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1 The influence of seagrass donor source on small-scale transplant resilience

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Abstract

1. Concern for conservation of seagrass habitat has prompted international transplantation-style restoration efforts. A recent review of these restoration efforts has highlighted the low success associated with small-scale restorations, yet scaling up transplantation effort may be too costly for underfunded regions. Small-scale transplant survival can be enhanced with alleviation of two underlying issues: restoration site selection and donor site selection.
2. To investigate appropriate donor source selection, donor site environmental influence on seagrass (*Halodule wrightii*) transplant survival was examined by transplanting donor cores from two environmentally disparate sites to a transplantation site with limited environmental uncertainties. Donor sites were chosen to represent either end of a benthic light gradient (high versus low) to elucidate seagrass resilience to transplantation stress, with respect to donor site conditions.
3. After total loss of the first trial, a second trial was conducted with stabilizing mesh placed over transplants to reduce stingray bioturbation. The second trial resulted in 100% survival of high light transplants after 12 months and moderate survival (30-60%) of low light transplants for the first six months.
4. At 18 months, the second trial ended after sediment burial from a hurricane. One year post-burial, a patch of *H. wrightii* recovered at the high light transplant site; after six

years the patch expanded to approximately 74 m^2, an area 37-fold larger than originally planted.

5. Results from this transplant experiment provide evidence that donor environment plays a role in transplant resilience. The transplants sourced from high light had 47% greater leaf area per shoot, were more resistant to transplantation stress, and recovered following an extreme event relative to low light transplants. Therefore, selection of donor plants with more resilience features, a transplantation site with limited environmental uncertainties, and adaptive intervention can enhance seagrass resilience at a small planting scale.

Keywords: estuary, coastal, restoration, reintroduction, macrophytes, benthos, urban development, nutrient enrichment

1. Introduction.

The increasing rate of global seagrass habitat loss, and the concomitant loss of many ecosystem services provided by and associated with these habitat-forming species, has raised conservation concerns worldwide (Butler & Jernakoff, 1999; Oceana, 2010; Orth, Harwell, & Inglis, 2006; Waycott et al., 2009). The recognition that seagrass loss readily translates to economic losses (Costanza et al., 1997; Duke & Kruczynski, 1992; Vassallo et al., 2013) has motivated international efforts to identify potential remediation tactics (e.g. Novagrass, Seagrass Restoration network, World Seagrass Association, among others). Causal mechanisms for seagrass loss are believed to stem primarily from increased turbidity of coastal waters due to enhanced watershed urbanization and nutrient pollution (Duarte, 2002; Short & Wyllie-Echeverria, 1996). Reductions in nutrient load from coastal watersheds have led to local recovery of seagrass beds (Cardoso et al., 2010; Tomasko et al., 2018; Vaudrey, Kremer, Branco, & Short, 2010), but on a global scale seagrasses are still declining (van Katwijk et al., 2016). Due to natural limitations of vegetative expansion and recruitment, amelioration of seagrass losses within historical seagrass habitat often requires transplantation for the purposes of reintroduction of a viable population following the return of amenable conditions (Fonseca, 2011; Orsenigo, 2018; van der Heide et al., 2007).

Transplantation of seagrass from donor sites is a common method of restoration (Bayraktarov et al., 2016) because it requires limited technical skill and is one of the few options available for restoration of a species with low or irregular reproduction. However, as indicated by a meta-analysis of transplantation efforts by van Katwijk et al. (2016), not all transplantation techniques have shown similar levels of success. Authors of the 2016 meta-analysis identified a positive relationship between restoration success and planting scale, that is likely explained by the incorporation of high transplant unit redundancy across a typically dynamic shallow subtidal environment. When examining small-scale restorations (< 1,000 transplant units), van Katwijk et al. (2016) found survival rates to be only approximately 37%, yet small scale restorations made up over half of all restoration effort. Donor seagrass transplantation can be costly, wherein the lowest estimate per hectare of planted area using the least technical methodologies is approximately US$35,000 (Bayraktarov et al., 2016, accounting for inflation). Such restoration costs hinder large-scale seagrass restoration projects in low monetary resource regions, possibly explaining the large reported percentage of small-scale transplant effort. Frequent reports of little to no success with small-scale restoration efforts are likely to lower confidence in funding future transplantation efforts as a seagrass conservation technique. For example, following an analysis of transplantation success rates in Australia, Ganassin and Gibbs (2008) gave the recommendation of lack of confidence for this conservation method to the NSW, Australia Department of Industry. Success rates of lower cost small-scale transplantations need to be improved for better promotion of this low-cost technique as a viable conservation method. Therefore, research efforts should be used to assist with alleviation of two problems that plague most small-scale restorations: improper restoration site selection and improper donor material selection (Fonseca, Kenworthy, & Thayer, 1998; Paling, Fonseca, van Katwijk, & van Keulen, 2009; Treat & Lewis, 2006; van Katwijk et al., 2009).

Of the two commonly encountered transplantation problems, improper restoration site selection can typically be remedied with thorough background research into the uncertainties and risks of potential restoration sites and basic knowledge of the local seagrass species' biological/physiological requirements and environmental tolerances (Fonseca, 2011; Thom et al., 2005). As extensively detailed by Thom et al. (2005), uncertainties that could affect the outcome of seagrass transplantation experiments include: lack of knowledge regarding transplantation site conditions; lack of knowledge regarding reasons for seagrass losses at site; poor knowledge regarding past and potential future disturbances and stressors; poor understanding of natural and anthropogenic controlling factors affecting habitat-forming processes (e.g. hydrology, future landscape development); unpredicted human interactions with a transplantation project affecting project performance (e.g. boating encounters, trampling); and natural climate variability effects. Addressing these uncertainties prior to transplantation should minimize transplant failure caused by improper site selection.

Alternatively, despite recommended guidelines for donor plant material selection based on reviews of trial results (see Paling, Fonseca, van Katwijk, & van Keulen (2009) and van Katwijk et al. (2009)), proper donor site selection remains a challenge for small-scale transplantation trials (Novak, Plaisted, Hays, & Hughes, 2017). Current guidelines for best donor selection practices recommend that genetically diverse donor material be collected from habitats that are environmentally similar to restoration sites (Calumpong & Fonseca, 2001; Fonseca et al., 1998; van Katwijk et al., 2009). However, seagrass declines have lowered the availability of viable source material and incorporation of genetic diversity in transplanting could require collections over distances with high environmental variability. Honing best practices for donor selection in regions with substantial seagrass decline requires further investigation to enhance success of small-scale transplantation trials.

In the north-central Gulf of Mexico (nGoM) region of the U.S., historical losses of shoalgrass (*Halodule wrightii*) have left behind discontinuous patches available for donor material collection (Handley, Altsman, & DeMay, 2007). These patches are often located in abiotically diverse environments based on degree of watershed influence (Handley et al., 2007). One defining environmental characteristic among these nGoM potential donor *H. wrightii* patches is variation in the light availability that can range from chronically turbid to predominantly clear (McCarthy, Otis, Mendez-Lazaro, & Muller-Karger, 2018). For seagrasses, light conditions and environmental variability can drive phenotypic responses that lead to distinct morphological and growth attributes (McDonald et al., 2016; Ralph, Durako, Enríquez, Collier, & Doblin, 2007). Such attributes can play a large role in the resilience features of natural seagrass populations, such as the self-facilitative positive feedback link where shoot density drives suspended particle deposition and environmental stabilization (Carr, D’Odorico, McGlathery, & Wiberg, 2010; van der Heide et al., 2007). Connections between morphological features and better establishment of seagrass transplants have been tentatively made (Novak et al., 2017), but the relationship between donor environment, donor morphology, and transplant establishment needs further investigation to determine if such a relationship can be used to estimate quality of seagrass transplant candidates.

Described herein are the results of experimental trials conducted to assess whether inherent environmental condition of the donor site influences survival of seagrass transplants in a small-scale transplantation. Donor source material was selected from two spatially disconnected seagrass populations at either end of a light availability gradient for transplantation into a pre-selected, mid-gradient site. Survival and morphology of transplanted shoots were monitored as well as physical conditions of the transplant site to determine the potential for small-scale restoration success. Evidence of a link between donor site environment and transplant resilience would, therefore, help restoration practitioners with limited donor choices to select the optimal donor material for greater opportunity of seagrass transplantation success. The techniques and findings from this study will enhance the accessibility of transplantation as an option for restoration and conservation activities pertaining to this vital habitat.

2. Methods.

2.1 Transplantation Species.

An appropriate candidate for seagrass transplantation in the nGoM is shoalgrass (*Halodule wrightii*), a tropical/subtropical species whose distribution extends throughout the GoM, Caribbean, Atlantic Coast of the U.S. and Bermuda (van Tussenbroek, Barba Santos, Wong, van Dijk, & Waycott, 2010). Shoalgrass is characterized as a pioneer species that readily colonizes unconsolidated sediments (Zieman, 1982) with a rapid vegetative expansion rate (Marbà & Duarte, 1998) that serves to expedite restoration site establishment. It is also ideal for transplantation into dynamic, estuarine influenced coastlines due to the species' wide salinity tolerance range (Lirman & Cropper, 2003; Mazzotti et al., 2007). Earlier establishment of a perennial, disturbance-tolerant species like shoalgrass is beneficial in areas like the Gulf of Mexico that are prone to climate variability related events (e.g. hurricanes, coastal flooding, etc.). Intrinsic plant properties promote positive feedbacks, such as density dependent wave attenuation (Suykerbuyk et al., 2016), and establishment of seedbanks during calm periods between storm related disturbance events serves as a vital recovery feature (Fonseca et al., 1998; Orth et al., 2006). The use of shoalgrass as the transplantation species will somewhat limit transplantation site uncertainties related to disturbances caused by climate variability and extreme events, because of the high growth rate and recovery features that define a pioneer seagrass species.

2.2 Transplantation site selection.

To limit the more predictable uncertainties related to transplant site characteristics, site selection was based on the following criteria for *H. wrightii* requirements: light quality regularly exceeded a conservative estimate for productivity requirements (14-33% surface irradiance (SI), Choice, Frazer, & Jacoby, 2014; Shafer, 1999; Steward, Virnstein, Morris, & Lowe, 2005); salinity and temperature ranges during the most active periods of growth were near optima (15-30 psu, Madden & McDonald, 2006; 25-30°C, Lee, Park, & Kim, 2007, respectively) between

April and September (as based on leaf elongation peaks (Dunton, 1994); limited runoff that would cause fluctuations in environmental stressors; a site that would be protected from future shoreline development, limited wave disturbance, and a suitable sediment type. Little Lagoon, Alabama (30.24°N, 87.78°W), a 12.6 x 0.8 km nearly enclosed lagoon system with a residence time of approximately 10 days (Monsen, Cloern, Lucas, & Monismith, 2002; Figure 1.) was selected based on the site selection requirements. Salinity in Little Lagoon typically ranges from 15-36 ppt and natural, unaltered shoreline encircles 68.3% of the lagoon (Jones & Tidwell, 2012). Pre-transplant inspection of a site on the western end of the lagoon found an average SI of 62% (±2.2 SE) across from a saltmarsh-vegetated shoreline, limited wave action due to protection from a nearby peninsula, and sandy sediments. This site is also within the bounds of the Bon Secour National Wildlife Refuge, providing protection against future coastal development and limiting unpredicted human interaction.

Historical declines of *H. wrightii* populations in Little Lagoon are believed to have been brought about by declines in water quality due to heavy agricultural and septic tank influence to the watershed (Davies, 2000; Liefer, MacIntyre, Su, & Burnett, 2014; Murgulet & Tick, 2009;Vittor and Associates, 2005). Development of sewer service utilities in the area and regular maintenance of the 25-m wide connection to the GoM to improve lagoon flushing have resulted in recently enhanced water quality of the undeveloped, westernmost portion of the lagoon (ADEM, 2010). A modest decadal increase in *H. wrightii* areal coverage (Vittor and Associates, 2005 & 2009) suggested improvements in water quality would allow for new seagrass growth in

Little Lagoon. For all the above reasons, the western Little Lagoon site was selected for the transplantation experiment.

2.3 Donor sites for experimental transplantation.

Donor sites were selected from coastal Alabama, where water column abiotic variability is dominated by the influence of the large discharge volume from the Mobile Bay plume that flows westward out of Mobile Bay to the Mississippi Sound (Dzwonkowski, Park, & Collini, 2015). The first donor site was a *H. wrightii* meadow located West of Mobile Bay on the western shore of the Point aux Pins (PaP) peninsula (30.38°N, 88.31°W; Figure 1) that experiences regular low light conditions (average light extinction coefficient (k)= 1.9 m^{-1}±0.15 SE). The second donor site was a *H. wrightii* meadow in Perdido Bay, near Keys Bayou (KB) (30.31°N, 87.48°W; Figure 1) that experiences higher light conditions (average k= 1.0 m^{-1}±0.06 SE). Donor cores were taken from meadow centres in areas of similar coverage (between 50 to 80%) and depths (0.4 to 0.7m) at both sites.

2.4 Transplantation Phase I.

Transplantation, as defined here, involves the translocation of cored seagrass plugs (with original sediment intact) taken from a donor seagrass meadow and replanted in the pre-selected transplantation site. On May 1, 2009, 720 transplant units (TUs) measuring 15 cm in diameter (0.018 m^2) were collected from both donor meadows and transplanted in Little Lagoon over a period of six hours using volunteer help. A checkerboard planting design was selected based on prior successful marsh restorations that had proven to be both cost-effective and enhanced self-facilitative properties of black needlerush (*Juncus roemerianus*) transplants (Sparks, Cebrian, Biber, Sheehan, & Tobias, 2013). We modified the scale to better suit the small shoalgrass morphology so that each transplant replicate was a 1.5 m x 1.5 m square, checkerboarded with five planted and four non-planted squares of 0.5 m sides (Figure 2). Nine TUs were placed in each designated planting square, so that a total of 45 TUs were planted per 2.25 m^2 treatment replicate (Figure 2). Two depths (shallow= 0.35 to 0.45 m; deep= 0.6 to 0.7 m) were chosen to examine differences in planting depth. In total, the experimental design consisted of four treatments (PaP shallow, PaP deep, KB shallow, KB deep) with four replicate blocks per treatment placed 5 m apart and the two depths approximately 7 m apart. All treatment replicates combined represented 12.96 m^2 of transplanted *H. wrightii* at the start of Phase I.

2.4.1 Data Collection Phase I.

Transplantation success was measured by counting surviving TUs (i.e. units with visible aboveground biomass) and measuring leaf area as a proxy for transplant condition (Lee et al., 2007; Ralph, Durako, Enríquez, Collier, & Doblin, 2007). Percentage survival was determined by snorkelling and visually counting all living TUs of each treatment replicate, represented as number of surviving TUs out of 45 total transplanted TUs per replicate. Leaf area was calculated by randomly collecting ten shoots within each treatment replicate and measuring the length and width of the second leaf. The second leaf was chosen to examine the youngest mature leaves that represented the more recent timeframe at each sample time, relative to transplantation. TU survival and collections for leaf area measurements were done monthly. Physical parameters were measured between 9 and 11am at each sampling round and consisted of depth (m), temperature (°C), and salinity (psu) using a handheld Hach 40D multiprobe; light attenuation (k m^{-1}) and % SI were calculated from µmol PAR m^{-2} s^{-1} measurements of incoming radiation, incident surface radiation, and light at depth using a LiCOR model 1400 datalogger fitted with 4π spherical sensors.

2.4.2 Statistical Analysis.

RStudio (R Core Team, 2019) and the lme4 package (Bates, Mächler, Bolker, & Walker, 2015) were used to perform a mixed model repeated measures analysis of the relationship between TU survival and treatment over time. Treatment, sampling time, and the interaction were included in the model as fixed effects with block as a random effect. Akaike's Information Criterion (AIC) values, along with likelihood ratio comparisons, were used to determine the best random effect and covariance correlation structures for the model. In the event of a significant interaction term, the four treatments across time were examined using the post-hoc interaction analysis (de Rosario-Martinez, 2015) package in R to investigate the effect that donor site conditions and planting depth might have on TU survival over time. Due to loss of leaf material over time, the relationship between Phase I leaf area and treatment was investigated using one-way ANOVAs at each of the time points, with the accepted α for each analysis lowered to 0.01. In the event of a significant treatment factor, a post-hoc Tukey analysis was conducted to distinguish treatment groupings. In all analyses, visual examination of studentized residuals was conducted to determine the existence of any major deviations from normality or underlying patterns.

2.5 Transplantation Phase II.

Massive mortality of Phase I began occurring within three months post-transplantation with loss of all TUs after five months. Disturbance by stingrays became apparent two months post-transplantation; therefore, bioturbation prevention measures were taken for the subsequent transplantation experiment that took place on May 5, 2011 and will be hereafter referred to as Phase II. However, the planting scheme for Phase II varied from that of Phase I (Figure 2). Although the same two donor sites were used in Phase II, three depths were planted rather than two (shallow= 30-40 cm; mid= 40-50 cm; and deep= 50-70 cm). Due to limited funding, only 120 TUs total were planted, using 60 TUs for each donor site treatment plot. Also, the size of the checkerboard patterned plot was reduced to 0.5 x 0.5 m and only a single TU was planted in each dark square for a planted area of 0.09 m^2 per treatment plot. Four plots were planted for each treatment spaced 1 m apart and grouped based on donor site in non-randomized fashion for a total transplanted shoalgrass area of 2.16 m^2. Plots were not randomized, given the possibility that the shoalgrass would vegetatively expand and coalesce into indistinguishable units, due to the planting proximity of this small-scale effort. The two groups of donor site plots were spaced 7 m apart, while depths were separated by 5 m. To deter bioturbation, biaxial geogrid (a durable, webbing-like material commonly used in soil stabilization activities) with a 9 x 9 cm aperture was placed over the TUs and anchored to the sediments with bent rebar. This type of bioturbation deterrent prevented exposure of the rhizosphere to large organisms, but still allowed free access to the benthic environment by smaller fauna.

2.5.1 Data Collection Phase II.

Phase II TU health and survival was measured using percentage survival of transplanted cores and leaf area per shoot, rather than leaf area of the second leaf only, as was done for Phase I. TUs were examined for visible aboveground biomass visually and between five and ten shoots were collected from each of the four plots per treatment for shoot area measurements. Leaf area shoot^{-1} was determined by measuring length and width of each leaf on a shoot, which were combined to determine an average per plot. Temperature and salinity data were collected at a nearby water quality station with a continuous YSI 6600 multiparameter water quality sonde (J. Anders, unpublished data). Light availability was determined monthly for the first four months, using the same techniques as described for Phase I. Lack of randomized treatment replication during Phase II prohibits the quantitative investigation of treatment effects over time for TU survival and leaf area shoot^{-1}.

2.6 Transplantation and Data Collection Phase III.

The landfall of Hurricane Isaac in August of 2012 created storm surge that deposited sediment directly onto the Phase II TUs, the result of washover from the sand dunes that separate Little Lagoon from the nGoM. This burial resulted in total loss of aboveground biomass in all transplantation plots for ca. two months after landfall of the storm. With no shoots visible after two months post-Isaac, Phase II of the Little Lagoon transplantation experiment was considered a total loss with no plans for further experimentation at this site. However, a chance outing to Little Lagoon in November of 2013 led to discovery of a *H. wrightii* patch precisely where KB shallow plantings had been transplanted in Phase II prior to Hurricane Isaac landfall (Figure S1). The discovery of surviving TUs warranted continued monitoring at this site and is referred to as Phase III. To date, monitoring has been conducted with Real Time Kinematics (RTK) global positioning with 1 cm accuracy to measure patch area and to follow expansion of the rediscovered patch.

3. Results

3.1 Transplant site environmental condition.

Benthic light conditions, salinity, and temperature were all well within tolerance limits for shoalgrass throughout both Phase I and II of the experiment (Table 1.). To better compare environmental conditions for the two phases, Phase II_a refers to the same seasonal timeframe as in Phase I, while Phase II_b references seasonal conditions over the entire second transplantation trial. On average, Phase II_a benthic light availability was slightly higher than in Phase I, while salinity and temperature conditions were comparable to those in Phase I. Light availability was not reliably obtained after September 2011, and therefore not considered after this time. However, salinity and temperature conditions remained within ranges tolerated by *H. wrightii* for the remainder of Phase II.

3.2 Phase I transplant survival.

The Phase I TUs suffered a total loss after approximately four months. For Phase I TU survival over time, a linear model consisting of a random intercept and compound symmetry covariance correlation structure with unequal variances was chosen as the best fit for the longitudinal data. This model provided statistical evidence of an interaction effect of treatment with time for TUs and negligible influence of the random Block factor, implying lack of any spatial effect on TU survival (Table 2). Treatment comparisons across time indicated treatment-specific survival up to total collapse of the transplantation experiment at the 4-month timepoint. On average, TUs from both donor sites in shallow treatments exhibited higher survival over time (54.1%) than those at deeper depths (33.6%) (Figure 3). Also, TUs originating from the high light KB site meadow exhibited higher survival over time (57.3%) than those from PaP (30.4%) (Figure 3). Second leaf area at time of collection for KB shoots was nearly 3-fold higher than shoots of PaP (6.9 cm^2 ±1.2 SD and 2.4 cm^2 ±0.1 SD, respectively). Following transplantation, analyses of second leaf area at each sampling date found consistently larger leaf area of KB shoots than those originating from PaP (Table 3, Figure 3). However, this leaf area declined over time in all treatments, as TUs gradually succumbed and leaf senescence progressed.

3.3 Phase II.

Percentage survival of TUs during Phase II resembled initial findings for Phase I treatment effects. TUs originating from low light PaP showed greater initial signs of distress, with deep plantings of PaP TUs lost after one month (Figure 4). Similarly, shallow and mid PaP TUs exhibited an initial decline in survivorship with aboveground biomass of shallow PaP plantings fully recovered after one year, while the mid-depth plantings fluctuated between 30 to 60% presence for the first year. Aboveground biomass of TUs that originated from the KB site maintained 100% presence for the first year, regardless of depth. These TUs eventually filled in the unplanted checkerboard areas over the following year and expanded outward from the original planted area. At the time of collection, shoots originating from KB had nearly double the leaf area per shoot as shoots from PaP (17.6 ±1.7 SD and 9.3 ±1.7 SD, respectively). Leaf area per shoot fluctuated with season among all treatments, with KB shoots' maintaining larger leaf area per shoot than PaP shoots up to1 year post-transplantation (Figure 4), although this relationship was not examined statistically due to lack of spatial randomization in Phase II experimental design. Further monitoring of Phase II transplantation was halted due to the landfall of Hurricane Isaac in August of 2012.

3.4 Phase III.

Current monitoring efforts indicate that the Phase III patch rapidly expanded from no visible aboveground shoot biomass in August 2012 to an area of ~74 m^2 as of July 2018, at an expansion rate of ~12 m^2 $year^{-1}$ based on the linear regression of patch area over time (Figure 5).

4. Discussion

Experimental efforts of seagrass transplant trials to date have reiterated the importance of scaling up the planting design for transplant redundancy and to enhance the inherent self-facilitative properties of seagrasses (Paulo et al., 2019; van Katwijk et al., 2016). Dynamic coastal environments also present difficulties when attempting to locate donor meadows with similar environmental conditions to the restoration site, as underscored in best practices recommendations (Calumpong & Fonseca, 2001; van Katwijk et al., 2009). While these transplant recommendations are well-reasoned and constructive guidelines based on the results of hundreds of restoration trials, restoration practitioners in regions with financial constraints and fewer transplant donor sources require a more adaptable approach tailored for small-scale transplantation success. Thus, the original goal of this experimental investigation was to examine the influence of donor source environment on small-scale transplant resilience to improve success and accessibility of seagrass restorations. Our transplantation of *H. wrightii* from two different donor sites with distinct light availability characteristics indicated enhanced resilience to transplantation stress and successful acclimation for transplants from a higher quality donor site. The Little Lagoon transplantation experiments provided evidence that small-scale restoration can be effectively accomplished if, in addition to well-established recommendations, the following actions are taken: select donor plants with resilience traits useful in transplantation site environment (e.g. larger morphology and seed production for Little Lagoon, AL); create a planting design that optimizes both spatial coverage and use of limited donor material to enhance self-facilitative properties of seagrass; and reduce uncertainties at the transplantation site with a thorough understanding of potential disturbances and future interactions (e.g. bioturbation protection).

The first important lesson learned from the Little Lagoon transplantation trials was adaptive control over restoration uncertainties, as recommended by Thom et al. (2005). The immediate decline of Phase I TUs caused by bioturbation was not anticipated, and likely accounted for a significant portion of TU loss both directly and indirectly. Stingray bioturbation is a common and substantial threat to seagrass transplants and regularly cited as an initial cause of decline for restoration attempts (Fonseca, 2011; Treat & Lewis, 2006). Because stingray densities were negligible in all previous site scouting trips, preventative measures were not included in our initial planting design. However, the transplants provided habitat for stingray prey (e.g. polychaetes, small fish, crustaceans) in a previously unvegetated area and subsequent stingray foraging uprooted whole TUs. The loss of individual TUs then limited the self-facilitation promoting features of the checkerboard planting design and left remaining isolated TUs more open to the site's physical forces than intended, leading to some sediment erosion. The decline of leaf area over time during Phase I is a potential response to the combined effects of hydrodynamic and bioturbation stress, in addition to accumulation of toxic hydrogen sulphide in the transplant site sediments over this same period (Christiaen, McDonald, Cebrian, & Ortmann, 2013). Adaptation to the Phase I methods to incorporate geogrid stabilizing mesh over the Phase II TUs, resulted in the prevention of losses from bioturbation and the rapid natural progression of shoot expansion into unplanted spaces promoted ecosystem engineering properties of the seagrass (van Katwijk et al., 2009). Furthermore, substantially higher survival of the Phase II shoots allowed for more insight into transplant resilience features between the two donor sources.

Donor *H. wrightii* shoots collected from an area with objectively higher quality environmental conditions were morphologically larger and consistently better transplant performers. The results of the Little Lagoon experiment suggest a connection between larger seagrass morphology having originated from more suitable environmental conditions and better transplant suitability, similar to the Novak et al. (2017) transplant experiment with *Zostera marina* populations. The more amenable light conditions of the KB donor site alleviated the higher carbon demands of a larger morphology, balancing the compensation of more photosynthetic area for loss of some photosynthetic potential from self-shading (Enríquez & Pantoja-Reyes, 2005). Alternatively, the lower carbon demands of a smaller morphology are better suited to less amenable light environments (Ralph et al., 2007), reflecting the PaP donor shoots and site conditions. The use of *H. wrightii* morphology as a preliminary indicator of transplant material suitability in an environmentally variable region is supported by our results. However, the connection between *H. wrightii* morphology and transplant success in this study may only reflect the uniqueness of the north-central Gulf Coast region. Here, shoalgrass meadows have varying levels of population connectivity due to the physical isolation of the shallow embayments and lagoonal habitats where most meadows are located (Handley, Altsman, & DeMay, 2007). Thus, the morphological attributes of the two populations could be related to the level of genetic diversity derived from a population’s degree of isolation (Connolly et al., 2018; Diekmann et al., 2005). Future transplant experiments should include sampling of donor genetics to determine the relative influence of genetic diversity on any connection between morphological features and transplant resilience.

Transplant failure of lower quality donor site (PaP) plants in this study is likely due to the condition of the donor shoots prior to transplant. Although light availability was within *H. wrightii* requirements at the Little Lagoon site during the experiment, TUs from the lower quality PaP site rapidly failed at both planting depths in Phase I. In Phase II, PaP TUs rapidly failed at the deep depth, languished at the mid depth, and required a year for presence recovery at the shallow depth. Since light limitation doesn't explain TU failure, inability of PaP shoots to acclimate is possibly related to the persistent low-light conditions of the donor site, combined with the stress of transplantation exerted on the donor shoots (Zimmerman, Reguzzoni, & Alberte, 1995). Seagrasses regularly exposed to low quality environmental conditions are less resilient to added stressors (Gurbisz & Kemp, 2014; Hemminga & Duarte, 2000; Koch & Erskine, 2001). When stressed, seagrasses are capable of relying on below-ground, non-structural carbohydrate reserves to supplement lowered photosynthetic capabilities (Hemminga & Duarte, 2000; Maxwell et al., 2014). However, *H. wrightii* has physiologically determined limitations for carbohydrate storage capabilities (Touchette & Burkholder, 2000), and stress associated with transplantation can lead to mortality if reserves were previously limited by sub-optimal growth conditions such as low light availability (Govers et al., 2015; Horn, Paling, & van Keulen, 2009; Park & Lee, 2007). Therefore, as suggested by Zimmerman, Reguzzoni, and

Alberte (1995), transplantation plans should consider the carbohydrate reserves of donor source material and how reserve fluctuations are potentially affected by recent seasonal stressors.

Resilience of transplants was further tested by a hurricane event that highlighted the importance of recovery features for restoration success. The re-establishment of the higher quality donor site (KB) plants following Hurricane Isaac burial indicated recovery features were present in KB shallow transplants but not in other transplants. Post-hurricane burial monitoring showed no indication of living TUs for either donor site after two months. Small collections of below-ground material from each of the planting locations after two months of burial appeared highly degraded or decomposed. It is for these reasons we suspect the newly established patch found at the KB shallow planting area in November 2013 was most likely derived from a seedbank. Seedbank formation occurs when seeds are deposited at or below the sediment with minimal dispersal distance from the parent plant (Darnell, Booth, Koch, & Dunton, 2015). Seedbanks are a vital recovery feature for *H. wrightii*, as limited carbohydrate storage potential makes this species vulnerable to severe or extended stress events (Biber, Kenworthy, & Paerl, 2009). The Little Lagoon *H. wrightii* transplant recovery implies that transplant restorations have a greater chance at overcoming a severe perturbation if practitioners select a donor population with evidence of recovery features, such as recent seed production or a viable seedbank that would be transferred to the transplantation site with the TUs.

In conclusion, our efforts have shown that monetary and seagrass donor resource limitations do not necessarily infer inevitable seagrass transplantation failure. With thorough background research on potential transplant site stressors, rigorous planning of planting design, and investigation into resilience potential prior to selection of donor populations, small-scale seagrass transplant success can be achieved. We determined that the donor site with higher light availability was the source of larger morphology *H. wrightii* transplants that were more resilient to transplantation and an extreme stochastic stress event. The adaptive planting design that used geogrid stabilizing mesh over a checkerboard TU planting scheme allowed for maximum coverage of planted space and protection from bioturbation, while natural ecosystem engineering feedback mechanisms enhanced vegetative growth into unplanted spaces. Furthermore, selection of a pioneer seagrass species resulted in rapid areal expansion of the recovered transplant plot at a rate of ~12 m^2 per year, similar to the reported rate of expansion of a larger scale *Z. marina* transplant patch (Paulo et al., 2019). Continued expansion of the Little Lagoon recovered patch will further enhance density-dependent resilience features associated with seagrass habitat by influencing hydrodynamic stability within the patch (Maxwell et al., 2016; Suykerbuyk et al., 2016; van der Heide, van Nes, van Katwijk, Olff, & Smolders, 2011). In May 2018, shoots in the recovered PaP shallow patch in Little Lagoon reproduced successfully, reinforcing the seedbank recovery feature and providing insight into what is considered a rare occurrence for *H. wrightii* in the nGoM (McGovern & Blankenhorn, 2007). Finally, although not specifically measured in this study, we recognize a need for reporting of donor meadow recovery times to investigate the effect of planting scale on degree of donor site impact. Size of mechanically extracted TUs, number of TUs collected from each meadow, and collection distance between individual TUs are all factors that will affect donor meadow recovery (Verduin, Paling, van Keulen, & Rivers, 2012). Improving small-scale transplant resilience may therefore be the best way to enhance restoration accessibility, while simultaneously lessening the burdens on valuable yet declining donor populations.

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Table 1. Environmental conditions during both Phase I and II restoration trials. Phase II_a represents only the same timeframe as Phase I for comparison purposes, while Phase II_b represents the entire monitored Phase II restoration timeframe.

Restoration period	Light Attenuation ($k\ m^{-1}$) Mean [CV%] (Range)	Benthic Irradiance (% SI) Mean [CV%] (Range)	Salinity (ppt) Mean [CV%] (Range)	Temperature (°C) Mean [SE] (Range)
Phase I **May-September 2009**	1.4 [28] (0.1-2.4)	52 [23] (27-84)	26.1 [13] (22-34)	29.1 [0.34] (24- 33)
Phase II_a **May- September 2011**	0.8 [38] (0.4-1.2)	81 [12] (67-92)	29.8 [5] (27-32)	30.1 [0.6] (27-32)
Phase II_b **May 2011- September 2012**	-	-	28.3 [8] (23-32)	23.8 [1.35] (10-32)

Table 2. Results for Phase I linear mixed effects model with compound symmetry and unequal variances. DF for all post-hoc interaction contrasts is 5.

Fixed Factors	DF	F	p
Treatment	3	$2.7*10^9$	<0.0001
Time	5	$7.6*10^9$	<0.0001
Treatment*Time	15	$6.5*10^8$	<0.0001
Random Factor			
Block	Intercept	Residual	
Standard Deviation	$3.2*10^{-7}$	5.8	
Post-Hoc Interaction Contrasts	χ^2	Holm-adjusted P	
KB shallow vs KB deep	$3.6*10^9$	<0.0001	
KB shallow vs PaP shallow	$3.4*10^9$	<0.0001	
KB shallow vs PaP deep	$4.8*10^9$	<0.0001	
KB deep vs PaP shallow	$1.7*10^9$	<0.0001	
KB deep vs PaP deep	$4.7*10^9$	<0.0001	
PaP shallow vs PaP deep	$1.5*10^9$	<0.0001	

Table 3. Results for Phase I single ANOVAs at each sampling time point (acceptable α= 0.01).

	Fixed Factor: Treatment			Random Factor: Block	
Sample Time Point	**DF** (num, den)	**F**	**P**	**Intercept**	**Residual**
June 2009	(3, 12)	102.9	<0.0001	0	4.5
July 2009	(3, 12)	74.1	<0.0001	0	5.0
August 2009	(2, 9)	38.2	<0.0001	0.05	2.1
September 2009	(1,6)	38.5	0.0008	0	1.2

Figure legends:

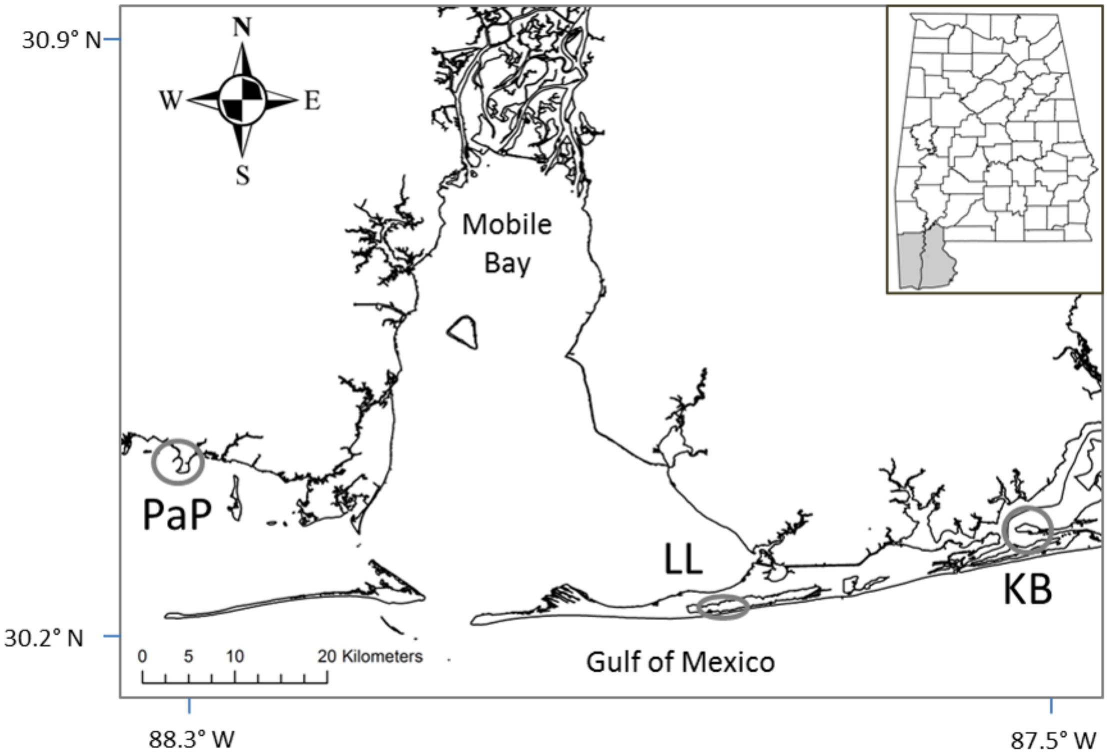
Fig. 1 Map of donor sites (PaP- Point aux Pins,AL; KB- Keys Bayou, FL) and restoration site (LL- Little Lagoon, AL)

Fig. 2 Transplantation schematic for Phase I (above) and Phase II (below) restoration trials. Phase I consisted of a randomized block design while Phase II was non-randomized and included geogrid stabilizing mesh over each planting plot.

Fig. 3 Phase I number of surviving TUs over time by treatment (±SE; top) and average leaf area over time (±SE; bottom) with multiple comparison post-hoc Tukey results. * At this timepoint PaP shallow had only one block replicate surviving, therefore the error bar represents standard deviation and is not included in the ANOVA comparison

Fig. 4 Phase II total number of surviving TUs over time by treatment (top) and average leaf area per shoot over time (±SD; bottom)

Fig. 5 Expansion of Phase III patch over time beginning in September, 2012 with ticks representing every September thereafter. Asterisks indicate use of lower precision technique to calculate patch area (i.e. Google Earth Pro©2016)



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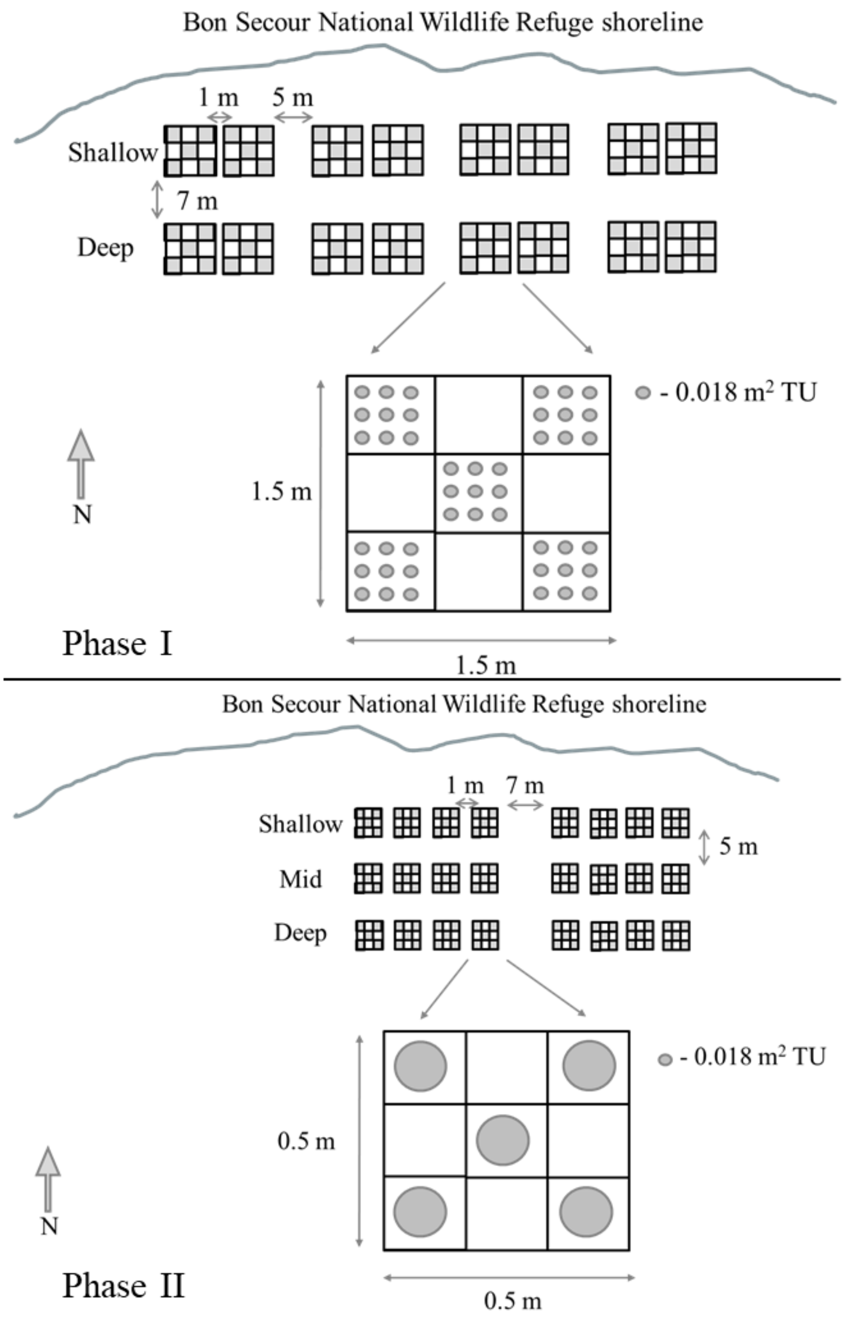

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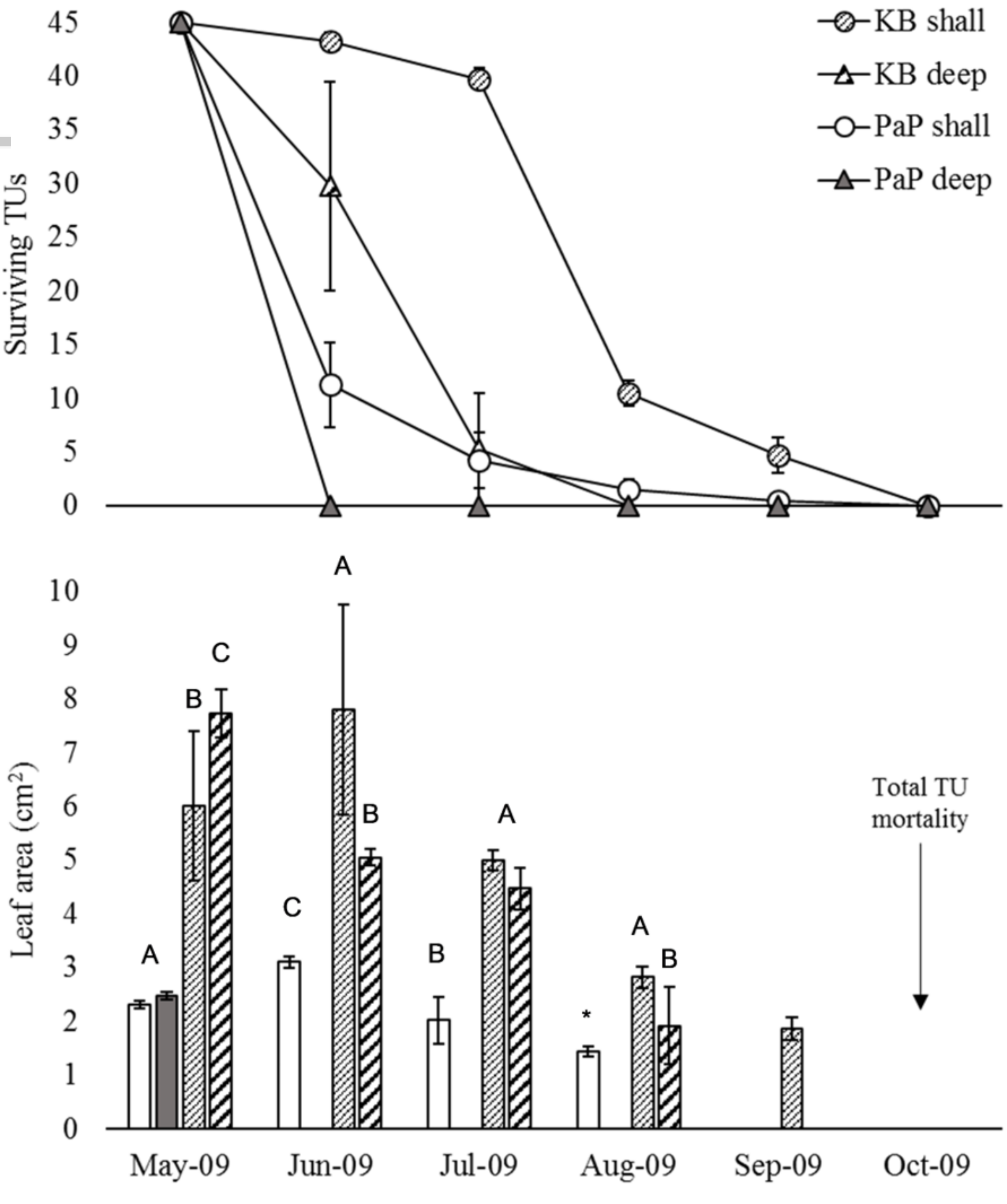

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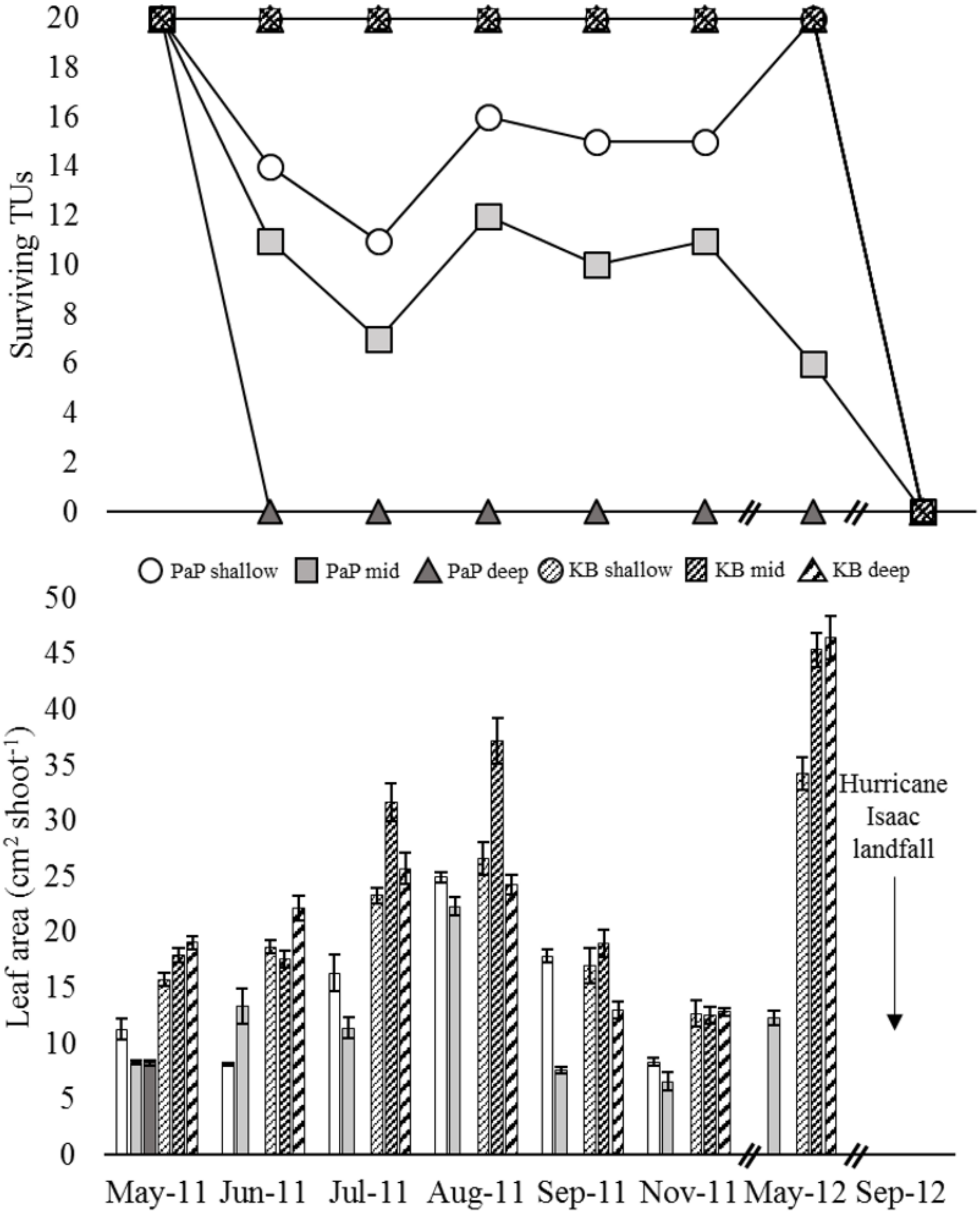

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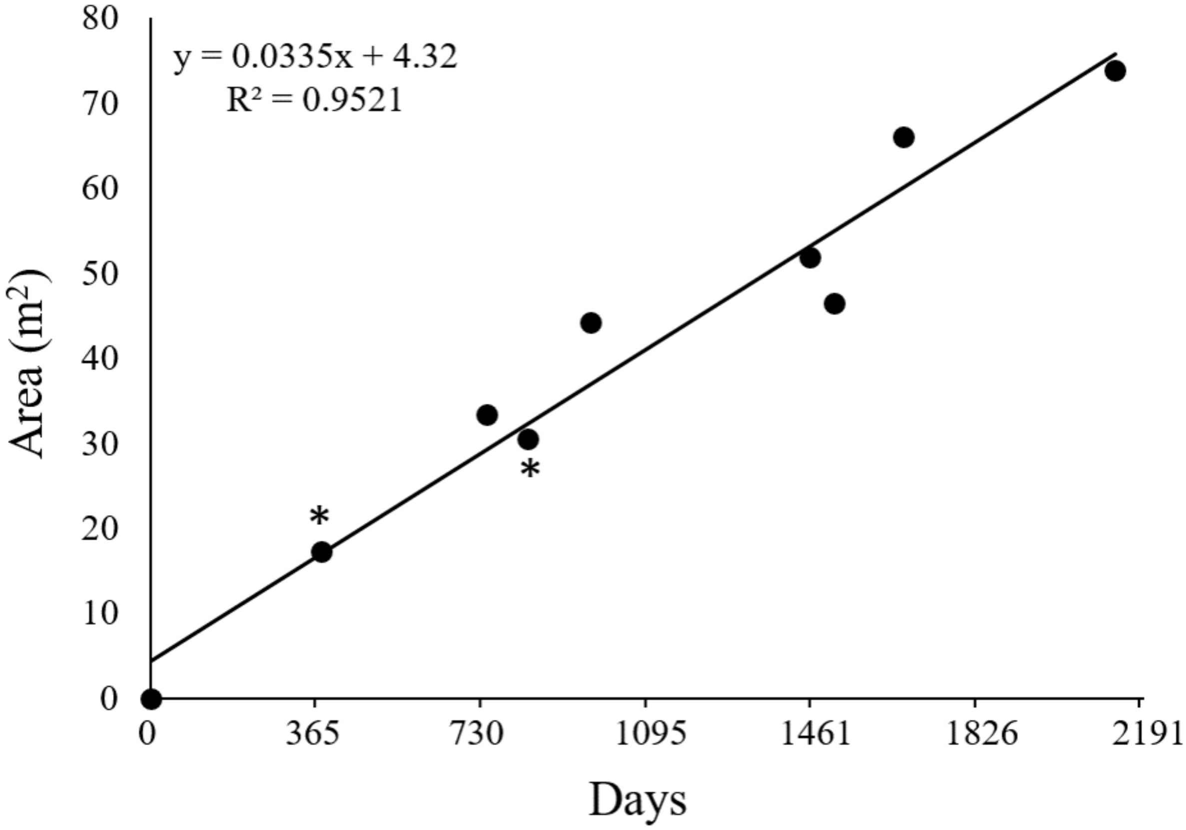

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