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Gulf of Mexico estuaries: Ecology of the nearshore and coastal ecosystems impacted by the deepwater horizon oil spill

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Gulf of Mexico estuaries: Ecology of the nearshore and coastal ecosystems impacted by the deepwater horizon oil spill

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Editorial: Gulf of Mexico estuaries: ecology of the nearshore and coastal ecosystems impacted by the Deepwater Horizon oil spill

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Editorial on the Research Topic
Gulf of Mexico estuaries: ecology of the nearshore and coastal ecosystems impacted by the Deepwater Horizon oil spill

The Gulf of Mexico: productive despite a legacy of stressors

Estuaries of the northern Gulf of Mexico (GoM), from the freshwater, tidally-influenced wetlands to the nearshore coastal waters, are among the most productive on the planet (Chesney et al., 2000). As a result, these areas provide some of the most ecologically, economically, and culturally significant natural resources in the United States. These areas have also been subjected to a long history of numerous anthropogenic and natural disturbances. GoM estuaries have been influenced by extensive changes to the upstream watersheds resulting in altered water quantity and quality; routinely impacted by increasingly larger hurricanes; extensively modified for commercial shipping, recreational/tourism opportunities, and urbanization; remain vulnerable to climate change via sea level rise, warming, species distribution changes, and other climate effects; and heavily exploited for natural resources ranging from the historically productive fisheries to economically important minerals and their processing and refinement (Chesney et al., 2000; Lellis-Dibble et al., 2008).

Among the largest recent disturbance in the area is the highly publicized *Deepwater Horizon* (DwH) oil spill, which released an unprecedented amount of crude oil and gaseous hydrocarbons that heavily impacted GoM ecosystems (Nixon et al., 2016). The influx of funding generated by penalties associated with DwH sparked renewed interest in chemical, physical, and biological processes and functions of these GoM ecosystems and allowed for detailed research that greatly increased our understanding (Lubchenco et al., 2012). Impact assessments frequently highlighted surprising resilience of faunal communities (Able et al.,

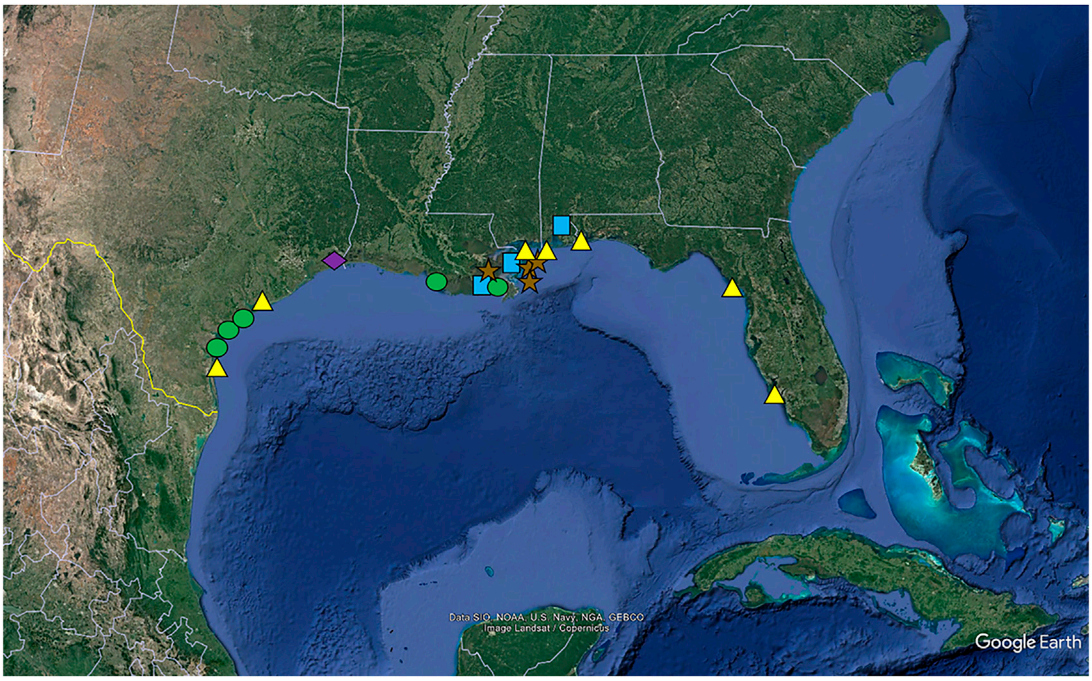

FIGURE 1
Approximate geographic locations of studies in the northern Gulf of Mexico included in this Research Topic using categories described in "Contributions of this Research Topic" section: 1) baseline studies (yellow triangles; Correia et al.; Rakocinski et al.), 2) non-oil stressors (blue squares; Lamb et al.), 3) spatial gradients (green circles; Husseneder et al.; Hu et al.), 4) oil effects (brown stars; Tatariw et al.; Berke et al.; Kiskaddon et al.; Snider et al.), and 5) restoration (purple diamond; Armitage et al.). Not depicted are Martin et al.; Carmichael et al. as they either represent a synthesis or Gulf-wide assessment.

2015; Olin et al., 2017) and food webs (McCann et al., 2017; Lewis et al., 2022), however to properly address oil impacts, previously unknown natural history knowledge was necessary. This included factors such as marsh fish physiology, demographics, site fidelity, and movement patterns (Vastano et al., 2017; Jensen et al., 2019), trophic interactions and energy flow pathways (Martin and Swenson, 2018; Keppeler et al., 2021; McDonald et al., 2022), and influences of other factors such as the multitude of confounding variables (Fodrie et al., 2014) and restoration efforts (Keppeler et al., 2023), all of which was attainable with DwH-facilitated funding.

Contributions of this Research Topic

Few article collections capture the complexities and multiple stressors GoM estuaries face. The aim of this Research Topic is to address this gap by illustrating the advances in ecological understanding and providing scientists and management practitioners with a roadmap for future studies that will contribute to the conservation of these coastal ecosystems for decades to come. In sum, the thirteen articles in this Research Topic detail our improved understanding of GoM ecosystem dynamics through documenting new insights into ecological processes and species distribution patterns, estuarine responses and resilience to various forms of disturbance and gradients, and finally providing critical baseline information that will improve future disturbance and restoration assessments (Figure 1).

GoM estuaries contain a mosaic of habitats, including sandy beaches, saltmarshes, mangroves, seagrass beds, and oyster reefs, to name a few. The productivity, complexity, and sensitivity of these different habitats to disturbance can be variable, further complicating efforts to produce overarching impact assessments. Despite this, important lessons have still emerged in the post-DwH research arena that merits attention and are highlighted in this Research Topic:

1) *Baseline information is critical to informing future disturbances.* A synthesis of post-DwH studies discusses an alarming lack of historical data which led to only ~3% of field-based studies employing preferred before-after-control-impact analytical approaches (Martin et al.). In light of this, numerous articles in this Research Topic present baseline data, including pertinent information on beach macrofauna (Rakocinski et al.) and economically important nekton assemblages (Correia et al.; Byrnes et al.) that can provide comparative information for future disturbances. These studies highlight an important lesson learned during this spill, that adequate preparation (e.g. , well-designed monitoring programs) is a key factor for future successful damage assessments.
2) *A legacy of disturbances affects GoM estuaries.* Centuries of exploitation and insults to ecosystems may produce synergistic effects. In this Research Topic, pollutants such as mercury are explored in this context, as mercury may become more bioavailable in the presence of stressors such as oil (Lamp et al.). Multiple stressors are known to unpredictably affect

ecosystems, and other pollutants (e.g., PFAS, microplastics, DDT, etc.) and their emergent effects when combined with other stressors require future consideration.

3) *Natural gradients exist, which can be leveraged for improved understanding.* Taking advantage of gradients can provide empirical evidence of the role of different environmental influences on the ecosystem. Here, several papers explore the influence of these gradients. For example, salinity effects on metazoan biodiversity is explored across Louisiana salinity zones, with detailed reports on species occurrences and distributions (Husseneder et al.). In addition, estuarine water aragonite saturation state is examined along a Texas climate gradient, with reported impacts attributable to freshwater discharge (Hu et al.). Given that future ecosystems will be inherently different, these studies also serve as important baseline information for future assessments.
4) *Oil impacts were context dependent.* Differential sensitivities may lead to changes in assemblages, altered energy flows, and modified rates of ecosystem functions. In this Research Topic, the role of mangrove expansion effects on biogeochemistry in oiled wetlands is reported (Tatariw et al.). Important changes to infauna community composition were found at a range of oil impacted sites in the Chandeleur Islands, Louisiana (Berke et al.), and this oil exposure impacted infaunal family richness but not phylogenetic diversity (Kiskaddon et al.). Dietary composition of seaside sparrows at oiled and unoiled sites were found to be strongly influenced following a major hurricane (Sinder et al.). Finally, analysis of a dolphin stranding database highlights the role of human activities, including oil exposure, in stranding occurrences (Carmichael et al.). These studies are not the first to illustrate variability in oil spill impacts (e.g., Fodrie et al., 2014; Martin et al., 2020), and this Research Topic highlights the complexity of such holistic oil spill damage assessments.
5) *Restoration remains a viable option for mitigating historic and ongoing damages.* Restored habitats may encourage rehabilitation of natural flora and fauna and thereby promote rejuvenated ecosystem properties. In this Research Topic, a case study is presented on coastal wetland restoration, finding that trophic relationships and food webs still varied between reference and restored areas, with key advice to practitioners to incorporate geomorphological heterogeneity and plant diversity in restoration practices (Armitage et al.). Moving forward, restoration activities such as those described in this study will be important for maintaining productive ecosystem services.

Historical and emerging threats to Gulf of Mexico estuaries

We conclude by outlining the known and potential emerging threats to GoM estuaries. Petroleum exploitation was a known liability based on previous spills elsewhere, however few could have predicted the magnitude and economic harm the DwH event caused. The next major disaster may or may not be a known threat and we posit here potential future disturbances:

1) Alterations to hydrology can have far-reaching impacts. Water-related Research Topic persist, including Mississippi River protection levees and diversions, hydrologic alterations implicated in the decline oyster fisheries in Florida and Mississippi, impediments (e.g., dams and other structures) that stifle connectivity among upstream and downstream ecosystem components, and canalization rerouting water flow (e.g., Florida Everglades).
2) Habitat loss through urbanization, development, and industry has already extensively occurred. This includes canal dredging, spoil bank creation, and modifications for navigation. Notably, a booming tourism industry also threatens many GoM estuaries and plans for increased human access continue.
3) Climate change alters GoM estuaries at large spatial scales. Examples abound of increased temperatures, rising sea levels, salinity changes from altered weather patterns, and species distribution changes, and climate changes interacts with other disturbances, often producing synergistic and/or unpredictable consequences.
4) The productivity of these ecosystems has long driven local economies, and culturally-important fisheries may be at risk of overexploitation. Working waterfronts have been priced out due to prime real estate, but fishing activities are now more advanced than ever. Activities such as trawling can have unintended impacts (e.g., bycatch, benthic damage).
5) The GoM contains many valuable mineral resources. Oil and gas exploration and production continues despite known risks, and other resources (phosphate, etc.) may pose additional risks.
6) Point- and nonpoint-source pollution continues to threaten water quality. Eutrophication from fertilizers originating from inland agriculture triggers plankton blooms leading to the hypoxic “dead zone”, and smaller localized sources (e.g., lawn, golf courses) may also have negative impacts. Harmful algal blooms occur almost annually in some areas and are linked to eutrophication.
7) Novel species have established, however inshore areas have yet to see a major reorganization of landscape-level communities or food webs as a result. To date, these are predominately species with low salinity tolerance or offshore, reef-associated fishes. Other novel species include climate-expanding species, however the next invader or climate vagrant may be the exception.

Predicting the next major disturbance is impossible; however, the knowledge attained in the wake of the DwH spill and summarized in this Research Topic will undoubtedly serve as critical baseline information to inform future disturbances. We encourage researchers to continue predictive, scenario-based research on the above factors, especially in the context of multiple, interacting stressors, as estuaries will undoubtedly continue to be subjected to contemporary and emerging, simultaneous challenges. It is our hope that information contained herein finds utility throughout future years and drives continued conservation of GoM’s vital, productive, valuable, and culturally-important estuaries.

Author contributions

All authors contributed to the conception, implementation, and execution of this Research Topic. CM wrote the first draft of this editorial, and JO, PL-D, and BR contributed to the manuscript revision and approved the submitted version. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Oil disturbance reduces infaunal family richness but does not affect phylogenetic diversity

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Infaunal organisms are susceptible to disturbances such as hypoxia and sediment contamination; changes in infaunal community structure are therefore often used as indicators of anthropogenic disturbance. Susceptibility to disturbance varies across taxa, either due to physiological factors or to behaviors or functional roles that increase exposure. Both sources of variability are likely to be heritable and shared among related taxa. Thus, we would expect oil disturbance to disproportionately affect related taxa and therefore decrease phylogenetic diversity (PD). We test this hypothesis for a shallow water marine infaunal community using a simulation approach that iteratively removes clades with shared vulnerability to oil exposure. Infauna were sampled at two sites in the Chandeleur Islands, LA, that reflect different exposures to crude oil after the *Deepwater Horizon* event. Seagrass and adjacent bare sediment habitats were sampled in 2015, 5 years after initial oil exposure, and again in 2016 after an acute re-oiling event. We found that strong correlation between PD and family richness masked any detectable PD patterns with oil exposure. For our full community tree, sensitivity analysis indicated that the removal of larger clades did not disproportionately reduce PD, against our prediction. For this pair of sites, PD did not provide a better metric for assessing the impacts of oil exposure than family richness alone. It is possible, however, that finer-scale taxonomic resolution of infaunal communities may better decouple PD from taxonomic richness. More work is needed to fully evaluate the impacts of disturbance on PD.

KEYWORDS

Deepwater Horizon, seagrass, sediment ecology, annelids, Gulf of Mexico

Introduction

Marine benthic invertebrate taxa facilitate many critical ecosystem functions, including serving as an important trophic link between the base of the food chain and higher trophic levels (Grebmeier et al., 2006; Davis et al., 2014). Sediment invertebrates play important roles in nutrient cycling, organic matter burial, and remineralization (Boudreau and Jørgensen, 2001; Middleburg 2019). Benthic macroinfauna are well-recognized biological indicators of ecosystem quality in coastal areas (Dutertre et al., 2013) and are commonly integrated into biological indices of ecosystem integrity and water quality (Dauvin and Ruellet, 2007). Benthic macrofaunal diversity is also a key variable in the development of coastal management plans (Carstensen et al., 2014). LA, United states, is a hotspot for coastal management activities focused on maintenance of coastal habitats, including emergent wetlands and barrier islands threatened by subsidence, sea level rise, extreme weather events, and oil spills (CPRA, 2017). However, there remain significant gaps in our understanding of benthic macroinfaunal community composition, dynamics, and resilience to perturbation at the base of marine food chains in coastal Louisiana.

Crude oil exposure, like the *Deepwater Horizon* (DWH) oil spill of 2010, is an ecosystem stressor capable of defaunating large areas of the seafloor and decreasing species diversity (Montagna et al., 2013), with long-lasting effects that may inhibit ecosystem recovery for decades after exposure (Bejarano and Michel, 2010; Pashaei et al., 2015). Montagna and Harper (1996) show that chronic oil exposure resulted in decreased abundance of harpacticoid copepods and amphipods and increased abundances of deposit feeding polychaetes and nematodes, which implies that related taxa may respond similarly to oil. In recent work by Han et al. (2017), Antarctic copepods of the genus *Tigriopus* exposed to a water accommodated fraction (WAF) of crude oil exhibited oxidative stress and reproductive impairment through upregulation of certain enzymes, but the effects differed among species within the genus. Reproductive impairment of certain families or genera of marine invertebrates when exposed to WAF (Scarlett et al., 2007; Lewis et al., 2008) further strengthens the hypothesis that oil exposure has the potential to alter population dynamics.

One metric that ecologists can use to detect heritable susceptibility to disturbance is an evaluation of Phylogenetic Diversity (PD). PD is defined as the sum of branch lengths in the phylogenetic tree connecting all members of a given community together, indicating diversity of evolutionary lineages of a given biological community (Faith, 1992). PD was first introduced as a tool for conservation practitioners to determine conservation priorities amongst diverse taxa based on quantitative measures of feature diversity held within related phylogenetic groups or clades (Faith, 1992). PD is commonly used to evaluate diversity in ecosystems, measure the impacts of ecosystem stressors on communities, and predict future biodiversity declines. If related taxa are susceptible to the same stressor and are lost from a community, then the resulting measure of PD will be lower than if taxa were lost at random (Faith 1992; Cadotte et al., 2008; Srivastava et al., 2012). The concept of shared susceptibility to stress based on evolutionary relatedness has been used to describe a broad array of taxa and contexts, including assessing urbanization impacts on birds (Carvajal-Castro etal., 2021), evaluating historical extinction patterns of mammals (Russell et al., 1998; Purvis et al., 2000), determining geographic distributions of threatened amphibians (Bielby et al., 2006), and identifying biodiversity hotspots and regions of potential biodiversity decline of plants (Davies et al., 2011; Yessoufou et al., 2012). These studies indicate that some incidences of stress can disproportionately impact some taxa over others (Russel et al., 1998; Purvis et al., 2000). However, recent research has shown that PD is less useful for detecting responses of macroinvertebrate communities to stress. Boda et al. (2021) found no phylogenetic signal of stream restoration impacts on macroinvertebrate communities using and Arbi et al. (2017) observed no differences between species-level indicators (richness, diversity) and measures of PD in macrobenthic communities in response to nutrient pollution.

The objective of this study is to evaluate whether PD captures changes in infaunal communities sampled from a site that was heavily impacted by oil versus those from a site that was less impacted. We used PD to examine whether oil exposure disproportionately affects benthic invertebrate families based on evolutionary relatedness and inherited susceptibility. We hypothesize that responses to crude oil would be conserved at the family level, resulting in lower PD overall. We also explicitly test the prediction that removal of related taxa rather than random taxa from a marine infaunal community phylogenetic tree would result in a substantially lower measure of PD as described for other biological communities.

Methods

Study design and infauna sampling

This study was conducted in 2015 and 2016 on the inshore side of the Chandeleur Islands, LA, a barrier island system that received patchy oiling during the DWH oil spill in 2010. Macroinfauna were collected in shallow water (<1 m depth) at two sites roughly 3.7 km apart, one moderately to highly oiled (29.894658N, −88.828155W) and the other only lightly oiled (29.864039N, −88.842443W; sites B and A, respectively, described by Berke et al., this issue). Sampling was conducted in the summer and fall of 2015 and 2016. During sampling efforts in summer 2016, a visible layer of oil was observed at the sediment surface in the nearby marsh at the previously oiled site. The origin of the oil is unknown, but it is possible that it was transported or unearthed during a storm event over the winter of 2016 (see Berke et al., in this issue for further details). We therefore concluded that the samples collected in the summer and fall of 2015 reflect long-term oil recovery after the DWH oiling event, and the samples collected in 2016 (especially in summer) reflect short-term recovery after acute re-oiling. At each shallow water site, we sampled two distinct habitat types: seagrass (*Ruppia* sp., most likely *R. maritima*) and unvegetated sediment. Due to a lack of baseline data prior to the DWH event, differences in infauna communities between sites is used as an indicator of oiling impact.

Infaunal community analysis of these sites and habitats by Berke et al. (this issue) showed that community structures were significantly different between the sites and habitats for all sampling efforts. Furthermore, after the summer 2016 re-oiling event, abundance, family richness, and Shannon diversity were significantly lower at the oiled site. Here, we use the same dataset as Berke et al. (this issue) to determine whether phylogenetic diversity calculations contribute to our understanding of the impacts of oil on these communities. Sampling methods are described in detail by Berke et al., (this issue). Briefly, infauna samples were collected using sediment cores (10 or 15 cm diam., 15 cm depth) from both habitat types at each site. Upon collection, sediment was sieved (500 μm) in the field and macroinfauna immediately preserved in 95% EtOH with Rose Bengal stain. All preserved animals were later sorted, enumerated and identified to species-level if possible, otherwise to genus-level. Juvenile crustaceans and mollusks too small for confident identification were not included in this analysis.

Phylogenetic tree construction

To estimate phylogenetic diversity, we constructed a maximum likelihood (ML) phylogeny based on 18S ribosomal RNA and mitochondrial cytochrome oxidase subunit 1 (COI). These genes are commonly used for phylogenetic reconstruction of the four phyla included in our dataset: Annelida (Struck et al., 2006; Struck et al., 2015), Arthropoda (Koenemann et al., 2010), Mollusca (Sharma et al., 2012; Galindo et al., 2016), and Echinodermata (Janies 2001; Okanishi and Fujita, 2013). Published 18S and COI gene sequences were obtained from GenBank for most identified families, and sequences were selected from the lowest level of identification (species level where possible) to more accurately represent their phylogenetic placement. We attempted to use 18S and COI genes from the same species, however, in a few taxa this was not possible (see Supplementary Material). For taxa with no publicly available 18S and COI sequences, sequences were generated using the DNeasy Blood and Tissue Kit (Qiagen©) for DNA extraction using the manufacturer's instructions, followed by PCR amplification using taxon-specific primers (Supplementary Table 2). PCR products were sent to Integrated DNA Technologies, Inc. for purification and sequencing using Sanger sequencing methods. Unfortunately, a few taxa had no 18S or COI sequence data available on GenBank at the time of analysis and sequencing attempts failed. We therefore omitted the families Cossuridae (Annelida), Mysidacea (Arthropoda), Tornatinidae (Mollusa), Cystiscidae (Mollusca), and Litiopidae (Mollusca), none of which were abundant in our samples. A complete list of identified families, associated GenBank accession numbers (if applicable), and DNA preparation protocols are provided in the Supplementary Material.

Sequences were trimmed and checked using Geneious v. 9.0.5 software. Each gene was aligned separately using MAFFT (Katoh and Standley, 2013); for COI, the L-INS-I algorithm with default settings was used. For 18S, the E-INS-I algorithm with default settings was used, and alignment positions comprising at least 75% gap sites were stripped from the alignment. Alignment ends were trimmed to exclude sites with poor coverage. The final gene alignments were concatenated in Geneious Pro to produce an alignment with 2,404 sites. Partitioned maximum likelihood (ML) tree reconstruction was performed using RAxML-NG v. 1.1.0-master on the CIPRES Science Gateway (Miller et al., 2010), under a gene-partitioned GTR + G model with 10 replicate tree searches and 1,000 non-parametric bootstrap replicates. Because there are limitations to the accurate reconstruction of deeper nodes based on only two genes (Hou et al., 2007; Heimeier et al., 2010; Zanol et al., 2010), particularly at the scale of multiple phyla, a constraint topology based on several more robust ML phylogenetic analyses and multiple genetic loci (Taylor et al., 2007; Bracken et al., 2009; Toon et al., 2009; Zrzavý et al., 2009; Bracken et al., 2010; Kocot et al., 2011; Okanishi et al., 2011; Smith et al., 2011; Weigert et al., 2014; Weigert et al., 2014; Struck et al., 2015; Verheye et al., 2015; Kocot et al., 2017) was generated in the Mesquite software package (v3.51; Maddison and Maddison, 2018) and applied in the ML analysis (Figure 1). Use of a constrained topology during tree building ensured that robustly supported phylogenetic relationships were retained while allowing for variable branch lengths to calculate PD. The tree was rooted using the sole deuterostome clade Echinodermata as the outgroup.

The final comprehensive family-level ML tree was pruned (i.e., branches removed) to obtain sub-trees that reflect the snapshot community composition found in each of the 16 community treatments (oiled/unoiled sites x seagrass/unvegetated x summer/fall x 2015/2016), hereafter referred to as "community trees." Topologies of all community trees are given in Supplementary Figures 1–16. PD and family richness were calculated for all samples (Supplementary Material) and each of the 16 community trees.

Calculating phylogenetic diversity

PD analyses were conducted separately by site, year, season, and habitat by community tree. PD and family richness were calculated for each individual sample and each of the 16 community trees using

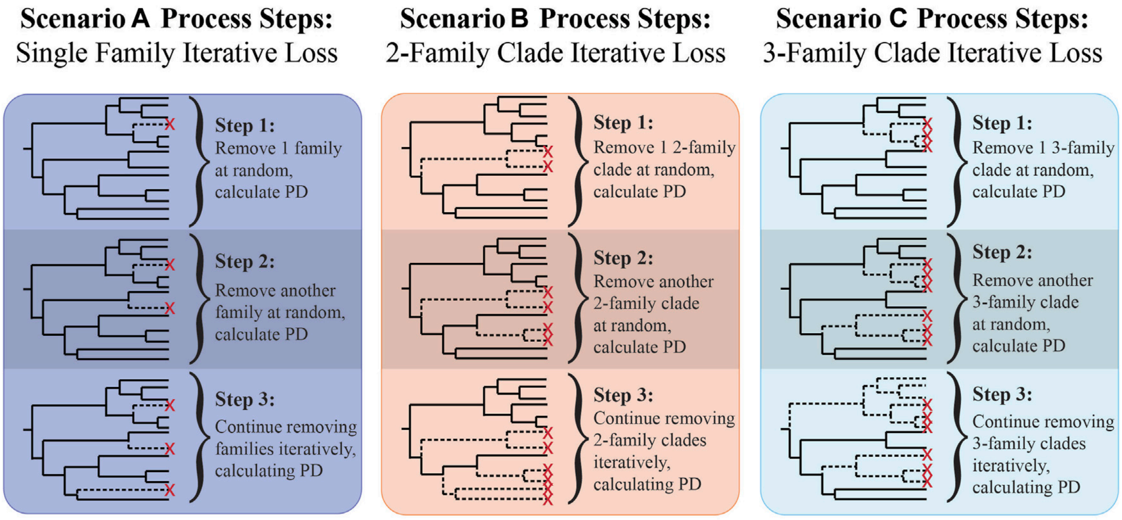

FIGURE 1
Illustration of scenario A, B, and C process steps used in the sensitivity analysis to test the effects of family clade size removal.

the PD function (Picante v1.8 package; Kembel et al., 2010) in R (R Core Team, 2018). Prior studies have shown that PD decreases nearly linearly with taxon richness (Flynn et al., 2011; Voskamp et al., 2017); we therefore needed to statistically determine whether the observed value of PD significantly departs from the expected value for a given number of families. A significantly lower observed PD value could indicate that an environmental stressor (i.e., oil) disproportionately impacted certain families. To achieve this, we used a bootstrapping method to construct a distribution of PD values generated through randomized taxa groups. In other words, we created a null distribution of PD for richness. This null distribution was created by randomly subsampling n = family richness (with replacement) from the comprehensive tree, then calculating PD for each subsample using the PhyloMeasures v2.1 and ape v5.3 packages in R (Tsirogiannis and Sandel, 2015; Paradis and Schliep, 2018). This subsampling was repeated 10,000 times for each family richness value to obtain a null distribution of PD values associated with a given family richness. We compared $PD_{observed}$ for each community against this bootstrapped null distribution for a given richness. One-tailed p-values were calculated as the proportion of the null distribution ≤ $PD_{observed}$ (a one-tailed test was appropriate because we hypothesized *a priori* that PD would be reduced following oil disturbance).

Sensitivity analysis for testing the effects of family clade removal on PD

To test our prediction that successive losses of related families would result in lower PD values, we implemented three extinction scenarios using our comprehensive family tree: A) single family loss: iterative random removal of one family branch; B) two-family clade loss: iterative random removal of clades containing two families; C) three-family clade loss: iterative random removal of clades containing three families (Figure 1). Starting with PD of the full tree, PD was calculated for the remaining branches at each removal step until no families or specified clade sizes remained. Each scenario was repeated for 100 iterations. For each iteration, the order of families removed was randomized. PD values for all 100 iterations were then plotted against family richness for each scenario. Upper bounds were used to highlight each scenario's lowest possible rate of PD loss; upper bounds were calculated by sequentially removing families or clades based on terminal branch length ordered from shortest to longest. We predicted that PD would decrease more rapidly when larger clades were removed, therefore scenario C would result in the greatest rate of PD decline with decreasing richness. We also predicted that if oiling disproportionately eliminated groups of related taxa (i.e., larger clades), the observed PDs from the oiled site would be closer to scenario C. If oiling did not result in loss of related taxa, observed PD values would more closely reflect scenario A.

Results

Calculating phylogenetic diversity

The comprehensive family-level constrained ML phylogenetic tree included 70 families across four phyla: Echinodermata, Arthropoda, Mollusca, and Annelida (Figure 2). Linear regression constrained through the origin for oiled and unoiled community

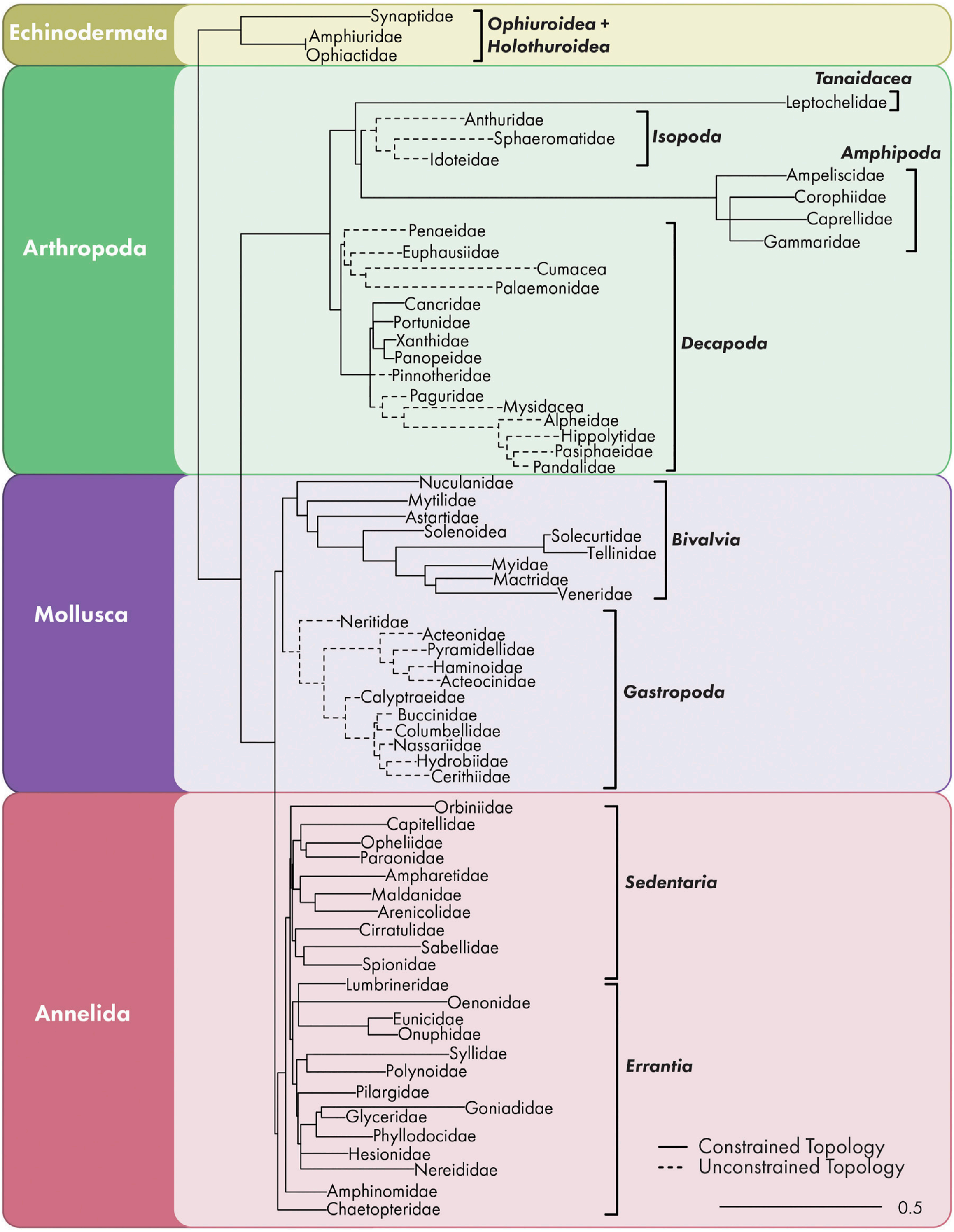

FIGURE 2
Comprehensive family-level ML phylogenetic tree showing all 70 families identified during sampling at both oiled and unoiled locations. Dashed branches indicate families were not constrained during tree building.

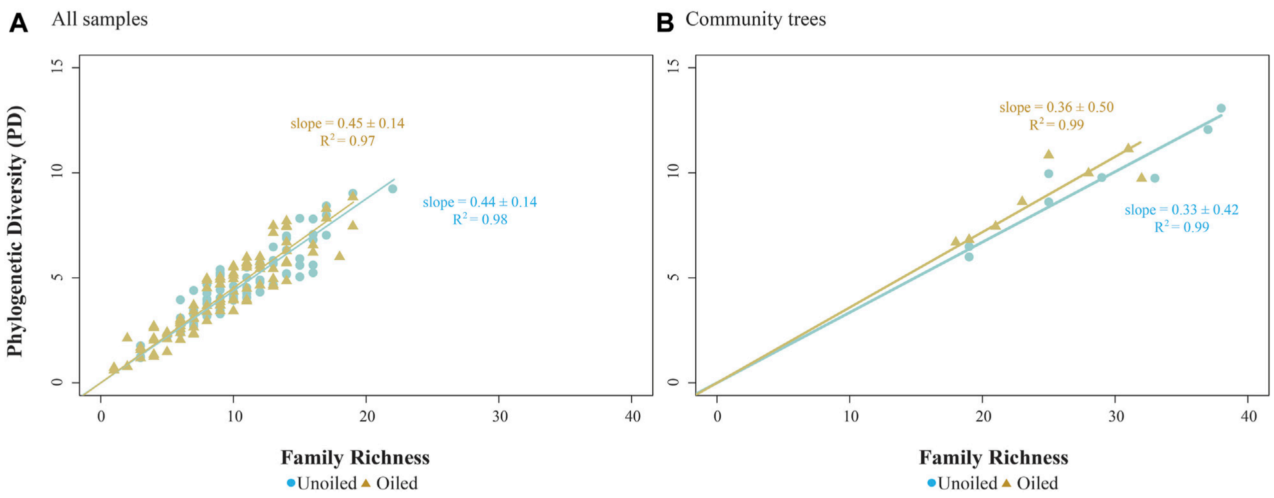

FIGURE 3
Scatterplot of PD and family richness values calculated for **(A)** all samples, and **(B)** each of the 16 community trees, separated by site. Linear regression lines constrained through the origin are given for each site, with slope ± 95% confidence interval and R^2 provided.

trees indicated strong correlation (slope = 0.33–0.36) between PD and richness (all $R^2 > 0.95$, Figure 3).

Family richness declined markedly following the summer 2016 reoiling event (Figure 4, see also Berke et al., this volume), but was otherwise similar for samples from both the oiled and unoiled sites. Samples from seagrass versus unvegetated habitats, however, showed more pervasive differences, with two-fold higher family richness in seagrass in some instances. As expected, the null PD distributions were strongly correlated with family richness (Figure 4). None of the observed PD values were significantly lower than the randomized null distributions of PD, indicating the observed values were not different than those generated by a random group of families. The only exception was summer 2015 in which observed PD in unvegetated habitat at the oiled site was significantly greater than the distribution created using randomized trees that maintained richness (Table 1).

Sensitivity analysis for the effects of clade removal on PD

We predicted that 1) PD would decline more sharply if larger clades were removed (i.e., that the PD would be lower for Scenario C than A), and 2) that PD for the oiled sites to fall within the range for Scenario C and below those of the unoiled sites, which would more closely follow Scenario A. In contrast, we found that the trends in PD decline were highly similar across all scenarios (Figure 5). Furthermore, the PD values from our community trees were within the range or slightly higher than our modelled results, regardless of oiling history.

Discussion

Effect of oil on macrofaunal community

This analysis confirmed that family richness and PD are tightly correlated for marine infaunal communities, as expected from studies in other biological communities (Flynn et al., 2011; Voskamp et al., 2017). We predicted that PD would be significantly lower than expected for a given richness at the oiled sites, assuming that related taxa would be removed. However, the data indicate that in most of our sampling events, the PD of each community showed no effect of oiling, and in the one event in which an effect was observed, it was captured by the simpler metric of richness and calculation of PD did not contribute to better detection of the effects of oiling. We did find a significantly higher PD for one sampling effort, the oiled site in the unvegetated habitat in summer 2015 (Figure 4). Examination of the community-level tree for that effort (Supplementary Figure 14) showed the presence of the Arthropoda family Leptochelidae, a family with a noticeably long terminal branch length. Some arthropods are commonly characterized with proportionally long terminal branches when phylogenies are resolved using 18S rRNA data (Jenner et al., 2009) which explains the higher PD observed in summer 2015. More favorable genes and phylogenetic analyses are still being determined for resolving the class Malacostraca.

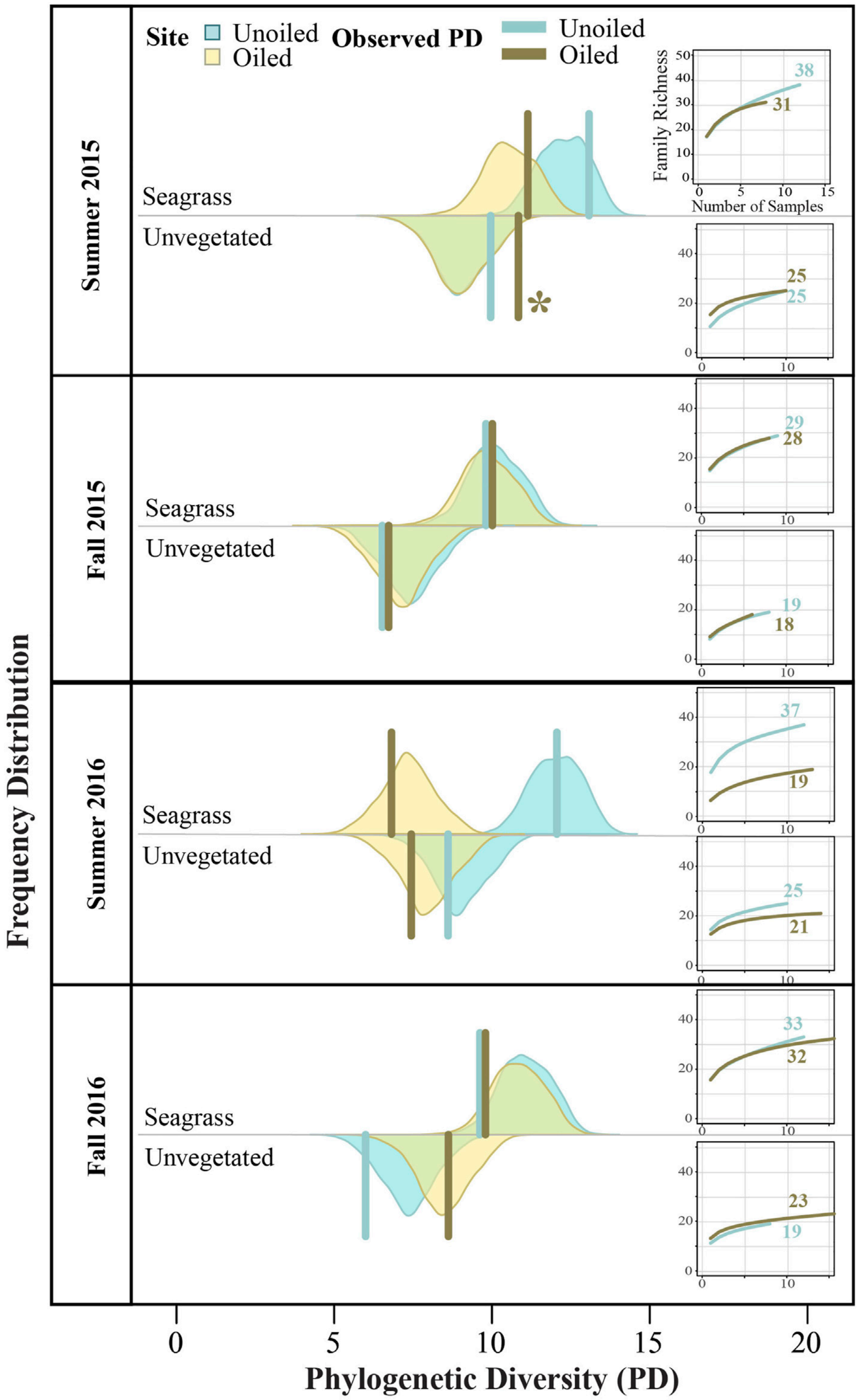

FIGURE 4
Observed community tree phylogenetic diversity (PD; solid bars) compared to null distributions (shaded areas) of PD values for the observed family richness generated for each season, year and habitat type for both sites. The asterisk (*) indicates significantly lower community PD than expected using one-tailed p-values calculated as the proportion of the null distribution ≤ $PD_{observed}$ (Table 1). Sample-based rarefaction curves and associated values of family richness are also shown for each site by sampling effort. Shaded colors are transparent, green shading indicates overlap of yellow and blue regions.

TABLE 1 Observed PD values and family richness by site, habitat, season, and sampling year for each of the 16 community trees.

			Unoiled site			Oiled site		
Habitat	Year	Season	Richness	Observed PD	*p*-value	Richness	Observed PD	*p*-value
Unvegetated	2015	Summer	25	9.96	0.15	25	10.84	0.01*
		Fall	19	6.49	0.16	18	6.69	0.32
	2016	Summer	25	8.61	0.31	21	7.44	0.27
		Fall	19	5.99	0.06	23	8.62	0.44
Seagrass	2015	Summer	38	13.08	0.17	31	11.14	0.27
		Fall	29	9.78	0.37	28	9.98	0.42
	2016	Summer	37	12.06	0.50	19	6.82	0.26
		Fall	33	9.74	0.05	32	9.73	0.09

One-tailed *p*-values indicate the significance of observed PD against a null distribution of randomized community PD. The asterisk * indicates significance at $\alpha = 0.05$.

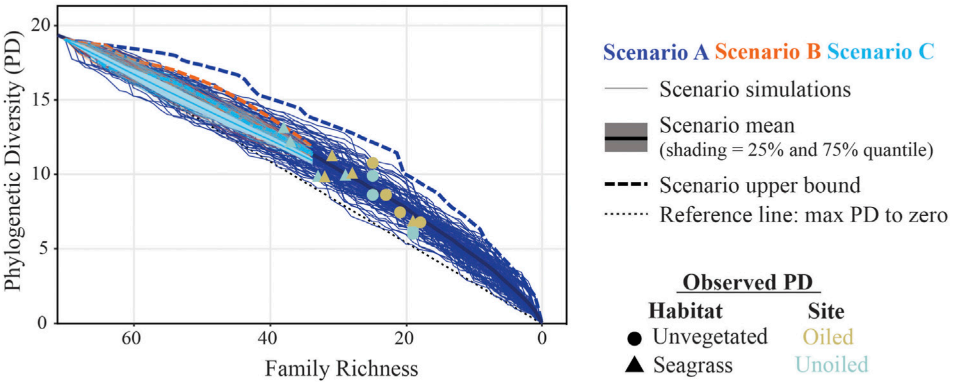

FIGURE 5
Clade loss scenarios modeling PD decline as a function of family richness. The three scenarios include: **(A)** single family, **(B)** two-family clade, and **(C)** three-family clade iterative PD loss. Iterations (*n* = 100 per scenario) and scenario mean (with 25 and 75% shaded quantiles) are plotted. The upper bounds of each scenario and a line through the origin are provided for reference. For all scenarios, the iterations fell below what would be expected from the slowest possible rate of PD loss based on removing branches/clades ordered shortest to longest total branch length (upper bound). Individual points reflect PD values from each community tree.

Using PD to assess disturbance to macrofaunal communities

Our prediction that the loss of related taxa would result in lower PD, as shown in other studies (Faith 1992; Cadotte et al., 2008; Srivastava et al., 2012), was not supported for our community tree. We found substantial overlap in possible PD values when simulating loss of single family, 2-family, and 3-family clades (Figure 5). This is consistent, however, with Parhar and Mooers' (2011) finding that PD estimates could not distinguish between random and targeted cladal extinction patterns. Phylogenetic tree topology can strongly affect how extinction of taxa from the tree decrease PD (Nee and May 1997; Malleit et al., 2018). For trees that are comb-shaped (short terminal branches) rather than bush-shaped (long terminal branches), the loss of a single taxon removes a greater proportion of the phylogenetic diversity in the tree, and phylogenetic diversity is more closely correlated with taxon richness (Nee and May 1997; Malleit et al., 2018). To better understand why our prediction was not supported, we examined our tree topology. Tree shape can be quantified using two parameters, ß and α (Maliet et al., 2018). ß quantifies variation in the species richness of clades, with higher values of ß indicating more even distributions of species among clades. ß < 0 indicates an unbalanced tree with large variation, and ß = −2 converges on a comb-like tree in which all branches connect at the spine of the tree. α describes the relationship between the age and richness of a clade, with α < 0 indicating that small clades contain evolutionarily distinct species, i.e., have long branches. Using ß and α, Maliet et al. (2018) show that PD in unbalanced trees ($\beta < -1$ or $a < 0$) can decrease almost as quickly as taxon richness, whereas PD loss is more gradual for more balanced trees. A post-hoc analysis of our full community tree using apTreeshape, a package that uses statistical analysis to compare tree topologies against null topologies generated from standard stochastic models of evolution (Bortolussi et al., 2006), results in a β value of −0.80 ± (1.22) and an α value of −0.15. These β and α values indicate that the community tree is unbalanced in favor of a comb-like structure, potentially explaining our finding of tight correlation between family richness and PD. In this case, PD may be less useful as a metric to assess disturbances because our community tree was strongly unbalanced when analyzed at a family level. We opted for a family-level analysis because a key goal of our study was to assess whether PD might be a useful metric in identifying effects of disturbances on infaunal communities. Many infaunal community studies identify organisms only to the family level (e.g., Gomez Gesteira et al., 2003; Hernández-Arana et al., 2003; Bernardino et al., 2012). Identification of infaunal taxa to species level is much more time consuming and requires much greater taxonomic expertise than identification to family-level. Additionally, a species-based tree would have required us to sequence every species rather than using available sequences from closely related species available on GenBank. This amount of effort was not feasible under the constraints of our study, and indeed would not be feasible for most community-level studies of environmental impacts.

That said, our results raise the question of whether species-level identification of taxa would result in a more balanced tree, and if so, whether PD might then be a useful metric for characterizing disturbances in sediment communities. This question is especially relevant as high-throughput DNA sequencing (HTS) is increasingly being used to survey diversity of microbial communities (Liu et al., 2021), fungal communities (Nilsson et al., 2018), microphytobenthos (An et al., 2020), meiobenthic communities (Broman et al., 2020), and macroinvertebrate communities (Carew et al., 2018; Mauffrey et al., 2020), among others. It would be interesting to know whether community trees based on HTS of sediments are more balanced than our family-level tree. Whereas HTS is increasingly used for meiofauna (Brannock et al., 2018; Broman et al., 2020), and has been shown to perform well for benthic monitoring (Lejzerowicz et al., 2015; Mauffrey et al., 2020), family richness using HTS can be much lower than that detected using traditional morphological analysis (Lejzerowicz et al., 2015). Additionally, Nascimento et al. (2018) found that large sample volumes are needed for robust analysis of sediment community diversity. However, this field is advancing rapidly, and HTS will likely become increasingly common for surveying macrofaunal communities as well as meiofauna. PD will be relatively easy to calculate for HTS data, and it appears that its usefulness depends strongly upon the tree topology.

PD is calculated from presence/absence data, but moderate oil stress can reduce abundances without completely removing taxa. Therefore, we explored the possibility of phylogenetic patterning in the distribution of effect sizes for changes in abundance at the oiled site (not shown). However, many families were rare, making it difficult to robustly estimate effect sizes and complicating the phylogenetic analysis. Berke et al. (this issue) provide further analysis of differences in infaunal communities between sites across sampling efforts.

Overall, for this pair of sites, PD did not provide a better metric for assessing the impacts of oil exposure on benthic macroinfaunal communities than family richness alone. It is possible, however, that finer taxonomic resolution of infaunal communities achieved through high throughput sequencing may better decouple PD from richness, justifying further study of the acute impacts of disturbance on PD.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://data.gulfresearchinitiative.org/, R4.x262.000:0010, R4.x262.000:0003.

Author contributions

All authors contributed to project planning and coordination. EK, KD, and KG, and SB developed the idea of the paper. EK and KG led and conducted all laboratory analyses. EK, KD, and JM analysed the data and, with KG, contributed to writing the final manuscript. SB and SB were invaluable in the manuscript review and revision process.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.950493/full#supplementary-material

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Shallow infaunal responses to the *Deepwater Horizon* event: Implications for studying future oil spills

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Infaunal sedimentary communities underpin marine ecosystems worldwide. Understanding how disturbances such as oil spills influence infauna is therefore important, especially given that oil can be trapped in sediments for years or even decades. The 2010 *Deepwater Horizon* (DWH) event was the largest marine oil spill in United States history, impacting habitats throughout the Northern Gulf of Mexico. We investigated infaunal community structure at two shallow sites in the Chandeleur Islands, LA, United States, over a 2-year period from 2015 to 2016 (5–6 years post-spill). One site was moderately contaminated with oil from the DWH spill, while the other was only lightly contaminated. Both sites featured patchy *Ruppia* seagrass meadows, allowing us to compare infaunal communities between sites for seagrass versus unvegetated sediment. The moderately-oiled site featured a significantly different community than that of the lightly oiled site; these differences were driven by altered abundance of key taxa, with some taxa being less abundant at the moderately oiled site but others more abundant. During our second year of sampling, a crude oil slick moved transiently through the moderately-oiled site, allowing us to directly observe responses to an acute re-oiling event. Virtually every taxonomic and community-level metric declined during the re-oiling, with effects more pronounced in seagrass beds than in unvegetated sediment. The sole exception was the snail, *Neritina usnea*, which we found exclusively at the more-oiled site. Our observations suggest that oil responses are driven more by key taxa than by entire guilds responding together. By identifying the families and genera that showed the largest signal at this pair of sites, we can begin laying groundwork for understanding which benthic taxa are most likely to be impacted by oil spills, both in the immediate aftermath of a spill and through longer-term contamination. While more studies will certainly be needed, this contribution is a step towards developing clear *a priori* hypotheses that can inform future oil-spill work. Such hypotheses would help to focus future sampling efforts, allowing resources to be directed towards those taxa that are most likely to be responding, and which are potential bio-indicators of oil exposure.

KEYWORDS

infauna, seagrass, oil spill, polychaeta, neritina, capitellidae, arenicolidae, bio-indicating species

1 Introduction

Oil spills, like climate change and habitat loss, are a hallmark of post-industrial human activities (Kennish, 2002; Jernelöv, 2010; Farrington, 2013; Barron et al., 2020; Rowe et al., 2020). Spills have occurred in every ocean of the world and can be surprisingly frequent (Eckle et al., 2012) –nearly one million gallons of oil are released in US waters annually (US Dept of Transportation, 2017). Oil can remain in habitats for decades or longer (Bejarano and Michel, 2010; Pashaei et al., 2015; Bejarano and Michel, 2016), yet its impacts are most often assessed in the immediate aftermath of the largest spills. Understanding how marine communities are influenced by oil contamination is important, not only for assessing long-term consequences of major disasters, but also for understanding how systems experiencing pervasive oil exposure might be affected.

Sedimentary infaunal communities are the foundation of marine ecosystems worldwide. Infauna contribute secondary productivity to marine food webs, and their activities drive bioturbation and sediment oxygenation, thereby influencing biogeochemical cycling at global scales (Meysman et al., 2006; Mermillod-Blondin, 2011; Solan et al., 2019). Infaunal communities often respond to oiling (Montagna and Harper, 1996; Joydas et al., 2012; Zabbey and Uyi, 2014; Washburn et al., 2016; Reuscher et al., 2017; Reuscher et al., 2020). However, more research has focused on immediate post-spill responses than on longer-term effects (for exceptions, see (Montagna and Harper, 1996; Zerebecki et al., 2021). While responses are usually negative, oiling can facilitate some taxa, most notably the polychaete family Capitellidae and perhaps other deposit-feeding organisms (Daan et al., 1994; Montagna and Harper, 1996). This has been interpreted as a trophic response: if oil provides organic enrichment, it may fuel microbial growth and thereby increase food availability for deposit feeders (Montagna and Harper, 1996).

The *Deepwater Horizon* (DWH) event was the third largest oil spill of all time, and the single largest marine spill in US history (Beyer et al., 2016). Between April and July 2010, roughly five million barrels of Macondo oil spewed from a broken riser pipe into the Gulf of Mexico. The resulting oil slick, covering nearly 65,000 km^2, was visible from space, yet most oil released was likely entrained at depth (Beyer et al., 2016). In shallow coastal habitats, oil can be transported into marine sediments by infaunal bioturbation and by sediment deposition. Even 5 years after the spill, DWH hydrocarbons were detectable in shallow-water sediments at sites in the Northern Gulf of Mexico (NGOM; Robertson and Baltzer, 2017; Duan et al., 2018; Tatariw et al., 2018, Robertson et al. in prep), making long-term impacts likely.

Assessing infaunal responses to oiling presents many challenges. Perhaps most significant is a broad lack of baseline data that would permit comparison between pre- and post-spill communities. Post-spill assessments must primarily rely on comparisons among sites with variable degrees of oiling, which in turn raises issues of how to distinguish oiling effects from other between-site differences. Ideally, studies would sample a large number of sites for a range of oiling intensities, allowing oil effects to be statistically disambiguated from other sources of variance. However, sampling on this scale is often not feasible due to logistical and funding constraints. Furthermore, every oil spill is chemically distinct, and the fractions of oil moving in different portions of the water column at different points of time during the spill can be chemically different. True replication may therefore be elusive for field studies. Another challenge is that taxonomically identifying infaunal samples requires extensive time and expertise. Better *a priori* hypotheses for which taxa were most likely to respond to oiling would allow future studies to target limited resources on examining the taxa most likely to be impacted.

Here we describe infaunal communities from a pair of sites located in the Chandeleur Islands, LA. One site was moderately contaminated with DWH oil (as determined by Shoreline Cleanup and Assessment Technique maps and published chemical analyses), the other was only lightly oiled but is otherwise physically similar. This pairing permits a focused assessment of taxonomic differences at sites that differ primarily in their oiling history. We sampled in summer and fall of 2015 and 2016, five and 6 years after the DWH spill. The more-oiled site experienced a reoiling event in summer of 2016, in which a tar slick moved transiently through the site. This affords a unique opportunity to document acute community responses to an oiling event, with comparisons to a control site where no known re-oiling occurred. Previous work has suggested that infaunal responses to oil can be mediated by both toxicity and organic enrichment (Daan et al., 1994; Montagna and Harper, 1996; Pashaei et al., 2015). If community-level effects of oiling are dominated by toxicity, then we would predict reduced abundance and diversity at oiled sites. Alternatively, if organic enrichment facilitates deposit feeders, then we might expect to see increased abundances for deposit feeding taxa (Daan et al., 1994; Montagna and Harper, 1996), with possible indirect effects at higher trophic levels. These impacts may affect

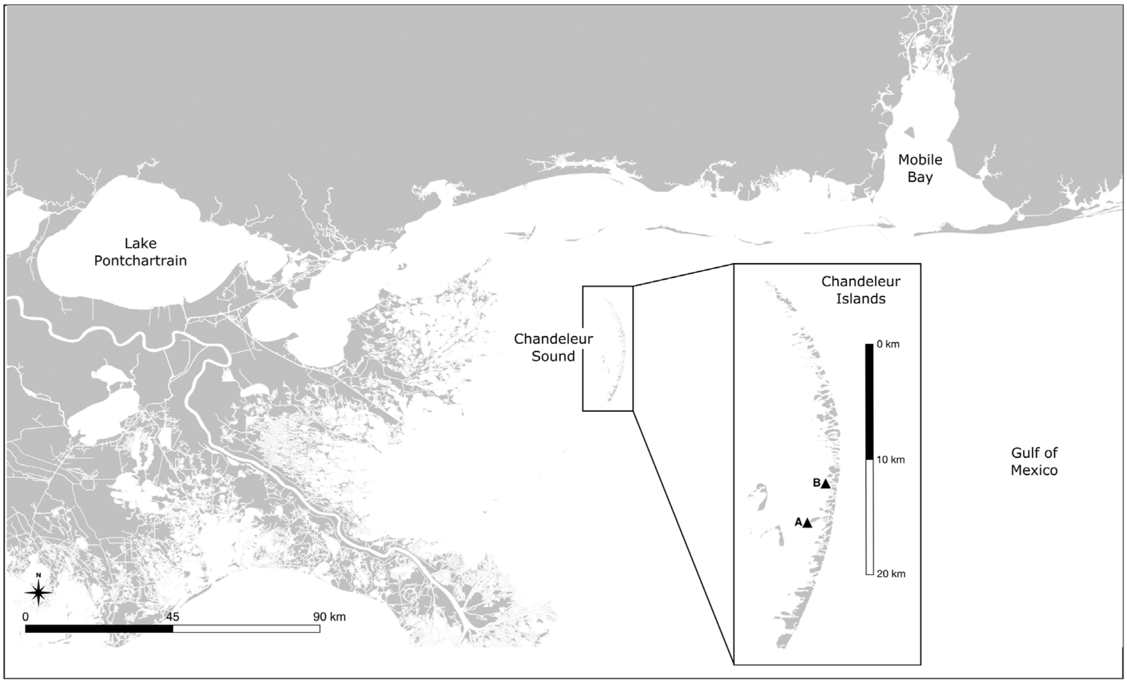

FIGURE 1
Map of the Northern Gulf of Mexico, showing the study sites in Louisiana's Chandeleur Islands (inset). Site A (29°51′36″N 88°50′24″W) showed low to no evidence of hydrocarbon contamination, while site B (29°53′24″N 88°49′48″W) showed elevated hydrocarbon concentrations throughout the upper 15 cm of sediment. The sites are 3.7 km apart and show similar physical characteristics.

entire functional groups in similar ways, or species within functional groups may show distinct responses. Species may share inherited traits making them more susceptible to oil disturbance, in which case larger taxonomic groups or evolutionary history may be useful in assessing impacts of oil on communities (addressed in Kiskaddon et al., this issue). By comprehensively evaluating the benthic communities at these sites, we provide insight into different strategies of assessing benthic community recovery, thus advancing *a priori* hypotheses to inform future work.

2 Methods

2.1 Sampling and site selection

The Chandeleur Islands are a chain of low-lying uninhabited barrier islands in the NGOM. Following the DWH spill, no cleanup activities were conducted at the Chandeleurs, making them an ideal location to study oiling effects. The distribution of oil across the barrier island chain was patchy, allowing us to select oiled-unoiled pairs of sites within close proximity. Sites were chosen in collaboration with the ACER consortium (Alabama Center for Ecological Resilience, (Tatariw et al., 2018; Zerebecki et al., 2021). Oiled sites were identified first, then matched to nearby less-oiled but physically similar sites to serve as controls. Both oiled and control sites were initially identified using National Oceanic and Atmospheric Administration (NOAA) Shoreline Cleanup and Assessment Technique (SCAT) maps (Michel et al., 2013), then confirmed by chemical analysis of sediment cores (Robertson and Baltzer, 2017; Tatariw et al., 2018). Here we present data from a single oiled/control pair of sites designated A and B; site A is the less-oiled “control”, while site B is more heavily oiled (Figure 1; Table 1). Both sites are shallow-water (30–50 cm depth) sandy mud flats fringing Chandeleur Sound with large *Ruppia* seagrass beds and areas of unvegetated sediment (Table 2). The seagrass *Thalassia testudinum* is also sporadically present. The sites are separated by only 3.7 km, and experience similar salinity, temperature, and dissolved oxygen concentrations (Supplementary Figure S1). Both sites experienced oiling during the DWH spill, but SCAT maps showed more spatially extensive oil at site B (see Zerebecki et al., 2021 Figure 1). SCAT maps are useful but are only one snapshot in time; sediment polycyclic aromatic hydrocarbon (PAH) levels provide more robust evidence of oiling history. Tatariw et al. (2018) assessed oil residues from marsh sediments near both sites and found that Site A (their ‘Site 1’) was only lightly oiled while Site B (their ‘Site 2’) was moderately to heavily oiled. These findings are congruent with data showing higher Benzo(a)pyrene concentrations at Site B in

TABLE 1 Sampling periods, dates, and sample sizes for *Ruppia* and unvegetated ('open') habitats at Site A (unoiled) and Site B (oiled).

		Ruppia		**Open**	
Sampling period	**Dates**	**Site A**	**Site B**	**Site A**	**Site B**
Summer 2015	June 29-July 1	12	8	10	10
Fall 2015	September 21–23	9	8	8	6
Summer 2016	June 13–15	12	14	10	14
Fall 2016	September 16–18	12	18	8	16

TABLE 2 Summary of environmental data for each site. Temperature and DO (means ± SD) were measured from HOBO oxygen sensors being used to measure sediment metabolism at each site (Dorgan et al. in preparation).

Site	**Lat/Lon**	**Temp °C**	**DO (mg/ml)**	***Ruppia* % sand**	**Open % sand**
A	29°51′36″N 88°50′24″W	29.1 ± 2.06	6.81 ± 2.70	50–93%	78–93%
B	29°53′24″N 88°49′48″W	29.5 ± 281	5.05 ± 3.64	48–87%	50–87&

marsh samples of summer 2015 (Robertson and Baltzer, 2017) and in intertidal saltmarsh, subtidal *Ruppia* seagrass (most likely *R. maritina,* hereafter referred to as *Ruppia*), and subtidal unvegetated mud samples of fall 2015 (A. Robertson ms in preparation and pers. Comm; Benzo(a)pyrene is commonly used as an indicator of long-term organic polyaromatic hydrocarbon pollution (Vane et al., 2020). Taken together, these data support the designation of site A as a "control" site suitable for comparison to the more-heavily oiled Site B.

Sampling was carried out during four time periods in the summer and fall of 2015 and of 2016 (Table 1). Within each site, we sampled *Ruppia* seagrass habitats as well as unvegetated mud (n = 8 to 18 per season/site/habitat, Table 1). Each sample consisted of either a 14.5 cm diameter x 15 cm deep sediment core or a 9.5 cm diam x 15 cm deep core, collected from mud submerged in 15–40 cm water depth. The larger cores were tied to a sediment metabolism study being conducted concurrently, which required larger cores to accommodate sensors (Dorgan et al. ms in preparation); the smaller cores were added to increase our sample size with less processing time. Abundances were standardized to the larger core using the formula $(A_{observed} \cdot 0.0071\ m^{-2}) \cdot 2.324 = A_{adjusted} \cdot 0.0165\ m^{-2}$. Each core was sieved in the field; organisms retained on a 500 µm mesh were transferred to 95% ethanol with Rose Bengal stain ($0.05\ g\ L^{-1}$). Organisms were later sorted, identified to the lowest taxonomic level possible, and counted.

In summer 2016, we discovered a large slick of crude oil at the oiled site, Site B. The slick measured roughly 50 cm wide by 5 + m long and was caught in vegetation at the edge of the marsh, immediately below the water line (Robertson et al. in preparation), and was closer to the *Ruppia* seagrass beds than it was to unvegetated mud. The slick may have been transported into the site at some point after our fall 2015 sampling, perhaps during a storm. Alternatively, it may have been buried below sediment surfaces and then exposed by scour during a storm. Either way, we interpret this as a reoiling event. The slick was no longer evident when we returned to the site in fall 2016, although oil was detected nearby (~650 m away) in Nov. 2016 and Feb. 2017 (Tatariw et al., 2018).

It is important to recognize that samples from a single pair of sites cannot statistically enumerate an "oil effect"—such estimation would require replicate random sampling from a theoretical population of "oiled" sites in comparison to a population of unoiled sites. Rather, this study provides an in-depth comparison of two sites that are known to differ primarily in their oiling history. Furthermore, the re-oiling event that we document is a truly unique opportunity to observe immediate responses to oiling in a single place and time.

2.2 Community structure

Non-metric Multidimensional Scaling: To compare community structure at oiled versus unoiled sites, we conducted a non-metric multidimensional scaling (NMDS) analysis using the R package 'vegan' (Oksanen et al., 2013). We used 'isoMDS' (k = 2) with a Bray-Curtis dissimilarity matrix obtained via 'vegdist'. Differences were assessed using permutational multivariate analysis of variance (PERMANOVA, 999 permutations) using the function 'adonis2', and assumptions of homogeneity of dispersions were tested using the function 'betadisper'. A three-way model for site × habitat × sampling period suffered from significant heterogeneity of dispersions, primarily associated with higher dispersion following the summer 2016 reoiling event. Unlike univariate ANOVA, there is no alternative to PERMANOVA when assumptions are violated (Anderson, 2014; Anderson et al., 2017). It is well known that infaunal communities are highly variable across seasons and years, making temporal variability the least biologically interesting model term. Thus, to focus more deeply on patterns for sites and habitats, we rejected the three-way model and focused instead on two-way PERMANOVAs (again evaluated for homogeneity of dispersion) for site × habitat effects within each sampling period.

Other Diversity Metrics: We assessed differences in standard diversity metrics (Shannon, Simpson, and Inverse Simpson diversity) plus total abundance, taxon richness, and functional richness. Taxon richness was estimated at the family level for taxa that could be reliably identified to family or lower; but at the level of ‘order’ when lower identification would have been prohibitively time-consuming (see Table 1). Statistical methods for these univariate metrics are detailed below.

2.3 Taxon-level analyses

To evaluate which taxa contribute most to differences revealed by NMDS, we examined patterns in abundance for the most abundant taxa observed. We also comprehensively explored the data for all individual taxa and for other levels of aggregation (i.e., genus, functional groups, feeding guilds) to ensure that we were not missing important patterns, but no consistent patterns emerged. Analyses presented here were conducted at the family level, but we have verified that aggregating taxa at the genus level does not change any interpretations.

2.4 Statistical methods for univariate analyses

2.4.1 General procedure

We examined community and taxon-level responses to oiling and habitat type using ANOVA. Abundance and diversity data were first evaluated for normality and square-root transformed if needed. We then examined a three-way model for site × habitat × sampling period, and examined residuals for heteroscedasticity. In many cases, residual patterning suggested a poor model fit. Rather than attempt three-way non-parametric tests, we employed separate two-way ANOVAs for site × habitat within each sampling period. This approach is reasonable because seasonal and inter-annual variability is expected in benthic habitats, and we were not primarily interested in estimating year or season effects. Non-significant interaction terms were dropped, and if the ‘habitat’ main effect was non-significant it, too, was dropped in favor of a one-way ANOVA for the ‘site’ main effect. All significant ANOVAs were followed by Tukey post-hoc tests (after adjusting for normality and checking residuals for heteroscedasticity).

If residual patterns suggested that two-way ANOVA model assumptions were violated, we instead performed the non-parametric Kruskal–Wallis test. Kruskal–Wallis cannot handle two-way interactions, so we pooled together data from both habitats and assessed the ‘site’ term only (after confirming that separate Kruskal–Wallis tests for each habitat would not have altered our interpretation, not shown).

2.4.2 Constraining the false discovery rate

Ecological data can be explored at many taxonomic or functional levels, leading to multiple comparisons in most community studies. This can compound the risk of Type I error–falsely rejecting a true null hypothesis. In the ecological literature, there is little consensus about when and how to offset this risk (reviewed Cabin and Mitchell, 2000; Moran, 2003; García, 2004); many published studies ignore the issue entirely. In this study, we were primarily interested in evaluating differences between sites. Because we may or may not expect to see an oil effect, this is where the greatest risk of false discovery lies. We also evaluated ‘habitat’ effects, because infaunal communities are usually different in seagrass versus unvegetated areas (Bostrom et al., 2006; Rodil et al., 2021). Given this strong *a priori* knowledge, we see false discovery in the ‘habitat’ term as being unlikely. We have therefore applied the Benjamini–Hochberg (B-H) method to adjust p-value interpretations for the ‘site’ main effect for all ANOVA and Kruskal–Wallis analyses. We report raw p-values throughout the main text; the B-H correction did not alter interpretation of most tests, and the few exceptions will be clearly indicated. A more detailed explanation of false discovery rate and our rationale is in Supplementary Appendix S1; results of the B-H procedure are in Supplementary Table S2.

3 Results

3.1 NMDS analysis

We used NMDS and PERMANOVA to evaluate community composition for *Ruppia* vs unvegetated habitats at oiled vs unoiled sites in summer and fall of 2015 and 2016. Oiled and unoiled sites cluster separately, as do *Ruppia* and unvegetated habitats within each site (Figure 2). Site, habitat, and site × habitat interactions were highly significant in all four sampling periods (Table 3; full statistics in Supplementary Appendix S2). However, site (oiled vs. unoiled) had the greatest explanatory power for both summer sampling periods (2015 R^2 = 0.25; 2016 R^2 = 0.21). For fall samples, the site and habitat terms captured similar variance in 2015 (site R^2 = 0.17; habitat R^2 = 0.19), but the habitat term captured more variance in fall 2016 (R^2 = 0.28) (Table 3 and Supplementary Appendix S2). Notably,

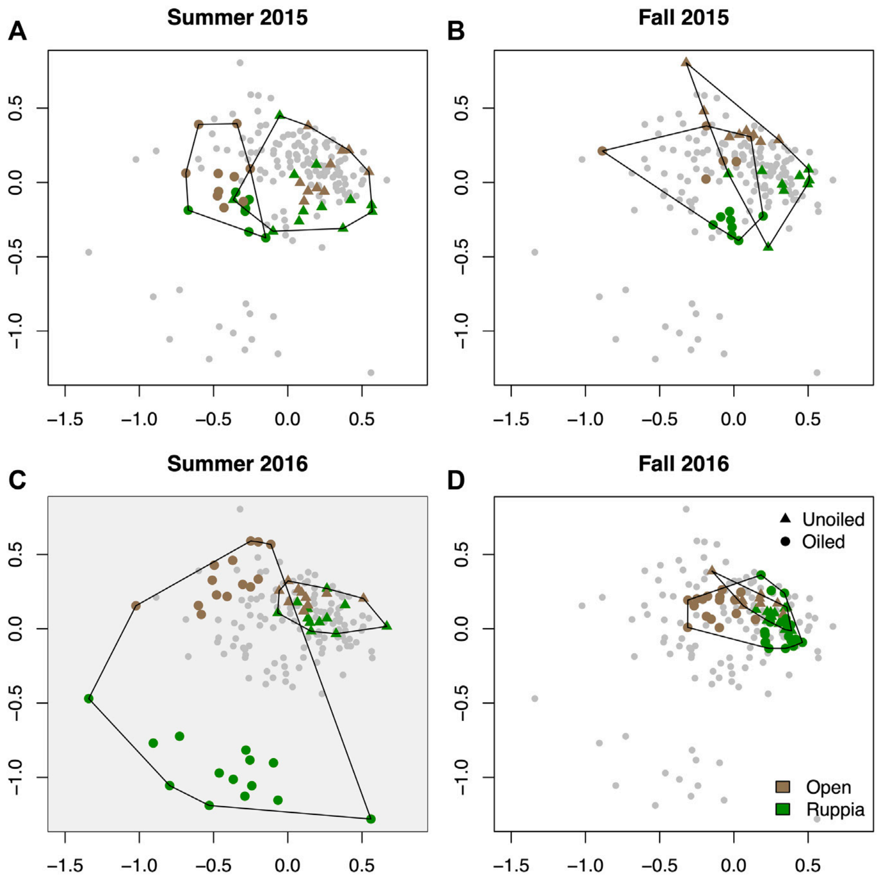

FIGURE 2
Nonmetric Multidimensional Scaling for infaunal communities in cores from unvegetated or *Ruppia* habitat at oiled vs. unoiled sites in each of the four sampling periods (**A**: Summer 2015; **B**: Fall 2015; **C**: Summer 2016; **D**: Fall 2016). A single ordination was performed using family-level abundance data from all four sampling periods (stress = 0.205). Each plot highlights samples from a single year/season. The convex hulls enclose all samples from a given site, while habitats are differentiated using color. In each sampling period, both Site and Habitat were significantly different by PERMANOVA (Table 2, Supplementary Appendix S2). The Summer 2016 panel is highlighted to emphasize the reoiling event observed at that time.

there was no overlap between the cluster of samples from the *Ruppia* habitat at the oiled site in Summer 2016 and samples from other habitats and sampling times (Figure 2).

3.2 Univariate analyses

3.2.1 Community-level responses

To explore community-level patterns more deeply, we examined total abundance, taxonomic richness, and Shannon diversity as a function of site and habitat within each sampling period. Total abundance was 2-fold–6-fold lower at the oiled site following the reoiling event in summer 2016 (Figure 3A; Table 3, Supplementary Appendix S2; site $F_{1,46}$ = 48.3, p << 0.0001; habitat $F_{1,46}$ = 0.52, ns; site x habitat $F_{1,46}$ = 11.04, p << 0.0001). However, neither 'site' main effects nor interaction terms were significant in other sampling periods (Table 3 and Supplementary Appendix S2). Unvegetated habitats had lower abundances than *Ruppia* habitats during both fall sampling periods, but not in summer samples (Table 3 and Supplementary Appendix S2).

Taxonomic richness was 2.6-fold lower at the oiled *Ruppia* site following the summer 2016 reoiling event (Figure 3B; Table 3; Supplementary Appendix S2; site $F_{1,46}$ = 39.81, p << 0.0001), but showed no site differences in other sampling periods. (Supplementary Appendix S2). Note that the 'site' term yielded a *p*-value of 0.03 for Summer 2015, but this was not significant after applying the B-H procedure (Supplementary Table S2). Overall, richness was higher in *Ruppia* than in unvegetated habitats for all four sampling periods, particularly at the less oiled site (Figure 3B, Supplementary Appendix S2).

Shannon diversity was 28% lower at oiled *Ruppia* habitats following the summer 2016 reoiling event (Figure 3C; Table 3, Supplementary Appendix S2 site $F_{1,138}$ = 10.2, p = 0.002). Overall,

TABLE 3 Summary of p-values for community-level analyses. PERMANOVA results from PERMANOVA apply to the NMDS analysis shown in Figure 2. ANOVA results accompany Figure 3. Full statistical tables are in Supplementary Appendix S2. When a $p < 0.05$ is marked (ns), this indicates that the p-value should not be considered significant after applying the Benjamini–Hochberg procedure (Supplementary Table S2).

	PERMANOVA	ANOVA		
	NMDS	$\sqrt{}$Abundance	Taxonomic richness	Shannon diversity
Summer 2015				
Site	0.001	0.16	0.03 (ns)	<< 0.0001
Habitat	0.002	—	0.0002	0.0016
Interaction	0.034	—	—	—
Fall 2015				
Site	0.001	0.77	0.15	0.0012
Habitat	0.001	<<0.0001	<<0.0001	0.0004
Interaction	0.034	—	—	0.13
Summer 2016 (re-oiling event)				
Site	0.001	<<0.0001	<<0.0001	0.0015
Habitat	0.001	0.47	0.3	0.16
Interaction	0.001	<<0.0001	0.0007	0.0052
Fall 2016				
Site	0.001	0.65	0.16	0.08
Habitat	0.001	<<0.0001	0.008	—
Interaction	0.004	—	—	—

however, Shannon diversity patterns were inconsistent across sites and habitats, driving significant interaction terms (Table 3 and Supplementary Appendix S2). Results for the Simpson and Inverse Simpson indices were broadly similar to the Shannon index (not shown).

3.2.2 Individual taxon responses

To understand which taxa contributed most to observed differences at the oiled site, we first identified the most abundant taxa overall. After examining a rank-abundance plot, we identified a breakpoint after the top ten taxa, after which abundances remain low and shallowly decreasing (Supplementary Figure S2). Of the top ten taxa, one was a catch-all group of minute gastropods that we could not identify to family; these were consequently not analyzed further. Another was the annelid family Maldanidae, which we also excluded because it was only abundant in 2015, and almost entirely absent from both sites in 2016. The eight remaining most abundant taxa were the annelid families Capitellidae, Spionidae, Nereididae, Paraonidae, and Ampharetidae; the gastropod *Neritina usnea* (the only neritid in our samples); the bivalve family Tellinidae; and the crustacean order Amphipoda (excluding the tube-building family Ampeliscidae, which was enumerated separately and was less common). In addition to these eight taxa, we examined patterns for the annelid *Arenicola cristata* (the only arenicolid in this region), given *a priori* knowledge about its ecologically important effects on sediment structure and surrounding communities (reviewed Berke, 2010; Volkenborn et al., 2010). Some of these focal taxa showed generally negative responses to oiling. Others showed generally positive responses, while a third group showed mixed or no response. Notably, however, all taxa except *N. usnea* declined in abundance following the summer 2016 reoiling event. Most of these appeared to rebound by fall 2016.

Negative Responses to Oiling: The annelid families Capitellidae, Spionidae, and Arenicolidae generally showed lower abundance at the oiled site (with some exceptions) (Figure 4). This pattern was strongest for *Ruppia* samples–indeed, all three taxa were entirely absent from oiled *Ruppia* following the 2016 reoiling event (S16 capitellid 'site' $F_{1,46} = 392.4$, $p << 0.0001$; spionid $F_{1,46} = 23.78$, $p << 0.0001$; arenicolid K-W $\chi^2_1 = 7.5$, $p = 0.005$; Tables 4-5; Supplementary Appendix S3).

For capitellids, summertime abundance was consistently lower at the oiled site. In fall samples, however, patterns were mixed. The largest effects were observed in *Ruppia* beds. At the oiled site, capitellid abundance in *Ruppia* was 7-to-8-fold lower than at the unoiled site sampling period except fall 2016. In unvegetated muds, capitellids were generally 2-to-6-fold less abundant at the oiled site in every sampling period except fall 2015 (Figure 4; Table 4, Supplementary Appendix S3).

Spionids were 5-fold less abundant in oiled *Ruppia* samples in both summer sampling periods, and 3-fold less abundant in oiled unvegetated samples in summer 2015. However, there were

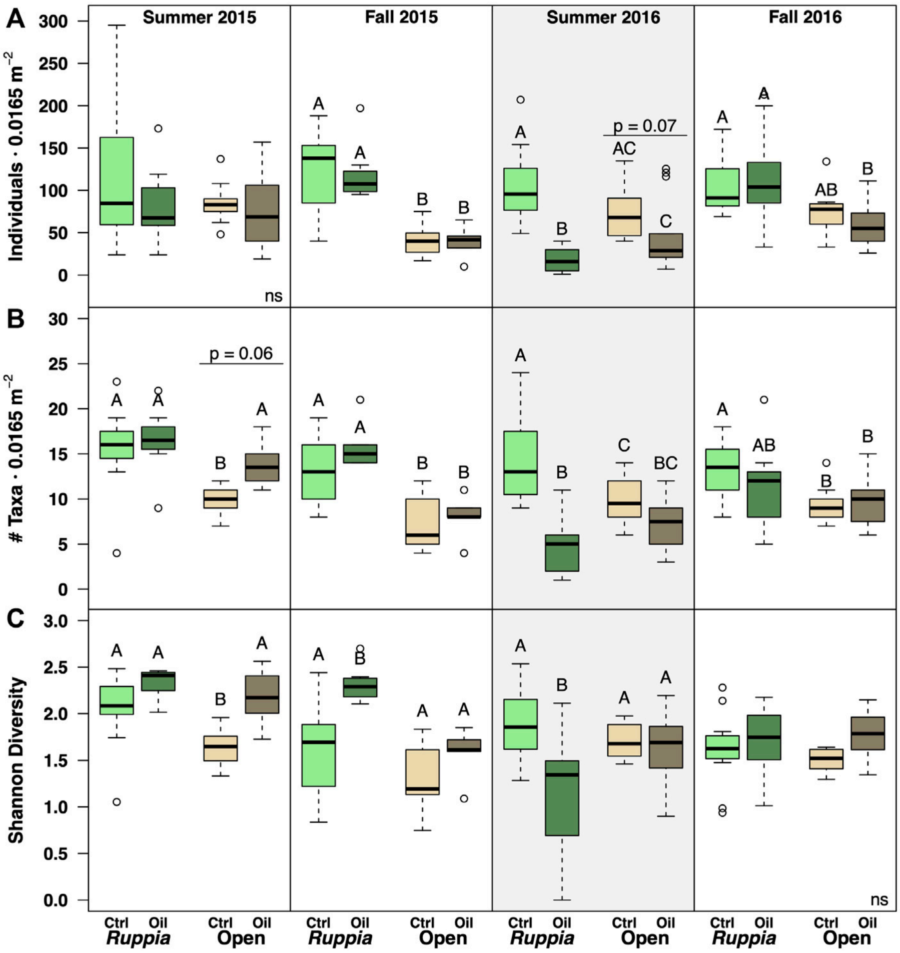

FIGURE 3
Diversity and abundance metrics for oiled vs. unoiled sites in unvegetated vs. *Ruppia* habitats for each sampling period. The Summer 2016 panels are highlighted to emphasize the reoiling event observed at that time. Letters indicate Tukey groupings; full ANOVA results are given in Table ANOVA. **(A)** Total abundance per core; **(B)** Taxonomic richness (taxa defined at the family level, or higher if ID'ing to family was not possible). **(C)** Shannon diversity index; other diversity indices showed similar patterns.

no site differences in fall samples (Figure 4; Table 4, Supplementary Appendix S3).

Positive Response to Oiling: The gastropod *N. usnea* was notably *only* found at the oiled site, being entirely absent from the control site. (Figure 5; Table 5, Supplementary Appendix S3). *N. usnea* was also primarily confined to *Ruppia* beds, with abundances ranging from 0.63 ± 1.41 to 18.25 ± 11.49 ind · 0.0165 m^{-2}.

The families Tellinidae (Bivalvia) and Nereididae (Annelida) were either more abundant at the oiled site or showed no pattern. The important exception, however, was that both taxa were less abundant in *Ruppia* beds at the oiled site following the summer 2016 reoiling event, when nereids were nearly 5-fold less abundant at the oiled site (ctrl 2.03 ± 1.8 vs. oiled 0.43 ± 0.85 ind · 0.0165 m^{-2}) and tellinids nearly 20-fold less abundant (ctrl 5.4 ± 4.5 vs. oiled 0.29 ± 0.60 ind · 0.0165 m^{-2}). Otherwise, Nereids were 3-fold more abundant at the oiled *Ruppia* site in both fall 2015 and 2016, and in unvegetated mud in 2015. Tellinids were at least 3-fold more abundant at the oiled site (both habitats) in summer 2015, but showed no 'site' response in fall samples.

Mixed Response to Oiling: The remaining most abundant taxa—Crustacean amphipods, and the annelid families Paraonidae and Ampharetidae—all showed mixed responses to oiling in general, but nonetheless declined in *Ruppia* after the 2016 reoiling event (Figure 6). For ampharetids, this pattern continued into fall 2016. In contrast, however, both ampharetids and paraonids were more abundant at the oiled site throughout 2015. Amphipods showed no clear patterns outside of the

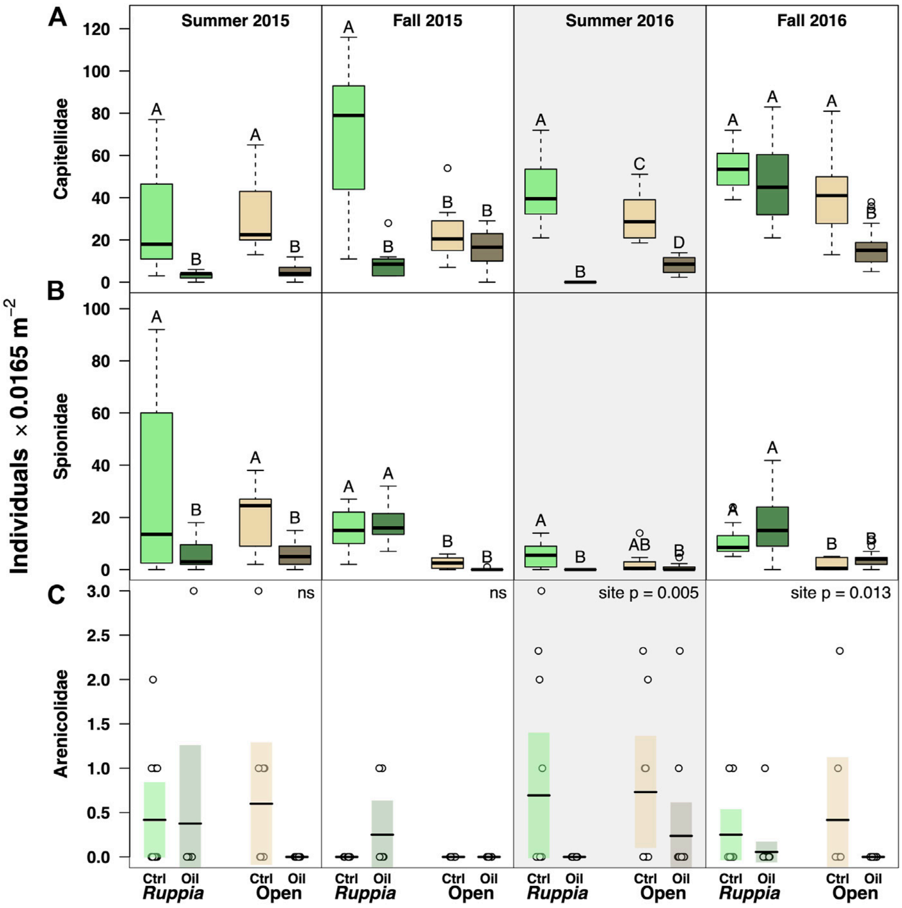

FIGURE 4
Abundance data for families that showed negative responses to oiling (all here are in phylum Annelida). The Summer 2016 panels are highlighted to emphasize the reoiling event observed at that time. For panels **(A,B)**, boxes with different letters are different by Tukey post-hoc tests. Arenicolid data **(C)** was heavily dominated by 0 values, making boxplots inappropriate visualizations–instead, we are showing raw data (circles) overlaid with means (horizontal bars) and 95% confidence intervals for the mean (shaded rectangles; in cases where no 95%CI is visible, all abundances were '0').

reoiling event (Figure 6; Tables 4-5, Supplementary Appendix S3).

4 Discussion

4.1 Major patterns

While community structure clearly differed between sites in every sampling period, our single most consistent finding is widespread negative responses to the reoiling event of summer 2016. The slick we observed was caught in marsh grass near the shoreline and was closest to the site's *Ruppia* habitats. It is therefore unsurprising that the strongest responses were observed in *Ruppia*–indeed, all univariate metrics, at both the community and taxon level, declined in *Ruppia* at the oiled site in summer 2016. The sole exception was *Neritina usnea* abundance, discussed more below. Unvegetated samples qualitatively showed a similar trend, and 'site' main effects were pervasively significant for summer 2016. These acute responses, however, appear to have been transient: the heavily oiled site looked generally similar to the less-oiled site in fall 2016 samples. We cannot know how long the slick remained at the site; we do know that it was gone during the fall 2016 sampling (for that matter, we cannot know that the control site never experienced re-oiling; we do know that we never observed such an event there, and oil contamination in sediments from the control site were lower.) This type of transient reoiling was also noted by Beyer et al. (2016), who reviewed information about the changing toxicity profiles of tar balls and other weathered oil. Understanding and predicting oil slick movement has long been a focus of oceanographic research, but most work has focused on short-term modeling in the cleanup phase of an oil spill (Kim et al., 2014; Lee et al., 2020). Understanding how small slicks move around marine habitats over scales of years and decades has, to our knowledge,

TABLE 4 Summary of ANOVA p-values for square-root transformed abundances of individual taxa (Figures 4–6). Full ANOVA tables are in Supplementary Appendix S3; statistics for taxa with non-normal abundances are in Table 5. In this table, all $p < 0.05$ remain significant after applying the Benjamini–Hochberg procedure (Supplementary Table S2).

	Decreasing		Increasing		Mixed	
	Capitellidae	Spionidae	Nereididae	Tellinidae	Ampharetidae	Amphipoda
	(Annelida)	(Annelida)	(Annelida)	(Bivalvia)	(Annelida)	(Crustacea)
Summer 2015						
Site	<<0.0001	0.0013	0.6	<<0.0001	<<0.0001	0.001
Habitat	—	—	<<0.0001	—	0.0015	0.14
Interaction	—	—	—	—	0.1	—
Fall 2015						
Site	<<0.0001	0.23	<<0.0001	0.39	0.0001	0.15
Habitat	0.09	<<0.0001	<<0.0001	—	0.0005	0.0008
Interaction	0.01	0.03	—	—	0.05	—
Summer 2016 (re-oiling event)						
Site	<<0.0001	<<0.0001	0.007	0.002	0.0003	<<0.0001
Habitat	0.0003	0.3	0.00067	0.0004	0.046	0.0013
Interaction	<<0.0001	0.01	0.027	0.008	0.09	0.09
Fall 2016						
Site	0.0002	0.03	0.09	0.35	0.19	0.9
Habitat	<<0.0001	<<0.0001	0.001	<<0.0001	0.01	<<0.0001
Interaction	0.02	—	0.0017	0.05	—	—

received less attention; slicks like the one we observed are probably too small to detect in satellite imagery, which could make them difficult to track and model. Evaluating how smaller oil slicks move in shallow marine environments, and how communities respond to their presence, would be an interesting target for further research.

4.2 Study limitations

The observational nature of this study makes it impossible to fully rule out the possibility that site differences were caused by some unknown factor(s), unrelated to oil. However, given the sites' similarity in terms of temperature, salinity, oxygen saturation, sediment type, and vegetation, we see oiling history as the most likely explanation. Quantitatively estimating what proportion of between-site variability is attributable to oil would naturally require a much larger study.

4.3 Taxa of special interest

Shifts in community structure appear to have been driven by strong responses in select taxa, some negative but others positive. The two most abundant taxa, polychaete families Capitellidae and Spionidae, clearly appear sensitive to oil at these sites. For capitellids this is surprising, given that *Capitella capitata* is a well-known indicator species with high tolerance for environmental pollutants (Dean, 2008). *C. capitata* can metabolize at least some PAHs (Forbes et al., 2001), and has dominated oil-contaminated muds in the Niger river delta (Zabbey and Uyi, 2014)and the Netherlands (Daan et al., 1994). Unfortunately, capitellids are one of the more challenging groups to identify beyond family, and genus-level identification would have been prohibitively time-consuming given the large scope of taxonomic work for this study. However, reexamining a subset of our samples showed that *Capitella cf. Capitata* is certainly present at these sites, comprising ≥50% of the individuals in Capitellidae overall. Interestingly, all capitellids at the oiled *Ruppia* site in fall 2016 were *C. cf. Capitata*, whereas the unoiled *Ruppia* site had a mix of capitellid species (Supplementary Figure S3). Together with the total absence of capitellids at the reoiled *Ruppia* site in summer 2016, this suggests that oil toxicity overwhelmed *C. cf. Capitata* during the reoiling event, but the species recruited opportunistically once the oil slick had moved away.

The lugworm *Arenicola cristata* also declined in samples from the oiled site. While not highly abundant, it did occur commonly (ranging from 0 to 3 worms per core), and is by far the largest polychaete we observed (most individuals 3–4 cm in length). Lugworms are well-known ecosystem engineers, building deep

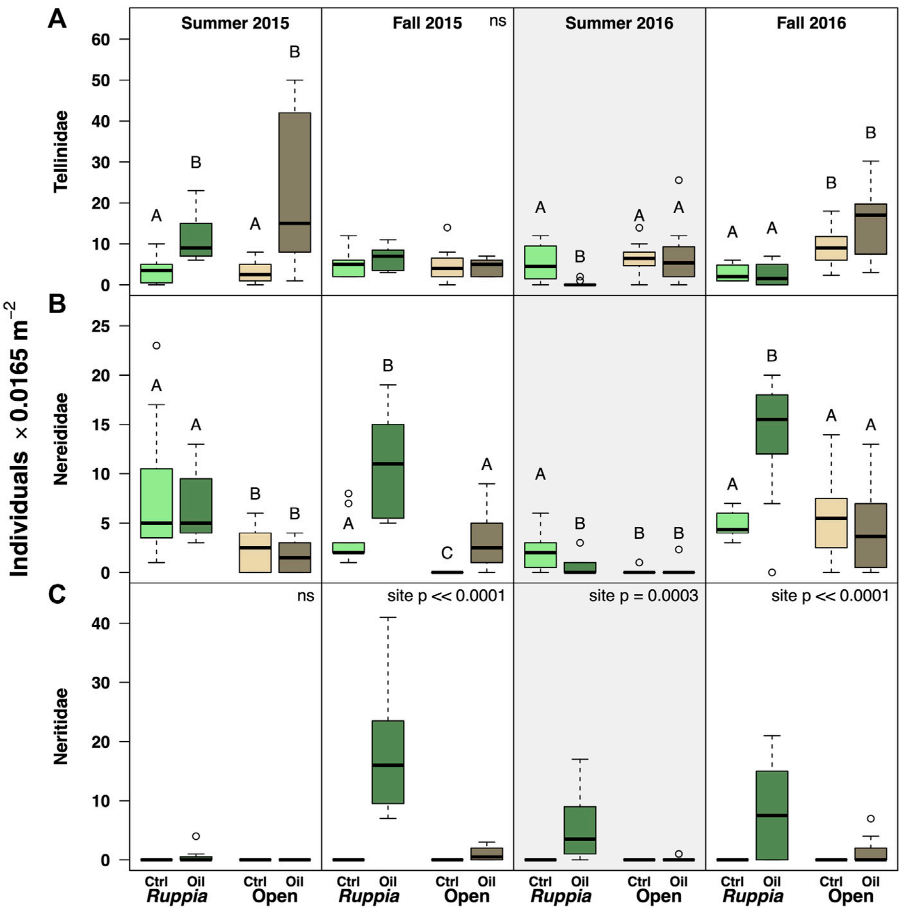

FIGURE 5
Abundance data for families that showed positive responses to oiling **(A)** Tellinidae (Mollusca: Bivalvia). **(B)** Nereididae (Annelida). **(C)** Neritidae (Mollusca:Gastropoda), here represented by *Neritina usnea*. The Summer 2016 panels are highlighted to emphasize the reoiling event observed at that time. Boxes with different letters are different by Tukey post-hoc tests.

TABLE 5 Summary of Kruskal–Wallis *p*-values for site differences for individual taxon abundances that could not be adjusted for normality. Full Kruskal–Wallis tables are in Supplementary Appendix S3. When a $p < 0.05$ is marked (ns), this indicates that the *p*-value should not be considered significant after applying the Benjamini–Hochberg procedure (Supplementary Table S2).

	Decreasing	Increasing	Mixed
	Arenicolidae (Annelida)	**Neritidae (Gastropoda)**	**Paraonidae (Annelida)**
Summer 2015	0.03 (ns)	0.11	0.0003
Fall 2015	0.11	<<0.0001	0.0002
Summer 2016 (re-oiling event)	0.005	0.0003	0.84
Fall 2016	0.013	<<0.0001	0.25

burrows of compacted sediment that they actively irrigate (Wells, 1966). The hydraulic forces generated by irrigation and defecation events drive porewater movement laterally at distances of 10–20 cm from the burrow (Volkenborn et al., 2010). Lugworms also have a unique funnel-feeding mechanism in which they pull surface sediment down into the burrow for feeding then later defecate on the surface. Together, their feeding and burrowing activities contribute to vertical mixing in the upper 10–20 cm of sediment (Volkenborn and Reise, 2007). Even modest changes to lugworm density may therefore have substantial changes on sediment

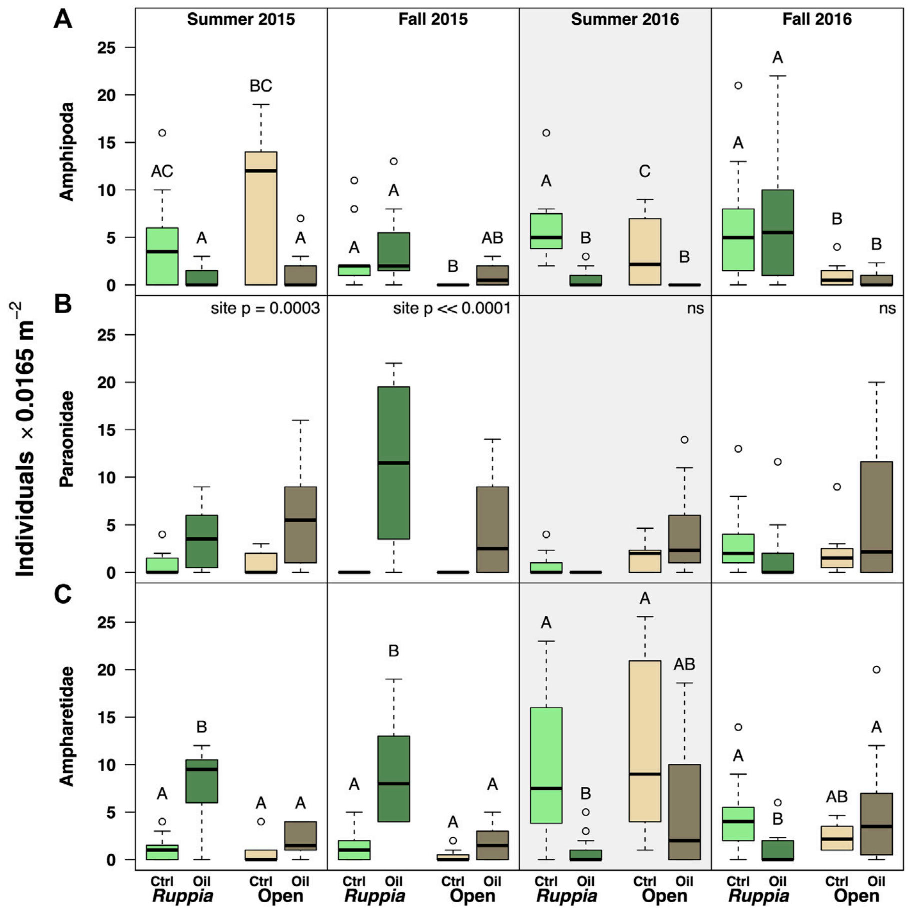

FIGURE 6
Abundance data for abundant taxa that showed mixed responses to oiling **(A)** Amphipoda (Arthropoda:Crustacea). **(B)** Paraonidae (Annelida). **(C)** Ampharetidae (Annelida). The Summer 2016 panels are highlighted to emphasize the reoiling event observed at that time. Boxes with different letters are different by Tukey post-hoc tests.

microbial communities and nutrient cycling, with possible cascading effects to other infauna. Experimentally excluding lugworms can increase polychaete diversity (Volkenborn and Reise, 2007); it is possible that tellinids and nereids increased at the oiled site in part due to the near-total absence of bioturbation and disturbance by lugworms. That said, baseline lugworm density is much lower here than that reported by Volkenborn and Reise (2007), so lugworm impacts may not be as extensive.

While several taxa increased in abundance at the oiled site, these effects were inconsistent over time, making interpretation difficult. However, the pattern is unequivocal, however, for *Neritina usnea,* which we observed *only* at the oiled site, even after the reoiling event. Moreover, while previous information on *N. usnea* in seagrass beds is scarce, densities at the oiled site greatly exceed published abundances for other *N. usnea* populations in *Halodule wrightii* beds from the northern Gulf of Mexico (Cebrian et al., 2013). This suggests that attention should be directed at evaluating the mechanisms driving this snail's positive response. *N. usnea* differs from other taxa surveyed in being comparatively large-bodied (~2–5 mm shell length) and entirely epibenthic, found primarily on seagrass blades. We considered the possibility that *N. usnea* larvae cannot access the control site, but *N. usnea* is similar to other taxa from these samples in having planktonic larvae (Heard and Lutz, 1982). An explanation invoking larval supply would thus need to explain why *N. usnea* larvae are uniquely unable to get to this site when all other pelagic dispersers are unhindered. A post-settlement explanation seems more likely; we could speculate that these snails may be exploiting a predation refuge at the oiled site (i.e. if oil-contaminated sediments are less attractive to mobile crustacean and fish predators, then predation risk could be lower at the oiled site). Our sampling methods would not capture mobile epibenthic fauna, but future work exploring epibenthic communities in oiled seagrass beds would be highly valuable.

5 Conclusion

Most studies of infaunal responses to oil spills have found evidence that oil toxicity generally reduces abundance/diversity (Montagna et al., 2013; Reuscher et al., 2020), with notable exceptions for some more tolerant opportunistic species (Daan et al., 1994; Bejarano and Michel, 2010; McCall and Pennings, 2012; Pashaei et al., 2015). Long term effects may be more complex. While high marsh fauna in the Chandeleurs were less abundant at oiled sites more than 5 years after the DWH (Zerebecki et al., 2021), more nuanced trophic cascades can also drive longer-term patterns. Montagna and Harper (1996) suggested a paradigm for long-term effects in which organic enrichment from oil can boost deposit-feeding taxa, even as toxicity continues to reduce abundance of more sensitive taxa. In contrast, the numerically most abundant subsurface deposit feeder in our study–Capitellidae–was less abundant at the oiled site. However, this did not extend to other deposit-feeding groups such as Paraonidae (or Orbiniidae, not shown), and organic enrichment could contribute to positive responses in Tellinidae (which switch between surface deposit and suspension feeding) and the omnivorous Nereididae. More broadly, we examined data for each of the major infaunal feeding modes, but found no clear patterns. Similarly, we found that for these sites, incorporating evolutionary relatedness by calculating phylogenetic diversity did not add to our understanding of oil impacts on infaunal communities; instead phylogenetic diversity was strongly correlated with taxon richness (Kiskaddon et al., this issue). Rather than grouping by feeding mode, we would recommend that future studies examine (at minimum) tellinid bivalves plus four polychaete families: Capitellidae, Spionidae, Nereididae, and Arenicolidae. A focus on *N. usnea* merits consideration as well when impacts of oiling is assessed in seagrass beds. These recommendations acknowledge that multiple sampling methodologies may need to be conducted to quantify adequately the targeted benthic taxa.

Given the reoiling event we observed, it is difficult to truly know how often and how recently these communities have experienced acute oil exposure. Long term responses to oiling may be more dynamic than realized, as oil continues to move about habitats. Deeply understanding the long-term effects of oil spills may require more active monitoring over long periods of time, with an eye towards quantifying the continued movement of smaller tar-balls and oil slicks. Outside of reoiling events, it seems clear that oiled sites can exhibit high levels of infaunal diversity. However, stable 'recovery' from an oil spill may not be possible as long as mobile oil remains in the habitat. At regional scales, the spatial and temporal extent of reoiling may determine how long it takes to fully return to pre-spill levels of ecosystem functioning.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://data.gulfresearchinitiative.org, doi:10.7266/N7CF9NFD.

Author contributions

SKB, KD, and SB planned the study. SKB and KD oversaw all field collection and taxonomic work. SKB, KD, EPK, KG, WC, ELK, and TC participated in field work and taxonomic work. SKB conducted the analyses and wrote the paper. KD, SB, and EPK provided valuable feedback and helped refine the draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.950458/full#supplementary-material

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Drift macroalgae positively influence seagrass-associated nekton communities of the northern Gulf of Mexico

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Drift macroalgae, often found in clumps or mats adjacent to or within seagrass beds, can provide additional food resources and habitat complexity, leading to increased animal abundance, but large concentrations can also inhibit faunal movements, smother benthic communities, and contribute to hypoxia, reducing nekton abundance. Despite its ubiquity, few studies have quantified drift macroalgal prevalence over large spatial scales or its effects on seagrass-associated nekton, hindering our understanding of the functional role of drift macroalgae in ecosystems. We quantified the relationship between drift macroalgal biomass and the seagrass-associated nekton community within five estuaries spanning 2000 km across the northern Gulf of Mexico. Overall, increases in macroalgal biomass within seagrass meadows significantly influenced community structure, increasing shrimp, crab, and fish abundances, but the effect varied by region. Relationships between species richness, diversity, organism size, and macroalgal biomass were not observed, suggesting that drift macroalgae provide additional habitat but not necessarily new niche space. Small nekton play a vital role in many local fisheries, providing valuable food resources for fish and invertebrates. Increased recruitment into macroalgae can benefit local fisheries by providing shelter and increased food resources, which may increase the survival, growth, and population size of recreationally and economically important species. While excess levels of drift macroalgae can negatively impact benthic plant and animal communities, particularly in eutrophic areas, the moderate levels observed during this survey were associated with positive effects on organismal abundance, suggesting that drift algal dynamics should be considered in habitat-based management strategies for coastal estuaries.

KEYWORDS

habitat complexity, diversity, abundance, Texas, Louisiana, Florida

Introduction

Despite comprising less than 10% of the ocean area, shallow coastal habitats, such as seagrass meadows, salt marshes, mangroves, and oyster reefs, provide extensive benefits that are both ecologically and economically important. Seagrass habitats alone contribute over \$19,000 ha^{-1} yr^{-1} to local economies (Costanza et al., 1997; Fourqurean et al., 2012; Tuya et al., 2014). When found within seagrass habitats, drift macroalgae can further increase phytobiomass aboveground by 3- to 100-fold (Morris and Hall, 2001), adding to habitat complexity that can support increased animal diversity (Kingsford, 1995). Drift macroalgae often originate as attached algae on seagrass leaves and other hard substrata before becoming uprooted by various physical disturbances (e.g., currents and waves) (Norton and Mathieson, 1983; Bell and Hall, 1997; Biber, 2002; Lirman et al., 2003). They are classified into three major groups: brown algae (Phaeophyceae), green algae (Chlorophyta), and red algae (Rhodophyta; Norton and Mathieson, 1983; Bell and Hall, 1997; Biber, 2002; Lirman et al., 2003), with rhodophytes being the dominant taxa in the northern Gulf of Mexico (Virnstein and Carbonara, 1985; Bell and Hall, 1997; Holmquist, 1997). Seagrass and associated macroalgae create productive ecosystems relative to neighboring unvegetated substrates, providing valuable nursery habitats (e.g., Carr, 1994; Jackson et al., 2001; Heck et al., 2003), enhancing local fisheries, increasing food resources and fish growth rates, and decreasing predation and competition (e.g., Orth et al., 1984; Fraser and Gilliam, 1987; Rooker et al., 1998; Nagelkerken et al., 2002; Jackson et al., 2015).

Drift macroalgae often increase the structural complexity of seagrass habitats (Morris and Hall, 2001) and provide additional refuge, leading to increases in infauna (Hull, 1987), epifauna (Isaksson and Pihl, 1992), and macrofauna, including small crustaceans and mollusks (Norkko et al., 2000; Raffaelli, 2000; Salovius et al., 2005), juvenile blue crabs (Wilson et al., 1990), and small shrimp and fish (Holmquist, 1994; Holmquist, 1997; Kingsford, 1995; Correia, 2021). Relationships between habitat complexity and nekton abundance and species richness have been the focus of many studies, with more complex habitats increasing niche space and leading to increases in the abundance and diversity of animals found there (e.g., Kingsford, 1995; Rozas and Minello, 1998; Minello, 1999). While macroalgae can enhance seagrass ecosystem services, at sufficiently high biomass, they can also lead to hypoxic conditions with resulting declines in animal abundance and species diversity (Hull, 1987; Bonsdorff, 1992; Raffaelli et al., 1998; Cummins et al., 2004; Zajac and McCarthy, 2015). Whether drift macroalgae have positive or negative impacts on the animal community is largely driven by the quantity and type of algae present, with lower drift algal biomass oftentimes leading to higher nekton abundance and species richness (e.g., Everett, 1994; Holmquist, 1997; Norkko et al., 2000; Arroyo et al., 2006) and high biomass leading to a decline in species richness (e.g., Bonsdorff, 1992; Raffaelli et al., 1998). However, understanding the threshold where the net impact of drift algal biomass transitions from positive to negative and the overall role that drift algae play in structuring animal communities remains an ongoing challenge.

Over the past few decades, seagrass cover has declined in the northern Gulf of Mexico, while drift algal abundance has remained constant or increased (Benz et al., 1979; Virnstein and Carbonara, 1985; Zieman et al., 1989; Biber and Irlandi, 2006; Kopecky and Dunton, 2006; Fredericq et al., 2009; Correia, 2021). With future climate projections indicating an increase in macroalgae in accordance with higher sea surface temperatures and ocean acidification (Brodie et al., 2014), contemporary studies are needed to assess both the presence and effects of drift macroalgae on seagrass-associated nekton communities. The purpose of this study was to investigate the relationships between drift macroalgal biomass and small nekton communities on a Gulf-wide scale. Previous comparisons across the northern Gulf of Mexico revealed significant differences in algal biomass across the region and sampling period (Correia et al., in press). Because macroalgae were often more abundant during the early summer months, this study primarily focused on data collected during May–June 2018. To determine how drift algal biomass influences nekton community structure, we performed a survey of drift macroalgae across five estuaries spanning 2000 km of the coastline in the northern Gulf of Mexico, measuring macroalgal biomass as well as the nekton community found within the seagrass-macroalgal mosaic. Specifically, we quantified (1) the relationship between algal biomass and nekton community composition (e.g., abundance, diversity, and taxon-specific variations) and (2) regional variation in these relationships across the northern Gulf of Mexico. An epibenthic sled was used during data collection to sample the smaller epibenthic nekton and invertebrates living within the algal-seagrass matrix, which are more likely to be influenced by variations in algal biomass. Because the seagrass percent cover and structure varied across each region, which could affect our interpretation of algal impacts on fauna, a subset of the data was also analyzed to compare nekton community changes across a range of algal weights when the areal coverage of the dominant seagrass turtle grass (*Thalassia testudinum*) exceeded 75% and canopy heights were between 200 and 400 mm (Correia, 2021). Based on previous findings, we hypothesized that algal biomass would influence the community composition of fauna, increasing the abundance and richness at intermediate algal biomasses.

Materials and methods

Study regions

Five estuaries in the northern Gulf of Mexico, each containing at least 20 sites within seagrass meadows, were surveyed during early (May–June) and late (August–September) summer of 2018 (Supplementary Figure S1). The estuaries sampled included Laguna Madre, TX (LM; $n = 20$; 26°08′N, 97°14′W), Corpus Christi Bay, TX (CB; $n = 20$; 27°51′N, 97°08′W), the Chandeleur Islands, LA (LA; $n = 20$; 29°54′N, 88°50′W), Cedar Key, FL (CK; $n = 25$; 29°05′N, 83°01′W), and Charlotte Harbor (CH; $n = 25$; 26°04′N, 82°14′W). These estuaries encompass two semi-enclosed bays with major freshwater inputs (CB and CH), two sites along the open coastline (CK and LA), and one semi-enclosed lagoon with no major freshwater inputs (LM). Sites were selected by overlaying a tessellated hexagonal grid (500 m edge length) on each estuary in ArcGIS (Moore, 2009; Neckles et al., 2012; Wilson and Dunton, 2012). One station was randomly generated within each of the 20–25 grid cells that contained more than 50% seagrass coverage, with a minimum of 500 m distance between sites (Belgrad et al., 2021). Across all regions, turtle grass (*T. testudinum*) was the dominant macrophyte taxa; however, manatee grass (*Syringodium filiforme*) and shoal grass (*Halodule wrightii*) were also common. At each sampling site, abiotic conditions (i.e., temperature, salinity, dissolved oxygen, and depth) were recorded, drift macroalgal biomass was measured, and seagrass cover/abundance and morphometrics [i.e., percent cover (0–100%), shoot height (mm), and shoot density] were assessed. Because we sampled synoptically using the same methods, we were able to evaluate algal biomass and distribution within and among locations across the northern Gulf of Mexico (Supplementary Table S1; Belgrad et al., 2021; Correia, 2021).

Drift algal biomass and nekton community assessment

Within each hexagon, drift algal biomass and nekton abundance within seagrass meadows were measured using an epibenthic sled, consisting of an aluminum frame (0.75 m wide and 0.6 m high) with two skids on either side (0.7 m in length), and a 2 mm stretch mesh net. The sled captured smaller epibenthic fish and invertebrates living within the algal-seagrass matrix. The sled was towed for 13.3 m at approximately 0.3 m sec^{-1}, covering an area of approximately 10 m^2. Macroalgae from benthic sled samples were bagged, frozen, and transported to the lab where they were later identified and weighed. Nekton within the sled were identified to the lowest possible classification and counted, and their lengths (i.e., total length for shrimp and fish; carapace width for crabs) were recorded.

Seagrass and algal vegetative sampling (quadrats)

Seagrass structural complexity was assessed using a 1 m^2 quadrat divided into 100, 10 cm × 10 cm cells. Four quadrats were haphazardly thrown at each sampling location, and the percent cover, shoot count, and canopy height of each seagrass species present in a quadrat were recorded. The seagrass percent cover by species and the cover of drift algae were measured by counting the number of grid cells within each quadrat that contained a particular vegetation type (0–100 grids $quadrat^{-1}$). Shoot count was calculated for each seagrass species by counting the number of shoots within a random quadrat grid cell. Canopy height was defined as the mean of three randomly selected shoot height measurements.

Statistical analysis

SAS© and Primer™ 7 were used for all statistical analyses. A combination of univariate and multivariate analyses was performed to assess nekton abundance, biodiversity, animal size, and community structure in relation to drift algal biomass. Variables were tested in SAS© for normality, and abundance was log-transformed to mitigate skewness and to achieve normality. Generalized linear mixed models (GLMMs) were used in SAS© to compare nekton abundance (N), species richness (S), and diversity (H’) across the region (CB, CH, CK, LA, and LM), to algal weight [g wet weight (ww); Horton and Lipsitz, 2012; Sokal and Rohlf, 2013]. The weights of algae collected in the epibenthic sled were then ranked, and multivariate analysis was performed in Primer to compare community differences across these weight classes (0–4), with 0 indicating no algae present ($n = 70$), 1 indicating 1–300 g ww of algae ($n = 77$, mean = 115 ± 83 SD), 2 indicating 301–500 g ww ($n = 25$, mean = 115 ± 83), 3 indicating 501–1,000 g ww ($n = 23$, mean = 755 ± 172), and 4 indicating over 1,000 g ww ($n = 25$, mean = 2,560 ± 1,630). The ranking system was necessary for Primer analysis because this software cannot analyze continuous data as a treatment. Species abundance was square-root-transformed to manage skewness and analyzed using a one-way PERMANOVA design with algal weight class (0–4) as the fixed factor. A canonical analysis of principal coordinates (CAP) plot was also performed to illustrate a pattern of difference across algal weight classes.

To account for the positive relationship between drift macroalgal biomass and increased seagrass percent cover (Correia, 2021; Correia et al., in press), a subset of the data was also analyzed to compare the effects of drift algal biomass to

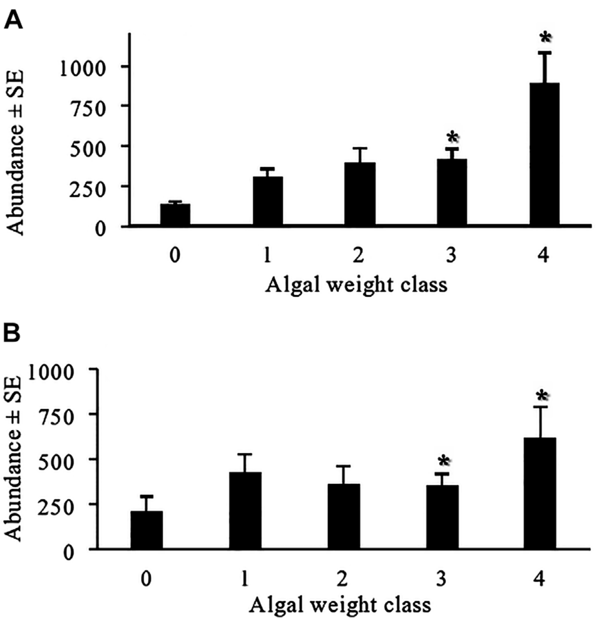

FIGURE 1
Average (+SE) small nekton abundance across drift macroalgae weight classes in the early summer benthic sled samples. Abundance measurements were collected from the epibenthic sled pull, which covered a 10 m^2 area. The asterisk (*) indicates that there is a significant difference in the algae weight class compared to that when no algae were present. The full data set **(A)** and subset in which seagrass was held constant **(B)** showed a similar pattern of increased abundance at algal concentrations greater than 500 g. In this data set, "0" indicates that no algae were present in the sled sample [n = 19, 22 (full, subset, respectively)], "1" indicates 1–300 g of algae (n = 37, 30), "2" represents 301–500 g of algae (n = 19, 15), "3" represents 501–1,000 g of algae (n = 19, 9), and "4" represents samples containing over 1,001 g of algae (n = 15, 8).

nekton community composition while holding seagrass constant (the turtle grass percent cover was greater than 75%, and the canopy heights were between 200 and 400 mm), encompassing 31.8% of the original data set. When analyzing the subset, community or diversity metrics were no longer significant among sampling periods (i.e., early vs. late summer, $p > 0.05$), and we used samples collected in both early and late summer for this analysis. Generalized linear mixed models (GLMMs) were again performed in SAS© on the data subset to compare nekton abundance (N), species richness (S), and diversity (H') across the region (CB, CH, CK, LA, and LM) and algal weight (g ww; Horton and Lipsitz, 2012; Sokal and Rohlf, 2013).

In Primer, similarity percentage (SIMPER) analysis identified which species contributed over 70% of the difference across algal weight classes; individual species (or groups of species based upon classification similarities) were compiled and reanalyzed to test for taxon-specific abundance and size differences across the algal weight. During taxon-specific analysis, seagrass variables were held constant and only samples containing greater than 75% turtle grass coverage and canopy heights of 200–400 mm were included. Analysis of taxon-specific abundance was also compared using GLMMs across the region (CB, CH, CK, LA, and LM) and algal weight (g ww) to identify taxon-specific changes across constant seagrass coverage and canopy height.

Results

Overall, the mean animal abundance was significantly different across algal weight ($p < 0.001$) and was higher when the algal biomass was greater than 500 g (Figure 1A; Table 1). While algal biomass led to increased nekton species abundance, no clear pattern was found with nekton species richness or diversity ($p = 0.92$; Table 1). Nekton species richness remained relatively stable across algal weight classes, with 49 unique species identified when no algae were present (n = 19), 53 species in 1–300 g ww (n = 37), 52 species in 301–500 g ww (n = 19), 42 species in 501–1,000 g ww (n = 19), and 56 species in areas with over 1,000 g ww of algae (n = 15). Shannon diversity also did not differ significantly across algal weight and only varied by region ($p = 0.03$; Table 1). When analyzing a subset of the data in turtle grass with a similar coverage and canopy height, similar patterns were observed for animal abundance, with significantly higher animal abundances seen at algal biomasses over 500 g ww, compared to those when no algae were present (Figure 1B; Table 1). Diversity was not significantly different across region ($p = 0.14$) or algal weight ($p = 0.71$; Table 1) in the data subset, and the richness again remained stable across all algal weight classes (Correia, 2021).

Macroalgal biomass significantly influenced overall community structure (Figure 2A; *Pseudo-F* = 3.124; p = 0.001). Pairwise comparisons across the different algal weight classes indicated that small benthic communities were significantly different when algae were present. When comparing algal weight classes to one another, all areas containing algae were significantly different from areas with no algae (Supplementary Table S2). Areas containing smaller concentrations of algae (i.e., Class 1–3) were also significantly different from areas containing over 1,000 g of algae (Class 4; Supplementary Table S2). When analyzing these relationships using a subset of the data to control for seagrass cover and canopy height, macroalgal biomass still significantly influenced community structure (Figure 2B; *Pseudo-F* = 2.24; p = 0.002). Pairwise comparisons across algal weight class indicated that all areas containing algae were significantly different from areas with no algae (Supplementary Table S2). When comparing algal weight classes to one another, areas containing 300–500 g (Class 2) of macroalgae were significantly different from areas containing over 500 g (Classes 3 and 4). Macroalgal concentrations below 300 g showed the most variability in the nekton community and were not significantly different from the other macroalgal classes (Supplementary Table S2).

TABLE 1 Animal diversity across region and algae weight. GLMMs were performed in SAS© to compare nekton abundance (N) and diversity (H′) across region (CB, CH, CK, LA, and LM) and algae weight [g wet weight (ww)]. General linear regression models for species abundance across region and algae weight during the summer 2018 sampling season, when seagrass was held constant, was also performed.

Full data set

Diversity variable	**SS**	***df***	***F* ratio**	**Prob > *F***
GLMM results across all regions and species abundance (N)				
Region (CB, LM, LA, CK, and CH)	6.19	4	1.40	0.2391
Algae weight (g ww)	21.28	1	19.27	<0.0001
Region*algae weight	4.40	4	1.00	0.4136
Shannon Diversity (H′)				
Region (CB, LM, LA, CK, and CH)	1.43	4	2.89	0.0259
Algae weight (g ww)	0.001	1	0.01	0.9161
Region*algae weight	0.14	4	0.28	0.8921

Subset of data	**SS**	***df***	***F* ratio**	**Prob > *F***
Species abundance (N)				
Region (CB, LM, LA, CK, and CH)	25.86	4	6.12	0.0003
Algae weight (g ww)	6.05	1	5.73	0.0192
Region*algae weight	5.31	4	1.26	0.2946
Shannon Diversity (H′)				
Region (CB, LM, LA, CK, and CH)	0.84	4	1.80	0.1374
Algae weight (g ww)	0.02	1	0.14	0.7140
Region*algae weight	0.26	4	0.55	0.6964

Taxon-specific responses to macroalgae

The largest sources of dissimilarity between communities across the different algal classes, as identified by SIMPER analysis, were nonpenaeid shrimp (Hippolytidae, Palaemonidae, Processidae), penaeid shrimp (Penaeidae), gobies (Gobiidae), pipefish (Syngnathidae), and pinfish (*Lagodon rhomboides*), which collectively made up over 70% of the dissimilarity. Hippolytidae (hippolytid shrimp) were the most abundant organisms collected, with over 1,000 individuals present in some samples. Because of this, Hippolytidae was analyzed separately from other nonpenaeid shrimp. All other nonpenaeid shrimp (Palaemonidae, *Palaemonetes* sp., *Periclimenes* sp., *Processa* sp., *Periclimenes americanus*, *Periclimenes longicaudatus*, *Palaemon floridanus*, *Palaemonetes pugio*, *Palaemonetes vulgaris*, *Tozeuma carolinense*, *Thor* sp., and *Thor dobkini*) were grouped together. Penaeid shrimp included Penaeidae, *Farfantepenaeus* sp., *Rimapenaeus* sp., *Farfantepenaeus duorarum*, *Farfantepenaeus aztecus*, and *Litopenaeus setiferus*. Gobies included Gobiidae, *Gobiosoma* sp., *Microgobius* sp., *Bathygobius* sp., *Ctenogobius* sp., *Gobionellus* sp., *Gobiosoma robustum*, *Ctenogobius boleosoma*, *Microgobius gulosus*, *Microgobius thalassinus*, *Gobiosoma bosc*, *Evothodus lyricus*, and *Bathygobius soporator*. Pipefish included Syngnathidae, *Syngnathus* sp., *Syngnathus floridae*, *Syngnathus louisiana*, *Syngnathus scovelli*, and *Anarchopterus criniger*.

There was a significant interaction between region and weight class for Hippolytidae shrimp abundance at $\alpha = 0.1$ ($p = 0.09$; Table 2). Other nonpenaeid shrimp abundances were also significantly different across region ($p < 0.001$) and algal weight class ($p = 0.03$; Table 2). The abundances of penaeid shrimp and gobies, however, only differed across region and were not related to algal biomass (Table 2). An overall positive trend in Hippolytidae shrimp was observed in Texas and Florida regions with increasing algal biomass, with a slight negative trend in the Chandeleur Islands, LA. At the highest algal biomass, a slight decline in Hippolytidae abundance was also observed in Laguna Madre, TX, Corpus Christi Bay, TX, and Charlotte Harbor, FL (Supplementary Figure S2). Other nonpenaeid shrimp followed a similar pattern, with an increase in shrimp abundance at all locations except Cedar Key, FL (Supplementary Figure S4). The degree of shrimp increase was regionally dependent (Supplementary Figure S4, S5). While organismal abundance increased, the overall length of shrimp and fish did not change with algal weight ($p > 0.05$; Supplementary Table S3, S4), indicating that the same organismal size classes were accessing structured habitats at all drift algal biomasses.

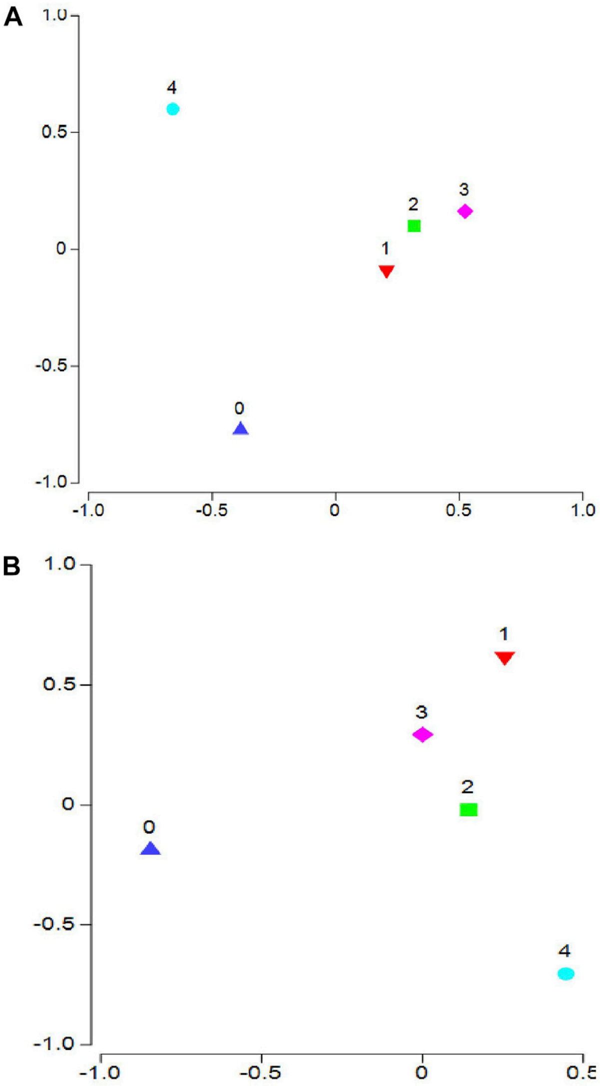

FIGURE 2
CAP plot of community structure across algae weight class. CAP plot comparing communities during the early summer across averaged algae weight classes. Comparisons were run using the complete data set **(A)** and a subset of the data, in which the turtle grass percent cover was >75% cover and the average canopy height was between 200 and 400 mm **(B)**. Classifications included microbenthic sled samples where no algae were present (0), along with areas containing up to 300 g (1), 301–500 g (2), 501–1,000 g (3), and over 1,000 g (4) of macroalgae.

Discussion

Across the northern Gulf of Mexico, the abundance of small epibenthic nekton were positively related to drift algal biomass, but the strength of the effect varied regionally. When the seagrass cover and canopy height were held constant, the same patterns were observed, strengthening our conclusion that changes in macroalgal biomass were the primary driver of organismal patterns. Diversity and species-specific size, however, did not change with macroalgal biomass, indicating an additive role within the seagrass landscape rather than a distinct habitat type, at least for the nekton sampled. Red and brown drift algae, which are common in the Gulf of Mexico and accounted for most of the drift algae in this study, are known to increase faunal abundances (Virnstein and Carbonara, 1985; Bell and Hall, 1997; Holmquist, 1997; Correia, 2021), and thus, our results are consistent with previous findings. In some instances, however, drift algae can smother seagrass beds, cause hypoxia, and ultimately reduce faunal abundance and diversity (Hull, 1987; Isaksson and Pihl, 1992; Norkko and Bonsdorff, 1996a; Norkko and Bonsdorff, 1996b; Raffaelli, 2000). This is commonly seen when nutrient enrichment leads to drastic increases in drift macroalgal biomass, reducing local dissolved oxygen and light concentrations. This effect appears to be dependent on algal biomass and may be species-specific (Hull, 1987; Raffaelli, 2000; Young, 2009). A slight decline in nonpenaeid shrimp was observed at the highest algal biomasses (exceeding 2,000 g ww) in Corpus Christi Bay, TX, Laguna Madre, TX, and Charlotte Harbor, FL, which could be an indicator of algal-mediated habitat degradation (Supplementary Figure S2). Our data are limited at the higher end of algal biomass, although making it difficult to identify a threshold at which algae might begin to have negative effects. Future studies should quantify abundance changes across a larger range of algal biomass to pinpoint such thresholds. With few exceptions, in this study, drift algae were associated with higher animal abundance, driven largely by shrimp species. We believe that this represents an effect of added three-dimensional structure within the seagrass-macroalgal mosaic, allowing more individuals to occupy a given area of seagrass.

Shallow vegetated habitats are commonly associated with increased abundance and diversity of organisms compared to adjacent bare substrates, leading to inferences that structure, regardless of type, is important when determining species occupancy per given area (Heck et al., 2003; Minello et al., 2003; Humphries et al., 2011). Previously, a positive relationship between increased turtle grass percent cover and drift macroalgae percent cover was found (Correia, 2021; Correia et al., in press), which could be influencing the results of this study, due to the overall increase in habitat complexity. However, by analyzing a subset of the data over a narrow range of turtle grass percent cover and canopy heights and coming to similar conclusions, it seems unlikely that seagrass percent cover alone explains the patterns noted between algal biomass and nekton abundance. The added complexity provided by drift macroalgae likely leads to positive changes in nekton abundance by the same mechanism. However, quantification of algal influences on flow, temperature, dissolved oxygen, and recruitment of small nekton organisms would provide additional information regarding the nature of the habitat provided by drift algae relative to seagrass alone.

TABLE 2 Selected species-specific abundance comparisons across region and algae weight when seagrass metrics were held constant. Only samples with greater than 75% turtle grass cover and canopy heights of 200–400 mm were used in this analysis.

	SS	*df*	*F* ratio	Prob > *F*
Hippolytidae				
Region (CB, LM, LA, CK, and CH)	20.93	4	2.07	0.0934
Algae weight (g ww)	2.95	1	1.17	0.2837
Region*algae weight	20.85	4	2.06	0.0945
Other nonpenaeid shrimp				
Region (CB, LM, LA, CK, and CH)	52.03	4	7.18	<0.0001
Algae weight (g ww)	8.89	1	4.90	0.0299
Region*algae weight	8.17	4	1.13	0.3506
Penaeid shrimp				
Region (CB, LM, LA, CK, and CH)	31.21	4	8.86	<0.0001
Algae weight (g ww)	1.80	1	2.05	0.1567
Region*algae weight	4.71	4	1.34	0.2647
Goby				
Region (CB, LM, LA, CK, and CH)	29.25	4	7.65	<0.0001
Algae weight (g ww)	1.98	1	2.07	0.1545
Region*algae weight	2.61	4	0.68	0.6056

Macroalgal blooms are more variable than other complex habitats found along the coast (e.g., seagrass meadow, coral reef, and mangrove forest) and are largely dependent on local flow, season, and water quality. Generally, red and brown macroalgae in the Gulf of Mexico are found at peak abundance during the summer (Pihl et al., 1996). Although algae can grow rapidly, storms and currents can displace them quickly (Pihl et al., 1999). Not surprisingly, the ephemeral nature of macroalgal communities makes this a good habitat for smaller, motile, and more opportunistic organisms that can successfully find and use the habitat type when available (Norkko et al., 2000; Camp et al., 2014).

Variation in the positive association between algal biomass and nekton abundance could be related to differences in algal species composition across each region or differences in life history characteristics of both penaeid and nonpenaeid shrimp found in these areas. Changes in macroalgae species composition significantly influenced local animal abundances, with some algal species containing higher abundances than others (Correia, 2021). Spyridia, for example, contained approximately 757 ± 335 individuals when listed as the primary algal taxon in Charlotte Harbor, FL, with 23 unique species identified. Conversely, locations primarily containing Hypnea had 57 ± 39.5 individuals and nine unique species present (Correia, 2021). However, regardless of region and macroalgal composition, which likely contributed to the variation in our study, higher algal abundances were associated with increased nekton abundances.

Across the northern Gulf of Mexico, the presence of drift macroalgae may have a large, positive effect on smaller nekton organisms, such as shrimp and gobies, with the timing of peak algal abundance often coinciding with the peak in the recruitment of juvenile nekton (Turner and Brody, 1983; Rogers et al., 1993). This increase in recruitment can be beneficial to local fisheries by providing additional food resources, increasing the survival, growth rates, and development of important prey and forage species. It is important, however, to continue to monitor changes in drift macroalgae biomass since they often indicate eutrophication and can quickly transition to nuisance species with well-documented negative effects on the seagrass ecosystems in which they are found.

Data availability statement

The data sets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: http://www.darnellseagrassecologylab.com/turtlegrass.

Author contributions

KD and MD conceived and proposed the project, and all authors assisted in planning, coordination, and execution of the project. KC, BB, and DS took samples in the Texas region. KD, MD, and CH collected samples in the Louisiana region, and SA, BF, MH, CM, and AM collected samples in the Florida regions. Data were QA/QCed by CH and KC and analyzed by KC. KC coordinated the first draft with writing input by DS. All co-authors contributed to the editing of the subsequent drafts of the manuscript.

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Conflict of interest

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.939296/full#supplementary-material

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Mercury biomagnification in a coastal Louisiana food web following the 2010 Deepwater Horizon oil spill

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The estuarine environments surrounding coastal Louisiana create favorable conditions for microbially mediated mercury (Hg) methylation and subsequent bioaccumulation by biota. In 2010, the Deepwater Horizon (DWH) oil spill released large amounts of oil which, despite having low Hg concentrations, had the potential to influence methylmercury (MeHg) bioavailability in the coastal zone. To explore this possibility, we assessed Hg concentrations and trophodynamics in the coastal Louisiana food web prior to and immediately following the DWH oil spill and compared these metrics with an adjacent coastal ecosystem in the northern Gulf of Mexico. We found no differences in MeHg concentrations between oysters collected in years prior to the spill (1986–2007) and those collected during or in the months immediately after the spill (May to December 2010). When comparing tissue MeHg concentrations and carbon and nitrogen stable isotope values across 13 species of bivalves, shrimp, crabs, fishes, and birds we found evidence of significant biomagnification within the coastal Louisiana food web driven by species' trophic position and their use of differing basal carbon sources. In addition, Hg trophodynamics also differed between two adjacent coastal ecosystems, post-spill coastal Louisiana (2010) and pre-spill coastal Alabama (2008–2009). While there was a higher trophic magnification factor in coastal Louisiana relative to coastal Alabama, food web baseline MeHg concentrations were higher in coastal Alabama. The high degree of biomagnification in coastal Louisiana, and significant regional variation, underscores the need to monitor Hg trophodynamics over space and time to better evaluate the short and long-term ecological consequences of events like the DWH oil spill.

KEYWORDS

methylmercury, Hg, stable isotope analysis, trophic magnification factor, trophodynamics

Introduction

Resulting from both natural and anthropogenic activities, mercury (Hg) has gained notoriety as a global pollutant and a threat to human health (Boening, 2000). The cycling of Hg in the environment is complex and difficult to measure, as it is present in several different chemical forms (Liu et al., 2011). While both organic and inorganic forms of Hg can be taken up actively or passively by the surrounding biota (Golding et al., 2002; Pickhardt and Fisher, 2007), methylmercury (MeHg) is particularly concerning due to its potential to bioaccumulate in organisms and biomagnify in food webs (Bloom, 1992; Boening, 2000; Bank et al., 2007). Biomagnification occurs when contaminant concentrations in consumers exceed the concentrations found in their prey (Borgå et al., 2012). Soil microbes that are generally associated with the reduction of sulfate and iron are primarily responsible for the transformation of Hg into a bioavailable form (i.e., MeHg) where it enters the food web (Compeau and Bartha, 1985; Golding et al., 2002). Larger organisms cannot readily take up MeHg directly from the water column or sediment, but are largely exposed to MeHg *via* their diet (Hall et al., 1997; Liu et al., 2011). In fish for example, MeHg tends to be sequestered in muscle tissue, and studies have demonstrated that nearly 60–100% of total Hg (THg) in fish muscle tissue is in the organic, MeHg, form (Cappon and Smith, 1981; Bloom, 1992; Harris, 2003). The lipophilic and protein-binding properties of MeHg make it difficult for organisms to eliminate MeHg, and chronic exposure can lead to the accumulation of MeHg concentrations high enough to have severe toxicological effects (Wiener and Spry, 1996; Leaner and Mason, 2001). Moreover, the relatively long residence time of MeHg in consumer tissues makes it more likely to biomagnify with trophic level, as higher trophic position predators must consume more prey biomass to satisfy their greater metabolic requirements (Harris, 2003; Chumchal et al., 2011).

Advances in the ecological use of stable isotopes as tracers of organic matter and nutrients through food webs now allow for the quantification of Hg and other contaminant dynamics and biomagnification in complex ecosystems (Jardine et al., 2006). This is because stable isotope analysis provides a reliable method of quantifying trophic positions and habitat usage, and, in turn, the trophodynamics of Hg and other heavy metals as well as organic contaminants and other lipophilic pollutants (Hallanger et al., 2011; Borgå et al., 2012; Masset et al., 2019). Carbon (^{13}C/^{12}C) and nitrogen (^{15}N/^{14}N) stable isotopes occur naturally and become assimilated in the tissues of organisms *via* the metabolic processing of nutrients from the organism's diet. As there is minimal fractionation of carbon stable isotope values ($\delta^{13}C$) from the basal carbon source to primary and subsequent consumers (~0.3‰) it can be a reliable tracer for consumer's habitat and/or basal resource use (Peterson and Fry, 1987). While $\delta^{13}C$ values are used in biomagnification studies less frequently than nitrogen stable isotope values ($\delta^{15}N$), several studies have found $\delta^{13}C$ values in consumers to be related to the location and/or sources of Hg accumulation (Power et al., 2002; Kidd et al., 2003; Cardona-Marek et al., 2009). $\delta^{15}N$ values are commonly used as a proxy for trophic position due a general increase in tissue $\delta^{15}N$ values with each trophic step (Pinnegar and Polunin, 1999). Therefore, $\delta^{15}N$ value-based estimates of trophic position are often combined with contaminant data to quantify a food web's trophic magnification factor (TMF) which is a measure of the average diet-to-consumer transfer of contaminants in cases where diet is the main route of exposure and consumers cannot readily eliminate contaminants from their tissue (Borgå et al., 2012). This TMF approach has been successfully applied to studies of Hg biomagnification across a wide range of aquatic, estuarine, and marine food webs (Bisi et al., 2012; Lavoie et al., 2013; Rumbold et al., 2018; Fonseca et al., 2019).

The sub-tropical, estuarine environment surrounding the Mississippi River delta produces an abundance of economically important species, such as oysters, crab, menhaden, shrimp, and drum (Day et al., 1982). However, it also creates a favorable environment for microbially mediated MeHg production and bioaccumulation (Delaune et al., 2008; Jonsson et al., 2014; Taylor et al., 2019). Coastal Louisiana is also an active area for oil exploration, refining, and petrochemical industries, which are known sources of heavy metals (Pardue et al., 1992; Delaune et al., 2008). Between April and July 2010, the Deepwater Horizon (DWH) oil spill released approximately 779 million liters of oil into Louisiana's coastal zone, in addition to the approximately 7.9 million liters of lipophilic chemical oil dispersants used in mitigation efforts (Kujawinski et al., 2011; McNutt et al., 2012). DWH oil itself had low concentrations of Hg (Wilhelm et al., 2007; Paris et al., 2012) and a study of coastal sediments bracketing the spill from 2009 to 2011 reported a mean THg concentration of 0.15 ± 0.03 parts per million (ppm) with no difference among years (Steffy et al., 2013). However, it is possible that oil and dispersants may have influenced Hg bioavailability at the base of coastal food chains *via* changes in sedimentation rates, microbial activity, and/or water chemistry (Perrot et al., 2019). In addition, in late April 2010, the State of Louisiana opened freshwater diversion structures in the Barataria Bay and Breton Sound basins to prevent oil from entering coastal marshes (Martínez et al., 2012). This practice, along with concurrent large rain events from multiple tropical cyclones, altered sea surface temperatures, river flux anomalies, and the quantity of fresh water entering the estuarine environment, had the potential to further influence the deposition of atmospheric Hg, MeHg production, and Hg bioaccumulation and biomagnification in Louisiana's coastal zone (Pickhardt and Fisher, 2007; Black et al., 2012; Kleindienst et al., 2015; Hastings et al., 2016; O'Connor et al., 2016).

Elevated Hg levels in marine and freshwater fishes have been reported in several economically important species across Louisiana which are regularly monitored for safe

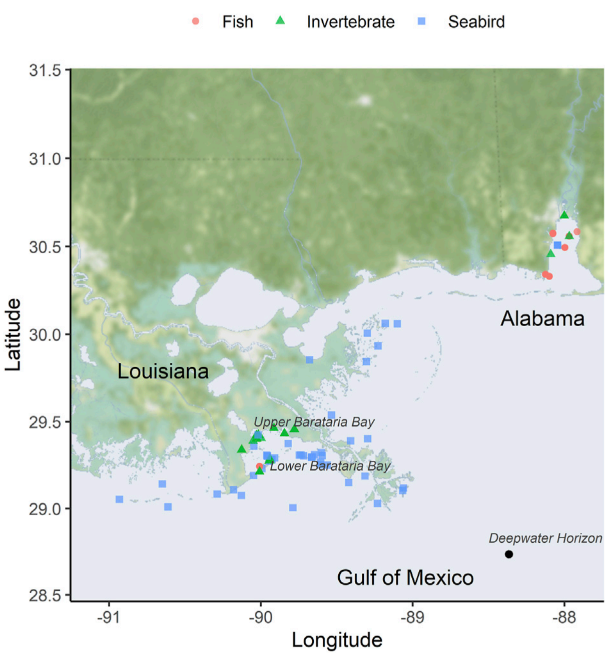

FIGURE 1
Study sampling locations in the northern Gulf of Mexico. Invertebrates, fishes, and seabirds around the Louisiana coast were collected in the aftermath of the 2010 Deepwater Horizon oil spill. Historic oyster samples were collected from the two locations in Barataria Bay by the Mussel Watch Program from 1986 to 2007. Upper Barataria Bay corresponds to Bayou St. Denis and Lower Barataria Bay corresponds to the Middle Bank. Alabama samples were collected by Showalter (2010) in 2008 and 2009.

seafood consumption (O'Connor, 1998; Kongchum et al., 2006; Katner et al., 2010; Apeti et al., 2012). In addition, past studies in coastal Louisiana have examined abiotic cycling of Hg in sediments and the water column, bioaccumulation in select species, and/or biomagnification in estuarine fishes (Bank et al., 2007; Katner et al., 2010; Black et al., 2012; Fry and Chumchal, 2012; Jonsson et al., 2014). The goal of this research was to build on these prior studies and provide a holistic assessment of Hg concentrations and Hg biomagnification through the coastal Louisiana food web prior to and immediately following the 2010 DWH oil spill. Specifically, our first objective was to assess the immediate effect DWH had on the amount of Hg incorporated into taxa at the base of a coastal Louisiana food web by comparing Hg concentrations of oysters collected after the oil spill (May–December 2010) to oysters collected by the Mussel Watch Program prior to the spill (1986–2007). Second, we assessed Hg concentrations and biomagnification potential across a coastal Louisiana food web following the DWH oil spill, from bivalves to seabirds, and identified drivers of Hg concentration using stable isotope analysis. Finally, we compared metrics of Hg trophodynamics and food web biomagnification between two adjacent coastal ecosystems in the northern Gulf of Mexico, specifically coastal Louisiana immediately following DWH oil spill (2010) and costal Alabama prior to the DWH oil spill (2008 and 2009).

Materials and methods

Oyster sampling in Louisiana

Following the Deepwater Horizon oil spill in 2010, the Natural Resource Damage Assessment (NRDA) collected eastern oysters (*Crassostrea virginica*; *n* = 38) from Upper and Lower Barataria Bay, Louisiana in September and November 2010 (Figure 1). Whole bodies of shucked oysters were homogenized and stored frozen (−20°C) prior to analysis. In addition to this NRDA dataset, we obtained pre-spill total Hg (THg) concentrations of eastern oysters from NOAA's National Status and Trends Mussel Watch Program (MWP; NCCOS 2016). The MWP annually monitors pollutants in invertebrates across the Gulf of Mexico. We selected data collected from 1986 to 2007 from two monitoring sites in Barataria Bay: Bayou Saint Denis (Upper Barataria Bay) and Middle Bank (Lower Barataria Bay; Figure 1). Composite samples (~20 individuals) were analyzed for THg in dry weight. Detailed information regarding sample collection and laboratory analysis can be found in Apeti et al. (2012).

Food web sampling in Louisiana

In addition to oysters, specimens of fish, crab, shrimp and adult seabird were collected from multiple locations in coastal Louisiana centered around Barataria Bay between May and December 2010 as part of NRDA and other DWH response efforts (Figure 1). Species collected included: blue crab (*Callinectes sapidus*), white shrimp (*Litopenaeus setiferus*), flathead grey mullet (also known as striped mullet; *Mugil cephalus*), white mullet (*Mugil curema*), Gulf menhaden (*Brevoortia patronus*), Atlantic croaker (*Micropogonias undulatus*), pinfish (*Lagodon rhomboides*), sand seatrout (*Cynoscion arenarius*), brown pelican (*Pelecanus occidentalis*), royal tern (*Thalasseus maximus*), black skimmer (*Rynchops niger*) and laughing gull (*Leucophaeus atriclla*). No collected individuals were visibly oiled, and all specimens were frozen (−20°C) prior to analysis.

Hg and stable isotope analysis of Louisiana samples

Homogenized whole-body tissue of oysters and crabs (excluding shell and carapace), white muscle tissue of shrimp

TABLE 1 Taxa-specific literature values of the average percentage of measured total mercury (THg) in dry muscle tissue that is present in the form of Methylmercury (MeHg). The percentages of THg that is MeHg averaged for each consumer type: bivalves (46%), crustaceans (84%), fish (76%), and seabirds (91%) were then used to convert all THg concentrations to MeHg concentrations.

Taxa	MeHg (%)	References
Bivalves		
Crassostrea virginica	46	Apeti et al. (2012)
Crustaceans		
Callinectes sapidus	95	Adams and Engel (2014)
Farfantepenaeus aztecus	72	Kannan et al. (1998)
All crustaceans	84	
Fish		
Micropogonias undulatus	69	Xu et al. (2013)
Mugil cephalus	90	Bebbington et al. (1977)
Brevoortia patronus	52	Senn et al. (2010)
Lagodon rhomboides	81	Kannan et al. (1998)
Cynoscion arenarius	85	Kannan et al. (1998)
Mugil curema	80	Nilson et al. (2001)
All fish	76	
Seabirds		
Pelecanus occidentalis	94	Ruelas-Inzunza et al. (2009)
Larus argentatus	80	Kim et al. (1996)
Sterna paradisaea	100	Kim et al. (1996)
All seabirds	91	

and fish, and breast muscle tissue of seabirds were rinsed in deionized water, lyophilized for 24 h, ground into a homogenous powder and weighed in preparation for Hg and stable isotope analysis. We prioritized muscle tissue when possible because Hg has a strong affinity for the sulfhydryl groups attached to proteins when methylated and is sequestered into muscle tissues where it accumulates over time (Cappon and Smith, 1981; Bloom, 1992; Hall et al., 1997; Liu et al., 2011). In addition, it has a longer isotopic turnover time relative to other tissues (e.g., blood, liver, etc.) and generally reflects a consumer's diet assimilated over a longer period of time (Post, 2002), which may vary from weeks to months depending on species.

Individual samples analyzed for total Hg (THg) concentrations were measured using a Nippon MA-3000 direct mercury analyzer following the US EPA Method 7473 (US EPA, 2007) at Southeast Missouri University. Approximately 10–20 mg of dried, homogenized tissue was analyzed per sample, with duplicate samples run every 10 samples to test for sample homogeneity. Two samples of standard reference materials (TORT-3) were run at the beginning of each run and after every 20 samples throughout the analysis. The mean percent recovery for TORT-3 was 98 ± 2%, and the method detection limit was 0.001. THg concentrations are reported in parts per million (ppm), dry weight.

THg concentration is commonly used as proxy of the bioavailable form (i.e., MeHg) in both applied and basic research. For example, the Louisiana Department of Environmental Quality's Mercury Initiative regularly monitors THg levels in fish muscle to develop and implement consumption advisories (LDEQ, 1993). In addition, studies of Hg across a wide range of aquatic (e.g., reviewed by Eagles-Smith et al., 2016) and avian (e.g., reviewed by Ackerman et al., 2016) taxa regularly rely on THg and/or convert between THg and MeHg as needed to explore spatial and temporal patterns in Hg concentrations and biomagnification. This includes prior studies specific to mercury bioaccumulation in Louisiana's estuarine food webs (Fry and Chumchal, 2012).

Therefore, we compiled data from prior studies that validated relationships between THg and MeHg concentrations in the tissues of the taxa examined in our study. For example, Apeti et al. (2012) found that tissue THg and MeHg concentrations in bivalves collected from the Central Gulf of Mexico sub-region had a strong positive correlation ($r = 0.92, p < 0.001$). Specifically, in Baritaria Bay they found that on average 46% of tissue THg was MeHg (Apeti et al., 2012; Table 1). Similarly, we compiled research that validated the percentage of THg that is MeHg in muscle tissue for blue crabs, penaeid shrimp, fishes, gulls, terns, and pelicans reflecting the taxa we sampled in Louisiana in 2010 (Table 1). Similar to Apeti et al. (2012), these studies of crustaceans (Kannan et al., 1998; Adams and Engel, 2014), fish (Bebbington et al., 1977; Kannan et al., 1998; Nilson et al., 2001; Senn et al., 2010; Xu et al., 2013), and seabirds (Kim et al., 1996; Ruelas-Inzunza et al., 2009) reported strong positive relationships between tissue THg and MeHg concentrations. The percentages of THg that is MeHg were then averaged for each consumer type: bivalves (46%), crustaceans (84%), fish (76%), and seabirds (91%; Table 1). These average percentages were used as conservative estimates to convert all THg concentrations to MeHg concentrations. This conversion was implemented as MeHg is the toxic species of Hg that bioaccumulates leading to deleterious organismal effects (Liu et al., 2011) and better satisfies the assumptions of TMF models (Borgå et al., 2012).

Individual samples were analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values using a Costech ECS 4010 elemental analyzer interfaced with a Delta XP continuous-flow stable isotope ratio mass spectrometer. Approximately 0.600 ± 0.025 mg of dried, homogenized tissue was analyzed per sample. Glutamic acid reference materials (USGS-40 and USGS-41) with high and low values were used to normalize the raw *d* values on a two-point scale. The analytical precision, based on standard deviations of repeated reference materials, were 0.1 and 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Reported in *d* notation in per mil units (‰), stable isotope ratios were calculated by using the following equation:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 1000$$

TABLE 2 Stable isotope values and Hg concentrations of consumers from coastal Alabama derived from Showalter (2010). Hg concentrations were converted from the original wet wt. measurement to dry wt. using moisture percentages estimated from moisture content measured in Louisiana species. Trophic position (TP) estimates were estimated using $\delta^{15}N$ of bivalves as the isotopic baseline to compare subsequent consumers. MeHg concentrations were estimated using literature values averaged across groups of seabirds, fishes, and species-specific invertebrates (Table 1). The total number (N) of individuals sampled is reported for each species along with mean ±1 standard deviation for each variable.

Common name (*Genus species*)	*N*	THg dw (μg g^{-1})	THg ww (μg g^{-1})	MeHg (μg g^{-1})	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TP
Rangia Clam (*Rangia cuneata*)	10	0.029 ± 0.012	0.026 ± 0.011	0.013 ± 0.006	−26.1 ± 2.4	9.6 ± 0.4	2.0
White Shrimp (*Litopenaeus setiferus*)	2	0.016 ± 0.008	0.011 ± 0.006	0.011 ± 0.006	−24.7 ± 2.7	12.2 ± 0.1	2.8 ± 0.1
Flathead Grey Mullet (*Mugil cephalus*)	5	0.09 ± 0.071	0.073 ± 0.058	0.090 ± 0.071	−19.9 ± 1.3	12.6 ± 0.5	2.8 ± 0.1
Gulf Menhaden (*Brevoortia patronus*)	5	0.034 ± 0.016	0.026 ± 0.012	0.026 ± 0.012	−24.2 ± 2.1	12.3 ± 0.6	2.8 ± 0.2
Atlantic Croaker (*Micropogonias undulatus*)	19	0.030 ± 0.014	0.03 ± 0.014	0.028 ± 0.013	−22.9 ± 2.4	13.5 ± 1.2	3.1 ± 0.3
Sand Seatrout (*Cynoscion arenarius*)	11	0.103 ± 0.025	0.078 ± 0.019	0.078 ± 0.019	−20.6 ± 1.4	15.3 ± 0.3	3.7 ± 0.1
Laughing Gull (*Leucophaeus atricilla*)	7	0.326 ± 0.368	0.230 ± 0.260	0.297 ± 0.335	−19.1 ± 1.4	10.4 ± 2.5	2.6 ± 0.5
Brown Pelican (*Pelecanus occidentalis*)	5	0.616 ± 0.203	0.399 ± 0.132	0.560 ± 0.185	−21.0 ± 1.8	13.1 ± 1.0	3.0 ± 0.3

where X is ^{13}C or ^{15}N, R is the ratio of heavy/light isotope respectively ($^{13}C/^{12}C$, $^{15}N/^{14}N$). The $R_{standard}$ values were based on the Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$. Samples with large C:N ratios (C:N > 3.5) were normalized for lipid content using Post et al.'s (2007) recommended mathematical correction:

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C:\ N$$

Hg and stable isotope analysis of Alabama samples

Hg and stable isotope data were obtained from a food web study conducted in coastal Alabama by Showalter (2010) for comparison with our data collected from Louisiana. Only species common to both regions were included in this comparison, except for rangia clams (*Rangia cuneata*) and eastern oysters, which represent common filter-feeding, primary consumers in Alabama and Louisiana, respectively. The resulting dataset included muscle tissue THg concentrations and $\delta^{13}C$ and $\delta^{15}N$ values for rangia clams (n = 10), white shrimp (n = 2), sand seatrout (n = 11), Gulf menhaden (n = 5), flathead grey mullet (n = 5), Atlantic croaker (n = 19), adult brown pelicans (n = 5), and adult laughing gulls (n = 7) collected in Mobile Bay and Mississippi Sound between June–October 2008 and May–July 2009 (Figure 1; Table 2). Detailed collection and analysis protocols for this dataset are found in Showalter (2010). THg concentration in the Alabama dataset were converted from wet weight (wt.) to dry wt. using the average moisture content in the muscle tissue of each species in the Louisiana study and then converted to MeHg concentrations using the method listed above (Table 1).

Trophic position calculations

Borgå et al. (2012) recommends converting $\delta^{15}N$ to relative trophic position (TP) when comparing TMFs across space and time to account for regional baseline differences. In accordance, we used the following single-baseline equation, from Post (2002), to estimate the TP of individual fish, invertebrates, and seabirds:

$$TP = \lambda + \left(\delta^{15}N_{consumer} - \delta^{15}N_{base}\right) / \Delta^{15}N_{foodweb}$$

where $\delta^{15}N_{base}$ represents the mean value of primary consumers, oysters in Louisiana and rangia clams in Alabama, and are assumed to have a TP of 2, represented as λ. Secondary and subsequent consumer values are represented as $\delta^{15}N_{consumer}$. The trophic enrichment factor, $\delta^{15}N_{foodweb}$, corrects for the fractionation that occurs when energy is transferred between trophic levels. We assumed a trophic enrichment factor of 3.4‰, as this value has been found to be robust across multiple food webs and is suggested when there is a lack of prior knowledge Post (2002).

Statistical analysis

To achieve our first objective, we compared THg concentrations in oysters collected before the oil spill (1986–2007) by MWP to those collected immediately following the spill in 2010 by NRDA. For these comparisons we pooled MWP THg concentrations from all years to collectively represent the pre-spill group. For this specific analysis THg concentrations were not converted to MeHg, because we were comparing the same organism and the relative difference in bioaccumulative potential for each species of Hg was assumed to be constant. Three *t*-tests were conducted with one to compare THg between areas (Upper and

Lower Barataria Bay) with pre- and post-spill samples pooled, and two more to compare pre- and post-spill THg but in separate areas (Upper and Lower Barataria Bay). For this analysis and subsequent analyses, we opted to use Bayesian estimation over frequentist or other estimation routines. Bayesian methods in general provide numerous advantages, such as exact inferences, robust small-sample size estimation, and incorporation of prior information. Furthermore, Bayesian probabilistic interpretation allows for understanding the magnitude of the effect rather than simply rejecting or failing to reject the binary outcome of a *p*-value. *t*-tests were run in JAGS that used a normal distribution for the THg response variable and normal vague priors for both groups and a vague uniform prior for the variance. Three MCMC (Markov chain Monte Carlo) chains were run each for 10,000 iterations with a burn-in of 2,000 iterations and thinning rate of three. Pairwise comparison between location (Lower vs. Upper) and time period (pre- vs. post-spill) were calculated as the posterior probability that mean estimates of THg concentration in one group are different in a one-way comparison with another group.

As part of our second objective, we used means parameterized one-way ANOVAs run in JAGS to compare MeHg, stable isotope values, and TP across all species collected in Louisiana. Descriptions of MCMC sampling and convergence were the same as those presented above for the *t*-tests. Pairwise differences were modeled as derived quantities and evaluated using the same criteria of difference described for the *t*-test above. Pairwise comparisons between species were calculated as the posterior probability that mean estimates of MeHg, stable isotope values and/or TP concentrations in one group are different in a one-way comparison with another group.

Next, we ran a linear regression in JAGS with the response variable of log (MeHg) concentrations regressed with two covariates (δ^{13}C and δ^{15}N values) to aid in identifying the ecological drivers of variation in MeHg within the coastal Louisiana food web. To directly compare the effect of each covariate, the covariates were standardized by mean centering and dividing by one standard deviation. Such standardizing allows for direct comparison of the estimated coefficients. Descriptions of MCMC sampling and convergence were the same as those presented above for the *t*-tests. δ^{13}C and δ^{15}N values were weakly correlated (r = 0.36), which allowed them both to be used in the model.

TP was not included in the above model given its inherent correlation with δ^{15}N values (r = 0.97). However, we did use TP values to examine biomagnification in the coastal Louisiana food web by calculating a TMF based on the equations outlined in Borgå et al. (2012). Specifically, TMF was calculated by taking the anti-log of slope coefficient estimated by a linear regression of log (MeHg) concentrations as the dependent variable and TP as the independent variable. To aid in the biological interpretation of the regression intercept TP was transformed prior to modeling by subtracting 1 from all TP estimates, such that the model intercept represents log (MeHg) at a TP of 1. For this model we used all available samples collected from coastal Louisiana in 2010. Borgå et al. (2012) suggests that positive slopes that produce TMFs >1 indicate the occurrence of biomagnification up the food web. Therefore, we concluded that biomagnification was occurring if 0 was not contained within the 95% credible interval of posterior β. Descriptions of MCMC sampling and convergence were the same as those presented above for the *t*-tests.

Finally, to achieve our third objective, we calculated and compared TMFs between coastal Louisiana immediately following DWH oil spill (2010) and costal Alabama prior to the DWH oil spill (2008 and 2009). However, several species were not sampled in both food webs and species common to both food webs had different sample sizes. Given that ANOVA results suggested differences in MeHg concentration among species, if a species with high THg concentrations were overrepresented in one state, the resulting TMF may disproportionately reflect the presence or weight (i.e., sample size) of that species. Therefore, we first modeled and compared TMFs between Louisiana and Alabama after we subsampled each food web for only overlapping (i.e., common) species, with rangia clams and eastern oysters considered as trophic position analogs. To complement this primary analysis, we conducted a secondary comparison of TMFs after further sub-setting these common species so that species had the same sample sizes in each food web. This was accomplished by randomly selecting the same number of species observations from each state for each species. This iterative approach allowed us to conservatively quantify TMFs while also assessing the potential influences that differences in species and/or sample sizes might have when comparing biomagnification between food webs.

As noted above, TMFs were calculated using linear regression between TP and log (MeHg) with model intercepts representing the log (MeHg) at a TP of 1. Although a factor of interest, state (Louisiana and Alabama), makes sense as a random effect that would unify the models, we wanted to avoid running a model with a random effect that included only two levels. Specifically, the expected coefficient shrinkage in a model with only two levels of a random effect could dampen any estimates, in addition to the fact that we are hypothesizing that Alabama and Louisiana samples may be different and not necessarily representative of random effects (in this case). To assess if our random subsampling approach influenced model estimates we ran 20 model iterations each with different random samples. Although the numerical coefficient estimates varied slightly with each iteration, they never changed in their significance and never overlapped with each other supporting the validity of this approach. Pairwise comparisons for both the "common species" and "common species/sample size" models between location (Louisiana and Alabama) were calculated as the posterior probability that mean estimates of intercept, slope, and TMF from one location are different in a one-way comparison with the other location.

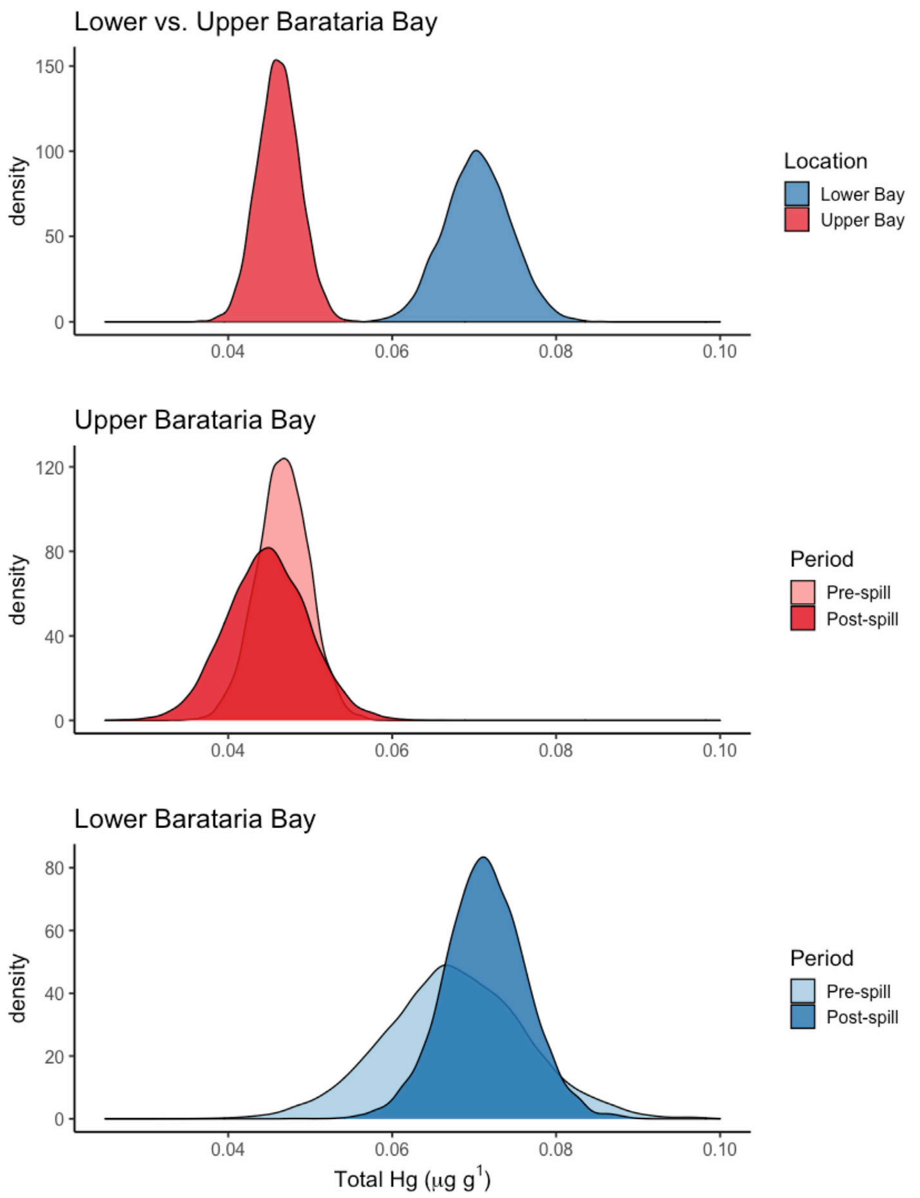

FIGURE 2
Posterior distributions estimated from Bayesian t-tests to compare THg dry wt. concentrations in oysters at two different locations in Barataria Bay before (pre-spill) and after (post-spill) the 2010 Deepwater Horizon oil spill.

Results

Oyster Hg: Pre- vs. post- spill

All *t*-test models converged (based on $\hat{R}$ values and traceplots) and resulted in 7,998 total posterior iterations. Model estimates are described with a posterior mean and 95% credible interval. When pre- and post-spill oysters were pooled, oysters collected from Lower Barataria Bay (0.070 μg g^{-1}; 95% CI: 0.063, 0.073) had credibly higher (posterior probability: 100%) mean THg concentrations relative to oysters collected from Upper Barataria Bay (0.046 μg g^{-1}; 95% CI: 0.041, 0.048; Figure 2). However, when comparing pre- and post-spill oysters Lower Barataria Bay had similar pre- and post-spill THg concentrations. The mean pre-spill oyster THg concentration was 0.07 μg g^{-1} (95% CI: 0.05, 0.09) while the post-spill mean oyster THg concentration was 0.06 μg g^{-1} (95% CI: 0.06, 0.08; Figure 2). The posterior probability that modeled pre-spill oyster THg concentrations were higher than post-spill THg concentrations in Lower Barataria Bay was 33%. In

TABLE 3 Stable isotope values and Hg concentrations measured in tissues of consumers collected in coastal Louisiana following 2010 Deepwater Horizon oil spill. Trophic position (TP) estimates were estimated using $\delta^{15}N$ of bivalves as the isotopic baseline to compare subsequent consumers. MeHg concentrations were estimated using literature values averaged across groups of seabirds, fishes, and species-specific invertebrates (Table 1). The total number (N) of individuals sampled is reported for each species along with mean ±1 standard deviation for each variable. Results of post hoc comparisons of each variable across species are represented by lettered superscripts. Species that do not share the same letter within the same column have pairwise comparisons with posterior probabilities ≥95%.

Common name (*Genus species*)	***N***	**THg (μg g^{-1})**	**MeHg (μg g^{-1})**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**TP**
Eastern Oyster (*Crassostrea virginica*)	38	0.049 ± 0.015	0.023 ± 0.007^{a}	−22.8 ± 2.2^{g}	8.5 ± 0.9^{f}	2.0 ± 0.0^{g}
Blue Crab (*Callinectes sapidus*)	9	0.202 ± 0.107	0.170 ± 0.09^{ac}	−17.4 ± 2.5^{ef}	10.8 ± 1.8^{d}	2.7 ± 0.4^{e}
White Shrimp (*Litopenaeus setiferus*)	5	0.013 ± 0.009	0.010 ± 0.007^{a}	−19.9 ± 1.3^{abcd}	10.6 ± 1.9^{dg}	2.7 ± 0.3^{ef}
White Mullet (*Mugil curema*)	10	0.005 ± 0.004	0.004 ± 0.003^{a}	−16.7 ± 0.6^{f}	9.9 ± 1.3^{d}	2.5 ± 0.3^{e}
Flathead Grey Mullet (*Mugil cephalus*)	10	0.002 ± 0.001	0.001 ± 0.001^{a}	−17.9 ± 1.0^{e}	10.1 ± 0.6^{d}	2.5 ± 0.2^{e}
Pinfish (*Lagodon rhomboides*)	10	0.081 ± 0.042	0.061 ± 0.032^{a}	−20.7 ± 2.4^{b}	11.8 ± 1.5^{eg}	3.0 ± 0.4^{cf}
Gulf Menhaden (*Brevoortia patronus*)	10	0.022 ± 0.005	0.017 ± 0.004^{a}	−20.3 ± 1.1^{ab}	12.3 ± 0.9^{ae}	3.1 ± 0.3^{acf}
Atlantic Croaker (*Micropogonias undulatus*)	11	0.035 ± 0.025	0.027 ± 0.019^{a}	−19.7 ± 0.7^{abcd}	13.1 ± 1.1^{ab}	3.3 ± 0.3^{abc}
Sand Seatrout (*Cynoscion arenarius*)	4	0.212 ± 0.042	0.161 ± 0.032^{acd}	−19.4 ± 0.4^{acd}	13.8 ± 0.3^{bc}	3.5 ± 0.1^{bd}
Brown Pelican (*Pelecanus occidentalis*)	11	0.598 ± 0.313	0.544 ± 0.285^{cd}	−19.5 ± 0.8^{acd}	13.3 ± 0.8^{bab}	3.4 ± 0.2^{ab}
Laughing Gull (*Leucophaeus atricilla*)	13	0.722 ± 0.396	0.657 ± 0.36^{d}	−19.2 ± 1.2^{d}	13.3 ± 1.7^{b}	3.4 ± 0.5^{b}
Royal Tern (*Thalasseus maximus*)	14	1.211 ± 0.912	1.102 ± 0.83^{b}	−19.5 ± 1.1^{cd}	13.4 ± 1.0^{b}	3.4 ± 0.3^{b}
Black Skimmer (*Rynchops niger*)	8	1.656 ± 2.448	1.507 ± 2.228^{b}	−19.9 ± 1.9^{abc}	14.7 ± 1.6^{c}	3.8 ± 0.5^{d}

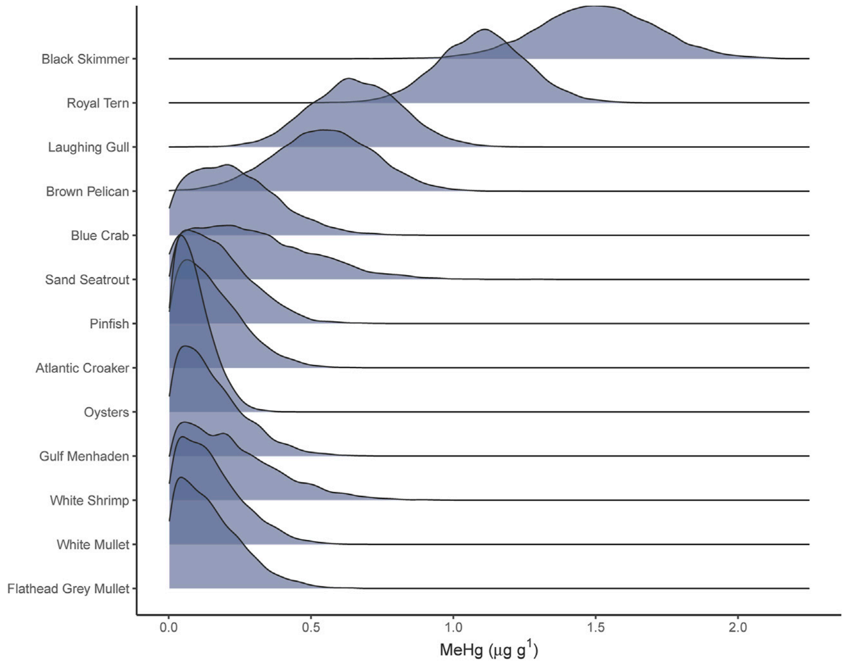

FIGURE 3
Posterior distributions estimated from Bayesian ANOVA to compare MeHg dry wt. concentrations across 13 species of bivalves, crustaceans, fish and seabirds in the aftermath of the 2010 Deepwater Horizon oil spill.

addition, Upper Barataria Bay also had similar pre- and post-spill THg concentrations. The mean pre-spill THg concentration of oysters was 0.05 μg g^{-1} (95% CI: 0.04–0.05) and post-spill was 0.05 μg g^{-1} (95% CI: 0.03–0.05; Figure 2). The posterior probability that modeled pre-spill oyster THg concentrations were higher than post-spill THg concentrations in Upper

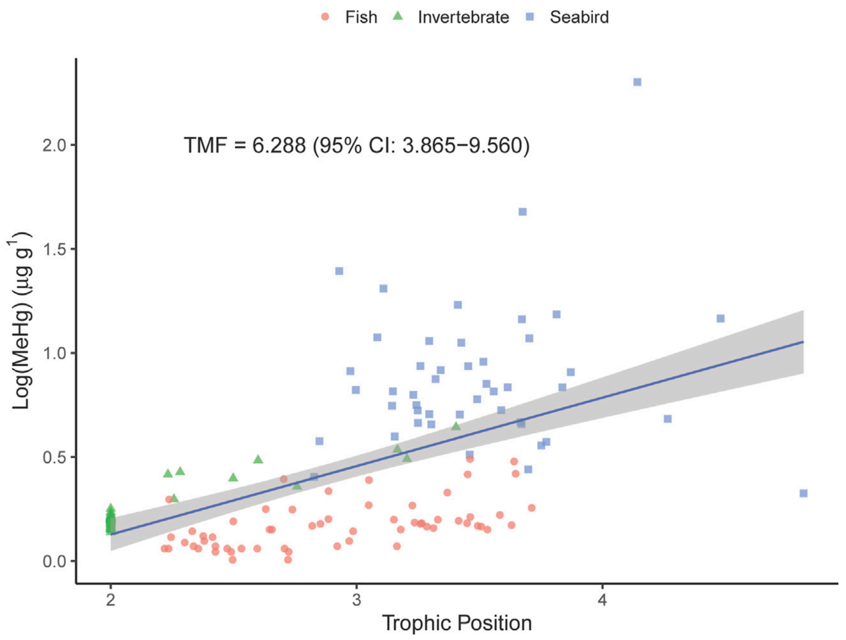

FIGURE 4
The relationship between consumers' MeHg (log-transformed) burden and trophic positions in Louisiana. The overall fit of the line is represented by the grey shaded area. Trophic magnification factors (TMF) are derived from the anti-log of the slope coefficient and indicate biomagnification is occurring when values exceed 1.

Barataria Bay was 63%. These relatively low posterior probabilities suggest there was no significant effect of the DWH oil spill on THg concentrations in oysters in either Lower or Upper Barataria Bay.

Hg and stable isotope values in coastal Louisiana

All ANOVA models converged (based on $\hat{R}$ values and traceplots) and resulted in 7,998 total posterior iterations per model. Posterior distributions estimated flathead gray mullet to have the lowest THg and MeHg concentrations and royal tern to have the highest THg and MeHg concentrations (Table 3; Figure 3). Nearly half (47%; 74 out of 156) of pairwise species comparisons of MeHg concentrations were found to have posterior probabilities ≥95% (Table 3). Posterior distributions estimated that eastern oysters had the lowest δ^{13}C values and white mullet had the highest δ^{13}C values (Table 3). Almost two-thirds (64%; 100 out of 156) of pairwise species comparisons of δ^{13}C values were found have posterior probabilities ≥95% (Table 3). Posterior distributions estimated that eastern oysters had the lowest δ^{15}N values and black skimmer had the highest δ^{15}N values (Table 3). Three-fourths (74%; 115 out of 156) of pairwise species comparisons of δ^{15}N values were found to have posterior probabilities ≥95% (Table 3). Similarly, posterior distributions estimated that eastern oysters had the lowest TP and black skimmer had the highest TP (Table 3), while 74% (116 out of 156) of pairwise species comparisons of TP were found to have posterior probabilities ≥95% (Table 3).

The linear regression that included δ^{13}C values (habitat proxy) and δ^{15}N values (trophic position proxy) converged (based on $\hat{R}$ values and traceplots) and resulted in 7,998 total posterior iterations. The intercept estimate was −1.31 (95% CI: −1.44, −1.18). The slope coefficient for δ^{13}C was −0.24 (95% CI: −0.38, −0.10) and the slope coefficient for δ^{15}N was 0.61 (95% CI 0.46, 0.74). The 95% credible interval around both slope estimates excluded zero, indicating a significantly non-zero slope for both δ^{13}C and δ^{15}N values. However, although the effect of δ^{13}C was negative and the effect of δ^{15}N was positive, the effect size of δ^{15}N was over twice that of δ^{13}C indicating a stronger relationship between MeHg and δ^{15}N values.

Trophic magnification factors in coastal Louisiana

The slope of the relationship between log (MeHg) and TP in the coastal Louisiana food web was estimated to have a mean value of 0.786 with an intercept of −2.757 (Figure 4). This intercept equates to a mean MeHg concentration of 0.063 μg g^{-1} (95% CI: 0.043, 0.093) at a TP of 1. The 95%

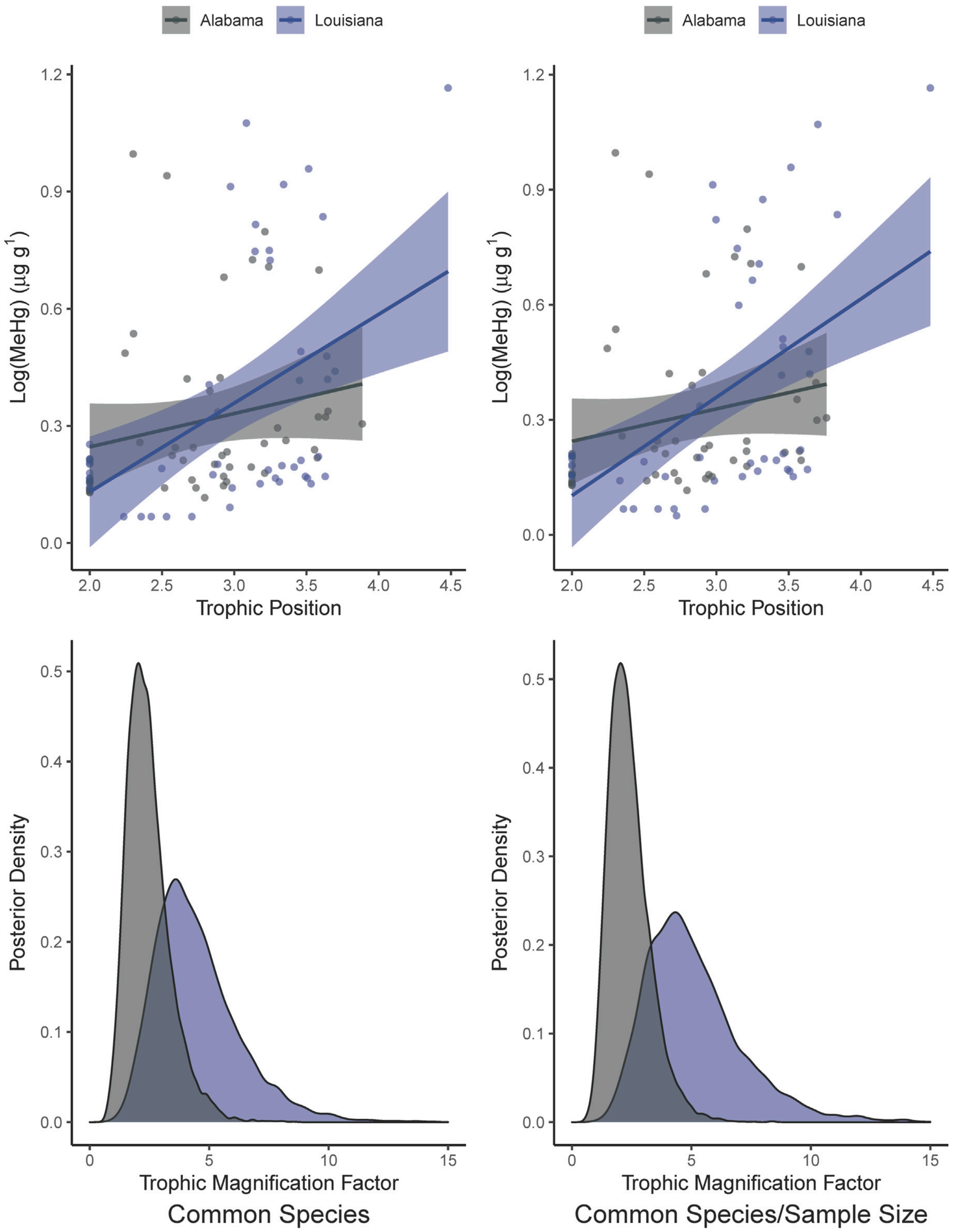

FIGURE 5
The relationship between consumers' MeHg (log-transformed) burden and trophic positions and estimated posterior distributions of trophic magnification factors (TMF) in Louisiana and Alabama using common species but differing samples sizes or common species and standardized samples sizes.

credible interval around the slope estimate (0.588–0.989) excludes 0, indicating a significantly non-zero slope. The mean antilog of the slope for the coastal Louisiana food web (or TMF) was 6.288 (95% CI: 3.865, 9.560), which is greater than the commonly used biomagnification threshold of 1.

Trophic magnification factors comparisons between Louisiana and Alabama

All regression models for Alabama and Louisiana TMF estimates converged (based on $\hat{R}$ values and trace plots) and resulted in 7,998 total posterior iterations for each model. For models that included common species, but differing sample sizes the intercept, slope, and TMF mean estimates were −1.94 (95% CI: −2.39, −1.50), 0.31 (95% CI: 0.09, 0.53), and 2.089 (95% CI: 1.193, 3.403), respectively for Alabama (Figure 5). The Alabama regression intercept equates to a mean MeHg concentration of 0.143 (95% CI: 0.092–0.226) μg g^{-1} at a TP of 1. The intercept, slope, and TMF mean estimates for Louisiana were -2.47 (95% CI: −2.83, −2.11), 0.60 (95% CI: 0.41, 0.80), and 4.093 (95% CI: 2.512, 6.233), respectively (Figure 5). The Louisiana regression intercept equates to a mean MeHg concentration of 0.084 (95% CI: 0.060, 0.120) μg g^{-1} at a TP of 1. Pairwise comparisons of model parameters indicated that intercept estimates were higher in Alabama relative to Louisiana (posterior probability: 97%), while slope and TMF estimates were higher in Louisiana relative to Alabama (posterior probabilities: 98% (Figure 5)).

For models that included common species and balanced sample sizes the intercept, slope, and TMF mean estimates were 2.01 (95% CI: 2.56, −1.44), 0.37 (95% CI: 0.06, 0.67), and 2.305 (95% CI: 1.108, 4.501), respectively for Alabama (Figure 5). The Alabama regression intercept equates to a mean MeHg concentration of 0.143 (95% CI: 0.081, 0.255) μg g^{-1} at a TP of 1. The mean intercept, slope, and TMF estimates for Louisiana were −2.63 (95% CI: 3.22, −2.01), 0.63 (95% CI: 0.33, 0.92), and 4.973 (95% CI: 2.002, 10.460), respectively (Figure 5). The Louisiana regression intercept equates to a mean MeHg concentration of 0.072 (95% CI: 0.036, 0.144) μg g^{-1} at a TP of 1. Pairwise comparisons of model parameters indicated that intercept estimates were generally higher in Alabama relative to Louisiana (posterior probability: 92%), while slope and TMF estimates were higher in Louisiana relative to Alabama (posterior probabilities: 90%; Figure 5).

Discussion

When comparing samples collected before and after the DWH oil spill we found little evidence to suggest that the DWH event, either directly or indirectly, led to changes in the concentration of Hg assimilated into the tissues of consumers at the base of the coastal Louisiana food web. Even so, our results indicate that there was significant biomagnification of Hg within the coastal Louisiana food web, driven by the consumers' trophic position and their use of differing basal carbon sources. Regional comparisons between Louisiana and Alabama further identified differing concentrations of MeHg and THg at the base of the food web as well as evidence of higher trophic biomagnification of Hg in the coastal Louisiana, relative to Alabama.

Mercury in Louisiana oysters

We found that spatial variation in oyster THg concentration within Barataria Bay, Louisiana was greater than temporal variation in THg prior to (1986–2007) and immediately after (2010) the DWH oil spill. Specifically, oysters collected from Lower Barataria Bay consistently had credibly higher concentrations of THg in their tissues relative to oysters collected from Upper Barataria Bay. Our results agree with Apeti et al. (2012), who prior to the spill in 2006 reported higher Hg concentrations in oyster tissue from Lower Barataria Bay (Middle Bank: THg: 0.047 ppm; MeHg 0.023) relative to Upper Barataria Bay (Bayou Saint Dennis: THg: 0.026 ppm; MeHg 0.011). Surface sediment THg concentrations in 2016 were also higher in Lower Barataria Bay (0.054 ppm) relative to Upper Barataria Bay (0.048 ppm) and MeHg concentrations were low (≤0.005 ppm) in both areas of Barataria Bay (Apeti et al., 2012). Following the spill in January 2011, surface sediment THg concentrations reported across four sites within Barataria Bay (0.043 ± 0.015 ppm; Keevan, 2012) were broadly similar to pre-spill THg concentrations reported by Apeti et al. (2012). These prior studies support the conclusions of our analyses, which found spatial differences in oyster THg within Barataria Bay both before and after the DWH oil spill and no credible difference in THg concentration between oysters collected from 1986 to 2007 and oysters collected in 2010.

Multiple factors had the potential to alter Hg deposition and dynamics in coastal Louisiana following the DWH oil spill. For example, while DWH oil itself had a low Hg concentration (Paris et al., 2012), harmful algal blooms formed from the lack of copepod grazing could have aided evasion of Hg into the atmosphere (Pandey et al., 2009; Walsh et al., 2015). Excessive rain events potentially increased the deposition of atmospheric Hg into the coastal ecosystem where dramatic changes in microbial activity and assemblages were occurring in response to the presence of hydrocarbons and dispersant substrates (Beazley et al., 2012; Scalise, 2013; Joye et al., 2014; Kleindienst et al., 2015; Engel et al., 2017). The increased flux of fresh water and dissolved organic carbon from the river could also have increased the amount of Hg in the coastal system (Aiken et al., 2003; Ravichandran, 2004; Wang et al., 2004; Taylor et al., 2019). Even so, our results indicate that these factors had no measurable effect on the amount of Hg incorporated into a common primary consumer (i.e., oysters) at the base of the coastal Louisiana food web directly after the DWH oil spill, at least at the spatial and temporal scope examined in this study.

The time needed for Hg to move through the food web along with the spatial separation among the site of the oil spill, our sample sites, and the location of river diversion may have also influenced our results. Hg deposited from the DWH oil and/or subsequent environmental conditions was most likely in the inorganic form and had to undergo microbial transformation before becoming bioavailable in the food web (Knightes et al., 2007). In addition, oyster tissues reflect longer-term assimilation of diet and Hg (Cunningham and Tripp, 1973). Thus, a potential temporal lag between Hg deposition, methylation, and tissue assimilation may have contributed to the lack of differences found between oysters collected before and after DWH oil spill. The offshore location (64 km southeast off the Louisiana coast) and depth of the spill (1,525 m) further decrease the likelihood that the spill had any immediate influence on Hg burden in primary consumers in the coastal Louisiana food web (McNutt et al., 2012). In addition, both the upper and lower sampling sites in Barataria Bay may have been too distant from the Davis Pond, LA river diversion to have experienced significant trophic responses to punctuated salinity changes following DWH (Fry and Chumchal, 2012). However, because there was no change in Hg concentrations in oysters at the bottom of the food chain, it is reasonable to assume the Hg burdens in subsequent higher trophic position consumers were not also influenced by DWH, and thus these data may be used as baseline values of Hg for this and future studies. However, it is important to note that pathways for accumulation and exposure in higher trophic predators could have varied if predators had to prey shift as a result of loss of preferred prey following the spill.

Mercury dynamics in the Louisiana coastal food web

We found that both a consumer's trophic position (i.e., $\delta^{15}N$) and, to a lesser extent, basal carbon source use (i.e., $\delta^{13}C$) were predictors of the tissue Hg concentration across species in the Louisiana coastal food web. With the exception of seabirds, none of the organisms sampled in Louisiana had an Hg value larger than the FDA advisory limit of 1.0 ppm nor the LDEQ limit of 0.500 ppm (Katner et al., 2010), which are consistent with concentrations of Hg found in fish species sampled from the same area by Fry and Chumchal (2012). Blue crabs, which are a commonly consumed species, had similar Hg burdens to blue crabs from the US Atlantic coast (Adams and Engel, 2014). The observed relationship between tissue Hg concentration, $\delta^{15}N$ values, and trophic position is not unexpected as multiple past studies have found biomagnification of Hg in upper trophic level aquatic predators which generally consume a larger biomass than that of its prey to sustain metabolic function (Lavoie et al., 2013). Most consumers cannot efficiently eliminate MeHg from tissues, so chronic consumption of contaminated prey (even at low levels) may cause the MeHg to accumulate in consumers and may lead to deleterious health consequences (Liu et al., 2011).

Despite their potential as bioindicators of ecosystem health, few studies have captured the long-term effects of chronic exposure to sub-lethal Hg concentrations in long-lived endotherms such as seabirds along the northern Gulf of Mexico coast because of their complex life histories, spatial movements, and species-specific metabolisms (Monteiro and Furness, 1995; Kim et al., 1996). The Hg burden in brown pelicans from this study was less than those measured in brown pelican tissues in both southeastern California and North Carolina, which were not associated with any severe adverse effects (Ruelas-Inzunza et al., 2009; Newtoff and Emslie, 2017). While there remains a disconnect between how the Hg burden directly relates to the health, and considering the emerging links between atmospheric pollutants and neurodegenerative diseases, monitoring Hg dynamics and toxicological effects should remain a priority (Levesque et al., 2011; Bondy, 2016).

In contrast to $\delta^{15}N$ values and trophic position, only a weak negative effect of a $\delta^{13}C$ values on MeHg concentrations was observed. Consumer $\delta^{13}C$ values in marine systems are often used to infer the importance terrestrial, nearshore/benthic, and offshore/pelagic resource use (France, 1995). For example, in coastal systems C_4 marsh grasses and benthic macroalgae generally have lower $\delta^{13}C$ values relative to coastal phytoplankton (Peterson et al., 1985; Peterson and Fry, 1987). Our results indicate consumers that assimilate higher proportions of terrestrial and/or benthic carbon sources are more likely to have lower Hg concentrations relative to species consuming water column/pelagic resources. This could be due to higher Hg bioavailability and/or biomagnification in the water column relative to the benthos or marsh platform. Similar conclusions have been drawn in Hg studies in other estuarine and lake systems, where the effect of basal carbon source use was stronger than the one in this study (Chen et al., 2014; Karimi et al., 2016).

In addition to differences in trophic position and basal resource use, the observed variability in Hg concentrations among coastal Louisiana consumers may also correspond with species-specific feeding strategies, residence time in coastal habitats, and tissue-specific differences in Hg. In Louisiana the two mullet species had the lowest MeHg concentrations (0.004 ± 0.003 and 0.001 ± 0.001 μg g^{-1}) relative to the other fishes (Table 3). In addition, the Hg concentrations found in the planktivorous small mullet in this study were lower than those found in past studies of Baritaria Bay (Fry and Chumchal, 2012). Moreover, our results indicate a range of variation in Hg concentrations among organisms with similar basal resource and/or trophic position. For example, $\delta^{13}C$ values, $\delta^{15}N$ values, and trophic position were generally similar between benthivorous blue crabs and mullet species, however Hg concentration was relatively higher in blue crabs. Evans et al. (2000) observed a similar trend between blue crabs and another benthic consumer, the pink shrimp (*Farfantepenaeus duorarum*), and concluded that the differentiating factor could be the residence time each consumer spent in contaminated areas. Alternatively, the higher concentrations observed in blue crabs could be due to the use of homogenized whole bodies including the hepatopancreas, which, as the primary organ responsible for elimination, is known to accumulate larger concentrations of Hg relative to other tissues (Adams and Engel, 2014). When compiling prior research that validated the relationships between MeHg and THg we endeavored to obtain data for the specific species and/or taxa we sampled in Louisiana (Table 1). While multiple studies have found strongly positive correlations between tissue MeHg and THg concentrations in these species (e.g., Kannan et al., 1998; Apeti et al., 2012; Adams and Engel, 2014), deviations from these assumed relationships may contribute to additional unexplained variation in the MeHg concentrations calculated for blue crab and other taxa in our study. It is of note that blue crabs sampled in Louisiana from this study had higher Hg burdens relative to those sampled by Adams and Engel (2014) on the Atlantic coast of Florida. While it lies beyond the scope of this study, elevated levels of Hg in blue crabs may have implications for long-term consumption by humans (Lincoln et al., 2011).

Trophic magnification of Hg in coastal Louisiana and Alabama

Based on findings from prior studies on select coastal and estuarine invertebrate and fish species, we expected that Hg would biomagnify up the coastal Louisiana food web to meso- and top predators (Bank et al., 2007; Fry and Chumchal, 2012). However, we did not anticipate the large magnitude of Hg transferred between trophic positions. Other studies that have utilized TMFs as biomagnification metrics generally have slopes closer to 1, even when the system is biomagnifying the contaminant (Bisi et al., 2012). Most of these studies, however, include only ectothermic species or rely solely on $\delta^{15}N$ values as a proxy for trophic position and have stronger linear fits (Rumbold et al., 2018; Fonseca et al., 2019). It is possible that these results could be influenced by the assumed trophic enrichment factor (3.4‰) we used in the TP calculations. Previous studies have demonstrated that observed trophic enrichment factors can in some cases be lower than the commonly assumed Post (2002) value (3.4‰) (Kobayashi et al., 2019). In addition, the uncertainty in the fitted linear regression could be a consequence of sampling across a large region with varying environmental conditions created by natural gradients imposed by riverine and marine processes. Regardless, these results provide new insight into the trophic dynamics of Hg in the coastal Louisiana food web with consideration of coastal seabirds. This biomagnification model establishes a baseline that may be used to inform future studies and monitoring efforts.

While both Louisiana and Alabama had TMF values greater than 1, Louisiana had a higher TMF. This result implies that MeHg is potentially biomagnifying at relatively higher concentrations per trophic transfer in the coastal Louisiana food web. On the contrary, Alabama had a higher baseline concentration of MeHg. One possible explanation for this differing MeHg baseline may be that the Alabama sample came from locations within Mobile Bay, which is directly influenced by the Mobile and Tensaw rivers. In contrast, Barataria Bay in Louisiana does not have a direct hydrological connection to the Mississippi River, and it has been suggested that the basin has historically less contamination than other coastal areas in Louisiana (Katner et al., 2010). Even so, Showalter (2010) found significant correlation between log [THg] and $\delta^{15}N$ values when considering all species in their study, which agrees with our results indicating Hg biomagnification in the Alabama coastal food web.

Our analyses indicate that variation in species composition and sample sizes have the potential to influence estimates of TMFs as well as comparisons of biomagnification between food webs. For example, while 95% CI overlapped across methods, mean estimates of TMF varied when using our full Louisiana dataset (6.288), Louisiana species also found in Alabama (2.305), and Louisiana species also found in Alabama at standardized sample sizes (4.973). In addition, standardizing both species and sample sizes between Louisiana and Alabama reduced the posterior probability that TMF differed (90%) relative to standardizing only by species (98%). This suggests that sub-setting species and/or sample sizes may be an important approach to consider when seeking to standardize TMF comparisons between food webs. Regardless, it is important to recognize that restricting/reducing species and sample sizes also has the potential to mask meaningful biological differences between food webs. Therefore, we recommend researchers calculate TMFs using multiple approaches (e.g., full datasets vs. standardized datasets) to allow for more comprehensive assessments when seeking to compare biomagnification between food webs.

Conclusion

This research provides an assessment of Hg concentrations and biomagnification through the coastal Louisiana food web prior to and immediately following the 2010 DWH oil spill. We found no evidence to suggest that the DWH event influenced Hg concentrations in oysters, a common indicator species at the base of the coastal Louisiana food web. However, we did observe significant biomagnification of Hg within the coastal Louisiana food web. Specifically, variation in Hg concentration among species was related to trophic position and the use of differing carbon sources. Regional comparisons between Louisiana and Alabama found different baseline concentrations of MeHg and

THg at the base of the food web, along with evidence of higher trophic biomagnification of Hg in the coastal Louisiana food web, relative to Alabama. Differences at the regional level underscore the need for development of trophic and contaminant baselines in consumers at multiple trophic levels over space and time in order to adequately evaluate short- and long-term ecological consequences of environmental stressors.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://data.gulfresearchinitiative.org/data/R6.x808.000:0071.

Ethics statement

Ethical review and approval was not required for the animal study because analyses were conducted on vertebrate tissue samples previously collected for other research projects and government monitoring efforts not associated with this research.

Author contributions

KL and MP designed the study; KL, MP, MK, and PL-D collected, collated, and processes samples; KL and RB analyzed samples; KL, SM, and RB analyzed data; KL and MP wrote the manuscript; KL, MP, and SM designed the graphics; MP and PL-D supervised the study; and all authors contributed to previous versions and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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A comparison of intertidal metazoan biodiversity among different salinity zones of Louisiana coastal marshes

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Tidal marshes are among the most valuable, productive, and vulnerable ecosystems with high biodiversity. Louisiana's saltmarshes are endangered by natural and man-made stressors, including oil pollution, saltwater intrusion, and land loss due to sea level rise and erosion. Freshwater diversions have been planned to restore sediment input from the Mississippi River to rebuild marsh habitats in South Louisiana. These proposed diversions will undoubtedly change salinity levels, which is a major controlling factor in the distribution of marsh organisms, including those in soil; however, detailed pre-event inventories are lacking. This study describes the diversity of metazoan meiofauna (organisms between 45 and 500 μm) and environmental DNA in marsh soil collected in 2018 from Barataria and Caillou Bay, Louisiana, across three salinity zones and four distances from the marsh edge. Diversity analyses using 18S rRNA gene metabarcodes identified salinity as a factor impacting soil metazoan composition. Nematoda and Mollusca were equally distributed across salinity zones. Gastrotricha, Bryozoa, Rotifera, and Platyhelminthes were more prevalent in low salinity while Kinorhyncha were not detected in low salinity. Annelida and insects were equally common in low and high salinity but less in mid salinity. Five nematodes (*Eumonhystera filiformis*, two *Prismatolaimus* spp., *Anoplostoma* sp., and *Prodorylaimus* sp.), two annelids (*Marionina southerni* and *Dendronereis aestuarina*), two *platyhelminthes* (*Rhynchoscolex simplex* and *Olisthanella truncula*), the gastrotrich *Chaetonotus novenarius* and four collembola and ostracods appear to be low salinity bioindicators and are expected to expand range with freshwater diversions. No frequently detected organisms were unique to mid or high salinity zones, but four Nematoda (*Meleidogyne spartinae*, *Prochaetosoma* sp., *Halalaimus* sp., and *Dichromadora* sp.), two Annelida (*Alitta succinea* and *Namalycastis jaya*), two Platyhelminthes (*Macrostomum kepneri* and *Mesorhynchus terminostylis*), and one Kinorhyncha (*Echinoderes* sp.) were never detected in low salinity zones. None of the frequently detected taxa were unique for a particular distance from the marsh edge or bay. This dataset will be useful as baseline for assessing how soil communities will change in response to salinity changes caused by freshwater diversions and saltwater

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intrusion as well as measuring the environmental impact of pollution and other stressors.

KEYWORDS

18S metabarcoding, next-generation sequencing, environmental DNA, meiofauna, soil organisms

Introduction

Tidal saltmarshes provide a number of ecosystem services which are valuable to humans, including coastal protection from storms, carbon sequestration, and denitrification (Barbier et al., 2011). These habitats also act as nurseries for commercially and recreationally important species (Boesch and Turner, 1984; Zimmerman et al., 2002; Mackenzie and Dionne, 2008). However, salt marshes across the globe are in decline due to a variety of factors (MEA, 2005; UNEP, 2006).

The marshes of Louisiana were initially built by yearly flooding of the Mississippi River over thousands of years (Fisk and McFarlan, 1955) but have lost an area the size of the state of Delaware since 1932 (Couvillion et al., 2017) due to flood control structures preventing these floods and sediment accretion. River sediment compacts readily causing subsidence, which together with sea-level rise leads to land loss and salt water intrusion (Delaune and Pezeshki, 1994; Yuill et al., 2009). In some areas of Louisiana marsh, subsidence may exceed 1 cm per year, which is among the highest rates of subsidence along the Gulf Coast of the United States (Penland and Ramsey, 1990). In addition, marshland is lost due to erosion exacerbated by hurricanes and oil spills (Palaseanu-Lovejoy et al., 2013; Rabalais and Turner, 2016). For example, oiling after the 2010 Deepwater Horizon oil spill of Louisiana's salt marshes, which are dominated by *Spartina alterniflora* (Loisel.) and *Juncus roemerianus* (Scheele), led in some areas to complete loss of vegetation, which resulted in shearing of the marsh edge and accelerated land loss after hurricanes (Lin and Mendelssohn, 2012; Mendelssohn et al., 2012; Silliman et al., 2012; Zengel et al., 2015; Khanna et al., 2017).

A comprehensive Louisiana Coastal Master Plan pledging $50 billion to 79 coastal restoration and protection projects through the next half century has been compiled by the Coastal Resource Management Agency to counteract the alarming rate of loss of Louisiana marshes (Coastal Protection and Restoration Authority of Louisiana, 2007; Coastal Protection and Restoration Authority of Louisiana, 2012; Peyronnin et al., 2013; Coastal Protection and Restoration Authority of Louisiana, 2017). Included in these projects are several sediment and freshwater diversions planned to divert flow from the Mississippi River into the marshes to promote land accretion and reduce saltwater intrusion into historically freshwater marshes (Elsey-Quirk et al., 2019). The first of these major sediment diversions is the Mid-Barataria Sediment Diversion, which was slated to begin construction in 2022 but is now delayed. Environmental impact studies of these diversions will require the establishment of baselines of the composition and distribution of organisms in the marsh. Because salinity is one of the dominant environmental variables in salt marshes (Chapman, 1977; Holland et al., 1990), detailed inventories of different salinity zones are vital to measuring effects of freshwater diversions, saltwater intrusion and other environmental disturbances on the ecology of the marsh community.

Invertebrate organisms are often used for ecological monitoring because they have a high diversity of species, life histories and niches and are responsive to environmental change such as salinity shifts. Therefore, Aker (Aker, 2020) established detailed year-long insect inventories across different salinity zones in Louisiana marshes and identified bioindicator taxa for salinity zones. In addition, sediment-dwelling infauna acts as a linkage between microbe and macrofauna food webs and between benthic and pelagic food webs (Coull, 1999; Posey et al., 2005; Rozas and Minello, 2011; Schratzberger and Ingels, 2018). Meiofauna are infauna typically ranging from 45 to 500 µm (Giere, 2009). The intergenerational time of many groups of meiofauna is quite short, with multiple generations per year, allowing rapid response in both diversity and abundance of major taxa to environmental changes (Warwick, 1981; Kennedy and Jacoby, 1999; Montagna et al., 2002; Cai et al., 2012; Ngo et al., 2013; Zeppilli et al., 2015). Therefore, meiofauna are often used as bioindicators of marsh health, especially with regards to pollution responses (Fleeger et al., 1982; Fleeger and Chandler, 1983; Fleeger, 1985; Fleeger et al., 2007; Fleeger et al., 2015; Semprucci et al., 2015; Bam et al., 2018). However, soil infauna is also likely to be affected by salinity changes. Meiofauna abundance has been shown to increase in salt marshes as salinity increases due to the influx of marine species (Montagna et al., 2002). Salinity was also a major factor impacting benthic infauna across marsh zones from tidal flats to high marsh of the Atlantic coast (MacKenzie et al., 2015; van Regteren et al., 2020).

While meiofauna have been studied in both Gulf and Atlantic coastal marshes, these studies tend to identify meiofauna to major taxonomic groups such as nematodes, harpacticoid copepods, and oligochaetes (Bell, 1979; Coull and Bell, 1979; Fleeger, 1985; Kennedy and Jacoby, 1999; MacKenzie et al., 2015; van Regteren et al., 2020) or a limited number of well described species (Fleeger et al., 2015; Fleeger et al., 2018; Fleeger et al., 2019). Meiofauna diversity in general remains poorly known at the species level (Snelgrove et al., 1997; Gielings et al., 2021). This is largely due to the effort and technical experience required to identify meiofauna to the species level with classical morphological methods. Traditional studies of meiofauna also are limited by capture of certain groups, especially the smaller, soft bodied organisms such as the Platyhelminthes, Rotifera, and Gastrotricha, due to sampling bias and preservation issues (Kennedy and Jacoby, 1999; Carugati et al., 2015; Leasi et al., 2018). To overcome these limitations, studies on meiofauna are increasingly using DNA metabarcoding methods for rapid, cost-effective identification of meiofauna over traditional methods (Bik et al., 2012; Brannock et al., 2014; Brannock and Halanych, 2015; Creer et al., 2016; Husseneder et al., 2022, reviewed in Gielings et al., 2021). Metabarcoding is used to assess diversity *via* high-throughput sequencing of amplified DNA markers and assign taxonomic information by comparison to sequences of identified organisms in reference databases (Taberlet et al., 2012). Metabarcoding captures and analyzes DNA from all living organisms in a sample without the need of isolating them, but also from genetic material shed into the environment (environmental DNA, Zaiko et al., 2018). The use of DNA sequencing methods detects even rare taxa and cryptic life stages and allows diversity comparisons even when only a fraction of the species is described and, thus, often exceeds conventional diversity assessments in terms of taxonomic resolution, precision and sensitivity (Zaiko et al., 2018). Thus, metabarcoding is an excellent tool in ecological monitoring (Chariton et al., 2015; DiBattista et al., 2020).

The purpose of this study was to describe metazoan community diversity consisting of meiofauna and environmental DNA at different salinity zones in Louisiana estuaries to detect changes in these communities caused by the planned freshwater diversion projects or other unforeseen environmental disturbances including saltwater intrusion and pollution. Communities were described from three salinity zones in two Louisiana bays, Barataria Bay and Caillou Bay, which previously were selected for the collection of baseline insect community data across the salinity zones (Aker, 2020). Samples were collected from four distances from the marsh edge to capture diversity and test whether there would be distance-dependent differences in metazoan composition. The timing of this study was critical for the collection of a baseline taxa and diversity inventory since Barataria Bay likely will be immediately affected by the Mid-Barataria Sediment Diversion project when it is initiated (Coastal Protection and Restoration Authority of Louisiana, 2007; Mccall and Pennings, 2012; Minowa and Garaffoni, 2017).

Materials and methods

Site determination and sample collection

Sampling sites were selected from two major Louisiana estuaries: Caillou Bay and Barataria Bay. Caillou Bay is to the southwest of the mouth of the Atchafalaya River, and Barataria Bay is to the East of the mouth of the Mississippi River. Monthly recordings by six Coastal Reference Monitoring Stations from 2014 to 2017 (CPRA, 2018, http://cims.coastal.louisiana.gov) were analyzed to choose sample sites in three salinity zones (low (~4.8 ppt), mid (~8.5 ppt), and high salinity (~13.9 ppt) for each bay (Supplementary Figure S1). Salinity differed significantly among the three zones but no differences were detected between corresponding zones in both bays (Aker, 2020). Sample sites were not exposed to oiling by the Deepwater Horizon oil spill in April 2010 as determined by landing data from the Environmental Response Management Application (NOAA, 2018) (https://erma.noaa.gov/gulfofmexico/). Within each zone in each bay, three replicate sites were selected by accessibility and proximity to the Coastal Reference Monitoring Station used to determine local salinity. The sites in Barataria Bay were also selected for proximity to the proposed Mid-Barataria Sediment Diversion project. Four soil samples were collected at each site along a transect at 0, 1, 5, and 10 m from the water edge. The starting point of transects (0 m into the marsh) was defined as the edge of the marsh, where the outermost plants begin to appear. Soil samples were collected using a coring device with removable 10.2 cm diameter × 30.5 cm long cylindrical acrylic cores (Rayle, 2021). Once the soil sample was collected, it was immediately capped and placed into a cooler with ice for transport. Soil cores were stored in a −20°C freezer until processing. Overall, 72 soil samples were collected (two bays * three salinity zones * four distances from the marsh edge * three replicates at each site).

Soil chemistry and analysis

Samples collected 5 and 10 m from the marsh edge ($n = 36$) were processed at the LSU Agcenter Soil Testing and Plant Analysis lab for chemistry analysis using routine methods described at http://www.lsuagcenter.com/portals/our_offices/departments/spess/servicelabs/soil_testing_lab. Parameters measured were Carbon (%), Nitrogen (%), carbon/nitrogen (C/N) ratios, Percent Organic Matter (%), Aluminum (ppm), Boron (ppm), Calcium (ppm), Chloride (ppm), Conductivity (dS/m), Copper (ppm), Iron (ppm), Magnesium (ppm), Manganese (ppm), pH, Phosphorus (ppm), Potassium (ppm), Salts (ppm), Sodium (ppm), Sodium Adsorption Ratio (SAR), Sulfur (ppm), Zinc (ppm). All soil chemistry data were tested for significant differences across the salinity zones, the different bays, and the different distances from the marsh edge using a three-way ANOVA including all 2- and 3-way interactions in the aov function in the stats package in R (Chambers et al., 1992). The Tukey HSD method in R (Yandell, 1997), was used to perform post hoc testing on all analyses. Spearman correlations were performed to test for correlation of sediment chemistry parameters and metazoan alpha-diversity.

Sample processing

Frozen soil cores were removed from the acrylic cores and an approximately 5 g section was cut from the top one-centimeter section of each core staying well above the anoxic zone using a handsaw disinfected with 10% bleach. The cut sections were thawed in 95% ethanol at 4°C, then rinsed through a 500 μm sieve onto a 45 μm sieve. The portion of the sample retained on the 500 μm sieve was stored in 95% ethanol at −20°C as voucher material. The portion of the sample retained on the 45 μm sieve was mixed with Ludox (Sigma-Aldrich, Munich, Germany) for 1 h and centrifuged at 45,000 rpm for 15 min at 25°C. The supernatants containing the organic portion including the meiofauna were collected onto a 45 μm sieve, thoroughly rinsed and stored in 95% ethanol at 4°C until DNA extraction (Rayle, 2021). Since the sediment samples were frozen and then thawed and agitated, fragments of macrofauna were captured along with meiofauna.

DNA extraction, polymerase chain amplification, and sequencing

Total DNA extractions were performed on three 0.25 g portions of the organic portion of each sample using DNeasy Powersoil kits (Qiagen, Hilden, Germany). All aliquots were checked for DNA concentration using the Invitrogen Qubit 4 Fluorometer (Thermo Fisher Scientific, Wilmington, DE) with the Qubit dsDNA BR Assay Kit. The NF1/18Sr2b primer set (Porazinska et al., 2009; Porazinska et al., 2010), with NextEra Illumina adapter sequences was used to amplify a portion of the 18S small ribosomal subunit gene, which is a universal marker for comparatively unbiased identification of nearly all eukaryote taxa (Creer et al., 2016; Jacquiod et al., 2016). The PCR mix consisted of 10.5 μl Taq polymerase master mix (New England BioLabs), 0.5 μl (at 5 μM concentration) of each of the forward and reverse primer construct, 1 μl extracted sample DNA (at 10 ng/μl), and 12.5 μl DNA-ase free water for a total of 25 μl for each reaction. All PCR products were sequenced at the University of New Hampshire Hubbard Center for Genome Studies on the Illumina Hiseq 2500 platform (Caporaso et al., 2012), using NextEra DNA Flex Library Prep kits (Illumina, San Diego, California). For all DNA aliquots, 2 × 250 base pair forward and reverse FASTQ files were produced. Sequence files and metadata are available in NCBI GenBank *via* the BioProject accession number PRJNA706429.

Bioinformatics

Initial bioinformatics steps were performed on the University of New Hampshire Hubbard Genome Center server using QIIME2 version 2020-2 (Bolyen et al., 2019). Sequences were subjected to rigorous quality control and ends with low Phred quality scores were trimmed using the DADA2 algorithm (Callahan et al., 2016). Due to the low quality of the reverse sequences, only forward sequences were used for further analyses. The resulting amplicon sequence variants (ASVs), which are unique representative sequences, were taxonomically classified by comparing them to the SILVA 132 database (Quast et al., 2013) using the BLAST algorithm (Camacho et al., 2009) with a <97% cutoff for unassigned sequences. This procedure resulted in 11,620 ASVs represented by approximately 15 million reads with 3,813 ASVs represented by approximately eight million reads assigned to eukaryote taxa. The separate entries in the ASV table for each DNA aliquot were merged into a single entry per sample using the q2-feature-table plugin. All ASVs were filtered to remove non-metazoa taxa, vertebrate environmental DNAs, and all taxa which might be contaminants using the filter-table method of the q2-taxa plugin.

These filtered ASVs were clustered at 97% using the cluster-features-de-novo method in the q2-vsearch plugin to account for the intraspecific variation present in metazoans and to avoid artificially inflating biodiversity (Bucklin et al., 2011; Phillips et al., 2019; Brandt et al., 2020). Clustering produced a table of operational taxonomic units (OTUs) by sample. The sequences were aligned using the mafft method (Katoh et al., 2002) and masked (Lane, 1991) to create a phylogenetic tree with the q2-fasttree plugin (Price et al., 2010). The OTU table was exported from the qiime format to the biom format for use in R with the biomformat package (McMurdie and Paulson, 2020). Read counts were manually converted into incidence per sample. Throughout this manuscript the term “incidence” refers to the presence of an OTU in a sample. If only one OTU is considered, incidence represents the number of samples in which the OTU was detected. For a group of OTUs incidence is the sum of the number of times the OTUs were detected in the dataset. Incidences were preferred over read counts because read counts are only weakly correlated to biomass or the number of individuals in multicellular metazoa (Egge et al., 2013).

The method rarecurve in the R package vegan (Oksanen et al., 2018) was used to generate alpha rarefaction curves featuring OTU richness plotted against sequencing depth for each sample. Additional sample and coverage-based rarefaction curves were generated in the R package iNEXT (Hsieh et al., 2016) using three metrics to measure effective diversity, i.e., richness, the exponential of Shannon’s entropy index, and the inverse of Simpson’s index. Effective diversity is the number of equally abundant OTUs required to reach the value of the metric at the number of samples or estimated coverage.

Diversity profiles were calculated with the R package SpadeR (Chao and Jost 2012; Chao et al., 2015) using the OTU incidence table. The diversity profile consisted of observed total incidences, total richness, coverage estimate, estimated coefficient of variation, incidences, and total OTUs for both the frequent (in >10 samples) and infrequent groups (in ≤10 samples) of OTUs. The coverage estimate is a measure of sampling completeness, which estimates the percentage of the actual community present in the environment that the sampling captured. The coefficient of variation is the measure of heterogeneity in the dataset. Richness values for the actual community were estimated using the homogenous model, the Chao2 model, the iChao2 model, and the incidence-based coverage estimator (ICE) model. The homogenous model estimates richness as though all OTUs have an equal chance of being detected. The Chao2 model uses OTUs detected in only one or two samples to estimate actual richness. The iChao2 model uses the OTUs detected in four or less samples to estimate actual richness and is less biased than the Chao2 model for more heterogeneous datasets. The ICE model uses the infrequent group of OTUs to estimate OTUs, and is an incidence based version of the abundance-based coverage estimator (Chao and Lee, 1992).

Taxonomically assigned OTUs were manually checked using the BLAST + algorithm against the NCBI GenBank database (Benson et al., 2011). All OTUs were given additional assignments based on the top BLAST hit, sorted by E-value and taxonomic strings were checked for accuracy against the World Register of Marine Species database (Horton et al., 2022). Taxa bar plots for each salinity zone were created using the OTU incidence table in the R package ggplot2.

Alpha-diversity, i.e., diversity within samples, was calculated using the incidence-based OTU richness and Faith's Phylogenetic Diversity (PD) measures in the q2-alpha plugin. The OTU richness is the number of OTUs in a sample, while the Faith's PD value is the total branch length of the phylogenetic tree of all OTUs within a sample (Faith, 1992). Both values were used to create boxplots in the ggplot2 package in R and tested for differences across the salinity zones, bays, and distances from the marsh edge using a Kruskal-Wallis procedure in the q2-diversity plugin (Kruskal and Wallis, 1952).

For calculating Beta-diversity, i.e., diversity among groups of samples, a distance matrix of Sørensen index values for each pair of samples in the dataset was created using the q2-diversity plugin (Sørensen, 1948). This index was chosen because it uses the composition of samples, rather than read counts, which avoids the issue of individual numbers not corresponding to read counts in metazoan taxa. The distance matrix was then tested using the Adonis procedure in the q2-diversity plugin, with the formula "salinity zone * bay * distance from marsh edge" and 10,000 permutations. The Adonis procedure is a multifactorial PERMANOVA test, which uses the sum of squares of the distances between centroids of groups of samples belonging to each factor and the overall centroid to calculate F-ratios and permutations of observations to determine significant differences. An assumption of the PERMANOVA test is that all groups have similar spread in the multivariate space. A PERMDISP test was used to determine the spread of these groups. Following all tests, p-values were corrected to account for the false discovery rate using the p.adjust function in the stats package in R (Benjamini and Hochberg, 1995). Beta-diversity, i.e., dispersion and differentiation of the metazoan composition of the samples from the different salinity zones, bays, and the distances from marsh edge, was visualized using Non-metric MultiDimensional Scaling (NMDS) ordination using the Sørensen index in the metaMDS method in the R package vegan (Oksanen et al., 2018). The ordination was plotted using the R package ggordiplot, available at https://github.com/jfq3/ggordiplots (Quensen, 2020).

Results

Sequence depth-, sample-, and coverage-based rarefaction of metazoan communities

Only the groups Metazoa, Fungi, and Archaeplastida were present in all samples with the majority of reads in most samples belonging to the Metazoa (Supplementary Figure S1). Metazoa also had the highest rate of clear taxonomic assignments (Supplementary Table S1). Therefore, the dataset was filtered for non-vertebrate Metazoa (893 ASVs with approximately four million reads). After clustering of ASVs, which were more than 97% similar, to control for intraspecific variation, the final total number of OTUs was 312.

Rarefaction curves of OTU richness versus sequencing depth for all samples leveled off, indicating that sufficient sampling depth was achieved for all samples to collect all metazoan OTUs present (Figure 1). In addition, though a wide range of both richness and sampling depth was detected, higher sequencing depth did not lead to higher OTU richness.

Sample-based rarefaction curves for groups of samples from each salinity zone began to level off at actual sample sizes, but the extrapolated portions of the richness curves continued to increase with additional samples (Figure 2). The curves for the effective diversity of the Shannon and Simpson Inverse indices barely increased, which indicates that all common OTUs within the actual metazoan community were collected (Figure 2). Sample-based rarefaction curves for the different bays and distances from the marsh edge also showed sufficient capture of common OTUs (Supplementary Figure S3).

Coverage-based rarefaction curves confirmed that the current number of samples collected represented the majority of OTU richness in the actual metazoan community for each salinity zone (92%–94% coverage, Figure 2), bay (94%, Supplementary Figure S3) and distance from the marsh edge (90%, Supplementary Figure S3), which is consistent with a high Coverage Estimate for the entire data set (96.7%, Supplementary Table S2). However, the dataset was heterogeneous with a high Coefficient of Variation (1.4, Supplementary Table S2). Only

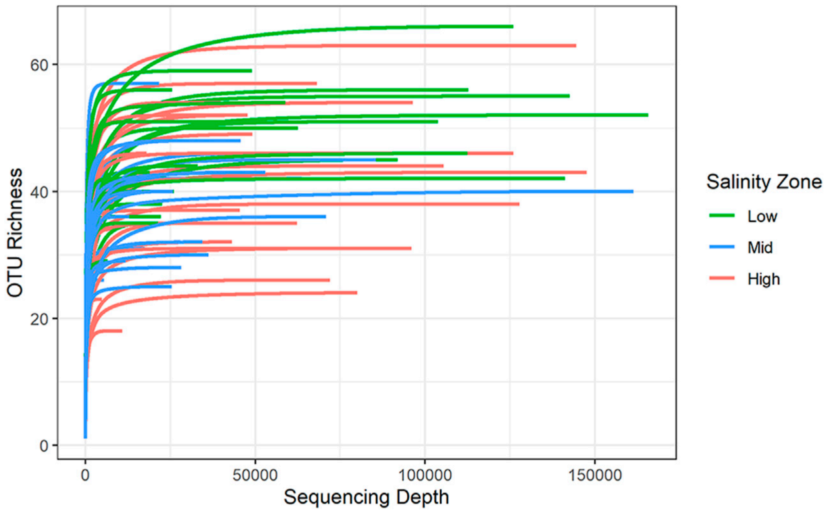

FIGURE 1
Alpha rarefaction curves show the number of OTUs detected in each sample when randomly sampling sequences at each given sequencing depth.

88 of the 312 OTUs were present in more than 10 samples but they represented the majority of the incidences (2,345 of the 2,993 incidences, Supplementary Table S2). The observed OTU richness (312) represented at least 58% (535 OTUs at the upper bound of the Chao2 model) to 92% (339 OTUs at the lower bound of the Homogenous Model, Supplementary Figure S4) of the total estimated metazoan OTU richness.

Alpha-diversity of the saltmarsh metazoan community

The OTU richness was significantly higher in samples from the low salinity zone than in those from the mid salinity zone [Kruskal-Wallis pairwise test, χ2(2) = 9.16, Benjamini Hochberg corrected $p < 0.01$], but samples from the high salinity zone did not differ in terms of metazoan richness from the other salinity zones (Figure 3). No significant differences in OTU richness were detected for samples collected from different bays or distances from the marsh edge or interaction of the three factors. Values for the Faith's Phylogenetic Diversity metric showed no significant differences for any factor or interaction of factors. Since Faith's PD was not significantly different across the three salinity zones (Kruskal-Wallis test, $p = 0.35$) the elevated richness in low salinity samples was not caused by the addition of widely phylogenetically different taxa.

Correlation between 21 soil chemistry variables (Supplementary Table S3) and OTU richness only produced significant results for carbon and nitrogen percentage, which were both positively correlated with metazoan richness ($p = 0.015$ and 0.011, Spearman rank). Faith's PD was not significantly correlated with any of the soil chemistry variables ($p > 0.05$).

Beta-diversity of metazoans from different bays, salinity zones, and distance from the marsh edge

Similarity among metazoan communities measured by the number of shared OTUs and Sørensen index values was lowest among salinity zones, followed by similarity between bays and among distances from marsh edge (Supplementary Table S4). Pairwise Sørensen index values indicated that the low and high salinity zone communities had the lowest similarity of any pair of the salinity zone communities because they shared the lowest number of OTUs (Supplementary Table S4).

All factors (salinity zone, bay, and distance from marsh edge) and all factor interactions had significant effects on the differentiation of metazoan composition of samples in the dataset (Adonis test with Benjamini-Hochberg correction, Table 1). Salinity accounted for more variation in the dataset than any other factor or interaction (20%, Table 1). Distance from the marsh edge and bay explained only 7% and 6% of variation, respectively. Nevertheless, the significant interactions indicate that the effect of salinity also depend on those minor factors.

Since significant differences in dispersion had been observed for salinity and bay (PERMDISP, Benjamini-Hochberg corrected $p < 0.05$), differences detected by Adonis within these factors may be a combination of differences in the multivariate spread of the groups and

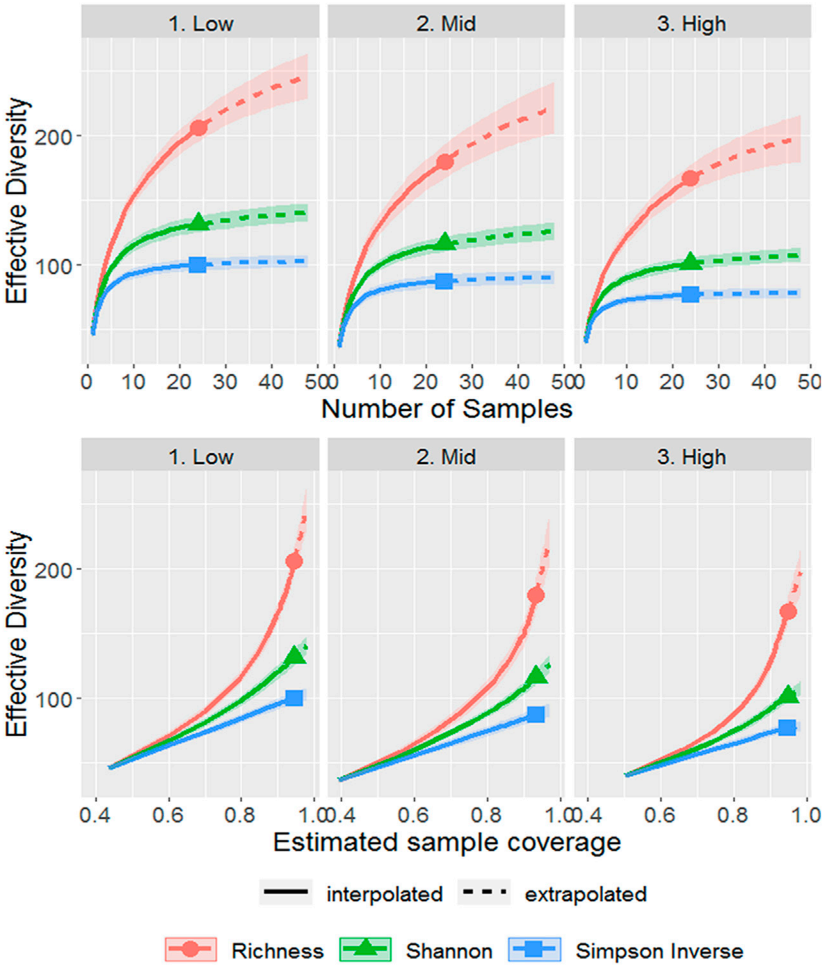

FIGURE 2
Sample-(top) and coverage-based (bottom) rarefaction curves for the metazoan community from marsh soil of three salinity zones (low, mid, and high salinity) calculated with three metrics of effective diversity (Richness, Shannon, and Simpson inverse indices). Extrapolation extended out to twice the number of samples. The shaded area around each curve represents the 95% confidence interval.

differences in the location of the groups in the multivariate space. To visualize contributions of the three factors in terms of spread and differentiation between groups in the multivariate space, we employed NMDS scaling (Figure 4). Plotting the Beta-diversity among samples from different salinity zones showed a distinct separation of samples from the low and high salinity zones, with samples from the mid salinity zone falling roughly between them (Figure 4 top). Samples from the mid salinity zone showed larger dispersion than the samples from the high and low salinity zones (pairwise PERMDISP, Benjamini Hochberg corrected $p < 0.05$). The large spread of samples in the mid salinity zone was mainly caused by samples from Caillou Bay which had significantly different multivariate spread from all other groups (pairwise PERMDISP, $p < 0.05$, Figure 4 bottom). Distance from the marsh edge showed no particular pattern (Rayle 2021).

Taxonomic assignment of metazoan infauna in Louisiana marshes

Each sample contained between 5 and 10 phyla. Nematodes, arthropods, and annelids were present in all samples (Figure 5). The phylum Nematoda made up the largest percentage of the 312 OTUs and the 2,993 total incidences (Supplementary Figure S5), followed by Arthropoda, Annelida, and Platyhelminthes. Platyhelminthes, rotifers, and mollusks were present in nearly all samples. Members of the phyla Nemertea, Tardigrada, Xenacoelomorpha, and Calcinea were rarely detected in samples (Figure 5; Supplementary Table S5).

The most common OTU in the dataset was assigned to the rotifer *Octotrocha speciosa* (detected in 99% of samples), followed by an OTU assigned to the arthropod *Chrysops flavidus* (deer fly in 96% of samples) and one assigned to the nematode *Spirinia parasitifera*

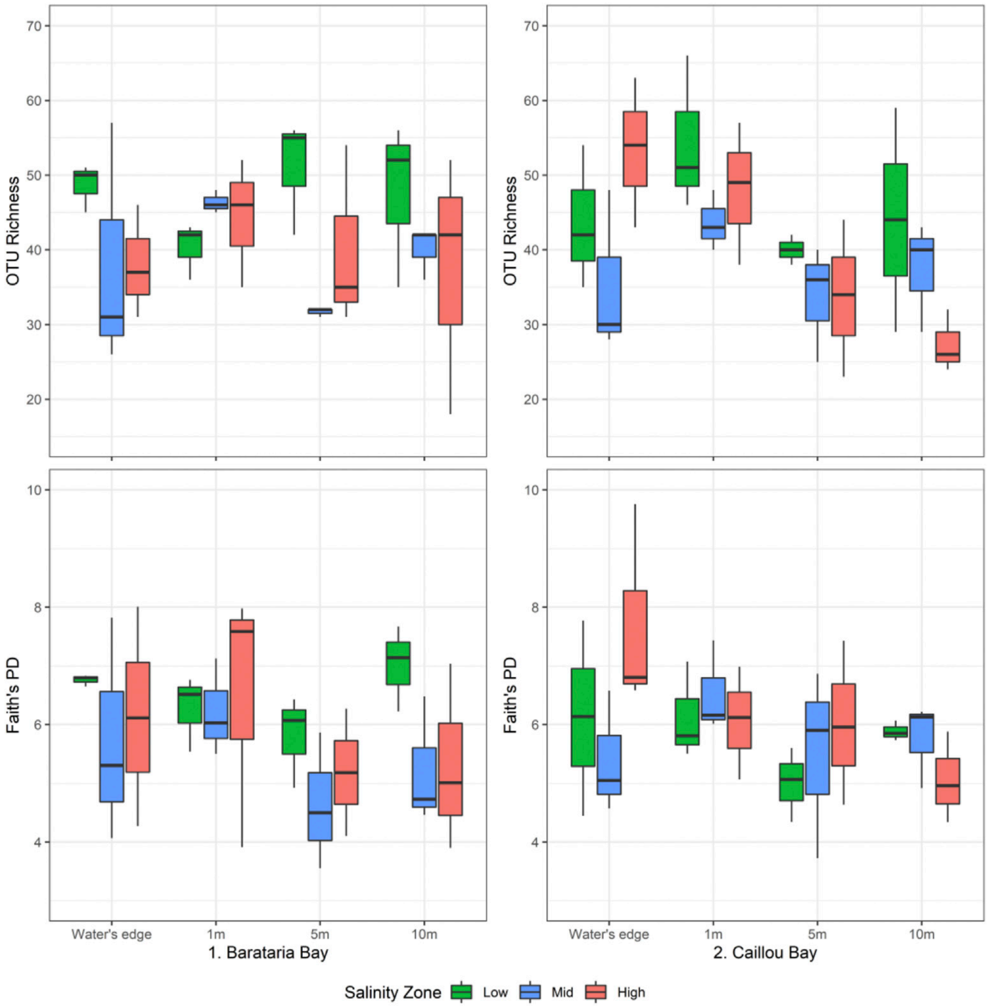

FIGURE 3
Boxplots of metazoan OTU richness (top) and Faith's PD values (bottom), divided by bay, salinity zone, and distance from marsh edge.

TABLE 1 Adonis test results for differentiation of metazoan communities among samples from different bays (Caillou and Barataria), distances from the marsh edge (0, 1, 5, and 10 m) and salinity zones (low, mid, and high). All *p*-values remained significant after the Benjamini-Hochberg correction.

Factor	Df	SumOfSqs	R2	F	Pr(>F)
Bay	1	0.8142	0.0599	7.6024	0.0001
Distance from marsh edge	3	0.9711	0.0714	3.0225	0.0001
Salinity zone	2	2.7142	0.1997	12.6720	0.0001
Bay:Distance	3	0.5914	0.0435	1.8407	0.0033
Bay:Salinity	2	1.1212	0.0825	5.2345	0.0001
Distance:Salinity	6	1.0878	0.0800	1.6930	0.0006
Bay:Distance:Salinity	6	1.1527	0.0848	1.7940	0.0006
Residual	48	5.1405	0.3782		
Total	71	13.5929	1.0000		

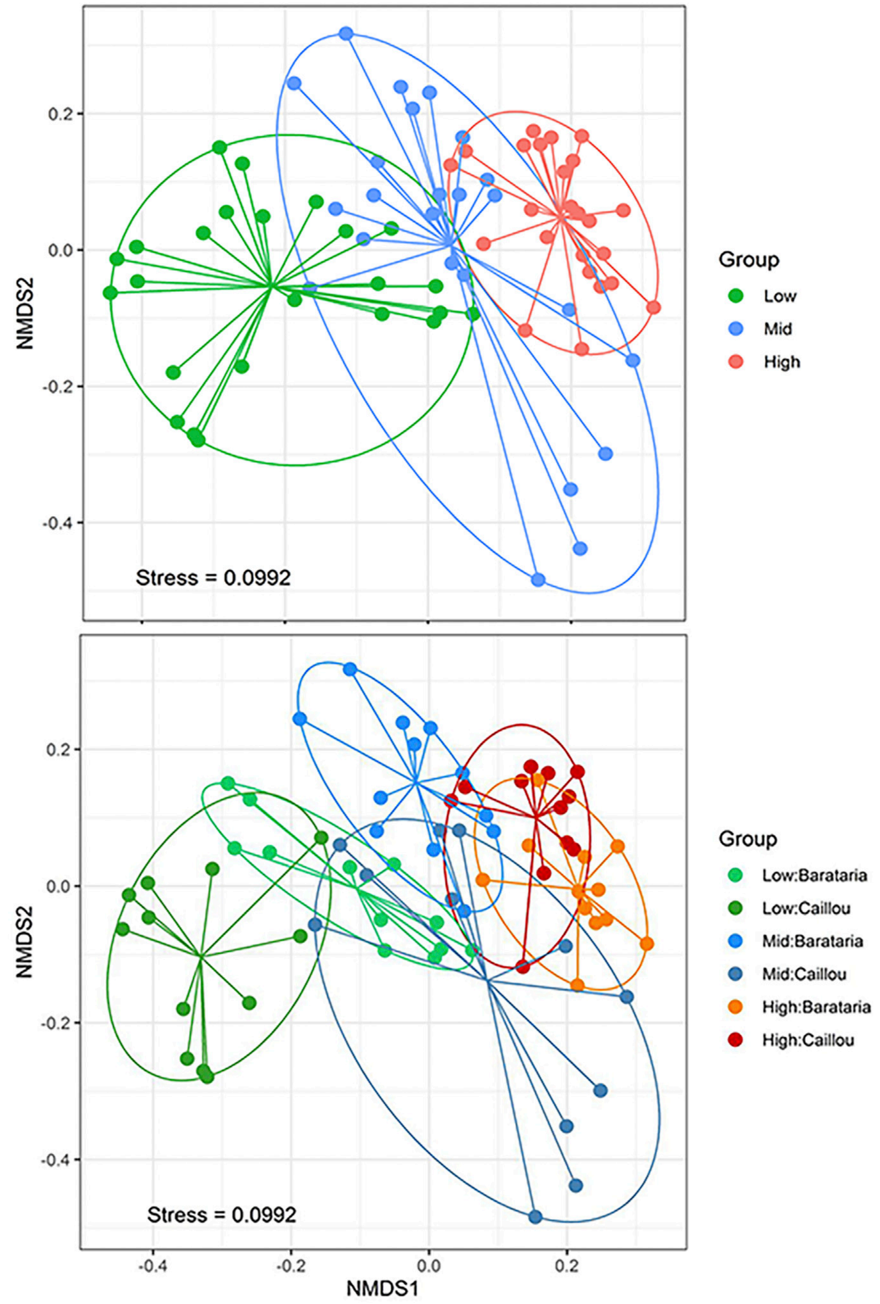

FIGURE 4
Plot of the first two dimensions of the NMDS ordination of the Sørensen distance matrix, with salinity zones (top) and from each bay (bottom). Dots represent the metazoan composition of individual samples. Solid ellipses represent the elliptical hulls of the groups. Solid, straight lines connect a sample to the group centroid. Stress denotes the goodness of fit of the regression of the original distance matrix values against the ordination distances.

(94% of samples, Supplementary Table S5). The frequent group (in >10 samples) consisted of 88 OTUs. The frequent group included 38 nematode OTUs of the orders Enoplida (9 OTUs), Monhysterida (8 OTUs), Tylenchida (7 OTUs), Chromadorida (4 OTUs) and Dorylamida (4 OTUs), Desmodorida (2 OTUs), Plectida (2 OTUs), Areolamida (1 OTU), and Rhabditida (1 OTU, Supplementary Table S5). The frequent group also contained 19 Arthropoda OTUs of which nine OTUs belonged to the crustacean orders Podocopida, Harpacticoida, Decapoda, and Tanaidacea, five OTUs belonged to the insect order Diptera, four

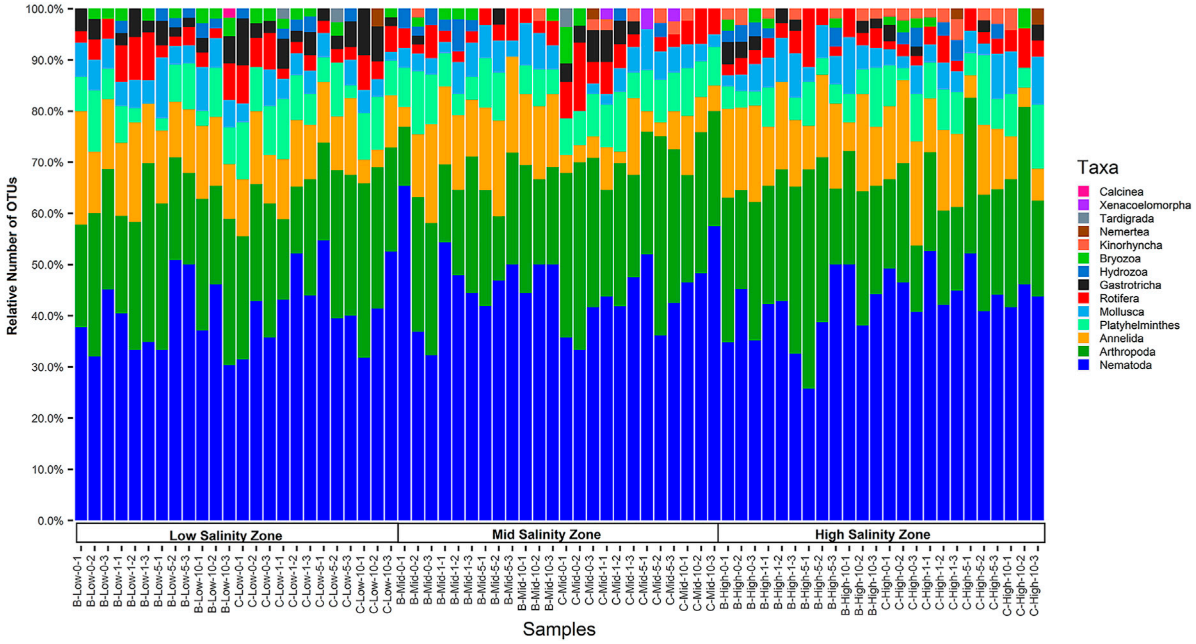

FIGURE 5
Taxa bar plots of the 72 samples collected from the three salinity zones of Barataria and Caillou Bays during July 2018. Each colored section represents the relative proportion of OTU incidences belonging to different phyla within that sample. Phyla are sorted within each bar by the overall highest to lowest relative percentage (from top to bottom). For the *x* axis, samples are labelled as B (Barataria) or C (Caillou), and Low, Mid, and High for the salinity zones. The number following the salinity zone designation indicates the distance from the edge of the marsh, designated as 0, 1, 5, or 10 m, followed by the transect replicate number.

to the Collembola, and one OTU belonged to the Acari (Supplementary Table S5). Of the 12 frequently detected annelid OTUs, half were assigned as the oligochaete order Haplotaxida the other half were polychaetes. Three Haplotaxida OTUs belonged to the family Naididae, including the most commonly detected annelid OTU in the dataset assigned to the species *Monopylephorus rubroniveus* (Naididae) and detected in 88% of samples. The remaining Haplotaxida OTUs were all assigned to members of the genus *Marionina*. Three polychaete OTUs of the order Spionida each were assigned to members of various genera in the family Spionidae, while the three Phyllodocida OTUs were assigned to the family Nereididae. The seven Platyhelminthes OTUs of the frequent group were mostly assigned to the order Rhabdocoela (5 OTUs), with one OTU assigned to the order Macrostomida and one assigned to the class Catenulida. In addition, top GenBank matches identified an ambiguous OTU as another member of the Macrostomida, *Macrostomum lignano*, which appeared in 63% of samples. Both OTUs of the Gastrotricha frequent group were assigned to the order Chaetonotida. The three frequently detected mollusk OTUs were members of the sea snails (orders Littorinimorpha and Caenogastropoda) and bivalves (Veneroida), the two Bryozoa OTUs were assigned to the orders Ctenostomatida and Plumatellida and the two Rotifera OTUs were assigned to the orders Flosculariaceae and Ploimida. The only hydrozoan in the frequent group was assigned to the order Limnomedusae, while the only kinorhynch OTU was assigned to the order Cyclorhagida (*Echinoderes* sp.). Assignments of OTUs from the infrequent group are also listed in Supplementary Table S5.

Taxa unique to salinity zones

Overall, metazoan DNA samples showed higher taxa richness and incidence in low salinity compared to mid salinity zones; however, samples from high salinity zones had high values similar to low salinity (Supplementary Table S5). The Nematoda and Mollusca were fairly equally distributed across salinity zones (Supplementary Table S5). Gastrotricha, Bryozoa, Platyhelminthes, and Rotifera were more prevalent in the low salinity zone (61%, 48%, 41%, and 41% of incidences, respectively) than in the other two salinity zones. Kinorhyncha (*Echinoderes* sp.) were never detected in the low salinity zone and were more commonly detected in the high (68% of incidences) than in the mid salinity zone (32%).

While most Arthropoda were detected in low salinity, insect diversity was U-shaped across the salinity zones with lower detection in mid salinity (97 incidences of 20 OTUs in 12 families) when compared to the low (106 incidences of 25 OTUs in 16 families) and high salinity zones (106 incidences of 24 OTUs in 15 families, Supplementary Table S5). Fifty-five percent of the insect OTUs detected were unique to a single salinity zone with most of them being unique in the low salinity zone. However, there were five Diptera OTUs of gall midges (Cecidomyiidae) and tabanids (*Chrysops* and *Tabanus* spp.) in the frequent group that were common among all salinity zones. These and several other OTUs were assigned to species known from Louisiana marshes, including *Cedusa obscura* (Derbidae) and biting midges (Culicoides, Cerapogonidae) (Supplementary Table S5). Similarly, the frequency of detection of the Annelida, across salinity zones was U-shaped with lower numbers of annelid incidence (26%) in the mid salinity zone compared to low and high salinity zones (~37% of total annelid incidence).

Many of the OTUs (113) were either unique to one of the salinity zones or were absent from one of the salinity zones (Supplementary Table S5). Twenty-three of the unique OTUs belonged to the frequent group (in >10 samples) and included nematodes, platyhelminths, annelids, arthropods, gastrotrichs, and kinorhynchs (Table 2). Two nematodes (assigned as *E. filiformis* and *Prismatolaimus* sp.) and one platyhelminth OTU (assigned as *R. simplex*) were unique to the low salinity zone. One gastrotrich (*C. novenarius*) was detected 15 times in the low salinity zone samples but was absent in mid salinity and only detected once in high salinity (Supplementary Table S5). A second *Prismatolaimus* sp. OTU, *Anoplostoma* sp., and *Prodorylaimus* sp. of the Nematoda, two annelids (assigned as *M. southerni* and *D. aestuarina*), four arthropods (Collembola assigned as ambiguous taxa and *Cryptopygus sverdrupi* as well as ostracods of the Cyprididae and *Cypria* sp.) and the platyhelminth assigned as *O. truncula* were present in low and

TABLE 2 Frequent OTUs with unique incidences in samples from different salinity zones. OTUs listed were not detected in at least one of the salinity zones (L = Low, M = Mid, H = High), and were detected at least twice in one or two salinity zones. No frequent OTUs were unique for mid or high salinity. Infrequent OTUs (in ≤10 samples) are listed in Supplementary Table S6.

Phylum	Order	Incidence	Lowest SILVA assignment	Top GenBank match	L	M	H
Unique to L							
Nematoda	Enoplida	16	Triplonchida; Ambiguous_taxa	*Prismatolaimus* sp. MCb6	16	0	0
Nematoda	Monhysterida	11	*E. filiformis*	*E. filiformis* strain	11	0	0
Platyhelminthes	—	11	*R. simplex*	*R. simplex* isolate K05_04	11	0	0
Unique to L + M							
Annelida	Haplotaxida	13	*M. southerni*	*Marionina nothachaeta* clone LM225	9	4	0
Annelida	Phyllodocida	11	*D. aestuarina*	*D. aestuarina*	10	1	0
Arthropoda	Podocopida	15	Cyprididae gen sp. Mexico	*Cypridopsis* sp. Ca1 isolate CYD_SMA1	10	5	0
Arthropoda	—	13	Collembola; Ambiguous_taxa	*Dicyrtomidae* sp. R3	8	5	0
Arthropoda	Entomobryomorpha	14	*C. sverdrupi*	*Isotoma viridis* isolate 6G1a1_JC448	10	4	0
Arthropoda	Podocopida	12	*Cypria* sp. QY-2003	*Physocypria* cf. *biwaensis* 32 IK-2017	9	3	0
Nematoda	Enoplida	22	Triplonchida; Ambiguous_taxa	*Prismatolaimus* sp. MCb2	18	4	0
Nematoda	Enoplida	19	*Anoplostoma* sp. 1093	*Anoplostoma* sp. 1093	10	9	0
Nematoda	Dorylaimida	12	*Prodorylaimus* sp. HHBM-2007a	*Prodorylaimus* sp. HHBM-2007a	10	2	0
Platyhelminthes	Rhabdocoela	18	*O. truncula*	*O. truncula*	12	6	0
Unique to L + H							
Gastrotricha	Chaetonotida	16	*Chaetonotus cf novenarius* TK151	*Haltidytes pseudosquamosus* voucher HA1	15	0	1
Unique to M + H							
Annelida	Phyllodocida	14	*A. succinea*	*A. succinea* isolate AlSu-02	0	1	13
Annelida	Phyllodocida	11	*N. jaya*	*Namalycastis abiuma* isolate naa185	0	6	5
Kinorhyncha	—	24	Cyclorhagida; Ambiguous_taxa	*Echinoderes* sp. MVS2014	0	8	16
Nematoda	Tylenchida	20	*M. spartinae*	*M. spartinae*	0	2	18
Nematoda	Desmodorida	19	*Prochaetosoma* sp. 3 HSR-2009	*Prochaetosoma* sp. OK-2015 isolate t2	0	1	18
Nematoda	Enoplida	15	*Halalaimus* sp. 1034	*Halalaimus* sp. 1034	0	6	9
Nematoda	Chromadorida	13	*Dichromadora* sp. 2 JH-2014	Cf. *Dichromadora* sp. 2 JH-2014	0	7	6
Platyhelminthes	Macrostomida	15	*M. kepneri*	*M. kepneri* voucher MTP LS 285	0	5	10
Platyhelminthes	Rhabdocoela	13	*M. terminostylis*	*Itaipusa sinensis* isolate YTP3	0	4	9

mid but absent from high salinity (Table 2). In contrast, two frequent group members of the annelids (*A. succinea* and *N. jaya*), a kinorhynch (assigned as an ambiguous Cyclorhagida), four nematodes (*Meloidogyne spartinae*, *Prochaetosoma* sp., *Halalaimus* sp., and *Dichromadora* sp.), and two platyhelminths (assigned as *M. kepneri* and *M. terminostylis*) were present in both the mid and high but not low salinity zones. Rare OTUs unique to salinity zones, including the polychaete *Manayunkia aestuarina*, are listed in Supplementary Table S6.

Annelid (e.g. *Marionina* spp.) and mollusk OTUs were much more commonly detected in samples from Barataria Bay, while gastrotrichs, platyhelminths and kinorhynchs (e.g., *Echinoderes* sp.) were more commonly detected in the Caillou Bay samples (Supplementary Table S5). Annelids, gastrotrichs, and hydrozoans more frequently detected at the marsh edge and at 1 m from the marsh edge while mollusks and nematodes were more common in the samples 1 m from marsh edge than further inland (Supplementary Table S5). None of the frequent group OTUs were uniquely found at a certain bay or distance from the marsh edge. Incidences of frequent and rare OTUs for each bay and distance in addition to salinity zone, are listed in Supplementary Tables S5, S7.

Discussion

Coastal marsh ecosystems, especially within Louisiana, are at risk due to numerous natural and anthropogenic environmental stressors (Yuill et al., 2009; Wong et al., 2010; Lin and Mendelssohn 2012; Kirwan et al., 2016). Assessment of the impact of these stressors on marsh communities and habitat remediation *via* restoration projects must take changes in salinity levels and resulting shifts in marsh communities into account. Therefore, detailed inventories of organisms relative to salinity zones are needed as baselines to measure impacts of environmental changes on marsh community health.

Soil meiofauna and macrofauna (collectively, infauna) have been studied for decades in Louisiana's coastal marshes and inventories based on morphological classification are relatively complete for major taxa (Fleeger, 1985; Fleeger et al., 2015; Fleeger et al., 2018; Fleeger et al., 2019). However, morphological identification is likely to underestimate meiofaunal diversity (Gielings et al., 2021) and only few studies included eukaryote metabarcoding to describe infauna diversity in Louisiana's tidal marshes (Husseneder et al., 2022). Moreover, species inventories of Louisiana's tidal marshes lack detailed metadata concerning salinity, which is expected to be one of the major factors changing due to sea level rise and freshwater diversions (Das et al., 2012). This project was designed to provide baselines of metazoan biodiversity based on meiofauna and environmental DNA across salinity zones to monitor changes in marsh habitat primarily due to salinity changes with saltwater intrusion or freshwater influx when the planned Mid-Barataria Sediment Diversion commences (Coastal Protection and Restoration Authority of Louisiana, 2007; Coastal Protection and Restoration Authority of Louisiana, 2012; Coastal Protection and Restoration Authority of Louisiana, 2017; Peyronnin et al., 2013). Freshwater flow has been shown to drastically change meiofauna populations (Montagna and Kalke 1992; Montagna et al., 2002).

In summary, our study showed that salinity, but not bay or distance from the marsh edge significantly impacted metazoan Alpha-diversity within samples. Salinity was also a major factor accounting for 20% of the variation in community composition among samples, with many frequent OTUs being uniquely present or absent in salinity zones. Bay and distance from the marsh edge as well as interactions among the factors only had minor impacts on community differentiation (all <8%).

Differences in metazoan communities among bays might be driven by differences in the plant communities between the bays. For example, *Marionina* spp. (Annelida), which are likely associated with Spartina stems (Healy and Walters, 1994), were more common in the Barataria Bay, which had more Spartina ground cover than Caillou Bay (Aker, 2020). Long-term impacts of the Deepwater Horizon oil spill might also explain some differences between bays. Taxa with slow recovery due to poor dispersal activity including the kinorynchs (Fleeger et al., 2018; Fleeger et al., 2019), might still endure reduced abundance in Barataria Bay, which had been more heavily impacted by the Deepwater Horizon oil spill than Caillou Bay (Michel et al., 2013). Kinorhyncha detected by metabarcoding made up less than 1% of the total OTUs and were assigned as *Echinoderes* sp. *Echinoderes* were an indicator for oil pollution, since they were absent from the heavily oiled marsh sites following the Deepwater Horizon oil spill (Fleeger et al., 2015; Fleeger et al., 2018). Even though our sites were not oiled, area wide population reduction for some species has been observed even at sites not directly oiled (Husseneder et al., 2022).

Previous studies reported distance effects with higher invertebrate densities near the marsh-water interface due to larval recruitment and food deposited by inundation (Kneib, 1984; MacKenzie et al., 2015) and clear differentiation of benthos communities across marsh zones from the tidal flats landward to the pioneer zone, low marsh and high marsh (MacKenzie et al., 2015; van Regteren et al., 2020). Dominance of annelids and mollusks in the pioneer zone and tidal flats reported by van Regentren et al. (2020) supports the higher frequency of detection of these phyla at our sample sites close to the marsh edge. In contrast, MacKenzie et al. (2015) found higher species richness in the high marsh compared to low marsh but this was due to sampling across larger distances and elevations compared to our study. Overall, distance effects were much less pronounced in our metabarcoding study and none of the frequently detected OTUs were uniquely present or absent at a certain distance. Our study used much shorter transects, which explains why less community differentiation was detected compared to other studies across marsh zones. Moreover, with the low elevation gain of Louisiana marshes even our sites furthest from the marsh edge (10 m) received frequent inundation.

Similar to our study, salinity was a major explanatory variable of benthic macro- and meiofauna community variation in previous studies. Generally, macro- and meiofauna diversity increases with higher salinity along marine salinity gradients (Broman et al., 2019). Studies in saltmarshes of the East coast and estuaries of the Texas Gulf coast found weak correlations of salinity to observed soil invertebrate communities (MacKenzie et al., 2015; Van Diggelen and Montagna, 2016). However, in our metabarcoding study, the relationship of overall metazoan diversity and salinity was U-shaped with the lowest values in mid salinity zones. In particular, insects and annelids had the lowest detection frequency in mid salinity. This pattern has been observed frequently in estuarine systems (Deaton and Greenberg, 1986).The mid salinity zone in our study averaged approximately 8.5 ppt, which is close to the "Artenminimum" zone between 5 and 8 ppt where overall species numbers are lowest in estuaries and bodies of brackish water but numbers of brackish water species are highest (Remane, 1931; Deaton and Greenberg, 1986). The low species diversity in the mid salinity zone has been hypothesized to occur because speciation rates in dilute brackish environments are lower than for freshwater and marine species and few animals acquire successful physiological adaptations to tolerate the range of conditions in this habitat (Deaton and Greenberg, 1986).

In traditional studies of tidal marsh fauna, nematodes are typically among the most abundant non-insect invertebrate group (Bell, 1979; Fleeger, 1985; Alves et al., 2013; MacKenzie et al., 2015), which was consistent with nematodes being the most frequently detected group in our study. Nematodes are known for their ability to tolerate extreme environmental conditions (Zeppilli et al., 2018). Previous studies showed a positive correlation of nematode diversity or abundance with salinity gradients of intertidal rivers and the Baltic Sea (Tran et al., 2018; Broman et al., 2019). However, no such relationship was detected with our incidence data across similar salinity ranges. The most frequently detected nematode, *S. parasitifera* a marine microbivore in the family Desmodoridae also commonly found in European estuaries (Soetaert et al., 1995; Hodda, 2011), was detected across all three salinity zones. Five nematode OTUs of the frequent group were never detected in samples from the high salinity zone: *E. filiformis,* two *Prismatolaimus* sp. OTUs, *Anoplostoma* sp., and *Prodorylaimus* sp. We predict that salinity reduction following the opening of the Mid-Barataria freshwater diversion will lead to range expansion of these OTUs into formerly high salinity areas. The opposite is expected for taxa predominantly found in high salinity, such as the plant-parasitic root-knot nematode *M. spartinae* (Plantard et al., 2007) and an OTU of the genus *Prochaetosoma*, which contains nematode species known from sub-tidal zones (Rho et al., 2010). Notably, some nematodes can survive in hypersaline zones with salinities higher than seawater (Zeppilli et al., 2018).

Arthropoda were the second most abundant group in the dataset after the nematodes. Similar to the nematode distribution, arthropod incidences were approximately evenly distributed across the different bays and distances from marsh edges, but Arthropods were more commonly detected in the low salinity zone than in the mid and high salinity zone. This was mainly caused by non-insect arthropods. Frequently detected non-insect arthropods that were possible bioindicators for low salinity included Collembola assigned as ambiguous taxa and *C. sverdrupi* as well as ostracods of the Cyprididae and *Cypria* sp.

Insects are typically among the most common organisms in the low and high marsh (MacKenzie et al., 2015; van Regteren et al., 2020). To confirm taxonomic assignments of the insect OTUs and compare incidences to organismal abundance, we compared our data to those of Aker (2020), who conducted a year-long sampling of insects (2018-2019) using sweep net techniques at the same sites and salinity zones as sampled in this study. While insect abundance cannot be directly compared between metabarcoding and sweep net collection studies due to differences in sampling strategy and data representation, broader insect community comparisons can be made. Aker's studies found that insect abundance increased dramatically with salinity while insect family-level biodiversity decreased as salinity increased, which mirrored the decrease in plant species. In contrast, the DNA-based incidences and number of insect OTUs and families was U-shaped across salinity zones with slightly lower numbers detected at the mid salinity zone and high salinity zones.

Over half of the insect OTUs detected were unique to a single salinity zone. Being unique for a salinity zone alone could not unequivocally identify these insect OTUs as salinity bioindicators due to their low detection rate; however, closely related species have been confirmed independently as salinity bioindicators (Aker, 2020). *Ischnura ramburii* (Coenagrionidae, Odonata) and *C. obscura* (Derbidae, Hemiptera) were identified as bioindicators of low salinity by Aker (2020). Therefore, the metabarcoded OTUs from the families Derbidae (*C. obscura*) and Coenagrionidae (ambiguous taxa) likely represent bioindicators of low-salinity insect communities because they were detected at the low salinity sites within a month of their peak abundances described by Aker (2020). Biting midges of the genus Culicoides (Ceratopogonidae) are abundant in marshes along the Atlantic (Kneib, 1984; MacKenzie et al., 2015) and Gulf coast (Aker, 2020). The *Culicoides* OTU detected predominantly in mid and high salinity zones is likely the highly abundant high salinity bioindicator identified in Aker's studies, *Culicoides mississippiensis*.

Fly larvae are common in saltmarsh macrofaunal samples but are typically rare in meiofauna assemblages (Kneib, 1984). In our study, five Diptera OTUs belonging to gall midges (Cecidomyiidae) and tabanids (*Chrysops* and *Tabanus* spp.) were common among all salinity zones. Gall midges of salt marsh succulents have been described from Australia

(Veenstra-Quah et al., 2007), but were not found in Aker's sweep net surveys because larvae are found in plant tissue and dispersing adults might only be present for a limited time. The detection rate of tabanids across samples and their wide distribution across salinity zones is remarkable and supports the prevalence of tabanid larvae in salt marsh sediment where they are top invertebrate predators (Kneib, 1984; Husseneder et al., 2022). All tabanid OTUs belonged to the known estuarine species of Tabanidae (*Tabanus nigrovittatus*, *T. acutu*s, *T. hinellus*, and *C. flavidus*) collected from the same coastal sites by Aker (2020) with sweep nets and Davis (2022) using tabanid targeted trap techniques (Hribar et al., 1991). *Tabanus nigrovittatus* is the saltmarsh greenhead horse fly that showed severe population crashes after the Deepwater Horizon oil spill and is thus regarded as a bioindicator of oil pollution (Husseneder et al., 2016; Husseneder et al., 2018; Husseneder et al., 2022). Although direct comparison of the different detection methods is difficult, the sweep net and metabarcoding inventories represent complementary aspects of the marsh community. The advantage of the use of metabarcoding over traditional insect surveys by collection is that insect taxa can be monitored without the need for catching and identifying intact organisms. This makes metabarcoding especially useful for high throughput detection of insects including those living in inaccessible niches or outside of the daily or annual activity period.

Similar to the insects, the frequency of detection of the third most numerous group, i.e., the Annelida, across salinity zones was also U-shaped with lower numbers of annelid incidence in the mid salinity zone compared to low and high salinity zones. The annelid species *M. southerni* and *D. aestuarina* were never detected in the high salinity zone samples and are likely low salinity bioindicators. The organisms that these OTUs represent may experience range expansions associated with decreased salinity resulting from the future Mid Barataria freshwater diversion. The polychaete *M. aestuarina*, which is commonly represented among both macrofauna and meiofauna in marshes of the Southeastern U.S. (Bell, 1979; Kneib, 1984) and is a well-known bioindicator species for the impact of oil spills (Fleeger et al., 2015; Fleeger et al., 2018) was detected in the high salinity zone but in rare incidences. Polychaetes typically show the highest abundance in the tidal flats with higher soil salinity and moisture than the low marsh where oligochaetes have their peak abundance (MacKenzie et al., 2015; van Regteren et al., 2020). The limited detection of Manayunkia is likely due to the processing of the samples, since this species was among the most frequent OTUs in unprocessed soil at three marsh islands in Barataria Bay surrounding Bay Jimmy while in samples sieved for meiofauna it belonged to the infrequent group (Rayle, 2021).

The Platyhelminthes were the fourth most commonly detected and numerous group of OTUs. The Platyhelminthes are not commonly detected or reported in traditional studies of meiofauna (Fleeger et al., 2018), presumably since traditional sampling and extraction methodology destroys the soft bodied members of the meiofauna such as the Platyhelminthes (Carugati et al., 2015). However, they are abundantly represented in environmental DNA studies (Fonseca et al., 2010). Detection frequency of platyhelminths decreased with increasing salinity. Two Platyhelminthes (*O. truncula* and *R. simplex*) were never detected in the high salinity zone and, thus, are likely indicators of reduced salinity, which might be able to expand into the areas currently designated as high salinity when the Mid-Barataria freshwater diversion opens. In contrast, two members of the genus *Macrostomum* were never detected in the low salinity zone and are, thus, potential candidates for indicators of saltwater intrusion. The remarkable ability of saline acclimation of intertidal infauna has been studied in the model *M. lignano*, which was the most abundant flatworm OTU in our study. *Macrostomum* was able to survive in high salinity through upregulation of antioxidant enzymes to combat oxidative stress from increased respiration rates to meet the energy demand for osmoregulation (Rivera-Ingraham et al., 2016). In low salinity *M. lignano* decreased respiration and activity in a hyposmotically induced metabolic shutdown similar to that in other intertidal invertebrates like the marsh periwinkle snail.

The Gastrotricha were the fifth most numerous but the seventh most commonly detected group and were more prevalent in the low salinity zone than in the other two salinity zones. Specifically, the OTU assigned as *C. novenarius* can be considered as a low salinity bioindicator which might experience a range expansion with freshwater diversions. The Mollusca were the sixth most numerous group of OTUs and the fifth most commonly detected. Most of the conspicuous mollusk species in southeastern US saltmarshes are large and are only represented by traces of their environmental DNA in our data (Kneib, 1984). Most mollusk OTUs were roughly evenly distributed across salinity zones. Kinorhyncha (*Echinoderes* sp.) were never detected in the low salinity zone and were most prevalent in the high salinity zone. Kinorhynchs of the genus *Echinoderes* are believed to have special osmoregulatory adaptations provided by an enlarged nephridial sieve plate that enables them to tolerate salinity fluctuations and hypersaline conditions (Zeppilli et al., 2018). Therefore, increasing kinorhynch populations may be an early indicator of high salinity through saltwater intrusion.

This metabarcoding study provided a comparison of compositional inventories and diversity of soil organisms among salinity zones in Louisiana tidal marshes based on sequence data from metazoan meiofauna and environmental DNA. The advantage of the use of metabarcoding over traditional surveys by collection is that taxa can be monitored based on their DNA without the need for having intact organisms, keys and expertise for time-consuming morphologically identification. This makes metabarcoding especially useful for high throughput detection of diversity including taxa that are not well described or difficult to collect because they are small, fragile, cryptic or inactive during the collection period (Gielings et al., 2021). However, the disadvantage is that it is unknown how long environmental DNA can be detected in the absence of living organisms (Harrison et al., 2019; Sakata et al., 2020), which might create background noise for monitoring current state and functional changes in ecosystems. In addition, number of sequence reads correlates only weakly with organism numbers or biomass (Egge et al., 2013) in multicellular organisms, so population abundance is more accurately determined by collections of organisms *via* traditional methods. Ideally, metabarcoding would not replace traditional sampling methods but rather add a complementary view of the marsh soil community with increased sampling depth and diversity detection. In summary, using environmental DNA we found numerous meiofaunal taxa in Louisiana salt marshes that are potentially limited in distribution by specific salinity conditions. These data are not only useful by providing potential indicator taxa for salinity changes from future events such as freshwater releases or sea-level rise, but also for predicting what meiofaunal communities may look like before these changes occur.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA706429.

Author contributions

LF and CH designed the study and obtained funding. LF supervised the field work, CH supervised the lab work and bioinformatics. BA and PR conducted the soil collection. PR performed DNA extractions and analyses. WT supervised next-generation sequencing and provided expertise in bioinformatics and meiofauna composition. CH, PR, BA, and LF wrote the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.950527/full#supplementary-material

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Enhanced susceptibility to oiling may limit denitrification recovery in marshes subjected to woody encroachment

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Coastal salt marshes provide valuable ecosystem services but are subjected to multiple concomitant stressors that may impact their ability to provide those services. Global climate change has led to the poleward expansion of mangroves into salt marshes on each continent where mangroves and marshes co-occur. In the northern Gulf of Mexico, warming winter temperatures have resulted in the expansion of *Avicennia germinans* (black mangrove) into forb-dominated salt marshes, resulting in a shift in ecosystem structure that can impact the ecosystem services marshes provide, including biogeochemical processes such as nitrogen removal. There have been limited studies addressing how mangrove expansion impacts nitrogen removal rates in salt marshes, but it is possible that mangroves enhance microbial nitrogen removal capacity through more efficient oxygen translocation to sediments. However, mangroves are more sensitive to oiling (such as occurred during the 2010 *Deepwater Horizon* spill) than marsh plants, such as *Spartina alterniflora*, which have a higher turnover. Thus, even if they enhance nitrogen removal, if they cannot withstand disturbances such as oiling, there still may be a loss of function associated with woody encroachment. We conducted a field study to assess the impact of woody encroachment in mediating biogeochemical recovery 7 to 8 years after the *Deepwater Horizon* oil spill. We collected sediments from *S. alterniflora*- and *A. germinans*-dominated plots in the Chandeleur Islands (LA, United States), a chain of barrier islands in the northern Gulf of Mexico subjected to a range of oiling following the spill. We compared nitrate reduction rates (denitrification and dissimilatory nitrate reduction to ammonium), microbial community composition, and denitrifier marker gene abundance at sites subjected to light and moderate oiling using a combination of isotope pairing on sediment slurries, 16S sequencing, and qPCR. We predicted that overall, denitrification rates and microbial functional capacity would be enhanced in mangrove-dominated sediments. We also predicted that these enhancements would be diminished at the more intensely oiled site due to the higher susceptibility of *A. germinans* to oiling. Denitrification potential rates were higher in mangrove sediments at the lightly oiled site, whereas dissimilatory nitrate reduction to ammonium potential rates were higher in marsh sediments. Indicator analysis of 16S rRNA data selected putative sulfur cycling taxa as indicators of marsh sediments, suggesting that changes in oxygen availability associated with encroachment may be driving the differences in process rates. There was no difference in process rates between plant types at the moderately oiled site, where heavily weathered oil residue was still present. Sediment nutrient stocks were lower in moderately oiled mangrove plots than in lightly oiled mangrove plots, suggesting that sediment fertility recovery following the spill may have been slower in the mangroves, contributing to a change in ecosystem function. This study shows that woody encroachment has the potential to impact both the biogeochemical services that marshes provide and their response to and recovery from disturbances.

KEYWORDS

woody encroachment, denitrification, oil spill, *S. alterniflora*, *A. germinans*, DNRA, 16S rRNA, microbial diversity

Introduction

Humans have dramatically increased the amount of reactive nitrogen (N) in the environment, with negative ecological consequences (Paerl and Whitall 2010; Fowler et al., 2013). Wetlands, including salt marshes, intercept and remove as much as a third of the excess N they receive through uptake, burial, and microbially mediated processes (Jordan et al., 2011), protecting vulnerable coastal ecosystems (Valiela and Cole 2002). However, disturbances such as sea-level rise, eutrophication, and warming temperatures have altered the composition and distribution of wetland vegetation (e.g., Donnelly and Bertness, 2001; Saintilan et al., 2014), with subsequent impacts on the valuable biogeochemical services wetlands provide (Kelleway et al., 2017; Charles et al., 2019). One widespread shift in marsh vegetation composition is the woody encroachment of mangroves into coastal salt marshes. Warming winter temperatures associated with climate change have resulted in the poleward migration of mangroves on all continents where marshes and mangroves co-occur (Osland et al., 2013; Armitage et al., 2015; Kelleway et al., 2017), with subsequent changes in faunal diversity (Smee et al., 2017; Scheffel et al., 2018), habitat structure (Guo et al., 2017), ecosystem nutrient stocks (Doughty et al., 2016; Simpson et al., 2019; Macy et al., 2020), and microbial community composition (Barreto et al., 2018). Despite the myriad impacts of woody encroachment on marsh structure and function, we still have a limited understanding of how mangrove expansion impacts the N cycle (but see Henry 2012; Steinmuller et al., 2020; Macy et al., 2020), particularly the removal of excess anthropogenic N.

Nitrogen cycling is tightly coupled to vegetation in coastal wetlands (Koop-Jakobsen and Giblin, 2009; Giblin et al., 2013). Plant root exudates provide a source of organic carbon (OC) for heterotrophic microbes (Spivak and Reeve, 2015). Additionally, oxygen (O_2) translocation to the rhizosphere alters redoximorphic conditions, promoting processes such as nitrification (Hamersley and Howes, 2005; Koop-Jakobsen and Giblin, 2009) and limiting sulfate reduction (Holmer et al., 2002). Mangrove expansion into marshes results in multiple physicochemical changes to sediments that could impact microbially driven biogeochemical processes (Perry and Mendelssohn, 2009). Pneumatophores are efficient at translocating O_2 to the subsurface, potentially altering subsurface redox conditions (Perry and Mendelssohn, 2009; Comeaux et al., 2012). Enhanced O_2 translocation also impacts microbial community structure, altering the functional capacity of sediment microbial communities (Barreto et al., 2018). Mangrove expansion can also impact the availability of N in sediments. Mangroves competitively utilize N compared to marsh plants (Simpson et al., 2013; Dangremond et al., 2020), potentially reducing porewater N concentrations (Macy et al., 2019). However, mangroves can have a higher leaf N content than marsh plants (Macy et al., 2020) that can accelerate litter decomposition and N cycling (Simpson et al., 2021). Therefore, it is likely that woody encroachment affects N removal rates in marsh sediments.

The dominant pathway for permanent N removal in coastal wetlands is denitrification, the microbially mediated stepwise reduction of nitrate (NO_3^-) to nitrous oxide (N_2O) and dinitrogen (N_2) gases (Knowles, 1982). Dissimilatory NO_3^- reduction to ammonium (DNRA) is a competing process for NO_3^- reduction in which N is retained as ammonium (NH_4^+) rather than removed (Burgin and Hamilton, 2007). The composition and productivity of marsh vegetation can influence whether N is removed by denitrification or retained by DNRA (Ledford et al., 2020). Increased oxygen translocation to the rhizosphere promotes coupled nitrification–denitrification (Reddy et al., 1989; Koop-Jakobsen and Giblin, 2009) and reduces sulfide accumulation, which inhibits both nitrification and denitrification (Sorensen et al., 1980; Joye and Hollibaugh, 1995). It is possible that the greater root O_2 translocation by mangroves compared to marshes (Perry and Mendelssohn, 2009) would promote denitrification over DNRA following encroachment. OC availability is another important factor that controls whether NO_3^- is removed or retained. Due to the higher energetic demand of DNRA, retention is favored under high sediment OC: NO_3^- ratios (Hardison et al., 2015; Kessler et al., 2018). Thus, if mangroves rapidly increase sediment OC pools, as has sometimes been observed (Doughty et al., 2016; Simpson et al., 2019), encroachment may promote DNRA over denitrification.

In addition to altering the physicochemical characteristics of marshes, mangrove encroachment may also affect ecosystem response to disturbance (e.g., Lin and Mendelssohn, 2012; McKee and Vervaeke, 2018; Armitage et al., 2020). Increased ecosystem susceptibility to disturbance has important implications for biogeochemical processes such as N removal, as salt marsh damage and loss can lead to a loss of biogeochemical function (Hinshaw et al., 2017). One type of disturbance that mangroves can be particularly vulnerable to is oil spills. The pneumatophore root structure easily traps oil residues in the sediment, which, coupled with the relatively slow turnover of aboveground biomass, contributes to long-term negative impacts on plant health (Lewis et al., 2011; Duke, 2016). Oiling also suffocates mangroves by coating leaves and pneumatophores, which can weaken and kill plants for years following initial oiling (Duke, 2016). Following the 2010 *Deepwater Horizon (DWH)* oil spill, ecosystem recovery in mixed mangrove–marsh systems was dominated by marsh vegetation (Lin and Mendelssohn, 2012; Shapiro et al., 2016), suggesting enhanced oil sensitivity in mangroves. Although oiling has a neutral (Kleinhuizen et al., 2017) or even positive (Levine et al., 2017a) effect on N removal in marsh sediments, it can lead to long-term losses in marsh N removal capacity (Hinshaw et al., 2017; Tatariw et al., 2021), highlighting a need to understand potential differences in oiling response in marshes and mangroves.

We measured NO_3^- reduction (i.e., denitrification and DNRA) rates and the microbial community structure (16S rRNA) in mangrove (*Avicennia germinans*)- and marsh (*Spartina alterniflora*)-dominated sediments at sites subjected to light and moderate oiling during the *DWH* spill. We hypothesized that denitrification would be favored over DNRA in *A. germinans* plots because 1) *A. germinans* is more efficient at translocating oxygen to the sediment, reducing sulfide inhibition and promoting coupled nitrification/denitrification, and 2) *A. germinans* litter has a lower C:N ratio, which favors denitrification over DNRA. We predicted that denitrification and DNRA rates would be lower at the moderately oiled site due to the detrimental effects of oiling on plant function and loss of plant C, with a greater impact in *A. germinans*, which is more sensitive to oiling than *S. alterniflora*.

Materials and methods

Site description

The Chandeleur Islands are a chain of low-lying (<2 m) barrier islands situated approximately 60 km south of Biloxi, Mississippi, in the Gulf of Mexico (Figure 1). The islands run north to south for 80 km and range 2–10 km in width. The dominant vegetation is *Spartina alterniflora* (smooth cordgrass) and *Avicennia germinans* (black mangrove). During the *DWH* oil spill, the islands were subjected to a range of very light to heavy oiling, as determined by the Shoreline Cleanup and Assessment Technique (SCAT) data from the summer of 2010 (EMRA, 2015). SCAT oiling intensities are determined by the distribution, width, and thickness of oil bands (Michel et al., 2013; Nixon et al., 2016). There were no clean-up efforts on the islands.

We used SCAT data to establish two sampling sites subjected to different oiling intensities during the *DWH* spill (Figure 1). Both sites were located along the western shore of the islands and had both *S. alterniflora* and *A. germinans* present. The southern site was subjected to light oiling during the spill ("lightly oiled"; 29.863750° −88.841466°), and the northern site was subjected to moderate oiling during the *DWH* spill ("moderately oiled"; 29.895448° -88.827780°). Three 1 × 1-m plots were established within each vegetation type at each site. Samples were collected from each plot on five dates (30 May 2017, 03 July 2017, 02 August 2017, 07 May 2018, and 13 June 2018). Salinities and nutrient concentrations from the water column adjacent to the shore were comparable between sites.

On each sampling date, triplicate sediment samples (0–5 cm) were collected for nitrate reduction potentials, microbial analysis, and oil residue analysis using a 60-ml syringe corer (i.d. 2.6 cm). A 10-ml syringe corer (i.d. 1.3 cm) was used to collect triplicate sediment samples (0–5 cm) for porewater nutrient extraction. Single cores were collected from each plot on each date to test for sediment C and N content and sediment chlorophyll a (chl-a) (0–1 cm). All samples were transported to the lab on ice.

Plant metrics

A. germinans height was measured on all dates but 30 May 2017 and 03 July 2017. The average plant height (m) for each plot was determined by measuring six points along the length of the plant perpendicular to the shoreline. This transect included the highest point of the plant (maximum plant height, m). Sediment cores (7.9 cm i.d.) were collected to a depth of 5 cm to determine plant belowground biomass for all dates but 30 May 2017 and 03 July 2017. Roots were separated from the sediment by rinsing with tap water through a 2-mm sieve. The remaining plant material was oven-dried at 60 °C to a constant weight, and

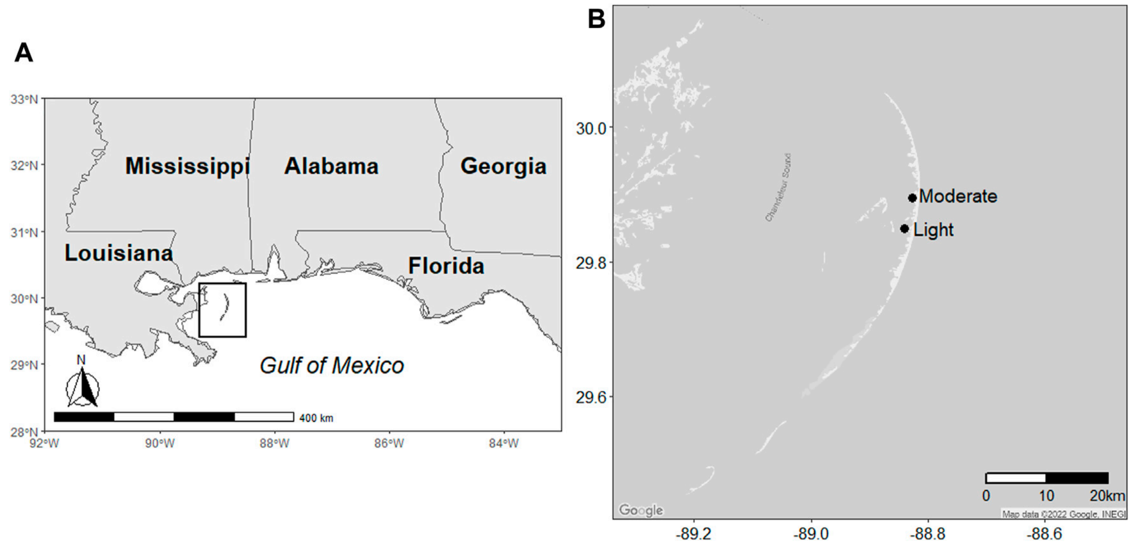

FIGURE 1
Map depicting the location of **(A)** the Chandeleur Islands and **(B)** the locations of the study sites.

the belowground biomass was determined on an areal basis (kg m^{-2}).

Sediment physicochemical analysis

A core (0–5 cm, i.d. 1.3 cm) was collected from each plot to determine porewater extractable NH_4^+. Porewater NH_4^+ was extracted with 2N potassium chloride (KCl) by incubation on a shaker table. After 24 h, the slurries were centrifuged, and the supernatant was filtered (0.45 µm nylon membrane filter) and frozen until analysis. Porewater NH_4^+ was determined fluorometrically (Holmes et al., 1999: Protocol B). Sediment chlorophyll a (chl-a) inventories were determined from the top 2 cm of the sediment from each plot (i.d. 1.3 cm). Sediments were freeze-dried, sediment dry weight was recorded, and chl-a was extracted with 90% acetone for 24 h. Chl-a concentrations were determined fluorometrically (Welschmeyer 1994). To determine the molar carbon to nitrogen ratio (C:N) of sediments, cores (5 cm × 1.3 cm) were oven-dried at 60°C, ground with a mortar and pestle, and then fumigated with 12N HCl overnight to remove carbonates. Following fumigation, samples were oven-dried again and ground prior to analysis on a Costech 4010 CHN analyzer.

Oil residue analysis

Oil-source fingerprinting was conducted on the leftover frozen microbial sediments. Profiles were determined from petroleum biomarker compounds, as measured via GC/MS analytical methods at Louisiana State University (Iqbal et al., 2008; Meyer et al., 2018). Source matching was done by visually comparing hopane and sterane biomarker compound concentrations in their respective m/z191 (hopanes), m/z 217 (sterane), and m/z 218 (sterane) ion chromatograms. The ion chromatograms of extracted field samples were compared to the equivalent data for biomarker compounds in the MC252 source oil.

Nitrate reduction potentials

Triplicate samples were collected from each plot for nitrate reduction (i.e., denitrification, anammox, and DNRA) potential assays as described in Tatariw et al. (2021). Two cores (0–5 cm, i.d. 2.6 cm) were homogenized for each replicate. Following overnight incubation at ambient water temperature, roughly one half of each sample was slurried with artificial sea water (ASW) adjusted to the average salinity of the sitewater at the time of sampling. The remaining sediments were stored at −80°C. Slurries were bubbled with dinitrogen (N_2) gas to produce anoxic conditions and siphoned into Exetainer vials (Labco). Following overnight incubation at the sitewater temperature to remove ambient NO_3^- and oxygen (O_2), slurries were spiked to ~50 µM $Na^{15}NO_3$ (98 atom %; Cambridge Isotope Laboratories, Inc.). One half of the slurry tubes were immediately spiked with zinc chloride ($ZnCl_2$, 50% W/V) to stop biological activity. The other half were incubated for ~6 h at ambient water temperature and then amended with $ZnCl_2$.

Denitrification and anaerobic ammonium oxidation (annamox) were measured based on the concentrations of $^{29}N_2$ and $^{30}N_2$ in slurry water using a membrane inlet mass spectrometer (MIMS) outfitted with a copper reduction column to remove excess oxygen (Nielsen, 1992; Kana et al., 1994; Eyre et al., 2002). DNRA was measured based on $^{15}NH_4$ production by means of hypobromite reduction (Thamdrup and Dalsgaard, 2002; Yin et al., 2014). Briefly, DNRA tubes were bubbled with N_2 to purge the $^{29}N_2$ and $^{30}N_2$ produced by denitrification and anammox. Samples were then amended with 200 µL sodium hypobromite, which converts NH_4^+ to N_2. The resulting $^{29}N_2$ and $^{30}N_2$ concentrations were measured on the MIMS. Following analysis, sediments in the tubes were dried to a constant weight to calculate NO_3^- reduction rates as µmol N kg dry weight^{-1} h^{-1}.

To determine the isotope enrichment of the slurries, slurry tubes with and without $^{15}NO_3^-$ addition were filtered and frozen for NO_{2+3}^- analysis as described earlier. Ambient NO_{2+3}^- was extremely low, and samples were ^{15}N enriched at >95% across all dates. The contribution of anammox to potential nitrate reduction was very small (<1%) and was excluded from further analysis.

DNA isolation and 16S rRNA amplicon sequencing

Total genomic DNA was extracted in triplicate from each core with an MP FastDNA™ Spin Kit for soil (MP Biomedicals, LLC) according to the manufacturer's instructions with the addition of 2-min ice incubations following the homogenization and 4°C centrifugation steps. The triplicate extractions were then pooled and purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research) and eluted in sterile ddH_2O (total volume 50 µL). Total DNA concentration was quantified spectrophotometrically using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) and stored at -20°C.

One of the three cores from each plot and date was selected for 16S rRNA sequencing for microbial community analysis (total 59 samples). Briefly, the hypervariable V3–V4 region of the 16S rRNA gene from total DNA was amplified with region-specific primers that included Illumina flowcell adapter sequences. Following library preparation, a 2 × 250-bp amplification run was conducted using Illumina MiSeq 2000 technology by the University of Tennessee Genomics Core (Knoxville, TN, United States).

Sequence processing

Raw paired-end FASTQ files were trimmed (cutadapt), dereplicated, denoised, and merged using DADA2 in QIIME2 (version 2018.8). Chimeric sequences were identified and removed using the consensus method in DADA2 (Callahan et al., 2016). Taxonomic assignment of amplicon sequence variants (ASVs) was performed using the QIIME2 q2-feature-classifier plugin using a SILVA 13_5 99% trained Naïve Bayes classifier for the V3–V4 region of the 16S rRNA gene (Quast et al., 2013). Multiple sequence alignment and phylogenetic reconstruction were carried out using MAFFT and FastTree. To account for the diverse sequence distribution across samples, ASV abundance data were transformed into relative abundance by dividing each ASV by the sum of a given sample. Amplicon sequences were randomly subsampled to an even depth of 53,963 reads per sample. Pielou's evenness and Shannon's diversity were calculated to assess alpha (i.e., within sample) diversity using the vegan package in R (Oksanen et al., 2015).

Indicator analysis

Multipatt and random forest modeling (RFM) were used to identify the ASVs driving community differences between oiling intensity (i.e., sites) and vegetation types. The taxonomic assignment for each identifier is listed in Supplementary Table S3. To reduce the noise caused by rare features (Hackstadt and Hess, 2009), ASVs consisting of more than 80% zeros were removed with METAGENassist (Arndt et al., 2012). ASVs that were selected by both indicator species and random forest modeling were designated as important species similar to Kolton et al. (2020). The multipatt function was used to identify indicator species using correlation to identify ASVs based on fidelity (present in all samples of a habitat) and exclusivity (only present in a habitat) in the indicspecies package in R with 999 permutations (De Cáceres and Legendre, 2009). A classification random forest analysis (Breiman, 2001) was used to identify significant ASVs by vegetation type and oiling intensity with the randomForest package in R (Liaw and Wiener, 2002). The RFM was run with 501 trees and cross-validated with leave-one-out validation. The 15 ASVs with the greatest mean decrease in accuracy were considered the most important.

qPCR on N cycling genes

Quantitative PCR (qPCR) was performed to assess the abundance of two genes in the denitrification pathway: *nirS* (nitrite reductase) and *nosZ* (nitrous oxide reductase). Total community abundance was determined by qPCR analysis of 16S rRNA. Each reaction included Platinum SYBR Green qPCR SuperMix-UDG with ROX (12.5 µl) (Invitrogen), 1 µl of forward and reverse primer (5 µM, IDT DNA Technology), 0.5 µl $MgCl_2$ (50 mM), and 5 ng of DNA template. The total reaction volume was adjusted to 25 µl with PCR grade water

(ThermoFisher). Genomic DNA isolated from boil preparations of *Vibrio fischeri* and *Pseudomonas stutzeri* were used for PCR amplification to generate single-copy gene plasmids for *nirS*, *nosZ*, and 16S rRNA standard curves. Samples were run in duplicate on a 7000 Sequence Detection System (ABI Prism) with the primer combinations and qPCR conditions listed in Supplementary Table S1. Following qPCR, random samples were run on a 1.2% sodium boric acid (SB) gel to assess correctly sized amplicons. PCR inhibition due to co-extracted humic material was tested by standard addition during reaction setup. DNA copy numbers were expressed as gene copies per gram of wet sediment as previously described in Lindemann et al. (2016) and Warneke et al. (2011).

Statistical analyses

Differences in NO_3^- reduction rates, sediment characteristics, and 16S alpha diversity between vegetation types and sites were tested using a linear mixed-effects model (LME; package *nlme*, Pinheiro et al., 2015) with site x vegetation type x sampling year as main factors and plot as a random factor. *A. germinans* height was tested with LME with site x year as a main factor and plot as a random factor. Significant interactions were tested using tests of simple effects. All variables except for *nirS* abundance were natural log-transformed to meet the assumption of normality. *NirS* abundance was square-root transformed. To determine the effect of site and vegetation type on beta diversity, we conducted a permutational multivariate analysis of variance (PERMANOVA) using the adonis() function in vegan (Oksanen et al., 2015).

Multivariate homogeneity of group dispersions was used to test for differences in variances between land-use types (function: Betadisper). PERMANOVA was performed on the distance matrix to test for differences in β-diversity between land-use types (function: Pairwise.adonis2) (Martinez Arbizu, 2017). Bioenv was used with a mantel test to identify the best environmental characteristics that correlated with community dissimilarities using Spearman's correlation (Clarke and Ainsworth, 1993). Non-metric multidimensional (NMDS) scaling was used to visualize differences in microbial communities between sites and vegetation types. NMDS was performed on a Bray–Curtis distance matrix using metaMDS. Vectors of environmental variables were fitted to the NMDS coordinates with envfit.

Results

Plant and sediment characteristics

The average *A. germinans* height was 1.2 ± 0.3 m and did not differ between sites (LME, $p = 0.391$) or years (LME, $p = 0.156$). Belowground biomass did not differ between sites for either plant type (LME, $p = 0.941$). The belowground biomass was 2X greater in *S. alterniflora* plots (1.1 ± 0.4 kg m^{-2}) than in *A. germinans* plots (0.5 ± 0.3 kg m^{-2}) (LME, $p < 0.001$).

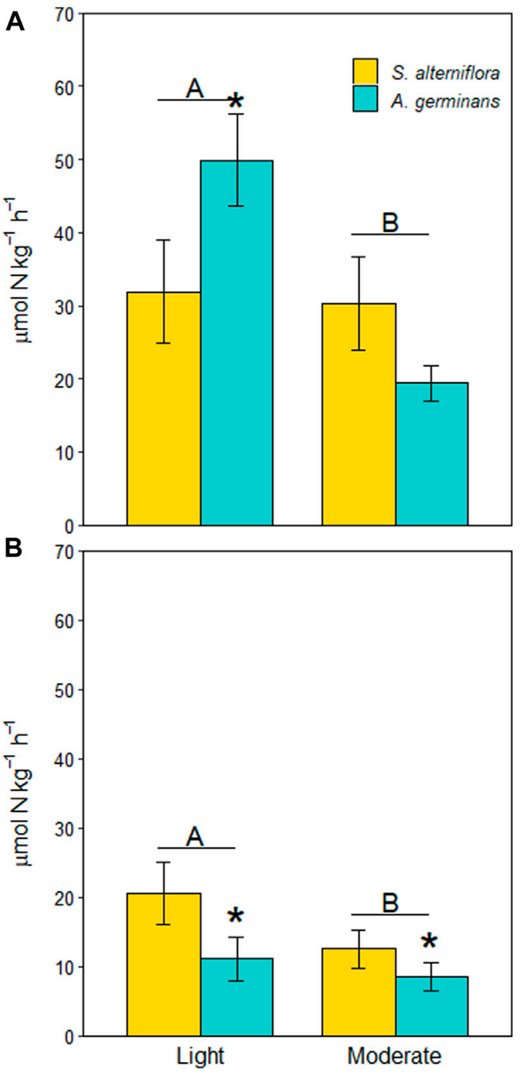

FIGURE 2
Denitrification **(A)** and DNRA **(B)** potential rates for each site and vegetation type (indicated by color). Significant differences between sites are indicated by capital letters. Significant differences between vegetation types within sites are indicated with an asterisk (*). Bars are standard error.

On average, extractable NH_4^+ was nearly twice as high at the lightly oiled site than the moderately oiled site (0.24 ± 0.20 vs. 0.15 ± 0.11 μmol g^{-1}) (LME, $p = 0.011$), and the effect of vegetation type depended on site (LME, $p = 0.006$). Extractable NH_4^+ concentrations were significantly lower (2.3X) in *A. germinans* plots at the moderately oiled site than in the lightly oiled site (TSE, $p < 0.001$), whereas in *S. alterniflora* plots there were no differences in concentrations between sites (TSE, $p = 0.719$). Temporal effects were also

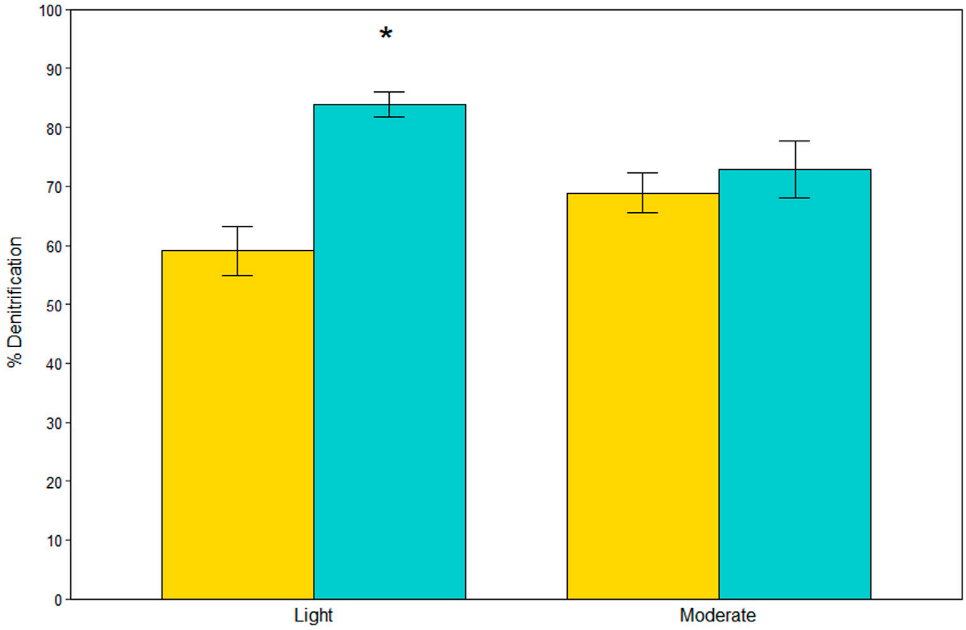

FIGURE 3
Percent (%) contribution of denitrification to total nitrate reduction for each site and vegetation type (indicated by color). Significant differences between vegetation types within sites are indicated with an asterisk (*). Bars are standard error.

dependent on site (LME, $p = 0.039$). In 2017, NH_4^+ concentrations at the lightly oiled site were more than double those at the moderately oiled site (0.30 ± 0.23 vs. 0.13 ± 0.09 μmol g^{-1}) (TSE, $p < 0.001$), whereas there were no differences between sites in 2018 (TSE, $p = 0.985$).

The effect of vegetation type was dependent on site for both sediment C and N (LME, $p = 0.005$; Supplementary Table S3). In *A. germinans* plots, sediments C and N were, respectively, 2.3X and 3X lower at the moderately oiled site (TSE, $p < 0.001$ and $p < 0.001$). In contrast, neither sediment C nor sediment N differed between sites in *S. alterniflora* plots (TSE, $p = 0.480$ and $p = 0.151$). Both C and N were higher in 2017 than 2018 (131 ± 95 vs. 36 ± 22 μmol C g sediment^{-1} and 8 ± 7 vs. 2 ± 1 μmol N g sediment^{-1}) (LME, $p < 0.001$ for both).

Sediment chl-a inventories differed between vegetation types; on average, inventories were 2.1X higher in *S. alterniflora* plots than in *A. germinans* plots (LME, $p < 0.001$). The effect of site on sediment chl-a depended on vegetation type (LME, $p < 0.001$). Chl-a inventories in *A. germinans* plots were lower at the moderately oiled site than at the lightly oiled site (38 ± 13 vs 83 ± 66 mg m^{-2}) (TSE, $p < 0.001$), whereas there were no differences in chl-a inventories between moderately and lightly oiled sites in *S. alterniflora* plots (144 ± 46 vs 105 ± 53 mg m^{-2}) (TSE, $p = 0.090$). Sediment chl-a inventories were consistent across years (LME, $p = 0.794$).

Oil residue analysis

Heavily weathered oil residues were detected in 10 of the 60 samples analyzed (Supplementary Table S2). All but one of the samples with detectable residues were from the moderately oiled site. The majority (7) of the samples with detectable residues were collected from *A. germinans* plots. Each collection date had at least one sample with detectable residues.

Potential nitrate reduction rates

Average denitrification rates were 1.6X higher at the lightly oiled site than at the moderately oiled site (Figure 2A, LME, $p = 0.013$), although the effect was largely driven by differences in *A. germinans* plots (site*vegetation, LME, $p = 0.006$). At the lightly oiled site, denitrification rates were 1.6X higher in *A. germinans* plots that in *S. alterniflora* plots (TSE, $p = 0.034$). There was no difference in denitrification rates between vegetation types at the moderately oiled site (TSE, $p = 0.319$).

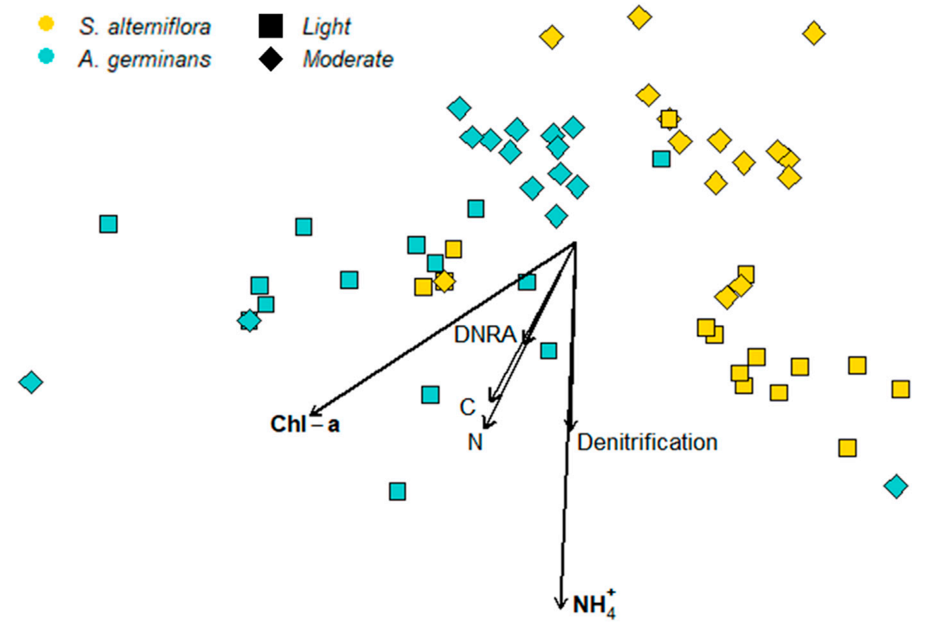

FIGURE 4
NMDS of the 16S microbial community with bioenv variables as vectors. Oiling intensity is indicated by symbol shape, with lightly oiled = squares, moderately oiled = diamonds. Turquoise symbols indicate *A. germinans* communities and goldenrod symbols indicate *S. alterniflora* communities.

DNRA rates were 1.5X higher at the lightly oiled site that at the moderately oiled site (Figure 1B, LME, $p = 0.037$). Unlike denitrification rates, the effect of vegetation type was independent of site (site*vegetation, LME, $p = 0.559$). Rather, DNRA rates were on average 1.7X higher in *S. alterniflora* plots than *A. germinans* plots across both sites (Figure 2B, LME, $p = 0.004$).

N removal dominated over DNRA, accounting for 71 ± 2% of NO_3^- reduction on average (Figure 3). The effect of site on percent denitrification depended on vegetation type (site*vegetation, LME, $p = 0.004$). At the lightly oiled site, percent denitrification was 1.4X higher in *A. germinans* plots than *S. alterniflora* plots (TSE, $p < 0.001$), whereas there was no difference between vegetation types at the moderately oiled site (TSE, $p = 0.370$).

On average, denitrification potential rates were 1.3X higher in 2018 than 2017 across both sites and vegetation types (LME, $p = 0.026$). Similarly, DNRA potential rates were significantly higher (2.2X) in 2018 than 2017 (LME, $p < 0.001$). Percent denitrification was 1.2X higher in 2017 than 2018.

16S rRNA community diversity

16S rRNA gene sequencing resulted in a total of 18,733,736 sequences in 59 samples. Among the 8,345,939 quality-filtered sequences, 7,942,475 (95.3%) were assigned to bacterial 16S rRNA ASV IDs. Quality-filtered reads ranging from 53,963 to 273,796 reads per sample accounted for a total of 796 16S rRNA ASVs. Fourteen of the 64 assigned phyla contributed to greater than 1% of relative abundance. Proteobacteria, Chloroflexi, and Bacteroidetes were the three most abundant phyla, accounting for 62% of ASVs.

The observed number of ASVs ranged from 1849 to 5,937 ASVs per sample. There was no difference in Shannon diversity between sites (LME, $p = 0.434$; Supplementary Figure S1), but microbial communities in *A. germinans* plots were more diverse than those in *S. alterniflora* plots across both sites (LME, $p = 0.018$). There was a marginal interaction between site and vegetation type (LME, $p = 0.052$) that showed a trend of higher diversity in *A. germinans* plots at the lightly oiled site. Like Shannon diversity, Pielou's evenness was significantly higher in *A. germinans* plots than *S. alterniflora* plots (LME, $p = 0.0247$; Supplementary Figure S1). The effect of site on Pielou's evenness depended on vegetation type (LME, site x vegetation, $p = 0.037$). In *A. germinans* plots, evenness was higher at the lightly oiled site (TSE, $p = 0.037$), whereas there was no difference in evenness between sites in *S. alterniflora* plots (TSE, $p = 0.970$). Shannon diversity was higher in 2018 than 2017 (LME, $p = 0.008$), but there was no difference in Pielou's evenness between years (LME, $p = 0.088$).

PERMANOVA revealed a significant site and vegetation interaction for beta diversity (PERMANOVA, $p < 0.004$), and

TABLE 1 *Bioenv* correlations. Italicized text indicates significant correlations.

Variable	NMDS1	NMDS2	R^2	*p*-value
Chl-a	−0.837	−0.546	*0.170*	*0.014*
Extractable NH_4^+	−0.041	−0.999	*0.227*	*0.002*
Sediment N	−0.439	−0.898	0.072	0.153
Sediment C	−0.474	−0.881	0.055	0.244
Denitrification rate	−0.028	−0.999	0.060	0.219
DNRA rate	−0.454	−0.819	0.022	0.567

pairwise comparisons (*pairwise.adonis*) between each site and vegetation combination confirmed that beta diversity differed between all site–vegetation combinations (PERMANOVA, $p < 0.006$; Figure 4). Beta dispersion did not differ between sites ($p = 0.111$) and vegetation types ($p = 0.752$), indicating that community differences between sites and vegetation types were driven by changes in microbial community structure and not changes in variance between the groups.

Bioenv selected chl-a, extractable NH_4^+, bulk N, and denitrification rates as the best sediment variables to correlate with community distances (Mantel test, $\rho = 0.19$, $p = 0.004$). Two of the fitted vectors for sediment variables were significantly correlated with community structure (Table 1). Sediment chl-a correlated with both NMDS axes ($R^2 = 0.170$, $p = 0.014$), and extractable NH_4^+ correlated with NMDS axis 2 ($R^2 = 0.227$, $p = 0.002$).

Indicator analysis

Seven of the 15 taxa were selected as indicators of the lightly oiled site (Figure 5). Four of these were indicators for *S. alterniflora* sediments. The *S. alterniflora* indicators included two taxa from the phylum Proteobacteria: genus *Erythrobacter* and family Desulfobulbaceae. *S. alterniflora* indicator taxa at the lightly oiled site also included *Eudoraea*, a genus in Bacteroidetes, and Gemm-2, a class in Gemmatimonadetes. Of the three indicator taxa selected for *A. germinans* at the lightly oiled site: two were from Proteobacteria; one was from the genus *Rhodovibrio* in Alphaproteobacteria; and the other was from Thiohalorhabdales in Gammaproteobacteria. The third *A. germinans* indicator at the lightly oiled site was the class AT-s2-57 in Acidobacteria.

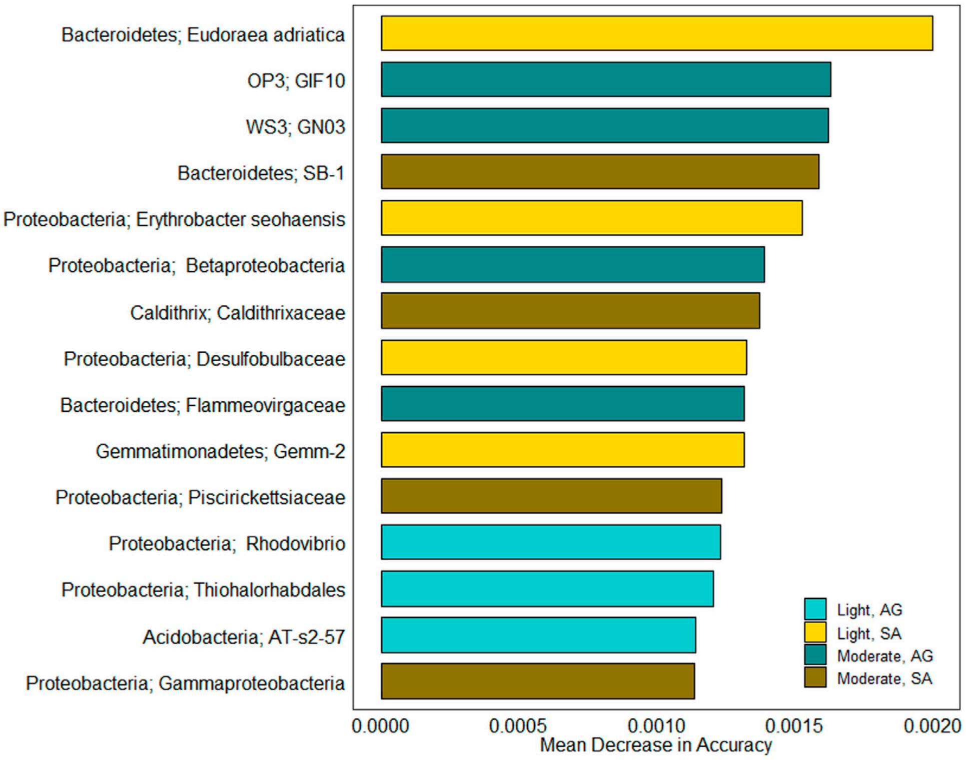

FIGURE 5
Indicator taxa driving community differences between oiling intensity and vegetation types. Bars depict mean decrease in accuracy for the 15 most important features as determined by random forest analysis. The color of each bar indicates for which combination of oiling intensity and vegetation type each taxon was an indicator.

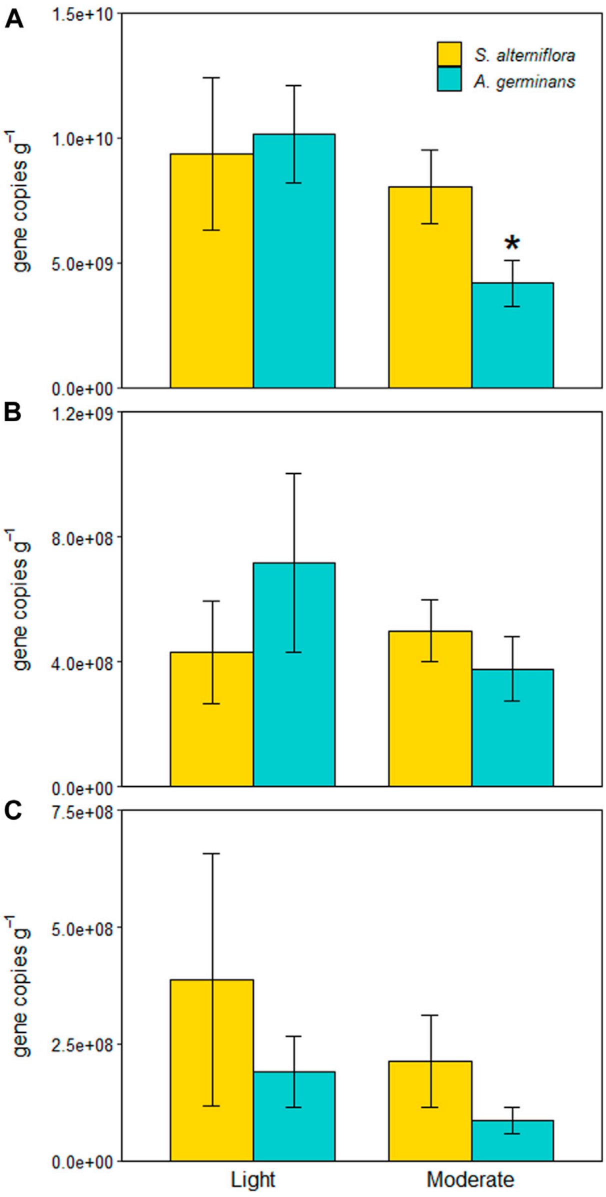

FIGURE 6
16S **(A)**, *nirS* **(B)**, and *nosZ* **(C)** marker abundances for each site and vegetation type (indicated by color). Significant differences between vegetation types within sites are indicated with an asterisk (*). Bars are standard error.

The eight indicator taxa selected for the moderately oiled site were evenly divided between *S. alterniflora* and *A. germinans*. Two *S. alterniflora* taxa were Gammaproteobacteria, including one from the family Piscirickettsiaceae. The remaining two *S. alterniflora* taxa were from Bacteroidetes (family SB-1) and *Caldithrix* (family Caldithrixaceae). The indicator taxa for *A. germinans* at the moderately oiled site were from four different phyla: Proteobacteria (class Betaproteobacteria), Bacteroidetes (family Flammeovirgaceae), OP3 (order GIF10), and WS3 (order GNO3).

qPCR

The effect of vegetation type on 16S abundance depended on site (LME, $p = 0.044$; Figure 6A). There was no difference in 16S abundance between *A. germinans* and *S. alterniflora* sediments at the lightly oiled site (TSE, $p = 0.981$), but at the moderately oiled site, 16S abundance was 1.3X lower in *A. germinans* plots than in *S. alterniflora* sediments (TSE, $p = 0.013$; Figure 6A). There was no difference in 16S rRNA abundance between sites in *S. alterniflora* plots, but 16S abundance was significantly lower (2.4X) at the moderately oiled site in *A. germinans* plots (TSE, $p = 0.018$). As with denitrification and DNRA potential rates, 16S amplicon abundance differed between years; however, in contrast to process rates, 16S abundances were 2X higher in 2017 than 2018 (LME, $p = 0.001$).

Neither nitrite reductase (*nirS*) nor nitrous oxide reductase (*nosZ*) functional markers differed significantly between sites (LME, $p = 0.723$ and $p = 0.378$) or vegetation types (LME, $p = 0.955$ and $p = 0.616$) (Figure 5B). Although not statistically significant (LME, $p = 0.290$ and $p = 0.817$), trends in *nirS* and *nosZ* abundances between sites and vegetation types mirrored those of denitrification potentials showing a decrease from the lightly oiled to the moderately oiled site in *A. germinans* plots compared to similar abundances at both sites in *S. alterniflora* plots. Neither nitrite reductase (*nirS*) nor nitrous oxide reductase (*nosZ*) functional markers differed significantly between years (LME, $p = 0.529$ and $p = 0.439$).

Discussion

We found that at the less oil-impacted site, mangrove sediments had a significantly higher N removal potential than marsh sediments, both in terms of denitrification potential rates and the proportion of NO_3^- being removed. Woody encroachment has been shown to alter the chemical, microbial, and redox characteristics of marsh sediments (Perry and Mendelssohn, 2009; Guo et al., 2017; Barreto et al., 2018; Simpson et al., 2019), all of which are important drivers of biogeochemical processes. Our data suggest that a combination of redox and microbial community changes associated with woody encroachment, rather than changes in nutrient availability, resulted in increased denitrification potential in mangrove sediments. Vegetation is an important driver of N removal in coastal wetlands, as root O_2 translocation to the sediment alleviates sulfide inhibition of coupled nitrification–denitrification (Koop-Jakobsen and Giblin, 2009). The mangrove pneumatophore root structure is efficient at O_2 translocation to the subsurface and can increase sediment redox potential (Andersen and Kristensen, 1988; Perry and Mendelssohn, 2009; Comeaux et al., 2012). Additionally, *A. germinans* prefers higher elevations, and better drainage can also increase sediment redox potential (Perry and

Mendelssohn, 2009). Although we did not directly measure changes in redox, the results of our microbial indicator analysis suggest that O_2 availability may differ between *Avicennia* and *Spartina* sediments. *A. germinans* indicator taxa included Acidobacteria, which are commonly found in soil and sediments and consist mainly of aerobic heterotrophs (Ward et al., 2009), whereas *S. alterniflora* indicators included Caldithrixales (phylum Caldithrix), a putative anaerobe that has been found in *Spartina* marshes (Bulseco et al., 2019). This is consistent with the findings of Barreto et al. (2018), who found that mangrove indicator taxa were more likely to be putative aerobes, whereas marsh indicator taxa were more likely to be putative anaerobes (Barreto et al., 2018). Furthermore, they also found that Acidobacteria and Caldithrix were indicators for mangrove and marsh sediments, respectively (Barreto et al., 2018).

The higher DNRA rates in *S. alterniflora* sediments also support the idea that higher redox potential in *A. germinans* sediments favors denitrification. DNRA is favored under highly reducing conditions or when there is a high organic C: NO_3^- ratio (Gardner and McCarthy, 2009; Hardison et al., 2015; Kessler et al., 2018). We did not observe differences in sediment C and N availability in sediments dominated by different vegetation types, possibly because high sediment deposition rates in the northern Gulf of Mexico can outweigh the influence of woody encroachment on nutrient stocks (Henry and Twilley, 2013). Rather, the selection of taxa associated with sulfur cycling suggests that sulfide accumulation associated with more reducing conditions in marsh sediments may be the driving factor of N retention via DRNA. Desulfobulbaceae (Deltaproteobacteria) was an indicator taxon for *S. alterniflora* that includes sulfate-reducing bacteria (Dyksma et al., 2018) and cable bacteria (Kjeldsen et al., 2019). Cable bacteria are filamentous bacteria capable of sulfide oxidation using oxygen or nitrate as electron acceptors (Murphy et al., 2020). They are found where sulfide accumulates, and using long-distance electron transport, they create sulfide-free suboxic zones (Larsen et al., 2015; Kjeldsen et al., 2019). Significantly, they can promote DNRA by increasing the Fe^{2+} pool by iron sulfide dissolution (Kessler et al., 2019). Another putative sulfide oxidizer selected as an indicator for *S. alterniflora* was Piscirickettsiaceae (Proteobacteria) (Borin et al., 2009). Piscirickettsiaceae has been shown to thrive in sulfidic environments (Pavlovska et al., 2021).

The two vegetation types also had different associated sediment microbial communities that were correlated with sediment-extractable NH_4^+ and chl-a inventories. While there were no vegetation effects on NH_4^+, chl-a was significantly lower in *A. germinans*, likely due to increased canopy shading (Guo et al., 2017). This suggests that even though there were no differences in substrate quantity (e.g., bulk C and N), there may have been differences in substrate quality that impacted microbial diversity and activity. *A. germinans* leaves have a higher N content than *S. alterniflora* leaves (Macy et al., 2019), and changes in litter quality associated with woody encroachment can increase decomposition rates in *A germinans*-dominated sediments compared to *S. alterniflora*-dominated sediments (Simpson et al., 2021). While we did not directly test for changes in litter quality, the microbial indicator analysis selected taxa associated with litter degradation in *S. alterniflora.* Gemmatimonadetes have metabolic pathways for carbohydrate and protein degradation (Baker et al., 2015) and have been found in *S. alterniflora* salt marshes (Bowen et al., 2012). *Erythrobacter* is a saprophyte that has been associated with *S. alterniflora* litter degradation (Buchan et al., 2003). Additionally, Bacteroidales SB-1 (phylum Bacteroidetes) and Caldithrixales (phylum Caldithrix) have been previously associated with *S. alterniflora* sediments (Bulseco et al., 2019; Emery et al., 2019). Thus, we suggest that the changes in litter quality associated with woody encroachment resulted in microbial community shifts that can influence other ecosystem functions, such as N removal.

Although woody encroachment enhanced N removal at the lightly oiled site, the effect did not persist at the moderately oiled site. We measured a similar effect on microbial diversity and sediment nutrient inventories in mangrove sediments that did not co-occur in marsh sediments, suggesting that ecosystem recovery, and by extension functional recovery, is slower in marshes impacted by woody encroachment. *Avicennia* species of mangroves are generally more sensitive to oil than other mangrove species (Lewis et al., 2011), whereas *S. alterniflora* is less sensitive to oiling than other marsh grasses (Michel and Rutherford, 2014). Although there were no differences in mangrove height or belowground biomass between the two sites, the majority (70%) of oil residues were detected in mangrove sediments at the moderately oiled site, possibly because pneumatophores trap oil in sediments (Duke, 2016). Re-suspension of oil residues, such as occurs during the slurrying process, can reduce denitrification rates (Levine et al., 2017a). Following the *DWH* spill, *S. alternifora* was faster to recover than *A. germinans* (Lin and Mendelssohn, 2012; Shapiro et al., 2016). The higher turnover and litter production of *S. alterniflora* (Mckee and Rooth, 2008) may have contributed to fewer differences in process rates and microbial community composition between sites in marsh sediments.

Another mechanism through which oiling may have impacted denitrification rates in *A. germinans* sediments is by altering sediment redox conditions. Buried oil residues can decrease the redox potential of rhizosphere sediments (Levine et al., 2017b). We found that the indicator taxa for *A. germinans* at the moderately oiled site selected more anaerobic taxa than the lightly oiled site. GIF10 (phylum OP3) is found in anoxic environments (Glöckner et al., 2010) and is associated with sulfate cycling (Liu et al., 2013). GNO3 is a member of the phylum Latescibacteria (WS3) found in marine environments (Baker et al., 2015; Farag et al., 2017). Latescibacteria are largely saprophytic (Farag et al., 2017) and have metabolic potential for anaerobic fermentation (Youssef et al., 2015). Thus, it is possible that altered redox conditions associated with oiling affected the N removal capacity of *A. germinans*-dominated sediments. While future research is necessary to elucidate the direct mechanism, our research indicates that oiling has a more prolonged negative effect on N removal rates in mangrove than marsh systems.

Enrichment of hydrocarbon-degrading microbial communities was documented following the *DWH* spill (Beazley et al., 2012; King et al., 2015; Engel et al., 2017). This was also reflected in our indicator analysis, as putative hydrocarbon degraders were selected as indicator taxa for both vegetation types, regardless of oiling intensity. These included *Erythrobacter seohaensis* (phylum Alphaproteobacteria) and *Rhodovibrio* (phylum Proteobacteria) (Yoon et al., 2005; Liu and Liu, 2013; Godoy-Lozano et al., 2018; Machado et al., 2019) at the lightly oiled site and the class Gammaproteobacteria (Proteobacteria), the family Piscirickettsiaceae (Proteobacteria), the family SB-1 (Bacteroidetes), and the class Betaproteobacteria (Hazen et al., 2010; Rivers et al., 2013; Hamdan et al., 2018; Emery et al., 2019; Tan and Parales, 2019) at the moderately oiled site. The indicator analysis also selected microbial taxa that have been identified as indicators of oiling recovery. At the lightly oiled site, a selected indicator was Group Gemm-2 in Gemmatimonadetes. Gemm-2 was found to be in decline after oiling in beach sediments and then returned to pre-oiling numbers following cleanup (Huettel et al. 2018). Flammeovirgaceae (phylum Bacteroidetes) was selected at the moderately oiled site and is a widely distributed, salt-tolerant, aerobic taxa that has been known to decline in abundance after oiling (Koo et al., 2015; Mishamandani et al., 2016; Rath et al., 2018). Regardless of whether these taxa reflect long-term microbial community shifts as a result of the *DWH* spill or the representation of indigenous hydrocarbon-degrading communities, these results indicate that both marsh and mangrove sediment communities have the functional capacity to degrade oil. This is important, as while the *DWH* spill was an extreme event, natural oil seeps are highly prevalent in the Gulf of Mexico (MacDonald et al., 2015), and the persistence of hydrocarbon-degrading communities in *A. germinans* sediments may enhance resilience to future oiling events.

Conclusion

This is one of the first studies to compare nitrogen removal rates between *A. germinans*-dominated sediments and the *S. alterniflora* marsh sediments they are replacing. We found that *A. germinans* expansion may enhance marsh N removal capacity, possibly through changes in sediment redox potential or litter quality, although additional research is needed to directly test these mechanisms. We also found that this effect did not occur in sediments subjected to moderate oiling from the *DWH* spill. Results from our indicator taxa analysis suggest that this may be due to changes in sediment redox potential or shifts in the functional microbial community following oiling. Both *S. alterniflora* and *A. germinans* sediment microbial communities included putative hydrocarbon-degrading taxa, regardless of oiling intensity, suggesting that these systems have the functional capacity to break down oil. However, the higher sensitivity of *A. germinans* to oiling may result in an overall slower recovery following a spill and therefore a lag in biogeochemical functional recovery that may not co-occur in salt marshes.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found here: https://data.gulfresearchinitiative.org, 10.7266/Z5MG1AFS, https://data.gulfresearchinitiative.org, 10.7266/4BSSB0AZ.

Author contributions

CT, AK, and BM were responsible for study conception and design. CT was responsible for manuscript preparation. CT and AK were responsible for fieldwork, sediment analyses, and nitrate reduction rates. PC was responsible for nucleic acid isolation. NF was responsible for 16S RNA sequence processing, and NF and CT were responsible for 16S data analysis. NF and PC were responsible for qPCR. EO was responsible for oil residue analysis. BM and PS received funding for the project.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.951365/full#supplementary-material

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Does compensatory mitigation restore food webs in coastal wetlands? A terrestrial arthropod case study on the Upper Texas Coast

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Compensatory wetland restoration is a critical component of holistic, ecosystem-level oil spill response strategies. An important goal of restoration is to rehabilitate food webs in impacted areas, but faunal assemblages and trophic dynamics are rarely included in post-restoration monitoring or assessments of success. Different approaches to wetland restoration, including variations in construction technique, may influence faunal recovery and trophic interactions. To explore these dynamics, we compared emergent plant communities, terrestrial arthropod assemblages, and trophic interactions in restored emergent marshes that were constructed in terrace and mound configurations and in reference areas in the Lower Neches Wildlife Management Area (Texas, United States). Plant community composition differed among all habitat types, with higher diversity on terraces and in reference marshes. Terrestrial arthropod abundance was similar among habitat types, but species composition was distinctly different among habitat types, especially at the herbivore level, where four of the eight herbivore species were found in either reference or restored sites, but not in both habitat types. Herbivores (primarily beetles and planthoppers) were more abundant than detritivores (midges and flies) in all habitat types. Predator (web-building and hunting spiders) abundance and species identity were similar among habitat types. Based on stable isotope analysis, trophic relationships differed among mounds, terraces, and reference areas. Herbivore diets were more variable at the reference sites than in either restored habitat type, aligning with higher plant diversity in reference areas. In contrast, detritivore diets were more variable at restored sites, where they were likely consuming food sources such as benthic algae or sediment organic matter. Predator diets were primarily comprised of herbivores in reference areas and detritivores in restored habitats. Overall, the restoration approaches supported abundant terrestrial arthropod assemblages, but species composition was different. In addition, trophic relationships differed between restored and reference areas, in part due to unique plant species assemblages at restored and reference sites. These results suggest that ecosystem restoration strategies that introduce geomorphological heterogeneity and plant diversity are more likely to support a diverse array of species and functions.

KEYWORDS

Spartina, Deepwater Horizon oil spill, Chenier Plains, Salt Bayou ecosystem, insect, spider, planthopper

Introduction

Coastal wetlands in the continental United States have been substantially reduced in area over the past 100 years (Dahl, 1990), primarily due to urban and agricultural development, hydrological alterations, and natural and anthropogenic subsidence (Mitsch and Hernandez, 2012). Although the rate of loss has slowed in recent years (Entwistle et al., 2018; Murray et al., 2022), coastal wetlands continue to be vulnerable to disturbance from development, storm events, and offshore oil spills (Morton and Barras, 2011; Turner et al., 2016; Hu et al., 2017). Oil spills have received extensive public attention, with acute and long-term effects across all trophic levels, from plants and insects to iconic megafauna (McCall and Pennings, 2012; Silliman et al., 2012; Haney et al., 2014; Lin et al., 2016; Zengel et al., 2016; Zengel et al., 2022). These highly visible impacts make it critical for state management agencies to have robust, multi-faceted, and effective oil spill response plans that address ecosystem recovery across multiple trophic levels (French McCay and Rowe, 2003). Compensatory wetland restoration that considers a range of ecosystem attributes is a critical long-term component of these response plans (Simenstad and Thom, 1996; Armitage, 2021).

Restoration efforts often focus on the creation of vegetated, low elevation marsh habitat that is of high near-term value to fisheries (e.g., La Peyre et al., 2007; Zeug et al., 2007). In the northern Gulf of Mexico, these projects frequently include mound or terrace formations (Rozas et al., 2005) (Figure 1) that create marsh edge habitat. Despite differences in plant species identity and biomass (Garbutt and Wolters, 2008; Mossman et al., 2012; Staszak and Armitage, 2013), previous investigations of ecosystem-level functions in these types of restored marshes indicate that many metrics, such as aquatic faunal density and carbon sequestration potential, can be similar among mound and terrace formations but are sensitive to the amount of aquatic habitat surrounding the emergent marsh

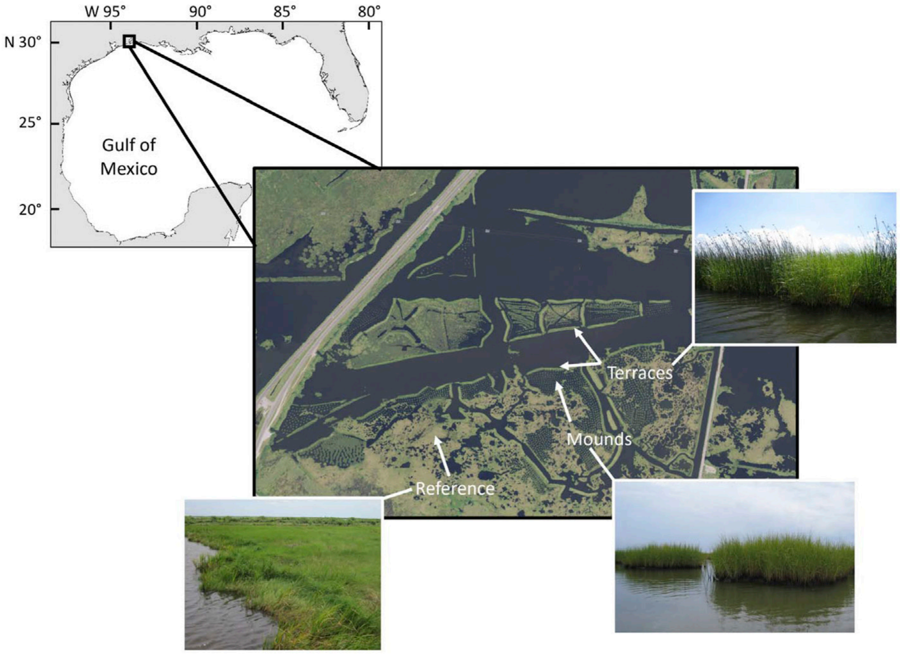

FIGURE 1
Map of study area in the Lower Neches Wildlife Management Area, depicting examples of restored mounds (planted with *Spartina alterniflora*), restored terraces (planted with *Spartina alterniflora* and *Schoenoplectus californicus*), and reference areas (primarily *S. alterniflora*, *Spartina patens*, and *Bolboschoenus robustus*). Inset on upper left indicates study area location in the northern Gulf of Mexico. Aerial image obtained from the United States Department of Agriculture (USDA); Texas NAIP Imagery, 2016–12-15.

features (Rozas et al., 2005; Madrid et al., 2012; Armitage et al., 2014).

An equally important but frequently overlooked goal of compensatory restoration, particularly from public and commercial perspectives, is to rehabilitate impacted wildlife and the food webs that support them. However, the response of lower trophic levels to compensatory restoration is usually assumed but seldom quantified and rarely considered in assessments of restoration success (Weinstein et al., 2005; Loch et al., 2020). In coastal marshes, terrestrial arthropods (insects, spiders, and their kin) are important grazers and prey items in food webs (Post and Greenlaw, 2006; Batzer and Wu, 2020; Ning et al., 2021). Many of these organisms are closely associated with marsh plants, and so faunal use of restored areas may also be affected by marsh plant identity (Talley and Levin, 1999; Wu et al., 2008). For example, insect diversity and abundance are often lower in wetlands invaded by *Spartina alterniflora* (Ning et al., 2021). Conversely, restoration of *S. alterniflora* within its native range can increase arthropod abundance and diversity (Gratton and Denno, 2005). For generalist species, plant identity may be less important than plant cover or density (Guiden et al., 2021), though plant structure may be linked to habitat suitability for some predators such as web-building spiders. In some cases, terrestrial arthropod assemblages and trophic interactions recover following habitat restoration (Gratton and Denno, 2006; Watts and Didham, 2006), but in others, food webs can be slow to approach reference conditions (Wozniak et al., 2006; Ning et al., 2021; Wu et al., 2021). On a decadal scale, the character of the food web changes over time, shifting from detrital-based to herbivore-driven as the restored site ages (Schrama et al., 2012).

Despite the large number of restoration projects on the northern Gulf of Mexico coastline, there have been few quantitative comparisons of the values of different construction and planting techniques for associated wildlife at lower trophic levels (but see Madrid et al., 2012; Armitage et al., 2014). Restoration projects on the upper Texas coast have used various combinations of these approaches, providing a unique opportunity to quantify food web development in response to a range of restoration techniques. Understanding food web structure in restored habitats will help resource managers and oil spill response technicians identify appropriate compensatory restoration techniques that are tailored to the taxa or resources that were impacted, maximizing the efficient use of oil spill response funds. Therefore, our broad objective was to identify compensatory wetland restoration techniques that boosted food web development. In this case study within a restored wetland in the early successional stages, we focused on terrestrial arthropod responses to three key aspects of restoration project design: construction type (terraces or mounds), plant species identity, and plant species diversity. Given the young age of the study site, we expected that detritivores would dominate the food web in restored areas (sensu Schrama et al., 2012).

Materials and methods

Study area

We conducted this study in the Lower Neches Wildlife Management Area (latitude 30° 0′ 5″ N, longitude 93° 51’ 49” W), Texas, United States (Figure 1). The study area consisted of reference (not actively planted or amended) and restored brackish marshes, where two types of restored marsh structures, mounds and terraces, were created in spring 2008. Both mounds and terraces were constructed with material excavated from adjacent bottom sediment. Mounds were circular formations (10–20 m in diameter) and were planted with the smooth cordgrass, *Spartina alterniflora* cv. Vermilion. Terraces were long, narrow structures (5 m x > 100 m) that were planted with *Spartina alterniflora* cv. Vermilion and *Schoenoplectus californicus*. Each of the three habitat types (reference, mounds, terraces) were distinct from each other (separated by at least 100 m) but were within the same hydrological management unit and experienced similar tidal influence. More detailed information about the study site and restoration project was reported by Armitage et al. (2014).

Plant and arthropod sampling

To examine whether reference and restored marshes differed in trophic structure, in summer 2010, we sampled five replicate sites within each of the three habitat types: reference sites, restored mounds, and restored terraces. Replicate sites were at least 30 m apart from each other. Sampling in these habitats provided an opportunity to examine the effect of different restoration configurations (i.e., mounds vs terraces) on plant and arthropod communities and food web structure.

The aboveground biomass of each emergent marsh plant species in each habitat type was determined by clipping all stems at the ground level in representative 10 cm x 20 cm quadrats at each site (n = 5 per habitat type). All plant samples were collected over a 2-day period in June 2010. Stems were rinsed, dried, and weighed to determine biomass (kg/m^2). Additional detail on plant community characterization is described in Armitage et al. (2014). For isotopic analyses, we collected fresh plants and plant detritus from each sampling site, all of which were immediately preserved in coolers with ice. Focal species included the dominant plants in each habitat, such as *S. alterniflora*, *Spartina patens*, and *Bolboschoenus robustus* in reference sites, *S. alterniflora* cv. Vermilion on mounds, and *S. alterniflora* cv. Vermilion and *S. californicus* on terraces. For each plant species, we haphazardly collected 6 leaves at each site for stable isotopic analysis. For plant detritus, it was infeasible to identity to species level and therefore detritus samples were pooled within sampling sites.

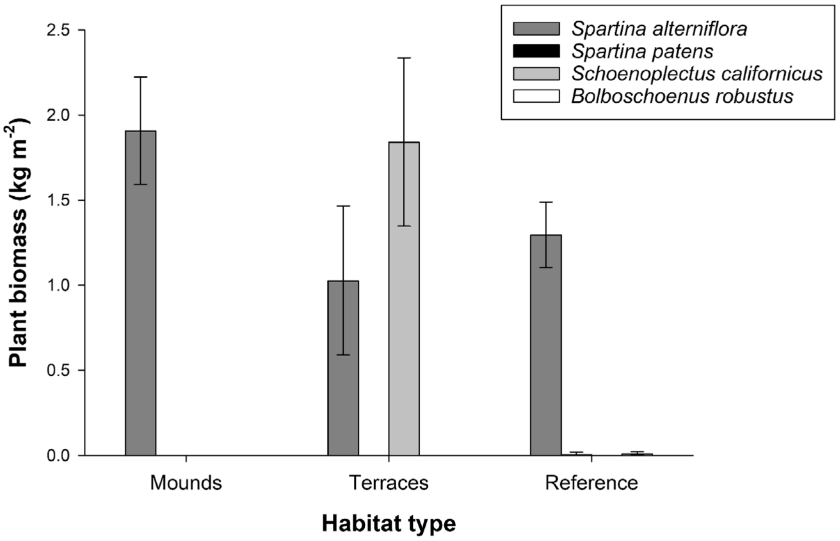

FIGURE 2
Plant biomass at two restored habitat types (mounds and terraces) and in reference areas. Error bars depict standard error.

To characterize arthropod assemblages, we used an insect vacuum fitted with a sampling head (7 cm in diameter), haphazardly selected a starting point within a sampling site, and vacuumed all arthropods from the bottom to the top of vegetation cylinder (13 cm in diameter). We sampled 10 non-overlapping vegetation cylinders per sampling site; all arthropods were collected over a 2-day period in July 2010. This sampling method is effective for capturing species directly associated with the vegetation (Buffington and Redak, 1998). Samples were placed on ice for transport to the lab for identification and processing. Arthropods were identified to the lowest practical taxonomic level. When possible, arthropods were assigned to a trophic level (herbivore, detritivore, or predator) based on the literature and identification guides (Arnett Jr, 2000; Gratton and Denno, 2005).

Stable isotope analysis

To examine trophic linkages among species in the restored wetlands, we analyzed the stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of plants, detritus, and common arthropods (herbivores, detritivores, predators) from each habitat type. Plant and detritus samples collected from the field were rinsed with water at the laboratory before stable isotope analysis. All samples were dried at 60°C for 48 h and ground using a mortar and pestle or electric mill. Plant samples from each site were analyzed separately for each species, and detritus was pooled within sampling sites, since detrital material could not be identified to species level. We pooled arthropod species from the same trophic level at each sampling site to provide enough material for stable isotope analysis; this approach yielded results that reflected the overall signature of each trophic level within each habitat type. Herbivores in our analysis included *Ischnodemus conicus* (Family Blissidae), *Megamelus* sp. and *Prokelisia* sp. (Family Delphacidae), and unidentified members of Families Chrysomelidae, Curculionidae, Elachistidae, Membracidae, and Miridae. Detritivores included members of Families Chironomidae, Drosophilidae, and Ephydridae. Predators included web-building and hunting spiders (Families Araneidae, Lycosidae, Salticidae, Tetragnathidae, and Thomisidae). To reduce analysis bias introduced by various fat content in animals (Post et al., 2007), we treated all arthropod samples with lipid extraction using an Accelerated Solvent Extractor (Dionex) at Texas A&M University at Galveston, Texas, United States. All plant and animal samples were sent to the University of California Davis Stable Isotope Facility for stable isotopic analysis of carbon (C) and nitrogen (N), using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer. Vienna Pee Dee Belemnite and air were used as standards for carbon and nitrogen, respectively.

Statistical analyses

Total plant biomass was analyzed with one-way univariate PERMANOVA following square root transformation using the adonis2 routine in the vegan package in R version 4.2.0 (R Core

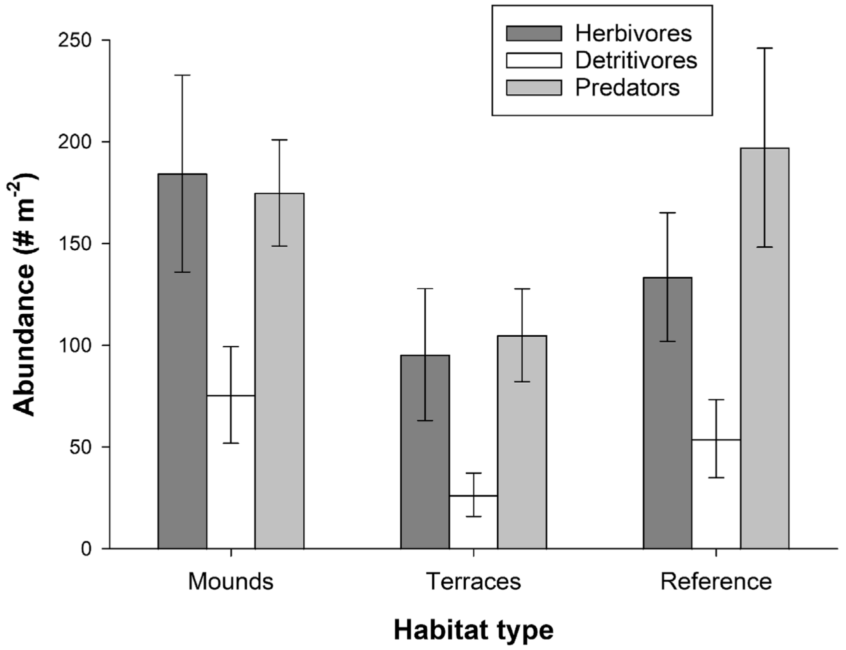

FIGURE 3
Abundance of terrestrial arthropod functional groups at two restored habitat types (mounds and terraces) and in reference areas. Error bars depict standard error.

Team, 2020), where habitat type (reference, mounds, or terraces) was the independent variable. Plant assemblages were analyzed with two-way multivariate PERMANOVA, where species identity and habitat type were the independent variables. Arthropod abundances were analyzed with separate one-way PERMANOVA for each functional group (herbivores, detritivores, predators), where habitat type was the independent variable.

Stable isotope values were used to evaluate trophic relationships for herbivores and predators in each of the three habitat types using Bayesian mixing models in the package MixSIAR (v 3.1.12, Stock et al., 2018) in R version 4.1.2 (R Core Team, 2020). We were not confident that we fully sampled all likely food sources for detritivores; therefore mixing models were not performed for this trophic group. Each model was run in three chains with 1,000,000 iterations and a burn-in of 500,000 and was thinned by 500 each iteration. For herbivores, different end members (food sources) were available in restored and reference sites, so separate models were run in each habitat type. For predators, end members were the same at all sites, so a single model was run to determine the contribution of herbivores and detritivores to predator diets in each habitat type. The biomass (of plants) or the abundance (of herbivores and detritivores) of each dietary source within each habitat type was incorporated into each model as an informative prior. Detrital biomass was not recorded, but based on studies in similar climate zones (Netto and Lana, 1999), it was assumed to be equivalent to live biomass of the species present at each sampling site. *Spartina alterniflora* and *S. patens* were not isotopically distinct from each other, so in the reference habitat type where both plants were present, they were combined into a single *Spartina* source in the model as recommended by MixSIAR. We used trophic discrimination factors of 0.4 ± 1.3 for carbon and 2.0 ± 1.8 for nitrogen as suggested by McCutchan et al. (2003) for terrestrial arthropod consumers.

To supplement the mixing model outputs, we constructed dual isotope graphs for visual assessments of trophic relationships. In addition, we analyzed absolute differences in $\delta^{13}C$ and $\delta^{15}N$ values for all three trophic groups among habitat types with one-way univariate PERMANOVA as described above.

Results

Plants

Marsh plant biomass was 50–100% higher in restored (mounds 1.9 ± 0.3 kg m^{-2}; terraces 2.9 ± 0.3 kg m^{-2}) than in reference areas (1.3 ± 0.2 kg m^{-2}; PERMANOVA df = 2, F = 5.58, $p = 0.007$). The distribution of biomass among species differed among habitat types (Figure 2; PERMANOVA df = 2, F = 6.42, $p = 0.002$). In restored mounds and in reference sites, *S. alterniflora* was the dominant species. In restored terraces, *S. alterniflora* and *S. californicus* were

TABLE 1 Relative abundance of terrestrial arthropod taxa collected in each habitat type (restored mounds and terraces, reference marsh). In most cases, identification was made to family level; when individuals could be identified to species, common names and families are indicated in parentheses. Black indicates common taxa (>5 m^{-2}), grey indicates uncommon (<5 m^{-2}), and white indicates that taxa was absent from that habitat type.

	Mounds	**Terraces**	**Reference**
Trophic levels			
Herbivores			
Chrysomelidae (leaf beetles)		▒	
Curculionidae (snout and bark beetles)			▒
Elachistidae (grass-miner moths)			▒
Ischnodemus conicus (planthoppers, F. Blissidae)	■	■	▒
Megamelus sp. (planthoppers, F. Delphacidae)		▒	▒
Membracidae (treehoppers)			▒
Miridae (plant bugs)	▒	▒	
Prokelisia sp. (planthoppers, F. Delphacidae)	■	■	■
Detritivores			
Chironomidae (midges)	▒	▒	▒
Drosophilidae (vinegar flies)	■	■	■
Ephydridae (shore flies)	▒	▒	
Predators			
Araneidae (orb weavers)	■	■	■
Lycosidae (wolf spiders)	▒	▒	■
Salticidae (jumping spiders)	▒	▒	▒
Tetragnathidae (long-jawed orb weavers)	▒	▒	▒
Thomisidae (crab spiders)		▒	▒

co-dominant. Small amounts of two other species, *S. patens* and *B. robustus*, were found in reference areas (Figure 2).

Arthropods

Arthropod abundance tended to be lower on terraces than on mounds or in reference areas, but there was high variability within habitat types and no statistical difference among any restored or reference areas (Herbivore PERMANOVA df = 2, F = 1.25, p = 0.321; Detritivores df = 2, F = 1.25, p = 0.322; Predators df = 2, F = 1.93, p = 0.179). Herbivores were more abundant than detritivores at all sites and had similar abundances as predators (Figure 3). Common herbivores included typical salt marsh taxa such as *Ischnodemus conicus* (true bugs, Family Blissidae) and *Prokelisia* spp. (planthoppers, Family Delphacidae) (Table 1). Detritivores

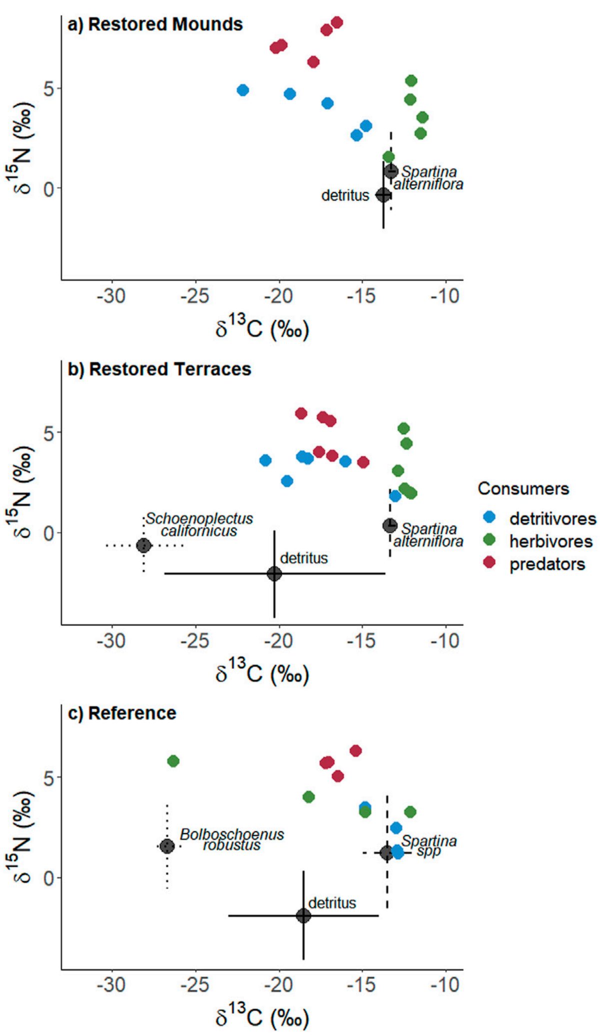

FIGURE 4
Dual isotope graphs of consumers and food sources in **(A)** restored mounds, **(B)** restored terraces, and **(C)** reference sites. Error bars depict standard deviation.

were less abundant and had lower species richness than herbivores, primarily comprised of cosmopolitan consumers of decaying plant material (Table 1). Predators were web-building and hunting spiders (Table 1).

Trophic relationships

Herbivore diets were more variable at the reference sites than in either restored habitat type (Figure 4). The dominant herbivore food source in all habitat types was *S. alterniflora* (or a combination of the two *Spartina* species at the reference sites) (Table 2). The mixing models suggested that detritus comprised a substantial portion of herbivore diets on mounds, but the mixing models may not have been able to differentiate between live and detrital *S. alterniflora* contributions (Figure 4A). Herbivore $\delta^{13}C$ signatures differed among habitat types but $\delta^{15}N$ signatures were consistent across sites (Table 3). Herbivore $\delta^{13}C$ (ranging from -11 to -13.5‰) was closely aligned with *S.*

TABLE 2 Mixing model estimates of end member contributions to herbivore and predator diets in reference and restored (mound or terrace) marshes (mean ± SD). Bolded values indicate the primary contributor to the diet, and blank cells indicate that a source did not occur in a given habitat type.

Trophic levels	Mounds	Terraces	References
Herbivores			
Detritus	38.3 ± 34.1	1.5 ± 3.4	26.3 ± 35.4
Spartina alterniflora	**61.7 ± 34.1**	**97.8 ± 4.3**	–
Spartina spp.	–	–	**73.3 ± 35.5**
Schoenoplectus californicus	–	0.8 ± 2.0	–
Bolboschoenus robustus	–	–	0.4 ± 3.6
Predators			
Herbivores	1.5 ± 3.9	3.2 ± 6.8	**76.0 ± 23.6**
Detritivores	**98.5 ± 3.9**	**96.8 ± 6.8**	24.0 ± 23.6

TABLE 3 Results from one-way PERMANOVA of absolute differences in $\delta^{13}C$ and $\delta^{15}N$ values for three trophic groups among reference and restored (mound or terrace) habitat types. *p*-values less than 0.05 are indicated in bold type.

	Df	SS	Pseudo-F	P
Herbivores				
$\delta^{13}C$				
Habitat type	2	1.36	5.20	**0.006**
Residual	12	1.57		
$\delta^{15}N$				
Habitat type	2	0.17	0.62	0.534
Residual	14	1.63		
Detritivores				
$\delta^{13}C$				
Habitat type	2	0.85	4.46	**0.040**
Residual	12	1.14		
$\delta^{15}N$				
Habitat type	2	0.66	4.14	**0.042**
Residual	12	0.95		
Predators				
$\delta^{13}C$				
Habitat type	2	0.11	2.32	0.159
Residual	12	0.29		
$\delta^{15}N$				
Habitat type	2	0.77	10.46	**0.005**
Residual	12	0.44		

alterniflora signatures in both restored habitats (Figure 4). In reference sites, herbivore δ^{13}C signatures were more variable (ranging from -12 to -26‰), reflecting a wider array of food sources such as *B. robustus*) or unsampled food sources (e.g., aquatic vegetation or algae).

Detritivore δ^{13}C and δ^{15}N signatures varied among habitat types (Table 3). In restored mounds and terraces, δ^{13}C signatures indicated that detritivore diets were variable, ranging from -13 to -22‰ (Figure 4). Detritivore isotopic ratios at mounded sites frequently fell outside the range of collected end member values, indicating the presence of additional food sources (e.g., benthic algae or sediment organic matter) that were not sampled; these missing end members prevented the accurate calculation of diet contributions with mixing models. In reference areas, detritivore δ^{13}C signatures were less variable (ranging from -13 to -15‰) and were more closely aligned with *Spartina* spp. signatures (Figure 4). Detritivore δ^{15}N signatures were lower in reference areas (average 2.1‰ ± 0.5 SE) than on mounds (3.9‰ ± 0.4) or terraces (3.2‰ ± 0.3), indicating that detritivores were consuming a more enriched organic end member in restored areas.

Predators showed a clear dietary shift with restoration (Table 2). Predator δ^{13}C signatures were similar in all habitat types but were more closely aligned with detritivores in restored habitat types and with herbivores in the reference areas (Table 3; Figure 4). Predator δ^{15}N signatures were enriched on mounds (average 7.3‰ ± 0.3 SE), relative to terraces (4.8‰ ± 0.4) and reference areas (5.7‰ ± 0.3) (Table 3). The higher predator δ^{15}N signature on mounds corresponded with higher detritivore δ^{15}N signatures in those areas.

Discussion

Despite some high-level similarities between restored and reference areas in terms of plant biomass and cover and terrestrial arthropod abundance (Figures 2, 3; Armitage et al., 2014), there were clear distinctions in species composition and trophic relationships in the restored sites. Terrestrial arthropod assemblages are notoriously heterogeneous over space and time (David et al., 2016; Hacala et al., 2019), but abundance does tend to be linked to plant assemblage characteristics. In habitats ranging from tidal marshes to terrestrial grasslands, restored sites with plant communities that resemble reference areas are more likely to develop similar faunal communities (Mortimer et al., 2002; Gratton and Denno, 2005; Gerber et al., 2008; Watts et al., 2008; Moir et al., 2010). Accordingly, the successful development of food webs in restored habitats is closely linked to plant and animal species identity (Gratton and Denno, 2006; Watts and Didham, 2006).

Based on the similarity in arthropod abundance between restored and reference areas, and the relatively young age (2 years) of the restored sites, it appears that terrestrial arthropods quickly colonized the restored areas. However, abundance metrics do not tell the full story about the restoration of arthropod assemblages (Burkhalter et al., 2013; Maoela et al., 2016; Hacala et al., 2019). In fact, arthropod taxonomic identities were distinctly different among habitat types, especially among herbivores. Four of the eight herbivore species were found in either reference or restored sites, but not in both types of sites. Only two herbivorous genera were found in all three habitat types; both of those species are known to be closely associated with *S. alterniflora*: the sap-feeder *Prokelisia* and the planthopper *Ischnodemus* (Denno and Grissell, 1979; Slater and Baranowski, 1990). Predator and detritivore assemblages were more similar between restored and reference areas, with four out of five predators and two out of three detritivores found in all habitat types. Notably, arthropod assemblages in restored areas were not dominated by detritivores as expected, despite the young age of the restored sites (Schrama et al., 2012). Asynchronous recovery of different trophic levels in arthropod assemblages is influenced by many factors, including dispersal ability, food availability for specialist species, and abiotic conditions, as well as plant identity and characteristics (Gratton and Denno, 2006; Simao et al., 2010; Dibble et al., 2013; Pétillon et al., 2014).

Emergent plant species identities varied among habitat types, with the fewest species in mounded areas and the most in reference areas. This variation in species composition was reflected in biomass and cover measures (Figure 2; Armitage et al., 2014) as well as in the detrital isotopic signatures (Figure 4). These results are typical in restored wetlands, where differences in plant species identity and biomass can persist for many years (Garbutt and Wolters, 2008; Mossman et al., 2012; Staszak and Armitage, 2013). In this study area, the emergent plant communities in restored areas were intentionally different from the reference area, and from each other. The goal of planting in coastal wetland restoration is typically to rapidly generate a plant canopy that meets some cover level stipulated in the associated permit (Cole and Shafer, 2002). Thus, the primary plant species in the restored sites (*S. alterniflora*) was chosen to jump-start plant canopy restoration, despite the relative rarity of *S. alterniflora* in other portions of the reference area, where *S. patens* and *B. robustus* dominated in terms of cover (Armitage, 2021). Although *S. alterniflora* was not abundant throughout the reference area, the Vermilion strain selected was a particularly robust variant that grows much taller than local strains (Fine et al., 2000). Thus, even at low abundance, *S. alterniflora* comprised a substantial portion of the emergent plant biomass at our study sites and was therefore a potentially important and readily available end member for both herbivores and detritivores in all habitat types.

The food sources supporting the terrestrial arthropod food web likely included more than just emergent marsh plants. In young restored sites, detritus can play a particularly important role in food web support (Schrama et al., 2012). In addition, marine subsidies can make substantial contributions to arthropod food webs in tidal wetlands (Paetzold et al., 2008), and aquatic vegetation, algae, and particulate organic matter were abundant in all habitat types (Armitage et al., 2014), though these sources likely varied in importance for different consumer groups. Accordingly, the variation in detrital $\delta^{13}C$ signatures in terraced and reference habitat types indicated that there was a wide array of organic matter sources available to consumers. These alternate food sources were not sampled in this study, preventing the use of mixing models to specifically characterize their importance in consumer diets. Regardless, consumer isotopic signatures can be useful in making relative comparisons in trophic relationships among habitat types, and provide insight into how similar (or dissimilar) the restored areas are to target reference conditions.

In general, isotopic signatures indicated that consumer diets differed between restored and reference areas, but each consumer group showed unique patterns. Herbivore dietary diversity was relatively low in restored habitat types, with $\delta^{13}C$ signatures closely clustered near *S. alterniflora* values. Although the mixing models suggested that detritus comprised a portion of herbivore diets on restored mounds, the detrital and live *S. alterniflora* $\delta^{13}C$ signatures were very close together, suggesting that the mixing models were unable to differentiate between live plant and detrital contributions. Based on the natural history of two common herbivores at this site (Armitage et al., 2013), it is unlikely that detritus was an important component of herbivore diets. *Prokelisia* is a sap-feeding specialist on *S. alterniflora* (Denno and Grissell, 1979) and *Ischnodemus conicus* typically lives cryptically under *S. alterniflora* basal leaf sheaths (Slater and Baranowski, 1990). Model outputs suggesting detrital consumption may have also been confounded by the sap-sucking nature of *Prokelisia*. Sap-suckers tend to have different trophic discrimination factors than insects that consume leaf tissue (McCutchan et al., 2003) but there was no way to separate out the two feeding methods in the mixing models. Evidence of strong herbivore affiliations with *S. alterniflora* was clear in terraced sites, where *S. californicus* was also an abundant plant, but $\delta^{13}C$ signatures indicated that herbivores avoided that species in favor of *S. alterniflora*. In the reference areas, a wider array of available emergent plant species corresponded with a wider spread (and lower absolute average) in herbivore $\delta^{13}C$ values, indicating more diet heterogeneity.

In contrast to herbivores, detritivore diet diversity was highest, as indicated by a wider spread in $\delta^{13}C$ values, in restored areas. Detritivore isotopic signatures were similar in mounds and terraces, despite differences in the signatures of available detritus. Detrital signatures in restored mounds were closely aligned with *S. alterniflora*. In contrast, the detritus on terraces was likely a mix between the two abundant plant species, *S. alterniflora* and *S. californicus*. Additional detritus from subtidal sources may have accumulated on terraces but not on mounds due to their smaller size and lower elevation profile. To some degree, detritivores in both sites were likely consuming some detrital vascular plant material, but unsampled end members were also likely to be relevant diet components in both restored areas. In particular, sediment organic matter may have been an important (but unsampled) end member for these consumers (Page et al., 2010). In contrast to restored areas, detritivores in reference areas appeared to have distinctly different diets, as detritivore signatures were closely aligned with *S. alterniflora*, suggesting that those taxa may have primarily consumed detritus from that plant species.

Based on isotope signatures and mixing models, predator diets were more closely aligned with detritivores in restored areas and herbivores in reference areas. The spider taxa in Gulf Coast tidal marsh ecosystems are typically generalists, utilizing either webs or active hunting strategies (Gratton and Denno, 2005). Some predatory taxa in these tidal ecosystems may engage in intraguild predation (Matsumura et al., 2004), but based on isotopic signatures, either herbivores or detritivores comprised the bulk of predator diets. As such, shifts in predator diets following restoration likely tracked changes in abundance or biomass of prey items within these two consumer groups (Pétillon et al., 2005; Gratton and Denno, 2006). Although detritivores were not the most abundant group (Figure 3), some of the taxa in restored areas had relatively large body sizes (e.g., shore flies in Family Ephydridae), and their biomass may have been substantial enough to offset their relative rarity in the arthropod assemblage. The co-occurring herbivore species in restored sites tended to be more cryptic or stationary (Denno and Grissell, 1979; Slater and Baranowski, 1990), and thus were less susceptible to predation from either passive web builders or active hunters. In contrast, in reference areas, members of Family Ephydridae were absent and other smaller taxa in Families Chironomidae and Drosophilidae were more common. Accordingly, the biomass of detritivores available to predators in reference areas was lower, relative to restored areas.

Implications for restoration

The intent of compensatory coastal wetland restoration is to mitigate for structural or functional loss of habitat within the watershed, often following major disturbance events such as the Deepwater Horizon oil spill (Van den Bosch and Matthews, 2017; Zengel et al., 2021). The establishment of a specified level of emergent plant cover is a standard metric of restoration success (Ruiz-Jaen and Aide, 2005; Matthews and Endress, 2008). However, plant cover rarely corresponds with a broad spectrum of functional characteristics such as species diversity, nutrient cycling, or trophic energy flow (Craft et al., 1999; Zedler and Lindig-Cisneros, 2000; Levrel et al., 2012). In this study, plant and terrestrial arthropod communities were distinctly different between restored and reference areas, and those differences contributed to dissimilarity in food web structure among habitat types. Terrestrial arthropods play important roles in controlling productivity and supporting higher trophic levels, including fish and avian predators (Post and Greenlaw, 2006; Batzer and Wu, 2020; Ning et al., 2021). In some cases, terrestrial arthropod abundances rapidly re-establish following habitat restoration (Gratton and Denno, 2005; David et al., 2016), but restored assemblages are often dissimilar in terms of diversity and distribution among trophic guilds (Pétillon et al., 2014; Ning et al., 2021; Farfán-Beltrán et al., 2022). Restored areas with plant assemblages that are distinct from those in reference areas are especially likely to yield persistent dissimilarities in associated fauna (Talley and Levin, 1999; David et al., 2016; Leonard et al., 2021).

The broad objective of this study was to identify compensatory wetland restoration techniques that would boost food web development. We focused on terrestrial arthropod responses to three key aspects of restoration project design: construction type (terraces or mounds), plant species identity, and plant species diversity. The two construction types each yielded unique terrestrial arthropod assemblages and food web structure. In contrast to expectations, neither restored habitat type had a food web supported primarily by detritus. Furthermore, neither restored habitat type closely resembled reference conditions. The unique assemblages and trophic relationships within each habitat type were likely linked to differences in plant communities and localized geomorphology (this study; Rozas et al., 2005; Armitage et al., 2014). Although neither restored site successfully achieved absolute parity with the reference area, all three habitat types contributed to ecosystem-level diversity at the lower trophic levels. This outcome highlights the importance of integrating heterogeneity into ecosystem restoration design to boost a diverse array of species and functions (Rozas et al., 2005; Armitage et al., 2007; Pétillon and Garbutt, 2008; Madrid et al., 2012; Weisberg et al., 2012; Lengyel et al., 2020; Armitage, 2021). Restoration approaches that strategically include topographic and vegetation heterogeneity are more likely to achieve a broader range of ecosystem benefits.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

AA and C-KH contributed to conception and design of the study. C-KH collected the data. AA and JG performed the statistical analyses. AA wrote the first draft of the manuscript. All authors contributed to manuscript revision and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

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Aragonite saturation states in estuaries along a climate gradient in the northwestern Gulf of Mexico

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In the northwestern Gulf of Mexico (nwGOM), the coastal climate shifts abruptly from the humid northeast to the semiarid southwest within a narrow latitudinal range. The climate effect plays an important role in controlling freshwater discharge into the shallow estuaries in this region. In addition to diminishing freshwater runoff down the coast, evaporation also increases substantially. Hence, these estuaries show increasing salinity along the coastline due to the large difference in freshwater inflow balance (river runoff and precipitation minus evaporation and diversion). However, this spatial gradient can be disrupted by intense storm events as a copious amount of precipitation leads to river flooding, which can cause temporary freshening of these systems in extreme cases, in addition to freshwater-induced ephemeral stratification. We examined estuarine water aragonite saturation state (Ω_{arag}) data collected between 2014 and 2018, covering a period of contrasting hydrological conditions, from the initial drought to multiple flooding events, including a brief period that was influenced by a category 4 hurricane. Based on freshwater availability, these estuaries exhibited a diminishing Ω_{arag} fluctuation from the most freshwater enriched Guadalupe Estuary to the most freshwater-starved Nueces Estuary. While Ω_{arag} values were usually much higher than the threshold level ($\Omega_{arag} = 1$), brief freshwater discharge events and subsequent low oxygen levels in the lower water column led to episodic corrosive conditions. Based on previously obtained Ω_{arag} temporal trends and Ω_{arag} values obtained in this study, we estimated the time of emergence (ToE) for Ω_{arag}. Not only did estuaries show decreasing ToE with diminishing freshwater availability but the sub-embayments of individual estuaries that had a less freshwater influence also had shorter ToE. This spatial pattern suggests that planning coastal restoration efforts, especially for shellfish organisms, should emphasize areas with longer ToE.

KEYWORDS

aragonite saturation state, freshwater inflow, subtropical estuaries, ocean acidification (OA), time of emergence

Introduction

It is widely known that acidification of the aquatic environment caused by both oceanic CO_2 uptake and respiration within the water column leads to changes in carbonate equilibria. In the marine system, the increase in total dissolved inorganic carbon (DIC) gives rise to elevated CO_2 partial pressure (pCO_2) and decreases both pH and carbonate saturation state (Ω) (Feely et al., 2010; Cai et al., 2011; Feely et al., 2018). All these changes could affect marine organisms on their metabolism, reproduction, and a host of other physiological functions (Andersson et al., 2015). Calcifying organisms, because of their need to synthesize calcium carbonate using the mineral base of natural waters, are especially susceptible to the changing carbonate chemistry (Orr et al., 2005; Doney et al., 2009; Spalding et al., 2017; Mcnicholl et al., 2020).

Based on thermodynamics, Ω in seawater is defined as the ratio of the ionic concentration product of Ca^{2+} and carbonate (CO_3^{2-}) and the stochiometric solubility constant ($K_{sp}{}'$, Eq. 1):

$$\Omega = [Ca^{2+}][CO_3^{2-}]/K_{sp}{}' \tag{1}$$

Using this definition, if $\Omega < 1$, the water is considered undersaturated (or corrosive), which hence favors carbonate dissolution, and if $\Omega > 1$ or the water is supersaturated, carbonate precipitation could potentially occur. The two most common carbonate minerals in the marine environment, that is, aragonite and calcite, have different solubilities, with aragonite being slightly more soluble (Mucci, 1983). Because of the implications for the early life stages of important calcifying species (for example, larval stage oysters, Waldbusser et al., 2014; Waldbusser et al., 2016, food web species (e.g., pteropods, Manno et al., 2017), and shallow water calcifiers (such as corals, Eyre et al., 2018)), aragonite saturation state (Ω_{arag}) is often used to describe the suitability of water carbonate chemistry (Feely et al., 2012; Jiang et al., 2015; Sasse et al., 2015; Davis et al., 2018; Negrete-García et al., 2019).

Ocean acidification has led to a multidecadal decrease in both pH and Ω_{arag} and has caused "shoaling" of saturation horizons, that is, the depths at which $\Omega_{arag} = 1$ have migrated upward as ocean acidification progresses over time (Feely et al., 2012). These changes pose challenges to coral reefs in the tropics and planktonic calcifiers such as pteropods, coastal aquaculture communities, and benthic communities (Juranek et al., 2009; Feely et al., 2012; Hales et al., 2016; Mclaughlin et al., 2018; Ross et al., 2020). In addition to the CO_2 level, both temperature and salinity play important roles in regulating Ω_{arag}. First, $K_{sp}{}'$ is a function of temperature, and it increases with decreasing temperature. Along with the increases in CO_2 solubility, Ω_{arag} decreases toward higher latitudes in the open ocean (Jiang et al., 2015). In addition, because of the high Ca^{2+} background concentration in ocean water (~10.3 mmol kg^{-1} at salinity 35), mixing caused by precipitation, river runoff, and glacial and sea ice melting also dilute seawater Ca^{2+}. Hence, Ω_{arag} in lower salinity waters is usually low. Therefore, salinity also affects Ω_{arag} based on the definition (Eq. 1). In addition, carbonate chemistry variation in the freshwater endmember could also exert important controls on Ω_{arag} in the low salinity range (Moore-Maley et al., 2018).

Numerous studies have examined both short-term variations and long-term trends of Ω_{arag} in both the open ocean and coastal regions (Gledhill et al., 2008; Franco et al., 2021; Gomez et al., 2021; Siedlecki et al., 2021). In recent years, increasing numbers of investigations have been conducted to understand Ω_{arag} dynamics in estuarine settings, using both field sampling (Feely et al., 2010; Wallace et al., 2014; Mccutcheon et al., 2019) and numerical methods (Da et al., 2021). Nevertheless, given the diversity of estuarine environments, knowledge of estuarine Ω_{arag} remains limited. Ω_{arag} in mixing-dominated estuaries can often be estimated using endmember mixing models (Moore-Maley et al., 2018; Simpson et al., 2022). However, there are also estuaries where freshwater discharge is smaller relative to the sizes of the receiving water bodies, and water residence time can be further prolonged by the sluggish exchange with the coastal ocean due to the presence of physical barriers. In this case, water chemistry can be modified further by evaporation and biogeochemical processes. Many of these estuaries are important habitats for ecologically and economically important species including shellfish. Hence, studying Ω_{arag} in this type of environment is important given that the response of these estuaries to changes in hydrological conditions may be significantly longer than those that have more efficient exchange with the coastal ocean (Yáñez-Arancibia and Day, 2004; Kim et al., 2014; Bishop et al., 2017).

In the northwestern Gulf of Mexico (nwGOM), there are seven major estuaries that lie along a climate transition zone from northeast to southwest: Sabine-Neches Estuary (SNE), Trinity-San Jacinto Estuary (TSJE), Lavaca-Colorado Estuary (LCE), Guadalupe Estuary (GE), Mission-Aransas Estuary (MAE), Nueces Estuary (NE), and Laguna Madre (LM) (Montagna et al., 2011b). These microtidal estuaries have similar geomorphic structures and physiograph (Montagna et al., 2007) with depths ranging from 0.5 to 2.5 m (Montagna et al., 2013), and they are separated from the Gulf of Mexico by barrier islands and peninsulas. Water exchange with the coastal ocean usually takes place through narrow channels and passages. Along this narrow latitudinal gradient, freshwater inflow into these estuaries decreases by a factor of two, yet the freshwater inflow balance, that is, freshwater inputs minus outputs, decreases by two orders of magnitude (Montagna et al., 2011a). Hence, these estuaries exhibit a general increase in both residence time and average salinity from north to south from a few weeks to over a year (Solis and Powell, 1999). In addition, hydrological conditions in this region have varied substantially as a result of broader climate patterns (Tolan, 2007), and extreme events (flooding caused by tropical storms and hurricanes) can result in

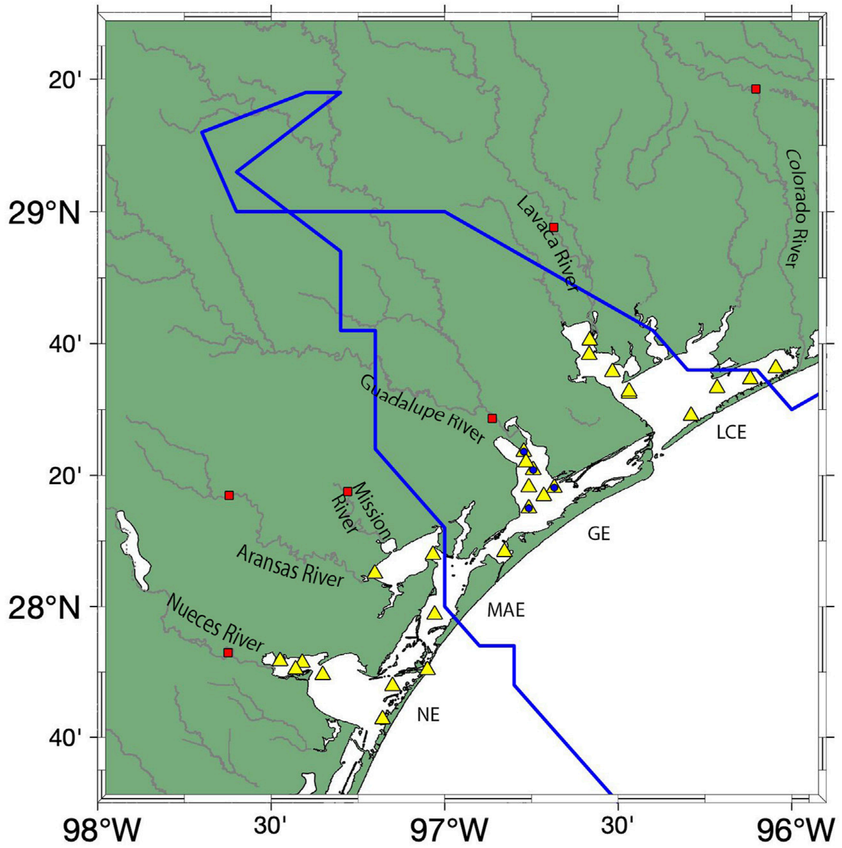

FIGURE 1
Estuarine sampling stations (yellow triangles). It is to be noted that the blue circles in the Guadalupe Estuary are the seasonal sampling stations. USGS streamflow gages are shown in red squares. The blue line presents the track of Hurricane Harvey in August 2017.

nearly complete, albeit episodic, freshening of these estuaries (Pollack et al., 2011; Du and Park, 2019; Walker et al., 2020).

Most of these estuaries, with the exception of NE and LM to the southern end, are important habitats for commercially important shellfish species, for example, blue crab, shrimps, and eastern oysters (Tunnell, 2017). However, large freshwater discharge events driven by hurricanes and storms have caused a significant decrease in the abundance of oysters (Pollack et al., 2011; Du et al., 2021). These systems experienced severe drought in the earlier portion of the study period and went through multiple large freshwater discharge events (Yao et al., 2022). To better understand these ecosystems that host this important species and the habitats that they create, we examined a previously poorly studied water chemistry parameter, Ω_{arag}, and other water quality data collected in four mid-coast estuaries in recent years. Then, using multidecadal change rates of Ω_{arag} from a prior study (Mccutcheon and Hu, 2022), we also explored timescales beyond which Ω_{arag} change will exceed its natural variability.

Materials and methods

Water column sampling was conducted in four nwGOM estuaries (LCE, GE, MAE, and NE, Figure 1) during multiple field expeditions (Table 1) in 2014–2018. In 2017, category 4 Hurricane Harvey made landfall in this studied area, and the total rainfall was as much as 152 cm in some areas (Walker et al., 2020). River discharge data were obtained from USGS streamflow gages (#08162000 for Colorado, #08164000 for Lavaca, #08188810 for Guadalupe, #08189500 for Mission, #08189700 for Aransas, and #08211500 for Nueces, Figure 1).

Water sampling and analytical methods were detailed in our previous studies (Yao and Hu, 2017; Mccutcheon et al., 2021). Briefly, water column salinity, temperature, and dissolved oxygen (DO) were measured using a calibrated multisonde. Both surface (within 0.5 m below surface) and bottom (within 0.5 m above sediment) water samples for carbonate chemistry analysis were taken using a van Dorn horizontal water sampler into 250 borosilicate bottles and preserved using 100 μL of saturated $HgCl_2$. The bottles were sealed using Apiezon®

TABLE 1 Sampling times at the four studied nwGOM estuaries.

Estuary name	Sampling duration	Sampling frequency	Comments
LCE	01/2014–10/2018	Quarterly	
GE	01/2014–10/2018	Quarterly	05–09/2017 sampling frequency every 2 weeks
MAE	05/2014–12/2018	Every 2 weeks	
NE	01/2014–10/2018	Quarterly	

grease, and the stoppers were secured using rubber bands and hose clamps, following the best practice in Dickson et al. (2007). Then, 20-ml scintillation vials were used for Ca^{2+} sample collection without preservation. All water samples were stored at 4°C in the dark until analysis, typically within 2 months of sample collection.

Total dissolved inorganic carbon (DIC) was analyzed using infrared detection (AS-C3, Apollo SciTech Inc.), and total alkalinity (TA) was analyzed using open-cell Gran titration (AS-ALK2, Apollo SciTech). Certified reference materials or CRMs (Dickson et al., 2003) were used throughout both TA and DIC analyses to ensure data quality. We used various approaches to measure pH. For samples prior to 28 September 2017 with salinity ≥20, we used a spectrophotometric method with purified m-cresol purple (mCP) (precision of ±0.0004, Liu et al., 2011); then, a calibrated Ross Orion high precision pH electrode was used to measure pH for samples with salinity less than 20 (precision ±0.01). From 28 September 2017, all pH samples were measured using the spectrophotometric method, and pH for samples with salinity <20 was calculated using the updated equation in Douglas and Byrne (2017), which covers a wider salinity range than that in Liu et al. (2011). Ca^{2+} concentration ($[Ca^{2+}]$) was titrated using ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) (Kanamori and Ikegami, 1980) with a precision of 0.2%, and a Metrohm® calcium ion-selective electrode was used for endpoint detection on a Titrando 888 automatic titrator. Salinity was measured using a calibrated benchtop salinometer.

For chlorophyll-a (Chl-a) sampling, water samples were filtered using GF/F filters and Chl-a was extracted using either 95% methanol (LCE, GE, and NE, Montagna et al., 2018) or 90% acetone (MAE, Mooney and Mcclelland, 2012). Chl-a concentration was determined using a Turner Trilogy fluorometer. Nutrient concentration in these estuaries was also measured following the techniques in Montagna et al. (2018) (phosphate, nitrate + nitrite or NO_x, and silicate for LCE, GE, and NE) and Mooney and Mcclelland (2012) for MAE (all macronutrients except silicate). All concentrations were reported in mass-based units, assuming the lab temperature was 22°C.

The Ω_{arag} was calculated using pH and DIC as input variables because of the likely presence of non-carbonate species that may contribute to alkalinity (Yao and Hu, 2017). The calculation was carried out using the MATLAB version of the program CO2SYS (Van Heuven et al., 2011). The dissociation constants of carbonic acid were from Waters et al. (2014), bisulfate dissociation constant from Dickson et al. (1990), and borate and salinity relationship from Lee et al. (2010) were used in the speciation calculations. Measured $[Ca^{2+}]$ and the values obtained from the $[Ca^{2+}]$ vs. salinity regression (for samples without $[Ca^{2+}]$ data) from each estuary were used to correct Ω_{arag}. Because the stoichiometric solubility constant of aragonite is only valid for salinity 5–44 (Mucci, 1983), calculated Ω_{arag} values from samples that had a salinity lower than 5 were excluded from the data analysis. The uncertainty for the calculated Ω_{arag} was ±0.18 using the error propagation approach in Orr et al. (2018), and the two different pH analytical approaches (potentiometric and spectrophotometric) yielded slightly different Ω_{arag} uncertainties (~0.01).

To better explore the correlation between the carbonate systems and environmental conditions, principal component analysis (PCA) was performed for all variables. Because of the different sampling frequencies (biweekly to monthly in MAE vs. seasonal in all other estuaries) and numbers of sampling stations in these estuaries, average data of sampled months for each estuary was used in this analysis. Then, all variables were log transformed using ln (X+1) after checking for normality and homoscedasticity, except the already logarithmically expressed parameter pH. Then, all variables were standardized with a normal distribution with a mean of 0 and a variance of 1 to ensure the same scale before PCA. The results were presented graphically as vector plots with the major dimensionalities and length representing the relationship and weight of the principal components. PCA was conducted in R studio based on package "factoextra" (Kassambara and Mundt, 2020). To further understand its spatiotemporal pattern, individual PCAs were performed for monthly data that was grouped by location, year, and season (e.g., March–May are used as spring), respectively.

In climate and ocean studies, the term "time of emergence" has been frequently used to describe the timescale for a secular trend to appear in time-series data (Hawkins and Sutton, 2012; Sutton et al., 2019; Turk et al., 2019). In a prior study, Mccutcheon and Hu (2022) calculated temporal trends of Ω_{arag} in nwGOM estuaries using water chemistry data collected by the Texas Commission on Environmental Quality

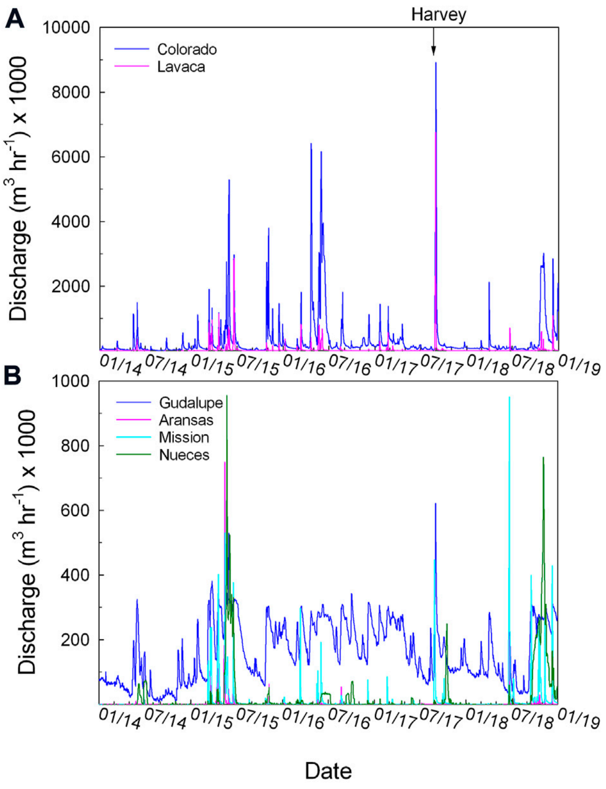

FIGURE 2
River discharge during the period of 2014–2018. The arrow points to the date of Hurricane Harvey landfall near San Jose Island, Texas, at the southern end of the Mission-Aransas Estuary. The Lavaca and Colorado rivers empty into LCE, the Guadalupe River empties into GE, the Mission and Aransas rivers empty into MAE, and the Nueces River empties into NE. Lavaca and Colorado rivers **(A)** and all four other rivers **(B)** are separately plotted to show the large differences in their discharge (note the vertical scales).

(TCEQ) that dated back to the 1970s. Based on Ω_{arag} values obtained in this study, we explored the time scales for these trends to emerge for both secondary and primary bays of each estuary using Eq. 2.

$$\text{ToE} = (\text{N}\times\text{noise})/\text{trend} \tag{2}$$

Noise represents natural variability or standard deviation of the observations; N is an arbitrarily chosen integer and 2 is often used (Sutton et al., 2019).

Results

Hydrological conditions

The six rivers that discharge into the studied estuaries showed large spatial and temporal variations. The Colorado River had the largest annual discharge on average (2.83×10^9 m^3 yr^{-1}) during the study period (2014–2018), more than 100 times higher than that of the Aransas River (2.12×10^7 m^3 yr^{-1}). The three larger rivers (Colorado, Lavaca, and Guadalupe) experienced extremely low discharge during the height of a dry period (August–September of 2014). On the other hand, the three southernmost rivers (Aransas, Mission, and Nueces) had nearly zero discharge most of the time although episodic precipitation events often led to ephemeral pulses of freshwater (Figure 2). Regional flooding events have caused widespread increases in the discharge during the period of May–June 2015, when the three southern rivers exhibited substantial increases from the baseflow conditions, so did the northern rivers. In comparison, Hurricane Harvey caused discharge increases that were more conspicuous in the three northern rivers and, to a less extent, the Mission River, as it sits near the center of the storm path. Neither Aransas nor Nueces rivers experienced significant hurricane-induced flooding.

Salinity in all estuaries showed large swings during the study period with little stratification. Following the initial drought in 2014, salinity had multiple decreases across the entire coastal area (mid 2015 and 2016, post-Harvey in 2017, and late 2018). Overall salinity of these estuaries ranged from 16.4 ± 8.3 in GE to 30.6 ± 4.9 in NE (Table 2; Figure 3). Despite the smaller overall freshwater input into GE compared to LCE, its salinity values were the lowest. During most of the sampling times, little water column stratification was observed in these shallow estuaries (average depth 1–2 m other than the Aransas Ship Channel station, ~6 m) except during the few flooding events including the short period (~1 month) after Hurricane Harvey (Walker et al., 2020). Based on the proximity to freshwater sources, each estuary can be divided into a primary bay that is connected to coastal water and one or two secondary bays that directly receive freshwater. As a result, salinity in primary bays was often greater than that in secondary bays, except in MAE, during the initial drought period when the secondary bays (Copano and Mesquite bays) were saltier than the primary bay (Aransas Bay) (Hu et al., 2015).

Nutrients and Chl-a

While phosphate, silicate (except MAE has no data), and ammonium had relatively small variations across these estuaries, average NO_x (NO_3 and NO_2) concentrations were higher by 6–8 times in LCE and GE than in MAE and NE, with larger variations as well (Table 2). Chl-a concentrations were also 2 to 3 higher in LCE and GE than in MAE and NE, with higher temporal variations. In both LCE and GE, the secondary bays generally had higher nutrient levels than the primary bays although this was not necessarily true for Chl-a. In comparison, the concentration differences between secondary and primary bays were much less for MAE and NE (Table 2).

TABLE 2 Mean and standard deviations of measured water chemistry variables in the four studied nwGOM estuaries during 2014-2018.

Estuary	Bay	S	DIC	TA	Ω_{arag}	pH	DO%	PO_4	SiO_4	NH_4	NO_x	Chl-a
Lavaca-Colorado Estuary (LCE)	S	17.5	2,189.4	2,384.8	3.13	8.03	107.8	1.8	67.5	1.5	8.2	10.1
		8.5	*472.9*	*578.8*	*1.51*	*0.24*	*19.9*	*2.0*	*47.8*	*2.4*	*18.3*	*8.6*
	P	22.8	2,281.8	2,565.8	3.66	8.14	103.9	0.9	40.7	1.8	4.7	13.6
		6.6	*236.5*	*263.6*	*1.35*	*0.20*	*28.0*	*0.9*	*31.6*	*4.0*	*12.0*	*10.0*
	All	19.9	2,230.8	2,465.9	3.39	8.08	106.0	1.4	55.5	1.6	6.6	11.7
		8.1	*387.7*	*473.0*	*1.46*	*0.23*	*23.9*	*1.7*	*43.0*	*3.2*	*15.7*	*9.3*
Guadalupe Estuary (GE)	S	11.2	2,524.4	2,762.0	4.32	8.10	107.3	2.4	128.5	2.1	13.8	23.9
		6.7	*552.7*	*620.9*	*2.82*	*0.33*	*35.6*	*2.4*	*78.3*	*4.7*	*29.6*	*16.3*
	P	18.3	2,429.7	2,695.6	3.84	8.12	101.9	1.3	85.8	1.7	2.3	11.6
		7.4	*299.8*	*314.9*	*1.19*	*0.17*	*16.5*	*1.2*	*59.7*	*3.3*	*5.4*	*7.4*
	All	14.5	2,480.3	2,731.1	4.08	8.11	104.6	1.8	106.9	1.9	8.0	17.7
		7.9	*454.7*	*502.6*	*2.16*	*0.27*	*28.1*	*2.0*	*72.0*	*4.0*	*21.8*	*13.9*
Mission-Aransas Estuary (MAE)	S	20.8	2,282.4	2,526.4	3.16	8.11	99.5	2.1	-	2.8	0.8	7.4
		9.6	*284.5*	*334.7*	*1.00*	*0.18*	*8.1*	*2.0*	-	*5.4*	*0.8*	*4.8*
	P	28.5	2,172.0	2,459.1	3.40	8.09	98.9	0.7	-	1.9	0.8	5.7
		6.4	*161.6*	*168.2*	*0.65*	*0.11*	*7.1*	*0.7*	-	*3.6*	*0.8*	*3.1*
	All	23.8	2,238.5	2,499.7	3.25	8.10	99.3	1.6	-	2.4	0.8	6.7
		9.3	*249.0*	*282.4*	*0.89*	*0.15*	*7.7*	*1.7*	-	*4.7*	*0.8*	*4.2*
Nueces Estuary (NE)	S	29.4	2,271.2	2,575.6	3.60	8.07	103.2	0.9	66.9	1.1	1.0	8.9
		5.4	*235.6*	*246.1*	*0.98*	*0.10*	*10.4*	*0.9*	*42.1*	*1.8*	*1.9*	*5.8*
	P	32.4	2,180.9	2,528.8	3.86	8.12	96.9	0.2	33.4	1.0	0.4	6.8
		3.1	*154.3*	*171.9*	*0.87*	*0.08*	*14.1*	*0.6*	*17.7*	*2.2*	*0.4*	*3.9*
	All	30.6	2,235.1	2,556.9	3.71	8.09	100.7	0.6	53.6	1.1	0.8	8.0
		4.9	*211.2*	*220.2*	*0.95*	*0.09*	*12.4*	*0.8*	*37.5*	*1.9*	*1.5*	*5.1*

DIC, TA, and nutrient concentrations have the unit of μmol kg^{-1}, Chl-a concentration is in the unit of μg kg^{-1}. pH (total scale) and Ω_{arag} were calculated for *in situ* conditions. Abbreviations: average values, S, secondary bay; P, primary bay; All; overall estuary average. Values in italics represent standard deviations.

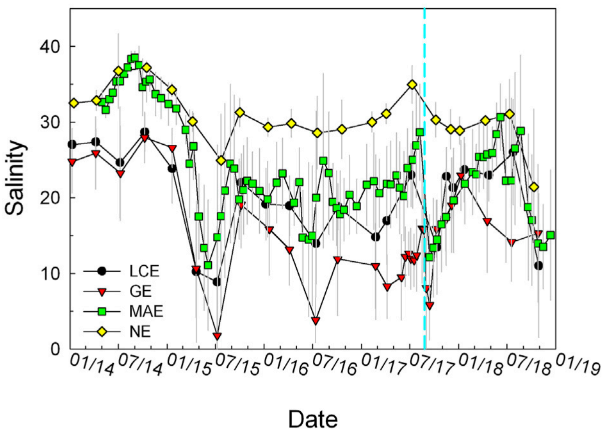

FIGURE 3
Salinity time series in the four studied estuaries during 2014–2018. These values are averages of all data from these stations as stratification occurred infrequently.

Carbonate chemistry

Average TA and DIC concentrations in these estuaries (2,466–2,731 and 2,231–2,480 μmol kg^{-1}, respectively, Table 2) were higher than the Gulf of Mexico surface water (Hu et al., 2018). GE had the highest values of all four estuaries on average. The values in the two northern estuaries (LCE and GE) had larger variations than in the two southern estuaries (MAE and NE). LCE was the only estuary that had lower TA and DIC levels in its secondary bay than in the primary bay, and all others had higher TA and DIC levels in the secondary bays despite their lower salinity (Table 2). In addition, these estuaries also had different TA and DIC vs. salinity relationships (Figures 4, 5). LCE and GE had varying freshwater endmember concentrations with a range of ~1,000 μmol kg^{-1}. Although GE and NE both had higher freshwater endmember values than others, most NE data points were clustered at a salinity greater than 25. In the mid-salinity range, apparent TA and DIC enrichment appeared,

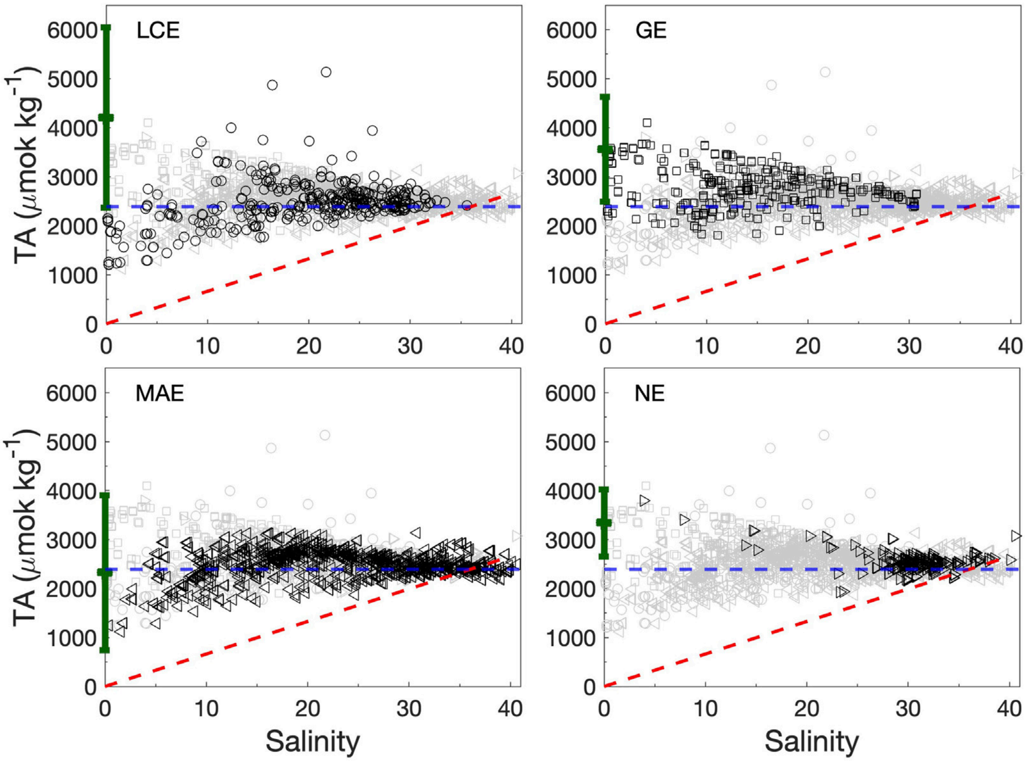

FIGURE 4
TA vs. salinity in the four studied estuaries during 2014–2018. Data from each estuary are shown in black symbols against the entire dataset (gray). The green error bar in each panel represents the river endmember value (±1 standard deviation) for the corresponding estuary (Yao et al., 2022). The horizontal dashed line (blue) represents the open ocean TA value (2,390 μmol kg^{-1} at salinity 36) (Hu et al., 2018). The red dashed line represents the precipitation-evaporation line based on the ocean endmember value.

especially in LCE and MAE. At high salinity conditions (>36), TA and DIC in MAE and NE both showed noticeable depletion (up to a few hundred μmol kg^{-1}) compared to what would be expected from the evaporation of the ocean endmember (Figures 4, 5).

[Ca^{2+}] exhibited excellent linear relationships with salinity in all estuaries. The intercepts of the linear regression between [Ca^{2+}] and salinity in these four estuaries were 0.67 (LCE), 0.99 (GE), 0.55 (MAE), and 1.16 mmol kg^{-1} (NE) (Figure 6).

Similar to TA and DIC, both pH and Ω_{arag} were also the highest in GE (average 8.11 and 4.08, respectively), and both LCE and GE had greater variations than MAE and NE as well (Figure 7). Large freshwater discharge events were usually accompanied by significant reductions in both pH and Ω_{arag} (Figure 7). However, reductions of pH/Ω_{arag} and salinity were not always synchronized. For example, during the end of the extremely dry period in mid-2015, both pH and Ω_{arag} reached their minimum values before the maximum freshening of these estuaries when the lowest salinity was reached (Figure 7).

The first two principal components PC1 and PC2 explained 34.2 and 26.3% of the data variability, respectively, and together explained 60.54% of the variance (Figure 8A). PC1 is correlated with salinity, pH, pCO$_2$, Ω_{Ar}, Ω_{Ca}, and PO_4^{3-}, which reveals the discharge-induced carbonate system change in nwGOM estuaries. Specifically, the inverse relationship between salinity/pH/Ω_{Ar} and pCO$_2$ suggests higher [CO_2*] brought by freshwater discharge. PC2 is indicative of metabolic processes, where DIC, TA, pH, Chl-*a*, and NO_x are negatively correlated to salinity. Given the significant contribution of salinity on both PC1 and PC2, it is clear that freshwater discharge played a crucial role in the estuarine carbonate system change.

According to individual PCAs, there is a clear spatial pattern of water chemistry in nwGOM estuaries. That is the decreasing data variance from GE to NE (Figure 8B), which coincides with their corresponding water discharge levels (Figure 2). For example, the high discharge level is reflected by the lowest salinity (Figure 3), causing the largest salinity range in GE. In contrast, the carbonate system in NE had the least variation because of its lowest river discharge and hence highest salinity level. Similarly, at the end of a 4-year drought, all variables in 2014 were found to have changed the least (Figure 8C), while the largest hydrological conditions shift occurred in 2015 when the first major storm came after the extreme drought. This change was even greater than in 2017 (the hurricane year), but the

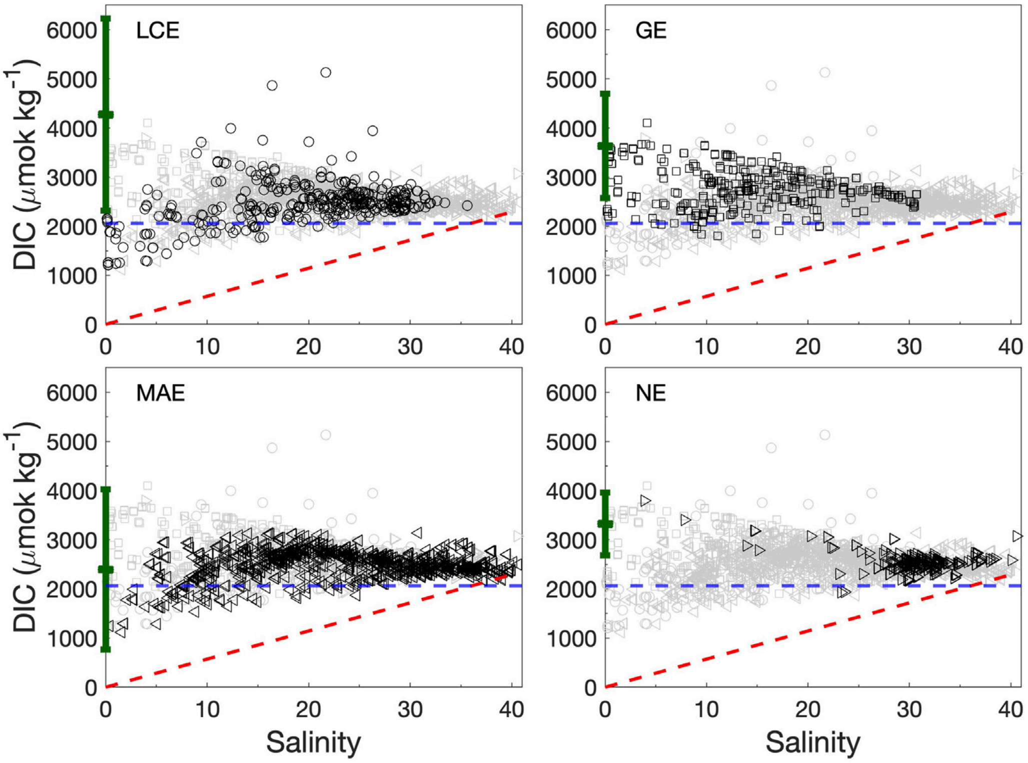

FIGURE 5
DIC vs. salinity in the four studied estuaries during 2014–2018. Data from each panel are shown in black symbols against the entire dataset (gray). The green error bar in each panel represents the river endmember value (±1 standard deviation) for the corresponding estuary (Yao et al., 2022). The horizontal dashed line (blue) represents the open ocean TA value (2060 μmol kg^{-1} at salinity 36) (Hu et al., 2018). The red dashed line represents the precipitation-evaporation line based on the ocean endmember value.

hydrological condition change mostly occurred in GE and LCE (Patrick et al., 2020; Walker et al., 2020). Nevertheless, the metabolic effect on water chemistry is similar between 2015 and 2018, as their scores are comparable in PC2. On the other hand, seasonal water variance is not distinct as their data ranges were mostly overlapping (Figure 8D), which can be attributed to the mild weather in this subtropical region, and temperature contributes less significantly to both PC1 and PC2 scores.

Time of emergence

All secondary bays, except that in the most freshwater-starved NE, had longer ToEs than their respective primary bays (Table 3). For the primary bays of MAE and LCE as well as the entire NE, the years that have elapsed (i.e., the period of datasets that were examined previously till 2015) have taken nearly 1/3–1/2 of the ToEs, indicating that the trends may merge from the variability during 2060–2080 if these trends were maintained the same. The secondary bay of LCE, the entire GE, and the secondary bays of MAE all had ToE on the order of more than 100 years.

Discussion

This study period (2014–2018) covered an initial extreme drought (before mid-2015), followed by several regional flooding events and subsequent flood relaxation periods that affected the entire nwGOM coast. In particular, LCE and GE were significantly affected by Hurricane Harvey due to hydrological condition changes (Yao et al., 2022). The large changes in salinity, from hypersaline to brief, yet nearly complete freshening of the estuarine waters, along with flood-induced productivity change as well as ephemeral hypoxia (%DO saturation decreases to less than 20%) in limited areas within some of the estuaries, provided an opportunity to examine the “baseline” and temporal changes in Ω_{arag}. Understanding Ω_{arag} dynamics under these contrasting conditions in the context of multidecadal Ω_{arag} trends found in these estuaries (Mccutcheon and Hu, 2022) is potentially important for managing and

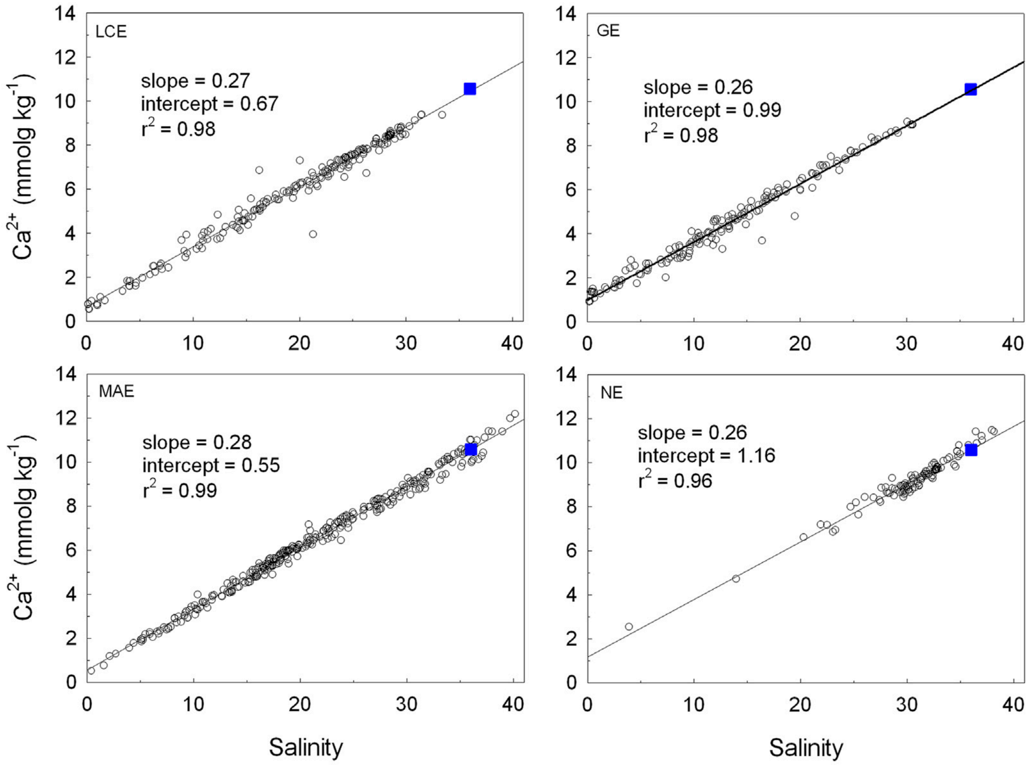

FIGURE 6
Linear regression between estuarine [Ca^{2+}] and salinity in the four studied nwGOM estuaries between 2014 and 2018. The blue square in each panel represents seawater condition (10.57 mmol kg^{-1} at salinity 36).

restoring economically valuable and ecologically important calcifying species such as the eastern oysters.

Non-mixing controlled estuarine total alkalinity and dissolved inorganic carbon

In mixing-dominated estuaries, TA and DIC often exhibit linear relationships with salinity (Wong, 1979; Cooley et al., 2007; Jiang et al., 2008). Given such linear relationships, Ω_{arag} dynamics and its long-term changes can be calculated using endmember concentrations along with known biogeochemical reactions (Moore-Maley et al., 2018; Simpson et al., 2022). However, TA and DIC in nwGOM estuaries are influenced by more factors than simple water column processes. These factors include evaporation, carbonate precipitation and dissolution, and perhaps more importantly, sedimentary processes, in addition to mixing, primary production, and respiration which are commonly encountered in typical water column studies. For example, evaporation-induced hypersalinity is often associated with TA depletion compared with the ocean endmember (Hu et al., 2015). Observations in both MAE and NE also revealed similar patterns at high salinity (Figure 4), indicating TA loss through reactions during prolonged water residence (both carbonate precipitation and sediment redox reactions, Hu, unpublished data). Because pCO_2 varies within a narrower range (~100 μatm) during high salinity periods (Yao and Hu, 2017), this relative depletion of TA also corresponded with DIC deficiency relative to ocean water as well (Figure 5). In the mid-salinity range, however, apparent enrichment of both TA and DIC also appeared (Figures 4, 5), which may be a combination of both evaporation and benthic contribution (Hu et al., 2015). Varying river endmember values at different hydrological conditions is another important factor that controls TA and DIC distribution along the salinity gradient (Cifuentes et al., 1990).

Nevertheless, the non-conservative behavior of TA and DIC can be attributed to the shallow water depths and long water residence time in these estuaries, conditions suitable for water-sediment interactions, especially during periods of low river discharge (Figure 2). Moreover, the poorly constrained groundwater input adds additional complexity to unraveling estuarine TA and DIC dynamics in these estuaries (Murgulet et al., 2018). Non-conservative TA and DIC behaviors are also observed in other small river estuaries (Oliveira et al., 2017; Stokowski et al., 2020).

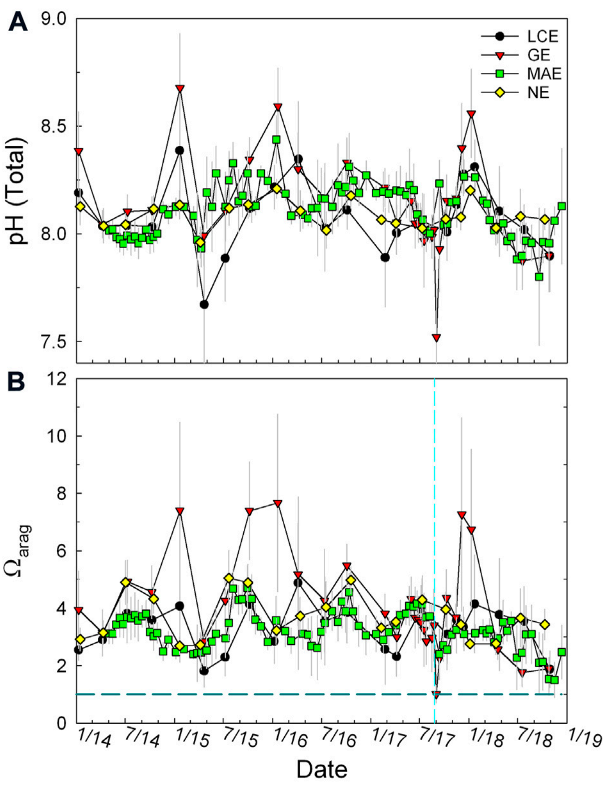

FIGURE 7
pH on a total scale **(A)** and Ω_{arag} **(B)** in the four studied estuaries during 2014–2018. Note in calculating Ω_{arag}, samples with S < 5 were not included as the solubility constant of aragonite (Mucci, 1983) is valid for salinity 5–44. These values are averages of all data from these stations as stratification occurred infrequently.

Controls on estuarine Ω_{arag}

According to the definition, Ω_{arag} is, to a large extent, controlled by carbonate system speciation in the oceanic environment. In river estuaries and areas influenced by melting ice where freshwater is prevalent (Reisdorph and Mathis, 2014; Beckwith et al., 2019), $[Ca^{2+}]$ vs. salinity often have a linear relationship. The calculated intercepts from the $[Ca^{2+}]$ vs. salinity regressions (Figure 6) corresponded to different drainage basin characteristics (for example, karst feature for the Guadalupe and Nueces river basins; increasing evaporation to the south) and also temporal variations that reflect either direct river input (high freshwater $[Ca^{2+}]$) or dilution by localized precipitation (low freshwater $[Ca^{2+}]$, Hu et al., 2020). Nevertheless, $[Ca^{2+}]$ decreases by a factor of 4 (GE and NE) to 5 (LCE and MAE) when salinity changes from 35 to 5. At the same time, the solubility constant of aragonite (K_{sp}) at salinity 35 is ~7.7 times the value when salinity is 5 when we chose the average temperature of 23.6°C across all sampling times and locations. Hence, the dilution effect (due to $[Ca^{2+}]$ decrease with salinity) can be canceled out by the decrease in solubility from the perspective of Ω_{arag} change. As a result, the large Ω_{arag} variations in these estuaries can be primarily attributed to changing $[CO_3^{2-}]$. In fact, the TA/DIC ratio was the single most important factor that explained $[CO_3^{2-}]$ variations across the salinity gradient (≥5 as the carbonic acid dissociation constants are valid for salinity 5–45, Waters et al., 2014) in all four estuaries (Figure 9), explaining ~80% of $[CO_3^{2-}]$ variations in LCE and GE and ~70% in MAE and NE. In addition, low salinity waters usually had a low TA/DIC ratio, especially in LCE, GE, and MAE (Figure 9), indicating high freshwater pCO_2 (Jiang et al., 2008). However, the highest $[CO_3^{2-}]$ appeared in the mid-salinity range (LCE and GE), corresponding to the highest TA/DIC ratios, indicating high primary productivity (see below). In comparison, temperature showed no relationship with $[CO_3^{2-}]$ in LCE but explained 16, 6, and 31% of $[CO_3^{2-}]$ changes in GE, MAE, and NE, respectively (data not shown). The slopes for both MAE and NE that had less freshwater input were positive, consistent with the open ocean (Jiang et al., 2015). However, the slope for GE was negative. Clearly, hydrological condition changes and biogeochemical processes outweighed the temperature effect on $[CO_3^{2-}]$ in these estuaries.

Along the salinity gradients, most estuarine waters with salinity greater than 30 had relatively confined Ω_{arag} values (2–5) compared to the entire dataset, although the values in both polyhaline (salinity 16–30) and mesohaline (5–16) waters had the largest variations (Figure 10A). High Ω_{arag} values often coincided with high pH values as the extreme pH value reached near 9 (Figure 10A). In many estuarine and coastal waters, high pH values are often observed along with high primary productivity sustained by abundant nutrients (Guo et al., 2012; Nixon et al., 2015). High pH levels are also observed in seagrass bed-influenced estuarine waters in Corpus Christi Bay (Mccutcheon et al., 2019) and Chesapeake Bay (Su et al., 2020), where the coupling between CO_2 consumption and pH increase is present. Both LCE and GE exhibited greater variance in pH (Table 2) and Ω_{arag} (Table 2; Figure 10A) compared with MAE and NE. While primary production driven by river nutrient input was certainly responsible for some of the high Ω_{arag} values because they did correspond to greater concentrations of Chl-a, although high Chl-a was not an *a priori* factor for high Ω_{arag} waters, especially in low salinity waters (Figure 10B, also see Figure 8A for the PCA results).

Another important reason for the high Ω_{arag} values in mid-salinity waters (<30) was high river TA levels, especially in the Guadalupe River that empties into GE. In fact, both the high intercept of $[Ca^{2+}]$ vs. salinity regression (0.99 mmol kg^{-1}, Figure 6) and the overall highest estuarine TA levels in GE yet the lowest average salinity among all studied estuaries (Table 2) point to the mineral-rich freshwater source, that is, the Guadalupe River, which is joined by the San Antonio River at its upper stream (*cf.* Edward Aquifer, Woodruff and Abbott, 1979). It is to be noted that even though the intercept of the

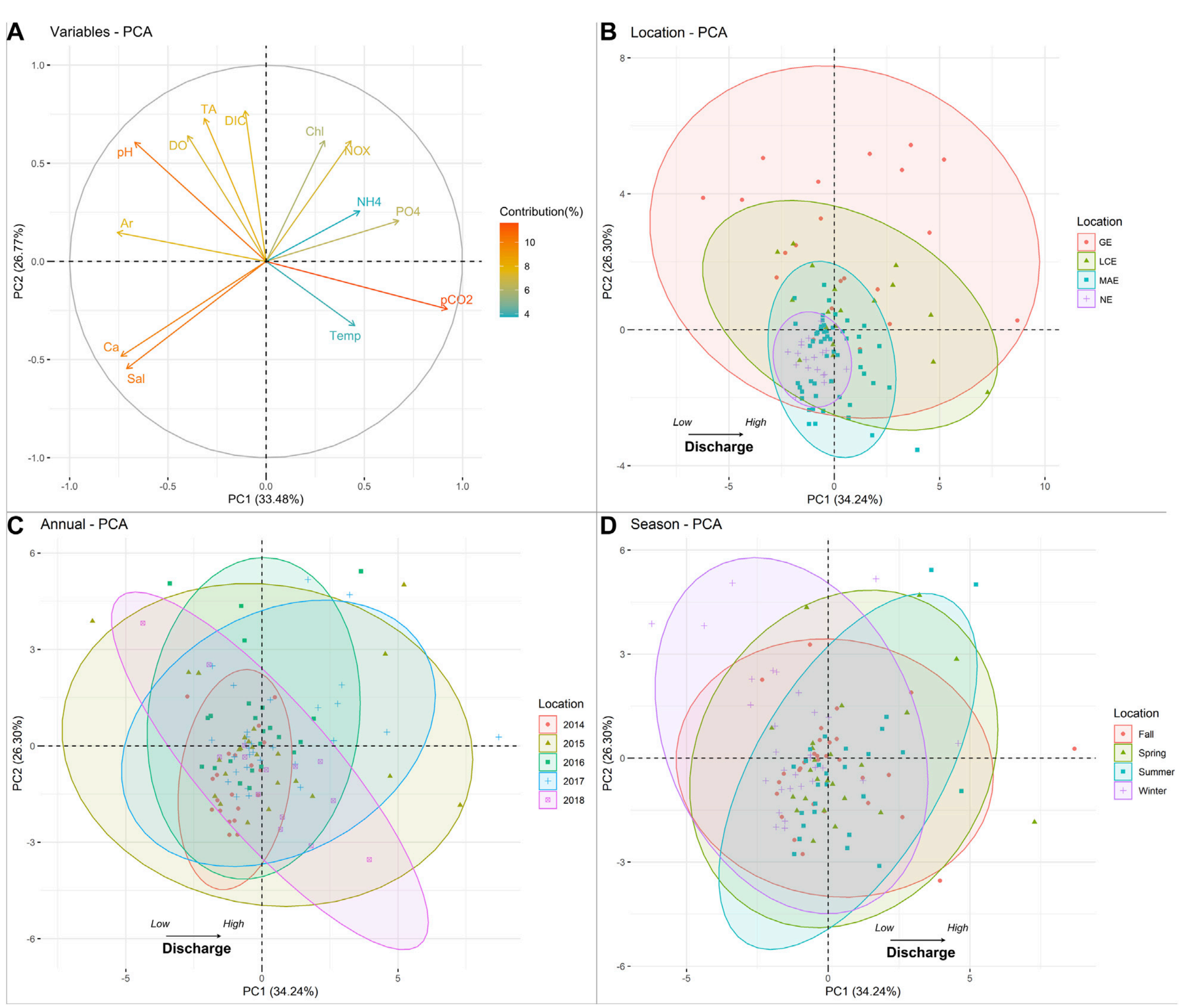

FIGURE 8
PCA analysis of water chemistry in nwGOM estuaries. **(A)** Variables loads for PC1 and PC2; **(B)** principal component of monthly variance between estuaries; **(C)** principal component of monthly variance between sampling years; **(D)** principal component of monthly variance between seasons. Abbreviations: water temperature (Temp), salinity (Sal), chlorophyll a (Chl), dissolved oxygen percentage (pDO), Ar (Ω_{Arag}), Ca ($[Ca^{2+}]$), ammonium (NH_4), nitrate and nitrite (NO_X), and orthophosphate (PO_4).

$[Ca^{2+}]$ vs. salinity regression in NE was slightly higher (1.16 mmol kg^{-1}, Figure 6), the high average salinity in NE suggests that freshwater discharge itself did not have strong control on estuarine Ω_{arag} (Figures 4–6). The largest freshwater influence in GE is expected given its highest freshwater discharge to volume ratio among all studied estuaries (Evans et al., 2012; Montagna et al., 2018).

A decrease in DO concentration is linked with respiration-produced DIC, which is known to cause reductions in estuarine water pH and carbonate saturation (Feely et al., 2010; Wallace et al., 2014; Ianson et al., 2016). Except in estuaries with persistent hypoxia (Mucci et al., 2011), the DO influence is usually relatively short-lived, that is, from diurnal to seasonal. Low oxygen conditions in the studied estuaries have been linked to two hydrological extremes that both contributed to water column stratification. In NE, where freshwater discharge is the lowest, hypoxia has been long observed in the southeast corner of Corpus Christi Bay (Ritter and Montagna, 1999; Applebaum et al., 2005). Mccutcheon et al. (2019) observed hypoxic conditions associated with the density flow of hypersaline water to southeastern Corpus Christi Bay from the highly evaporative Laguna Madre, inhabited by abundant seagrasses. On the other hand, due to the abrupt increase in freshwater discharge after Hurricane Harvey, hypoxia was also observed to last for nearly a week in the upper GE (Hu et al., 2020; Walker et al., 2020). No hypoxia was observed in either MAE or LCE

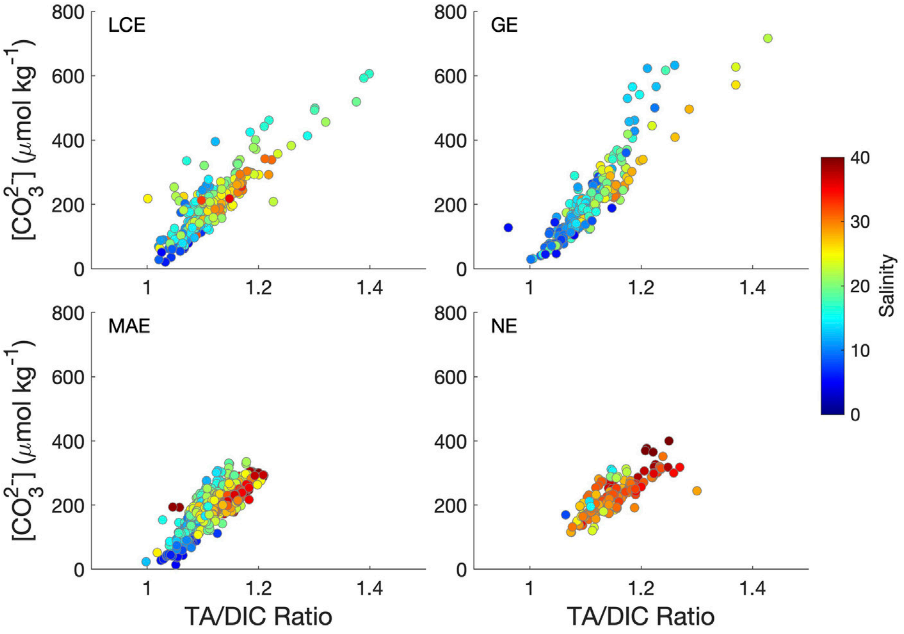

FIGURE 9
Carbonate ion concentration vs. TA/DIC ratio in the four studied nwGOM estuaries during 2014–2018. Samples with S < 5 were not included as the carbonate dissociation constants are valid for salinity 5–45 (Waters et al., 2014). The r^2 values for these regressions are 0.76 (LCE), 0.82 (GE), 0.68 (MAE), and 0.69 (NE).

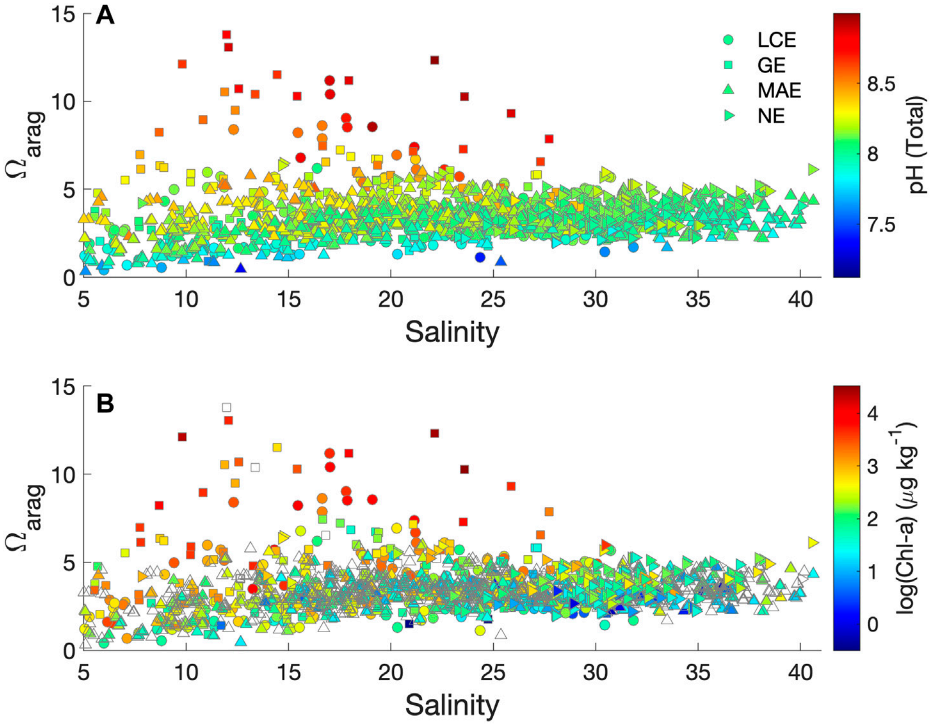

FIGURE 10
Ω_{arag} vs. salinity and pH or chlorophyll-a in the four studied nwGOM estuaries. Color infill represents pH. **(A)** and Chlorophyll-a concentration in log scale **(B)**. Open symbols represent no chlorophyll data.

TABLE 3 Time of emergence for Ω_{arag} for primary (P) and secondary (S) bays of the four studied nwGOM estuaries.

Estuary	Bay	ToE (years)	Years elapsed
LCE	S	135	31
	P	97	35
GE	S	282	38
	P	119	38
MAE	S	251	39
	P	90	39
NE	S	71	34
	P	76	39

Years elapsed represent the average year of data (ending in 2015) examined in Mccutcheon and Hu (2022) for the long-term trends. Note in the prior study, GE was not divided into primary and secondary bays, although trends in this study were calculated for upper (secondary) and lower (primary) GE.

although brief stratification did occur during the episodic high freshwater discharge period (Bruesewitz et al., 2013). Overall, DO saturation had a significant correlation ($p < 0.001$) with Ω_{arag}, but the relationship was dominated by the few low DO data points (not shown). However, low and high DO conditions occurred infrequently because the water column was not far from saturation (%DO = 101.5 ± 16.4), and only a small fraction of DO data (~2% of a total of 1870 measurements) had less than 75% saturation.

State of estuarine Ω_{arag} in the context of long-term change

Using historical data collected by TCEQ, Hu et al. (2015) and Mccutcheon and Hu (2022) explored multidecadal changes in estuarine carbonate chemistry in the nwGOM estuaries. These studies found decreases in pH, alkalinity, and Ω_{arag} in most estuaries. Most of the long-term stations (with >20 years of record) in these estuaries have Ω_{arag} trends of ~ -0.01 – -0.07 years^{-1} (Mccutcheon and Hu, 2022) amid the general warming trend of ~0.04°C yr^{-1} (Montagna et al., 2011). The latter would have led to a slight increase in Ω_{arag} (10^{-3} yr^{-1}) but far from countering the calculated decreasing trends. Nevertheless, the ToE calculations reveal interesting patterns on the timescale of "state" change for Ω_{arag} in these estuaries. Longer ToE can be primarily attributed to larger standard deviations (Table 3), or variabilities, in areas that have greater freshwater discharge as the trends are not significantly different between each pair of primary and secondary bays (Mccutcheon and Hu, 2022). Clearly, this ToE calculation did not consider other factors that may control Ω_{arag} evolution in these estuaries, including projected increasing drought in the southwestern United States that may decrease freshwater discharge in the long run (Cook et al., 2015), sea level rise, and coastal erosion that may create a more open environment that could facilitate more efficient estuarine-coastal water exchange. Either way, these changes could reduce the variability in estuarine biogeochemical parameters, including Ω_{arag}, and make the actual ToE shorter than estimated here. In fact, both the coastal ocean and the open ocean have much shorter ToE, ranging from 1 to 4 decades (Sutton et al., 2019; Turk et al., 2019). Hence, if the projected drought were held true, the distinctions between ToE values estimated for these estuarine and coastal/oceanic waters may diminish.

Nevertheless, despite the potential overestimation of ToE based on historical trends, these ToE values for Ω_{arag} may be used as a semi-quantitative indicator for planning restoration efforts of calcifying species (oysters). Based on the current Ω_{arag} level and the ToE (Tables 2, 3), it appears that restoration activities may emphasize estuaries with greater freshwater input (in particular GE), and for estuaries with less freshwater, areas closer to the freshwater source rather than downstream may be considered. This consideration actually coincides with previous studies that suggested a positive correlation between freshwater input and secondary productivity (Kim and Montagna, 2012), including oysters (Buzan et al., 2009), even though these studies examined the issue from the perspective of nutrient-driven production.

Conclusion

Using data collected in 2014–2018 that covered a period of varying hydrological conditions, from drought to flood, we examined Ω_{arag} in four estuaries in a climate transition zone. Our results indicate that river discharge exerted dominant control on Ω_{arag} variations in all estuaries, through both nutrient export and subsequent surface production (high Ω_{arag}) and stratification-caused hypoxia (low Ω_{arag}), all of which were responsible for carbonate equilibrium changes. Larger Ω_{arag} variations occurred in estuaries with greater freshwater discharge and in bays within an estuary that had greater freshwater influence. Even though episodic flood events can depress Ω_{arag} to extreme levels (<1), the frequency of such occurrences was relatively low in these shallow, well-mixed environments. Using Ω_{arag} temporal trends in a previous study and the current Ω_{arag} levels, time of emergence calculations indicate that the estuaries with less freshwater input (primary bay of MAE and the entire NE) may start seeing a change of Ω_{arag} that exceeds the current background variations within the next few decades, while the estuaries with more abundant freshwater input at present (secondary bay of MAE, LCE, and GE) have substantially longer ToE (i.e., century scale). Future changes in the hydrological cycle, climate change, and related changes in ocean conditions may all alter the ToE.

We suggest that ToE may be a useful tool for planning coastal resource restoration activities.

Data availability statement

Data used in this study can be downloaded from Biological and Chemical Oceanography Data Management Office (BCO-DMO) (DOI:10.26008/1912/bco-dmo.835227.1 and DOI: 10.1575/1912/bco-dmo.784673.1).

Author contributions

XH conceived the research idea, carried out the data analysis, and led the manuscript writing. HY, MM, LD, and CS participated in the fieldwork, analyzed the carbonate system parameters, and reported data. MW and PM were both in charge of analyzing nutrient and chlorophyll-a samples as well as data reporting. HY conducted the PA analysis. XH, MW, and PM all secured funding at various stages of the sampling period. All authors contributed to the writing of this manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Changes in the morphology of widgeon grass (*Ruppia maritima*) with the onset of reproduction and impacts on fish assemblages at the Chandeleur Islands, LA

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Seagrass beds are important submerged coastal habitats that support nearshore communities. *Ruppia maritima* (widgeon grass) is a widespread seagrass species that undergoes dramatic changes in morphology at the onset of reproduction. The goal of this study was to compare fish assemblages associated with reproductive and non-reproductive *R. maritima*, recognizing the morphological change undergone by the plant when flowering. During the peak reproductive season in August and September 2021, *R. maritima* meadows at the northern extent of the Chandeleur Islands, Louisiana were sampled to describe the spatial distribution and morphology of reproductive plants and investigate habitat use by fish assemblages. We assessed spatial trends in *R. maritima* presence and occurrence of reproductive plants and evaluated differences in shoot morphology. We calculated total fish density, Shannon diversity, and species richness to describe fish assemblages in reproductive and non-reproductive meadows. Additionally, general additive models were used to predict drivers of fish assemblage metrics. Results indicate that *R. maritima* was distributed along the entire length of the sampled area, but reproductive plants were only located in the central, protected portion of the island. Reproductive plants were more morphologically complex with longer shoots, greater surface area, and more leaves, but this did not impact fish assemblages. Rather, fish abundance was related to *R. maritima* biomass. This study provides information on patterns and drivers of habitat use by fish in *R. maritima*-dominated ecosystems that can be used to inform management and restoration.

KEYWORDS

seagrass, Chandeleur Islands, Ruppia maritima, seagrass reproduction, fish assemblage

Introduction

Seagrasses are submerged angiosperms that inhabit marine environments, and include over 60 species across 13 genera (Hartog and Kuo, 2006). The majority of seagrass genera occur at temperate and tropical latitudes in the northern and southern hemispheres (Hartog and Kuo, 2006), and species' distributions are determined by taxon-specific light, nutrient, salinity, temperature, pH, and substrate requirements (Hemminga and Duarte, 2000; Hartog and Kuo, 2006; McKenzie et al., 2016). Seagrasses may exhibit changes in geographical range or localized abundance as a result of changes in underlying abiotic parameter baselines (Cho et al., 2009; Fourqurean et al., 2003; Johnson et al., 2003).

Seagrass expansion can occur through asexual or sexual processes. Clonal propagation (asexual reproduction) through fragmentation or rhizome extension is considered the primary mechanism for meadow formation and maintenance (Olesen et al., 2004); however, seagrasses also have the capacity for sexual reproduction, with species being either monecious (i.e., both male and female organs on a single plant; e.g., *Ruppia maritima*) or dioecious (i.e., separate male and female plants; e.g., *Thalassia testudinum*) (Hartog and Kuo, 2006). Whereas some species produce seeds that germinate on the shoot (e.g., *T. testudinum*), the majority of seagrasses (42 species) produce seeds that can remain dormant for extended periods of time and create seed reserves in the sediment that provide a buffer against disturbance (Darnell et al., 2021); in highly disturbed environments, dormant seeds can be essential to the recolonization process (Olesen et al., 2004; Orth et al., 2006).

Seagrass beds support faunal communities that are highly diverse and productive (Castillo-Rivera et al., 2002) and include valuable recreationally and commercially fished species (Bertelli and Unsworth, 2014; Nordlund et al., 2018). Seagrasses provide shelter from predators for small cryptic species and serve as productive feeding grounds (Heck et al., 2003; Vaslet et al., 2012; Nordlund et al., 2018). Animals often rely on seagrass habitats for particular life stages, most importantly as nursery habitat for juveniles. McDevitt-Irwin et al. (2016) conducted a meta-analysis of 51 studies and found that seagrasses tended to support greater abundances of juveniles with higher survival rates compared to marsh, reef, mangrove, and other benthic habitats. Juveniles of many species utilize the abundant food and resources within seagrasses to achieve faster growth rates prior to moving to more competitive adult habitats (McDevitt-Irwin et al., 2016).

Many drivers of fish assemblages have been identified and include local environmental conditions as well as the species and morphology of the seagrasses present, and at a broader scale, climate patterns. Distinct fish assemblages among seagrass species can be linked to differences in plant morphology (Rotherham and West, 2002; Nakaoka, 2005; Kiggins et al., 2019). Morphological characteristics (e.g., leaf number and length and shoot density) create microhabitats that may be favored by certain species because of the food, shelter, and/or other ecological benefits provided. Hyndes et al. (1996), for example, reported that whiting species (Sillaginidae) in southwestern Australia showed preference for *Zostera* spp. Because the less dense canopy allows for easier movement when compared to the other local seagrasses *Posidonia australis* and *P. sinuosa*. More recently, Belgrad et al. (2021) reported that abundances of several nekton species were influenced by both shoot density and canopy height of turtlegrass (*T. testudinum*) (Belgrad et al., 2021).

Ruppia maritima is a widely distributed seagrass with a broad tolerance to environmental conditions that allows it to thrive in waters ranging from fresh to hypersaline in both the northern and southern hemispheres from tropical to temperate latitudes (Orth and Moore, 1988; Reyes and Merino, 1991). *Ruppia maritima* is an early successional species that is often the first species to colonize an area following a disturbance event (Cho et al., 2009). This is due to high rates of shoot turnover and the species' reliance on sexual reproduction that creates a persistent seed bank buried in the sediment (Kilminster et al., 2015). In some areas, *R. maritima* is perennial, whereas in other areas, plants are predominantly annual (Malea et al., 2004). *Ruppia maritima* is monecious, having both male and female reproductive structures on a single plant, and when reproductive, the morphology of *R. maritima* shoots change dramatically; whereas non-reproductive *R. maritima* shoots have several narrow (1–2 mm) leaves ranging from 5 to 20 cm in length (Kantrud, 1997; Hartog and Kuo, 2006), reproductive shoots branch extensively and can reach a length of 2.5 m (Hartog and Kuo, 2006) (Figure 1).

Ruppia maritima plants at temperate latitudes undergo one annual reproductive cycle, typically in the summer months (Bigley and Harrison, 1986), while those found in more tropical regions usually complete two reproductive cycles (Pulich, 1985; Orth & Moore, 1988). Cho and Poirrier (2005) reported that *R. maritima* in Lake Pontchartrain, LA, flowers in spring (March to May) and again in late summer/early fall (August to October). The change in plant growth form with the onset of reproduction increases structural complexity and has the potential to impact its use as habitat, as the reproductive shoots may indirectly provide increased opportunities for food and shelter through the creation of additional microhabitats. *Ruppia maritima* is a known habitat for fish, and Kanouse et al. (2006) reported fish densities ranging from 10 to 102 individuals per meter square in *R. maritima*-dominated brackish ponds in Louisiana. It is critical to understand patterns in the distribution of reproductive plants as a predictor of the habitat value of *R. maritima*, especially in areas experiencing changes in seagrass distribution and shifts in species composition.

The Chandeleur Islands, LA, United States, a chain of barrier islands in the northern Gulf of Mexico, represent an area of shifting seagrass species distribution and composition. The islands are suffering chronic land loss due to a lack of

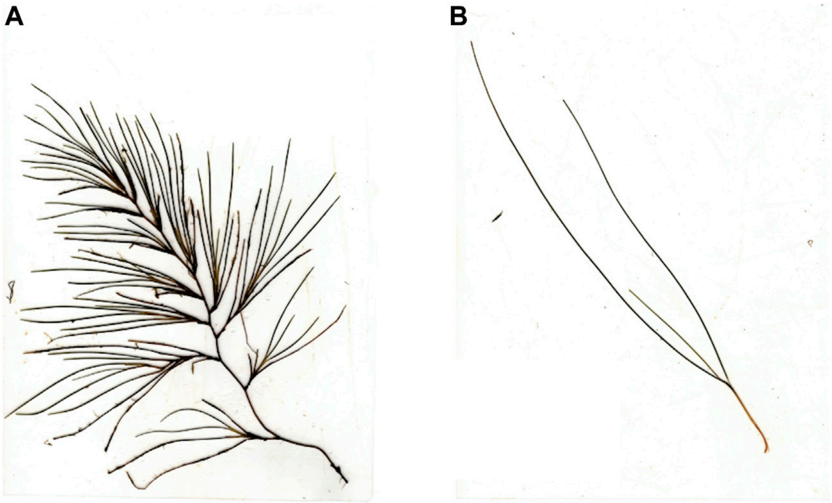

FIGURE 1
Comparison of non-reproductive and reproductive *R. maritima* shoots **(A)** reproductive *R. maritima* and **(B)** non-reproductive *R. maritima*.

sediment input, hurricane damage, and rising sea levels (Moore et al., 2014). Additionally, the *Deepwater Horizon* oil spill in 2010 impacted sediment and vegetation along the islands with both showing increased levels of total polycyclic aromatic hydrocarbons, the long-term impacts of which are unknown (Kenworthy et al., 2017). The back barrier shelf to the west of the Chandeleur Islands is protected from wave action which allows for the proliferation of extensive seagrass meadows (Ellinwood, 2008). There are five species of seagrass that grow along the leeward protected side of the islands: *R. maritima*, shoal grass (*Halodule wrightii*), turtlegrass, manatee grass (*Syringodium filiforme*), and star grass (*Halophila engelmannii*). *Ruppia maritima*, shoal grass, and turtlegrass are the three most abundant species along the islands, with manatee grass and star grass much less abundant (Kenworthy et al., 2017). Seagrass meadows at the Chandeleur Islands represent the only mixed meadows of these five species along 1,000 km of coastline from Perdido Key, Florida to the Texas Coastal Bend, United States (Darnell et al., 2017). Seagrass cover has decreased along the island chain from 15,758 acres in 1969 to only 2,614 acres in 2011 (Pham et al., 2014; Handley and Lockwood, 2020). The observed decline is linked to a reduction of shallow protected areas through the combined processes of changes in island geomorphology and storm-related land loss (Darnell et al., 2017). For example, in the aftermath of Hurricane Katrina, the Chandeleur Islands lost approximately 70% of their land mass and 20% of the seagrass cover (Bethel and Martinez, 2008). In the wake of these disturbances, there has also been an observed shift in seagrass species composition, with increase in cover of *R. maritima* and decrease in cover of other species such as turtlegrass (Kenworthy et al., 2017). Large seed banks and rapid growth are characteristics that allow *R. maritima* to thrive after large disturbances and outcompete other seagrass species (Poirrier and Handley, 2007; Cho et al., 2009). As disturbances increase in frequency, as projected with impacts of climate change (Collins et al., 2019), it is likely that *R. maritima* will continue to increase in cover at the Chandeleur Islands, with unknown impacts to the seagrass-associated communities.

The goal of this study was to understand the distribution of *R. maritima*, describe the morphology of reproductive plants at the Chandeleur Islands, and quantify the impacts of reproductive and non-reproductive *R. maritima* on habitat association by fishes. Specific objectives are as follows:

Objective 1: Evaluate reproductive *R. maritima* plant homogeneity across the Chandeleur Islands.

Objective 2: Compare fish assemblages between reproductive and non-reproductive *R. maritima.*

Methods

Study area

The Chandeluer Island chain stretches 72 km, with the northern end located 35 km south of Biloxi, MS and the southern end lying in an arch 25 km northeast of Venice, LA (Poirrier and Handley, 2007). Much of the land mass of the Chandeleur Islands is in the northern islands, with North Chandeleur Island being the northernmost and largest island (Figure 2).

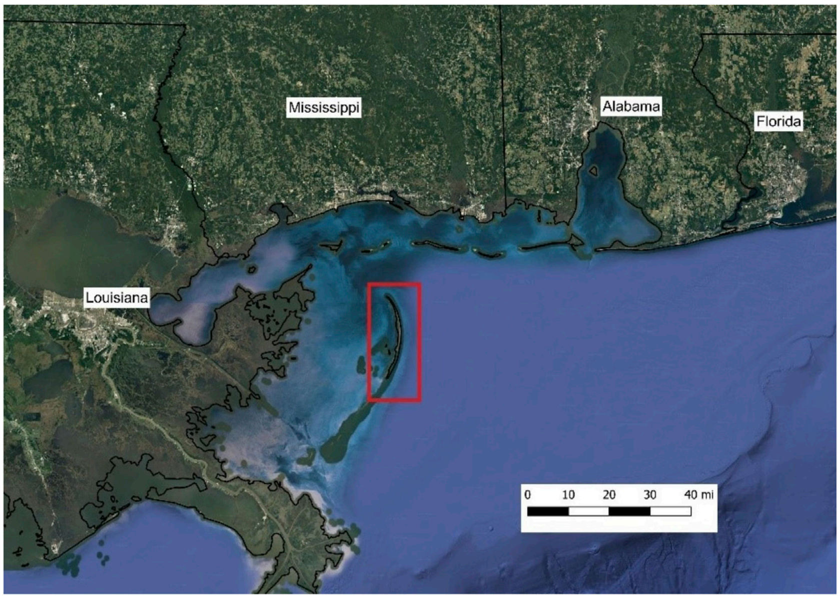

FIGURE 2
Map of the northern Gulf of Mexico with the North Chandeleur Island, LA study site bounded by the red box (*Google Maps*, 2021).

Objective 1: Evaluate reproductive R. maritima plant homogeneity across the Chandeleur Islands

To describe the distribution and morphology of reproductive and non-reproductive *R. maritima* at the Chandeleur Islands, LA, *R. maritima* was surveyed across its extent at the islands during late summer (August/September) 2021. Prior to sampling, sites with historic presence of reproductive and non-reproductive *R. maritima* were identified using seagrass monitoring data collected in previous years (K. Darnell, unpublished data). A total of 27 sites were chosen where *R. maritima* previously occurred, while ensuring sites were distributed across the entire area of seagrass occurrence at the Chandeleur Islands.

At each sampling site, a YSI handheld meter (Pro 2030; YSI Inc.) was used to measure bottom salinity, dissolved oxygen, and temperature; water depth was measured using a pole marked in 5 cm increments; and light irradiance at the surface and at depth was measured using two four-π (spherical quantum) sensors and a data logger (LI-1500, LI-Cor.). Light attenuation coefficients (k_d) were calculated using the following equation:

$$k_d = -[\ln(I_z/I_0)]/0.58$$

where I_z is irradiance at depth, I_0 is irradiance at the surface, and 0.58 represents the vertical distance (m) between the sensors.

At each site, percent cover of each seagrass species and bare sediment were quantified in three quadrats (50-cm × 50-cm). Additionally, the percent cover of reproductive and non-reproductive *R. maritima* was quantified. One seagrass core (9.5-cm diameter × 15-cm depth) was collected within *R. maritima* in each quadrat. Quadrats were only sampled at sites with seagrass present and cores were only collected from quadrats with seagrass cover. Cores were placed in plastic bags and transported to the Gulf Coast Research Lab (GCRL), United States where they were frozen until processing. Five reproductive and five non-reproductive plants were retained from the site for image analysis, with care taken to collect all aboveground (leaf and flower) and belowground (root and rhizome) tissue for each plant.

Seagrass cores were rinsed over a 500-μm sieve and plants were separated by species. The number of shoots of each species was counted, but only *R. maritima* plants were retained. The number of shoots, the reproductive status of each shoot, and the number of branching nodes per plant were recorded. Epiphytes were removed from leaves by gently scraping both sides of each leaf with a razor blade; epiphytes were then placed in a drying oven for 48 h at 60°C before being weighed to obtain a dry weight. Shoots were classified as reproductive or non-reproductive and separated. Reproductive shoots were defined as those with branching leaves and/or the presence of inflorescences. The aboveground and belowground tissues were separated, and the aboveground and belowground tissues for each shoot type (reproductive or non-reproductive) were grouped and placed in a drying oven for 48 h at 60°C, after which they were weighed to obtain a dry weight. Dried biomass was used to calculate a core-level root to shoot ratio (RSR), which is a useful proxy for plant condition and productivity, with higher values indicating greater biomass and energy in belowground structures (Hitchcock et al., 2017). Measurements from the 9-cm diameter core were then extrapolated to obtain bed characteristics per square meter.

Digital images (600 dpi) were taken of the handpicked plants using a flatbed scanner (Epson WF-3640). Prior to scanning, epiphytes were removed from each shoot and the aboveground and belowground tissues were separated. The aboveground biomass was scanned to produce a JPEG format image. Total leaf area was calculated using ImageJ (Version 1.53) with a threshold processing procedure (Easlon and Bloom, 2014). Additionally, total shoot length and the number of branching nodes were calculated from the image. Total leaf surface area, number of branching nodes, and shoot length were used to quantify overall plant complexity. Each individual shoot's epiphyte, and aboveground and belowground biomass were dried separately in a drying oven for at least 48 h at 60°C, after which they were weighed to obtain dry weights. This information was used to calculate an individual plant RSR.

Sampling sites were mapped using QGIS (version 3.18.1) to display the spatial distribution of *R. maritima* along the Chandeleur Islands. Sites with reproductive *R. maritima* were also mapped to show the spatial distribution of reproductive plants. Each map was then evaluated for qualitative trends such as spatial clustering. The mean and standard error were calculated for abiotic parameters across all sites to characterize the distribution of site conditions across the study area.

Several metrics were selected to compare plant morphology between reproductive and non-reproductive shoots and included surface area, shoot length, leaf number, and RSR. Metrics were compared using unpaired two-sample t-tests ($\alpha = 0.05$). Prior to testing each metric, the data were evaluated for violations of the parametric testing assumptions. If assumptions were violated the sample was bootstrapped with resampling and the test statistic calculated. This was repeated for 10,000 permutations and the reported test statistic was represented by the mean of bootstrapped test statistic distribution (R version 4.1.3).

Objective 2: Compare fish assemblages between reproductive and non-reproductive *R. maritima*

Fish were sampled at the Chandeleur Islands during a 6-day period from September 6–11, 2021, at the height of the *R. maritima* reproductive season in this area. This sampling time frame captures the greatest contrast in plant complexity between reproductive and non-reproductive plants. Fish were sampled using a throw trap at 22 sites along the island/Throw trapping provides a targeted approach to sampling patchy habitat that is not possible with other gears such as a benthic sled or trawl which cover larger areas (Jordan et al., 1997; Camp et al., 2011). The throw trap consisted of a 1-m × 1-m × 0.6-m high aluminum frame. Nylon mesh (3.175 mm) was sewn onto the frame sides as well as extended above the frame and attached to floats to extend the trap height to 1.5 m. Sampling was conducted in *R. maritima* patches across a range of reproductive plant coverage. Patches with desired characteristics (e.g., target cover of reproductive and non-reproductive plants) were identified from those sampled in the survey for plant distribution and morphology and marked with PVC poles prior to sampling. Sampling depth was limited to <1.5-m due to the height of the throw trap, and sites were separated by at least 15-m to minimize effects of disturbance. Thirty sites were selected to span a range of reproductive cover values.

After trap deployment, all vegetation within the throw trap was removed and retained for processing. A bar seine was used to remove organisms from within the trap. The bar seine (90-cm wide × 50-cm high) consisted of a PVC frame with handles and 3.175-mm mesh stretched between the handles. The pattern for seining organisms was modeled from Shakeri et al. (2020) and involved three sweeps from each side of the trap, with sweeps continuing until three consecutive sweeps yielded no additional fish. Collected fish were placed into plastic bags and stored on ice until returned to GCRL, where they were frozen until processing.

Seagrass collected from the throw trap was returned to GCRL and sorted for nekton, then the plant biomass was spun for 90 s using an industrial-sized salad spinner to remove excess water and sorted and weighed by species to obtain a species-specific wet weight (g). Individual seagrass species wet weights were then combined to get a total wet weight. Finally, a random subset of reproductive and non-reproductive *R. maritima* plants (at least 3 reproductive and 3 non-reproductive plants per throw trap sample) were imaged and dried as described above to quantify total leaf area and biomass.

Fish were identified to species using taxonomic keys and the number of individuals of each species was counted. All individuals were measured for standard length (SL, mm) (except for Dwarf Seahorses, which were only measured for total length), total length (TL, mm), and weighed. Fish assemblages were compared across sites using density, Shannon diversity, and species richness. Density was calculated as the number of individuals per square meter. Shannon diversity (H') was calculated using standard methods (Freeman et al., 1984), and species richness was calculated as the number of unique species.

General Additive Models (GAM) were formulated to evaluate drivers of fish assemblage metrics (Shannon diversity, species richness, and density) with three potential predictor variables: water depth (cm), biomass of vegetation recovered from throw trap (g), and the proportion of reproductive *R. maritima* in the throw trap. Prior to running the GAMs, the predictor variables were plotted against each other to identify any significant relationships. Biomass of vegetation recovered from throw trap and the proportion of reproductive *R. maritima* in the throw trap were found to co-vary with a positive relationship. AIC scores were compared between models with biomass of vegetation recovered

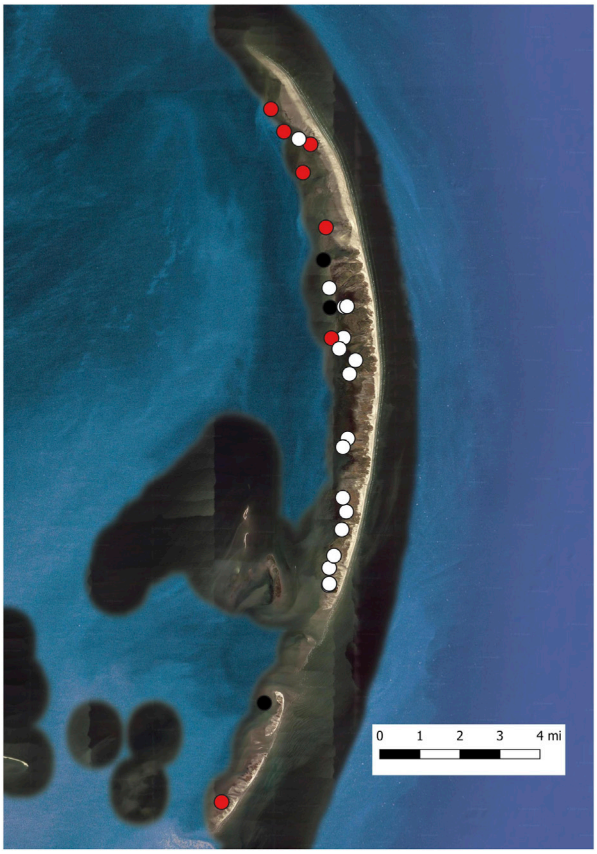

FIGURE 3
Presence of reproductive *R. maritima* at the Chandeleur Islands, LA during August and September 2021. White symbols indicate sampling stations with reproductive *R. maritima* present, while red symbols indicate stations where only non-reproductive *R. maritima* was present. Black symbols indicate stations where *R. maritima* was completely absent.

from throw trap and the proportion of reproductive *R. maritima* in the throw trap, and the final GAM formulations only included biomass of vegetation recovered from throw trap and depth as predictor variables. Poisson distributions were used to model species richness and density, while a Gaussian distribution was used for modeling Shannon diversity.

Results

Objective 1: Evaluate reproductive R. maritima plant homogeneity across the Chandeleur Islands

Across the 27 sites sampled between August 5 and 3 September 2021, mean (±SE) depth was 97.2 ± 7.60 cm, mean salinity was 18.97 ± 0.67 ppt, mean temperature was 30.8 ± 0.44°C, mean dissolved oxygen was 7.82 ± 0.52 mg/L, and mean light attenuation coefficient was 1.08 ± 0.06 m^{-1}. Abiotic parameters were within the known ranges for seagrass meadows at the Chandeleur Islands (Darnell et al., 2017; Hayes, 2021).

TABLE 1 Mean (±SE) morphological metrics for individual reproductive and non-reproductive hand-collected *R. maritima* plants. Values with "*" indicate that the value is significantly greater ($p < 0.05$) than the compared value.

Plant metric	Reproductive	Non-reproductive
Shoot Length (mm)	229.37 ± 8.83 *	150.72 ± 7.04
Total Surface Area (mm^2)	1,507.92 ± 126.06*	310.36 ± 21.65
Root-Shoot Ratio	0.192 ± 0.025	1.033 ± 0.078 *
Leaf Number	25.04 ± 1.84 *	3.125 ± 0.09

FIGURE 4
Sites sampled for fish assemblages within R. maritima during September 2021. Each white circle represents a general sampling area, with the number inside the white circle indicating the number of samples taken in that area.

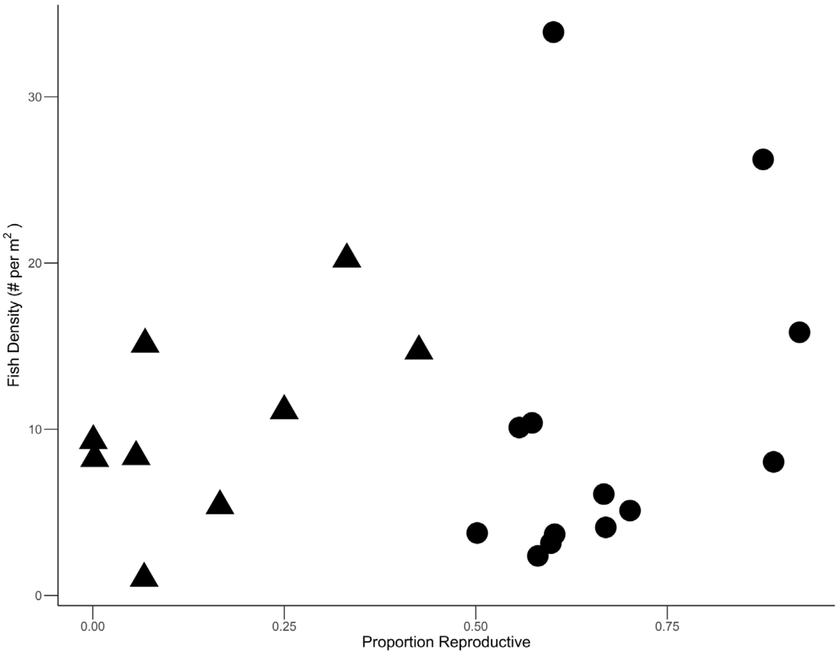

FIGURE 5
Fish density per meter square plotted against the proportion of *R. maritima* cover that was reproductive. Triangles indicate sites that were majority non reproductive cover while circles are those with majority reproductive cover.

Of the sites sampled, 24 (88.9%) had *R. maritima* present, and 17 of the sites (63.0%) had reproductive plants (Figure 3). Among 72 quadrats sampled across the 27 sites, mean (±SE) percent cover of non-reproductive *R. maritima* was 44.5 ± 3.5%, mean percent cover of reproductive *R. maritima* 21.5 ± 3.2%, and mean canopy height was 216.8 ± 6.3 mm. Sites with reproductive plants were primarily located near the latitudinal center of the islands, with sites near the northern and southern limits of the islands dominated by non-reproductive plants (Figure 3).

A total of 65 cores were collected across the sites with *R. maritima* present. Mean (±SE) overall shoot density was 3,866.71 ± 293.01 shoots per m^2, mean non-reproductive *R. maritima* shoot density was 3,449.39 ± 268.69 shoots per m^2, mean reproductive *R. maritima* shoot density was 804.15 ± 174.32 shoots per m^2, and mean core RSR was 1.097 ± 0.111. Within the cores, the density of non-reproductive shoots was more than four times greater than the density of reproductive shoots (t = 8.2589, $p < 0.05$). Out of the 65 cores, only 19 cores had measurable epiphyte cover (0.068 ± 0.041 g for all cores combined). Mean epiphyte biomass for cores with only non-reproductive shoots (n = 10) was 0.098 ± 0.075 g and epiphyte biomass for cores containing both non-reproductive and reproductive shoots (n = 9) was 0.035 ± 0.026 g.

A total of 74 reproductive and 112 non-reproductive plants were hand-collected to assess differences in plant morphology. Reproductive plants had significantly longer leaves (t = 7.054, $p < 0.05$), greater total surface area (t = 11.509, $p < 0.05$), and a greater number of leaves (t = 15.005, $p < 0.05$), while non-reproductive plants had a higher RSR (t = 10.440, $p < 0.05$), indicating a larger contribution of belowground biomass (Table 1).

Objective 2: Compare fish assemblages between reproductive and non-reproductive R. maritima

A total of 22 sites were sampled to investigate habitat use by fishes. Sites were predominant located near the center of the island chain where most reproductive *R. maritima* plants were observed (Figure 4). Mean (±SE) depth was 71.6 ± 2.8 cm, salinity was 19.46 ± 0.37 ppt, temperature was 28.81 ± 8.64°C, and dissolved oxygen was 8.15 ± 0.02 mg/L. Mean (±SE) total percent cover of seagrass was 69.1 ± 2.9%, percent cover of non-reproductive *R. maritima* was 38.9 ± 5.2%, percent cover of reproductive *R. maritima* was 30.2 ± 4.3% and total *R. maritima* wet biomass was 371.58 ± 27.61 g per m^2.

Across all sites, a total of 224 individual fish were collected across 15 species. Mean (±SE) fish density was 10.2 ± 1.7 per m^2,

TABLE 2 Count and mean (±SE) morphological metrics for fish collected, NA values indicate data not collected or not applicable for metric.

Common name	Scientific name	Count	Standard length (mm)	Total length (mm)	Individual wet weight (g)
Darter Goby	*Ctenogobius boleosoma*	107	24.05 ± 0.66	30.81 ± 0.76	0.29 ± 0.02
Blackcheek Tonguefish	*Symphurus plagiusa*	33	32.21 ± 1.5	34.03 ± 1	0.38 ± 0.03
Gulf Pipefish	*Syngnathus scovelli*	23	62.3 ± 4.4	64.96 ± 4.59	0.25 ± 0.05
Rough Silverside	*Membras martinica*	20	46.7 ± 0.93	57.5 ± 1.21	1.43 ± 0.1
Code Goby	*Gobiosoma robustum*	11	23.45 ± 1.48	28.64 ± 1.81	0.33 ± 0.05
Pinfish	*Lagodon rhomboides*	7	72.29 ± 8.59	90.71 ± 11.49	14.3 ± 3.35
Chain Pipefish	*Syngnathus louisianae*	6	70 ± 7.45	73 ± 7.93	0.30 ± 0.11
Scaled Sardine	*Harengula jaguana*	5	47.4 ± 1.17	59.6 ± 1.29	2.26 ± 0.19
Speckled Seatrout	*Cynoscion nebulosus*	4	38.75 ± 4.64	47.75 ± 6.02	1.14 ± 0.33
Atlantic Threadfin	*Opisthonema oglinum*	2	46.5 ± 3.5	56 ± 4	1.79 ± 0.31
Dwarf Seahorse	*Hippocampus zosterae*	2	NA	18.5 ± 0.5	0.03 ± 0.02
Bay Anchovy	*Anchoa mitchilli*	1	30	37	0.27
Freckled Blenny	*Hypsoblennius ionthas*	1	25	28	0.12
Gulf Killifish	*Fundulus grandis*	1	19	24	0.14
Silver Perch	*Bairdiella chrysoura*	1	70	86	7.29

TABLE 3 General additive model outputs for models with dependent variables A) fish density, B) species richness, and C) Shannon diversity. Values with "*" indicate a significant relationship ($p < 0.05$) with the predictor variable.

	Variable	EDF	χ2	*p*
A)	Depth (cm)	1	1.88	0.161
	Wet biomass (g) of *R. maritima* recovered from throw trap	1	6.992	0.008*
B)	Depth (cm)	1	0.021	0.885
	Wet biomass (g) of *R. maritima* recovered from throw trap	1	0.287	0.592
C)	Depth (cm)	1	0.008	0.931
	Wet biomass (g) of *R. maritima* recovered from throw trap	1.227	0.117	0.759

species richness was 3.4 ± 0.2, and Shannon diversity was 0.97 ± 0.07. Fish density showed no relationship with the proportion of reproductive *R. maritima* (Figure 5). The five most abundant species accounted for 86.6% of all individuals collected and included Darter Goby (n = 107), Blackcheek Tonguefish (n = 33), Gulf Pipefish (n = 23), Rough Silverside (n = 20), and Code Goby (n = 11). Mean weights, total lengths, and standard lengths for all species are listed in Table 2.

Three GAMs were formulated to identify potential drivers of the fish assemblage metrics of density, species richness, and Shannon diversity. The first model with total number of fish as the response variable identified the wet biomass *R. maritima* of vegetation recovered from throw trap ($\chi 2 = 4.506$, $p < 0.05$) as the only significant predictor variable with a positive relationship (Table 3A), and the second and third models identified no significant predictor variables for species richness and Shannon diversity (Tables 3B, C).

Discussion

This study described the spatial distribution of reproductive *R. maritima* across the Chandeleur Islands and compared seagrass-associated fish assemblages between reproductive and non-reproductive plants. We conducted extensive seagrass surveys along the length of the Chandeleur Islands and used throw trapping to describe habitat associations by fish, and found that reproductive and non-reproductive plants displayed distinct morphologies, but the morphology of reproductive plants did not significantly impact species richness or Shannon diversity. The more robust measurement of total wet biomass of *R. maritima* recovered from the throw trap, however, was related to fish density, with more fish associated with greater plant biomass.

Ruppia maritma occurred along the entire distribution of seagrass at North Chandeleur Island. Given the robustness of *R. maritima* to environmental stress, the absence of the species at only three of the sampled sites is likely linked to localized physical disturbance (e.g., wave action). The only other species present at the stations sampled was turtlegrass (*Thalassia testudinum*). Turtlegrass is a climax species that requires relatively stable environmental conditions and low levels of physical disturbance (Hartog and Kuo, 2006). Turtlegrass occurred in centralized portions of North Chandeleur Island that likely represent areas with relatively more stable environmental and physical conditions than the northern and southern tips of the island.

Similar to turtlegrass, reproductive *R. maritima* plants were primarily restricted to the center of the island's back shelf. The complex reproductive shoots have reduced structural tissue and rely on support from the surrounding water (Kantrud, 1997), making them susceptible to physical disturbances such as wave action. The central portion of the island represents an area with increased sheltering from wind driven wave action that dominates the system (Moore et al., 2014). The most northern and southern portions of the islands are characterized by reduced island elevation and more exposed shallow waters (Kahn, 1986; Miselis and Plant, 2021). It is possible that *R. maritima* is reproductive at the northern and southern tips of North Chandeleur island, but that shoots cannot sustain the physical disturbance and are dislodged, suggesting that the environmental requirements for sexually reproductive *R. maritima* are stricter than those of non-reproductive plants (de los Santos et al., 2016; Koch et al., 2006).

Core samples showed distinct differences between reproductive and non-reproductive shoot densities, with about one fourth of the shoots being reproductive. The production of flowers, fruits, and seeds is energetically costly, and as a result, the number of plants undergoing reproduction varies across space and time, which can lead to not all plants undergoing sexual reproduction (e.g., Bigley and Harrison, 1986; Strazisar et al., 2015; von Staats et al., 2021).

R. maritima exhibits morphological plasticity across its range in distribution (Cho et al., 2009; Lopez-Calderon et al., 2010; Ito et al., 2013; Martínez-Garrido et al., 2017), and the current study represents the first known description of *R. maritima* reproductive shoot morphology at the Chandeleur Islands. Results from this study of increased surface area, aboveground biomass and leaf number for reproductive shoots relative to non-reproductive shoots are consistent with descriptions for the species (Bigley and Harrison, 1986; Cho and Poirrier, 2005). In this study, the mean shoot length for reproductive shoots was 229.37 mm, which is much shorter than the maximum described shoot length of 2.5 m (Hartog and Kuo, 2006). The observed difference may be genetic, linked to environmental drivers such as salinity, and/or the presence of physical disturbances that limit the length of the fragile reproductive stems (Richardson, 1983). While plasticity in reproductive output for *R. maritima* is not well understood, studies of other species report increased reproductive effort for plants in more physically disturbed environments (Lee et al., 2005; Mishra and Apte, 2020). Epiphyte cover on seagrasses at the Chandeleur Islands can be high (Hayes 2021); however, among the cores collected for this study, less than one third (29.2%) showed measurable levels of epiphyte cover. This may be due to the sloughing off of older leaves and/or rapid growth of plants with the onset of reproductive plants which may not provide sufficient time for epiphytes to colonize to a measurable amount.

This study describes potential drivers of habitat use by fish in reproductive and non-reproductive *R. maritima* and suggests that total plant biomass rather than plant morphology most influences fish density in *R. maritima* meadows, despite the distinct morphological differences between reproductive and non-reproductive plants. These results indicate that the increase in biomass that occurs at the onset of reproduction is largely driving the relationship between fish density and proportion of *R. maritima* cover that was reproductive (Figure 5). Similar relationships have been described for nekton communities of *R. maritima* in brackish ponds in Louisiana (Kanouse et al., 2006), but our findings are contrary to studies with other seagrass species (e.g., *Halophila ovalis, Halophila beccarii, Amphibolis griffithii, Posidonia sinuosa, Posidonia australis, Zostera capricorni*) where interspecific differences in plant morphology/complexity have variable impacts on fish assemblages (Rotherham and West, 2002; Hyndes et al., 2003; Hori et al., 2009). GAMs for species richness and Shannon diversity showed that no predictor variables significantly affected richness or diversity, further suggesting that influence of the morphology of the reproductive plants is marginal when compared to the overall influence of seagrass presence for shaping the fish community. This conclusion aligns with previous studies investigating relationships between seagrass presence and biomass and animal abundance, diversity, and richness (Wyda et al., 2002; Heck et al., 2003; Strayer et al., 2003).

The lack of significant predictors among GAMs for species richness and Shannon diversity implies that other predictors are driving the relationships. One possible driver may be prey availability, which is linked to the function of seagrass as foraging grounds (K. Heck et al., 2003; Heck et al., 1997). Invertebrates were collected during the present study but were found to be outside the size range of those consumed by the fishes collected. Future studies designed to collect infauna and smaller invertebrates could help elucidate the relationship between prey availability and habitat use. Additionally, other environmental drivers such as salinity (Matheson et al., 1999), turbidity (Blaber and Blaber, 1980) and the availability of dissolved nutrients (Deegan et al., 2002) may impact the distribution of fishes within seagrass habitats due to physiological requirements of individual species. Although salinity was measured in the present study, there was little variation in the measured values which limited power in analyses.

The fish collected within *R. maritima* meadows in this study were predominately small benthic species with individuals under 100-mm total length. The majority of individuals were adults of their respective species, with notable exceptions being spotted seatrout (*Cynoscion nebulosus*) and pinfish (*Lagodon rhomboides*) which were primarily young-of-year individuals (*FishBase,* 2022). The family Gobiidae made up the majority (52.7%) of all species collected. Gobiidae are benthic-associated fish and are known to live within seagrass meadows during all life stages where they feed on meiofaunal prey and use the structure provided by seagrasses as shelter from predators (Carle and Hastings, 1982; Ara et al., 2010). Although adult pinfish are known to be abundant in seagrass beds at the Chandeleur Islands (e.g., Hayes 2021), they were collected in low numbers during this study. This may be due to the life history characteristics of the species, where adults leave seagrass beds between May and October to spawn (Faletti et al., 2019), which could have led to a decrease in abundance within seagrass beds at the time of this study (September).

Interestingly, the fish collected in this study included no tropical species. Recent studies have reported the occurrence of tropical fishes in Chandeleur Islands seagrass meadows, indicating tropicalization, or a northward movement species to this area (Fodrie et al., 2010; Hayes, 2021). The previous studies collected relatively low abundances of tropical species (e.g., Lane Snapper (*Lutjanus synagris*) 0.056 per m^2 and Gag Grouper (*Mycteroperca microlepis*) 0.002 per m^2 (Hayes, 2021)), and these were sampled using trawls and benthic sleds which cover a greater area than the throw trap. The absence of tropical species in our small-scale (1 m^2) samples suggests that, although they may be present in seagrass meadows at the Chandeleur Islands, tropical species are not likely abundant in *R. maritima* meadows.

One potential limitation of the current study is the throw trap sampling method which may underrepresent the abundance of highly mobile species (Kushlan, 1981; Freeman et al., 1984) such as members of family Mugilidae. Despite this limitation, throw traps are known to have high rates of accuracy when describing fish assemblages (Jordan et al., 1997). Additionally, the removal of all vegetation within the throw trap contributed to our clearing efficiency, as animals were recovered from the collected plant material during processing.

Conclusion

R. maritima occurred along the entire latitudinal range of seagrass distribution at North Chandeleur Island. Despite the widespread distribution of non-reproductive plants, there was a tendency for reproductive plants to be located along the central portion of the island. Where reproductive plants occurred, their density was lower than that of non-reproductive shoots, but reproductive shoots had significantly greater shoot lengths, number of leaves, and surface area. Non-reproductive plants showed significantly greater RSR, likely due to the substantial increase in aboveground biomass with the onset of reproduction for flowering plants. These results of distinct spatial and morphological characteristics for reproductive *R. maritima* plants suggest that the ecosystem functions of *R. maritima* may change with the onset of reproduction.

Fish assemblages within *R. maritima* beds at the Chandeleur Islands are represented by an abundant group of small benthic species. Total seagrass biomass was the primary driver of habitat use, suggesting that future studies to investigate the role of *R. maritima* in structuring fish communities in similar study systems may be better served to focus effort on robust measures of seagrass complexity (e.g., biomass) rather than more laborious measures of plant complexity such as cores and image analyses. Assitionally, future studies designed to collect infauna and smaller invertebrates could help elucidate the relationship between prey availability and habitat use.

At the Chandeleur Islands, *R. maritima* has increased in cover by colonizing disturbed areas along the islands and displacing climax species such as *T. testudinum*. Future studies should also compare *R. maritima* to other co-occurring seagrass species to better understand possible functional differences for associated fishes.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Human influence on bottlenose dolphin (*Tursiops truncatus*) strandings in the northern Gulf of Mexico

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Human activity affects marine mammal stranding rates in two major ways; through human interaction (HI) that may lead to mortality and through search and response efforts that enable carcass detection. To better quantify the combined effects of these interacting human influences, we analyzed strandings for bottlenose dolphins (*Tursiops truncatus*) in the northern Gulf of Mexico (nGOM), an area of high cetacean strandings. To identify hotspots of human influence, we first determined the number of total and HI-related bottlenose dolphin strandings normalized to shoreline length in each nGOM U.S. state, which represent major response areas. To estimate the effects of response effort on stranding numbers (for HI and non-HI strandings), we used the *Deepwater Horizon* oil spill (DWHOS) as an established benchmark to compare periods of lower (pre-spill) and higher (post-spill) response effort. Strandings in Alabama waters were used as a case study to detail spatial and temporal variation due to human influences during the 39-year period of retrospective study. We found an increase in strandings from Louisiana through Alabama following the DWHOS. Non-oil related HI strandings increased in total number in AL, and they increased as a proportion of total strandings in Alabama (16%) and the Florida panhandle (12%). The increase in HI-related strandings in Alabama was driven by mortality of many types, but particularly fishery interactions and cases of apparent intentional harm. The Alabama case study clearly detected lower stranding numbers during periods of low or intermittent response coverage. Our findings are consistent with an overall increase in stranding numbers due to a combination of increased stranding occurrence and response effort following the DWHOS. Importantly, we provide evidence that HI-related standings increased independently from the DWHOS, with ongoing increases in at least one hotspot (Alabama). These findings provide a first step to parsing out different effects of human influences on stranding data for a common coastal cetacean. Our approach establishes baselines for future damage and recovery assessments, identifies areas where resources can be focused for management and education, and highlights the power of response and monitoring agencies to positively influence stranding datasets.

KEYWORDS

human interaction, mortality, search effort, deepwater horizon oil spill, marine mammals

Introduction

Human activity can directly lead to stranding of live or dead aquatic animals. The most commonly reported anthropogenic drivers of marine mammal strandings (animals alive and in distress or dead washed ashore or floating) include various types of pollution, fisheries interactions, and boat strikes, which can have a range of effects that tend to be poorly defined even when animals are recovered dead (e.g., Desforges et al., 2018; Harvell et al., 1999; Laist et al., 2001; Nowacek et al., 2007; Read et al., 2006). Marine mammals may also depredate commercial and recreational fisheries species and become entangled in fishing line or caught incidentally in nets and drown (Zollett and Read, 2006; Read, 2008; Deming et al., 2020). In some locations, these human interactions include intentional or unintentional harassment and associated changes in natural behavior and feeding or cause purposeful injury to marine mammals (Spradlin et al., 2001; Samuels and Bejder, 2004; Vail, 2016; Collins et al., 2020). As a result, increased interaction with humans can increase the rate of marine mammal strandings, especially among coastal and estuarine species that are in close proximity to human activities such as fishing and tourism (Cunningham-Smith et al., 2006; Powell et al., 2018; Collins et al., 2020).

Stranding rates of marine mammals are also indirectly influenced by humans through search and response efforts. Across the globe, marine mammal stranding networks respond to live and dead stranded marine mammals, with varying levels of consistency and coverage (Chan et al., 2017; Simeone and Moore, 2018; Gómez-Hernández et al., 2022). The presence of a stranding network necessarily increases the likelihood that data will be collected on stranded animals to determine potential causes of death, including human interactions (HI). In the U.S., local stranding networks collect at least basic demographic data and submit them to a national database (NOAA National Marine Fisheries Service's Marine Mammal Health and Stranding Response Program (MMHSRP)), which is one of a few such databases world-wide (Chan et al., 2017; Gómez-Hernández et al., 2022). Stranding data, however, have been historically limited by insufficient funding and infrastructure for comprehensive and enduring response, which can result in poor monitoring, incomplete documentation, and inconsistent data collection (Hofman, 1991; Wilkinson, 1991; Gómez-Hernández et al., 2022). These factors can result in underreporting despite the existence of a stranding response network (e.g., Kemper et al., 2005; Wells et al., 2015; Lloyd and Ross, 2015). Hence, variation in stranding response effort and potential effects on spatial or temporal demographics of stranding patterns must be considered to accurately quantify stranding numbers and clearly define hotspots of stranding activity.

How to best quantify monitoring efforts for demographics studies is an ongoing challenge in ecology (e.g., Symons et al., 2018; McBride-Kebert et al., 2019). Effects of effort on demographics data for live animals have been well studied in fisheries and some targeted marine mammal research (McCluskey and Lewison, 2008; McBride-Kebert et al., 2019; Tiongson et al., 2021), but these approaches typically cannot be applied to marine mammal stranding data. Stranding data typically rely on opportunistic public reporting rather than regular targeted monitoring of a given location. Furthermore, because marine mammals are not a commercial or recreational fishery in the U.S., many traditional metrics of effort such as fishing licenses, boats on the water, and catch reports are not options for effort assessment. Comparable data on public access or use are not broadly available for many locations where marine mammals strand to historically relate strandings to frequency of human presence. Despite these limitations, use of opportunistic, anecdotal, or traditional ecological knowledge has been successfully used and is growing in popularity to at least yield best estimates of long-term or large-scale patterns for some fisheries and marine mammal studies (e.g., Symons et al., 2018; Giovos et al., 2019; IJsseldijk et al., 2020). Moving forward, studies that apply stranding data will benefit from efforts to identify and test alternative metrics and approaches to account for monitoring or response efforts (e.g., Wells et al., 2015). Such approaches will be essential to the future of ecological monitoring, particularly for long-lived, difficult to study species and under changing climate regimes where understanding historical trends are critical to detecting and managing future change (Harvey et al., 2018; Symons et al., 2018).

The potential effect of response effort on marine mammal stranding numbers was recently evidenced following the *Deepwater Horizon* oil spill (DWHOS) in the northern Gulf of Mexico (nGOM). The DWHOS is recognized as a major source of HI-related mortality, responsible for the largest and longest-running cetacean unusual mortality event (UME) in the Gulf of Mexico in U.S. history, which included loss of more than 1,000 bottlenose dolphins (Litz et al., 2014; Table 1). During 2010, as part of NOAA's Natural Resource Damage Assessment there was an increase in the number of observers monitoring and cleaning local beaches and increased funding for stranding network operations in the region (Wilkin et al., 2017). These increased resources led to greater stranding response through increased search effort and development of more formalized stranding response protocols for the nGOM, both of which may have influenced stranding numbers (Williams et al., 2011;

TABLE 1 Marine mammal stranding occurrences designated as unusual mortality events (UMEs) in nGOM states that occurred 1990–2016 [Texas = TX, Louisiana = LA, Mississippi = MS, Alabama = AL, Florida panhandle (Escambia-Jefferson counties)] = FL.

					Factors associated with mortality					
Event	**Dates**	**State**	**Number stranded**	**Cause**	**Abiotic factors**	**Biotoxin**	**Prey shortage**	**Pollution**	**Disease**	**Sources**
1990 nGOM UME	January-May	TX LA MS AL FL	159 47 52 42 44	Undetermined	Cold temperatures		X		suspect morbillivirus	Hansen, 1992; Litz et al., 2014; Worthy, 1998
1992 TX UME	January-May	TX	119	Undetermined	Freshwater			X	suspect morbillivirus	Colbert et al., 1999; Litz et al., 2014; NOAA Fisheries, 2017; Worthy, 1998
1993 TX UME[a]	December 1993-June 1994	TX	240	Morbillivirus					X	Lipscomb et al., 1996; Litz et al., 2014; NOAA Fisheries, 2017; Worthy, 1998
1996 MS UME	November-December	MS	31	Undetermined		suspected	X			Litz et al., 2014; NOAA Fisheries, 2017
1999 FL Panhandle UME	August 1999-May 2000	FL	162	Biotoxin		X	X			Litz et al., 2014; NOAA Fisheries, 2017; Schwacke et al., 2010; Twiner et al., 2012
2004 FL Panhandle UME	March-April	FL	107	Biotoxin		X	X			Litz et al., 2014; NOAA Fisheries, 2017; Schwacke et al., 2010; Twiner et al., 2012
2005 FL Panhandle UME	September 2005-April 2006	FL	93	Biotoxin		X	X			Litz et al., 2014; NOAA Fisheries, 2017; Schwacke et al., 2010; Twiner et al., 2012
2007 TX UME	February-March	TX LA	61 5	Undetermined			X		suspect *Brucella*	Litz et al., 2014; NOAA Fisheries, 2017
2008 TX UME	February-March	TX	113	Undetermined		X	X		suspect *Brucella*	Fire et al., 2011; Litz et al., 2014; NOAA Fisheries, 2017
2010 nGOM UME	February 2010-July 2014	LA MS AL FL	539 274 140 121	DWHOS/ Undetermined	Cold temperatures, freshwater	X	X	X	X	Carmichael et al., 2012; Litz et al., 2014; NOAA Fisheries, 2017
2011 TX UME	November 2011-March 2012	TX	126	Undetermined		X				NOAA Fisheries, (2017)

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[a] AL and MS were not officially included in 1993 TX UME, but also experienced an increase in strandings (31 for AL, and 32 for MS) associated with morbillivirus during July-December 1993 (Lipscomb et al., 1996).

Pitchford et al., 2018). The historical carcass recovery rate for cetacean species in the nGOM is estimated at 2%, largely due to poor detection among populations of unknown animals (Williams et al., 2011; Wells et al., 2015), but the region also includes known periods of marine mammal stranding response gaps, including little to no coverage in Louisiana, Mississippi, Alabama and parts of western Florida prior to the DWHOS (Worthy, 1998; Venn-Watson et al., 2015). These observations provide well-known and documented temporal and spatial inflection points with potential to help parse out the net effects of human influence on strandings in terms of mortality and response effort.

In this study, we sought to better quantify human influence on strandings rates of the bottlenose dolphin (*Tursiops truncatus*) in the nGOM in terms of human interactions with potential for contributions to mortality (HI) and response effort. *T. truncatus* was selected because it is prevalent in coastal waters globally, interactions with people are well documented, and it is the most commonly-stranded marine mammal in the nGOM, comprising approximately 85% of cetacean strandings in the region (Litz, 2015; Russell et al., 2022). We hypothesized that the proportion of HI-positive strandings would increase through time relative to increased stranding response effort, particularly following the DWHOS. We also hypothesized that hotspots of human influence would be detectable as areas with a high density of HI-positive cases due to increased HI events, regardless of effort. To test these hypotheses, we determined the number of total and HI-positive bottlenose dolphin strandings normalized to shoreline length in each nGOM state. To estimate the effects of response effort on stranding number (for total strandings and HI-positive strandings), we used the DWHOS as an established benchmark to compare periods of lower (pre-spill) and higher (post-spill) response effort. Strandings in Alabama waters were used as a case study to detail spatial and temporal variation due to human influences during the 39-year period of retrospective study.

Materials and methods

Publicly available demographic (Level A; NOAA, 2020) data for *T. truncatus* stranded in the nGOM from the Texas-Mexico border through Jefferson County, Florida were accessed from NOAA's MMHSRP database as of 8 November 2017 for the years 1978 through 2016. For the state of Alabama case study, we additionally documented the names of designated responding organizations from Level A data for each stranding.

Human interaction-positive strandings

To determine the number of HI-positive strandings, data were categorized as HI-positive (definitive evidence of HI present) or HI-negative (no definitive evidence of HI, including cases where HI status could not be determined or the carcass was not entirely examined) based on documentation in the MMHSRP. The initial determination of HI status for stranded dolphins within each state is made in the field, typically by a primary responder or response team and corroborated by NOAA personnel when data are verified in the MMHSRP (e.g., Moore and Barco, 2013). Strandings prior to 2001, when HI status was first formally recorded in the MMHSRP database, were updated as HI-positive or HI-negative based on whether HI was mentioned in the "additional comments" section of the Level A data.

To determine the density of HI-positive strandings along the nGOM coast, the annual number of total and HI-positive strandings were standardized using the linear distance of shoreline, separated by state (Figure 1). The linear shoreline distance of each state (Texas, Louisiana, Mississippi, Alabama, Florida panhandle) was determined from the National Environmental Sensitivity Index Shoreline dataset (NOAA Office of Response and Restoration, 2017). Vector line data used from this dataset included all natural and manmade shoreline boundaries of the contiguous nGOM states where strandings could wash ashore, ending at the eastern boundary of Jefferson County, Florida where there is an historical break in stranding response that separates the panhandle from peninsular Florida (Litz et al., 2014). We analyzed HI data including and excluding data from animals stranded during the DWHOS UME that were classified as visibly oiled in the MMHSRP to remove bias associated with this acute event in the HI data.

For the state of Alabama case study, we further related temporal and spatial variation in HI strandings to HI type by classifying HI-positive strandings into five groups: fishery interaction, mutilation, boat collision, incidental take, and other. For these analyses, one visibly oiled animal that stranded during the DWHOS UME was included and categorized as "other." Strandings were then plotted through time and mapped using ArcGIS 10.3 (ESRI, 2014) to detect local patterns relative to HI type.

Stranding response effort

Because stranding response efforts in the U.S. are state-specific, we accounted for response effort by state and normalized to shoreline distance as described above and further divided the data into pre-DWHOS (1978–2009) and post-DWHOS (2010–2016) coverage periods, representing known periods of lower and higher effort, respectively (Wilkin et al., 2017). We additionally analyzed data with and without respect to known UMEs to eliminate the effect of these acute events, particularly associated with the DWHOS.

We took advantage of additional data on stranding response effort in the state of Alabama to conduct a more detailed case

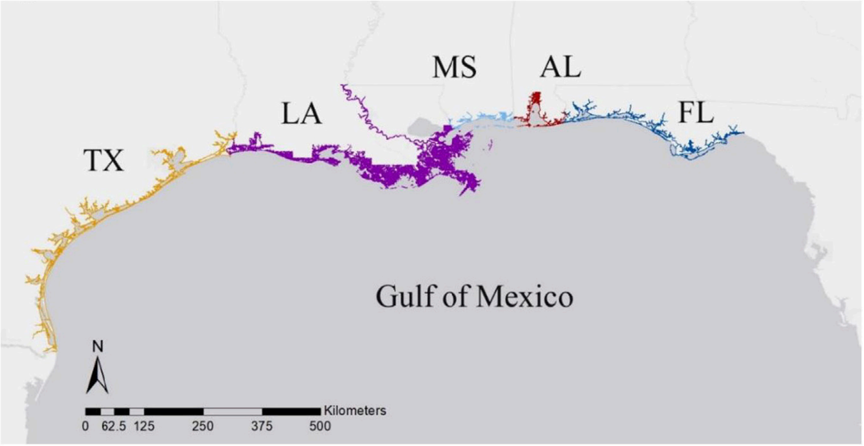

FIGURE 1
Linear coastline of each state in the northern Gulf of Mexico, where Texas = TX, Louisiana = LA, Mississippi = MS, Alabama = AL, and the Florida panhandle = FL.

study to extend our approach to account for interannual variation in response effort in this state. We compared annual stranding counts for all cetaceans (90% *T. truncatus*) and the occurrence and types of HI (100% *T. truncatus*) to the level of stranding response effort, indicated by the response organization in the MMHSRP and known relevant metrics of effort (i.e., whether there was a formal stranding agreement holder designated by NOAA NMFS, if response was opportunistic or conducted by trained volunteers or paid staff members, if responders included full-time designated personnel, and if dedicated state or federal funding was awarded; Supplementary Table S1).

Based on these metrics, we defined three distinct levels of stranding response effort in Alabama, including: 1) low effort (periods of minimal response when response was opportunistic and there was no formal stranding response designee or dedicated stranding response funding for the state; 1978–1986, 2007–2008), 2) moderate effort (fully volunteer response designees with limited dedicated funding; 1987–2006), and 3) high effort (full-time paid response designees with regular dedicated funding; 2009–2016). In some years, designees responded for part of a year or multiple groups responded to strandings, with different levels of effort. In these cases, the maximum estimated level of effort was assigned for the full year (Supplementary Table S1). Through time, the location of designated response organizations also varied from the eastern to the western sides of Mobile Bay, the major body of water in Alabama. Hence, we were additionally able to test possible effects of effort on the local geographic distribution of stranding numbers, assuming greater response effort may occur in areas nearer responding organizations.

Data analysis

To compare stranding numbers among states and between pre- and post-DWHOS periods for the entire nGOM, we performed Multivariate Analysis of Variance (MANOVA) with marginal *p*-values on annual stranding count data, normalized by linear shoreline distance (total, HI-positive, and non-oiled HI-positive data). A Levene's test (Levene, 1960) was performed to test for homoskedasticity, and generalized least squares (GLS) models in R *nlme* v3.1-124 were applied to compensate for heteroskedasticity. Model selection was performed using a corrected Akaike information criterion estimator (AICc; Burnham and Anderson, 2004). We selected a GLS model with variance weighted by state for the total annual stranding data and weighted by state and coverage period (pre-v. post-DWHOS) for HI-positive and non-oiled HI-positive strandings, normalized by linear shoreline distance and reported as mean annual strandings per 100 km for pre- and post-DWHOS periods.

After performing the MANOVA on the selected GLS models, post-hoc two-sided Welch's t-tests (Welch, 1947) and Westfall method of multiple comparisons (Westfall, 1997) were performed to determine differences between pre- and post-DWHOS periods (low v. high effort) within states and between states, respectively. All GLS models were selected in R using *MuMIn* v1.156, with post-hoc analyses performed in R *car* version 2.3 and R *multcomp* v1.4-6. To additionally determine if there were differences in the proportion of HI-positive and non-oiled HI-positive strandings (% of total) for all the states within the two (pre-v. post-DWHOS) periods, the test for equality of proportions with continuity correction was used

TABLE 2 The total number (and percent HI-positive) of *T. truncatus* strandings compared to linear shoreline distance of nGOM states (shown in Figure 1) during the 39-year study period, separated by time periods pre- (1978–2009) and post- (2010–2016) DWHOS. FL panhandle includes shoreline from the Alabama-Florida border through Jefferson County, FL.

State	Shoreline linear distance (km)	Total stranded (%HI)		
		Pre-DWHOS 1978–2009 (32 y)	Post-DWHOS 2010–2016 (7 y)	Total study period 1978–2016 (39 y)
TX	14226	3645 (4.6)	867 (6.3)	4512 (4.9)
LA	85497	626 (5.1)	625 (8.3)	1251 (6.7)
MS	3037	870 (4.4)	402 (4.2)	1272 (4.3)
AL	2604	412 (3.1)	221 (16.3)	633 (7.7)
FL panhandle	7305	808 (3.3)	191 (12.0)	999 (5.0)

for each respective category. We then used a chi-square test statistic and Holm-corrected pair-wise comparison of proportions post-hoc test with five groups. All analyses were performed in R v3.4.1, *SingleCandle*.

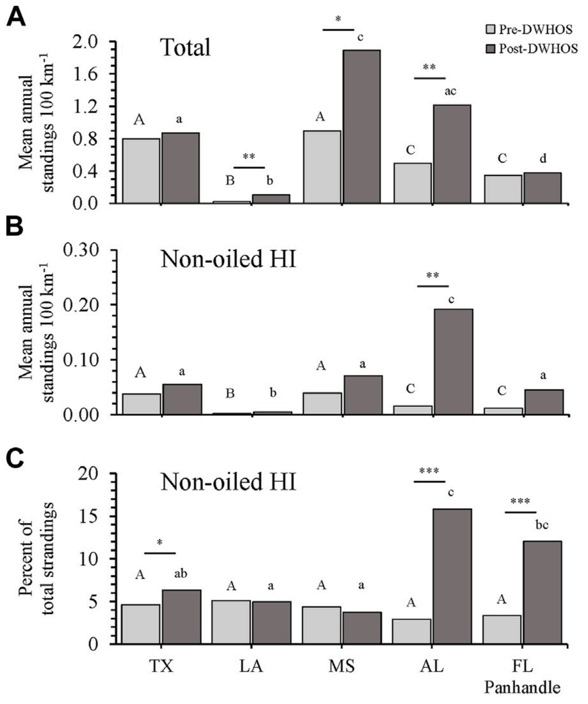

FIGURE 2
Total **(A)** and non-oiled HI-positive **(B)** mean annual strandings normalized to linear shoreline distance (100 km^{-1}) and non-oiled HI-positive cases as a percentage of total strandings **(C)**, separated by state and pre- (1978–2009) and post- (2010–2016) DWHOS coverage periods. Capital and lower-case letters indicate significant differences among locations for pre-DWHOS and post-DWHOS coverage periods, respectively. Asterisks indicate differences between coverage periods within each state, where *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

For the Alabama case study, regression analysis was used to detect changes in total cetacean strandings or HI-positive *T. truncatus* strandings through time relative to estimated level of response effort (low, moderate, high) using StatPlus: mac v6. Data were analyzed including and excluding UMEs to help distinguish changes in effort from acute events (e.g., Table 1). To detect differences in stranding distributions relative to the primary location of response agencies, stranding location data were divided geographically into 0.2-degree longitude bins, separated by location and level of effort, and a matrix was used to compare locations in R v3.4.1, *SingleCandle*.

Results

Total strandings in the northern Gulf of Mexico

There were a total of 8,667 *T. truncatus* strandings along the 112,669 linear km nGOM shoreline from 1978–2016 (Figure 1; Table 2). Texas had the highest number of total strandings along the second longest shoreline, and Alabama had the highest proportion (8%) of total HI-positive strandings along the shortest shoreline during the 39-year study period (Table 2). Accordingly, the mean number of strandings per 100 km differed by state (MANOVA; $F_{4,185} = 52.69$, $p < 0.0001$) and pre- or post-spill coverage period (MANOVA; $F_{1,185} = 12.28$, $p < 0.01$; Supplementary Tables S2, S3), with a significant interaction between state and coverage period (MANOVA; $F_{4,185} = 5.00$, $p < 0.001$).

Prior to the DWHOS, when response efforts were lower, Texas and Mississippi had greater mean annual strandings per 100 km of shoreline than other states (Westfall; $p < 0.02$ for all comparisons; Figure 2A). Significant increases in strandings in Mississippi and Alabama post-spill (Westfall; $p < 0.01$ for all comparisons) resulted in a higher number of strandings in these states compared to others during this 2010–2016 period (Westfall; $p < 0.02$). Although strandings increased in

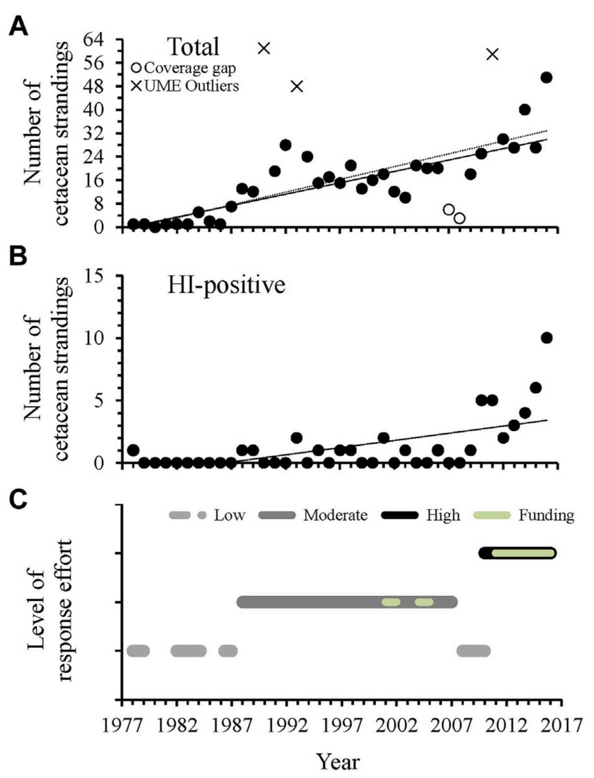

FIGURE 3
The total number of Alabama cetacean strandings **(A)** and HI-positive strandings, including a visibly oiled animal (n = 1 in 2010) **(B)** compared to estimated level of stranding effort and dedicated response funding **(C)** in Alabama waters through time. For total strandings, the solid black line ($y = 0.78x - 1535.20$ $R^2 = 0.57$, $F_{1,34} = 44.89$, $p < 0.001$) excludes the documented UMEs that occurred in 1990, 1993 and 2011 (Table 1), which were outliers to the regression. The dotted black line ($y = 0.87x - 1723.70$, $R^2 = 0.71$, $F_{1,26} = 35.44$, $p < 0.001$) additionally excludes data for 2007 and 2008 that coincided with a recent gap in stranding response and were outliers to the regression when UMEs were excluded. For HI-related strandings, $y = 0.12x - 234.02$, $R^2 = 0.38$; $Freg_{1,37} = 22.48$, $p < 0.001$, with an exponential increase since 2012 ($y = 1.96e^{0.39(x-2012)}$, $R^2 = 0.99$; $Freg_{1,3} = 338.43$, $p < 0.001$). Detailed metrics for level of effort assignments are provided in Supplementary Table S1.

Louisiana post-spill, Louisiana had fewer annual strandings relative to shoreline length than other states during both periods (Westfall; $p < 0.001$ for all comparisons; Figure 2A).

Non-oiled human interaction-positive strandings in the northern Gulf of Mexico

Mean annual HI-positive strandings, excluding oiled animals, also differed by state (MANOVA; $F_{4,175} = 23.75$, $p < 0.001$) and pre- or post-spill period (MANOVA; $F_{1,175} = 55.08$, $p < 0.001$) and had a significant interaction between state and coverage period (MANOVA; $F_{1,175} = 14.31$, $p < 0.001$; Supplementary Tables S2, S3). As with total strandings, Texas and Mississippi had higher HI-positive strandings compared to other states prior to the DWHOS, when effort was lower (Westfall; $p < 0.02$ for all comparisons). Following the spill, there was a 13-fold increase in mean annual HI-positive strandings per 100 km in Alabama ($t_{6.16} = -4.68$, $p = 0.0031$). No other states had an increase in mean annual non-oiled HI-positive strandings between pre- and post-spill periods (Figure 2B).

When considering non-oiled HI-positive strandings as a proportion (%) of total strandings, there was no difference in HI prevalence among states during the pre-spill period ($\chi^2_4 = 5.48$, $p = 0.24$), but there was a difference among states during the post-spill period ($\chi^2_4 = 45.73$, $p < 0.0001$) (Figure 2C). Alabama had a higher proportion of HI-positive strandings than Louisiana ($z = 4.45$, $p < 0.0001$), Mississippi ($z = 4.57$, $p < 0.0001$), and Texas. Similarly, the Florida panhandle had a higher proportion of HI-positive strandings than Louisiana ($z = 2.45$, $p = 0.0062$) and Mississippi ($z = 2.94$, $p = 0.0016$). Texas ($\chi^2_1 = 4.12$, $p = 0.042$), Alabama ($\chi^2_1 = 55.18$, $p < 0.001$) and Florida panhandle ($\chi^2_1 = 22.78$, $p < 0.001$) showed significant relative increases in HI between pre- and post-spill periods, with Alabama and Florida having the highest percentage of HI-positive strandings (16% and 12%, respectively; Table 2 and Figure 2C).

Alabama case study

A total of 709 reported cetacean strandings occurred in Alabama from 1978 through 2016. Of the reported cetaceans, 633 strandings (89%) were identified as *T. truncatus* and 49 of those were documented as HI-positive. The total number of reported strandings increased through time at a rate of approximately 1 cetacean per year during the entire 39-year period of documented strandings (Figure 3A). During this same period, HI-positive strandings also increased through time, but at a lower rate due to low documented numbers of HI (≤ two per year) until 2010, when reports more than doubled in number during 2010 and 2011 (Figure 3B). Since 2012, HI-related strandings have increased exponentially, doubling in number during each of the last 2 years of this study (Figure 3B).

Outliers to the regression comparing total stranding numbers through time were found during 1990, 1993, and 2011 (Figure 3A), consistent with periods of documented UMEs in the GOM even when UMEs were not specifically declared for AL waters (Table 1). Additional UMEs that occurred in the GOM during 1996 and 2010–2013 did not result in statistical outliers in AL. When data were reanalyzed excluding UME outliers, 2 years (2007, 2008) had lower than expected numbers of strandings and occurred during a period of known low stranding response effort (Figures 3A, C). Strandings during this period were comparable to those during the low coverage periods from 1978–1992 in

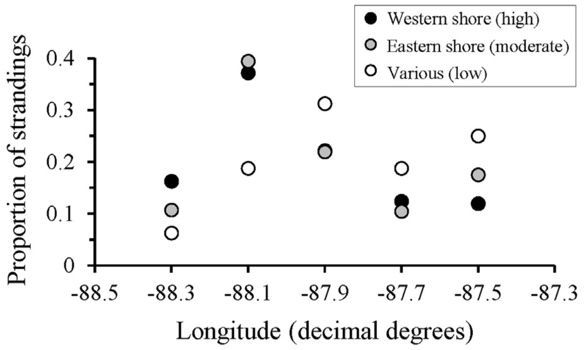

FIGURE 4
The proportion of *Tursiops truncatus* strandings that occurred along the Alabama coastline, grouped into 0.2-degrees longitude binds, separated by location of the major response agency and associated level of response effort (locations and effort metrics are detailed in Supplementary Table S1).

Alabama, when response was limited and opportunistic (Supplementary Table S1). Stranding numbers were higher under moderate and high levels of effort from 1987–2007 and since 2010, respectively (Figure 3C). The geographic distribution of strandings was highly correlated between periods of moderate and high response effort (moderate compared to high effort: $r = 0.94$, $p = 0.02$), regardless of location of the response organization (Figure 4 and Supplementary Figure S1). When response effort was low, however, stranding locations were not correlated to stranding locations during moderate or high effort periods ($r < 0.28$, $p > 0.64$).

HI-positive strandings in Alabama were most frequently reported along Dauphin Island, Fort Morgan and the nGOM coastlines (Figure 5A) and included strandings in all categories throughout Alabama waters. Most HI-positive strandings were categorized as fishery interactions, which were documented since 1993 and increased in recent years (Figure 5B). Other types of strandings (categorized as ‘other’) included visibly oiled ($n = 1$), shot with a field arrow ($n = 1$), trapped behind a barrier ($n = 1$), rope entanglement of the fluke that could not be confirmed as a fishery interaction or not ($n = 1$), and blunt trauma from an unknown source ($n = 1$; Figure 6). Since 2010, five individuals were also documented to simultaneously exhibit two types of HI (Figure 5A, multicolored circles and Figure 5B, hashed bars).

Discussion

Human interactions with marine mammals pose a threat of increasing global concern for conservation (Obusan et al., 2016; Avila et al., 2018; Liu et al., 2019). Our study supports the idea that human activities also can provide for better response to and documentation of strandings, if effort is directed toward monitoring and reporting of stranded animals (see also Wilkin et al., 2017; Moore et al., 2018; Pitchford et al., 2018). We found that Alabama is a hotspot of HI-positive strandings along the nGOM coast, having the highest density and percentage of HI-positive strandings since 2010, not related to the DWHOS. This increase has included more HI-positive reports associated with many types of mortality, but particularly fishery interactions and cases of apparent intentional harm. Higher numbers of non-oiled HI-positive strandings in 2010-2011 provide quantitative evidence that greater effort associated with DWHOS Natural Resource Damage Assessment response, which provided for more vessels on the water and personnel monitoring beaches (Wilkin et al., 2017), likely contributed to increased reporting of strandings. Better awareness from local citizens also likely contributed to increased reporting during this period (Wilkin

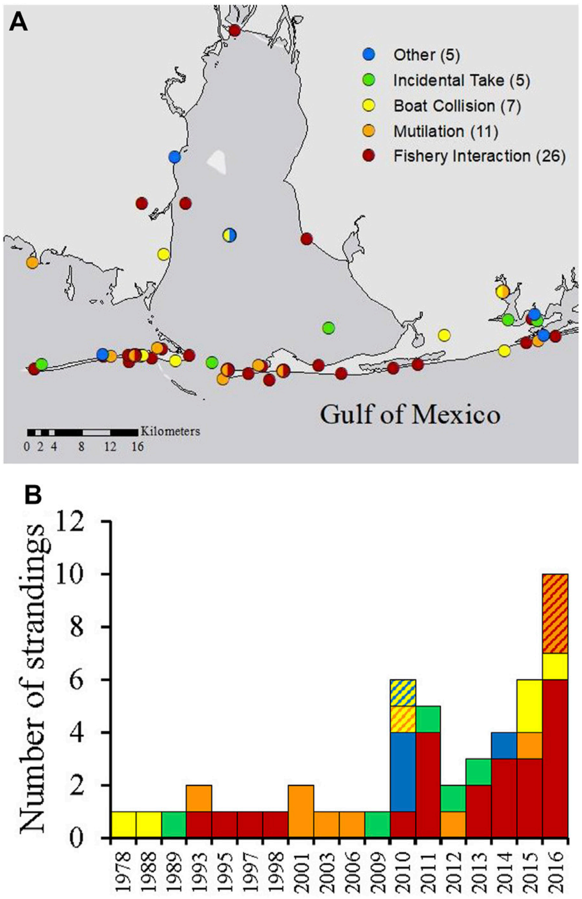

FIGURE 5
Location **(A)** and total annual numbers **(B)** of HI-positive strandings in Alabama, separated by HI type. Strandings that included more than one type of HI ($n = 5$) are indicated by multicolored circles **(A)** and muticolored hashed bars **(B)**.

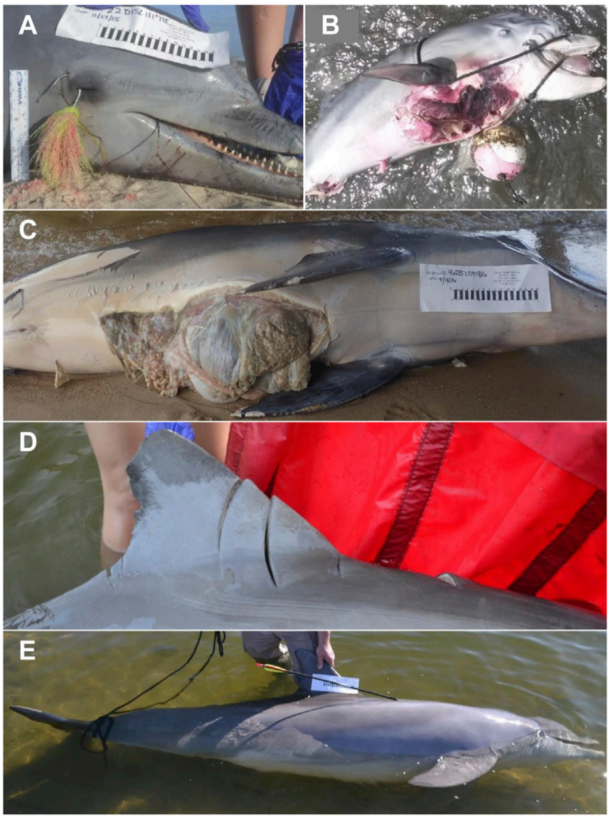

FIGURE 6
Examples of HI-positive dolphin stranding cases in Alabama, including fishery interaction [hook and line, **(A)**; crab pot entanglement, **(B)**; mutilation (ventrum sliced; **(C)**; boat collision (acute trauma; **(D)**; purposeful harm (shot with arrow; **(E)**].

et al., 2017). The subsequent drop in HI-positive strandings in Alabama in 2012 is consistent with a return to pre-DWHOS (baseline) levels, further suggesting that the exponential increase in HI-positive strandings since 2012 is primarily due to increased stranding occurrence rather than effort alone; a critical finding that was only possible because of our assignment of effort levels to Alabama data. Of note, the higher proportion of HI-positive reports in Alabama cannot be attributed to greater capability for HI detection compared to other states because the ALMMSN at DISL was the newest stranding network on the nGOM coast and unlike most neighboring states did not have a full-time veterinarian on staff during the study period. Identifying areas of high HI-positive strandings provides an opportunity to direct resources toward efforts to change behavior and reduce negative interactions in areas of greatest need, while increasing the positive aspects of human participation in monitoring and stranding response. For Alabama, this analysis also afforded the unique opportunity to quantify the additional effects of effort on stranding numbers and detect a meaningful increase in HI-positive stranding occurrence in recent years.

Gulf-wide, our findings indicate a regionally specific increase in strandings along the nGOM coast from Louisiana through Alabama, with the greatest number of HI-positive strandings to the east. While the overall increase in stranding numbers is likely due to a combination of increased stranding occurrence and response effort following the DWHOS, we provide evidence that HI-positive standings increased independently from the DWHOS and in locations of the nGOM most distant from the spill site. Ongoing or residual effects of the DWHOS are possible throughout the nGOM, and HI-positive cases in particular may remain higher than historical numbers due to increased awareness and better-trained and prepared investigators. The additional resources provided to stranding networks throughout the Gulf of Mexico during and following the DWHOS, have helped build long-term capacity for enhanced response through funding for trained personnel and equipment as well as development and coast-wide use of standardized comprehensive protocols that include HI assessment (Wilkin et al., 2017). Furthermore, the Alabama case study suggests stranding numbers documented during the early years of stranding response (prior to 1990) may be artificially low due to poor, intermittent coverage, as evidenced by the similarity to stranding numbers during known periods of low coverage in recent years. As a result, the historical baseline for total annual stranding numbers may not be accurately definable for the nGOM, and small differences between pre- and post-spill periods may not be highly meaningful. Also, because HI status can most readily be determined in fresh carcasses by properly-trained personnel, these numbers likely represent a minimum count of HI-positive strandings. Therefore, the dramatic increases observed in total strandings in Alabama and Mississippi and HI-positive strandings in Alabama and the Florida panhandle warrant additional attention from managers and stranding networks as they continue to assess dolphin population recovery and plan for future conservation throughout the nGOM. These data will be particularly important to determine relationships between HI and causes of mortality, given that many animals may experience HI throughout their life, with evidence detectable at stranding, but HI may not be the proximate cause of death.

This study provides the first regional-scale analysis of anthropogenic drivers of bottlenose dolphin stranding reports in the nGOM and localized data in the state of Alabama. Our findings of this study highlight the need for high quality regional and local data collection and analysis of patterns in marine mammal stranding events. Had we not normalized data by area of shoreline for each state or considered the effects of effort through time, it is likely the substantial increase in HI-related strandings in an area of relatively small shoreline like Alabama or the Florida panhandle (compared to all of Florida) would be overlooked. Of note, the types of HI-positive strandings in Alabama represent the wide range of HI types, which are often described but rarely quantified in publications, for bottlenose dolphin strandings in U.S. waters (Samuels and Bejder, 2004; Byrd et al., 2014; Vail, 2016; Powell et al., 2018; Maze-Foley et al., 2019; Collins et al., 2020), further emphasizing the broad nature of HI in this local area. Our analytical approach provides a method for other researchers to similarly evaluate anthropogenic impacts, including historical assessment of response effort, on marine mammal strandings and identify areas of high occurrence for other species and geographical locations. The HI-positive stranding increases documented here were serendipitously detectable in part due to the increased effort enabled by DWHOS response. These results evidence the benefits of sufficient funding to stranding networks as well as potential importance of some human influences on stranding numbers. Stranding networks and managers will benefit from taking advantage of the increased information derived from post-DWHOS stranding response efforts to better define the net effects of human influences on dolphin strandings and work collaboratively toward solutions to human-caused mortality in areas of high occurrence (Balmer, 2007).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: The data analyzed in this study were obtained from the NOAA's MMHSRP and Office of Response and Restoration. Requests to access the MMHSRP dataset should be directed to MMHSRP NationalDB@noaa.gov. The Office of Response and Restoration's National Environmental Sensitivity Index Shoreline data are available at https://response.restoration.noaa.gov/esi_download.

Ethics statement

Ethical review and approval was not required for the animal study because work was conducted on dead animals under a NOAA NMFS stranding agreement to the DISL.

Author contributions

RC, MH, NW, and MR contributed conception and design of the study; MH and NW organized the dataset; RC, MH, and NW performed the statistical analysis; RC, MH, NW, and MR designed figures and tables; RC and MH wrote the first draft of the manuscript; RC, MH, and NW wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.951329/full#supplementary-material

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Diet and disturbance: Seaside Sparrow resource use driven by oiling and Hurricane Isaac

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The 2010 Deepwater Horizon (DWH) disaster led to extensive oil deposition in Louisiana's coastal wetlands, followed two years later by Hurricane Isaac. These disturbances led to changes in saltmarsh invertebrate communities, potentially affecting higher-level predators and the saltmarsh food web. Seaside Sparrows (*Ammospiza maritima*) are ubiquitous, year-round residents of the coastal saltmarsh affected by the DWH spill, where they consume diverse invertebrates and may be considered an indicator species for the ecosystem's integrity. We used DNA metabarcoding to evaluate prey consumed by Seaside Sparrows to understand how sparrows responded to residual contamination from the DWH oil spill and ecosystem disturbance caused by Hurricane Isaac. To do so, we evaluated metrics of diet (prey richness, diversity, overall diet composition) and resource use (total niche width, individual specialization) from 2011 to 2017 on oiled, unoiled, and reference sites. We found that while diet composition varied across years and site type, Hurricane Isaac had an even greater effect on the richness and diversity of prey consumed. Resource use—as measured by the total niche width of the populations and degree of individual specialization—was most stable on unoiled sites compared to unoiled and reference sites. Finally, we analyzed resource use for each combination of site type and year (i.e.: "2014 oiled sites"), which indicated a strong correlation between individual specialization and total niche width: as total niche width increased, individuals became more specialized, following the predictions of the Niche Variation Hypothesis.

KEYWORDS

Seaside Sparrow, DNA metabarcoding, niche variation hypothesis, diet, hurricane, disturbance, Deepwater Horizon (DWH)

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1 Introduction

In an effort to understand the resource use of populations, Van Valen (1965) proposed the niche variation hypothesis (NVH), suggesting that in the absence of interspecific competition, the overall suite of resources used by a population—or the total niche width (TNW)—will broaden as individuals take advantage of available resources. The NVH posits that unique phenotypes of individuals (or behavioral differences; Werner and Sherry 1987; Toscano et al., 2016) will drive them to consume unique prey—that is, individuals will specialize on resources suited to their unique morphology or cognitive ability and the overall diversity of prey consumed by the population will increase, leading to a broader TNW (Van Valen 1965). Since the width of a trophic niche is driven by the number of resources used, specialist species are often assumed to have narrow TNW, while generalist species have broader TNW (though see Peers et al., 2012).

Dietary niche is one important component in determining how species cope with ecosystem disturbance (Graham et al., 2011; Cloyed et al., 2021). Trophic generalists consume a variety of prey and can adapt to changing prey communities through foraging plasticity, with individuals switching between food sources when resources are scarce. This flexibility can allow generalist species to persist despite changes in their environment (Swihart et al., 2003). Trophic specialists, on the other hand, may struggle to survive if their preferred resources become unavailable (Clavel et al., 2011). The evolution of these trophic strategies may be driven by a variety of pressures, including intra- and interspecific competition, resource availability, and environmental factors. Ecosystem heterogeneity is one such factor, with spatially and temporally stable ecosystems favoring the evolution of specialists and more heterogenous or unpredictable environments leading to the evolution of generalists (Kassen, 2002). Disturbance can create environmental heterogeneity by altering habitat and the structure of prey communities through time and space, ultimately influencing diets of higher predators (Waide, 1991; Alves et al., 2016; Trevelline et al., 2018). In ecosystems where disturbances are frequent, selection should favor generalist strategies over specialist strategies (Waide, 1991).

Traditionally, ecological studies assumed all individuals in a generalist population sample the full suite of resources used by the population (Colwell and Futuyma, 1971; Pielou, 1972). However, it has become increasingly appreciated that individual variation and specialization within generalist populations may be more common—and important—than previously recognized (Durell, 2000; DeSantis et al., 2022). Within a generalist population, individuals may specialize to take advantage of distinct resources (type A generalists, as predicted by the NVH); individuals may share the same, broad set of resources (type B generalists); or the population may be composed of individuals that fall somewhere in between these two extremes (Bolnick et al., 2003).

Here, we present a case study on the response of the Seaside Sparrow (*Ammospiza maritima*) to anthropogenic and natural disturbances, for which we used DNA metabarcoding to identify prey consumed and evaluate resource use. Seaside Sparrows are year-round residents of the coastal saltmarshes of southeast Louisiana, an area that experiences frequent large-scale storms (Keim et al., 2007). These saltmarshes were also affected by the 2010 Deepwater Horizon (DWH) oil spill disaster, which led to the oiling of 1,105 km of wetlands, with the majority (1,055 km) in Louisiana (Nixon et al., 2016). This created justifiable concern for the coastal ecosystem, as Louisiana is an important stopover site for migratory birds and home to many resident species, including approximately 3.5 million non-migratory Seaside Sparrows, comprising ~55% of their global population (Remsen et al., 2019). Only two years later, in August 2012, Hurricane Isaac made landfall. The storm inundated coastal Louisiana for several days and was later found to have redistributed oil that had come ashore from the DWH disaster (Turner et al., 2014; Zengel et al., 2015). Invertebrate communities changed following both saltmarsh oiling and hurricane disturbance (McCall and Pennings, 2012; Zengel et al., 2015; Bam et al., 2018). Accordingly, resources used by Seaside Sparrows may have changed as a result: for example, sparrows may have had access to fewer species of prey, or the reduced availability of prey may have led to an increase in competition. That is, competition might be more intense on sites with reduced prey (i.e. poorer quality sites, like oiled sites) compared to sites with more robust invertebrate communities (i.e. higher quality sites, like unoiled sites).

To assess the effect of oil contamination and hurricane disturbance on the coastal saltmarsh food web, we quantified the diets of Seaside Sparrows from 2011 to 2017 on sites with varying degrees of oil contamination by evaluating dietary diversity, richness, and overall diet composition. We compared the degrees of individual specialization to the total niche width (TNW) of Seaside Sparrows on sites throughout the study period to determine if sparrow resource use follows the predictions of NVH, that is, whether individuals specialize when competition is reduced.

2 Methods

2.1 Study sites

Sites were chosen as part of a larger project studying the effects of the Deepwater Horizon oil spill and were based on the degree of shoreline oiling as determined by the National Oceanic and Atmospheric Administration (NOAA, 2013) Shoreline Cleanup Assessment Technique (SCAT; NOAA 2013; Michel et al., 2013). SCAT maps from 3 August 2010 were used to identify oiled (n = 5) and unoiled (n = 6) sites in Bays Batiste and Sansbois, LA (both located within the larger Barataria Bay). After

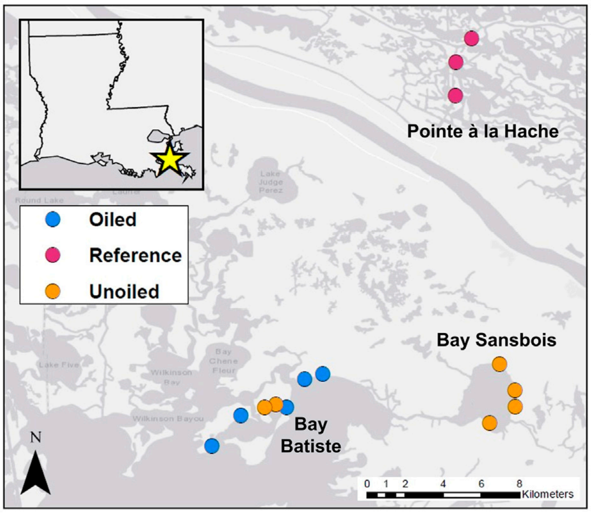

FIGURE 1
Study sites in southeastern Louisiana. Main study sites are located in Bay Batiste and nearby Bay Sansbois. Additional sites were added on the northeastern side of the Mississippi River in 2013, after Hurricane Isaac affected the entire area in 2012.

Hurricane Isaac (2012), additional sites (n = 3) were added near Pointe à la Hache, LA on the northeast side of the Mississippi River to serve as an outgroup in the event that the hurricane redistributed oil in Barataria Bay (Figure 1).

Seaside Sparrows exhibit high site fidelity, with some birds even returning to their original capture location after Hurricane Isaac (Stouffer et al., 2013). To date, no research has been conducted on the individual breeding territory or home range size of Seaside Sparrows in Louisiana; throughout their range, territory size varies from 1,203 m^2 (Oak Beach, New York; Post and Greenlaw, 1975) to 36,000 m^2 (Ochopee, Florida; Werner and Woolfenden, 1983), with variation between these sizes at different locations (Baker, 1973; Werner, 1975; Post et al., 1983; DeRagon, 1988; Marshall and Reinert, 1990). Additionally, Seaside Sparrows will forage outside of their territory during nesting periods to provision for their young, but the distance travelled was rarely over 100 m from the center of their territory in New York (Post, 1974). Given this, sites in this study were selected to minimize the possibility of individuals traveling between locations; our closest sites were approximately 1 km apart.

2.2 Sample collection

Adult Seaside Sparrows were collected in August 2011 and June 2012–2017. Birds were euthanized and dissected to collect tissues for multiple studies (Olin et al., 2017; Perez-Umphrey et al., 2018; Bonisoli-Alquati et al., 2020; Moyo et al., 2021). In all years except 2013, stomach contents were immediately removed from the stomach in the field before being flash frozen and stored in liquid nitrogen, then transferred to long-term storage at -80°C. In 2013, stomachs were flash frozen whole, with the contents inside. For samples collected in 2013, the contents were removed in the lab using clean scalpels and forceps. A total of 168 stomach content samples were collected throughout the study period (2011: n = 9, 2012: n = 19, 2013: n = 25, 2014: n = 27, 2015: n = 22, 2016: n = 33, 2017: n = 33).

2.3 DNA extraction and amplification

DNA extraction followed Vo & Jedlicka (2014); a detailed description can be found in Snider et al. (2022). The entire sample was extracted to avoid subsampling, which can lead to reduced prey detection and incomplete description of the diet (Oehm et al., 2011; Jedlicka et al., 2013). In brief, stomach contents were first weighed, and samples over 50 mg were divided into multiple extractions. For samples split into multiple extractions, the final DNA elutions were combined. All extractions included a negative control. DNA concentration was quantified with the Denovix Fluorescence dsDNA High Sensitivity Assay kit (Denovix Inc. Wilmington, DE) and samples below 0.2 ng/μL were concentrated in a vacuufuge (Savant DNA 120 SpeedVac Concentrator, Thermo Scientific) for 20 min on medium heat.

PCRs targeted two markers: COI, an amplicon from the mitochondrial genome, and 18s, an amplicon from nuclear DNA that codes for 18s rRNA. These markers were selected to complement each other, because together they detect prey across a wide range of taxa (da Silva et al., 2019). Generally, COI is useful for high-resolution prey identification (Clarke et al., 2017) but is limited in the breadth of prey it can detect. 18s is a more conserved marker that is less suited for high-resolution prey identification (Tang et al., 2012) but is able to identify a wider breadth of prey. Seaside Sparrows are a generalist species, and despite consuming both plants and animals they are thought to primarily feed on insects and other invertebrates during the summer, when samples were collected (Martin et al., 1961; Post, 1974; Post and Greenlaw, 2006). With this *a priori* knowledge of the diet, the Zeale et al. (2011) arthropod-specific primer set was chosen to identify arthropod prey, coupled with the Jarman et al. (2013) 18s primer set to identify non-arthropod organisms (i.e., da Silva et al., 2019).

PCR reactions and thermocycler conditions for COI followed Jedlicka et al. (2016). PCR reactions consisted of 1X Phusion GC Buffer, 200 μM dNTPs, 5% DMSO, 0.5 μM of each primer modified with Illumina overhangs (ZBJ-ArtF1c and ZBJ-ArtR2c; Zeale et al., 2011), 1 U Phusion High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA, United States), 0.004–4.25 ng DNA, and nH2O to 20 μl. COI thermocycler conditions followed an initial denaturation step of 98°C for

2 min; 35 cycles of 98°C for 8 s, 48.5°C for 20 s, 72°C for 30 s; and a final extension step of 72°C for 7 min. PCR reactions for 18s consisted of 1X NEBNext Ultra II Q5 master mix, 0.5 µM of each primer modified with Illumina overhangs (SSU3′F and SSU3′R; Jarman et al., 2013), 0.01–8.5 ng DNA, and nH2O to 10 µl. Thermocycler conditions for 18s followed an initial denaturation step of 98°C for 30 s; 35 cycles of 98°C for 15 s, 45°C for 30 s, 72°C for 30 s; and a final extension step of 72°C for 2 min. Both COI and 18s primers were modified to include Illumina-specific overhangs to anneal sample-specific indexes during library preparation (Illumina, 2013). PCRs were performed in triplicate, with resulting PCR products pooled before remaining library preparation steps in an effort to increase prey detection (Jedlicka et al., 2016; Alberdi et al., 2019). Negative controls were included with each PCR.

2.4 Library preparation and sequencing

Libraries were prepared following a two-step PCR protocol (Illumina, 2013). In the first PCR, detailed above, DNA was amplified by primers modified with Illumina-specific overhangs. In the second PCR, unique combinations of Illumina Nextera XT indexes (v2) (Illumina, San Diego, CA, United States) were annealed to each sample using an enrichment PCR (detailed in: Illumina 2013). For additional details on library preparation, see Snider et al. (2022). Since 18s amplicon size differed from COI, the SPRI cleanups were 2.1x and 1.5x SPRI:product concentrations, respectively. The samples used in this study were split across two sequencing lanes: the first included all COI amplicons, while the second contained all 18s amplicons. Both pooled libraries were sequenced at the Genomics Core Facility at the Pennington Biomedical Research Center (Baton Rouge, LA, United States) and quantified on an Agilent Bioanalyzer. Both libraries were spiked with 15% PhiX before being sequenced (2 × 250 bp) on an Illumina MiSeq platform with v2 reagent kit (Illumina). Reads were de-multiplexed by the sequencing facility and raw reads are available on the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra).

2.5 Bioinformatics and statistical analysis

De-multiplexed reads were imported into QIIME2 version 2021.2 (Bolyen et al., 2019) and processed. QIIME2 is a wrapper and the following programs were used within the QIIME2 pipeline: first, primer sequences were trimmed and any reads not containing these sequences were removed using Cutadapt (Martin, 2011). Next, trimmed reads were quality filtered with DADA2 (Callahan et al., 2016): chimeras were removed, Phred score quality cutoffs were implemented (COI: 30, 18s: 25), paired end reads were merged, and identical reads were collapsed into amplicon sequence variants (ASVs). The Phred cutoffs differed by marker because read quality was lower with the 18s primer set, likely because this marker is intended to cover a broader range of organisms. Finally, singletons—or ASVs that appeared only once in the dataset—were removed before taxonomy was assigned (Burgar et al., 2014).

To assign taxonomy to ASVs, two naive Bayes classifiers were trained (Bokulich et al., 2018). The 18s classifier was based on a custom 18s dataset of plant, algae, and metazoan sequences and the COI classifier was comprised of arthropod orders previously found to make up Seaside Sparrow diet (Snider et al., 2022). Both training datasets were retrieved from NCBI's GenBank using RESCRIPt (Robeson II et al., 2021); training datasets were then trimmed before each classifier was trained in order to improve ASV identification, and function *feature-classifier fit-classifier-naive-bayes* was used to train the classifiers. Once ASVs were assigned taxonomic IDs with their respective classifiers, unassigned reads were removed, as were ASVs that matched to Aves and Mammalia. Next, taxonomic ranks were edited in the following way: for COI, identifications were edited to follow Zeale et al. (2011) so that species-level IDs were made when they matched a reference at 99.4–100%, genus at 95.0–99.3%, family at 91.1–94.9%, and order at 86.0–91.0%; any taxonomic assignment with less than 86% confidence was removed. For 18s, reads were edited to follow Holovachov et al. (2017), so that species-level IDs were made when they matched a reference at 99.0–100%, genus at 96.5–98.9%, family at 90.0–96.4%, and order at 84.0–89.9%; any taxonomic assignment with less than 86% confidence was removed. Next, ASVs identified in negative controls were removed, duplicate ASVs were collapsed, and resulting read tables were imported into R (R Core Team, 2019), using R Studio (version 2022.02.3; RStudio Team 2020) for further analysis. To create the combined dataset, duplicate ASVs that were detected by both markers were collapsed within each sample.

Datasets were first visualized with an NMDS plot, accompanied by the permutational ANOVA, *Adonis*, to test for significance. Both the NMDS and *Adonis* were performed on a presence-absence matrix of all ASVs in the diet using Bray-Curtis dissimilarity in *vegan* (Oksanen et al., 2019). In addition to evaluating year and site type, the NMDS and *Adonis* were also performed to evaluate "bay" (that is, the bay each site was located in: Batiste, Sansbois, or Pointe à la Hache) to assess any possible role that location may play in similarities between diets. The function *pairwise.adonis* (Martinez Arbizu, 2017) was used as a post-hoc test to evaluate which group, if any, was significantly different from the others. For each year and site type, diversity indexes were calculated: Shannon-Wiener diversity index (H) and Simpson's diversity index (D) were calculated using the *vegan* package (Oksanen et al., 2019). For both Shannon and Simpson's diversity indexes, means were compared across years using a Kruskal–Wallis rank sum test, as the data were not normally distributed (Shapiro-Wilk, Shannon: $W = 0.95$, p-value $= 3.18 \times 10^{-5}$; Simpson: $W = 0.93$, p-value $= 6.45 \times 10^{-7}$). The mean diversity metrics were then compared across

TABLE 1 Sample size, TNW, and both metrics of individual specialization (WIC/TNW and IS) of Seaside Sparrows, broken down by year and site type. Sparrows that did not produce metabarcoding results are not included in the sample size. The average measure of total niche width (TNW) and individual specialization were calculated by WIC/TNW and through the PSicalc function (IS). All values of WIC/TNW and IS have $p < 0.05$. Note that reference sites were not established until 2013.

Site type	Year	Sample size	TNW	WIC/TNW	IS
References	2013	3	1.42	0.14	0.37
	2014	3	0.90	0.76	0.61
	2015	3	1.70	0.42	0.35
	2016	8	1.80	0.05	0.25
	2017	9	2.70	0.53	0.28
Oiled	2011	4	0.90	0.59	0.73
	2012	7	1.76	0.47	0.49
	2013	12	1.63	0.28	0.42
	2014	12	2.28	0.31	0.23
	2015	6	1.05	0.54	0.72
	2016	12	2.39	0.36	0.31
	2017	10	2.26	0.19	0.33
Unoiled	2011	5	0.96	0.25	0.78
	2012	12	2.39	0.39	0.31
	2013	9	2.21	0.15	0.32
	2014	11	2.01	0.28	0.33
	2015	11	2.18	0.30	0.29
	2016	11	2.34	0.34	0.25
	2017	12	2.80	0.44	0.30

year and site type using a pairwise Wilcoxon rank sum test with the Benjamini–Hochberg (BH) *p*-value adjustment.

Next the *RInSp* package (Zaccarelli et al., 2013) was used to calculate dietary richness (function *pop.diet*, "average" method) and total niche width (TNW) (*WTdMC* function). TNW refers to the entire suite of resources used by a population (Roughgarden, 1972); TNW values were calculated for each combination of site type and year, then the values were compared with a two-way ANOVA, with site type and year as factors (TNW ~ Site Type + Year). A Tukey's HSD test was used to evaluate the factors. TNW can be broken down into two components: the between individual component (BIC) and within individual component (WIC), such that BIC + WIC = TNW (Roughgarden, 1972). BIC refers to the variation in diet between individuals, while WIC refers to the variation in diet within individuals (Bolnick et al., 2003). WIC/TNW, or the ratio of the individual/population's resource use, is one way to measure individual specialization (Roughgarden, 1972); the metric produced by *RInSp* uses the Roughgarden (1979) formula, which incorporates the Shannon diversity index (Zaccarelli et al., 2013). Another measure of individual specialization (IS), based on Bolnick et al. (2002), was calculated (function *PSicalc*). IS describes individual specialization as the "mean proportional similarity (PSi) between individuals and population" (Bolnick et al., 2002). Both metrics of individual specialization (WIC/TNW and IS) range from zero to one, where a value close to zero represents very little overlap between individuals and the population, and a value close to one represents complete overlap (Bolnick et al., 2002).

For both WIC/TNW and IS, the *RInSp* package (Zaccarelli et al., 2013) produces a single value for each combination of year and site type. To assess the significance of these values compared to the null hypothesis that individuals are sampling equally from the full suite of resources, a Monte Carlo resampling procedure was run with 9,999 permutations (Zaccarelli et al., 2013; Lunghi et al., 2020). WIC/TNW and IS were calculated for each combination of year and site type (Table 1). A linear regression model was used to test whether there was any relationship between TNW and IS or TNW and WIC/TNW.

3 Results

3.1 Seaside Sparrow diet

Overall, 126 ASVs were detected in Seaside Sparrow gut contents. 121 ASVs were identified as Metazoa, four ASVs as Viridiplantae (green plants), and one ASV as Fungi. A majority of the metazoan ASVs were Arthropoda (100/121), while the remainder were Platyhelminthes (7/121), Mollusca (7/121), Nematoda (2/121), Annelida (1/121), Acanthocephala (1/121), Cnidaria (1/121), and Chordata (1/121). Of the 100 ASVs that were Arthropoda, 73 were identified as Insecta, 15 as Arachnida, and 10 as Malacostraca.

The most common ASV was Insecta, with a 70.81% frequency of occurrence (FOO), meaning that Insecta occurred in 70.81% of samples (Table 2). Of the 18 ASVs detected in at least 10% of samples, eight were insects, including the orders Diptera (FOO = 51.55%), Hemiptera (FOO = 39.75%), and Lepidoptera (FOO = 31.68%). Additional insect ASVs were identified to the family-level as Chironomidae (FOO = 34.78%), Crambidae (FOO = 19.88%), Dolichopodidae (FOO = 16.15%), and Tabanidae (FOO = 11.80%). Four types of Arachnids (including the families Salticidae and Lycosidae), two types of Malacostraca (including the family Ocypodidae), and one gastropod were also detected. Only two of the most common ASVs (defined as >10% frequency of occurrence) were identified as plants.

3.2 Resource use

A total of 160 birds (of the 168 collected) passed all filtering and quality control steps for at least one marker and were

TABLE 2 The most commonly occurring (>10% frequency of occurrence, or "FOO") ASVs detected in Seaside Sparrow diet. Note that each row is one ASV, independent of the other rows.

Kingdom	Phylum	Class	Order	Family	FOO (%)
Metazoa	Arthropoda	Insecta			70.81
Metazoa	Arthropoda	Malacostraca	Decapoda	Ocypodidae	59.63
Metazoa	Arthropoda	Insecta	Diptera		51.55
Metazoa	Arthropoda	Insecta	Hemiptera		39.75
Metazoa	Arthropoda	Malacostraca	Decapoda		39.13
Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	34.78
Metazoa	Arthropoda	Insecta	Lepidoptera		31.68
Metazoa	Arthropoda	Arachnida	Araneae		30.43
Viridiplantae	Streptophyta	Magnoliopsida			30.43
Metazoa	Arthropoda	Arachnida	Araneae	Salticidae	26.09
Metazoa	Arthropoda	Arachnida	Araneae	Lycosidae	25.47
Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	19.88
Metazoa	Arthropoda				16.15
Metazoa	Arthropoda	Insecta	Diptera	Dolichopodidae	16.15
Metazoa	Mollusca	Gastropoda	Littorinimorpha	Barleeiidae	15.53
Metazoa	Arthropoda	Arachnida	Araneae	Linyphiidae	11.80
Metazoa	Arthropoda	Insecta	Diptera	Tabanidae	11.80
Viridiplantae	Streptophyta				10.56

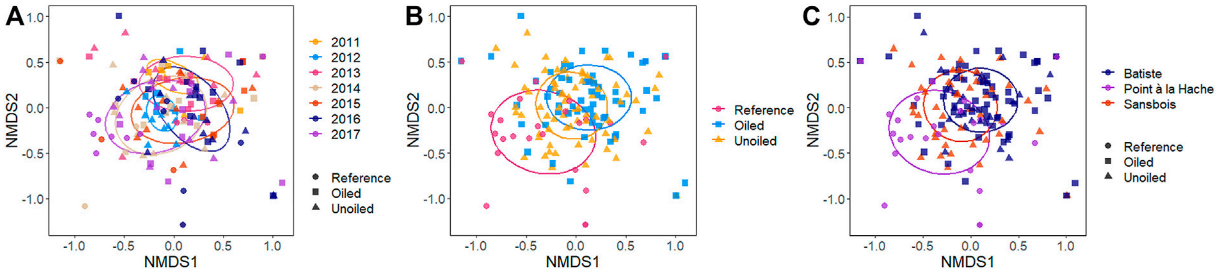

FIGURE 2
NMDS plot of all diet points coded to show the spread of diet between years **(A)**, site type **(B)**, and bay **(C)**. Samples that are more similar in composition are closer together. Three outlier points have been removed for the sake of visualization. Stress = 0.17. Ellipses show 95% confidence level.

included in the combined dataset (Table 1). *Adonis* indicated both year and site type (oiled sites) to be significant drivers of diet based on the presence/absence of ASVs (year: pseudo F = 2.79; $R^2 = 0.12$; df = 6, 153; $p = 0.001$; site type: pseudo F = 5.40, $R^2 = 0.06$, df = 2, 157 $p = 0.001$). Bay also appeared to be a significant driver of diet composition based on *Adonis* (pseudo F = 5.75, $R^2 = 0.07$, df = 2, 157, $p = 0.001$). The NMDS plots, which illustrate each individual bird's diet composition as a single point based on the ordination of all birds, show high overlap among years, with differences in the spread of points so that 2011 and 2012 are nested within later years (Figure 2A). Post-hoc tests show that diet composition in 2012 was significantly different from all other years (all adjusted *p*-values = 0.02, Supplementary Table S1), 2013 was significantly different from 2014, 2016, and 2017 (adjusted *p*-value = 2014: 0.04; 2016, 2017: 0.02; Supplementary Table S1), and 2014 was significantly different from 2017 (adjusted *p*-value = 0.04, Supplementary Table S1). The NMDS plot of site type shows reference points are clustered separately from both oiled and unoiled points (Figure 2B) and looks very similar to the NMDS plot organized by bay (Figure 2C). Post-hoc tests indicate that diet composition is significantly different among all treatments (all adjusted *p*-values = oiled vs. unoiled: 0.02, oiled vs. reference: 0.003, unoiled vs. reference: 0.003; Supplementary Table S2), as well

TABLE 3 Mean Shannon diversity indexes for each year were compared with a pairwise Wilcoxon rank sum test with the Benjamini–Hochberg (BH) *p*-value adjustment. In this table, all site types (unoiled, oiled, and reference) are pooled within the year they were collected. Significant values are in italics and indicate a statistically significant difference in the mean diversity between two years.

	2011	2012	2013	2014	2015	2016
2012	*0.04*	-	-	-	-	-
2013	0.54	0.07	-	-	-	-
2014	0.99	*0.04*	0.54	-	-	-
2015	0.54	0.11	0.94	0.54	-	-
2016	0.82	0.17	0.82	0.54	0.96	-
2017	0.07	0.54	*0.01*	*0.01*	*0.02*	*0.01*

TABLE 4 Mean Simpson diversity indexes for each year were compared with a pairwise Wilcoxon rank sum test with the Benjamini–Hochberg (BH) *p*-value adjustment. In this table, all site types (unoiled, oiled, and reference) are pooled within the year they were collected. Significant values are in italics and indicate a statistically significant difference in the mean diversity between two years.

	2011	2012	2013	2014	2015	2016
2012	0.07	-	-	-	-	-
2013	0.54	0.32	-	-	-	-
2014	1	0.06	0.37	-	-	-
2015	0.54	0.22	0.77	0.54	-	-
2016	0.77	0.25	0.77	0.56	1	-
2017	0.07	0.54	*0.03*	*0.02*	0.06	*0.02*

as all bays (all adjusted *p*-values = 0.003, Supplementary Table S3). Kruskal–Wallis rank sum tests indicated a significant difference in both Shannon and Simpson diversity between years (Shannon: chi-squared = 22.68, df = 6, *p*-value = 0.0009; Simpson: chi-squared = 19.92, df = 6, *p*-value = 0.003). There were no differences in either index among site type (chi-squared = 4.20, df = 2, *p*-value = 0.12; chi-squared = 3.49, df = 2, *p*-value = 0.17). Pairwise Wilcoxon rank sum tests showed similar trends for both Shannon and Simpson diversity, with low diversity in 2011, an increase in 2012, followed by a drop in 2013 and little change until diversity increased in 2017 (Tables 3 and 4, Figures 3A,B). Richness shows a similar trend, with a drop in the number of ASVs detected in 2013, despite the addition of reference sites that year (Figure 3C).

While there was no statistical difference in TNW between site type (ANOVA; TNW: df = 2; F = 2.21; $p = 0.16$), year was a significant factor in determining TNW (ANOVA; TNW: df = 6; F = 4.56; $p = 0.018$). Tukey's HSD indicated that 2011 was significantly different from 2016 ($p = 0.046$) and 2017 ($p = 0.009$). Across oiling status, TNW was significantly negatively correlated with individual specialization, when individual specialization was calculated with *PSicalc* (IS: Multiple $R^2 = 0.74$, Adjusted $R^2 = 0.73$, $F_{1,17} = 48.89$, $p = 2.17 \times 10^{-6}$; Figure 4D), meaning that, as TNW increases, individuals become more specialized. When calculated as WIC/TNW, there was no significant correlation with TNW (Multiple $R^2 = 0.07$, Adjusted $R^2 = 0.02$, $F_{1,17} = 1.30$, $p = 0.27$; Figure 4E).

4 Discussion

We used DNA metabarcoding to evaluate resource use by Seaside Sparrows from 2011 to 2017 on oiled, unoiled, and reference sites, with the goal of understanding how sparrows responded to the residual contamination left from the Deepwater Horizon oil spill and from ecosystem disturbance caused by Hurricane Isaac. Our DNA metabarcoding study is the most detailed account of the diet of any animal following the

FIGURE 3
Diversity and richness trends in Seaside Sparrow diet over time. In figures **(A,B)**, points represent population means and shading represent ±1 standard error. In figure **(C)**, the total number of ASVs detected per year is represented by the gray dashed line, but note that duplicate ASVs are only counted once in the overall total richness. For example, an ASV detected in both oiled and unoiled sites would only be counted once toward the total ASVs in a year. The dashed black line in all figures represents the approximate time of Hurricane Isaac making landfall.

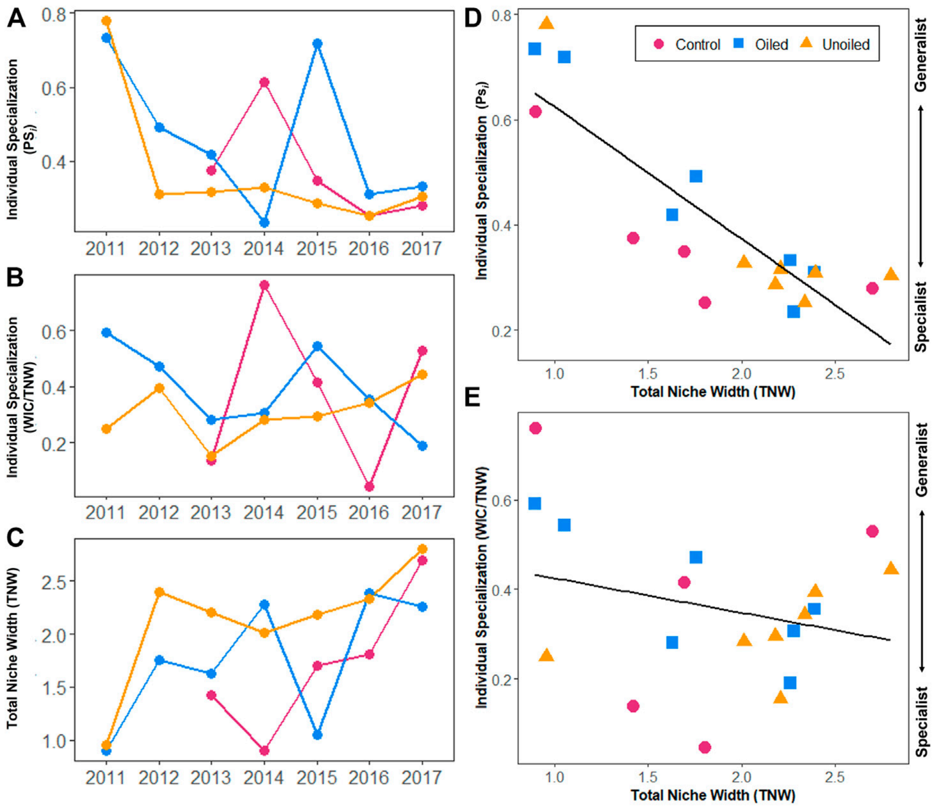

FIGURE 4
Individual specialization (IS), calculated by *PSicalc* **(A)** and WIC/TNW **(B)**, was most stable on unoiled sites throughout time, as was total niche width (TNW), **(C)**. There is a strong correlation between TNW and IS (when is calculated by *PSicalc*), meaning as a sample group's TNW increases, individuals become more specialized **(D)**. As IS values approach one, the more individual diet overlaps with the population, where one represents complete overlap. As the values approach zero, the less an individual's diet overlaps with the population and the more specialized individuals are. However, when IS calculated by WIC/TNW, there is no significant correlation with TNW **(E)**.

Deepwater Horizon spill, and the most comprehensive assessment of Seaside Sparrow diet to date. Previous studies on Seaside Sparrow diet and resource use have used traditional methods, manually observing foraging or stomach contents (Post, 1974; Post and Greenlaw, 2006; Post and Greenlaw, 2020) or isotopic approaches (Johnson, 2017; Olin et al., 2017; Johnson et al., 2019; Moyo et al., 2021). Our use of metabarcoding allows for a more detailed evaluation of diet compared to these approaches, providing a more nuanced evaluation of resource use than previously attained.

4.1 Seaside Sparrow diet

While we detected three of the prey groups identified previously *via* morphology on nearby Grand Isle, Louisiana [Orthoptera (33%), moth larvae (26%), and spiders (18%) by volume (cited without attribution in Post and Greenlaw 2020)], Orthoptera were less common than expected; we only found Orthoptera in six (out of 160) samples. We detected three Lepidoptera families (Crambidae, Eriebidae, and Tortricidae) and while metabarcoding cannot determine if these moths were consumed as adults or larvae, we expect both life stages were consumed based on previous work (Post 1974) and field observations (A. Snider, personal observation). Finally, we detected five spider families; the two families most commonly consumed (Lycosidae and Salticidae) are ground-dwelling spiders, while web-building spiders (i.e., Linyphiidae and Araneidae) (Döbel et al., 1990) were consumed less frequently.

Interestingly, we found some families of prey that could represent secondary ingestion, i.e., prey ingested by the sparrow's prey, or unintentional ingestion through consuming a parasitized prey item (da Silva et al., 2019). For example, we detected mites that could be parasites of both insect (Arrenuridae) or plant resources (Eriophyidae). We also detected several families that are presumed to be parasites of sparrows, including feather mites (Proctophyllodidae) and internal parasites such as Archiacanthocephala, Microphallidae, Acuariidae, and several

Trematodes. Although we did not detect some prey taxa reported previously (including amphipods), or only detected them minimally (isopods) (Post et al., 1983; Post and Greenlaw, 2006), our findings were broadly similar to prior Seaside Sparrow diet studies throughout their range (Post 1974; Post and Greenlaw 2020). Additionally, due to primer bias, some taxa may have been present, but were poorly amplified. For instance, ants (Formicidae) were not detected in our study, but this taxon does not amplify well with the Zeale primer set (da Silva et al., 2019), and may be consumed more frequently than we were able to determine. This could have implications for our estimates of resource use if ants are a regular component of Seaside Sparrow diet, as ant abundance was significantly reduced in 2013 (Bam et al., 2018).

4.2 Resource use

The overall alpha diversity (both for Shannon and Simpson diversity metrics) dropped sharply in 2013, corresponding with the first field season after Hurricane Isaac. We interpret this as mortality of non-aquatic prey across all sites caused by storm surge that left the marsh completely underwater for several days. Alpha diversity then stayed low and remained relatively stable from 2013 to 2016, with a sharp increase in 2017. A similar trend was seen with the overall richness of diet, with the richness declining in 2013 and increasing—possibly due to ecosystem recovery or stabilization—by the end of the sampling period in 2017. Assuming that Seaside Sparrow diet is a reflection of the available prey, both alpha diversity and richness corroborate previous findings that Hurricane Isaac altered the invertebrate community in the saltmarsh (Bam et al., 2018; Chen et al., 2020). Additionally, although TNW was not significantly different in 2013, other studies conducted post-DWH in this region have found various indicators of disturbance to the ecosystem in 2013. These include increased alkanes and polycyclic aromatic hydrocarbons in marsh sediment (Turner et al., 2014), corresponding signs of this increased contamination in different species (Paruk et al., 2016; Perez-Umphrey et al., 2018), altered invertebrate communities (Bam et al., 2018), and altered patterns of resource use (Olin et al., 2017).

We did, however, find that TNW, WIC/TNW, and IS were less consistent over time on oiled and reference sites compared to unoiled sites. Oiled and reference sites appear to have greater fluctuations across time, but these fluctuations were not synchronized. For example, there was a steep reduction in TNW on oiled sites in 2015 but not at unoiled sites; previous work has highlighted similar anomalies in vertebrate diet on these study sites in 2015 (Moyo et al., 2021). However, with no major storms or weather incidents, it is unclear what might have driven these changes.

In evaluating overall diet composition, we found that diets from oiled, unoiled, and reference sites were all significantly different from each other. In NMDS plots, the clustering of sparrows on reference sites was further from birds on both oiled and unoiled sites, likely due to the underlying ecosystem differences on either side of the Mississippi River. Reference sites were located in marshes that are slightly less brackish than those on the southwest side of the river (oiled and unoiled sites); these differences in salinity influence the plant communities on these sites which likely leads to differences in the invertebrate community and thus, sparrow diet. This could potentially explain the tighter clustering of diet in 2011 and 2012, as reference sites had not yet been established and no samples were collected from these sites in these years.

Based on data from Louisiana State University's Coastal Emergency Risks Assessment (CERA, 2022: https://cera.coastalrisk.live/), all study sites were flooded for approximately the same length of time (2–3 days) as Hurricane Isaac passed through the region (for more details on the flooding of sites west of the Mississippi river, see Stouffer et al. (2013)). Due to the counterclockwise rotation of Hurricane Isaac, sites on the east side of the Mississippi River (reference sites) experienced a greater degree of flooding compared to the oiled and unoiled sites on the west side: at peak flood depth, reference sites were inundated with over 4 m of water, compared to a peak depth of 2–3 m at oiled and unoiled sites. Since the marsh was completely submerged at all study sites for approximately the same length of time (thus affecting prey communities in the same way), we do not expect that this difference in flood depth is a contributing factor to any differences detected between site type in this study.

The difference in diet composition between bays makes it difficult to determine the underlying cause of what is driving the difference in diet composition. Sites were selected because of their degree of oiling, but the oil each site received is directly related to their location within the larger Barataria Bay system. Untangling whether diet is driven by hydrologic processes within each bay or residual contamination is an area of research that would likely benefit a host of ongoing studies taking place in this system and provide context to previous work that took place in Barataria Bay (Olin et al., 2017; Moyo et al., 2021). While Seaside Sparrows were physiologically impacted by contamination on oiled sites (Bonisoli-Alquati et al., 2016; Perez-Umphrey et al., 2018; Bonisoli-Alquati et al., 2020), diet composition might be impacted for years beyond the initial disturbance as the saltmarsh community—and prey species—recover. To know whether prey communities are inherently different between locations, or if differences are due to the various recovery processes of different species (Zengel et al., 2016a; Zengel et al., 2016b; Zengel et al., 2017; Bam et al., 2018), would require long-term community monitoring data that is not currently available. If a sparrow foraged on one site, then was captured on another during this study, this would probably cause the two sites to appear more similar in overall diet composition; since the birds in this study were not banded, there would be no way to know whether or not this occurred. In a concurrent banding study at nearby sites from 2012 to 2017, we recorded 199 recapture events representing 137 unique individuals. Over the 6 years of banding data, only 25 of those recaptures were a bird that had moved between sites, but a bird never was recaptured on the opposite side of the Mississippi River (Snider et al., unpublished data). As the banding study was conducted over the course of at least 4 months each year (March-June, as well as October 2012), with low movement among sites, we expect the likelihood of birds moving among sites does not pose a significant issue in the data presented here. Finally, we found evidence for a relationship between IS and TNW that appears to support the NVH. Assuming that oiled sites are poor quality and low in resources compared to unoiled sites, our findings indicate that individuals broaden the scope of their diets when resources are scarce: when individual specialization is measured by *PSicalc*, individuals were more generalized when TNW was low. Further, individuals on unoiled sites remained specialized and stable after 2011, in conjunction with high, stable TNW. This suggests that in higher quality areas, presumably due to reduced competition or a wider variety of resources available, individuals can specialize on unique resources. This increase in individual specialization broadens the TNW of the population, as predicted by the NVH (Bolnick et al., 2007).

Both oiled and unoiled sites showed more generalized individuals (when measured by *PSicalc*) in 2011. If oiling was the driving factor behind reduced TNW in 2011, we would expect unoiled and oiled sites to be different. This small TNW for both site types in 2011 could be influenced by a low sample size, or perhaps additional abiotic factors that were not considered when setting up the experimental design. Overall, if we assume that unoiled sites represent higher quality habitat, and that the years post-2013 represent more stable environmental conditions on these sites, it appears that Seaside Sparrows are type B generalists (sensu Bolnick et al., 2007): a generalist population made up of relatively specialized individuals when environmental conditions are stable.

Empirically, populations have responded to reduced resources with mixed results (niche width increase: Manlick and Pauli, 2020, Jesmer et al., 2020, Rahman et al., 2021; niche width decrease after disturbance: di Lascio et al., 2013). Our findings, however, agree with previous work from the same geographic area showing that Seaside Sparrow total niche width was smaller on oiled sites compared to unoiled sites in 2015 and 2017 (Moyo et al., 2021); the unoiled and oiled sites presented in this study are located in the same bays (Sansbois and Batiste) as those presented in Moyo et al. (2021). This is interesting given the different temporal scales represented by our studies. DNA metabarcoding of stomach contents provides a snapshot of sparrow diet, likely representing diet on the scale of hours or even single foraging bouts (Snider et al., 2022), whereas the blood stable isotope approach used by Moyo et al. (2021) summarizes diet over a scale of days (Podlesak et al., 2005). While DNA metabarcoding showed Seaside Sparrows had roughly the same TNW on oiled and unoiled sites in 2016, Moyo et al. (2021) showed that sparrows on unoiled sites had a wider TNW than those on oiled sites in 2016. This underscores the need for further work to assess how different approaches to studying diet may provide inconsistent results, as well as how these methods can be used in conjunction to provide comprehensive interpretations (Hoenig et al., 2022).

As pointed out by Bolnick et al. (2003), these measures of resource use (TNW, WIC/TNW, IS) need to be carefully interpreted in the context of temporal variation: while these measures can indeed be driven by behavioral or phenotypic variation between individuals, they can also be influenced by stochastic events, like patchy resource distribution (e.g., insect hatches, Hansen et al., 2020). Using blood plasma for a stable isotope study, Woo et al. (2008) found that these tissue samples—which describe diet on the scale of weeks—can suffer from temporal biases, so the stomach contents used here for DNA metabarcoding (which likely reflect diet on the scale of hours) almost certainly contain these same biases. When possible, long-term studies should be used to evaluate the diet from the same individuals repeatedly to account for this (Bolnick et al., 2003), which could easily be accomplished by collecting fecal samples from individuals throughout time. Future work in this system will benefit from the integration of multiple approaches, such as using both stable isotope and DNA-based methods together, allowing for a more holistic approach of resource use across multiple time scales.

Our findings on the resource use of Seaside Sparrows fit into the larger context of previous work on the resource use by generalists, such as that of Bolnick et al. (2003), who found support across several populations and species that generalist populations tend to be comprised of heterogenous individuals (i.e., type B generalists). Our description of Seaside Sparrow resource use could also be influenced by some key factors, including high rates of ecosystem disturbance, low interspecific competition, and low resource diversity. First, areas of frequent disturbance should favor generalists (Kassen, 2002) and it is likely that this is an important factor in the Seaside Sparrow's southern range. On average, southeast Louisiana experiences a tropical storm or hurricane every 3 years (Keim et al., 2007). Second, Seaside Sparrows are often abundant in the Louisiana saltmarshes, with few other terrestrial bird species present. Under the NVH, Seaside Sparrow individuals should be able to take advantage of the available resources through individuals specializing on unique resources (Van Valen, 1965). Finally, saltmarshes are highly productive habitats; however, they are surprisingly low in species diversity (Odum, 1988; Silliman, 2014). In low diversity environments, like saltmarshes, individuals in generalist populations are more specialized (Tinker et al., 2008; Robinson and Strauss 2020). Given these factors, Seaside Sparrows may have evolved a generalist dietary strategy, where individuals are able to take advantage of unique resources in less disturbed areas, using dietary plasticity as an adaptation to periodic ecosystem disturbance.

Data availability statement

Data are publicly available through NCBI Sequence Read Archive at https://www.ncbi.nlm.nih.gov/sra, BioProject: PRJNA629391, Accession Numbers: SAMN14775163-SAMN14775290 and SAMN31697890-SAMN31698083. Further inquiries can be directed to the corresponding author.

Ethics statement

Seaside Sparrows were handled and collected under USFWS permits 22648 and MB095918-0, LDWF permits LNHP-12-023 (2012), LNHP-13-059 (2013), LNHP-14-051 (2014), LNHP-15-033 (2015), LNHP-16-048 (2016), and LNHP-17-039 (2017). Methods were approved by the Institutional Animal Care and Use Committee of the Louisiana State University AgCenter, permits AE2011-04 (2011-2014), A2012-05 (2012-2014), and A2015-04 (2015-2018). 2011 Seaside Sparrows were collected under collection permits USFWS MB679782 and LDWF LNHP11-068.

Author contributions

Conceived the research idea and design: PS, ST, and AS. Collected data and performed field work: AP-U, AB-A, SW, AS. Wrote first draft of manuscript: AS. Provided substantial input and editing to manuscript: ST, PS, AB-A, SW, and AP-U. Developed and designed methods: AS. Analyzed the data: AS. Contributed substantial materials, resources, or funding: PS and ST.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor PL declared a past co-authorship with the authors.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.978325/full#supplementary-material

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Towards relevant ecological experiments and assessments of coastal oil spill effects: Insights from the 2010 Deepwater Horizon oil spill

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Introduction

The health of coastal environments is at risk due to exploitation of economically valuable minerals, including oil and natural gas. Impacts to biota occur both directly (e.g., chemical toxicity, foraging/growth changes, behavioral alterations; McDonald et al., 2022) and indirectly (e.g., environmental conditions, cleanup/mitigation activities, food web alterations; McCann et al., 2017; Lewis et al., 2022). Despite acknowledgement of adverse effects, our understanding of oil spill impacts has been driven by unplanned disasters, including Exxon Valdez (Peterson et al., 2003), Ixtoc (Soto et al., 2014), Arthur Kill (Burger 1994), Falmouth Harbor (Reddy et al., 2002), and numerous smaller spills (Rozas et al., 2000; Roth and Baltz 2009). Smaller spills are frequent, with hundreds reported monthly in the United States (USCG National Response Center reports >20,000 calls per year) and many are sufficiently small enough to not initiate management response. In Louisiana (United States), oil spill response efforts are coordinated by the Louisiana Oil Spill Coordinator's Office (LOSCO) and in 2021, 90 spills triggered on-scene response activities by LOSCO (https://data.losco.org/).

On 20-April-2010, an explosion aboard the *Deepwater Horizon* (DwH) drilling platform off the Louisiana coast created fires resulting in the uncontrolled release of petroleum into the Gulf of Mexico (GoM). The depth, ~1500 m, was an obstacle preventing the immediate capping of the damaged wellhead, which leaked for 87 days. A significant portion of the five million+ barrels released (McNutt et al., 2012) inundated wetlands throughout Louisiana, Mississippi, Alabama, and Florida (Michel et al., 2013; Nixon et al., 2016). The volume, temporal and spatial heterogeneity in oil coverage, disproportionate weathering and unknown organism tolerances, plus unintended impacts from mitigation activities, all limited accurate damage assessments. To better understand DwH impacts, the Gulf of Mexico Research Initiative (GoMRI) was established and administered approximately $500 M USD, largely for DwH research, data archival efforts, and outreach over a 10-year period (Shepherd et al., 2016).

Here, we draw on archived GOMRI studies of DwH impacts in nearshore, coastal ecosystems to: 1) highlight the volume and diversity of information obtained *via* funding, 2) discuss major uncertainties and variability in impact determinations, and 3) provide recommendations to improve future environmental assessments. Given continued and growing coastal development and exploitation of nearshore resources in GoM waters, unexpected disasters will likely reoccur, and discussion of major challenges will guide future experimental and empirical determinations.

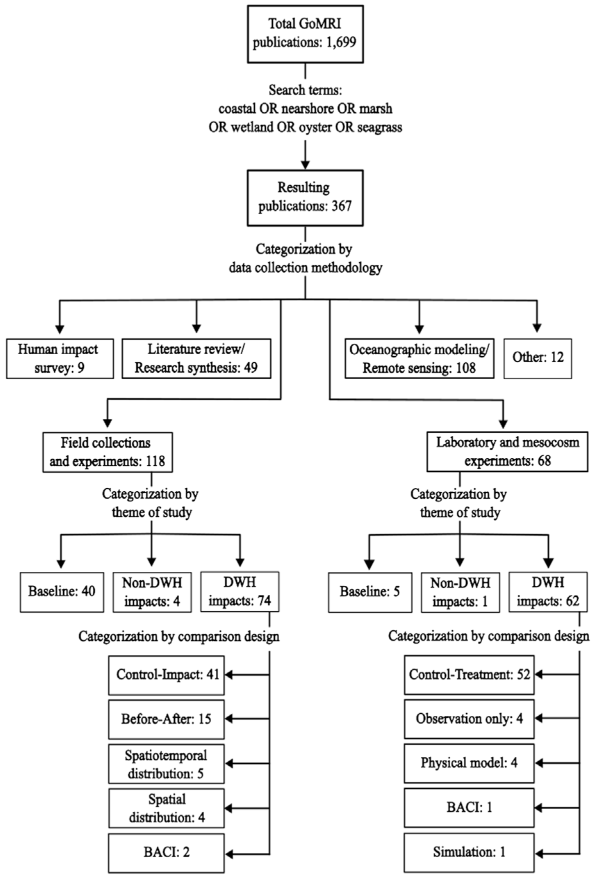

FIGURE 1
Publications deposited in the online GoMRI publication repository database were searched for nearshore studies using the Boolean search terms coastal OR nearshore OR marsh OR wetland OR oyster OR seagrass. The resulting 367 publications were then categorized by data collection methodology, with publications that could be described as "Field collections and experiments" and "Laboratory and mesocosm experiments" subsequently categorized by general study theme. Field collection/experiment and lab/mesocosm studies that investigated DWH impacts were then further categorized by comparison design, resulting in five general comparison categories for each of these two collection methods. Note: Although not listed out in this figure, the 'Other' collection method category consisted of the following method descriptions: public outreach (4 publications), perspective (2 publications), dataset (1 publication), and technical advancement (1 publication). Additionally, DWH was a study theme in all other collection method categories, with 9 DWH themed publications in "Human impact survey," 36 publications in "Literature review/Research synthesis," 25 publications in "Oceanographic modeling/Remote sensing," and 7 publications in "Other."

What have we learned? Success stories post-Deepwater Horizon

Numerous revelations resulted from GoMRI-funded research. The GoMRI enterprise, as a funding instrument, was successful in creating an unprecedented network of researchers focused on coastal issues ranging from oil/human impacts and petroleum safety to ecological effects. GoMRI funding supported over 1,500 peer-reviewed articles, of which almost 400 focused on inshore/nearshore ecosystems (Figure 1). These studies were used in our assessment below to further identify variability in study designs.

GoMRI funding improved our understanding of the region's physical, biological, and geochemical importance-a demonstrable success story. This included collections of the first baseline inventories in decades, critical documentation that can serve as baseline reference sites for future stressors. Other noteworthy contributions highlighted the enhanced understanding of deep sea population dynamics and community resilience following this disturbance, quantification of nearshore ecosystem properties, and improved understanding of oil chemistry, especially as it relates to physical/meteorological forcings, weathering/degradation, and effects in coastal ecosystems (Hsing et al., 2013; Felder et al., 2014; Prouty et al., 2016; Rabalais and Turner 2016; Murawski et al., 2021; Overton et al., 2022).

The unprecedented findings were driven by large, collaborative research teams ("consortia"). Funding for consortia supported over 4,000 scientists and support personnel, including faculty (1,106), postdoctoral researchers (367), graduate (1,205) and undergraduate (1,050) students, and research staff (718). The consortia concept was novel and intended to create multidisciplinary teams, and linked researchers from 43 states and 18 different countries. These consortia also facilitated rapid responses to other disasters including the Hercules gas well blowout (Joye et al., 2014) and Hurricanes Isaac and Harvey (Curcic et al., 2016; Steichen et al., 2020), as well as ongoing research collaborations not related to DwH. As a result of GOMRI funding, we now have a greater understanding of the benefits of large and diverse research networks, and the roadblocks historically limiting product generation.

Lessons learned: Improving oil impact assessments

Incorporating confounding variables

Due to the mensurative, observational nature of field measurements, the possibility of unconsidered/unmeasured drivers exists and potentially obfuscated DwH impact assessments (*sensu* Osenberg and Schmitt 1996; Stewart-Oaten et al., 1992). In other words, the lack of controlled comparisons reduces the ability to assign causality. Potentially confounding variables include the inconsistencies in oiling intensity, weathering, and inconsistent analytical methods, including often-overlooked indirect connections (e.g., food web alterations, changes in connectivity, habitat loss, and other environmental responses). Here, we provide a few examples of possible confounding variables that should be considered in future assessments.

DwH oil (e.g., Sweet Louisiana Crude, MC-252, etc.) was a unique blend of >10,000 chemical compounds, ranging from more toxic, lighter aromatics to less toxic, heavier, and stable compounds (Overton et al., 2022). Lighter compounds (e.g., naphthalenes) precipitate rapidly during the weathering process (*via* wave action, ultraviolet light degradation, and other chemical processes), while more stable compounds (e.g., asphaltenes) resist weathering. Remedial management actions following the release of oil offshore were largely focused on increasing oil weathering (e.g., Corexit dispersant application to increase oil surface area and expedite microbial degradation, burning of surface oil, and application of booms for oil collection). As oil moved inshore, its chemical composition changed, with some exceptions. Oil protected inside of tar balls and later resuspended from buried sediments were less weathered, as documented in remobilized oil after Hurricane Isaac in 2012 (Zengel et al., 2015; Bam et al., 2018). Overall, however, oil that arrived inshore became highly weathered and compositionally different than when initially released at the wellhead. This variable oil weathering is an important aspect that merits consideration in future impact assessments and accounting for during lab-based trials, as important differences in responses can result (Martin 2017). Moreover, oil characterization techniques (total petroleum hydrocarbons, fingerprinting, etc.) varies considerably among studies, making cross-study comparisons difficult. Compounds measured in total petroleum estimates can also vary among labs and employed instruments have variable detection limits and accuracy.

Several non-oil factors can also introduce confounding variables. Prevailing meteorological and hydrological conditions controlled DwH oil delivery to coastal wetlands, and deposition may thus occur in areas already prone to erosion from wind/wave energy or in areas where recruitment of specific benthic organisms may be prevalent. To keep oil out of coastal wetlands, the State of Louisiana diverted Mississippi River water into estuaries, an action of great importance because salinity is a key driver of biological composition, and this also caused physiological stress to estuarine organisms (e.g., oysters, Powers et al., 2017). Crews conducting remedial (Martinez et al., 2012) or cleaning activities of oiled marshes often trampled vegetation causing additional harm unnecessary in lightly oiled areas (Zengel et al., 2015). At higher trophic levels, cessation of commercial/recreational fishing likely had important cascading effects throughout GoM food webs (Fodrie et al., 2014; Schaefer et al., 2016; Martin et al., 2020). Thus, not all effects were due to oil toxicity.

Of the total 177 DwH-related GoMRI publications that focused on nearshore habitats (excluding literature reviews), field-based and laboratory/mesocosm comparisons respectively comprised just 42% and 38% of published research (Figure 1), making the need for consideration of previously mentioned study concerns even more important. Revealing field patterns is often difficult due to multicollinearity (Graham 2003), but reduced realism in laboratory environments offers a philosophical tradeoff for determining true effects (Diamond, 1986). We suggest that pluralistic approaches can take advantage of positive aspects of each technique (e.g., pairing field and lab to account for variability while retaining realism) and provide more definitive results.

Appropriate study designs

The unpredictable spatial/temporal heterogeneity of unplanned disturbances make robust assessments difficult (Stewart-Oaten et al., 1992; Schmitt and Osenberg 1996). DwH studies often lacked unimpacted baseline information (only ~23% of field studies incorporated baseline data, Figure 1) thus relying on control-impact comparisons, many of which lacked appropriate controls and/or sufficient experimental designs to conduct impact assessments. Few field assessments employed before-after-control impact (BACI) studies, or their suggested alternative approaches, often preferred for determining impacts (~3%, Figure 1). Given the lack of baseline data, characterizing temporal variability in oiling is critical as many comparisons were made years after DwH. Many field studies (~52%) inferred oiling status from anecdotal or broad oil categorization assessments (e.g., Shoreline Cleanup Assessment Team, SCAT; Michel et al., 2013) to determine treatment identities. In other cases, researchers leveraged prior available information to serve as surrogate baseline (pre-impact) data. These before-after studies represented only ~20% of publications in our survey of nearshore GoMRI publications (Figure 1). Given the numerous anthropogenic stressors affecting GoM estuaries, coupled with oil response/mitigation impacts (described above), before-after comparisons should be considered as the successional trajectory of many ecosystems change naturally (Schmitt and Osenberg 1996). The presence of multiple, interacting, and often non-additive stressors also challenge the power of studies sacrificing time or space components. Thus, other stressors (e.g., dredging, climate factors, etc.) may be incorrectly attributed to oil impacts. This supports the use of paired laboratory experiments to investigate potential confounding factors.

Similarly, the design of laboratory assessments requires careful consideration. Oil has a variety of effects, making it difficult to design rigorous laboratory studies. For example, degrading oil can produce air-borne chemicals that influence non-oil treatments leading to questionable replicate independence (Hurlbert, 1984). Moreover, oil can interact with plastics used in tanks to produce novel toxic chemicals that may impact the survival of experimental organisms (de Soysa et al., 2012). Oil treatments should be reflective of natural conditions, with relevant concentrations, compositions, and weathering to mimic impacted areas (Overton et al., 2022). Trial duration should also be considered, as oil effects in contained systems may overestimate exposure by reducing oil dilution or preventing emigration that occurs in nature. Representative species that minimize handling stress, an important experimental artifact, while maximizing ecological relevance (e.g., species that are appropriate as indicators, or have some other intrinsic value due to abundance or importance) should be considered. Done appropriately, lab studies provide great insight and supplement field observations, thereby lending increased confidence in mensurative field patterns (Diamond, 1986; Schmitt and Osenberg 1996).

Discussion: A way forward and future considerations

In the years following DwH, researchers have made significant contributions to our understanding of nearshore GoM ecosystems, and in the process made advances in diverse areas of study. Petroleum exploitation in the GoM will only increase due to international tension, and thus it is in the best interest of researchers, managers, conservationists, and the general public alike to reflect on the DwH experience, both positive and negative, to better improve the response to future spills. Given this, we conclude with these recommendations based on our assessment of work to date on nearshore GoM ecosystems.

- The novel use of large consortia stimulated multi-institution collaboration, and future efforts should determine when and why these efforts were successful (or not), including best practices, obstacles, and optimization of group dynamics and participation.
- While a greater understanding of oil chemistry has been achieved, significant variability in measurement techniques, groupings (e.g., total petroleum hydrocarbons), and analytical

detection limits can be improved by adopting standardized approaches. This will facilitate future cross-study comparisons and meta-analyses.
- Attribution of oil concentrations in field conditions can be problematic without appropriate caution. For example, large scale oil patterns (e.g., SCAT) may be inappropriate when used to determine small plot impacts or inappropriate oil treatments due to temporal factors when oil concentrations are known to change.
- Field assessments are hampered by multiple unmeasured/unconsidered variables and these additional stressors require consideration and acknowledgement.
- A system of environmental monitoring, including experiments, to determine baseline conditions across a broad geographic area should be implemented to avoid lack of pre-disturbance information which led to suboptimal designs during DwH. This will facilitate preferred designs (e.g., BACI) and is especially true given the continued shifting baselines and frequency of disturbances.
- Laboratory studies should seek appropriate species, methods, and oil concentrations/weatherings to maintain realism.
- The pairing of observational field data with appropriate lab studies can generate robust assessments.

Author contributions

CM framed the study with input from JV and BR. CM wrote the initial draft of the text. AM created and edited the figure. All authors contributed portions of the text, reviewed, edited, and revised content.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.1092097/full#supplementary-material

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Establishing a benthic macrofaunal baseline for the sandy shoreline ecosystem within the Gulf Islands National Seashore in response to the DwH oil spill

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Sandy shorelines present a first line of defense against the catastrophic effects of storms and oil spills within the coastal zone of the northern Gulf of Mexico. Immediately following the DwH oil spill prior to any spill related impacts, we conducted a rapid response survey of the sandy shoreline benthic macrofauna from throughout the National Park Service - Gulf Islands National Seashore (GINS) in Mississippi and Florida. To characterize pre-spill macrofaunal assemblages, we surveyed seven barrier island or peninsular areas comprising nine exposed and 12 protected shoreline sites. A comparable benthic macrofaunal inventory had been conducted 17 years earlier using a parallel study design. The primary objective of this study was to distinguish hierarchical spatiotemporal scales of macrofaunal variation within the 1993 and 2010 GINS data. We hypothesized that the 1993 GINS macrofaunal inventory baseline was stable, despite multiple disturbances by large storms within the intervening 17-year period. Additionally, the relative importance of hierarchical spatial scales of macrofaunal dissimilarity was examined so suitable scales of macrofaunal variation could be identified for assessments of stressor effects at commensurate scales. An Implicit Nested Mixed Model PERMANOVA using Type 1 sequential Sum of Squares delineated variation components of nested scales which ranked Station > Shore Side > Site > Habitat > District > Year. The Year main factor had the smallest effect on macrofaunal variation, confirming that the 1993 GINS macrofaunal inventory can serve as the foundation for a robust baseline including both the 1993 and the 2010 macrofaunal data for the GINS. A literal Hierarchical Nested Mixed Model PERMANOVA using Type 1 sequential Sum of Squares (SS) partitioned effects among nested factors and their interactions. Definitive macrofaunal variation was expressed for all combinations of two levels for each of the three spatially nested fixed factors, District, Shore Side, and Habitat. Variation in macrofaunal dissimilarity for combined levels of fixed factors reflected corresponding differences in the macrofauna. The use of sandy shoreline macrofaunal assemblages as ecological indicators would fulfill the need to focus on cumulative effects of oil spills and should be eminently tractable when responses and impacts are considered on commensurate scales.

KEYWORDS

benthic macrofauna, sandy shoreline, ecological indicators, shifting baseline syndrome, DWH oil spill

1 Introduction

As a major type of land margin feature, sandy shorelines comprise 75 percent of the coastline worldwide (Lercari and Defeo, 2003). Sandy shorelines form wherever inorganic sediments are transported to the sea by riverine discharge, distributed and deposited by longshore currents and wave action. They may stretch great distances often in conjunction with the formation of barrier island chains acting as coastal bulwarks. As such, sandy shorelines provide the first line of defense against catastrophic effects of storms and oil spills within the coastal zone (Beyer et al., 2016). Sandy shorelines provide important ecosystem services, including coastal protection, biological diversity, and trophic support of seabirds and fishes (Amaral et al., 2016; Maslo et al., 2019) as well as recreation and tourism (Klein et al., 2004; Schlacher et al., 2007; Amaral et al., 2016). However, environmental stressors increasingly threaten ecosystem services associated with sandy shorelines worldwide (Schlacher et al., 2007; Defeo et al., 2008).

Landscape-scale disturbances often afflict sandy shoreline ecosystems. Both prolonged press stressors and temporary pulse stressors (sensu Holling, 1973) influence sandy shoreline ecosystems over wide-ranging spatiotemporal scales spanning from several to thousands of km and from weeks to centuries (Defeo et al., 2008). Direct geomorphological alterations to sandy shorelines include the creation of shipping channels (Morton, 2008) which disrupt sediment transport processes (Fanini et al., 2009; Jones et al., 2011). Erosion and inundation due to storms and sea level rise due to global warming increasingly impinge upon sandy shorelines (Defeo et al., 2008). Consequently, the ecological condition and resilience of sandy shoreline ecosystems is a major concern (Jones et al., 2011).

Spatiotemporal scaling of variation in benthic macrofauna is increasingly regarded as key to understanding environmental impacts on sandy shore ecosystems (Rakocinski et al., 1998; Defeo and McLachlan, 2005; Rodil et al., 2012; Veiga et al., 2014; Pandey and Thiruchitrambalam, 2019). Morphological and behavioral adaptations have made resident macrofauna resistant to frequent natural disturbance within dynamic sandy shoreline benthic habitats (McLachlan and Jaramillo, 1995; Jones et al., 2011; Veiga et al., 2014). Thus, natural spatial patterns in sandy shoreline macrofaunal assemblages can serve as baseline indicators of excessive environmental disturbance and stress if reference conditions can be defined at the appropriate spatiotemporal scales (Bessa et al., 2013). Sandy shore macrofaunal assemblages are negatively affected at different spatiotemporal scales of impact by various stressors, including copper pollution (Arntz et al., 1987; Ramirez et al., 2005), oil contamination (Rabalais and Flint, 1983; Short et al., 2004; Owens et al., 2008), tar pollution (Shiber, 1989; Abu-Hilal and Khordagui, 2007), beach renourishment (Goldberg, 1988; Rakocinski et al., 1996; Peterson and Bishop, 2005; Jones et al., 2008; Leewis et al., 2012; Lercari and Defeo, 2003; Leewis et al., 2012; Soga and Gaston, 2018), physical structures (Kraus and McDougal, 1996; Jaramillo et al., 2002), coastal erosion (Masalu, 2002), storms and hurricanes (Rakocinski et al., 2000), and El Nino events (Castilla, 1983). Despite such studies, the spatiotemporal limits of natural variation in sandy shoreline benthic macrofauna remain poorly known (Defeo et al., 2008). Defining boundaries and threshold limits of natural macrofaunal variation within sandy shoreline ecosystems is both challenging and important.

The frequency and extent of sampling needed for defining proper baseline conditions will vary among ecosystems and biomes. Whether and how the shifting baseline syndrome (SBS) applies to defining the limits of natural variation in sandy shore macrofaunal assemblages is a primary concern (Bessa et al., 2013). The SBS refers to how background environmental degradation may elicit continuously changing reference conditions (Pauly, 1995; Soga and Gaston, 2018). The SBS problem challenges resource managers and policymakers. Unacknowledged SBS effects may confound the detection of contemporaneous macrofaunal impacts. The SBS challenge can be met by monitoring reference indicators over a sufficient time frame for detecting effects of shifting background conditions. Long-term comparisons can help determine the proper temporal scale for using monitoring data to define a dependable reference condition. Moreover, reference variation needs to be framed at the correct scale for assessing responses to different environmental stressors (Defeo and McLachlan, 2005). Greater flexibility requires more widespread and frequent sampling over longer time frames. The correct nested scale of macrofaunal variation needs to agree with the extent of exposure to the stressor of concern (Rakocinski et al., 1998).

Occurring within the northern Gulf of Mexico between April and September of 2010, the Deepwater Horizon (DwH) oil spill ranks as the biggest marine oil spill (Beyer et al., 2016). Baseline macrofaunal assemblages of sandy shoreline habitats within the Gulf Islands National Seashore (GINS) of the US National Park Service (NPS) needed to be characterized with respect to the DwH oil spill. This extensive natural sandy shoreline ecosystem offers an appropriate context for environmental assessment (Rakocinski et al., 1993; Rakocinski et al., 1996; Rakocinski et al., 1998; Rakocinski et al., 2000). A previous extensive seasonal macrofaunal inventory of the GINS in 1993 provided an operational baseline for the assessment of storm effects in 1996. However, use of the 1993 macrofaunal inventory as a baseline in the face of the 2010 DwH oil spill was questionable given the plausibility of baseline shifts over the intervening 17-year period. Notwithstanding the specialized nature of sandy shoreline macrofaunal taxa within a frequently disturbed setting, it was not known whether sandy shore assemblages were resilient enough to have completely recovered from the major large-scale hurricanes, Ivan and Katrina, occurring 5–6 years prior to the 2010 DwH oil spill. Together, these two major storms impacted both GINS districts in Florida and Mississippi. It was also essential to characterize the macrofaunal baseline with respect to the DwH oil spill at different hierarchical spatial scales corresponding to environmental factors. Thus a pre-DwH GINS macrofaunal survey was conducted right before the oil spill in spring of 2010. The 2010 pre-DwH macrofaunal survey was used to address the question of whether baseline macrofaunal assemblages shifted between 1993 and 2010 when considered at various spatial scales. For macrofaunal assemblages to serve as reliable indicators, they need to be examined within a hierarchical spatiotemporal context (Defeo and McLachlan, 2005).

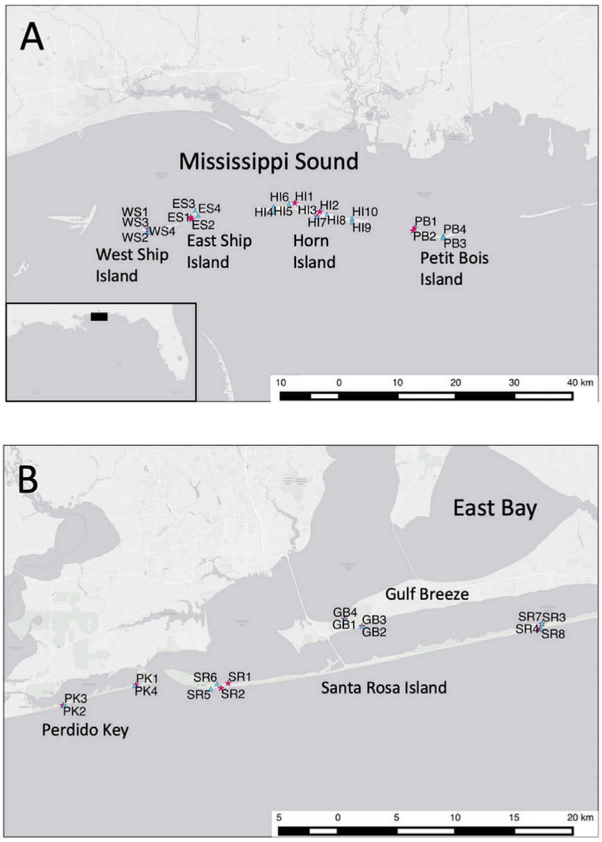

FIGURE 1
(A) Map of western district of the Gulf Islands National Seashore (GINS) study area, showing sites sampled for the 1993 macrofaunal inventory (red stars) and the 2010 macrofaunal pre-oil DwH survey (blue triangles); **(B)**. Map of eastern district of the GINS study area, showing sites sampled for the 1993 macrofaunal inventory (red stars) and the 2010 macrofaunal pre-oil DwH survey (blue triangles).

To determine whether the 1993 GINS baseline was still valid, we examined hierarchical spatiotemporal scales of natural variation in macrofaunal assemblages of sandy shorelines for the combined 1993 and 2010 macrofaunal GINS datasets. We hypothesized that the 1993 GINS macrofaunal inventory baseline was stable, notwithstanding multiple disturbances by large storms within the intervening 17-year period. Additionally, the relative importance of variability in macrofaunal assemblage structure was examined across hierarchical spatial scales reflecting meaningful subdivisions of the sandy shore environment. Thus, essential scales of macrofaunal variation could be identified and validated for matching up with scales of stressor effects. Finally, a robust baseline was characterized using both datasets.

2 Study area

The US NPS Gulf Islands National Seashore (GINS) embodies natural shoreline located across two disjunct subregions separated by Mobile Bay, including a western district (i.e., Mississippi district) in Mississippi and an eastern district (i.e., Florida district) in the west Florida panhandle near the Alabama border (Figure 1). The two districts encompass land margin ecosystems including barrier islands and embayments separated by ~ 100 km of intervening coastline. Barrier-islands include West Ship, East Ship, Horn, and Petit Bois Islands within the western Mississippi district, as well as Perdido Key and Santa Rosa Islands within the eastern Florida district. Four embayments intersecting or entirely within the GINS include Mississippi Sound in the western district, as well as smaller Big Lagoon, Santa Rosa Sound, and Pensacola Bay in the eastern district. The GINS region is influenced by major watersheds, including the Pascagoula River in the western district and the Escambia River in the eastern district. Roughly 85,000 acres of natural habitats exist within 169 km of GINS shoreline, comprising both high-energy exposed and protected medium grain quartz sand beaches, as well as adjacent grass beds and saltmarshes. Protected beaches within this region are subsidized by organic material from neighboring grassbed and salt marsh habitats.

3 Materials and methods

3.1 Field sampling

A quantitative seasonal inventory of the GINS sandy shoreline macrofauna was conducted in 1993 (Rakocinski et al., 1998). Seventeen years later, during the DwH oil spill event and before any oil impacts occurred, a pre-DwH survey of the GINS sandy shoreline macrofauna was conducted in May 2010 (Figure 1). The study design for the 2010 DwH survey was predicated on the 1993 GINS macrofaunal inventory, which comprised 148 station events including 52 collections in spring and summer, in addition to 22 station events in summer and winter. To attain a spatially balanced design for this study, the 1993 data represented spring macrofaunal samples taken from 5 April to 18 May at 51 stations among 17 sites (i.e., 10 protected, seven exposed) and the 2010 data represented samples taken from 1 May to 28 May in 2010 at 42 stations among 21 sites (i.e., 12 protected, nine exposed). In both years, at least one site was sampled from exposed and protected sites from each barrier island or mainland area where natural sandy shoreline occurred within the GINS (Figure 1). In 1993, nine sites represented the western district, including one exposed and one protected site each at West Ship Island, East Ship Island, and Petit Bois Island, as well as two protected and one exposed sites from Horn Island. Eight sites represented the eastern district in 1993, including one exposed and one protected site each from Perdido Key, two protected sites from Gulf Breeze, and two protected and two exposed sites from Santa Rosa Island. In 2010, thirteen sites represented the western district, including one exposed and one protected site each from West Ship Island, East Ship Island, and Petit Bois Island, as well as seven protected and six exposed sites from Horn Island. Eight sites represented the eastern district in 2010, including one exposed and one protected site each from Perdido Key, two protected sites from Gulf Breeze, and two protected and two exposed sites from Santa Rosa Island. Locations for many sites were similar for both surveys, but each site-event was regarded as independent, especially given the 17-year gap between the 1993 inventory and the 2010 survey. Many of our study sites were subsequently exposed to crude oil from the DwH spill.

Benthic macrofauna were sampled from the upper 20–25 cm of sediment using a 0.016 m^2 stainless steel box-corer (12.5 cm on a side; 1/64 m^2), with the top covered by 0.5 mm stainless mesh (Saloman and

Naughton 1977). In 1993, eight box-core samples were taken from each of three stations located within the intertidal swash zone, and from subtidal habitat at 5 and 15 m from the shoreline. In 2010, five box-core samples were taken from each of two stations located within the intertidal swash zone and the subtidal zone at 8 m from the shoreline. Box-core samples were spaced evenly by 1–2 m, parallel to the shoreline. Small macrofaunal organisms were removed by elutriating sediment five times through a 0.5 mm sieve using a dilute formalin solution. Remaining material was washed through a 1.0 mm sieve to recover larger heavy organisms like bivalves. This procedure is effective in removing more than 95% of the organisms (Rakocinski et al., 1991). Processed samples were labeled, fixed with 5%–10% formalin (i.e., depending on the amount of organic material), and returned to the laboratory. Collection information recorded for each event included latitude and longitude, salinity, dissolved oxygen (DO) (mg l^{-1}), pH, and water temperature (°C) at 1 m depth, as well as meteorological conditions, including wind speed and direction, tide stage, sea state, and cloud cover.

3.2 Lab processing

In the laboratory, macrofauna from each sample were sorted into major groups and transferred to 70% ethanol. Next, specimens were identified to the lowest practical taxonomic level (i.e., typically species) using existing literature and enumerated. Letters were used to represent undescribed species. A voucher collection of all nominal taxa was deposited in the USM GCRL zoology museum. A complete list of taxa for this study is presented in the Supplementary Appendix.

3.3 Data analysis

Macrofaunal community patterns and the relative importance of spatiotemporal scales of macrofaunal variation indicated by the combined 1993 and 2010 datasets were examined to define a robust baseline. Macrofaunal data represented 17 intertidal and 34 subtidal stations from 17 sites sampled in spring of the 1993 GINS inventory and 21 intertidal and 21 subtidal stations from 21 sites sampled during the 2010 DwH GINS survey. To match sampling effort at stations between 1993 and 2010, macrofaunal data were used from up to five randomly selected box-core samples for each of the 51 spring 1993 station events. The resulting data matrix for this study represented 159 macrofaunal taxa obtained from 459 box-core samples and 93 station events.

Statistical analyses were conducted using individual box-core samples, whereas some ordinations and graphical procedures were conducted at the station level, in terms of average abundances, or at the site level, in terms of centroids. Prior to calculation of Bray-Curtis dissimilarity among box-core samples or station events, macrofaunal abundances were fourth root transformed in PRIMER-e v7 to deemphasize the influence of large disparities in abundances. All macrofaunal taxa were included as 1) it is acceptable to retain rare taxa in nMDS (Anderson et al., 2008), and 2) to enable full sensitivity to the detection of baseline shifts or responses to stressors.

A preliminary station-level nMDS was used to assess macrofaunal dissimilarities among habitat zones defined by swash intertidal, 5 m subtidal, 8 m subtidal and 15 m subtidal stations. A Hierarchical Group-Average cluster analysis (Clarke and Gorley, 2015) classified station events as defined by habitat zone. Resulting group centroids were plotted for 5m, 8m, and 15 m zones along with similarity envelopes depicting group-average subdivisions superimposed within 2-D nMDS space to validate intertidal and subtidal habitat designations.

Salient macrofaunal groupings were identified from the Similarity Profile (SIMPROF) permutation procedure based on a Hierarchical Group-Average cluster analysis using an index of association for the 30 'most important species' as defined by a per-sample threshold percentage in PRIMER-e 7 (Clarke and Gorley, 2015). The 30 'most important' taxa were classified into eight coherent (i.e., statistically indistinguishable across all samples) indicator groups (i.e., SFGv groups) by Type 3 SIMPROF tests. Affinities of the 30 selected taxa were illustrated by plotting the relative positions of species coordinates along with their group identities within a follow-up nMDS ordination.

A Hierarchical Nested Mixed Model PERMANOVA using Type 1 sequential Sum of Squares (SS) was used to evaluate progressively inclusive sources of variation in macrofaunal dissimilarity. Type 1 SS indicates that variation attributed to specific terms in the model is exclusive and model-order-dependent. As such, successive terms explained remaining variation at larger scales, after preceding terms accounted for the smaller scales (Anderson et al., 2008). Accordingly, progressively inclusive scales ended with year as the broadest scale of variation within the hierarchically nested model. Entry order of terms into the nested mixed model followed the hierarchical arrangement of Group (box-cores for station events) as a random factor at the smallest scale, nested within habitat as a fixed factor (intertidal or subtidal), further nested within Site as a random factor, Side as a fixed factor (protected or exposed), District as a fixed factor (west or east) and terminating at the largest scale with Year as a fixed factor (1993 or 2010). Higher order interaction terms among the four fixed factors and all testable interactions involving random factors were also included within the Hierarchical Nested Mixed Model. Follow up pairwise t-tests between levels of a factor of interest within each level of an interacting factor were made for fixed factors exhibiting significant interactions within the model.

nMDS ordinations of station level or site level means in PRIMER-e v7 (Clarke and Gorley, 2015) illustrated differences in macrofaunal dissimilarity for combinations of factor levels. To determine whether differences in dispersions of sample coordinates contributed to between-group differences, homogeneity of multivariate dispersion (PERMDISP) tests compared deviations of sample coordinates from centroids between levels of fixed factors and their interactions in ordination space. Principal Coordinate Analysis (i.e., PCO or metric MDS) of the dissimilarity matrix of station means facilitated PERMDISP tests of homogeneity of multivariate dispersions of coordinates for groups relative to their centroids (Anderson et al., 2008). Pairwise t-tests were used to examine whether cumulative deviations from centroids differed among levels of fixed factors or interactions. Multiple comparisons among levels were corrected for family-wise error using Holm's sequential Bonferroni method (Holm, 1979). To determine whether locations of sample coordinates differed among combined levels of the three fixed factors, District, Side, and Habitat, confidence intervals for locations of group centroids were ascertained by bootstrap resampling and plotted along with 96% confidence envelopes within nMDS ordination space (Clarke and Gorley, 2015).

To partition components of variation in macrofaunal dissimilarity exclusively among the main factors of concern, a complimentary hierarchical mixed model PERMANOVA involving crossed random factors based on unique elements and using Type 1 sequential Sum of Squares (SS) provided an implicitly nested model design (sensu Schielzeth and Nakagawa, 2013). Group (station-events) and Site served as random factors, which together with four fixed factors, Habitat, Side, District and Year, entered the model sequentially in the order of increasing scale. Unique elements within the random factors precluded complete crossing within the PERMANOVA design, resulting in the lack of interaction terms and *de facto* partitioning of the total variance into components attributable exclusively to the main factor terms. Total Sum of Squares was identical between this model and the hierarchical nested mixed model, however components of variation and significance levels differed between models for terms occurring in both models.

The Similarity Percentages (SIMPER) procedure in PRIMER-e v7 (Clarke and Gorley, 2015) helped identify which major taxa were driving differences among PERMANOVA factor levels. SIMPER outputs were used to interpret macrofaunal differences between years for the two districts, and between habitats of protected and exposed sites within the western and eastern districts. Resulting tables included differences between the top 20 numerically dominant taxa, or fewer if those dominant taxa provided ≥90% of the cumulative total abundance. Summary tables of abundances and percentages of total numbers for all taxa occurring within the eight combinations of District, Side and Habitat across both macrofaunal surveys were constructed, summarized, and compared in terms of sample effort, total density, species richness, and major taxonomic groups.

4 Results

A total of 47,571 macrofaunal organisms were obtained from 255 box core samples representing 51 station events at 17 sites for the spring 1993 inventory and from 204 box-core samples representing 42 station events at 21 sites for the spring 2010 pre-impact DwH survey. Taxonomic classification, numbers of specimens, and percent total number for all macrofaunal taxa occurring among eight combined levels of District, Side, and Habitat are presented in Supplementary Appendix S1A–S1H. The baseline dataset comprised 159 taxa, including 68 polychaete, 21 amphipod, 17 bivalve, nine decapod, nine gastropod, six isopod, six cumacean, five echinoderm, five nemertean, three mysid, two tanaid and six miscellaneous taxa (Supplementary Appendix S1I). Species richness (S) per square meter varied from 16.67 to 83.33 among the eight groups representing combined levels of District, Side, and Habitat (Supplementary Appendix S1I). The number of samples ranged between 30 and 85 box-cores, and effort was proportionate with inherent taxonomic diversity across the eight subdivisions. Species richness (S) typically reached higher levels at protected sites than at exposed sites and in subtidal habitat than in intertidal habitat (Supplementary Appendix S1I). When normalized to sample area (i.e., m^{-2}), S was higher within subtidal habitat at protected sites within the eastern district than within the western district (S = 83.33 vs 61.03 m^{-2}, respectively). In contrast, S was higher within intertidal habitat at exposed sites within the western district than within the eastern district (S = 25.0 vs 16.67 m^{-2}, respectively). Total density varied from 2,120.83 m^{-2} to 15,254.41 m^{-2}, and total densities from protected and exposed subtidal habitats differed substantially and inversely between districts: subtidal densities at protected sites were more than twice as high in the western district (15,254.41 m^{-2} vs 5,937.50 m^{-2}); whereas subtidal densities at exposed sites were more than fivefold higher in the eastern district (13,629.17 m^{-2} vs 2,523.55 m^{-2}).

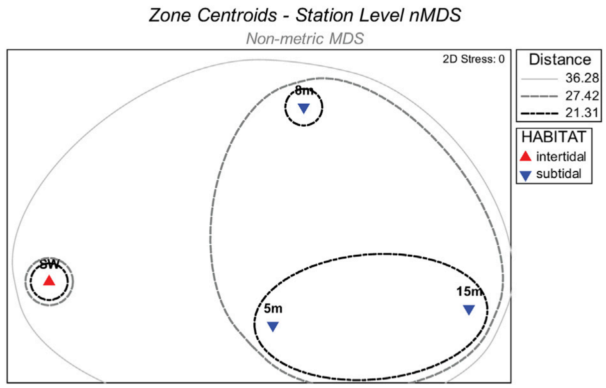

FIGURE 2
Non-metric multidimensional scaling (nMDS) centroids for station groups defined by seaward distance (i.e., swash, 5m, 8m, or 15 m) along with overlayed similarity envelopes delineated by group average cluster analysis branches.

Intertidal and subtidal habitat designations for nMDS and PERMANOVA were confirmed by a station-level nMDS ordination for the four habitat zones as defined by seaward distance (i.e., swash, 5 m, 8 m, or 15 m). Centroids for the zones along with accompanying similarity envelopes showed that the swash zone macrofauna was uniquely different from the subtidal zones (Figure 2). Moreover, the degree of macrofaunal dissimilarity among the three subtidal zones reflected survey year more than distance from shore, as shown by grouping of the centroids for the 5 m and 15 m zones. Thus, intertidal (i.e., swash zone) and subtidal (i.e., 5 m 8 m, and 15 m) habitat designations were used for further analysis.

Average total densities ranged from 1,673.75 within western exposed intertidal habitat to 15,254.41 within western protected subtidal habitat across the eight combinations of District, Side, and Habitat (Supplementary Appendix S1I). The 30 'most important' taxa selected by PRIMER-e collectively made-up 94.2 percent of the total number of macrofaunal organisms. And the selected taxa formed eight coherent (i.e., statistically indistinguishable) indicator groups (i.e., SFGv groups), as determined by Type 3 SIMPROF tests (Table 1). Taxa belonging to mutually exclusive SFGv groups within nMDS space are less likely to co-occur, whereas taxa within the same groups are more likely to co-occur. These SFGv groups also likely correspond with distinct ecological conditions within the sandy nearshore environment, as illustrated by their relative positions within 2-D nMDS space (Figure 3).

The Hierarchical Nested Mixed Model PERMANOVA using Type 1 SS distinguished multiple sources of meaningful variation and effects among the 459 box-core samples (Table 2). All four of the main fixed factor terms were significant. However, the significance level of the Year main factor was comparatively weak. The Year main factor explained only 2.8 percent of the variation in faunal dissimilarity, which was the lowest of any significant effect. As the Year factor represented the highest level within the hierarchical nested model, it

TABLE 1 Eight coherent SFGv indicator groups designated by Type 3 SIMPROF tests from among the 30 'most important' taxa as selected by PRIMER-e. SFGv indicator groups exemplify co-occurring taxa. (Avg–average abundance; SD–standard deviation; % Abun–percent of total abundance; cumulative abundance for the 30 selected taxa = 94.2%.

	Taxon	Order	Class	Avg	Sd	% Abun
SFGv1:a						
AHUN	*Acanthohaustorius uncinus*	Amphipoda	Malacostraca	0.564	4.324	0.544
AYSZ	*Almyracuma* sp.	Cumacea	Malacostraca	0.091	0.826	0.088
HMFL	*Heteromastus filiformis*	Capitellida	Polychaeta	0.379	1.788	0.366
LACL	*Laeonereis culveri*	Phyllodocida	Polychaeta	0.898	3.411	0.866
LTRB	*Leitoscoloplos robustus*	Orbiniida	Polychaeta	0.464	3.254	0.448
MRSZ	*Micropthalmus sczelkowii*	Phyllodocida	Polychaeta	0.381	2.725	0.368
PETR	*Parastarte triquetra*	Veneroida	Bivalvia	0.342	3.093	0.330
SFGv1:b						
MTSW	*Metamysidopsis swifti*	Mysidacea	Malacostraca	3.089	29.552	2.981
SFGv1:c						
HSSA	*Haustorius jayneae*	Amphipoda	Malacostraca	4.196	8.957	4.049
SCAR	*Scyphacella arenicola*	Isopoda	Malacostraca	0.131	0.859	0.126
SFGv1:d						
DNSP	*Donax* sp.	Veneroida	Bivalvia	3.253	15.729	3.138
EMTL	*Emerita talpoida*	Decapoda	Malacostraca	0.244	1.070	0.235
SCSQ	*Scolelepis squamata*	Spionida	Polychaeta	23.985	91.272	23.142
SFGv1:e						
ANDP	*Ancinus depressus*	Isopoda	Malacostraca	0.163	1.130	0.158
BWFL	*Bowmaniella dissimilis*	Mysidacea	Malacostraca	0.216	1.083	0.208
CNDL	*Goniocuna dalli*	Veneroida	Bivalvia	1.022	4.450	0.986
LTFR	*Leitoscoloplos fragilis*	Orbiniida	Polychaeta	0.490	2.901	0.473
SNAM	*Americhelidium americanum*	Amphipoda	Malacostraca	0.089	0.604	0.086
SPSL	*Spilocuma salomani*	Cumacea	Malacostraca	0.436	1.493	0.420
SFGv1:f						
BRWF	*Brania wellfleetensis*	Syllidae	Polychaeta	0.680	6.029	0.656
NPSM	*Nephtys simoni*	Phyllodocida	Polychaeta	0.091	0.508	0.088
SFGv1:g						
CPCP	*Capitella capitata*	Capitellida	Polychaeta	0.590	2.944	0.570
KLSA	*Kalliapseudes* sp. A	Tanaidacea	Malacostraca	2.505	12.670	2.417
PYSA	*Polygordius* sp. A	Polygordiida	Polychaeta	1.235	8.613	1.192
SSLB	*Sphaerosyllis labyrinthophila*	Phyllodocida	Polychaeta	1.131	8.499	1.091
SSTA	*Sphaerosyllis taylori*	Phyllodocida	Polychaeta	3.771	19.752	3.639
SFGv1:h						
EXDM	*Exosphaeroma diminutum*	Isopoda	Malacostraca	4.277	11.871	4.126
LPSA	*Lepidactylus* sp. A	Amphipoda	Malacostraca	33.481	101.482	32.305
PRFL	*Paraonis fulgens*	Cirratulida	Malacostraca	8.898	23.979	8.585
SHBM	*Spiophanes bombyx*	Spionida	Polychaeta	0.536	1.887	0.517

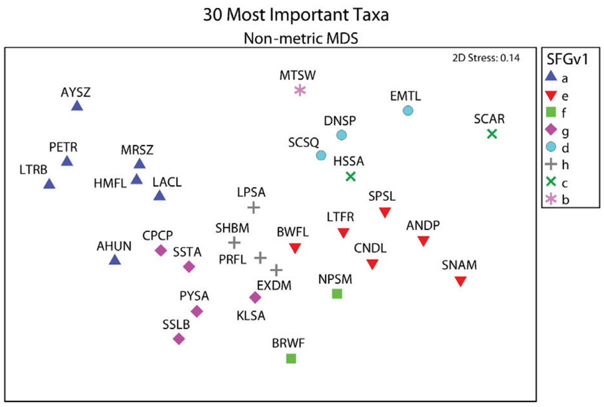

FIGURE 3
Eight coherent SFGv indicator groups designated by Type 3 SIMPROF tests from among the 30 'most important' taxa selected by PRIMER-e. SFGv indicator groups exemplified co-occurring taxa as illustrated by their assigned symbols and relative positions within 2-D nMDS space. Letters in legend correspond to SFGv group identities. Specific taxa for taxon codes presented in Table 1.

was given the best chance to show a difference when using the Type 1 sequential SS method, because none of the macrofaunal variation had been attributed to other factors. Accordingly, the nMDS 2-D ordination of station events showed better separation of districts than years (Figure 4A). Moreover, site centroids of station coordinates in 2-D nMDS space illustrated notable macrofaunal affinity based on island identity, regional proximity, and shore side (i.e., exposed vs protected), notwithstanding year (Figure 4B) Four two-way interactions involving fixed factors were also significant, including Year × Habitat, District × Side, District × Habitat, and Side × Habitat. In addition, the three-way District × Side × Habitat interaction was marginally significant (P = 0.036).

When classified into eight groups representing combined levels of the three spatially hierarchical fixed factors, the nMDS 2-D ordination showed clear separation of station event groups (Figure 5A). Locations for the eight groups were clearly distinct, as shown by representative bootstrapped means and 96% confidence envelopes within nMDS space (Figure 5B). Protected sites showed greater separation within nMDS space than exposed sites. Macrofaunal dissimilarity between districts was also greater for protected sites than for exposed sites. The widest separation

TABLE 2 Results of the Hierarchical Nested Mixed PERMANOVA using Type 1 sequential Sum of Squares. Nested factors are random. Year, District, Side, and Habitat are fixed factors, each comprising two levels. Bold–*p* < 0.05.

Factor	df	SS	MS	Pseudo-F	P	Variation component
Year	1	26,230	26,230	2.292	**0.030**	8.08 (0.028)
District	1	81,430	81,430	6.765	**0.001**	17.667 (0.062)
Side	1	249,550	249,550	20.806	**0.001**	32.672 (0.115)
Habitat	1	96,061	96,061	13.452	**0.001**	20.337 (0.071)
Year × District	1	19,874	19,874	1.7463	0.107	8.8165 (0.031)
Year × Side	1	19,592	19,592	1.7075	0.119	8.5917 (0.030)
Year × Habitat	1	20,192	20,192	2.8784	**0.006**	11.097 (0.039)
District × Side	1	54,339	54,339	4.5139	**0.001**	19.888 (0.070)
District × Habitat	1	23,935	23,935	3.3473	**0.005**	12.719 (0.045)
Side × Habitat	1	48,634	48,634	6.8268	**0.001**	19.954 (0.070)
Site(District × Side)	31	352,200	11,361	2.9254	**0.001**	24.969 (0.088)
Year × District × Side	0	---	---	---	---	No test
Year × District × Habitat	1	9,057.9	9,057.9	1.3092	0.260	6.4523 (0.023)
Year × Side × Habitat	1	9,039.7	9,039.7	1.2976	0.255	6.3312 (0.022)
District × Side × Habitat	1	15,364	15,364	2.1671	**0.036**	12.9 (0.045)
Year × Site(District × Side)**	0	---	---	---	---	No test
Site(District × Side) × Habitat	31	212,570	6,857.2	1.779	**0.003**	23.125 (0.081)
Year × District × Side × Habitat	0	---	---	---	---	No test
Group (Site(District × Side) × Habitat)	17	66,736	3,925.6	5.9827	**0.001**	25.571 (0.090)
Year × Site(District × Side) × Habitat**	0	---	---	---	---	No test
Year × Group (Site(District × Side) × Habitat)	0	---	---	---	---	No test
Residual	366	240,150	656.16			25.616 (0.090)
Total	458	1,545,000				284.7867 (1.000)

Bold values indicate significant probabilities that are less than .05; i.e., $p < .05$.

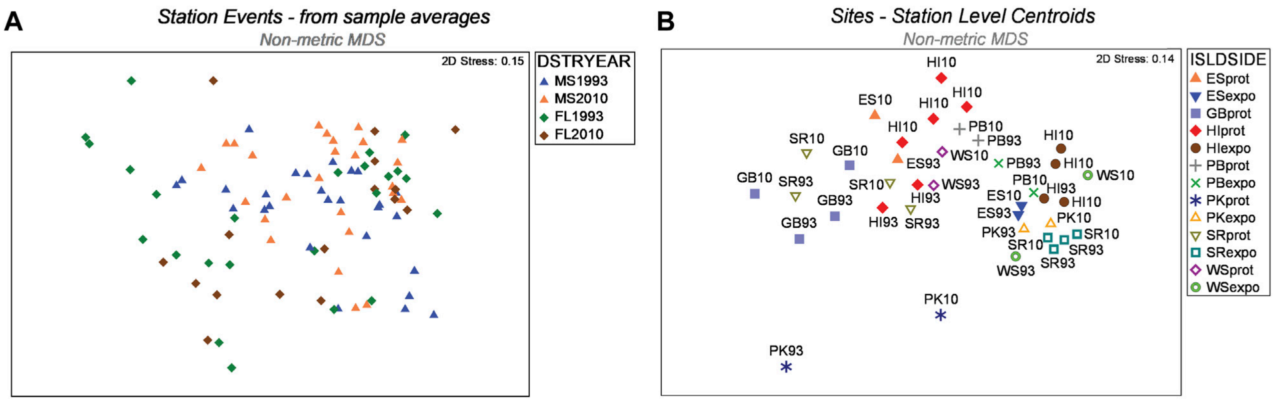

FIGURE 4
(A) Ordination of nMDS coordinates for station events. Coordinates overlap less between districts than between years (legend codes: DSTRYEAR - District/Year; MS–western district; FL eastern district; **(B)**. Site centroids of station coordinates in 2-D nMDS space illustrating macrofaunal affinity based on island identity, region, and shore side (i.e., exposed vs protected legend codes: ISLDSIDE, Island/Side; ES, East Ship Island; GB, Gulf Breeze; HI, Horn Island; PB, Petit Bois Island; PK, Perdido Key; SR, Santa Rosa Island; WS, West Ship Island; prot, protected; expo, exposed).

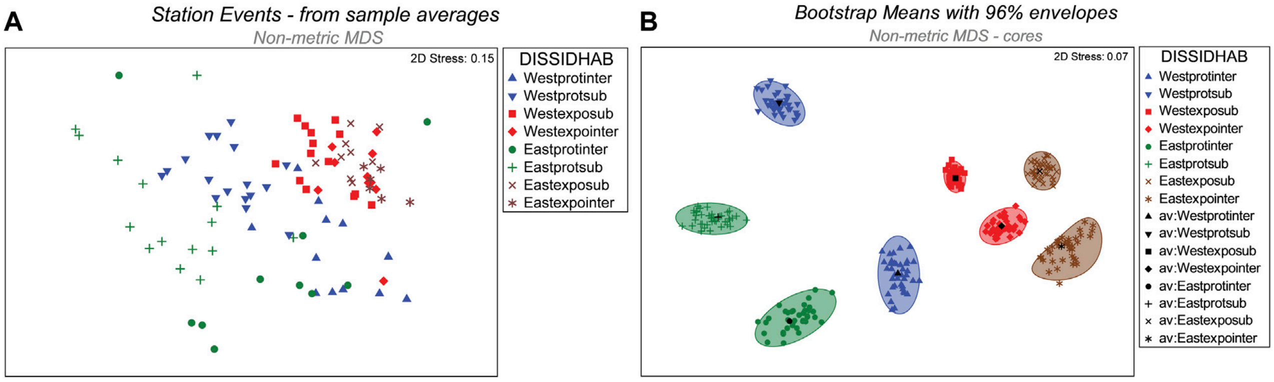

FIGURE 5
(A) Ordination of nMDS coordinates representing station events. Coordinates for the eight combinations of District, Shore side, and Habitat segregate within 2-D ordination space. **(B)**. Plots of group centroids with 96% confidence envelopes as determined by bootstrap resampling for eight combined levels of District, Side, and Habitat. (legend codes: DISSIDHAB - District/Side/Habitat; West–western district; East -eastern district; prot–protected site; expo–exposed site; inter–intertidal habitat; sub–subtidal habitat; av, group centroid).

within nMDS space occurred between intertidal and subtidal habitats of protected sites within the Western district.

Follow up pairwise t-tests between factor levels of interest for significant interactions revealed that assemblages were modestly different between years within intertidal habitat, whereas assemblages were markedly different between years within subtidal habitat (Table 3). Notwithstanding between-year differences within habitats, the variance component of the Year × Habitat interaction only accounted for four percent of the total variance in assemblage structure (Table 2). Differences between the two districts as well as differences between exposed and protected sites were significant for both intertidal and subtidal habitats (Table 3; Figure 5B). And the two districts were modestly different for exposed sites and markedly different for protected sites (Table 3). Furthermore, the districts were moderately different between exposed intertidal and exposed subtidal habitats; and districts were markedly different between protected intertidal and protected subtidal habitats (Table 3; Figure 5B). Follow up differences were not of concern for significant interactions involving nested random factors. Nevertheless, significant interactions involving nested random terms collectively explained 26 percent of the variation in faunal dissimilarity (Table 2).

In addition to locations of centroids, significant PERMANOVA results can signify differences in dispersions of nMDS coordinates between groups (Anderson et al., 2008). But PERMDISP tests of homogeneity of multivariate dispersions of nMDS coordinates were non-significant for Year (P = 0.992) and Habitat (P = 0.102) (Supplementary Appendix S2A). However, PERMDISP tests were strongly significant for District (P = 0.001) and Side (P = 0.001). Thus, both locations and dispersions of nMDS coordinates differed between both levels of the District and Side factors. Furthermore, nMDS dispersions also clearly differed among combined levels for four of six possible interactions between two fixed factors, specifically for Year by Habitat (P = 0.003), District by Side (P = 0.001), District by Habitat (P = 0.001), and Side by Habitat (P = 0.001). And dispersions differed for the three-factor combination of District by Side by Habitat (P = 0.001) (Supplementary Appendix S2A). Follow up pairwise t-tests showed that dispersions for subtidal habitat differed between 1993 and 2010 (P = 0.008), and dispersions in 2010 differed between

TABLE 3 Pairwise t-tests between factor levels of interest for significant interactions within the Hierarchical Nested Mixed Model PERMANOVA (Number of permutations = 998–999). Bold–*p* < 0.05.

Interaction	Comparison	t	P
Year × Habitat	1993 intertidal, 2010 intertidal	1.632	**0.035**
	1993 subtidal, 2010 subtidal	1.635	**0.009**
District × Habitat	West intertidal, East intertidal	2.265	**0.001**
	West subtidal, East subtidal	2.335	**0.001**
Side × Habitat	Protected intertidal, Exposed intertidal	3.553	**0.001**
	Protected subtidal, Exposed subtidal	4.115	**0.001**
District × Side	West exposed, East exposed	1.910	**0.012**
	West protected, East protected	2.686	**0.001**
District × Side × Habitat	West exposed intertidal, East exposed intertidal	1.523	**0.039**
	West protected intertidal, East protected intertidal	2.280	**0.003**
	West exposed subtidal, East exposed subtidal	1.729	**0.021**
	West protected subtidal, East protected subtidal	2.514	**0.001**

Bold values indicate significant probabilities that are less than .05; i.e., *p* < .05.

TABLE 4 Results from the Implicit Nested PERMANOVA model using a crossed design between unique coded random and fixed factors and Type 1 sequential Sum of Squares. Year, District, Side, and Habitat are fixed factors, each comprising two levels. Output terms were constrained to main factors by design, which facilitated designations of their exclusive variance components. Bold–*p* < 0.05.

Factor	Status	df	SS	MS	Pseudo-F	P	Variation component
Year	Fixed	1	26,230	26,230	1.9836	0.059	7.759 (0.048)
District	Fixed	1	81,430	81,430	5.8672	**0.001**	17.452 (0.107)
Side	Fixed	1	249,550	249,550	18.014	**0.001**	32.555 (0.200)
Site	Random	34	447,560	13,164	1.7553	**0.001**	21.705 (0.133)
Habitat	Fixed	1	95,807	95,807	12.783	**0.001**	20.290 (0.125)
Group	Random	54	404,220	7,485.6	11.408	**0.001**	37.217 (0.229)
Residual		366	240,150	656.16			25.616 (0.157)
Total		458	1,545,000				162.594 (0.999)

Bold values indicate significant probabilities that are less than .05; i.e., *p* < .05.

intertidal and subtidal habitat. Salient differences in dispersions among the four combinations of District and Side included that between exposed sites of Western vs Eastern districts (P = 0.004). Salient differences in dispersions among the four combinations of District and Habitat included that for subtidal habitats of Western vs Eastern districts (P = 0.001). Salient differences in dispersions among the four combinations of Side and Habitat included that for intertidal habitats of protected vs exposed sites (P = 0.001), and for subtidal habitats of protected vs exposed sites (P = 0.001). Upon correction for family-wise significance, dispersions differed for eight of 28 combined levels of District, Side, and Habitat, including salient differences for subtidal habitats of protected sites between western vs eastern districts (P = 0.001) and for subtidal habitats between protected vs exposed sites within the eastern district (P = 0.001).

The Implicit Nested Mixed Model PERMANOVA using Type 1 sequential Sum of Squares partitioned variation entirely among the main factors at decreasing spatiotemporal scales (Table 4). The Total Sum of Squares was identical between the implicit nested model and the nested mixed model, however components of variation and significance levels of the terms differed between models. At the largest scale, Year was non-significant in the implicit nested model. However, all subsequent terms were strongly significant (P = 0.001). Moreover, when ranked in order of decreasing magnitude, variation components indicated: Group (Station level) > Shore Side > Site > Habitat > District > Year. Thus, the degree of variation attributable to the factor levels did not directly follow the inherent spatiotemporal hierarchy.

The Similarity Percentage (SIMPER) procedure helped to interpret macrofaunal differences between years for each District and between districts for each combination of Side and Habitat (Supplementary Appendix S3A). Nine of 20 top taxa across both years were shared between districts. Of the top 20 taxa in the western district, eight taxa were more abundant in 1993 and five taxa were more abundant in 2010, whereas of the 20 top taxa in the eastern district, 13 taxa were more abundant in 1993 and five taxa were more abundant in 2010. The most dominant taxon, *Scolelepis squamata*, was markedly more abundant in 2010 than in 1993 in the eastern district, whereas the abundance of this polychaete was more similar between years within the western district. Conversely, the haustoriid amphipod, *Lepidactylus* sp. A, was more abundant in 1993 than in 2010 in both districts. Another haustoriid amphipod, *Haustorius jayneae*, was more abundant in 1993 within the western district, while abundances of this amphipod were comparable between years within the eastern district. And the polychaete, *Paraonis fulgens*, was more abundant in 1993 in both districts.

Notable differences in abundances of the top 20 taxa across both districts occurred within intertidal and subtidal habitats of protected sites (Supplementary Appendix S3A). The top 20 numerically dominant taxa within intertidal habitat at protected sites made up 85.79 percent of the total abundance. Percent average dissimilarity of intertidal habitat at protected sites between districts was 74.20. Greater abundances of *Lepidactylus* sp. A occurred within intertidal habitat of protected sites within the eastern district. Conversely, greater relative abundances of *Scolelepis squamata* and *Emerita talpoida* occurred within intertidal habitat of protected sites within the western district. The top 20 numerically dominant taxa within subtidal habitat at protected sites made up 51.38 percent of the total abundance between districts. Percent average dissimilarity of subtidal habitat of protected sites between districts was 80.52. Various salient taxa were more abundant within subtidal habitats at protected sites within the western district, including *Exosphaeroma diminutum*, *Kalliapseudes* sp. A, *Scolelepis squamata*, *Paraonis fulgens*, *Sphaerosyllis taylori*, and *Spilocuma watlingi*. Conversely, *Laeonereis culveri* was more abundant within subtidal habitat at protected sites within the eastern district. Taxonomic classification, numbers of specimens, and percent total number for all macrofaunal taxa collected from intertidal and subtidal habitats of protected sites within both districts are presented in Supplementary Appendix S1A, B, E, F.

The top 13 numerically dominant taxa within intertidal habitat at exposed sites made up 90.39 percent of the total abundance (Supplementary Appendix S3A). Percent average dissimilarity of intertidal habitat at exposed sites between districts was 47.56. Six of the 13 dominant taxa occurred exclusively within one district, five of which occurred solely within the western district. Most notably, the diminutive brooding bivalve, *Goniocuna dalli*, was abundant within intertidal habitat of exposed beaches within the western district, while absent from the eastern district. Conversely, the anomuran crab, *Emerita talpoida*, was more abundant within intertidal habitat of exposed beaches within the eastern district than in the same habitat within the western district. The top 20 numerically dominant taxa within subtidal habitat at exposed sites made up 84.24 percent of the total abundance (Supplementary Appendix S3A). Percent average dissimilarity of subtidal habitat at exposed sites between districts was 58.15. Nine of the 20 dominant taxa occurred exclusively within one district, seven of which occurred solely within the western district. Four of the salient taxa, including *Paraonis fulgens*, *Haustorius jayneae*, *Ancinus depressus*, and *Leitoscoloplos fragilis*, were more abundant within the subtidal habitat of exposed beaches within the western district, whereas four other salient taxa, including *Scolelepis squamata*, *Metamysidopsis swifti*, *Donax* sp., and *Emerita talpoida* were more abundant within the subtidal habitat of exposed beaches within the eastern district. Taxonomic classification, numbers of specimens, and percent total number for all macrofaunal taxa collected from intertidal and subtidal habitats of exposed sites within both districts are presented in Supplementary Appendix S1C, D, G, H.

5 Discussion

For macrofaunal assemblage structure to serve as robust indicator of environmental degradation, the boundaries of natural variation need to be defined and validated (Ysebaert and Herman, 2002). Properly defined baseline boundaries should facilitate detection of egregious macrofaunal impacts and recovery. This is especially challenging for sandy shore macrofaunal taxa that are adapted and subjected to varying degrees of natural disturbance on landscape and habitat scales (Jones et al., 2011), in addition to intermittent meteorological disturbances on regional scales (Rakocinski et al., 2000). Using sandy shore macrofauna as indicators of temporal environmental shifts, Bessa et al. (2014) inferred sustained effects of human pressures over a 10-year period at an urban beach. So, it is noteworthy that sandy shoreline macrofaunal assemblages appeared relatively stable on a 17-year temporal scale between spring 1993 and just before impingement by the DwH oil spill, in spring 2010. The Year factor within the implicit nested PERMANOVA model explained only 4.8 percent of the variation in faunal dissimilarity, compared to other main factors which collectively accounted for an additional 79.5 percent of the variation. Indeed, statistically indistinguishable dispersions of nMDS coordinates between spring 1993 and 2010 also confirm the lack of a substantial macrofaunal baseline shift over the 17-year intervening period. A significant Year by Habitat interaction within the hierarchical nested mixed model PERMANOVA denoted between year differences in both locations and dispersions of sample coordinates for subtidal habitat, and likely reflected the broader range of seaward distances for samples from subtidal habitat in 1993 (i.e., 5m and 15 m) than in 2010 (i.e., 8 m). Hence our combined dataset expanded baseline boundaries of natural variation in the sandy shore macrofaunal assemblage structure. Accordingly, the combined GINS data for 1993 and 2010 provide a robust baseline for natural sandy shore habitat within this region.

The lack of a major baseline shift in macrofaunal assemblage dissimilarity between 1993 and 2010 was notable given the history of disturbances by major storms within the intervening 17-year period. Most notably, two of the most severe hurricanes within the region impinged on our study area 5–6 years prior to 2010. Hurricane Ivan directly impacted the eastern district of GINS in 2004 and hurricane Katrina directly impacted the western district in 2005. Effects of hurricanes on sandy shore macrofauna have been documented within the northern Gulf of Mexico region, including the GINS (Saloman and Naughton, 1977; Rakocinski et al., 2000). Fall samples from the eastern district of our 1993 GINS inventory provided a baseline for comparing with macrofaunal samples taken in 1996, 1 year after hurricanes Opal and Erin consecutively struck in 1995 (Rakocinski et al., 2000). Effects on macrofaunal species richness, total density, densities of indicator species, and assemblage structure were still evident in fall 1996. Moreover, the degree of inferred impact corresponded with the proximity of storm disturbance on a landscape scale. Such a correspondence between macrofaunal impact and disturbance could indicate major effects of over washed sediments. The recovery of impacted shoreline macrofaunal assemblages also corresponds to landscape and habitat scale factors related to the frequency and magnitude of exposure to disruptive hydrology. Accordingly, assemblages of organisms adapted to withstand wave swept conditions, such as those populating exposed sandy shorelines and intertidal habitats, are quite resilient (Rakocinski et al., 2000). Essentially comparable macrofaunal assemblages between 1993 and 2010 implies that the sandy shore macrofauna can fully recover from regional effects of major storms within 5–6 years within our study area. Fortuitously, the 1993 GINS macrofaunal inventory provided an excellent foundation for establishing a baseline, because there had been a preceding period of 20 years without direct impacts of major storms within either district (https://www.nhc.noaa.gov/outreach/history/).

Another strong indication of an inherently stable sandy shoreline macrofauna was evinced by centroids for sites representing specific islands. Although replication was insufficient to include island as a hierarchical level within PERMANOVAs, notable fidelity in macrofaunal assemblage composition was implied by the proximity of site centroids representing specific islands in nMDS space, despite any between-year differences. High site-specific macrofaunal similarity within the 15-m subtidal zone was also apparent in a former study within this system (Rakocinski et al., 1998), for which site level fidelity in macrofaunal similarity was noted over a six- or 7-year period. In the present study, site level fidelity in macrofaunal similarity was inferred across a much longer 17-year period. More variable centroids in nMDS space for protected sites than for exposed sites reflected differences in inherent assemblage diversity. Nevertheless, the relatively close placement of centroids for proximate protected sites across the 17-year period also revealed location-specific macrofaunal fidelity. Characteristic environmental factors related to morphodynamics, as well as to physical abiotic and biotic conditions likely favored many of the same macrofaunal taxa between the two time periods (Bae et al., 2018).

The Implicit Nested Mixed Model PERMANOVA facilitated the partitioning of variance in macrofaunal dissimilarity among the main factors representing the nesting of spatiotemporal scales. As such, decreasing amounts of variance were attributed to Group (Station level random factor), Side (Landscape level fixed factor), Site (Location level random factor), Habitat (Seaward location fixed factor), District (Region level fixed factor), and finally Year (Survey level fixed factor). The Group factor delineated subjects as represented by sets of individual box-core samples at the station level. The Type 1 sequential Sum of Squares option ensured that variance ascribed to each successive hierarchical level referred only to that which was not already explained at less inclusive levels within the model. A different approach to evaluating the influence of hierarchical factors on the same sandy shore macrofauna was taken by Rakocinski et al. (1998), based on a cluster analysis using the Group Average sorting strategy and a Principal Coordinate analysis. Based on 23 common taxa from the 15 m subtidal habitat obtained in 1986/87 and 1993, the sequence of factors influencing macrofaunal similarity was Side (Landscape), followed by District (Region), Year (1986/87 vs 1993), Site (Location), and Season (spring, summer, autumn, winter). Our present study calculated dissimilarity on the entire macrofauna, included intertidal in addition to subtidal habitat, and only contained samples taken during the spring season. Despite these and other incommensurate differences between studies, the order of three major sources of macrofaunal variation ranked the same. In both studies, Side explained more variation than District, which explained more than Year. Thus, the landscape scale was more important than region, and year was the least important explanatory factor of those in common within both studies. The hierarchical placement of Season could not be compared, as only the spring 1993 samples were commensurate with 2010 pre DwH samples. But as season was the least important level noted by Rakocinski et al. (1998), data from all four seasons of the 1993 GINS inventory can also be considered as part of the valid baseline. Indeed, the subordinate role of season as a source of macrofaunal variation was noted in Rakocinski et al. (1998): "... dominant taxa occurred more consistently across seasons at individual stations than across stations within any given season." Understanding the relative importance of nested spatiotemporal scales of macrofaunal variation facilitates environmental assessments by helping to correctly match different stressors to their spatiotemporal scales of reference (Morrisey et al., 1992; Rakocinski et al., 1998; Defeo and McLachlan, 2005; Veiga et al., 2014; Pandey and Thiruchitrambalam, 2019).

An important rationale for characterizing variation in macrofaunal assemblages at multiple nested spatiotemporal scales is to appropriately match effects of stressors to ecological responses (Defeo et al., 2008). Corresponding with scales of variation in macrofaunal assemblages, effects of disturbance and stress are expressed on various scales (Defeo and McLachlan, 2005). For example, the largest spatial scale of hurricane impact is typically expressed at the district level within our study area. The regional scale of impacts from major storms is a product of several factors, including 1) the separation of the GINS districts by about 100 km of coastline encompassing the Mobile River and Mobile Bay estuarine system; 2) impacts focused on the right front quadrants of hurricanes; and 3) a typical hurricane impact area of around 180 km. Effects of storms will also vary relative to how disturbance is manifested at smaller scales with respect to landscape and habitat features (Rakocinski et al., 2000). Effects of the DwH oil spill were also expressed across multiple scales, including the most inclusive spatial scale covering the entire GINS study area (Nixon et al., 2016). However, actual oiling of surface sediments occurred patchily on exposed and protected beaches over several years (Owens et al., 2008; Michel et al., 2013; Clough et al., 2017). Due to the disposition of the Macondo well, Florida beaches experienced heavier oiling than Mississippi beaches (Nixon et al., 2016). And incoming oil was relatively weathered in the eastern district (Snyder et al., 2014). Thus, effects of the DwH spill on macrofaunal assemblages would have been expressed at several spatial scales for which meaningful variation was observed in this study, including habitat, landscape, and district levels. Moreover, recurrent exposure to various stages of weathered oil would have continued to affect the sandy shoreline ecosystem over multiple years. It would be insightful to know when the sandy shoreline macrofauna fully recovered from the DwH event with respect to the limits defined by this study.

Despite superficial homogeneity, sandy shoreline ecosystems comprise dynamic benthic habitats that vary seasonally, regionally, and geographically (Defeo and McLachlan, 2013). Spatial variation in sandy shoreline macrofauna has been attributed to differences in shoreline morphodynamics in connection with the degree of wave exposure and attendant sediment dynamics (Oliver et al., 1979; Shelton and Robertson, 1981; Dexter, 1983; Knott et al., 1983; McLachlan et al., 1984; Eleftheriou, 1988; Fleischack and de Freitas, 1989; Raffaelli et al., 1991; Rakocinski et al., 1991; 1993; Jaramillo and McLachlan, 1993). Landscape related variation in sandy shoreline macrofaunal assemblages corresponds with different disturbance regimes in connection with sediment properties, including sediment grain size and organic content (Defeo and McLachlan, 2005; Pandey and Thiruchitrambalam, 2019). Protected shoreline habitats may also receive high subsidies of organic input from other nearby habitats like grass beds and salt marshes (Rakocinski et al., 1998). Known scale-related patterns in sandy shore macrofaunal variability include effects of local patchiness, habitat zonation, landscape related morphodynamics, and regional discontinuities (McLachlan et al., 1993; Rakocinski et al., 1993; Rakocinski et al., 1996; Rakocinski et al., 1998). In the present study, definitive macrofaunal subdivisions were defined for each of the eight combinations of levels for the three fixed factors, District, Side, and Habitat. Subdivisions were exemplified by distinct bootstrapped mean dissimilarity and 96% confidence envelopes for each of the eight factor combinations within nMDS space. Greater separation of bootstrapped means for protected sites than for exposed sites in nMDS space reflected greater macrofaunal dissimilarities at the landscape level. Discernable baseline macrofaunal assemblages were delineated by combined levels of District, Side and Habitat. Corresponding differences in macrofaunal assemblages include rare taxa which depend on adequate sample effort to obtain. Thus, assessments of environmental impacts require properly defined references. Accordingly, a complete listing of the macrofaunal data for the eight combined levels of District, Side and Habitat, along with a summary table are provided (Supplementary Appendix S1A–I).

Sandy shoreline benthic habitats worldwide consist of comparable functional groups of macrofauna, due to the general prerequisite for adaptation to wave swept conditions, including the ability to burrow and filter-feed within moving water (Rodil et al., 2014). Consequently, benthic macrofaunal assemblages of sandy shorelines are quite resilient, and thus provide good indicators of biotic integrity in the face of catastrophic damages like those presented by the DwH oil spill. However, resource managers often avoid using shoreline macrofaunal assemblages in assessments of oil spills, because macrofauna are considered too variable to serve as reliable indicators. Indeed, published assessments of the effects of the DwH oil spill on the sandy shoreline ecosystem using macrofaunal assemblages as indicators appear deficient (Beyer et al., 2016; Clough et al., 2017). Effective management of sandy shoreline ecosystems requires the development and communication of sound research tools by scientists (Scapini and Fanini, 2011). Incorporating more integrative ecological indicators like macrofaunal assemblages would help address the need to focus on the cumulative effects of oil spills (Bjorndal et al., 2011). Using macrofauna as indicators for sandy shoreline ecosystems should be eminently tractable when responses and impacts are compared on commensurate scales. Although the present study is limited to a narrow seasonal window and infrequent sampling, it provides a spatially extensive macrofaunal reference for the sandy shoreline ecosystem of the GINS at two time points spanning a 17-year period for a region of the northern Gulf of Mexico facing multiple environmental threats (Defeo et al., 2008). In conclusion, this study validates a macrofaunal baseline reference for future assessments of environmental impacts and trends, delineation of salient spatiotemporal scales, and the management of benthic habitats within the GINS sandy shoreline ecosystem.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: http://www.usm.edu/gulf-coast-research-laboratory/museum-collections.php.

Author contributions

CR, RH, and SL contributed to the conceptualization and design of the study. RH, CR, and SL helped to obtain funding for the study. CR and SL supervised the project. SL, KV, and CR conducted field work to obtain samples for the 2010 and 1993 GINS macrofaunal surveys. KV managed the project, including sample processing and data entry. KV and SL verified taxonomic identifications. SL created and curated the data. CR conducted the data analyses, prepared the figures, and wrote the first draft and revision. All authors reviewed and approved the draft. This paper is dedicated to the memory of RH.

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Conflict of interest

Publisher’s note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.951341/full#supplementary-material

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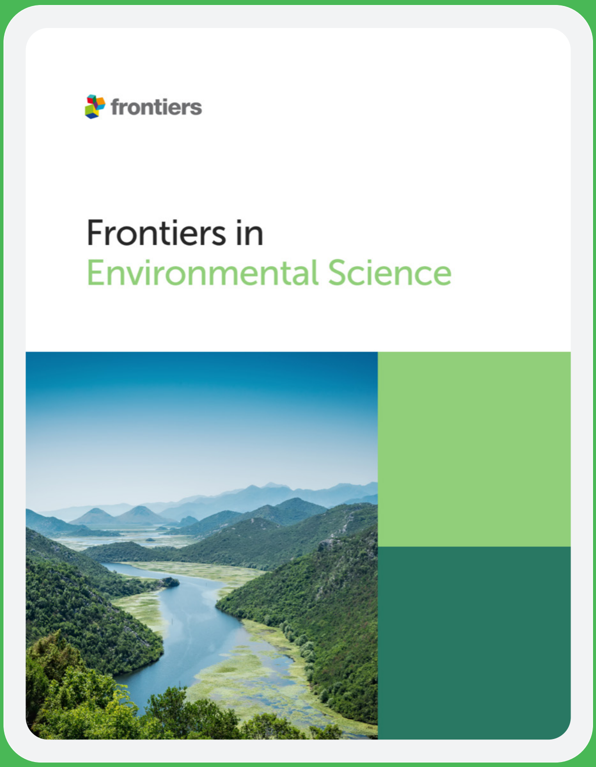

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