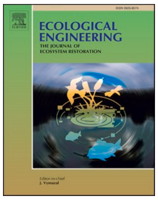
Ecological Engineering 200 (2024) 107167

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



# A simple approach to estimating the nutrient and carbon storage benefits of restoring submerged aquatic vegetation, applied to *Vallisneria americana* in the Caloosahatchee Estuary, Florida, USA

Brondum M. Krebs [a], Nicole Iadevaia [b], Jennifer Hecker [b], James G. Douglass [c,*]

[a] College of the Coast and Environment, Louisiana State University, 93 S Quad Dr, Baton Rouge, LA 70803, USA
[b] Coastal and Heartland National Estuary Partnership, 1050 Loveland Blvd. Port, Charlotte, FL 33980-1836, USA
[c] Department of Marine and Earth Sciences, The Water School, 10501 FGCU Blvd S, Florida Gulf Coast University, Fort Myers, FL 33965, USA

ARTICLE INFO

*Keywords:*
*Vallisneria americana*
Nutrient storage
Carbon storage
Caloosahatchee
Submerged aquatic vegetation
Restoration

ABSTRACT

Carbon and nutrient storage are important ecosystem services of submerged aquatic vegetation (SAV) and may be enhanced by SAV restoration. This study demonstrates an approach to quantifying the nutrient and carbon storage potential of SAV restoration, focusing on the SAV *Vallisneria americana* in the oligohaline reaches of Florida's Caloosahatchee River Estuary (CRE). The variables of habitat area, plant size, plant density, and tissue nutrient stoichiometry are considered, and estimates are made both for storage in living tissue and for deposition in sediments. System-specific parameter values are obtained from a combination of abundance surveys, historical accounts of abundance and distribution, and field and mesocosm studies of tissue stoichiometry responses to nutrient addition. These are integrated with literature values for sediment deposition rates of C, N, and P in other SAV systems to estimate carbon and nutrient storage for current conditions and various restoration scenarios. Calculations indicate that under a restoration scenario assuming a return to the abundance documented in 1998–1999, *V. americana* tissues could act as a substantial sink for macro-elements in the CRE, representing 28.4 mt-C, 2.6 mt-N, and 0.16 mt-P, and depositing 897 mt-C $y^{-1}$, 68.5 mt-N $y^{-1}$, and 3.87 mt-P $y^{-1}$ in meadow sediments. However, at current low shoot densities and small shoot sizes, these benefits are two to three orders of magnitude less. In addition to the large difference between the restoration and current-conditions scenarios, propagation of uncertainty around parameter estimates within each scenario leads to wide ranges of uncertainty around model outputs. More system-specific empirical studies would help constrain parameter estimates and improve the model. Overall, these findings emphasize the sensitivity of C, N, and P storage and deposition rates to SAV habitat conditions, and the importance of reversing declines in SAV density through restoration, and other conservation measures.

## 1. Introduction

Agricultural and urban development, coupled with loss of natural land cover and alteration of watershed hydrology, has increased nutrient loading to aquatic ecosystems worldwide (Paerl, 2009; Withers et al., 2014; Le Moal et al., 2019). As water column nutrients rise, excessive primary production (eutrophication) occurs. Eutrophication is a principal cause of global biodiversity loss and functional impairment of aquatic systems (Diaz and Rosenberg, 2008; Janse et al., 2015; Glibert, 2020). Impacts to ecosystems often include loss of native aquatic plants, excessive algae blooms, spikes in biochemical toxins, dissolved oxygen (DO) depletion, aquatic fauna mortality, and detrimental effects to human health and economy (Dorgham, 2014; Heil and Muni-Morgan, 2021; Shi et al., 2021). Globally, public health costs associated with algal biotoxins reach into the billions of U.S. dollars (GESAMP, 2001). In the U.S., annual economic loss due to freshwater eutrophication is around 2.2 billion USD (Dodds et al., 2009). As excess nutrients reach coastal areas, further detriment to natural resources and their associated economic values is incurred (Hoagland and Scatasta, 2006; Ralston et al., 2017). This is a particular threat to the coastal counties of Southwest Florida, where natural resources generate \$13.6 billion in total output, \$3.8 billion in regional income, \$146 million in local and state tax revenues, and support >148,000 jobs annually (Cortez et al., 2020).

---

* Corresponding author.
*E-mail address:* jdouglass@fgcu.edu (J.G. Douglass).

https://doi.org/10.1016/j.ecoleng.2023.107167
Received 30 May 2023; Received in revised form 16 November 2023; Accepted 7 December 2023

In Florida, rapid population expansion (>100,000 people year$^{-1}$) and urbanization increasingly strain hydrologic systems (Heil and Muni-Morgan, 2021). An estimated 4.9 × $10^7$ kg-N year$^{-1}$ and 6.3 × $10^6$ kg-P year$^{-1}$ are input to the Florida aquatic environment from septic wastes, and even greater amounts are derived from residential and commercial fertilizer applications (Badruzzaman et al., 2012). Hence, >25% of Florida's rivers, lakes, streams, and canals exceed total nitrogen thresholds for recreation and healthy aquatic life, and are classified as impaired by the Florida Department of Environmental Protection Clean Water Act 303(d) list (FDEP, 2020; 2021). Waterbodies deemed impaired by the FDEP in the Southwest Florida's Caloosahatchee River Basin rose by 36% from 2018 to 2020, predominantly because of increases in nutrient impairments. Reversing this eutrophication trend is a top priority for managers.

Efforts to combat eutrophication can be generalized into two approaches: 1) preventing nutrients from entering aquatic systems, and 2) sequestering or removing nutrients from aquatic systems. Plant-mediated techniques (phytoremediation) have applications in both approaches. Upland and riparian plant communities are effective means to attenuate run-off and prevent nutrient loading at the terrestrial-aquatic interface (Parkyn et al., 2005), while treatment wetland and aquatic plant communities are increasingly used to remove nutrients from enriched waters (Mitsch et al., 2014; Quilliam et al., 2015).

Submerged aquatic vegetation (SAV) in particular, is increasingly recognized as a valuable sink for dissolved nutrients (Fourqurean et al., 2012; Vasanthi et al., 2015; Dahl et al., 2020). SAV absorbs and biologically stores water column nutrients through its shoots and associated epibiota, and porewater nutrients through its roots and rhizomes (Straile, 2015; Yasin et al., 2017; Zhang et al., 2018). Additionally, SAV canopies reduce wave and current energy, increasing rates of sedimentation, including the deposition and burial of particle-bound nutrients (Fonseca and Fisher, 1986; Reddy et al., 2021). In the saturated soils of SAV beds, rhizosphere microbial associations facilitate biogeochemical processes, e.g., denitrification and anaerobic ammonium oxidation, that render nutrients into less-bioavailable forms (Fig. 1) (Pilon-Smits, 2005; Reddy and DeLaune, 2008; Wenzel, 2009). Monetary cost per acre (± SE) to replace denitrification services in SAV habitat has been estimated at \$2999 ± 695 acre$^{-1}$ y$^{-1}$, which is \$1400–2500 greater than the equivalent area of intertidal and subtidal unstructured habitat (Piehler and Smyth, 2011). Unfortunately, SAV abundance is declining worldwide, often a result of chronic eutrophic conditions and other anthropogenic stressors exceeding the resilience of these plant communities. Areas undergoing SAV loss may experience positive feedbacks, allowing phytoplankton and benthic algal blooms to outcompete SAV for light resources, further compounding eutrophication and SAV loss problems (Orth et al., 2006; Burkholder et al., 2007). However, where SAV loss has occurred for reasons not entirely related to eutrophication, restoration efforts can improve water quality and activate positive feedbacks in the opposite direction, aiding further SAV recovery (Orth et al., 2020). This may be the case in Florida's Caloosahatchee River Estuary (CRE).

The CRE is a drowned river valley estuary in Lee County, Southwestern Florida (26.684, −81.831). The tidally influenced estuary extends 42 km from a water control structure at its head (the S-79 lock and dam) to the Gulf of Mexico at San Carlos Bay (Doering et al., 2006). The Caloosahatchee River was historically fed by a 3440 km$^2$ watershed but periodic inputs from an additional 11,300 km$^2$ now occur via a canal and lock system extending the river eastward to central Florida's Lake Okeechobee (SFWMD, 2021). The expanded watershed, and changes in land use patterns and hydrology within the watershed, have altered natural flow regimes and increased variability in physical and chemical characteristics of the CRE (Doering et al., 2002; Stevens et al., 2010; Douglass et al., 2020a).

The natural variation in estuarine salinity associated with south Florida's seasonal weather cycle (November–April: dry season, May–October: wet season) has been magnified by these changes, to the detriment of estuarine biota like oyster reef communities and submerged aquatic vegetation (Tolley et al., 2006; Volety et al., 2014; Douglass et al., 2020a). The formerly abundant SAV *Vallisneria americana* is among the species most heavily impacted, because it is a freshwater

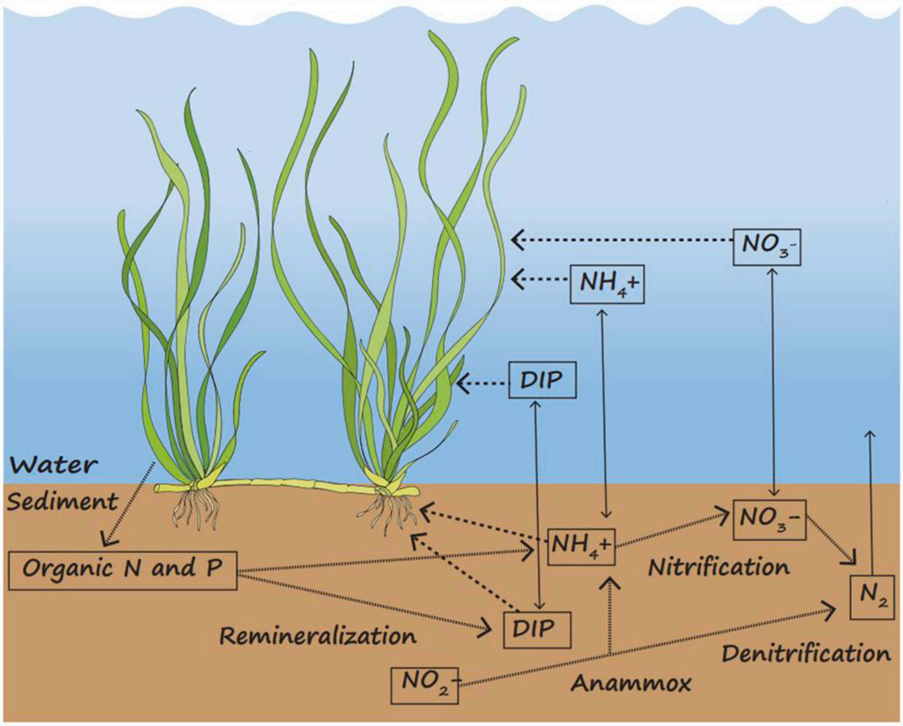


**Fig. 1.** Conceptual model of macronutrient and SAV biochemical interactions in the water column and sediment. DIP: Dissolved Inorganic Phosphorus, Anammox: anaerobic ammonia oxidation. Illustration Mischa Schultz, 2023.

plant restricted to the oligohaline reaches of the estuary by low tolerance of saline conditions. The taxonomic identity and diversity of *Vallisneria* has recently been reappraised, and Florida populations including those in the CRE are likely to actually be *V. neotropicalis* (Martin and Mort, 2023). However, because of remaining uncertainty, and for consistency with prior studies in the CRE, we continue to refer to it here as *V. americana*. The combination of altered hydrology and droughts in 1999–2001, 2007–2009, and 2011 created high salinity conditions in the upper CRE that nearly extirpated *V. americana* from the system (Doering et al., 2001; Douglass et al., 2020a). Costly efforts are underway to manage and stabilize the estuarine salinity regime, e.g., via construction of the C-43 freshwater reservoir in the historic watershed of the CRE (SFWMD, 2009), and concurrent efforts are being made to restore *V. americana* by replanting (Center for Coastal Ecology, Mote Marine Laboratory., 2007; Ceilley, 2018; Johnson Engineering, 2019). Estimating the ecological benefits of *V. americana* in the CRE would help with cost-benefit assessments for these restoration efforts. In this study, we focused on characterizing the potential of *V. americana* to combat eutrophication by serving as a biological sink of macronutrients. Carbon storage, another valuable ecosystem service of SAV (Fourqurean et al., 2012, 2023; Duarte et al., 2013), was also estimated.

## 2. Methods

### 2.1. Overview

The elements of our estimate of potential nutrient storage and deposition rates for *V. americana* in the CRE were: 1) Tissue nutrient stoichiometry data (% C, % N, % P) for *V. americana* grown in nutrient poor, nutrient replete, and estuarine field conditions. These data were obtained from mesocosm and field experiments originally intended as direct assessments of *V. americana* on surrounding water quality. Artifacts of nutrient addition levels, confinement, and scale limited the degree to which the water quality effects observed in the experiments could be extrapolated to the estuary, but the tissue stoichiometry results were nonetheless useful. 2) *V. americana* abundance data from a 2020 field survey in the CRE (Douglass et al., 2020b) and 1998–1999 abundance data from South Florida Water Management District monitoring in the system (Bortone and Turpine, 2000; Douglass et al., 2020a). 3) Potential post-restoration *V. americana* abundance in the CRE based on bathymetry, light requirements, historical reports, and literature values for shoot density and biomass. 4) Literature values for organic matter deposition rates and nutrient stoichiometry in SAV beds versus unvegetated areas. Once the four elements were assembled, a range of nutrient storage and deposition rate estimates was generated based on two scenarios: current *V. americana* abundance and full restoration to 1998–1999 *V. americana* abundance. Each scenario was run with three sub-scenarios: centered estimate, conservative estimate, and optimistic estimate. The conservative and optimistic sub-scenarios were based on the bounds of 95% confidence intervals around parameter means, as well as other types of uncertainty range estimates detailed in Table 1.

### 2.2. Mesocosm experiment setup and conditions-

The mesocosm experiment was conducted outdoors at Florida Gulf Coast University's Emergent Technologies Institute from 21-October-20 to 18-December-20. It compared vegetated (V) and unvegetated (O) substrates at ambient (A) and enriched (N) nutrient levels, resulting in four unique treatments: VA, VN, OA, and ON, each with five replicates. Each vegetated treatment tank (VN, VA) was planted with 30 shoots of narrow leaf *V. americana* typical of the CRE (Lowden, 1982), which were obtained from an aquatic restoration nursery (Sea and Shoreline, LLC). Wet weights of the plant material were determined as in McAskill and Douglass (2017) and standardized across vegetated treatments with a mean initial planting wet biomass of 27.16 g, ± 0.55 g SD in each 416-l outdoor mesocosm. Water column nutrient enrichment of treatments

**Table 1**
Aspects of nutrient calculations and sources of derived data.

| Parameter | Scenario | Sub-Scenario | Value | Origin/Derivation |
|---|---|---|---|---|
| *V. americana* Shoot Density (shoots $m^{-2}$) | Current abundance | Centered Estimate | 4.7 | Mean from summer 2020 field surveys |
| | | Conservative Boundary | 1.66 | Mean - 2 standard error |
| | | Optimistic Boundary | 7.74 | Mean + 2 standard error |
| | Full Restoration | Centered Estimate | 257 | Mean from 1998 to 1999 monthly surveys |
| | | Conservative Boundary | 208 | Mean - 2 standard error |
| | | Optimistic Boundary | 306 | Mean + 2 standard error |
| Biomass per Shoot (g DW $shoot^{-1}$) | Current abundance | Centered Estimate | 0.02 | Mean of shoot samples from 2021 field study |
| | | Conservative Boundary | 0.014 | Mean - 2 standard error |
| | | Optimistic Boundary | 0.026 | Mean + 2 standard error |
| | Full Restoration | Centered Estimate | 0.1 | 1998 mean from Bortone & Turpin 2000 |
| | | Conservative Boundary | 0.02 | Seasonal minimum from Bortone & Turpin (2000) |
| | | Optimistic Boundary | 0.2 | Seasonal maximum from Bortone & Turpin (2000) |
| Biomass (g DW $m^{-2}$) | Both | All | – | Product of shoot density and biomass per shoot |
| Carbon Fraction of DW | Both | All | 0.33 | Mean from elemental analysis of 2021 field samples |
| Tissue C:N | Both | Centered Estimate | 10.8 | Mean from 2021 field study |
| | | Conservative Boundary | 31.9 | Mean from oligotrophic mesocosm experiment |
| | | Optimistic Boundary | 10.8 | Mean from 2021 field study |
| Tissue C:P | Both | Centered Estimate | 177 | Mean from 2021 field study |
| | | Conservative Boundary | 274 | Mean from oligotrophic mesocosm experiment |
| | | Optimistic Boundary | 93.2 | Mean from eutrophic mesocosm experiment |
| Carbon (g $m^{-2}$) | Both | All | – | Product of biomass per area and carbon fraction |
| Nitrogen (g $m^{-2}$) | Both | All | – | Carbon biomass divided by tissue C:N |
| Phosphorus (g $m^{-2}$) | Both | All | – | Carbon biomass divided by tissue C:P |
| Habitable Area ($km^2$) | Both | Centered Estimate | 3.34 | Benthic area < 1 m deep, derived from bathymetry and shoreline length (see Methods) |
| | | Conservative Boundary | 1.67 | 50% of centered estimate |
| | | Optimistic Boundary | 5.01 | 150% of centered estimate |

(*continued on next page*)

**Table 1** (*continued*)

| Parameter | Scenario | Sub-Scenario | Value | Origin/Derivation |
|---|---|---|---|---|
| Total C Storage (kg) | Both | All | – | Product of habitat area in $m^2$ and C mass per $m^2$ |
| Total N storage (kg) | Both | All | – | Product of habitat area in $m^2$ and N mass per $m^2$ |
| Total P storage (kg) | Both | All | – | Product of habitat area in $m^2$ and P mass per $m^2$ |
| C deposition (g $m^{-2}y^{-1}$) | Both | Centered Estimate | 269 | Midpoint of literature values; near 307.2 g $m^{-2}y^{-1}$ from Hillman et al. 2020 for intermediate salinity SAV |
| | | Conservative Boundary | 53 | Duarte et al., 2013 estimate for seagrass meadows |
| | | Optimistic Boundary | 590 | Reddy et al., 2021 rate for SAV treatment wetlands |
| N deposition (g $m^{-2}y^{-1}$) | Both | Centered Estimate | 20.5 | Reddy et al., 2021, median |
| | | Conservative Boundary | 9 | Reddy et al., 2021, minimum |
| | | Optimistic Boundary | 32 | Reddy et al., 2021, maximum |
| P deposition (g $m^{-2}y^{-1}$) | Both | Centered Estimate | 1.16 | Dierburg et al., 2002, median for SAV treatment wetland |
| | | Conservative Boundary | 0.66 | Dierburg et al., 2002, minimum |
| | | Optimistic Boundary | 1.66 | Dierburg et al., 2002, maximum |
| Adjusted C, N, and P deposition (g $m^{-2}y^{-1}$) | Both | All | – | Unadjusted deposition rate multiplied by ratio of *V. americana* biomass $m^{-2}$ in focal scenario to mean *V. americana* biomass $m^{-2}$ in CRE in 1998–1999. |
| Total C (kg $y^{-1}$) | Both | All | – | Product of habitat area and adjusted C deposition rate |
| Total N (kg $y^{-1}$) | Both | All | – | Product of habitat area and adjusted N deposition rate |
| Total P (kg $y^{-1}$) | Both | All | – | Product of habitat area and adjusted P deposition rate |

was achieved by administration of 100 g Osmocote™ N:P:K 19:6:12 slow-release fertilizer (Douglass et al., 2007; Spivak et al., 2009). The fertilizer granules were administered via mesh bags and allowed to continuously soak in the water. This equated to a higher than ecologically relevant nutrient dose, but it allowed determination of potential *V. americana* tissue % N and % P in a nutrient-replete environment.

### 2.3. Field experiment setup and conditions-

The field experiment took place in the upper CRE near Marsh Point (26.6792° N, −81.8542° W) from 09-June-21 to 04-July-21. The study area was characterized by oligohaline waters high in colored dissolved organic matter (CDOM) and total nutrients (1.1 mg/L TN and 0.11 mg/L TP, Florida Department of Environmental Protection, 2009). The mean water depth was shallow (< 1 m below MLLW), and the SAV beds occurred near a mangrove-lined shoreline. The treatments in the field experiment were analogous to those in the mesocosm experiment (VN, VA, ON, OA), each with six replicates. Naturally established patches of short-stature *V. americana* (vegetated plots) alternated with areas of bare mud/sand bottom (non-vegetated plots). Treatment plots (0.65 $m^2$) were separated by at least two meters, as nutrient enrichment signals were found to be insignificant beyond 1.5 m from the slow-release fertilizer source in a similar seagrass enrichment study (Douglass et al., 2007). Shoot height and abundance were recorded in each vegetated plot ($n = 6$ per treatment) at the beginning, middle, and end of the study.

### 2.4. Experiment data collection and processing-

All shoots from mesocosm vegetated treatments were destructively sampled at the end of the experiment. For the field experiment, shoot samples were collected from each plot at the beginning, middle, and end of the study (i.e., 09-June, 22-June, 04-July). In the lab, *V. americana* plant tissue was scraped and rinsed with deionized water to remove sediment and epibiota and was separated into aboveground and belowground portions. Solid plant material samples were dried at 60 °C until a stable weight was achieved. After drying, plants were transferred to a precision balance to record dry weight (DW). To determine elemental stoichiometry, dried plant tissues were powdered by mortar and pestle, sealed in scintillation vials, and subsequently analyzed by Florida International University's Blue Carbon Analysis Lab (FIU BCAL) via CE Flash 1112 Elemental Analyzer & UV-2101 Shimadzu Spectrophotometer, for tissue composition (Campbell and Fourqurean, 2009).

### 2.5. *V. americana* field abundance and potential habitat area assessment-

Based on salinity regime data and reports of *V. americana* extent in the CRE during benign, low-salinity conditions (Hoffacker, 1994; Bortone and Turpine, 2000; Buzzelli et al., 2017; Douglass et al., 2020a), we considered the potentially habitable area for *V. americana* to extend from the S-79 lock and dam to the US 41 Business (Edison) bridge at Fort Myers (Fig. 2). Low optical water quality in this tidal oligohaline portion of the CRE restricts SAV to shallow depths <1 m below MLLW (Douglass et al., 2020b). Therefore, for the study area southwest of I-75, we restricted potential habitat to the 1 m depth contour using bathymetric data collected by the South Florida Water Management District in 2002 (Hansen, 2015). Recent shoreline perpendicular SAV transect surveys in that region observed depths that corresponded well with the Hansen (2015) bathymetry (Douglass et al., 2020b). The study area northeast of I-75, extending to the S-79 lock and dam, currently lacks complete bathymetry data, and was not included in the Douglass et al. (2020b) transect surveys. Thus, an estimated habitat area for that uppermost estuarine segment was extrapolated from detailed bathymetry of two SAV restoration sites in that area that were surveyed in 2018 (Johnson Engineering, 2019). At those sites, the average distance from the shoreline to the 1 m depth contour was 30 m, and it was assumed that 30 m would be the average width of potential SAV habitat for the entire I-75 to S-79 estuarine segment. Shoreline distance was manually calculated for the segment, including insular landmasses, but ignoring tributaries, canals, and other fine scale shoreline features (Fig. 2). The resulting total shoreline distance (52.42 km) was multiplied by the habitable area width (30 m) to produce an approximate habitable area (1.77 $km^2$) which was added to the habitable area from the lower estuarine segment (1.57 $km^2$) to get 3.34 $km^2$. To account for the uncertainty in these bathymetry-based habitat area estimates, 50% of the estimated area was used for conservative sub-scenario models, and 150% of the estimated area was used for optimistic sub-scenario models (Tables 1, 2). The optimistic scenario habitat area estimate (5.01 $km^2$) was similar to the 4.68 $km^2$ area of dense *V. americana* beds in the estuary estimated by Hoffacker (1994) based on 1993 surveys. All geospatial analyses were performed with ArcGIS Pro software.

Historic reference condition *V. americana* density and shoot biomass data for the oligohaline CRE, used to establish the "full restoration" scenario, were derived from South Florida Water Management District

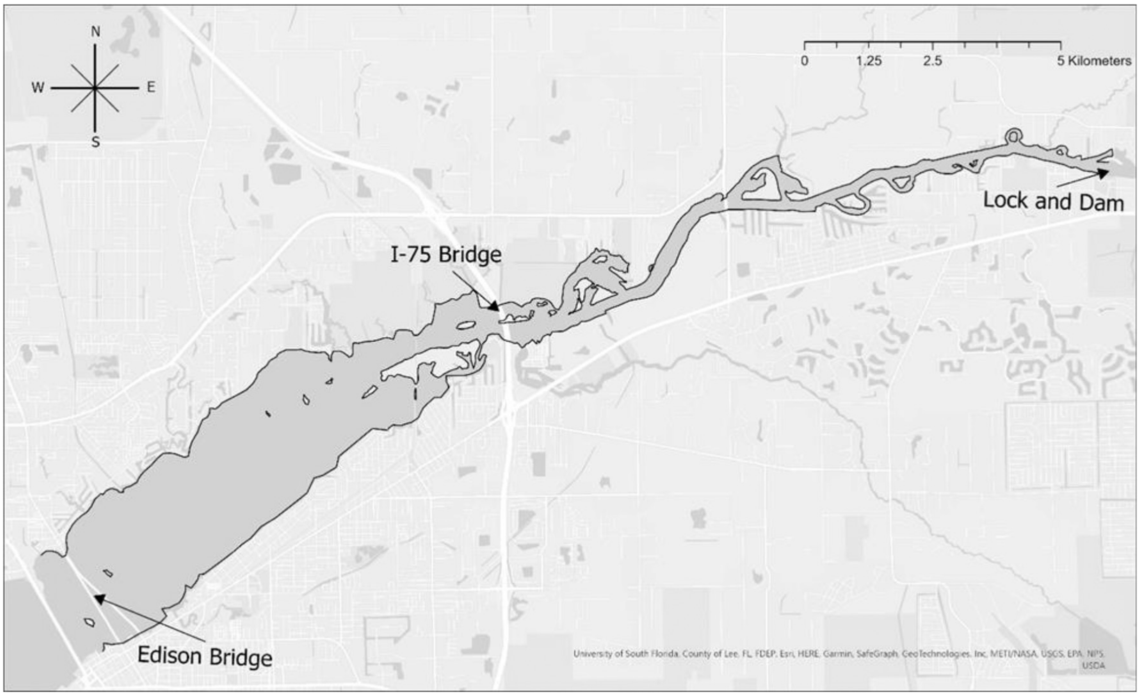


**Fig. 2.** Map of the region of the Caloosahatchee River Estuary included in this study, bounded by the black outlined shoreline. The southwest and northeast portions of the study region are separated by the I-75 bridge. See text regarding methods of estimation of habitable area for *Vallisneria americana* in each portion.

**Table 2**
Summary of growth conditions and plant characteristics from the mesocosm and field experiments at their final sample dates. PAR is photosynthetically active radiation.

| Experiment | Treatment | Time of Year | Temp. Range (C) | Mean PAR (micromoles $m^{-2}$ $s^{-1}$) | Mean Final Shoot Height (cm) | Mean C % Mass | Mean Tissue C: N | Mean Tissue C: P |
|---|---|---|---|---|---|---|---|---|
| Mesocosm | Ambient | Oct-Dec | 8–28 | 40–90 | 9.7 | 32.74 | 31.9 | 272.8 |
| Mesocosm | Enriched | Oct-Dec | 8–28 | 40–90 | 9.3 | 29.81 | 12.7 | 93.2 |
| Field | Ambient | Jun-Jul | 26–33 | 10–50 | 5.8 | 36.72 | 10.7 | 193.3 |
| Field | Enriched | Jun-Jul | 26–33 | 10–50 | 6.0 | 34.87 | 10.9 | 174.4 |

monitoring data and associated reports (Bortone and Turpine, 2000, Buzzelli et al., 2017, Douglass et al., 2020a). In particular, shoot density (shoots $m^{-2}$) and shoot biomass (g DW $shoot^{-1}$) were derived from four sites in the oligohaline CRE that were monitored monthly from 1998 through 1999. The centered estimate sub-scenario values for shoot density and shoot biomass were based on annual mean values from this period, and the conservative and optimistic sub-scenario values were based on winter minimum and summer maximum values from the period, respectively.

Current *V. americana* shoot density in the oligohaline CRE was determined with 2020 data from a US EPA-funded monitoring effort that surveyed 21 shoreline-perpendicular transects in the area, from just offshore of the 1 m depth contour landward to the shoreline (Douglass et al., 2020b). Current shoot biomass data was determined from the mean DW per shoot of *V. americana* samples collected from all dates and all vegetated treatments in the summer 2021 field experiment described in section 2.3 of this manuscript. For both shoot density and shoot biomass, mean values were used for centered estimate sub-scenarios, and values from the lower and upper boundaries of 95% confidence intervals around the mean were used for the conservative and optimistic sub-scenarios, respectively.

### 2.6. *Nutrient storage calculations for V. americana biomass-*

The quantity of nutrients retained in living tissue by current and restored *V. americana* populations in the CRE was calculated in a multi-step process. First, the % mass of carbon of a typical *V. americana* shoot in the CRE was determined by taking the mean % mass of carbon from all 2021 field samples, as determined by a BCAL Flash 1112 elemental analyzer (see Section 2.4). This was then multiplied by mean DW per shoot to get a carbon mass per shoot, which was in turn coupled with tissue C:N and tissue C:P mass ratios to determine nitrogen and phosphorus content per shoot. Conservative nutrient content estimates of *V. americana* tissue were determined from the vegetated ambient (VA) treatment's C:N and C:P mass ratios in the mesocosm experiment, where the plants were grown in sterile sand and low-nutrient municipal water. The optimistic (high-end) nutrient content estimate for P was obtained from the enriched (VN) treatment's C:P ratio in the mesocosm experiment. The optimistic N content estimate was obtained from the field experiment's C:N mass ratio, where N content was similarly high in both the VA and VN treatments and higher than in the mesocosm experiment (Table 2). Values for tissue C, N, and P content were multiplied by scenario-specific shoot density and shoot biomass estimates to determine carbon and nutrient storage $m^{-2}$. This was multiplied by the bathymetry-derived habitable area estimates to determine total C, N, and P storage in *V. americana* biomass in the CRE.

### 2.7. *Nutrient Storage Calculations for V. americana bed sediments-*

Nutrients and carbon are deposited and retained in the sediments of SAV beds at rates that can lead, over time, to much greater storage of those elements in SAV sediments than in living SAV tissues. Thus, we estimated the contribution of CRE SAV beds to C, N, and P deposition rates in the surficial sediment layers (10 cm). Because direct measurements of these deposition rates are not available for *V. americana* beds in the CRE, literature values from a range of marine, estuarine, and freshwater SAV systems were used. For sediment C deposition, data were derived from SAV treatment wetlands in Florida (Reddy et al., 2021), marine and estuarine seagrass meadows (Duarte et al., 2013), and from estuarine to freshwater SAV in Louisiana (Hillmann et al., 2020). The high deposition rate reported for SAV treatment wetlands (Reddy et al., 2021) was used for our optimistic scenarios, and the relatively low rate from marine seagrass meadows (Duarte et al., 2013) for the conservative scenarios. For the centered estimate scenarios we used the mean of these values, which was similar to the rate estimated for freshwater-estuarine transitional SAV systems in Louisiana (Hillmann et al., 2020). Deposition rates for N were taken from the median, minimum, and maximum values reported in Reddy et al. (2021). This N burial rate is approximately the same as the one that would be derived by dividing the carbon burial rate by the 10:1C:N ratio of refractory sediment organic matter reported by Reddy and DeLaune (2008) in a similar SAV study.

Phosphorus storage rates were derived from a freshwater SAV mesocosm experiment by Dierburg et al. (2002), which quantified P accumulation in freshwater SAV sediments in south Florida over 8-months (0.44–1.11 g $m^{-2}$). To produce an annual mass-by-area storage rate estimate, the median P storage over 8 months was extrapolated to 12 months (1.16 g-P $m^{-2}$ $y^{-1}$), as no seasonal variation in P storage rate was reported and a linear relationship was assumed (Dierburg et al., 2002). The conservative and optimistic P deposition rate values for those sub-scenarios were derived from Dierberg et al. 2002's minimum and maximum observed rates, respectively.

Recent evidence suggests a positive relationship between SAV aboveground biomass (a function of shoot size and shoot density), canopy cover, and soil carbon storage in seagrass meadows (Fourqurean et al., 2023; McHenry et al., 2023). Thus, we attenuated our C, N, and P sediment storage rate estimates by factors equal to the ratio of focal scenario SAV biomass (g DW $m^{-2}$) to full restoration scenario SAV biomass (1998–1998 observed mean SAV biomass for the study area; 25.7 g DW $m^{-2}$).

## 3. Results

### 3.1. Plant characteristics in field and mesocosm studies-

Caloosahatchee strain *V. americana* shoot heights in the 2021 field (6.11 ± 0.35 SE cm) and mesocosm experiments (9.56 ± 1.23 SE cm) were <10% of maximum shoot heights documented elsewhere in Florida (up to 134 cm) (Hauxwell et al., 2007) and diminutive compared to historic CRE shoot heights reported by Bortone and Turpine (2000). No reproductive shoots were observed in the field or mesocosms. The mean shoot + biomass at our summer 2021 field experiment site was 0.02 ± 0.003 SE g-DW. This is an order of magnitude smaller than the 1998 summer shoot biomass from the same area reported in Bortone and Turpine (2000). The shoots assessed in the 2021 field study had a % C of 33.15% ± 0.92 SE or 6.82 ± 0.06 mg-C.

### 3.2. Nutrient storage estimates for *V. americana* living biomass in the CRE-

Tissue C:N and C:P were highest in the mesocosm ambient treatment, suggesting nutrient limitation in the municipal water used in that treatment. In the mesocosm enriched treatment where the water column was hypereutrophic, tissue C:N and C:P were less than half their values in the ambient treatment (Table 2). Interestingly, the lowest tissue C:N values were observed in the field experiment (10.7), suggesting that nitrogen levels were replete in the field. Tissue P content was highest in the enriched mesocosm treatment, with C:P at 93.2. In the field experiment, there were no significant differences in tissue nutrient stoichiometry (C, N, P) between ambient and enriched treatments ($p > 0.05$). Transect surveys of the upper CRE during Summer 2020 (Douglass et al., 2020b) yielded a mean shoot density at the <1 m depth stratum of 4.7 ± 1.5 SE shoots $m^{-2}$ and found no SAV below 1 m depth. The approximate area of habitable stratum determined by geospatial and bathymetry analysis was 3.34 $km^2$. Therefore, the total number of shoots in the upper estuary at current density and in all available habitat was estimated at 15,698,000. In a fully restored scenario with shoot density at 257 shoots $m^{-2}$, there would be a total 858,380,000 *V. americana* shoots in the estuary. Combination of tissue nutrient stoichiometry values and shoot size and abundance values yielded the C, N, and P storage estimates presented in Table 3a.

### 3.3. Nutrient storage rate estimates of sediments in CRE *V. americana* beds-

Total nutrients deposited and buried annually in SAV bed sediments in the CRE were estimated at 896,790 kg-C $y^{-1}$, 68,470 kg-N $y^{-1}$ and 3841 kg-P $y^{-1}$ for the fully-restored scenario (Table 3b). However, adjusting this estimate down according to the relatively low shoot density and small shoot size of *V. americana* currently observed in the CRE reduced the estimates of burial efficiency by two orders of magnitude (Table 3b).

## 4. Discussion

In the design of ecological experiments there is often a trade-off between control and realism. Small scale laboratory and mesocosm experiments emphasize control but may generate conditions and responses that are not representative of ecosystem processes. Conversely, observational studies and field experiments emphasize realism yet are subject to uncontrolled environmental influences. By combining information from each type of study, we were able to parameterize simple scenarios and estimate the carbon and nutrient storage potential of *V. americana* beds in the Caloosahatchee River Estuary (CRE). Among the insights from this exercise was the confirmation that *V. americana* has appreciable stoichiometric plasticity. Its ability to incorporate excess nutrients into tissues contributes to its nutrient storage and removal potential (Gerloff & Krombholz, 1966). While tissue P content was highest in hypereutrophic mesocosm conditions, as predicted, it was somewhat surprising that the highest tissue N content (by a small margin) was observed in ambient field conditions. This suggests that *V. americana* in the CRE is saturated with respect to tissue N incorporation such that further N storage by the plants could only be achieved by an increase in plant abundance or biomass.

Indeed, a second insight from the study is that nutrient storage estimates based on current *V. americana* abundance and shoot size in the CRE are stunningly low in comparison with estimates based on the historic baseline conditions. Note that in the restoration scenarios (Table 3), increasing shoot density and shoot size from current to historic means increased estimates of *V. americana* biomass in the system 286-fold, driving equally large changes in the estimated amounts of C, N, and P stored. In comparison, different values for tissue stoichiometry ratios had less influence on storage estimates, although they could still affect nutrient storage estimates substantially.

A third insight from the exercise is that rates of carbon and nutrient removal by sediment deposition could lead to sediment storage of these materials greatly exceeding storage in living tissues. However, these sediment storage rates are also likely to depend on *V. americana* biomass and density. Furthermore, they could be affected by hydrodynamic and biological processes, including resuspension, decomposition, and microbially mediated nutrient cycling (Koch et al., 2001; Duarte et al., 2013). These factors vary within landscapes and across regions, therefore our efforts to quantify C, N, and P storage processes for a particular location using literature values from other regions are somewhat fraught (Bijak et al., 2023). Recent literature supports the existence of SAV

**Table 3**

(3a) Tissue nutrient storage estimates for different scenarios in the Caloosahatchee River Estuary (CRE). Scenario 1 (Current *Vallisneria* density) is based on 2020–2021 shoot density and biomass in the CRE, while Scenario 2 (Full *Vallisneria* restoration) uses shoot density and biomass from a 1998–1999 period prior to extensive die-offs. Derivation of parameter values for "Centered Estimate," "Conservative Boundary," and "Optimistic Boundary" sub-scenarios is detailed in Table 1. (3b) Sediment carbon and nutrient storage estimates for *V. americana* beds in the CRE for current conditions (scenario 3), and full restoration conditions (scenario 4). Derivation of parameter values for "Centered Estimate," "Conservative Boundary," and "Optimistic Boundary" sub-scenarios is detailed in Table 1.

3a

| Scenario | Sub-Scenario | Shoot Density (shoots $m^{-2}$) | Biomass per Shoot (g DW $shoot^{-1}$) | Biomass (g DW $m^{-2}$) | Carbon Fraction of DW | Tissue C:N | Tissue C:P | Carbon (g $m^{-2}$) | Nitrogen (g $m^{-2}$) | Phosphorus (g $m^{-2}$) | Habitable Area ($km^2$) | Total C Storage (kg) | Total N storage (kg) | Total P storage (kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1- Current *Vallisneria* density | Centered Estimate | 4.7 | 0.02 | 0.09 | 0.33 | 10.8 | 177 | 0.03 | 0.003 | 0.0002 | 3.34 | 104 | 9.64 | 0.59 |
| | Conservative Boundary | 1.66 | 0.014 | 0.02 | 0.33 | 31.9 | 274 | 0.01 | 0.0007 | 0.00004 | 1.67 | 12.9 | 1.19 | 0.07 |
| | Optimistic Boundary | 7.74 | 0.026 | 0.20 | 0.33 | 10.8 | 93.2 | 0.07 | 0.006 | 0.0004 | 5.01 | 334 | 30.9 | 1.89 |
| 2- Full *Vallisneria* restoration | Centered Estimate | 257 | 0.1 | 25.7 | 0.33 | 10.8 | 177 | 8.52 | 0.789 | 0.048 | 3.34 | 28,455 | 2635 | 161 |
| | Conservative Boundary | 208 | 0.02 | 4.16 | 0.33 | 31.9 | 274 | 1.38 | 0.128 | 0.008 | 1.67 | 2303 | 213 | 13.0 |
| | Optimistic Boundary | 306 | 0.2 | 61.2 | 0.33 | 10.8 | 93.2 | 20.3 | 1.88 | 0.115 | 5.01 | 101,642 | 9411 | 574 |

3b

| | | | Ranges from Literature | | | Adjusted Values | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Scenario | Sub-Scenario | Biomass (g DW $m^{-2}$) | C deposition (g $m^{-2}y^{-1}$) | N deposition (g $m^{-2}y^{-1}$) | P deposition (g $m^{-2}y^{-1}$) | C deposition (g $m^{-2}y^{-1}$) | N deposition (g $m^{-2}y^{-1}$) | P deposition (g $m^{-2}y^{-1}$) | Habitable Area ($km^2$) | Total C (kg $y^{-1}$) | Total N (kg $y^{-1}$) | Total P (kg $y^{-1}$) |
| 3- Current *Vallisneria* density | Centered Estimate | 0.09 | 269 | 20.5 | 1.16 | 0.98 | 0.07 | 0.00 | 3.34 | 3280 | 250 | 14.2 |
| | Conservative Boundary | 0.02 | 53 | 9 | 0.66 | 0.05 | 0.01 | 0.00 | 1.67 | 80.0 | 13.6 | 1.00 |
| | Optimistic Boundary | 0.20 | 590 | 32 | 1.66 | 4.62 | 0.25 | 0.01 | 5.01 | 23,146 | 1255 | 65.1 |
| 4- Full *Vallisneria* restoration | Centered Estimate | 25.7 | 269 | 20.5 | 1.16 | 269 | 20.5 | 1.16 | 3.34 | 896,790 | 68,470 | 3874 |
| | Conservative Boundary | 4.16 | 53 | 9 | 0.66 | 8.58 | 1.46 | 0.11 | 1.67 | 14,327 | 2433 | 178 |
| | Optimistic Boundary | 61.2 | 590 | 32 | 1.66 | 1405 | 76.2 | 3.95 | 5.01 | 7,038,953 | 381,774 | 19,805 |

biomass - carbon deposition relationships over large scales, justifying the biomass-scaling approach we have taken in the estimates here (Fourqurean et al., 2023). Yet, studies also acknowledge high variability among individual SAV meadows (Bijak et al., 2023). Even unvegetated benthic habitats can have substantial sediment storage rates of C, N, and P in hydrodynamic environments favorable for deposition, although they tend to be less effective at these ecosystem functions than SAV and are more vulnerable to surficial disturbance (Hansen and Reidenbach, 2013; Marba et al., 2015; McHenry et al., 2023). In addition to the effect of SAV biomass on deposition and storage rates, plant morphology (i.e., large-bodied SAV spp.) and temporal stability of meadows have been shown to be useful predictors of sustained sediment carbon storage (Fourqurean et al., 2023; Bijak et al., 2023). Future studies directly measuring sediment storage of C, N, and P in this system, and quantifying the relationships between shoot density, shoot size, historical SAV cover, and sediment C, N, and P deposition in estuarine SAV systems generally would be helpful for improving models like ours.

Along with its effects on C, N, and P storage, SAV habitat can also offer significantly greater denitrification services than unvegetated benthic habitats (Piehler and Smyth, 2011). Future modeling of estuarine *V. americana* nutrient removal potential should also incorporate denitrification among the other nutrient removal mechanisms, and assess its relationship with SAV density.

## 5. Conclusion

Through a combined approach of mesocosm, field experiments, and literature review, we produced a first approximation of nutrient storage potential of restored *V. americana* in the Caloosahatchee River Estuary. Approximately 28.4 mt-C, 2.6 mt-N and 0.16 mt-P in standing plant biomass and 897 mt-C $y^{-1}$, 68.5 mt-N $y^{-1}$ and 3.87 mt-P $y^{-1}$ in SAV bed sediments could be stored with successful restoration of 3.34 $km^2$ of *V. americana*. Unfortunately, we estimate that <1% of these potential benefits are currently realized in the CRE, due to the small size and low density of *V. americana* shoots in the current CRE versus in its historic (1998–1999) condition. Coordinated efforts to improve optical water quality conditions and stabilize salinity regimes in the upper estuary, in conjunction with active restoration measures such as replanting, will be essential if the potential nutrient and carbon storage benefits of CRE SAV are to be realized. There is a need for continued work on these types of projects as the re-establishment of SAV beds can help return impacted or degraded habitats to levels that are self-sustaining and positively impact the surrounding water quality (Orth et al., 2020). To encourage funding for further restoration efforts, it is critical to quantify the benefits provided by such projects. Outcomes from this type of work can have direct applications for evaluating the effectiveness of seagrass for nutrient removal in stormwater treatment systems, canals, and natural water bodies. It also has implications for the National Pollutant Discharge Elimination Systems (NPDES) permit compliance, Basin Management Action Plans (BMAPs), and wet detention pond design Best Management Practices (BMPs).

## 6. Glossary

Phytoremediation: treatment of environmental pollutants and contaminants via the ecological functions of plants and plant systems.

Mesocosm: medium scale, semi-controlled experimental replicate of an ecological system.

## Funding

This work was supported by the Coastal and Heartland National Estuary Partnership.

## Disclosure

We know of no conflicts of interest associated with this publication, and there has been no financial interest in this research that could influence its outcome.

## CRediT authorship contribution statement

**Brondum M. Krebs:** Data curation, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft, Writing – review & editing. **Nicole Iadevaia:** Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Jennifer Hecker:** Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. **James G. Douglass:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

## Declaration of Competing Interest

James Douglass reports financial support was provided by Coastal and Heartland National Estuary Partnership. James Douglass reports financial support was provided by US Environmental Protection Agency. James Douglass reports a relationship with Johnson Engineering Incorporated that includes: funding grants. One of the suggested reviewers, William Mitsch, is a former colleague of James Douglass at Florida Gulf Coast University and on the editorial board of Ecological Engineering.

## Data availability

Data will be made available on request.

## Acknowledgments

We thank David Ceilley for his contributions to the project's conception, Sea & Shoreline LLC for their plant donations, The Water School at Florida Gulf Coast University for providing logistical and administrative support, and Rhonda Mason for editorial assistance. Numerous staff, students, and volunteers from Florida Gulf Coast University assisted with this work.

## References

Badruzzaman, M., Pinzon, J., Oppenheimer, J., Jacangelo, J.G., 2012. Sources of Nutrient Impacting Surface Waters in Florida: a Review. J. Environ. Manag. 109, 80–92. https://doi.org/10.1016/j.jenvman.2012.04.040.

Bijak, A.L., Reynolds, L.K., Smyth, A.R., 2023. Seagrass Meadows Stability and Composition Influence Carbon Storage. Landsc. Ecol. https://doi.org/10.1007/s10980-023-01700-3.

Bortone, S.A., Turpine, R.K., 2000. Tape Grass Life history Metrics Associated with Environmental Variables in a Controlled Estuary. Seagrasses, 1st ed. CRC Press (ISBN 9780429128035).

Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and Eutrophication. J. Exp. Mar. Biol. Ecol. 350, 46–72. https://doi.org/10.1016/j.jembe.2007.06.024.

Buzzelli, C., Doering, P., Chen, Z., Wan, Y., 2017. Component Study 7: Relationships between Salinity and Survival of *Vallisneria americana* in the CRE. Final Assessment of the Response of the Caloosahatchee River to Low Freshwater Inflow in the Dry season, Executive Summary. SFWMD, pp. 95–107.

Campbell, J.E., Fourqurean, J.W., 2009. Interspecific variation in the elemental and stable isotope content of seagrasses in South Florida. Mar. Ecol. Prog. Ser. 387, 109–123. https://doi.org/10.3354/meps08093.

Ceilley, D.W., 2018. Tape Grass (*Vallisneria americana*) Restoration and Seed Stock Enhancement in the C-43 (Caloosahatchee River) with Plantings and Exclosure Cages. Johnson Engineering Inc. Final Report, South Florida Water Management District, West Palm Beach, FL.

Center for Coastal Ecology, Mote Marine Laboratory., 2007. *Vallisneria americana* Restoration in the Caloosahatchee River, Lee County, Florida. Mote Marine Laboratory Technical Report Number, 1230 CHNEP, 1-13.

Cortez, C., Seidel, V., Diamond, C., Mandell, E., 2020. Economic Valuation of the Coastal & Heartland National Estuary Partnership Area. The Balmoral Group, Winter Park, FL.

Dahl, M., Asplund, M.E., Björk, M., 2020. The influence of hydrodynamic exposure on carbon storage and nutrient retention in Eelgrass (*Zostera marina* L.) Meadows on the Swedish Skagerrak coast. Nat. Sci. Rep. 10, 1–13. https://doi.org/10.1038/s41598-020-70403-5.

Diaz, R.J., Rosenberg, R., 2008. Spreading Dead zones and Consequences for Marine Ecosystems. Science 321, 926–929. https://doi.org/10.1126/science.1156401.

Dierburg, F.E., DeBusk, T.A., Jackson, S.D., Chimney, M.J., Pietro, K., 2002. Submerged Aquatic Vegetation based Treatment Wetlands for Removing Phosphorus from Agricultural Runoff: Response to Hydraulic and Nutrient Loading. Water Res. 36, 1409–1422. https://doi.org/10.1016/s0043-1354(01)00354-2.

Dodds, W.K., Bouska, W.W., Eitzman, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T., Thornburgh, D.J., 2009. Eutrophication of U.S. Freshwaters: Analysis of potential Economic Damages. Environ. Sci. Technol. 43, (1), 12–19. https://doi.org/10.1021/es801217q.

Doering, P.H., Chamberlain, R.H., McMunigal, J.M., 2001. Effects of simulated Saltwater Intrusions on the growth and Survival of Wild Celery, *Vallisneria americana*, from the Caloosahatchee Estuary (South Florida). Estuaries 24 (6a), 894–903. https://doi.org/10.2307/1353180.

Doering, P.H., Chamberlain, R.H., Haunert, D.E., 2002. Using Submerged Aquatic Vegetation to establish Minimum and Maximum Freshwater Inflows to the Caloosahatchee Estuary, Florida. Estuaries 25 (6), 1343–1354. https://doi.org/10.1007/BF02692229.

Doering, P.H., Chamberlain, R.H., Haunert, K.M., 2006. Chlorophyll a and its use as an Indicator of Eutrophication in the Caloosahatchee Estuary, Florida. Florida Scientist 69, 51–72.

Dorgham, M., 2014. Effects of Eutrophication. In: Ansari, A., Gill, S. (Eds.), Eutrophication: Causes, Consequences and Control. Springer, Dordrecht.

Douglass, J.G., Duffy, J.E., Spivak, A.C., Richardson, J.P., 2007. Nutrient versus consumer control of community structure in a Chesapeake Bay eelgrass habitat. Mar. Ecol. Prog. Ser. 348, 71–83. https://doi.org/10.3354/meps07091.

Douglass, J.G., Chamberlain, R.H., Wan, Y., Doering, P.H., 2020a. Submerged vegetation responses to climate variation and altered hydrology in a subtropical estuary: interpreting 33 years of change. Estuar. Coasts 43, 1406–1424. https://doi.org/10.1007/s12237-020-00721-4.

Douglass, J.G., Adhikari, P., Urakawa, H., Bartleson, R., 2020b. Enhanced Water Quality and Seagrass monitoring in the Caloosahatchee Estuary. US Environmental Protection Agency Grant #01D00420, Unpublished data.

Duarte, C., Kennedy, H., Marbà, N., Hendriks, I., 2013. Assessing the capacity of seagrass meadows for carbon burial: current limitations and future strategies. Ocean Coast. Manag. 83, 32–38. https://doi.org/10.1016/j.ocecoaman.2011.09.001.

Florida Department of Environmental Protection, 2009. Nutrient TMDL for the Caloosahatchee Estuary (WBID 3240A, 3240B and 3240C). Bureau of Watershed Management, Tallahassee, FL.

Florida Department of Environmental Protection, 2020. 2020 Integrated Water Quality Assessment for Florida: Sections 303(d), 305(b), and 314 Report and Listing Update. Technical Report. Division of Environmental Assessment and Restoration.

Florida Department of Environmental Protection, 2021. Water Shed Assessment Lists. Accessed: 01/13/2022. https://floridadep.gov/dear/watershed-assessment-section/content/assessment-lists.

Fonseca, M.S., Fisher, J.S., 1986. A comparison of canopy friction and sediment movement between four species of seagrass with reference to their ecology and restoration. Mar. Ecol. Prog. Ser. 29 (1), 15–22. https://doi.org/10.3354/meps029015.

Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marba, N., Holmer, N., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krouse-Jensen, D., McGlathery, K.J., Serrano, O., 2012. Seagrass ecosystems as a globally significant carbon stock. Nat. Geosci. 5, 505–509. https://doi.org/10.1038/ngeo1477.

Fourqurean, J.W., Kennedy Rhoades, O., Munson, C.J., Krause, J.R., Altieri, A.H., Douglass, J.G., Heck Jr., K.L., Paul, V.J., Armitage, A.R., Barry, S.C., Bethel, E., Christ, L., Chrsitiansen, M.J.A., Dodillet, G., Dutton Frazier, T.K., Gaffey, B.M., Glazner, R., Goeke, J.A., Grana-Valdes, R., Kramer, O.A.A., Linhardt, S.T., Martin, C. W., Martinez Lopez, I.G., McDonald, A.M., Maine, V.A., Manuel, S.A., Marco-Mendez, C., O'Brien, D.A., O'Shea, O., Patrick, C.J., Peabody, C., Reynolds, L.K., Rodriguez, A., Rodriguez Bravo, L.M., Sang, A., Sawall, Y., Smulders, F.O.H., Thompson, J.E., van Tussenbroek, B., Wied, W.L., Wilson, S.S., 2023. Seagrass abundance predicts surficial soil organic carbon stocks across the range of *Thalassia testudinum* in the Western North Atlantic. Estuar. Coasts 46, 1280–1301.

Gerloff, G.C., Krombholz, P.H., 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. Limnology and Oceanography 11 (4), 529–537. https://doi.org/10.4319/lo.1966.11.4.0529.

GESAMP (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection) and Advisory Committee on Protection of the Sea, 2001. Protecting the Oceans from Land-based Activities - Land-based sources and Activities Affecting the Quality and Uses of the Marine, Coastal and Associated Freshwater Environment. Rep. Stud. GESAMP 71, 162.

Glibert, P.M., 2020. From hogs to HABS: Impacts of Industrial Farming in the US on Nitrogen and Phosphorus and Greenhouse Gas Pollution. Biogeochemistry 150 (2), 139–180. https://doi.org/10.1007/s10533-020-00691-6.

Hansen, M., 2015. Single-Beam bathymetry sounding data of the caloosahatchee river, Florida (2002). In: XYZ Format: Archive of Bathymetry Data Collected in South Florida from 1995 to 2015 U.S. Geological Survey Data Series-1031, U.S. Geological Survey, St. Petersburg, Florida.

Hansen, J.C., Reidenbach, M.A., 2013. Seasonal growth and senescence of a *Zostera marina* seagrass meadow alters wave-dominated flow and sediment suspension within a coastal bay. Estuar. Coasts 36, 1099–1114. https://doi.org/10.1007/s12237-013-9620-5.

Hauxwell, J., Frazer, T.K., Osenberg, C.W., 2007. An annual cycle of biomass and productivity of *Vallisneria americana* in a subtropical spring-fed estuary. Aquat. Bot. 87, 61–68. https://doi.org/10.1016/j.aquabot.2007.03.003.

Heil, C.A., Muni-Morgan, A.L., 2021. Florida's harmful algal bloom (HAB) problem: escalating risks to human, environmental and economic health with climate change. Front. Ecol. Evol. 9, 1–38. https://doi.org/10.3389/fevo.2021.646080.

Hillmann, E.R., Rivera-Monroy, V.H., Nyman, J.A., LaPeyre, M.K., 2020. Estuarine submerged aquatic vegetation habitat provides organic carbon storage across a shifting landscape. Sci. Total Environ. 717, 1–12. https://doi.org/10.1016/j.scitotenv.2020.137217.

Hoagland, P., Scatasta, S., 2006. The Economic Impacts of Harmful Algal Blooms. Ecological Studies, 189, Ecology of Harmful Algal Blooms, pp. 391–402.

Hoffacker, V.A., 1994. Caloosahatchee River Submerged Grass Observation during 1993. W. Dexter Bender and Associates, Inc. Letter-report and map to Chip Meriam. SFWMD.

Janse, J.H., Kuiper, J.J., Weijters, M.J., Westerbeek, E.P., Jeuken, M.H.J.L., Bakkenes, M., Alkmade, R., Mooij, W.M., Verhoeven, J.T.A., 2015. Globio-aquatic, a global model of human impact on the biodiversity of inland aquatic ecosystems. Environ. Sci. Pol. 48, 99–114. https://doi.org/10.1016/j.envsci.2014.12.007.

Johnson Engineering, 2019. Baseline Aquatic Fauna & Submerged Aquatic Vegetation (SAV) Assessment for Caloosahatchee River Submerged Aquatic Vegetation Restoration Project. Report to Sea and Shoreline LLC and Angler Action Foundation (56 pp).

Koch, M.S., Benz, R.E., Rudnick, D.T., 2001. Solid-phase phosphorus pool in highly organic carbonate sediments of North-eastern Florida Bay. Estuar. Coast. Shelf Sci. 52, 1–13. https://doi.org/10.1006/ecss.2000.0751.

Le Moal, M., Gascuel-Odoux, C., Menesguen, A., Souchon, Y., Etrillard, C., Levain, A., Moatar, F., Pannard, A., Souchu, P., Lefebvre, A., Pinay, G., 2019. Eutrophication: a new wine in an old bottle? Sci. Total Environ. 651 (1), 1–11. https://doi.org/10.1016/j.scitotenv.2018.09.139.

Lowden, R.M., 1982. An approach to the taxonomy of Vallisneria L. (Hydrocharitaceae). Aquat. Bot. 13, 269–298.

Marba, N., Arias-Ortiz, A., Masque, P., Kendrick, G.A., Mazarrasa, I., Bastyan, G.R., Garcia-Orellana, J., Duarte, C.M., 2015. Impact of Seagrass loss and subsequent Revegetation on Carbon Storage and stocks. J. Ecol. 103, 296–302. https://doi.org/10.1111/1365-2745.12370.

Martin, A.P., Mort, M.E., 2023. *Vallisneria* (Hydrocharitaceae): novel species, taxonomic revisions, and hybridization. Aquat. Bot. 188, 103669.

McAskill, S., Douglass, J.G. 2017. Salinity and Temperature Alter *Pomacea maculate* herbivory rates on *Vallisneria americana.* J. Molluscan Stud., 83, (4), 481–483. Florida Gulf Coast University (M.S. Thesis).

McHenry, J., Rassweiler, A., Hernan, G., Dubel, A.K., Curtain, C., Barzak, J., Varias, N., Lester, S.E., 2023. Geographic variation in organic carbon storage by seagrass beds. Limnol. Oceanogr. 68, 1256–1268. https://doi.org/10.1002/lno.12343.

Mitsch, W., Zhang, L., Waletzko, E., Bernal, B., 2014. Validation of the ecosystem services of created wetlands: two decades of plant succession, nutrient retention, and carbon storage in experimental riverine marshes. Ecol. Eng. 72, 11–24. https://doi.org/10.1016/j.ecoleng.2014.09.108.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck Jr., K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F. T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. BioScience 56, 987–996. https://doi.org/10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2.

Orth, R.J., Lefcheck, J.S., McGlathery, K.S., Aoki, L., Luckenbach, M.W., Moore, K.A., Oreska, M.P.J., Snyder, R., Wilcox, D.J., Lusk, B., 2020. Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services. Sci. Adv. 6 https://doi.org/10.1126/sciadv.abc6434.

Paerl, H.W., 2009. Controlling eutrophication along the freshwater-marine continuum: dual nutrient (N and P) reductions are essential. Estuar. Coasts 32, 593–601. https://doi.org/10.1007/s12237-009-9158-8.

Parkyn, S.M., Davies-Colley, R.J., Cooper, A.B., Stroud, M.J., 2005. Predictions of stream nutrient and sediment yield changes following restoration of forested riparian buffers. Ecol. Eng. 24 (5), 551–558. https://doi.org/10.1016/j.ecoleng.2005.01.004.

Piehler, M.F., Smyth, A.R., 2011. Habitat specific distinctions in estuarine denitrification affect both ecosystem function and services. Ecosphere 2 (1), 1–17.

Pilon-Smits, E., 2005. Phytoremediation. Ann. Rev. Plant Biol. Earth Atmos. Aquatic Sci. Collection 56, 15. https://doi.org/10.1890/ES10-00082.1.

Quilliam, R.S., van Niekerk, M., Chadwick, D.R., Cross, P., Hanley, N., Jones, D.L., Vinten, A.J.A., Willby, N., Oliver, D.M., 2015. Can macrophyte harvesting from eutrophic water close the loop on nutrient loss from agricultural land? J. Environ. Manag. 152, 210–217. https://doi.org/10.1016/j.jenvman.2015.01.046.

Ralston, E.P., Kite-Powell, H., Beet, A., 2017. An Estimate of the cost of acute food and water borne health effects from marine pathogens and toxins in the United States. J. Water Health 9 (4), 680–694. https://doi.org/10.2166/wh.2011.157.

Reddy, K.R., DeLaune, R.D., 2008. Biogeochemistry of Wetlands: Science and Applications (1st Edition). CRC Press.

Reddy, K.R., Hu, J., Villapando, O., Bhomia, R.K., Vardanyan, L., Osborne, T., 2021. Long-term accumulation of macro- and secondary elements in subtropical treatment wetlands. Ecosphere 12, 11. https://doi.org/10.1002/ecs2.3787.

Shi, L., Du, X., Liu, H., Chen, X., Ma, Y., Wang, R., Tian, Z., Tian, S., Zhang, S., Guo, H., Zhang, H., 2021. Update on the adverse Effects of *Microcystins* on the Liver. Environ. Res. 195 https://doi.org/10.1016/j.envres.2021.110890.

South Florida Water Management District, 2009. Caloosahatchee River Watershed Protection Plan. https://www.sfwmd.gov/sites/default/files/documents/ne_crwpp_main_123108.pdf.

South Florida Water Management District, 2021. Lake Okeechobee. Accessed: 02/04/2022. https://www.sfwmd.gov/our-work/lake-okeechobee.

Spivak, A.C., Canuel, E.A., Duffy, J.E., Douglass, J.D., Richardson, J.P., 2009. Epifaunal community composition and nutrient addition alter sediment organic matter composition in a natural eelgrass *Zostera marina* bed: a field experiment. Mar. Ecol. Prog. Ser. 376, 55–67. https://doi.org/10.3354/meps07813.

Stevens, P.W., Greenwood, M.F.D., Idelberger, C.F., Blewett, D.A., 2010. Mainstem and backwater fish assemblages in the tidal caloosahatchee river: implications for freshwater inflow studies. Estuar. Coasts 33, 1216–1224. https://doi.org/10.1007/s12237-010-9318-x.

Straile, D., 2015. Zooplankton biomass dynamics in oligotrophic versus eutrophic conditions: a test of the PEG model. Freshw. Biol. 60 (1), 174–183. https://doi.org/10.1111/fwb.12484.

Tolley, S.G., Volety, A.K., Savarese, M., Walls, L.D., Linardich, C., Everham, E.M., 2006. Impacts of salinity and freshwater inflow on oyster-reef communities in Southwest Florida. Aquat. Living Resour. 19, 371–387. https://doi.org/10.1051/alr:2007007.

Vasanthi, D., Karuppasamy, P.K., Santhanam, P., Dinesh Kumar, S., Malarvannan, G., 2015. Phytoremediation to remove nutrients and textile dye effluent using seagrass (Cymodocea rotundata). Adv. Biol. Res. 9 (6), 405–412. https://doi.org/10.5829/idosi.abr.2015.9.6.96110.

Volety, A.K., Haynes, L., Goodman, P., Gorman, P., 2014. Ecological condition and value of oyster reefs of the Southwest Florida shelf ecosystem. Ecol. Indic. 44, 108–119. https://doi.org/10.1016/j.ecolind.2014.03.012.

Wenzel, W.W., 2009. Rhizosphere processes and management in plant assisted bioremediation (phytoremediation) of soils. Plant Soil 321, 382–408. https://doi.org/10.1007/s11104-008-9686-1.

Withers, P.J., Colin, N., Jarvie, H.P., Doody, D.G., 2014. Agriculture and Eutrophication: where do we go from Here? Sustainability 6 (9), 5853–5875. https://doi.org/10.3390/su6095853.

Yasin, H., Usman, M., Rashid, H., Nasir, A., Randhawa, I.A., 2017. Alternative approaches for solid waste management: a case study in Faisalabad Pakistan. Earth Sci. Pakistan 1 (2), 7–9. https://doi.org/10.26480/esp.02.2017.07.09.

Zhang, Z., Li, W., Ashraf, M.A., 2018. Allelopathic effects of various aquatic plants in eutrophic water areas. J. Coast. Res. 82 (1), 137–142. https://doi.org/10.2112/SI82-019.1.