Appendix 5C-2: Evaluation of Inundation Depth and Duration for Cattail Sustainability: In Situ Study

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SUMMARY

Typha species (cattails) are the dominant emergent macrophyte in the emergent aquatic vegetation (EAV) cells of the Everglades Stormwater Treatment Areas (STAs). The structural complexity of EAV plants such as cattails reduces flow velocity, which enhances settling of suspended solids and particulate P improving runoff water quality (Chimney and Newman 2006). However, high flows during the wet season can result in excessive inundation and deep-water conditions that can stress the cattail communities of these cells. Chen and Vaughan (2014) concluded that persistent deep-water conditions in Cell 7 of STA-1E had an adverse effect on the health of the cattail community, which may have caused the decline in the cells ability to reduce P in the water column. Uneven topography and vegetation resistance to flow within treatment cells also create deep areas even when target depths (32–46 centimeters [cm]) are maintained. Inundation exceeding the optimal water depth and duration can cause physiological stress on *Typha* species. During Water Year 2016 (WY2016; May 1, 2015–April 30, 2016) to WY2018, daily average water depths in the inflow region of STA-3/4 Cell 2A were consistently higher than those in the outflow region, with 52% of the daily water depths >76 cm compared to only 16% in the outflow region of the cell. The inflow region of this cell experienced water depths >91 cm for 40 consecutive days in WY2016; three periods in WY2017 for 50, 29, and 35 consecutive days; and three periods in WY2018 for 19, 19, and 67 consecutive days. The outflow region also experienced deep conditions, but at a lesser extent than the inflow region.

During the 2015 wet season, no significant differences were observed in total cattail shoot density between the inflow and outflow regions. However, total cattail densities during the 2016 and 2017 wet seasons were significantly lower in the inflow plots than in the outflow plots of this cell, indicating a declining cattail population in the deeper areas of the cell over time. Similarly, juvenile shoot density from the inflow plots were lower during all monitoring seasons, with significantly lower density during the 2016 and 2017 seasons. Cattail density decline is attributed primarily to the period of prolonged water depths during the 2016 and 2017 seasons particularly in the inflow region that experienced a total of 114 days at a water depth ranging from 91 to 136 cm in WY2017 and another 105 days at a water depth ranging from 91 to 154 cm in WY2018. The higher number of juveniles, dead cattail plants, and higher amount of necromass in the outflow region suggest a higher turnover rate in the outflow region of this cell.

Leaf elongation rates were consistently higher for cattail plants in the inflow region, with higher rates measured early in the wet season (June–July). Elongation rates decreased toward the end of the wet season (October–November). Data indicates that deep and prolonged inundation periods, such as those experienced

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in wet seasons of 2016 and 2017, resulted in increased leaf elongation rates, especially in the deeper areas within the inflow region.

Photosynthetic rates between inflow and outflow regions across all of the three monitoring years were not significantly different (probability factor [p] > 0.05). However, there was a slight increase in photosynthetic rate, stomatal conductance, and water use efficiency (WUE) toward the end of the wet season, indicating a possible recovery due to lower water levels in the cell and less stressful conditions for the cattail plants.

Total live biomass did not differ between the inflow and outflow plots for all of the three biomass sampling years. However, the combined inflow and outflow region measurements indicate a significant decline in all biomass components over time. There was also a noticeable decrease in the belowground biomass:leaf ratio in the inflow region over the three-year period, suggesting that the root and rhizomes of the cattail population were stressed more than shoots in the deeper region of this cell. Total phosphorus (TP) and total nitrogen (TN) concentrations were generally higher in the inflow than in the outflow region, which is consistent with a nutrient concentration gradient from inflow to outflow reported in other studies in the STAs (Villapando and King 2018). For the different cattail biomass components, the highest TP concentrations in the inflow plots over the three-year period were observed in the shoot base, followed by rhizomes. Outflow plot TP concentrations from all biomass components remained constant over time, except for shoot base from the 2017 sampling with an outflow TP concentration of 155% of the 2014 sampling. Total carbon (TC) concentrations in all biomass components remained nearly constant over time, with concentrations slightly higher in the outflow region of the cell.

Results from this study will be important considerations in the experimental design of the second phase of the Cattail Project (Test Cell Study) and would be complementary with the results of the Test Cell Study to better define the inundation depth and duration thresholds of *Typha domingensis* in the STAs.

INTRODUCTION

Fluctuating water levels, a common occurrence in wetlands, can influence growth and productivity of emergent macrophytes depending on the amplitude of these water level fluctuations (Edwards et al. 2003, Deegan et al. 2007). In the Everglades STAs, the target depth in EAV cells is ~38 cm; however, this depth is often exceeded during high flow events in the wet season while attaining much more shallow depths during the dry season.

Cattail species (*T. domingensis* and *T. latifolia*) are generally flood-tolerant plants that can exist under a wide range of hydrological conditions. Optimal water depths where cattails reach their highest density and biomass range between 20 and 60 cm depending on the study. Several studies have reported that *T. latifolia* and *T. domingensis* reach peak densities at water depths around 22 cm (Grace 1989, Waters and Shay 1992, Redwine 2008). Similarly, Miao and Zou (2012) reported that *T. domingensis* plants grown in 20-cm water depth produced significantly greater biomass than those grown in 60-cm water depth after a year. However, other studies suggest that the optimal water depth for *T. domingensis* ranges from 30 to 60 cm (Newman et al. 1996, Grace and Wetzel 1998, White et al. 2007). Based on a three-year mesocosm study, *T. domingensis* communities grown in 40-cm water depths were healthy and maintained their high productivity and ecological functions (Miao 2014).

Declines in cattail have been observed at deeper water levels. Deegan et al. (2007) reported that *T. domingensis* declined when water levels fluctuated around an initial depth of 60 cm, but there was no change in biomass at three water fluctuations treatments (static, ± 15, and ± 30 cm, each cycling over a 40-day period). However, biomass decreased by 52% when the amplitude of water fluctuation increased to ± 45 cm (Deegan et al. 2007). Chen and Vaughan (2014) reported that increasing inundation depths from 30 to 80 cm significantly increased necromass and the belowground biomass ratio, but did not affect leaf, belowground, and total biomass in the cattails in STA-1 East in South Florida. Inundation exceeding the

optimal depth and duration causes increasing physiological stress to cattail plants, reducing growth and anchorage capacity by decreasing biomass allocation to rhizomes and roots (Grace and Wetzel 1982). Several studies suggest that *T. domingensis* is stressed at water depth greater than 60-cm with an inundation duration ranging from a few weeks to more than a year (Grace 1989, Miao and Zou 2012). Grace (1989) reported that *T. domingensis* density decreased at water depths greater than 58 cm, however, plant height increased even at an inundation of 110 cm. However, Chen and Vaughan (2014) reported that the increased stress on the cattail community by deeper inundation depths decreased shoot density and leaf elongation rates, suggesting that deep water condition adversely affect their reproduction and propagation. Miao and Zou (2012) reported that mortality of *T. domingensis* plants in a mesocosm study using a flow-through system, with low phosphorus (P) water from STA-1 West (STA-1W), increased approximately 50% after one year when subjected to an inundation depth of 60 cm.

Other studies in the Everglades STAs found that increasing inundation depths from 40 to 137 cm for six weeks significantly decreased growth, biomass, photosynthesis, and belowground non-structural carbohydrate storage of *T. domingensis* (Chen et al. 2010, 2013). When water depths from different treatments returned to 40 cm for a 4-week recovery period, damage to roots and belowground biomass to cattail plants stressed at water depths < 90 cm was reversed, however, damage to cattail plants stressed at 137-cm water depth was not reversed. Increasing inundation also reduces the anchorage capacity of cattails, sometimes resulting in floating cattail mats as plants respond to deep inundation by decreasing biomass allocation to rhizomes and roots and increasing allocation to shoots (Grace 1989, Chen et al. 2010, Miao and Zou 2012).

In the Everglades STAs, prolonged deep water conditions may result in the formation of large cattail floating tussocks (Chimney et al. 2000). In time and under continuous deep flooding conditions, the occurrence of large stands of floating cattails that eventually will develop into a floating decaying mat or tussock increases (Chen and Vaughan 2014, Pietro 2016, Diaz 2018). Harris and Marshall (1963) reported that cattail plants died after they were continuously flooded for 2 to 4 years at water depths ranging from 30 to 100 cm.

One of the operational challenges for STA management has been to maintain optimal water levels despite changing magnitude and duration of inflows. Such challenges have occurred during periods of very high rainfall, as experienced in June and September 2017, as well as during periods of regulatory Lake Okeechobee releases to the Everglades. During these periods, EAV cells in STA-3/4 (Cells 1A, 2A, and 3A) experienced high inflow resulting in high water depths. For example, daily inflow water depths in Cell 2A during June 2017 ranged from approximately 122 to 152 cm. Some of these high water depths occurred for extended periods of time, negatively affecting cattail communities, particularly in the inflow region of the cells. During high flow events, vegetation density, plant community architecture, and microtopography are important parameters contributing to flow resistance, which in EAV cells with dense cattail stands can result in increased water depths at the inflow region of the cells (Lal 2017).

To reduce high flow events, the A-1 Flow Equalization Basin (FEB), a 15,000-acre aboveground storage reservoir, was constructed immediately north of STA-3/4 and started operations in Water Year 2016 (WY2016; May 1, 2015–April 30, 2016). The primary objective of this reservoir is to temporarily store stormwater runoff, reducing high flows to this STA during the wet season, and provide a source of water during the dry season to decrease the frequency of dryout conditions.

The primary objective of the first phase of this cattail sustainability study is to identify field conditions such as water depth, duration, and frequency of inundation affecting the health of cattail communities in selected STA cells. Results gathered from the In Situ Study will complement and guide the experimental design of the next phase of this study (Test Cell Study), to establish an inundation depth and duration threshold for cattail sustainability. The Test Cell Study is expected to start by June 2019. Three hypotheses will be evaluated: (1) there is an inundation duration threshold for cattail sustainability at a specific inundation depth, in terms of survival, growth, and propagation, (2) the inundation period threshold is

longer at relatively shallow inundation depth than at deeper inundation conditions, and (3) longer inundation durations than the threshold result in a decline in plant density, the ability to propagate, and biomass. The results of these studies will help identify the depth and duration threshold for cattail sustainability that will assist in the development of water level management strategies in the STAs. Data presented in this report correspond to three monitoring seasons (2015, 2016, and 2017) and relate to STA-3/4 Cell 2A.

METHODS

STA-1W Cell 2A and STA-3/4 Cell 2A, both emergent aquatic vegetation (EAV) cells, were originally selected for the In Situ Study. Monitoring in STA-1W Cell 2A, was discontinued after one year due to widespread decline of the cattail community and the presence of cattail floating mats throughout the cell (Diaz 2018). Monitoring in STA-3/4 Cell 2A continued during the wet seasons of 2016 and 2017. STA-3/4 Cell 2A has an effective treatment area of 1,014 hectares with a healthy *T. domingensis* population prior to the initiation of the In Situ Study (**Figure 1**). Prior to this study, STA-3/4 Cell 2A experienced a decline in cattail coverage and density, particularly in the inflow region. Water levels in the cell were drawn down for vegetation rehabilitation in 2011 and 2013, resulting in improved cattail coverage and health, particularly after the six months of low water levels in the spring of 2013 (Chimney 2014).

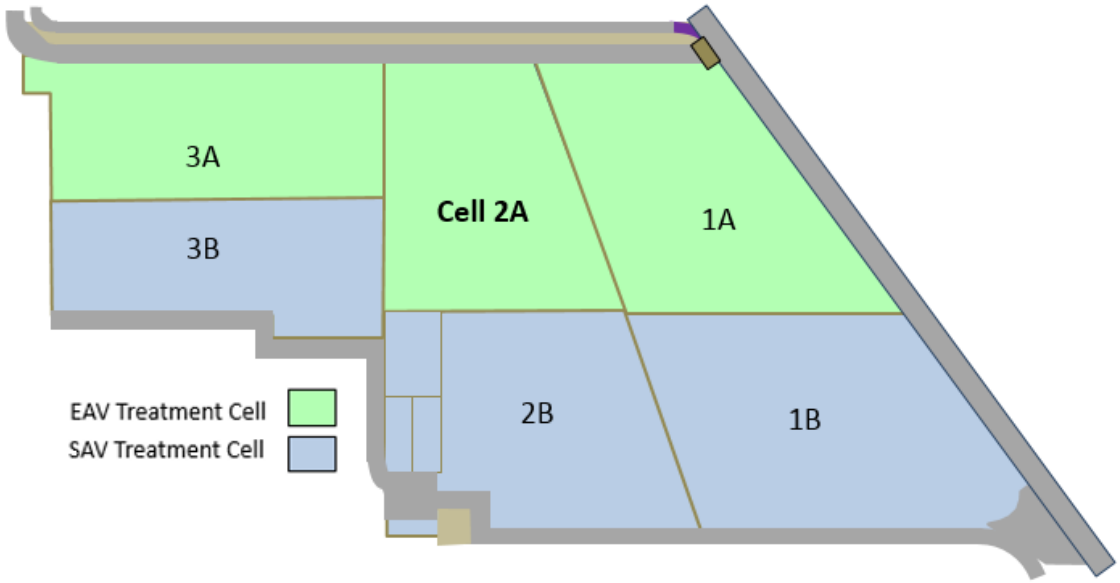

Figure 1. Schematic map of STA-3/4 Cell 2A location where the in situ cattail monitoring and measurements for the Cattail Study were conducted from the 2015 through 2017 wet seasons.

Fifteen 2-meter (m) x 3-m plots were established in STA-3/4 Cell 2A during summer 2014 (**Figure 2**). There were three monitoring events in 2015: June, August, and October, representing wet season conditions. For 2016 and 2017, the first and second monitoring events for each year were conducted in July and November 2016 and 2017, respectively, due to high water depths during most of the wet season (July–October). The third monitoring event for the 2016 season was conducted in January 2017 and the third event for the 2017 season was conducted in January 2018. For each monitoring event, field measurements included plant density (adult and juvenile), photosynthesis, leaf elongation, and water depth using a graduated polyvinyl chloride (PVC) pole. Field observations included visible cattail damage and presence of floating mats, presence of other emergent or floating aquatic plants within the plots, and photo documentation of each plot.

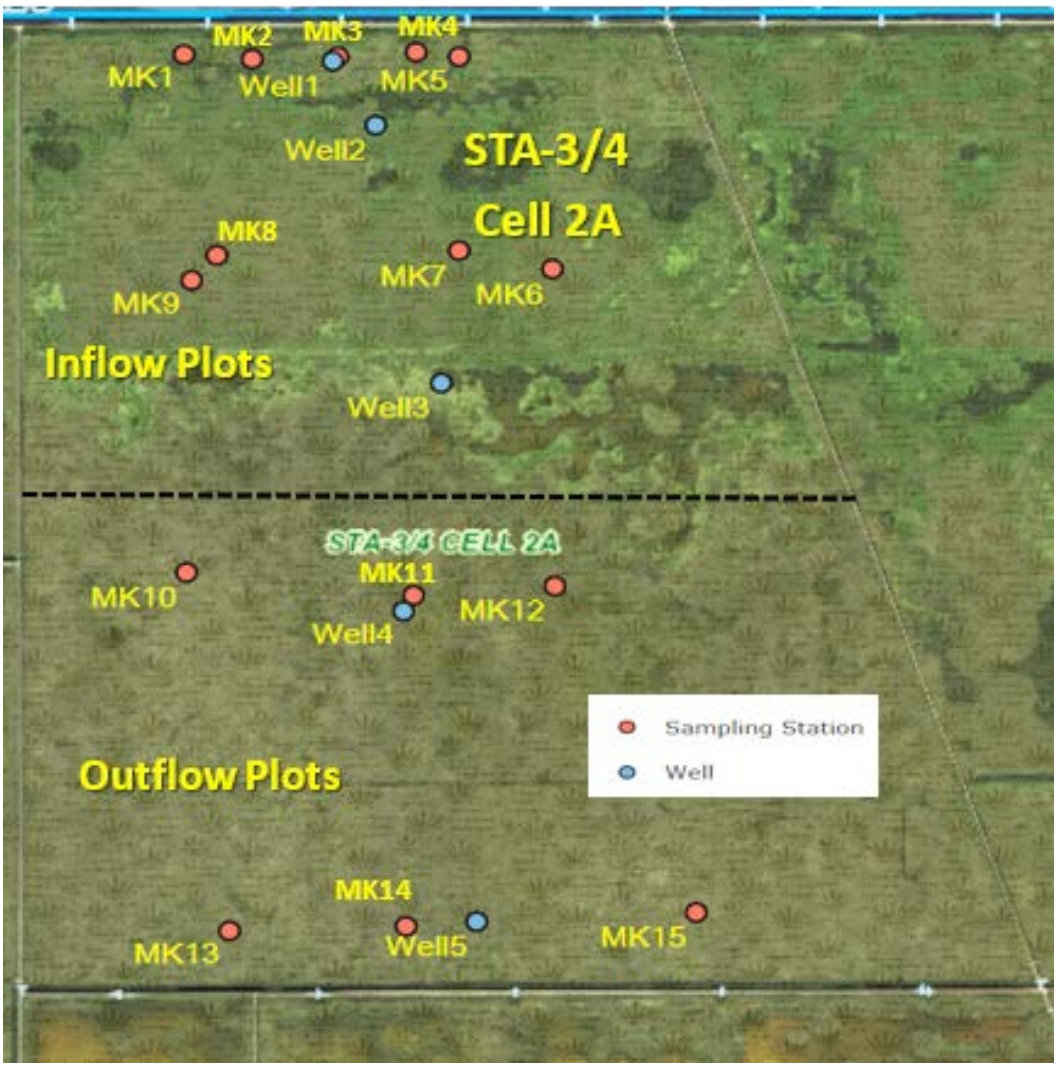

Figure 2. STA-3/4 Cell 2A survey plot (red) and water level logger (blue) locations.

The number of cattail plants within each plot was categorized into four groups: (1) live adults (> 1.5 m in height), (2) live juveniles (< 1.5 m in height), (3) live adults with flower or seed stalk, and (4) dead plants. Cattail shoot density was estimated as the number of plant shoots per square meter (shoots/m^2). Leaf elongation was measured on five recently emerged leaves from five healthy rooted cattail plants in each plot. After the cattail plants were selected, the selected young leaves were tagged with a brightly colored flagging tape. The leaf height from the base of the plant to the tip of the leaf was measured using a graduated pole. The length of the labeled leaves was measured and remeasured between 7 to 10 days later. Leaf elongation rate was calculated by dividing the change in height by the number of days between measurements in centimeters per day (cm/d).

Leaf gas exchange was measured using a LI-6400XT Portable Photosynthesis System (Li-COR, Lincoln, Nebraska). The same five healthy adult plants selected for leaf elongation were used for gas exchange measurements. Measurements were generally collected over a 4- to 5-day period, with 3 to 4 plots completed per day. Ideally, all selected plant leaves would show no sign of chlorosis, herbivory, or other malformations, but depending on how stressful the conditions in the plot were, these criteria could not always be met. A correction factor was applied to these readings to ensure an accurate representation for photosynthesis of the plant. When calculating the photosynthetic rate and stomatal conductance, the LI-

6400XT assumes the leaf being measured fills the entire area of the chamber. However, it was common for some of the cattail leaves measured in this study to be narrower than the chamber. When this situation occurred, a note was made on the field data sheet and the chamber area value was manually reduced to compensate for the narrow leaf. The adjustment was made to the area parameter in the photosynthetic rate equation. Once selected for measurement, the leaf was gently arced so that the LI-6400XT chamber could be clamped 6 to 18 inches from the tip of the leaf. The chamber was held in place to allow the gas exchange values to stabilize before collecting a reading. This process was repeated for each selected plant in the plot. The presence of anomalies (e.g., narrowness, chlorosis, herbivory, and malformed) was noted on the field data sheets.

Samples also were collected for plant biomass, including aboveground and belowground, and live and dead materials from a 0.25-square meter (m^2) quadrat (0.5 m x 0.5 m) in an area adjacent to the experimental plot to avoid destruction of the cattail within the plot. For STA-3/4 Cell 2A, a saw was used to cut along the quadrat perimeter to the bedrock (soil layer from this cell is shallow). All belowground material from the quadrat was carefully excavated by hand and placed in a labeled rice bag together with the aboveground biomass for transportation to the laboratory. Baseline or initial biomass samples were collected in November 2014. A second biomass sampling was collected at the end of the first monitoring season in October 2015, and a final biomass sampling collected toward the end of the study in November 2017. Plant biomass processing included washing and separation of the different plant components into leaf, shoot base, root, rhizome, and dead biomass. Processed plant biomass samples were dried at 70 degrees Celsius (°C) for a minimum of two weeks and weighed. A sub-sample of each biomass component was submitted to the South Florida Water Management District's (SFWMD or District) Laboratory for analysis of TC, TN, and TP.

Daily stages from WY2015 to WY2018 were estimated from the SFWMD corporate environmental database, DBHYDRO, by averaging the daily average stage at the inflow (tailwater [TW]) stages of structure G-377 and outflow (headwater [HW]) of structure G-378. Daily average water depths for each cell region were calculated by subtracting the average ground elevation in National Geodetic Vertical Datum of 1929 (NGVD29, Piccone et al. 2014) from the daily stage values. The phrase "prolonged water depths" was used throughout this report whenever the daily average water depth in the cell was >76 cm for more than 50 consecutive days.

In addition, continuous water depths at 30-minute intervals were monitored with five *Solinst* water level logger stations deployed at Wells 1 to 5 across the cell in July 2015 (**Figure 2**). Data from Water Level Loggers 1, 2, and 3 (Wells 1, 2, and 3, respectively) were averaged to represent water depths in the inflow region, and Water Level Loggers 4 and 5 (Wells 4 and 5, respectively) were averaged to represent water depths in the outflow region of the cell.

Data analysis were analyzed with JMP® statistical software (Version 13.1.0, SAS Institute Inc. 2015, Cary, North Carolina). Experimental plots were grouped into inflow and outflow regions due to general observation of deeper water depths in the inflow region than in the outflow region of this cell. Summary statistics and frequency distribution for water depth were calculated. Plant biomass, cattail density leaf elongation and gas exchange parameters (photosynthetic rate, stomatal conductance and water use efficiency) were tested for normality and for equal variances. The Shapiro-Wilk W Test was used to determine normality. Parameters that met these assumptions were compared using a parametric pooled t-test. Parameters that did not meet these assumptions were compared using the non-parametric Wilcoxon Rank Sum Test. For multiple comparisons, the parameters that met the assumptions for parametric analysis were compared using the Tukey-Kramer honest significant difference test. Parameters that did not meet the assumptions for parametric analysis were compared using Dunn Allpairs test. Statistical significance was determined at $\alpha = 0.05$.

RESULTS AND DISCUSSION

DAILY WATER DEPTHS

Daily average water depths in the inflow region of STA-3/4 Cell 2A were consistently higher than in the outflow region during the entire monitoring period (**Figure 3**). Annual average water depths in the inflow region from WY2015 to WY2018 (May 1, 2014 to February 13, 2018) were 67, 77, 85, and 90 cm, respectively, and in the outflow region, they were 48, 54, 59, and 68 cm, respectively (**Table 1**). There are several explanations for the increases in the average water depths. In WY2016 the Central Flow-Way (Cell 2A and 2B) was not 100% operational due to vegetation management activities. In WY2017, the Central Flow-Way was the only flow-way of the three in STA-3/4 that was 100% operational with no restriction, thus the majority of the flow in WY2017 was treated in these two cells in WY2017. In WY2018, South Florida was affected by two large storms that significantly increased the total flow volume treated by the STAs. Daily average water depths during this period were generally greater than the target depth of 38 cm for EAV cells.

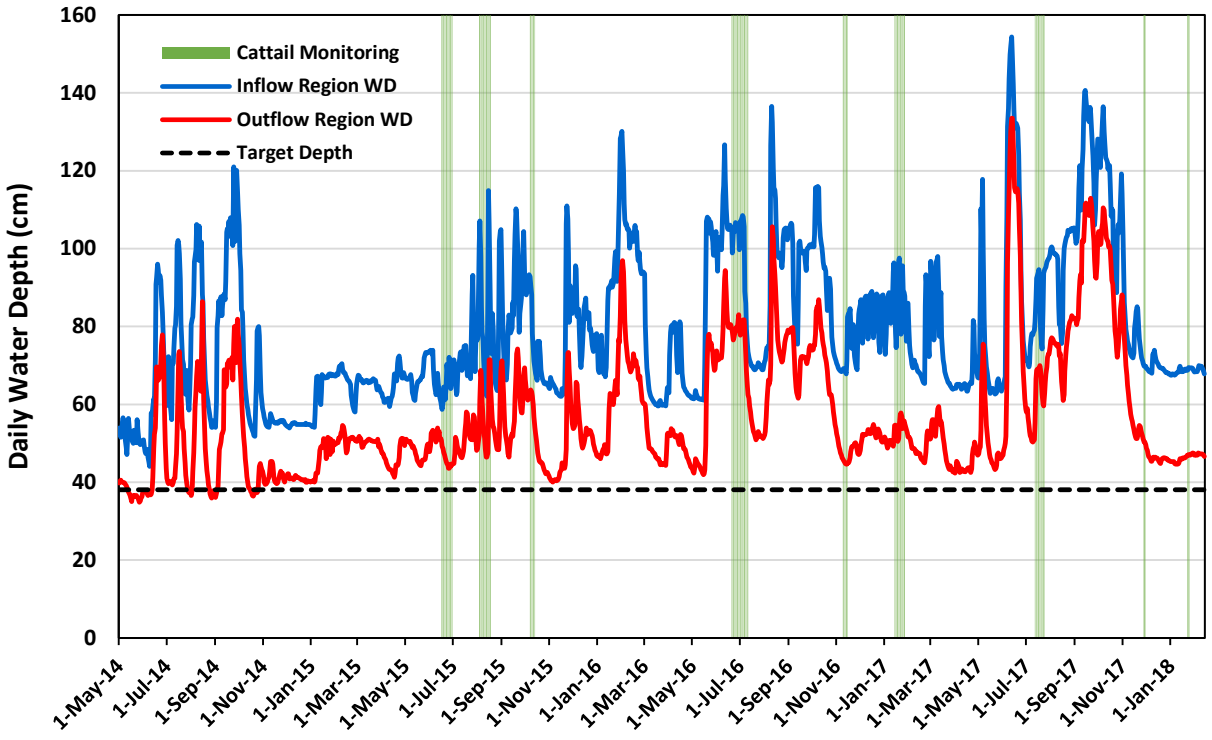

Figure 3. Daily water depths in STA-3/4 Cell 2A from WY2015 to WY2018 showing hydrologic events and cattail monitoring periods.

Table 1. Summary statistics for water depths at the inflow and outflow marsh regions of STA-3/4 Cell 2A.

Water Year [a]	Inflow Water Depth (cm)				Outflow Water Depth (cm)			
	Mean	Standard Deviation	Minimum	Maximum	Mean	Standard Deviation	Minimum	Maximum
WY2015	67	15	44	121	48	11	35	86
WY2016	77	15	59	130	54	10	40	97
WY2017	85	16	61	137	59	14	42	106
WY2018	90	24	63	154	68	24	43	134

a. Data range: May 1, 2014– February 13, 2018.

In the inflow region, 44% of the daily average water depths ranged from 61 to 76 cm, with 17.4% of the daily average water depths ranging from 76 to 91 cm and 26.4% of the daily average water depths exceeding 91 cm (**Table 2**). In contrast, in the outflow, region 66.4% of the daily average water depths ranged from 38 to 61 cm, with only 12.5% of the daily average water depths exceeding 76 cm (**Table 2**). Daily average water depths recorded from the five *Solinst* water level loggers deployed in the marsh were consistent with the daily water depth values from the DBHYDRO database with greater depths in the inflow region than in the outflow region. All daily average water level logger data from July 17, 2015, to September 10, 2017, in the inflow region exceeded 61 cm with 64% exceeding 76 cm. In contrast, 48% of the daily water depths in the outflow region ranged between 38 to 76 cm and only 28% exceeded 76 cm.

Table 2. Frequency of occurrence of water depth ranges at the marsh inflow and outflow regions of STA-3/4 Cell 2A in WY2015–WY2018.

Water Year [c]	Water Depth Range Categories (cm) [a]				
	< 38	38–61	61–76	76–91	> 91
	Days within Each Depth Range Category [b]				
Inflow					
WY2015	0 (0.0%)	155 (42.5%)	136 (37.2%)	34 (9.3%)	40 (11.0%)
WY2016	0 (0.0%)	20 (5.5%)	190 (51.9%)	83 (22.7%)	73 (19.9%)
WY2017	0 (0.0%)	0 (0.0%)	145 (39.7%)	90 (24.7%)	130 (35.6%)
WY2018	0 (0.0%)	0 (0.0%)	132 (45.7%)	34 (11.8%)	123 (42.5%)
Outflow					
WY2015	53 (14.5%)	263 (72.1%)	42 (11.5%)	7 (1.9%)	0 (0.0%)
WY2016	0 (0.0%)	286 (78.1%)	71 (19.4%)	5 (1.4%)	4 (1.1%)
WY2017	0 (0.0%)	227 (62.2%)	76 (20.8%)	54 (14.8%)	8 (2.2%)
WY2018	0 (0.0%)	143 (49.5%)	52 (18.0%)	36 (12.4%)	58 (20.1%)

a. Water depth ranges are based on stages at inflow and outflow structures.

b. Number of days per range category with their respective percentage in parenthesis.

c. Data range: May 1, 2014–February 13, 2018.

CATTAIL DENSITY

Cattail density measurements were averaged for inflow and outflow regions during the 2015, 2016, and 2017 wet seasons. For the 2015 wet season, the average total number of shoots/m^2 was not significantly different between the inflow and outflow regions ($p > 0.05$, **Figure 4A**). However, the number of adult shoots was significantly higher in the inflow plots and the number of dead shoots significantly higher in the outflow plots ($p < 0.05$). The total number of adult plants in the inflow region was generally higher than in the outflow region during the first-year monitoring events (June, August, and October 2015). In contrast, the total number of juvenile plants and dead plants were consistently higher in the outflow region with densities increasing toward the end of the monitoring season (October 2015). Average inflow region water depths from the 2015 wet season ranged from 67 cm in May–June 2015 to 84 cm at the end of the third monitoring event in mid-October 2015. Average outflow region water depths ranged from 48 cm in May–June 2015 to 58 cm at the end of the third monitoring event in mid-October 2015 (**Table 2**). The average cattail shoot density during the 2015 wet season was 9.3 and 8.9 shoots/m^2 in the inflow and outflow plots, respectively, which are within the range of cattail densities reported in STA-3/4 Cell 2B (Toth and Galloway 2009, Chen and Vaughn 2014).

Total number of cattail shoots in the 2016 wet season in plots within the inflow region (mean = 7.9 shoots/m^2) were significantly lower ($p < 0.05$, **Figure 4B**) than in plots from the outflow region (mean = 9.8 shoots/m^2). Total number of juveniles and dead cattail plants were also significantly lower ($p < 0.05$) in the inflow region than in the outflow region plots. Total number of adults decreased during 2016, particularly during the June 2016 event, with densities slightly higher in the outflow region in November 2016 and January 2017, in contrast to the 2015 season. The juvenile population was considerably higher in the outflow region, with the highest density observed in June 2016, then a considerable decline in November 2016, and followed by an increase in late January 2017. The difference between the 2015 and 2016 wet seasons were the prolonged deeper water depths in the inflow region in 2016. Other studies indicate that *Typha spp* adapt to deep water conditions through changes in morphology such as leaf height (Grace 1989). Average inflow water depths during the 2016 wet season ranged from 93 cm in May–June 2016 to 89 cm at the end of the second monitoring event in mid-November 2016 (deep inundation period of 198 consecutive days). Average water depths decreased to 83 cm from November to the end of the third monitoring event in late January 2017. In contrast, average outflow water depths from the 2016 wet season ranged from 69 cm in May–June 2016 to 51 cm at the end of the third monitoring event in late January 2017.

The total number of cattail shoots during the 2017 wet season within plots from the inflow region (mean = 6.2 shoots/m^2) were also significantly lower ($p < 0.05$, **Figure 4C**) than in plots from the outflow region (mean = 9.4 shoots/m^2). Total number of adult, juvenile, and dead cattail plants from plots in the inflow region were significantly lower than in the outflow plots ($p < 0.05$). The total number of adult plants in the inflow region decreased to the lowest densities observed in this three-year study while the adult density in the outflow region remained constant. We hypothesize that prolonged water depths during the 2016 and 2017 wet season resulted in a decrease of the adult cattail population in the inflow region of the cell. As inundation depth increases, the *T. domingensis* community tend to adapt to the new conditions producing fewer but larger ramets to escape the deeper waters (Grace 1989). The juvenile densities were consistently higher in the outflow plots, with densities decreasing from the July 2017 event to November 2017 and increased toward the end of the third event in January 2018. The juvenile plants in these plots are primarily propagated through rhizomes, and probably grow better through the water column in the shallower outflow region of this cell. Grace (1989) reported prolonged inundation periods adversely affect growth and reproduction of *Typha* species. Dead plant density remained higher in the outflow region, but overall remained constant in both inflow and outflow regions throughout the study, except for a notable spike in the outflow plots during the January 2018 event. Like the 2016 wet season, prolonged deeper water depths in the inflow region during the 2017 wet season may have decreased the overall cattail density in the inflow plots of this cell (**Figure 3**). Inflow water depths ranged from 88 cm in May–July 2017 to 103 cm at the

end of the second monitoring event in late November 2017 (deep inundation period of 214 consecutive days). Average water depths decreased to 69 cm from early December 2017 to the end of the third monitoring event in late January 2018. In contrast, average outflow water depths from the 2017 wet season ranged from 68 cm in May–July 2017 to 81 cm in in late November 2017 to 46 cm at the end of the third monitoring season in late January 2018.

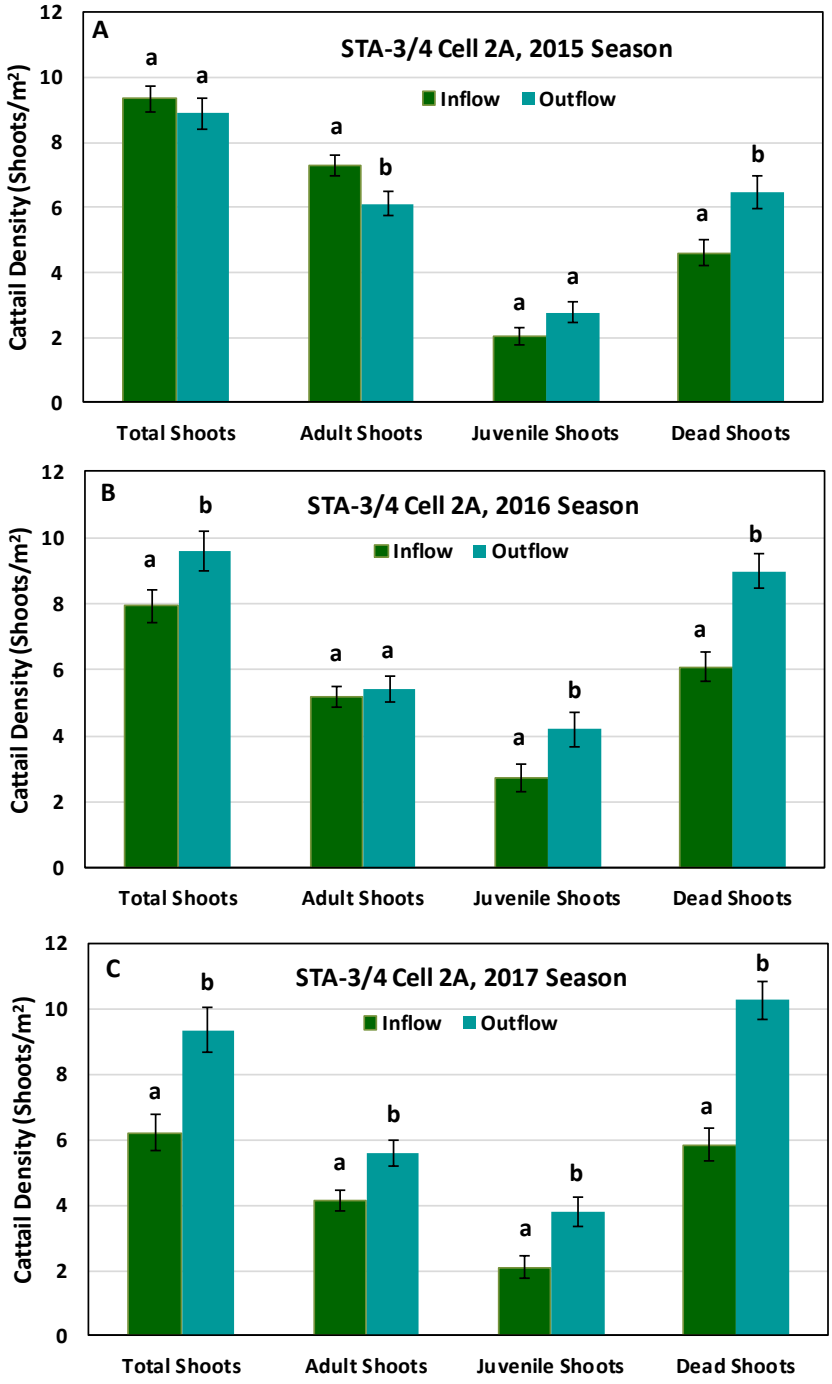

Figure 4. Cattail density parameters (mean ± standard error) in STA-3/4 Cell 2A: (A) 2015, (B) 2016, and (C) 2017 seasons. Mean differences with the same letter are not significantly different at the 0.05 probability level using the pooled t-test and Wilcoxon rank sum test.

LEAF ELONGATION

Leaf elongation rates in the deeper monitoring plots of the inflow region were consistently higher than in the shallower plots of the outflow region (**Figure 5**), however only the elongation rates for the 2015 season between the inflow (7.53 cm/d) and outflow (6.28 cm/d) regions were significantly different ($p < 0.05$, **Table 3**). Measurements during the 2015 wet season ranged from 7.1 to 7.8 cm/d in the inflow region and from 5.8 to 7.1 cm/d in the outflow region, with the highest rates observed during the months of June and August. Daily average water depths during the leaf elongation monitoring period in the inflow region during the 2015 wet season ranged from 68 cm in June 2015 to 81 cm in August 2015, compared to 46 and 56 cm during the same months in the outflow region, respectively. Leaf elongation rates during the 2016 wet season ranged from 5.4 to 10.2 cm/d in the inflow region and from 4.8 to 6.9 cm/d in the outflow region, with the highest elongation rate measured in June 2016 (**Figure 5**). Water depths from May 18, 2016, to July 6, 2016, averaged 105 and 77 cm in the inflow and outflow regions, respectively. This prolonged inundation period (50 consecutive days) may have resulted in increased leaf elongation rates measured in the inflow region during this period. To survive inundation, some species physically escape from the high water environment by increasing shoot growth (Voesenek et al. 2003). Leaf elongation rates during the 2017 monitoring season were slightly lower than in the previous two seasons, with rates decreasing from 6.3 cm/d in July 2017 to 5.4 cm/d in January 2018 in the inflow region, and from 6.3 cm/d in July 2017 to 3.6 cm/d in January 2018, in the outflow region. The average water depth in the inflow region during the shoot elongation monitoring periods in the 2017 wet season ranged from 69 cm in November 2017 to 91 cm in July 2017 compared to 48 cm and to 69 cm during the same months in the outflow region, respectively.

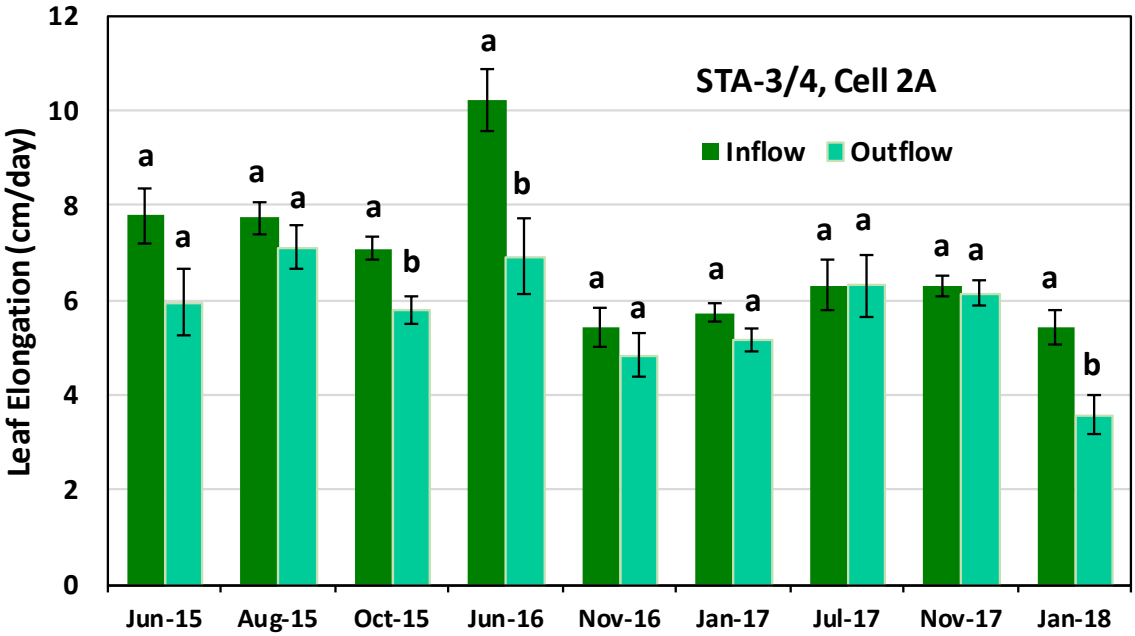

Figure 5. Leaf elongation rate (mean ± standard error) of data collected during the wet seasons of 2015, 2016, and 2017 from STA-3/4 Cell 2A. Mean differences with the same letter are not significantly different at $p = 0.05$ using the pooled t-test and Wilcoxon rank sum test.

Table 3. Cattail leaf elongation and gas exchange parameters from the inflow and outflow regions during the 2015, 2016, and 2017 wet seasons in STA-3/4 Cell 2A. [a]

Monitoring Zone	Leaf Elongation (cm/d)	Photosynthetic Rate (µmol $CO_2/m^2/s$)	Stomatal Conductance (mmol $H_2O/m^2/s$)	Water Use Efficiency (µmol CO_2/mmol H_2O)
2015 Monitoring [b]				
Inflow	7.53 ± 0.25 [c]	14.3 ± 0.80	0.44 ± 0.03	35.4 ± 1.5
Outflow	6.28 ± 0.30	14.5 ± 1.05	0.37 ± 0.04	38.4 ± 2.0
2016 Monitoring				
Inflow	7.33 ± 0.46	18.9 ± 0.68	0.58 ± 0.06	38.7 ± 2.1
Outflow	5.68 ± 0.55	19.9 ± 0.79	0.62 ± 0.07	41.0 ± 2.4
2017 Monitoring				
Inflow	6.02 ± 0.29	20.4 ± 0.96	0.78 ± 0.09	38.8 ± 3.2
Outflow	5.34 ± 0.34	21.1 ± 1.20	0.90 ± 0.11	40.8 ± 4.0

a. Key to units: cm/d – µmol $CO_2/m^2/s$ – micromoles carbon dioxide per square meter per second; µmol CO_2/mmol H_2O – micromoles carbon dioxide per millimole water; centimeters per day; and mmol $H_2O/m^2/s$ – millimoles water per square meter per second.

b. Means ± standard error.

c. Significant at the 0.05 probability level using the pooled t-test and Wilcoxon rank sum test.

Chen and Vaughan (2014) reported that increasing inundation depth from 30 to 80 cm to the cattail community in a South Florida STA added additional stress to the cattail community resulting in a decrease in shoot elongation. However, the data from this study indicates that deeper water conditions in the inflow region of this cell may have stimulated shoot growth rates of juvenile leaves, possibly as an effort to restore gas exchange between roots and the atmosphere (Bailey-Serres and Voesenek 2008). Other studies have indicated that some species, including *Typha* species, experience certain changes in morphology, especially leaf height, to survive growing in deeper waters (Grace 1989, Voesenek et al. 2003).

GAS EXCHANGE RATES

This analysis focuses on three parameters that are good physiological stress indicators: net photosynthetic rate, transpiration (i.e., stomatal conductance), and water use efficiency (WUE) (Kadlec and Wallace 2009, Liao and Lin 2001).

Photosynthetic rate is the rate at which the plant is able to fix carbon for growth. Low photosynthetic rates can indicate that a plant is stressed, while higher values show that the plant is growing under more favorable conditions. Photosynthetic rates across all three wet seasons were not significantly different ($p > 0.05$) between the inflow and outflow regions in this cell (**Table 3**). However, the data indicates that rates tended to be higher towards the end of the growing season (October/November) than early in the growing season (June/July) of each year (**Figure 6**). Chen et al. (2010) reported significant decreases in photosynthetic rates of *T. domingensis* at inundation depths of 91 and 137 cm, compared to those exposed to a flooding depth of 40 cm. Similar results were reported in a field study with decreases in photosynthetic rates of *T. domingensis* with increasing inundation depths from 30 to 80 cm (Chen and Vaughan 2014). The slight increases in gas exchange parameters observed in this study toward the end of the growing season was likely due to lower water levels in the cell coupled with slightly cooler temperatures, which may be less stressful for the cattail.

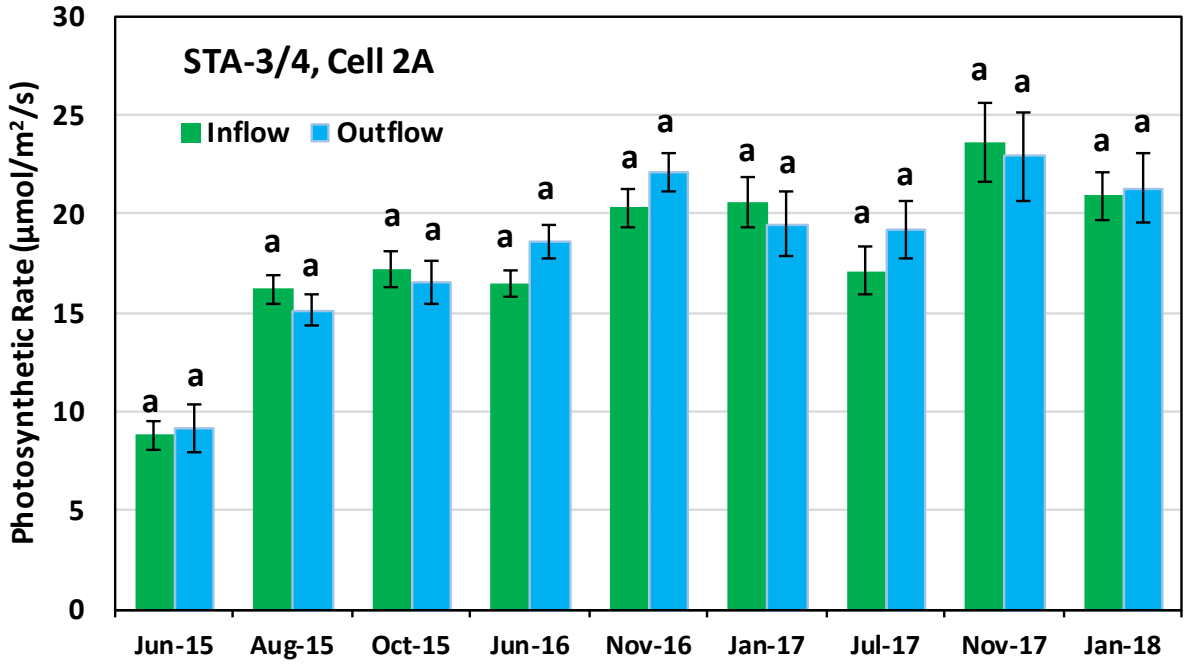

Figure 6. Photosynthetic rates (mean ± standard error) of *T. domingensis* in STA-3/4 Cell 2A during the 2015, 2016, and 2017 wet seasons. Mean differences for each event with the same letter are not significantly different at the 0.05 probability level using the pooled t-test and Wilcoxon rank sum test.

Transpiration, as measured by stomatal conductance, is the rate of water vapor exiting through the stomates in the leaves. Transpiration rates across all three wet seasons were not significantly different ($p > 0.05$) between the inflow and outflow regions in this cell (**Table 3**). An evaluation of the stomatal conductance between the inflow and outflow regions for each event showed that August 2015 was the only event where the value from inflow region was significantly higher ($p < 0.05$, **Figure 7**). Stomatal conductance from the June and October 2015 events were also slightly higher in the inflow region of the cell. For the 2016 and 2017 wet seasons, stomatal conductance was higher in the outflow region and tended to increase toward the end of the growing season (October/November) and decrease in the January events. Higher transpiration rates generally indicate a better physiological function, with higher rates generally observed during the rapid growth rate period of the plant coupled with higher temperatures, while lower stomatal conductance values indicate that the water demand for the plant is declining usually toward the end of the growing season (Cronk and Fennessy 2001). During the 2016 and 2017 wet seasons, the cell experienced deeper water depth conditions over prolonged periods from mid-July through mid-November (**Figure 3**) that primarily affected the inflow region of this cell. Despite the deeper water depths and more difficult growing conditions during the wet season in the inflow plots there was no reduction of transpiration.

WUE is defined as the ratio of carbon assimilation (photosynthesis) to the rate of transpiration. WUE rates between the inflow and outflow regions were not significantly different ($p > 0.05$) for any of the three wet seasons (**Table 3**). The only statistically significant ($p< 0.05$) difference in WUE between the inflow and outflow sites was found in the November 2016, event ($p < 0.05$, **Figure 8**). WUE rates from individual events were generally lower at the end of the growing season (November), especially during the 2016 and 2017 wet seasons than in the earlier part of the growing season, and increasing again in the January events, when the transpiration values decreased.

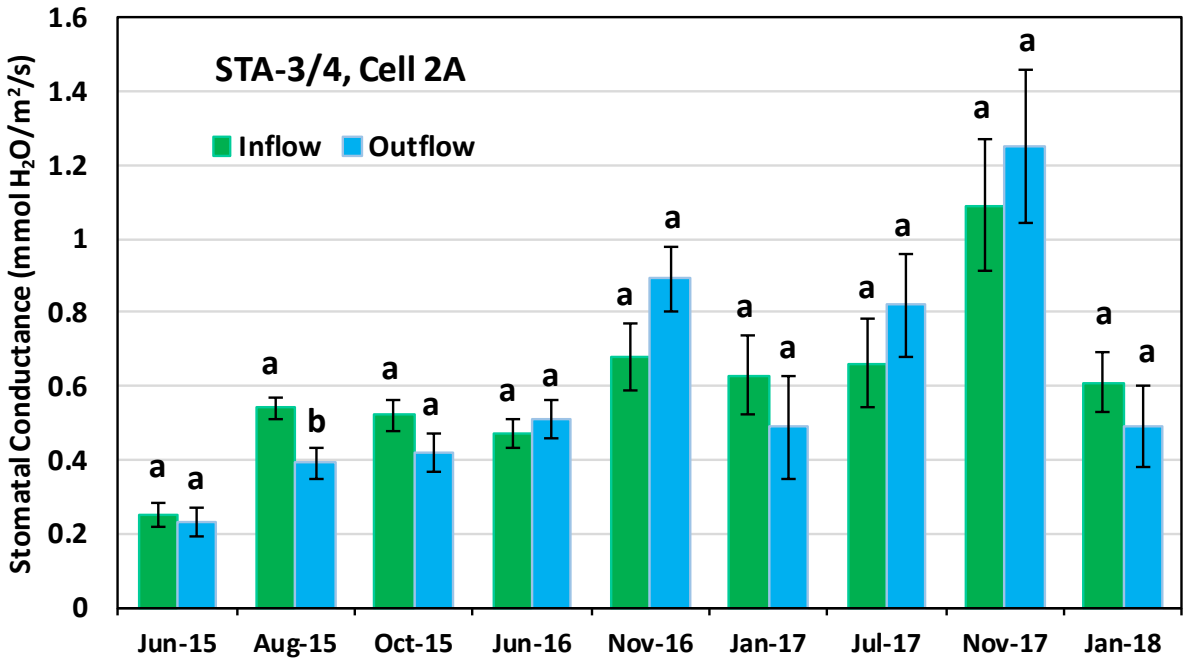

Figure 7. Stomatal conductance (mean ± standard error) of *T. domingensis* in STA-3/4 Cell 2A during the 2015, 2016, and 2017 wet seasons. Mean differences for each event with the same letter are not significantly different at the 0.05 probability level using the pooled t-test and Wilcoxon rank sum test.

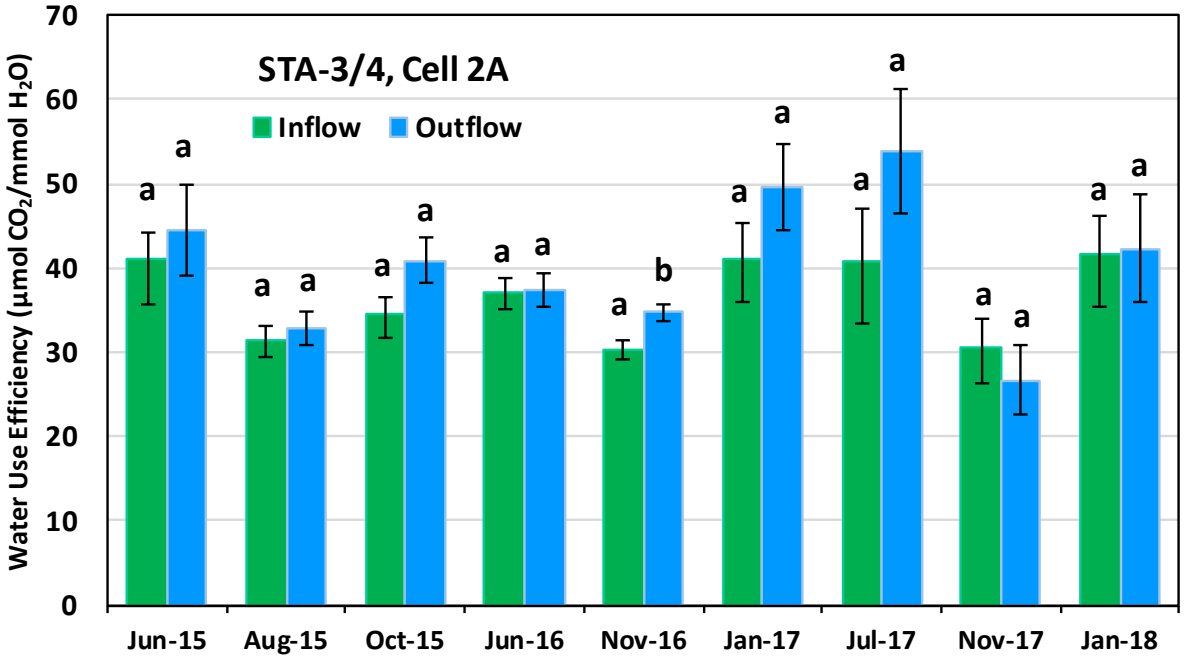

Figure 8. WUE (mean ± standard error) of *T. domingensis* in STA-3/4 Cell 2A during the wet seasons of 2015, 2016, and 2017. Mean differences for each event with the same letter are not significantly different at the 0.05 probability level using the pooled t-test and Wilcoxon rank sum test.

CATTAIL BIOMASS

Inflow versus Outflow Biomass

Biomass of the different plant components in the initial sampling (November 2014) was slightly higher in the outflow region than in the inflow region, except leaf biomass. However, the differences were not significant ($p > 0.05$). Similar results were observed in the second (October 2015) and third (November 2017) harvesting events with no significant differences ($p > 0.05$) between plots in the inflow and outflow regions, except the root and belowground biomass in the second biomass sampling ($p < 0.05$, **Table 4**). Total dead biomass (necromass) was consistently higher in the outflow region plots of this cell, with values from the November 2014 significantly higher ($p < 0.05$). The higher number of juveniles and dead cattail plants in the outflow region plots reported in the *Cattail Density* section earlier in this appendix plus the presence of considerably more necromass may indicate that there is a higher turnover rate in outflow region of this cell. Likewise, Chen and Vaughan (2014) also reported that leaf and total biomass from a cattail community from STA-1 East in South Florida were not significantly affected by inundation depths.

Table 4. Cattail biomass (mean ± standard error) in grams per square meter (g/m^2) from the inflow and outflow regions collected in November 2014, October 2015, and November 2017 in STA-3/4 Cell 2A.

Zone	Live Biomass Components [a]				Belowground Biomass	Total Live Biomass	Necromass	Belowground/ Leaf Ratio
	Leaf	Root	Rhizome	Shoot Base				
November 2014								
Inflow	1,084 ± 191	65 ± 20	93 ± 21	692 ± 107	850 ± 132	1,934 ± 301	2,520 ± 388 [b]	0.84 ± 0.11
Outflow	892 ± 144	119 ± 21	118 ± 32	837 ± 173	1,074 ± 196	1,965 ± 302	4,903 ± 475	1.31 ± 0.25
October 2015								
Inflow	931 ± 133	43 ± 7 [b]	50 ± 12	168 ± 45	261 ± 47 [b]	1,192 ± 155	2,193 ± 438	0.31 ± 0.08 [b]
Outflow	842 ± 151	94 ± 19	87 ± 19	249 ± 54	430 ± 57	1,272 ± 182	3,140 ± 536	0.57 ± 0.08
November 2017								
Inflow	478 ± 91	39 ± 7	72 ± 19	117 ± 53	228 ± 60	706 ± 128	1,565 ± 408	0.49 ± 0.10
Outflow	388 ± 59	58 ± 11	54 ± 16	143 ± 33	255 ± 43	643 ± 92	2,521 ± 500	0.68 ± 0.09

a. Means ± standard error.

b. Significantly different at the 0.05 probability level using the pooled t-test and Wilcoxon rank sum test.

Aboveground biomass between the inflow and outflow regions were not significant ($p > 0.05$); however, the biomass values from the inflow region plots were consistently higher than in the outflow region plots, suggesting that the cattail plants from the inflow plots produced larger ramets to survive and escape the deeper water conditions from the inflow region (**Figure 9**). Grace (1989) reported that *Typha* species tend to adjust to deeper water depths through changes in shoot and ramet morphology producing fewer but larger ramets. Total belowground biomass differences between the inflow and outflow regions were significant only during the October 2015 sampling ($p < 0.05$), with biomass values consistently lower in the inflow region plots. Other studies have reported that increasing water depth decreases the biomass allocation to rhizome and roots reducing the anchorage capacity of cattail plants (Grace and Wetzel 1982, Miao and Zou 2012).

This study also showed a decrease in the belowground biomass: leaf ratio in all sampling events for the inflow region plots of this cell, with a significant ($p < 0.05$) difference only during the second biomass sampling (**Table 4**). A decrease in the belowground biomass: leaf ratio suggests that root and rhizomes of

T. domingensis were stressed more substantially than shoots in the inflow region of this cell (Chen et al. 2010). The daily water depths in the inflow region were consistently deeper than in the outflow region of this cell (**Figure 3**). The higher water depths in the inflow region were more noticeable during the cattail monitoring events in 2016 and 2017, where the average water depths in the inflow region ranged from 83 to 93 cm and 69 to 103 cm for years 2016 and 2017, respectively, compared to outflow water depths ranges of 51 to 69 cm and 46 to 81 cm, for the same years, respectively. Belowground biomass in the first, second, and third biomass samplings was 21, 39, and 11% lower in the inflow plots than in the outflow plots, indicating that the cattail community in the inflow region were under more stress due to the prolonged deeper water depths in that region of the cell. Chen et al. (2010) on a study of the effect of flooding depth on *T. domingensis* reported a decrease of approximately 80% of root and rhizome biomass on cattail plants flooded for a period of six weeks at 137 cm.

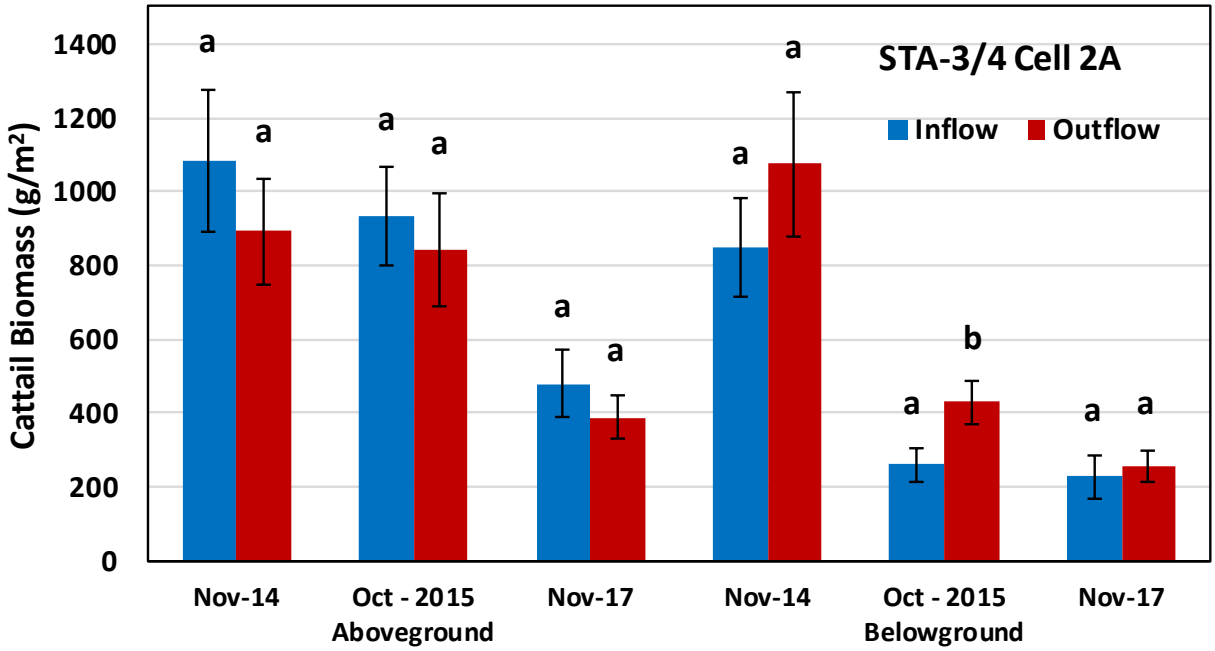

Figure 9. Cattail biomass (mean ± standard error) in grams per square meter (g/m^2) from the inflow and outflow regions collected in November 2014, October 2015, and November 2017. Mean differences with the same letter for each event are not significantly different at the 0.05 probability level using the pooled t-test and Wilcoxon rank sum test.

Temporal Trends in Biomass and Necromass

Comparison of plant biomass from the three years of sampling showed a significant decrease in all components over time ($p < 0.05$, **Figure 10**). Aboveground biomass in the second and third year was 89 and 44% of the initial aboveground biomass in 2014, with the difference between the first and second sampling not significant ($p > 0.05$). Belowground biomass in the second and third year was 40 and 25% of the belowground biomass in the initial year of sampling; with biomass values from the second and third years not significantly different ($p > 0.05$). Total live biomass significantly decreased from the 2014 sampling (1,947 grams per square meter [g/m^2]) to 1,224 and 681 g/m^2 in the 2015 and 2017 samplings, respectively. There was also a notable change in biomass distribution in terms of aboveground and belowground biomass. During the initial year, biomass was approximately evenly distributed at 52% leaf and 48% belowground biomass. This distribution shifted in 2015 during which 73% was in leaves and only 27% in belowground biomass. In 2017, 65% of the biomass was in leaves and 35% was in belowground

biomass. Total biomass from the initial sampling was slightly lower than the total biomass reported by Chen and Vaughan (2014), and slightly higher than the biomass reported in Water Conservation Area 2A by Miao and Sklar (1998). Total biomass from the second and third harvests were lower than both studies.

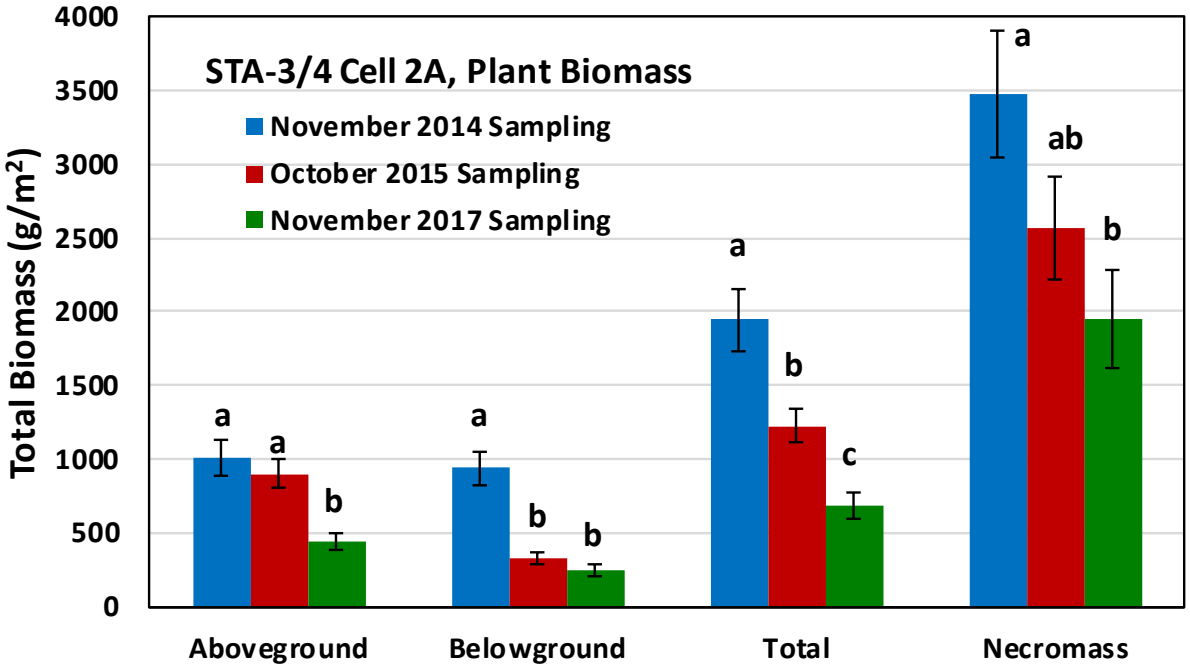

Figure 10. Total biomass (mean ± standard error) of samples collected November 2014, October 2015, and November 2017 biomass from STA-3/4 Cell 2A. Mean differences with the same letter are not significantly different at the 0.05 probability level using the Tukey-Kramer honest significant difference Test and Duns Allpairs test.

Biomass Nutrient Content

Plant biomass nutrient concentrations in the November 2014 sampling were not significantly different between inflow and outflow regions ($p > 0.05$), however, TP concentrations were generally higher in the inflow than in the outflow region with the highest concentration measured in the shoot base from the inflow region (**Figure 11A**). TN behaved differently with concentrations in the root, rhizome, and necromass higher in the outflow region, and concentrations in the leaves and shoot base higher in the inflow region (**Figure 12A**). TC concentrations were slightly higher in the outflow than in the inflow, with concentrations in the roots significantly different between the inflow and outflow regions (**Figure 13A**, $p < 0.05$).

TP concentrations from all the biomass components in the October 2015 sampling were significantly higher ($p < 0.05$) in the inflow region than in the outflow region, with highest concentration measured in the shoot base from the inflow region (**Figure 11B**). Likewise, TN concentrations in all plant components were also higher in the inflow region than in the outflow region, with concentrations in the rhizome, shoot base, and necromass significantly higher (**Figure 12B**, $p < 0.05$). TC concentrations were not significantly different between inflow and outflow regions, except in shoot base ($p < 0.05$), with concentrations generally higher in the outflow region (**Figure 13B**).

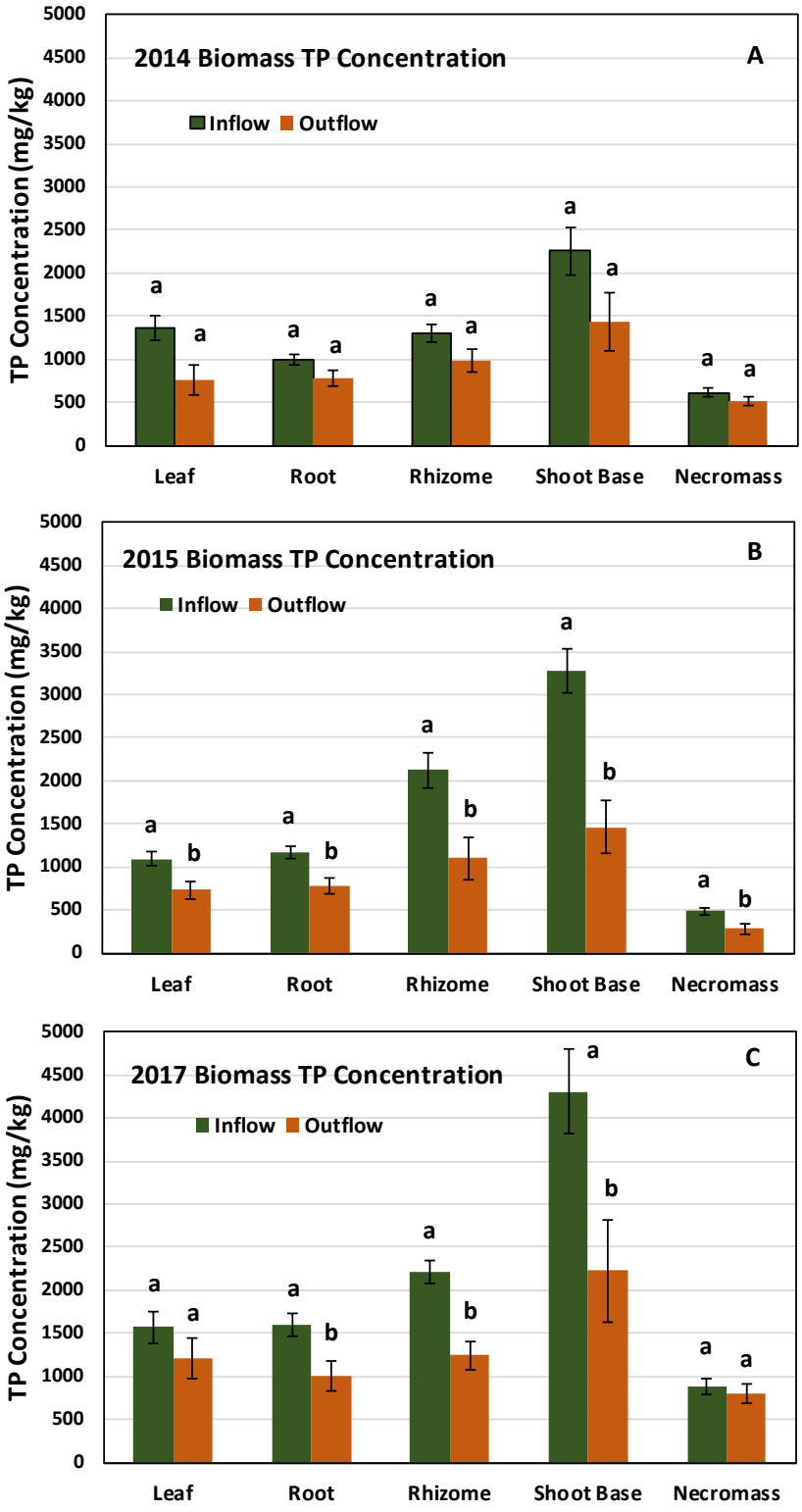

Figure 11. Cattail biomass TP concentration (mean ± standard error) in milligrams per kilogram (mg/kg) from the inflow and outflow regions collected in November 2014, October 2015, and November 2017. Mean differences with the same letter are not significantly different at the 0.05 probability level using the pooled t-test and Wilcoxon rank sum test.

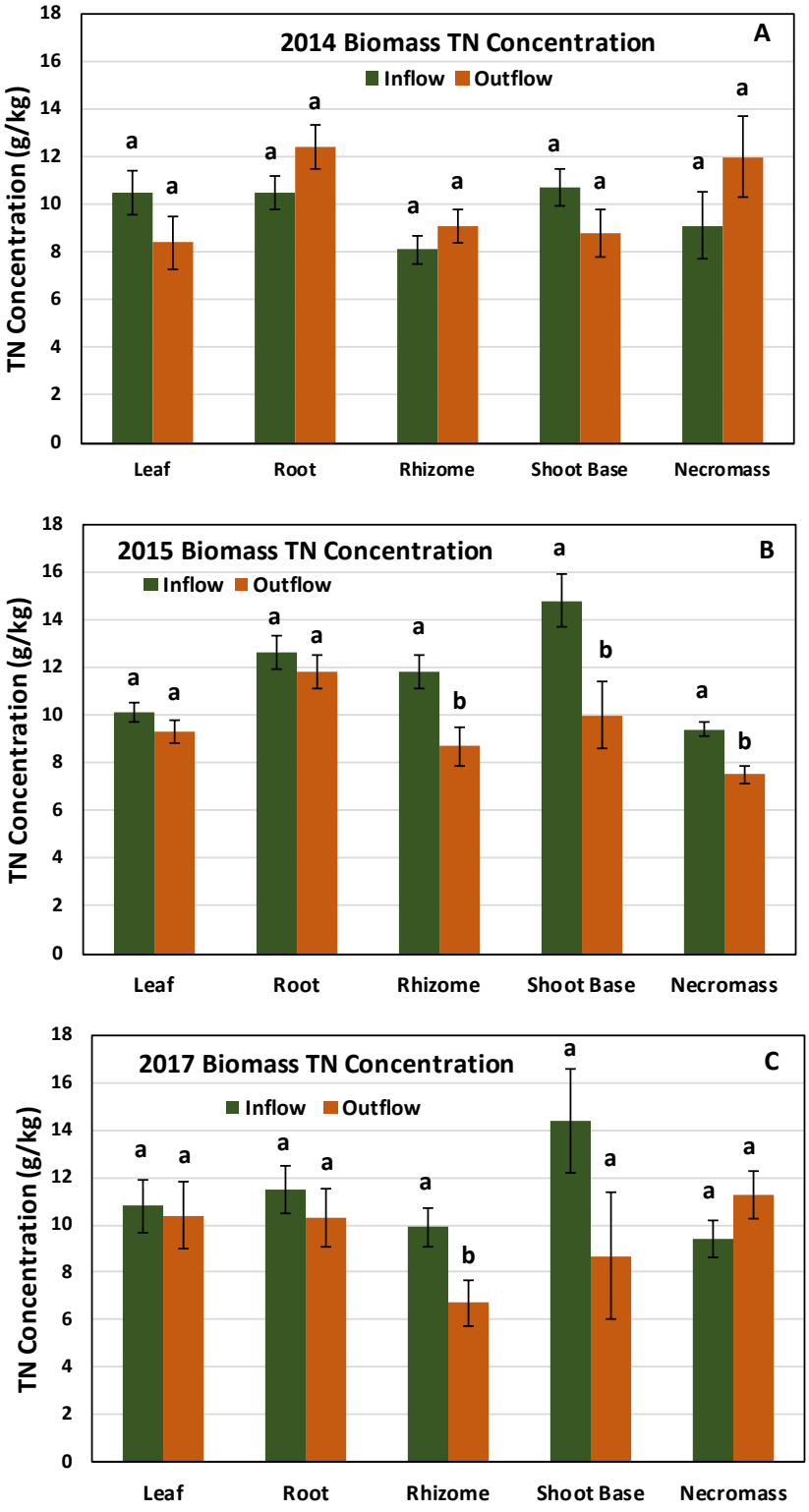

Figure 12. Cattail biomass TN concentration (mean ± standard error) in grams per kilogram (g/kg) from the inflow and outflow regions collected in November 2014, October 2015, and November 2017. Mean differences with the same letter are not significantly different at the 0.05 probability level using the pooled t-test and Wilcoxon rank sum test.

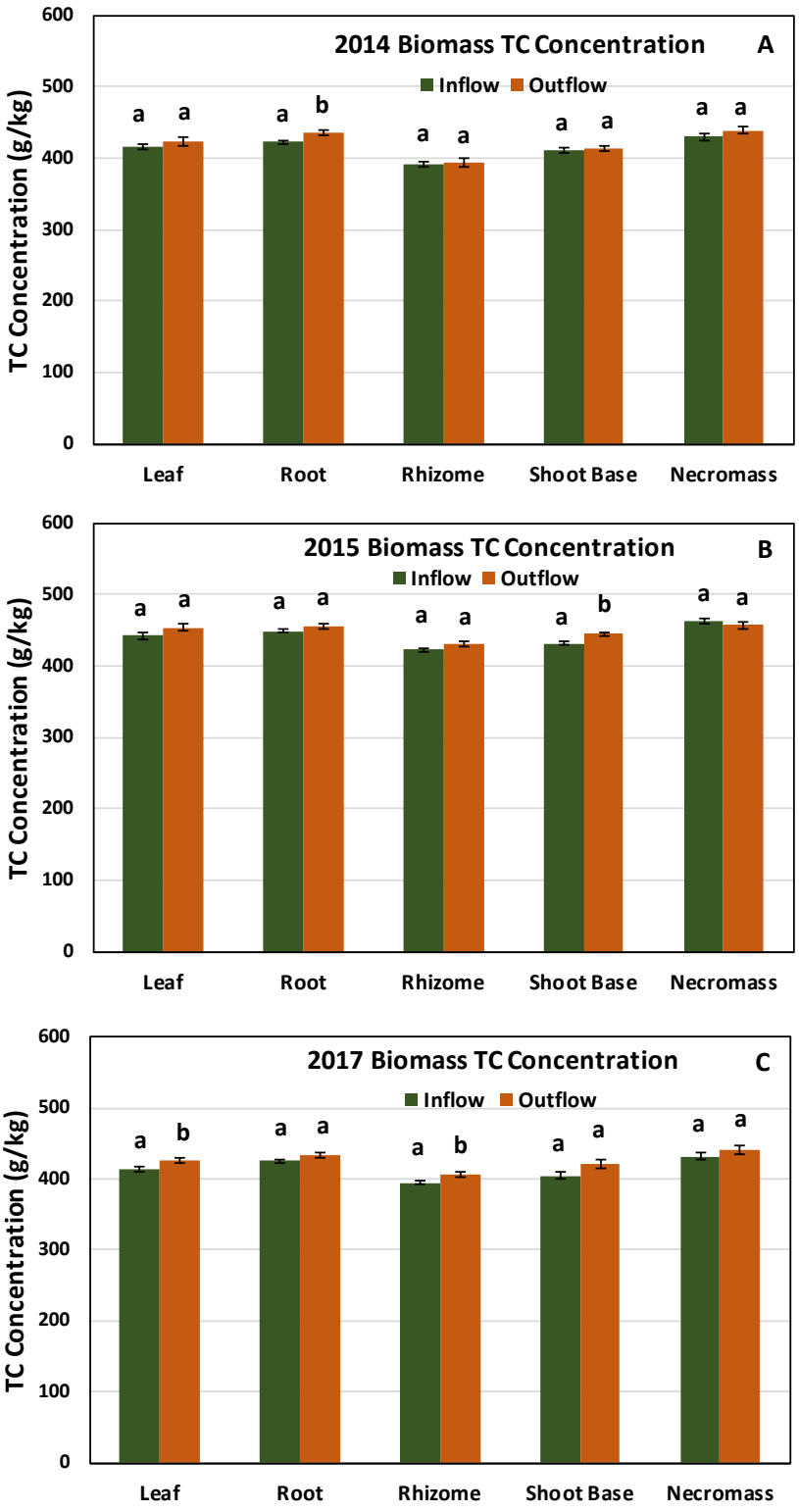

Figure 13. Cattail biomass TC concentration (mean ± standard error) from the inflow and outflow regions collected in November 2014, October 2015, and November 2017. Mean differences with the same letter are not significantly different at the 0.05 probability level using the pooled t-test and Wilcoxon rank sum test.

TP concentrations from all biomass components of the November 2017 sampling were also higher in the inflow than in the outflow region, with TP concentrations in the roots, rhizome, and shoot base significantly higher (**Figure 11C**, $p < 0.05$) in the inflow region. Likewise, TN concentrations in all plant components from the inflow region were also higher than in the outflow region, except in the necromass, with concentrations in the rhizomes significantly higher (**Figure 12C**, $p < 0.05$) in the inflow region. TC concentrations were generally higher in the outflow region of the cell, with concentrations in the leaves and rhizomes significantly different (**Figure 13C**, $p < 0.05$).

Total P concentrations in all biomass components showed a noticeable decline in concentration from the inflow to the outflow region. Water nutrient concentrations are generally higher in the inflow region of the cell where, in EAV cells, the cattail population is the first line of defense to remove nutrients from the water column. Villapando and King (2018) reported a strong TP concentration gradient from inflow to outflow in the Evaluation of P Sources, Forms, Flux and Transformation Processes in the STAs (P Flux Study), with concentration reductions as high as 80% in some flow events.

For the different cattail biomass components, the highest TP concentrations in the inflow plots during the three-year period were measured in the shoot base, followed by rhizomes, roots, leaves, and necromass. Shoot base and rhizome biomass from the inflow region showed a considerable increase in TP concentration over time (**Figure 11**). Shoot base TP concentrations from inflow region in the 2015 and 2017 biomass samplings were 145 and 190% of the initial biomass sampling in 2014. Rhizome TP concentrations from the inflow region in the 2015 and 2017 biomass samplings were 163 and 170% of the 2014 biomass sampling. Outflow TP concentrations from all biomass components remained constant over time, except shoot base from the 2017 biomass sampling with an outflow TP concentration of 155% of the 2014 biomass sampling.

Shoot base and rhizome were the only biomass component that showed a considerable increase in TN concentration over time (**Figure 12**). Shoot base TN concentrations from the inflow region in the 2015 and 2017 biomass samplings were 138 and 135% of the initial biomass sampling in 2014. Rhizome TN concentrations from the inflow region in the 2015 and 2017 biomass samplings were 146 and 122% of the 2014 biomass sampling. Root biomass from the outflow region was the most noticeable component that showed a decrease in TN concentration over time. Root TN concentration from the outflow region was 95 and 83% of the initial biomass sampling in 2014. TC concentrations in all biomass components remained nearly constant over time, with concentrations slightly higher in the outflow region (**Figure 13**).

CONCLUSIONS

Results from this study, suggest that the cattail community in the deeper inflow region was under higher stress than in the outflow region of this cell particularly during the extended periods of deep-water conditions that occurred in the 2016 and 2017 wet seasons. The cattail plants in regions of deeper water responded with increased elongation rates and taller ramets to increase the above water plant surface that provide oxygen to the roots through the lenticels and aerenchymous tissues (Grace 1989, Brix 1993). However, even with the increase in plant height to adjust to the deeper water conditions, total cattail density (adult and juvenile) and total belowground biomass of the cattail community in the deeper inflow region declined after the 2015 season. A decrease in the belowground biomass: leaf ratio suggest that the roots and rhizomes of the cattail plants were under higher stress than the shoots in the deeper inflow region of the cell. Results from the in situ study are still inconclusive to properly address the three hypotheses being evaluated by the Cattail Study. We expect that results from the different water depths and duration of inundation treatments from the second phase of the Cattail Study will provide enough information to properly answer these hypotheses and better define the inundation depth and duration thresholds of *Typha domingensis* in the STAs.

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