The Effects of Rollover Pass Closure on Tidal Wetland Plant Assemblages and Associated Fauna
22-045-012-D109

Final Report
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Project Background

Rollover Pass was a constructed channel on the Bolivar Peninsula that linked the Gulf of Mexico with Rollover Bay and East Bay and was originally opened by Texas Game and Fish Commission in 1954 to increase bay water salinity and provide access for marine fish to East Bay. Although Rollover Pass was a popular location for recreational fishing, the Pass contributed to accelerating erosion and exacerbated hurricane damage to adjacent residential and commercial developments. Further, the Pass led to loss of emergent and submerged aquatic vegetation and a shift towards more marine nekton and benthic faunal species. Rollover Pass was closed in December 2019, and in a previous project (CMP Cycle 24), Texas A&M University at Galveston (TAMUG) recorded plant, water, and nekton characteristics prior to and one year after the closure. Near the Pass, salinity declined immediately after closure. However, the responses of plant and animal assemblages will likely occur more slowly, over several years.

TAMUG used CMP Cycle 26 funds to assess longer-term (3+ years) ecosystem responses to the Pass closure. The monitoring plan is aligned with pre-closure surveys conducted by Texas Parks and Wildlife Department personnel and CMP Cycle 24 surveys by TAMUG personnel. In the current cycle, TAMUG added targeted sampling to track changes in *Phragmites australis*, a nuisance species that may proliferate in response to lower salinity. In addition, TAMUG continued to monitor changes in the salinity regime, which has implications for the recovery of freshwater marsh habitat in the area. Currently there is little information on the emergent or submerged plant communities or nekton (e.g., flounder, shrimp) resources utilizing this area of Bolivar Peninsula or East Bay; yet this information is critical to understanding whether the restored ecosystem can support the multiple threatened species (e.g., piping plover and sea turtles) that utilize this area.

This project will inform future resource management plans for this area and revisions to freshwater inflow guidance for East Galveston Bay. The collected data will determine the efficacy of the pass closure management action and will inform future management and restoration actions in the area.

Task 1 Summary: Project Planning and Data Collection

Deliverables	Due Date	Date submitted/completed
1. Map of project sites	12/31/21	Completed 12/31/21
2. TAMU animal use permit	12/31/21	Completed 12/31/21
3. Notification of initial sampling	3/31/22	Completed 4/7/22
4. Final sampling and update	12/31/22	Completed 11/8/22

Major accomplishments and findings

1. A map of the four project survey sites is included in Appendix A.
2. The PI was issued a TAMU Animal Use Permit (IACUC 2021-0119) for work on this project in May 2021.
3. For this cycle, initial sampling of all sites was conducted in March-April 2022. Sites had been previously sampled from 2019-2021 as part of an earlier funding cycle (Cycle 24). Major findings summarized across both project periods are included in the Data Analysis Report (Appendix A).
4. The final sampling was completed in November 2022. Findings are summarized in the Data Analysis Report (Appendix A). Reported data include information on emergent plants (identity and cover), aquatic vegetation (biomass), and aquatic fauna (identity and abundance). Soil samples were analyzed for salinity. Data from water salinity-temperature loggers were downloaded from a subset of sites (see Problems or Obstacles section below). Data on *Phragmites* reproductive output (seed head biomass, seed count, germination rates) were collected in lab studies.

Problems or obstacles

Field work in Fall 2022 experienced multiple delays due to weather and other logistical issues (e.g., sampling during the fall duck hunting season restricts the days/hours that we can access the site). We were eventually able to complete the field work in early November 2022.

Task 2 Summary: Data analysis

Deliverables	Due Date	Date submitted/completed
1. Data analysis report	6/30/24	Completed 6/30/24 (See Appendix A)

Major accomplishments and findings

1. The narrative in Appendix A describes the key findings and data analyses.

Problems or obstacles

Changes in personnel (e.g., the graduate student assigned to the project graduated in May 2023) delayed the processing of soil samples from the November 2022 sampling event. As a result, salinity measurements from those soils may have decreased accuracy.

Due to changes in personnel and training of new technicians on the project, sample processing and data analysis took longer than expected. As a result, we requested and were granted a no-cost extension through June 2024 to complete the final analyses, presentations, and reporting.

Task 3 Summary: Data Dissemination, Education and Outreach

Deliverables	Due Date	Date submitted/completed
1. Graduate and undergraduate students recruited	10/31/22	Completed 10/31/22
2. Notification of TAMU website launch	12/31/21	Completed 12/31/21
3. Notification of data upload to public database	6/30/24	Completed 1/10/24
4. Copies of presentations	12/31/23	Completed 1/10/23
5. Stakeholder meeting notes	6/30/24	Completed 3/13/2024

Major accomplishments and findings

1. This project provided support for four graduate students, including one (A. Barnes Rhodes) who focused on this project for her thesis research (thesis defended May 2023). The other graduate students assisted with field sampling coordinate sample processing. In addition, two undergraduate students assisted in the field with sample collection. Three conference presentations were given at regional and national scientific conferences. Two presentations were led by a graduate student (Barnes Rhodes) and included an undergraduate co-author (Starr).
2. In December 2021, the PI Armitage's institutional website was updated with a summary of project goals and an acknowledgement of the funding source: https://www.tamug.edu/armitage/Current_Projects.html. The final report will be publicly available on a GLO server.
3. The loss and failure of loggers limited the amount of water salinity data we were able to obtain and add to the public database (see Problems and Obstacles below). All reasonably reliable and available data on salinity has been uploaded to the public database (https://www.waterdatafortexas.org/coastal).
4. The research team gave three presentations on project findings at scientific conferences:

Armitage, A.R., A. Barnes, A.E. McDonald. October 2022. Delayed coastal wetland vegetation responses to a large-scale tidal restoration project on the Texas Upper Coast. Gulf Estuarine Research Society biennial meeting, Ocean Springs, MS.

Barnes, A., A. Starr, A.R. Armitage. October 2022. *Phragmites australis* fitness and morphology along a salinity gradient in the Anahuac National Wildlife Refuge. Gulf Estuarine Research Society biennial meeting, Ocean Springs, MS.

Barnes, A., A. Starr, A.R. Armitage. December 2022. *Phragmites australis* fitness and morphology along a salinity gradient in the Anahuac National Wildlife Refuge. Restore America's Estuaries 2022 Summit, New Orleans, LA.

5. PI Armitage gave a presentation to the Galveston Bay Estuary Program Monitoring & Research Subcommittee on 3/13/2024. Discussions with stakeholders identified additional potential sources of data and discussed long-term plans for continuation of the study.

Problems or obstacles

Water salinity loggers retrieved in November 2022 were heavily fouled by colonizing organisms (barnacles, oysters, algae), and data could only be retrieved from two of the four loggers. Persistently low water levels decreased the quality and quantity of data we were able to retrieve from those two loggers. We requested additional data from one site from our project partners at the Texas Parks & Wildlife Department. However, their logger also experienced failure, so we were unable to acquire additional high quality water salinity data from their logger. Accordingly, we used an alternate method to extract salinity measurements from soil samples (see Appendix A).

Task 4 Summary: Project Monitoring and Reporting

Deliverables	Due Date	Date submitted/completed
1. Quarterly progress reports and requests for reimbursement	Quarterly	Quarterly
2. Draft final report	6/15/24	6/15/24
3. Final report	6/30/24	6/30/24
4. Project closeout form	6/30/24	6/30/24

Major accomplishments and findings

1. All quarterly progress reports have been submitted.
2. The draft final report was submitted to the project manager prior to 6/15/24.
3. The revised final report was submitted to the project manager by 6/30/24.
4. The Project closeout form was submitted to the project manager by 6/30/24.

Problems or obstacles

A no-cost extension was granted to extend the project end date to 6/30/24 (See Tasks 1-3 for explanation). All tasks were completed by that end date. On occasion, turnover in support personnel delayed the submission of accurate reimbursement requests.

Appendix A: Data Analysis Report (Task 2)

Approach

<u>Study sites</u>

Rollover Pass (29.508287, -94.500271) was located on Bolivar Peninsula east of Galveston Island, allowing tidal flow into East Galveston Bay (Figure 1). To assess how the closure of the pass impacted surrounding wetland plant communities, four sites occurring along a natural salinity gradient were selected. Sites 1, 2, and 3 were located northeast of the pass along Oyster Bayou within Anahuac National Wildlife Refuge, while Site 4 was located approximately 1-km from the eastern bay-side of the Pass. Sites were accessed by airboat following appropriate safety and permitting protocols (Figure 2).

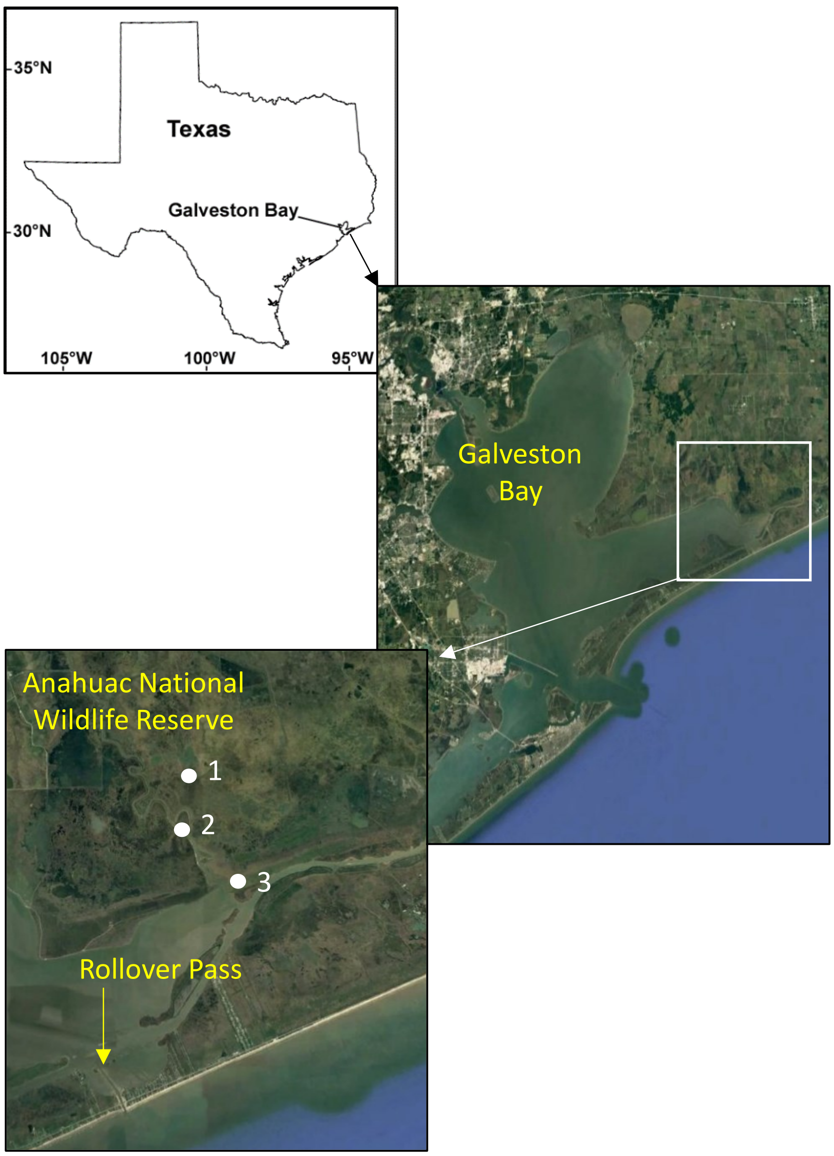

Figure 1. Area map depicting sites along tributaries within Anahuac National Wildlife Refuge for monitoring water quality, plant communities, and nekton.

Figure 2. Airboat used to access study sites.

Salinity

Salinity data were obtained from soil samples in order to assess the salinity conditions that plants were exposed to at each site. Salinity samples were collected only from sites 1-3 in spring and fall 2022 due to logistical constraints. Three soil cores (27 mm in diameter, 50 mL volume) were collected at each of the three *Phragmites* sampling stations using a 60-cc syringe (9 cores per site) (Figure 3). All soil cores (5 cm deep) were taken within 5 meters of the tidal channel. Samples were temporarily stored in a cooler for transport to the lab. Cores were weighed to determine wet weight, and then dried at 60°C for 72 hours to constant mass before recording dry weight (Pennings and Richards 1998). The difference between the wet weight and the dry weight was the amount of water (g) in the soil; this value was converted to volume (1 g = 1 ml) for salinity calculations (see below). The percent water content in the soil was determined by the following equation: $\frac{(wet\ weight - dry\ weight)}{dry\ weight} \times 100$.

The dried samples collected for soil water content were used to determine soil salinity (9 samples per site); ten grams of dried soil were subsampled for each station to use in salinity calculations. Subsamples of soil were rehydrated with 25 mL of deionized water and stored at room temperature on the shaker table in the lab for at least 12 hours to fully rehydrate the soil. Once rehydrated, soil was homogenized by physically mixing the sample with a scooping tool. A drop of the supernatant was transferred to a refractometer to record the supernatant salinity (Pennings and Richards 1998). Water volume (ml) in the soil sample (1 g = 1 ml) was determined using the above percent water content equation. Calculations were based on the weight of subsampled soil (10 g). The following equation was used to determine soil sample salinity: $(\frac{supernatant\ salinity \times 25\ ml\ water \div 10g}{water\ volume\ (ml) in\ sample \div 10g})$.

Soil salinity was analyzed with separate one-way Analysis of Variance (ANOVA) for each season, where site was the independent variable, and the dependent variable was soil salinity. All data were assessed for homogeneity of variances (all assumptions were met without the need for transformations) and Tukey's method of multiple comparisons was used to determine significant ($p < 0.05$) differences among sites. Soil salinity analyses were performed using R version 4.2.2 (R Core Team 2022).

Figure 3. Collecting a soil core to measure soil salinity.

<u>Emergent vegetation</u>

At each site, a permanent 50-m transect was established perpendicular to the shoreline. At three stations along the transect (0, 25, and 50 m from the water's edge), four 0.5 m^2 quadrats were haphazardly placed, with two to the left of the transect and two to the right (Figure 4). Within each quadrat, percent cover for each plant species present was recorded based on a bird's eye visual estimate. As part of a previous funding cycle, these sites were sampled near the closure date (Fall 2019) and post closure in Fall 2020, Spring 2021, and Fall 2021. In the current funding cycle, additional site sampling occurred in Spring and Fall 2022. The intent of these sequential funding cycles was to assess multi-year responses to Pass closure; thus all sampling dates are included in this report.

Each of the sites had different starting plant communities due to the natural tidal gradient and had different elevation profiles, so changes over time were analyzed separately at each site. To analyze the changes in plant community composition over time at each site, percent cover of each species was used in separate one-way Analyses of Similarity (ANOSIM) based on Bray-Curtis similarity matrices for each season, where the factor was year (Fall: 2019, 2020, 2021, 2022; Spring: 2021, 2022). ANOSIM generates an R-statistic that is essentially an indicator of effect size; where values < 0.25 indicate substantial overlap among groups, values $0.25 \leq 0.75$ indicate that groups are somewhat distinct from each other, and values > 0.75 indicate distinct separation between groups. Nonmetric multidimensional scaling (MDS) ordination was used to represent average dissimilarities among years in Euclidean two-dimensional space. In addition, changes in plant assemblages were analyzed with permutational multivariate analyses of variance (PERMANOVA), where year (2019, 2020, 2021, 2022) was the independent variable and a p-value less than 0.05 indicated significant differences among years. Theses analyses were performed with PRIMER v.6 (PRIMER-E Ltd., Plymouth Marine Laboratory, United Kingdom).

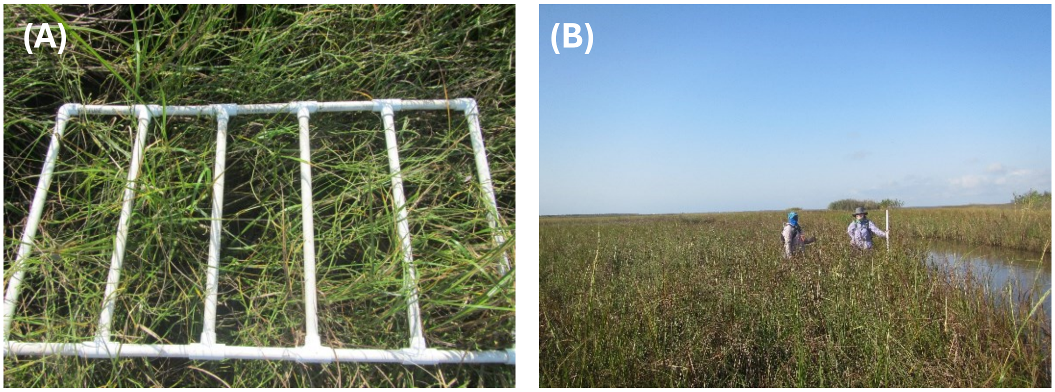

Figure 4. (A) Quadrat for recording plant cover at a representative site. (B) Field crew at a permanent transect marker.

<u>*Phragmites*</u>

To track the response of a nuisance species (*Phragmites australis*) that may proliferate in response to lower salinity, additional targeted sampling was conducted in Fall 2022. *Phragmites* measurements were collected only at Sites 1-3; there was no *Phragmites* present at Site 4. Three *Phragmites* monitoring stations were established at each of the three survey sites (nine stations total). The distribution of *Phragmites* was patchy at all sites, so stations were selected based on the presence of a *Phragmites* stand at least five meters in diameter. To standardize soil and inundation conditions, all sampling stations were located within five meters of a tidal channel.

Ten representative *Phragmites* plants were haphazardly selected from each sampling station (30 plants per site). Plant height was recorded as the length of the plant culm from ground level to the base of the highest green leaf blade (Figure 5A). In addition, the number of live leaves (defined as leaves with any green tissue) per culm was recorded. For each of the selected culms, the youngest three blades were collected and transported to the lab for further analysis. To assess relative photosynthetic activity as a proxy for plant fitness, chlorophyll *a* content was measured *in situ* on five haphazardly selected representative *Phragmites* plants at each sampling station (15 plants per site) using a chlorophyll meter (Konica Minolta SPAD502-Plus; Figure 5B). One reading was taken on each of the three youngest leaves per culm and an average value was calculated for each culm. Chlorophyll *a* values are reported in instrument-specific units of micro specified pigment units [mSPU] of plant carotenoid pigments per µg chlorophyll *a*.

Phragmites reproductive fitness was assessed by measuring inflorescence characteristics and by performing a germination test. Five representative reproductive plants from each sampling station were haphazardly selected and the inflorescence lengths were measured (15 plants per site) from the base of the rachilla (the first spikelet on the panicle) to the tip of the longest spikelet. Each inflorescence was then clipped, placed in a large plastic bag, and taken to the lab for analysis. Once in the lab, the spikelet branches were separated from the rachilla, and the biomass of the spikelets was recorded as a measure of seed mass per plant. The number of seeds per inflorescence was also recorded. To assess seed viability, all of the spikelets (whole florets) from each inflorescence were placed between moist sheets of filter paper and stored in a dark refrigerator (4°C) for 60 days (Kettenring and Whigham 2009). A germination trial was then conducted where the cold-stratified seeds were incubated at 19°C in moist filter paper for 3 weeks. The germination rate was calculated as the percent of seeds that sprouted after that incubation period; this value represents the percent germination per station.

To determine differences in *Phragmites* characteristics among sites, one-way ANOVA were used, where site was the independent variable, and the dependent variables were the response metrics (i.e., plant height, leaf size, chlorophyll *a* content, inflorescence length, number of seeds that germinated). All data were assessed for homogeneity of variances (all assumptions were met without the need for transformations) and Tukey's method of multiple comparisons was used to determine significant ($p < 0.05$) differences among sites. Pearson's Chi-squared test was used to determine differences in percent germination per inflorescence among sites. Statistical analyses were performed using R version 4.2.2 (R Core Team 2022).

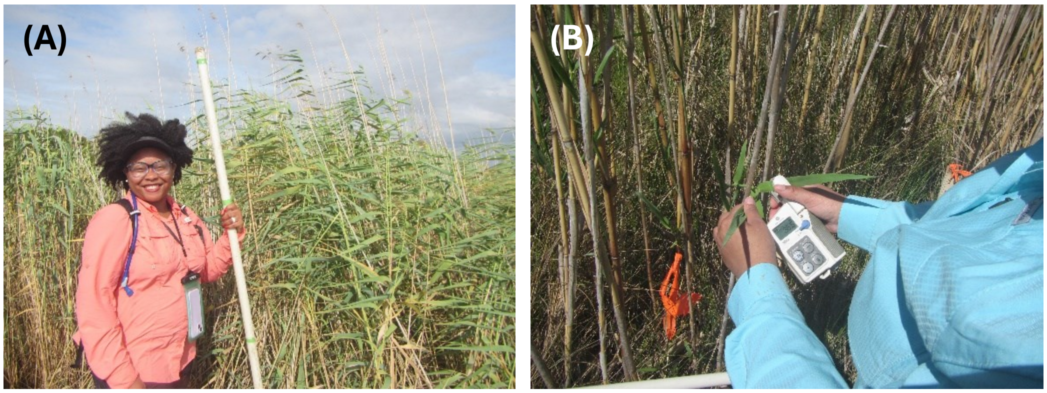

Figure 5. (A) Recording *Phragmites australis* height in the field. (B) Using a SPAD meter to record relative chlorophyll *a* content in *Phragmites* leaves.

<u>Nekton</u>

At each sampling event, nekton were collected by tossing a 1-m diameter cast net (10 mm mesh) from the shoreline of each site (Figure 6). Three replicate casts were conducted at each site on a subset of sampling dates (October 2020, March 2021, March 2022, October 2022). Collected nekton were identified to the lowest practical taxonomic level in the field and then released.

Figure 6. Using a cast net to sample nekton in the channel adjacent to the study site.

Results

<u>Salinity</u>

In the spring, soil salinity at Site 3 was 75% higher than the inland site (Site 1) and 38% higher than the salinity at Site 2 ($F_{2,24} = 26.817$, $p < 0.001$; Figure 7A). A similar pattern emerged in the fall, when soil salinity at the most seaward site (Site 3) was 86% higher than at Site 1 and 63% higher than Site 2 ($F_{2,24} = 49.149$, $p < 0.001$; Figure 7B).

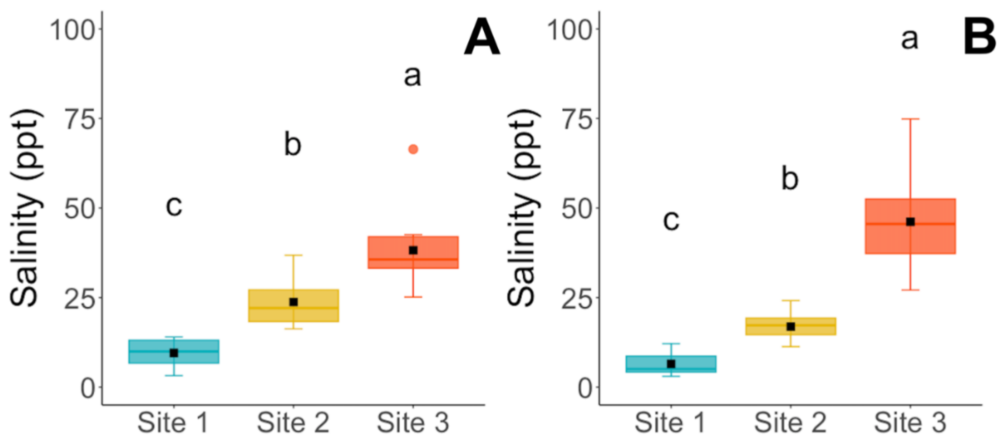

Figure 7. Differences in soil salinity (ppt) among sites in (A) Spring 2022 and (B) Fall 2022. Letters above bars denote significant differences among sites based on Tukey HSD post hoc tests. Error bars denote SD. The lines inside each boxplot represent the median value and the black squares inside each boxplot represent the mean value. The dot denotes an outlier in the data.

<u>Emergent vegetation</u>

Plant community assemblages changed over time at some sites, but changes were mostly a reorganization in the relative abundance of existing species, without evidence of directional shifts in species composition. Overall, there was no substantive change in dominant species identity nor was there a distinct directional change towards a more diverse, less salt tolerant community at any of the sites.

Site 1

At the site furthest from the Pass, ANOSIM indicated a high degree of overlap among plant communities each fall (R = 0.145) (Figure 8). PERMANOVA suggested that plant communities did vary among years, with a somewhat different community in 2019 (pseudo-F = 3.2493, p = 0.007). There was a large amount of overlap among plant communities in 2020-2022. *Distichlis spicata* was somewhat more common in 2019 than in later years (Table 1).

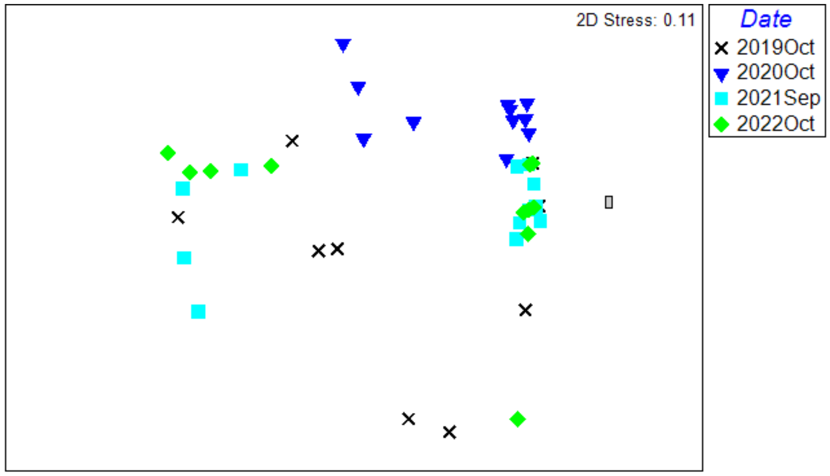

Figure 8. Nonmetric multidimensional scaling ordination plot of plant community changes over time in four successive fall sampling events at Site 1.

Table 1. Percent cover of plant species recorded in four successive fall sampling events at Site 1.

	2019	2020	2021	2022
Baccharis halimifolia	0	0	0.8	0.4
Distichlis spicata	21.3	1.4	0	0
Iva frutescens	16.7	6.7	20.8	51.0
Schoenoplectus pungens	0	5.3	0	0
Schoenoplectus robustus	7.3	0	3.8	9.4
Spartina patens	63.8	72.9	56.3	54.2
Symphyotrichum tenuifolium	0	0.4	0	1.0
Vigna luteola	0	0.3	3.3	0

In the spring, ANOSIM indicated a high degree of overlap among plant communities at Site 1 ($R = 0.113$) (Figure 9). PERMANOVA indicated that plant assemblages did not differ between spring 2021 and spring 2022 (pseudo-$F = 1.9823$, $p = 0.148$; Table 2).

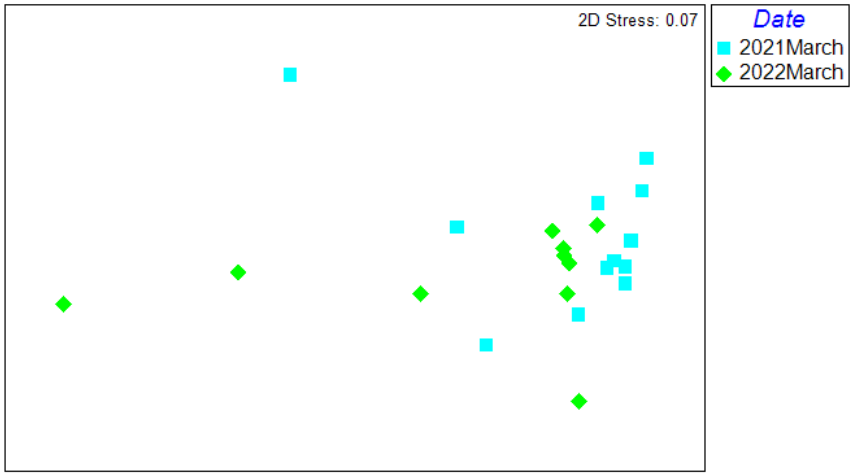

Figure 9. Nonmetric multidimensional scaling ordination plot of plant community changes over time in two successive spring sampling events at Site 1.

Table 2. Percent cover of plant species recorded in two spring sampling events at Site 1.

	2021	2022
Chloracantha spinosa	0.8	0
Cuscuta sp.	0.8	0
Distichlis spicata	3.3	0.1
Iva frutescens	2.3	3.3
Schoenoplectus robustus	4.0	3.4
Spartina alterniflora	0	0.5
Spartina patens	79.6	72.1
Symphyotrichum tenuifolium	0.1	0

Site 2

At Site 2, ANOSIM indicated a high degree of overlap among plant communities each fall (R = 0.250) (Figure 10). PERMANOVA suggested that plant communities did vary among years, with years 2019 and 2022 most similar to each other (pseudo-F = 5.1072, p = 0.001; Table 3).

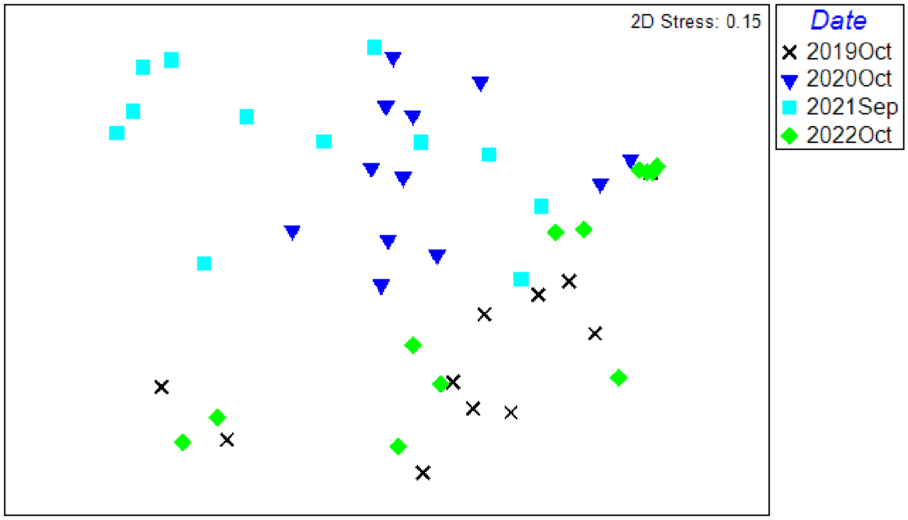

Figure 10. Nonmetric multidimensional scaling ordination plot of plant community changes over time in four successive fall sampling events at Site 2.

Table 3. Percent cover of plant species recorded in four successive fall sampling events at Site 2.

	2019	2020	2021	2022
Distichlis spicata	0	0.2	0	0.8
Ipomoea sagitta	2.1	0	0	0
Juncus roemerianus	0	0.2	0	0
Schoenoplectus americanus	7.9	0	0	6.1
Schoenoplectus robustus	0	16.3	19.6	0
Spartina alterniflora	37.9	27.5	13.2	44.6
Spartina cynosuroides	4.2	1.4	5.1	10.0
Spartina patens	21.7	15.8	9.2	13.8
Symphyotrichum tenuifolium	5.8	0.4	8.0	8.9

In the spring, ANOSIM indicated that the plant communities did not differ among years at Site 2 ($R = -0.025$) (Figure 11). PERMANOVA confirmed that plant assemblages did not differ between spring 2021 and spring 2022 (pseudo-$F = 0.3064$, $p = 0.817$; Table 4).

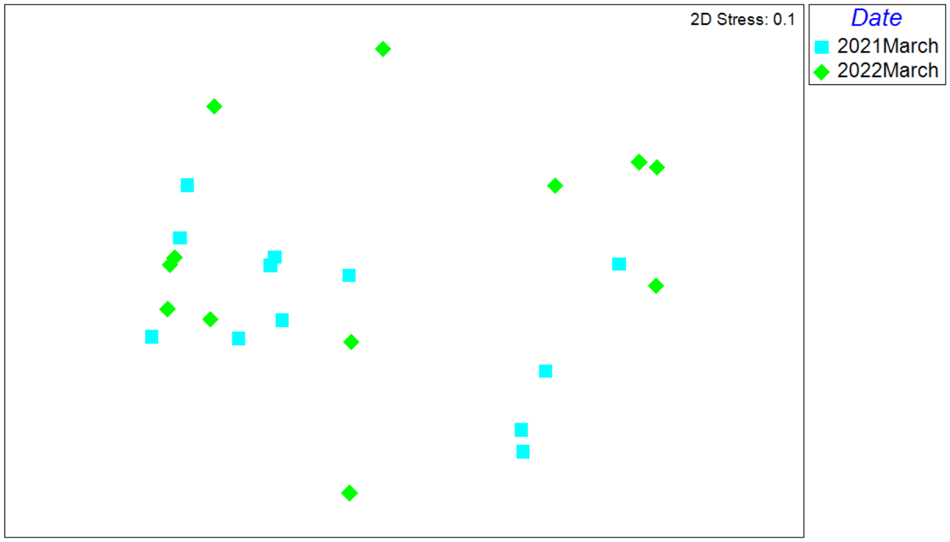

Figure 11. Nonmetric multidimensional scaling ordination plot of plant community changes over time in two successive spring sampling events at Site 2.

Table 4. Percent cover of plant species recorded in two spring sampling events at Site 2.

	2021	2022
Distichlis spicata	0.2	0.1
Schoenoplectus robustus	20.7	7.6
Spartina alterniflora	25.5	22.6
Spartina cynosuroides	3.8	5.8
Spartina patens	14.2	15.1
Symphyotrichum tenuifolium	0.9	1.8

Site 3

At Site 3, ANOSIM indicated some differences among plant communities each fall ($R = 0.454$) (Figure 10). PERMANOVA confirmed that plant communities varied among years, with 2021 distinct from the other years, largely driven by lower *Spartina alterniflora* abundance (pseudo-F = 13.627, $p = 0.001$; Table 5).

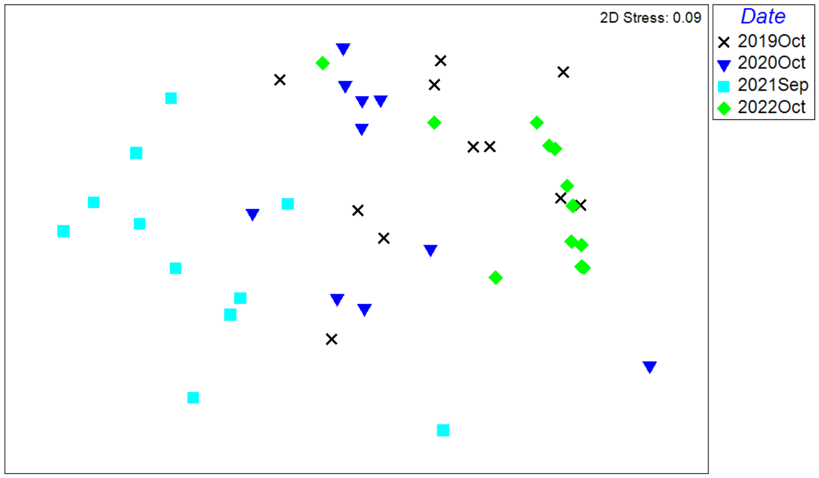

Figure 12. Nonmetric multidimensional scaling ordination plot of plant community changes over time in four successive fall sampling events at Site 3.

Table 5. Percent cover of plant species recorded in four successive fall sampling events at Site 3.

	2019	2020	2021	2022
Schoenoplectus robustus	12.1	15.8	43.7	2.1
Spartina alterniflora	42.1	20.4	4.4	51.7
Spartina cynosuroides	1.3	0	0	0
Symphyotrichum tenuifolium	43.7	37.1	11.7	31.7

In the spring, ANOSIM indicated that the plant communities were distinct each year at Site 3 (R = 0.443) (Figure 13). PERMANOVA confirmed that plant assemblages differed between spring 2021 and spring 2022, largely due to higher Schoenoplectus robustus cover in 2022 (pseudo-F = 8.381, p = 0.001; Table 6).

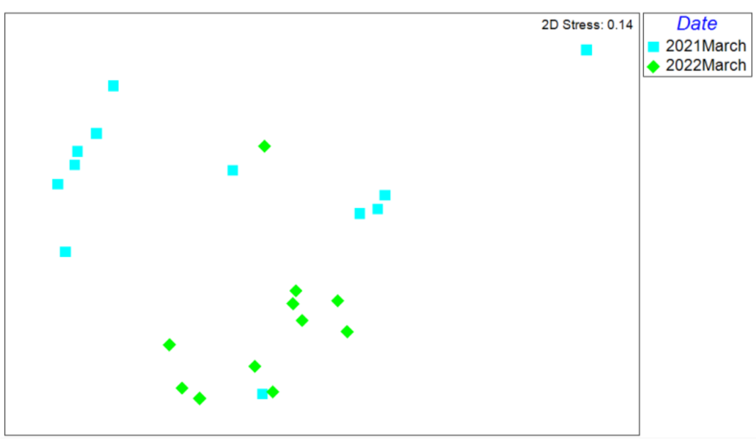

Figure 13. Nonmetric multidimensional scaling ordination plot of plant community changes over time in two successive spring sampling events at Site 3.

Table 6. Percent cover of plant species recorded in two successive sampling events at Site 3.

	2021	2022
Schoenoplectus robustus	2.4	26.3
Spartina alterniflora	45.0	40.8
Spartina patens	7.3	3.3
Symphyotrichum tenuifolium	5.6	3.4

Site 4

At Site 4 nearest to Rollover Pass, ANOSIM indicated that plant communities did not change among years in the fall (R = 0.0) (Figure 14). PERMANOVA confirmed that plant communities were similar among years (pseudo-F = 0.895, p = 0.455; Table *7*).

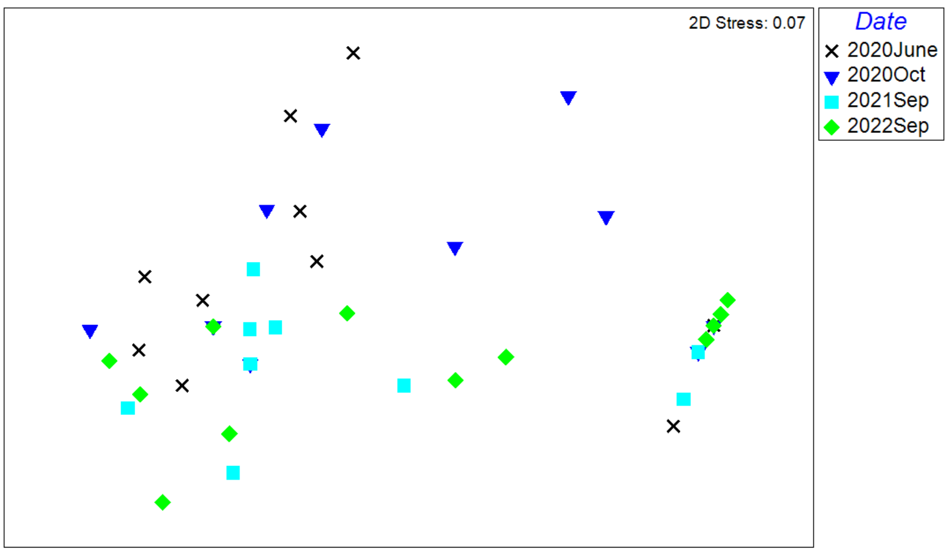

Figure 14. Nonmetric multidimensional scaling ordination plot of plant community changes over time in four successive fall sampling events near Rollover Pass at Site 4.

Table 7. Percent cover of plant species recorded in four successive fall sampling events near Rollover Pass at Site 4.

	2019	2020	2021	2022
Avicennia germinans	0.5	4.2	0	0
Batis maritima	10.0	3.3	2.1	2.6
Distichlis spicata	22.5	20.4	27.9	34.2
Spartina alterniflora	21.7	31.3	24.6	30.0

In the spring, ANOSIM indicated that the plant communities did not differ among years at Site 4 (R = 0.036) (Figure 15). PERMANOVA confirmed that plant assemblages did not differ between spring 2021 and spring 2022 (pseudo-F = 1.553, p = 0.249; Table 8).

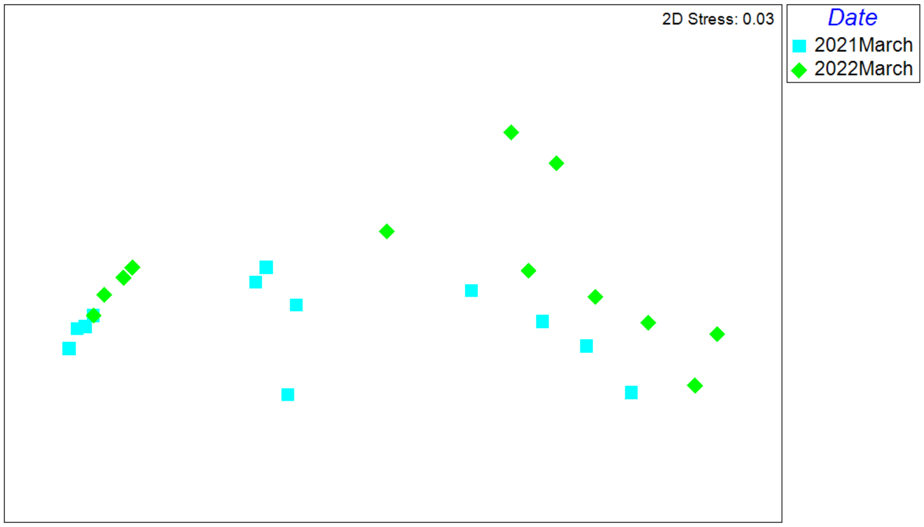

Figure 15. Nonmetric multidimensional scaling ordination plot of plant community changes over time in two successive spring sampling events at Site 4, nearest Rollover Pass.

Table 8. Percent cover of plant species recorded in two successive spring sampling events at Site 4, nearest Rollover Pass.

	2021	2022
Avicennia germinans	0.5	0
Batis maritima	0	3.0
Distichlis spicata	22.1	37.9
Salicornia sp.	0	0.5
Spartina alterniflora	42.9	25.2

Phragmites

In the fall, *Phragmites* plants were 21-27% taller at Site 1 than at the other two sites ($F_{2,87}$ = 21.578, p < 0.001; Figure 16). *Phragmites* leaves were larger at Site 1 compared to those at Site 3, though there was substantial variability and overlap among sites ($F_{2,351}$ = 3.964, p = 0.020). There was no significant difference in *Phragmites* culm density among sites ($F_{2,6}$ = 3.49, p = 0.099). Plants at the intermediate site (Site 2) had 11.08% more chlorophyll a content than those at Site 3 ($F_{2,42}$ = 5.64, p = 0.007).

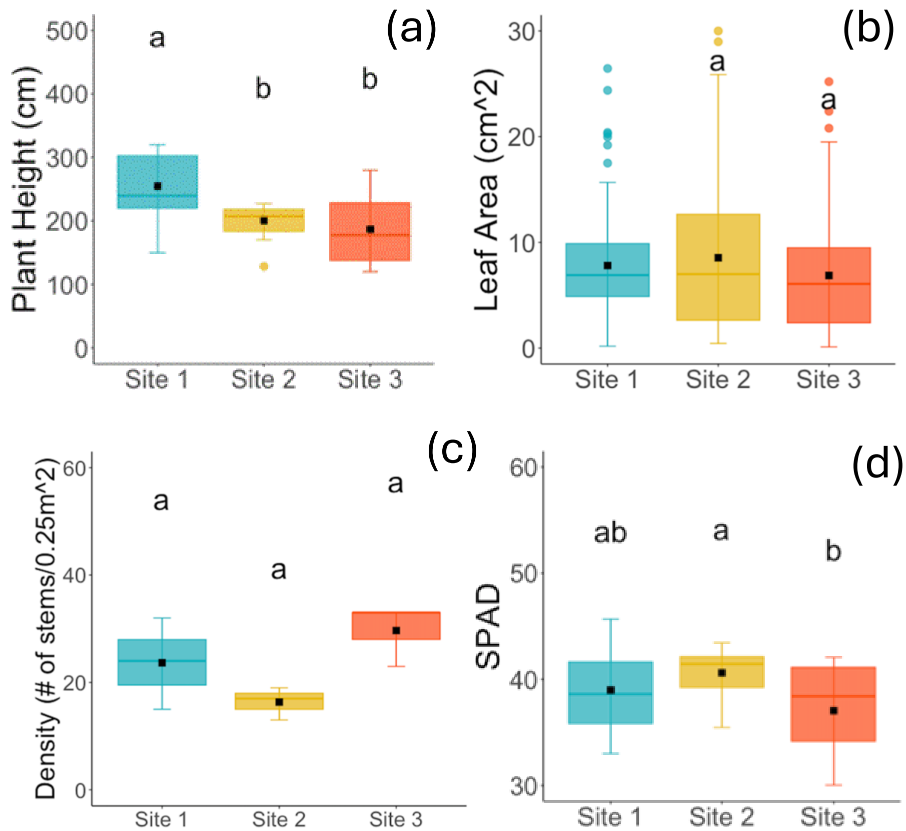

Figure 16. Differences in *Phragmites* (a) plant height, (b) leaf area, (c) culm (stem) density, and (d) chlorophyll *a* (SPAD) content among sites in Fall 2022. Letters above bars indicate significant differences among sites based on Tukey HSD post hoc tests. Error bars denote SD. The lines inside each boxplot represent the median value and the black squares inside each boxplot represent the mean value. Dots denote outliers.

Inflorescences at Site 1 were 48% taller than those at the seaward site (Site 3) and 32.5% taller compared to those at Site 2 ($F_{2,28}$ = 10.446, p < 0.001; Figure 17A). Plants at Site 2 had lower average seed counts per inflorescence than plants at the other sites; although seed counts were about 35% lower at Site 2, this difference was only significant between Sites 1 and 2 ($F_{2,28}$ = 3.35, p = 0.050; Figure 17B). Likewise, seed biomass was lowest at Site 2, but this difference was only significant between Sites 2 and 3 ($F_{2,28}$ = 4.236, p = 0.025; Figure 17C). Seed germination rates were very low, less than 1% in most cases (Figure 17D). Site 1 was the only site where any seeds germinated, but only three inflorescences from Site 1 produced seeds that germinated.

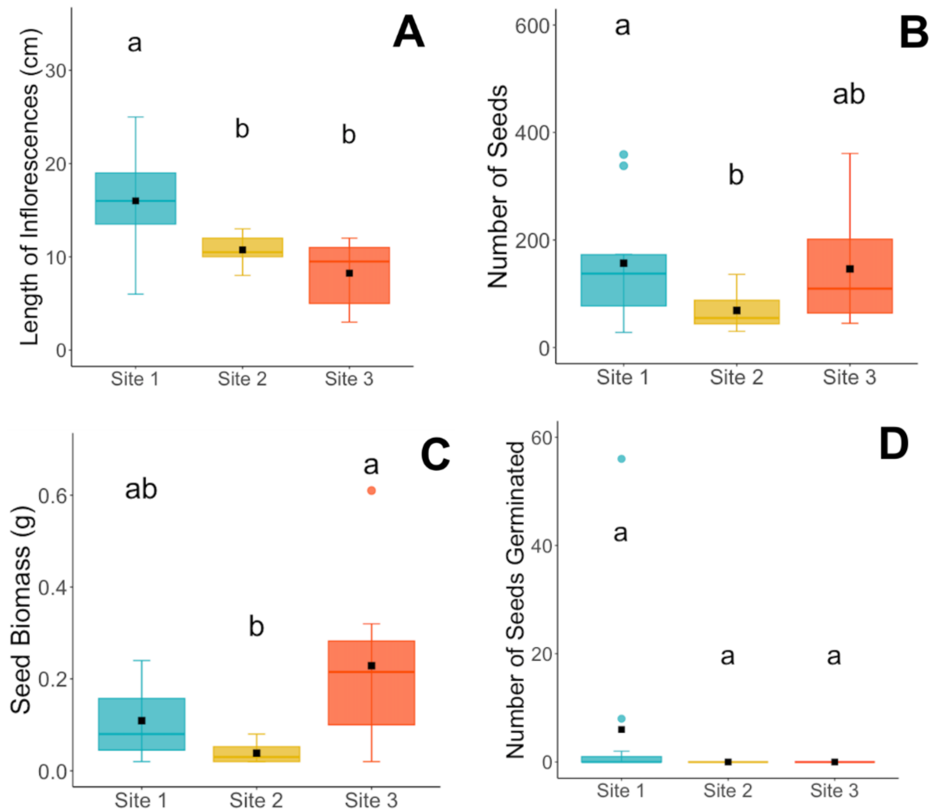

Figure 17. A comparison of (A) *Phragmites* inflorescence height/length, (B) number of seeds per inflorescence, (C) seed biomass per inflorescence, and (D) the number of seeds that germinated per inflorescence among sites. Differing letters indicate significant differences based on Tukey HSD post hoc tests. The lines inside each boxplot represent the median value and the black squares inside each boxplot represent the mean value. Dots denote outliers in the data. Error bars denote SD.

<u>Nekton</u>

Nekton were variable across sites and over time. Most of the species encountered were salt tolerant, and the most common species (*Brevoortia patronus*, *Palaemonetes pugio*, and *Farfantepenaeus* spp.) occurred at both saline and fresher sites (Table 9). The sparse nekton abundances we detected indicate that the sampling approach used did not fully characterize the nekton assemblages, and that a larger temporal and spatial scale is needed to assess the trajectory and dynamics of nekton response to Rollover Pass closure.

Table 9. Total number of nekton collected, pooled over three cast net tosses at each site. nd = no data were collected due to logistical constraints.

Site 1		**Date**			
Species		Oct-20	Mar-21	Mar-22	Oct-22
Anchoa mitchilli		0	1	0	0
Brevoortia patronus		71	2	22	36
Farfantepenaeus duorarum		1	0	0	1
Site 2					
Species		Oct-20	Mar-21	Mar-22	Oct-22
Brevoortia patronus		2	0	4	0
Cynoscion nebulosus		1	0	0	0
Farfantepenaeus aztecus		1	0	0	0
Farfantepenaeus duorarum		1	0	0	0
Litopenaeus setiferus		1	0	0	0
Palaemonetes pugio		0	33	2	0
Site 3					
Species		Oct-20	Mar-21	Mar-22	Oct-22
Micropogonias undulatus		0	3	0	0
Farfantepenaeus aztecus		5	0	0	1
Farfantepenaeus duorarum		1	0	0	0
Palaemonetes pugio		1	17	1	0
Site 4					
Species	Jun-20	Oct-20	Mar-21	Mar-22	Oct-22
Anchoa mitchilli	1	0	0	nd	0
Brevoortia patronus	43	0	0	nd	0
Micropogonias undulatus	0	0	1	nd	0
Farfantepenaeus aztecus	1	0	0	nd	0
Farfantepenaeus duorarum	2	6	0	nd	0
Litopenaeus setiferus	1	0	0	nd	0
Palaemonetes pugio	1	11	0	nd	0

Summary and Conclusions

Our evaluation of the near-term ecosystem responses within East Bay to the closure of Rollover Pass indicated that ecosystem responses were gradual and non-directional. Although the upstream sites did have lower soil salinity, all sites were relatively saline. Accordingly, few freshwater species appeared or expanded in any sites over the course of the study, indicating that the colonization of freshwater tolerant species did not contribute to the differences between years in an ecologically meaningful way. Changes in emergent plant communities were largely attributable to fluctuations in abundance of existing species, and there was no clear shift towards species characteristic of freshwater marshes. Likewise, all of the fish and invertebrate species present were salt tolerant. Overall, these data indicate that ecosystem responses to the closure of Rollover Pass are occurring gradually, and any additional changes may occur slowly over the coming years.

Phragmites stands were present at each of the three sites along Oyster Bayou, but plants at the site furthest inland (Site 1) were somewhat healthier based on the plant height, leaf surface area, leaf chlorophyll a content, inflorescence length, and number of seeds per inflorescence. These results are consistent with previous field and greenhouse studies in other regions demonstrating that increasing salinity negatively correlates with several aspects of *Phragmites* health (Hellings and Gallagher 1992; Lissner and Schierup 1997; Burdick et al. 2001; Saltonstall 2002; Chambers et al. 2003; Swearingen and Saltonstall 2010; Hazelton et al. 2014). Thus, the reduction of saltwater input after Rollover Pass closure may boost some measures of *Phragmites* fitness, particularly at downstream sites with higher baseline salinities. However, the threat of *Phragmites* behaving invasively throughout the Anahuac NWR appears to be minimal, as the persistently brackish waters in the ANWR (McDonald 2022) and saline soils (this study) may limit the potential for proliferation and competitive displacement of other emergent marsh species in the refuge.

Closure of Rollover Pass had relatively modest effects on the salinity gradient in the ANWR (McDonald 2022, this study). Given that eradication is likely to be ineffective (Uddin et al. 2020) and the relatively low abundance of *Phragmites* at the site level (McDonald 2022), no action to manage *Phragmites* is indicated at this time. However, in the future, additional water management actions that reduce salinity may facilitate the spread of *Phragmites*. Thus, *Phragmites* management should be considered in future water management decisions, including increases in the volume of freshwater released from upstream into the ANWR. As suggested by the 2019 Texas Coastal Resiliency Master Plan, The Marshland Restoration Project at Anahuac National Wildlife Refuge (Project ID R1-43) aims to restore freshwater flows to the refuge to restore the natural salinity gradients and improve wetland habitats (Bush 2019). Such coastal management practices could eventually make this environment more conducive to *Phragmites* spread, though rapid proliferation remains unlikely as long as some tidal exchange with east Galveston Bay persists.

Appendix B: References cited

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