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TITLE

Phase I Submerged Aquatic Vegetation (SAV) Pilot Restoration Site, Lynnhaven River, Virginia – Adaptive seagrass restoration comparing stoic *Zostera marina* & opportunistic *Ruppia maritima*

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ABSTRACT

Nearshore ecosystems provide coastal protection and essential habitat for fisheries but are under threat from a myriad of stressors including rising temperatures that often compromise the ability of habitat-forming, foundation species to maintain populations. Habitat restoration is a powerful tool to help mitigate the loss of foundation species, however, many efforts focus on reintroducing a single, declining or lost species instead of incorporating alternatives that are more conducive to current and future conditions. Globally, seagrass meadows are declining, and many are experiencing dominant species shifts from stable to opportunistic species. Here, we experimentally tested if widgeongrass (*Ruppia maritima*) – an opportunistic, generalist species that can withstand wide ranges of salinities and temperatures – can be planted by seed and if planted in conjunction with eelgrass (*Zostera marina*) can increase seagrass restoration success, and how seagrass identity alters bed structure and function. We found widgeongrass can be restored via direct seeding and the pilot restoration area was increased by 170% by planting widgeongrass in shallow waters unsuitable for eelgrass due to high temperatures. Seagrass identity was observed to alter bed structure and function. Widgeongrass had higher faunal diversity and two times more animals per plant biomass than eelgrass, whereas eelgrass produced three times greater plant biomass as well as significantly more recycled nitrogen available within the sediment. These results demonstrate that planting widgeongrass from seed is an effective way to enhance temperate seagrass restoration and the principle of using generalist species alone or in conjunction with historically dominant taxa in restoration is broadly transferrable to other restoration scenarios. Overall, we provide evidence that using opportunistic, generalist species in habitat restoration is a proactive and effective approach as these species can increase foundation species diversity which, for our study, increased restoration area and more generally, increases the ability for restored beds to promote facilitation cascades, stability, and grass persistence through changing environments.

KEYWORDS

Coastal ecosystems, habitat restoration, seagrass, broadcast seeding methods, widgeongrass, *Ruppia maritima*, eelgrass, *Zostera marina*, marine heatwaves, opportunistic species, generalists, facilitation, proactive, climate change

INTRODUCTION

Reversing the decline of coastal ecosystems through habitat restoration can be a powerful technique to help mitigate loss of functions including shoreline protection, nutrient cycling, and habitat for many ecologically and economically important species (Lotze et al., 2006, Halpern et al., 2008). Direct planting of key habitat-forming foundation species like corals, mangroves, and seagrasses has been a widely accepted and longstanding approach for coastal conservation. While coasts are inherently stressful and dynamic systems, they are currently undergoing unprecedented changes including human-driven eutrophication, shifting temperature trends, and increases in extreme disturbance events. Because of this, initial success of coastal restoration practices still vary widely among habitat types, and efforts resulting in long-term persistence are still rare (van Katwijk et al., 2016, Suding, 2011). The additional combined effects of novel human and climate disturbances may specifically contribute to low success when declining dominant species are the focus of restoration efforts, as opposed to incorporating alternative species that are more conducive for current and future conditions.

Seagrasses are an example of an important habitat in rapid global decline (Orth et al., 2006) that need novel mitigation approaches to conserve key services including nursery and foraging habitat, high primary productivity, sediment retention, and carbon sequestration (Gattuso et al., 2018, Orth et al., 2020). Restoration efforts result in enhanced plant survival and persistent recovery occurs when carefully selected sites meet minimum viable thresholds for the species of interest and when plantings are done in high densities (seeds or transplants) at large enough spatial scales to enable positive feedback mechanisms (van Katwijk et al., 2016). However, in areas undergoing long-term or irreversible environmental shifts, local sites that meet the minimum conditions for viable restoration may no longer exist for seagrasses impacted by climate change (Kilminster et al., 2015, Turschwell et al., 2021). In these situations, when the goal is to restore lost ecosystem functions, opportunistic, generalist species that are more likely to thrive and persist in the altered environment may offer a solution. Such non-target species may not only provide short term restoration success, but through their ecosystem engineering capabilities, may also provide longer-term restoration success to lost or other species. For example, the accidental introduction of the non-native macrophyte *Hydrilla verticillata* to the Potomac River, Virginia, ultimately resulted in many positive outcomes for the system (De Mutsert et al., 2017). Hydrilla not only re-established large areas of vegetation but also facilitated the recovery of many native plant species, resulting in very stable high diversity meadows (Patrick et al., 2018). Use of non-native species in restoration is generally not advisable (may need a reference here), but native species with related qualities to successful invaders may provide similar benefits. We identify widgeongrass, *Ruppia maritima*, as a potentially ideal species that can be used for restoration in these situations throughout many regions worldwide.

Widgeongrass is a generalist species that is distributed across broad latitudinal ranges in both hemispheres and tolerates a wide range of salinities and temperatures (Verhoeven, 1979, Short et al., 2007). While widgeongrass is still threatened by humans impacts like other seagrasses, it's highly resilient and has demonstrated the ability to both rapidly recover and expand its coverage where formerly dominant species like eelgrass, *Zostera marina*, have declined (Cho and May, 2008, Cho and Poirrier, 2005, Johnson et al., 2003, Richardson et al., 2018). There have been efforts to restore widgeongrass (Ailstock et al., 2010, Cho and Biber, 2010), however, it is less frequently used than dominant or 'climax' species. Few studies have tested widgeongrass restoration outcomes in the field under realistic environmental conditions. Importantly, several knowledge gaps remain in how to precisely plant and promote long-term survival as well as establish widgeongrass effectively at larger, restoration spatial scales (Cho et al., 2009). Furthermore, we also lack a clear understanding of how ecosystem structure and function may be altered when planting widgeongrass in areas where it hasn't been historically dominant.

The lower Chesapeake Bay, United States provides an ideal place to evaluate the use of widgeongrass in seagrass restoration. Historically this region has been dominated by eelgrass, the most wide-ranging seagrass in the Northern Hemisphere where its lower latitudinal range is just south of the Chesapeake Bay. Due to increasing temperature trends from climate change (Orth et al., 2010, Lefcheck et al., 2017, Johnson et al., 2020), eelgrass meadows have been declining in the Chesapeake Bay and is in danger of possibly being extirpated if waters continue to warm (Orth et al., 2017, Wilson and Lotze, 2019, Arnold et al., 2017, Hammer et al., 2018). Environmental researchers and managers including the U.S. Army Corps of Engineers are concerned about the long-term stability of eelgrass populations in the Chesapeake Bay and have recommended a multi-species restoration strategy focused on two species, eelgrass and widgeongrass (USACE 2013). Widgeongrass is a native, opportunistic or 'pioneer' species and much more tolerant to increasing water temperatures than eelgrass (Moore et al. 2013). Yet, to date, lower Chesapeake Bay and Virginia's coastal bays seagrass restoration efforts have focused on eelgrass. Widgeongrass has not been used in any significant large-scale restoration efforts (Orth et al., 2010). By using both eelgrass and widgeongrass, the risk of overall seagrass restoration failure would be significantly reduced.

Here, we conducted a manipulative field experiment in the lower Chesapeake Bay with widgeongrass and eelgrass and then operationalized our findings into a pilot, proactive restoration study to ask: (1) what planting technique promoted the highest survival and growth for widgeongrass, (2) how does grass species alter seagrass structure and function, (3) do experimental findings and trends scale up at effective restoration spatial scales, and lastly, (4) what are implications of our findings for the broader use of widgeongrass or generalist species in seagrass restoration. This seagrass restoration effort was a significant part of a larger restoration plan for the Lynnhaven River, VA (USACE 2013). It is an effort that also represents the first large-scale attempt to restore widgeongrass in the Chesapeake Bay. Widgeongrass' ability to survive increasing water temperatures compared to eelgrass was the deciding factor to include it in our large-scale restoration pilot due to concerns over the long-term viability of eelgrass in this changing environment. It was also assumed that widgeongrass could aid the survival of eelgrass due to improvements in water clarity and quality, such improvements would reduce stress on eelgrass and aid in its survival (Lefcheck et al., 2017). Widgeongrass may also survive and expand if restored eelgrass experiences a total die-off from water temperatures rising past its thermal limit (Moore et al., 2014, Shields et al., 2019), thus increasing the chances of restoration success and minimizing the chances of overall failure.

2| MATERIALS AND METHODS

Study Site

Our study was conducted in Broad Bay in the Lynnhaven River System in Virginia Beach, Virginia, USA from October 2020 – August 2021 for the field experiment and our pilot restoration was conducted October 2021 and data collected April 2022 (Figure 1). The Lynnhaven River is a heavily human-influenced system (Sisson et al., 2010) where local practitioners are actively managing the area with goals of improved water quality and habitat, with the latter using coastal and nearshore habitat restoration efforts including seagrasses. During our study from March to December, Broad Bay's average temperature (°C) ranged from 11.67 ± 2.94 SD – 28.7 ± 1.52 SD, turbidity (NTU) 3.22 ± 1.34 SD – 14.44 ± 7.57 SD, and salinity (ppt) 17.19 ± 0.96 SD – 23.80±1.02 SD (CBNERR-VA VIMS, 2022). In May 2020, small patches of wild widgeongrass were observed ~2.5 km south of our study site (https://www.vims.edu/research/units /programs/sav/access/maps/index.php), and eelgrass was observed in the area until 2012 (https://www.vims.edu/research/units/programs/sav/access/maps/index.php). Our study site is located along an undeveloped shoreline adjacent to First Landing State Park (36.90418 latitude, -76.03084 longitude) where water depth varies from 50 – 150 cm depth between tidal exchange and the benthic substrate is bare, sandy sediment. Our site was determined on available, non-commercial fishery leased space as well as meeting suitable seagrass environmental criteria (Marion and Orth, 2010a, Marion and Orth, 2010b, Moore et al., 2014). Since only small patches of widgeongrass were present in Lynnhaven River, we also conducted trial plots of each of our experimental treatments (described below) at a second site where both widgeongrass and eelgrass naturally grow to evaluate the importance of environmental conditions relative to our experimental manipulations. Our second site was located along the southern shoreline of Goodwin Island, part of the Chesapeake Bay National Estuarine Research Reserve (CBNERR) located near the mouth of the York River (37.21896 latitude, -76.39322 longitude). Natural seagrass beds composed of either or both widgeongrass and eelgrass are observed around the entirety of the Goodwin Island, with bare sediment patches throughout (see Supplemental materials for environmental characteristics).

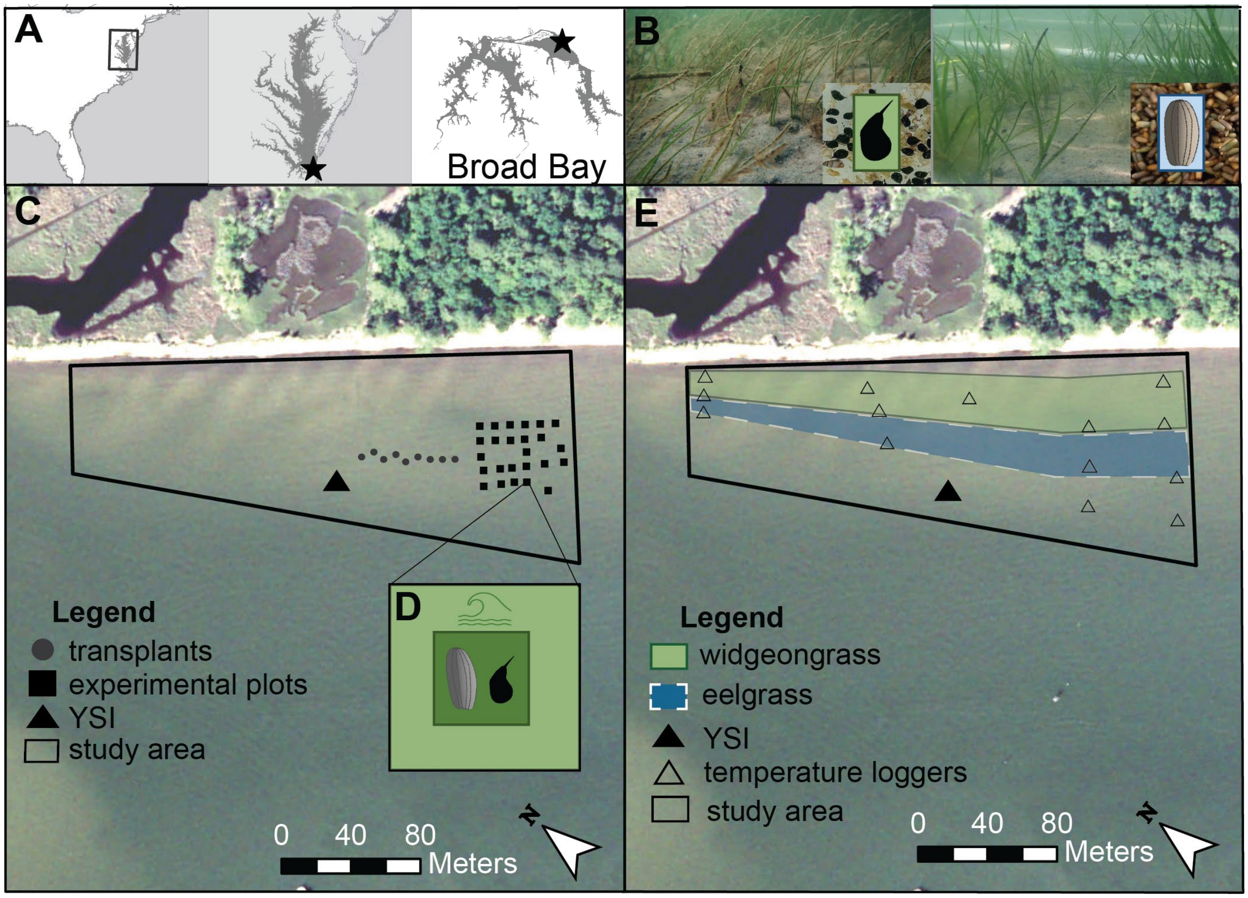

Figure 1. Study site in Broad Bay, Lynnhaven River, VA, USA (**A**). Example image of widgeongrass (left) and eelgrass (right) seed and plant morphology (**B**). Study site specifics for 2020-2021 experiment (**C**) and 2021- 2022 pilot restoration (**E**). Total study area parameters (black outline) were constricted to non-commercial fishery leased area where experimental and habitat restoration were further constricted by low tide depths, water temperature ranges, and water clarity (**E**). Environmental conditions were measure by both YSI EXO2 multi-parameter water quality sonde station and Onset® HOBO® temperature loggers. Black squares represent $4m^2$ experimental plots that are a minimum five meters apart from one another and were randomly assigned one of the five treatments: control, eelgrass planted in fall, widgeongrass planted in fall, widgeongrass planted in spring, or widgeongrass planted in spring + seed given a 48-hour freshwater shock prior to planting. Experimental plots representation (**D**) with either widgeongrass or eelgrass treatments having seeds broadcasted into the center $1m^2$ to allow a 1m buffer perimeter to measure any effects from seeds that dispersed from wave action; controls received no seeds.

Experimental Set-Up and Monitoring

In October 2020, we established 30, $4m^2$ experimental plots $\geq$ 5m apart at our Broad Bay experimental site. We then assigned one of five treatments with five replicates each: control (no seeds), eelgrass seeds planted in fall, widgeongrass seeds planted in fall, widgeongrass seeds planted in spring, or widgeongrass seeds that were given a 48-hour freshwater shock prior to planting in spring (Figure 1). A 48-hour freshwater shock cracked at least 50% of the widgeongrass seed shells and mimicked a spring freshet which has been identified as a cue for widgeongrass germination (Ailstock et al., 2010). Fall treatment plots were seeded in October 2020 and spring treatment plots were seeded in March 2021. Because there was no naturally growing seagrass directly at our study site and therefore, some uncertainty on the environmental suitability for seagrass survival, we also transplanted widgeongrass and eelgrass shoots in five,

$1m^2$ plots per species to identify site suitability versus our seeding manipulations and/or seed viability. Transplants came from grasses near the northern side of the York River mouth, in Mobjack Bay.

To monitor ambient environmental conditions, both sites had an YSI EXO2 multi-parameter water quality sonde station within 50 – 130 m from our experimental plots collecting several measurements including water temperature, turbidity, salinity, and depth at approximately 15-minute intervals during the experimental duration except from January to March. These data were collected and reviewed by the CBNERR. In Broad Bay, additional secchi discs measurements were periodically taken, and 30-minute interval temperature data loggers (Onset® HOBO®) were stationed along the border of potential suitable restoration area and along three distances from the shoreline (Figure 1) to help determine environmental barriers (e.g., light availability for widgeongrass and eelgrass upper temperature limits). Light requirements for widgeongrass in our study have not been well-studied and so we had no true threshold, but it's known to be more sensitive than eelgrass which has a light threshold requiring 22% of light through the water (Tango and Batiuk, 2013). We used 25°C and 30°C as our upper temperature limits for eelgrass as these temperatures have been shown to be stressful for eelgrass in the lower Chesapeake Bay where water clarity was simply monitored without a known threshold for this particular system (Shields et al., 2019).

Experimental Broadcast Seeding

Widgeongrass and eelgrass seeds were collected and stored using similar procedures described in (Orth et al., 2003, Ailstock et al., 2010, Orth et al., 1994); both species' seeds were harvested from local beds located in the polyhaline (salinity range 18-30 ppt), southern Chesapeake Bay area at the same location of our transplants. Reproductive shoots were hand collected in May and July 2020 for eelgrass and widgeongrass, respectively, and modified seed viability tests followed Orth et al. 2003 procedures. A portion of the widgeongrass seeds were stored in 1L glass jars with 33 ppt saltwater made from deionized water and Instant Ocean® Sea Salt and placed in a dark refrigerator at 4°C until we seeded our spring experimental plots in March 2021 (Ailstock et al., 2010). For each experimental plot minus controls, which were left as $4m^2$ bare sediment, we broadcast seeded approximately 500 seeds within the $1m^2$ center, i.e., hand sprinkled seeds in the water column, leaving a one-meter buffer area to measure grass from seeds that dispersed from wave action before they settled into the sediment (Figure 1). Previous research had shown that eelgrass seeds did not move far from where they settled on the sediment surface (Orth et al. 1994) allowing for an accurate assessment of seedling abundance from sprinkled seeds within and adjacent to the experimental plot. We assumed widgeongrass seeds would behave similarly. To determine the best seeding technique for widgeongrass, we compared plot percentage survival till the end of the first growing season, total plot areal cover, and shoot density.

Experimental seagrass structure and function measurements

Bed characteristics: area, shoot density, and canopy height

For bed structure characteristics, initial shoots or seedling estimates, i.e., the percentage of seedlings sprouted from seeds planted, were measured in early April 2021 and plots were monitored monthly for areal coverage, shoot density, and canopy height until our final data collection at the peak growth season – late June for eelgrass and late July for widgeongrass. We measured not only the 1 m2 plot, but also ae 4m^2 area subdivided into 16 0.25m^2 subplots for each plot to account for some seed movement once they settled on the sediment surface, and to not miss any ecological effects from our planted seeds. For each subplot, we recorded shoot density using a haphazardly placed 0.01 m^2 quadrat and took 5 randomized shoot heights to the nearest centimeter. Final seagrass plot area coverage was then measured as the total number of subplots with at least 5% seagrass cover at peak growth season, multiplied by 0.25. Because our plot size of interest is 1m^2, for average shoot density, canopy height, and percent macroalgae cover, we took the average of four of the 16 subplots that had the highest seagrass percent cover, i.e., where most seeds settled.

Macrofauna for habitat provision (maybe say community faunal dynamics)

To measure potential differences in habitat provision, epi-benthic and -phytic invertebrate fauna were collected from our fall widgeongrass and eelgrass treatment and control plots to measure differences in community composition, i.e., guild diversity, abundance, and biomass. For each plot, we placed a mesh bag 20 cm in diameter within a representative area of the plot collecting the top benthic surface and aboveground plant material and froze each sample until later processed in the lab. Once thawed, all invertebrates were counted and identified to one of the following groups or guilds: sponge (Porifera), free squirt tunicate (Tunicata), mud snail (*Ilyanassa obsoleta*), *Bittium* spp. snail, mobile amphipod (Amphipoda), sessile amphipod (Caprellidae), worms (Annelida), shrimp (non-Brachyura Decapoda), and blue crabs (*Callinectes sapidus*). Groupings were determined based on taxonomy as well as specific habitat and/or niche use. Associated aboveground plant biomass was also collected from each sample. Fauna and plant materials were dried for four days at 60°C and weighed to the nearest 1/10th milligram. Faunal community composition metrics were calculated as a ratio to the amount of aboveground plant biomass.

Primary production

To compare potential differences in primary production between seagrass species, we collected one biomass core from each plot during their respectively peak growth seasons. Cores were 20 cm in diameter and went 10 cm into sediment or when no more roots were observed. Core placement was in a location that represented the four subplots with the highest seagrass cover. Each core was sieved *in situ* to remove sediment and plant material was frozen and later processed in the laboratory. In the laboratory, plant material was thawed and then separated into above and below biomass with epiphytic algae scraped off blades. Seagrass material was dried for four days at 60°C and weighed to the nearest 1/10th milligram.

Sedimentary nitrogen removal and recycling

Sediment cores were collected in triplicate from our fall widgeongrass and eelgrass treatments and control. Core collection tubes were fashioned from plastic 50 mL falcon tubes to collect the top 5 cm of sediment in treatment plots. Cores were kept on ice until returned to the lab and homogenized. To measure the rates of denitrification (nitrogen removal) and dissimilatory nitrate reduction to ammonia (DNRA; nitrogen recycling), sediment slurry incubation experiments were conducted with ~1g of the composited sediment from each core. The sediments were transferred in 12mL exetainer vials (Labco^{LTD}), sealed with screw caps with rubber septa, and flushed with helium (He) gas for 5 min. The tubes with sediments were pre-incubated overnight and then reflushed with He for 5 min. After injecting 100 nmoles $^{15}NO_{3-}$ (99 atm%, Cambridge Isotope Laboratories, Inc.), the tubes were incubated at room temperature and the activities were killed at 0, 1, and 2 hours by adding saturated ZnCl solution. The production of $^{29,30}N_2$ gas over time was measured using a gas bench isotope ratio mass spectrometer (IRMS, Delta V Plus, Thermo Fisher Scientific, Waltham, MA) to calculate denitrification rates described by (Song and Tobias, 2011). To determine DNRA rates, ammonium was extracted from incubation samples using 5mL of 2M KCl. The extract was then diluted with Milli-Q (MilliporeSigma, Burlington, MA, USA) and poured into a new exetainer tube. Each extract was then amended with 200 µL of a hypobromite solution, which converts all NH_4+ to N_2 (Yin et al., 2014), inverted, and incubated for at least 15 min before being run on a membrane inlet mass spectrometer (MIMS, Balzers Prisma, Pfeiffer, Aßlar, Germany). The concentration of excess $^{29}N_2$ and $^{30}N_2$ produced by the addition of the hypobromite solution was calculated as described by Fortin et al (2021) with the exception that a single air equilibrated DI water standard, held at the same temperature as the samples, was used. The concentration of $^{29}N_2$ and $^{30}N_2$ were used to calculate the concentration of $^{15}NH_4^+$ present in each sample and the production of $^{15}NH_4^+$ over time was used to determine DNRA rates.

Seagrass Restoration Pilot Study

For our proactive restoration pilot study, suitable habitat to plant seagrass was constricted by various environmental characteristics observed during the experiment including water temperature, clarity, and physical disturbances within available space for restoration (Moore et al., 2014, Shields et al., 2019, Orth and Moore, 1988). As mentioned above in the Introduction, we planned to use both seagrass species, but exact methodology was driven from experimental observations and results as well as how many seeds were available from the seed harvest in summer of 2021 for specifically determining seed dispersal density. Overall, grass species were planted into two monocultures directly adjacent to one another (Figure 1). Widgeongrass was restricted to an area close to the shoreline, which had a low tide depth from 30-50 cm and eelgrass was planted in areas with a low tide depth from 50-150 cm. Due to the bathymetry of the benthic slope from the shoreline to the middle of the river, tidal depth change declines with proximity to shoreline which maximizes water clarity time during the day and, also allows temperatures to reach over viable levels for eelgrass for more days and hours within each day oppose to areas further from the shoreline. Furthermore, we observed both wave action from boats and uprooting disturbances likely from local bioturbators such as stingrays and crabs that were seen at the study site during the experiment. By planting widgeongrass closer to the shoreline in the shallows, our expectations were to decrease access to large-bodied bioturbators to maximize widgeongrass persistence. For our two major environmental thresholds, i.e., 22% of light through the water and upper temperature limits of 25°C and 30°C, light penetrated 68 – 99.9% of the water throughout the year at 0.5-1.0 m depth and 0.2 – 95.6% of the water throughout the year at 1.0 – 1.5 m depth. Lastly, in the selected area to plant widgeongrass, temperatures above 25°C were observed for 133 days and reached 30°C for total of 40 days whereas our area selected to grow eelgrass did encounter above 25°C for 119 days and reached 30°C for total of 30 days.

Based on available harvested seed material as well as experimental plot survival, final total areal coverage, and final shoot density, we seeded a total area of 3,837 m^2 with 90 seeds per meter-square and 3,924 m^2 with 50 seeds per meter-square for widgeongrass and eelgrass, respectively (Table 1; Figure 1). To compare and test the scalability of our experimental outcomes to the larger spatial scale of restoration efforts, we compared initial spring shoots percentage estimates.

Statistical Analyses

Data were analyzed with R version 4.2.1 (Team, 2022). One-way ANOVAS were conducted to assess main effects and Tukey's post hoc multiple comparisons tests. Normality of the residuals and heterogeneity of variances were checked prior to data analyses using Kolmogorov-Smirnov normality test as well with the Performance R package. To determine any differences in guild diversity we used effective Shannon hill number using the hillR R package.

RESULTS

Transplant shoots of widgeongrass and eelgrass all survived during the duration of our experiment providing evidence our study site is conducive for widgeongrass and eelgrass to grow and survive. Our trial plots along Goodwin Island reflected similar responses to our Broad Bay plots (see Supplemental material for more details), showing that any environmental differences between these two sites were not significant enough to influence or alter our measure responses.

Widgeongrass seeding techniques

For the three different planting techniques of widgeongrass, plots with seeds planted in the fall with no pre-seed treatment was determined as the best technique as it had the highest plot survival and largest percent cover area growth, i.e., areal cover and shoot density within the first growing season (Table 1).

Table 1. To determine which seeding technique had the most optimal outcome from our experiment, we compared average treatment plot percentage survival till the end of the first growing season as well as plot-level total areal cover (m^2) and shoot density ($0.01m^2$). Averages are from the five replicate plots per treatments with standard errors.

treatment	% survival	area cover	shoot density
eelgrass	100	3.1 ± 0.34	11.55 ± 0.64
widgeongrass planted in fall	75	2.05 ± 0.68	6.95 ± 2.26
widgeongrass planted in spring	100	0.25 ± 0.18	3.00 ± 2.76
widgeongrass 48-hour freshwater shock + planted in spring	25	0.15 ± 0.10	0.15 ± 0.15

Structure and functional comparison between widgeongrass and eelgrass

Plant structure metrics were altered by our treatments, i.e., total bed area cover ($F_{(3,15)} = 12.14$, R^2-adj = 0.65, $p < 0.01$), shoot density ($F_{(3,15)} = 9.0$, R^2-adj = 0.64, $p < 0.001$), and canopy height ($F_{(3,9)} = 83.0$, R^2-adj = 0.95, $p < 0.001$; Figure 2). Specifically, widgeongrass with no pre-seed treatment and eelgrass planted in the fall had similar areal growth (m^2) (average 2.05 ± 0.68 SE fall widgeongrass and 3.10 ± 0.34) while spring widgeongrass plots produced negligible coverage (average 3.0 ± 2.78 SE spring widgeongrass and 0.15 ± 0.15 SE spring widgeongrass with freshwater shock). Fall widgeongrass and eelgrass had the highest shoot density ($0.01m^2$) (average 6.95 ± 2.26 SE fall widgeongrass and 11.55 ± 0.64 SE eelgrass). Lastly, eelgrass had a significantly higher canopy height (cm) than any widgeongrass plot (average 5.85 ± 0.74 SE fall widgeongrass, 28.60 ± 1.56 SE cm eelgrass).

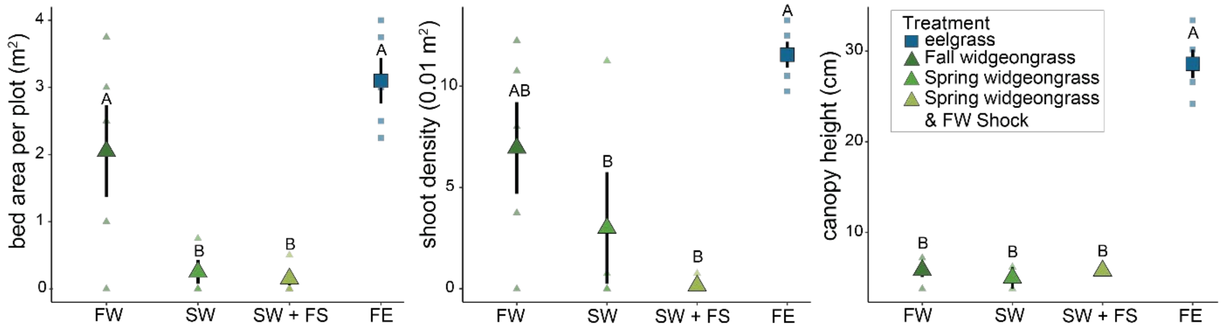

Figure 2. Average total bed area cover is altered by planting season ($F_{(3,15)} = 12.14$, R^2-adj = 0.65, $p < 0.01$). Average shoot density is altered by both planting season and seeding method ($F_{(3,15)} = 9.0$, R^2-adj = 0.64, $p < 0.001$). Average canopy height is altered by seagrass species ($F_{(3,9)} = 83.0$, R^2-adj = 0.95, $p < 0.001$. A and B indicate significantly different treatments from another based on TukeyHSD comparisons. X- axis abbreviations match treatment legend: FW = fall widgeongrass, SW = spring widgeongrass, SW +FS = spring widgeongrass + 48-hour freshwater shock, FE = eelgrass (only seeded in fall). Averages are from the five replicate plots per treatments with standard errors.

Widgeongrass had the higher faunal guild diversity (effective Shannon) (average 2.15 ± 0.08 SE fall widgeongrass, 1.04 ± 0.13 SE eelgrass) and abundance (average 40.73 ± 7.33 SE fall widgeongrass, 11.50 ± 2.04 SE eelgrass) than eelgrass per plant biomass, but no differences were found in faunal biomass comparisons (Figure 3). We tested total biomass (g) from the total abundance of individuals present per sample per plant biomass present ($F_{(1,8)} = 0.37$, R^2-adj = -0.08, $p = 0.56$) as well as average individual weight of each organism per plant biomass present (average 0.01 ± 0.00 SE fall widgeongrass, 0.07 ± 0.04 SE eelgrass). While the effect was not statistically significant ($F_{(1,8)} = 1.9$, R^2-adj = 0.1, $p = 0.2$), individuals on average were eight times larger in eelgrass plots than widgeongrass (Figure 3). We only compared fall widgeongrass and eelgrass plots for faunal composition metrics as fall widgeongrass was determined as our most optimal seeding technique. No fauna were found in control plots.

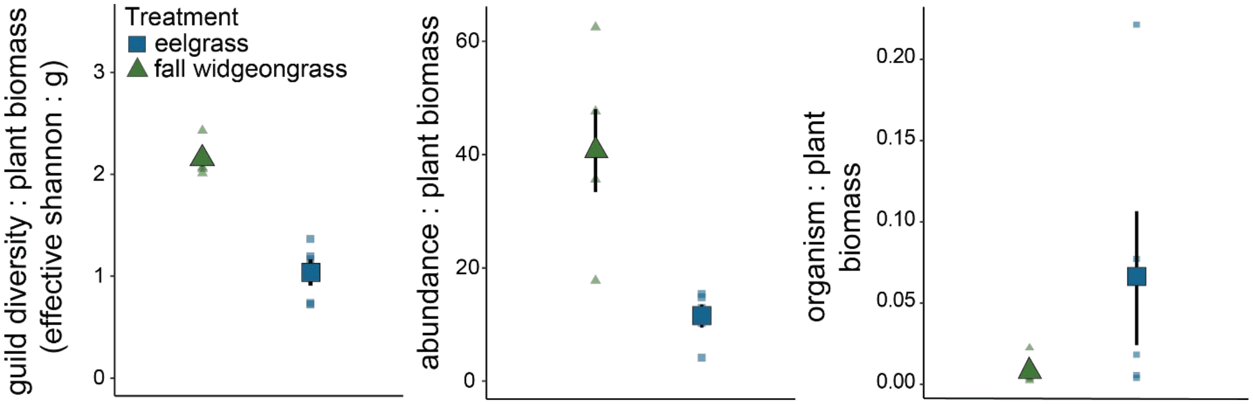

Figure 3. Average guild diversity is altered by seagrass species ($F_{(1,8)} = 55.0$, R^2-adj = 0.86, $p < 0.001$) as well as average total abundance per plant biomass within 0.02m^2 samples ($F_{(1,8)} = 14.7$, R^2-adj = 0.60, $p < 0.001$). Average individual organism biomass per plant biomass was not altered by seagrass species ($F_{(1,8)} = 1.9$, R^2-adj = 0.1, p = 0.2). Averages are from the five replicate plots per treatments with standard errors.

For plant biomass, widgeongrass produced significantly less biomass than eelgrass (average 0.48 ± 0.12 SE fall widgeongrass, 2.37 ± 0.43 SE eelgrass; Figure 4). The ratio of nitrogen recycling (dissimilatory nitrate reduction to ammonia or DNRA) to removal (denitrification or DNF) was influenced by the presence of seagrass opposed to bare sediment controls, but the direction was affected by grass identity (average 1.11 ± 0.20 SE fall widgeongrass, 3.68 ± 0.83 SE eelgrass, 2.24 ± 0.63 SE bare sediment). DNRA:DNF decreased in widgeongrass and increased in eelgrass plots compared to bare sediment (Figure 4), but the effect was not significant at an alpha of 0.05 ($F_{(2,10)} = 3.41$, R^2-adj = 0.29, p = 0.07).

Specifically, Nitrogen removal (DNF) from the sediment microbial community was not affected by either grass species ($F_{(2,10)} = 2.31$, R^2-adj = 0.18, p = 0.15), while nitrogen recycling (DNRA) was altered by seagrass and direction was dependent on species ($F_{(2,10)} = 4.33$, R^2-adj = 0.36, p = 0.04; Figure 4). Again, we only compared fall widgeongrass and eelgrass plots to our control plots as fall widgeongrass was determined as our most optimal seeding technique.

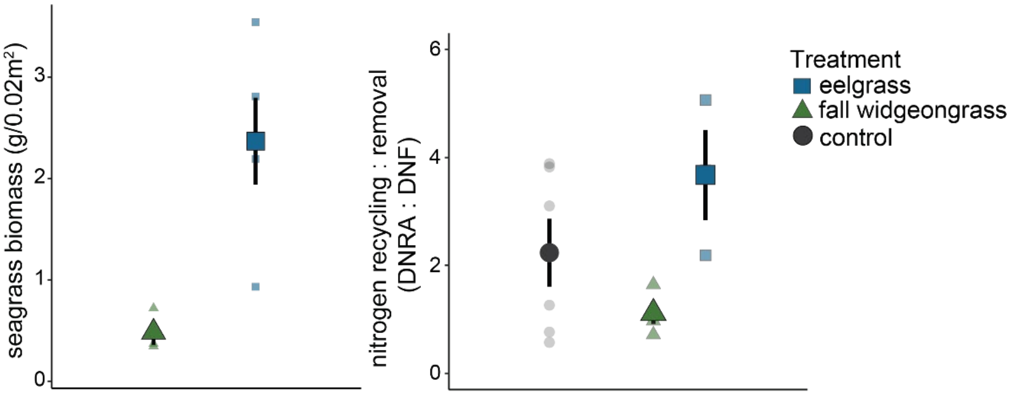

Figure 4. Total plant biomass is significantly altered by seagrass species ($F_{(1,6)} = 10.71$, R^2-adj = 0.58, p = 0.02). Nitrogen recycling to removal or dissimilatory nitrate reduction to ammonia (DNRA) to denitrification (DNF), is somewhat affected by treatment ($F_{(2,10)} = 3.41$, R^2-adj = 0.29, p = 0.07) where only seagrass species differs from one another to an extent (Tukey-HSD p-adj = 0.06). Nitrogen metrics are in μmol Nm^{-2}d^{-1}. Averages are from the five replicate plots per treatments with standard errors.

Pilot restoration

Average initial spring shoots percentage estimates for our restoration monocultures (widgeongrass = 2.13% ± 0.3 SE, eelgrass = 16.98 ± 2.6 SE) paralleled our experiments (widgeongrass = 0.68% ± 0.5 SE, eelgrass = 15.92% ± 2.2 SE) where widgeongrass initial shoot estimates increased (Figure 5). Furthermore, while widgeongrass initial shoot estimates were lower than our study's eelgrass estimates, they were within range of previous restoration efforts conducted in the area (Figure 5). During initial shoot counts for the pilot restoration, the experimental arena and transplant plots were excluded to avoid overestimation.

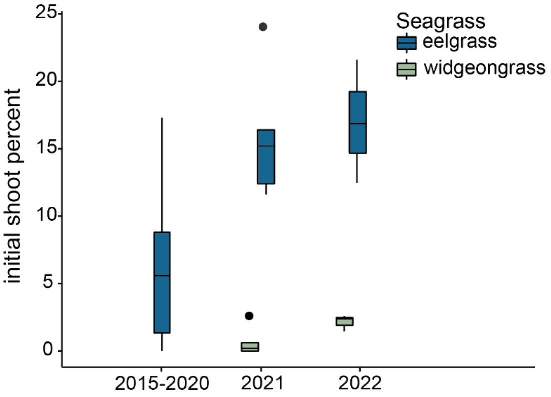

Figure 5. Standard boxplots comparing all initial shoot or seedling percentage estimates, i.e., the percentage of seedlings sprouted from seeds planted, from all eelgrass restoration efforts in the lower Chesapeake Bay, USA from 2015-2020 (n = 69) and our study's estimates from our 2021 experimental plots as well as 2022 pilot restoration. Estimates from eelgrass restoration from 2015-2020 varied in restoration spatial scale and density of seeds planted ranged from 25-100 seeds dispersed per meter-square. Our experimental plots (n= 5 per species) seeded in 2021 had 500 seeds per meter-square dispersed, and for our 2022 pilot restoration we seeded 50 eelgrass seeds per meter-square and 90 widgeongrass seeds per meter-square. We had three replicate estimates for each species for our 2022 pilot restoration estimates where total seeded area was evenly divided into three areas.

DISCUSSION

We show that incorporating generalist seagrasses can enhance habitat restoration in a changing environment by maximizing total habitat area restored and increasing biodiversity. In our seagrass system, we found that widgeongrass can be grown from broadcast, hand seeding at both the experimental and restoration spatial scale and these methods are just as effective as when used to plant eelgrass in our study area. We did demonstrate that season of planting was important for widgeongrass restoration, with fall seeding being the optimal period for our area. Our experimental results demonstrate that while both species cover similar bottom area within their first growing season, there are structural and functional differences, primarily in habitat provision for invertebrate fauna and primary production. For the restoration pilot, initial seedling establishments for both species mirrored experimental observations, suggesting our techniques and experimental findings scale up to effective restoration size. Furthermore, total area planted, or suitable restoration habitat, was greatly increased by planting widgeongrass in shallow areas at the pilot site where high temperatures would have been unsuitable for eelgrass. These results support the premise that widgeongrass is a valuable, candidate species for global seagrass conservation in the face of climate change because it's distributed worldwide, tolerant to wide ranges in salinity and temperature, and can grow easily from planting seeds and establish beds at an effective restorable scale. Broadly, we provide evidence that using opportunistic habitat-forming species in restoration is a proactive and effective approach as these species can increase foundation species diversity and increases restoration area, which can then promote facilitation cascades (Valdez et al., 2020), stability (Lefcheck et al., 2017, van Katwijk et al., 2016), and overall grass persistence through changing environments.

Broad-cast seeded widgeongrass can establish at effective restoration spatial scales

Our study builds from previous mesocosm studies that have shown the potential for using widgeongrass seeds for restoration by testing broadcast seeding survival in the wild and at both small and large spatial scales. Widgeongrass establishment, growth, and survival over its first growing season was highest when seeds were planted in the fall with no pre-seed treatment (i.e., no winter storage or freshwater shock). Specifically, experimental widgeongrass plots planted in the fall opposed to spring showed to expand 10 times more in total areal growth and all pre-treated seeded plots had very low survival rates by the peak of growing season (Table 1). Practically, this is an advantageous finding for restoration practitioners because the most effective method requires the least maintenance and time. The difference in areal growth between widgeongrass fall and spring treatments might result from length of growing time. While initial shoot or germination percentages for fall treatment plots were low (< 1 %) at the time we seeded spring plots, those few established shoots might explain the observed growth differences (areal coverage and shoot density). The spring treatments that had an additional pre-seeded treatment of a freshwater shock prior to planting, either didn't survive to the peak of growing season or had very few shoots, likely due to low or no root establishment into the sediment (*observed during the field experiment*). Poor root establishment has been a main cause for low survival of widgeongrass, particularly efforts that planted post-germinated seeds or full shoot transplants (Cho and Biber, 2010).

Our study uniquely operationalized experimental findings into a pilot restoration and found that our results were scalable – making widgeongrass planted from seed a viable option for seagrass restoration efforts. Experimental initial shoot percentage estimates, i.e., the percentage of seedlings sprouted to seeds planted, were 23 times higher for eelgrass than widgeongrass. Despite this difference in initial seagrass shoots in late spring, both species grew to similar areal cover with comparable shoot densities by their peak growth seasons. The vast difference between the species' initial shoot percentages was likely due to a later widgeongrass seedling emergence timing, which was observed during the experiment. More so, widgeongrass' estimates for both the experiment and pilot restoration were within the average estimates for all eelgrass restoration efforts conducted in the lower Chesapeake Bay from 2015-2020 (Figure 5). For the pilot restoration, initial shoot estimates mirrored the experimental estimates which provides evidence that our experimental results are scalable. One difference, however, was widgeongrass' estimates were four times greater at the restoration scale than the experimental estimates (Figure 5). One hypothesis for this result is that the increase in total widgeongrass areal cover enabled seagrass positive feedback mechanisms including increases in root stabilization from physical disturbances (e.g., wave action and bioturbators).

Strategically, we only focused on mechanistically understanding the most optimal seed-based planting approach for two important reasons. First, we were able to directly compare widgeongrass' initial shoot percentage and survival with eelgrass broadcast-seeding outcomes which has been extensively studied and practiced in the lower Chesapeake Bay (study area) for decades. Second, when seed-based, habitat restoration is possible, it is inherently more effective, efficient, less expensive, and overall sensible for large-scale restorations than other more labor-intensive approaches (e.g., seedling plugs, transplantation). As with any restorative introduction, ecological and genetic diversity implications of the transplanted material need to be considered, but among restoration strategies for plants, seeds can be most easily transported to areas that lack reproductive widgeongrass for seagrass restoration efforts.

Widgeongrass & eelgrass were structurally and functionally different

Our experimental results show that both seagrass species grew to a comparable areal size with similar shoot density within its first growing season, but differed in structural complexity which, importantly, can translate to differences in faunal habitat provisioning. Specifically, widgeongrass shoots grew in several dense patches opposed to eelgrass having a rather even monoculture across its treatment plots. In addition, widgeongrass canopy height was approximately five times shorter with naturally thinner leaves than eelgrass (Figure 2). These structural differences are likely explained by species' life history and leaf morphology with, for the latter, widgeongrass having wiry, branching shoots and eelgrass having wider, ribbon-like leaves. For life history, widgeongrass vertical, canopy growth typically doesn't grow to its full potential in its first growing season and the species' shoot density is more highly dependent on sexual reproduction (seed) oppose to an eelgrass seedling's ability to expand radially up to 30 cm via runners in one growing season (Kantrud, 1991). The structural complexity differences observed between the two species likely explains their faunal composition differences (Figure 3). Nearshore faunal communities are known to be dependent on habitat type as well as its structural complexity and morphology (Boström and Bonsdorff, 2000). For example, many tropical coral reef fishes have high site fidelity to reefs that have refuge areas matching their body size and shape (Eggleston et al., 1990, Eggleston et al., 1997). For our two seagrass species, widgeongrass' higher structural complexity is mirrored by higher faunal diversity and abundances compared to eelgrass (Figure 3). Meanwhile, eelgrass appeared to support fewer but larger individuals, likely due to its fast growing, tall canopy that provides ample refugia (Figure 3).

Primary production and nitrogen recycling were altered by seagrass species and can be explained by both life history characteristics and age of plot-size 'beds' when functions were measured. Primary production or the total biomass produced at each species' peak of growing season, was five times less per area for widgeongrass than eelgrass. The difference in biomass is expectedly from widgeongrass' leaf morphology being very thin, as mentioned above, and has a less extensive root and rhizome system than eelgrass. The latter is generally the case when comparing opportunistic to more stable, dominant grasses, as the two life history strategies have different energy allocation strategies for growth and establishment. While widgeongrass will almost always have less biomass per area than eelgrass due to these inherent differences in morphology and growth strategy, the magnitude of the difference may decrease with time. In our study system widgeongrass' shoots tend to grow taller (20 – 50 cm in length, *personal observations*) starting their second growing season in comparison to their first established shoots, i.e., our final experimental measurements. More so, in areas like our study site where widgeongrass produces reproductive shoots annually, these shoots are significantly taller than non-reproductive shoots (up to one meter in length, *personal observations*). For sediment microbial nitrogen activities, eelgrass plots seem to favor DNRA than widgeongrass plots most likely due to the higher amount of plant biomass present (Figure 4). Importantly, nitrogen sediment microbial activities have been shown to undergo vast changes during seagrass bed development, and early observations should be reviewed cautiously when being interpreted for more established seagrass beds or other generalities (Giblin et al., 2013). However, our findings are helpful in the context of seagrass restoration when planting in a completely seagrass denuded area. Specifically, initial eelgrass beds increasing available nitrogen to uptake infers efficient nutrient recycling for optimal eelgrass growth under oligotrophic or nutrient poor conditions only. But in eutrophic or nutrient-rich conditions, like areas that are typically in need of coastal management, this positive feedback may experience a directional shift by promoting macro- and epi-algal growth, and thus resource competition with grasses. Lastly, in the same context, the small reduction in available nitrogen observed in our widgeongrass plots compared to bare sediment may be evidence in how the presence of widgeongrass can increase the chances for successful establishment of the targeted, lost species, i.e., eelgrass, by directing ambient nutrient conditions more conducive for grass than algal growth.

Integrate opportunistic, generalist species for proactive seagrass restoration

Using native, generalist species in seagrass restoration enables efforts to enhance their success by forming critical habitat in areas where conditions are becoming unconducive for the historically dominant seagrasses and with its presence, potentially facilitating the recovery of the lost, targeted species (Lefcheck et al., 2017, van Katwijk et al., 2016, Derksen-Hooijberg et al., 2018). In addition, by using native, alternative species, restoration efforts can best mimic the natural, local seagrass composition. For our pilot restoration, we were able to revegetate 170% more space with widgeongrass in a seagrass denuded area where our historically dominant species, eelgrass, would not have been planted due to high water temperatures. We also mimicked natural extant seagrass meadows of the lower Chesapeake as they are undergoing dominant species shifts from eelgrass to widgeongrass (Richardson et al., 2018). Importantly, the use of opportunistic species in restoration, particularly widgeongrass, has been controversial among seagrass experts due to their unpredictable, ephemeral nature (Trussell et al., 2006). However, at the most extreme, in areas where the lost species may never be able to establish again, planting and maintaining opportunistic species through routine restoration management can still provide habitat and other critical seagrass services; such management approaches are common practice in habitat restoration and conservation (Török et al., 2021, Rinkevich, 2005, Van Kooten et al., 2005, Beck et al., 2011). Conservatively, we argue by planting opportunistic species complimentary with the target, ‘more stable’ species in restoration efforts (our pilot restoration), restored beds will be able to maintain seagrass habitat and their functions by proactively creating seagrass meadows that are more resistant to rapid environmental changes like rising temperatures. Specifically, the persistence of grass, regardless of species, is key in seagrass ecosystems as they are known habitats that shift into two alternative states of stable plant communities or bare sediment (Knowlton, 2004). This is due to their persistence relying on positive density dependence mechanisms including enough individuals for successful reproduction (Van Tussenbroek et al., 2016), density of algal grazers present (Lefcheck et al., 2017), and effective sediment retention that calms water turbidity (Orth et al., 2020). Therefore, as human activities and climate change impacts continue to spread and amplify unconducive conditions for declining species, using alternative species that can undergo rapidly shifting environments will have a much higher chance of success as they can restore ecosystem function and desired services and create conditions conducive for the reintroduction of the lost dominant species.

Currently in coastal restoration, efforts often focus on the re-establishment of a single climax or dominant foundation species that is declining or lost in order to restore community assemblage and ecosystem functions. When the declining species is no longer available for restoration needs, practitioners' alternative approaches typically include genetically-modified versions of the lost species (Caruso et al., 2021), non-native species most similar to the lost species (Török et al., 2021, Patrick et al., 2018), or artificial structure (Baine, 2001). Our study emphasizes that by using opportunistic or generalist species into coastal restoration, managers are reintroducing habitat that simultaneously mirror habitat compositions that are naturally evolving (Turschwell et al., 2021, Cho et al., 2009), can undergo rapid global change, and most effectively restore the structure and function when the use of the lost species is no longer possible. Current and future coastal habitats need to be managed in a way that make them adaptable and more resilient to the increases in stress from rapid environmental shifts due human-drive global change. Paralleling facilitation research (Silliman et al., 2015, Angelini et al., 2011), integrating opportunistic species within targeted restoration will likely maximize restored habitat success due to the presence of positive feedback cascades which are key for habitat resilience in stressful, dynamic environments like coastal ecosystems.

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SUPPLEMENTAL METHODS

Site Characteristics

For our Goodwin Island site, from March to December 2021 monthly average temperatures (°C) ranged from 8.04 ± 1.22 SD – 28.0 ±0.83 SD, turbidity (NTU) ranged from 3.49 ±1.90 SD – 7.38 ± 7.50SD, and salinity (ppt) ranged from 16.65 ± 0.74 – 21.51 ± 0.63 SD. Temperatures above 25°C were observed for 52 days and reach 30°C for seven days.

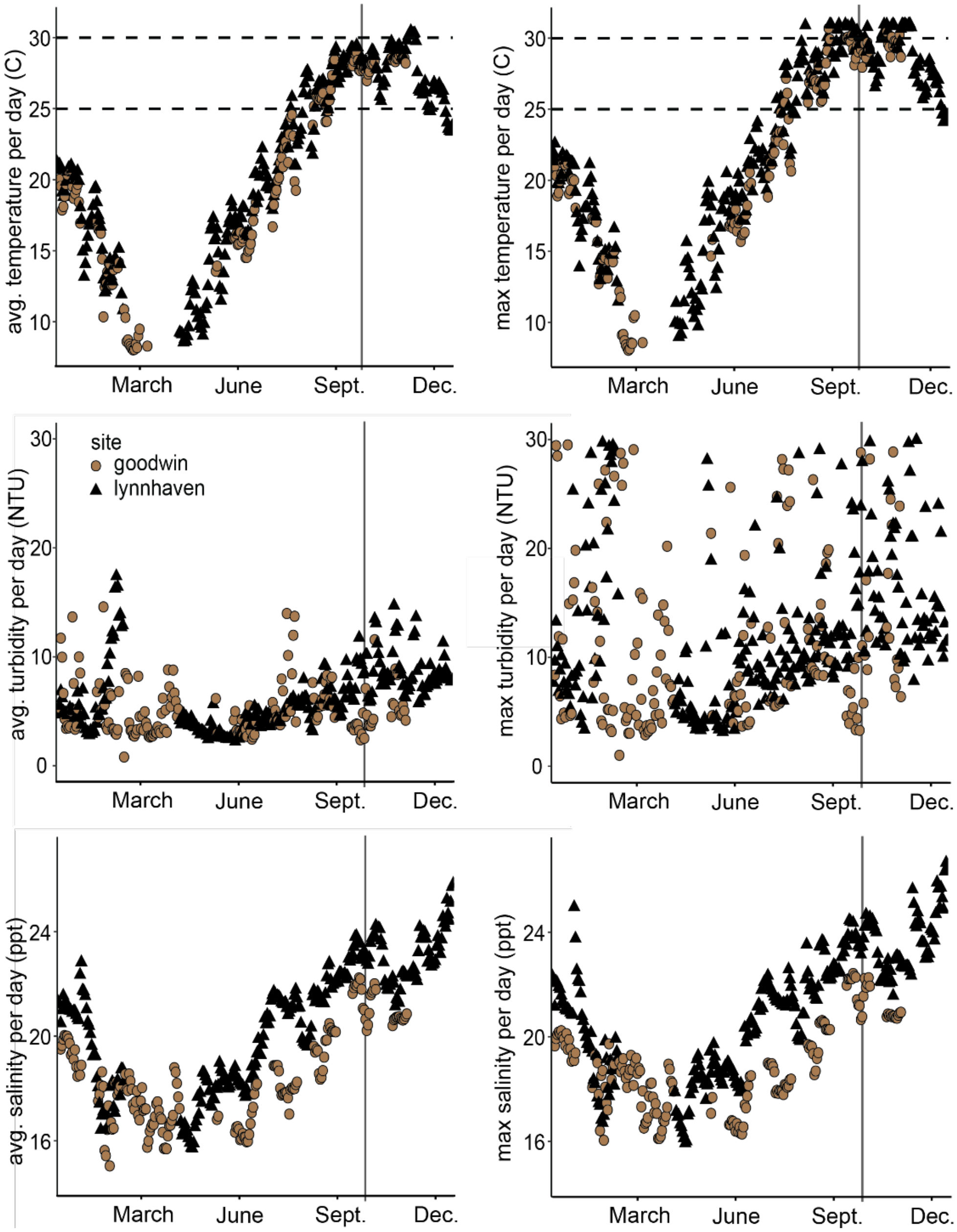

Figure S1. Data are during the experimental study from October 2020 (denoted with the vertical grey line) to the end of September 2021. Dashed horizontal lines in the first row are upper, viable temperature thresholds for eelgrass (25°C and 30°C). Average (avg.) daily measurements of temperature, turbidity, and salinity are formed from 15-minute intervals.

Seed Collection to Viability Test

Collection of both seagrasses as well as initial seed storage followed standard methods of the Submerged Aquatic Vegetation (SAV) laboratory at Virginia Institute of Marine Science (VIMS) (Orth et al., 1994, Orth et al., 2008, Orth et al., 2003). The tops of reproductive shoots that contained mature seeds were hand-harvested in late May and July for eelgrass and widgeongrass, respectively. Vegetative material with seeds were held in 3.8 m^3, aerated, flow-through outdoor seawater tanks at VIMS until the majority or all seeds release was complete. Floating, decomposing vegetative material with no seeds was removed from the surface of the tanks weekly with particular care to widgeongrass. Due to widgeongrass seed shape, seeds could easily stick to decomposing vegetative material, making seeds vulnerable to decomposing as well as inefficient collection (Ailstock et al., 2010). Seeds were subsequently sieved from decomposing plant material and held in the tanks until initiation of the experiments. For eelgrass, a seed was considered viable if it had a rigid seed coat and a high fall velocity (Orth et al., 2003). Appropriate numbers of viable seeds were obtained via direct counts or sub-sampled estimation of sieved material. We did not test the viability of widgeongrass seeds. As a proxy, we estimated monthly germination percentages on ~100 seeds from October to March where the seeds would be placed in deionized water in a shallow, glass petri-dish and the number of germinated seeds, i.e., number of unemptied cracked seeds, were counted for 14 consecutive days. Germination percentage increased each month with over 70% of seeds germinated in March 2021.

Sediment Microbial Core Characteristics

Sediment percent water, percent organics and bulk density were determined by weight. Wet sediment was dried at 60 C and reweighed to determine percent water content. Dried sediment was then muffled at 500 c for 4 hours and reweighed to determine percent organic content. Bulk density was determined by weighing the amount of wet sediment to fill 2mL in a 15mL falcon tube. To collect sediment extractable nutrients, 2M potassium chloride (KCl) was added to 4g of sediment, shaken for 1 hour before decanting and filtering the extract with a 0.45 μM Whatman Puradisc membrane filter (GE Healthcare Life Sciences). Extracted samples were analyzed for inorganic nutrients NO_3^-, NO_2^-, and NH_4^+ on a Lachat QuikChem 8000 automated ion analyzer (Lachat Instruments, Milwaukee, WI, USA).

SUPPLEMENTAL RESULTS

Widgeongrass seeding techniques for pilot restoration

Table 1. Goodwin Site. To determine which seeding technique had the most optimal outcome from our experiment, we compared plot percentage survival till the end of the first growing season as well as plot-level total areal cover (m^2) and shoot density ($0.01m^2$). area cover and shoot densities are averages from the five replicate plots per treatments with standard errors.

treatment	% survival	area cover	shoot density
eelgrass	100	2.75 ± 0.16	3.75 ± 0.54
widgeongrass planted in fall	100	2.50 ± 0.73	1.70 ± 0.97
widgeongrass planted in spring	80	1.17 ± 0.30	1.45 ± 0.35
widgeongrass 48-hour freshwater shock + planted in spring	80	1.30 ± 0.51	0.85 ± 0.26

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SUPPORTING WATER QUALITY MONITORING AND ANALYSIS

INTRODUCTION - Supporting Water Quality Monitoring and Analysis

The overall goal for this studies water quality monitoring effort was to document and assess water quality conditions at the seagrass experimental and pilot restoration site within Broad Bay, of the Lynnhaven River, VA system. Beginning in October 2020, the experimental portion of the project focused on identifying preferred planting techniques (seeds vs shoots, fall vs spring), which supported a scaled up pilot restoration effort beginning in summer 2021. CBNERR-VA relied on two complementary approaches to document water quality conditions: (1) establishment and operation of a local continuous (15 min interval) fixed water quality station (CMON) at the Broad Bay study site; and (2) high resolution surface water quality mapping (DATAFLOW) as part of Virginia's Dept. of Environmental Quality Shallow Water Quality Monitoring Program administered by CBNERR-VA. This summary report serves as a companion report to that submitted by the Institute's Submerged Aquatic Vegetation Program & others entitled 'Incorporating Generalist Seagrasses Enhances Habitat Restoration in a Changing Environment'.

METHODS - Supporting Water Quality Monitoring and Analysis

Seagrass Water Quality Monitoring

CBNERR-VA established and maintained a single water quality CMON station (36.904, -76.032) adjacent to the Broad Bay seagrass experimental and pilot study site (Figure WQ1). A sign identified piling platform, consisting of a 25 cm (10 in) diameter treated piling and 10.2 cm (4 in) biofoulant treated PVC deployment tube, specific for this study, was permitted and installed on June 9, 2020 and June 30, 2020, respectively. The station was equipped with a YSI EXO2 multi-parameter sonde, set to a 15 min sampling interval, and was deployed over critical study periods. Measured parameters include: depth (unit: m, range: 0-9.1, ± 0.02), water temperature (°C, -5-50, ± 1%), specific conductance (mS/cm, 0-100, ± 1%), DO (%sat, 0-500, ± 1%), pH (SU, 0-14, ± 0.1), turbidity (NTU, 0-4000, ± 2%), and chlorophyll fluorescence (ug/L, 0-400, linear $r^2 \geq 0.999$ across dye range); note: DO (mg/L, 0-50, ± 0.1) and salinity (ppt, 0-70, ± 1%) are calculated parameters. The sonde was deployed at a fixed depth off the bottom (0.25 m) in order to reflect environmental conditions directly influencing seagrass beds; previous measurements indicated that the water column is relatively well mixed. Two EXO2 sondes were dedicated to this effort so as to eliminate data loss between sonde switch-out and maintenance periods. Sonde switch-outs typically occurred at 3-4 week intervals dependent on the level of biofouling. Sonde deployment periods were July 7-December 3, 2020 to capture initial experimental plant seedings and growth, March 2-December 1, 2021to capture primary polyhaline seagrass growth period, and June 22-December 2, 2022 to provide additional information beyond the scope of the project. All calibrations and maintenance on EXO model sondes were completed in accordance with the Xylem (YSI, Inc.) operating manual methods. Collected data were managed by the CBNERR-VA Environmental Data Center and included Tier I quality assurance checks (e.g., time gap, sensor span, spike, out of the water), data and metadata archive, and timely dissemination to project partners. Data were visually examined for sensor flat-line and drift by graph inspection.

As part of Virginia's Dept. of Environmental Quality Shallow Water Quality Monitoring Program, CBNERR-VA collects high spatial resolution tidal tributary and coastal embayment water quality data on a rotational basis; see representative Lynnhaven system cruise tract and verification station locations in Figure WQ2. Specific to this project, information derived from this monitoring program was used to develop the relationship between on-site water quality conditions and water clarity (see Section below), and more generally, provides detailed information for the broader system including areas for potential future restoration. The sampling interval of the DATAFLOW mapping system is 3-4 seconds with water quality measurements made every 15-30 linear meters based on vessel speeds of 10-20 knots. The DATAFLOW system utilizes a YSI 6600V2 sonde or EXO2 multi-parameter sonde equipped with a flow-through chamber. Measured parameters and sensor specs are similar to those reported above for CMON stations. All calibrations and maintenance on 6600V2 and EXO model sondes are completed in accordance with the Xylem (YSI, Inc.) operating manual methods. The sonde transmits data collected from the sensors directly to a ruggedized laptop computer using a data acquisition system created with LabView software (National Instruments, Inc). An integrated Garmin GPS with sounder provides time stamped GPS and water depth information at the time of sensor data collection. In addition to the continuous underway sensor measurements, near surface (0.25-0.5 m) verification samples and supplemental information (9 stations) is collected along the Lynnhaven Polyhaline (LYNPH) cruise track. Verification sample parameters include total suspended solids (TSS), volatile suspended solids (VSS), chlorophyll, and pheophytin, with supplemental information including vertical profiles of photosynthetic active radiation (PAR), as well as a vertical profile for temperature, dissolved oxygen, salinity and pH. DATAFLOW cruises occurred at monthly intervals over the polyhaline seagrass growing period (March-November). Collected data and interpolated maps are managed by the CBNERR-VA Environmental Data Center.

Seagrass Water Quality Thresholds - Supporting Water Quality Monitoring and Analysis

Water temperature and water clarity represent two critical water quality parameters that influence seagrass population dynamics, health and geographic extent (Orth et al. 2017, Arnold et al. 2017, Shield et al. 2019). In order to best target seagrass restoration areas that increase chances of success and support post-restoration assessments, local pilot project water quality site conditions were evaluated as to Chesapeake Bay Program (CBP) accepted temperature and water clarity seagrass thresholds. Temperature thresholds of 25 °C and 30 °C were applied based on stress and mortality thresholds for the restoration of eelgrass (*Zostera marina*) (Jasinski et al. 2021).

CBP derived minimum light requirements for polyhaline bay grasses is 22% light through water (PLW) with a bimodal temporal application period to reflect the high spring (March 1-May 31) and fall (September 1-November 30) growth pattern of eelgrass (Tango and Batiuk 2013). Although light requirements of widgeongrass (*Ruppia maritima*) have not been as well studied, this species generally has higher light requirements compared to eelgrass, causing a clear zonation in the Chesapeake Bay where it grows in monoculture in the shallows, mixes with eelgrass in the mid-depths, and does not grow in the deeper depths where eelgrass can survive (Orth and Moore 1988). Light attenuation measurements were taken using a PAR LI-COR light sensor at established cruise verification stations during monthly high-frequency surface water quality sampling in the Lynnhaven system using the DATAFLOW sampling protocol conducted by CBNERR-VA in 2019-2021; see Figure WQ2 for cruise tract and verification station locations.

Attenuation of light may be described by equation 1, where I_z is light intensity at depth Z (m), I_0 light intensity just below waters surface, and K_d as light attenuation coefficient (m^{-1}).

$$I_z = I_0\, e^{-K_d Z} \qquad \text{eq. 1}$$

In order to assess water clarity characteristics at the pilot restoration site, a linear model was developed by pairing high-frequency surface water quality sampling measurements of K_d and turbidity in the Lynnhaven system (2019-2021) and applied to local CMON data collected over this project period. Using CBP reported minimum water clarity requirements, expressed as percent light through water (PLW=22%; calculated as 100 $e^{-K_d Z}$) for polyhaline salinity regimes, critical K_d values for 0.5, 1.0, 1.5 and 2.0 m application depths were 3.03, 1.51, 1.01 and 0.76 m^{-1}, respectively.

SUMMARY OF FINDINGS - Supporting Water Quality Monitoring and Analysis

The objectives of the water quality monitoring portion of this study was to: (1) provide a basic physical and chemical characterization of the Broad Bay seagrass experimental and pilot restoration site; (2) document water quality conditions that could influence seagrass growth/health which ultimately would impact planting success or failure; and (3) provide supporting information to guide local seagrass restoration efforts.

General Study Site & Water Quality Conditions.

Tidal exchange with Broad Bay is somewhat limited given the limited and shallow channels providing tidal flow access; mean tide range within the Broad Bay canal is on the order of 0.4-0.5 m (1.3-1.6 ft). Water depth at the CMON station averaged 1.2 m (3.9 ft) during 2020-2022 measurement periods; see Table WQ1 for summary water level and quality information and Figures WQ 3 A-C for water depth time series. Minimum and maximum depths on the order of 0.5-0.6 m (1.6-2.0 ft) and 2.0-2.3 m (6.6-7.5 ft), respectively, were observed over the three year deployment intervals. Seasonal water temperature fluctuation patterns, best demonstrated by 2021 where measurements encompassed a full spring-fall cycle, were typical of shallow water Bay environments; see Figures WQ4 A-C for water temperature time series. Of relevance to this project were elevated summer temperatures (≥ 30 °C, ≥ 86 °F) that will be discussed in further detail below. Average salinity levels (> 20 ppt) observed over the measurement periods were representative of a polyhaline conditions with somewhat reduced levels being sustained most notably in late fall 2020 and early-mid spring 2021; see Figures WQ5 A-C for salinity time series. Observed instantaneous minimum salinity levels were on the order of 14 ppt and still within the optimum salinity range reported for eelgrass.

pH at the study site was mildly basic, with yearly deployment averages ranging from 7.8-7.9 SU and instantaneous minimum and maximums ranging between 7.3 and 8.5 SU; see Figures WQ6 A-C for pH time series. Reflecting the buffer capacity of high salinity water, study site pH levels were well within the Virginia's tidal waters numerical criteria for pH (6.0-9.0 SU). Providing a relative measure of water clarity, turbidity is a key measurement parameter to guide seagrass restoration project siting and understand seagrass response over time. Average turbidity levels, expressed in Nephelometric Turbidity Units (NTU), ranged from 5.6-7.5 NTUs with maximum instantaneous levels approaching 100 NTUs; see Figures V7 A-C for turbidity time series. Turbidity levels in excess of 15 NTUs are generally considered detrimental to seagrass health. The use of turbidity to generate estimates of water column light attenuation will be discussed in further detail below. Serving as a key indicator of ecosystem health, dissolved oxygen levels (measured both as concentration and % saturation) did not indicate any significant acute or chronic issues at the study site; see Figures V8 A-C for % saturation time series. Deployment average percent (%) saturation of dissolved oxygen levels ranged between 89-95% and hypoxic conditions, defined as oxygen levels between 1-30% saturation, occurred rarely (0.01% of measurements in 2020). It should be noted that the study site has the potential of receiving drainage waters from emergent and forested wetlands which are characterized by low dissolved oxygen and high acidity (low pH) waters.

Seagrass Water Quality Thresholds

Local pilot project water quality site conditions were evaluated as to CBP accepted temperature and water clarity seagrass thresholds. Summary statistics for % of time water temperature met or exceeded CBP thresholds of 25 °C (77 °F) and 30 °C (86 °F) are provided in Table WQ2; analyses are provided for available data over the seagrass growing period (Mar-Nov) and summer season (Jun-Aug). Within the Chesapeake Bay region, summer heat stress mortality of eelgrass beds has been documented and represents a critical challenge to local and regional seagrass restoration efforts. Individual measurements of summer water temperature exceeded the 25 °C (77 °F) threshold between 91-100% over the 3 year deployment period. Exceedance of the 30 °C (86 °F) threshold was more moderate at 12-29% over the summer season. Temperature is not considered a limiting factor for widgeongrass in the Chesapeake Bay region, as it can tolerate higher temperature than eelgrass (Evans et al. 1986), and therefore these temperature thresholds only apply to eelgrass and can be viewed as overly protective for widgeongrass.

Paired Lynnhaven system high-frequency surface water quality sampling measurements of K_d and turbidity over the 2019-2021 sampling period, presented in Figure V9, resulted in a significant linear relationship ($K_d = 0.11 \times NTU + 0.783$; $r^2=0.78$). Estimated K_d values over the CMON station deployment periods, along with multiple application depth threshold levels, are provided in Figures WQ10 A-C. Summary statistics for % of time study site K_d estimates exceeded depth thresholds over the spring and fall growing periods are presented in Table WQ3. Generally, local water clarity was not conducive for seagrass establishment and growth at depths ≥ 1.5 m.

K_d estimates typically met the 1.5 m threshold less than 1% of the time (exception occurred in fall 2021) and the 2 m threshold was never met. There was significant improvement in complying with water clarity thresholds at the 0.5 and 1.0 m application depths; near 100% at 0.5 m and ~ 70% at 1.0 m. It should be noted that while local site substrate is relatively coarse textured, sediment resuspension was observed in the nearshore shallows landward of the CMON station under moderate wave conditions driven by wind and vessel traffic. This point is illustrated in Figure WQ11, where elevated turbidity levels are generated during low water stands; the weighting of elevated turbidity at shallow water depths was observed for all yearly deployments. Nearshore sediment resuspension should be assessed in selecting local seagrass restoration sites given the requirement for shallow (≤ 1.0 m) water to meet PLW requirements, the relative openness of Broad Bay allowing for increased wind fetch, and high recreational use of its waterways.

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Table WQ1. Deployment period summary statistics for selected CMON station measured parameters; values represent study period 15-min data.

Parameter	2020 (Jul 7 19:15 – Dec 3 11:45)	2021 Mar 2 12:15 – Dec 1 14:00	2022 (Jun 22 10:45 – Dec 2 14:30)
Water depth m	Average: 1.20 Min, Max: 0.57, 2.13 Std Dev.: 0.25 Count: 14,275	Average: 1.15 Min, Max: 0.46, 2.04 Std Dev.: 0.24 Count: 26,262	Average: 1.20 Min, Max: 0.58, 2.26 Std Dev.: 0.25 Count: 15,664
Temperature °C	Average: 23.3 Min, Max: 9.6, 33.2 Std Dev.: 5.8 Count: 14,273	Average: 21.4 Min, Max: 7.3, 32.5 Std Dev.: 6.4 Count: 26,263	Average: 23.2 Min, Max: 9.4, 33.2 Std Dev.: 6.0 Count: 15,661
Salinity ppt	Average: 20.2 Min, Max: 14.5, 25.3 Std Dev.: 1.5 Count: 14,242	Average: 21.6 Min, Max: 14.3, 26.7 Std Dev.: 2.4 Count: 26,253	Average: 24.1 Min, Max: 19.9, 28.9 Std Dev.: 1.3 Count: 15,659
pH SU	Average: 7.8 Min, Max: 7.3, 8.5 Std Dev.: 0.1 Count: 14,275	Average: 7.8 Min, Max: 7.3, 8.4 Std Dev.: 0.2 Count: 26,267	Average: 7.9 Min, Max: 7.5, 8.4 Std Dev.: 0.1 Count: 15,664
Turbidity NTU	Average: 7.2 Min, Max: 1.7, 95.4 Std Dev.: 3.5 Count: 12,720	Average: 5.6 Min, Max: 0.7, 68.0 Std Dev.: 3.3 Count: 26,241	Average: 7.5 Min, Max: 2.6, 50.0 Std Dev.: 3.4 Count: 15,649
$DO_{\%sat}$	Average: 89.6 Min, Max: 29.2, 237.2 Std Dev.: 15.4 Count: 14,263	Average: 93.1 Min, Max: 43.2, 177.5 Std Dev.: 12.8 Count: 26,263	Average: 95.2 Min, Max: 50.4, 185.3 Std Dev.: 12.5 Count: 15,657
[DO] mg•L^{-1}	Average: 6.9 Min, Max: 2.0, 15.5 Std Dev.: 1.5 Count: 14,273	Average: 7.4 Min, Max: 3.0, 13.3 Std Dev.: 1.7 Count: 26,267	Average: 7.2 Min, Max: 3.4, 12.1 Std Dev.: 1.2 Count: 15,664

Table WQ2. Temperature (°C) summary statistics for % of time water temperature met or exceeded CBP thresholds. Statistics are provided for available data over seagrass growing period (Mar-Nov) and summer season (Jun-Aug). Data collection starting points are noted as they, particularly 2020 and 2022, occurred after the start of seagrass growing period.

		Full Deployment Period		Summer June-August	
Year	Deployment Period	≥ 25 °C	≥ 30 °C	≥ 25 °C	≥ 30 °C
2020	July 7 – Nov 30	47.4	10.9	100.0	28.9
2021	Mar 2 – Nov 30	39.5	4.0	91.4	11.5
2022	Jun 22-Nov 30	56.0	5.5	96.7	12.5

Table WQ3. Summary statistics for % of time estimated K_d values exceeded CBP application depth thresholds over the seagrass growing period (Mar-Nov) and summer season (Jun-Aug). ND denotes no available data. Any variation to standard spring (March 1-May 31) or fall (September 1-November 30) intervals are identified in footnotes.

Application Depth m	K_d Threshold m^{-1}	2020		2021		2022	
		Spring	Fall[1]	Spring[2]	Fall	Spring	Fall
0.5	< 3.03	ND	99.9 %	99.9 %	99.9 %	ND	99.8 %
1.0	< 1.51	ND	70.8 %	95.6 %	68.0 %	ND	69.3 %
1.5	< 1.01	ND	0.2 %	0.7 %	13.1 %	ND	0 %
2.0	< 0.76	ND	0 %	0 %	0 %	ND	0 %

1. Deployment period ends: November 17.
2. Deployment period begins: March 2, 2021.

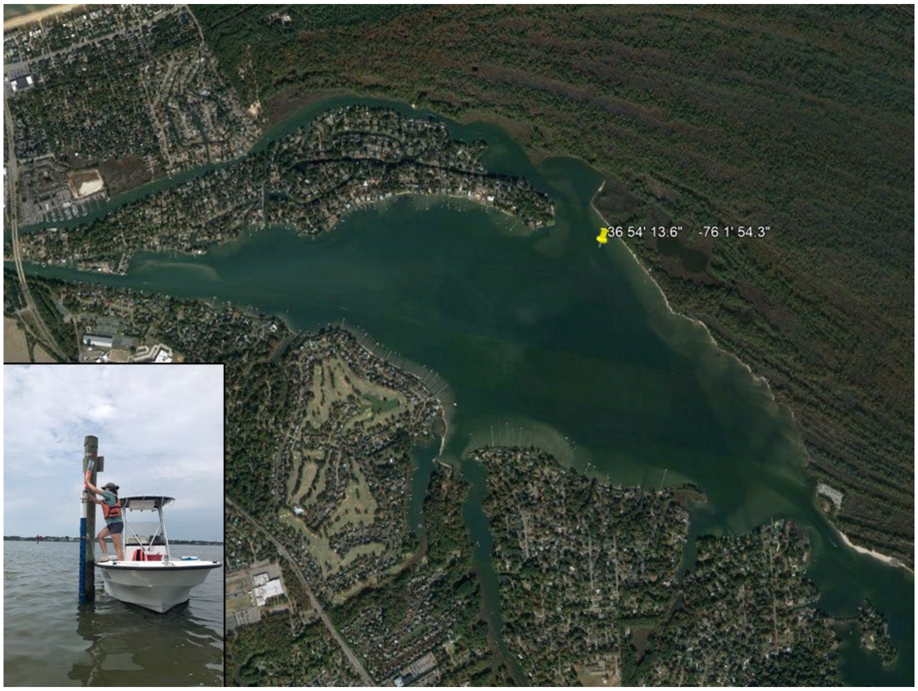


Figure WQ1. Broad Bay seagrass experimental and pilot study site CMON station location image. Inset: Routine deployment of EXO sonde.

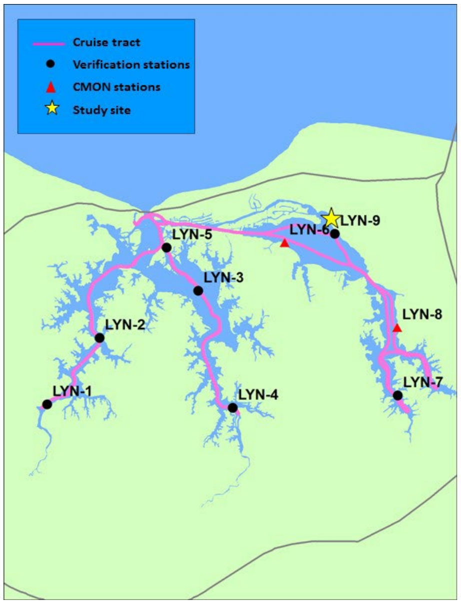


Figure WQ2. Representative (2019-2021) Lynnhaven system DATAFLOW cruise tract and verification station locations. Note Virginia's Dept. of Environmental Quality Shallow Water Quality Monitoring Program CMON stations were deployed 2019-2020.

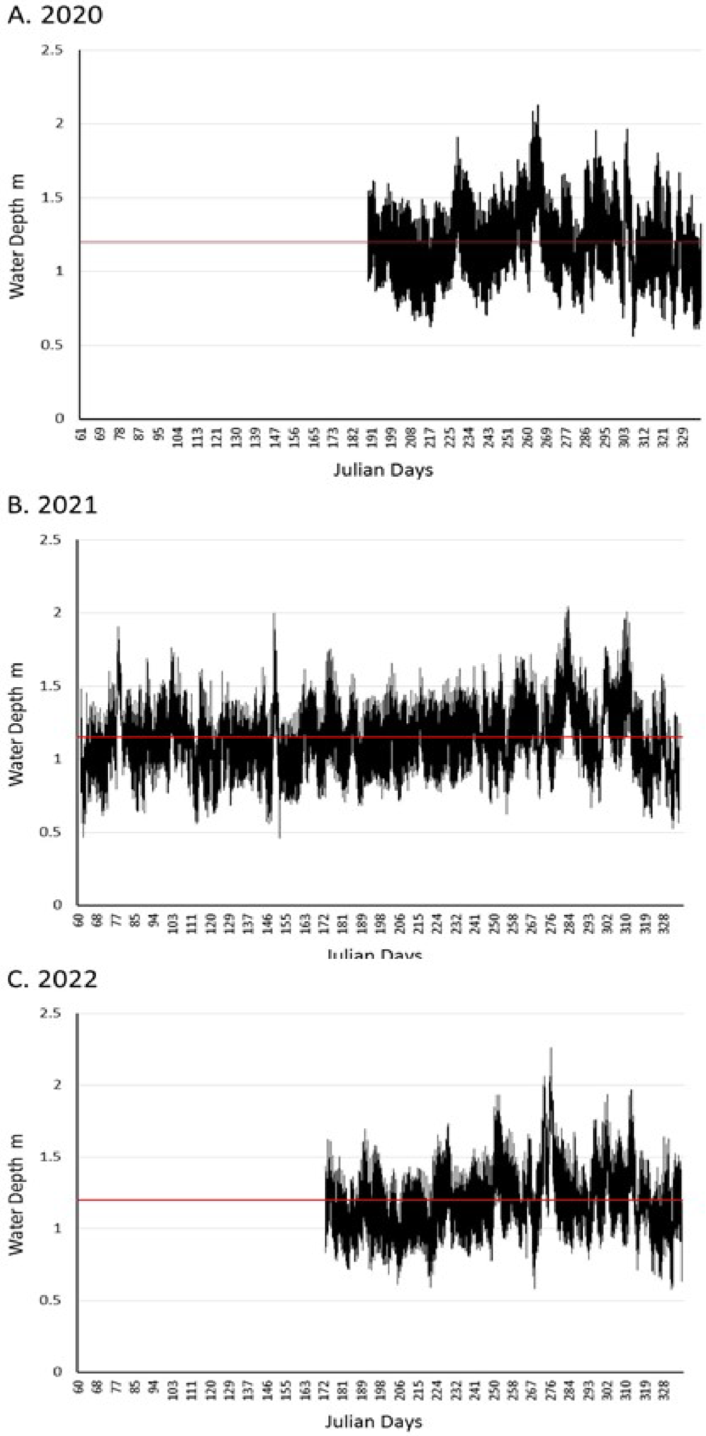
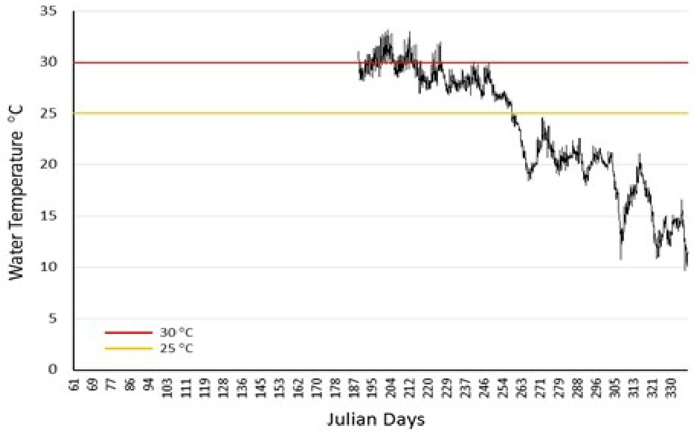
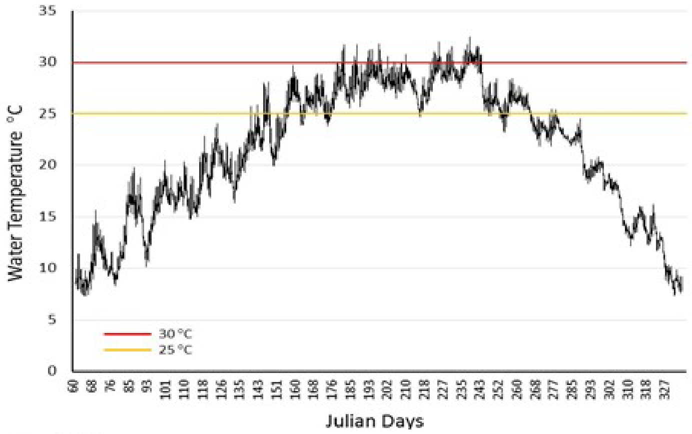


Figure WQ3. Time series of CMON station water depth (m) for 2020 (A), 2021 (B) and 2022 (C) deployment periods. Average yearly deployment period mean is depicted by red line. Note that graphics start at the beginning of the polyhaline seagrass growing period (March 1) and that 2020 was a leap year.

A. 2020



B. 2021



C. 2022

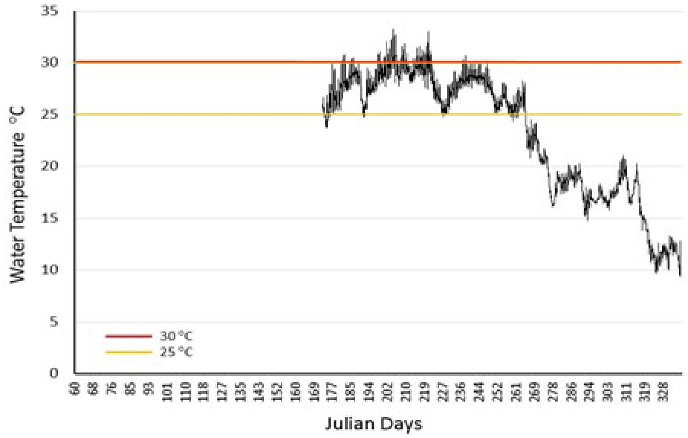


Figure WQ4. Time series of CMON station water temperature (°C) for 2020 (A), 2021 (B) and 2022 (C) deployment periods. Maximum seagrass temperature thresholds (25 °C and 30 °C) are depicted by yellow and red lines.

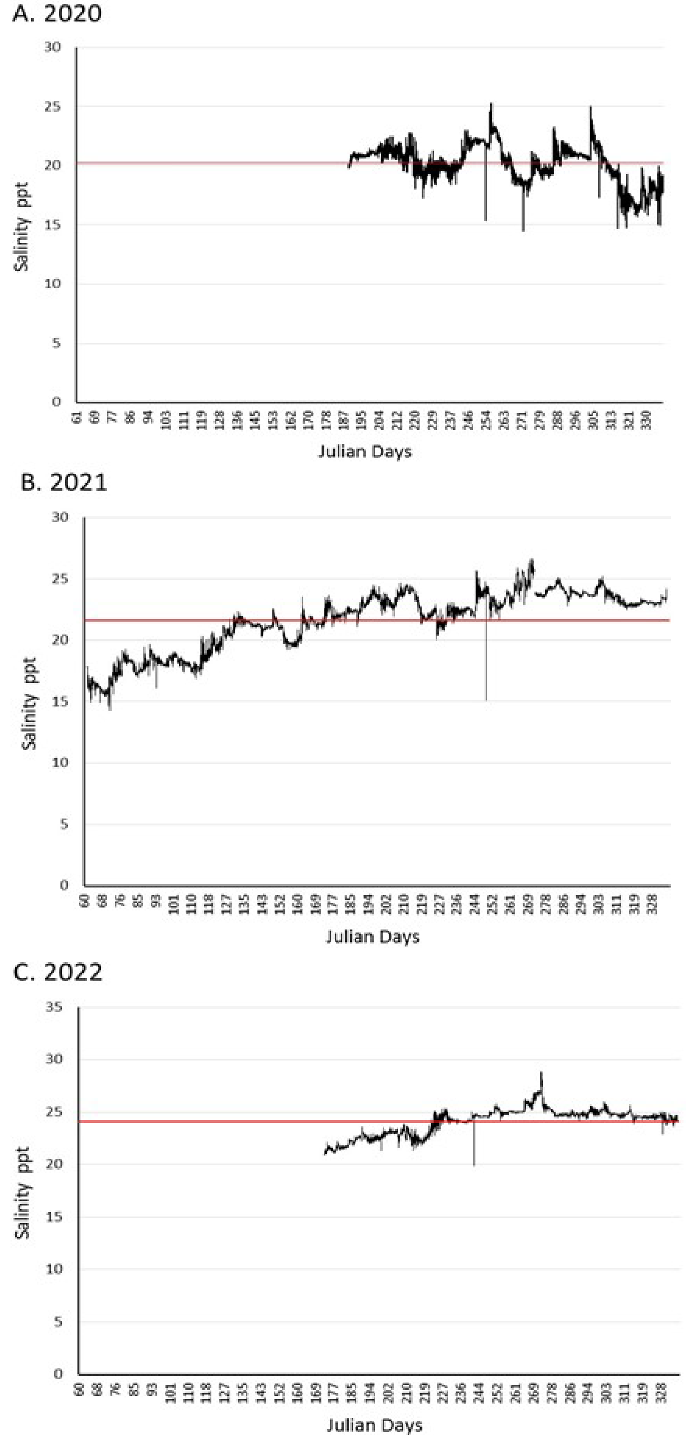
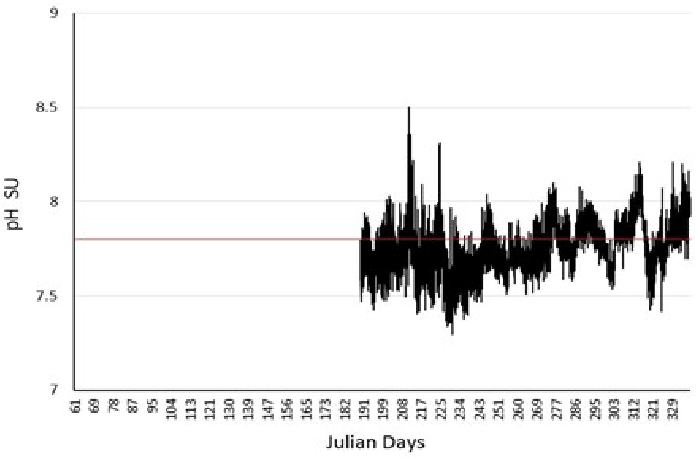
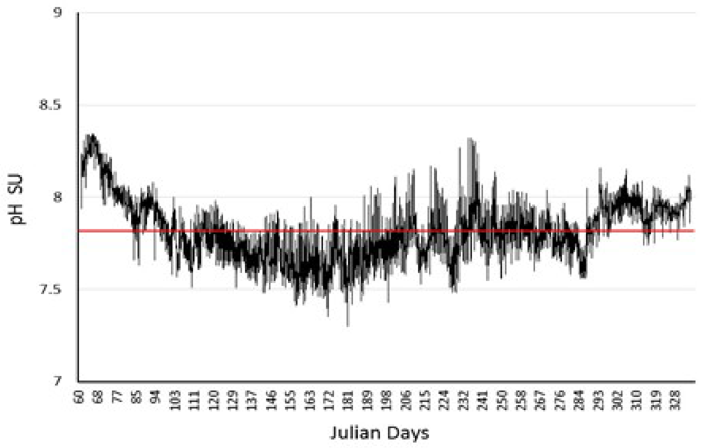


Figure WQ5. Time series of CMON station salinity (ppt) for 2020 (A), 2021 (B) and 2022 (C) deployment periods. Average yearly deployment period mean is depicted by red line.

A. 2020



B. 2021



C. 2022

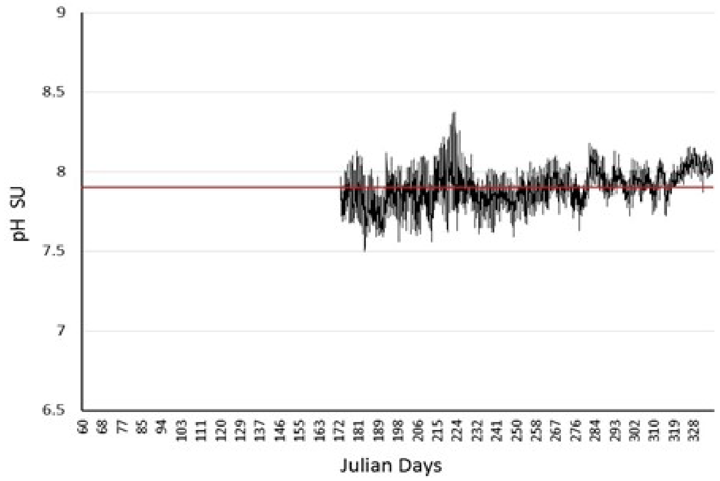
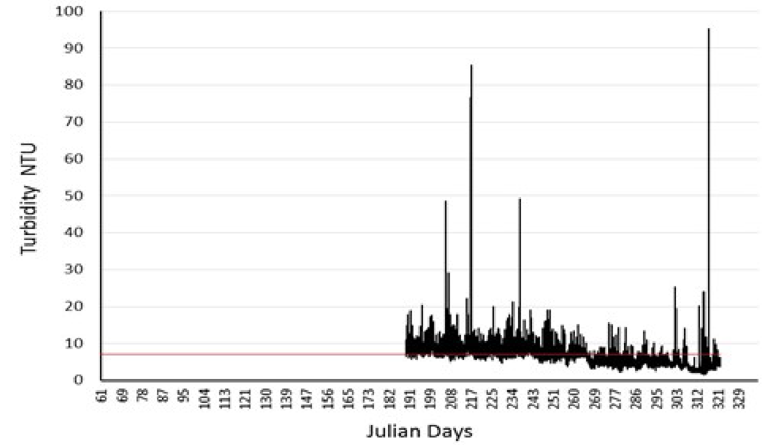
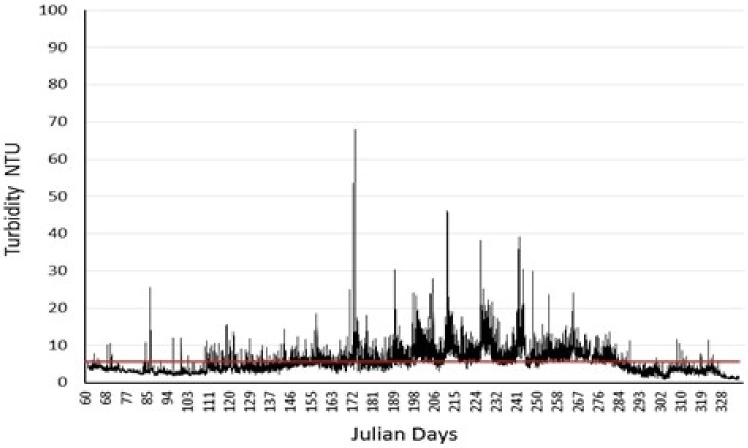


Figure WQ6. Time series of CMON station pH (SU) for 2020 (A), 2021 (B) and 2022 (C) deployment periods. Average yearly deployment period mean is depicted by red line.

A. 2020



B. 2021



C. 2022

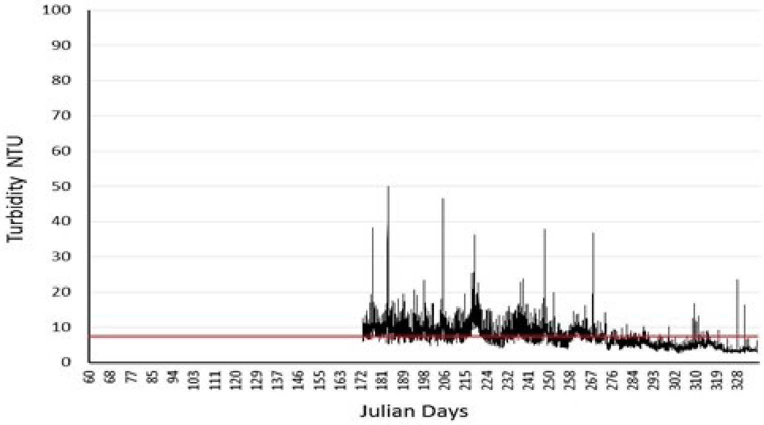


Figure WQ7. Time series of CMON station turbidity (NTU) for 2020 (A), 2021 (B) and 2022 (C) deployment periods. Average yearly deployment period mean is depicted by red line.

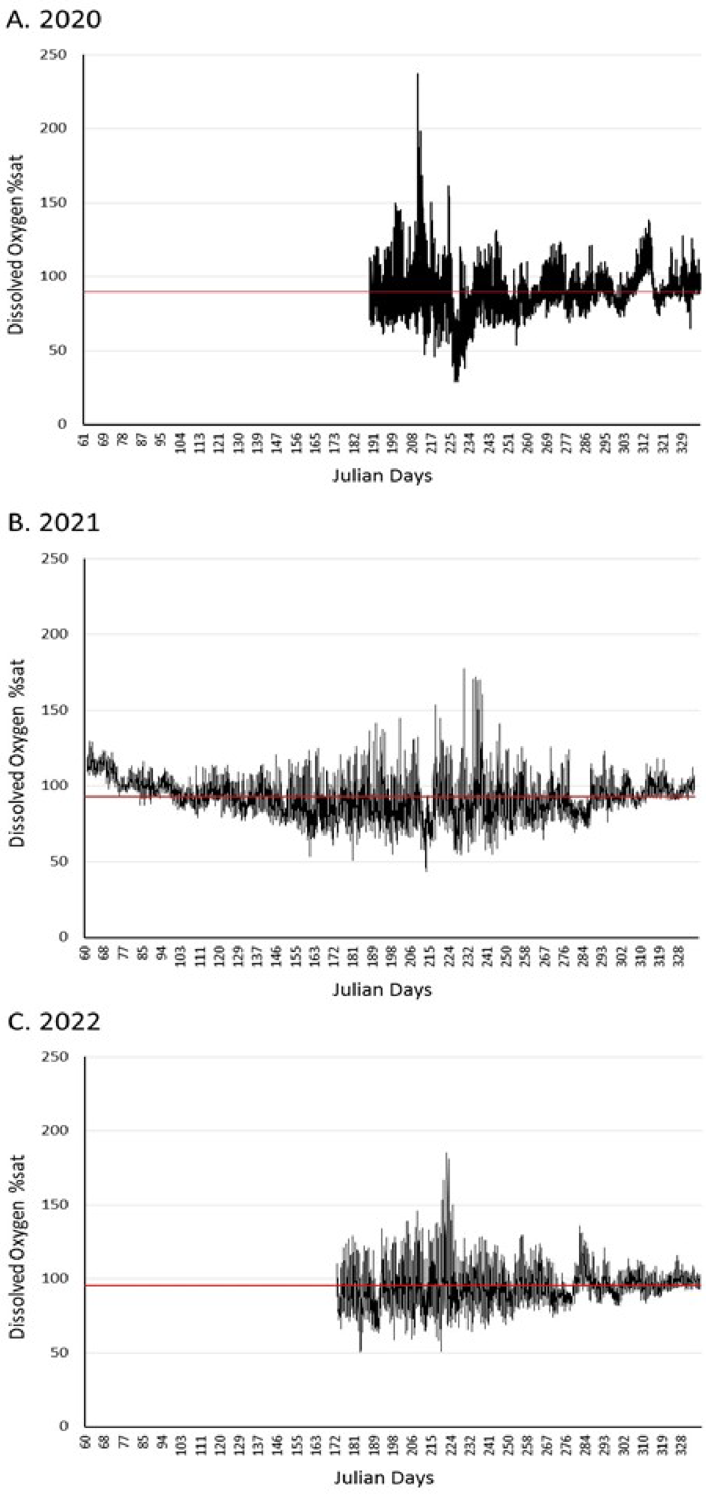


Figure WQ8. Time series of CMON station dissolved oxygen % saturation for 2020 (A), 2021 (B) and 2022 (C) deployment periods. Average yearly deployment period mean is depicted by red line.

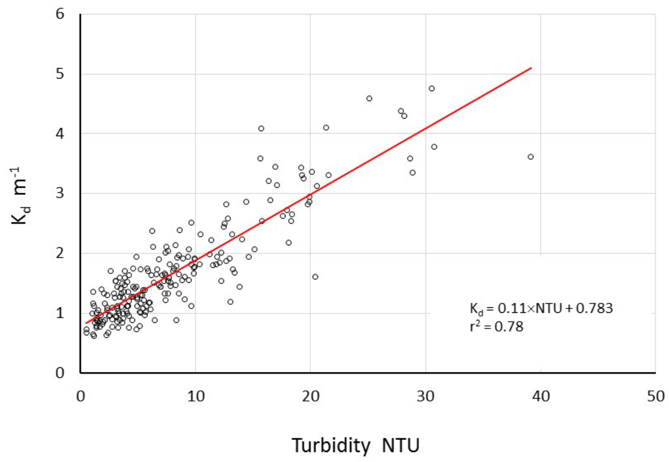


Figure WQ9. Linear regression relationship between field measured K_d (m^{-1}) and turbidity (NTU) at Lynnhaven system DATAFLOW verification stations from 2019 through 2021. Slope: 0.11, Y-intercept: 0.783, N=217, r^2=0.78.

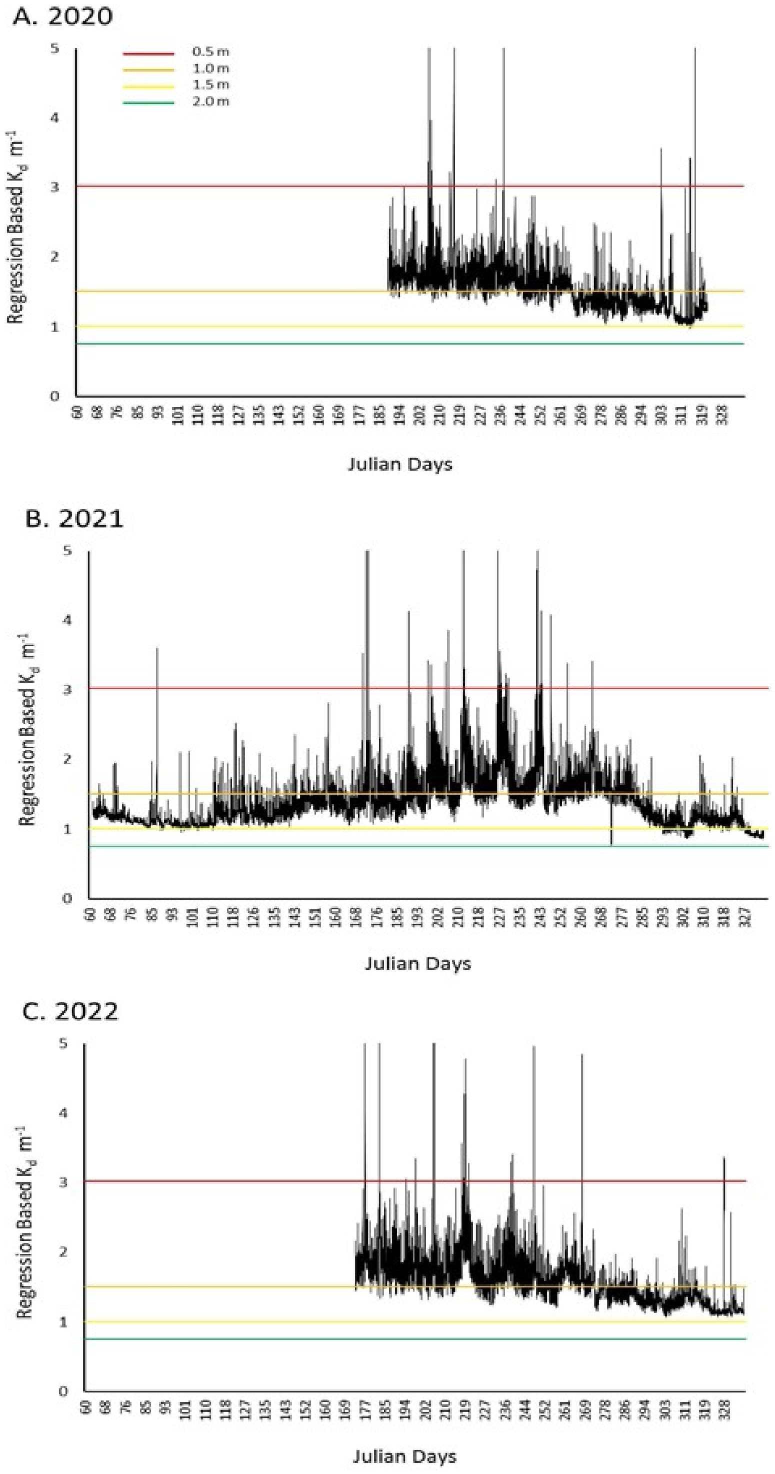
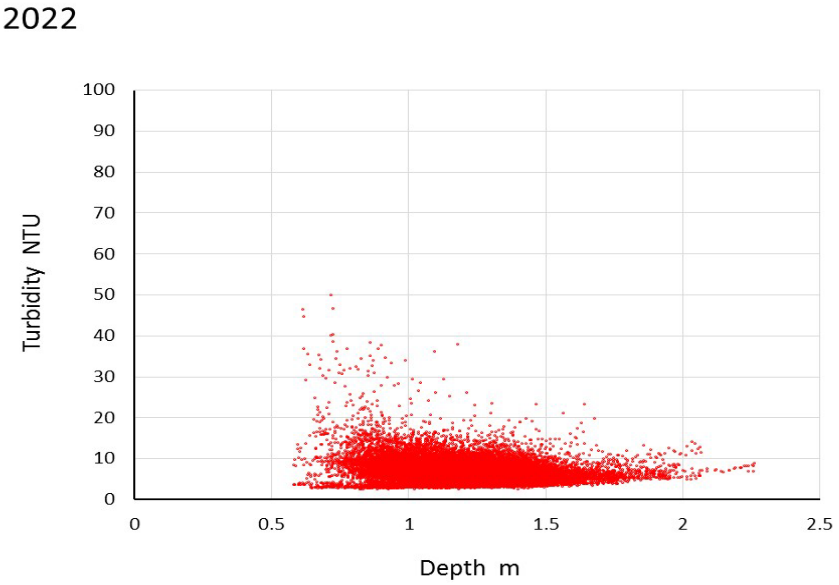


Figure 10. Regression based K_d values over 2020 (A), 2021 (B) and 2022 (C) CMON station deployment periods. Depth applications of critical K_d values based on CBP polyhaline PLW levels (22%) are highlighted.



2022

Figure 11. Turbidity (NTU) in relation to CMON station water depth. Sampling years 2020 and 2021 show a similar clustering pattern of elevated NTU levels associated with low water stands.