a greater mean coverage of water stargrass (45.0%), and a decrease in coverage of alligatorweed (0.8%).

The second group contained Sites 2 and 3. Mean site scores were negatively associated with PC axis I and positively associated with PC axis II in the summer (July) and fall (October) (**Figure 4**). The stronger negative association with PC axis I can be attributed to greater overall salinity concentrations compared to Site 1 mean scores. Additionally, changes in salinity levels exhibited seasonal variation among Site 2 and 3. Fall (October) mean site scores had a stronger negative association with PC axis I compared to the summer (July) due to an increase in salinity concentrations at both sites from summer (July) to fall (October). Mean salinity at Site 2 increased from 2.92 ppt to 9.13 ppt, whereas at Site 3 mean salinity increased from 1.86 ppt to 14.78 ppt from July to October (**Table 3**). The weaker associations of Sites 2 and 3 with PC axis II can be attributed to a lack of submergent vegetation and differences in emergent vegetation along the marsh edge (**Figure 4**). Water stargrass was absent from Site 2 and 3, and coontail (*Ceratophyllum demersum*) was the only submerged vegetation type at Site 2, where it was rare compared to Site 1. Along the marsh edge, alligatorweed and water hyacinth were absent at Site 2 and 3, which instead included Graminoids such as common reed (*Phragmites australis*), saltmarsh bulrush, and smooth cordgrass.

Site	Summer (July)	Fall (Oct.)
1	0.34 ± 0.001	0.39 ± 0.000
2	2.92 ± 0.004	9.13 ± 0.071
3	1.86 ± 0.230	14.78 ± 0.036

Table 3. Average salinity values (ppt; \pm standard error) measured at each site during faunal community sampling.



Phragmites australis)], water hyacinth [WH (Eichornia crassipes)], cordgrass [CG (Spartina alterniflora)], saltmarsh bulrush [BR Figure 4. Principal components analysis bi-plots for measured environmental conditions (left) and general habitat characteristics (Ceratophyllum demersum)], water lettuce [WL (Pistia stratiotes)], water stargrass [SG (Heteranthera dubia)], common reed [R (right) for each site (1, 2, and 3) based on mean site scores $(\pm SD)$ during summer (S) and fall (F) sampling events. Vegetation abbreviations for the environmental conditions bi-plot include alligatorweed [AW (Alteranthera philoxeroides)], coontail [CT [Scirpus spp.)], duckweed [DW (Lemna minor)], and vine [V (Mikania scandens and Vigna luteola, in aggregate)].

3.2.2 Faunal abundance

A total of 6,385 individuals represented by 23 families and approximately 34 species were observed. Crustaceans were the most abundant taxonomic group among all sites and comprised 65.9% of the total catch. Fishes characterized 34.1% of all individuals, though they were the most taxa rich faunal group with 16 families and 27 species observed. Mollusks were the least common faunal group, with Atlantic Rangia (Rangia cuneata) characterizing 0.1% of the total catch. Among life-history guilds, estuarine-residents characterized 77.4% of all individuals observed. Estuarine-dependent species were less represented at 22.4% and freshwater species were rare at 0.2% (Table 4).

Table 4. Number of Families, taxa richness, counts (#), and relative abundance (%) of guilds among faunal groups at all sites. Species guilds include freshwater (F), estuarine-resident (R), and estuarine-dependent (D).

		11.				Specie	s Guild			
				F	13	R	1	D	To	tal
Faunal Group	Families	Taxa Richness	#	%	#	%	#	%	#	%
Mollusks	1	1	0	0.0	6	0.1	0	0.0	6	0.1
Crustceans	6	6	6	0.1	3949	61.8	250	3.9	4205	65.9
Fish	16	27	8	0.1	985	15.4	1181	18.5	2174	34.0
Total	23	34	14	0.2	4940	77.4	1431	22.4	6385	

Per site taxa richness was similar during both sampling events for Site 1 and Site 2, ranging from 16-17. Richness was lower at Site 3, ranging from 8-13. At all sites, diversity and species evenness were lower in the summer (July) compared to the fall (October). At Site 1, diversity and species evenness were lower, ranging from 0.57-0.92 and 0.20-0.33, respectively. Diversity at Site 2 was 1.36-1.58 and species evenness was 0.49-0.56. Site 3 was similar, with diversity observed at 1.13-1.47 and species evenness ranging from 0.54-0.57 (Table 5).

Across sites and seasons, grass shrimp was the most dominate taxa representing from 40.1-88.1% of organisms collected. Other crustaceans were uncommon at Sites 1 and 2. However, at Site 3 White Shrimp (*Litopenaeus setiferus*) represented 9.3% of the catch in the summer (July)

and 7.9% in the fall (October). Among fishes, the estuarine-dependent Bay Anchovy (Anchoa mitchilli) characterized a large portion of the assemblages at Sites 2 and 3, ranging from 30.9%-34.4% and 8.8%-15.2%, respectively. Sailfin Molly (Poecilla latipinna) was most abundant at Site 2 where it accounted for 6.5-11.5% of the assemblage. The most abundant fishes at Site 1 included Western Mosquitofish (Gambusia affinis) and Naked Goby (Gobiosoma bosc). Taxa only observed at Site 1 included Red Swamp Crayfish (Procambarus clarkii), Hogchoker (Trinectes maculatus), Bluegill (Lepomis macrochirus), White Crappie (Pomoxis annularis), Rio Grande Cichlid (Herichthys cvanoguttatus), Golden Topminnow (Fundulus chrysotus), Bluefin Killifsh (Lucania goodei), Channel Catfish (Ictalurus punctatus), and Tadpole Madtom (Noturus gyrinus). Species only observed at Site 2 included Mexican Tetra (Astvanax mexicanus), Sheepshead Minnow (Cyprinodon variegatus), Rainwater Killifish (Lucania parva), and Pinfish (Lagodon rhomboides). Taxa unique to Site 3 included White Shrimp, Hardhead Catfish (Ariopsis felis), Highfin Goby (Gobionellus oceanicus), and Spotted Seatrout (Cynoscion nebulosus; Table 5).

					S	ite 1			刼	e 2			S	e3	100
				Sur	nmer	-	all	Sun	mer	Fa		Sum	mer	Fa	-
Family	Scientific Name	Common Name	Guild	#	0/0	#	9/6	#	0/0	#	0,6	#	9,6	#	0/0
Mactridae	Raneia cuneata	Atlantic Rangia	D	1	0.1	2	0.3	0	0.0	1	0.1	0	0.0	1	0.3
Crustaceans			i			i.		•						ł.	
Cambaridae	Procambarus clarkii	Red Swamp Crayfish	щ	9	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Palaemonidae	Paleon sp.	grass shrimp	R	1511	88.1	621	78.6	610	48.2	741	40.1	126	69.2	339	58.0
Panopeidae	Rhithropanopeus harrisii	Estuarine Mud Crab	D	0	0.0	0	0.0	7	0.2	2	0.1	6	4.9	6	1.5
Penaeidae	Farfantepenaeus actecus	Brown Shrimp	D	0	0.0	0	0.0	0	0.0	30	1.6	0	0.0	6	0.3
	Litopenaeus setiferus	White Shrimp	D	0	0.0	0	0.0	0	0.0	60	3.2	17	9.3	46	1.9
		juvenile penaeid shrimp	D	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	80	1.4
Portunidae Fish	Callinectes sapidus	Blue Crab	D	4	0.2	2	0.6	7	0.2	9	0.3	4	22	45	7.7
Achiridae	Trinectes maculatus	Hogchoker	R	1	0.1	9	0.8	0	0.0	0	0.0	0	0.0	0	0.0
Ariidae	Ariopsis felis	Hardhead Catfish	D	0	0.0	0	0.0	0	0.0	0	0.0	5	11	0	0.0
Atherinopsidae	Menidia andens	Mississippi Silverside	R	5	0.3	5	0.6	13	1.0	16	6.0	0	0.0	21	3.6
Centrarchidae	Leponus mere rochirus	Bluegill	Į.	5	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Pomoxis annularis	White Crappie	ц	7	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Characidae	Astyance mexicanus	Mexican Tetra	щ	0	0.0	0	0.0	0	0.0	1	0.1	0	0.0	0	0.0
Cichlidae	Herichthys cyanoguttatus	Rio Grande Cichlid	щ	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0
Clupeidae	Brevoortia patromis	Gulf Menhaden	D	0	0.0	0	0.0	39	3.1	8	0.4	0	0.0	9	1.0
Cyprinodontidae	Cyprinodon variegatus	Sheepshead Minnow	R	0	0.0	0	0.0	4	0.3	0	0.0	0	0.0	0	0.0
Engraulidae	Anchoa mitchilli	Bay Anchovy	D	4	0.2	4	0.5	436	34.4	571	30.9	16	8.8	89	15.2
Fundulidae	Fundulus chrysotus	Golden Topminnow	R	0	0.0	2	6.0	0	0.0	0	0.0	0	0.0	0	0.0
	Fundulus grandis	Gulf Killifish	R	8	0.5	1	0.1	15	12	7	0.1	0	0.0	0	0.0
	Fundulus pulvereus	Bayou Killfish	K	5	0.3	0	0.0	13	1.0	0	0.0	0	0.0	0	0.0
	Lucania goodei	Blue fin Killifish	R	1	0.1	9	0.8	0	0.0	0	0.0	0	0.0	0	0.0
	Lucania parva	Rainwater Killifish	R	0	0.0	0	0.0	0	0.0	ŝ	0.2	0	0.0	0	0.0
Gobiidae	Gobionellus oceanicus	Highfin Goby	R	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	6	0.3
	Gobiosoma bosc	Naked Goby	R	31	1.8	74	9.4	28	22	4	2.4	1	3.8	8	1.4
	Gobiosoma robustum	Code Goby	K	0	0.0	e	0.4	0	0.0	0	0.0	0	0.0	1	0.3
	Microgobius gulosus	Clown Goby	R	0	0.0	4	0.5	1	0.1	e	0.2	0	0.0	0	0.0
Ic tal uridae	Ictalurus punctatus	Channel Catfish	щ	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0
	Noturus gyrimus	Tadpole Madtom	ц	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Poeciliidae	Gambusia affinis	Western Mosquitofish	R	114	9.9	31	3.9	18	1.4	146	1.9	1	0.5	0	0.0
	Poecilia formosa	Ama zon Molly	R	10	9.0	0	0.0	1	0.1	1	0.1	0	0.0	0	0.0
	Poecilia latipinna	Sailfin Molly	R	10	9.0	0	0.0	82	6.5	212	11.5	0	0.0	0	0.0
Sciaenidae	Cynoscion nebulosus	Spotted Seatrout	D	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	0.9
Sparidae	Lagodon rhomboides	Firefish	D	0	0.0	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0
Syngnathidae	Syngnatinus louisi anate	Chain Pipe fish	Я	0	0.0	19	2.4	1	0.1	0	0.0	0	0.0	0	0.0
			Total#	1716		790		1266		1847		182		584	
			Taxa Richness	17		16		16		17		00		13	
			SDI	0.57		0.92		1.36		1.58		1.13		1.47	
			X	0.20		0.33		0.49		0.56		0.54		0.57	-63

The AOVP did not detect significant differences in abundance of taxa among sites (F = 1.74, p = 0.19), seasons (F = 0.04, p = 0.84), or any combinations of interactive effects. However, average sample abundance (mean ± standard error) among sites was highest at Site 2 (129.7 ± 30.0), intermediate at Site 1 (83.5 ± 20.7), and considerably lower at Site 3 (32.0 ± 8.1). Among seasons, average sample abundance was similar in the summer (July = 75.3 ± 16.3) and fall (October = 89.5 ± 21.1). The AOVP detected significant differences in abundance among habitat types (F = 20.8, p > 0.001), with greater abundances in ME (150.8 ± 24.9) compared to SB (31.3 ± 6.9; Figure 5).

3.2.3 Assemblage structure

Results of the ANOSIM demonstrated that assemblage structure significantly differed among sites (R = 0.413, p < 0.001). Site 1 and Site 2 were 71.9% different. Five taxa accounted for 51.5% of assemblage dissimilarities and included Bay Anchovy (19.2%), grass shrimp (14.7%), Western Mosquitofish (7.2%), Naked Goby (6.3%), and Sailfin Molly (4.1%). Between Sites 1 and 3, assemblages were 77.2% different. Five taxa accounted for 53.3% of dissimilarities, including, grass shrimp (19.9%), Naked Goby (9.0%), Bay Anchovy (8.8%), White Shrimp (7.8%), and Western Mosquitofish (7.8%). Assemblages between Site 2 and 3 were 71.2% different. Five taxa accounted for 47.7% of dissimilarities and included Bay Anchovy (16.3%), grass shrimp (13.7%), White Shrimp (6.7%), Naked Goby (6.0%), and Blue Crab (5.0%).

Biotic assemblages among seasons were not significantly different at Site 1 (R = 0.074, p = 0.089), Site 2 (R = 0.051, p = 0.157), or Site 3 (R = 0.121, p = 0.066).

3.2.4 Habitat associations

From the CCA analysis, Axes I and II explained 23.8% of the variability in the Guadalupe Delta biotic assemblage from environmental parameters, site, and season. Within the explained variation, environmental parameters explained 30.1%, site explained 4.7%, and season explained 1.0%. The two-and three-way interactions among environmental parameters, site, and season explained 33.0% of assemblage variation. Habitat parameters and sites strongly associated with CCA Axis I included Site 1 (0.79), salinity (-0.72), water hyacinth (0.67), pH (-0.63). Stronger associations with CCA Axis II were water stargrass (0.64), Site 2 (-0.59), bulrush (0.55), and SB (0.53; Figure 6).

Bay Anchovy and Gulf Menhaden (*Brevoortia patronus*) exhibited a strong relationship with increased salinities. Blue Crab, Estuarine Mud Crab (*Rhithropanopeus harrisii*), and Spotted Seatrout were associated with higher salinity as well as SB habitats. In contrast, Red Swamp Crayfish was strongly associated with lower salinities and Hogchoker, Chain Pipefish (*Syngnathus louisianae*), and Bluefin Killifish were also associated with habitats with lower salinities and greater water stargrass coverage. Bayou Killifish (*Fundulus pulvereus*), Gulf Killifish (*Fundulus grandis*), and Western Mosquitofish were associated with ME habitats with lower salinities (**Figure 6**).



Figure 5. Average trap-net sample abundance among sites (top), seasons (middle), and marsh edge (ME) and shallow non-emergent vegetated bottom (SB) habitats (bottom).





4.0 **DISCUSSION**

Variation in environmental conditions among sites and sampling events observed during this study emphasize the importance of conducting these types of studies at multiple spatiotemporal scales. The PCA exhibited a longitudinal salinity gradient within the Delta that is a large factor in explaining the biotic variation observed. Site 1 was characterized by lower salinity levels and was characterized as a freshwater environment (salinity: 0-0.5 ppt) during both seasons. Site 2 and 3 exhibited greater salinities and were characterized as oligohaline (0.5-5.0 ppt) in the summer (July) and mesohaline (5-18 ppt) in the fall (October). The salinity regime observed was similar to previous research in the upper Guadalupe Estuary that also found longitudinal variation in salinities based on the proximity to freshwater inflow contributions (Longley 1994). Site 1 received nearby freshwater inflows via Traylor Cut, while Sites 2 and 3 were more distant from river inflows, and thus, more influenced by saltwater/tidal conditions.

Additionally, the magnitude of freshwater inflow played an obvious role in structuring salinity gradients within the Delta, and subsequently influencing temporal patterns in faunal assemblage structure at each site. Freshwater inflow calculations for the Guadalupe Delta require summing discharge data from multiple gages within the lower Guadalupe and lower San Antonio River basins along with rainfall/runoff model estimates, diversions, and return flows from ungaged coastal watersheds (TWDB 2010). Although detailed freshwater inflow estimates were not calculated as part of this study, contributing river hydrology can be used to provide a general representation of inflow conditions observed during the study period (**Figure 7**). Spring vegetation surveys occurred in early May 2019 during a wet period in the Guadalupe/San Antonio River basin (**Figure 7**), contributing to low salinity levels at all sites (max: 2.92 ppt at Site 2). Summer sampling occurred in late July following a typical summer decline in precipitation within the drainage area and near median flows in the contributing basin. Although salinity levels were similar at Site 1, they increased slightly at Site 2 and 3 during summer (July). Finally, fall sampling occurred at the end of October following a period of below-median flows in the basin (**Figure 7**), contributing to substantial increases in salinity at Sites 2 and 3.

Although varying considerably and dependent upon inflow conditions, long-term longitudinal gradients of salinity within the Delta are important in structuring vegetation communities. Emergent vegetation composition along the marsh edge was similar to previous studies (Longley 1994) in that Site 1 was strongly associated with freshwater taxa (e.g., alligatorweed, Beggar's ticks, swamp smartweed, and water hyacinth). Site 2 represented a mixture of freshwater (swamp smartweed) and salt-tolerant (wiregrass [*Spartina patens*]) taxa, resulting in the highest vegetation diversity at this site. Site 3 was dominated by salt-tolerant taxa (common reed, smooth cordgrass) that were previously documented as major components to emergent assemblages in the relic tributaries and brackish marshes (Longley 1994). Further, submergent vegetation was more common at Site 1. The submergent macrophyte taxa observed (coontail and water stargrass) are more common within freshwater environments, though they can occur under oligohaline conditions, explaining their presence in the summer (July) at Site 2, though at lower densities. By fall (October), submerged macrophytes at Site 2 had died, presumably from increased salinity, resulting in a considerable benthic layer of detritus at some throw-trap sites.



Figure 7. Hydrograph from the USGS gage on the lower Guadalupe River at Victoria, Texas and the lower San Antonio River at Goliad, Texas for the period of January 1 – December 2, 2019. Approximate timing of sampling events in the Guadalupe Delta are represented by red bars.

The Guadalupe Delta fauna also exhibited longitudinal patterns, driven in-part by the salinity and environmental/vegetation gradient described above. Estuarine-resident taxa dominated the communities observed, which is typical in coastal marsh environments (Ley et al. 1999; Akin et al. 2003). Grass shrimp was the most abundant taxa among all sites and seasons. This has been observed in previous studies and is likely due to their ability to tolerate a wide range of environmental conditions (Akin et al. 1993). The CCA supports this by displaying a weak association between grass shrimp and physiochemical parameters. Among sites, the greater abundance of grass shrimp at Site 1 was also a main contributor to lower diversity and species evenness, as well as dissimilarities in assemblage structure. Previous research has found that grass shrimp are more abundant in submerged vegetation (Rozas and Odum 1987), which may explain why this species was so dominant at Site 1.

Additional differences in faunal assemblage composition among sites supports the role of environmental conditions as ecological drivers in estuarine systems. Site 1 contained more freshwater species such as Bluegill, Channel Catfish, Tadpole Madtom, and White Crappie. Additionally, Red Swamp Crayfish was only observed at Site 1 and demonstrated a strong association with freshwater in the CCA. Interestingly, the CCA also displayed estuarineresidents Hogchoker and Chain Pipefish to be strongly associated with freshwater environments and water stargrass. Both these species can occupy a range of salinity levels, and Chain Pipefish are known to associate with aquatic vegetation. This suggests that physical habitat, such as submerged vegetation coverage, may have a greater influence on the distribution of species tolerant to a wide breadth of water quality conditions (Rozas and Hackney 1984).

Total taxa abundance is a commonly used metric to assess biological productivity (Montagna et al. 2013). Abundance was higher in emergent marsh edge habitats compared to more open-water areas. Marsh edge habitat is likely more productive due to emergent vegetation providing refuge for small-bodied organisms (Rozas and Minello 1997). For example, the CCA exhibited small bodied fish such as Sailfin Molly and Western Mosquitofish to be strongly associated with marsh edge habitats. Additionally, at Site 1, grass shrimp were also found in large abundances in marsh edge samples. Total abundance among sites was highly variable. Despite the AOVP failing to detect significant differences, Site 2 exhibited noticeably higher abundances than other sites. It was also the most diverse site from a vegetation standpoint. Mesohaline environments are often the most productive within estuarine systems (Longley 1994), due to their dynamic nature. These sites exhibit a constantly changing environment depending upon seasonality and freshwater inflow conditions. As a result, a wide variety of organisms, both freshwater and estuarine-dependent, can utilize such habitats, leading to increased diversity and productivity.

The assemblage structure of Site 2 and 3 differed from Site 1 due to a greater abundance of estuarine-dependent species. Previous research suggests species that utilize estuaries during parts of their life cycle are more abundant in oligohaline and mesohaline habitats (Akin et al. 2003) similar to Sites 2 and 3. This was supported by the CCA, which displayed a strong association between estuarine-dependent species such as Estuarine Mud Crab, White Shrimp, Bay Anchovy, and Gulf Menhaden and higher salinity levels. Bay Anchovy, in particular, has previously been observed at greater abundances in estuarine habitats with increased salinity concentrations (Rakocinski et al. 1992; Akin et al. 2003), and in this analysis was a main contributor to the observed dissimilarities in assemblage structure when comparing Sites 2 and 3 to Site 1.

Significant seasonal differences in faunal communities were not detected in this analysis which was based on one summer (July) and one fall (October) event. However, strong seasonal patterns in abundance of shrimp, crabs, and juvenile finfish have been documented in previous studies of Texas estuaries (Akin et al. 2003). The abundance of some estuarine species with known seasonal migratory patterns, such as Blue Crab, may have been underrepresented due to the timing of collection events (Ward 2012). Additional data under varying seasons and environmental conditions will help in distinguishing annual life history patterns from inflow-related dynamics, and thus, establishing a baseline of typical conditions within the Guadalupe Delta.

5.0 SUMMARY

The upper portion of the Guadalupe Delta nearest river inflows consistently exhibited freshwater characteristics, whereas the mid and outer regions were more dynamic and varied from oligohaline to mesohaline conditions. Over the long-term, the resulting salinity patterns create a longitudinal gradient in wetland plant communities that favors diversity near the middle of the delta, with freshwater and salt-tolerant taxa on each end.

This habitat diversity is directly reflected in the faunal communities, supporting that salinity regimes have a strong influence on taxa abundance and composition. Freshwater species were more strongly associated with Site 1 (near inflow areas) whereas estuarine-dependent species were associated with habitat conditions at Sites 2 and 3. Seasonal differences in assemblages were not observed due to the limited dataset.

Additional research is required to better quantify typical ontogenetic shifts in habitat utilization by estuarine-dependent species in this area. However, this study provides an abundance of data on the specific biota present within the Guadalupe Delta and begins to define typical seasonal patterns in habitat utilization. Although the results from this study provide the initial steps to understanding how differences in environmental conditions influence the biotic communities of the Guadalupe Delta at varying spatiotemporal scales, additional long-term monitoring is required in order to relate ecological variability to specific environmental variables such as freshwater inflows.

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APPENDIX A. SCOPE OF WORK

Exhibit A Scope of Work

Seasonal Ecological Assessment in the Upper Guadalupe Delta

Technical Approach

As the Senate Bill 3 (SB3) environmental flows process unfolded in Texas, it became evident that a major data gap in the understanding of the role of freshwater inflow to the bays and estuaries along the Texas Gulf Coast lies in the lack of ecological data at the interface between rivers and bays. This often led to disconnects between riverine and estuarine recommendations. A solid understanding of the faunal communities in Texas estuaries and in the open bay is known through the commercial and recreational fisheries sampling conducted by the Texas Parks and Wildlife Department (TPWD) and others over the years. However, a lacking component has been and continues to be those tidal/delta areas so infrequently sampled but yet so vitally important as nursery habitat in the estuaries.

Changes in freshwater inflow patterns to the estuary and other factors may affect estuarine salinity and marsh inundation patterns which play an important role in determining wetland vegetation community structure, vegetation productivity, and habitat utilization by organisms. As summarized during the Matagorda Bay Health Evaluation (MBHE 2006), there are two major biological data sources for the Texas Gulf coast: the TPWD coastal fisheries database and the National Marine Fisheries Service (NMFS) drop trap sampling data. However, the two databases differ in regard to their ability to define organism density relationships as a function of physical habitat and salinity. The NMFS drop trap data provides an advantage over the TPWD coastal fisheries database because NMFS drop trap sampling was conducted in many different physical habitat types including coastal marshes (edge and interior), submerged aquatic vegetation, oyster reefs and shallow non-vegetated bottom. The TPWD gear types are primarily used to sample open bay bottom and are not used within coastal marsh areas. Therefore, the NMFS drop trap approach was incorporated for all MBHE sampling (MBHE 2007).

As part of the MBHE project, multiple sites were sampled for marsh productivity and habitat utilization within Matagorda Bay and the Colorado River delta in 2006 and 2007. However, since that still represented only a snap shot across two water years, NMFS drop trap data collected over many years from Galveston, Lavaca, and San Antonio bays were also compiled to assess physical habitat suitability criteria for key MBHE designated species. Subsequently, during an extended drought in 2008, additional throw trap data at specific locations were sampled to add to the overall throw trap database (MBHE 2008). Finally, as part of a Senate Bill 3 (SB3) adaptive management study for the Colorado / Lavaca Bay BBASC, additional throw trap sampling was conducted in 2014 in both the Colorado River delta and Lavaca River delta to further assess the MBHE habitat suitability criteria (QEA 2015). Overall, the observations in 2014 were consistent with previous

> TWDB Contract No. 1800012267 Exhibit A, Page 1 of 7

years' data and supported the importance of marsh edge habitat. In each data set (including 2014) more juvenile finfish and shellfish were collected in marsh edge (defined as low estuarine marsh within 5 m of open water) habitat than in shallow non-vegetated bottom (e.g. open water) areas (QEA 2015).

An additional key ecological component of all rounds of MBHE sampling (2006-2008) was the collection of marsh biomass data to compare back to habitat utilization and freshwater inflow. In each report, end-of-season above-ground biomass measurements were assessed relative to the freshwater inflow during the entire growing season and compared to other years and locations. The results of these three years of seasonal above-ground biomass productivity data indicate that not only were the monotypic low estuarine marsh communities more productive during a high inflow year for the Colorado River, with corresponding low salinities at the sampling sites (2007), but that the mixed high estuarine marsh communities were more productive as well.

The proposed seasonal field sampling effort will build upon the existing literature for adjacent Texas bays as briefly summarized above and provide baseline data on habitat as well as habitat utilization by aquatic organisms within the Guadalupe Delta project area. A reconnaissance investigation and three seasonal sampling events will occur in spring, summer, and fall to cover nearly the length of the vegetation growing season along the Texas coast and encompass a time period when aquatic organisms inhabit these nursery areas. The geographic study area of this project includes the upper Guadalupe Delta region extending from approximately the existing salt water barrier into the upper portion of San Antonio Bay.

Following the Guadalupe-Blanco River Authority's (GBRA's) issuance of a Request for Qualifications and subsequent evaluation ranking of each submittal, BIO-WEST, Inc. was selected to assist GBRA with establishing a seasonal ecological assessment program in the upper Guadalupe Delta. Funding for this project will be executed via a Texas Water Development Board (TWDB) grant to address a priority research need identified in the SB3 Guadalupe, San Antonio, Mission, and Aransas Rivers and Mission, Copano, Aransas, and San Antonio Bays Basin and Bay Area work plan. Field work conducted as part of this seasonal ecological assessment will assist in establishing a baseline condition of wetland plant productivity and an understanding of how aquatic organisms, such as fish and shellfish, use this habitat within the upper Guadalupe Delta. It is anticipated that following the successful completion of this contract, the results will provide stakeholders and the Texas Commission on Environmental Quality (TCEQ) with valuable baseline ecological data for consideration upon future review of freshwater inflow standards for San Antonio Bay.

Objectives

The key objectives of this seasonal field study are to:

- Initiate establishment of baseline conditions of marsh productivity for the upper Guadalupe Delta region.
- Evaluate the role of salinity and inundation relative to marsh vegetation community dynamics within the project area.

TWDB Contract No. 1800012267 Exhibit A, Page 2 of 7 Quantify aquatic organism species abundance and community composition within shallow habitats in the study area in relation to physical habitat and salinity.

This field effort is focused on the wetland plant community and on aquatic organisms (not including the American oyster) within shallow non-vegetated bottom (SNB) and marsh edge (ME) habitats. Aquatic organisms expected to be sampled include juvenile shellfish and finfish.

Methodology

This project prioritizes baseline data collection focusing on marsh productivity (habitat and utilization). This scope of work is divided into two tasks: (1) field data collection and sample processing, and (2) data analysis and documentation.

Task 1 – Reconnaissance and Field Data Collection

Upon contract award, a reconnaissance investigation of the upper Guadalupe Delta region will be conducted in coordination with GBRA to establish study sites for subsequent seasonal sampling. It is anticipated that up to three study locations (upper, mid, lower) will be selected ranging longitudinally along the upper delta starting near the saltwater barrier and extending towards San Antonio Bay. Exact locations will be determined during the reconnaissance field trip.

Wetland Plant Productivity

Once sites are established, two seasonal wetland plant productivity sampling trips (Spring and Fall to bracket the growing season) will be conducted at each established location. To perform this, one transect per location (upper, mid, lower) will be established ranging from the marsh edge through low estuarine marsh (LEM) and high estuarine marsh (HEM). Each transect per location will be selected to be representative of the variability in salinity, elevation and vegetation types typically found at each of the locations. Wetland vegetation community structure will be quantified along each transect within vegetated habitats in close proximity to the throw-trap sampling stations (discussed below). During each event, one 1 m² vegetation-monitoring plot will be randomly assigned in LEM and HEM along each transect and sampled for plant species dominance, plant community composition, stem density and recruitment, utilizing stem count methods. This monitoring plot will remain constant over the study period and photo-documented during each sampling period.

In addition, triplicate vegetation plots (1 m²) will be randomly selected in the LEM to HEM plant communities along each transect during the spring and fall survey. This results in six individual vegetation plots (3 HEM, 3 LEM) per Spring and Fall per transect, resulting in a total of twelve vegetation plots per transect over the study period. The randomized triplicate design per trip, per transect will allow a statistical comparison of variability within and between sampling periods. Species within each plot will be identified and the density of the top three dominant species will be estimated. Standing plant biomass within each plot will be clipped at ground level, placed into a bag and shaken to homogenize the sample. Total wet weight will be measured on a hanging field scale. For the first sampling event, one-half weight of the sample will be placed in bags returned and oven-dried at 60°C

TWDB Contract No. 1800012267 Exhibit A, Page 3 of 7 to constant weight. The remaining one-half weight of the homogenized sample will be placed into galvanized mesh bags and staked into place within the plot area and left to decompose. This material will be collected at the second sampling event, placed into bags and returned to be dried and weighed. Standing crop sampling will be repeated at the second sampling event following the same methods detailed above but with only one-half of the homogenized harvested standing crop returned to be dried and weighed. These components will identify changes in the standing crop of marsh communities within the project area over the period of one growing season and assess the quantity and timeframe plant biomass is made available to delta aquatic organisms.

Sampling within the wetland complexes for wetland vegetation community structure and plant primary productivity will involve the use of an access vehicle (shallow-draft boat, airboat or truck), equipment for vegetation plot sampling and monitoring, and two to four personnel. The vegetation sampling would coincide with the throw-drop trap sampling in the fall to maximize efficiency.

Throw-Trap Sampling

Two biological sampling events (summer and fall) will be conducted within two identified habitats (Shallow non-vegetation bottom [SNB] and Marsh Edge [ME]) along the established marsh transects within the selected study locations. A throw-trap is a type of sampling device originally used by the National Marine Fisheries Service to sample aquatic organisms along the Gulf Coast. The throw-trap sampling technique is effective for sampling small fishes and large



macroinvertebrates such as the crab, shrimp and juvenile fish species. To be consistent with other recent efforts in Texas bays, the throw-drop trap used in this study will enclose a 1 m² area and be approximately 1.5m in height (picture to the right). A large dip net will be used within the throw-drop trap for the collection of organisms. The dip net will be swept along the length of the estuarine substrate a minimum of 10 times to ensure a nearly complete enumeration of all fish and macroinvertebrates caught within the trap. If organisms are collected in the 10th sweep, additional sweeps will be conducted until 3 successive empty sweeps are made. Using this procedure, key species will be collected, then counted and measured either in the field or upon return to the laboratory. Throwtrap sampling involves the use of a shallow-draft boat or airboat, throw-trap sampler, dip net and three to four scientists.

Throw-trap sampling will include three-replicate 1 m² samples in SNB and ME habitats at each established transect in the study area. Information on wetland vegetation cover present in the throw-trap samples will be collected within ME habitats. Sampling events will be conducted in late Summer (July/August) and Fall (late September/October) to capture periods when several of the key recreational and commercial species reside in San Antonio Bay. The sampling design will include equal sample numbers from each of the two identified habitat types and will occur (to the degree practicable) during high-tide to allow access by aquatic organisms to all habitats during the period of sampling. This sampling

> TWDB Contract No. 1800012267 Exhibit A, Page 4 of 7

method is limited to areas that are shallow enough to completely enclose using the throwtrap sampler (no more than 1.5m deep) and those areas that are inundated so as to be accessible to aquatic organisms. During throw-trap sampling, vegetation measurements and conventional physico-chemical parameters will be taken. The measurements taken within the enclosure and in the adjacent 1-meter area surrounding the trap will include water depth, substrate, identification of dominant vegetation type and percentage coverage. The physico-chemical parameters will include water temperature, salinity, pH, dissolved oxygen, and observations of local conditions taken at the surface and near the bottom at all sites.

Assuming three study locations, each with three transects, the total study effort would produce 108 throw-trap samples after two sampling periods (54 from SNB and 54 from ME). By nature, tidal wetland systems are periodically inundated and the tidal range in San Antonio Bay is approximately 1 to 2 feet. Sampling events will be conducted during daylight hours and designed to occur during local high-tide on each day of the sampling event in order to sample habitats while they are equally available to all organisms (in terms of water depth) and to maintain consistency between sampling events.

Deliverables:1) Raw data in electronic format 2) Digital photographs of field sampling activities

Task 2 – Data reduction, Analysis, and Documentation

The baseline data, methods, and results of this study will be documented in a final data report. It is anticipated that the report will be data oriented as this study represents some of the first data of this type to be collected in these areas. Data reduction and characterization will include baseline descriptive statistics of wetland plant communities and biomass (Nursery habitat) and aquatic organism life stages, community composition, and habitat utilization in areas of the delta with limited to no sampling over the years. It needs to be noted that with only one year of data, it will not be possible to conduct detailed statistical analysis of freshwater inflow to ecological responses in a holistic manner. The primary focus of this baseline data is to start the process of filling data gaps and acquiring data for the evaluation of freshwater inflow standards into the future.

Deliverables:1) QA/QC data presented in electronic format (spreadsheets)

- GIS dataset of sampling locations and vegetation transects
 - Monthly Progress Reports
 - 4) Draft and Final report
 - 5) Final Presentation

PROJECT MANAGEMENT:

Project management, contracting, task coordination, communication, and schedule are included in this category.

> TWDB Contract No. 1800012267 Exhibit A, Page 5 of 7

Project reporting:	Electronic reporting of project progress and invoicing will be submitted on a monthly basis.
Communication:	The project team anticipates frequent communication via phone or email with GBRA and TWDB. Additionally, one project meeting / presentation of data findings with TWDB, GBRA, and the GSA-BBASC is included under project management. The anticipated meeting date is early summer 2019.

Schedule:

- Field Work: The project will start immediately upon contract execution. A reconnaissance field effort will be conducted in 2018 following contract approval and formal notice to proceed. Three sampling events are scheduled to occur within the project area over the vegetative growing season (spring, summer, fall). As noted above, the throw-trap sampling will be conducted during optimal (or maximum) high-tide events when the maximum area of the sampling sites are inundated, allowing access to organisms and the measurement of water quality and depth across sample transects. Vegetation plots may be sampled at any time during the tidal cycle, though low-tide periods may allow for less problematic sampling procedures. Recommended sampling periods that encompass optimal high-tide events will be designated from NOAA predicted tidal charts. However, the current conditions and the short-term tidal predictions for the sampling region will be monitored on the TWDB website from 2 to 14 days prior to each sampling effort. This will enable the project team to determine if conditions will be appropriate and if any adjustment in the sampling schedule is warranted.
- Report:
 Upon completion of the final sampling event, the project team will prepare and submit a draft report to TWDB. The draft report is due to TWDB no later than 60 days before the contract expiration date. Upon receipt of the draft report, TWDB will have 30 days for review and my send the report out for external review. The final report is due to TWDB within 30 days of receiving review comments from the TWDB.

TWDB Contract No. 1800012267 Exhibit A, Page 6 of 7

References

- MBHE. 2006. Progress Report. MBHE Habitat Assessment. Prepared for Lower Colorado River Authority and San Antonio Water System. December 2006.
- MBHE 2007. Habitat Assessment. Final Report. Prepared for Lower Colorado River Authority and San Antonio Water System. June 2007.
- MBHE 2008. High Salinity Period Sampling (2008). Prepared for Lower Colorado River Authority and San Antonio Water System. December 2008.
- QEA 2015. Studies to Evaluate Achievement of Freshwater Inflow Standards and Ecological Response. Final Report to Texas Water Development Board. Prepared by Anchor QEA project team. August 2015.

TWDB Contract No. 1800012267 Exhibit A, Page 7 of 7 **APPENDIX B. COMMENT RESPONSES TO TWDB REVIEW**

Comment Responses to TWDB Comments on Draft Final Report "Seasonal Ecological Assessment in the Upper Guadalupe Estuary". TWDB Contract #1800012267

The project team appreciates the comments and suggested revisions provided by TWDB reviewers. Authors have responded to each comment and summarized edits made to the report in blue text below each reviewer comment.

REQUIRED CHANGES

General Draft Final Report Comments:

The draft report states that data and results from the fall sampling event will be included in the final report. Please remember to include this information and update all sections of the report accordingly. The report begins by referencing spring salinities and then changes to referencing summer salinities after Page 9. Although vegetative sampling was in May and faunal sampling was in July, salinity values are referred to as *summer* in the first two paragraphs of Page 12 instead of *spring*, even though the values apparently come from Table 1. Please likewise clarify, in the table caption for Table 1, the season associated with the data in Table 1.

Fall vegetation biomass data were added to the report.

In reference to salinity values, there were two water quality datasets that were analyzed. Salinity data associated with vegetation sampling took place in spring (May) and fall (Oct). This is referred to in Table 1 and prior to page 9 in the vegetation results. Salinity data associated with faunal sampling occurred in summer (July) and fall (Oct). This separate dataset is referenced in the faunal community results and analysis. To make this clearer, we have inserted a table (Table 3) in section 3.2.1 summarizing salinity data from faunal community sampling. To avoid confusion, we have also inserted the month in parentheses following each mention of spring, summer, and fall throughout the report.

Specific Draft Final Report Comments:

1. Cover page: Please add "Contract #1800012267" and the following statement to the cover page of the final report:

Pursuant to Senate Bill 1 as approved by the 85th 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.

Revised cover page to include this information.

1. Table of Contents, page ii - iii:

a. Please move the Scope of Work summary to the end of the report, possibly as an Appendix, and list it in the Table of Contents according to its location within the in the report. Additionally, as currently presented in the report, it is out of order with respect to the List of Figures and List of Tables.

Moved Scope of Work to Appendix A at end of report.

b. Please remove the "I." following the subheadings for "List of Figures" and "List of Tables".

Removed.

2. Section 3.2.1, Page 10, Paragraph 1, First sentence: Please verify that the total variation explained equals 39.1% given that Axis I explains 26% and Axis II explains 13.1%.

Changed 38.1% to 39.1%.

3. Section 3.2.1, Page 12, Paragraph 1, First sentence: Please clarify the source of the salinity values referenced in this sentence. If using the values in Table 1, summer salinity should be corrected as 0.33 ppt, not 0.34 ppt. If Table 1 is not the source of the value, then provide the source.

Inserted a reference to new Table 3 which provides mean salinity values from faunal community sampling.

4. Section 3.2.1, Page 12, Paragraph 2: Please reference Table 1 or clarify the source of the salinity values reported in this paragraph.

Inserted a reference to new Table 3 summarizing salinity values from faunal sampling.

5. Section 3.2.2 Faunal abundance, Page 13, Paragraph 2: Please clarify the source of uncertainties in the values for abundance, for example, "129.7±30.0".

Inserted "(mean ± standard error)" in this sentence to clarify that uncertainty values are standard error.

Figures and Tables Comments:

1. Table 1, Page 8: Please specify in the table caption that mean salinity is reported here.

These are not mean values. This is a single measurement from each site.

2. Figure 4, Page 11: Please include the notation that the plot for general habitat characteristics is on the right. Please also clarify the sentence as follows, "... and for general habitat characteristics (right) for each site (1, 2, and 3) based on mean site scores..." Please also correct the notation for the water stargrass (WS), which appears to be represented by "SG" on the plot.

Inserted these clarifications as suggested.

3. Table 3, Page 13: Please specify in the table caption that the data represents the total number of families collected among all sites.

Added "at all sites" to end of first sentence in caption.

References/Literature Cited Comments:

1. Please verify that the references for Lassuy (1983) and Muncy (1984) were used in this report. They are missing from the main body of the report.

These citations were removed from Literature Cited section.

SUGGESTED CHANGES Specific Draft Final Report Comments:

1. The TWDB can provide a clean copy of the Scope of Work to sue in the final report.

Thank you. If the copy provided in Final report is not acceptable, we will be glad to insert a different version.

Figures and Tables Comments:

1. Figure 4, Page 11: Please consider providing a better resolution images and tables for Figures 4 (page 11) and 6 (page 17) and for Tables 3 (page 13) and 4 (page 14).

These are highest resolution that can be provided. Decreases in resolution arise from converting from Word to PDF.