South Atlantic Regional Fish Monitoring of Restored Oyster Habitat along the Southeastern US Coast

Introduction

Oyster reefs are a critical component of coastal systems in the southeast United States. They are most commonly found within estuaries which are where the ocean and rivers meet, creating brackish water. Within this brackish water, they provide structural habitat for a variety of vertebrates and invertebrates, provide food, help maintain water quality through filtration, and mitigate shoreline erosion (Grabowski and Peterson 2007, Callihan et al. 2016, Baggett et al. 2014, zu Ermgassen et al. 2016, Hancock and zu Ermgassen 2019).

Oyster reefs are a well-studied, crucial nursery habitat for many commercially and recreationally important species (SCDNR, Callihan et al. 2016, zu Ermgassen et al. 2016, Hancock and zu Ermgassen 2019). Species ranging from blue crab to flounder and many more rely on oyster reefs at some stage of their life cycle (NRC). Juvenile species seek refuge from predators within the reef structure (SCDNR, zu Ermgassen et al. 2016, Hancock and zu Ermgassen 2019). Many of these fish feed on larvae and larval animals within the oyster reefs, and this allows them to grow and reproduce, or eventually become prey for larger fishes (SCDNR). Oyster reefs serve as a nursery habitat in another form such that some species attach their eggs to the underside of oyster shells (SCDNR, zu Ermgassen et al. 2016). In comparison to unstructured sediment, which usually replaces destroyed oyster reef habitat, the use of oyster reefs as a nursery habitat leads to enhanced fish production. (Hancock and zu Ermgassen 2019).

Oysters have multiple ecological and socioeconomic roles. They are an important food source for humans and wildlife alike. For instance, the American oystercatcher relies on oysters as a main food source. In 2017, commercial oyster landings for GA, SC, and NC totaled around $8.4 million in fishery value which is still significantly below historic harvest values (NOAA National Marine Fisheries Service). Commercial finfish and crustacean fishermen benefit from oyster reefs and the utilization of the reef by commercially valuable species (Callihan et al. 2016). Recreational fishermen benefit from the increased opportunity to target and catch reef-associated species such as black drum, blue crab, red drum, sheepshead, southern flounder, spotted seatrout, stone crab, and striped bass (Callihan et al. 2016). Not only are the fish communities associated with oyster reefs valued by recreational and commercial fishermen, but they are also culturally important. Lower-income families depend on sustenance fishing around oyster reefs. From native American shell rings through use of oyster shell tabby as building material to today's community oyster roasts, oysters have long been associated with the region's coastal heritage.

BROADER GOALS

The Conservancy is dedicated to working towards healthy oyster populations. Selected as one of the four priority marine conservation strategies for the South Atlantic, the Conservancy's overall program goal is to increase oyster populations by 10% across the region. Strategies to reach that goal include implementing on-the-ground restoration projects, piloting new restoration techniques, revising permitting regulations to enable greater restoration, supporting policies that recognize the ecologic and economic role of oysters, and increasing funding for conservation and restoration work.

The water filtering capacity of oysters is another tremendous benefit (NRC, Callihan et al. 2016, Baggett et al. 2014, Grabowski and Peterson 2007, zu Emgassen et al. 2013). An individual oyster can filter plankton, nitrogen, and other pollutants from as much as 50 gallons of water per day, providing enormous benefit to coastal waters that are increasingly impacted by runoff and pollution (NRC). Oyster reefs serve as natural breakwaters – their physical structure absorbs the energy of waves and storms, creating calmer waters, trapping sediment, and mitigating erosion (NRC, Grabowski and Peterson 2007). They also play a key role in protecting and building salt marshes whose roots are crucial for stabilizing the shoreline (NRC, Baggett et al. 2014, Grabowski and Peterson 2007).

Oyster reefs are a globally imperiled marine habitat with degradation primarily due to anthropogenic factors (Baggett et al. 2014, zu Emgassen et al. 2013). The 2011 Shellfish at Risk report (Beck et al. 2011) highlighted the dramatic loss of shellfish habitat across the world citing 85% of oyster habitat has been lost globally and that a majority of the natural oyster populations are in poor condition. In the South Atlantic region (North Carolina through Florida), the report showed that between 50% and 90% of historic oyster populations have been lost. This loss is attributed to overharvesting, disease and habitat loss. Given the importance of oyster reef habitat and oysters, the scale of this decline has major consequences for the health of our coastal waters and the economies of those South Atlantic communities.

Oyster reefs highlight a range of stakeholders, in addition to the oyster fishers, that benefit from oyster habitat such as a variety of researchers, sustenance farmers, land owners, and the public that eats from, fishes and swims in the habitat (Hancock and zu Ermgassen 2019). A variety of organizations, including state and federal natural resource agencies, universities and non-profit organizations, are working to restore oyster populations and reef habitats due to their ecological and socioeconomic values (Baggett et al. 2014, zu Ermgassen et al. 2016). This includes the incorporation of oyster reefs in living shorelines which are designed to provide natural shoreline stabilization and protection. Scale, long-term monitoring and assessment of these projects are, however, often limited, and thus, less impactful on a system scale and generally focused on shoreline change and reef health (as oyster production). Many studies have been done on a state by state basis, however, studies on an increased scale that are able to be compared, are limited. There is a need to generate additional scientific data, on a regional level, quantifying the role that restored oyster reefs play in fish productivity, including habitat value to commercially, recreationally and ecologically-important fish species. Developing a larger database on nekton use of restored oyster reef (e.g., living-shoreline habitats) and appropriate unstructured control sites has been a challenge for the coastal ecology and habitat-restoration communities.

In 2015, the Nature Conservancy (Conservancy) initiated a three-year project to evaluate fish communities associated with restored oyster reefs in five locations the southeast United States. With primary funding support from Boeing, the Conservancy's project focused on developing and implementing a regional fish productivity monitoring protocol to document the connection between restored oyster reefs, important fish species, and the marine food web.

Outcomes from this project will likely expand our body of knowledge about why larger scale oyster restorations, including living shorelines, are important, and these outcomes will be shared with key stakeholder groups across the southeast. Moving from smaller scale restoration projects to ecosystem-level success will require increases in the size and number of sites, funding, monitoring, and public support. This project was designed to help further quantify and communicate the link between fish communities and restored reefs across the region. The opportunity to combine the results of restored-oyster-reef sampling from numerous sites across the southeast US region enabled us to aggregate the larger amount of data needed to compare biotic communities using these habitats with those of unstructured habitats.

Federal grant programs dedicated to restoring fish habitats have been a key source of funding for on-the-ground restoration. Competition for these funds requires applicants to demonstrate how the project will benefit commercial and recreationally important fishes, either directly through use of the reef and/or indirectly through food chain associations. The lack of direct analysis that links fish use of these reefs has been cited as a reason why oyster reef restoration projects have not ranked as high as other restoration projects, such as dam removal. Hopefully, with these additional data, oyster reef restoration projects will be recognized for their ecological and economic importance and thus, ranked higher for funding opportunities. Additionally, engaging with oyster reef stakeholders through quantifying the benefits of oyster reef restoration sites to fisheries can be used to reinforce the value of oyster restoration with recreational and commercial fishers who can become greater advocates for the work (Hancock and ze Ermgassen 2019).

Methods

Project Composition and Locations

The project team was comprised of Conservancy staff and technical experts from the University of North Carolina at Chapel Hill, South Carolina Department of Natural Resources, and University of Georgia Marine Extension and Georgia Sea Grant. Monitoring was conducted at five sites across North Carolina (NC), South Carolina (SC) and Georgia (GA) (Table 1, Figure 1). Several of these Conservancy supported restoration sites were classified as living shorelines with oyster reef components that differed in design. To augment the monitoring dataset, previous sampling data from restored reefs in SC at Fort Johnson and Bears Bluff were included. These two additional sites were installed and monitored by the South Carolina Department of Natural Resources, a member of the project team, and supported by funding from NOAA via the Southeast Aquatic Resources Partnership (SARP) and USFWS through its State Wildlife Grants (SWG) Program. This report focuses on regional analyses of the monitoring data collected across all seven restoration sites. State specific data analyses were provided in annual grant reports.

Table 1: Overview of the living shoreline and oyster restoration sites included in fish monitoring project regional analyses. Monitoring at italicized sites was conducted outside the scope of this project.

State	**Site Name**	**Installation Year**	**Latitude**	**Longitude**
NC	Point Peter Road	2010, 2011	35°46’14.27”N	75°44’29.14”W
SC	North Island	2011	33°14’10.61”N	79°11’26.02”W
SC	Palmetto Plantation	2012	33°5’19.78”N	79°25’29.63”W
SC	*Fort Johnson*	2011, 2012	32°45’4.14”N	79°54’16.37”W
SC	*Bears Bluff*	2011, 2012	32°38’43.13”N	80°15’23.30”W
GA	Little St. Simons Island	2013	31°15’36.75”N	81°18’8.13”W
GA	Sapelo Island	2010	31°26’3.20”N	81°16’52.50”W

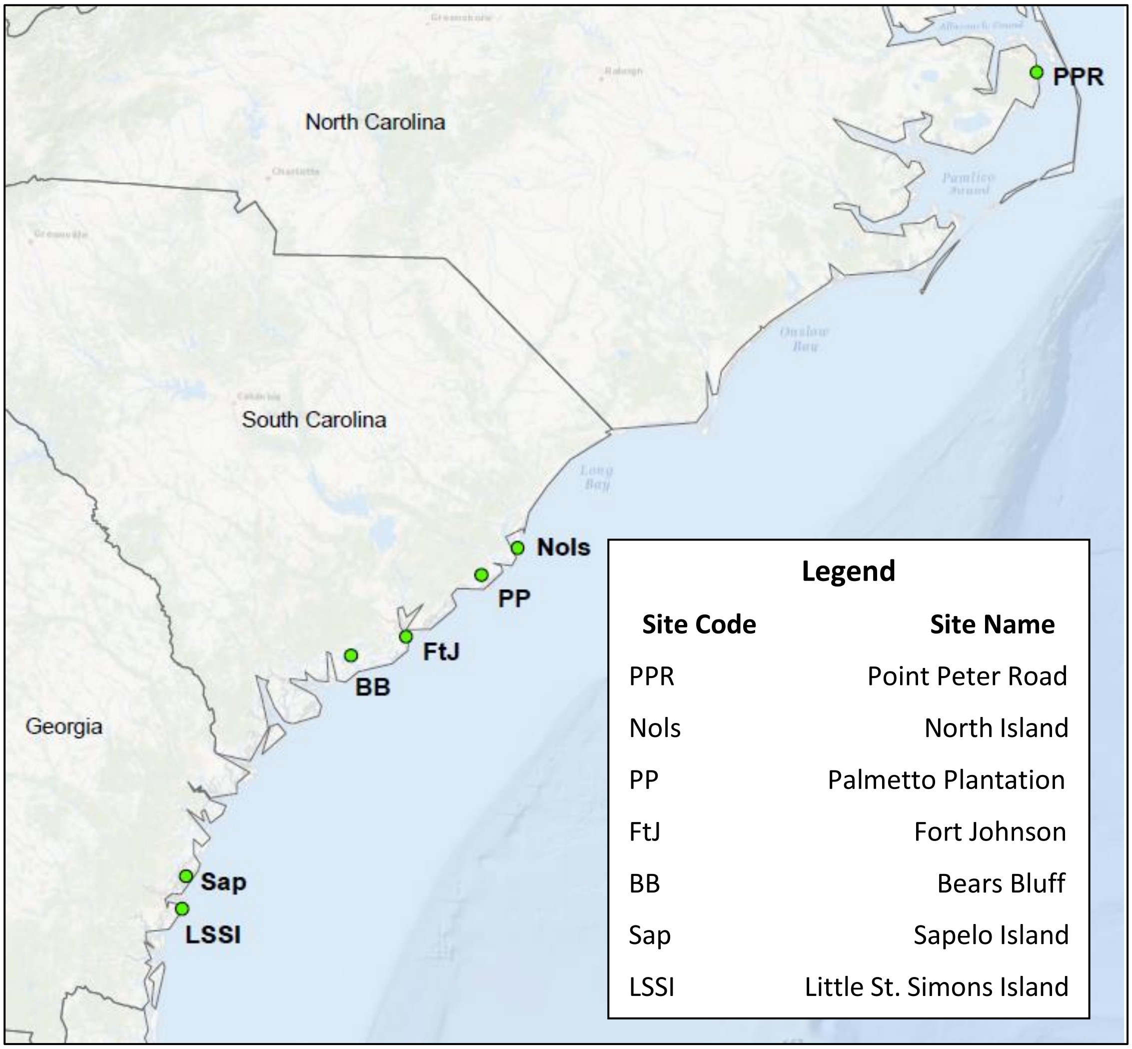

Figure 1. Map of study sites.

Reef Materials

The materials used to construct restored reefs differed among the sites (Table 2). Control sites were unstructured or 'non-reef' reference plots where the habitat type was bare sediment with no structure. NC and SC control sites were bare sediment areas adjacent to the monitored built reef site. GA control sites were a mix of bare sediment and natural reef sites. In 2015 and 2016, sampling in Georgia also included nearby natural oyster reefs.

Table 2. Types of reef material used at study sites

State	Site	Reef Material
NC	Point Peter Road	Bagged shell
		Marl (Class B)
SC	Bears Bluff	Coated pots
	Fort Johnson	Coated pots
	North Island	Oyster castle
	Palmetto Plantation	Oyster castle

GA	Little St. Simons Island	Bagged shell
		Natural oyster reef
	Sapelo Island	Bagged shell
		Natural oyster reef

Data Collection

Nekton sampling data was collected from a total of 7 study sites from three states in the southeast Atlantic United States: NC, SC & GA (Table 3). Sampling was conducted seasonally between 2012-2017. The analysis incorporated spring, summer, and fall samples. Organisms were identified to the lowest taxonomic level possible with total length recorded. Species were generally released except for a few cases when individuals were collected, according to permit conditions, to generate voucher specimens to support appropriate taxonomic identifications. Collectively, 4 different gear types were employed to account for variation in reef structure, location and tidal regime (Table 3). A description of the monitoring methods is available in the project's practitioners' guide: Sampling Nektonic Organisms Around Restored Oyster Reefs in the South Atlantic (Stone and Brown 2018).

Table 3. Description of study sites by state and environmental parameters of sampling events

State	Site Name	Year	Season	Mean Water temp (^{o}C)	Mean Salinity (psu)	Nature of reefs (subtidal vs. intertidal)-
NC	Point Peter Road	2016	Spring	29	12.1	Subtidal
			Summer	27.9	10.5	
			Fall	20.7	10.1	
		2017	Spring	16.8	6.5	
			Summer	25.7	5.4	
			Fall	20.7	10.1	
SC	Bears Bluff	2012	Summer	27.7	27.3	Intertidal
		2013	Summer	26.2	23.1	
			Fall	20.9	33.1	
	Fort Johnson	2012	Spring	21.3	21.3	
			Summer	27.7	27.7	
			Fall	24.4	24.4	
		2013	Summer	28.1	26.7	
			Fall	22.9	26.4	
		2014	Spring	20	25.6	
			Fall	22.1	27.8	
		2015	Spring	21.2	28.5	
			Summer	27.2	30.5	
			Fall	24.4	24	
		2016	Spring	22.6	31.4	
	North Island	2015	Spring	17.1	31.5	
			Summer	27.4	31.7	
			Fall	20.9	33.1	

		2016	Spring	17.1	31.5	
			Summer	27.8	32.3	
			Fall	21.1	6.1	
	Palmetto Plantation	2015	Spring	24.1	33.1	
			Summer	29.6	32.5	
		2016	Spring	24.1	33.1	
			Summer	31.1	34.6	
			Fall	20.1	27.2	
GA	Little St. Simons Island	2015	Spring	32.1	35.6	Intertidal
			Summer	30.1	35.8	
			Fall	24.6	27.6	
		2016	Spring	26	21.1	
			Summer	30.7	28.8	
			Fall	24.6	27.6	
	Sapelo Island	2015	Summer	31.3	28.7	
			Fall	25	27.2	
		2016	Spring	25.6	20.8	
			Summer	31.3	28.7	
			Fall	16.9	23.2	

Data Aggregation

For this regional assessment, data were combined into a single database. We refined the database to include only finfishes, crustaceans, and squid (Table 4) to comprise the nektonic community. These are the primary, secondary, and tertiary consumers of interest within the food web. Our analysis included a total of 76 finfish species and 15 invertebrate species. Twenty-six of the sampled species are managed at the state (NC, SC, and GA) and/or regional (Atlantic States Marine Fisheries Commission (ASMFC), South Atlantic Fish Management Council (SAFMC) level (Table 4).

Table 4. Taxonomic name, common name and management status for all species sampled across the restored oyster-reef habitats across the project area.

Scientific Name	Common Name	Managed			
		NC	SC	GA	ASMFC/SAFMC
Cephalopods					
Lolliguncula brevis	Atlantic Brief Squid				
Crustaceans					
Alpheus heterochaelis	Bigclaw Snapping Shrimp				
Callinectes sapidus	Blue Crab	X	X	X	
Callinectes similis	Lesser Blue Crab				
Callinectes spp.	Crab				
Eurypanopeus depressus	Flatback (Depressed) Mud Crab				
Menippe spp.	Stone Crab		X		
Palaemonetes pugio	Daggerblade Shrimp				
Palaemonetes spp.	Grass Shrimp				
Palaemonetes vulgaris	Common Grass Shrimp				

Scientific Name	Common Name	Managed			
		NC	SC	GA	ASMFC/SAFMC
Panopeus herbstii	Atlantic Mud Crab				
Penaeus aztecus	Brown Shrimp	X	X	X	SAFMC
Penaeus setiferus	White Shrimp	X	X	X	SAFMC
Rhithropanopeus harrisii	White-Tipped Mud Crab				
Elasmobranchs					
Hypanus sabinus	Atlantic Stingray				
Gymnura micrura	Smooth Butterfly Ray				
Rhinoptera bonasus	Cownose Ray				
Sphyrna tiburo	Bonnethead Shark				ASMFC
Teleosts					
Ameiurus catus	White Catfish				
Anchoa hepsetus	Striped Anchovy				
Anchoa mitchilli	Bay Anchovy				
Anchoa spp.	Anchovy				
Archosargus probatocephalus	Sheepshead		X	X	
Bairdiella chrysoura	Silver Perch				
Blennidae spp.	Blenny				
Brama brama	Atlantic Pomfret				
Brevoortia tyrannus	Atlantic Menhaden		X		ASMFC
Centropristis striata	Black Sea Bass		X		SAFMC
Chaetodipterus faber	Atlantic Spadefish		X		SAFMC
Chasmodes bosquianus	Striped Blenny				
Chilomycterus schoepfii	Striped Burrfish				
Chloroscombrus chrysurus	Atlantic Bumper				
Citharichthys spilopterus	Bay Whiff				
Conodon nobilis	Barred Grunt				
Ctenogobius boleosoma	Darter Goby				
Cynoscion nothus	Silver Seatrout				
Cynoscion nebulosus	Spotted Seatrout	X	X	X	ASMFC
Cyprinodon variegates	Sheepshead Minnow				
Cyprinus carpio	European Carp				
Diapterus auratus	Irish Mojarra				
Diapterus plumieri	Striped Mojarra				
Dorosoma cepedianum	American Gizzard Shad				
Dorosoma petenense	Threadfin Shad				
Elops saurus	Ladyfish				
Etropus crossotus	Fringed Flounder				
Eucinostomus argenteus	Silver Mojarra				
Fundulus heteroclitus	Mummichog				
Fundulus majalis	Striped Killifish				

Scientific Name	Common Name	Managed			
		NC	SC	GA	ASMFC/SAFMC
Gambusia holbrooki	Eastern Mosquitofish				
Gobiesox strumosus	Skilletfish				
Gobiosoma bosc	Naked Goby				
Harengula jaguana	Scaled Herring				
Hypsoblennius hentzi	Feather Blenny				
Labrisomus haitiensis	Longfin Blenny				
Lagodon rhomboides	Pinfish				
Larimus fasciatus	Banded Drum				
Leiostomus xanthurus	Spot		X		ASMFC
Lepisosteus osseus	Longnose Gar				
Lutjanus griseus	Grey Snapper		X		SAFMC
Menidia menidia	Atlantic Silverside				
Menticirrhus americanus	Southern Kingfish	X	X		
Menticirrhus saxatilis	Norther Kingfish	X			
Micropogonias undulatus	Atlantic Croaker		X		ASMFC
Morone americana	White Perch				
Morone saxatilis	Striped Bass	X	X		ASMFC
Mugil cephalus	Striped (Jumping) Mullet	X			
Mugil curema	White Mullet				
Mugil spp.	Mullet				
Oligoplites saurus	Leatherjacket				
Opisthonema oglinum	Atlantic Thread Herring				
Opsanus tau	Oyster Toadfish				
Orthopristis chrysoptera	Pigfish				
Paralichthys dentatus	Summer Flounder		X		ASMFC
Paralichthys lethostigma	Southern Flounder	X	X		
Paralichthys spp.	Flounder		X		
Peprilus paru	American Harvestfish				
Poecilia latipinna	Sailfin Molly				
Pogonias cromis	Black Drum		X		ASMFC
Pomatomus saltrix	Bluefish		X		ASMFC
Prionotus spp.	Searobin				
Prionotus tribulus	Bighead Searobin				
Prionotus evolans	Striped Searobin				
Sciaenops ocellatus	Red Drum	X	X	X	ASMFC
Scomberomorus cavalla	King Mackerel		X		SAFMC
Scomberomorus maculatus	Spanish Mackerel		X		ASMFC & SAFMC
Selene vomer	Lookdown				
Seriola zonata	Banded Rudderfish		X		SAFMC
Sphoeroides maculatus	Northern Puffer				

Scientific Name	Common Name	Managed			
		NC	SC	GA	ASMFC/SAFMC
Sphoeroides spengleri	Bandtail Puffer				
Stellifer lanceolatus	Star Drum				
Stephanolepis hispidus	Planehead Filefish				
Symphurus plagiusa	Blackcheek Tonguefish				
Symphurus pusillus	Northern Tonguefish				
Syngnathus floridae	Dusky Pipefish				
Syngnathus fuscus	Northern Pipefish				
Syngnathus louisianae	Chain Pipefish				
Syngnathus spp.	Pipefish				
Synodus foetens	Inshore Lizardfish				
Trachinotus spp.	Pompano				
Trinectes maculatus	Hogchoker				

Arithmetic means were computed for replicates of a given treatment for each season (1-to-2-day sampling event) for each site and for each gear type. To standardize sampling effort across all sites, we adjusted the abundance and summed total length of each species to account for gear efficiency and volume of water sampled. Drop and throw traps were considered 85% efficient for all nekton (Fonseca et al. 1990, Nestlerode 2004, Hovel et al. 2002) and seines and gill nets 75% efficient for most nekton (McIvor and Odum 1986). We then computed the abundance density (# individuals/m^3 for each species) and summed total-length density (combined mm of all individual/m^3 for each species) for cubic meter sampled (Table 5). Our analyses examined the similarities of the communities sampled (species and their density) based upon 3 response metrics: the presence or absence, abundance, and summed total length (TL) of all species.

Table 5. Description of gear type and catch efficiency and volume of water column sampled

State	Gear type	Mesh size (cm)	Volume of water column sampled/ replicate (m^3)	Catch Efficiency
NC	Seine net	0.32	20.00	75%
	Gill net	4.0, 7.0, & 10.0	820.22	75%
SC	Drop net	0.635	350.00	85%
GA	Lift net (2015)	0.32	43.95	85%
	Lift net (2016)	0.32	36.60	85%

Data analysis

We examined the similarity of the communities sampled from all sites in NC, SC, and GA using PRIMER (v7, Clarke 2015) and PERMANOVA (v 6.1.11, Anderson 2001). To do this, we employed a nonmetric multidimensional scaling (nMDS) technique that uses ordination to summarize patterns in the structure of multivariate datasets using the Bray-Curtis similarity index (Shepard 1962, Kruskal 1964, Legendre and Legendre 2012). PRIMER offers a graphical and statistical description of the relationships among biotic communities (Clarke 1993). One-way and two-way PERMANOVA tests were used to explore further differences between communities comparing the following factors: (1) structured (reef) vs unstructured (non-reef) habitats; (2) type of reef material (bagged shell, marl, coated pots, oyster castle, natural oyster reef); (3) site (PPR, Nols, PP, FtJ, BB, Sap, LSSI); (4) state (NC, SC, and GA); (5) season (Fall, Spring, Summer); and (6) year (2012, 2013, 2014, 2015, 2016, 2017). PERMANOVA extends the resemblance-based methods of PRIMER to allow for more complex statistical modeling of multivariate data; it is a nonparametric, multivariate analysis of variance based on permutations of Bray-Curtis or other similarity matrices (Anderson 2001). Data were prepared for PRIMER and PERMANOVA analyses using a fourth-root transformation to down-weight the importance of highly abundant species prior to analysis.

The nMDS graphs presented here display communities with the greatest degree of similarity closest to one another; thus, communities with the least in common are plotted furthest apart. Each point represents the biotic community (as described in methods), at one site, sampled from one specific treatment (reef or reference plot), in a specific season. PRIMER software randomly selects a point from the database, then randomly selects subsequent points, plotting them spatially, according to their degree of similarity or dissimilarity. Each analysis typically completes 999 permutations of this random-selection and spatial-plotting process. By using numerous permutations with randomly selected points, results reveal clear patterns in community composition and define the relationships between communities, which are assumed to be unburdened by random error. Because a series of permutations is initiated by one randomly selected point, results and their graphic representation can slightly differ among runs of the same analysis, even using the exact same database. Therefore, differences in the way points are plotted can be observed; however, community relationships persist.

Within any given cluster of points exists a centroid (not plotted) from which one can visualize the degree to which communities relate to one another. Note that dissimilarity can be just as relevant as similarity when examining communities. For example, we would not want to suggest that two communities are similar because they *lack* the same species, but because they contain the same species. The more similar the ratio of abundance or summed total length of in-common species results in communities being plotted relatively close in proximity. The nMDS graphs are color- or icon-coded to emphasize the similarities and dissimilarities in describing community structure.

Results

Presence-absence of species

The patterns of results in which we considered only the presence or absence of species differed little from the results in which we examined the abundance density of species in communities. Because measures of community abundance and total-length density better characterize community composition, we present results of these assessments only. We considered the tests of comparing simply the presence and absence of species because it is the most basic comparison of the structure of biotic communities and can sometimes offer insight where more-detailed response metrics are confounded by experimental variables. For this assessment, our database was sufficient such that communities were more fully described by the abundance and summed total length of each species.

Abundance density

The abundance density of biotic community assemblages of organisms using reef and non-reef habitats clearly differed significantly by site (p= 0.001) and by state (p= 0.001) (Fig 2 and 3). Community structure was significantly similar among the various reef materials used in oyster reef restoration (p= 0.001), with important species of recreational interest (Spotted seatrout *Cynoscion nebulosus,* Red drum *Sciaenops ocellatus,* Southern flounder *Paralichthys lethostigma,* Striped bass *Morone saxatilis,* White perch

Morone americana, Summer flounder *Paralichthys dentatus)* contributing to shaping those communities (Fig 4 and 5).

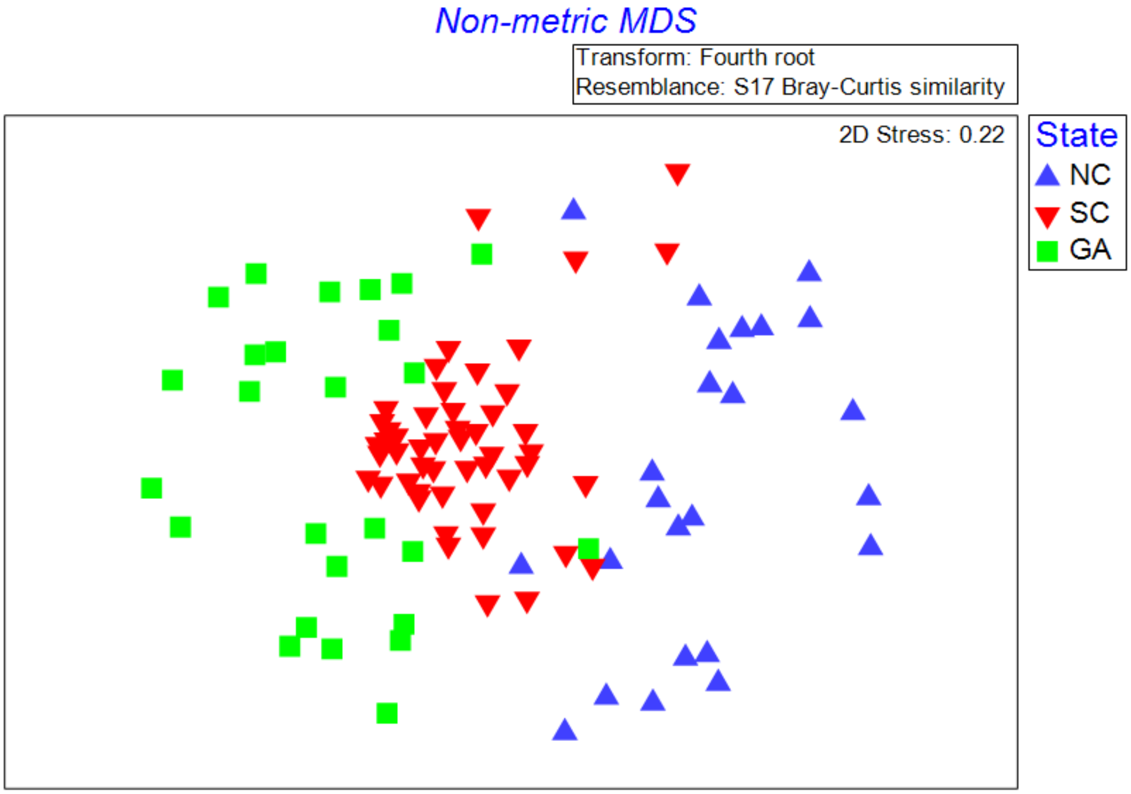

Figure 2. nMDS plot showing the similarities of nekton communities, based upon the abundance density of its species, by state.

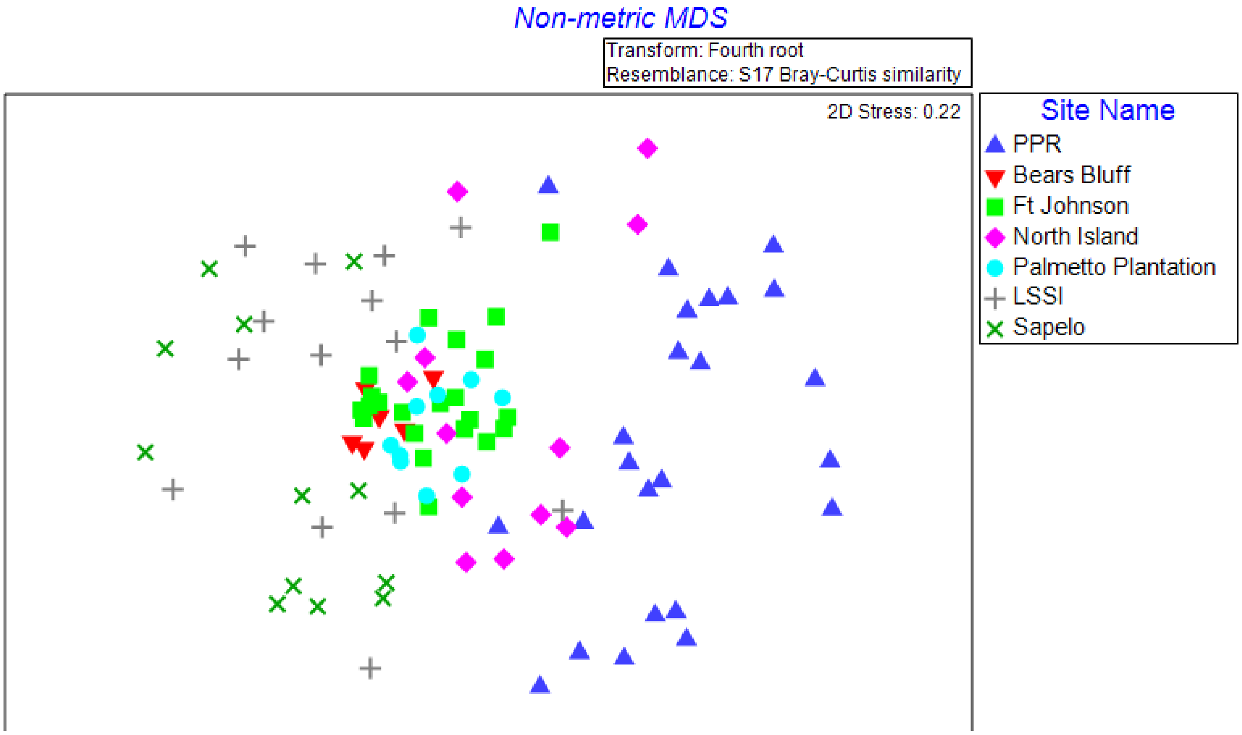

Figure 3. nMDS plot showing the similarities of nekton communities, based upon the abundance density of its species, by site.

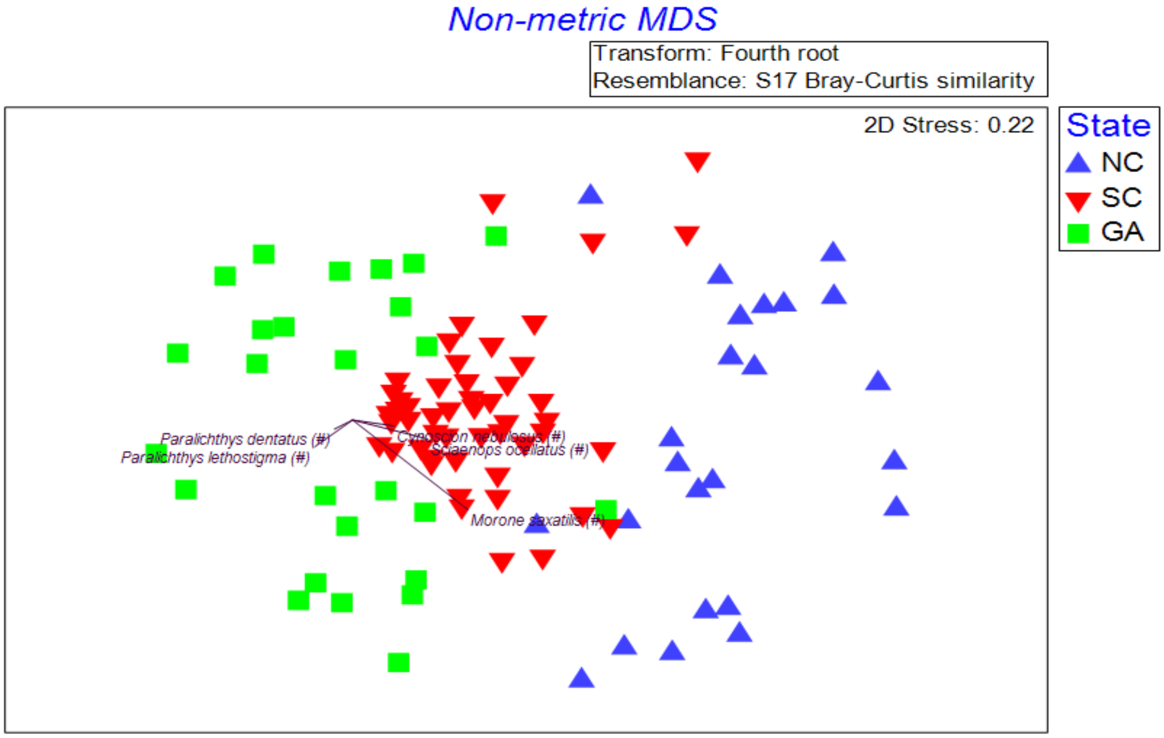

Figure 4. nMDS plot showing the similarities of nekton communities, based upon the abundance density of its species, by state, with vectors showing the role of important recreational fishes (Spotted seatrout *Cynoscion nebulosus,* Red drum *Sciaenops ocellatus,* Southern flounder *Paralichthys lethostigma,* Striped

bass *Morone saxatilis,* White perch *Morone americana,* Summer flounder *Paralichthys dentatus)* in shaping those communities.

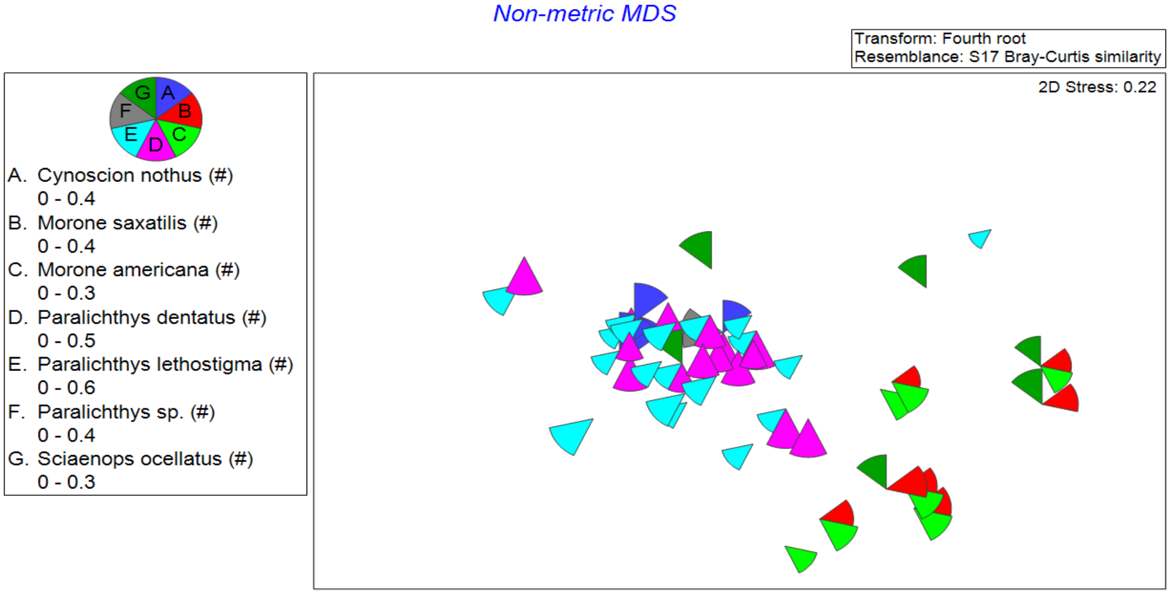

Figure 5. Contribution of selected recreational species of interest to the composition of communities sampled on restored oyster reefs (Spotted seatrout *Cynoscion nebulosus,* Striped bass *Morone saxatilis*, Red drum *Sciaenops ocellatus,* Summer flounder *Paralichthys dentatus,* Southern flounder *Paralichthys lethostigma,* Flounder *Paralichthys sp.)*

Perhaps most important to learn was that biotic communities differed between structured and unstructured habitats (p= 0.04), even though these communities were only 50% similar in many of the individual comparisons (Fig 6). It is clear that some sites supported communities that were quite similar and dissimilar when examining by structured and unstructured habitats (Fig 7). A summary of all of the one-way and two-way PERMANOVA tests that we performed may be found in Table 6. One-way PERMANOVA tests showed only marginally significant differences between the structured and unstructured habitats; however, this factor was significant in two-way PERMANOVAs with site and state, and when nested within site and state (Table 6). Additional tests would likely explain how the structure of these communities differed among sites and states. Because each PRIMER and PERMANOVA test starts at a randomly selected point, we have reported here multiple p-values for the tests that we repeated several times, as the full range of results is most informative in describing relationships among the communities we sampled. You will note that multiple tests yield highly similar results.

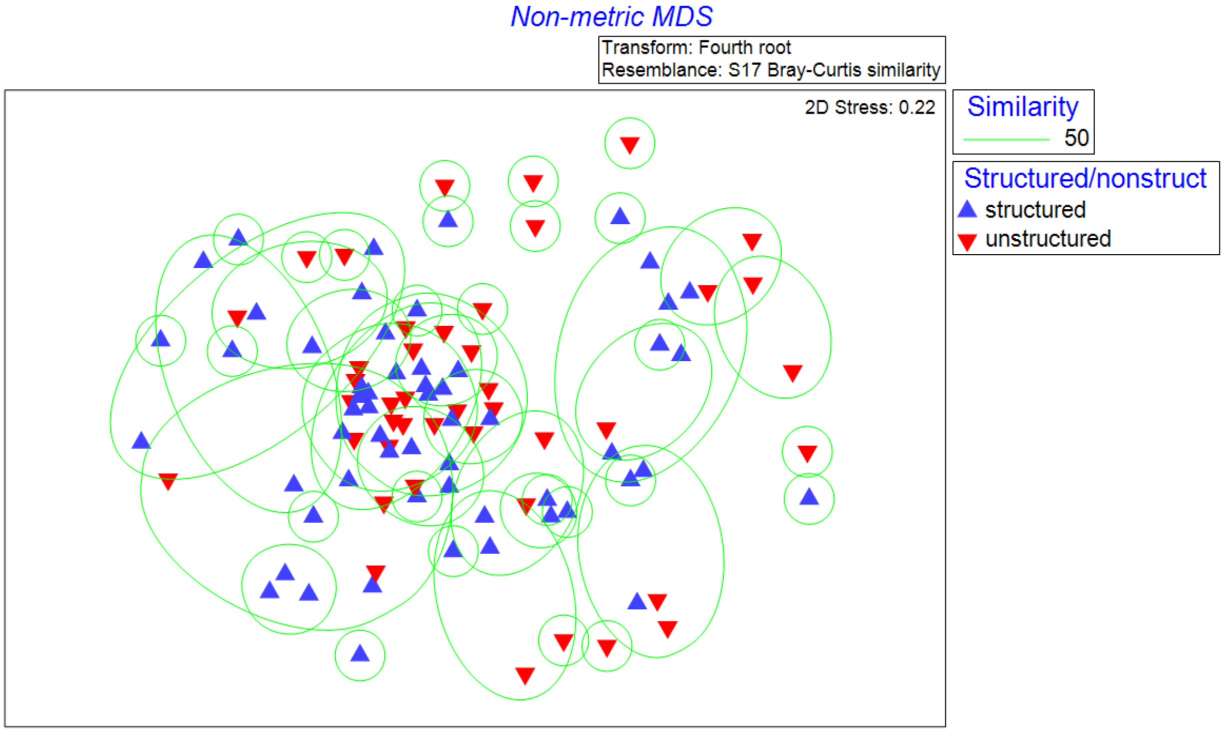

Figure 6. nMDS plot showing abundance density of sampled communities by structured and unstructured habitats with communities that are up to 50% similar in composition encircled in green.

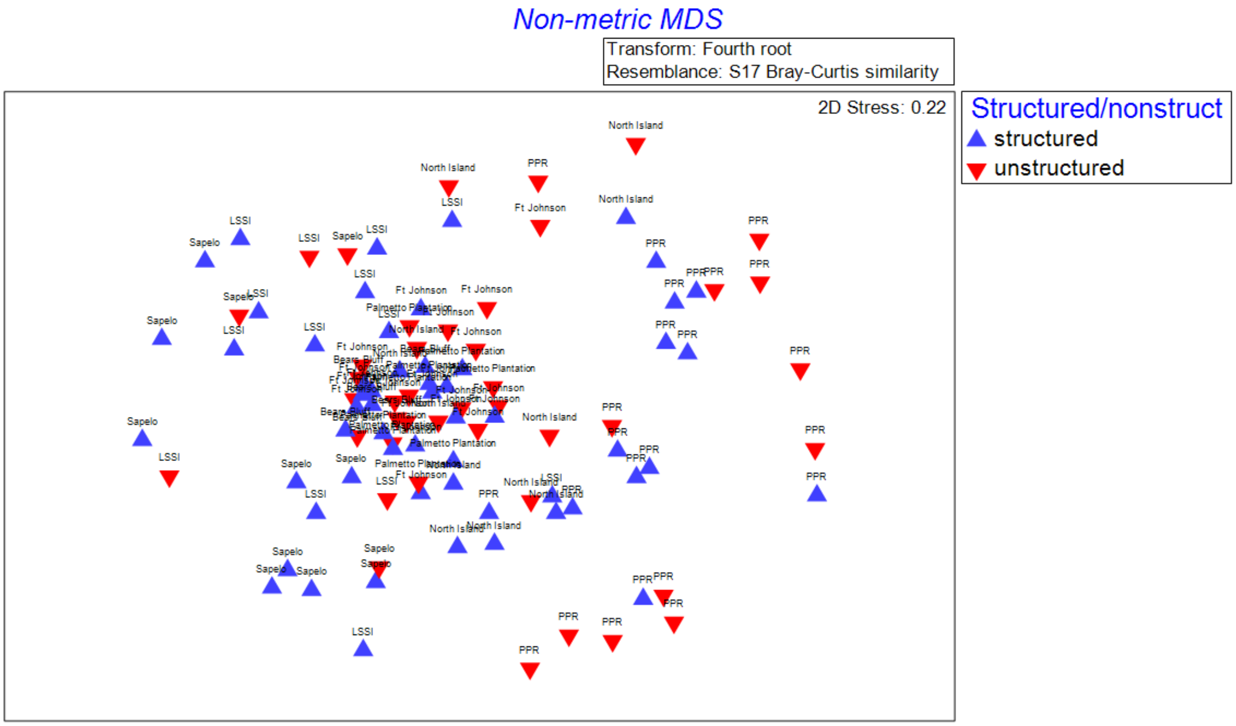

Figure 7. nMDS plot showing abundance density of sampled communities by structured (blue) and unstructured (red) habitats with study site location labeled.

Table 6. Results of one-way and two-way PERMANOVA tests used to compare the similarity of community structure of restored oyster reef habitats and non-reef reference plots. These tests used the following measures of community composition: (1) abundance density; (2) summed total-length density; and (3) fish-only abundance density. Mean p-values are shown when multiple permutations were considered.

PERMANOVA TEST variable(s)	**ABUNDANCE Density**	**SUMMED TOTAL-LENGTH Density**	**Fish-only ABUNDANCE Density**
State	0.001	0.001	0.001
Site	0.001	0.001	0.001
Structured vs unstructured	0.040	0.038	0.055
Reef material (all treatments)	0.001	0.001	0.001
Reef material (only structured treatments)	0.001	0.001	0.001
Year	0.001	0.001	0.001
Season	0.001	0.001	0.002
Reef material **State** **Reef material x State** **Reef material nested in State**	0.001 0.001 0.019 0.008	0.001 0.001 0.041 0.023	0.001 0.001 0.478 0.206

PERMANOVA TEST variable(s)	ABUNDANCE Density	SUMMED TOTAL-LENGTH Density	Fish-only ABUNDANCE Density
Reef material	0.001	0.001	0.001
Site	0.001	0.001	0.001
Reef material x Site	0.135	0.335	0.443
Reef material nested in Site	0.125	0.416	0.420
Struc vs Unstruc	0.011	0.007	0.019
State	0.001	0.001	0.001
State x Str v Unstr	0.028	0.067	0.153
Struc vs Unstruc nested in State	0.007	0.021	0.196
Struc vs Unstruc	0.008	0.005	0.015
Site	0.001	0.001	0.001
Site x Str vs Unstr	0.584	0.828	0.894
Struc vs Unstruc nested in Site	0.189	0.408	0.685
Site	0.001	0.001	0.001
Yr	0.002	0.001	0.001
Site x Yr	0.170	0.042	0.461
Site	0.001	0.001	0.001
Season	0.001	0.001	0.001
Site x Season	0.001	0.001	0.002
Season	0.001	0.001	0.001
Reef material	0.001	0.001	0.001
Season x Reef mat	0.969	0.832	0.836
Subtidal v tidal	0.001	0.001	0.001
Struc vs Unstruc	0.025	0.020	0.034
Tide x Str v Unstr	0.017	0.050	0.543
Year	0.001	0.001	0.001
Reef material	0.001	0.001	0.009
Yr x Reef material	0.999	0.991	0.997

The abundance density of biotic community assemblages using reef and non-reef habitats clearly differed significantly by the type of material used to construct reefs (p= 0.001) (Fig 8). Reef material type was a significant factor explaining the difference in community assemblages, both when bare-sediment control samples were included and when excluded from this analysis (Table 6). Note that points representing communities sampled on bare sediment have a greater degree of dispersion from their centroid (are more broadly dispersed) than those of structured habitats (Fig 8). In two-way PERMANOVAs, community assemblages differed significantly (p=0.001) by reef material type, as well as by state (p=0.001) or site (p=0.001); however, this difference was reduced when communities associated with various reef materials were nested within state (p=0.008) and did not differ significantly when nested within sites (p=0.125).

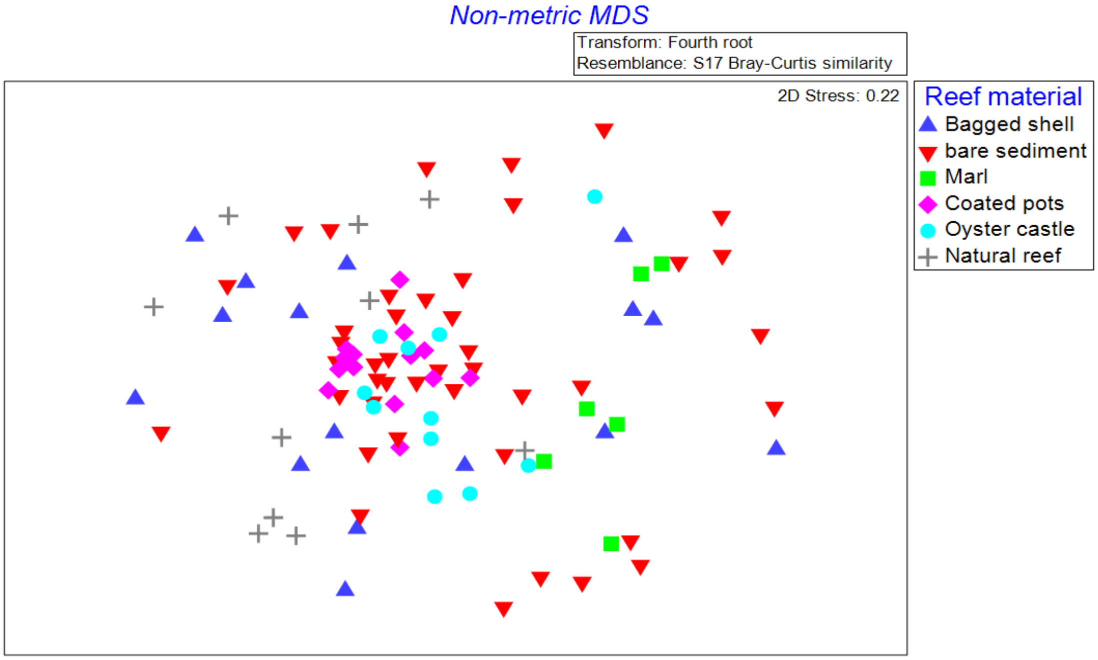

Figure 8. nMDS plot showing abundance density of sampled communities by reef material type.

As expected, the composition of communities differed significantly between seasons (p= 0.001) (Fig 9), as well as an interaction between site and season (p= 0.001), making clear that there were seasonal differences in the biotic communities observed at all study sites. Some of these seasonal differences in community composition could be due to the life history of species that use oyster reefs and other estuarine habitats in succession as individuals transition from juveniles to adults; growth through an individual's life is typically accompanied by a significant increase in size. Community composition also differed significantly between years (p= 0.001). Although year-to-year differences are clearly defined (Fig 10), some of this statistical difference is due to the fact that all sites were not sampled in all years. We combined the results of multiple nekton-sampling efforts to provide more powerful comparisons of the communities that use structured and unstructured habitats.

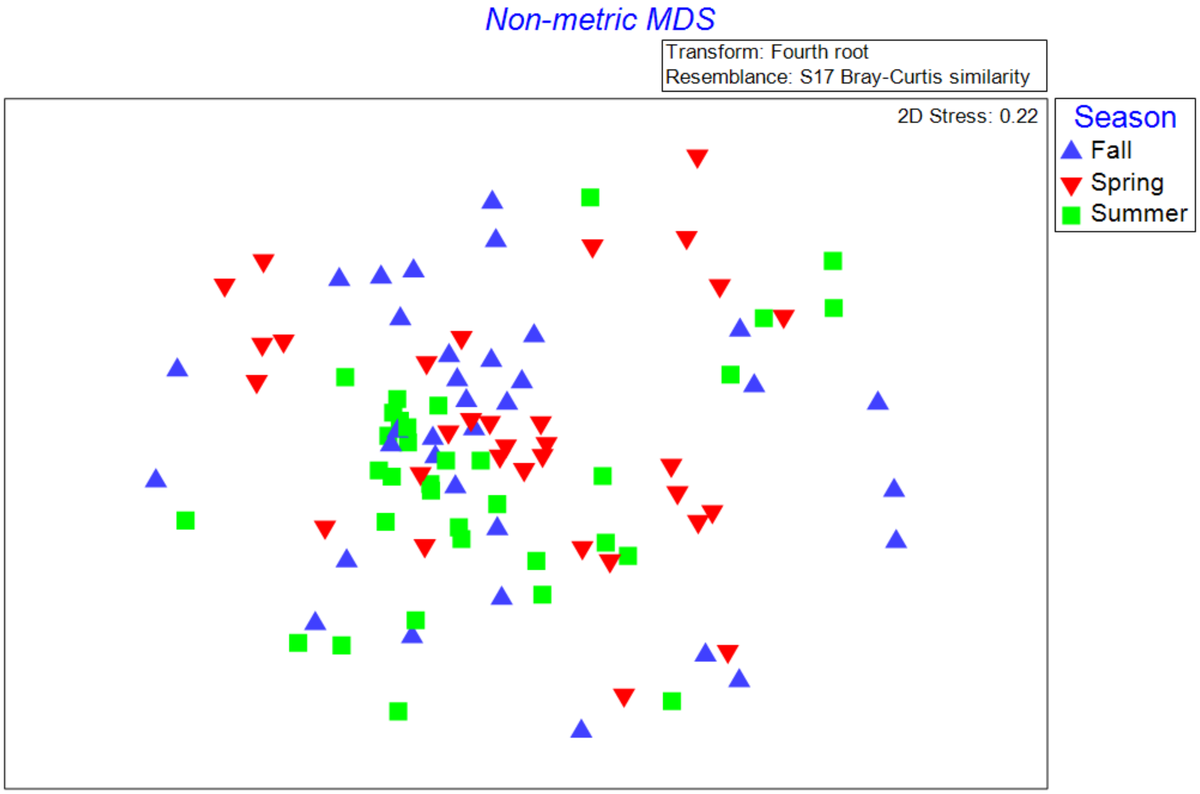

Figure 9. nMDS plot showing the abundance density of sampled communities by season sampled across the years of 2012 through 2016 (Note: not all sites were sampled in each year- see Table 2 for details).

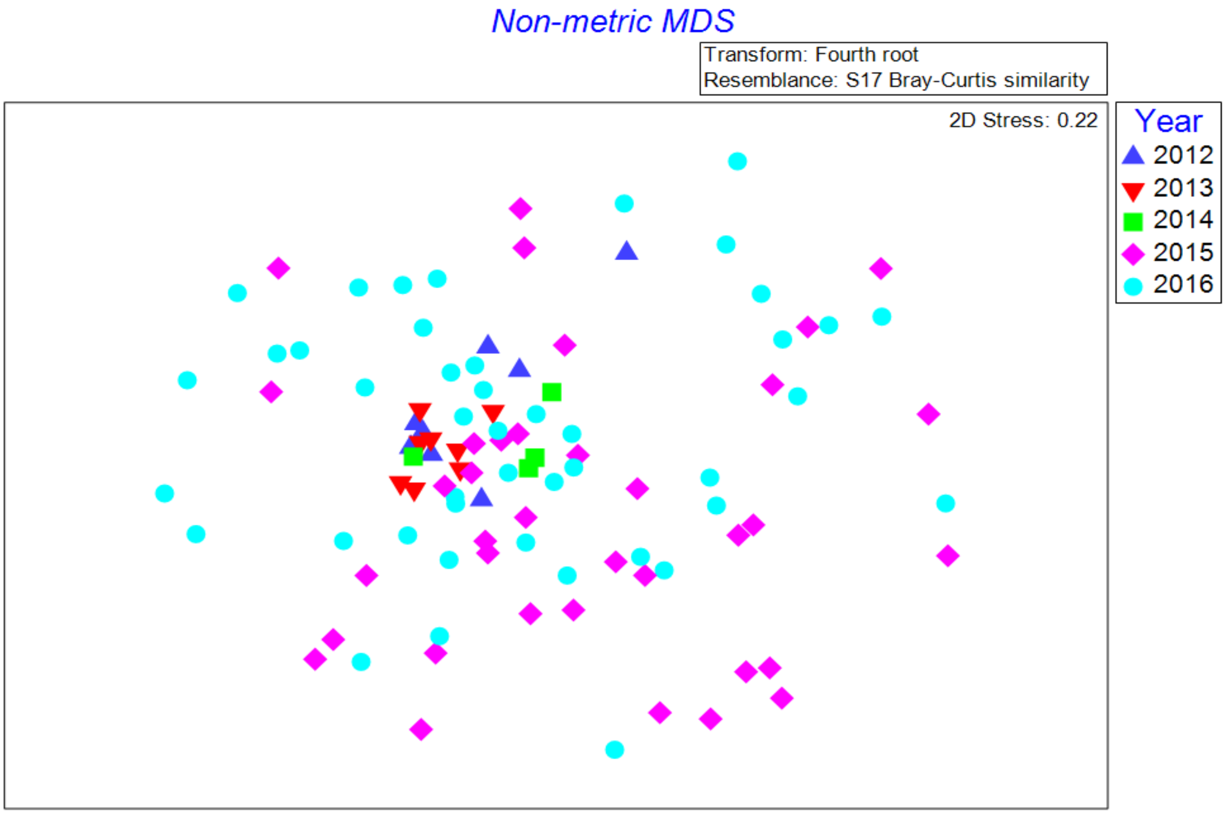

Figure 10. nMDS plot showing the abundance density of sampled communities by year sampled (Note: not all sites were sampled in each year- see Table 2 for details).

In addition to the tests of abundance density of the nekton communities (Table 3) sampled at our sites in the region, we also examined separately only the fish communities of our samples. Because fishes were largely responsible for driving the patterns described above, it was not surprising to find these same patterns when considering only fishes. PERMANOVA tests of the abundance density of fish-only followed patterns similar to that of the sampled biotic communities; results of these tests may be found in Table 6.

Summed total-length density

We conducted separate analyses on the density of summed total length of each species in sampled communities to compare with their abundance density. The total length of individual organisms represents the volume of biomass (or carbon in trophic level and food web terms) that is supported by various habitat types. In this regional assessment of the nektonic communities using restored oyster reef and unstructured habitats, we found great similarity between the abundance and summed total length of species in the communities we sampled.

The biotic community assemblages of organisms using structured versus unstructured habitats clearly differed significantly by site (p= 0.001) (Fig 11) with sites showing approximately the same degree of dispersion from centroids as in the analogous abundance density analysis (Fig 4 and 11). Community structure was also significantly similar among the three states (p= 0.001) (Fig 12), with important species of recreational interest contributing to shaping those communities.

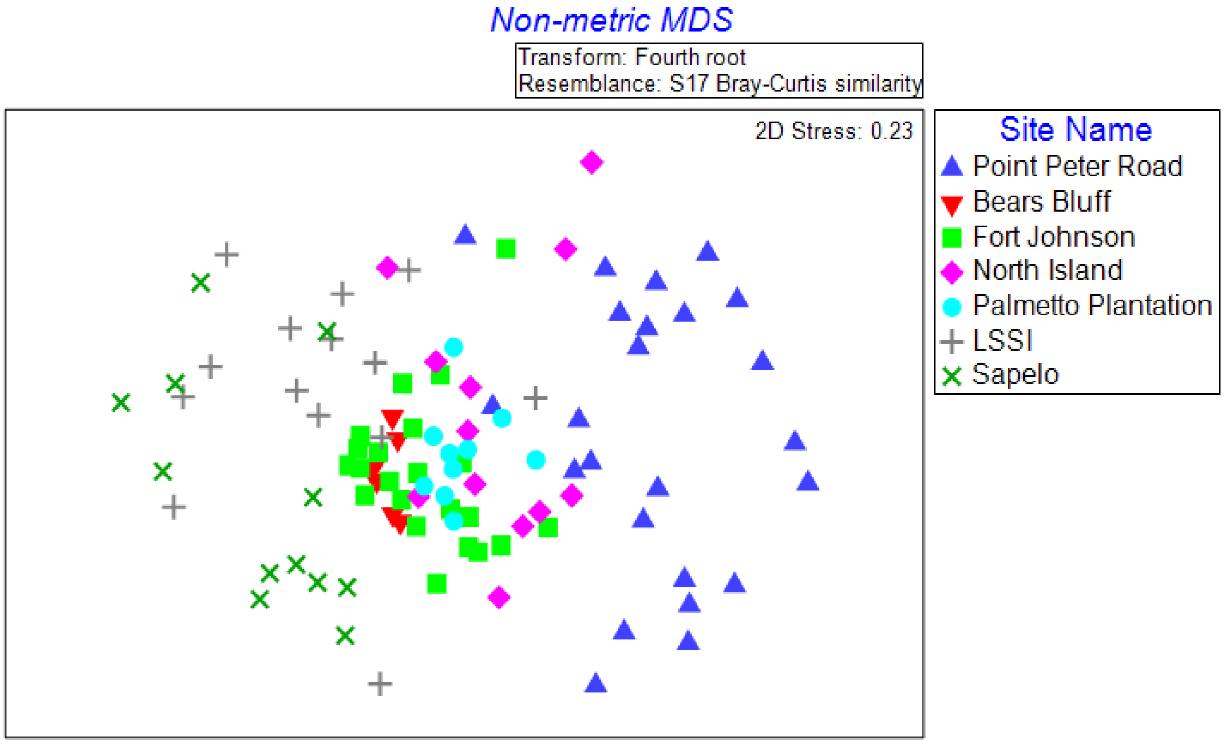

Figure 11. nMDS plot showing the similarities of nekton communities, based upon the density of the summed total length of individuals of its species, by study site.

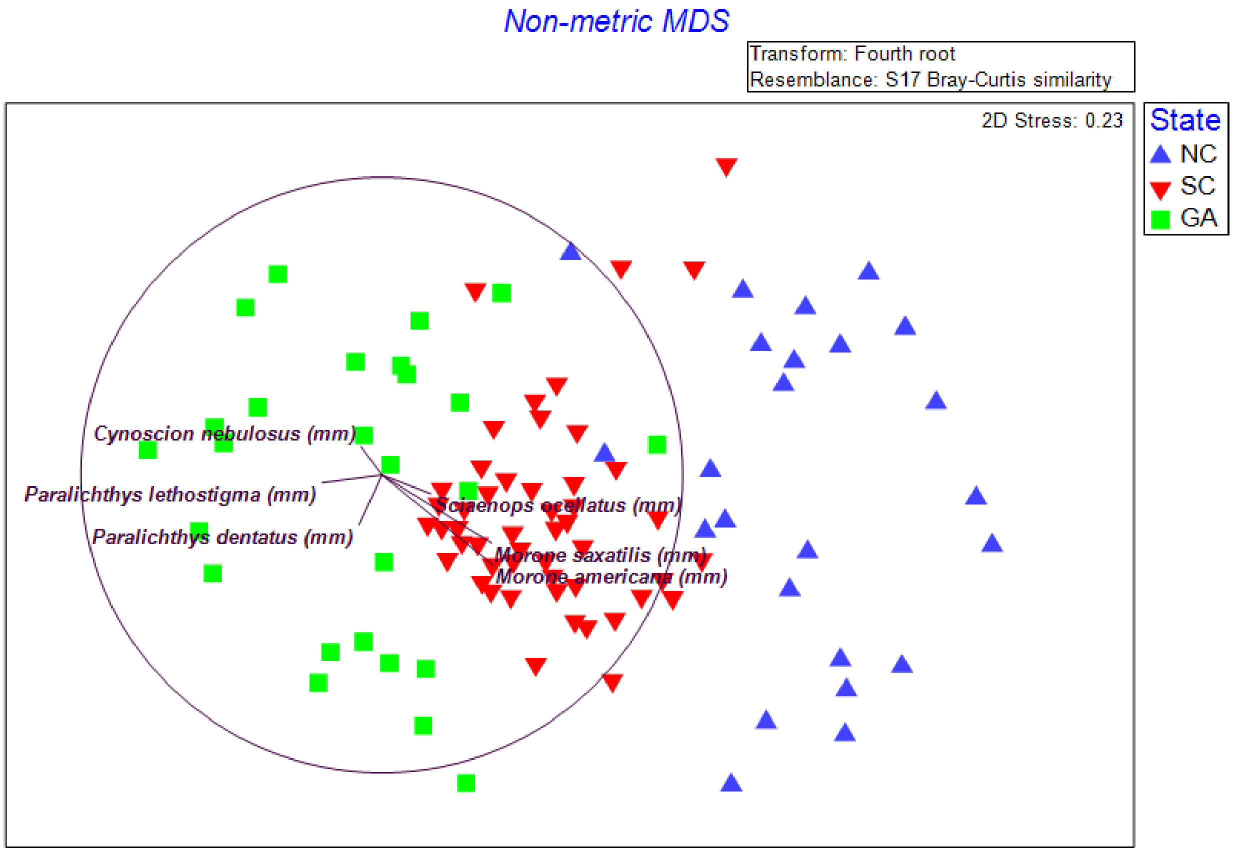

Figure 12. nMDS plot showing the similarities of nekton communities, based upon the density of the summed total length of individuals of its species, by state, with vectors showing the role of important recreational species (Spotted seatrout *Cynoscion nebulosus,* Red drum *Sciaenops ocellatus,* Southern flounder *Paralichthys lethostigma,* Striped bass *Morone saxatilis,* White perch *Morone americana,* Summer flounder *Paralichthys dentatus*, Flounder *Paralichthys* sp.) in shaping those communities.

As observed in the abundance density of nekton, community similarities when considering the summed total length density of all individuals differed between structured and unstructured habitats (p= 0.038) in one-way PERMANNOVAs (Figs 13 and 14), with species of flounder and spotted sea trout clearly favoring structured habitats (Fig 13). The green spheres in Figure 14 show the communities that were at least 25% similar and indicate that the degree to which communities were similar was somewhat lower when examining the summed total length of individuals across species than when examining their abundance. This lower degree of similarity is also reflected in a two-way PERMAOVA tests of structured vs unstructured habitats differed (p=0.007) with states (p=0.001), showing a marginal interaction of these factors (p= 0.067) and PERMAOVA tests of structured vs unstructured habitats differed (p=0.005) with sites (p=0.001), showing no interaction of these factors (p= 0.408). Two-way PERMANOVAs found the communities associated between structured and unstructured habitats to differ significantly within each state (p=0.021), but not within each site (p=0.408) when tested in a nested test design. As with tests of the abundance density of communities, it is likely that the small sample size at each study site is sufficient to discern differences between structured and unstructured habitats, as the larger, combined dataset infers that the communities using these habitats do differ (p=0.038).

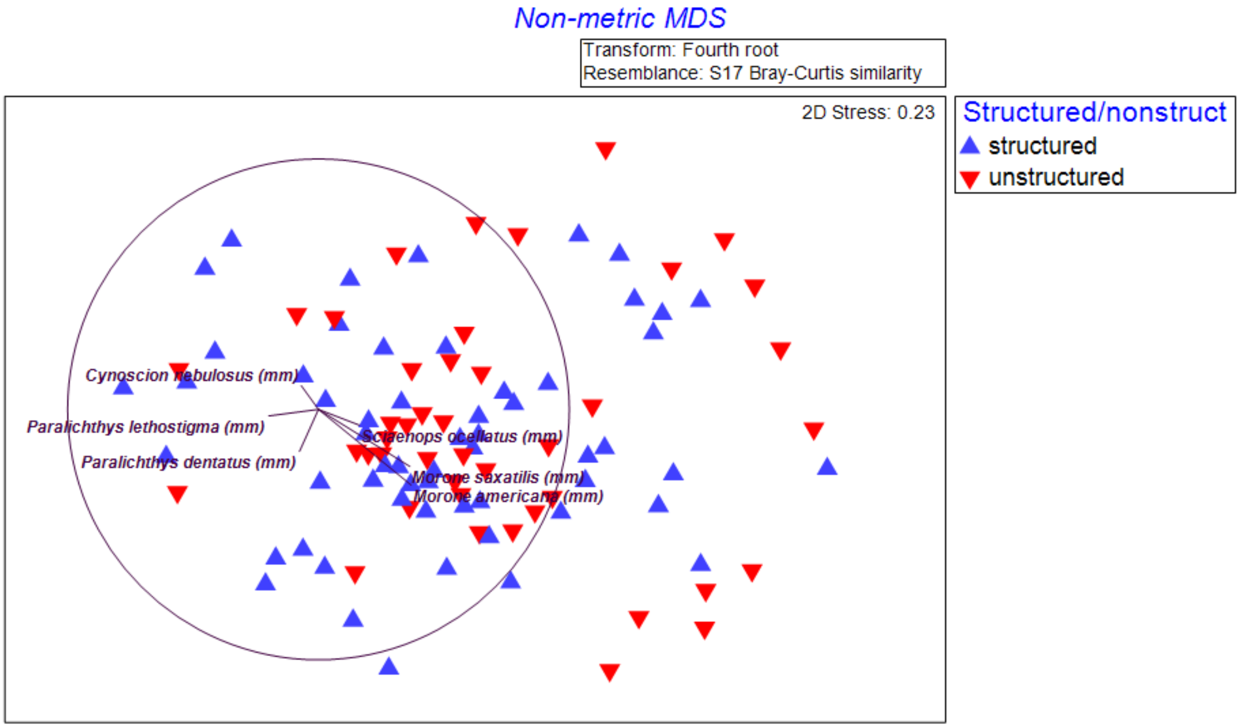

Figure 13. nMDS of the summed total length density of communities by structured (blue) and unstructured (red) habitats, with vectors showing the relative contribution of important recreational species to those communities (Spotted seatrout *Cynoscion nebulosus,* Red drum *Sciaenops ocellatus,* Southern flounder *Paralichthys lethostigma,* Striped bass *Morone saxatilis,* White perch *Morone americana,* Summer flounder *Paralichthys dentatus,* Flounder *Paralichthys* sp.).

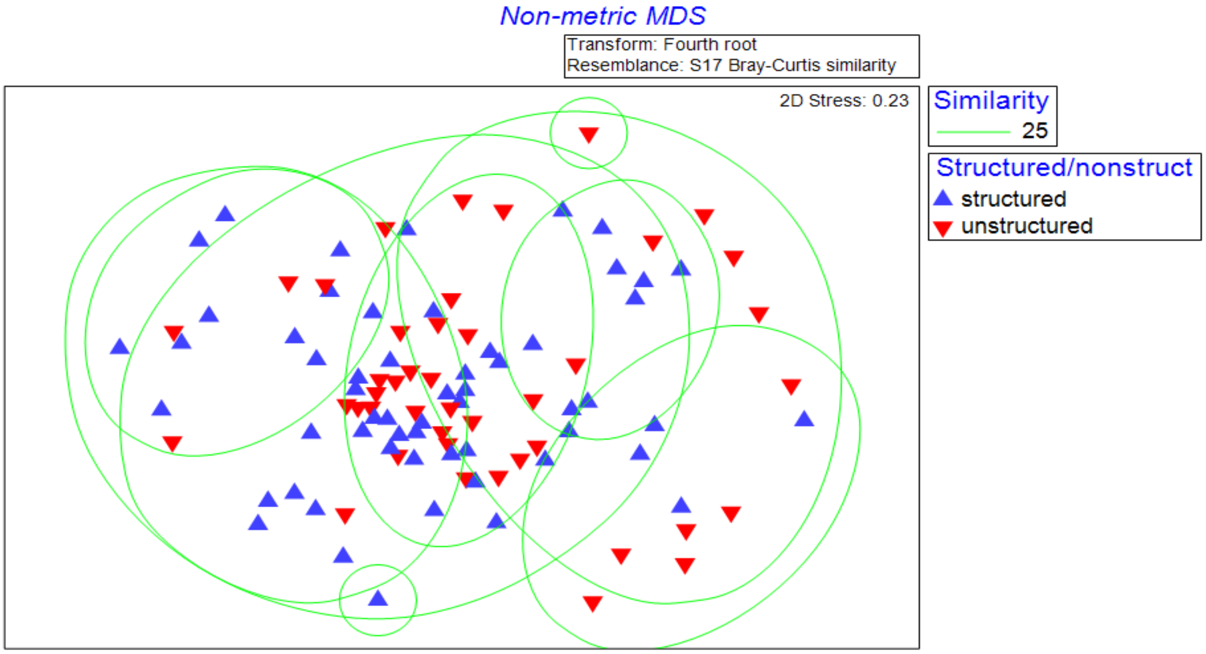

Figure 14. nMDS plot of the summed total length density of communities by structured (blue) and unstructured (red) habitats, with communities that were at least 25% similar encircled in green.

Similar to our examination of communities by their abundance density, the summed total length density of biotic community assemblages clearly differed significantly by the type of material used to construct reefs (p= 0.001), whether bare sediment control treatments were included or excluded from tests (Fig 15) (Table 6). In two-way PERMANOVAs, community assemblages again differed significantly (p=0.001) by reef material type, as well as by state (p=0.001) or site (p=0.001); however, this difference was reduced when communities associated with various reef materials were nested within state (p=0.03) and did not differ significantly when nested within sites (p=0.416).

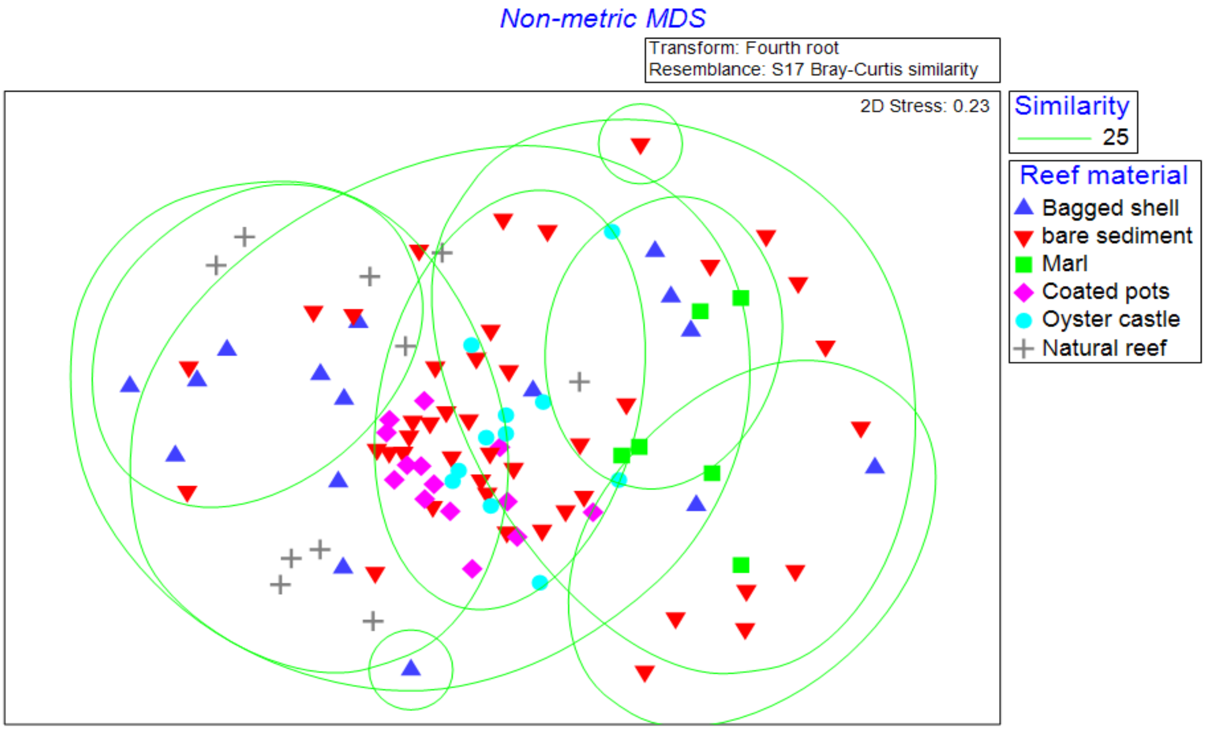

Figure 15. nMDS plot showing the similarities of nekton communities, based upon the density of the summed total length of individuals of its species, by type of reef material.

There were clear and significant (p= 0.001) differences in communities in each of the seasons (Fig 16) and years (p= 0.001) (Fig 17) sampled. Two-way PERMANOVA tests indicated a significant interaction (p= 0.001) between site and season in describing community composition. This is logical, because not only do species utilized different estuarine habitats as they transition from juveniles to adults, but the increase in size over time.

Differing from measures of abundance density, for summed total length density we found a slight interaction between site and year (p= 0.049), meaning the total length of species differed between years. This difference was likely cause by the very large common carp that we found in and released from gill nets at Point Peter Road, NC during spring of 2016, when high levels of precipitation caused the salinity to drop quite low (Table 2).During this sampling event, we caught 9 carp individuals that were all over one-half a meter long (range: 59 to 84 cm).

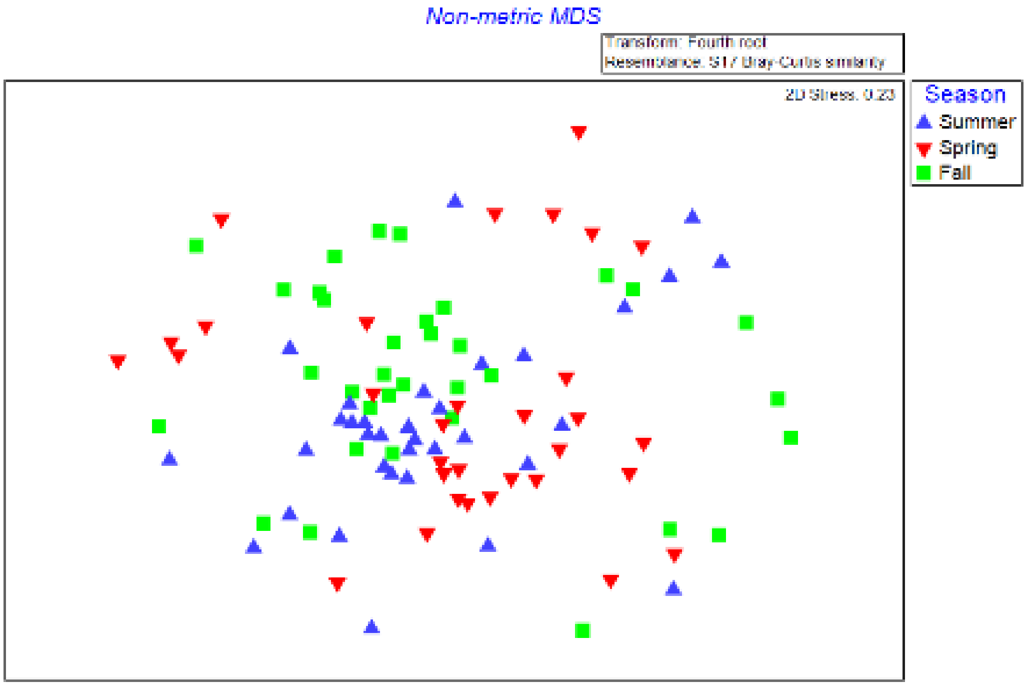

Figure 16. nMDS plot showing the summed total-length density of sampled communities by season sampled across the years of 2012 through 2016 (Note: not all sites were sampled in each year- see Table 2 for details).

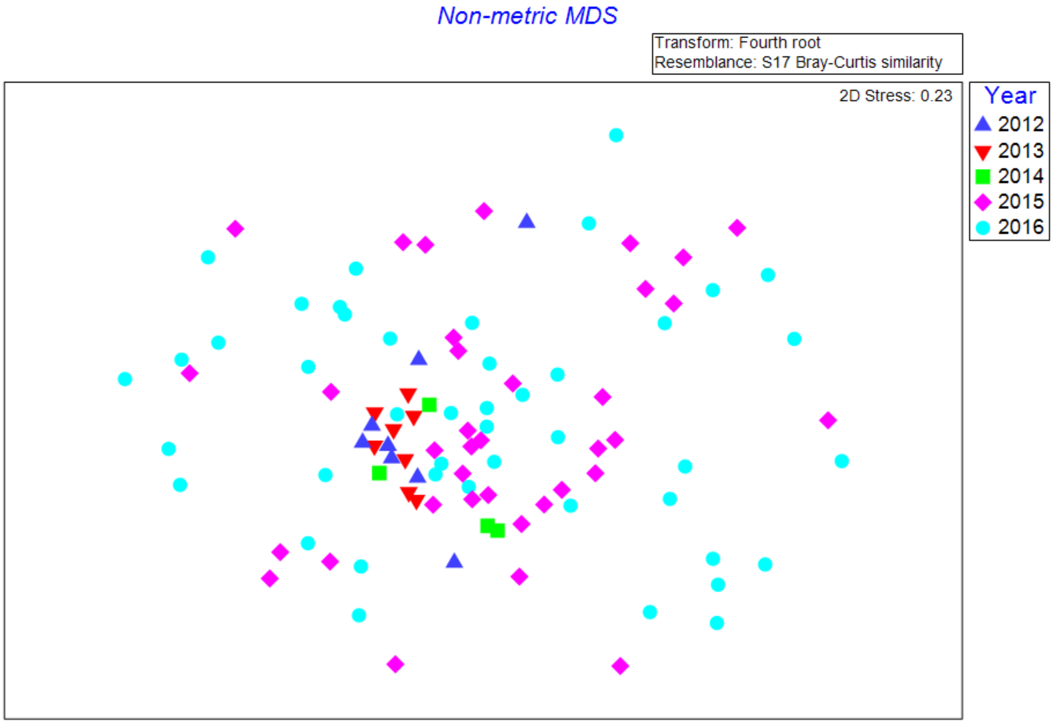

Figure 17. nMDS plot showing the summed total-length density of sampled communities by year sampled (Note: not all sites were sampled in each year- see Table 2 for details).

Discussion

This study was created due to the need to acquire additional scientific data quantifying the role restored oyster reefs play in fish communities while also analyzing restored oyster reefs value to commercially and recreationally important fish species on a regional scale. To do this, we monitored restored sites in NC, SC, and GA and coupled it with additional sampling data around restored sites in SC. Several of the restored sites are living shorelines which are being increasingly used across the region as a natural way to stabilize the shoreline while also mitigating erosion and maintaining a healthy estuarine habitat. Living shorelines serve a variety of purposes but are mainly installed to add shoreline stability and reduce erosion by absorbing energy, trapping sediment and enabling vegetation to grow. As an alternative to bulkheads and rip rap, they are promoted for their additional values as ecological habitat by providing crucial nursery habitat for recreationally and commercially important species, improving water quality, and providing food for numerous species. The sites chosen to be monitored varied by state based on location, reef structure, season and tidal regime. Despite these differences, the analysis shows significant variation in fish communities at structured vs. unstructured sites on multiple scales. This project increased our direct understanding of the link between fish communities and their utilization of oyster restoration sites in the southeastern estuaries.

The study included 76 species of finfish and 15 species of invertebrates from the structured and unstructured sites. Of these, 26 are managed species either by the individual states, by ASMFC and/or by SAFMC reflecting their importance to commercial and/or recreational harvest. These managed species included species of crustaceans, elasmobranches, and teleosts highlighting not only the importance of the species found but also the diversity of species. While oyster reefs are located in inshore coastal/tidal systems, 8 of the total caught species are managed by the SAFMC which focuses on offshore fisheries. This demonstrates a link between inshore and offshore habitats and emphasizes the importance of protecting and restoring oyster reefs not only to benefit inshore species, but also offshore species. Many of the species found are economically important to the area given their management status and recreational and commercial value. Oysters, blue crab, shrimp, flounders, king mackerels, and snappers are all listed as key South Atlantic commercial species, and all of these species were found in our study within the restoration sites (NMFS 2018). Additionally, blue crab and shrimp values are tremendous for GA, SC, and NC (NOAA National Marine Fisheries Service). In 2017, Blue crab were valued around $32.9 million in landings for all three states (NOAA National Marine Fisheries Service). Similarly, white shrimp were valued around $35.6 million in landings in 2017 for all three states (NOAA National Marine Fisheries Service). Inshore recreational fishing is important for contributions to state economies also. Atlantic croaker, spot, black sea bass, bluefish, king mackerel, red drum, sheepshead, Spanish mackerel and spotted sea trout are all species that have been listed as some of the key South Atlantic recreational species, and we commonly found these within the restoration sites (NMFS 2018). When analyzing differences among the types of oyster reef restoration material, there was a similar pattern of community composition with important species of recreational interest contributing to shaping those reef communities. These results can be used to communicate with recreational anglers about the value of oyster restoration, and hopefully build a coalition of support and funding for these activities and studies. Hancock and zu Ermgassen (2019) found that southern kingfish and striped bass (species found in this study) both spend a majority of their time on oyster reefs (52% and 93% respectively) and therefore, these fish derive most of their growth from the oyster habitat from disproportionally feeding on oyster reefs.

As Hancock and zu Ermgassen (2019) explain, simply understanding the loss and extent of oyster reef habitat does not generate incentive to fund and support oyster reef restoration at the necessary large scale. Highlighting a direct economic value that is received when these oyster restoration sites are implemented encourages the restoration effort and gives stakeholders a tangible gain to society (Hancock and zu Ermgassen 2019). The economic value from the recreationally and commercially managed fish species that were found to use oyster restoration sites can be transferred to the oyster restoration sites giving these sites an economic value that can begin to be quantified. When an economic value is placed on oyster restoration sites, this will also hopefully attract funding and public support which is needed to continue sampling and growing the database. Not only were many of the species economically-important but also ecologically-important. Prey species help maintain the balance in a fish community by occupying a central position in the food web. They prey upon the base of the food web and are preyed upon by larger, higher trophic level predators which are responsible for keeping the whole ecosystem in balance. Since prey species are mostly unmanaged and therefore can be overlooked, it is important to note that 22 prey species were found in this study. This emphasizes the important role that restored oyster sites play in maintaining the stability of the ecosystem and food web in the region.

The results demonstrate significant differences in nekton communities between structured and unstructured sites across the entire dataset, regionally. This continued to be true when evaluating states separately. The significant difference on a state by state comparison between structured and unstructured habitats is not surprising given the distance between the states and the diverse habitats found across the region. The variation existed when using presence-absence data, abundance density data and summed total length density data. We focused our analysis on the latter because they more fully describe the data and offer more insight. The statistical difference between unstructured and structured sites is not present when looking at individual sites. This could also be due to the small sample size of the communities within any one site, versus the larger and statistically more powerful and significant dataset testing all the communities of structured and unstructured habitats.

Next Steps

While we were able to build our knowledge on the role restored oyster reefs play within and to fish communities, the small individual site data highlights the need for continued sampling and demonstrates the value of combining data for a more powerful analysis. This assessment emphasizes the value of a large and diverse database to better understand the ways in which fish and other nekton use restored estuarine habitats. Continued sampling would provide more data for a larger dataset making the results between individual sites more statistically reliable. If sampling were to continue, trying to standardize the sampling methods would be beneficial, so that we all use the same nekton sampling gear and the results can be easily compared. Along with more data to compare within states, sites, and types of reef material, another opportunity for additional analysis would be to better understand what defines the community structures and why the communities are different based on the species represented. Examining these communities based on trophic level and relationship between benthic and more sessile organisms (mud crabs) with transient species would be helpful data to include in the future. The biodiversity index could be used as a potential tool to quantitatively measure diversity while also considering the evenness of species. For example, does greater biodiversity equate to greater abundance? Future studies would also ideally include a broader suite of environmental factors such as depth, proximity to inlets, by age of reef, etc.

In addition to recreational and commercial fishers, both the data and the study results could be useful for coastal and ocean organizations many of which conduct studies and set management plans for the oyster populations and nekton communities. It is important to share these results with coastal managers and decision-makers to continue the momentum on promoting and protecting living shorelines due to the important role they play for fish communities and to encourage additional studies. This helps the stakeholder groups that are involved in oyster restoration make a case for this type of work and set meaningful goals for the restoration project. For example, The Nature Conservancy is using the raw data to update the fish production component of the oyster calculator, a tool used to provide insight into how ecosystem services can be used to set oyster habitat restoration objectives. Sharing these results with public and private organizations that fund coastal habitat restoration is important given that some entities, such as NOAA National Marine Fisheries Service, require that habitat restoration projects demonstrate a connection to commercially and recreationally valuable fish. This project benefited from private dollars to support regional finfish monitoring around restored oyster reefs. Many existing funding programs do not provide money for ongoing monitoring or to expand efforts beyond oyster heath. As mentioned earlier, currently, little to no larger scale reef monitoring has been done in the southeast where data can be compared. Monitoring of fish communities within oyster reefs has mainly been done on a state-by-state basis with data difficult to compare. Continued and increased monitoring and additional analysis are vital for this study to succeed in the longer term. It is crucial to get this data out to regional stakeholders and funders, so they can visualize the benefits in larger scale restoration projects economically and ecologically.

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