CONNECTICUT SEA GRANT PROJECT REPORT

Please complete this progress or final report form and return by the date indicated in the emailed progress report request from the Connecticut Sea Grant College Program. Fill in the requested information using your word processor (i.e., Microsoft Word), and e-mail the completed form to Syma Ebbin syma.ebbin@uconn.edu, Research Coordinator, Connecticut Sea Grant College Program. Do NOT mail or fax hard copies. Please try to address the specific sections below. If applicable, you can attach files of electronic publications when you return the form. If you have questions, please call Nancy Balcom at (860) 405-9107.

Please fill out all of the following that apply to your specific research or development project. Pay particular attention to goals, accomplishments, benefits, impacts and publications, where applicable.

Name of Submitter: **Craig Tobias**

Date of Report submission: **10/20/24**

Project #: **(R/CMC-19-CTNY), RFA 21101 EPA Award # LI-00A00284**
Check one: [ ] Progress Report [x] Final report

Duration (dates) of entire project, including extensions: From **[03/01/2021] to [08/31/2024]**.

Project Title or Topic: **IMPROVING EELGRASS RESTORATION SUCCESS BY MANIPULATING THE SEDIMENT IRON CYCLE**

Principal Investigator(s) and Affiliation(s):

1. **Craig Tobias / University of Connecticut (PI)**
2. **Jamie Vaudrey / University of Connecticut**
3. **Chris Pickerell / Cornell Cooperative Extension**

A. COLLABORATORS AND PARTNERS: None to date

B. PROJECT GOALS AND OBJECTIVES:

1. Evaluate the efficacy of lowering sediment porewater sulfide (H_2S) concentrations *in situ* through iron (Fe) additions.

2. Test whether lower amounts of H_2S resulting from iron additions translate into higher survivorship of transplanted eelgrass.

C. LISS CCMP IMPLEMENTATION ACTIONS: (*List the top 3 primary CCMP Implementation Actions that this project addresses. LISS CCMP Implementation Actions can be found at https://longislandsoundstudy.net/2021/01/ccmp-implementation-actions-supplemental-documents/*

1. HW-1: Complete projects that result in restoration of coastal habitat.
2. HW-2: Develop a list of current and new or innovative restoration techniques.
3. SM-1: Regularly update and refine the high-priority science needs relating to the understanding and attainment of management objectives and ecosystem targets.

D. PROGRESS: (*Summarize progress relative to project goals and objectives. Highlight outstanding accomplishments, outreach and education efforts; describe problems encountered and explain any delays.)*

Project scope of work included: Field site selection and characterization, benchtop short-duration iron dosing experiments, a mesocosm long-term iron dosing experiment, two transplant experiments with associated post-emergence geochemical and plant monitoring.

Site selection: Based upon transects completed in year one in the Niantic River Estuary, three sites were chosen for seagrass transplants. The criteria used for site selection was the spatial distribution of porewater H_2S concentrations measured in areas where seagrass once grew but no longer does. Sites were designated high, medium, and low sulfide corresponding to >500µM, 100-500µM, and <100µM respectively.

Benchtop short-duration iron dosing experiments – High sulfide sediments were amended in sealed tube experiments using two different iron mineral types (siderite and magnetite) and grain sizes at multiple iron loading concentrations. The effect on residual porewater sulfide concentration was measured after one week.

Mesocosm long-term iron dosing experiment - Mesocosm experiments were conducted using high sulfide sediments amended with a single standardized addition of siderite and/or magnetite to test the duration of sulfide dampening in porewaters on monthly timescales. Iron was added using a methodology that would mimic the procedure used during the in-situ transplant experiments.

Transplants: Two eelgrass transplant efforts were conducted by our Cornell partners in October 2022 and October 2023. All three sites (low, medium, and high sulfide) were successfully transplanted. The transplant design consisted of the following iron treatments: no iron addition; 15% of the mesocosm iron dose (low iron); the mesocosm dose (medium iron); and 5 times the mesocosm dose (high iron). Magnetite was used in the transplant amendments. The transplant activity was facilitated by a public outreach activity, The Seagrass Meadows workshop, held just prior to each transplanting. The activity attracted > 20 volunteers who spend four hours weaving donor seagrass strands into transplant discs.

Post transplant monitoring – Divers surveyed the eelgrass transplants in the Spring of 2023 and Spring 2024 to assess the first and second transplant efforts respectively after a period of eight months. Porewaters were sampled from each treatment and analyzed for sulfide, ferrous iron, and pH. Plant survival was assessed qualitatively in 2023 using methods from the Long Island Sound Unified Water Study, and assessed quantitatively in 2024 using video transect.

Summary of Findings (see attached thesis):

Under laboratory and mesocosm conditions, fine-grained magnetite was more effective than siderite at dropping and maintaining low sulfide concentrations in sediment porewater. This effect occurred within one week at loadings as low as 0.004 g Fe cm^{-3} sediment in sealed tubes. Magnetite additions at a loading of 0.016 g Fe cm^{-3} sediment lowered porewater sulfide in mesocosms from 900 μM to non-detectable levels for six months. Magnetite added with eelgrass transplants in-situ, decreased porewater sulfide by magnitudes proportional the magnetite loading. The decreased sulfide lasted for eight months, persisting throughout plant emergence in the spring and early growth in the summer of 2023. The sulfide lower effect at higher iron loadings was also measured in situ 2024. The effect of the iron additions on transplant success was however, mixed and uncertain. Both transplant years were affected by conditions deleterious to eelgrass survival that were unrelated to sediment geochemistry. Early onset high water temperatures combined with low irradiance and turbidity in 2023 led to complete eelgrass mortality at all sites / all treatments. One-third of established beds died in the Niantic River Estuary. Qualitative plant surveys indicated that overwinter plant survival scored consistently higher in the highest iron treatments prior to the system-wide dieback. In 2024, all the transplant areas were affected by extensive macroalgal blooms that induced high mortality at all sites. Mortality was complete at the high sulfide site. With the exception of the medium iron addition at the medium sulfide site, which had the highest blade count, the video transects in 2024 showed no other effect of iron loading on eelgrass survival in the low and medium sulfide sites. Plant survival was too low generally at all sites, from the macroalgal loading, to discern any iron effect on transplant success if one existed. While the results from this project clearly demonstrate that iron additions can be used at field scale to lower porewater sulfide on the timescale suitable to support eelgrass restorations, it remains unclear if / how this tool could be used to improve transplant success, given all other confounding environmental conditions that limit eelgrass survival.

E. **PROJECT PUBLICATIONS, PRODUCTS, PRESENTATIONS AND PATENTS**: *(Include published materials with complete references, as well as those which have been submitted but not yet published and those in press. Please attach electronic versions of any journal articles, reports, and abstracts not previously provided.)*

Journal Articles (List URLs): **none to date – in prep**

Conference Papers: **none to date**

Proceedings or book chapters: **none to date**

Web sites, Software, etc.: **none**

Technical Reports/Other Publications: **This report**

Other Products (including popular articles): **none to date**

Publications planned / in progress: **Shannon Jordan Masters Thesis, Journal Article – in prep.**

Patents: *(List those awarded or pending as a result of this project.)* **none**

Presentations and Posters: *(Include name and date of the conference or meeting, whether it was a talk or poster, if it was invited, and who the presenter was.*)

LIS Research Conference, 2022 - Poster

LIS STAC Presentation, 2022 - Webinar

Coastal and Estuarine Research Federation Conference, 2023 - Talk

F. **FUNDS LEVERAGED:** *(If this Sea Grant funding facilitated the leveraging of additional funding for this or a related project, note the amount and source below.)*
none

G. **STUDENTS: (***Document the number and type of students supported by this project.)*
*Note: **"Supported"** means supported by Sea Grant through financial or other means, such as Sea Grant federal, match, state and other leveraged funds. **"New"** students are those who **have not** worked on this project previously. **"Continuing"** students are those who **have** worked on this project previously. If a student volunteered time on this project, please use section G, below.*

Total number of **new*** K-12 students who worked with you: **none**

Total number of **new** undergraduates who worked with you: **none**

Total number of **new** Masters degree candidates who worked with you: **none**

Total number of **new** Ph.D. candidates who worked with you: **none**

Total number of **continuing**** K-12 students who worked with you: **none**

Total number of **continuing** undergraduates who worked with you: **One**

Total number of **continuing** Masters degree candidates who worked with you: **One**

Total number of **continuing** Ph.D. candidates who worked with you: **none**

Total number of volunteer hours:

160 - Marine Meadows Workshops

*(Note: *__New__ students are those who have not worked on this project previously. **__Continuing__ students are those who have worked on this project previously.)*

In the case of graduate students, please list student names, degree pursued, and thesis or dissertation titles related to this project.

Student Name: **Shannon Jordan**

Degree Sought: **Masters Oceanography**

Thesis or Dissertation Title: **IMPROVING EELGRASS RESTORATION SUCCESS BY MANIPULATING THE SEDIMENT IRON CYCLE**

Date of thesis completion: **April 04, 2024**

H. VOLUNTEER HOURS:

(List the number of hours provided to the project by volunteers, i.e., individuals who were not compensated in any way or for whom involvement is not part of their paid occupation. This could be students or citizens. What was their contribution?)

160 - See above re: the Marine Meadows Workshops

I. PICTORIAL: Please provide high resolution images/photos of personnel at work, in the field or laboratory, equipment being used, field sites, organism(s) of study. Attach images as separate files (do not embed). Include links to websites associated with the research project. Please include proper photo credits and a caption with date, location, names of people, and activity. These images are useful to document your project in future CTSG publications, websites and presentations.

See photos at the end of this report.

J. **HONORS AND AWARDS**: *(List any honors or awards received during the reporting period, for anyone working on the project. This can be for best paper or poster, university awards, etc.*) Specify:

None to date

a) Name of person or group receiving recognition: **na**

b) Name of award or honor: **na**

c) Group or individual bestowing the award or honor: **na**

d) What it was for: **na**

e) Date: **na**

K. **DATA MANAGEMENT PLANS:** Proposals funded in 2014-2016 and later cycles are required to have a data management plan in place. All environmental data and information collected and/or created must be made visible, accessible, and independently understandable to general users, free of charge or at minimal cost, in a timely manner (typically no later than two years after the data are collected or created). This is a reminder that your CTSG funded research data needs to be archived and accessible as outlined in the data management plan you submitted with your proposal. If there have been any modifications, adjustments or new information available regarding the location, timing, type, formatting and metadata standards, content, sharing, stewardship, archiving, accessibility, publication or security of the data produced please elaborate here.

FOR FINAL DEVELOPMENT AND RESEARCH GRANT REPORTS, PLEASE COMPLETE THIS SECTION:

L. PROJECT OUTCOMES AND IMPACTS

RELEVANCE OF PROJECT: *(Describe briefly the issue/problem / identified need(s) that led to this work.)*

Considerable effort has been made to improve water quality in Long Island Sound embayments to a point where many should be suitable for eelgrass restoration. Yet there remains a disconnect between the acreage of habitat that ‘should’ support eelgrass based on water quality criteria and the amount of eelgrass either in existence or which has been successfully restored. This project tested whether sediment geochemistry (high sulfide) was being overlooked as an impediment to eelgrass transplant success, and whether sediments could be amended to lower sulfide and improve restoration success.

RESPONSE: *(Describe briefly what key elements were undertaken to address the issue, problem or need, and who is/are the target audience(s) for the work.)*

The target audience for the work includes scientists and seagrass restoration practitioners. Restoration practitioners (Cornell Cooperative Extension) were partners on the project.

RESULTS: *(Summarize findings and significant achievements in terms of the research and any related education or outreach component; cite benefits, applications, and uses stemming from this project, including those expected in the future. Include qualitative and quantitative results.)*

Results from the work clearly showed that sediments could be amended with iron in a tractable way as part of eelgrass transplants. These amendments would not be cost prohibitive at scale. The amendments lowered porewater sulfide concentrations in-situ long enough for plants to remerge in the spring/summer following fall transplants. However, the work also reinforced the reality that multiple factors can conspire to make eelgrass transplants fail. In challenging transplant years (high temperature, turbidity, or macroalgae), controlling one aspect of sediment geochemistry, which we were successful at doing, does not alleviate other stressors. In good transplant years, it remains unresolved whether lower the sulfide could give plants an edge to improve survivability. Neither of our two transplant years were ‘good’.

Consider the following as they apply to your research and any related outreach/education.

- What new tools, technologies, methods or information services were developed from this work? Have any been adopted / implemented for use and by whom?

  This project developed a tool for implementing iron amendments in-situ that lowers porewater sulfide. It identified the best mineral type and appropriate dosage. The tool can be implemented by restoration practitioners in further tests.

- What are the environmental benefits of this work? Have policies been changed? How has conservation (of ecosystems, habitats or species) been improved?

  Additional implementation under water column conditions more favorable to restoration success will need to happen to fully determine whether, and under what conditions, lowering sulfide using iron would be beneficial for eelgrass restorations. More broadly, sulfide toxicity has negative effects beyond eelgrass. The benefits of lowering sulfide using iron amendments has implications for improving sediment conditions for infauna which can further oxygenate and improve benthic habitat. If the iron amendments indeed accelerate organic matter diagenesis through iron reduction, this pathway could represent a significant source of alkalinity that might offset local acidification.

- What are the social payoffs of this work? Who has benefited from this work? Have attitudes / behaviors of target audience changed? Elaborate. Have policies been changed?

  Social payoffs from this work have yet to be realized. Restoration practitioners will have to be willing to incorporate this tool into more restorations. The outcome of those restorations will dictate whether or not the approach becomes widely applied and results in policy, or 'best practices' changes.

- What are the economic implications / impacts of this work? (Where possible, please quantify.) Have new businesses been created /or existing businesses retained as a result of this research? Have new jobs been created or retained? Are new businesses or jobs anticipated?

  Eelgrass restorations implemented at scale represents an untapped economic potential. Developing / improving methods that improve success can and will drive realization of that potential.

J. Stakeholder Summary (This is an abstract of your research and findings written for a lay audience)

Considerable effort has been made to improve water quality in Long Island Sound embayments to a point where many should be suitable for eelgrass restoration. Yet there remains a disconnect between the acreage of habitat that 'should' support eelgrass based on water quality criteria and the amount of eelgrass either in existence or which has been successfully restored. This project tested whether sediment geochemistry (high sulfide) was being overlooked as an impediment to eelgrass transplant success, and whether sediments could be amended to lower sulfide and improve restoration success. Results from this project clearly showed that sediments could be amended with iron in a tractable way as part of eelgrass transplants. These amendments would not be cost-prohibitive at scale. The iron amendments lowered porewater sulfide concentrations in the field long enough for plants to remerge in the spring/summer following fall transplants. The results were inconclusive however, with regard to gauging if lowered sulfide concentrations translated into more successful eelgrass transplant survival. The work reinforced the reality that multiple factors can conspire to make eelgrass transplants fail. In challenging transplant years (high temperature, turbidity, or macroalgae), controlling one aspect of sediment geochemistry, which we were successful at doing, does not alleviate other stressors. In good transplant years, it remains unresolved whether the lower the sulfide could give plants an edge to improve survivability. Neither of our two transplant years were 'good'. More field implementations coupling eelgrass restoration techniques with iron amendments are needed to determine when and under what conditions iron amendments could improve plant survivability.

Counter-clockwise from upper left: Marine Meadows workshop participants; eelgrass transplant discs; UConn Masters student Shannon Jordan sampling transplant sites for porewater chemistry.

UConn Masters student Shannon Jordan overseeing the iron dosing experiment.

Potential for Improved Eelgrass Restoration Success Through Manipulation of the Sediment Iron Cycle

Shannon Jordan

B.S., University of Connecticut, 2020

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Arts or Science

at the

University of Connecticut

2024

APPROVAL PAGE

Master of Arts or Science Thesis

Potential for Improved Eelgrass Restoration Success Through Manipulation of the Sediment Iron Cycle

Presented by

Shannon Jordan, B.S.

Approved by

Major Advisor: Craig Tobias

Associate Advisor: Jamie Vaudrey

Associate Advisor: Pieter Visscher

University of Connecticut
2024

Table of Contents

Introduction

Seagrasses provide a broad host of ecosystem services to the shallow estuaries and coastal waters of every continent excluding Antarctica (Nordlund et al., 2016; Orth et al., 2006). Well-established meadows support high biodiversity and complex food webs (Borowitzka et al., 2006; Duffy et al., 2005). Seagrass ecosystems enhance secondary production by providing strata for colonization, grazing material, habitable sediments, foraging grounds, and refuge for diverse assemblages of species across life stages (Gillanders, 2006; Unsworth & Cullen-Unsworth, 2014). Through functions both direct and indirect, seagrass ecosystems contribute to the health and success of ecologically and commercially significant fisheries, many of which operate in open water far beyond their perimeters (Unsworth et al., 2018; Waycott et al., 2009). The global reach of seagrass systems is well illustrated by the fact that even deep-sea trenches harbor communities that largely subsist on material exported from seagrass beds (Wolff, 1976, 1979).

Often referred to as 'ecosystem engineers', the structural characteristics of seagrasses serve several unique functions in their surrounding environment. A complex network of roots and rhizomes effectively stabilize sediments from below while flexible, upright blades alter above-ground hydrology sufficiently to dampen water velocity, enhance particle settling and associated water clarity (Ward et al., 1984). The seagrass bed morphology thus resists erosion and facilitates organic carbon burial and sequestration (Duarte et al., 2005; Duarte & Chiscano, 1999). Globally, seagrass meadows bury on the order of 27.4 Tg C annually despite occupying only 0.2 % of the global ocean's area. Organic carbon reserves in seagrass beds are currently estimated at 19.8 Pg C. This important blue carbon repository has the potential to be released following loss of seagrass ecosystems (Fourqurean et al., 2012).

In the last century, dramatic declines in seagrass coverage have been observed in coastal ecosystems worldwide (Orth et al., 2006; Waycott et al., 2009). The global trend of rapidly declining seagrass coverage is attributed to compounded stress from multiple anthropogenic sources including eutrophication, habitat destruction, overfishing, disease, and prolonged thermal stress related to climate change (Duarte, 1995; Duffy et al., 2005; Lotze et al., 2006; Short & Wyllie-Echeverria, 1996). Eelgrass (*Zostera marina*) loss in Long Island Sound, USA (LIS) is a microcosm of the global trend of seagrass decline over the past century (Orth et al., 2006). Current eelgrass coverage in LIS and its adjoining estuaries is less than 15 % of the historical extent (NYS, 2009). Eelgrass decline in this region has largely been attributed to poor water quality, including high nutrient loading and suspended sediment content; these conditions favor nuisance macroalgae and epiphytes on the blades of eelgrass, which are comparatively slow-growing and dependent on high light availability (Thom et al., 2008). While coverage in Connecticut and New York fluctuates on time scales ranging from annual to decadal, small increases in Eastern LIS appear to be offset by larger losses LIS-wide (Tiner, 2012; Vaudrey et al., 2013). Recent surveys suggest an overall decline in eelgrass coverage in the past five years (Bradley & Paton, 2018). Watershed and nutrient management over the past several decades have improved water quality in coastal embayments sufficiently to foster eelgrass repopulation, but recovery has been slow. Given the poor trajectory of reestablishment amidst this progress, there has been a push to increase eelgrass acreage through restoration (NYS, 2009). Current goals aim to double the 2012 eelgrass acreage in LIS by 2035.

The success of eelgrass restoration is mixed. Traditionally, light availability has been a primary criterion for predicting the suitability of a habitat to support eelgrass restoration. However, there are numerous water quality and habitat factors that govern the resilience of existing beds as well as the efficacy of restoring beds (Short et al., 2011). The complexity of interactions among plants, water, and sediment often confounds the key agents of restoration success or failure. Recent water quality improvements in LIS embayments have mitigated stress associated with light limitation, improving the prospect of successful eelgrass restoration efforts in this region. However, sediment geochemistry lags water column responses of improved management by decades (Vaudrey et al., 2010) and thus presents a potential impediment to restoration success at some or many sites. In the highly organic, reducing environment characteristic of sediments which have seen substantial eelgrass die-off, one such factor could be high sediment sulfide levels.

Dissolved sulfide, present as hydrogen sulfide (H_2S) or hydrogen mono-sulfide (HS^-) in the pH range typical of coastal sediment porewater, is a well-studied phytotoxin which severely inhibits mitochondrial function and access to important metal-containing enzymes (Koch et al., 1990; Lamers et al., 2013). Seagrasses exposed to high sediment sulfide demonstrate impairment of photosynthetic activity, growth, reproductive success, and higher mortality (Dooley et al., 2013; Goodman et al., 1995; Holmer, Frederiksen, et al., 2005; Lamers et al., 2013; Mascaro et al., 2009). The negative effects of high sulfide exposure on eelgrass health are particularly pronounced in meristem and young root cells, areas of especially active growth. While tolerance thresholds vary with maturity and species, decreased growth has been demonstrated at concentrations as low as 100µM (Holmer & Bondgaard, 2001). Paradoxically, mature eelgrass beds often host high loads of organic matter and thus sustain elevated levels of porewater sulfide. Mature plants primarily mitigate sulfide stress through transport of oxygen to the root and rhizome, where it is released to create an oxidized microzone (Hasler-Sheetal & Holmer, 2015; Jensen et al., 2005; Pedersen et al., 2004; Smith et al., 1984). Eelgrass’s ability to buffer exposure in this way is proportional to the biomass and leaf area of shoots (Smith et al., 1984). As such, expansion of beds and establishment of young plants may be restricted in sulfidic sediments. Here lies a conundrum whereby areas which once hosted vibrant eelgrass beds may contain sediments whose geochemistry inhibits restoration, even if suitable habitat conditions have been reestablished.

The primary source of sulfide in marine sediments is bacterial sulfate reduction; this process involves the anaerobic oxidation of low molecular weight organics by sulfate, which is reduced to sulfide (Burdige, 2011; Canfield & Farquhar, 2012; Isaksen & Finster, 1996). While sulfate reduction (SR) has a poor thermodynamic yield, SO_4^{2-} concentrations in seawater exceed those of more energetically favorable electron acceptors by orders-of-magnitude (Burdige, 2011; Millero, 2005). As such, the SR pathway tends to dominate anaerobic respiration in marine sediments where organic matter content is high and more thermodynamically efficient electron acceptors are not present in sufficient quantity. Ferric iron (Fe^{3+}) is one such oxidant that occurs in solid and colloidal phases associated with sediment particles. Iron and sulfate reducing bacteria compete for the same low molecular weight organic substrates (Lovley & Phillips, 1987). Iron reduction is typically limited in marine settings by the low availability of solid phase Fe^{3+} relative to the abundant sulfate ion available for SR. Sediments with high iron mineral content, however, preferentially support the reduction of ferric (Fe^{3+}) iron to the ferrous (Fe^{2+}) form, as this reaction yields double the free energy change ($-\Delta G$) of SR (Stumm & Morgan, 1996). This pathway can dominate anaerobic sediment respiration and outcompete sulfate reduction when Fe^{3+} containing minerals are in abundance (Lovley & Phillips, 1986; Roden & Wetzel, 1996).

The Fe^{2+} produced during iron reduction is highly reactive with sulfide sourced from SR. Thus, in sediments which support both iron and sulfate reduction, iron monosulfide (FeS) and/or pyrite (FeS_2) formation is prevalent (Berner, 1984). Due to the high binding affinity and poor solubility of these minerals, their precipitation represents a significant sink for free sulfide ions (Berner, 1982; Canfield, 1989). The presence of ferrous and ferric iron thus represents a control exerted on sulfide production and accumulation in highly organic sediments. Ultimately, these mechanisms can be appropriated for the purpose of decreasing sulfide stress on younger or recently transplanted macrophytes with limited capacity for root O_2 release that relieves sulfide stress on more mature plants.

Iron amendments to sediments can be an effective method of lowering porewater sulfide concentrations and improving plant metrics such as growth and density (Chambers et al., 2001; Holmer, Frederiksen, et al., 2005; Marba et al., 2007). Iron decreases porewater sulfide concentrations through two main mechanisms; decreasing microbial sulfide production from SR and/or through scavenging free sulfide in porewaters. Augmenting iron reduction in marine sediments by providing the rate limiting Fe^{3+} reactant may permit iron reducers to compete with sulfate reducers more effectively for organic substrates. Further, the Fe^{2+} produced during iron reduction would likely scavenge sulfide from porewater and precipitate it as solid phase iron-sulfide minerals. Holmer et al. (2005b) and Chambers et al. (2001) demonstrated the effect of iron additions on lowering free sulfide in sediment and enhancing the growth of mature *Posidonia oceanica* and *Thallasia testidinum* plants. However, this past work was done on a limited scale with liquid injections of iron solutions and would be unfeasible for a larger ecosystem-scale eelgrass restoration. To date there have been no reports of adding iron to sediments specifically for improvement of eelgrass restoration success in a transplant context.

The overall goal of this study was to test whether eelgrass transplant survivorship in highly organic sediments can be improved by augmenting sediment iron (Fe^{3+}) reduction to mitigate sulfide toxicity. Here we evaluate 1) the efficacy of treating sediment with solid phase iron minerals for the purpose of reducing porewater sulfide; and 2) whether the effect of the iron addition improves the survivorship of eelgrass transplants. The work assessed two iron minerals: siderite ($FeCO_3$); and magnetite (Fe_3O_4,). Siderite's 2^+ valence permits binding to existing sulfide. Magnetite's mixed 2^+ and 3^+ valences can bind to existing sulfide and provides oxidized iron substrate for iron reducing bacteria to competing with sulfate reducers. We hypothesized that both magnetite and siderite will be effective at lowering porewater sulfide, and that magnetite will provide a more persistent dampening of sulfide and improved eelgrass transplant success. Experiments were conducted at the laboratory scale, in mesocosms, and *in situ*.

Methods

The scope of work was approached in five phases; I) identify and geochemically characterize sediment habitats that previously supported eelgrass but no longer do; II) conduct short-term laboratory sediment-iron amendment experiments to identify mineral forms and doses effective at lowering porewater sulfide; III) conduct long-duration iron amendments to plant-free sediments in mesocosms to assess the duration of sulfide dampening in porewaters; IV) transplant of eelgrass *in situ* into plots with and without sediment iron amendments; V) monitor post-transplant eelgrass and sediment chemistry.

Study Site

Field characterization of sediments and eelgrass transplants (project phases 1 and 4) was conducted in the Niantic River Estuary (NRE). The NRE is a shallow estuarine embayment with limited freshwater input located in Southeastern CT, USA (41°20’ N, 72°11’ W). Mean depth is 2.6 m and salinity ranges annually between 21 to 27 PSI. Water residence time, influenced by tidal constriction at the mouth of the estuary, ranges between 25 and 27 days (Colin & Kremer, 2002; Marshall, 1960). Historically, eelgrass was widespread in Long Island Sound and shallow coastal areas of the North Atlantic but experienced extensive loss due to wasting disease in the 1930’s. Eelgrass recovered in the decades following this event but in NRE, has seen a significant overall decline since the mid 1970’s with a sharp decline in 2012 (Vaudrey et al., 2019). Currently eelgrass extent in the NRE is less than half of its peak recorded coverage despite improvements in N reduction and improvements in water clarity.

Phase I: Habitat Characterization

The objective of Phase I was to identify eelgrass-free habitats that historically supported eelgrass growth, currently meet water quality standards supportive of eelgrass, and contain sediments spanning a range of porewater sulfide concentration. Using eelgrass monitoring surveys in the Niantic River from 1985 to 2020 (DENC, 2021) and porewater sulfide mapping data generated in 2014 (Plummer et al., 2015), three areas were selected for characterization of porewater sulfide and ferrous iron. These three areas were identified as low (L), medium (M), and high (H) sulfide as inferred by Plummer et al. (2015). Porewater at sites L, M, and H were initially characterized at 10 m intervals along a 100 m transect at each site. Sediments were collected using a single ponar grab at each 10 m interval and subcored to a depth of 10 cm using 60 ml syringe cores. Ten cm depth was selected because it approximated the depth interval over which eelgrass is transplanted. Each subcore was transferred to a 50 ml polyethylene centrifuge tube, sealed without headspace to minimize air exposure, and stored on ice immediately. Two replicate syringe cores were collected from each grab. Tubes were stored on ice for transport. Immediately upon return to the lab, the tubes were centrifuged at 5,000 RPM for 5 min to extract porewater. Porewaters were 0.45 µm (polyethersulfone-PES) filtered for Fe^{2+} and sulfide analysis using the ferrozine and methylene blue methods respectively (Cline, 1969; Stookey, 1970). A more detailed description of methodology can be found in the 'Analytical Methods' section.

Results from the 10 m interval surveys were used to identify zones in the L, M, and H sites with consistently low, medium, or high levels of sulfide large enough to establish transplant areas. Here we define low (LoS) as 0 to 100 μM sulfide, medium (MeS) as 100 to 500 μM sulfide, and high (HiS) as > 500 μM. Sampling transects were repeated at each intended transplant area at a finer scale to match the 5 m x 5 m spatial scale of the transplant treatments (Phase IV). Porewater sulfide and ferrous iron measurements were made using the same sampling and analysis procedures described above. Two sediment porewater samples were retrieved from the north, east, south, and west corners of a 5 m x 5 m square established within the intended transplant areas (Figure 1).

Phase II: Short Duration Iron Amendments

Phase II aimed to quantify the effect of mineral species, grain size, and mass of iron on porewater sulfide concentrations. Highly sulfidic sediments were collected by ponar grab in Bakers Cove, CT adjacent to the University of Connecticut Avery Point campus. Sediments were homogenized and distributed into open-topped 375 cm^2 LDPE containers filled to a depth of 10 cm. Sediment containers were submerged in flow-through seawater tanks at ambient temperature ranging from 20 to 22 °C. Sediments were left undisturbed for a two-week period during which redox gradients and high sulfide content were re-established. Syringe cores (60 ml) were then used to sample the sediment to a depth of 10 cm. Each core was pushed into a 50 mL centrifuge tube in segments between which pre-weighed amendments of different iron minerals were incrementally added. Six alternating additions of sediment and iron were done until the tube was filled to zero headspace. This approach was done rather than stirring iron into the sediment to minimize sulfide oxidation that might arise from oxygen exposure during stirring. Any air pockets were removed during filling by vigorous tapping of the tubes. Once sealed, treatments were re-submerged in the flow-through tank at ambient temperature and left undisturbed for an incubation period of 2 weeks. Tubes for the control treatment were prepared identically to the iron amended sediments except no iron was added. The form and amount of iron amendment treatments were as follows: Fe_3O_4, $FeCO_3$ (coarse), $FeCO_3$ (fine), ½ Fe_3O_4 + ½ $FeCO_3$ (coarse), ½ Fe_3O_4 + ½ $FeCO_3$ (fine) at masses of 0.20 g, 0.50 g, 1.0 g, 2.0 g, 2.5 g, 5.0 g, 10.0 g, and 20.0 g per 50 mL centrifuge tube (Figure 2). These masses were added across two separate trials, with phase A including 0.20 to 2.0 g additions and phase B including additions of 2.5 g and greater. For standardization, these loadings are hereafter reported as g-Fe cm^{-3} sediment. The coarse and fine siderite ($FeCO_3$) treatments were differentiated as < 2 mm (fine) or < 2.8 mm (coarse). Magnetite (Fe_3O_4) grain size was < 0.149 mm mesh. Siderite and magnetite were sourced from Sidco Inc. and chemicalstore.com respectively. Porewater pH and sulfide were measured in sediments collected from the LDPE containers during preparation of the amendments (T_0) and on all amended and control tubes of sediment following two-week incubations. For sampling, porewater was separated via centrifugation and processed for sulfide analysis as described later in the *Analytical Methods - Porewater* section. Porewater pH was measured using an Orion Star 3 pH meter. Porewater sulfide results were separated by trial for statistical analysis (one and two-way ANOVA) due to significant differences in mean control concentrations across trials and high inter-trial variability, which prohibited normalization.

Phase III: Tank Mesocosm Experiment

Phase 3 aimed to evaluate how long different iron mineral treatments of similar mass affect porewater sulfide concentrations under simulated transplant conditions. Three 1.3 m x 0.7 m x 0.5 m flow-through mesocosms were filled to a depth of 15 cm with high-sulfide homogenized sediments collected from Baker Cove. Flowing seawater was gently introduced to the tank to minimize resuspension. The final water depth was 20 cm above the sediment surface and maintained at a constant height by a central drainage standpipe. Tanks were covered with shade-cloth and allowed to stabilize for two weeks, with flowing seawater. After two weeks, treatments and control plots were established within the tanks. Each mesocosm included one control plot (Figure 3). The three iron treatment levels tested consisted of $FeCO_3$, Fe_3O_4, and $FeCO_3$ + Fe_3O_4. Triplicates of each of the three treatment types were randomly assigned by number to tanks #1-3 (Figure 3). All minerals were fine grained. Each iron addition was made to a 490 cm^2 area within a mesocosm to a depth of 10 cm, treating a sediment volume of 5067 cm^3. The method of addition mimicked eelgrass transplant procedures. To make the iron additions, a 25 cm diameter by 20 cm tall ring was placed into the sediment. Iron treatments received 82.4 grams of bulk iron mineral (rather than iron equivalents), which was added into the confines of the ring and hand-mixed into the submerged sediment. The ring was left for several minutes to allow suspended sediments to settle in the treatment zone, and then the ring was removed. The mass of iron added (82.4 g) was determined based on the sediment of the treatment zone and results from Phase 2 experiments.

The no-iron control treatments underwent ring placement and sediment manipulation, as done for the iron treatments, but no mineral amendment was made to these sediments. Prior to initiation of any of the treatments, background (T_0) sediment sampling was conducted. For T_0 sampling in each plot, nine syringe cores of sediment were collected from each treatment area along a predetermined sampling axis bisecting the treatment area (Figure 3a). Each subsequent sampling time point occurred along its own sampling axis. Sediment samples were transferred to 50 mL centrifuge tubes, sealed with no headspace, and immediately transported to the laboratory for porewater extraction and preservation for sulfide analysis. Iron mineral additions followed completion of T_0 sample collection. Time series sediment samples were collected from each treatment at 26-days, 101-days, and 169-days post iron addition along a time-specific sampling axis (Figure 3b). At each time point, eight ‘inner’ samples were retrieved with a syringe core from within each treatment zone and a single ‘outer’ sample was collected 4 cm outside of the perimeter of each treatment zone. Additionally, three background samples were collected from untreated, unmixed sediments throughout the tanks at each time point. Cavities left by the collection of each sample at each time point were plugged by 50 mL centrifuge tubes filled with seawater in an effort to retain the redox profile and structural integrity of sediment within plots. As was done with T_0 samples, sediments collected by syringe core at each time point were transferred to 50 mL centrifuge tubes, sealed without headspace, and centrifuged immediately (5000 rpm, 5 min). Porewaters were then extracted, 0.45 μm PES filtered, preserved with 5 % $ZnCl_2$ and refrigerated for sulfide analysis. Porewater sulfide concentrations underwent statistical analyses (one-way ANOVA) for differences between iron species and timepoint.

Phase IV: Eelgrass transplants with in situ iron amendments

Phase 4 combined the iron amendment approach used in the Phase 3 mesocosm studies with transplanting adult eelgrass in the NRE. Transplants for Phase 4 occurred November 3-7, 2022, and again repeated in October 2023. The transplant approach in Phase 4 consisted of transplanting adult eelgrass collected from a donor bed into three plots in the NRE corresponding to low (LoS), medium (MeS) and high (HiS) sulfide. Each station's plot contained four subplots, three of which received iron amendment at the time of transplanting and one control subplot (NoFe) which received transplants with no iron addition (Figure 4). Each of the three iron-treatment subplots were amended with one of three different masses of iron. The low iron treatment (LFe) was amended with an amount of iron equivalent to 15 % of the mesocosm additions in Phase 3 (12.4 g per transplant unit). The medium (MFe) and high (HFe) iron amendments were equivalent to (84.2 g per transplant unit), and 5x times (412 g per transplant unit) the mesocosm iron load, respectively (Figure 4). Iron additions and transplants occurred at the same time and were conducted by Cornell Cooperative Extension of Suffolk County. Twenty-five transplant units were placed in each subplot (Figure 4). Each transplant unit consisted of 10 plants woven into a 25 cm diameter burlap disc. For the full transplant design each site (HiS, LoS, MeS) had 100 transplant discs, for a total of 1,000 plants at each station. Each of the transplant units was prepared within 72 hours of transplanting.

In the field, similar to the mesocosm additions, a 25 cm diameter ring was pushed into the sediment for each transplant unit. A pre-weighed amount of iron was then poured into the sediment and hand-mixed within the ring before pushing the transplant unit into the treated sediment. Control plantings used the ring and hand-mixed the sediment but did not add iron. Transplants were left undisturbed over the course of the winter season.

Phase V – Post transplant monitoring

The objectives of post-transplant monitoring were to assess the iron effect on sulfide after six months in the field, and to qualitatively evaluate the relative iron effect on plant overwintering and reemergence. Phase 5 was executed from April to July 2023 for the 2022 transplant effort and in June 2024 for the 2023 transplant effort. Plant survival was assessed in July 2023 using qualitative metrics consistent with the Unified Water Study (Save the Sound, 2017). Plant survival was assessed quantitatively in June 2024 using video to enumerate the number of eelgrass blades visible in standardized timed transects.

For the assessment of iron effect, initial screening of porewater samples from the low iron load subplots indicated little change in sulfide, so efforts were concentrated on sampling the NoFe, MFe, and HFe subplots at each of the sulfide sites (HiS, MeS, LoS). Porewater was collected by diver using 60 ml syringe cores. At each subplot, cores were collected from within iron treatment zones under the transplant disc ('inner'), and from adjacent, untreated sediment ('outer') at each site. Syringe cores were inserted into the treatment zone at a 45° angle to minimize disturbance of burlap discs during collection of 'inner' samples. Full syringe cores were capped until divers surfaced with 8 replicates from each subplot (4 inside the treatment area inner and 4 outside the treatment area). Sediments were immediately transferred to 50 mL centrifuge tubes in the field, without headspace and stored on ice. Upon return from the field, samples were immediately centrifuged and porewaters were analyzed for sulfide, Fe^{2+} and pH as described in the 'Analytical Methods' section. Sediment sampling occurred between late May and early August of 2023.

Plant re-emergence was evaluated by UConn divers in May and June of 2023. At this time, plots were located, and preliminary assessment of transplant survival was made. Relative plant abundance (high, medium, low) was determined at each site by subplot. These assessments were made independently by two divers. By late June, transplants across all stations (HiS, LoS, MeS) and treatments (NoFe, LFe, MFe, HFe) experienced total mortality. This loss reflected a region-wide poor eelgrass year brought on by higher than usual water temperatures and higher than normal turbidity. As a result, collection of plant samples for quantitative growth and biomass measurements, and quantitative video surveys were unsuccessful.

Analytical Methods – Porewater

Porewaters were isolated from sediments packed into centrifuge tubes with no headspace via centrifugation (5000 rpm, 5 minutes). Ferrous iron and H_2S concentrations were measured using modified methods of Cline (1969) and Stookey (1970) respectively. All porewaters were 0.2 μm PES syringe-filtered prior to analysis. For Fe^{2+} analysis, 0.9 ml of filtered porewater was transferred into a microfuge tube containing 100 μL of ferrozine reagent as described by Stookey. Absorbance of the reacted sample was measured spectrophotometrically at a wavelength of 562 nm within 6 hours following reaction with ferrozine reagent. Absorbances were converted to Fe^{2+} concentrations using a calibration curve (prepared in oxygen free DIW) using ferrous ammonium sulfate standards ranging between 0 and 50 μM. For sulfide analysis, 1 ml of porewater was 0.2 μm filtered immediately following centrifugation into microfuge tube containing 100 μL of 5 % $ZnCl_2$ solution to fix the sulfide present in the sample as ZnS. Sulfide analysis on these fixed samples was performed within 60 days using a modification of the methylene blue method (Cline, 1969; Kiel Reese et al., 2011). The methylene blue reaction was conducted by adding one of three concentration-dependent diamine reagents directly to the microfuge tube to ensure full reaction of any ZnS adhered to the tube walls during storage. To determine the proper diamine reagent concentration and sample: reagent ratio, split samples were screened for approximate concentrations prior to full analysis. Based on the results of the screening, the duplicate expected low concentration samples were reacted undiluted with the appropriate low concentration diamine reagent without dilution and absorbance measured at 760nm to determine sulfide concentration. Duplicate samples with expected high concentration (based on the screening) were reacted with the high concentration diamine reagent. Very high concentration samples were reacted with the high concentration diamine reagent for 25 minutes and then transferred into 12 ml oxygen-free exetainers containing pre-weighed volumes of N_2-sparged ASTM Type I water (obtained from a Milli-Q Ultrapure Water System) for dilution. The concentrations of diluted samples were corrected based on the ratio of the reacted sample volume and volume of water in the exetainer. All samples, standards, and matrix spikes of concentrations above 40 µM required dilution and were treated in this manner. For each analytical run, 5 matrix spikes were preformed, and one analytical duplicate was run every 10 samples at minimum.

Results

Phase I – Site characterization

Three sites within the NRE were selected for transplants based on their historical support (but current lack) of eelgrass and porewater sulfide concentration; also taken into consideration were safety of dive operations and the absence of shellfishing designations. Sulfide concentrations from eight cores collected over 25 m^2 at each site, were averaged from 0-10 cm of sediment depth. At LoS, average porewater sulfide concentration was below detection (2.5 µM). MeS and HiS had average ± standard deviation sulfide concentrations of 147 ± 70 µM and 2133 ± 657 µM, respectively (Table 1). Intersite differences in sulfide concentration exceeded intrasite variance by more than one order of magnitude.

Phase II – Short term iron species and dose effects on sulfide and pH

The no-iron control treatment for Phase 1 showed sulfide loss in both the high and low iron loading trials, likely due to some oxidation during tube loading. Regardless, the iron additions resulted in lower sulfide concentrations relative to controls for all mineral types, grain sizes, and loadings (Figure 5a, c). In Trial A, which included the lowest loading masses, lower final sulfide concentrations were measured in magnetite relative to siderite ($P < 0.001$, Holm-Sidak method), and with fine grained siderite vs. coarse grained siderite ($P < 0.001$, Holm-Sidak method). In Trial B, the higher loadings caused near complete removal of sulfide relative to starting values; however, there was not a statistically significant loading effect on sulfide reduction across these higher mass loadings ($p=0.234$, two-way ANOVA).

The largest reduction in sulfide occurred using magnetite relative to siderite, resulting in complete removal over the two-week incubation period (Figure 5a, c). Magnetite treatments consistently dropped sulfide to levels that ranged from < 2.5 µM (undetectable) to 40 ± 20 µM, depending on the magnetite load (phase II results are reported as mean ± standard error). Final sulfide concentrations in control treatments were 771 ± 264 µM and 348 ± 108 µM for the low and high range iron loading experiments respectively. For magnetite treatments over 0.01 g cm^{-3} of iron in sediment, a complete loss of sulfide was observed in porewater. For magnetite loadings below 0.01 g cm^{-3}, sulfide measured at the end of the experiment was between 2 to 5 % of that in the control.

Siderite was also effective at reducing concentrations of sulfide but showed a dependence on grain size and load. The coarse- and fine-grained siderite treatments showed similar effects on sulfide at loadings above 0.05 g cm^{-3}. T_{final} sulfide concentrations for siderite added at the higher loads (0.05 to 0.04 g cm^{-3}) was 0 to 27.5 % of the T_{final} controls for coarse siderite and 3.5 to 13.7 % for fine siderite. Coarse siderite's ability to decrease sulfide at lower additions was loading dependent and variable. Coarse grained siderite at the three lowest loadings (0.004 to 0.02 g cm^{-3}) decreased porewater sulfide to 32.7 to 46.5 % of the no-iron control concentration but showed a rapid drop to 5.1 % at the 0.04 g cm^{-3} loading. In the high loading experiment (0.05 to 0.4 g cm^{-3}), porewater sulfide in coarse siderite treatments dropped 72.5 to 100 % relative to the control. Fine siderite maintained effective removal across all loadings. Below 0.05 g cm^{-3}, the fine-grained siderite was more than twice as effective at lowering the sulfide than the coarse-grained size fraction. At the lowest loading (0.004 g cm^{-3}), fine siderite still decreased porewater sulfide by 85 % compared to the control. High loads of either mineral (above 0.1 g cm^{-3}) completely removed sulfide, but siderite had less of an effect at lower loads (below 0.05 g cm^{-3}) than magnetite.

The 1:1 mixture of magnetite to siderite lowered porewater sulfide concentrations by amounts similar to the magnetite-only treatment when the loading was above 0.04 g cm^{-3}. Below loadings of 0.04 g cm^{-3}, the siderite to magnetite mixture resulted in no additional drop in porewater sulfide relative to the fine siderite alone.

For pH, the no-iron control treatments showed little change in porewater pH during the incubation (Figure 5b, d). The ending pH values were 6.66 ± 0.05 and 6.38 ± 0.03 for the high and low load experiments respectively. Similarly, fine siderite additions shifted pH by 0.15 pH units or less across all loadings. The magnetite treatments raised the pH at all levels of loading, but there was no clear relationship between the pH change and the mass of added magnetite. For the highest load (0.4 g cm^{-3}), the average pH increased by 0.47 units (Figure 5 b, d). Smaller increases were measured at magnetite loads below 0.05 g cm^{-3}. The siderite to magnetite mixture showed pH effects in between the siderite only and magnetite only treatments of similar load.

Small pH increases of 0.21 to 0.27 were measured at the lowest loadings (0.004 to 0.04 g cm^{-3}) and increases between 0.34 to 0.51 units were seen at the higher loadings (0.05 to 0.4 g cm^{-3}).

Phase III – Long term magnetite effects on sulfide in mesocosms

Over the 169-day mesocosm experiment, porewater sulfide concentrations increased in sediments that did not receive iron amendments (Figure 6a, b). Starting concentrations in the no-iron control and background sediments outside the treatment rings ranged from 702 ± 66 to 891 ± 104.7 µM (phase III results are reported as mean ± standard error). Sulfide concentrations in control and unmixed background sediments increased approximately linearly to day 101 peaking at 2149 ± 203 and 1750 ± 361 µM, respectively. Sulfide concentrations in these groups at day 169 were 2-fold higher than starting values (Figure 6a); statistically significant differences were seen in background plots ($P = 0.008$) as well as controls ($P = <0.001$, Kruskal Wallace One-Way ANOVA) between timepoints.

Within iron treatment zones there was a differential response in sulfide concentration with respect to magnitude and duration as a function of mineral type (Figure 6a). In addition to suppressing sulfide to lower concentrations, magnetite showed a longer duration of effect than siderite alone and the mixture of magnetite and siderite (Figure 6a). Complete loss of measurable sulfide was observed in magnetite treated sediments within the first 26 days. The initial average sulfide concentration of 896 ± 195 µM dropped below detection despite rising concentrations in sediments not treated with iron. Sulfide suppression in magnetite treatments was sustained through the remainder of the experiment, with average concentrations measured at 72 ± 60 µM by day 101 and 24 ± 11 µM by day 169 (Figure 6a); the mean sulfide concentration for magnetite remained significantly different from control and background samples by day 169 (P <

0.001). All post-addition means for this treatment were found to differ significantly from the T_0 mean ($P < 0.001$, Kruskal Wallace one-way ANOVA on ranks).

The sulfide scavenging capacity of siderite was less effective and less persistent over time than that of magnetite. The siderite effect on sulfide was realized only at the 26-day timepoint. The average sulfide concentration of siderite-only treatments dropped by 1/3, from an initial average of 377 ± 85 µM prior to amendment to 245 ± 85 µM at 26 days post treatment (Figure 6a). Though removal was not as pronounced as that seen with magnetite, siderite additions still appear to have offset increases observed in untreated sediments during this initial period in the experiment. However, by day 101 following amendment, sulfide concentrations within siderite treatments averaged 2128 ± 164 µM, comparable to controls (2149 ± 203 µM) and exceeding sediments sampled beyond the perimeter of treatment zones (1750 ± 361 µM) (Figure 6a). Thus, after 101 days the effect of siderite on sulfide was negligible and no additional reduction of sulfide was seen from day 101 to 169. There was no significant difference between the 101 and 169 day means for siderite compared to that of the control and background groups ($P = 1.000$, Kruskal Wallace one-way ANOVA on ranks).

The effect of the magnetite + siderite mixture on sulfide concentrations was most similar to the magnetite only treatment. Mean sulfide concentrations dropped from a T_0 concentration of 647.2 ± 98 µM to 4 ± 2 µM at 26 days post-amendment (Figure 6a). As with the magnetite only treatments, the effect of Fe_3O_4 + $FeCO_3$ amendment persisted despite increasing sulfide concentrations in background and control sediments; by day 169, significant differences remained between the mean sulfide concentration of this treatment and background ($P = 0.007$) as well as control groups ($P < 0.001$). All post-addition means for this treatment were found to differ significantly from the T_0 mean ($P < 0.001$, Kruskal Wallace one-way ANOVA on ranks).

Average sulfide concentrations were 113 ± 48 µM and 137 ± 41 µM for days 101 and 169 respectively compared to 72 ± 60 and 24 ± 11 µM for the magnetite only treatment (Figure 6a). Overall, both magnetite-containing treatments caused more than a 100-fold drop in porewater sulfide concentrations that was persistent for at least 169 days.

Samples taken 3 cm beyond the perimeter of treatment zones (outer samples) revealed that the effect of iron addition on porewater sulfide concentrations is localized. Across timepoints, outer samples were comparable to background and inner-amendment zone control samples (Figure 6b). While outer samples from magnetite containing treatments were higher in sulfide concentration than their inner-plot counterparts, they were consistently > 30 % lower than untreated background samples taken at the same timepoints. The magnitude of this difference varied by timepoint, being most pronounced at the 169-day timepoint; here, outer magnetite samples were 63 % lower than background samples, while control samples were 36 % lower (Figure 6b). Siderite only treatments across timepoints indicated no iron effect in outer samples.

Phase IV & V – 2022/2023 Transplants

The results of first post-transplant monitoring (transplanted fall 2022, and sampled summer 2023) showed variation between sites, treatments, and zone of sampling (inner versus outer transplant zone) for all porewater geochemical parameters (H_2S, Fe^{2+}, and pH). The ‘outer’ samples provided baseline measurements of these parameters unaffected by the transplanting. The ‘inner’ NoFe controls relative to the ‘outer’ samples evaluated the effect of the transplant activity alone in the absence of iron additions. The ‘inner’ iron treatments (MFe and HFe) relative to the ‘outer’ samples evaluated the effect of transplanting and iron. The ‘inner’ iron treatments (MFe and HFe) relative to the ‘inner’ NoFe control samples evaluated the effect of iron alone. Since porewater could not be recovered from centrifugation of sandy sediments at the

Low sulfide (LoS) site, geochemical comparisons here are restricted to the High (HiS) and Medium (MeS) sites (Figure 4).

Sulfide concentrations between inner and outer NoFe control samples showed no effect from transplanting alone on sulfide at the MeS site but did reveal 40 % lower sulfide in the inner NoFe control zones relative to the outer samples at the HiS site (Figure 7a). Controls at HiS displayed porewater sulfide concentrations that were 4-6 times higher than those at MeS, with mean concentrations of 1699 ± 347 µM and 2485 ± 228 µM inside and outside the treatment areas respectively (phase IV and V results are reported as mean ± standard error).

The iron effect of sulfide was mixed between the MeS and HiS sites. Relative to the inner NoFe controls the medium level iron addition (MFe) did not lower sulfide concentrations at the MeS site but did at the HiS site. Relative to the inner NoFe controls, the highest iron loading (HFe) lowered sulfide at the MeS and HiS sites. The sulfide decrease caused by the highest iron loading at MeS however was coincident with similarly lower sulfide concentrations measured in the outer samples adjacent to the HFe zone. Mean sulfide concentrations in the HFe treatment zone at MeS (149 ± 90) were 66 % lower than NoFe, 70 % lower than MFe treatments, and were less spatially variable than both. Sulfide concentrations adjacent to (outside) the HFe treatment area, however, were similarly low compared to NoFe or MFe treatments.

At the HiS site where sulfide concentrations were up to 2000uM higher than MeS, both the MFe and HFe treatments were clearly associated with lower sulfide concentrations relative to the control 'inner' NoFe and the outer undisturbed sediments. Within the MFe treatment at HiS, inner and outer samples had means of 1135 ± 238 µM and 2041 ± 240 µM, respectively (Figure 7a). While both inner and outer samples in this treatment were lower than the site's outer control

(2485 µM), the magnitude of this difference was threefold lower within the iron amendment zone than in adjacent sediments.

The HFe loading had a pronounced effect on sulfide concentrations at HiS. Treatment zone sulfide concentrations (HiS, HFe) averaged 611 ± 247 µM, compared to 1957 ± 250 µM measured outside transplant zones in the same plot (Figure 7a). Sulfide concentrations within HFe treatment zones at HiS were 64 % lower than those measured within the site's NoFe controls. These differences represent a ~900 µM and 1300 µM drop in sulfide induced by the MFe and HFe treatments respectively at the HiS site. In summary, the greatest reduction of sulfide in the field measured after six months (69 % relative to adjacent sediments) was realized at HiS with a magnetite loading of 412 g magnetite per treatment zone (0.08 g cm^{-3} iron in sediment).The observed effect of magnetite amendment on porewater pH in the field was similar to that observed in laboratory experiments, with higher pH coincident with high iron load despite smaller overall magnitudes of change. The pH of porewaters from no-iron controls at MeS averaged 7.27 ± 0.03 within transplant zones and 7.34 ± 0.04 in adjacent sediments. Lower pH values were seen in unamended sediments at HiS, where inner and outer samples averaged 7.14 ± 0.01 and 7.09 ± 0.04, respectively. Lower dose magnetite amendments, LFe and MFe loadings (up to 84.2 g Fe per treatment zone), had little effect on pH at both the MeS and HiS (Figure 7b).

The greatest differences in the porewater pH of treatments relative to controls was observed at the highest magnetite loading (412 g per transplant zone). At MeS, the mean pH of inner and outer samples was 7.47 ± 0.06 and 7.29 ± 0.03 respectively (Figure 7b). Porewater pH within the transplant zone was 0.20 units higher in the treatment than the controls despite comparable outer values, indicating the influence of magnetite. At HiS, the pH of outer-transplant samples (7.25 ± 0.03) was 0.16 units higher than those at the site's control plot (7.09 ±

0.04), indicating that some of the increases observed in the treatment's inner-transplant average relative to the control might not be attributable to magnetite. Regardless, the plot's inner sample average of 7.69 ± 0.13 remained high relative to the 7.14 ± 0.01 observed within the transplant zone of controls (Figure 7b).

Dissolved Fe^{2+} did not consistently correspond to levels of magnetite addition. Dissolved Fe^{2+} concentrations in unamended sediments at MeS were generally higher than those at HiS, where Fe^{2+} averaged 5.09 ± 2 µM within control transplant zones and 6.57 ± 2 µM in adjacent sediments. Comparatively, inner control samples at the HiS averaged 2.84 ± 0.5 µM, while outer control samples were below detection (< 2.0 µM Fe^{2+}).

There was no clear effect of iron additions raising the porewater Fe^{2+} concentrations. Only the highest iron addition at HiS increased Fe^{2+}, with concentrations inside the treatment zone averaging 5 µM compared to 2 µM outside (Figure 7c). This difference, as well as higher Fe concentrations outside the treatment zones relative to inside the treatment zones measured at MeS, were all within the range of Fe^{2+}measured at these sites during site characterization (Figure 7c; Table 1).

Qualitative plant surveys conducted by divers in spring/summer 2023 following the fall 2022 transplants revealed better plant re-emergence associated with higher iron treatments. The highest scores for plant survival occurred with the highest iron loading across all three sites (Figure 8). The greatest difference in plant rankings (i.e., over-wintering success) between HFe loading and controls was seen at HiS (Figure 8). Before biomass could be harvested and growth measured in summer 2023, there was 100 % mortality of all grass at all sites and treatments, as well as extensive mortality in existing beds in the Niantic. We attribute this loss to anomalously early onset high water temperature, high turbidity from record rainfall, and decreased incident irradiance (< 90 % of the mean 2018-2022 June light levels) caused by the Canadian wildfire plumes in the early summer. These factors coincided with a critical growth period.

The results of second post-transplant monitoring (transplanted fall 2023, and sampled summer 2024) revealed widespread mortality of plants due to extensive macroalgal growth at all sites. Only the medium sulfide and low sulfide sites had plants that survived through the spring 2024, and were thus the only sites sampled for porewater geochemistry and plant survival. The highest iron addition at the medium sulfide site dropped the porewater sulfide concentration by half (Fig. 9A, T-test, p=0.07), and raised the porewater pH from 7.05 to 7.21 relative to the untreated sediment (Fig 9C, T-test, p=0.03). Porewater sulfide was below detection in all iron loading treatments at the low sulfide site. There was no difference in porewater ferrous iron between the highest iron loading and no iron loading at the medium and low sulfide sites (Fig. 9B).

Video transects of plant survival in summer 2024 from the fall 2023 transplant showed extensive macroalgal coverage at all sites, particularly the high sulfide site where no plants survived. Very low plant numbers ranging from 2.9 to 3.6 blades per transect were measured at the low and medium sulfide sites (Figure 10). The low iron amendment at the medium sulfide site had the highest blade survival per transect (3.6). It was significantly higher than the no iron amendment treatment at that site (T-test, p=0.03). Blades per transect were not different among any of the other iron / no-iron amendments at the medium sulfide site (ANOVA, p=0.22), nor at the low sulfide site (ANOVA, p=0.55; Figure 10).

Discussion

Results from benchtop, mesocosm, and field scale experiments support the conclusions that: 1) solid phase iron mineral amendments are effective at lowering sulfide concentrations in sediment porewaters; 2) the efficiency and longevity of this removal is influenced by iron species, grain size and loading; and 3) this effect can persist through the *Zostera marina* growing season. While iron mineral addition effectively reduced high porewater sulfide concentrations in controlled settings as well as in the field, it remains unclear whether this result translates to improved eelgrass restoration success.

Comparison of efficacy between this study and preexisting publications is obscured by several factors that differ among studies; these include ambient sulfide concentration of study sites, duration of monitoring, number and type of iron additions made, as well as frequency in the case of repetitive additions. These factors and others calculated from them have been standardized and presented in Table 2 to provide context for discussion.

1.) Solid phase iron mineral amendments are effective at lowering sulfide concentrations in sediment porewaters.

Decreases in sulfide concentration did not respond linearly to loading in this study, but Phase II experiments identified fine-grained magnetite as an effective means of scavenging sulfide at loadings as low as 0.05 g cm^{-3} Fe (Figure 5a, c). Phase III mesocosm experiments further demonstrated that 0.02 g cm^{-3} Fe of the same material was sufficient to bring sulfide concentrations below detection within the volume of sediment which typically surrounds transplanted eelgrass (Figure 6a).

The rapid lowering of sulfide observed in benchtop and mesocosm Fe additions (Figure 5, 6) mimics results of other studies where sediment iron additions have resulted in removal of porewater sulfide (Holmer, Duarte, et al., 2005; Marba et al., 2007; Ruiz-Halpern et al., 2008). In some of those studies, Fe^{3+} chelate (Fe-EDDTA) or Fe^{2+} (in the form of $FeCl_2$) solution was injected directly into sediments with sulfide concentrations lower, higher, and comparable to those used in this study (Table 2). These injections contained iron equivalents of 4.6 to 48 mol m^{-3} total Fe per sediment, significantly less than the mineral addition used in field amendments here (211 and 1,054 mol m^{-3} Fe). Solid iron granules (298 mol Fe m^{-3}) were used by Chambers et al. (2001), a mass slightly higher than our MFe treatments and 3.5-fold lower than our HiFe treatments in the field. Porewater sulfide concentrations were not monitored following addition in that study and thus cannot be compared directly to our results; however, Chambers et al. (2001) noted that sulfide was not detectable by scent in treated sediments and documented a shift in d34S of plant tissues suggestive of lowered sulfide in the rootzone. Additional evidence for sulfide removal was presented as differences between chromium reducible sulfur (assumed to be pyrite) content between iron amended and control plots (Table 2). The efficiency of porewater sulfide removal in response to iron mineral amendment in this study ranged from complete removal in closed centrifuge and flowthrough mesocosm experiments (2 weeks and 6 months post-amendment, respectively) to 64 % removal within in-situ field experiments (8 months post-amendment). Despite varied approaches across experiments, these results are consistent with the broad range of outcomes achieved in previous studies employing a variety of methods involving liquid and solid phase amendments (Chambers et al., 2001; Holmer, Duarte, et al., 2005; Marba et al., 2007; Ruiz-Halpern et al., 2008).

In this field study, fine grained magnetite mineral added to OM rich sediments at 211 and 1,054 mol m^{-3} Fe per sediment decreased sulfide concentrations by 33 % and 64 % respectively compared to a 40 % reduction in sulfide measured by Marba et al. (2007) and an 86 % reduction measured by Ruiz-Halpern et al. (2008). While sulfide levels were reduced below detection by treatment in Holmer et al. (2005), the extremely low initial concentration of 13 µM renders comparison with our results largely uninformative. Overall, the solid phase amendments made here were similar at lowering porewater sulfide concentrations when compared to liquid injections, though liquid injections used significantly less iron. The 20x more Fe used here reduced average sulfide concentrations by 1,346 µM compared to the maximum loss of 1,422 µM measured following 15 rounds of liquid Fe injection (Ruiz-Halpern et al., 2008). While the amount of iron required for successful mineral amendment was high relative to liquid amendment, a single dose of the mineral phase produced a scavenging effect within the range of the higher frequency injections (Table 2), and similar to other experiments using a single dose of solid Fe (Chambers et al. 2001). Although higher Fe loads were used in this study, at most, the additions doubled the background iron content of the sediment. Iron content characteristic of sediments within the shoreline to the continental shelf range between 2.55 and 4.03 % by weight, compared to a global range of 0 to 10 % (Pasquier et al., 2022; Thamdrup, 2000). Local Long Island Sound sediments have typical Fe content of 3.1 % (Hardisty et al., 2018). The highest iron load used in the transplant phase in this study added another 3.9 % Fe to the sediments.

It is unsurprising that studies employing liquid injection methodology display greater sulfide scavenging than solid phase additions despite the presence of less excess iron. Interaction between solid phase iron and porewater sulfide is more limited by particle size and mixing than iron supplied to the sediments via liquid injection, and the scavenging potential of each solid granule is restricted to its surface area (Graham et al., 2020). The mineral addition approach, however, is more practical in a restoration context, where treatment areas are significantly larger in scale and injection by divers is unfeasible. The results presented here indicate that relative to liquid injections, a significantly greater mass of iron is required to successfully influence high porewater sulfide concentrations through solid phase amendment; however, the latter method of iron amendment is effective and demonstrates promise for larger scale sediment remediation efforts.

2.) The efficiency and longevity of this removal is influenced by iron species, grain size and loading.

The Fe(II,III)-bearing minerals reduced sulfide in porewater more efficiently at lower loadings than minerals containing Fe(II) alone. Magnetite and combined magnetite-siderite treatments were more effective and more prolonged in suppression of sulfide relative to siderite alone in benchtop and mesocosm experiments (Figure 6a). This outcome is attributed to the mixed valence states of iron present in magnetite, which would support a rerouting of organic matter respiration away from sulfate reduction and towards iron reduction (Nielsen et al., 2008; Sun et al., 2020). Enhancement of iron reduction in the magnetite treatments is further supported by the pH increase observed in benchtop and field sediments following amendment (Figure 5b, d, 7b). Iron reduction raises pH more than any other organic matter respiration pathway, exceeding that of sulfate reduction by ~14-fold (Soetaert et al., 2007). However, the methodology employed in this study was insufficient to determine whether organic matter reduction was rerouted through microbial iron reduction pathways, or whether sulfide produced from SR chemically reduced Fe^{3+} (Hansel et al., 2015). Sulfidization, or chemical Fe(III) reduction by sulfide, is theoretically only thermodynamically favorable to biologically mediated reduction under conditions more alkaline than those typical of organic rich coastal marine sediments (Flynn et al. 2014). In practice, however, factors other than free energy yield (i.e. crystalline structure and bioavailability) have been observed to influence the actual favorability of this process (Hansel et al., 2015). Thus, the possibility of sulfidization in iron-treated sediments here demands consideration.

The elevated pH measured at the highest magnetite addition in the lab and field (Figure 5b,d; Figure 7b) also suggested little reoxidation of Fe(II) and/or reduced sulfur, which would present as a decrease in pH (Boudreau & Canfield, 1993; Moses & Herman, 1991). Re-oxidation of Fe^{2+} formed during iron reduction may have been further limited by complexation with organic ligands, which are abundant in nearshore sediments (Burdige, 2011; Nielsen et al., 2008). This result indicates that augmentation of the biological iron cycle and/or chemical oxidation of iron by sulfides facilitates the formation of FeS mineral phases which are persistent and stable at least on the timescale of the *Z. marina* growing season (Blok et al., 2018; Jørgensen et al., 2019).

While the superior efficiency of magnetite relative to siderite can be explained mechanistically, it may also be confounded by the effects of both differential iron equivalency as well as grain size. Magnetite (Fe_3O_4) is composed of roughly 70 % iron compared to siderite's ($FeCO_3$) 50 % composition; this difference, however, appears insufficient to explain the magnitude of difference observed between scavenging performances, particularly in mesocosm experiments (Figure 6). Siderite's ability to reduce porewater sulfide concentrations was markedly influenced by grain size (Figure 5a, c). Almost half of the initial sulfide removal effect from siderite was lost with an increase in siderite grain size from size 10 to size 7 mesh (representing particles < 2 mm and > 2.8 mm, respectively). It follows that the same grain size effect would have been observed in magnetite additions (grain size < 0 < 0.15 mm) had variable size classes been compared. Variable size fractions of magnetite were not commercially available for comparison of magnetite grain size on sulfide removal. Finer grain sizes pose a challenge with respect to handling in the field and homogeneous mixing within the transplant zone, but this can be ameliorated by premixing with sand and using the mixture in transplanting instead of direct addition of pure iron mineral in the field. Regardless of gaps existing in direct comparison, these results parameterize iron-based amendment of highly sulfidic sediments using methods and materials that are accessible, affordable, and conducive to large scale restoration efforts.

3.) The sulfide lowering effect of iron can persist through the *Zostera marina* growing season.

The longevity of sulfide control from singular solid phase magnetite amendment extends to the six-month timescale needed for *Z. marina* overwintering and re-emergence of new shoots (Blok et al., 2018). This conclusion was evidenced by results from mesocosm and field transplant experiments (Figure 6a, 7a). Phase III mesocosm experiments using magnetite showed strong porewater sulfide removal over the 169-days (Figure 6a). The shorter duration of sulfide suppression observed in the siderite treatments reflected saturation of Fe^{2+} with accumulated sulfide such that the mineral's ability to complex additional sulfide, produced by continued sulfate reduction, was exhausted early on. This mechanism suggests that short-term direct complexation of sulfide with added Fe^{2+} (siderite) can be achieved, but the use of mixed valence magnetite is needed for sulfide suppression on the timescale of seasonal eelgrass lifecycles. This improved longevity could be attributable to excess surface area improving Fe^{2+} scavenging as a result of smaller magnetite grain size. Alternatively, and more likely, it reflects a combined effect of the small magnetite grain size and the favored induction of iron reduction of magnetite Fe^{3+} over sulfate reduction.

Mesocosm plots amended with an equal measure of Fe_3O_4 and $FeCO_3$ were nearly as long-lived as those amended with magnetite alone with respect to suppression of sulfide concentration (Figure 6a). This result was unexpected, as siderite-only treatments showed temporally limited scavenging capacity. The persistence of the effect observed here further demonstrates the importance of mixed valence iron, even in small amounts, in the long-term suppression of sulfide concentrations in organically enriched sediments. The totality of results presented here favor magnetite as the mineral of choice for even short-term control of porewater sulfide. The similar sulfide-lowering and pH-raising results measured in porewaters following the second transplant experiment showed that this geochemical effect resulting from iron additions is reproducible.

Conclusions

This work was concerned with two central inquiries; 1) whether high sulfide concentrations in sediment porewater can be efficiently lowered through solid phase iron amendment and, if so, 2) whether this modification results in improved eelgrass transplant success. Investigation of the first central question confirmed that high sulfide concentrations in sediment porewater can be efficiently lowered through solid phase iron amendment, as evidenced by laboratory, mesocosm and field scale sediment amendments. Mineral type emerged as an influential factor, with maximal outcomes occurring when iron was present in mixed valence states (as in magnetite) rather than Fe(II) alone. Grain size also exerted influence on the magnitude of sulfide removal by siderite mineral additions; the same is likely true of magnetite, and future investigation of this matter would further serve to characterize the ideal mineral for use in sediment remediation studies to come. The solid phase mineral additions implemented here are feasible in the context of eelgrass transplantation; this finding may inform approaches in future investigations of the factors governing restoration success, especially in sediments of high organic matter content.

Investigation of the extent to which iron-mediated sulfide removal might influence survival and establishment of transplanted eelgrass returned inconclusive results. Despite being able to lower sulfide at the field transplant scale, eelgrass transplant success hinges on multiple factors beyond sediment geochemistry, and beyond the control of this study. The eelgrass transplanted in 2022 that emerged in spring 2023 was met with unusually high turbidity and early onset on high water temperatures. Both of these factors led to transplant mortality at all sites as well as a 30% mortality of existing beds in the Niantic River Estuary. The eelgrass transplanted in 2023 that emerged in spring 2024 was met with extensive macroalgal blooms at all sites that smothered transplants at all locations. We can state conclusively that lowering porewater sulfide on the timescale of eelgrass transplanting can be done. It can be incorporated into transplant methodology and executed at field scale. We cannot state, however, that this manipulation of sediment geochemistry will necessarily lead to improved eelgrass transplant success because of all the other factors contributing to success or demise. Improving the sulfide environment in favor of eelgrass, did not supersede the three problems we encountered of high turbidity, high temperature, and macroalgal competition. The ability to integrate iron additions into transplant schemes for the purpose of lower porewater sulfide should be considered as a new tool. A complete evaluation of its utility for improving eelgrass transplant success, however would require its application under conditions / sites / times where other environmental factors do not severely hinder plant survival. Unfortunately, our field tests did not align with such circumstances. Nevertheless, data presented here provides a roadmap for the characterization of iron amendments which can be expected to successfully control porewater sulfide concentrations in situ.

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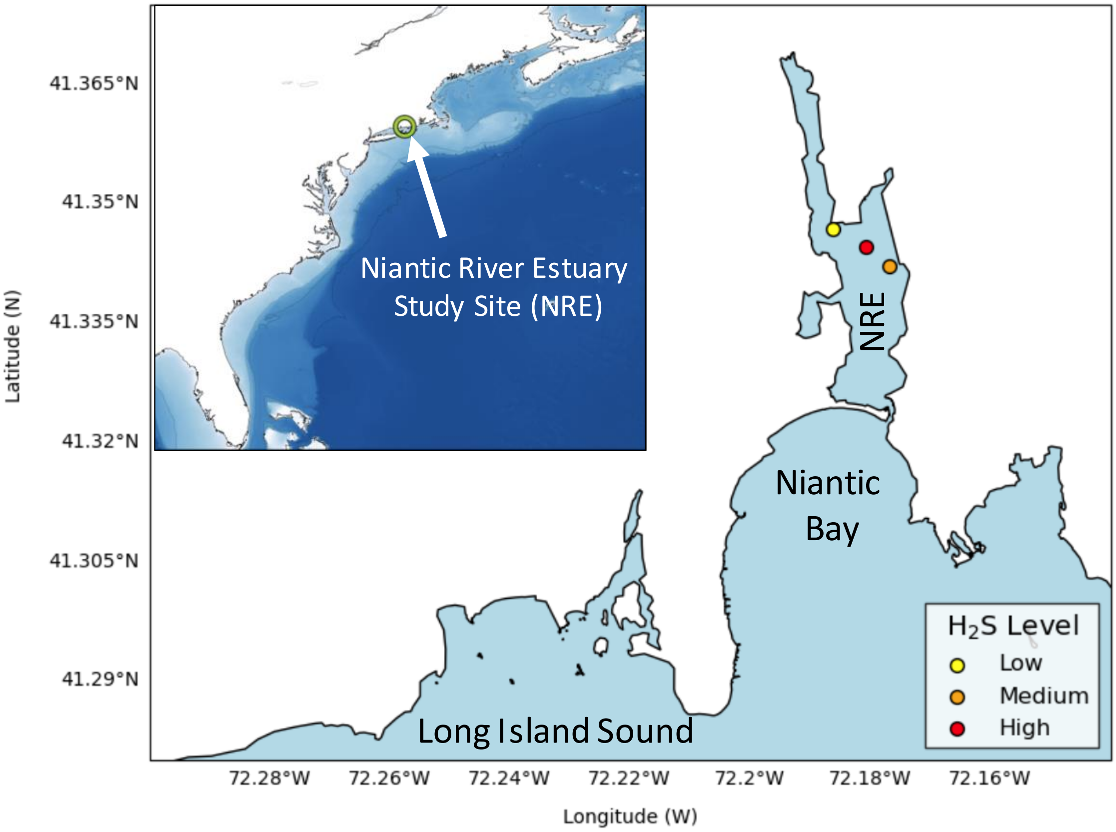


Text

Figure 1. Site location and orientation of transplant areas in the Niantic River Estuary (NRE). The three transplant areas are distinguished by low (<100µM), medium (100-500µM), and high (>500µM) porewater H_2S concentrations. All areas historically supported eelgrass beds but are currently unvegetated.

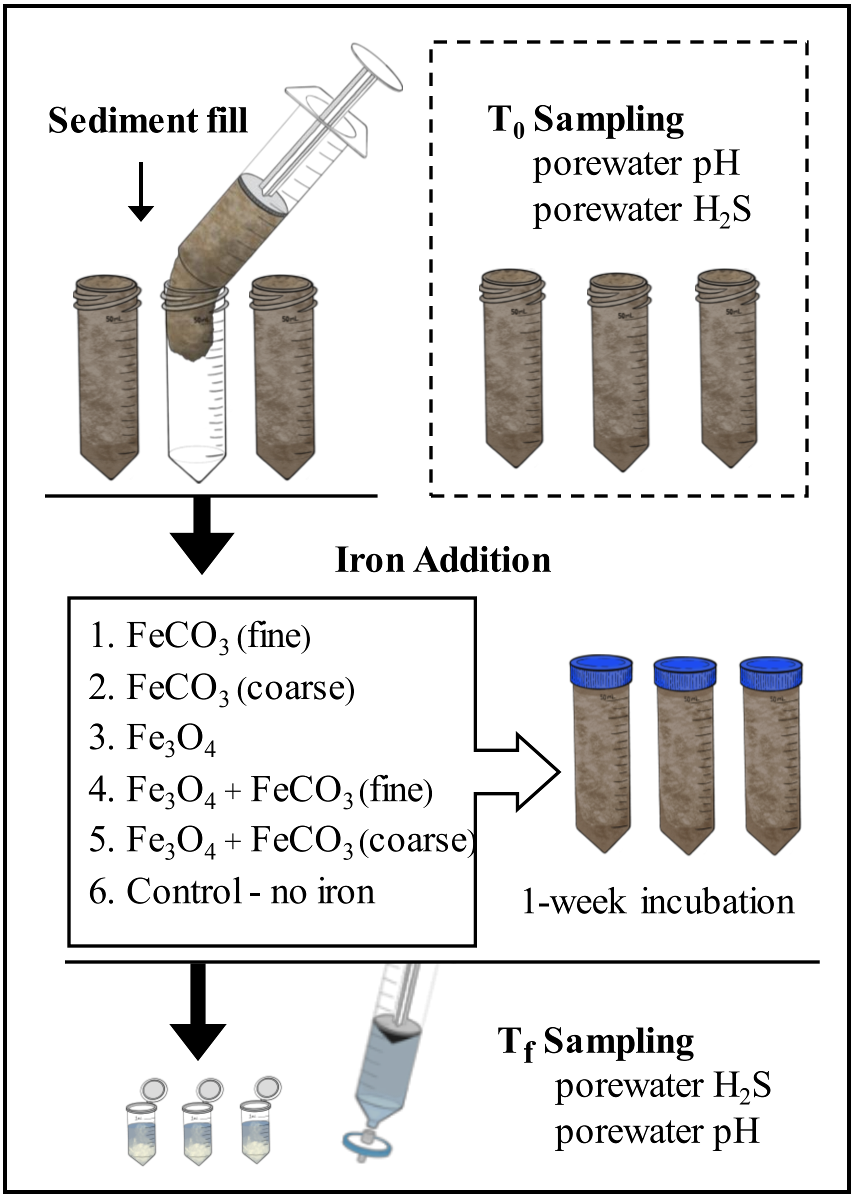


Figure 2. Closed system iron addition experiment. Tubes were filled with high sulfide sediments, amended with iron treatments, and sealed. Porewater pH and H_2S was measured for (T_0) on the day of iron addition to treatments. The same metrics were collected for controls and treatments following week-long incubations (T_f) in flow through seawater tanks at ambient temperature.

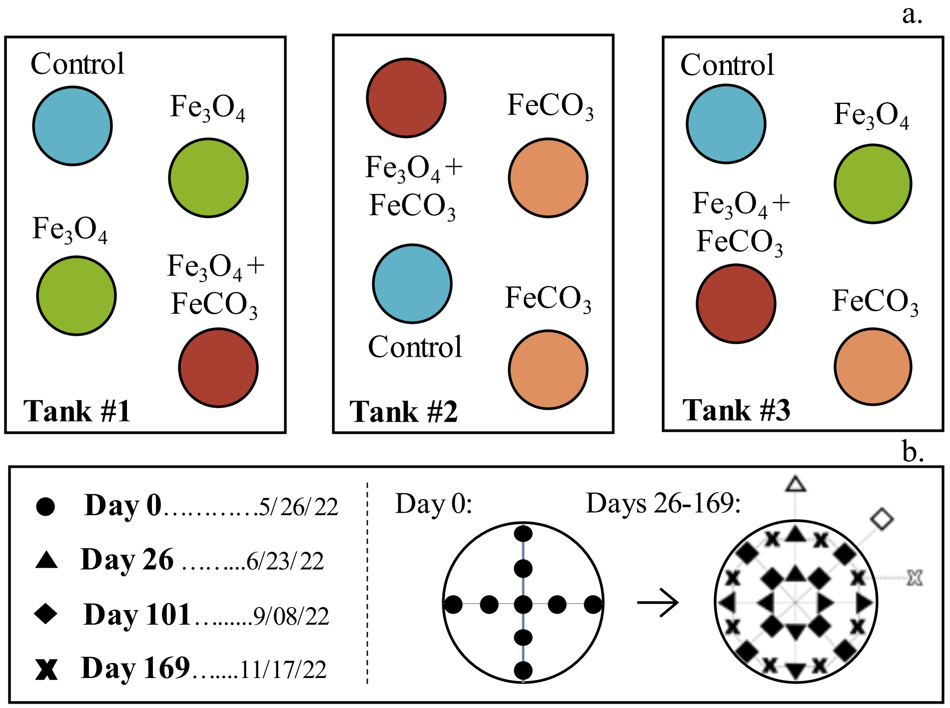


Figure 3 Tank mesocosm iron addition experimental design (a) and sampling scheme (b). Iron treatments were distributed among 3 flow-through mesocosm tanks (#1, #2, #3). Sediments were sampled within each plot on days 0, 26, 101 and 169 along offset axes between timepoints (indicated by shapes in panel b). Samples were also collected from a distance of 1.5 inches outside of each plot's perimeter following iron addition ('outer' samples are illustrated as hollow shapes for days 26-169 in panel b).

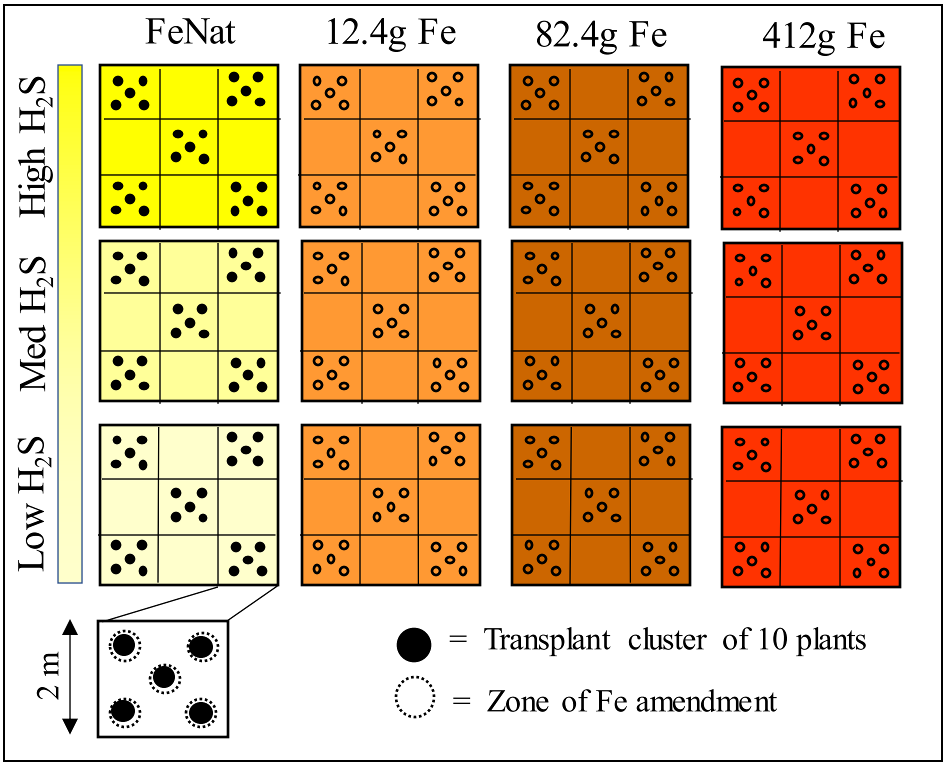


Figure 4. Experimental design for field transplants along a natural H_2S gradient. Three levels of iron amendment were made in areas of low, medium and high H_2S concentration (figure 1). FeNat are control plots, which reflect natural iron content and no addition. Treatment titles (12.4, 82.4, 412) describe the mass of iron (gFe) added to individual transplant clusters. Each 6m x 6m plot consisted of 25 clusters, each containing 10 plants.

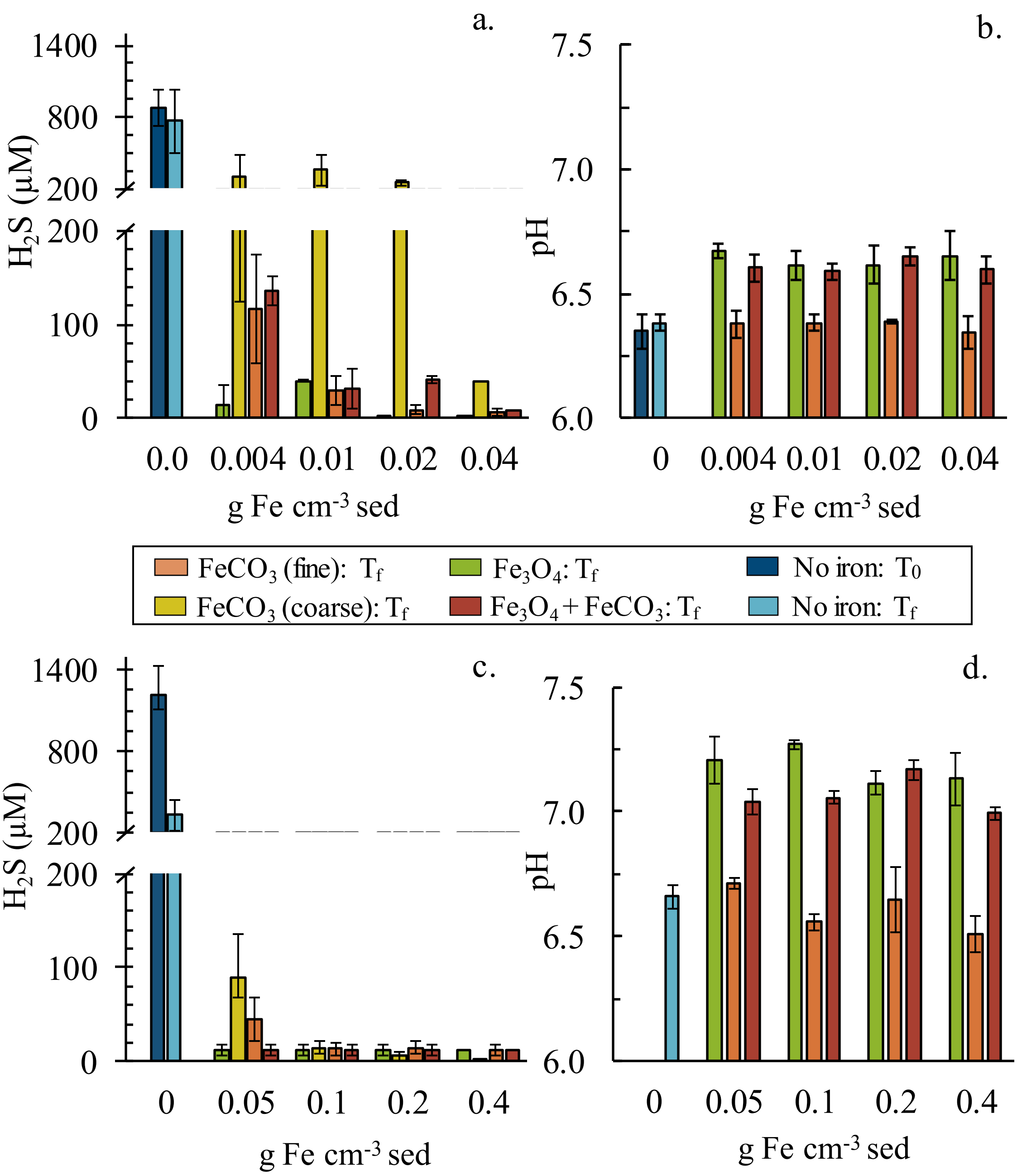


Figure 5. Iron dose and species effect on porewater H$_2$S (a) and pH (b) after 1 week incubation. No Iron T0 samples received no iron and values were measured on the day of iron addition. No iron Tf treatments received no iron and were measured after incubation (a, b). Fe$_3$O$_4$ is magnetite and FeCO$_3$ is siderite. Size fractions represent retention by (coarse) and passage through (fine) a sieve of mesh size #10. Colors in all panels apply to the central legend. Error bars denote standard error (n=4).

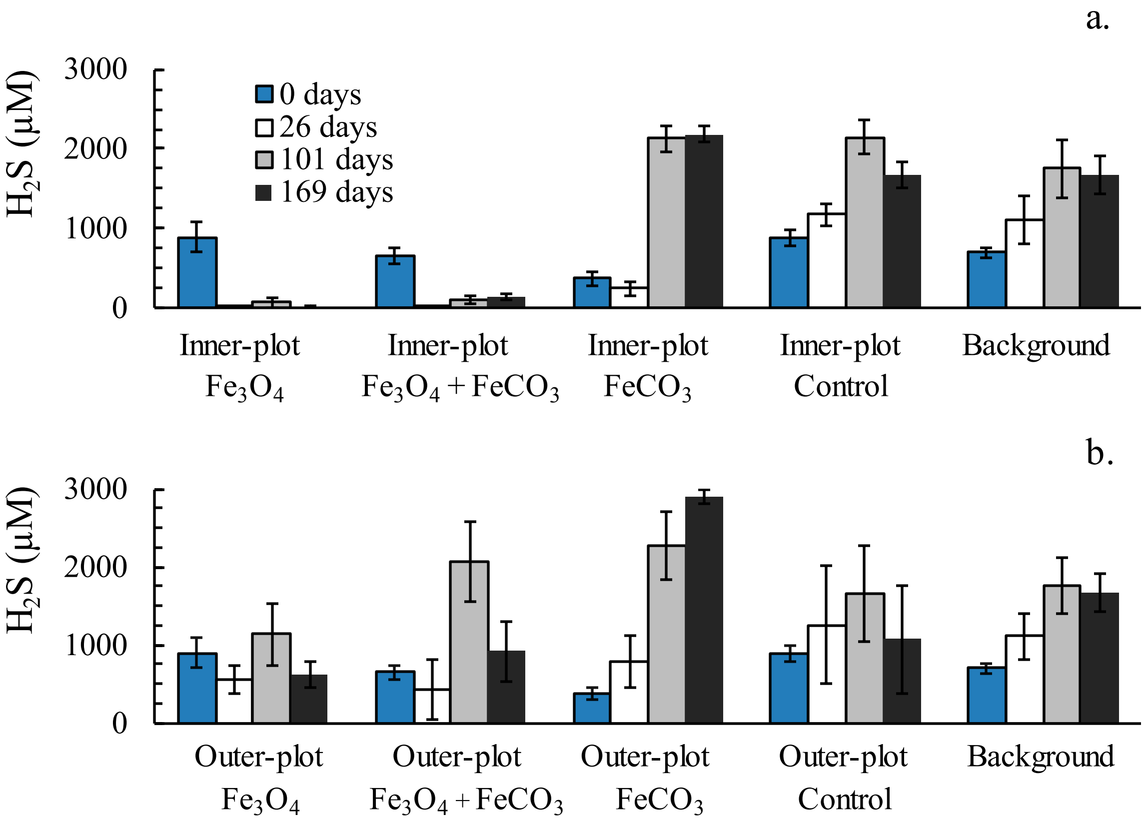


Figure 6. Evolution of porewater H_2S (µM) in tank mesocosms from May to November 2022 following iron addition. Blue columns represent average porewater H_2S values in each plot prior to iron addition; these values are averaged and presented as the day 0 value for background (a). Background samples (n=9) were taken from unmixed sediment adjacent to treatment plots at each sampling interval. Control plots were mixed in the same fashion as treatment plots. Error bars denote standard error (n=27). Samples taken 1.5 inches outside of each plot's perimeter (n=9) were collected at each sampling interval (b).

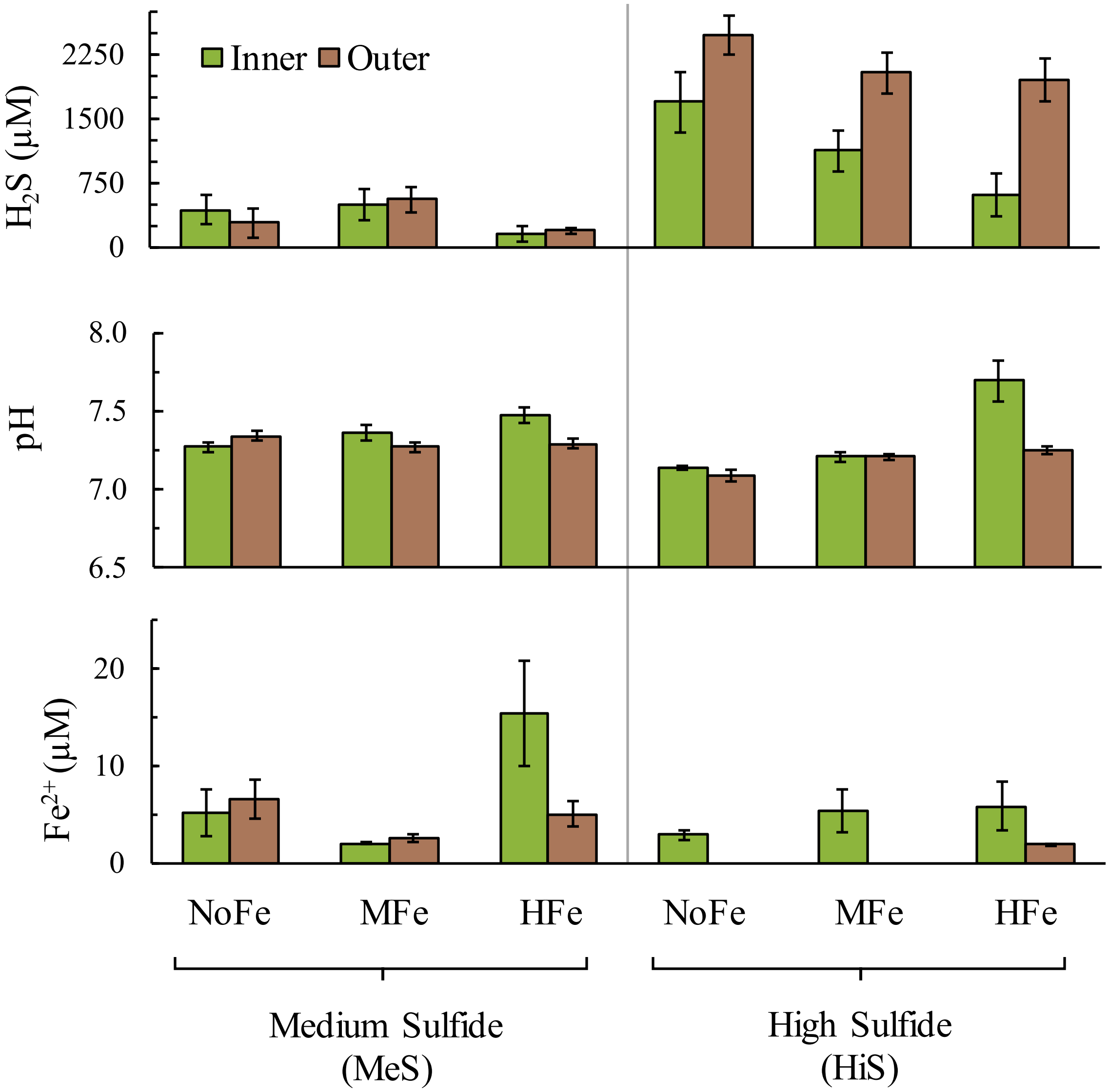
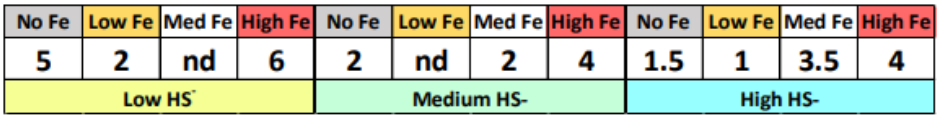


Figure 7. In situ porewater geochemistry following iron treatments and eelgrass transplants in the Niantic River Estuary. Porewater pH, HS and Fe^{2+} (μM) concentrations measured within (green) and adjacent to (brown) transplant treatment zones. Results are shown only for the high (H) and medium (M) sulfide sites, as porewater could not be retrieved from the low site sediments via centrifugation. Sampling occurred between late June and early August 2023, a minimum of 8 months following transplant and amendment with iron. Error bars denote standard error (n=8).



No Fe	Low Fe	Med Fe	High Fe	No Fe	Low Fe	Med Fe	High Fe	No Fe	Low Fe	Med Fe	High Fe
5	2	nd	6	2	nd	2	4	1.5	1	3.5	4
Low HS^{-}				Medium HS-				High HS-			

Figure 8. Qualitative assessment of plant survival taken in spring 2023. The amount of eelgrass is each plot was ranked by two divers and combined results are presented as numerical scores for each plot across the three stations. A score of 1 = absence of eelgrass, 2 = some grass, 3 = multiple plants in multiple transplant units, and nd = no data from either diver. Fe is iron and HS^{-} is sulfide. The table shows the sum of those two sets of rankings. The highest Fe loading had the highest plant score in all sulfide environments. The greatest difference in plant rankings (i.e. over wintering success) between high iron and no iron loadings was seen in the high sulfide environment.

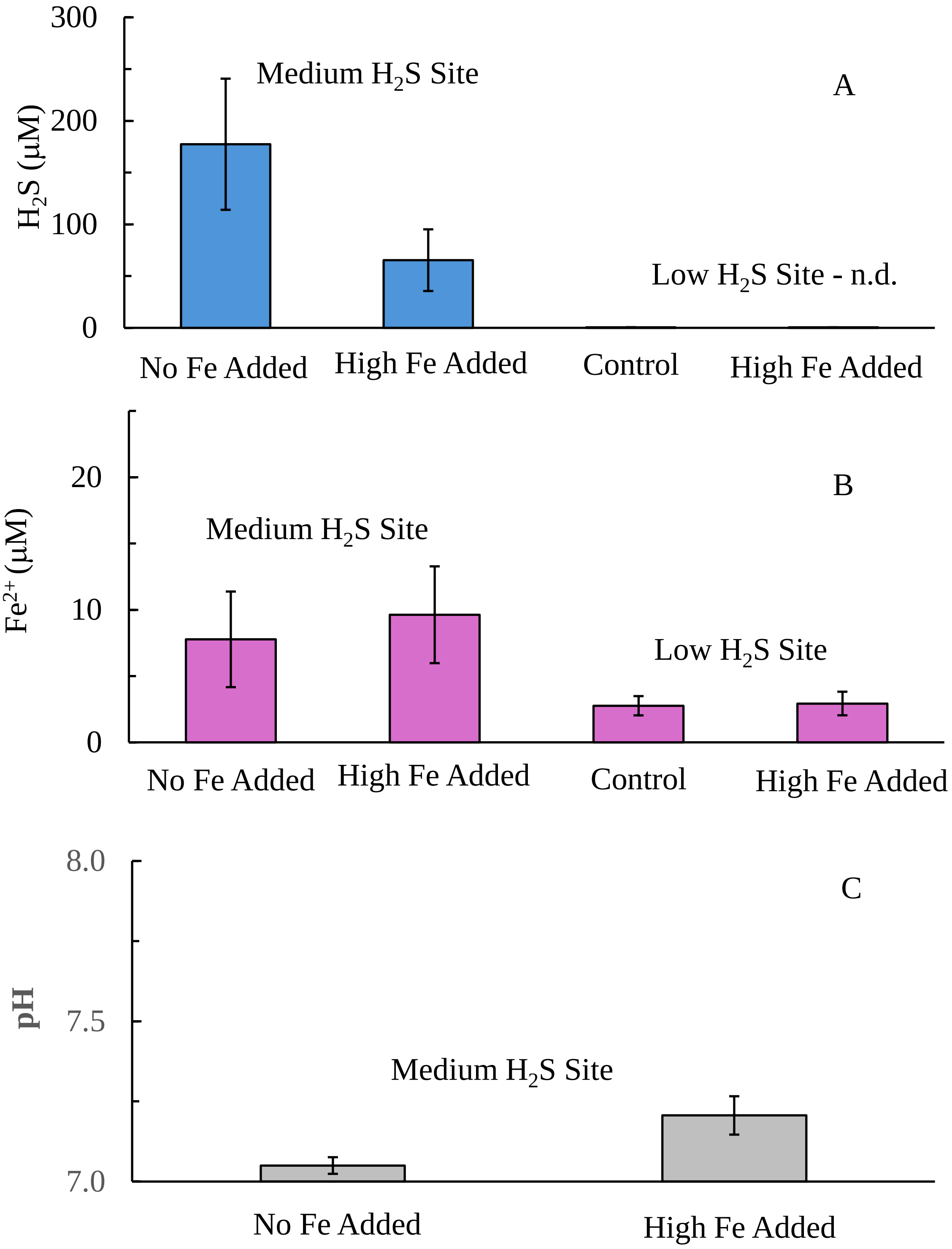


Figure 9. Porewater H_2S (A) , Fe^{2+} (B), and pH (C) measured in spring 2024 following second field iron addition experiment (Fall 2023). Data shown from sites that had transplant survival (medium and low H_2S sites). No pH data is available for the sandy low H_2S site due to low porewater yield. H_2S high iron was lower (T-test, p=0.07, n=9) and pH was higher at the Medium H_2S site with iron addition (T-test, p=0.03, n=9)

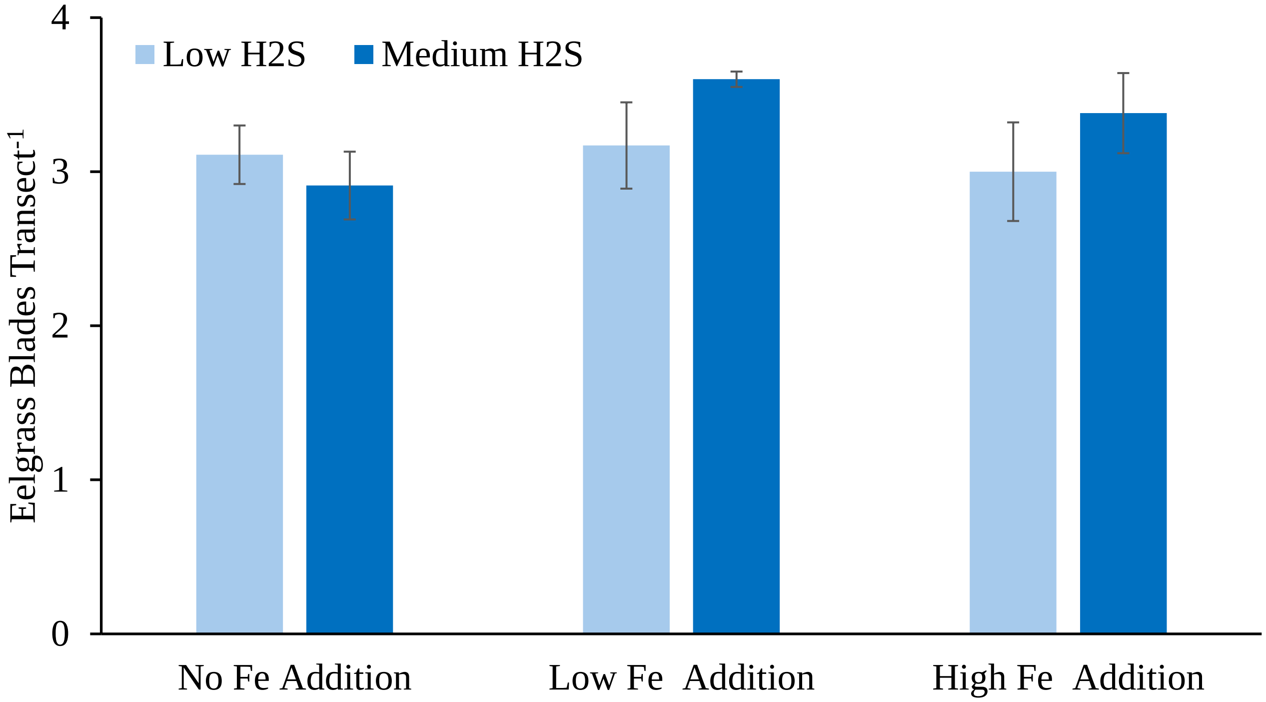


Figure 10. Eelgrass blades per transect measured in spring 2024 following second field iron addition experiment (Fall 2023). No difference in eelgrass blade abundance between treatments was measured at the Low H_2S site (ANOVA, p=0.55), nor at the Medium H_2S site (ANOVA, p=0.22).