1 EVALUATING A NOVEL BIODEGRADABLE LATTICE STRUCTURE FOR 2 SUBTROPICAL SEAGRASS RESTORATION

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25 Highlights:

- Seagrass restoration is expensive and often unsuccessful.
- Traditional and novel physical restoration techniques were compared.
- Biodegradable starch (BESE) lattices yielded better growth.
- BESE may improve restoration in hydrodynamically active environments.
- 30

31 Abstract

32 While attention in coastal ecosystem restoration has increased over the last two decades, 33 the success rate of efforts remains relatively low. To increase success rates, physical 34 restoration techniques often utilize supporting or protective materials to provide a stable 35 surface for transplantation, and in some cases reduce herbivory and hydrodynamic 36 disturbances. In this study, we evaluated the effectiveness of traditional (staples, burlap) 37 and novel (BESE elements, a biodegradable potato starch lattice) physical restoration 38 techniques on the growth of transplanted Halodule wrightii seagrass. A first experiment 39 revealed that seagrass planted in both two-stacked BESE structure without planting holes 40 and four-stacked BESE with holes had significantly higher shoot count and blade length 41 than four-stacked BESE without holes, with the latter design losing all seagrass shortly 42 after deployment as shoots could not float through the structure. In a second experiment, 43 the BESE lattice treatment (four-stacked with holes) had three times the shoot count and 44 equal to greater blade length compared to traditional methods of physical restoration 45 (staples and burlap), likely due to BESE providing some protection from hydrodynamic 46 activity. However, disturbances, possibly including herbivory and hydrodynamic activity 47 (culminating with Hurricane Irma), prevented long term study, illustrating the importance of stochastic abiotic factors in seagrass planting success. Overall our study
demonstrates the effectiveness of using BESE lattice designs and similar physical
techniques in the restoration of seagrass beds.

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52 **Keywords:** *Halodule wrightii*; seagrass; marine; restoration; coastal; Indian River 53 Lagoon; shoots; subtropical; success

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55 **1. Introduction**

56 In the past two decades, greater attention on coastal ecosystem restoration 57 (Basconi et al., 2020; Zhang et al., 2018) has better identified the challenges of this field 58 (i.e. costs, stressors, site characteristics, etc.). While regulations attempt to improve 59 conditions for natural regrowth (passive restoration), active restoration is often necessary 60 to remediate environments either too damaged or too low in the desired species 61 population to passively improve (Basconi et al., 2020; Rinkevich, 2005). As a result, 62 active coastal ecosystem restoration is often expensive and frequently fails to achieve 63 success (Bayraktarov et al., 2016; Zhao et al., 2016).

64 Restoration of seagrass ecosystems is often hindered by multiple and often 65 elevated/unnatural physical disturbances (Castillo et al., 2000; Hauxwell et al., 2004; 66 McFalls et al., 2010). For example, natural seedling establishment is typically low due to 67 hydrodynamic disturbances (Infantes et al., 2011) which can dislodge, erode, or bury 68 transplanted seagrasses before they can establish (Katwijk et al., 2009; Paulo et al., 69 2019). Herbivory has also been established as a significant factor in seagrass and other 70 submerged aquatic vegetation (SAV) restoration, with many manatees, sea turtles, and 71 fish capable of overwhelming grasses, especially in polluted systems already under stress (Bourque & Fourqurean, 2013; Hauxwell et al., 2004; Ravaglioli et al., 2018; Tomas et al., 2005; Tuya et al., 2017). As climate change may exacerbate both hydrodynamic activity (Duarte et al., 2013) and herbivory impacts (Heck et al., 2015) on seagrass beds, finding a means to reduce these physical disturbances is an important strategy to reducing stress on seagrass and improving the success of restoration efforts.

77 Physical restoration techniques utilize supporting or protective materials that 78 provide a stable surface for transplantation, and in some cases reduce herbivory and 79 physical disturbances (K. Hammerstrom et al., 1998; Temmink et al., 2020; Wear et al., 80 2010). These protective materials maintain seagrass patch size/density (important for 81 the long term survival of the plants [Maxwell et al., 2017; Silliman et al., 2015]), thereby 82 providing seagrass transplants the time and support to overcome transplantation stress 83 and becme established. Among physical restoration techniques applied to seagrasses, 84 staples affixed to seagrass rhizomes have been used for decades as an anchor while the 85 grass establishes (Bird et al., 1994; Heise & Bortone, 1999). Burlap is a another cost-86 effective anchoring surface for transplants, either as a stabilizing surface for sediment, or 87 as strips facilitating natural recruitment of seedlings in a hydrodynamically active 88 environment (Irving et al., 2010; Wear et al., 2010). Artificial seagrass (plastic strips 89 imitating seagrass) and exclusion cages have also found success in reducing fish and turtle herbivory, giving plants a sufficient window of opportunity to establish themselves 90 91 (Hammerstrom et al., 1998; Tuya et al., 2017).

92 The disadvantages of many physical restoration techniques are that the materials 93 used either provide limited protection (e.g., staples and burlap do not provide 94 aboveground protection from hydrodynamic activity or herbivory) or require regular

95 maintenance which may result in pollution of the surrounding environment. For example. 96 both cages and artificial grass accumulate algae and epiphytes, blocking light, and 97 artificial seagrass is often plastic-based, persisting and polluting the environment (Tuya 98 et al., 2017). Thus, low-maintenance biodegradable materials with aboveground 99 structure to protect from hydrodynamic activity and herbivory are a potential solution to 100 both maximizing resources and minimizing long-term debris accumulation at restored 101 Biodegradable Elements for Starting Ecosystems (BESE-elements, BESE BV, sites. 102 Culemborg, Netherlands, hereafter called BESE) are carbon neutral, biodegradable 103 potato starch-based lattices. BESE lattices can be used belowground as an anchor for 104 transplants and/or stacked into vertical structures aboveground to protect plants from 105 currents and herbivory. Studies have shown promise in trials restoring oyster, mangrove, 106 and saltmarsh habitats (BESE-Ecosystem Restoration Products, 2018; D'Angelo, 2018; 107 Temmink et al., 2020), but despite this potential, only one study has been published using 108 these structures in seagrass systems (Temmink et al., 2020). Additionally, Temmink et 109 al. (2020) did not examine subtropical environments (conducted in temperate and tropical 110 environments) and used Thalassia testudinum instead of Halodule wrightii, the latter 111 being an early successional seagrass species better suited for restoration in the southern 112 USA (Biber et al., 2013; Furman et al., 2019).

Based on the potential gaps of previous studies investigating the effectiveness of physical restoration techniques, the purpose of this study was to *i*) explore the effectiveness of different BESE lattice designs on promoting *H. wrightii* growth (measured via shoot count and blade length), *ii*) compare the relatively novel BESE lattices to traditional physical restoration techniques (staples and burlap), and *iii*) to determine the mechanisms (protection from physical disturbances, herbivory, etc.) behind their success or failure in establishing a seagrass community. We also aimed to identify the fish and invertebrate communities that utilize (consume, colonize, etc.) the seagrass/structures to inform the design of future restoration efforts. We hypothesized that seagrass within the BESE lattice would have increased success relative to staples and burlap planted units due to better protection from wave action and macro-herbivores (similar to Temmink et al., 2020).

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126 **2. Materials and Methods**

127 **2.1 Site Description**

This seagrass restoration study was conducted in Stuart, FL, within the Indian 128 129 River Lagoon (IRL) (Figure 1). The value of seagrasses, and the consequences of their 130 removal, have been especially relevant to the IRL. In 2009, estimates found that the IRL 131 was worth \$3.7 billion dollars in ecosystem services and revenue, with a large part of 132 these services coming from the (historic) 72,400 acres of seagrass in the lagoon, 133 estimated at \$2 billion annually (USEPA, 2009). "Superblooms" of toxic algae (caused 134 by poor water quality) subsequently covered tens of thousands of acres, destroying up to 135 95% of seagrass in the lagoon, and precipitating a phase shift of the environment into a 136 "toxic phytoplankton-dominated system" (Barile, 2018; Lapointe et al., 2020). The 137 seagrass and its associated communities have yet to recover (Barile, 2018; Lapointe et 138 While algae and bare sediment have largely replaced seagrass and al., 2020). 139 macroalgae in this area, there is a functioning seagrass bed directly across the study site, 140 indicating that seagrass establishment was possible.

141 The specific location of the experiment is a permitted ovster and seagrass 142 restoration site (27°12'33.4" N, 80°12'11.9" W) approximately 3.2 miles northwest of the 143 St. Lucie Inlet, and is maintained by the Florida Oceanographic Society (FOS) of Stuart, 144 FL. Efforts to restore seagrass at multiple sites in the southern IRL have been undertaken 145 by FOS. This system is an ecotone between temperate and sub-tropical habitats, 146 fostering above-average biodiversity (Lapointe et al., 2020). The IRL is a microtidal 147 system, with limited transport occurring locally around inlets (Weaver et al., 2016). Due 148 to its narrow (2-4 km wide) and shallow (average depth is 1.2 meters) characteristics, the 149 IRL is considered a restricted lagoonal system (Lapointe et al., 2020). The currents in 150 the IRL are wind-driven but extremely fetch limited except from the southeast or northwest 151 (Colvin et al., 2018). In 2017, average monthly water temperatures in the area of the 152 restoration site (Stuart, FL) ranged from 20.8 ± 0.2 °C to 30.6 ± 0.3 °C (mean ± standard 153 error), salinity ranged from 18.75 ± 1.11 ppt to 37.48 ± 0.76 ppt, Secchi depth ranged 154 from 0.70 ± 0.04 m to 1.37 ± 0.15 m, and dissolved oxygen ranged from 4.93 ± 0.16 mg.L⁻ 155 ¹ to 6.90 ± 0.42 mg.L⁻¹ (Florida Oceanographic Society, 2017). The experimental site had 156 a depth of approximately 0.61 m at mean low water (MLW).

157 2.2 Optimizing BESE design for seagrass restoration (Exp. 1)

The first study was an eight-week experiment (June 30^{th} to August 22^{nd} , 2017) to confirm the survivability of seagrass at the restoration site, as well as compare multiple BESE lattice designs (Figure 1). There were three replicates of three different designs: two-stacked (connecting two individual lattices to form a vertical structure, dimensions are approximately 1 m x 0.5 m x 4 cm, buried 4 cm in the sediment) and four-stacked with and without holes (dimensions approximately 1 m x 0.5 m x 8 cm, the lower 4 cm buried in the sediment). Four-stacked with planting holes had 25 cm circular cutouts in the top 165 three layers of BESE so seagrass shoots could be unimpeded by the lattice. While there 166 were no controls (seagrass not planted using physical restoration techniques), the staples 167 approach was considered a pseudo control in this experiment, as it appeared to provide 168 the least structural protection from hydrodynamic activity and herbivory. Twenty-five 169 shoots of seagrass were planted in each plot. All shoots were adult plants obtained from 170 the seagrass nursery lagoon at the FOS Coastal Center. Seagrasses were affixed to 171 BESE lattices via 0.404 mm (26 gauge) flower wire, and the BESE lattices were buried 172 approximately 4 cm in the sediment and anchored with 60.96 cm (24 in.) metal rods. 173 Plants were minimally exposed to air (x<10 minutes) to avoid desiccation and stress. 174 Seagrass metrics (shoot count and blade length) were taken 24 and 48 hours after 175 deployment, as well as five additional times over an approximate two month period (x < 176 14 days between sampling periods). Based on the survival of two BESE designs, a larger 177 project comparing novel BESE lattices to traditional physical restoration approaches was 178 subsequently deployed in August 2017 (Figure 1).

179 **2.3 Comparison of physical restoration techniques (Exp. 2)**

180 The second study was a two-week experiment (August 17th through August 31st) 181 to compare the BESE lattice (four-stacked with planting holes design) to staples 182 (seagrass attached directly to staples with flower wire) and burlap approaches (seagrass 183 attached to a burlap square with flower wire) (Figure 1). The four-stacked with planting 184 holes BESE design was chosen based on the favorable shoot and length measurements 185 found early in the first study (up until mid-July) versus the two-stacked and four-stacked 186 designs. Twelve plots with seagrass of approximately 0.5 m x 1 m (BESE was slightly 187 smaller at 0.45 m x 0.95 m) were arranged in randomized blocks approximately 0.5 m 188 apart. Twelve plots without seagrass were placed adjacent to the restoration site acting

189 as a control. The plot size and spacing of plots for all techniques tested was equivalent 190 and based on that described in Duarte et al. (2015), with a compromise between 191 restoration success and availability of seagrass plants and permitted space. We 192 expected the plots to remain independent for the duration of the study based on the 193 reported mean horizontal elongation rate of *H. wrightii* varying greatly in the literature, 194 (Duarte et al., 2013; Gallegos et al., 1994), the disturbed nature of the area/site being 195 restored, the distance between seagrass shoots (approximately 0.6 m when accounting 196 for the space between the plants and the edge of the plot), and the short period of 197 observation for the study.

198 All plots contained 50 shoots inside two 0.25 m diameter circles (Figure 1), 199 equivalent to approximately 510 shoots m^2 , approximating the historic densities of H. 200 wrightii seagrass in the IRL (Berninger, 2016). The total amount of seagrass used was 201 600 shoots. The burlap was installed with the 25.4 cm metal staples also used for the 202 stapled plots. The BESE lattice treatment was approximately 8 cm in height (four-203 stacked) and had two 25 cm diameter holes cut out to the bottommost lattice (used as a 204 point to attach the seagrass). This BESE design (four-stacked with planting holes) was 205 chosen based on the favorable shoot and length measurements found early in the first 206 study (up until mid-July) versus the two-stacked and four-stacked designs. BESE lattices 207 were installed at a 4 cm sediment depth and reinforced via 0.64 cm (0.25 in.) thick, 91.44 208 cm (three ft.) long rebar.

209 Seagrass metrics (shoot count and blade length) were taken one, five, and 14 days 210 after deployment. To assess the influence of herbivory on seagrass growth and survival 211 during the study, seagrass blades were tethered to the benthos to imitate restored 212 seagrasses, based on the methods of Holzer et al., (2011). Ropes with pre-measured H. 213 wrightii blades attached via wooden clothespins were placed adjacent to each block (n= 214 four strands per rope). After 24 hours, the blades were removed and compared based 215 on their original length and proximity to an adjacent oyster reef. To assist in confirming 216 the presence of herbivory and identity of herbivores, approximately three hours of time-217 lapse footage of four tethers placed parallel to each other were also recorded during 218 daylight hours using a GoPro placed on the seabed at level with the tethers (Hero 3 Silver, 219 GoPro, San Mateo, CA).

220 2.4 Seagrass Metrics and Nutrient Analyses

221 Seagrass shoot count (seagrass shoots defined as a unit of several leaves or 222 blades according to Short & Coles [2001]), and blade/leaf lengths (substrate to leaf tip 223 according to Arrington, [2008]) were quantified in both experiments. Up to five shoots 224 were randomly selected for blade length, with the longest blade being measured. For the 225 local site description/background data, samples of surface water were collected for total 226 dissolved phosphorus (TDP) and sediment samples were collected for total phosphorus 227 (TP), total nitrogen (TN), and total carbon (TC). Surface water TDP samples were 228 collected in plastic LPDE bottles, filtered (0.45 micron), preserved with sulfuric acid to a 229 pH to x < 2 and stored at 4 °C until analysis (USEPA, 1993). Bulk sediment samples of 230 the top 10 cm were collected via plastic corers, dried for 72 hours at 65 °C, and ground 231 using a ball mill.

Surface water TDP and sediment TP were measured in the University of Florida Wetland Biogeochemistry Laboratory (Gainesville, FL), while sediment total carbon (TC) and nitrogen (TN) were measured by the University of Florida Stable Isotope Mass Spectrometry Laboratory (Gainesville, FL). Water samples for TDP were digested with 236 potassium persulfate in an autoclave and analyzed via a Shimadzu UV-1800 237 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) using EPA method 365.1 238 (USEPA, 1993). TC/TN samples were run on an ECS 4010 CHN analyzer (Costech 239 Analytical Technologies, Inc., Valencia, CA, USA) (dry combustion method) (Nevins et 240 al., 2020). Sediment TP was determined by ashing the sample followed by dissolution 241 with 6 M HCL (following Andersen, 1976) before an analysis of soluble P using a 242 Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) (Liao et 243 al., 2014; USEPA, 1993).

244 **2.5 Fish Count Analysis**

Fish identity and counts were conducted utilizing the full video taken of the herbivory tethers, with the recording being paused every 10 seconds to determine fish quantity and species. This approach utilizing video cameras was found to be similarly effective as direct visual observations and has been used to determine fish herbivory in past studies (Bennett et al., 2015; Eggertsen et al., 2019). The focus of the fish observations were on pinfish (*Lagodon rhomboides*), filefish (family *Monacanthidae*), and parrotfish (family *Scaridae*), common seagrass consumers in Florida (Heck et al., 2015).

252 2.6 Statistical Analyses

Seagrass metrics (shoot count and blade length) for both experiments were calculated using a linear mixed model. Factors included treatment type, date, and the interaction between treatment and date, with subject/plot number set as a random variable. Both seagrass shoot count and length were log and/or square root transformed as necessary to improve normality. For significant effects, multiple comparison analysis was completed via a Fishers LSD test. Tests were completed in JMP 15.2.1 (SAS Software, Cary, NC, USA) with significance set to α = 0.05. Marginal significance was set between α = 0.05 and α = 0.10, consistent with multiple other ecology studies (Koyama et al., 2018; Nielsen et al., 2003; Stephan et al., 2000). To best determine model fit, residuals and qq plots were visually inspected. All statistical methods were accomplished in consultation with the Institute of Food and Agricultural Sciences (IFAS) Statistical Consulting Unit.

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266 **3. Results**

267 **3.1 Site Water and Sediment Characteristics**

268 Surface water TDP during the studies fluctuated between 0.072 ± 0.002 mg.L⁻¹ 269 and 0.104 ± 0.003 mg.L⁻¹. After Hurricane Irma made landfall in southeastern Florida as 270 a category 3 cyclone, samples were taken < 10 m from the experimental site revealed a 271 higher surface water TDP ($0.163 \pm 0.013 \text{ mg}$.L⁻¹), likely caused by surface runoff or sewer 272 overflow. Precipitation measurements from Cangialosi et al. (2018) confirmed the high 273 levels of runoff, finding that the maximum precipitation reported during Irma was 55.02 274 cm near Fort Pierce, just north of the restoration site. Bulk sediment concentrations were 275 1518 \pm 246 mg.kg⁻¹, 121.2 \pm 6.9 mg.kg⁻¹, and 48.98 \pm 4.35 mg.kg⁻¹, for TC, TN, and TP, 276 respectively. These levels are all relatively low but expected considering the nature of 277 the site as a bare sand bed with little organic matter.

278 **3.2 Optimizing BESE design for seagrass restoration (Exp. 1)**

The effects of design type, date, and the interaction between type and date were significant for shoot count (Table 1). The four-stacked plots rapidly declined in shoots, with most shoots absent during and after the first sampling period (Figure 2). The twostacked design initially decreased in shoot count until late July, subsequently increasing until August 22nd (54 days after deployment), while the four-stacked with planting holes design steadily decreased over the study period. However, the two-stacked design did not exhibit significantly higher shoot counts than the four-stacked with planting holes design until 54 days after deployment (t= 5.16, p < 0.0001). At the end of the study, mean shoot counts were 0 shoots in the four-stacked design, 2.3 ± 1.5 shoots for the fourstacked with planting holes design, and 25.0 ± 9.3 shoots in the two-stacked design. By 54 days post deployment, all designs had lost the majority of their shoots, likely due to increased hydrodynamic activity.

291 Over time, the mean blade length of all plots declined from 14.8 ± 3.9 cm after 292 deployment to 3.8 ± 1.0 cm in mid-July (Figure 3). Mean blade lengths were 0 cm for the 293 four-stacked design (due to an absence of shoots), 2.3 ± 0.3 cm for the four-stacked with 294 planting holes, and 3.2 ± 0.3 cm for the two-stacked design during the last date length 295 was measured (54 days after deployment). The effects of design type, date, and type x 296 date were significant for blade length (Table 1). Blade length was initially significantly 297 higher in the four-stacked with planting holes design than the two-stacked design (t=3.18, 298 p=0.0031; and t=3.12, p=0.0037 for one and two days after deployment, respectively) 299 After two days post deployment, the differences in length became (Figure 3). 300 insignificant.

301 Despite substantial declines in blade length, there was still survival and growth of 302 seagrass shoots in multiple plots at the restoration site. For example, one plot of two-303 stacked BESE increased the number of shoots from 20 to 43 shoots, almost 50% increase 304 over a two-month period. Due to this observed survival and growth, the second 305 experiment was deployed to compare BESE to the traditionally used physical restoration 306 techniques (staples and burlap).

307 **3.3 Comparison of physical restoration techniques (Exp. 2)**

308 Seagrass shoot count and blade length declined for all treatments over the course 309 of the two weeks, likely due to transplant stress and wave action (Figures 3). Direct 310 observations showed rhizome exposure in multiple plots, including almost all plots in the 311 burlap treatment. This elevated exposure in the burlap treatment likely contributed to 312 significant differences in shoot count and length between the staples, burlap, and BESE 313 treatments during the two weeks of observation. The effects of treatment, date, and the 314 interaction between treatment and date were significant for shoot count, while the effects 315 of date and treatment (marginally significant at p=0.0934, Table 2) were significant for 316 blade length. The BESE treatment had significantly higher shoot count than both burlap 317 (t= 5.35, p= 0.0002) and staples (t= 3.35, p= 0.0070). BESE also had a significantly 318 higher blade length than burlap (t= 2.24, p=0.0492), with marginal significance for blade 319 length compared to staples (t= 2.01, p= 0.0721). By the last sampling period, the BESE 320 treatment contained 64 shoots (totaling 70% of the total shoots observed), or three times 321 as many shoots as the staples and eight times as many as the burlap treatment. Within 322 two weeks of this last sampling period, Hurricane Irma made landfall and subsequent 323 observations in December revealed a complete loss of seagrass in all plots, likely due to 324 observed burial and high hydrodynamic activity at the site.

In the time-lapse footage, over 600 fish, a majority (> 90%) pinfish (*Lagodon rhomboides*), were observed passing by the tethers, not appearing to interact with the seagrass. There were fish interactions such as apparent feeding on the macroalgae attached to the BESE lattices. Measurements of herbivory via blade length of grass over 24 hours (to account for nighttime herbivory) did not suggest herbivory as a significant factor. Losses did appear to be related to distance from the adjacent oyster reefs (Figure 4). The herbivory tethers placed at approximately one to three meters from the reef had significantly lower blade lengths after 24 hours (t= 3.17, p= 0.0020; t= 5.44, p < 0.0001; and t= 3.13, p= 0.0022 for one, two, and three meters, respectively), while tethers four and five meters from the reef were not significantly lower after 24 hours. However, these results were not consistent with observations of shoot counts or blade lengths between plots.

337

338 4. Discussion

339 **4.1 Comparison of Methods and Abiotic Factors**

340 While brief in length, the results of both experiments suggest the effectiveness of 341 BESE lattices as a physical restoration technique enhanced the survival of H. wrightii seagrasses in the IRL. The first experiment demonstrated that the two-stacked BESE 342 343 lattices appeared to be more effective than other designs over time. The aboveground 344 BESE may have physically abraded the grass blades due to its relatively sharp structure 345 (Temmink et al 2020). The four-stacked plots that did not have planting holes were not 346 an adequate design for *H. wrightii*, as it did not allow the shoots to float and move through 347 the lattice and all plants quickly died off (due to burying/smothering). BESE designs that 348 did not smother H. wrightii may not completely prevent direct herbivory of shoots, 349 especially in environments where small fish or crustaceans are the primary concern. 350 However, the four-stacked with planting holes design could theoretically provide some 351 protection from macro-herbivores like manatees. Instead, the BESE lattices do appear 352 to provide a surface that accumulates sediment and/or prevents erosion. This sediment 353 accumulation/erosion protection effect was also found in the single published study 354 evaluating BESE lattices in seagrass, where it was determined that the BESE mimicked

a mature seagrass rooting mat, improving productivity compared to stapled controls(Temmink et al., 2020).

357 Temmink et al. (2020) used designs similar to the two-stacked and four-stacked 358 with holes from the present study, finding that the two-stacked design (which is 359 belowground) was equal or superior to aboveground designs. The aboveground BESE 360 treatment in Temmink et al. (2020) also had similar limitations to the four-stacked with 361 holes treatment used here, where for example, their lattice was 6 cm thick (three-stacked) 362 but only provided a 10 cm diameter circle for the thicker T. testudinum blades to freely 363 grow. While mean maximum lateral growth was still 36 ± 12 cm over the course of 22 364 months in the study by Temmink et al. (2020), both growth and shoot counts were 365 significantly lower than the belowground BESE plots. Given the similar lack of shoots of 366 the thinner and more flexible H. wrightii in the current study, it appears that aboveground 367 BESE lattices may inhibit H. wrightii growth. However, it has also been demonstrated 368 that 80% of flow velocity is attenuated within and downstream of an aboveground BESE 369 lattice design, possibly capturing sediments and organic matter (D'Angelo, 2018). Thus, 370 having a primarily belowground design with limited aboveground lattice (perhaps a three-371 stacked design, with two-stacks underground and one stack above the sediment) could 372 optimize sediment capture without limiting/damaging aboveground growth (as the 373 aboveground lattice would not cut/directly smother the grass).

The second experiment of this current study demonstrated the effectiveness of BESE, and to a lesser extent staples, over burlap in reducing the mortality of *H. wrightii* transplants. Burlap buried at 2 cm did not perform well in this environment, with the belowground biomass becoming quickly exposed, killing the seagrass, possibly due to 378 the burial depth being too shallow, or the currents/wave action being too high for the 379 technique. This observation is similar to the study of Katwijk et al., (2009) who suggested 380 this as a potential disadvantage of netting or fabric in high hydrodynamic environments. 381 Planting the burlap deeper in the sediment would assist in reducing the potential negative 382 effects of using burlap and other cloth/netting in further applications. While the staples 383 and burlap may not have performed better than the BESE lattice in this environment, their 384 accessibility and low cost necessitates further study in environments with lower levels of 385 physical disturbance.

386 The relatively shallow depth of the restoration site may have exacerbated the 387 impacts of wave and wind action on the seagrasses. The improvements in the shoot 388 count and length could be the result of the BESE lattice buffering wave action, anchoring 389 and protecting the grass. Wind patterns may have contributed to the accelerated decline 390 of seagrasses in the second experiment. Higher wind velocity (taken at NOAA station 391 TRDF1 in Trident Pier, approximately 70 miles north of the field site) was observed during 392 the second experiment (2.91 \pm 0.08 m s⁻¹ for the trial/first experiment, 3.47 \pm 0.24 m s⁻¹ 393 during the second experiment) combined with a northeast direction (average of 156 394 degrees, facing the restoration site) indicating a potentially higher level of physical 395 disturbance at the field site later in the summer. Wind-driven disturbance has also been 396 identified as a potential issue in the restoration of Tampa Bay seagrass beds (Yates et 397 al., 2011). Therefore, it appears that wind driven disturbance, and perhaps the structure 398 of the BESE lattice (which could have buffered hydrodynamic activity), may have been 399 major factors in the differences of shoot count and blade length between the different 400 restoration techniques.

401 **4.2 Effects of Herbivory**

402 As previously stated, herbivory is often found to significantly affect aquatic 403 vegetation restoration attempts (Bourgue & Fourgurean, 2013; Hauxwell et al., 2004; 404 Paulo et al., 2019; Ravaglioli et al., 2018; Tomas et al., 2005; Tuya et al., 2017). In this 405 present study, attempts to observe herbivory via the tethers and time-lapse footage 406 vielded no conclusive results, perhaps indicating herbivory was not a significant factor 407 compared to other physical disturbances. This conclusion is somewhat puzzling as known 408 seagrass consumers were present in large numbers (i.e. pinfish). There was a possibility 409 that resident herbivores in the adjacent oyster reef may have fed on the seagrass at night, 410 or that the oyster reef itself might have reduced blade lengths by increasing hydrodynamic 411 activity, given the significant decline in the length of herbivory tethers (Figure 4). 412 However, Gruninger (2019) found that nearby oyster reefs did not have a significant effect 413 on seagrass grazing pressure within the same proximity as our experiments. The nearby 414 seagrass bed may have reduced herbivory for the study site, where comparatively there 415 were far fewer seagrass shoots for a shorter period of time. However, other locations in 416 the southern IRL may face grazing pressures (Gilmore, 1995), and herbivory should still 417 be considered as a potential factor in future restoration efforts.

418 **4.3 Recommendations for Application and Future Studies**

Based on the positive performance of BESE in this study, it is recommended that lattices of this or similar type be applied to restoration efforts in areas with limited erosive hydrodynamic activity, including seagrass scars and fringing seagrass plots (plots at the edge of seagrass environments or restoration efforts). Smaller scale projects (x < 1000 shoots) could especially benefit from BESE as they lack some of the positive geomorphological feedbacks (i.e. sediment stabilization, improvement to local water 425 quality) inherent to larger efforts (Katwijk et al., 2016; Maxwell et al., 2017). However, 426 based on the observations of rapid burial at multiple plots in this study, prevailing winds 427 and shifting sand deposits should be included in future site considerations for BESE 428 applications. For example, in Florida, it is recommended to select sites on the central 429 Gulf coast or protected banks on the Atlantic coast that will avoid dominant wind patterns 430 that can damage plants and shift sediments at the site. The central Gulf coast of Florida 431 is an environment "favorable to seagrasses" and is home to one of the largest seagrass 432 communities in the US (Barry et al., 2017). However, extensive boat scarring in the Gulf 433 threatens otherwise healthy seagrass beds, a disturbance that produces environments of 434 elevated hydrodynamic activity that can erode adjacent healthy seagrass beds if not 435 otherwise remediated (Hammerstrom et al., 2007). Therefore, conducting studies of 436 seagrass scars in this region of Florida provides ample opportunities for the testing of 437 BESE in a subtropical environment relatively isolated from the effects of eutrophication 438 and other confounding variables/disturbances.

439 BESE and other physical restoration approaches should also be combined with 440 other common restoration techniques to improve the survivability of seagrasses 441 introduced to new environments. Seagrass is especially vulnerable to stressors during 442 transplantation, where the composition of the local sediment may be nutrient limiting, 443 reducing the probability of a successful restoration effort. Fertilization is a popular 444 restoration technique used to ameliorate issues caused by nutrient limitation (Armitage & 445 Fourgurean, 2016; Peralta et al., 2003). Combining the possible protective effects of 446 BESE lattices and the supplemental nutrients of fertilizers could increase the growth of 447 seagrass (especially in the low sediment TN and TP observed at the site) and improve

448 survivability during disturbance events. However, it is also important to remember that 449 the root cause of the much seagrass decline (i.e., urban waterways/estuaries) centers 450 around eutrophication. Until this core issue is resolved, seagrasses will likely continue to 451 decline, and thus, restoration will remain difficult regardless of the deployment of different 452 active restoration approaches.

453

454 **5. Summary and Conclusions**

455 Human disturbances are degrading seagrass ecosystems, and current attempts to 456 restore these coastal habitats are expensive and often unsuccessful. This study 457 investigated the effectiveness of multiple physical restoration techniques on a pioneer 458 seagrass species in a disturbed subtropical coastal system. While the study here was 459 not a long-term comparison among techniques, initial results found that novel BESE 460 lattices could reduce the rate of decline in seagrass shoot count and blade length relative 461 to traditional (staples and burlap) restoration techniques. Herbivory and hydrodynamic 462 activity (dominant wind and wave patterns) both may have made the local environment 463 unsuitable for seagrass establishment by the end of the study (based on declines in 464 shoots and length in all plots). However, the differences in seagrass parameters even in 465 such a relatively harsh environment reveal the promise of BESE in protecting seagrass 466 from wind and wave action. Future studies should investigate the effectiveness of other 467 BESE designs (i.e. wider or rounder lattices more compatible with seagrass) across a 468 wider range of systems and conditions, and in concert with other methods used to improve 469 restoration success (i.e. fertilization) over longer periods of time. The application of 470 staples and burlap should be further tested in different hydrodynamic environments to

determine which technique is more suitable in varying levels of wave and current
disturbance. By taking these steps, it may be possible to add new physical restoration
techniques to the portfolio of management options to effectively re-establish seagrass
and other coastal ecosystems and begin reversing their overall decline.

475

476 Funding Sources:

This work was supported by the Soil and Water Sciences Biogeochemistry Laboratory and Florida Seagrant. Seagrass restoration by the Florida Oceanographic Society was supported by the South Florida Water Management District Indian River Lagoon License Plate funding.

481

482 **Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

486

487 Acknowledgments:

A special thanks to Dr. C. Angelini for her assistance in the experimental design and assistance with writing. The authors acknowledge Sophia Barbour and Devin Leonard from the University of Florida's Wetland Biogeochemistry Laboratory for assistance with the analysis of surface water and soil nutrients. Special thanks to James Colee from the IFAS statistical consulting unit for confirming the quality of the data and the subsequent analyses. Many thanks to the staff and volunteers at the Florida Oceanographic Society, for permission to use their site and their seagrass nursery, and
for support in the deployment of the experiments. We also thank Lori Morris from the St.
Johns River Water Management District for help in seagrass identification and monitoring
techniques.

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Yates, K., Greening, H., & Morrison, G. (2011). In USGS circular 1348: Integrating

717 Table 1. Mixed model effect test results for shoot count and blade length measured during

the BESE design/first experiment.

		Va	riable			
		Shoot Count		Length cm		
Source						
Parameter	DF	F statistic	P value	DF	F statistic	P value
Treatment	2	54.90	< 0.0001	2	1069	< 0.0001
Date	7	14.25	< 0.0001	5	128.9	< 0.0001
Treatment x Date	14	4.461	< 0.0001	10	34.52	< 0.0001

Table 2. Mixed model effect test results for shoot count and blade length measured during

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	v	v

the physical restoration comparison/second experiment.

Variable								
	Shoot				Length			
Source	Count				ст			
Parameter	DF	F statistic	P value	DF	F statistic	P value		
Treatment	2	189.1	< 0.0001	2	134.9	< 0.0001		
Date	2	9.841	0.0043	2	3.034	0.0934		
Treatment x Date	4	4.722	0.0045	4	1.880	0.1398		



743 Figure 1. Location of field site (a), deployment of BESE design experiment at low tide

- (b), designs for BESE design/first experiment (c), and design for the physical restoration comparison/second experiment (d).



Figure 2. Average shoot count (a) (initial 25 shoots) and blade length (b) from the BESE
design/first experiment. Asterisk designates the significant difference between
the two-stacked and four-stacked with holes designs, points represent the
mean of three replicates (± SE).



Figure 3. Average shoot count (a) (initial 50 shoots per plot) and blade length (b) during the physical restoration comparison/second experiment. Letters designate significant differences between treatments for the same sample dates, points represent the mean of six replicates (\pm SE). Blade length in the BESE treatment was marginally significant (longer blade length with *t*= 2.01 and *p*= 0.0721) versus staples, designated with an asterisk.



Figure 4. Average blade length of herbivory tethers based on approximate distance from the nearest restored oyster bar (labeled "approximate distance" due to the approximately 0.5 meter length of the tether) during the last sampling period of the physical restoration comparison/second study. Asterisks designate tethers that were significantly lower in length after deployment versus before deployment, points represent the mean of four replicates (± SE).

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