

SEASONAL ECOLOGICAL ASSESSMENT IN THE UPPER GUADALUPE ESTUARY

FINAL REPORT

Prepared for

Texas Water Development Board
Contract #2100012475

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Pursuant to House 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.

August 25, 2022

Melissa Lupher, Ph.D.
Texas Water Development Board
1700 North Congress, Agency Code 580
Austin, TX 78701

Re: TWDB Contract No. 2100012475
Final Report – Guadalupe Delta Study

Dear Melissa:

The project for the Seasonal Ecological Assessment in the Upper Guadalupe Estuary is complete and we submit the Final Report.

The reports include a Table of Contents, Scope of Work, photos of study sites and List of Literature Cited as set out in Contract No. 2100012475.

Please contact me should you require any additional copies.

Sincerely,



Nathan E. Pence
Executive Manager of Environmental Science

NEP:mcn

Enclosure as stated



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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. Initial surveys were conducted in 2019 to establish baseline conditions of the vegetation and faunal assemblages within the Delta. Continuation of seasonal monitoring was performed in 2021, which included the initiation of avian community surveys. To bracket the growing season, species composition and relative abundance, and biomass of the marsh vegetation community were quantified from multiple plots along fixed transects at each site in spring and fall. To establish a baseline of avian community composition, seasonal timed point counts were utilized to quantify species abundance, diversity, and describe differences in assemblage structure among sites. To target seasons when key economically important faunal species are utilizing shallow estuarine areas, nekton sampling was conducted using throw-traps in summer and fall. 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 quantified 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.

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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. Among the measured vegetative communities, observations were generally in line with those observed during 2019 sampling with some exceptions. Site 1, as in 2019, was characterized by emergent freshwater marsh plants including alligatorweed, broadleaf arrowhead, and southern cattail. Submerged aquatic vegetation such as coontail and water stargrass were also common at Site 1. Contrary to 2019 sampling, this site exhibited the highest species diversity among sites in 2021. Site 2, near the middle of the Delta, exhibited the least diverse plant community and included a mixture of salt-tolerant species such as saltmarsh bulrush and smooth cordgrass. Lastly, Site 3, near the tip of the Delta, was dominated by salt-tolerant taxa such as smooth cordgrass and common reed. Higher species diversity was observed across all sites in 2021, as compared to 2019 sampling. This is most likely an artifact of an increased vegetative survey effort in 2021. Despite the implementation of updated survey protocol, general trends in species assemblage were consistent with 2019 sampling.

Avian community survey results were typical of a Texas Gulf Coast estuarine ecosystem, with a diverse array of shorebirds and migratory birds utilizing the variety of habitat types present at the Delta. Avian diversity was highest at Site 1, where marshes and nearby woodland and grassland areas are present providing habitat for both shorebirds and passerines associated with more upland environments. As the salinity increases in the lower portions of the Delta, saltmarsh species are predominant. Avian abundance and richness were greater at Site 2 than Site 3. This may be driven in part by the slightly more inland and channelized aspects of Site 2 providing greater refuge for foraging and breeding activities.

The observed salinity gradient and variation in habitat conditions influenced the spatial distribution of the Delta fauna. Estuarine-residents dominated faunal assemblages across sites during both years and grass shrimp was the most abundant taxa, which is typical in Texas coastal marshes. Estuarine-dependent taxa were also consistently most represented at Site 2 and Site 3. Among years, freshwater taxa were the least abundant guild across sites and most taxa were exclusively found at Site 1. Lastly, estuarine-dependent taxa collected in 2021 that were not observed in 2019 included big claw river shrimp, star drum, black drum, and skillettfish, suggesting that the sampling time period within seasons may affect observations.

Patterns in total faunal density were highly complex and varied based on interactions between site, season, and habitat type. Total density was generally higher in the summer within Marsh Edge (ME) habitats, with the exception of Site 2, where density was substantially higher in the fall when salinity concentrations exhibited mesohaline conditions (salinity: 5.0 to 18.0 ppt). Despite stronger associations between salinity and biological productivity shown at Site 2, other environmental factors also appear to influence faunal trends. Total density was generally higher in ME habitats at Site 1 compared to Site 3, suggesting that the suitability of physical habitat conditions may also affect productivity. Vegetation cover was generally lower in ME habitats at Site 3 compared to the other sites when tide levels were similar. Based on the importance of vegetation for

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small-bodied organisms, biological productivity at Site 3 may be lower due to sub-optimal ME conditions with less cover.

Despite lower productivity observed at Site 3, patterns in assemblage structure show that this area within the Delta harbors a more distinct faunal community. Dissimilarities in assemblage structure at Site 3 compared to others can best be explained by greater abundances in estuarine-dependent crustaceans. Higher abundance of estuarine-dependent taxa also explains differences in assemblage structure between Site 1 and Site 2. Greater abundances of estuarine-dependent taxa at Site 2 and Site 3 aligns with their positive associations with salinity observed during this study and previous research that also found species that use estuaries for part of their life cycle are more prevalent in oligohaline (salinity: 0.5 to 5.0 ppt) and mesohaline environments. Lastly, strong seasonal differences in assemblage structure within sites are most likely due to migratory patterns and reproductive timing of estuarine-dependent taxa.

Results from this study supported previous findings, while also providing new insights into potential mechanisms driving biotic assemblages. Analyses conducted demonstrated meaningful functional relationships between estuarine organisms and environmental parameters, providing a better understanding on the ecology of the Guadalupe Delta. These data, however, likely represent a subset of the environmental conditions experienced in the Delta. The high variability implicit with estuaries makes it difficult to accurately quantify and describe the complexities of these systems. 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.

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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, such as freshwater inflows, tidal influences, and predator/prey interactions, among others. Among these, freshwater inflows are recognized as a major driver of estuarine dynamics (Longley 1994; Alber 2002; Quigg et al. 2009), influencing environmental parameters like 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. In particular, there are limited ecological data at the interface between rivers and bays (i.e., tidal/delta areas), which are important nurseries for economically and ecologically important species within these estuarine areas (Longley 1994). Developing an understanding of functional relationships between freshwater inflows and biological productivity is an essential component for 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.

Therefore, the overarching goal of this study is to examine how freshwater inflow dynamics influence marsh productivity in the upper Guadalupe Estuary, as well as to develop and validate freshwater inflow recommendations. Initial surveys were conducted in 2019 to establish baseline conditions of the vegetation and faunal assemblages within the Guadalupe Delta. Following additional monitoring, these data will be used to evaluate spatiotemporal patterns in these assemblages (BIO-WEST 2020). Continuation of seasonal monitoring was conducted in 2021, which included the initiation of avian community

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surveys. This report summarizes spatiotemporal trends in biotic communities observed during 2021 sampling efforts. In addition, data collected in 2019 and 2021 were used to examine how environmental variation influences marsh productivity, biotic assemblage structure, and taxa-specific habitat associations.

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 (hereafter '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).

Three sites were established in 2019 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. 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 periphery of Lucas Lake, which is directly connected to the upper open-water portions of San Antonio Bay (**Figure 1**).

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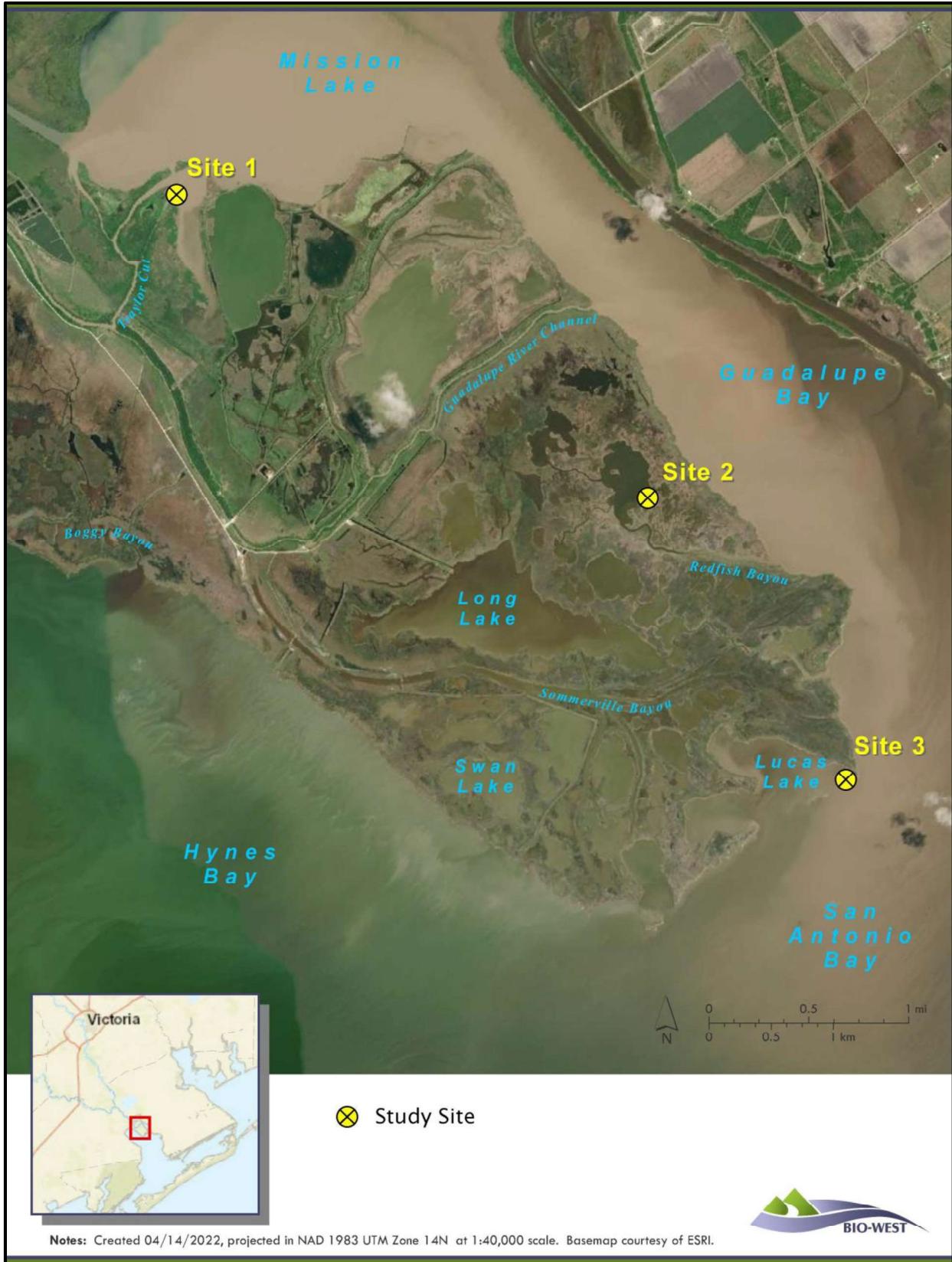


Figure 1. Study sites in the Guadalupe Delta.

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2.2 Vegetation Community

Similar to 2019 vegetation community sampling, each site was visited twice, once in the spring (April) and once in the fall (November) of 2021. Number of transects, transect length and number of plots per transect, however; were changed from 2019 to 2021 to increase sample sizes and provide a better representation of the marsh vegetation community. In 2021, number of transects per site increased from 1 to 3, transect length was increased from 25 meters to 50 meters and number of temporal monitoring plots per transect was increased from 3 to 10. During sampling, 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 50 meters (m) inland from the shoreline. Along each transect, 10 plots (1 m²) were established for temporal monitoring of the vegetation community. At 5-meter intervals along the transect, plots were selected at a random distance from the transect line in a perpendicular direction, from 0–5 meters on either side.

For each temporal monitoring plot, dominant taxa, percent cover estimates for dominant taxa, and vegetation height were collected. Common and scientific names for wetland plants follow Stutzenbaker (1999). Plant species richness 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 [µS/cm], and salinity [ppt]) were measured with a YSI ProDSS water-quality sonde in the water column at each site at the time of surveys.

2.3 Avian Community

To establish a baseline of avian communities across study sites and available habitats, timed point counts were conducted at each site during the spring (April), summer (July), and fall (November) of 2021. Six timed point counts were conducted per site during each monitoring event to get representative samples among habitats present, including three counts located in proximity to emergent vegetated marsh edge (ME) and three in non-emergent vegetated bay bottom (SB). The selection of time point count locations occurred in the field at the time of each sampling event and was influenced by the seasonal variation in accessibility and availability of habitat types (**Figure 2**). Timed point counts were conducted for a fixed 10-minute period. During timed point counts, all avian species observed (identified either aurally or visually), number of individuals, habitat associations at the time of observation, and relevant climate parameters were recorded (Verner 1985; USDA 1997).

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Figure 2. An example of timed point count locations (spring 2021) at each study site in the Guadalupe Delta.

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2.4 Faunal Community

Aquatic fauna was surveyed within wadeable habitats using a 1 m² throw-trap (**Figure 3**), which is effective for sampling macrocrustaceans (e.g., crabs, shrimp) and small-bodied fishes in shallow estuarine environments (Jordan et al. 1997; Rozas and Minello 1997; MBHE 2007). To assess seasonal variation in faunal communities, sampling was conducted in summer (July) and fall (November) of 2021. Shallow non-emergent vegetated bay bottom (SB) and emergent vegetated marsh edge (ME) habitats were sampled within three transects per site during each event. Within each throw-trap sample, habitat measurements were taken. Temperature (°C), pH, dissolved oxygen (DO) concentration (mg/L) and percent saturation (%), specific conductance (µS/cm), and salinity (ppt) were measured with a YSI ProDSS water-quality sonde. Additionally, emergent and submergent macrophyte composition and coverage (%) was estimated within each throw-trap and the presence/absence of floating periphyton and vegetation was recorded. To collect organisms, a large dip-net was swept along the length of the substratum within each throw-trap a minimum of 10 times. All biota collected from each sample were 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 3. Throw-trap sampling at Site 3 during the fall 2021 monitoring event.

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2.5 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 to 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). For the assessment of the avian community, site and seasonal occurrence, taxa richness, relative abundance, and Shannon Diversity Index were calculated. In addition, an avian taxa list per dominant habitat type per seasonal event was compiled.

Faunal taxa relative abundance (%), richness, evenness, and diversity were calculated for each site per season to summarize data collected in 2021. Taxa diversity was calculated based on the Shannon Diversity Index (Shannon 1948), which was then used to calculate taxa evenness. 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). The amphidromous big claw river shrimp (*Macrobrachium carcinus*) was also classified as estuarine-dependent, since this species relies on brackish environments to complete larval development, before migrating back to freshwater environments (Bowles et al. 2000). Lastly, grass shrimp (*Palaemon* sp.) were evaluated in aggregate due to the large numbers collected and difficulty in efficiently identifying to species-level.

Throw-trap data from 2019 and 2021 were used for all statistical analyses. Along with the habitat parameters quantified during sampling, additional data were obtained to represent patterns in tides and freshwater inflows. Water level data (m) was obtained from the nearest NOAA Tides and Current Monitoring Station (Seadrift, TX) to characterize tide conditions during sampling (NOAA 2022). River discharge (cms) data was also obtained from the nearest USGS stream gage to provide a general representation of inflow conditions during the month of each sampling event (USGS 2022).

Principal components analysis (PCA) was used to describe differences in environmental characteristics based on throw-trap data and median tide level during each discrete sampling interval. Continuous environmental variables were $\log(x)$ transformed, proportional environmental variables were $\arcsin(\sqrt{x})$ transformed, and categorical variables were coded as dummy variables. Dissolved oxygen parameters were omitted from the PCA due to their strong correlation ($r > 0.7$) with time of day, meaning that variation in DO was related to the sampling process, limiting their utility to make reliable comparisons in environmental conditions. Specific conductance was also removed from the PCA due to its strong correlation with salinity. A multivariate adaptive regression splines (MARS) model was also used to predict salinity trends and examine its associations with spatial, tidal, and freshwater inflow factors (Friedman 1991). Salinity was $\log(x)$ transformed and predictor variables included in the model were distance (km) from freshwater inflow sources (based on site location), median tide level during sampling intervals, and median discharge during the month of sampling. Prediction performance of

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the MARS model was evaluated via 10-fold cross-validation (mean $R^2 \pm SE$). For each fold, a subset of data excluded from model training (i.e., out-of-sample data) was used to simulate how well the model predicts new data (i.e., generalization error) (Hastie et al. 2009).

Spatiotemporal and habitat differences in marsh productivity were assessed using total density (ind./m²) and examined with a linear mixed effects model (LMM; $\alpha = 0.05$). For this analysis, replicates were based on the sum of densities among taxa for each throw-trap sample and were $\log(x+1)$ transformed. Fixed effects included sites, seasons, and habitat type (ME vs. SB), as well as all possible two- and three-way interactions. Throw-trap sample number nested by transect were included as random intercepts to control for non-independent data points (Harrison et al. 2018). To test for differences in faunal community structure between sites, seasons, and their interactive effect, analysis of similarities (ANOSIM, $\alpha = 0.05$, 10,000 permutations) was used. Taxa abundances were $\log(x+1)$ transformed and taxa that occurred at <5% of throw-trap samples were omitted to limit the statistical influence of rare taxa that may have low detectability. To further investigate the best supported main- or interactive effects, mean Bray Curtis distances were calculated and visualized with a dendrogram built based on hierarchical clustering procedures. For the most dissimilar groups displayed by the dendrogram, similarity percentages were also calculated to discriminate the contribution of individual taxa among assemblage groups assessed (Oksanen et al. 2022a).

Lastly, species-habitat associations were evaluated using canonical correspondence analysis (CCA). Taxa abundances were $\log(x+1)$ transformed and taxa that occurred at <5% of throw-trap samples were removed. Environmental parameters used in the PCA were also used for this analysis and transformed in the same manner. Relative importance of environmental parameters, site, and season were tested using variance-partitioning techniques in CCA (Borcard et al. 1992). All statistical analyses were performed in R 4.1 with the package's 'earth' (Milborrow 2021), 'lmerTest' (Christensen 2020), 'pdp' (Greenwell 2018), 'stats' (R-core package), 'tidyverse' (Wickham 2021), and 'vegan' (Oksanen 2022b).

3.0 Results

3.1 Vegetation Community

3.1.1 Community Composition

All three sampling sites presented low-lying tidal marsh and showed signs of regular and fluctuating levels of inundation. Salinity measurements recorded during each sampling event suggest that sites exhibit the following conditions: 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 dominant herbaceous species. In contrast to 2019 sampling, species overlap among sites was relatively higher with common reed (*Phragmites australis*) found across all three sites and smooth cordgrass (*Spartina alterniflora*) and wiregrass (*Spartina patens*) observed at Sites

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2 and 3. It is important to note that both higher degrees of species overlap across sites and species richness were observed in 2021 (26 species observed across all sites) as compared to 2019 (14 species observed across all sites). This is most likely a factor of the more robust sampling design implemented in 2021 (i.e., 10 quadrats/transect from 3 quadrats/transect). While 2021 vegetation represents repeated measures (overlapping with 2019 transects), the study was expanded to generate larger sample sizes and to better characterize community composition.

Table 1. Salinity (ppt) recorded at each site during each vegetation sampling event.

Site	Spring (April)	Fall (November)
1	0.5	0.4
2	22.9	1.2
3	28.8	2.6

Site 1 had the highest species diversity among sites, with 13 species documented in 2021. This community was composed almost entirely of herbaceous freshwater littoral and semiaquatic species (**Table 2**). The non-native alligatorweed (*Alternanthera philoxeroides*) was the most dominant species at Site 1 during both sampling seasons, followed by common reed. By fall, alligatorweed dominance decreased moderately as broadleaf arrowhead (*Sagittaria latifolia*), southern cattail (*Typha domingensis*), and non-native wild taro (*Colocasia esculenta*), and water hyacinth (*Eichhornia crassipes*) increased in dominance. Several less-common species were observed interspersed throughout the mosaic of non-native species which characterize Site 1. This included climbing hempweed (*Mikania scandens*), manyflower marsh-pennywort (*Hydrocotyle umbellata*), oppositeleaf spotflower (*Acmella repens*), and several other species represented by the families Apiaceae (lilies), Lemnoideae (duckweeds), Brassicaceae (searockets), and Commelinaceae (dayflowers).

Site 2 had the lowest species diversity, with 8 species identified from sampling plots. The community was comprised mostly of graminoid species, rushes and grasses, as well as several herbaceous species (**Table 2**). Saltmarsh bulrush (*Scirpus maritimus*) and smooth cordgrass (*Spartina alterniflora*) were widespread throughout the community. Dominance between species was captured differently between spring and fall sampling, but this may be an artifact of community heterogeneity rather than loss or expansion of individual species. With some 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.

In contrast to 2019 sampling wherein Site 3 was composed almost entirely of a homogenous stand of smooth cordgrass, saltmarsh bulrush dominated in 2021. In addition, more woody species such as sea myrtle (*Baccharis halimifolia*) were observed (**Table 2**). This site was noted as having the largest variation in salinity between spring and fall sampling events, ranging from 28.77 to 2.56, respectively (**Table 1**). Given the plant

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community present, low salinity observed at this site in the fall sampling event likely represents an atypical situation caused by above average spring rainfall throughout the watershed combined with low tide conditions at the time of survey. Weather conditions as a driver for higher variation in salinity at this site is further evidenced by data collected in 2019, where large variation in salinity was also observed between spring and fall events.

Table 2. Percent dominance of plant species identified from sampling plots at three sites in the Guadalupe Delta during spring (April) and fall (November) 2021. Only plants which were identified to the species-level were included in this table. All plants which were not identified to species-level were observed at <1% dominance.

Site	Common Name	Scientific Name	Dominance (%)	
			Spring	Fall
1	Alligatorweed	<i>Alternanthera philoxeroides</i>	67	33
	Broadleaf arrowhead	<i>Sagittaria latifolia</i>	0	7
	Climbing hempweed	<i>Mikania scandens</i>	<1	4
	Common reed	<i>Phragmites australis</i>	21	13
	Manyflower marsh-pennywort	<i>Hydrocotyle umbellata</i>	0	<1
	Oppositeleaf spotflower	<i>Acmella repens</i>	<1	0
	Southern cattail	<i>Typha domingensis</i>	0	8
	Swamp smartweed	<i>Polygonum hydropiperoides</i>	<1	0
	Wild taro	<i>Colocasia esculenta</i>	3	15
	Water hyacinth	<i>Eichhornia crassipes</i>	6	15
2	Common reed	<i>Phragmites australis</i>	25	<1
	Marsh fleabane	<i>Pluchea odorata</i>	0	<1
	Saltmarsh bulrush	<i>Scirpus maritimus</i>	25	55
	Smooth cordgrass	<i>Spartina alterniflora</i>	50	19
	Water hyssop	<i>Bacopa monnieri</i>	0	10
	Soft-stem bulrush	<i>Schoenoplectus tabernaemontani</i>	0	7
	Wiregrass	<i>Spartina patens</i>	0	7
3	Bermuda grass	<i>Cynodon dactylon</i>	0	<1
	Big cordgrass	<i>Spartina cynosuroides</i>	11	0
	Common reed	<i>Phragmites australis</i>	8	32
	Bigleaf marsh-elder	<i>Iva frutescens</i>	17	0
	Marsh fleabane	<i>Pluchea odorata</i>	0	<1
	Saltmarsh bulrush	<i>Scirpus maritimus</i>	61	17
	Sea myrtle	<i>Baccharis halimifolia</i>	0	16
	Smooth cordgrass	<i>Spartina alterniflora</i>	4	12
Wiregrass	<i>Spartina patens</i>	0	23	

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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 4**). Only plant species with available data were included. These trends are consistent with those reported during 2019 sampling.

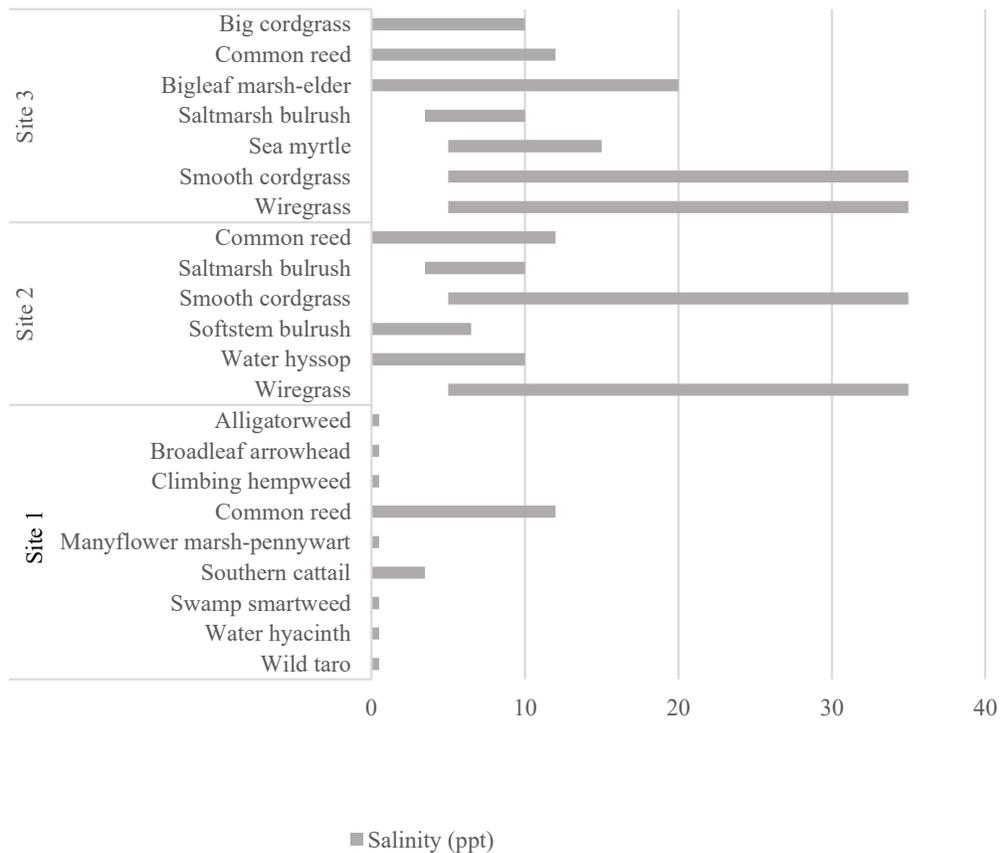


Figure 4. Reported salinity tolerance ranges for observed dominant species at each site. Salinity tolerances are based on data and information from Stutzenbaker 1999, Burdick and Konisky 2003, and USDA 2000.

3.2 Avian Community

Point counts were conducted within either emergent vegetated marsh or non-emergent vegetated bay bottom. However, given the radius of avian detectability (approximately 160-meters) and habitat heterogeneity present in the Delta, species were observed across six different habitat types. Therefore, species observations were reported by site and associated habitat type including, emergent marsh, open water, shoreline, mud flat,

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woodland, and grassland (**Table 3**). Unfortunately, fall survey data was lost due to a data transfer error related to the digital in-field data collection application utilized for this portion of the study. The following analysis is represented by bird community data from the spring and summer sampling events, only.

Table 3. Seasonal occurrence, site occurrence, count (#), relative abundance (%) and dominant habitat type of the avian communities observed during spring and summer sampling. Habitat types included emergent marsh (EM), open water (OW), shoreline (SL), mud flat (MF), woodland (WL), and grassland (GL).

Taxa	Season		Site			Count	Relative Abundance (%)	Dominant Habitat Type						
	Spring	Summer	1	2	3			EM	OW	SL	MF	WL	GL	
American bittern		X	X			3	0.2	X	X					
American white ibis	X	X	X	X	X	57	4.3	X	X					
Barn Swallow	X	X	X	X	X	339	25.8	X	X	X				
Black-bellied whistling duck	X	X	X			17	1.3	X	X					
Black rail	X				X	1	0.1	X						
Black tern	X	X		X	X	16	1.2		X					
Black-necked stilt	X	X	X	X	X	13	1.0	X		X				
Blue-winged teal	X		X	X	X	22	1.7	X						
Boat-tailed grackle		X	X	X	X	19	1.4	X	X					
Brown-headed cowbird		X	X			4	0.3	X	X					
Brown pelican	X		X	X	X	8	0.6	X	X					
Carolina wren		X		X		1	0.1	X						
Caspian tern	X			X	X	5	0.4	X		X				
Cattle egret	X	X	X	X	X	199	15.1	X	X	X				
Clapper rail		X	X	X	X	7	0.5	X						
Cliff swallow		X		X		1	0.1	X						
Common gallinule	X			X		14	1.1	X						
Common tern	X		X			4	0.3		X					
Eastern kingbird		X		X		2	0.2	X						
Forster's tern	X	X	X	X		13	1.0	X	X		X			
Great blue heron	X	X	X	X	X	7	0.5	X	X					
Great egret	X	X	X	X	X	6	0.5	X	X					
Greater yellowlegs		X	X			3	0.2					X		
Great-tailed grackle	X		X	X	X	129	9.8	X				X		
Killdeer		X	X			1	0.1	X						
Laughing gull	X	X	X	X	X	25	1.9	X	X	X	X			
Least bittern	X	X	X	X	X	7	0.5	X		X				
Least sandpiper	X			X		2	0.2	X						
Least tern		X	X			5	0.4	X	X	X				
Lesser yellowlegs	X			X	X	15	1.1	X						
Little blue heron	X	X	X	X		6	0.5	X	X					
Mourning dove		X	X		X	7	0.5	X	X				X	

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Continuation of **Table 3.**

Taxa	Season		Site			Count	Relative Abundance (%)	Dominant Habitat Type							
	Spring	Summer	1	2	3			EM	OW	SL	MF	WL	GL		
Neotropic Cormorant	X	X	X	X	X	9	0.7	X	X						
Northern cardinal	X	X	X	X		5	0.4	X					X	X	
Northern rough-winged swallow		X		X		3	0.2		X						
Orchard oriole	X		X			5	0.4						X		
Osprey		X	X			1	0.1		X						
Pectoral sandpiper	X			X		1	0.1	X							
Purple gallinule	X		X			3	0.2	X							
Red-bellied woodpecker		X	X			1	0.1						X		
Red-winged blackbird	X	X	X	X	X	182	13.8	X							X
Roseate spoonbill	X	X	X		X	8	0.6	X	X						
Royal tern		X	X			2	0.2					X			
Sandwich tern	X			X		4	0.3		X						
Sedge wren	X			X		1	0.1	X							
Semipalmated plover		X	X			3	0.2					X			
Short-billed dowitcher		X	X			4	0.3					X			
Snowy egret	X	X	X	X	X	54	4.1	X	X	X	X				
Sora	X			X		1	0.1	X							
Tree swallow	X		X	X		23	1.8	X							
Tricolored heron	X	X	X	X	X	25	1.9	X	X						
Turkey vulture	X	X	X	X	X	7	0.5	X	X						
Western sandpiper	X		X			3	0.2	X							
White-faced ibis	X		X	X		3	0.2	X	X						
Willet	X	X	X		X	6	0.5	X	X		X				
Yellow-crowned night heron	X	X	X			3	0.2	X					X		

In total, 1,315 individuals represented by 56 species were observed during spring and summer sampling events. The avian community was typical of an ecosystem presenting a mosaic of saltwater influenced marsh, shoreline, and mudflat habitat (Foster et al. 2009). All sites were characterized by an abundance of shorebird and/or migratory bird species, with relatively high species overlap between sites. The most common species observed was barn swallow (25.8%; *Hirundo rustica*), followed by cattle egret (15.1%; *Bubulcus ibis*), and red-winged blackbird (13.8%; *Agelaius phoeniceus*). These three species were present across all three sites and accounted for 55% of all species observations. The remarkably high representation of migratory birds such as barn swallow is most likely an artifact of seasonality wherein isolated sampling events incidentally capture higher numbers of migratory birds, subsequently leading to an over-representation of these species. While all observed avian behavior was recorded during surveys, it was not included in this report. This is because the high degree of variation in behavior across species, habitat type, and season prevented statistical analysis given the current sample size.

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The most notable species, the cryptic and imperiled black rail (*Laterallus jamaicensis*), was observed during the spring at Site 3 within emergent marsh. While marsh-associated species were present at Site 1, this site was characterized by an increased prevalence of passerines (sparrows and similar small birds) due to the presence of nearby woodland and grassland areas. Site 1 exhibited both the highest taxa richness (43) and Shannon diversity (4.23) (**Table 4**). The highest individual count (653) and second highest taxa richness (36) was observed at Site 2. This was largely driven by the higher numbers of barn swallow and cattle egret present. Marsh-associated species were generally observed across all three sites, however, among the more saline areas surveyed (Sites 2 and 3), species counts, taxa richness, and relative abundance were considerably higher at Site 2. Point count locations at Site 3 were commonly subject to higher winds and more wave action from the upper open-water portion of San Antonio Bay during surveys. Conversely, Site 2 was characterized by a more channelized wind-protected marsh habitat mosaic which likely provides the avian community with greater refuge for foraging and breeding activities.

Table 4. Avian count (#), taxa richness (#), relative abundance (%), and Shannon diversity by site.

Site	Count	Taxa Richness	Relative Abundance (%)	Shannon Diversity
1	535	43	40.7	4.23
2	653	36	49.7	3.09
3	127	25	9.7	4.04

3.3 Faunal Community

3.3.1 Habitat and Environmental Conditions

Throw-trap sampling was conducted in SB and ME habitats at three sites to quantify the Delta faunal community. The data collected was used to analyze spatiotemporal trends in environmental variation and faunal assemblage structure, examine influences on biological productivity, and assess species habitat associations. Principal components axes I and II explained 36.0% of the variation in environmental parameters among 150 throw-trap samples in 2019 and 2021. Axis I explained 18.3% of the variation and described a water chemistry and tidal gradient. Strongest loadings for PC axis I were median tide level (-0.94), salinity (-0.65), pH (-0.50), and water temperature (0.91). Axis II explained 17.7% of the variation and mostly represented a vegetation gradient. Strongest loadings were pH (-0.50), duckweed (0.68), and water hyacinth (0.76) (**Figure 5**).

Among each site-season-year combination, mean sample scores from the PCA distinguished two different sub-groups along PC I and PC II. Principal Component I displayed seasonal differences in water chemistry and tide level across seasons and within sampling events (**Figure 5**). Median water temperature was higher in summer (28.35-32.8 °C) than fall (20.57-24.03 °C). Median salinity concentration was higher in the fall at Site 2 (5.1 ppt) and Site 3 (8.6 ppt) and was similar across seasons at Site 1 (~0.3 ppt). Moreover, median tide level was higher during fall sampling in 2019 (0.24-0.37 m) and 2021 (0.24-0.37 m). In summer, water level was higher in 2019 (0.12-0.15 m) compared to 2021 (0.03-0.10 m).

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Principal Component II distinguish Site 1 from Site 2 and Site 3 based on vegetation composition (**Figure 5**). Site 1 ME habitats had high coverage of water hyacinth (median = 70.0%) and duckweed (family: Lemnoidae) presence, both of which didn't occur at Site 2 and Site 3. Vegetation taxa at these two lower sites were not strongly association with PC I or PC II. During summer surveys, when median tide level was lower, vegetation was minimally available to sample within ME habitats.

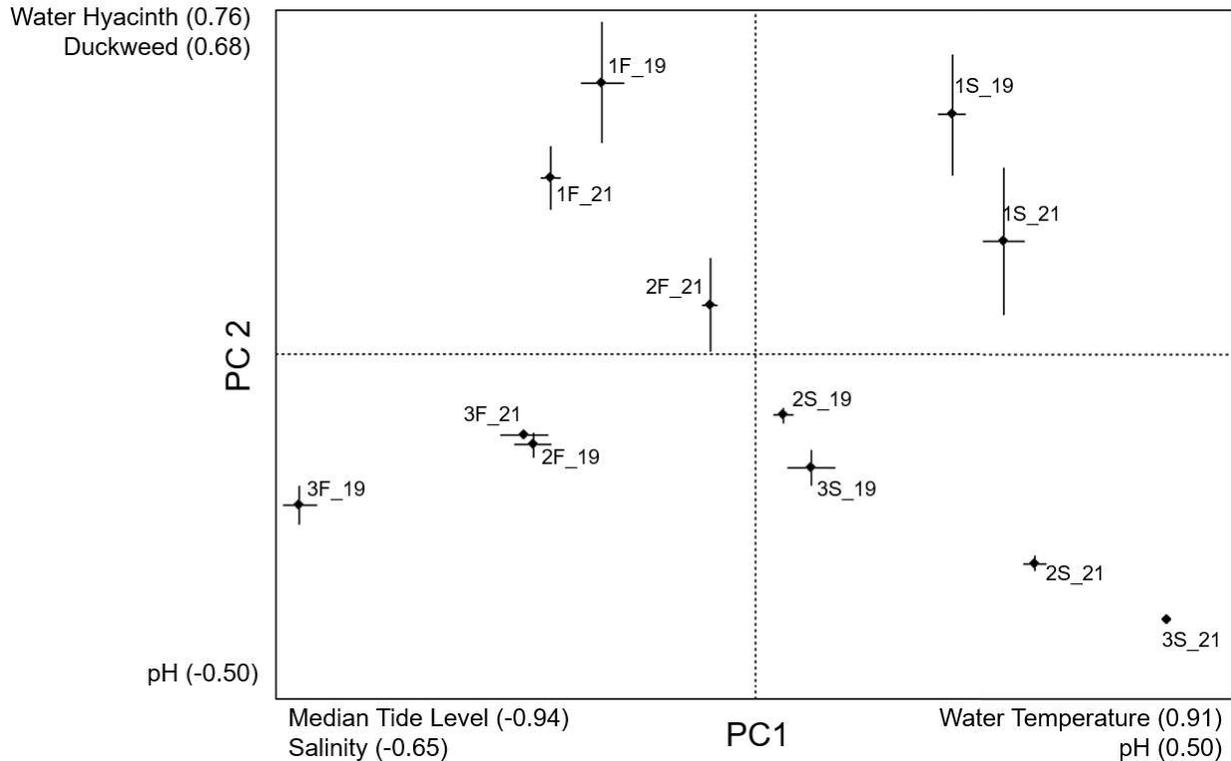


Figure 5. Principal components analysis displaying environmental variation among sites during seasonal sampling in the Guadalupe Delta in 2019 and 2021 based on mean (\pm standard error) PC scores. Only environmental parameters with a stronger correlation ($r \geq 0.50$).

Results from 10-fold cross-validation showed the MARS model accurately predicted salinity concentration and was able to generalize to new data ($R^2 = 0.93 \pm 0.01$). Among predictors, relative importance was higher for distance from freshwater inflow sources (100.0%) and median monthly discharge (61.6%), followed by median tide level (36.9%). Partial dependence plots showed that salinity was positively associated with distance and tide level. Salinity concentration increased with increasing distance from freshwater inflow sources and as median tide level increased from 0.03 to 0.37 m. In contrast, salinity concentration was negatively associated with discharge, decreasing as median monthly discharge increased from 20 to 74 cms (**Figure 6**).

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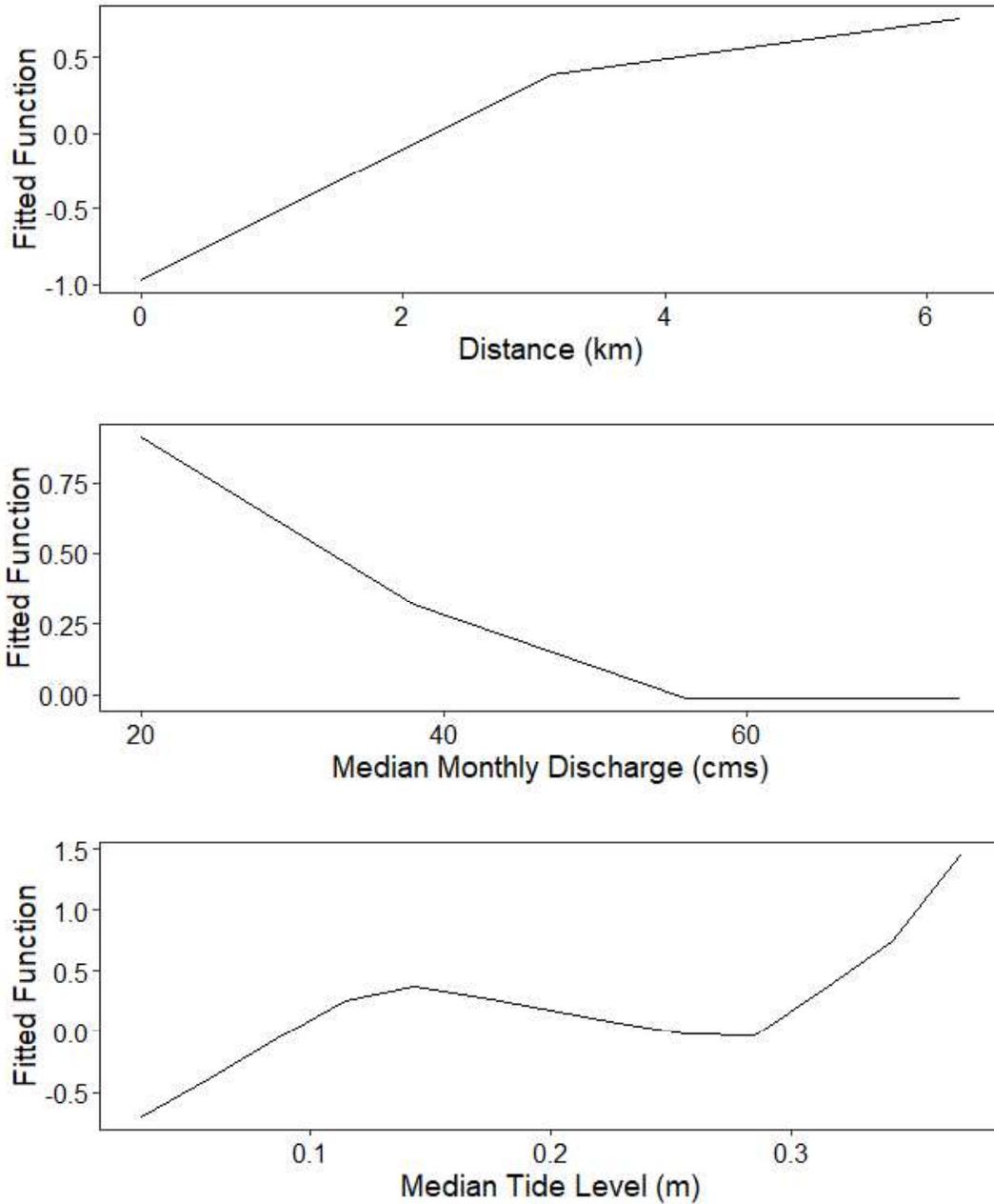


Figure 6. Multivariate adaptive regression splines partial dependence plots displaying functional relationships of salinity concentration with distance from freshwater inflow sources, median monthly discharge, and median tide level.

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3.3.2 Faunal Abundance

A total of 6,279 individuals represented by 21 families and 36 taxa were observed during 2021 sampling. Overall, crustaceans (54.1%) and fishes (45.9%) were the most abundant faunal groups. Mollusks (< 0.1%) were rare and characterized by a single Atlantic rangia (*Rangia cuneata*). Fishes were the most taxa rich, represented 15 families and 27 taxa. Among life-history guilds, estuarine-resident taxa (69.4%) were more abundant than estuarine-dependent (29.3%) and freshwater (1.3%) taxa. Moreover, estuarine-residents were mostly crustaceans (67.8%) and estuarine-dependents were dominated by fishes (78.1%) (**Table 5**).

Table 5. Number of Families, taxa richness, counts (#), and relative abundance (%) of guilds among faunal groups at all sites. Species guilds include freshwater (FW), estuarine-resident (ER), and estuarine-dependent (ED).

Faunal Group	Families	Taxa Richness	FW		ER		ED		Total	
			#	%	#	%	#	%	#	%
Mollusks	1	1	0	0.0	1	0.0	0	0.0	1	0.0
Crustaceans	5	8	39	46.4	2954	67.8	402	21.9	3395	54.1
Fishes	15	27	45	53.6	1402	32.2	1436	78.1	2883	45.9
Total	21	36	84	1.3	4357	69.4	1838	29.3	6279	

Taxa richness in 2021 was generally higher in summer (15-20) than fall (11-16). Among sites, taxa richness was more variable at Site 1 (12-20) compared to Site 2 (16-17) and Site 3 (11-15). Taxa evenness did not vary within sites and was similar between sites (0.4-0.6). Seasonal differences in diversity also varied minimally within sites. Diversity was higher at Site 2 (1.7), compared to Site 1 (1.1-1.3) and Site 3 (1.3). Among life history guilds, freshwater taxa were the least represented across sites (0.0-7.1%). Estuarine-residents typically dominated faunal assemblages and abundances of estuarine-dependents varied based on site and season. Estuarine dependent taxa were more abundant in the summer at Site 1 (37.0%). In contrast, estuarine-dependents were more abundant in the fall at Site 2 (27.3%) and Site 3 (55.7%) (**Table 6**).

Grass shrimp was the most dominate taxa across all sites and seasons (38.6-72.0%), while other crustaceans were uncommon at Sites 1 (0.4-8.1%) and Site 2 (1.6-3.1%). At Site 3, estuarine mudcrab (*Rhithropanopeus harrisi*; 30.9%) and blue crab (*Callinectes sapidus*; 41.0%) were the second most abundant taxa in summer and fall, respectively. Most fishes represented <10% of assemblages and the majority of individuals were characterized by several taxa. Gulf menhaden (*Brevoortia patronus*; 36.5%) was the second most abundant taxa in summer at Site 1. At Site 2, bay anchovy (*Anchoa mitchilli*; 24.9%) ranked second in abundance in fall and sailfin molly (*Poecilia latipinna*; 12.4-18.3%) was more represented relative to other fish taxa across seasons. Fishes in total were much less represented at Site 3 (8.3-11.2%).

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Table 6. Counts (#), relative abundance (%), taxa richness, taxa evenness, and Shannon diversity of faunal communities among sites and seasons. Total counts and relative abundance are also summarized across freshwater (FW), estuarine-resident (ER), and estuarine-dependent (ED) life-history guilds.

Family	Taxa	Guild	Site 1				Site 2				Site 3			
			Summer		Fall		Summer		Fall		Summer		Fall	
			#	%	#	%	#	%	#	%	#	%	#	%
Mollusks														
Mactridae	Atlantic rangia	ER	0	0.0	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0
Crustaceans														
Cambaridae	Crayfish	FW	0	0.0	20	6.8	0	0.0	19	0.9	0	0.0	0	0.0
Palaemonidae	Big claw river shrimp	ED	0	0.0	0	0.0	0	0.0	0	0.0	2	0.4	0	0.0
	Grass shrimp	ER	919	49.5	213	72.0	567	49.3	821	38.6	302	55.6	132	43.3
Panopeidae	Estuarine mudcrab	ED	2	0.1	0	0.0	14	1.2	6	0.3	168	30.9	10	3.3
Penaeidae	Penaeid shrimp spp.	ED	0	0.0	0	0.0	0	0.0	0	0.0	6	1.1	0	0.0
	Brown shrimp	ED	0	0.0	0	0.0	0	0.0	1	0.0	0	0.0	1	0.3
	White shrimp	ED	0	0.0	0	0.0	0	0.0	8	0.4	0	0.0	11	3.6
Portunidae	Blue crab	ED	5	0.3	4	1.4	4	0.3	31	1.5	4	0.7	125	41.0
Fishes														
Achiridae	Hogchoker	ER	0	0.0	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0
Atherinopsidae	Mississippi silverside	ER	5	0.3	2	0.7	124	10.8	30	1.4	12	2.2	0	0.0
Centrarchidae	Bluegill	FW	25	1.3	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0
	Sunfish spp.	FW	5	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Redspotted sunfish	FW	3	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Centropomidae	Snook	ED	0	0.0	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0
Clupeidae	Gizzard shad	FW	1	0.1	0	0.0	3	0.3	0	0.0	0	0.0	0	0.0
	Gulf menhaden	ED	677	36.5	11	3.7	91	7.9	4	0.2	0	0.0	7	2.3
	Threadfin shad	FW	5	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Cyprinodontidae	Sheepshead minnow	ER	2	0.1	0	0.0	65	5.7	16	0.8	8	1.5	0	0.0
Engraulidae	Bay anchovy	ED	3	0.2	13	4.4	75	6.5	531	24.9	1	0.2	4	1.3
Fundulidae	Bayou killifish	ER	8	0.4	0	0.0	0	0.0	80	3.8	0	0.0	1	0.3
	Bluefin killifish	ER	29	1.6	1	0.3	2	0.2	2	0.1	0	0.0	0	0.0
	Golden topminnow	ER	31	1.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Gulf killifish	ER	3	0.2	0	0.0	5	0.4	0	0.0	7	1.3	0	0.0
	Rainwater killifish	ER	0	0.0	0	0.0	0	0.0	1	0.0	1	0.2	0	0.0
	Skilletfish	ED	0	0.0	0	0.0	0	0.0	0	0.0	2	0.4	0	0.0
Gobiidae	Code goby	ER	1	0.1	0	0.0	8	0.7	0	0.0	13	2.4	0	0.0
	Highfin goby	ER	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.3
	Naked goby	ER	0	0.0	1	0.3	6	0.5	20	0.9	15	2.8	0	0.0
Ictaluridae	Blue catfish	FW	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0
Loricariidae	Vermiculated sailfin catfish	FW	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Poeciliidae	Sailfin molly	ER	25	1.3	0	0.0	143	12.4	390	18.3	0	0.0	0	0.0
	Western mosquitofish	ER	106	5.7	28	9.5	38	3.3	169	7.9	1	0.2	1	0.3
Sciaenidae	Black drum	ED	0	0.0	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0
	Star drum	ED	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	12	3.9
Sparidae	Pinfish	ED	0	0.0	0	0.0	3	0.3	0	0.0	0	0.0	0	0.0
Total			1856		296		1150		2129		543		305	
FW			40	2.2	21	7.1	3	0.3	19	0.9	1	0.2	0	0.0
ER			1129	60.8	246	83.1	959	83.4	1529	71.8	359	66.1	135	44.3
ED			687	37.0	29	9.8	188	16.3	581	27.3	183	33.7	170	55.7
Taxa Richness			20		12		17		16		15		11	
Taxa Evenness			0.4		0.4		0.6		0.6		0.5		0.5	
Shannon Diversity			1.3		1.1		1.7		1.7		1.3		1.3	

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Taxa only observed at Site 1 included hogchoker (*Trinectes maculatus*), redspotted sunfish (*Lepomis miniatus*), snook (*Centropomus undecimalis*), threadfin shad (*Dorosoma petenense*), golden topminnow (*Fundulus chrysotus*), and vermiculated sailfin catfish (*Pterygoplichthys disjunctivus*). Taxa unique to Site 2 included Atlantic rangia, black drum (*Pogonias cromis*), and pinfish (*Lagodon rhomboides*). Lastly, big claw river shrimp, skilletfish (*Gobiesox strumosus*), highfin goby (*Gobionellus oceanicus*), blue catfish (*Ictalurus furcatus*), and star drum (*Stellifer lanceolatus*) were only observed at Site 3 (**Table 6**).

The LMM model supported meaningful differences in total density among site ($F = 26.20$, $P < 0.001$), habitats ($F = 109.63$, $P < 0.001$), and site-season interactions ($F = 5.02$, $P = 0.008$). Strong support was also suggested for site-season-habitat interactions ($F = 12.51$, $P < 0.001$); therefore, data interpretations were restricted to this three-way interactive effect. In general, mean total density was higher in ME than SB habitats among each site-season combination. Seasonal differences in total mean density varied more at Site 1 and Site 2, but were similar for Site 3. Mean density in SB habitats was similar across sites in summer (6.2-71.5 ind./m²) and fall (6.5-44.6 ind./m²). In summer, mean total density in ME habitats decreased from Site 1 (187.6 ind./m²) to Site 3 (70.2 ind./m²). Mean total density in ME habitats during fall was substantially higher at Site 2 (314.8 ind./m²) than Site 1 (45.9 ind./m²) and Site 3 (67.6 ind./m²) (**Figure 7**).

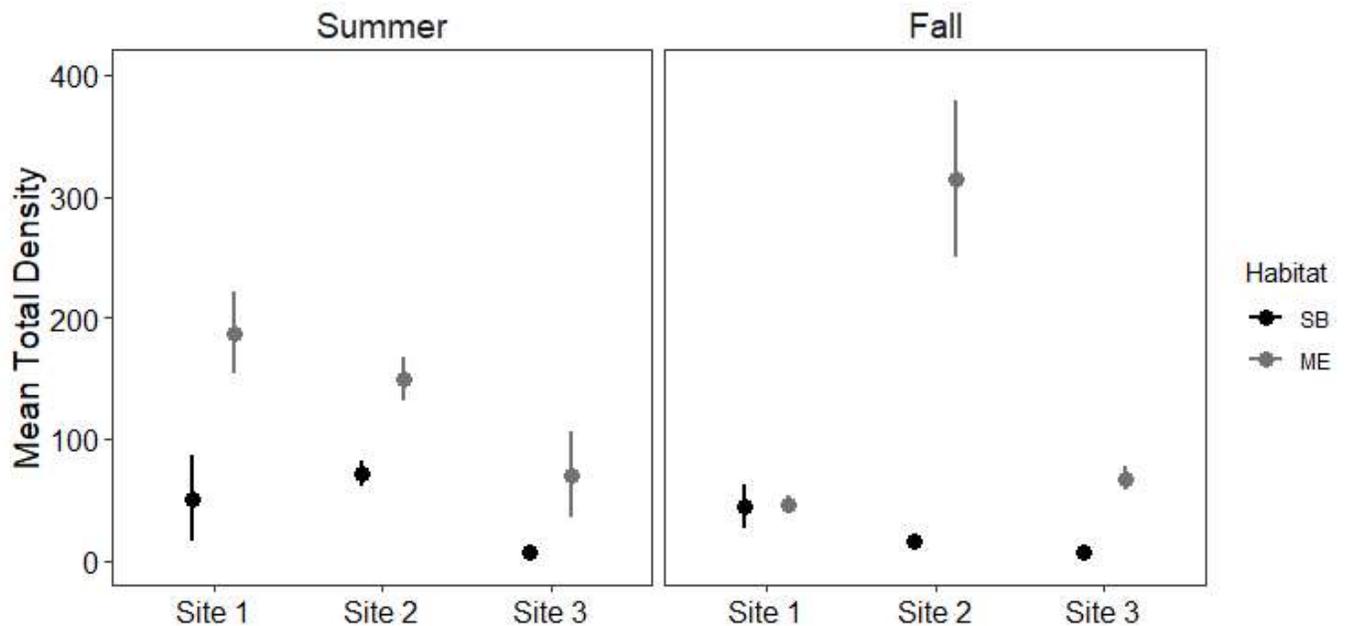


Figure 7. Mean total density per throw-trap sample among each site-season-habitat combination. Error bars represent ± 1 standard error from the mean.

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3.3.3 Assemblage Structure

Statistical procedures used to test for differences in assemblage structure were based on 18 taxa and 141 sites from sampling in 2019 and 2021. Results from ANOSIM strongly supported meaningful differences in assemblage structure for main effects site ($F = 13.16$, $P < 0.001$) and season ($F = 4.09$, $P < 0.001$). Interactive effects between site and season were also well supported ($F = 2.74$, $P = 0.001$). Based on this, independent evaluations for each main effect were not conducted. Hierarchical cluster analysis displaying mean dissimilarities between site-season combinations showed assemblage structure was more similar at Site 1 and Site 2 compared to Site 3. Within sites, dissimilarities between seasons were larger at Site 2 and 3. Moreover, the reversed leaf segment for Site 2-fall in the dendrogram suggests that assemblage structure was more heterogeneous within this group (**Figure 8**).

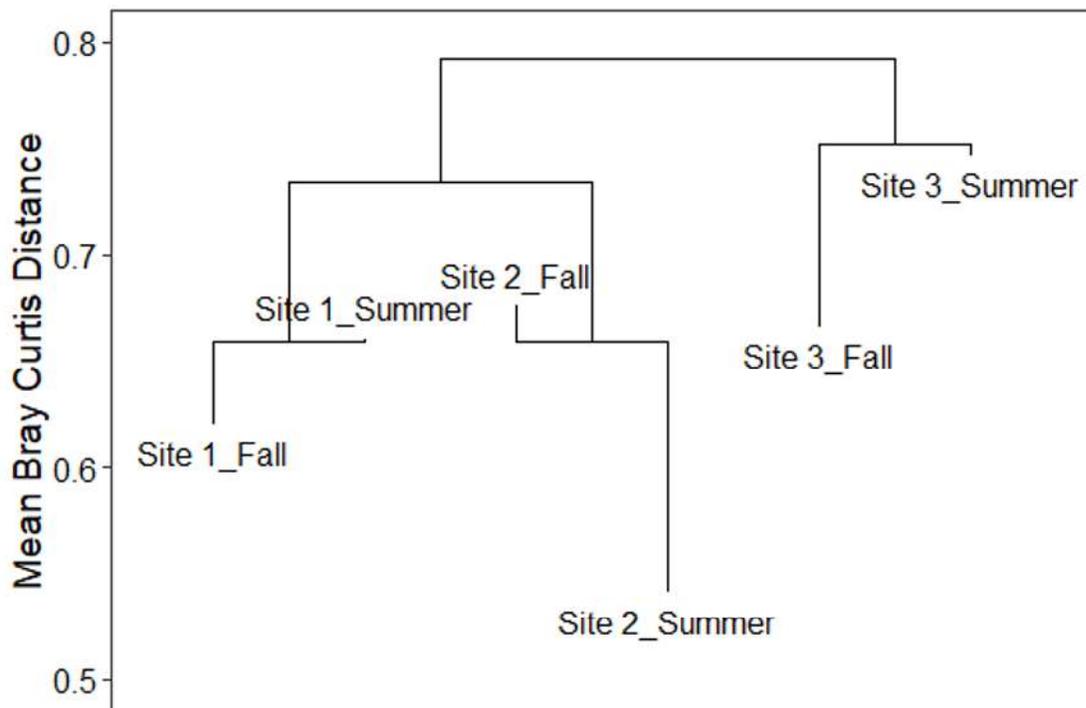


Figure 8. Dendrogram displaying dissimilarities in faunal assemblage structure grouped by each site-season combination via hierarchical cluster analysis using mean Bray Curtis distance. Reversed leaf segments demonstrate heterogeneous assemblage structure within a particular group.

Differences in assemblage structure between Site 1 and Site 3 were mainly due to percent dissimilarities in grass shrimp (24.8-28.7%), estuarine-dependent crustaceans (7.2-22.2%), and naked goby (*Gobiosoma bosc*; 7.93-12.31%). Assemblage dissimilarities between Site 2 and Site 3 groups were mostly contributed by grass shrimp (16.6-21.0%) and bay anchovy (17.9-20.1%). As stated previously, estuarine-dependent taxa were more abundant at Site 2 and Site 3 in fall, during which, bay anchovy, blue crab, and white shrimp (*Litopenaeus setiferus*) accounted for about 30% of assemblage differences. Among seasons, Site 2 differences were contributed by grass shrimp (20.1%), bay anchovy (16.1%), sailfin

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molly (10.73%), and Mississippi silverside (*Menidia audens*; 9.22%). Site 3 differences were mostly due to dissimilarities of grass shrimp (25.6%), blue crab (17.7%), bay anchovy (13.4%), white shrimp (11.8%), and estuarine mudcrab (10.4%).

3.3.4 Habitat Associations

From the CCA analysis, Axes I and II explained 43.30% of the variability in the Guadalupe Delta biotic assemblage from environmental parameters, site, and season. Within the explained variation across all eigen, pure effects were highest for environmental parameters (31.4%), marginal for site (14.5%) and habitat type (12.8%), and low for season (5.1%). Pure effects of two- and three-way interactions explained 25.6% of assemblage variation. Habitat parameters and sites strongly associated with CCA Axis I included salinity (0.70), Site 1 (-0.64), water hyacinth (-0.60), duckweed (-0.56), and ME (-0.54). Associations with CCA Axis II were weaker ($r < 0.50$), but relatively stronger for Site 2 (0.41), ME (-0.48), phragmites (-0.45), and spartina (-0.42; **Figure 9**).

Most species were strongly associated with ME habitats except for bay anchovy and gulf menhaden. Species most prevalent at Site 1 were associated with ME habitats that had greater coverages in water hyacinth and lower salinities, which included bluefin killifish (*Lucania goodei*), golden topminnow, crayfish (*Procambarus* sp.), and chain pipefish (*Syngnathus louisianae*). Bay anchovy and gulf menhaden were associated with higher salinities. White shrimp, brown shrimp (*Farfantepenaeus aztecus*), and blue crab were also strongly associated with higher salinities, as well as with ME habitats with greater coverages of phragmites or spartina. Taxa that with weaker associations among CCA covariates included grass shrimp, Mississippi silverside, naked goby, sailfin molly, and sheepshead minnow (*Cyprinodon variegatus*; **Figure 9**).

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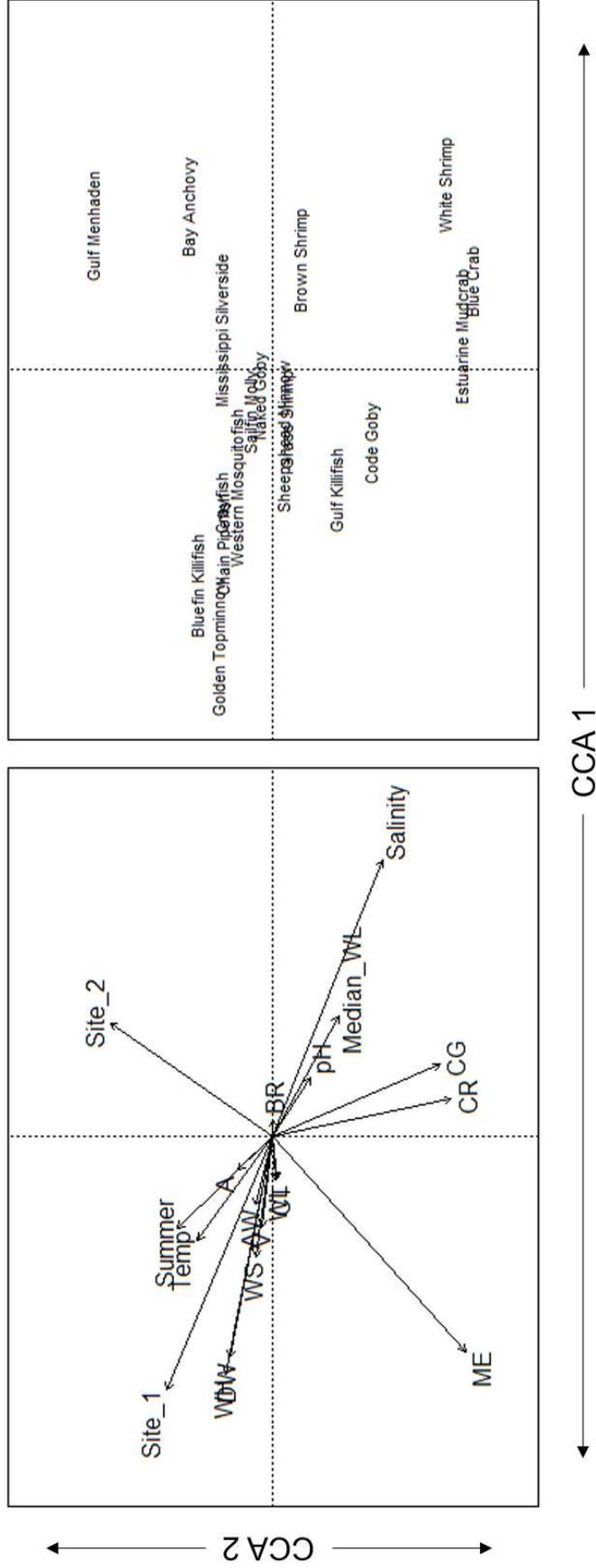


Figure 9. Canonical correspondence analysis bi-plots for environmental parameters, sites, and seasons (left plot) and faunal taxa (right plot). Median_WL denotes median tide level. Vegetation abbreviations for the environmental conditions bi-plot include alligatorweed [AW (*Alternanthera philoxeroides*)], coontail [CT (*Ceratophyllum demersum*)], water lettuce [WL (*Pistia stratiotes*)], water stargrass [SG (*Heteranthera dubia*)], common reed [R (*Phragmites australis*)], water hyacinth [WH (*Eichhornia crassipes*)], smooth cordgrass [CG (*Spartina alterniflora*)], saltmarsh bulrush [BR (*Scirpus spp.*)], duckweed [DW (*Lemna minor*)], and vine [V (*Mikania scandens* and *Vigna luteola*, in aggregate)].

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4.0 Discussion

4.1 Habitat and Environmental Variation

Environmental conditions observed during 2019 and 2021 sampling efforts in the Guadalupe River Delta demonstrated the highly complex nature of estuarine systems (Akin et al. 2003; Nichols et al. 2010). Spatiotemporal trends in physiochemical parameters displayed strong environmental gradients with increased site distance from freshwater inflow sources, as well as high levels of intra- and inter-seasonal variation within sites. PCA results showed discrete difference in vegetation composition within ME habitats for both sampling years. ME habitats at Site 1 were generally dominated by water hyacinth, while Site 2 and Site 3 habitats typically contained bulrush or common reed. That being said, vegetation taxa present at the two lower sites were not strongly associated with either PC axis, which appears to be best explained by median tide level during sampling. ME habitats with high vegetation cover were available for aquatic biota at Site 1 across all tide levels observed. In contrast, vegetation cover at the two lower sites was minimally available or unavailable at lower tide levels, suggesting that tidal regimes have a strong impact on the physical condition of ME habitats in areas farther away from freshwater inflow sources (**Figure 5**).

The PCA also demonstrated a strong salinity gradient within the Delta. Salinity concentrations at Site 1 were similar across all sampling events and was characterized as a freshwater environment (salinity: 0.0-0.5 ppt). Differences in mean PC scores within sites 2 and 3 support that salinity concentrations were highly variable over the study period, generally representing oligohaline (0.5-5.0 ppt) or mesohaline (salinity 5.0-18.0 ppt) environments. Moreover, sites 2 and 3 exhibited polyhaline conditions (salinity: 18.0-30.0 ppt), further supporting that salinity dynamics in these areas are highly variable (**Figure 5**). The MARS model was able to accurately predict trends in salinity and provides further evidence that supports salinity levels increased with increasing distance from freshwater inflows. Partial dependence plots show that variation in salinity concentrations at the two lower sites depend on freshwater inflows and tide level. Data also suggests that freshwater inflows and tide level have an interactive effect on salinity. For example, maximum salinity concentrations during faunal sampling occurred at Site 3 in 2019 (14.99 ppt), which was when median monthly discharge was lowest (20 cms) and median tide level was highest (0.37 m). (**Figure 6**). Longitudinal salinity gradients within estuarine systems are a well-documented phenomena and trends displayed by the MARS model further demonstrate that salinity levels are affected by freshwater inflow and tidal regimes (Day et al. 1989; Longley 1994; Beseres-Pollack et al. 2011; Palmer et al. 2011).

4.2 Vegetation, Avian, and Faunal Communities

Vegetation community compositions observed in 2021 were generally similar to 2019 surveys. Despite the increase in species diversity, vegetation assemblages exhibited similar trends, with sampling plots represented similarly across sites in terms of ratios between freshwater species and facultative halophytes. Dominance was captured differently between 2019 and 2021 sampling, including both new dominant species and previously

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unreported species. Differences in dominance may be a function of heterogeneity in sampling methodology and the increased vegetative sampling effort. Conversely, this may also suggest that marsh vegetation communities within the Delta fluctuate largely on both an inter-seasonal and inter-annual basis. Regardless, this work in conjunction with 2019 sampling supports the presence of a distinct longitudinal gradient of species composition following a pattern of long-term salinity conditions.

Avian community sampling was added to the 2021 data collection effort and provided a baseline for the assessment of future fluctuations in avian abundance, diversity, and habitat associations in the Delta. The avian diversity and presence of imperiled species (i.e., black rail) within marsh habitat indicates how important these areas are to sustaining robust communities. The salinity gradient and concomitant heterogeneity in vegetation and faunal community compositions likely affect avian abundance and drive avian distribution within the Delta (Armitrage et al 2007; VanDusen 2012).

Estuarine-residents dominated faunal assemblages across sites during both years and grass shrimp was the most abundant taxa (**Table 6**; BIO-WEST 2020), which is typical in Texas coastal marsh environments (Longley 1994; Ley et al. 1999; Akin et al. 2003). Estuarine-dependent taxa were also consistently most represented at Site 2 and Site 3, with the exception of gulf menhaden being the second most abundant taxa at Site 2 in summer 2021. Among years, freshwater taxa were the least abundant guild across sites and most taxa were exclusively found at Site 1. Lastly, estuarine-dependent taxa collected in 2021 that were not observed in 2019 included big claw river shrimp, star drum, black drum, and skiliffish (**Table 6**; BIO-WEST 2020), suggesting that the sampling time period within seasons may affect observations, though detectability or specific environmental factors unaccounted for may also influence taxa occurrence.

Results from this study suggest that patterns in faunal community indices, assemblage structure, and total density were mostly associated with the trends in environmental conditions described in section 4.1. Taxa richness did not show any consistent spatial or seasonal trends. Faunal diversity also didn't display seasonality, instead showing spatial differences, with higher levels of diversity at Site 2 (**Table 6**). Moreover, the strongly supported three-way interactive effect displayed by the LMM suggests that patterns in total density are highly variable and complex, depending on season, site location, and habitat type. Total density was generally higher in the summer within ME habitats, with the exception of Site 2, where density was substantially higher in the fall within ME habitats (**Figure 7**). Trends in diversity and total density provides supporting evidence that biological productivity was highest at Site 2. Higher productivity can be partially explained by mesohaline conditions that commonly occur at Site 2 (**Figure 6**), which are known to be more productive environments within estuarine systems, harboring a wide variety of organisms (Longley 1994). Habitat associations for the most abundant fauna at Site 2 helps demonstrate this phenomenon, which included estuarine-resident taxa with no strong habitat associations (e.g., grass shrimp, sailfin molly) and estuarine-dependent taxa (e.g., bay anchovy) more associated with higher salinities (**Figure 9**).

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Despite stronger associations between salinity and biological productivity shown at Site 2, other environmental factors also appear to influence faunal trends. Total density was generally higher in ME habitats at Site 1 compared to Site 3 (**Figure 7**), suggesting that the suitability of physical habitat conditions may also affect productivity. Vegetation cover was generally lower in ME habitats at Site 3 compared to the other sites when tide levels were similar and never exceeded 50% total coverage. Emergent vegetation is important refuge habitat that provides structure for small-bodied organisms (Rozas & Minello 1997; Castellanos & Rozas 2001), suggesting that biological productivity at Site 3 may be lower due to sub-optimal ME conditions with less cover. Despite lower productivity observed at Site 3, patterns in assemblage structure show that this area within the Delta harbors a more distinct faunal community. Dissimilarities in assemblage structure at Site 3 compared to others can best be explained by greater abundances of estuarine mudcrab, blue crab, and penaeid shrimp, which suggests Site 3 is an important habitat unit for estuarine-dependent crustaceans. Higher abundance of estuarine-dependent taxa also explains differences in assemblage structure between Site 1 and Site 2 (**Figure 8**). Greater abundances of estuarine-dependent taxa at Site 2 and Site 3 aligns with their positive associations with salinity observed during this study and previous research that also found species that use estuaries for part of their life cycle are more prevalent in oligohaline and mesohaline environments (Akin et al. 2003).

Interestingly, high abundances of gulf menhaden were observed at Site 1 in summer 2021, which was the only sampling event where an estuarine dependent species was prevalent. Greater abundance of gulf menhaden may be explained by higher median monthly discharge in summer 2021 (**Table 6; Figure 6**). Past studies have also observed higher abundances of estuarine-dependent species in upper reaches of estuaries during periods of increased freshwater inflow, supporting that in addition to salinity, other environmental factors may also be affecting faunal assemblage patterns (Grimes & Kingsford 1996; Akin et al. 2003; Gonzales et al. 2021). Moreover, results of ANOSIM indicated strong seasonal differences in assemblage structure within sites, most likely due to migratory patterns and reproductive timing of estuarine-dependent taxa (Zimmerman & Minello 1984; Akin et al. 2003). At Site 3 in 2021 for example, estuarine mudcrab and blue crab were more abundant during their peak spawning seasons, which are presumed to be in summer and fall, respectively (Hood 1962; Ward 2012).

5.0 Summary

In summary, the vegetation communities sampled in 2021 exhibited a gradient of freshwater-associated to saltwater-associated species assemblage similar to that reported in 2019. Areas closer to the freshwater influence of the Guadalupe River were characterized by emergent freshwater marsh plants and non-native species, with more saline lower sites generally dominated by facultative halophytes. The expanded scope of this study in 2021 likely contributed to the increased vegetative species overlap and diversity observed among sites. However, this effort represents a more robust baseline of the vegetation communities for future seasonal investigations.

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The avian community assessment demonstrated the high-level of diversity present within the Delta. This estuary provides a wide array of habitat types which support migratory and resident species from a variety of foraging guilds. Further investigation into inter-seasonal and inter-annual trends (including quarterly sampling) would provide a more accurate evaluation of the temporal and spatial distribution of avian species within the Delta. These studies could further refine avian species occurrence, abundance, distribution, seasonality, and habitat associations by strategically deploying bio-acoustic recorders and extending monitoring across four seasons.

Results from faunal sampling demonstrated the utility of a multi-year dataset and the importance of repeated sampling to capture interannual and seasonal variation in environmental conditions within the Delta. The salinity regime represented a longitudinal gradient and salinity concentrations at the lower sites were dependent on freshwater inflows and tide level. Availability of vegetation cover within ME habitats across tide levels most likely explains higher faunal densities at Site 1 and Site 2 compared to Site 3. Moreover, higher salinity levels that facilitated mesohaline environments was likely why faunal density was substantially higher at Site 2 in the fall compared to other sites. Salinity regimes also played a role in structuring dissimilar faunal assemblages across sites, while seasonality influenced variability in assemblages within sites.

Statistical analyses used for this study supported previous findings, while also providing new insights into potential mechanisms driving biotic assemblages in the Guadalupe Delta. A future objective of this project that would assist in guiding freshwater inflow criteria evaluation and validation, and/or refinement is to construct predictive models to quantify variation in biotic assemblages based on freshwater inflows dynamics. Previous studies that used a variety of statistical models to predict trends in estuarine fish communities demonstrated that predictive performance was highly variable and depended on the study system, community or species metric that was examined, ecological characteristics of the study species, as well as the modeling approach used (Francis et al. 2005; França et al. 2011; Francis et al. 2011; França & Cabral 2019). Preliminary regression-based models were developed during initial data exploration associated with this report to assess whether faunal trends could be estimated accurately, though exhibited low predictive performance ($R^2 < 0.50$), suggesting that a larger dataset is likely needed or that other factors associated with the data (e.g., structure) need to be accounted for. The high variability implicit with estuarine systems makes it difficult to identify functional relationships when sampling replicates are inadequate (Montagna et al. 2013). Therefore, multiple faunal metrics and modeling approaches will be explored for future development of predictive models when larger sample sizes are available. Faunal sampling across four seasons in future studies could provide further insight into the relationship between seasonal salinity variability and the behavior of transitory delta species.

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