

COASTAL RECREATIONAL FISHING LICENSE

FINAL REPORT

DEVELOPING METHODOLOGY FOR ASSESSING FISH USE IN STRATEGIC HABITAT AREAS

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Description of Work:

Identification of specific critical areas for important fishery species is a means of prioritizing conservation, enhancement, and restoration of coastal habitats. This, in turn, will enhance fishing opportunities in coastal North Carolina. The division completed a GIS-based spatial analysis in coastal watersheds to identify a network of high-quality habitat areas, referred to as Strategic Habitat Areas (SHAs) in 2017. Analyses were done for each of the four Coastal Habitat Protection Plan (CHPP) coastal regions (Figure 1). Field sampling was necessary to validate fish use and habitat condition of the SHAs selected by the GIS analysis and/or refine the SHA boundaries if necessary.

This CRFL project began as a pilot study within one SHA region (White Oak River Basin, Region 3; Figure 2) to determine the most ecologically sound and effective method to verify the quality of SHAs and define habitat metrics. Multiple gears were used to sample the shellfish and finfish communities in or adjacent to three coastal fish habitats (wetlands, submerged aquatic vegetation (SAV), and shell bottom) along with several metrics for these habitats. Sampling was conducted both inside and outside of designated SHAs.

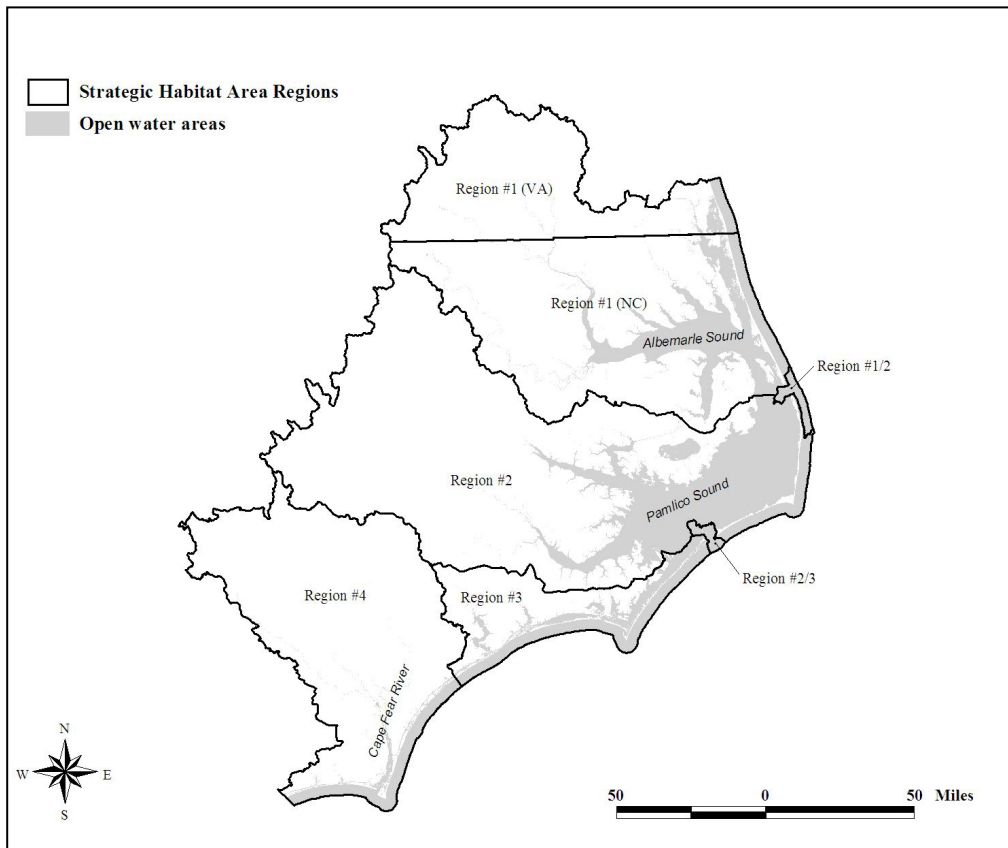


Figure 1. Regional boundaries for Strategic Habitat Areas delineations.

Region 3 includes waters in Carteret and Onslow counties, as well as a small amount of Jones and Craven counties, and the entire watershed is contained within the White Oak River Basin. This region lacks extensive riverine systems and consists primarily of estuarine waters and small to moderate sized sounds such as Core, Bogue, and Stump sounds. New River is the largest river. Submerged Aquatic Vegetation (SAV) and wetlands (marsh and forest) are extensive, and intertidal and subtidal oyster reefs and ocean hard bottom are also present. Oyster, clam, bay scallop, blue crab, shrimp, southern flounder, red drum, spotted seatrout, weakfish, and spot were determined to be priority fishery species in this region, and an important nursery area for gag grouper and black sea bass. There were 48 discrete SHA units selected within Region 3.

Region 4 is the southernmost region and includes riverine and estuarine waters in Pender, New Hanover, and Brunswick counties, as well as portions of Duplin, Sampson, Bladen, and Cumberland counties. It includes the Cape Fear River system upstream to approximately Lillington, the historical anadromous fish spawning grounds of Smiley Falls (approximate fall line). The estuarine waters include multiple small tidal creeks and sounds and extensive intertidal oyster reefs and marsh. Relatively small areas of SAV occur in the northern portion of the region. The priority fisheries species of the Cape Fear River Basin include eastern oyster, hard clam, blue crab, shrimp, bay scallop, southern, red drum, spotted seatrout, kingfishes, and spot. The Cape Fear River system is vital to anadromous species, including striped bass, American shad, river herring, and sturgeon, that migrate upstream for spawning; while the nearshore provides important habitat for gag grouper, black sea bass, sheepshead, and mackerels. There were 43 discrete SHAs selected within Region 4.

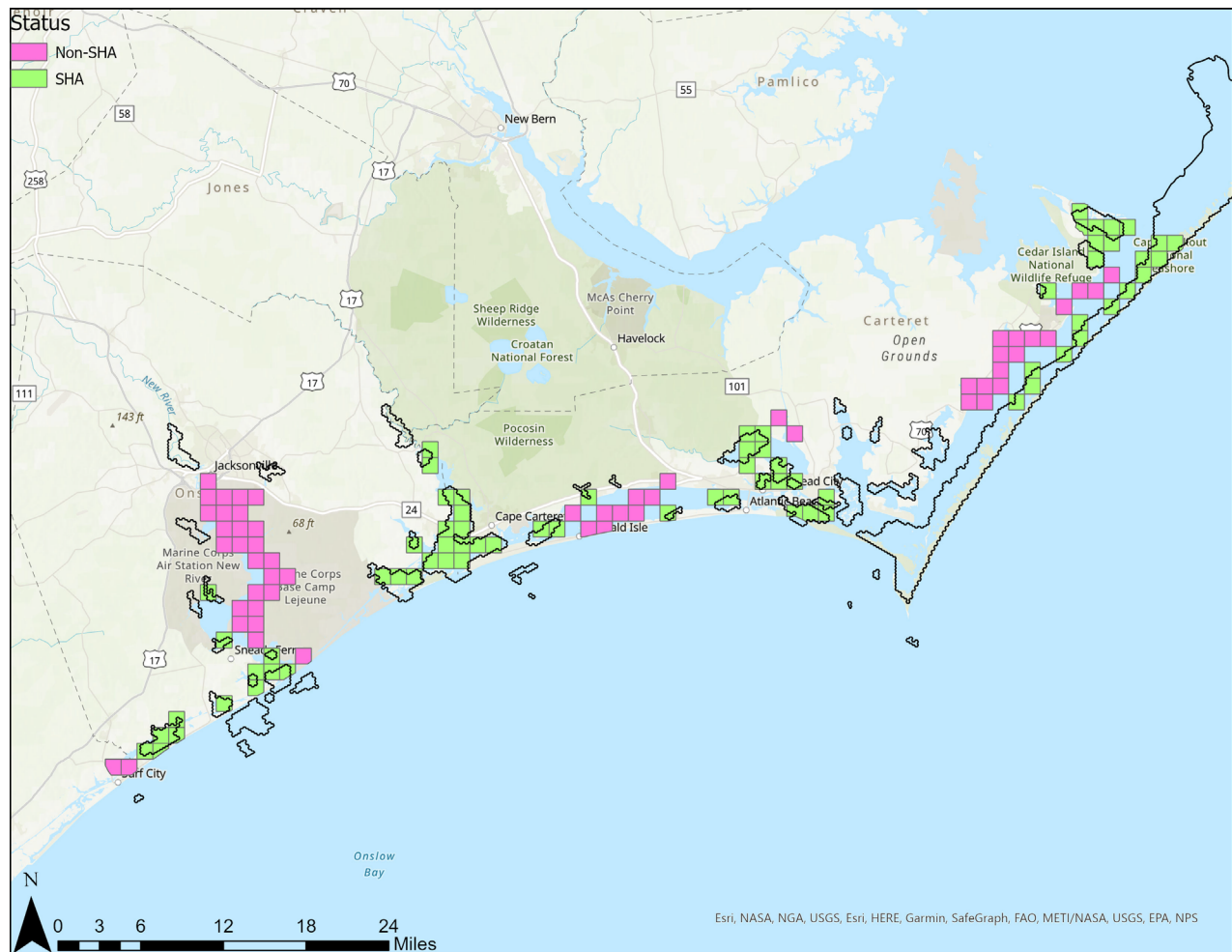


Figure 2. Region 3 Strategic Habitat Areas (SHAs) sampling area with one nautical mile (nmi) square grids stratified by SHA and Non-SHA. SHA polygons outlined in black.

Project Status/Work Accomplished:

After two full seasons of sampling in SHA Region 3 (Figure 2), a deviation from the original scope of work along with a no-cost extension was requested and approved in June 2019. This allowed for expansion of one year of sampling into an additional SHA region (Cape Fear River Basin, Region 4; Figure 3). In early 2021, a no-cost extension was approved to conduct a second year of sampling in Region 4 to complete field verification. The sampling schedule for both regions is shown in Table 1. As part of these extensions, we will also provide recommendations on any methodology changes needed to field verify Regions 1 and 2 and suggest if/how the sampling protocol could be used or modified to evaluate nursery areas.

Table 1. Revised grant timeline, with no-cost extensions, July 2017- June 2022.

Six month period	Six month period	Task
	7/1/2017-12/30/2017	Planned; bought gear

1/1/2018-6/30/2018	7/1/2018-12/30/2018	Sample R3, Year 1
1/1/2019-6/30/2019	7/1/2019-12/30/2019	Sample R3, Year 2
1/1/2020-6/30/2020	7/1/2020-12/30/2020	Sample R4, Year 1
1/1/2021-6/30/2021	7/1/2021-12/30/2021	Sample R4, Year 2
1/1/2022-6/30/2022		Data entry, analysis, report writeup

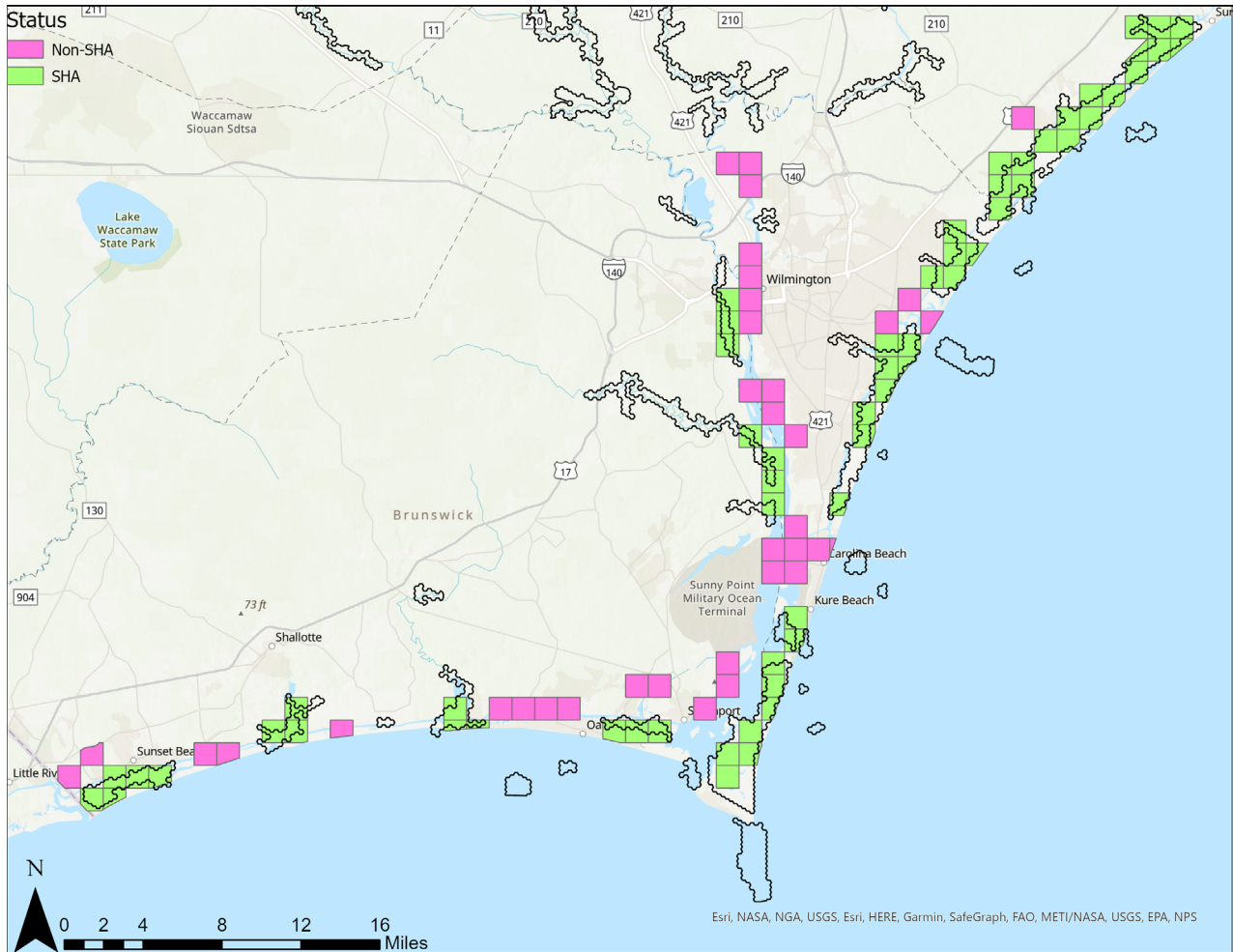


Figure 3. Region 4 sampling area with one nautical mile (nmi) square grids stratified by SHA and Non-SHA. SHA polygons outlined in black.

Study objectives

- 1) Conduct extensive field sampling of target fish species in three fish habitats inside and outside of SHAs to verify habitat condition and biological productivity
- 2) Develop indicator metrics for validating SHAs based on target species use and habitat metrics

- 3) Produce a standard operating procedure (SOP) for monitoring and potentially modifying SHAs in the future based on indicator performance

Objective 1: Field sample to verify habit condition and fish productivity

In both regions, ArcGIS was used to create the sampling universe. A stratified random sampling methodology was used with one nautical mile (nmi) square grid overlaid on the region sampling area. It was then modified according to accessibility and ability to sample. The ArcMap Sampling Design Tool with the grid layer for SHA Region 4 was then used to randomly select 16 proportionally allocated SHA and non-SHA sites (1.0 x 1.0 nmi² grids) for each of the nine monthly sampling periods (Figures 2 and 3). Because the grids were randomly selected, not all SHAs were sampled. Additionally, in Region 4, the sampling grid did not extend upstream of approximately Lyon Thorofare on the Cape Fear River and Cowpen Branch on the Northeast Cape Fear River, excluding 16 SHAs due to difficulty in sampling with the different gears in that area.

From 2018-2019, 252 sites were sampled in Region 3, with 126 sites in SHAs, and 126 sites in non-SHAs (Table 2). September 2018 sampling was lower than other months due to Hurricane Florence. Other minor deviations were due to weather or staff limitations. Program documentation was developed and data entered into DMF’s Biological Database (BDB) under Program 215: Assessing fish use in SHAs.

Table 2. The number of Strategic Habitat Areas Region 3 sites sampled (Strategic Habitat Areas (SHAs) and Non-SHAs) during the 2018 and 2019 sampling seasons.

Month	2018			2019			Grand Total Sampled
	SHAs Sampled	Non-SHAs Sampled	Total Sampled	SHAs Sampled	Non-SHAs Sampled	Total Sampled	
February	2	2	4	4	4	8	12
March	5	6	11	8	8	16	27
April	7	8	15	8	8	16	31
May	8	8	16	8	8	16	32
June	7	7	14	8	8	16	30
July	8	8	16	8	8	16	32
August	8	8	16	8	8	16	32
September	1	1	2	8	8	16	18
October	8	8	16	8	8	16	32
November	0	0	0	4	4	8	8
Totals	54	54	108	72	72	144	252

Region 4 sampling occurred in 2020-2021, however due to the Covid-19 pandemic, sampling was modified. Sampling began on March 1, 2020, but was suspended from March 26th until June 2nd, 2020. Before sampling was suspended in March only ten of the proposed 16 sampling sites were completed and when sampling resumed in June only 14 of the proposed 16 sampling sites were completed. While sampling was suspended due to Covid-19, the

technicians wrote detailed sampling SOPs and completed further quality control of all project data in the Biological Database making corrections as needed. From July 1st through November 15th, 2020 sampling was completed, meeting the 16 sites/month goal. The 2021 sampling season began on February 15th, 2021 continuing to meet the 16 sites/month sampling goal during this reporting period, except in July due to staff vacancies. From 2020-2021, 244 sites were sampled in Region 4, with 147 sites in SHAs, and 97 sites in non-SHAs (Table 3).

Table 3. The number of Strategic Habitat Areas Region 4 sites sampled (Strategic Habitat Areas (SHAs) and Non-SHAs) during the 2020 and 2021 sampling seasons.

Month	2020			2021			Grand Total Sampled
	SHAs Sampled	Non-SHAs Sampled	Total Sampled	SHAs Sampled	Non-SHAs Sampled	Total Sampled	
February	0	0	0	5	3	8	8
March	5	5	10	10	6	16	26
April	0	0	0	10	6	16	16
May	0	0	0	10	6	16	16
June	7	7	14	10	6	16	30
July	8	8	16	8	3	11	27
August	8	8	16	10	6	16	32
September	10	6	16	11	6	17	33
October	10	6	16	10	6	16	32
November	10	6	16	5	3	8	24
Totals	58	46	104	89	51	140	244

At each site, fish sampling was done using breder traps, gill nets and bottom trawls. Where intertidal wetlands, oyster reef, and shallow SAV are present within a grid, four breder traps are set in each habitat type, alternating perpendicular and parallel orientation to the habitat edge. Four gill nets (2", 3", 4", 5" stretched mesh) are set within 100 ft of shore where water depth is roughly three ft at MLW. Breder traps and gill nets are set at low tide and soak for at least three and four hours, respectively. For both traps and gill nets, the collected fish are identified to species and counted. Target species are measured and weighed, and total biomass of all fish will be recorded. Trawling is done on a separate day or at the end of the sampling day on the falling tide.

Habitat metrics were collected at low tide (Table 4) while traps and gill nets are soaking using quadrat sampling. Wetland metrics include habitat type (fringe or isolated), connectivity to other habitats, observable erosion, plant species, shoot count, maximum and average shoot height, and other fauna present. Oyster reef metrics include reef type (fringe or isolated), connectivity to other habitats, shellfish species present, percent cover shell, total number of live oysters, length of 30 oysters, rugosity, and other fauna present. SAV metrics

include habitat type (fringe or isolated), connectivity to other habitats, SAV species present, percent cover, shoot density, maximum and average shoot height. Overall habitat condition metrics include water quality, extend of hardened shoreline and eroded edge, and presence of shoreline basins, marinas and/or boat ramps, rock or wood structures perpendicular to shore, low profile (riprap) or wood structure parallel to shore, and vertical structures (i.e. bulkheads), and the presence of shell bottom, marsh, and SAV.

Table 4. Habitat metrics collected at each site where the habitat existed.

Wetlands	Oyster reef	SAV
Habitat type (fringe or isolated)	Habitat type (fringe or isolated)	Habitat type (fringe or isolated)
Connectivity to other habitats	Connectivity to other habitats	Connectivity to other habitats
Plant species present	Shellfish species present	SAV species present
Percent cover	Percent cover	Percent cover
Plant height	Abundance and size frequency live oysters	shoot density
Fauna present	Fauna present	Plant height
Visible erosion	Rugosity	

All data were coded, submitted, and quality controlled. All sampling data for Region 3 and 4 have been submitted for inclusion in the BDB (n=2,195 collections).

Habitat

Analysis of the Region 3 and 4 habitat data confirm greater overall extent (# of sites and acres) of the habitats investigated in this study (marsh, shell bottom, and SAV) in the SHA grids compared to the non-SHA grids (Table 5, Figures 4-9). SHAs compared to non-SHAs also had much greater acreage of habitat complexes (two or more structured habitats rather than one habitat only). In both regions, non-SHAs had a higher number of sites and acres with wetlands only. Regions 3 and 4 had no sites with only shell. Region 3 SHAs and non-SHAs had similar numbers of sites/acres with SAV, while Region 4 had none. These results verify that the GIS analysis accurately selected areas with an abundance of diverse habitats.

Table 5. Total acreage of structured habitats (wetlands, shell bottom, SAV) within SHA and non-SHAs and mean acreage in SHA and non-SHA grids.

Area	Total Structured Habitat Area (acres)	Mean Area (acres/grid)
Region 3 SHA	1681.2	10.2
Region 3 Non-SHA	690.0	18.5

Region 4 SHA	1926.0	30.1
Region 4 Non-SHA	502.0	13.2

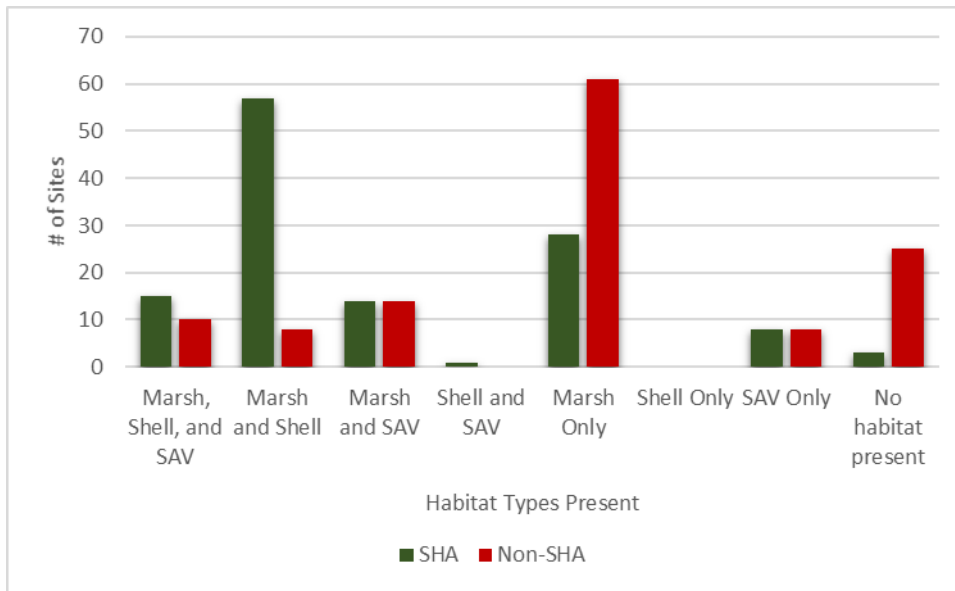


Figure 4. Number of sites with different habitat combinations present within the SHA and non-SHA sampling grids in Region 3, 2018-2019.

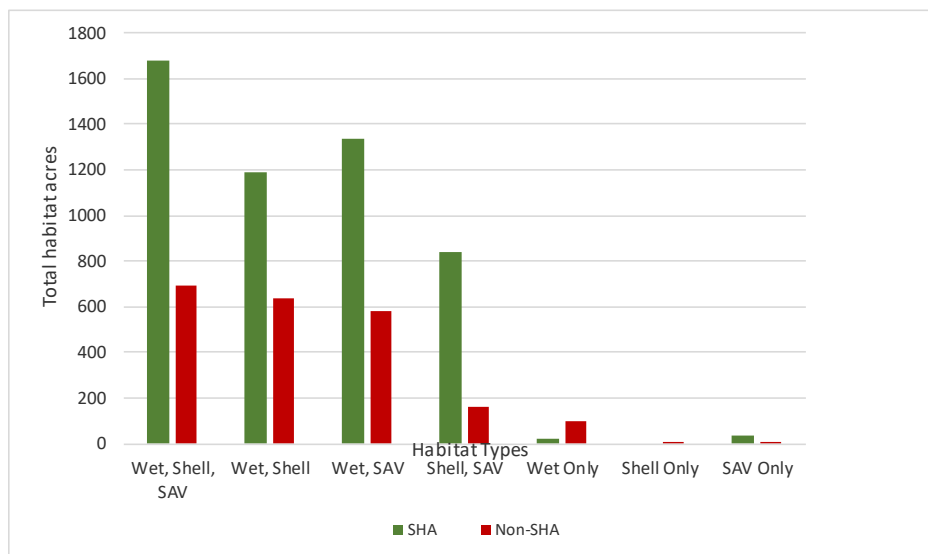


Figure 5. Acres of habitats present within SHA and non-SHA sampling grids in Region 3, 2018-2019.

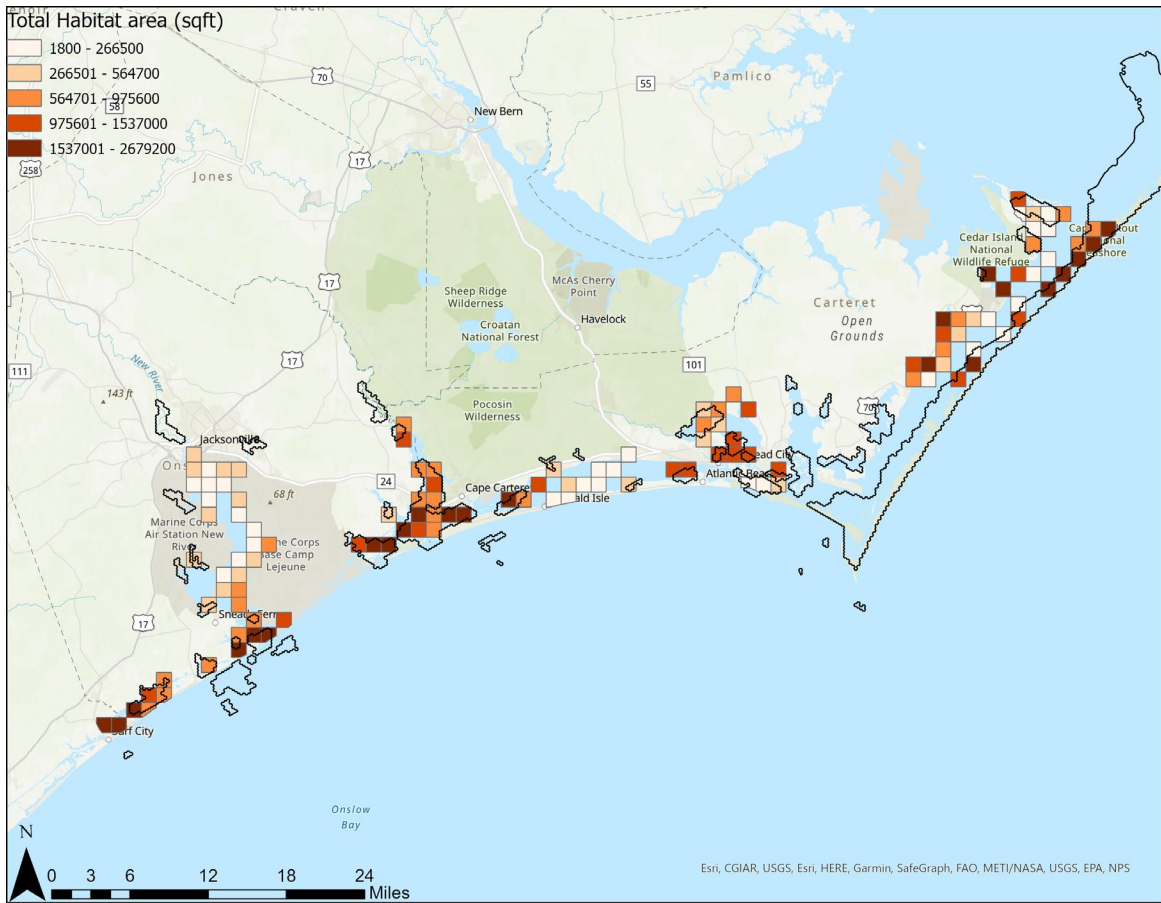


Figure 6. Acres of mapped wetlands, SAV, and shell bottom in Region 3 sampling grids.

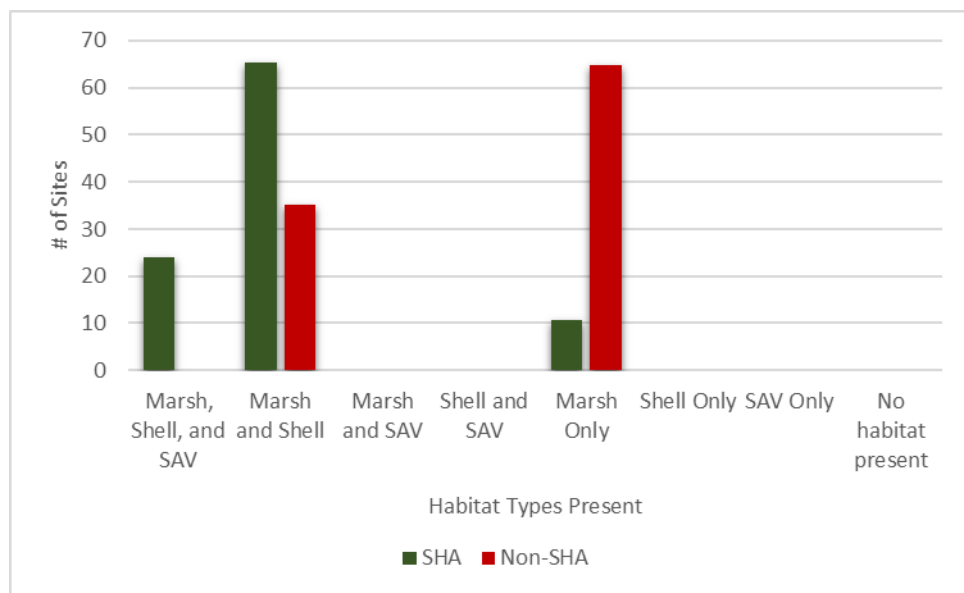


Figure 7. In Region 4, number of sites with different habitat combinations present within SHA and non-SHA sampling grids, 2020-2021.

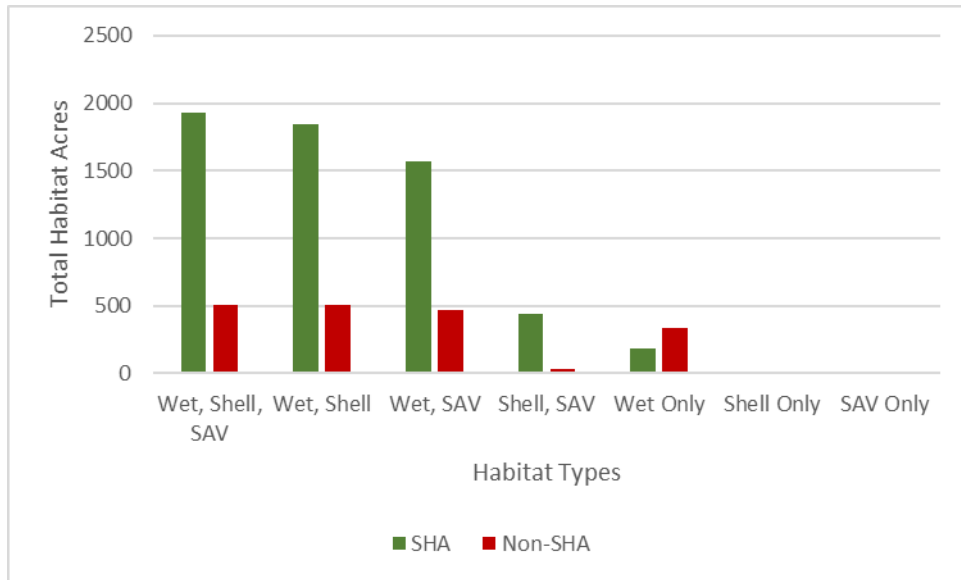


Figure 8. In Region 4, acres of habitats present within SHA and non-SHA sampling grids, 2020-2021.

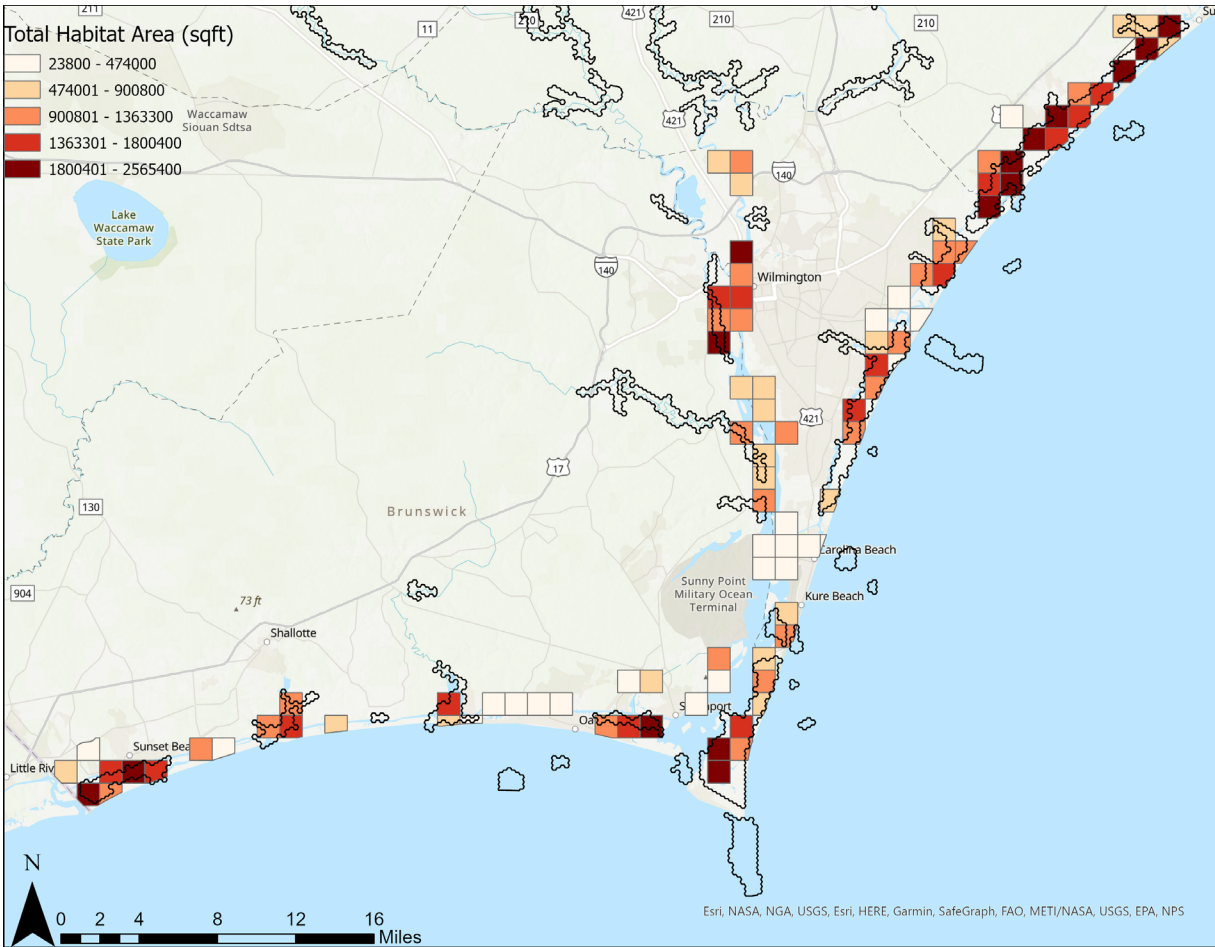


Figure 9. Acres of mapped wetlands, SAV, and shell bottom in Region 4 sampling grids.

Shoreline alterations

In Region 3, the extent of the eroded edge and percent hardened shoreline (Figures 10 and 11) was greater in non-SHAs than SHAs. This is the expected response to disturbed or altered habitat (Table 10). There were more SHA sites with 0-25% eroded edge than non-SHA sites, and there were more non-SHA sites with 26-100% eroded edge. In terms of shoreline hardening, SHA sites had more sites than non-SHA sites with low amounts of hardening ranging from 0-50%, whereas non-SHAs had more sites than SHAs with hardening 51-100%. There was mixed or small difference between SHAs and non-SHA shorelines modified with docking facilities or shoreline stabilization (Figure 12).

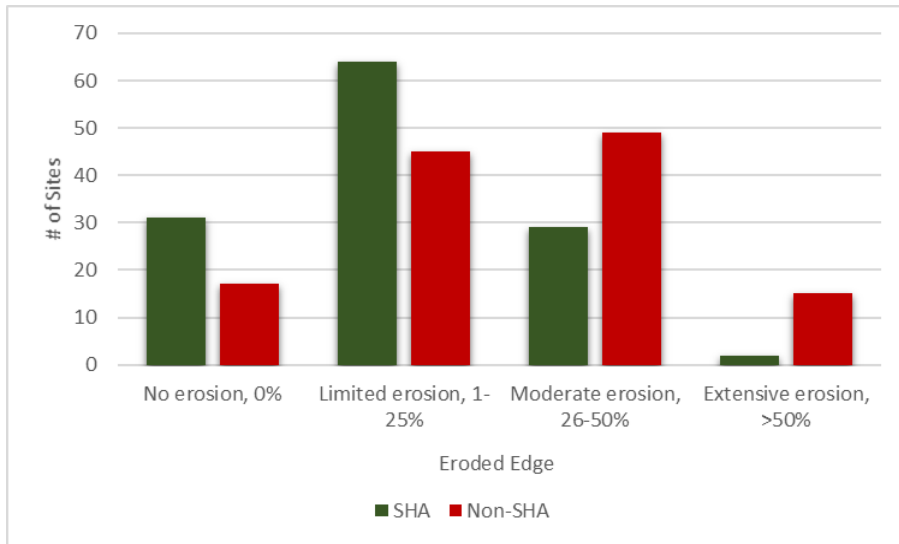


Figure 10. In Region 3, number of sites with varying extent of eroded edge within SHA and non-SHA sampling grids, 2018-2019.

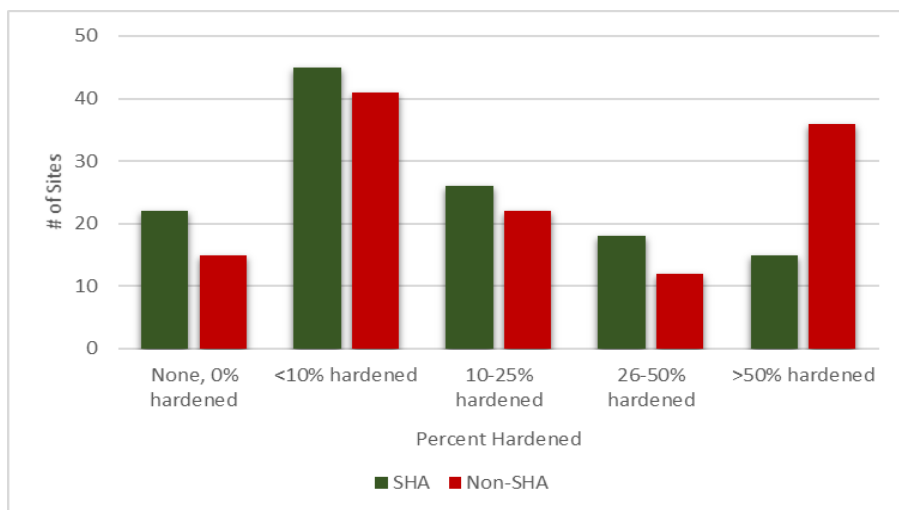


Figure 11. In Region 3, number of sites with varying extent of hardened shoreline or shoreline with engineered structures (eg. bulkheads, riprap) within SHA and non-SHA sampling grids, 2018-2019.

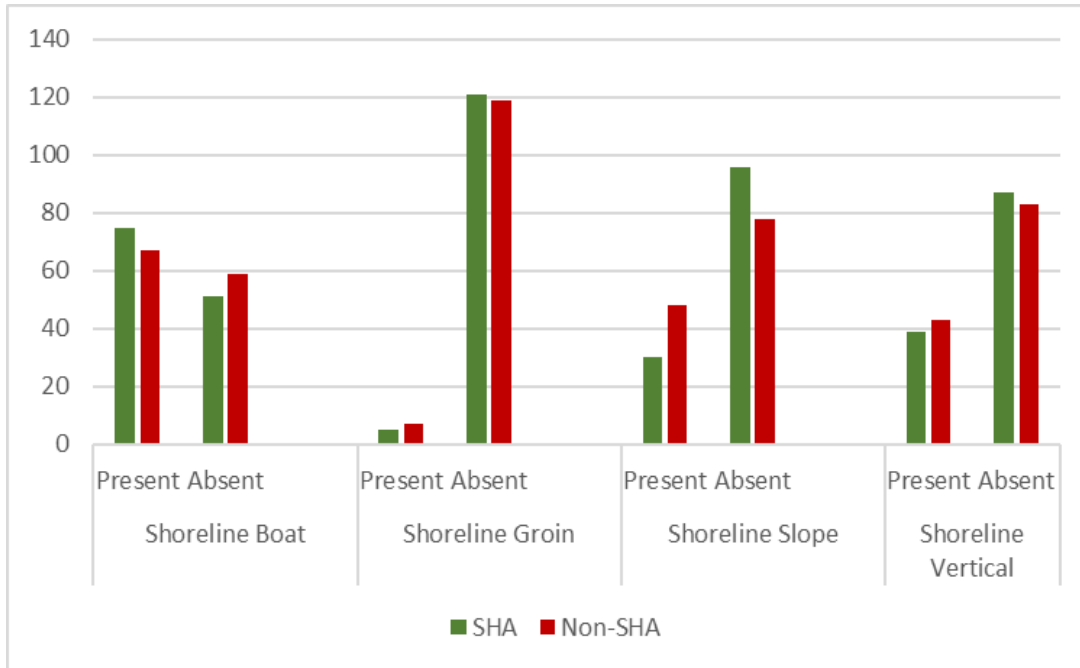


Figure 12. In Region 3, number of sites with varying types of shoreline alterations within SHA and non-SHA sampling grids, 2018-2019. Boat = marinas, channels, docking facilities; Groin = shore perpendicular structures; Slope = non-vertical shoreline stabilization structures such as riprap; Vertical = vertical shoreline stabilization structures such as bulkheads.

In Region 4, a similar number of SHA and non-SHA sites had no visible erosion (Figure 13). SHAs had a slightly greater number of sites with limited eroded edge (1-25%), and fewer sites with moderate (26-50%) and extensive (>50%) eroded edge. Except for “no eroded edge”, this is consistent with the expected response. There was minimal difference in the percent of shoreline hardening between SHAs and non-SHAs where less than 10%. Sites with 10-25% were more common in SHAs than non-SHAs. Non-SHA sites had a larger proportion of sites with more extensive hardened shoreline (25-100%) than SHAs (Figure 14).

There was an inconsistent relationship between SHA status and types of engineered shoreline (Figure 15). Docking facilities, groins, and bulkheads occurred to slightly greater extent in SHAs than non-SHAs, but sloped shorelines occurred more in non-SHAs. This is not consistent with what was expected, however the differences were not great. The results suggest that SHAs in Region 4 were more developed and thus altered than SHAs in Region 3 and not significantly different from non-SHAs in Region 4.

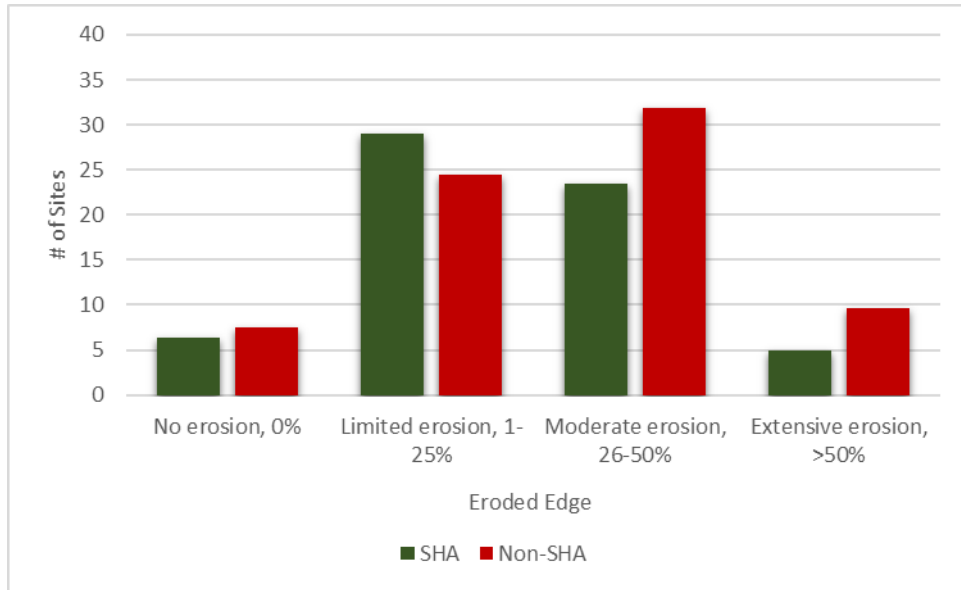


Figure 13. In Region 4, number of sites with varying extent of eroded edge within SHA and non-SHA sampling grids, 2020-2021.

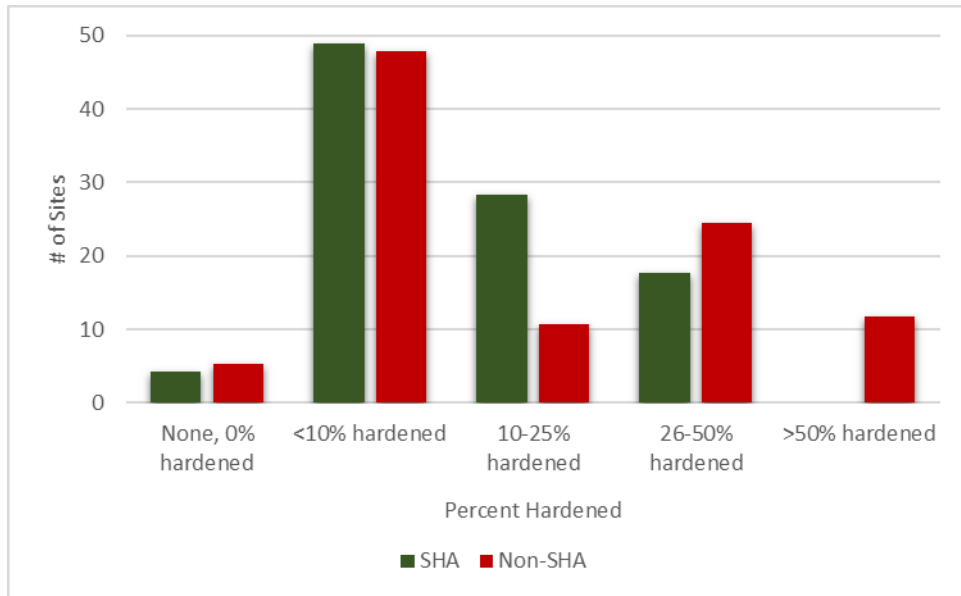


Figure 14. In Region 4, number of sites with varying extent of hardened shoreline or shoreline with engineered structures (eg. bulkheads, riprap) within SHA and non-SHA sampling grids, 2020-2021.

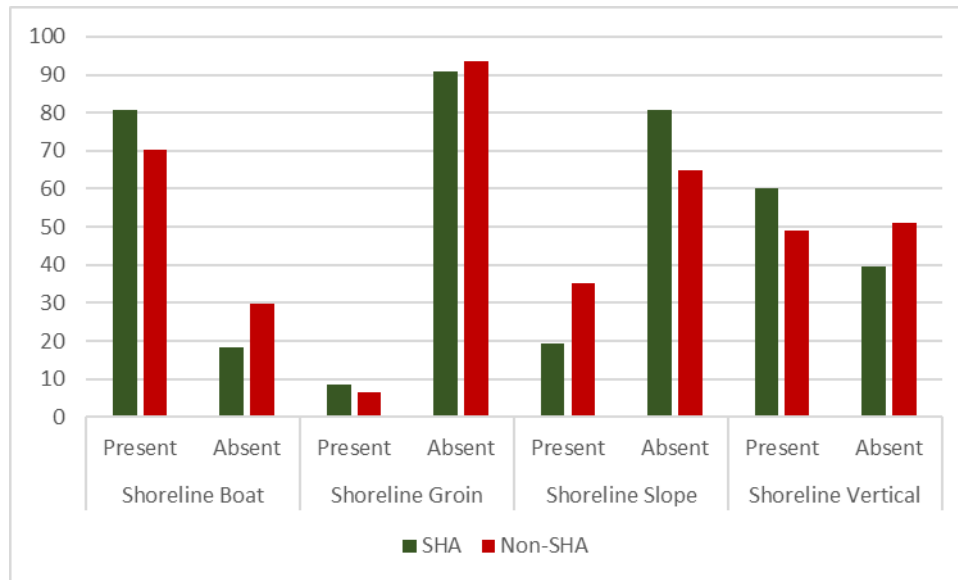


Figure 15. In Region 4, number of sites with varying types of shoreline alterations within SHA and non-SHA sampling grids, 2020-2021. Boat = marinas, channels, docking facilities; Groin = shore perpendicular structures; Slope = non-vertical shoreline stabilization structures such as riprap; Vertical = vertical shoreline stabilization structures such as bulkheads.

Fish Community

Several metrics were calculated to assess and compare fish community structure in SHAs and non-SHAs. Table 6 summarizes by gear type, whereas Table 7 combines gears. Multigear mean standardization or mean standardized catch (MSC) was calculated to allow standardized catch per unit effort data from different sampling gears to be combined (Gibson-Reinemer et al. 2016).

Trawl nets had the highest total number of species and total number of individuals per stratum relative to other sampling gear used (Table 6). Gill nets had the highest total biomass. Despite the differences, using three gear types provided a more complete characterization of fish use in an area since the gears target fish with different life history stages and habitat preferences. For example in Region 3, 36% of the species caught in trawls, 47% of the species caught in gill nets, and 24% of the species caught in Breder traps were only caught in that one gear. In Region 4, 65% of species caught in trawls, 48% caught in gill nets, and 33% caught in Breder traps were only caught in that one gear. Using multiple sampling gears is therefore necessary to accurately assess the diversity of the fish community at different life stages. A complete list of species collected in Regions 3 and 4 is provided in Appendices A and B.

Table 6. Total number of collected species, individuals per stratum, and biomass per stratum by region and SHA status.

Sample Gear	Metric	Region 3		Region 4	
		SHA	Non-SHA	SHA	Non-SHA
Breder trap	# of species	50	42	46	37
	# individuals per stratum	6,376	3,486	9,108	2,609
	Biomass per stratum (kg)	9.0	3.1	23.2	8.0
Trawl net	# of species	87	55	82	53
	# individuals per stratum	17,059	19,373	15,160	6,335
	Biomass per stratum (kg)	72.1	72.9	65.1	33.6
Gill net	# of species	59	62	58	40
	# individuals per stratum	1,645	2,804	1,333	658
	Biomass per stratum (kg)	330.3	447.8	454.4	235.6

Table 7. Number of species caught in only one gear type, by region.

Sample Gear	# of species collected	Region 3		Region 4		
		# of spp unique to single gear	% species unique	# of species collected	# spp unique to single gear	% species unique
Trawl Net	99	36	36	93	60	65
Gill Net	79	37	47	69	33	48
Breder Trap	63	15	24	52	17	33
Total	156	88	56	146	110	75

Looking at results with gears combined, Region 3 results were as expected from literature on fish community assessments. SHAs had greater richness, abundance (MSC), and slightly greater evenness than non-SHAs (Table 8). The three diversity indices used all found greater diversity in SHAs than non-SHAs, although differing in extent. The formulas give different weight to rare species, with the Hill-Shannon diversity index being intermediate between Shannon and Hill-Simpson. The Hill-Shannon diversity index may therefore be the best overall diversity index. Simpson Dominance is a measure of dominance of a few species in contrast to evenness in a population. Region 3 non-SHAs had a slightly greater dominance index.

Table 8. Preliminary analysis of P215 Strategic Habitat Area Region 3 (2018-2019) and 4 (2020-2021) sampling data for fish community diversity indices. Expected response is what is expected in the literature with decreasing habitat suitability.

Diversity Index	Region 3		Region 4		Expected Response
	SHA	Non-SHA	SHA	Non-SHA	
Richness	130	108	126	84	↓
Evenness	0.57	0.56	0.56	0.68	↓
Total MSC ¹	161.39	147.61	190.86	71.14	↓
Shannon Diversity	2.79	2.63	2.72	3.00	↓
Hill-Shannon Diversity	16.27	13.82	15.21	20.12	↓
Hill-Simpson Diversity	8.12	7.97	7.53	12.24	↓
Simpson Dominance	0.12	0.13	0.13	0.08	↑

¹ MSC = Mean Standardized Catch, added across all gears

In Region 4, results were mixed (Tables 6, 7, 8). The SHAs had much higher MSC and richness than non-SHAs, especially when compared to the differences observed in Region 3. However evenness, and the three diversity indices were lower in the SHAs. To determine if the higher diversity in non-SHAs was being driven by the environmental variability within the Cape Fear River, data was rerun with river grids excluded. Results found that the non-SHAs still had higher diversity indices and the SHAs still had higher abundance and more dominant species structure (less even).

In Region 4, MSC in SHAs was over 2.5 times greater than in non-SHAs, and species richness was 50% greater in SHAs than non-SHAs (Table 8). Because that much greater MSC in Region 4 SHAs was not distributed evenly among species, Region 4 SHAs were less even, which resulted in lower scores than Region 4 non-SHAs on the diversity indexes that weight for evenness. The large differences in MSC and species richness indicate that the Region 4 SHAs are providing disproportionate benefits to fish despite scoring lower than non-SHAs on diversity indexes.

Species abundance (mean standardized catch) for the top 25 species in SHAs for both regions is shown in Table 9. In Regions 3 and 4 SHAs, 90% of the top ten species occurred in both regions, including Pinfish, Spot, Atlantic Menhaden, Atlantic Croaker, and Blue Crab. Silver Perch, Brown and White Shrimp, Southern Flounder, and Bluefish were within the top 20 species in both regions. In comparing species abundance in SHAs and non-SHAs, most species in SHAs had higher abundance than in non-SHAs.

Table 9. Total MSC in Regions 3 and 4, ranked by MSC (top 25 species).

Region 3				Region 4			
Scientific name	Common Name	SHA MSC	Non-SHA MSC	Scientific name	Common Name	SHA MSC	Non-SHA MSC
Lagodon rhomboides	Pinfish	41.9	17.9	Leiostomus xanthurus	Spot	59.0	12.3
Leiostomus xanthurus	Spot	31.3	36.1	Lagodon rhomboides	Pinfish	21.7	3.0
Palaemonetes spp.	Grass Shrimps	10.1	7.6	Anchoa spp.	Anchovies	14.0	9.9
Brevoortia tyrannus	Atlantic Menhaden	9.2	24.0	Palaemonetes spp.	Grass Shrimps	12.5	7.8
Anchoa spp.	Anchovies	9.2	18.3	Micropogonias undulatus	Atlantic Croaker	12.2	6.0
Fundulus heteroclitus	Mummichog	8.5	2.8	Fundulus heteroclitus	Mummichog	11.7	4.1
Micropogonias undulatus	Atlantic Croaker	5.6	7.2	Nassarius spp.	Black Mud Snails	10.7	0.5
Callinectes sapidus	Blue Crab	5.4	6.0	Brevoortia tyrannus	Atlantic Menhaden	6.7	3.5
Nassarius spp.	Mudsnails	3.4	2.2	Bairdiella chrysoura	Silver Perch	6.0	1.6
Farfantepenaeus aztecus	Brown Shrimp	3.3	2.5	Callinectes sapidus	Blue Crab	3.5	2.4
Pomatomus saltatrix	Bluefish	3.0	4.6	Paralichthys lethostigma	Southern Flounder	2.7	0.9
					Atlantic Sharpnose		
Eucinostomus argenteus	Spotfin Mojarra	2.6	0.5	Rhizoprionodon terraenovae	Shark	2.1	0.8
Carcharhinus limbatus	Blacktip Shark	2.6	1.0	Pogonias cromis	Black Drum	2.1	0.8
	Sheepshead						
Cyprinodon variegatus	Minnnow	2.5	0.0	Sciaenops ocellatus	Red Drum	1.9	1.5
Bairdiella chrysoura	Silver Perch	2.2	1.4	Mugil cephalus	Striped Mullet	1.8	2.4
	Atlantic Thread						
Opisthonema oglinum	Herring	1.9	0.7	Farfantepenaeus aztecus	Brown Shrimp	1.8	1.6
Penaeus spp.	Penaeus Shrimps	1.9	0.0	Lepisosteus osseus	Longnose Gar	1.6	1.6
Paralichthys lethostigma	Southern Flounder	1.4	1.0	Cynoscion nebulosus	Spotted Sea Trout	1.5	0.8
Orthopristis chrysoptera	Pigfish	1.2	0.4	Litopenaeus setiferus	White Shrimp	1.4	1.6
Litopenaeus setiferus	White Shrimp	1.0	0.8	Pomatomus saltatrix	Bluefish	1.3	1.1
Elops saurus	Ladyfish	0.8	0.7	Synodus foetens	Inshore Lizardfish	1.0	0.2
Synodus foetens	Inshore Lizardfish	0.6	0.3	Citharichthys spilopterus	Bay Whiff	1.0	0.2
Gastropoda	Gasstropods	0.6	0.1	Eucinostomus argenteus	Spotfin Mojarra	0.9	1.0
Sciaenops ocellatus	Red Drum	0.6	0.3	Elops saurus	Ladyfish	0.8	0.5
Tozeuma carolinense	Arrow Shrimp	0.5	0.1	Orthopristis chrysoptera	Pigfish	0.8	0.4

Abundance and diversity indices within individual grids in Regions 3 and 4 are shown in Figures 16-19. While this can be useful to visually assess fish community spatially within the region and verify productivity in and outside of SHAs, sampling was not stratified by waterbody or season, so individual grids were not resampled over time, and values may be misleading. At a glance, SHAs appear to have equal or higher values in most areas. One exception is in Region 3, in the upper New River (Northeast Creek vicinity). This area was not selected as a SHA but had high abundance and diversity values. Structured habitat area was not predictive of abundance, richness, diversity, or evenness (very small R-squared values), though given the lack of seasonal controls in the study design, that result is unsurprising. Additional analysis of the collected habitat data (eg. marsh, oyster, and SAV density) or landscape characteristics could provide further indication of in-situ conditions.

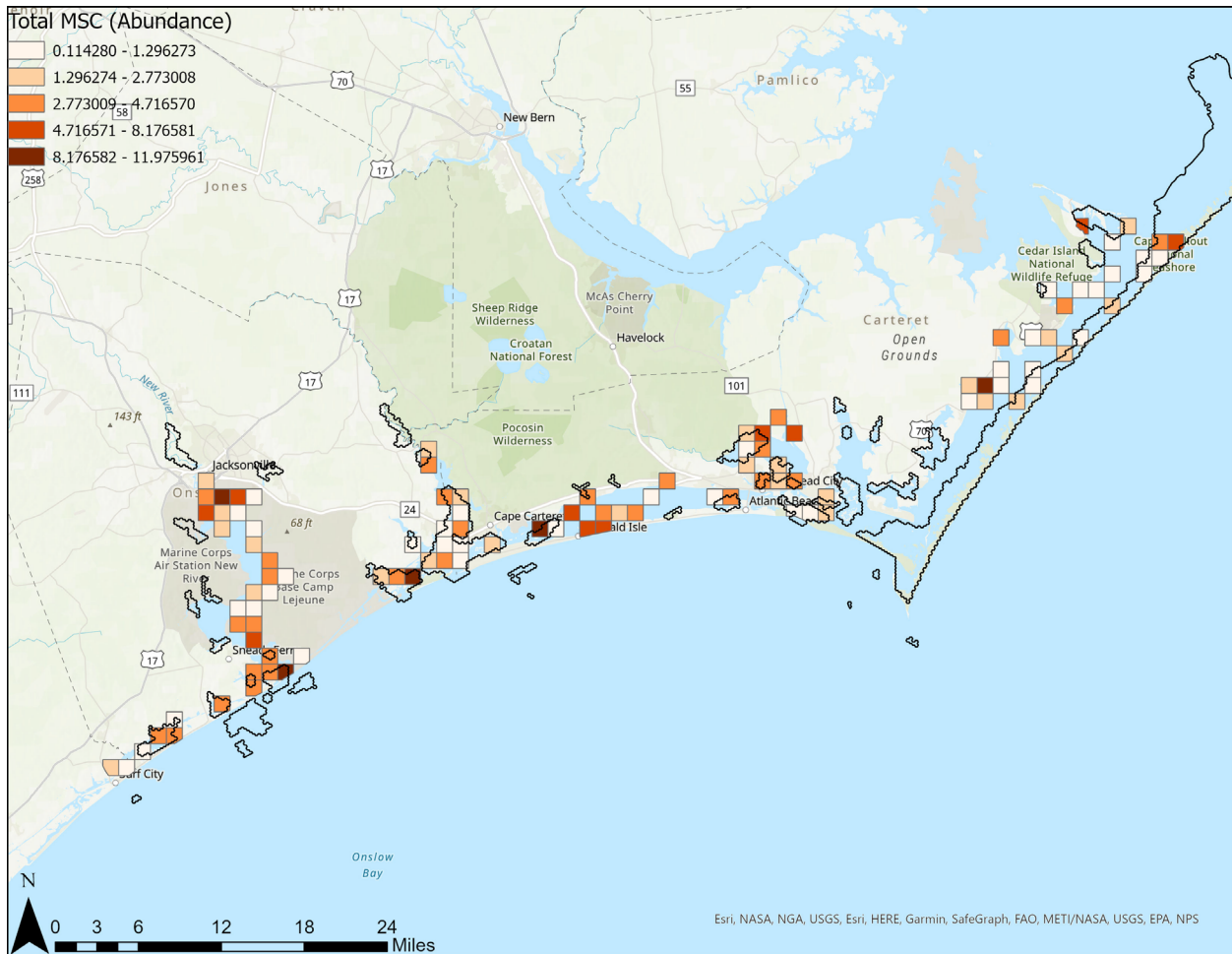


Figure 16. Mean Standardized Catch in Region 3 sampling grids.

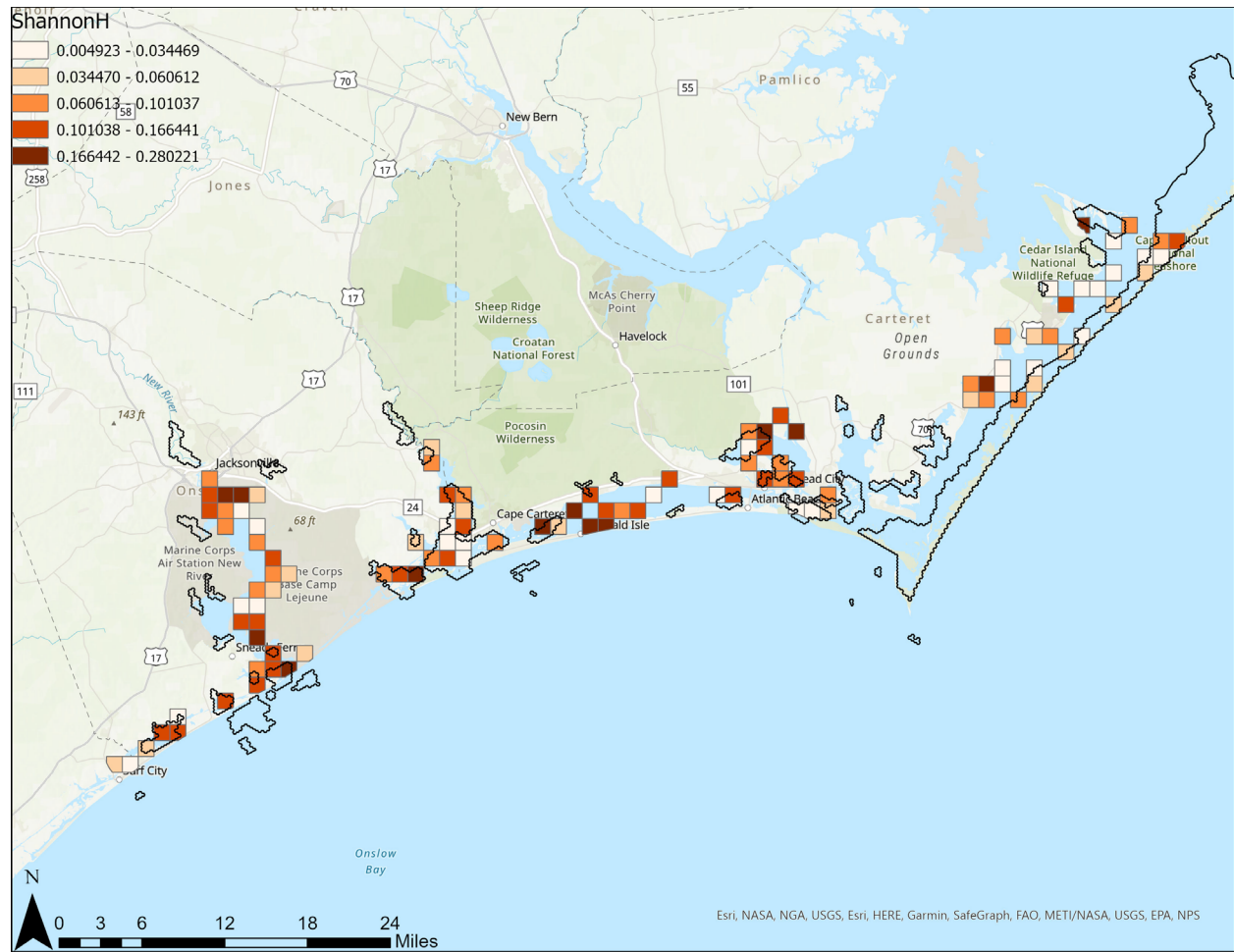


Figure 17. Shannon Diversity Indices in Region 3 sampling grids.

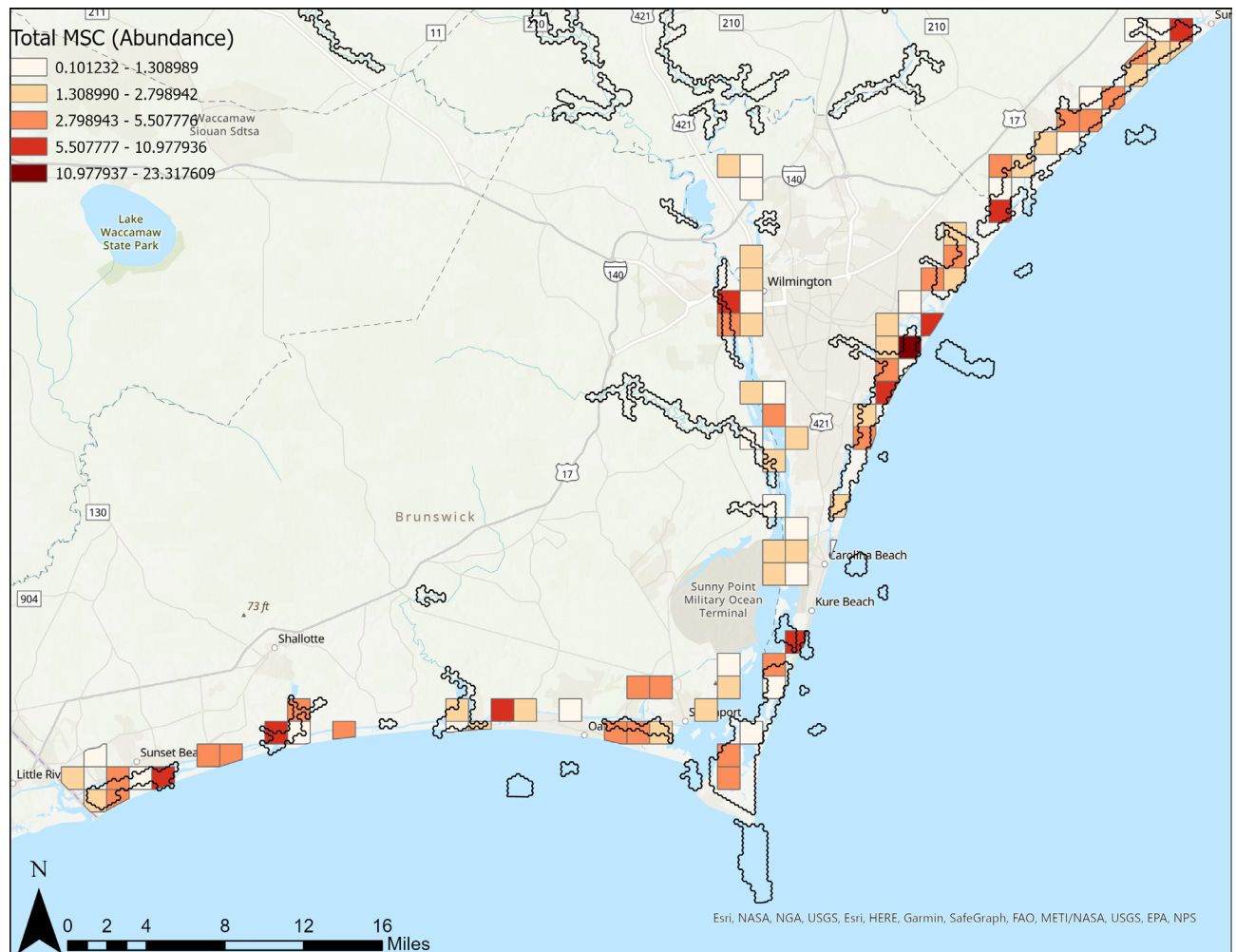


Figure 18. Mean Standardized Catch in Region 4 sampling grids.

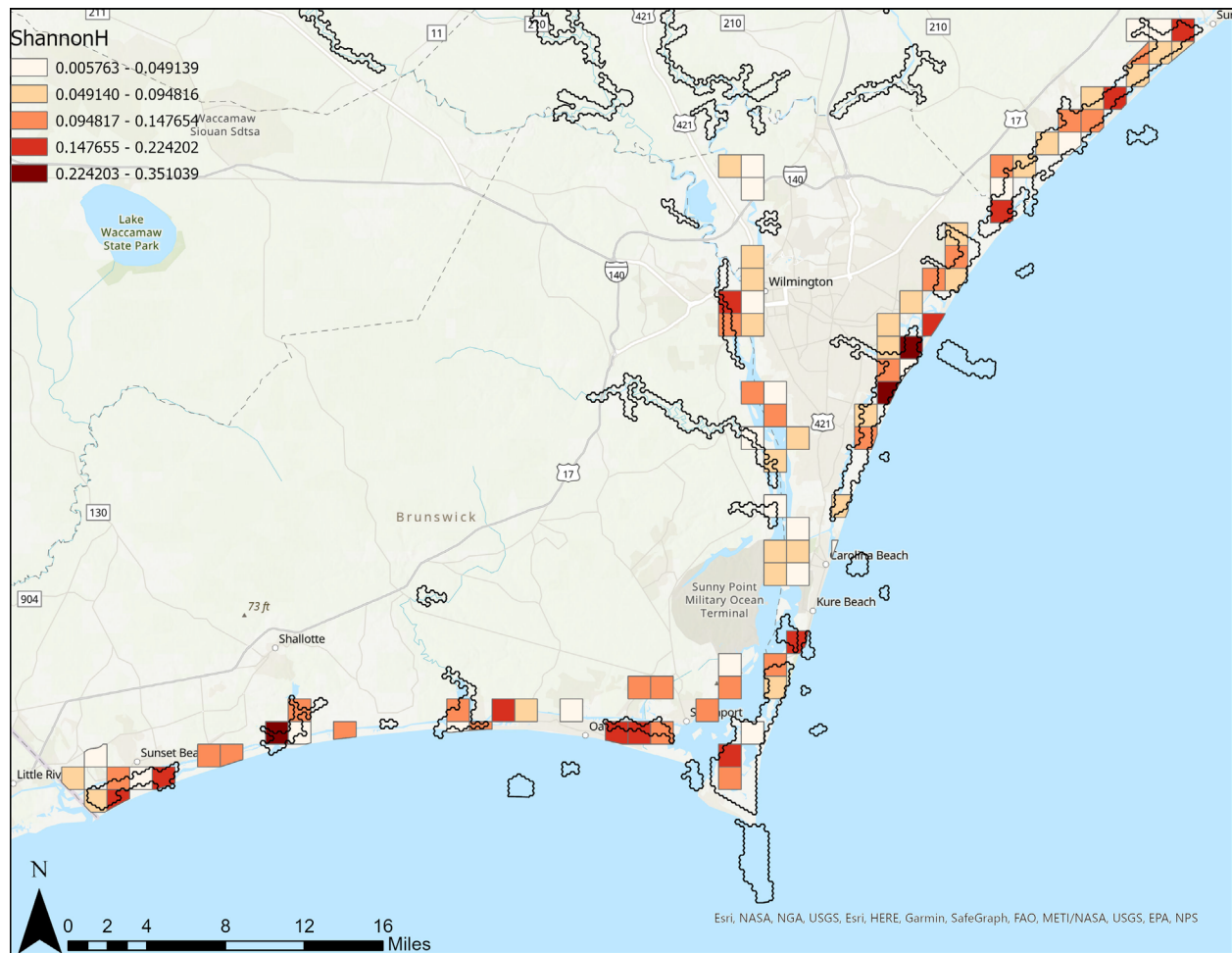


Figure 19. Shannon Diversity Indices in Region 4 sampling grids.

A rank abundance curve is another means of comparing community structure in SHAs and non-SHAs, where the x-axis is the rank of species abundance (1 having the highest abundance), and the Y-axis is the species abundance (mean standardized catch). The high MSC in the Region 4 SHA was due to an extremely high catch of spot. Removing spot from the data reduced the magnitude of difference in diversity indices and dominance between SHAs and non-SHAs in Region 4, although non-SHAs still scored higher on diversity indices and lower on the dominance index. All strata exhibited low evenness, as noted by the steep slope.

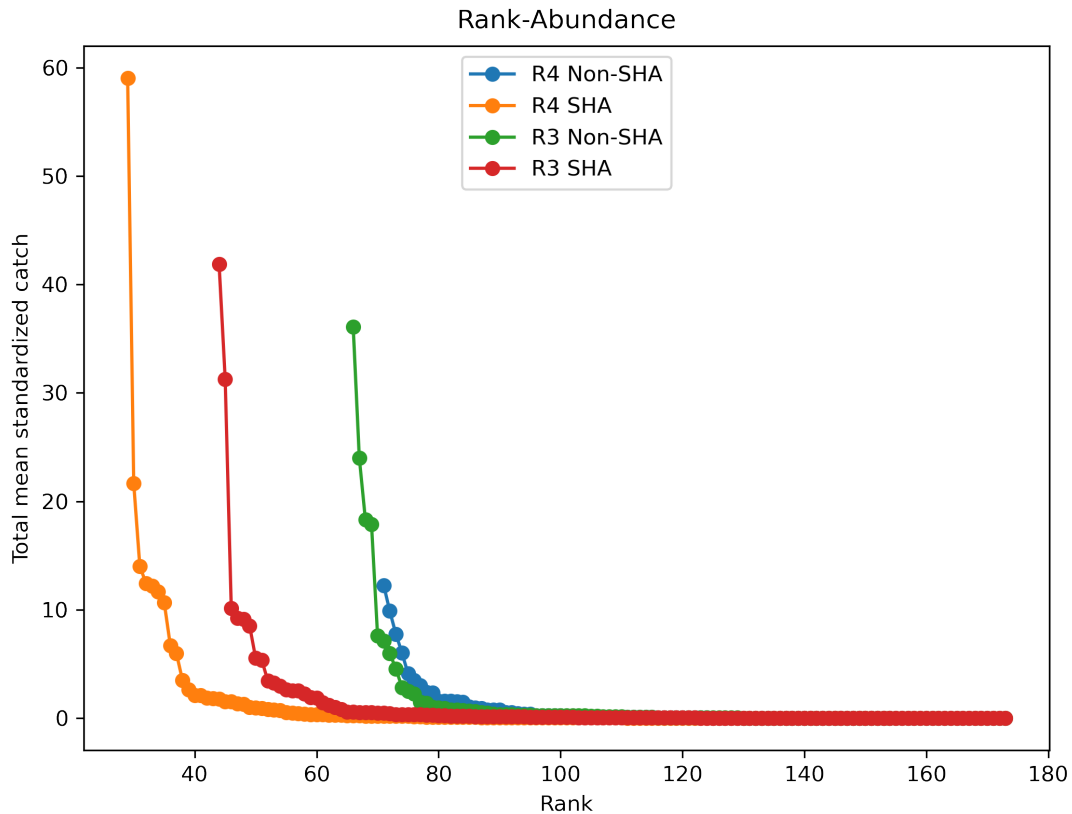


Figure 20. Difference in rank-abundance by region and SHA status.

Overall, with two years of sampling per region, SHAs in both Regions 3 and 4 supported more fish (greater total abundance) and more species (richness) than the non-SHAs. Region 3 SHAs had greater diversity indices than non-SHAs, but a reverse pattern was observed in Region 4, likely because of the much greater abundance in Region 4 SHAs. However the difference in diversity between the SHAs and non-SHAs was not extremely large. In conclusion, SHAs in Regions 3 and 4 were highly productive and supported a greater number of less common species, indicating that SHAs function largely as expected.

Objective 2: Develop fish and habitat indicator metrics

Individual ecological indicators that appear useful based on this project include:

- A measure of total abundance (mean standardized catch, CPUE)
- Richness
- Evenness
- Hill-Shannon Diversity
- Acreage of structured habitat within a system
- Eroded edge
- Shoreline hardened (possibly)
- Mean alteration score (an index of alterations)

The multimetric index, which takes into account both fish and habitat metrics, has been extensively developed and applied to assess ecological status and is considered more accurate and comprehensive than any single metric index such as those discussed above (Pérez-Domínguez et al. 2012; Smoliński and Całkiewicz 2015). With assistance from DMF's stock analyst, the Region 3 data were used to develop an SOP for a multi-metric index of habitat condition (Smolnski and Calkiewicz 2015). Development of the multimetric index includes five steps in general: (1) determining a list of candidate metrics; (2) selecting metrics by reducing redundancy among candidate metrics and identifying the metrics that show a significant response to a pre-defined proxy of human disturbance; (3) defining the scoring system for each selected metric; (4) defining the combination rule that produces the final multimetric index; and (5) validating the multimetric index.

The set of candidate metrics and the potential direction of each metric responding to the overall human disturbance was determined on a basis of expert opinions. The list contained 21 metrics that inform the overall fish community (global metrics) and habitat conditions including the metrics previously discussed, as well as feeding guilds of fish species (Table 10). The selection of metrics involves reducing the redundancy among candidate metrics and modeling of each metric. A total of nine metrics were selected by the modeling exercise and expert opinion (Table 10). A five-grade scoring system ranging from 1 (the worst ecological status) to 5 (the best ecological status) was defined for each selected metric, and a score was assigned to each observation based on this scoring system of the metric (Delpech et al. 2010; Smoliński and Całkiewicz 2015).

The final multimetric index was calculated for each observation by combining the scores of all selected metrics, in which scores of all metrics were summed and divided by the maximal potential score (five times the number of selected metrics; Delpech et al. 2010; Smoliński and Całkiewicz 2015). The final multimetric index ranges between 0 and 1, with a value of 0 for the worst ecological status and a value of 1 for the best ecological status. A fitted GLM model was used to predict the metric values in three disturbance levels based on alteration scores calculated during the SHA GIS-based analysis ([SHA final reports](#), Figures 24 and 25). The multimetric index effectively validated most of the nominated SHAs in CHPP Region 3 and 4, with higher index values in SHAs than the non-SHAs. These results suggest the multimetric index can perform as an effective indicator for human disturbance. For additional information, see Appendix B. After reviewing all data, some modifications appear needed to the Region 3 analysis and for the Region 4 analysis. Because the multimetric index is designed to incorporate both fish and habitat data, at least one global metric should be included, such as abundance or a diversity measure. Presence of riprap and vertical structures is duplicative of extent of hardened shoreline and therefore should be excluded. Presence of shoreline boating facilities should be excluded since it did not appear to have a consistent effect on SHAs.

Development of the multimetric index could continue with modifications for Region 3 and 4. The same method, with minor modification could be applied to other SHA regions or the evaluation of other nominations or designations once sampling has been conducted. However, to be comparable across regions, similar metrics should most likely be used.

Table 10. List of candidate metrics and their expected response to increasing human disturbance. Bolded metrics = nine selected for multi-metric index.

Candidate metric	Variable type	Expected response	Model Selected
<i>Global</i>			
1 Total abundance	Count	↓	No
2 Total number of species (richness)	Count	↓	No
3 Shannon's diversity	Continuous	↓	No
4 Evenness	Continuous	↓	No
5 Simpson's dominance	Continuous	↑	No
<i>Habitat conditions</i>			
6 Water clarity	Continuous	↓	No
7 Presence of shoreline basins, marinas and/or boat ramps	Binary	↑ (present)	Yes
8 Presence of rock or wood structures perpendicular to shore	Binary	↑ (present)	No
9 Presence of low profile rock (riprap) or wood structure parallel to shore	Binary	↑ (present)	Yes
10 Presence of vertical structures (i.e. bulkheads)	Binary	↑ (present)	Yes
11 Presence of shell habitat	Binary	↓(present)	Yes
12 Presence of marsh habitat	Binary	↓(present)	Yes
13 Presence of submerged aquatic vegetation	Binary	↓(present)	Yes
14 Extent of hardened shoreline	Categorical	↑	Yes
15 Extent of eroded edge	Categorical	↑	Yes
<i>Feeding guilds</i>			
16 Abundance of piscivorous species	Count	↓	No
17 Number of piscivorous species	Count	↓	No
18 Abundance of invertivorous species	Count	↓	Yes
19 Number of invertivorous species	Count	↓	No
20 Abundance of omnivorous species	Count	↑	No
21 Number of omnivorous species	Count	↑	No

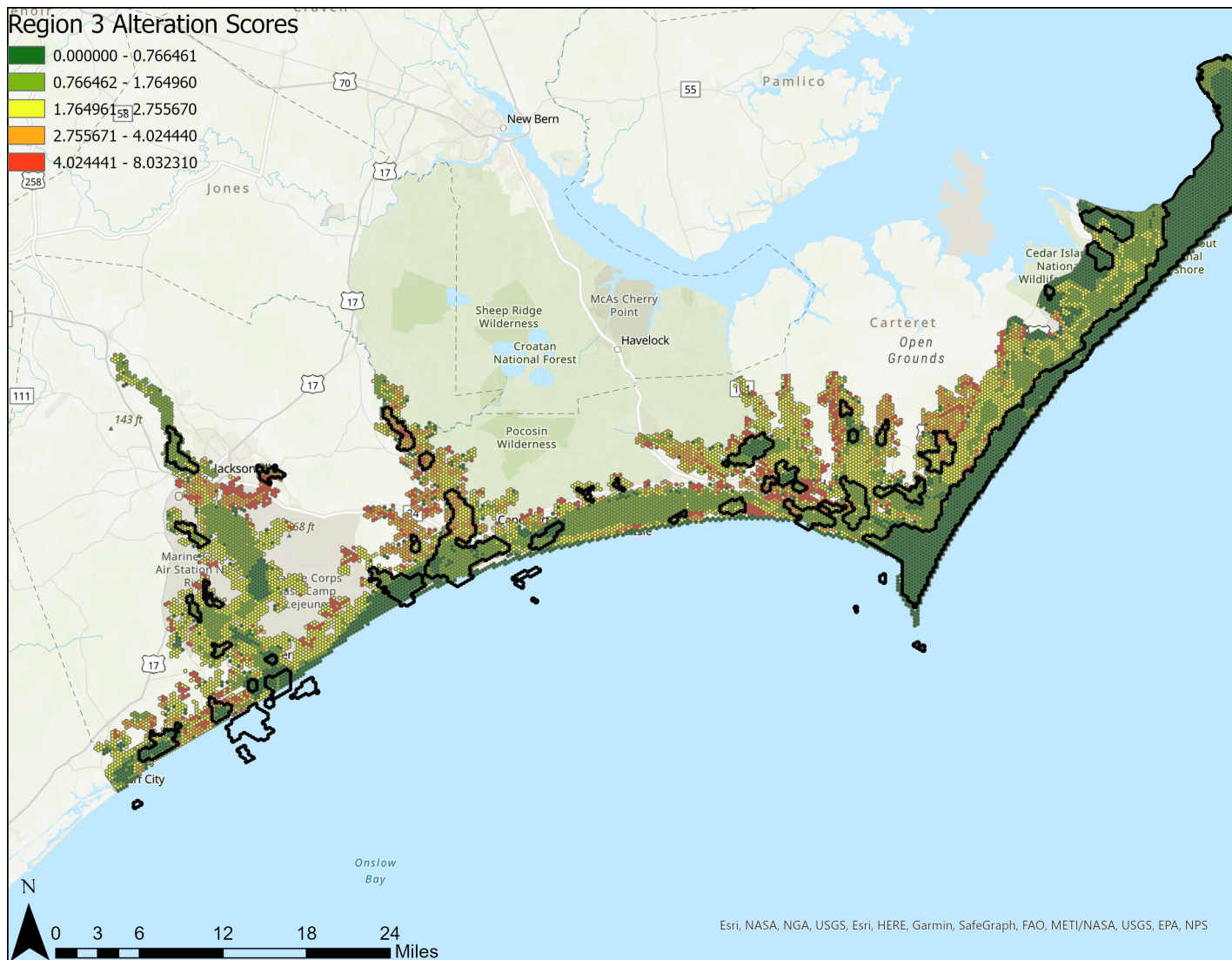


Figure 24. Alteration scores within Region 3 SHAs, with lowest scores (green) representing least alteration and highest priority for protection.

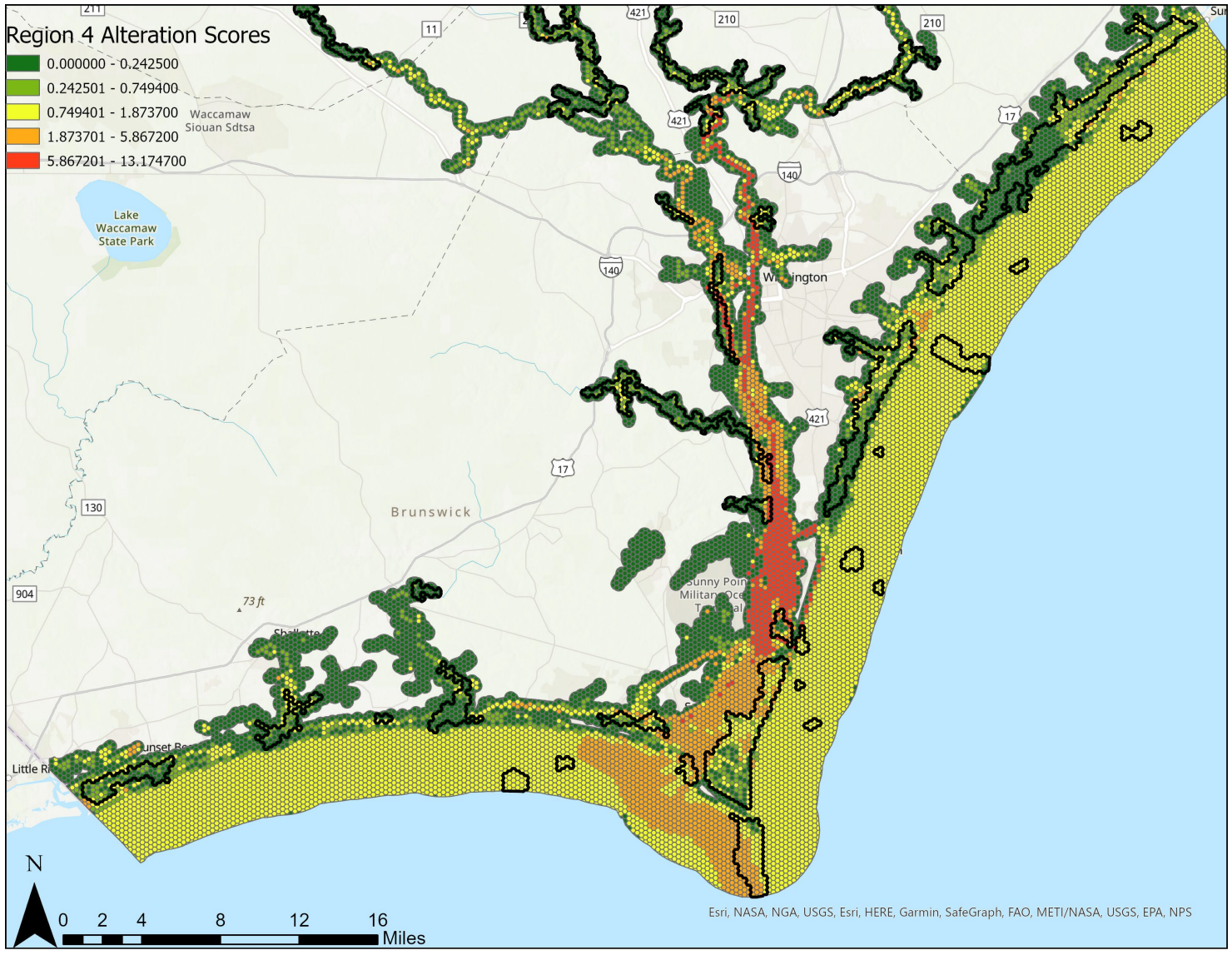


Figure 25. Alteration scores within Region 4 SHAs, with lowest scores (green) representing least alteration and highest priority for protection

Objective 3: Produce SOP for future monitoring and possible modification of SHA nominations based on indicator performance

Program documentation was completed for this project to provide direction for future sampling. It was approved and is located in the FIMSS folder on the DMF Lan. Specific SOPs are also complete and available.

Sampling in the Cape Fear River (Region 4) has been beneficial as it revealed the need to adjust some monitoring methods in riverine systems due to the different shoreline profiles and wetland types. Due to these differences, upstream riverine grids were excluded from the sampling universe. In regions dominated by this habitat type additional methodologies and gears will need to be considered. For example, there was generally not a shallow area to set Breder traps, and logs in the river made trawling difficult. Fyke nets would be able to capture fish leaving the wetlands, but require more labor and time to set. While the gear type can vary between areas, it should be effective and appropriate for the site conditions and at least two different gear types to capture different life stages of a variety of species.

In Regions 1 and 2, that are dominated by large sounds and rivers and where existing fish sampling occurs almost year-round, we recommend that fish data collected from existing programs substitute for the fish sampling conducted in Regions 3 and 4, but be augmented with sampling in closer proximity to structured habitats, particularly during periods of recruitment. This could consist of Breder traps or seine nets. Additionally, habitat metrics collected during this project should be added. This will require sampling in or near structured habitats. Sampling in habitat areas that already receive robust protection from fishing and development, and water quality impacts should be of secondary importance since data are not likely to result in any additional improvements.

Region 1

In Region 1, existing and future fish data from Programs 100, 135, and 150 could potentially be used, with supplemental collection and analysis of habitat metrics. These programs rely on trawls, gill nets, and seines. In addition to habitat presence that can be determined from mapping efforts and regularly collected environmental parameters, data could be collected on eroded edge, percent hardened shoreline, wetland characteristics, and water clarity. All of the Roanoke and Chowan river systems were nominated as SHAs due to the understanding that protecting only pieces of the rivers will be inadequate in systems where connectivity is critical for adults migrating upstream to spawn and resulting larvae and juveniles migrating downstream. Because of this, sampling could exclude the rivers and focus on the mouths of those two rivers mouths and the remaining portion of Region 1. Since there are no SHAs within the center of Albermarle Sound, the grid system could exclude that area.

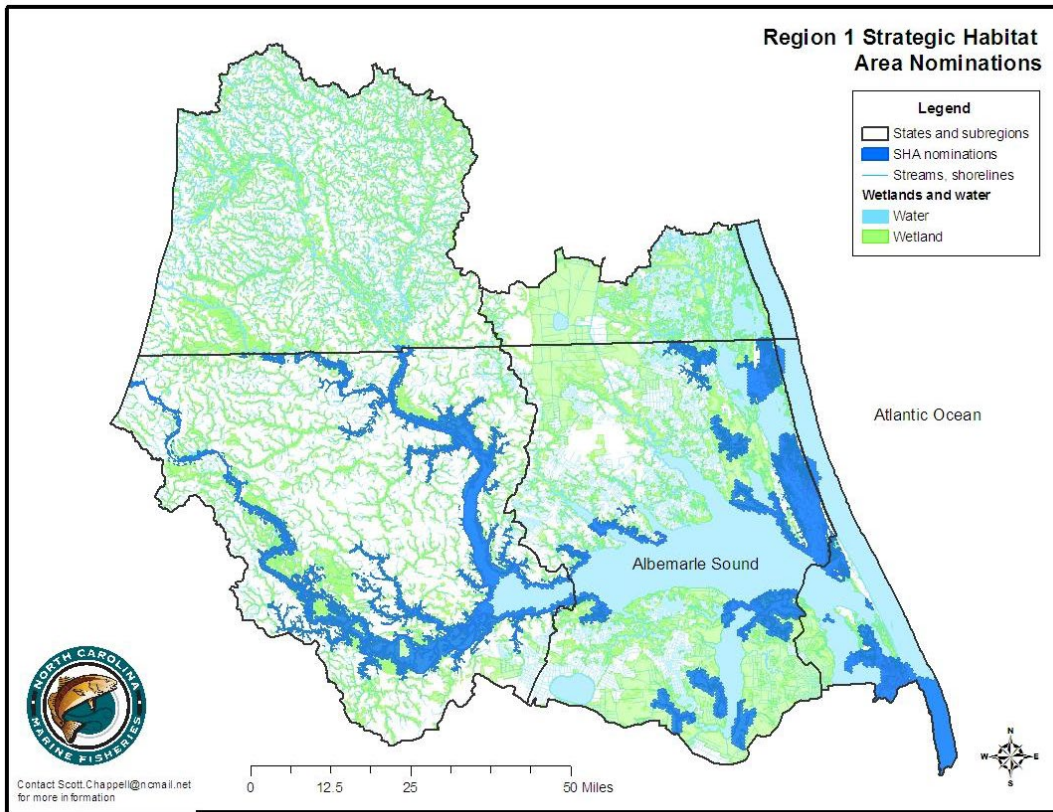


Figure 26: Region 1 Strategic Habitat Area nominations.

Region 2

Existing and future fish data from Programs 120, 915, 610, 611, and 195 could potentially be used for the multimetric index. Because Program 195 only samples twice a year in the open waters of Pamlico Sound, Neuse and Pamlico rivers, and there are minimal SHAs within these open waters, it may not be needed for SHA analysis. For programs 120 and 915, in addition to habitat presence that can be determined from mapping efforts, and regularly collected environmental parameters, data could be collected on eroded edge, percent hardened shoreline, wetland characteristics, and water clarity. SHAs around the perimeter of the sound primarily consist of subtidal natural and restored oyster reefs (cultch planting and oyster sanctuaries). Sampling metrics and frequency could be reviewed and modified if necessary to obtain adequate fish and oyster habitat metrics. Additional subtidal reefs that were not nominated as SHAs will also need to be sampled. Supplemental collection and analysis of habitat metrics will be needed.

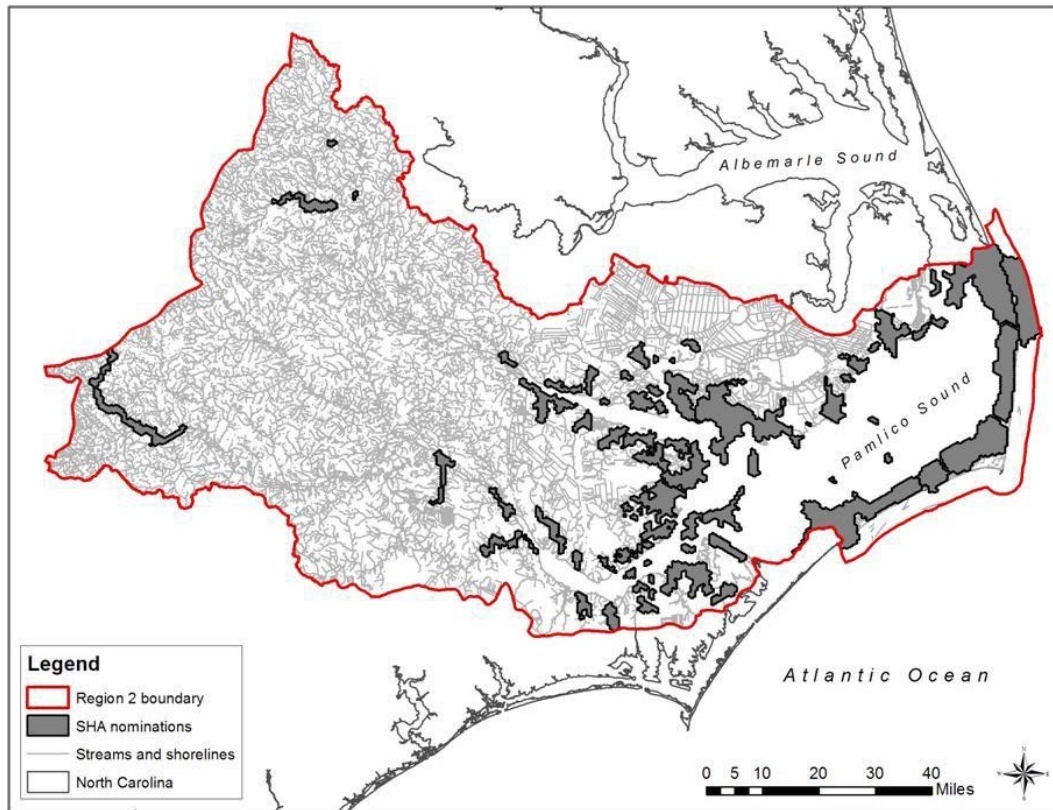


Figure 27: Region 2 Strategic Habitat Area nominations.

Deviations:

In 2022, a No-cost Extension was requested and approved due to staff turn over, with final report due June 30, 2023. Due to loss of stock assessment staff that began the multi-metric analysis, we are not able to complete that portion of the project. However, because the analysis requires collecting all the initial data and that data had informative trends, we recommend not pursuing completion of the multi-metric analysis at this time. Additionally, we did not include recommendations for sampling to determine nursery function of Pamlico Sound, as this would best be determined by a DMF group, considering these results as well as other fishery management information regarding nursery area evaluations.

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APPENDIX A. Species collected by region and gear

Table A.1. Species collected in Region 3, by gear 2018-2019.

Species	Common name	Gear
<i>Alosa aestivalis</i>	blueback herring	G
<i>Alosa mediocris</i>	hickory shad	T,G
<i>Alosa sapidissima</i>	American shad	G
<i>Alpheidae</i>	snapping shrimps - family	T
<i>Alpheus heterochaelis</i>	bigclaw snapping shrimp	T
<i>Ameiurus nebulosus</i>	brown bullhead	T,G
<i>Amphipoda</i>	amphipods	T,B
<i>Anchoa spp.</i>	anchovies	B
<i>Ancylosetta ommata(=quadrocellata)</i>	ocellated flounder	G
<i>Anguilla rostrata</i>	American eel	B
<i>Anomura paguridae</i>	hermit crabs	T,G,B
<i>Archosargus probatocephalus</i>	sheepshead	T,G
<i>Armases(=Sesarma) cinereum</i>	squareback marsh crab	B
<i>Ascidicea</i>	tunicates	T,G
<i>Aurelia aurita</i>	moon jellyfish	T
<i>Bairdiella chrysoura</i>	silver perch	T,G,B
<i>Brevoortia tyrannus</i>	Atlantic menhaden	T,G
<i>Bryozoa, ectoprocta</i>	bryozoans	T
<i>Busycon carica</i>	knobbed whelk	G
<i>Busycon spp.</i>	whelks (Busycon)	G
<i>Callinectes sapidus</i>	blue crab	T,G,B
<i>Callinectes similis</i>	lesser blue crab	T,B
<i>Caranx hippos</i>	crevalle jack	T,G,B
<i>Carcharhinus acronotus</i>	blacknose shark	G
<i>Carcharhinus isodon</i>	finetooth shark {w}	G
<i>Carcharhinus limbatus</i>	blacktip shark	G
<i>Centropristis striata</i>	black sea bass	T
<i>Chasmodes bosquianus</i>	striped blenny	B
<i>Chilomycterus schoepfii</i>	striped burrfish	T,G
<i>Chione cancellata</i>	cross-barred venus	T
<i>Citharichthys spilopterus</i>	bay whiff	T
<i>Cnidaria</i>	jellyfish	T,G
<i>Crassostrea virginica</i>	eastern oyster	T
<i>Ctenogobius boleosoma</i>	darter goby	B
<i>Ctenogobius shufeldti</i>	freshwater goby	T,B
<i>Ctenophora</i>	comb jellies	T,B
<i>Cynoscion nebulosus</i>	spotted seatrout	G,B
<i>Cynoscion regalis</i>	weakfish	T,G

<i>Cynoscion spp.</i>	seatrouts	G
<i>Cyprinidae</i>	minnows	B

Table A.1. Species collected in Region 3, by gear 2018-2019, continued.

Species	Common name	Gear
<i>Cyprinodon variegatus</i>	sheepshead minnow	B
<i>Dasyatidae</i>	stingrays	G
<i>Dasyatis americana</i>	southern stingray	G
<i>Dasyatis sabina</i>	Atlantic stingray	G
<i>Diapterus auratus</i>	Irish pompano {w}	T,B
<i>Diplodus holbrookii</i>	spottail pinfish	G
<i>Dorosoma cepedianum</i>	gizzard shad	G
<i>Dorosoma petenense</i>	threadfin shad	T,G
<i>Echinodermata</i>	Enchinoderms	T
<i>Echinoidea</i>	sea urchins & sand dollar	T
<i>Elopomorpha ang. anguilloidei</i>	eels	T
<i>Elops saurus</i>	ladyfish	G
<i>Ensis directus</i>	Atlantic jackknife	T
<i>Etropus crossotus</i>	fringed flounder	T
<i>Eucinostomus argenteus</i>	spotfin mojarra	T,B
<i>Eucinostomus spp.</i>	Eucinostomus mojarra	B
<i>Farfantepenaeus aztecus</i>	brown shrimp	T,G,B
<i>Farfantepenaeus duorarum</i>	pink shrimp	T,B
<i>Fundulus heteroclitus</i>	mummichog	B
<i>Fundulus majalis</i>	striped killifish	B
<i>Gambusia holbrooki</i>	eastern mosquitofish	B
<i>Gastropoda</i>	gastropods	T
<i>Glyceridae</i>	bristleworm	T,B
<i>Gobiidae</i>	gobies	T,B
<i>Gobiosoma bosc</i>	naked goby	T,B
<i>Halichoeres bivittatus</i>	slippery dick	T
<i>Hippocampus erectus</i>	lined seahorse	G
<i>Hydrozoa</i>	Hydrozoa	T
<i>Hypsoblennius hentz</i>	feather blenny	T,B
<i>Ictalurus punctatus</i>	channel catfish	T,G
<i>Lagodon rhomboides</i>	pinfish	T,G,B
<i>Leiostomus xanthurus</i>	spot	T,G,B
<i>Lepisosteus osseus</i>	longnose gar	G
<i>Lepomis cyanellus</i>	green sunfish	B
<i>Lepomis macrochirus</i>	bluegill	T,B
<i>Limulus polyphemus</i>	horseshoe crab	G
<i>Litopenaeus setiferus</i>	white shrimp	T,G,B

<i>Littorina spp.</i>	periwinkles	T,B
<i>Lobotes surinamensis</i>	Atlantic tripletail	G
<i>Loligo pealeii</i>	longfin squid	T
<i>Lolliguncula brevis</i>	Atlantic brief squid	T

Table A.1. Species collected in Region 3, by gear 2018-2019, continued.

Species	Common name	Gear
<i>Lucania parva</i>	rainwater killifish	B
<i>Lutjanus griseus</i>	gray snapper	T,B
<i>Majidae</i>	spider crabs	T,G
<i>Malaclemys terrapin</i>	diamondback turtle	G
<i>Menidia beryllina</i>	inland silverside	T,B
<i>Menidia menidia</i>	Atlantic silverside	T,B
<i>Menidia spp.</i>	Menidia silversides	B
<i>Menippe mercenaria</i>	Florida stone crab	T
<i>Menticirrhus americanus</i>	southern kingfish	G
<i>Menticirrhus saxatilis</i>	northern kingfish	G
<i>Mercenaria spp.</i>	quahogs	T
<i>Microgobius thalassinus</i>	green goby	T
<i>Micropogonias undulatus</i>	Atlantic croaker	T,G,B
<i>Micropterus salmoides</i>	largemouth bass	B
<i>Morone saxatilis</i>	striped bass	G
<i>Mugil cephalus</i>	striped mullet	G,B
<i>Mugil curema</i>	white mullet	T,G,B
<i>Mugil spp.</i>	mulletts	G,B
<i>Mustelus canis</i>	smooth dogfish	G
<i>Mycteroperca microlepis</i>	gag	T,G
<i>Mycteroperca spp.</i>	Mycteroperca groupers	T
<i>Myrophis punctatus</i>	speckled worm snake eel	T,B
<i>Mytilidae</i>	Mussels (Mytilidae)	T
<i>Nassarius spp.</i>	mudsnails	T,B
<i>Nudibranchia</i>	sea slugs (Nudibranchs)	T,B
<i>Oligoplites saurus</i>	leatherjack	G
<i>Opisthonema oglinum</i>	Atlantic thread herring	T,G
<i>Opsanus tau</i>	oyster toadfish	T,B
<i>Orthopristis chrysoptera</i>	pigfish	T,G,B
<i>Ovalipes ocellatus</i>	lady crab	G,B
<i>Palaemonetes spp.</i>	grass shrimps-Palaemonete	T,B
<i>Paralichthys albigutta</i>	Gulf flounder	T,G,B
<i>Paralichthys dentatus</i>	summer flounder	T,G,B
<i>Paralichthys lethostigma</i>	southern flounder	T,G,B
<i>Penaeus spp.</i>	prawn shrimps	T,B

<i>Peprilus paru</i>	harvestfish	T
<i>Peprilus triacanthus</i>	butterfish	T,G,B
<i>Periclimenaeus schmitti</i>	Tortugas bigclaw shrimp	T
<i>Phalacrocorax auritus</i>	double-crested cormorant	G
<i>Pleuronectiformes Pleuronectoidei</i>	flounders	T,G
<i>Pogonias cromis</i>	black drum	G

Table A.1. Species collected in Region 3, by gear 2018-2019, continued.

Species	Common name	Gear
<i>Pomatomus saltatrix</i>	bluefish	T,G
<i>Prionotus carolinus</i>	northern searobin	T,G
<i>Prionotus evolans</i>	striped searobin	T
<i>Prionotus spp.</i>	Prionotus searobins	G
<i>Prionotus tribulus</i>	bighead searobin	T,G
<i>Rachycentron canadum</i>	cobia	G
<i>Rhinoptera bonasus</i>	cownose ray	G
<i>Rhizoprionodon terraenovae</i>	Atlantic sharpnose shark	G
<i>Sciaenops ocellatus</i>	red drum	G
<i>Scomberomorus maculatus</i>	Spanish mackerel	G
<i>Selene vomer</i>	lookdown	T
<i>Sessilia</i>	barnacle	T
<i>Sphoeroides maculatus</i>	northern puffer	T
<i>Sphyraena borealis</i>	northern sennet	T
<i>Sphyrna tiburo</i>	bonnethead hammerhead	G
<i>Squilla empusa</i>	mantis shrimp	T
<i>Stellifer lanceolatus</i>	star drum	T
<i>Stephanolepis hispidus</i>	planehead filefish {u}	T,G,B
<i>Stephanolepis hispidus</i>	planehead filefish {w}	T,G,B
<i>Stomolophus meleagris</i>	cannonball jellyfish	G
<i>Symphurus plagiusa</i>	blackcheek tonguefish	T,B
<i>Symphurus urospilus</i>	spottail	T
<i>Syngnathus fuscus</i>	northern pipefish	T,B
<i>Synodus foetens</i>	inshore lizardfish	T,G
<i>Synodus spp.</i>	Synodus lizardfishes	T,G
<i>Tozeuma carolinense</i>	arrow cleaner shrimp	T
<i>Trachinotus carolinus</i>	Florida pompano	G
<i>Trachinotus falcatus</i>	permit	G,B
<i>Trachypenaeus constrictus</i>	roughneck shrimp	T
<i>Trichiurus lepturus</i>	Atlantic cutlassfish	T
<i>Trinectes maculatus</i>	hogchoker	T,G
<i>Tylosurus crocodilus</i>	houndfish	G
<i>Urosalpinx cinerea</i>	Atlantic oyster drill	T,B

Table A.2. Species collected in Region 4, by gear 2020-2021.

Species	Common name	Gear
<i>A. mandibulata crustacea</i>	crustaceans	T
<i>Acanthilia(=Iliacantha) intermedia</i>	granulose purse crab	T
<i>Alosa mediocris</i>	hickory shad	G
<i>Alpheus heterochaelis</i>	bigclaw snapping shrimp	T,B
<i>Amia calva</i>	bowfin	G
<i>Anchoa spp.</i>	anchovies	T,B
<i>Anguilla rostrata</i>	American eel	T
<i>Anomura paguridae</i>	hermit crabs	T,G,B
<i>Arbacia punctulata</i>	Atlantic purple sea urchi	G
<i>Archosargus probatocephalus</i>	sheepshead	T,G,B
<i>Armases(=Sesarma) cinereum</i>	squareback marsh crab	B
<i>Ascidicea</i>	tunicates	T
<i>Astroscopus guttatus</i>	northern stargazer	T
<i>Bairdiella chrysoura</i>	silver perch	T,G,B
<i>Bivalvia veneroida</i>	clams (Veneroida)	T
<i>Brevoortia tyrannus</i>	Atlantic menhaden	T,G,B
<i>Bryozoa, ectoprocta</i>	bryozoans	T
<i>Busycotypus canaliculatus</i>	channeled whelk	G
<i>Callinectes sapidus</i>	blue crab	T,G,B
<i>Cancer spp.</i>	Cancer rock crabs	T
<i>Cancridae</i>	rock crabs	T
<i>Carangoides(=Caranx) ruber</i>	bar jack	T
<i>Caranx hippos</i>	crevalle jack	T,G
<i>Caranx latus</i>	horse-eye jack	G
<i>Carcharhinus acronotus</i>	blacknose shark	G
<i>Carcharhinus limbatus</i>	blacktip shark	G
<i>Centropristis striata</i>	black sea bass	G
<i>Chaetodipterus faber</i>	Atlantic spadefish	G
<i>Chasmodes bosquianus</i>	striped blenny	B
<i>Chelonia mydas</i>	green sea turtle	G
<i>Chilomycterus schoepfii</i>	striped burrfish	T,G
<i>Chloroscombrus chrysurus</i>	Atlantic bumper	T
<i>Citharichthys spilopterus</i>	bay whiff	T,G,B

<i>Cnidaria</i>	jellyfish	T
<i>Crassostrea virginica</i>	eastern oyster	T
<i>Ctenogobius boleosoma</i>	darther goby	T,B
<i>Ctenogobius shufeldti</i>	freshwater goby	T,B
<i>Ctenophora</i>	comb jellies	T
<i>Cynoscion nebulosus</i>	spotted seatrout	T,G,B
<i>Cynoscion nothus</i>	silver seatrout	T,G
<i>Cynoscion regalis</i>	weakfish	G
<i>Cyprinodon variegatus</i>	sheepshead minnow	B
<i>Dactyloscopus spp.</i>	sand stargazers	T

Table A.2. Species collected in Region 4, by gear 2020-2021, continued.

Species	Common name	Gear
<i>Dasyatis americana</i>	southern stingray	G
<i>Dasyatis sabina</i>	Atlantic stingray	G
<i>Dorosoma cepedianum</i>	gizzard shad	G
<i>Echinacea</i>	sea urchins	G
<i>Elopiformes</i>	tarpons - order	T
<i>Elopomorpha ang. anguilloidei</i>	eels	T
<i>Elops saurus</i>	ladyfish	T,G
<i>Enneacanthus gloriosus</i>	bluespotted sunfish	T
<i>Esox niger</i>	chain pickerel	T
<i>Etropus spp.</i>	Etropus flounders	T
<i>Eucinostomus argenteus</i>	spotfin mojarra	T,B
<i>Eucinostomus lefroyi</i>	mottled mojarra	B
<i>Evorthodus lyricus</i>	lyre goby	B
<i>Farfantepenaeus aztecus</i>	brown shrimp	T,B
<i>Farfantepenaeus duorarum</i>	pink shrimp	T
<i>Fundulus heteroclitus</i>	mummichog	B
<i>Fundulus majalis</i>	striped killifish	B
<i>Gambusia holbrooki</i>	eastern mosquitofish	B
<i>Gastropoda</i>	gastropods	T
<i>Glyceridae</i>	bristleworm	T
<i>Gobiidae</i>	gobies	B
<i>Gobiosoma bosc</i>	naked goby	T,B
<i>Gymnura micrura</i>	smooth butterfly ray	G
<i>Hippocampus erectus</i>	lined seahorse	T
<i>Hydrozoa</i>	Hydrozoa	T
<i>Hypsoblennius hentz</i>	feather blenny	B
<i>Ictalurus furcatus</i>	blue catfish	T,G
<i>Isopoda</i>	isopods	T
<i>Lagodon rhomboides</i>	pinfish	T,G,B
<i>Leiostomus xanthurus</i>	spot	T,G,B
<i>Lepisosteus osseus</i>	longnose gar	T,G

<i>Lepomis macrochirus</i>	bluegill	T,G,B
<i>Limulus polyphemus</i>	horseshoe crab	G
<i>Litopenaeus setiferus</i>	white shrimp	T,B
<i>Littorina spp.</i>	periwinkles	B
<i>Lobotes surinamensis</i>	Atlantic tripletail	G
<i>Lolliguncula brevis</i>	Atlantic brief squid	T
<i>Lutjanus griseus</i>	gray snapper	B
<i>Macrobrachium spp.</i>	river shrimps	T,B
<i>Majidae</i>	spider crabs	T,G
<i>Menidia beryllina</i>	inland silverside	B
<i>Menidia menidia</i>	Atlantic silverside	T,B
<i>Menippe mercenaria</i>	Florida stone crab	T,G

Table A.2. Species collected in Region 4, by gear 2020-2021, continued.

Species	Common name	Gear
<i>Menticirrhus americanus</i>	southern kingfish	T,G
<i>Menticirrhus littoralis</i>	Gulf kingfish	G
<i>Menticirrhus saxatilis</i>	northern kingfish	G
<i>Mercenaria spp.</i>	quahogs	T
<i>Micropogonias undulatus</i>	Atlantic croaker	T,G,B
<i>Micropterus salmoides</i>	largemouth bass	G
<i>Morone americana</i>	white perch	G
<i>Morone saxatilis</i>	striped bass	T,G
<i>Mugil cephalus</i>	striped mullet	T,G,B
<i>Mugil curema</i>	white mullet	G,B
<i>Myrophis punctatus</i>	speckled worm snake eel	T
<i>Nassarius spp.</i>	mudsnails	T,B
<i>Nudibranchia</i>	sea slugs (Nudibranchs)	T
<i>Ophiothricidae</i>	Ophiothricidae B. stars	T
<i>Opisthonema oglinum</i>	Atlantic thread herring	T,G
<i>Opsanus tau</i>	oyster toadfish	T,G
<i>Orthopristis chrysoptera</i>	pigfish	T,G,B
<i>Palaemonetes spp.</i>	grass shrimps-Palaemonete	T,B
<i>Paralichthys albigutta</i>	Gulf flounder	G
<i>Paralichthys dentatus</i>	summer flounder	T,G
<i>Paralichthys lethostigma</i>	southern flounder	T,G,B
<i>Parthenopidae</i>	elbow crabs	T
<i>Penaeidae</i>	penaeid shrimps	T
<i>Persephona spp.</i>	Persephona purse crabs	T
<i>Pleuroploca gigantea</i>	horse conch	G
<i>Poecilia latipinna</i>	sailfin molly	B
<i>Pogonias cromis</i>	black drum	G
<i>Pomatomus saltatrix</i>	bluefish	G
<i>Porcellanidae</i>	porcelain crabs - family	T

<i>Porifera</i>	sponges	T
<i>Portunus spinimanus</i>	blotched swimming crab	T
<i>Portunus spp.</i>	Portunus swimming crabs	T,B
<i>Prionotus carolinus</i>	northern searobin	T
<i>Prionotus scitulus</i>	leopard searobin	G
<i>Prionotus spp.</i>	Prionotus searobins	T
<i>Prionotus tribulus</i>	bighead searobin	T,G,B
<i>Rachycentron canadum</i>	cobia	G
<i>Rhizoprionodon terraenovae</i>	Atlantic sharpnose shark	G
<i>Sciaenidae</i>	drums	T
<i>Sciaenops ocellatus</i>	red drum	T,G
<i>Scomberomorus maculatus</i>	Spanish mackerel	G
<i>Selene vomer</i>	lookdown	T
<i>Sphoeroides maculatus</i>	northern puffer	T,G

Table A.2. Species collected in Region 4, by gear 2020-2021, continued.

Species	Common name	Gear
<i>Sphyrna barracuda</i>	great barracuda	G
<i>Sphyrna tiburo</i>	bonnethead hammerhead	G
<i>Stephanolepis hispidus</i>	planehead filefish {u}	G,B
<i>Stephanolepis hispidus</i>	planehead filefish {w}	T,G,B
<i>Stomolophus meleagris</i>	cannonball jellyfish	T
<i>Symphurus plagiusa</i>	blackcheek tonguefish	T,B
<i>Syngnathus fuscus</i>	northern pipefish	T,B
<i>Syngnathus louisianae</i>	chain pipefish	B
<i>Synodus foetens</i>	inshore lizardfish	T,G
<i>Trachemys(=Chrysemys) scripta scri</i>	yellowbelly turtle	T
<i>Trachinotus carolinus</i>	Florida pompano	G,B
<i>Trachinotus falcatus</i>	permit	B
<i>Trichiurus lepturus</i>	Atlantic cutlassfish	G
<i>Trinectes maculatus</i>	hogchoker	T,G
<i>Uca spp.</i>	Uca fiddler crabs	B
<i>Urophycis regia</i>	spotted hake	T
<i>Xanthidae</i>	mud crabs	T,B

APPENDIX B: A multimetric index for assessing the ecological status of North Carolina coastal waters and validating nominations for Strategic Habitat Areas

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1. Objectives

Strategic Habitat Areas (SHAs) are defined as specific locations of individual fish habitats or systems of habitats that have been identified to provide exceptional habitat functions or that are particularly at risk due to imminent threats, vulnerability, or rarity. Extensive field sampling of target fish species in three fish habitats inside and outside of SHAs has been conducted through fishery-independent survey programs operated by the North Carolina Division of Marine Fisheries. The SHAs have been nominated using the GIS MARXAN analysis that is primarily based on habitat information (Deaton et al. 2006; Jensen et al. 2014). The multimetric index based on both fish and habitat metrics has been extensively developed and applied to assess ecological status and has been deemed more accurate and comprehensive than any single metric index (e.g., Pérez-Domínguez et al. 2012; Smoliński and Całkiewicz 2015). The objectives of this study are to (1) develop a multimetric index for assessing ecological status of the Coastal Habitat Protection Plan (CHPP) Region 3; (2) validate the SHA nominations from the MARXAN analysis using this multimetric index. The ultimate goal is to help produce a standard operating procedure (SOP) for monitoring and potentially modifying SHAs in the future based on the multimetric index.

2. Methods

2.1 Data

Data collected from Program 215 were used for the multimetric index development. Sampling was conducted in CHPP Region 3 in 2018 and 2019 from February 15–November 15. The sampling region was divided into one nautical mile by one nautical mile grids. Grids were identified as SHAs or Non-SHAs based on the amount of SHA within the grid. Grids were excluded to create a buffer to account for any edge effect of the SHAs, to avoid known areas of high turtle interactions (Management Area D1), and areas where sampling was determined to be unsafe/unattainable. A stratified random sample of 16 grids (eight SHA and eight non-SHA) was randomly selected each month. Weather and mechanical issues resulted in not completing all

samples during some months. Variables associated with environment, habitat and fish were collected. In this analysis, a total of 255 observations (or grids) and 33 variables (including environmental variables and potential metrics) were initially considered.

2.2 Multimetric index development

Development of the multimetric index includes five steps in general: (1) determining a list of candidate metrics; (2) selecting metrics by reducing redundancy among candidate metrics and identifying the metrics that show a significant response to a pre-defined proxy of human disturbance; (3) defining the scoring system for each selected metric; (4) defining the combination rule that produces the final multimetric index; (5) validating the multimetric index.

2.2.1 A list of candidate metrics

In this study, the set of candidate metrics and the potential direction of each metric responding to the overall human disturbance was determined on a basis of expert opinions. The list contained 21 metrics that inform the overall fish community (global metrics), habitat conditions and feeding guilds of fish species (Table 1). These metrics fall into three types: the numerical metrics (the metrics with count, e.g., INVEA, or continuous values, e.g., CLAR), the categorical metrics (the metrics with multiple categorized levels, e.g., HARD and EROD) and the binary metrics (the metrics with presence or absence, e.g., SAV).

2.2.2 Selection of metrics

Selection of metrics involves reducing the redundancy among candidate metrics and modeling of each metric. In this study, we used correlation analysis to exclude the redundant numerical metrics. The Spearman correlation was calculated among all candidate numerical metrics. The pair of metrics that had a correlation coefficient ≥ 0.8 were identified for high inter-correlation (Smoliński and Całkiewicz 2015). Between the pair of metrics with a high correlation, we rejected the metric that had poorer data quality, had more limited information (with a larger number of missing data), or showed weaker response to human disturbance according to the expert opinions or the metric modeling.

The metric modeling was applied to numerical and binary metrics. We used the generalized linear model (GLM) to examine the relationship between a metric and the human disturbance

along with environmental variables. A total of eight environmental variables were included in this study as essential variables that affect sampling protocol (e.g., sampling season) and fish community (e.g., salinity; Table 2). The mean alteration score (MAS) was used as a proxy of human disturbance. The MAS was calculated for each sampling grid during the GIS SHA nomination process and a higher score indicates a more altered condition and more human disturbance (Deaton et al. 2006). Both Poisson and negative binomial GLMs were initially considered for the metrics with count data (e.g., TA and TR). The negative binomial GLM was used when the Poisson GLM exhibited over-dispersion (Zuur et al. 2009, 2012). The Gaussian GLM was applied to the metrics with continuous data (e.g., DOMI and CLAR). Data of the metrics were log-transformed before fitting the GLM. In the case of data having zero observations, a small positive value (0.001) was added to the observations before log-transformation. The logistic GLM was applied to the metrics with binary data (e.g., SHRBT and SAV), and the zero-inflated GLM (ZIF; Zuur et al. 2012) was applied to the metrics with excess zeros (e.g., PISCR with 80% zero observations). In the ZIF, a Poisson or negative binomial GLM sub-model was used to model the positive count data and a logistic GLM sub-model was used to model the presence or absence of an observation.

A stepwise forward selection procedure was used to select the variables from the environmental variables and MAS that had significant impact on a metric (Li et al. 2016). The selection procedure started with a null model including only the intercept. The significant variables were identified and added to the null model based on Akaike Information Criterion (AIC) and Chi-square test (Akaike 1974; Burnham and Anderson 2002). At each step, the variable that most reduced the AIC value (i.e., the decrease in AIC was more than three) or had a *p*-value less than the significance level of 0.05 was added to the null model. This process was repeated until inclusion of an additional covariate would not substantially improve model performance. Those metrics that had MAS selected as a significant variable in the model were identified showing a significant response to MAS and were thus included in the final multivariate index (Smoliński and Całkiewicz 2015).

Two categorical metrics were examined in this study, i.e., the extent of hardened shoreline (HARD) and the extent of eroded edge (EROD). These two metrics were determined to be

important in response to human disturbance based on expert judgement; thus, they were included in the final multimetric index.

2.2.3 Scoring system for each selected metric

A five-grade scoring system ranging from 1 (the worst ecological status) to 5 (the best ecological status) was defined for each selected metric, and a score was assigned to each observation based on this scoring system of the metric (Delpech et al. 2010; Smoliński and Całkiewicz 2015). For a numerical metric, the previously fitted GLM model was used to predict the metric values given three disturbance levels: low disturbance (minimal MAS value = 0.0002), medium disturbance (mean of minimal and maximal MAS values = 2.1431) and high disturbance (maximal MAS value = 4.2859); thus, the least disturbed grids in the data were designated as the reference (Breine et al. 2007; Smoliński and Całkiewicz 2015). The environmental variables that were selected in the model were fixed at the mean (for continuous variables) or the mode (for categorical variables) of the variable, which helped reduce the impacts of these variables on the detection of the relationship between the metric and the MAS.

A nonparametric bootstrap was used to produce the prediction distribution under each disturbance level for the numerical metric (Delpech et al. 2010; Smoliński and Całkiewicz 2015). In this bootstrap procedure, a total of 5,000 datasets were generated by randomly sampling the original dataset with replacement, and the metric values were predicted under the three disturbance levels using the GLM fitted to each dataset. By examining the prediction distributions under the three disturbance levels for a metric, the discriminant ability of a metric for different disturbance levels can be evaluated and the score thresholds can be defined. In this study, the 10% and 90% quantile of a prediction distribution were calculated and used to define the score thresholds for a metric. Specifically, for a numerical metric, the 90% quantile of the prediction distribution for high disturbance level, the 10% and 90% quantile of the prediction distribution for medium disturbance level, and the 10% quantile of the prediction distribution for low disturbance level were defined as thresholds for assigning scores from 1 to 5.

For a categorical metric such as HARD and EROD, these two metrics have already been categorized into 5 and 4 levels in the data based on the percentage of hardened and eroded, respectively. Therefore, instead of using GLM modeling and bootstrap, their score thresholds can

be defined directly based on the levels recorded in the data (Cabrera et al. 2012). For a binary metric, a score of 1 and a score of 5 can be assigned to presence or absence, depending on the response of the metric to increasing human disturbance and the definition of the five-grade scoring system (Cabrera et al. 2012).

2.2.4 The final multimetric index: a combination of multiple metrics

The final multimetric index was calculated for each observation by combining the scores of all selected metrics, in which scores of all metrics were summed and divided by the maximal potential score (five times the number of selected metrics; Delpech et al. 2010; Smoliński and Całkiewicz 2015). The final multimetric index ranges between 0 and 1, with a value of 0 for the worst ecological status and a value of 1 for the best ecological status.

2.3 Validation of the multimetric index

A linear regression for the multimetric index scores against the MAS for all observations was conducted to validate the performance of the index. A strong negative relationship between the index scores and the MAS indicates the ability of the index for tracking the human disturbance. Additionally, we compared the index with the SHA nominations from the GIS MARXAN analysis for CHPP Region 3.

3. Results

Among the 21 candidate metrics initially evaluated, the Simpson's dominance (DOMI) was highly correlated with both Shannon's diversity (SHAN) and evenness (EVEN) with a correlation coefficient of -0.98 and -0.85 respectively. The metrics SHAN and EVEN were excluded because the information in both metrics were imbedded in the single metric DOMI. The abundance (PISCA) and richness (PISCR) of piscivorous species were also highly correlated with a correlation coefficient of 0.99. Although both metrics had a high percentage (around 80%) of zero observations, the positive observations of the metric PISCA were greatly skewed with extreme values (e.g., one observation of 1156 and the majority less than 100), and thus PISCA was excluded from further consideration.

According to metric modeling, six habitat related metrics ((i.e., SHRBT, SHRSL, SHRVT, SHELL, MARSH, SAV) and one feeding guilds related metric (i.e., INVEA) showed significant

response to the MAS, and thus were included in the final multimetric index (Table 3). Along with the two categorical metrics HARD and EROD that were pre-determined to be included, there were a total of nine metrics in the final multimetric index (Table 4).

Among the environmental variables, sampling season (SEA), location (LON and LAT), and water depth (DEPTH) were often selected in the GLM models as significant variables, suggesting the high importance of sampling protocol. Salinity was a significant variable in the models for the metric describing the abundance of invertivorous species (INVEA) and for most habitat related metrics (Table 3).

For the numerical metric INVEA that was included in the final multimetric index, the prediction distribution for the medium disturbance level substantially overlapped at the tails with those for low and high disturbance levels. This limited discrimination power between three disturbance levels made assigning five scores using these thresholds less feasible (Figure 1). Especially, the 90% quantile of the prediction distribution for high disturbance level was so close to the 10% quantile for the medium disturbance level that thresholds set between these two values would not be discriminative enough for assigning a score. Therefore, instead of assigning five scores, we dropped the prediction distribution for the medium disturbance level and set thresholds for assigning three scores based on the 90% and 10% quantiles of the prediction distributions for the high and low disturbance levels, respectively (Table 4; Figure 2).

The score thresholds for the six binary metrics and the two categorical metrics (HARD and EROD) were defined based on the levels recorded in the data. After assigning thresholds for a five-grade scoring system, the final multimetric index value for each observation was calculated.

The regression between the final multimetric index and MAS showed a significant negative relationship, with a p -value ≤ 0.001 (Figure 3). The multimetric index also effectively tracked the nominated SHAs in CHPP Region 3 by GIS MARXAN. The median value of the multimetric index for the nominated SHA grids (median = 0.7) was approximately 16.7% higher than the one for non-SHA grids (median = 0.6). The 95% confidence interval of the multimetric index values for the SHA grids did not overlap with the one for non-SHA grids, suggesting their difference was significant (Figure 4). These results suggest the multimetric index can perform as an effective indicator for human disturbance.

4. Discussion

The framework established in this study provides a useful and universal tool for developing a multimetric index for fish habitat evaluation. It shows an effective way to summarize complex information about habitat quality and to communicate with stakeholders and managers (Smoliński and Całkiewicz 2015). Although the multimetric index was developed for a specific region (i.e., the CHPP Region 3) in the current study, this framework is flexible and can be improved by including other important metrics when data become available for this region or can be adopted to other regions by adjusting the list of candidate metrics. For the CHPP Region 3 that was tested in this study, ongoing effort is warranted to refine the list of candidate metrics, for example, to explore more fish-based metrics such as the total abundance of certain commercially important species or certain species that are sensitive to pollution.

Pérez-Domínguez et al. (2012) reviewed 20 studies and found most multimetric indices included 9–10 metrics with a maximum of 16 (Franco et al. 2009) and a minimum of 4 (Delpech et al. 2010). A large number of metrics may raise concerns over overfitting problems (Pérez-Domínguez et al. 2012). The multimetric index developed in this study included a total of nine metrics, which falls within a reasonable range across studies.

The final set of metrics included in the multimetric index in this study is not in good balance with the habitat-related metrics dominating the multimetric index and no global fish metrics being selected. This result may be explained by the way MAS was developed. Information on habitat alteration was heavily used in the calculation of the MAS, along with the information on human activity. The final multimetric index can be improved by either choosing an alternative proxy of human disturbance that better focuses on human activity or refining global fish metrics to include metrics that describe abundance and richness of certain species of interest, e.g., the species of economic importance or the species sensitive to pollution.

In this study, we tested including TA in the final multimetric index because it is essential to include a global fish metric based on expert judgement, and the metric TA showed marginal significance to the MAS (p -value = 0.08); however, the discriminative power of TA was not good enough to provide score thresholds, with prediction distributions for all three disturbance levels overlapping with each other. We also tested assigning four scores instead of three scores

as applied in this study for the metric INVEA. To assign four scores, we used the prediction distributions for all three disturbance levels and only dropped the 10% quantile for the medium disturbance level that was very close to the 90% quantile of the prediction distribution for high disturbance level (Figure 1). The resulting multimetric index was similar to the one with three scores for INVEA.

The GLM analysis is usually restricted to the pool of variables for selection, and this pool of variables is subjective and is based on expert opinions. In the cases when the environmental variables are relatively more important in explaining the variance in the metric, the proxy of human disturbance may not be selected in the GLM model. When the pool of variables for selection changes, especially when including different environmental variables and alternative disturbance proxy, the sets of metrics selected in the final multimetric index could change dramatically; thus, expert judgement is essential in this framework.

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Tables

Table 1. List of candidate metrics and their expected response to increasing human disturbance.

Candidate metric	Abbreviation	Variable type	Expected response
<i>Global</i>			
1 Total abundance	TA	Count	↓
2 Total number of species (richness)	TR	Count	↓
3 Shannon's diversity	SHAN	Continuous	↓
4 Evenness	EVEN	Continuous	↓
5 Simpson's dominance	DOMI	Continuous	↑
<i>Habitat conditions</i>			
6 Water clarity	CLAR	Continuous	↓
7 Presence of shoreline basins, marinas and/or boat ramps	SHRBT	Binary	↑ (present)
8 Presence of rock or wood structures perpendicular to shore	SHRGR	Binary	↑ (present)
9 Presence of low profile rock (riprap) or wood structure parallel to shore	SHRSL	Binary	↑ (present)
10 Presence of vertical structures (i.e. bulkheads)	SHRVT	Binary	↑ (present)
11 Presence of shell habitat	SHELL	Binary	↓(present)
12 Presence of marsh habitat	MARSH	Binary	↓(present)
13 Presence of submerged aquatic vegetation	SAV	Binary	↓(present)
14 Extent of hardened shoreline	HARD	Categorical	↑
15 Extent of eroded edge	EROD	Categorical	↑
<i>Feeding guilds</i>			
16 Abundance of piscivorous species	PISCA	Count	↓
17 Number of piscivorous species	PISCR	Count	↓
18 Abundance of invertivorous species	INVEA	Count	↓
19 Number of invertivorous species	INVER	Count	↓
20 Abundance of omnivorous species	OMNIA	Count	↑
21 Number of omnivorous species	OMNIR	Count	↑

Table 2. List of environmental variables and the proxy of human disturbance for the generalized linear model (GLM) analysis.

Variable name	Abbreviation	Variable type
<i>Environmental variable</i>		
<i>Sampling protocol related</i>		
1 Season	SEA	Categorical
2 Latitude (°)	LAT	Continuous
3 Longitude (°)	LON	Continuous
4 Water depth (m)	DEPTH	Continuous
<i>Affecting fish community</i>		
5 Sediment size	SED	Categorical
6 Bottom water temperature (°C)	TEMP	Continuous
7 Bottom water salinity (ppt)	SAL	Continuous
8 Bottom dissolved oxygen (mg/L)	DO	Continuous
<i>Proxy of human disturbance</i>		
1 Mean alteration score	MAS	Continuous

Table 3. Generalized linear model (GLM) analysis for metrics that showed a significant response to the mean alteration score (MAS).

Predictor variables selected in model	Df	AIC	Pr(Chi)
SHRBT (Logistic GLM)			
NULL		337.71	
MAS	1	313.97	< 0.001
SHRSL (Logistic GLM)			
NULL		308.56	
MAS	1	301.78	0.003
SHRVT (Logistic GLM)			
NULL		315.71	
MAS	1	304.92	< 0.001
SAL	1	295.24	0.001
LAT	1	292.65	0.03
SHELL (Logistic GLM)			
NULL		318.31	
SAL	1	288.02	< 0.001
LAT	1	275.29	< 0.001
MAS	1	262.61	< 0.001
SED	3	258.17	0.02
LON	1	255.96	0.04
MARSH (Logistic GLM)			
NULL		229.61	
MAS	1	222.43	0.002
DEPTH	1	215.96	0.004
SAV (Logistic GLM)			
NULL		293.3	
SAL	1	265.48	< 0.001
MAS	1	247.54	< 0.001
LON	1	245.18	0.04
LAT	1	235.38	0.001
SED	3	232.72	0.03

INVEA (Negative binomial GLM)			
NULL		3470.57	
SEA	3	3403.79	< 0.001
SAL	1	3381.35	< 0.001
DO	1	3377.04	0.01
DEPTH	1	3370.28	0.003
MAS	1	3363.91	0.004

Table 4. The metrics included in the final multimetric index and the scoring system for each metric.

Metrics	Scores				
	1 (worst ecological status)	2	3	4	5 (best ecological status)
SHRBT	Present				Absent
SHRSL	Present				Absent
SHRVT	Present				Absent
SHELL	Absent				Present
MARSH	Absent				Present
SAV	Absent				Present
HARD	>50% hardened	26–50% hardened	10–25% hardened	<10% hardened	0% hardened
EROD	>50% erosion	26–50% erosion		1–25% erosion	0% erosion
INVEA	< 181		181–581		> 581

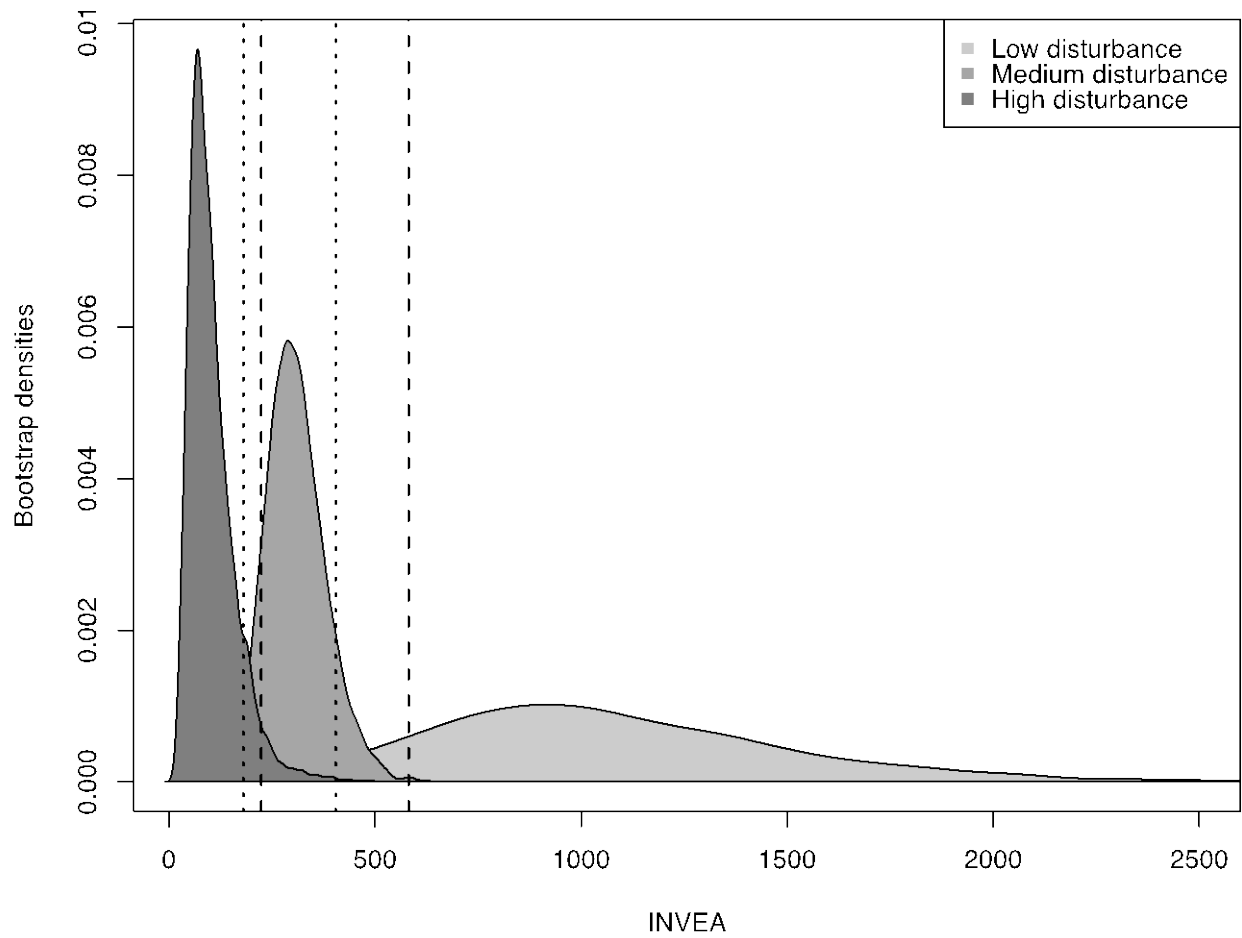


Figure 1. Prediction distributions of the abundance of invertivorous species (INVEA) under low, medium and high levels of the mean alteration score (MAS). The dash line is the 10% quantile and the dotted line is the 90% quantiles of the prediction distributions.

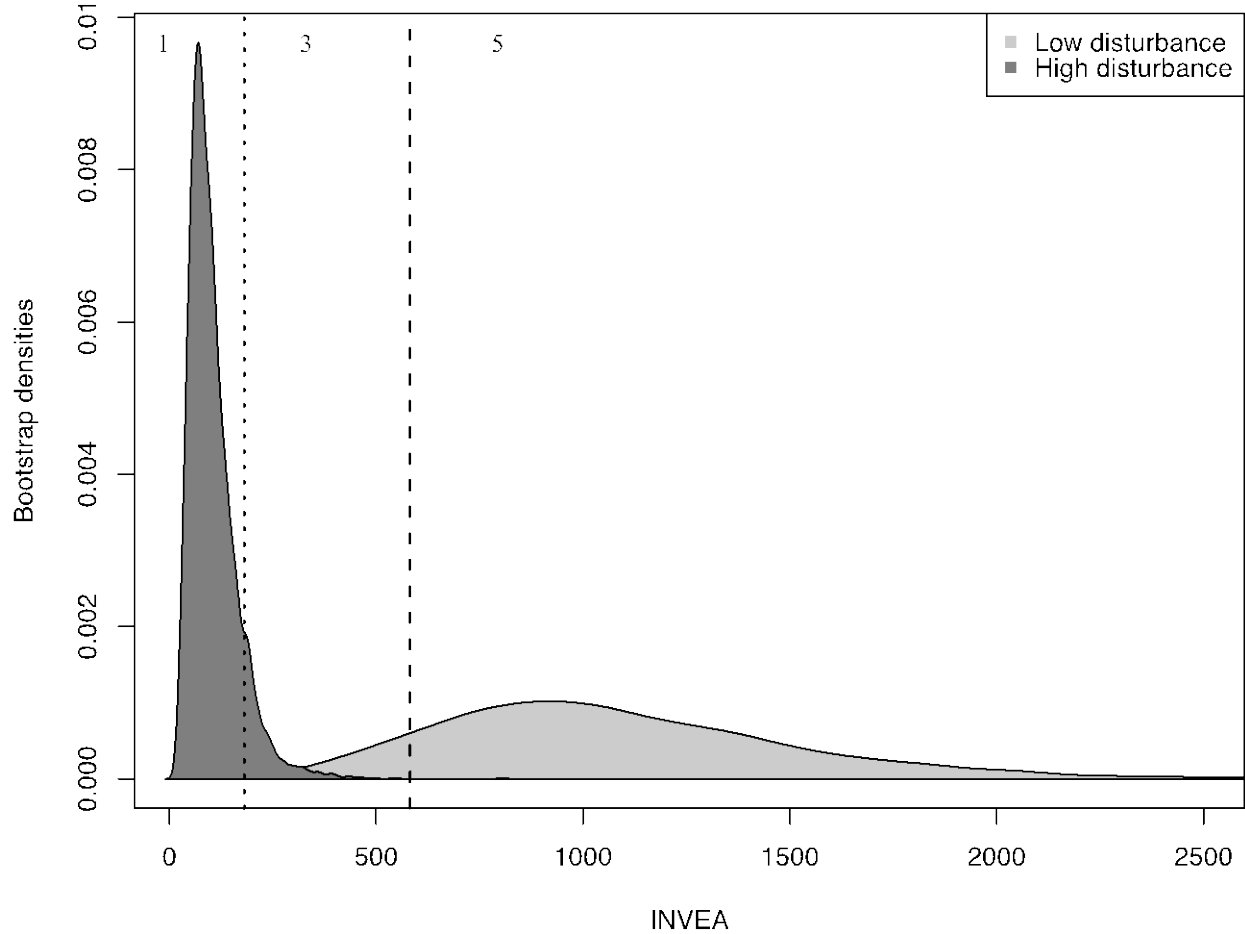


Figure 2. Prediction distributions of the abundance of invertivorous species (INVEA) under low and high levels of the mean alteration score (MAS). The dash line is the 10% quantile and the dotted line is the 90% quantiles of the prediction distributions; these two lines mark the thresholds for assigning scores. The numbers are the assigned scores.

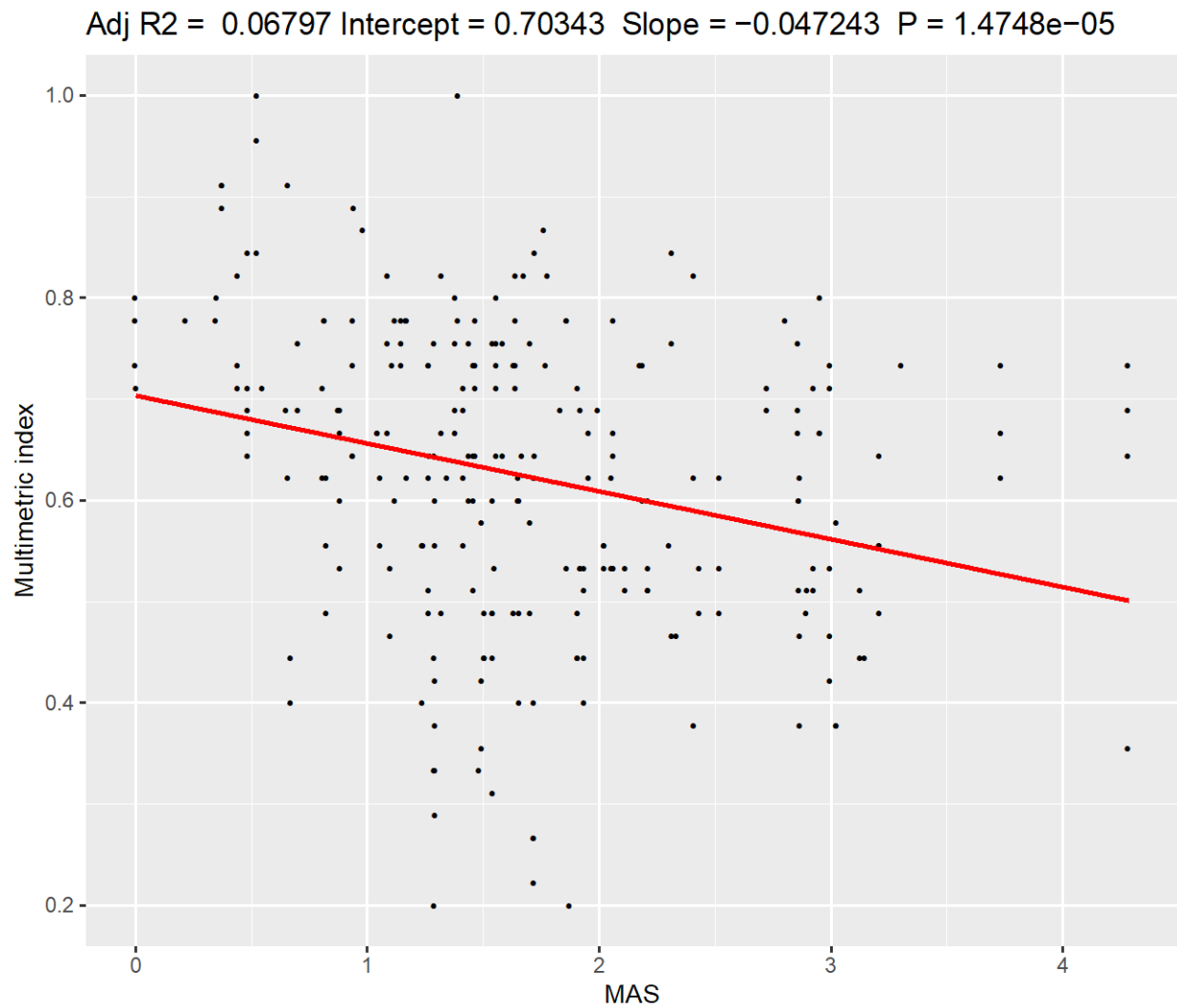


Figure 3. Linear regression of the multimeric index values against the mean alteration score (MAS). Dots are observations in the dataset; the solid line is the fitted values and the dash lines are the standard deviations.

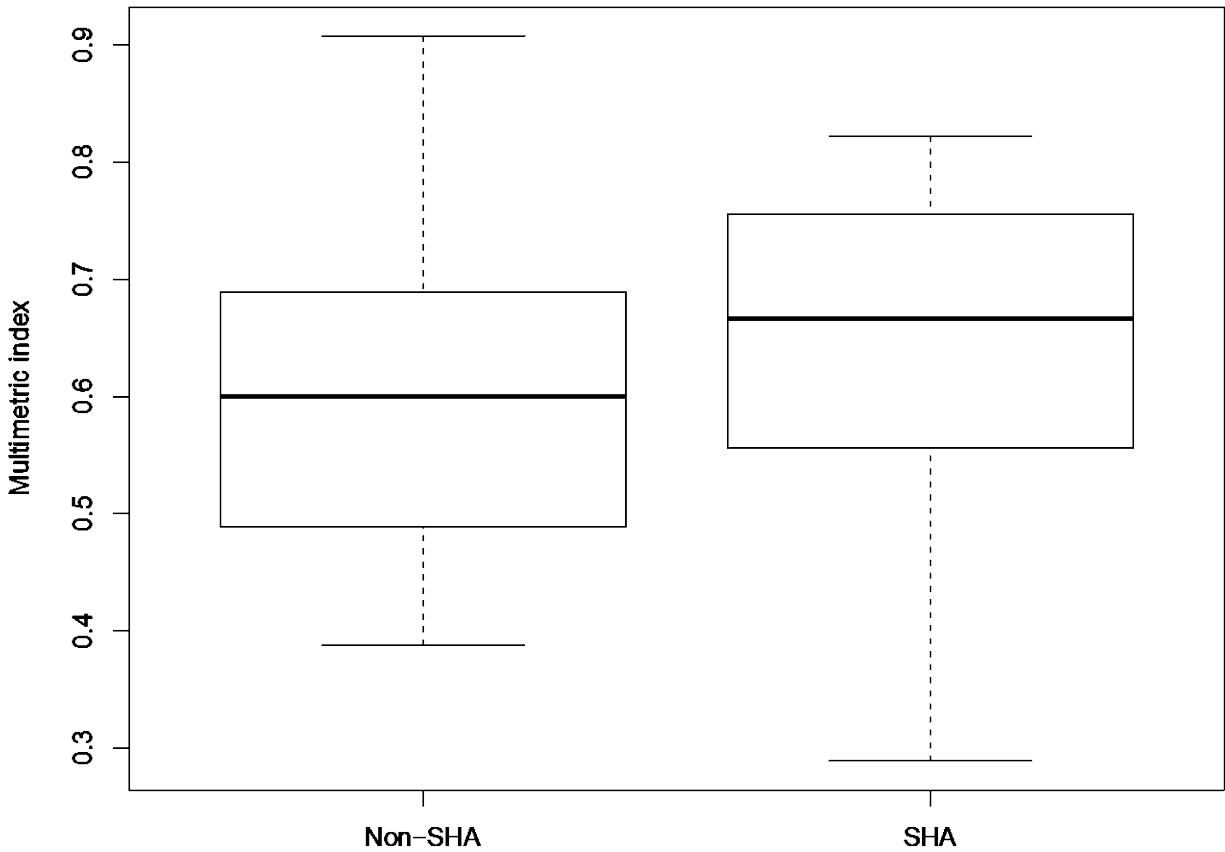


Figure 4. Multimetric index values for the Strategic Habitat Areas (SHAs) grids and the non-HAS grids based on the GIS MARXAN analysis represented as boxplots. The middle bolded line is the median; the upper and lower outlines of the box are the 75% and 25% quantiles respectively; the upper and lower bars are the 97.5% and the 2.5% quantiles respectively.