COASTAL RECREATIONAL FISHING LICENSE
FINAL PERFORMANCE REPORT

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Grant Number: 2010-H-004

Grant Title: Informing habitat enhancement in Pamlico Sound

Grant Award Period: June 15, 2010 to December 31, 2013

Project Costs:

Description	Budgeted	Expenditures
Personnel Costs	$46,181.00	$43,247.00
Non-Personnel costs	$90,216.00	$47,305.45
Total Direct Costs	$95,422.00	$90,553.23
Indirect Costs	$14,312.00	$10,629.93
Total Direct Costs	$109,734.00	$101,183.16

Final Project Summary:

The global decline of many marine species has prompted the use of habitat enhancement such as artificial reefs to stem population declines. Inadequate scientific information to guide site selection is one of the most common causes of unsuccessful habitat enhancement. In this collaborative study among NC State University, NC Division of Marine Fisheries (DMF), and UNC-CH, we addressed 3 CRFL Strategic Plan Management goals with relevance to "habitat" and "people" by applying a hierarchical optimization approach to selecting the most suitable sites for placement of artificial reefs, oyster sanctuaries, and general habitat enhancement in Pamlico Sound. Specifically, we used 4 steps: (1) statistical analyses of long-term salinity data in Pamlico Sound to produce sound-wide salinity GIS layer, (2) hydrodynamic modeling to produce oyster larval and gag postlarval settlement GIS layers, (3) GIS-based analyses to exclude unsuitable sites based on 7 GIS layers (e.g., substrate, navigational channels) and quantify the suitability of non-excluded sites using 12 additional layers (e.g., salinity, larval dispersal, proximity to boat ramps), and (4) field verification of water quality and substrate characteristics at selected sites (based on 3) to ensure minimum water quality and substrate standards. Model predictions indicated that ~39% of Pamlico Sound was unsuitable for habitat enhancement primarily due to shallow water depth limitation near shore and the presence of aquatic vegetation along the Outer Banks. The most suitable locations for oyster sanctuaries were in the southwestern (Neuse and Pamlico Rivers) and northern (Roanoke Island) portions of Pamlico Sound, whereas the most suitable locations for artificial reefs in support of reef fish were near inlets and along the Outer Banks region. In both instances, the most suitable sites were typically clustered. The GIS-based hierarchical approach used in this study for site selection was an effective method for (1) narrowing vast water bodies to a manageable number of sites for further ground-truthing, (2) identifying habitat enhancement "hot spots" where the most suitable cells are clustered, and (3) designing enhancement strategies to aid in applied management decisions. To this end, the results from this work are currently being integrated into NC DMF future habitat enhancement plans, as well as Army Corps of Engineer oyster sanctuary construction. More broadly, the results from this work were presented at 2 regional scientific meetings, 1 international workshop, and will be further disseminated through 4 peer-reviewed manuscripts (2 currently in review, 2 in prep).

Informing habitat enhancement in Pamlico Sound

Introduction

The global decline of many recreationally- and commercially-exploited marine species has prompted the use of habitat enhancement, such as artificial reefs, as a management tool for stemming population declines (Beck et al. 2011). Inadequate scientific support for site selection is one of the most common causes of unsuccessful habitat enhancement efforts such as artificial reefs (Kennish et al. 2002). With the focus on relocating efforts of the North Carolina Artificial Reef Program from offshore to estuarine waters, as well as recommendations of the NC Oyster Fishery Management Plan (2008) and goals established in the NC Coastal Habitat Protection Plan (Street et al. 2005), there is a need to identify new, suitable locations to expand estuarine enhancement efforts, which consists of oyster sanctuaries and artificial reefs.

Exclusion mapping, where cartographic information is used to exclude unsuitable areas, is one of the most popular methods used by managers and scientists to select sites for artificial reef deployment or habitat restoration (Kaiser 2006). In NC, several criteria including substrate suitability, user conflict, accessibility and, in the case of oyster sanctuaries, historical data on oyster bottoms documented in Winslow's (1889) report have been used to exclude unsuitable sites and identify suitable sites for habitat enhancement. Although exclusion mapping is very useful for identifying and excluding sites where conflicts are likely to arise (e.g., navigation, commercial fishing activities, platforms), this process does not provide managers with the physical and biological characteristics to determine the likely success of habitat enhancement. For example, exclusion maps may identify the same suitable sites for artificial reefs aimed at enhancing gag grouper (*Mycteroperca microlepis*) and sanctuaries aimed at enhancing oyster (*Crassostrea virginica*) broodstocks, but in reality, biophysical characteristics such as larval dispersal may result in very different optimal site selection portfolios for each species. Incorporating exclusion mapping and biophysical characteristics is critical for identifying optimal habitat enhancement locations that complement the existing enhancement portfolio. Effective habitat enhancement could increase young-of-the-year abundance and lifetime production of several commercially and recreationally important species such as gag, black sea bass (*Centropristis striata*), gray snapper (*Lutjanus griseus*), sheepshead (*Archosargus*

probatocephalus), and stone crab (*Menippe mercenaria*) (Gwak 2003, Peterson et al. 2003, Rindone and Eggleston 2011, Pierson and Eggleston 2014).

A number of criteria have been identified as important in artificial reef site selection, including currents (Baynes and Szmant 1989), wave action (Duzbastilar et al. 2006), proximity to natural habitat and migration corridors (Eggleston et al. 1997; Spieler et al. 2001), accessibility by fishermen (National Artificial Reef Plan 2007), substrate characteristics (Mathews 1985), and larval supply (Barber et al. 2009). In estuarine environments, salinity is a major driver of biological processes (Kennedy 1996). Moreover, very few studies have integrated exclusion mapping with physical and biological factors, and none that we are aware of have integrated exclusion mapping and larval/postlarval connectivity to evaluate the suitability of sites for habitat enhancement (National Artificial Reef Plan 2007, Barber et al. 2009).

Key information to maximize the long-term ecological function and structural integrity of habitat restoration and enhancement efforts, such as artificial reefs and rip-rap for oyster reef sanctuaries in Pamlico Sound, includes ensuring that: (1) water quality is adequate to meet the needs of the ecological community, (2) the bottom sediment characteristics are such that the restored habitat will not sink to a large degree or be destroyed, and (3) the reef is located in area that will receive recruitment of the target species to subsequently function as juvenile habitat in the case of artificial reefs and broodstock in the case of oyster sanctuaries.

Objectives

In this study, we: (1) conducted statistical spatial interpolation analyses of available long-term salinity data in Pamlico Sound using NC DMF Program 195 to produce a sound-wide salinity GIS layer used in 4 below; (2) applied hydrodynamic and particle dispersal modeling to predict larval connectivity between areas of larval release and subsequent settlement; (3) applied GIS-based analyses to exclude unsuitable sites based on 7 GIS layers such as substrate, presence of Submerged Aquatic Vegetation (SAV), military restricted areas, and bathymetry; (4) applied GIS-based analyses to quantify the suitability of non-excluded sites (from 3) using 12 threshold layers such as salinity (from 1), larval connectivity (from 2), and proximity to boat ramps and enhancement material stock piles; and (5) conducted field verification of water quality (primarily dissolved oxygen) and substrate characteristics at selected sites based on 3 and 4 to remove any sites that did not meet minimum water quality and substrate standards. Steps 1-5 were used

independently to identify optimal locations for placement of oyster sanctuaries and artificial reefs. Optimal locations for oyster sanctuaries and artificial reefs were combined to identify locations that were most suitable for overall habitat enhancement that may be of benefit to multiple species and user groups.

The objectives of this study were particularly relevant to 3 CRFL Strategic Plan Management Goals related to "Habitat" and "People":

Management Goal - Habitat

Objective 1*: Improve effectiveness of existing programs.*

-Strategy H.1.1 In cooperation with other DENR agencies and university scientists, conduct coastal habitat status assessments using standard indicators of estuarine and near shore ocean conditions.

-Example Priority Research Need: Analyze and interpret existing data to use in coastal habitat assessments.

Objective 3*: Enhance habitat and protect it from physical harm.*

-Strategy H.3.3 Conduct research on restoration techniques in order to improve the quality and function of created or enhanced habitat

-Example Priority Research Need: Develop a mechanism and strategy that conducts research on restoration techniques in order to improve the quality and function of created or enhanced habitat

-Strategy H.3.7 Solicit collaborative research on larval transport and dispersal patterns and incorporate results into siting oyster habitat restoration projects and no-take oyster sanctuaries.

-Example Priority Research Need: Conduct research on larval transport and dispersal pattern results into siting oyster habitat restoration projects and no-take sanctuaries.

Management Goal – People

Objective 1*: Provide fishermen increased access to fisheries resources and enhancement structures.*

-Strategy P.1.4 Coordinate the siting of artificial fishing reefs and oyster reefs with the locations of access structures to increase utilization by the recreational fishing community.

-Example Priority Research Need: Build/enhance artificial reefs inshore.

Methods

1. Statistically analyze long-term salinity data to produce sound-wide salinity GIS layer

We developed a process-based, spatial statistical model to produce a GIS salinity layer for Pamlico Sound using observational data collected by NC DMF Program 195. NC DMF P195 was initiated in 1987 as a deep-water (> 2m), fishery-independent trawl survey within Pamlico and Croatan sounds, as well as the Neuse, Pamlico, and Pungo Rivers. P195 is a stratified random sampling program that uses paired 9.1 m "Mongoose" trawls with a 1.9 cm cod-end and a tow time of 20 minutes to sample a total of 54 stations in June and again in September in each year. In addition to sampling fish, crabs and shrimp, P195 records abiotic data at each station, including water depth, substrate type (mud, sand, muddy, fine mud, etc.), and salinity (Eggleston et al. 2004, 2009). P195 sampling stations are located within 1 min x 1 min grid cells that are stratified by water body and depth (5 strata total). For each sampling period, the number of randomly chosen sampling stations in each stratum is proportional to its surface area to ensure that sampling effort per unit area is equal among the strata. We obtained P195 data for the period 1987-2008 and conducted a QA/QC of the salinity data prior to statistical analyses. Because salinity varied at spatiotemporal scales finer than those in the P195 sampling, a statistical model was developed to: (1) determine the best variables for predicting salinity patterns in NC within a given time period (June & Sept.), and (2) predict salinity for areas that were not sampled to generate spatial distribution maps of bottom salinity throughout Pamlico Sound, NC.

First, we fit multiple regression models to observed salinity data for each time period to evaluate the efficacy of various chemical (e.g., nutrient concentration/flux, etc.), physical (temperature, water column stratification intensity, etc.), landscape (e.g. distance to nearest inlet) and climatic (e.g., fresh water influx from four rivers, hurricane incidence) variables for predicting salinity. We used Akaike's information criterion (AIC) to select the best set of variables that explained space/time variation in salinity, and then characterized the spatial correlation in the regression residual errors to calculate more precise salinity predictions. Most environmental variables, such as salinity, are spatially correlated, such that data from points close in space are more similar than data from points farther apart. The spatial correlation in the regression residuals for each time period was modeled with the best fitting semi-variogram (a non-linear curve that describes the decay of spatial correlation with increasing distance between

points). The resulting statistical model was used to predict salinity at 4,000 stations in Pamlico Sound, each separated by 1.85 km. Long-term mean salinity predicted from statistical modeling was used to generate a salinity map and corresponding threshold-salinity GIS layer (see 4 below).

We were unable to statistically analyze and develop maps of dissolved oxygen (DO) in Pamlico Sound due to (i) relatively short duration of the DO measurements taken by NC DMF Program 195, as well as numerous gaps in these data measurements over time. For the time period 1987-2008, there were 11 years where DO measurements were taken; however, missing observations occurred in over half of these years. Moreover, there were concerns about the lack of calibration of the DO probe during many of the Program 195 cruises. To address the potential negative impacts of DO on site selection for habitat enhancement, we conducted field ground-truthing of DO at optimal sites (see below).

2. Hydrodynamic and particle-dispersal modeling to provide a larval connectivity GIS layer

We used ADCIRC 3D (ADvanced CIRCulation), a non-linear finite-element hydrodynamic model (Luettich and Westerink 2004, Reyns et al. 2006, 2007) coupled with a particle-tracking model (Haase et al. 2012) to simulate dispersal and settlement of two model species: (i) larval oysters (*Crassostrea virginica*), which spawn from May-October, with major spawning peaks in late May in Pamlico Sound when water temperatures reach 20°C (Eggleston et. al. 2011), and (ii) postlarval gag grouper (*Mycteroperca microlepis*) that ingress through the inlets of Pamlico Sound (Ocracoke, Hatteras and Oregon) from mid-April to mid-May (Adamski et al. 2011). Gag postlarvae settle within seagrass beds, oyster reefs, and other hard substrates such as artificial reefs (Ross and Moser 1995).

Hydrodynamic model—ADCIRC solves the shallow water form of the momentum equations over the entire CAPES (Croatan-Albemarle-Pamlico-Estuarine-System) domain. ADCIRC has produced flow fields that are in good agreement with observed currents in the southern portion of the Pamlico Sound (e.g. Neuse River Estuary, Luettich et al., 2002), as well as in our study region within Pamlico Sound (Reyns et al. 2006, 2007, Haase et al. 2012). ADCIRC was used to recreate Pamlico Sound circulation patterns under field conditions from May-October 2006, 2007 and 2008 during times of oyster larval dispersal and gag postlarval ingress. Our previous field validation of ADCIRC, using the blue crab (*Callinectes sapidus*) as a

model species whose postlarvae are transported from the continental shelf through the inlets and across Pamlico Sound to nursery habitats, indicated that circulation under the combined effects of wind and tides best matched observed current patterns and the distribution of postlarval and early juvenile blue crabs (Reyns et al. 2006, 2007). Accordingly, we used the combined effects of wind and tides to recreate circulation of Pamlico Sound in this study. A high-resolution unstructured grid comprised of 63,921 nodes and 120,422 elements depicting Pamlico Sound and adjacent coastal waters was extracted from a much larger grid extending from the coast of Nova Scotia (northern boundary), to the coast of South America (southern boundary) to 60° W (eastern boundary). The larger grid was needed to accurately simulate tidal forcings. The extracted Pamlico Sound grid was connected to the Atlantic Ocean through inlets with a grid resolution between 25 m and 1 km depending on bathymetry and geometry within the estuarine system. The model was forced with tides and winds during the primary periods that oyster larvae are dispersing and gag postlarvae are ingressing in Pamlico Sound (May to Oct.). We assumed that wind fields were spatially uniform over the entire CAPES domain (Eggleston et al. 2010), and therefore, used hourly wind velocities measured at the Cape Hatteras Meteorological Station. Tidal forcings included the K_1, O_1, M_2, S_2, and N_2 tidal constituents. Wind and tidally forced current velocities were computed over 7 variable-depth layers (i.e., 3D) and output at hourly intervals. A 10 day initiation was applied to wind and tidal forcing to allow time for the model to "spin-up" before particle-tracking simulations were run (see below). Baroclinic forcing was ignored due to the well-mixed water column typical of Pamlico Sound (Reyns et al. 2006, 2007; Haase et al. 2012).

Particle-tracking and behavioral models—A particle-tracking model designed to predict the movement of particles based on advection and programed organismal behavior were coupled to ADCIRC to predict dispersal and settlement of oyster larvae and gag postlarvae. Oyster larvae were programmed to be passive surface drifters based on a sensitivity analysis suggesting that oyster larval dispersal in Pamlico Sound is predominately dependent on when and where larvae are released from as opposed to various forms of larval behavior (Puckett et al. in review). Gag postlarvae were programmed to ingress during night-time flood tides only, which is a common behavioral strategy employed by many larval and early juvenile finfish and crustacean species to promote ingress and up-estuary transport (Forward and Tankersley 2001). During day and/or ebb tides, postlarvae were assumed to maintain their position near-bottom. During night-time flood

tides, postlarvae were assumed to ascend to the surface and disperse in the direction of currents until the end of the flood tide or sunrise.

Simulations—We ran 2 separate oyster larval dispersal simulations during May and July of 2006-2008 to simulate periods of primary (June) and secondary (September) peaks in oyster settlement in Pamlico Sound. In the first simulation, we released particles from the 10 existing oyster sanctuaries in Pamlico Sound (e.g., Puckett and Eggleston 2012) and tracked their dispersal over a 21 day period, which is the typical pelagic larval duration of oyster larvae during summer (North et al. 2008). The number of particles released from the sanctuaries was based on empirically estimated fecundity at 6 sanctuaries (Mroch et al. 2012) and interpolated fecundity at the remaining 4 sanctuaries. In all, the dispersal and fate of 9,240,000 larvae were simulated over the 3 year period. The map of particle locations after the 21 day period was used to create the *oyster larval settlement GIS layer* (see 4 below). In the second simulation, we were interested in identifying the natal location of oyster larvae that settled within the oyster sanctuaries (i.e., locations that act as larval sources for sanctuaries). To address this question, we again released 9,240,000 particles over the three year period from the existing oyster sanctuaries, but rather than releasing the particles on day 1 as in the first simulation, we released particles on day 21 and subjected particles to reversed current velocities in direction and time. The map of particle locations at day 1 was used to create the oyster *larval source GIS layer* (see 4 below). To simulate gag postlarval ingress through inlets, we ran simulations during 7 days (centered around new moon) in early- to mid-May of 2006-2008. We released 6,300,000 particles over the three year period. The same number of particles was released from each of the three inlets. Particles were distributed evenly across the width of each inlet and allowed to ingress for a 5 day period using night-time flood tide transport only. The map of particle locations at day 5 was used to create the gag larval settlement GIS layer (see 4 below).

3 and 4. Apply GIS-based analyses to exclude sites and quantify the suitability of non-excluded sites using threshold layers

Using GIS technology (ESRI ArcGIS 9.0), we created a grid covering the waters of Pamlico Sound, where each of the 5,987 grid cells were 1km x 1km (1km^2; Figure 1). The GIS-created grid matched the spatial extent of the hydrodynamic and particle tracking grid used to simulate oyster larval dispersal and gag postlarval ingress. We assembled 19 GIS layers (Table

1) relevant to biological, physical, and socioeconomic considerations of habitat enhancement: (1) bathymetry, (2) substrate type, (3) submerged aquatic vegetation (SAV), (4) nursery areas, (5) leased bottoms, (6) navigational channels, (7) military zones, (8) salinity, (9) oyster larval settlement (see 2 above), (10) oyster larval source (see 2 above), (11) gag postlarval settlement (see 2 above), (12) location of historic oyster reefs, (13) location of oyster cultch-planting sites, (14) material stock pile sites, (15) national pollutant discharge elimination system (NPDES), (16) boat ramps (access by recreational fishermen), (17) location of natural oyster reefs, (18) location of existing artificial reefs, and (19) location of existing oyster sanctuaries. Each of these layers was projected onto the grid of Pamlico Sound such that each cell contained a "value" for each layer. Layers were partitioned into two categories: (1) "exclusion" layers—binary, 0 or 1 layers used to exclude unsuitable sites, and (2) "threshold" layers—layers assigned thresholds and weights to quantify the suitability value of non-excluded cells (Table 1). For example, bathymetry was an exclusion layer because a grid cell was either of sufficient depth (for safe navigational clearance) or not. In contrast, salinity was a threshold layer whereby cells were divided into optimal, suitable, and unsuitable thresholds. In the case of salinity, cells with a long-term average salinity from 10-15 (for oysters; gag salinity thresholds were different) were considered optimal and scored as a 1, cells with salinity from 5-10 or 15-25 were considered suitable and scored a 0.5, and cells with a salinity <5 or >25 were considered unsuitable and scored a 0. The relative importance of each threshold layer to siting oyster sanctuaries, artificial reefs, or both was quantified by assigning a weight to each layer (higher weight = more important). We convened an expert panel consisting of academics (NCSU/CMAST), non-profits (NC Coastal Federation and the Nature Conservancy), and state employees (NC DMF) to determine layer thresholds and weights.

Exclusion layers—Exclusion layers were binary and used to exclude non-suitable cells based on the following criteria for each layer:

1) Bathymetry: excluded if the depth of an entire $1km^2$ cell was < 2m, which is required for navigational clearance (Figure 2)
2) Substrate: excluded if the substrate within a cell was NOT classified as "hard" or "firm without vegetation", which is necessary to prevent hard substrate used for habitat enhancement from sinking. Cells that were not mapped in the NC DMF shellfish mapping program were not excluded (Figure 3).

3) Submerged Aquatic Vegetation (SAV): excluded if a cell contained SAV because habitat enhancement is not permitted in the presence of SAV (Figure 4).
4) Nursery Areas: excluded if a cell contained primary or special secondary nursery areas because habitat enhancement is not permitted in the presence of nursery areas (Figure 5).
5) Shellfish Leased Areas: excluded if a cell contained leased area to prevent user conflicts (Figure 6).
6) Navigational Channels: excluded if a cell contained the Intracoastal Waterway to prevent navigational hazards to large vessels. We did not exclude cells on the basis of trawling lanes because nearly all cells without SAV were accessible to trawlers (Figure 7).
7) Military zones: excluded if a cell contained military protect area because habitat enhancement is not permitted in military zones (Figure 8).

Threshold layers—Threshold layers were scored from optimal to unsuitable and weighted based on the layer's relative importance to habitat enhancement. Layer-specific thresholds and weights are provided in Table 1.

1) Salinity: salinity thresholds for oyster sanctuaries and artificial reefs were based on Kennedy (1996; Figure 9) and Casey et al. (2010; Figure 10), respectively. Salinity was weighted relatively high due to its importance to biological processes (e.g., larval survival, growth, and predation) in estuarine environments.
2) Oyster Larval Settlement: cells were scored on a continuous scale from 1, where cell(s) contained the greatest number of oyster larval settlers over all simulations, to 0, where cells contained no oyster larval settlers over all simulations (Figure 11). Oyster larval settlement from existing sanctuaries was weighted relatively high due to the desire for new oyster sanctuaries to maximize settlement and provide larval connections with existing sanctuaries. This layer was not used for site selection of artificial reefs.
3) Oyster Larval Source: cells were scored on a continuous scale from 1, where the natal origin of oyster larvae settling within oyster sanctuaries was greatest, to 0, where the natal origin of oyster larvae settling with sanctuaries was 0 over all simulations (Figure 12). The natal location of oyster larvae settling within existing

sanctuaries was weighted relatively high due to the desire for new oyster sanctuaries to provide larval subsidies to existing sanctuaries. This layer was not used for site selection of artificial reefs.

4) Gag Postlarval Settlement: cells were scored on a continuous scale from 1, where cell(s) contained the greatest number of postlarval gag settlers over all simulations, to 0, where cells contained no postlarval gag settlers over all simulations (Figure 13). Gag postlarval settlement from inlets was weighted relatively high due to the desire for new artificial reefs to maximize recruitment. This layer was not used for site selection of oyster sanctuaries.
5) Historic Oyster Reefs: thresholds for historic oyster reefs for siting oyster sanctuaries were based on the notion that several existing sanctuaries have been sited on historic reefs (Figure 14). This layer was not used for site selection of artificial reefs.
6) Oyster Cultch-Planting Sites: thresholds for cultch-planting sites for siting oyster sanctuaries and artificial reefs were based on buffer distances around cultch-planting sites similar in magnitude to dispersal distances of oyster larvae or ingressing gag postlarvae (Figure 15 and 16). The location of cultch-planting sites was weighted relatively low for site selection of both oyster sanctuaries and artificial reefs, but was included to select sites for habitat enhancement that provide and receive biological contributions to existing restoration practices.
7) Material Stockpile Sites: thresholds for material stockpile sites were based on buffer distances of the vessels at each site, as well as the amount of material vessels were capable of transporting per load (Figure 17). The location of material stockpile sites was weighted relatively low for site selection of both oyster sanctuaries and artificial reefs, but was included due to the economic and logistical constraints associated with transporting large amounts of hard substrate throughout Pamlico Sound.
8) National Pollutant Discharge Elimination System (NPDES): thresholds for material stockpile sites were based on whether cells contained NPDES (Figure 18). The location of NPDES sites was weighted relatively low for site selection of

both oyster sanctuaries and artificial reefs, but was included due to the desire to locate habitat enhancement in areas not directly negatively impacted by pollution.

9) Boat Ramps: thresholds for material stockpile sites were based on buffer distances of 10 nautical miles, which is the average travel distance reported for inshore recreational vessels by Ramos et al. (2006; Figure 19). The location of boat ramps was weighted relatively low for site selection of both oyster sanctuaries and artificial reefs, but was included because one goal of habitat enhancement is to provide recreational fishing opportunities.
10) Natural Oyster Reefs: thresholds for natural oyster reefs for siting oyster sanctuaries and artificial reefs were based on buffer distances around natural reefs similar in magnitude to dispersal distances of oyster larvae or ingressing gag postlarvae (Figure 20 and 21). The location of natural oyster reefs was weighted relatively low for site selection of both oyster sanctuaries and artificial reefs, but was included to select sites for habitat enhancement that provide and receive biological contributions to existing oyster reefs.
11) Artificial Reefs: thresholds for existing artificial reefs for siting oyster sanctuaries and artificial reefs were based on buffer distances around artificial reefs similar in magnitude to dispersal distances of oyster larvae or ingressing gag postlarvae (Figure 22 and 23). The location of artificial reefs was weighted relatively low for site selection of both oyster sanctuaries and artificial reefs, but was included to select sites for habitat enhancement that provided and received biological contributions to existing artificial reefs.
12) Oyster Sanctuaries: thresholds for existing oyster sanctuaries for siting artificial reefs were based on buffer distances around oyster sanctuaries similar in magnitude to dispersal distances of gag postlarvae (Figure 24). This layer was not included in site selection for oyster sanctuaries because it was considered redundant with the Oyster Larval Settlement and Larval Source layers above. The location of oyster sanctuaries was weighted relatively low for site selection of artificial reefs, but was included to select artificial reefs that provided and received biological contributions to existing oyster sanctuaries.

Suitability and site selection— Using the GIS-raster calculator, the suitability of each cell (S_j) for siting oyster sanctuaries and artificial reefs was calculated in a two-step process as:

$$C_j = \sum_{x=1}^{12} (L_{xj} \cdot W_x)$$

$$S_j = C_j \cdot E_j ,$$

where C_j is the cumulative value of cell j calculated as the product of the score of cell j in threshold layer x and the weight of layer x summed across all 12 threshold layers, and E_j is the binary (0 or 1) score for cell j based on all 7 exclusion layers. On a scale of 0 to 1, cell suitability scores for oyster sanctuaries and artificial reefs were ranked from highest (most suitable) to lowest (least suitable). The most suitable sites were validated with empirical field measurements (see below). In addition to the enhancement-specific discrepancies for oyster sanctuaries and artificial reefs, we also averaged suitability scores for each cell for each enhancement type to generate combined suitability scores in an effort to identify optimal locations for habitat enhancement in general.

5 and 6. Field verification of water quality and substrate at optimal sites selected from 3 and 4 with subsequent removal of sites that did not meet minimal standards

To empirically validate the suitability of sites selected in 3 and 4 above, we conducted field observations of the top four oyster sanctuary sites during August-October 2012 to assess water quality and substrate characteristics. Visual surveys by divers at each site were used to assess the presence/absence of SAV or oyster reef substrate and to determine the firmness of sediment. Divers also moored YSI-6600 water quality instruments equipped with optical DO probes at each of the four locations with continuous monitoring over the 3 month period. Following field verification, sites that did not meet minimal water quality standards (e.g., extended periods of anoxia, muddy substrate, presences of SAV or oyster reef) were removed from analyses and the remaining sites re-ranked.

Results

1. Statistically analyze long-term salinity data to produce sound-wide salinity GIS layer

The final process-based spatial statistical model using observational data collected by NC DMF Program 195 to map the distribution of salinity throughout Pamlico Sound and its

tributaries explained 89% of the spatiotemporal variability in salinity in a withheld dataset. Low standard errors in time periods for which freshwater influx was the main explanatory variable for salinity indicated that these conditions were well represented by the mean function. In contrast, high standard errors during time periods with the passage of hurricanes illustrated the complex interplay of freshwater influx, hurricane storm surge, and/or other wind-forcing events on mean salinity. The process-based model was used to map spatiotemporal variation in salinity across 20 years of variable hydrologic and climatic conditions in Pamlico Sound, which identified system-state (e.g., prolonged drought or flood period) as an important determinant of system-wide salinity response to freshwater influx. The salinity map generated during 1995, which was an "average" year relative to the 20 year period investigated, was used for site selection (see 3 and 4 below; Figure 25). The statistical modeling of salinity was part of a MS thesis at NC State University (C. Durham 2009). For those interested, this thesis can be accessed on-line and contains a detailed description of the statistical modeling and associated SAS computer programming code in the Appendix (http://repository.lib.ncsu.edu/ir/handle/1840.16/1/browse?type=author&order=ASC&rpp=20&value=Durham%2C+Christina+Louise).

2. Hydrodynamic and particle-dispersal modeling to provide a larval connectivity GIS-layer

Oyster larval dispersal from oyster sanctuaries in Pamlico Sound was generally directed from southwest to northeast in alignment with the prevailing southwesterly winds during the summer oyster spawning season. The distance larvae dispersed from sanctuaries was dependent on the location of the sanctuary and the date during which larvae were released. On average, oyster larvae dispersed distances of ~15km from their natal sanctuary over a 21 day period (Figure 11). Predicted larval settlement from sanctuaries was concentrated along the southwestern and northern portions of Pamlico Sound with little settlement predicted to occur in the middle of Pamlico Sound. The natal location of oyster larvae predicted to settle within sanctuaries was concentrated in the southwestern and northern portions of Pamlico Sound with very little contribution coming from the Outer Banks region (Figure 12).

Gag postlarvae ingressing through inlets on night time flood tides around the new moon dispersed ~5km, on average, over a 5 day period (Figure 13). Predicted settlement patterns formed a plume on the sound-side of Ocracoke and Hatteras inlets. Settlement of postlarvae

ingressing through Oregon inlet was predicted to occur south of the inlet. The hydrodynamic and particle dispersal modeling were components of a Ph.D. dissertation (Puckett 2013) and a manuscript that is currently under review (Puckett et al. in review).

3 and 4. Apply GIS-based analyses to exclude sites and quantify the suitability of non-excluded sites using threshold layers

Of the 5,987 km^2 grid cells in Pamlico Sound (Figure 1), ~39% within Pamlico Sound (2,348 cells) were unsuitable for habitat enhancement based on the 7 exclusion layers (Figure 26). Unsuitable cells were concentrated near shore due to water depth limitations and along the Outer Banks due to the presence of SAV.

Oyster sanctuaries—The optimal location of oyster sanctuaries among non-excluded cells was primarily driven by three threshold layers: salinity, oyster larval settlement, and oyster larval source that had a combined weight of 73% (Table 1). Overlap between optimal salinity thresholds, the locations of peak larval settlement, and larval sources occurred in the southwestern (Neuse and Pamlico Rivers) and northern (Roanoke Island) portions of Pamlico Sound where site suitability for oyster sanctuaries was predicted to be highest (Figure 27; see Figures 9, 11, and 12 for salinity, settlement, and source layers). Of the non-excluded cells, suitability scores for oyster sanctuaries on a scale from 0 to 1 ranged from 0.12 (least suitable) to 0.79 (most suitable); modal suitability was ~0.35 (Figure 28). Suitability scores for the top 50 and top 14 sites clustered in the SW and N portion of Pamlico Sound were > 0.73 and > 0.77, respectively (Figure 29; Appendix 1).

Artificial reefs—The optimal location of artificial reefs (using gag grouper as the focal species) among non-excluded cells was primarily driven by two threshold layers: salinity and postlarval gag settlement, that had a combined weight of 67.5% (Table 1). Location of material stockpile sites and boat ramps was weighted more heavily for siting artificial reefs than for siting oyster sanctuaries. Overlap between optimal salinity thresholds and locations of peak larval settlement occurred near the inlets and along the Outer Banks region of Pamlico Sound where site suitability for artificial reefs was predicted to be highest, despite somewhat long distances from public boat ramps and material stockpile sites (Figure 30; see Figures 10, 13, 17, and 19 for salinity, settlement, boat ramp and stockpile site layers). Of the non-excluded cells, suitability scores for artificial reefs on a scale of 0 to 1 ranged from 0.03 (least suitable) to 0.85 (most

suitable); modal suitability was ~0.2 (Figure 31). Suitability scores for the top 50 and top 14 sites clustered near the inlets of Pamlico Sound and were > 0.51 and > 0.59, respectively (Figure 32; Appendix 2).

Combined: oyster sanctuaries and artificial reefs—The optimal location of habitat enhancement to provide benefits associated with both oyster sanctuaries and artificial reefs was concentrated in and around the Neuse and Pamlico Rivers, near Ocracoke and Hatteras inlets, and near Roanoke Island (Figure 33). Of the non-excluded cells, suitability scores for habitat enhancement on a scale of 0 to 1 ranged from 0.12 (least suitable) to 0.62 (most suitable); modal suitability was ~0.35 (Figure 34). Suitability scores for the top 50 and top 14 sites in Pamlico Sound clustered in the lower Neuse River, near Roanoke Island, and near Ocracoke inlet were > 0.49 and > 0.52, respectively (Figure 35; Appendix 3).

5 and 6. Field verification of water quality and substrate at optimal sites selected from 3 and 4 with subsequent removal of sites that did not meet minimal standards

Visual surveys by divers at each site confirmed the absence of essential fish habitat in the form of either SAV or oyster reef substrates. The substrate and water quality characteristics of the optimal four sites for oyster sanctuaries are provided below, and illustrate the importance of ground-truthing modeling predictions.

1) Southern Roanoke Island (RI; Coordinates: 35.86434N;75.70082W; Figure 29): Salinity at RI was variable, but generally near 15 psu, which is optimal for oysters (Figure 36a). Unlike the other three sites, dissolved oxygen at RI was rarely hypoxic (<2 mg/L) and never anoxic (0 mg/L) during the observational period (Figure 36b). The sediment was characterized as muddy sand and, when combined with generally normoxic conditions, **appears to be a good candidate for construction of an oyster sanctuary.**

2) Lower Neuse River Estuary (NR; Coordinates: 34.98521N; 76.67873W; Figure 29). Salinity at NR was between 16-19 psu, which is suitable for oysters (Figure 36a). Dissolved oxygen was problematic at NR. For example, there was a prolonged (10-14 day) anoxic and hypoxic upwelling event during field observations (Figure 36b). Given the intensity of hypoxic upwelling

and the sometimes prolonged duration of these events, combined with relatively muddy substrate, **this site was deemed unacceptable for oyster sanctuary construction**.

3) Neuse River Estuary Mouth (NRm; Coordinates: 35.02812N; 76.56788W; Figure 29): Salinity at NRm was 24-27 psu during the majority of the observational period, which is nearly unsuitable (due to disease and predation) for oysters (Figure 36a). Dissolved oxygen at NRm was similar to that observed at NR. For example, there was a prolonged (10-14 day) anoxic and hypoxic upwelling event during field observations (Figure 36b). Given the intensity of hypoxic upwelling and the sometimes prolonged duration of these events, combined with relatively muddy substrate, **this site was deemed unacceptable for oyster sanctuary construction**.

4) Pamlico River Mouth (PRm; Coordinates: 35.34585N; 76.45502W; Figure 29): Average salinity at PRm was suitable for oysters at 17 psu (Figure 36a). Dissolved oxygen was hypoxic for several consecutive days during September, but not unsuitable for oyster tolerances to prolonged hypoxia (Figure 36b). **The organic-rich, muddy sediment at PRm was not ideal for oyster sanctuary construction.**

Following field verification, we ranked the cluster of suitable sites nearby southern Roanoke Island as the best in terms of suitability for oyster sanctuary construction. We did not validate optimal sites for artificial reefs due to their proximity to inlets where there is high tidal flushing and relatively high DO, and the amount of soft substrate is reduced.

Conclusions

In this study, we applied a GIS-based hierarchical approach to provide scientific support for the placement of oyster sanctuaries, artificial reefs, and habitat enhancement in general. By integrating (i) statistical modeling, (ii) hydrodynamic and particle tracking modeling, and (iii) GIS-based modeling, we incorporated 19 GIS layers in the site selection process that spanned biological, socioeconomic, and logistical considerations relevant to habitat enhancement. In doing so, we were able to narrow a vast water body down to a manageable number of sites for more detailed study (e.g., field verification). Moreover, we identified habitat enhancement “hot spots” where the most suitable cells for habitat enhancement were clustered. The GIS-based

decision support tool for site selection developed herein should be effective in designing enhancement strategies to aid in applied management decisions. To this end, the results from this work are currently being integrated into the NC Division of Marine Fisheries future habitat enhancement plans, as well as U.S. Army Corps of Engineer oyster sanctuary construction plans as part of a mitigation project.

Caveats regarding model predictions—The modeling approach used in this study was extensive and required highly specialized skills in a number of technical disciplines, but it is worth noting that several important processes relevant to site selection and the success of habitat enhancement were not included. For instance, we were unable to include highly dynamic ecological processes and rates, such as maps of dissolved oxygen, predictions of food availability (e.g., plankton for oysters), or post-settlement processes (e.g., growth or survival). To overcome this shortcoming, we conducted field verification. Adapting the framework used herein to include more dynamic processes and variability within processes (e.g., salinity) should be the focus of future revisions to the model. We also focused our modeling efforts on a single species for each specific enhancement type. While each species is certainly important for the respective enhancement type, the success of habitat enhancement will depend on the development of a multi-species community. As the number of species modeled increases, so too will the complexity of the model and the processes underlying its output. Conducting habitat enhancement that is simultaneously optimal for only two species, oyster and gag does not seem overly feasible due to the minimal overlap of the most suitable sites for each species. As the number of species included in the model increases, the overlap of suitable habitat enhancement sites between all species will likely be further reduced. We speculate that decisions on site selection would come down to a handful of critical species.

Data sharing—All data and progress made in relation to this study has been submitted to the NC DMF in both written hard copy and PDF format, with mandatory updates to NC DMF submitted in writing to the appropriate program manager. Moreover, the results from this study have been and will continue to be used to guide restoration plans developed by the northeast working group of the NC Oyster Steering Committee. More broadly, results from this study were presented at two regional scientific meetings: The Tidewater Chapter of the American Fisheries Society and The Southeastern Estuarine Research Society, as well as to an international working group examining linkages between habitat-specific demographic rates of fishery species and

their population dynamics (ICES Fisheries Habitat Working Group; http://www.ices.dk/community/groups/Pages/WGVHES.aspx). Moreover, we have submitted 2 peer-reviewed manuscripts (Durham et al. in preparation and Puckett et al. in review) spawned from this research and are in the preparation stage of 2 additional manuscripts.

Future work—We plan to develop a more user-friendly version of the modeling framework developed in this study by adapting the current model within a graphical user interface such that layer weights and thresholds can be altered and updated using a sliding scale for each layer. We are also in the planning stages of designing a study to place experimental reefs (small-scale) at the most suitable sites to test model validity.

In conclusion, this study builds on efforts by others to apply and refine hierarchical models for siting artificial reefs (Mathews 1985, Baynes and Szmant 1989, Duzbastilar et al. 2006, National Artificial Reef Plan 2007, Barber et al. 2009). This study is one of very few that have integrated exclusion mapping with physical and biological factors, and one of the first we are aware of that have integrated exclusion mapping and larval/postlarval connectivity to evaluate the suitability of sites for habitat enhancement. Providing a scientific basis for siting artificial reefs should enhance the likelihood of success, as well as save time and money in this process.

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Table 1. List of 19 GIS layers used in determining suitability of sites in Pamlico Sound for placement of oyster sanctuaries and artificial reefs (using Gag as focal species). Exclusion layers were binary (suitable [score = 1] or unsuitable [0]) whereas threshold layers were assigned thresholds (e.g., optimal [score = 1], suitable [0.5], and unsuitable [0]) and subsequently weighted based on the layer's relative importance. An expert panel was used to assign thresholds and weights. Layer abbreviations are as follows: Submerged Aquatic Vegetation (SAV), National Pollutant Discharge Elimination System (NPDES). Source abbreviations are as follows: National Oceanic and Atmospheric Administration (NOAA), Division of Marine Fisheries (DMF), Department of Environment and Natural Resources (DENR), United States Geological Survey (USGS), Wildlife Resource Commission (WRC)

			Thresholds		**Weight**	
Layer	**Source**	**Type**	**Oyster**	**Gag**	**Oyster**	**Gag**
Bathymetry	NOAA	Exclusion	-	-	-	-
Substrate	DMF Shellfish mapping program	Exclusion	-	-	-	-
SAV	DMF Shellfish mapping program	Exclusion	-	-	-	-
Shellfish Leases	DMF Shellfish mapping program	Exclusion	-	-	-	-
Nursery Areas	DMF	Exclusion	-	-	-	-
Military Zones	NC DENR	Exclusion	-	-	-	-
Navigational Channels	USGS	Exclusion	-	-	-	-
Salinity (psu)	Durham et al. in review	Threshold	Optimal: 10-15 Suitable: 6-10; 15-25 Unsuitable: <6; >25	Optimal: >19 Suitable: 15-19 Unsuitable: <15	30%	27.5%
Oyster Larval Settlement	This study	Threshold	Continuous (0-1)	-	25%	-
Oyster Larval Source	This study	Threshold	Continuous (0-1)	-	18%	-
Gag Postlarval Settlement	This study	Threshold	-	Continuous (0-1)	-	40%
Historic Oyster Reefs	Winslow's	Threshold	Optimal: "Solid shell"	-	5%	-

	historical data		Suitable: “Scattered shell”			
Oyster Cultch-planting Sites (km)	DMF	Threshold	Optimal: <15 Suitable: 15-20 Unsuitable: >20	Optimal: <2.5 Suitable: 2.5-5 Unsuitable: >5	5%	1%
Material Stockpile Sites (km)	DMF	Threshold	Optimal: <5 Suitable: 5-10 Unsuitable: >10	Optimal: <5 Suitable: 5-10 Unsuitable: >10	5%	10%
NPDES	NC OneMap	Threshold	Optimal: “No NPDES” Unsuitable: “NPDES”	Optimal: “No NPDES” Unsuitable: “NPDES”	4%	3%
Boat Ramps (nm)	WRC	Threshold	Optimal: <10 Unsuitable: >10	Optimal: <10 Unsuitable: >10	3%	10%
Natural Oyster Reefs (km)	DMF Shellfish mapping program	Threshold	Optimal: <15 Suitable: 15-20 Unsuitable: >20	Optimal: <2.5 Suitable: 2.5-5 Unsuitable: >5	3%	1%
Artificial Reefs (km)	DMF	Threshold	Optimal: <15 Suitable: 15-20 Unsuitable: >20	Optimal: <2.5 Suitable: 2.5-5 Unsuitable: >5	2%	5%
Oyster sanctuaries (km)	DMF	Threshold	-	Optimal: <2.5 Suitable: 2.5-5 Unsuitable: >5	-	2.5%

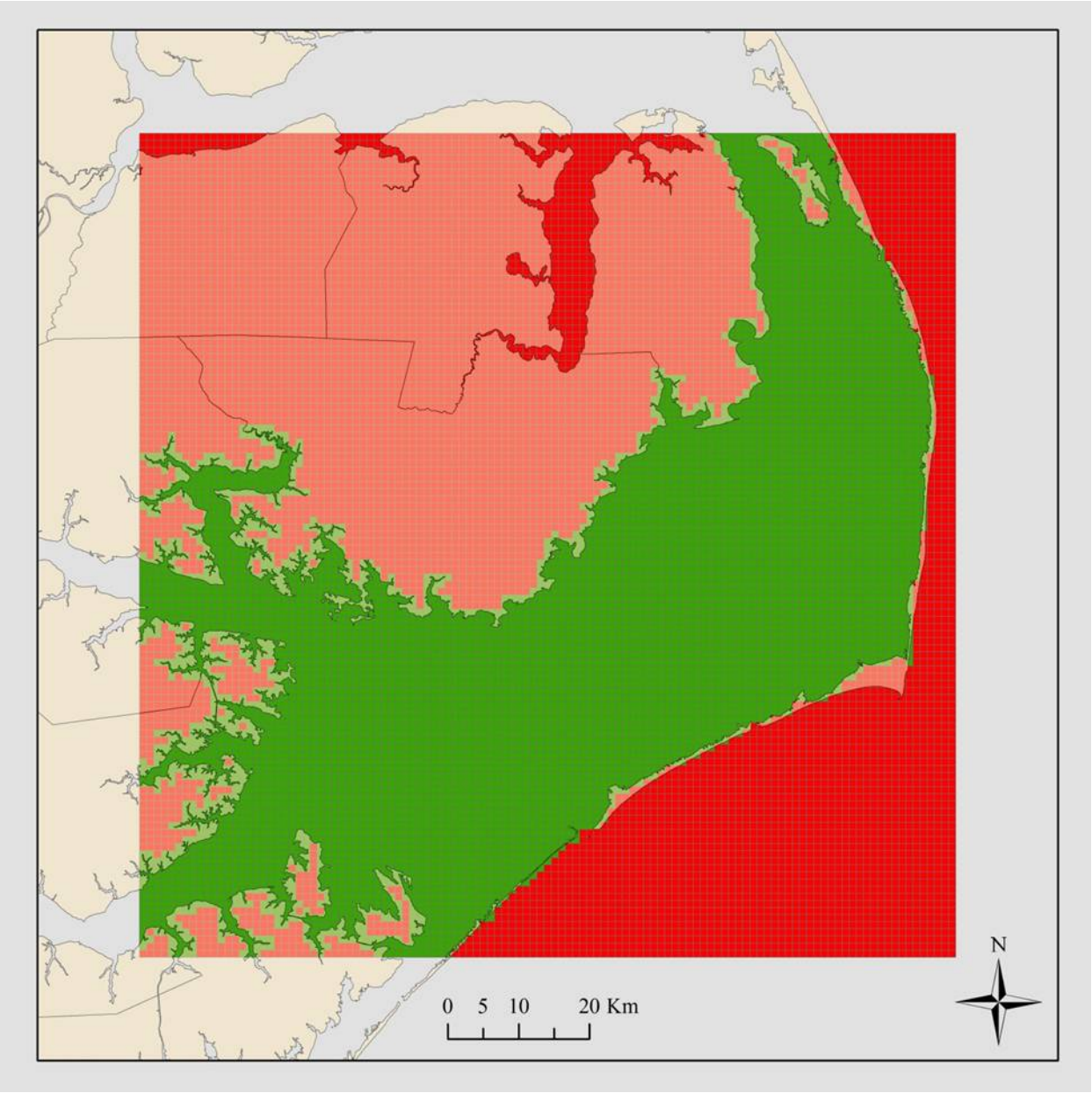

Figure 1. Grid of Pamlico Sound consisting of 5,987 km^2 cells (green).

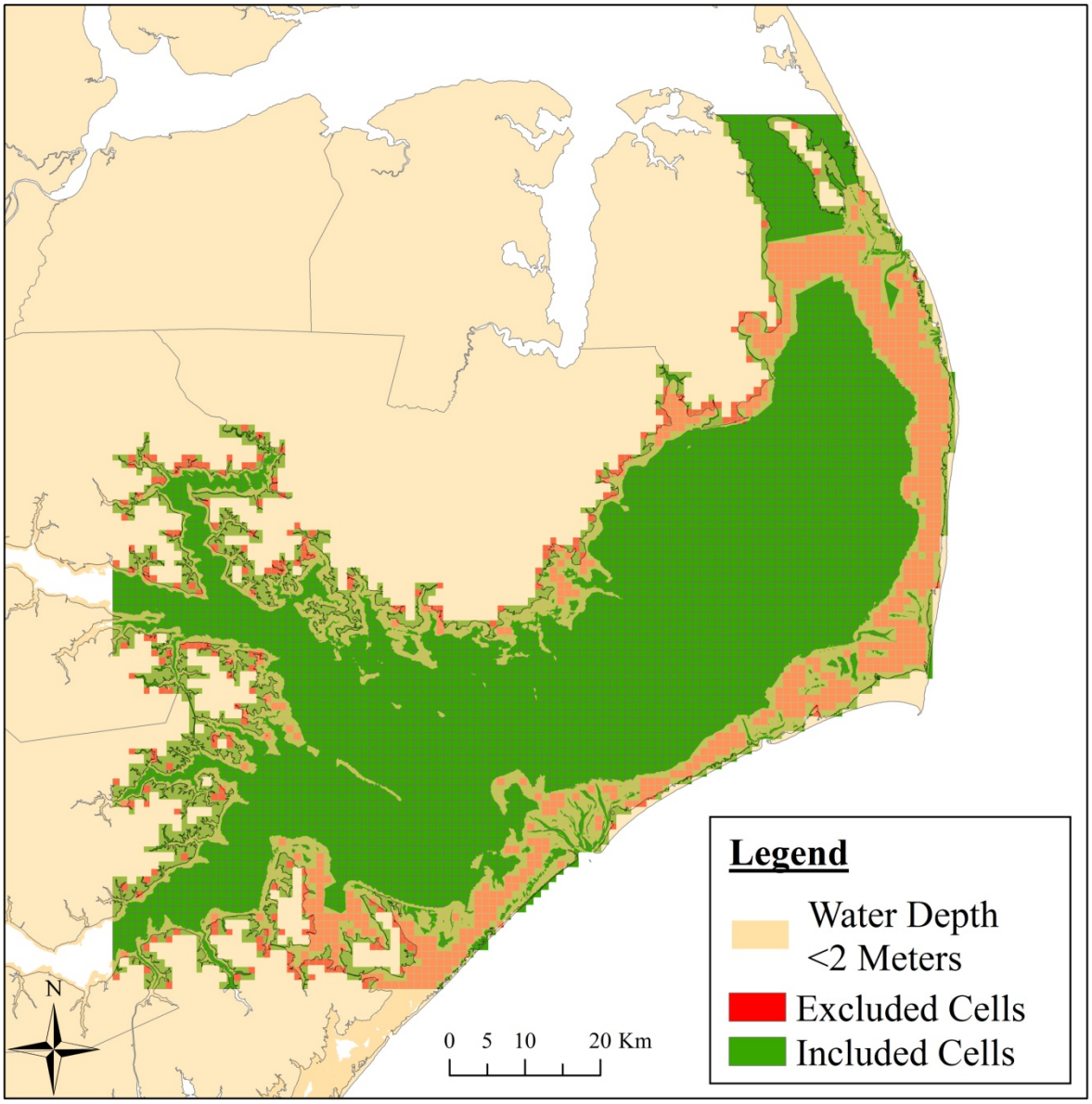

Figure 2. Pamlico Sound bathymetry exclusion layer. Depths < 2m (red) were excluded from consideration. Data from National Oceanic and Atmospheric Administration.

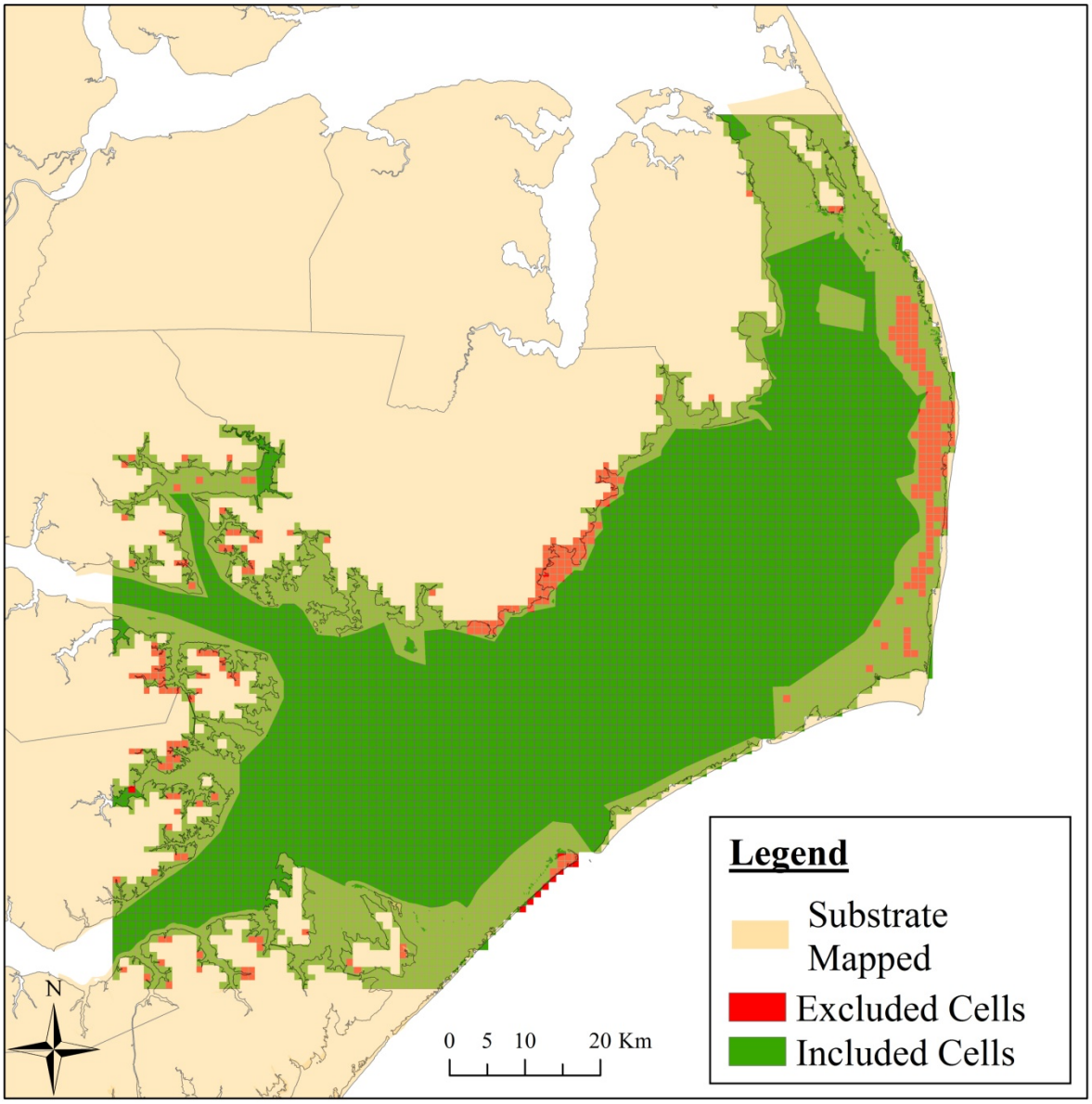

Figure 3. Pamlico Sound substrate exclusion layer. Substrate types of “not hard” or “firm with vegetation” (red) were excluded. Areas of Pamlico Sound that were not mapped for substrate type (main body of sound) were not excluded. Data from NC Division of Marine Fisheries.

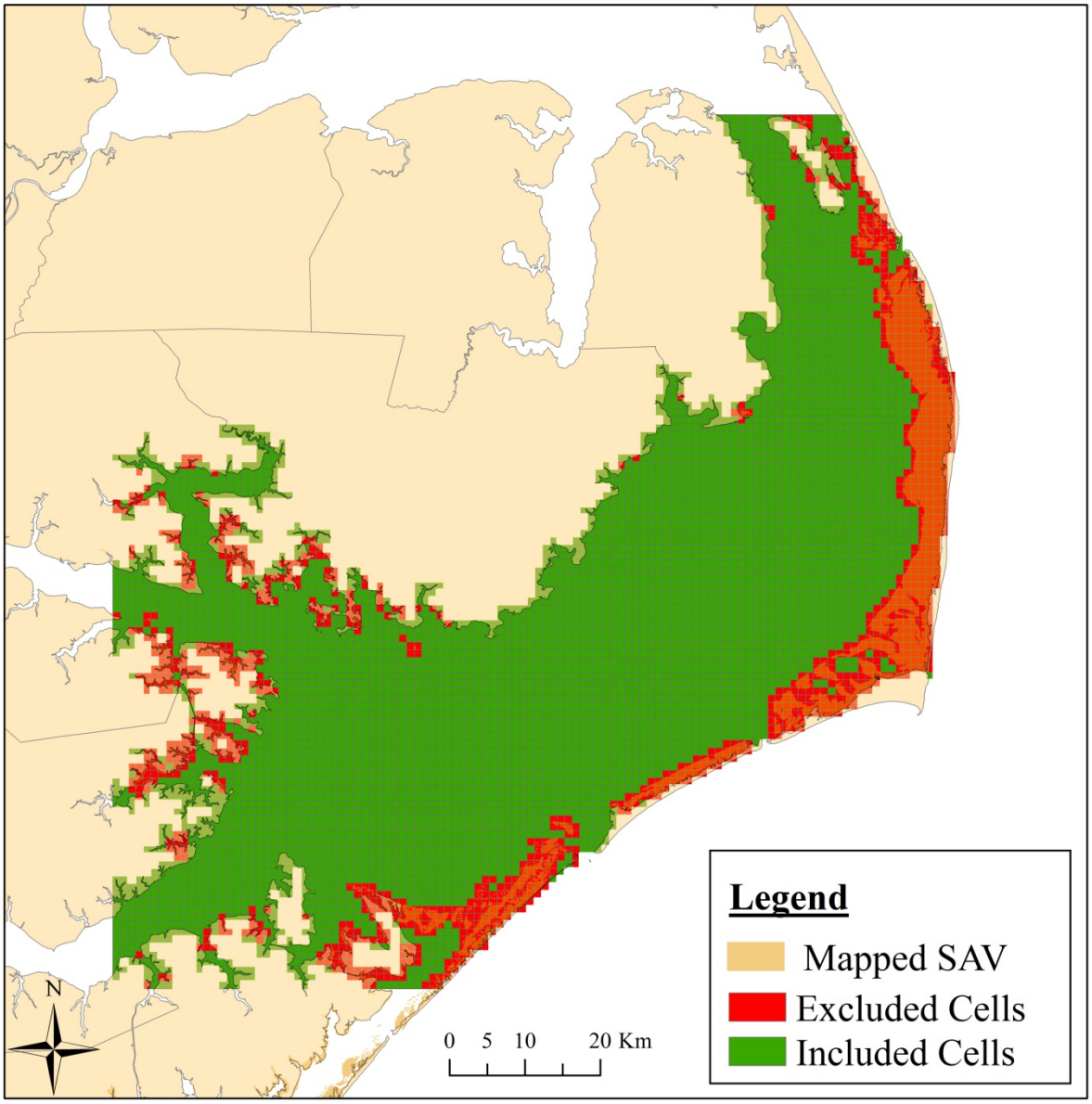

Figure 4. Pamlico Sound submerged aquatic vegetation (SAV) exclusion layer. Cells containing (SAV; red) were excluded. Data from NC Division of Marine Fisheries.

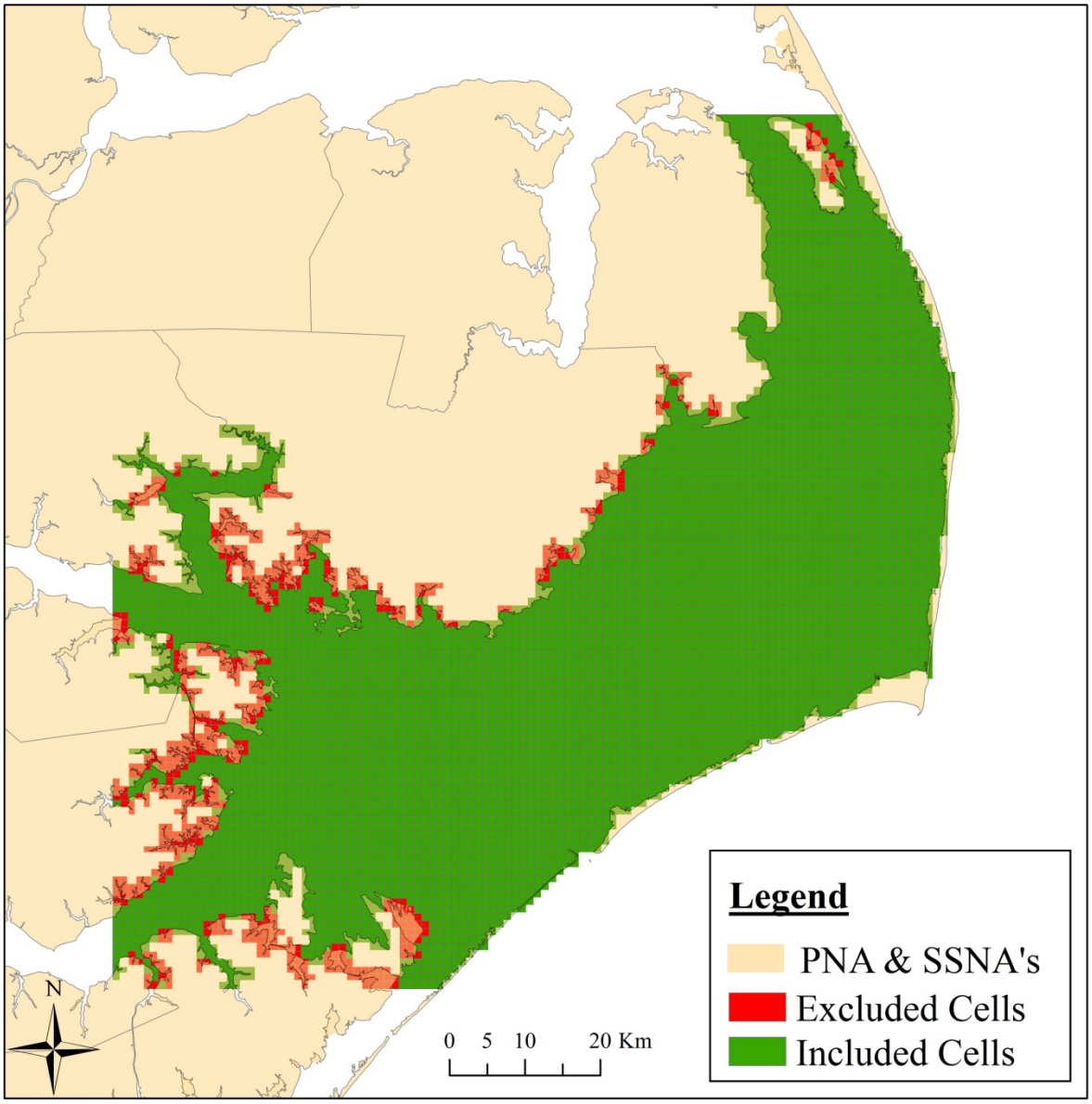

Figure 5. Pamlico Sound nursery area exclusion layer. Cells containing “primary” or “special secondary” nursery areas (red) were excluded. Data from NC Division of Marine Fisheries.

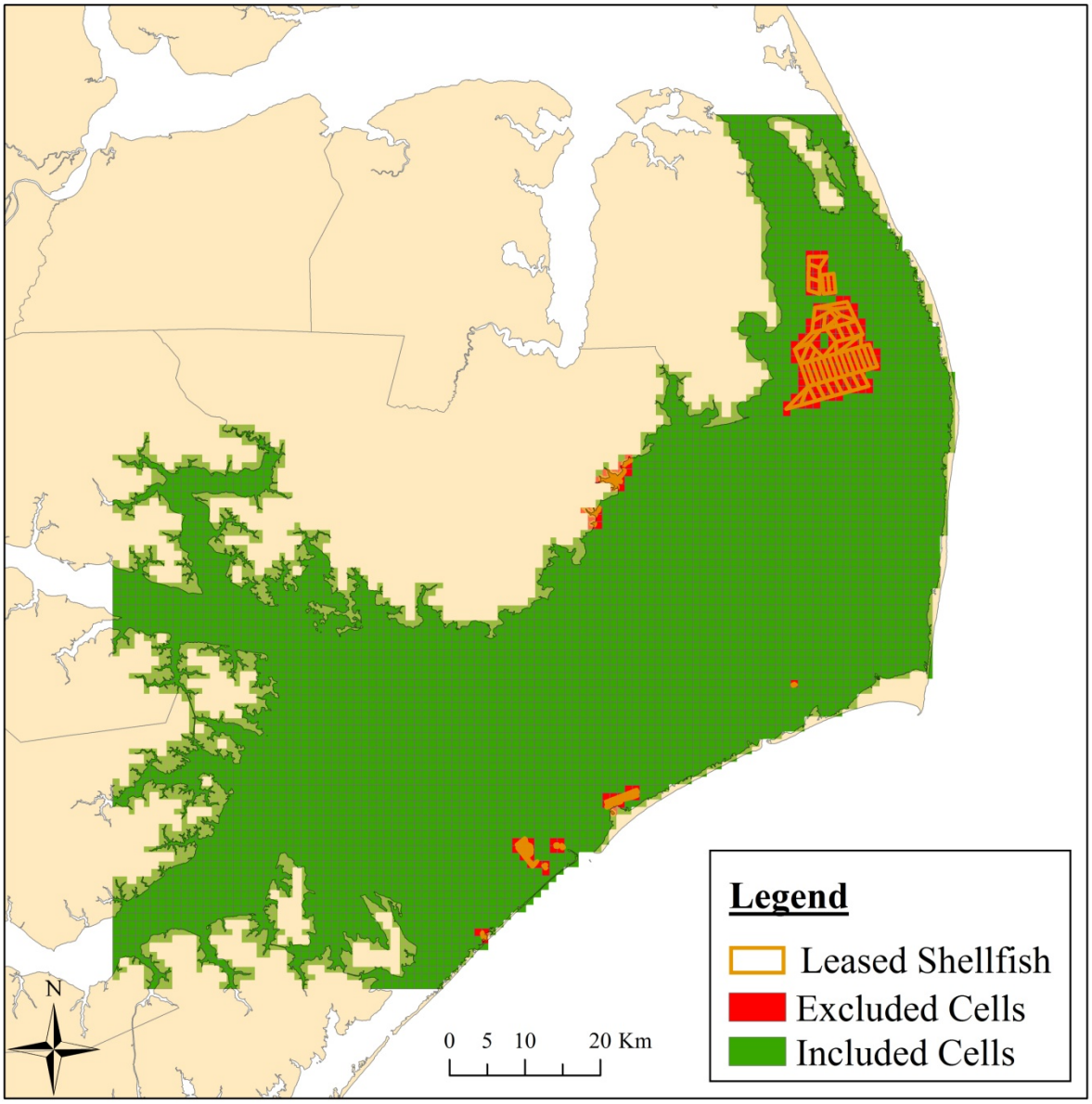

Figure 6. Pamlico Sound shellfish lease area exclusion layer. Cells containing shellfish leases (red) were excluded. Data from NC Division of Marine Fisheries.

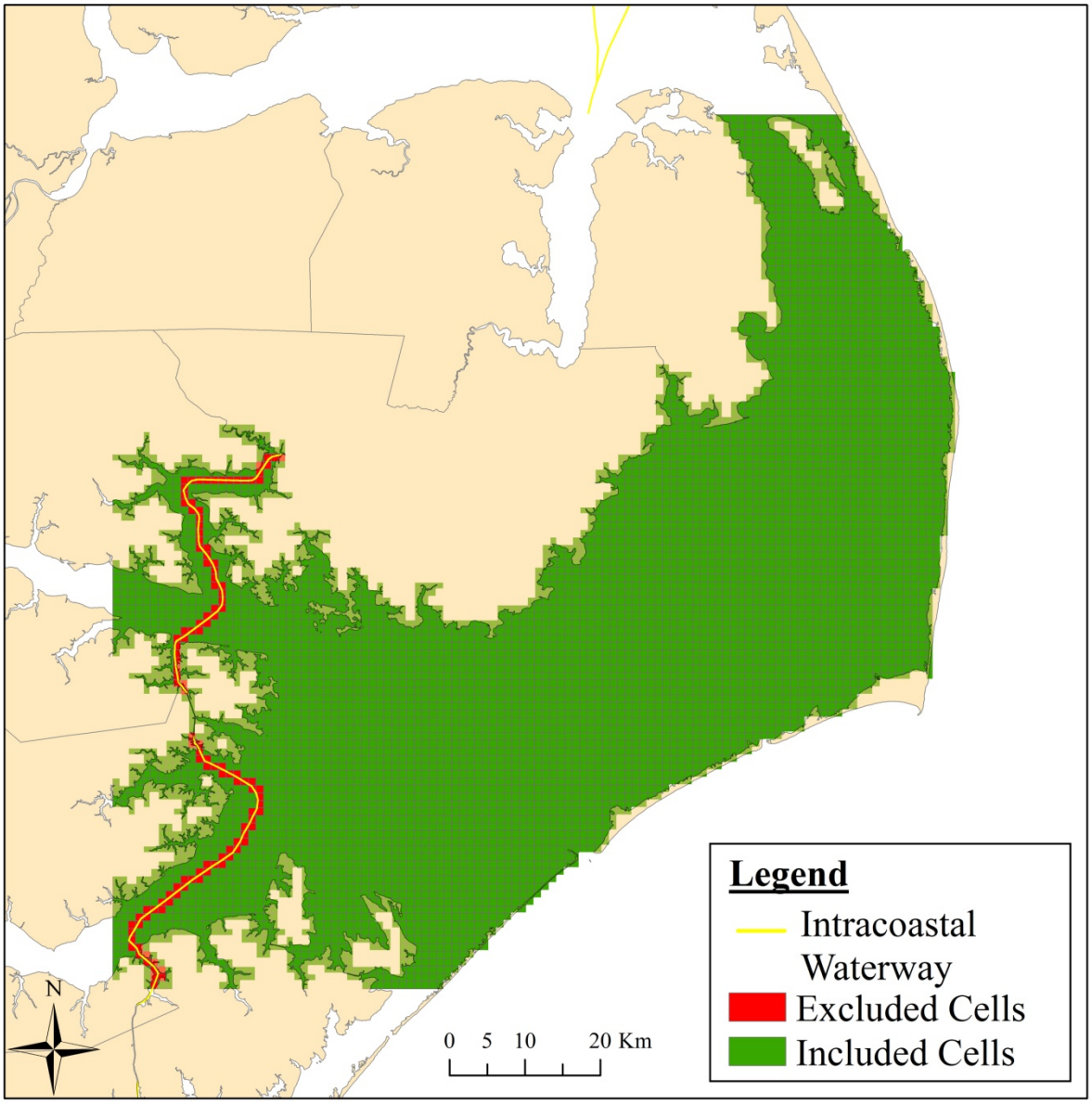

Figure 7. Pamlico Sound navigational channel exclusion layer. Cells containing intracoastal waterway (red) were excluded. Data from US Geological Survey.

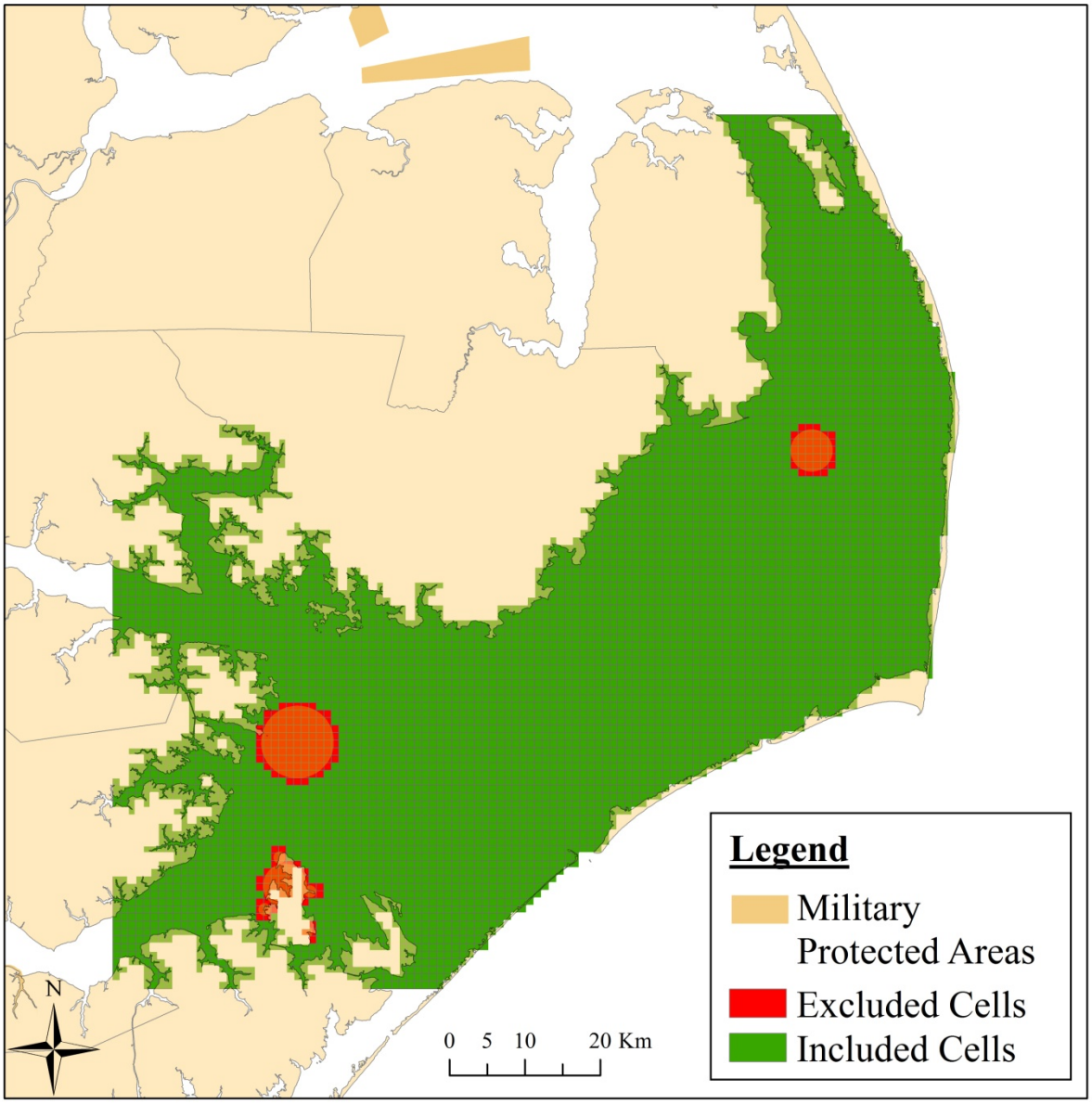

Figure 8. Pamlico Sound military restricted zones exclusion layer. Cells containing military restricted areas (red) were excluded. Data from NC Department of Environment and Natural Resources.

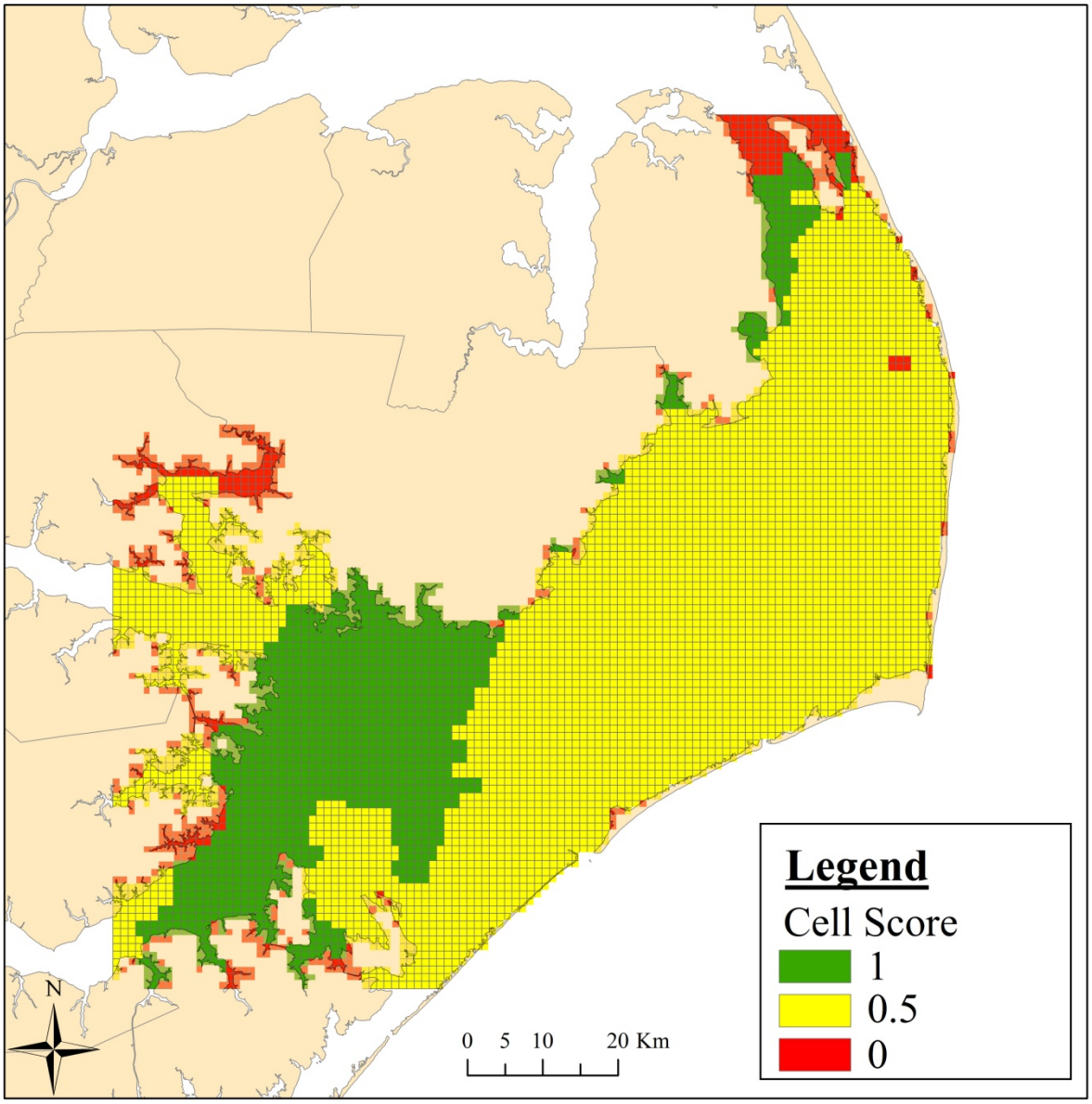

Figure 9. Pamlico Sound salinity threshold layer for oyster sanctuaries. Threshold scores were 1 for cells with average salinity between 10-15 psu, 0.5 for cells with average salinity between 6-10 and 15-25 psu, and 0 for cells with average salinity between <6 and > 25 psu. Salinity thresholds were based on Kennedy (1996). For layer weighting, see Table 1.

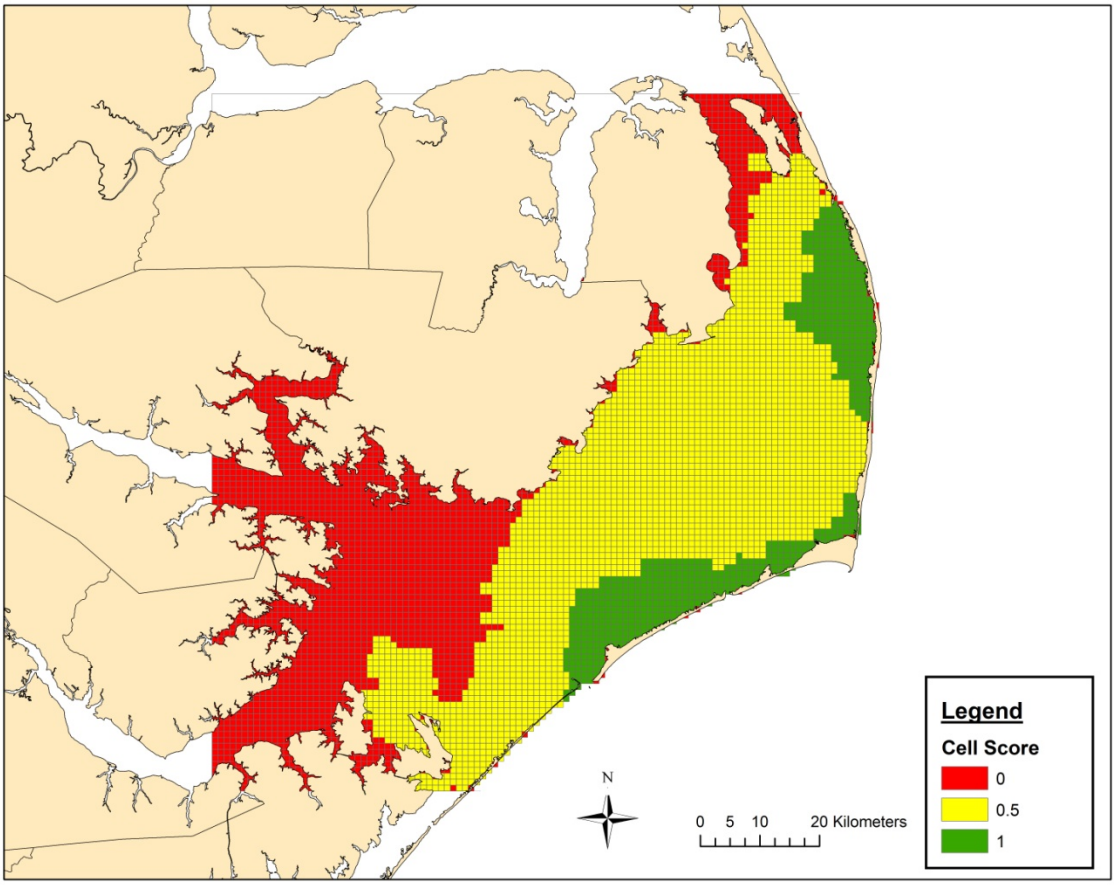

Figure 10. Pamlico Sound salinity threshold layer for artificial reefs. Threshold scores were 1 for cells with average salinity >19 psu, 0.5 for cells with average salinity between 15-19 psu, and 0 for cells with average salinity between <15 psu. Salinity thresholds were based on Casey et al. 2010. For layer weighting, see Table 1.

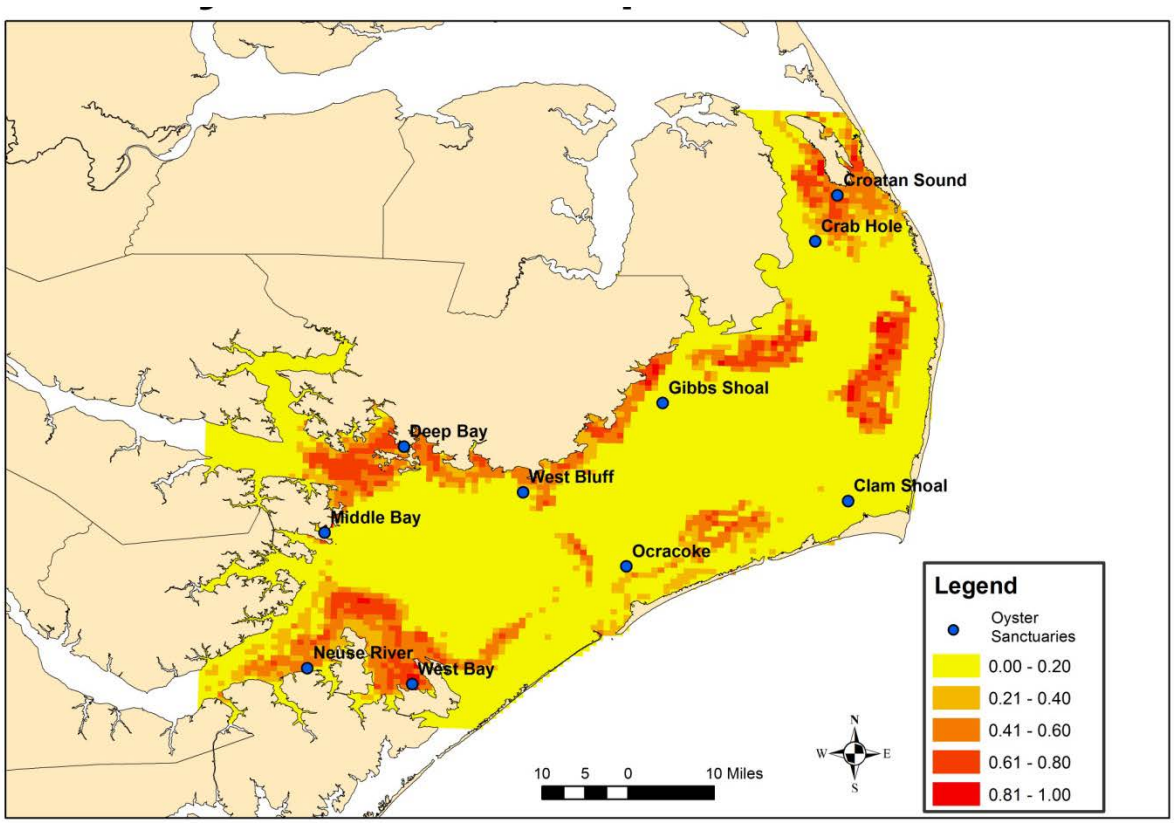

Figure 11. Pamlico Sound oyster larval settlement threshold layer for oyster sanctuaries. Cell scores were continuous from 1 (max settlement, red) to 0 (no settlement, yellow) and based on density of settlers in each cell standardized to cell with max settlement density. Existing oyster sanctuaries are labeled. For layer weighting, see Table 1.

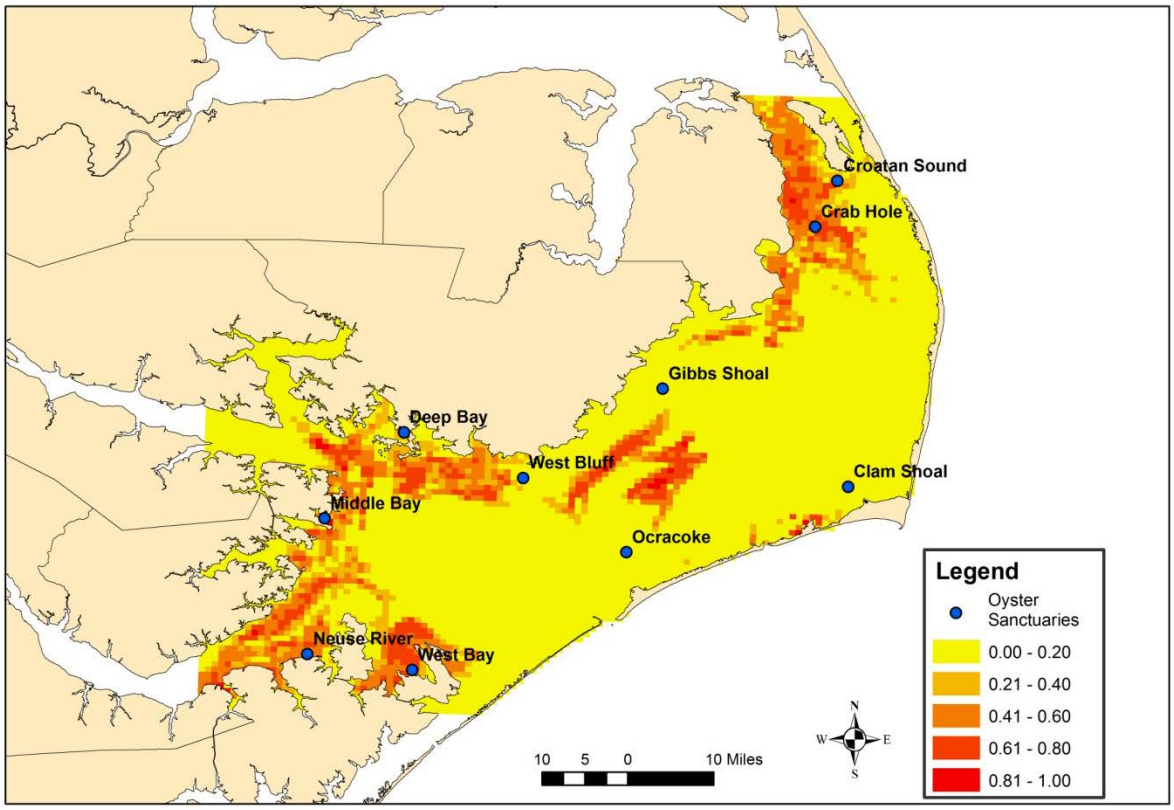

Figure 12. Pamlico Sound oyster larval source threshold layer for oyster sanctuaries. Cell scores were continuous from 1 (max source of larvae to sanctuaries, red) to 0 (no source of larvae to sanctuaries, yellow) and based on density of larvae originating from a cell that subsequently settle within existing oyster sanctuaries. Cell scores were standardized to cell with max larval source density. Existing oyster sanctuaries are labeled. For layer weighting, see Table 1.

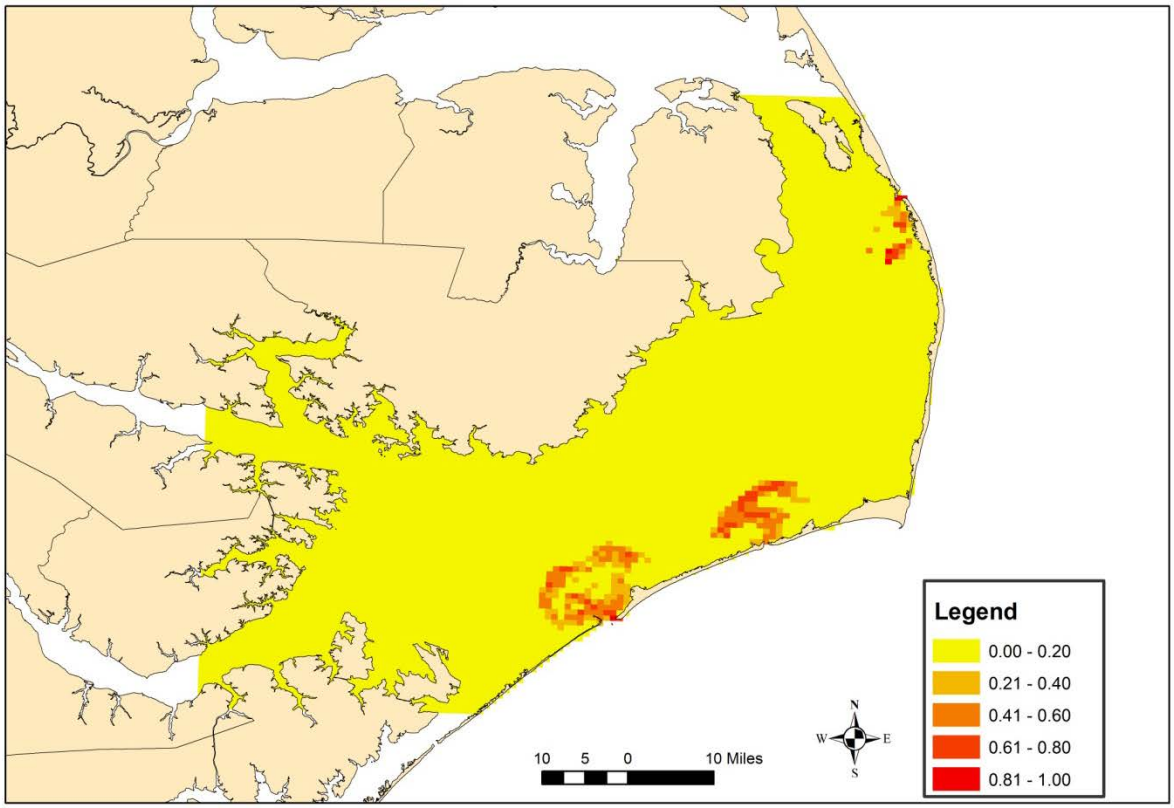

Figure 13. Pamlico Sound gag postlarval settlement threshold layer for artificial reefs. Cell scores were continuous from 1 (max settlement, red) to 0 (no settlement, yellow) and based on density of settlers in each cell standardized to cell with max settlement density. For layer weighting, see Table 1.

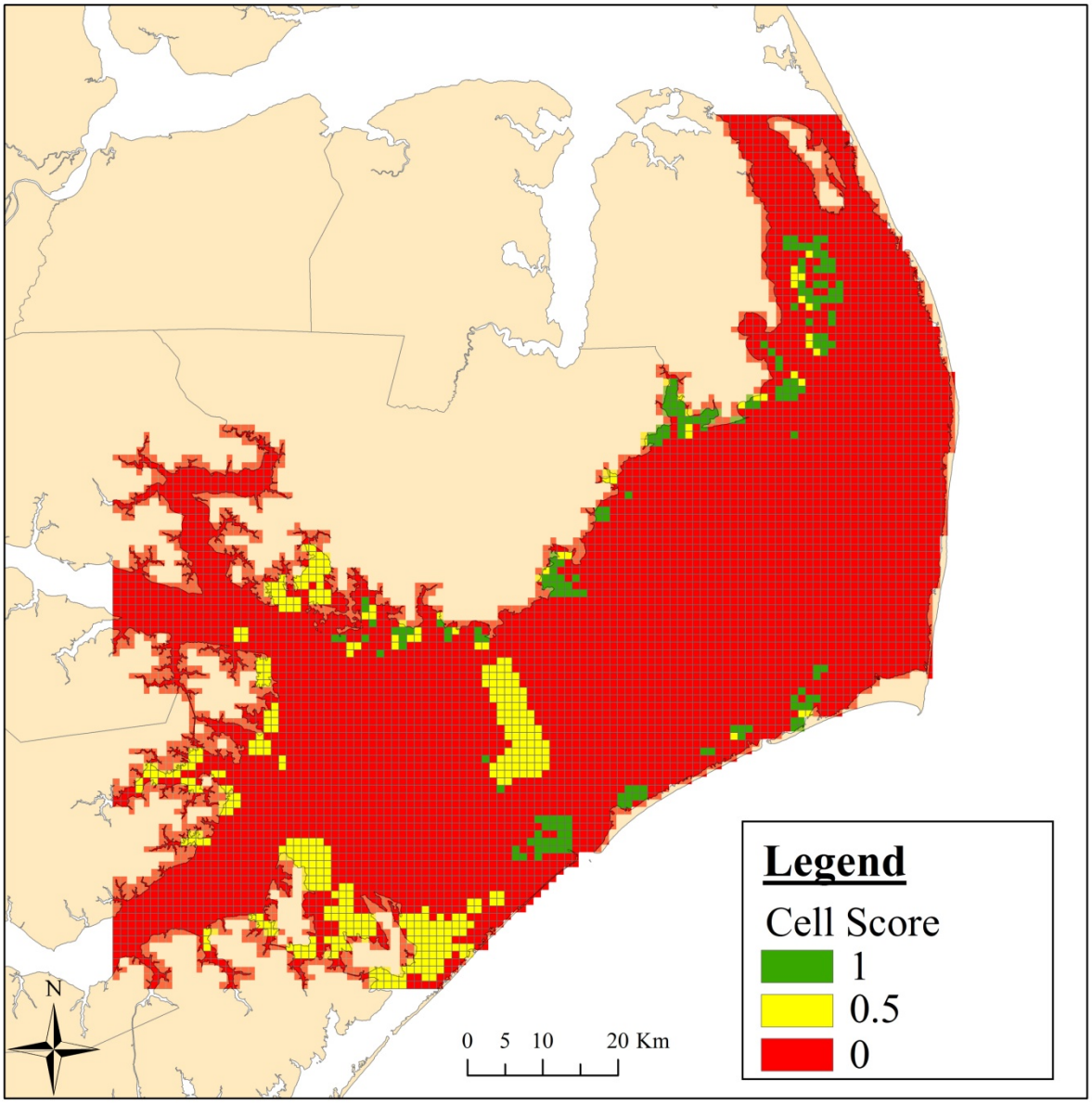

Figure 14. Pamlico Sound Winslow historic oyster reef threshold layer for oyster sanctuaries. Threshold scores were 1 for cells that contained “Winslow reef with solid shell” (green), 0.5 for cells that contained “Winslow reef with scattered shell” (yellow), and 0 for cells that contained “Winslow reef, but leased” or “non-Winslow reef” (red). Thresholds were based on use of Winslow historic reefs to site existing oyster sanctuaries. For layer weighting, see Table 1.

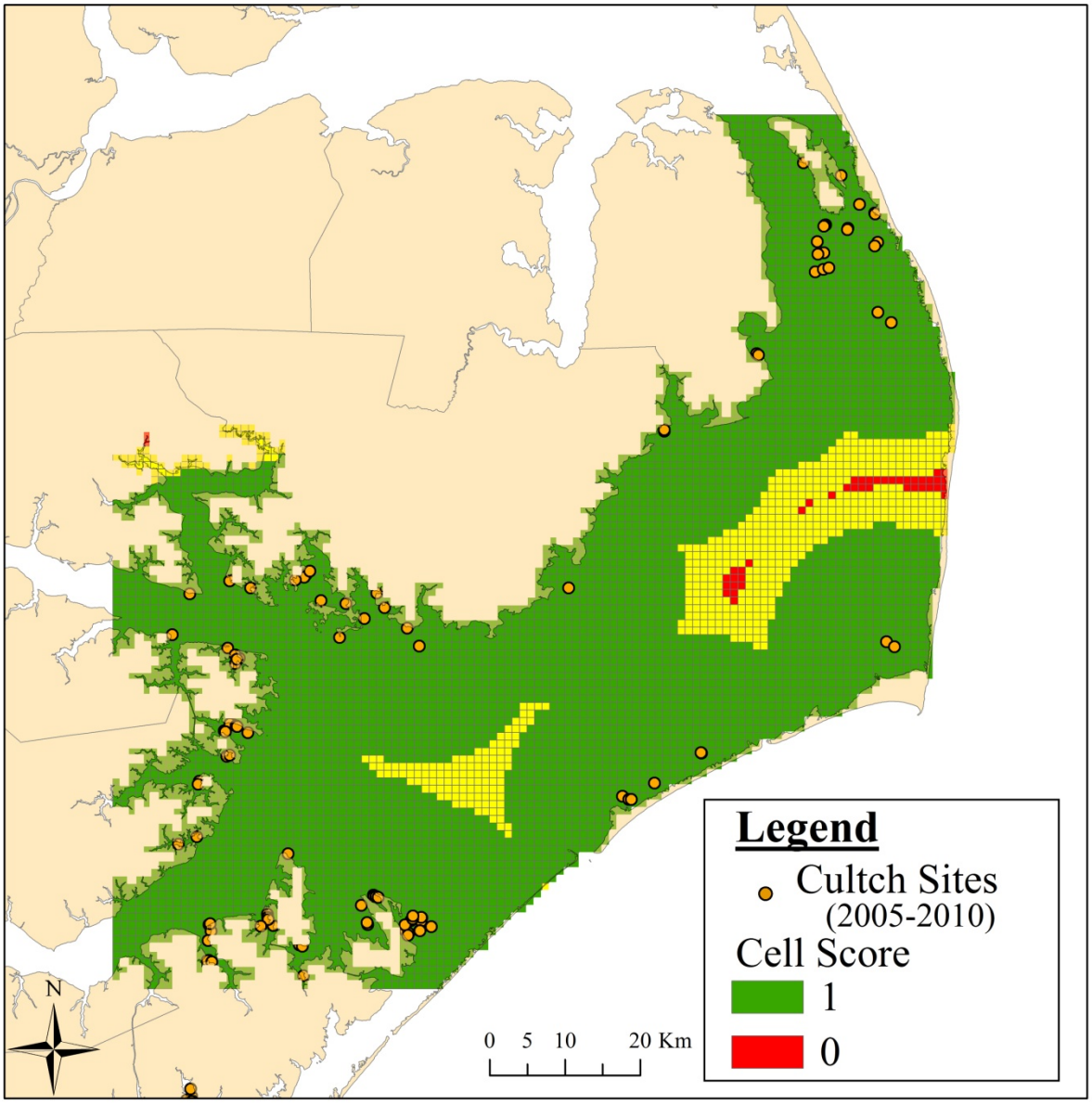

Figure 15. Pamlico Sound cultch-planting site threshold layer for oyster sanctuaries. Threshold scores were 1 for cells within a 15 km buffer distance of cultch-planting sites (green), 0.5 for cells between a 15-20 km buffer distance of cultch-planting sites (yellow), and 0 for cells beyond a 20 km buffer distance of cultch-planting sites (red). Buffer distance thresholds were based on average dispersal distances of oyster larvae such that new oyster sanctuaries provide and receive biological contributions to existing cultch-planting sites. For layer weighting, see Table 1.

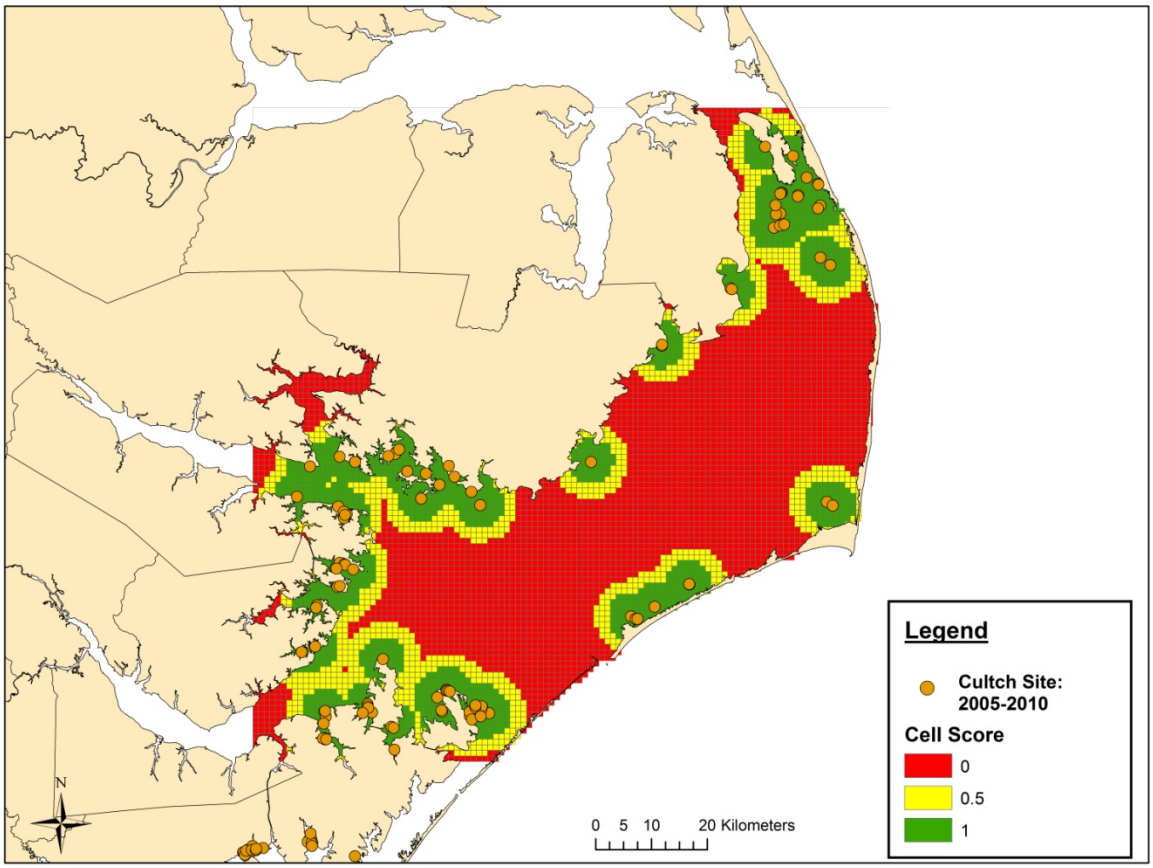

Figure 16. Pamlico Sound cultch-planting site threshold layer for artificial reefs. Threshold scores were 1 for cells within a 2.5 km buffer distance of cultch-planting sites (green), 0.5 for cells between a 2.5-5 km buffer distance of cultch-planting sites (yellow), and 0 for cells beyond a 5 km buffer distance of cultch-planting sites (red). Buffer distance thresholds were based on average dispersal distances of ingressing gag postlarvae such that new artificial reefs provide and receive biological contributions to existing cultch-planting sites. For layer weighting, see Table 1.

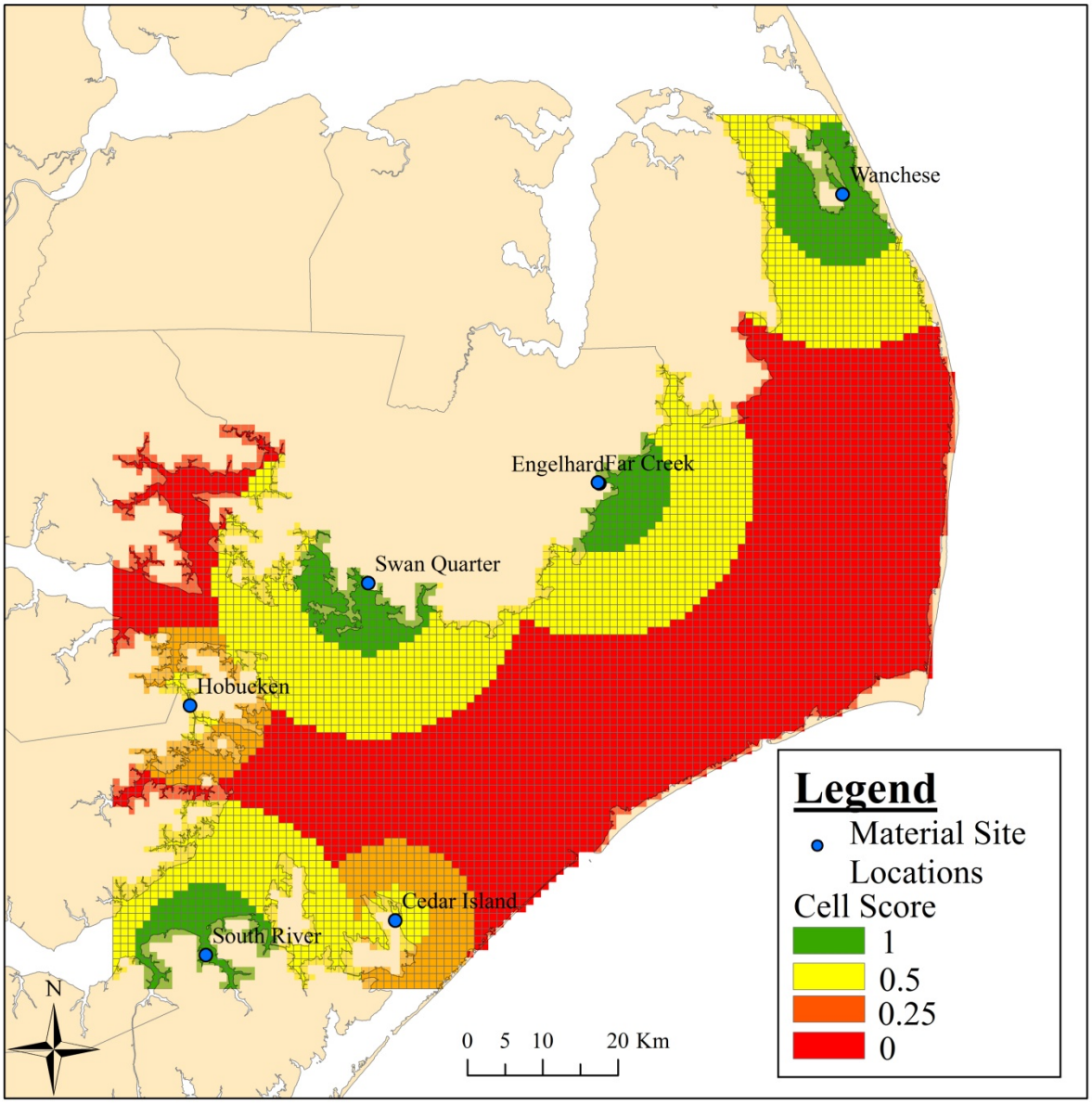

Figure 17. Pamlico Sound material stockpile site threshold layer for oyster sanctuaries and artificial reefs. Threshold scores were 1 for cells within a 5 km buffer distance of material stockpile sites (green), 0.5 for cells between a 5-10 km buffer distance of material stockpile sites (yellow), and 0 for cells beyond a 10 km buffer distance of material stockpile (red). There were exceptions to buffer distance thresholds at two sites that were only accessible by smaller barges due to shallow water depths, whereby cell scores were 0.5, 0.25, and 0 for buffer distances of <5km, 5-10 km, and >10km, respectively. Buffer distance thresholds were based on barge ranges and time considerations for travel between enhancement site and stockpile site for refill of materials. For layer weighting, see Table 1.

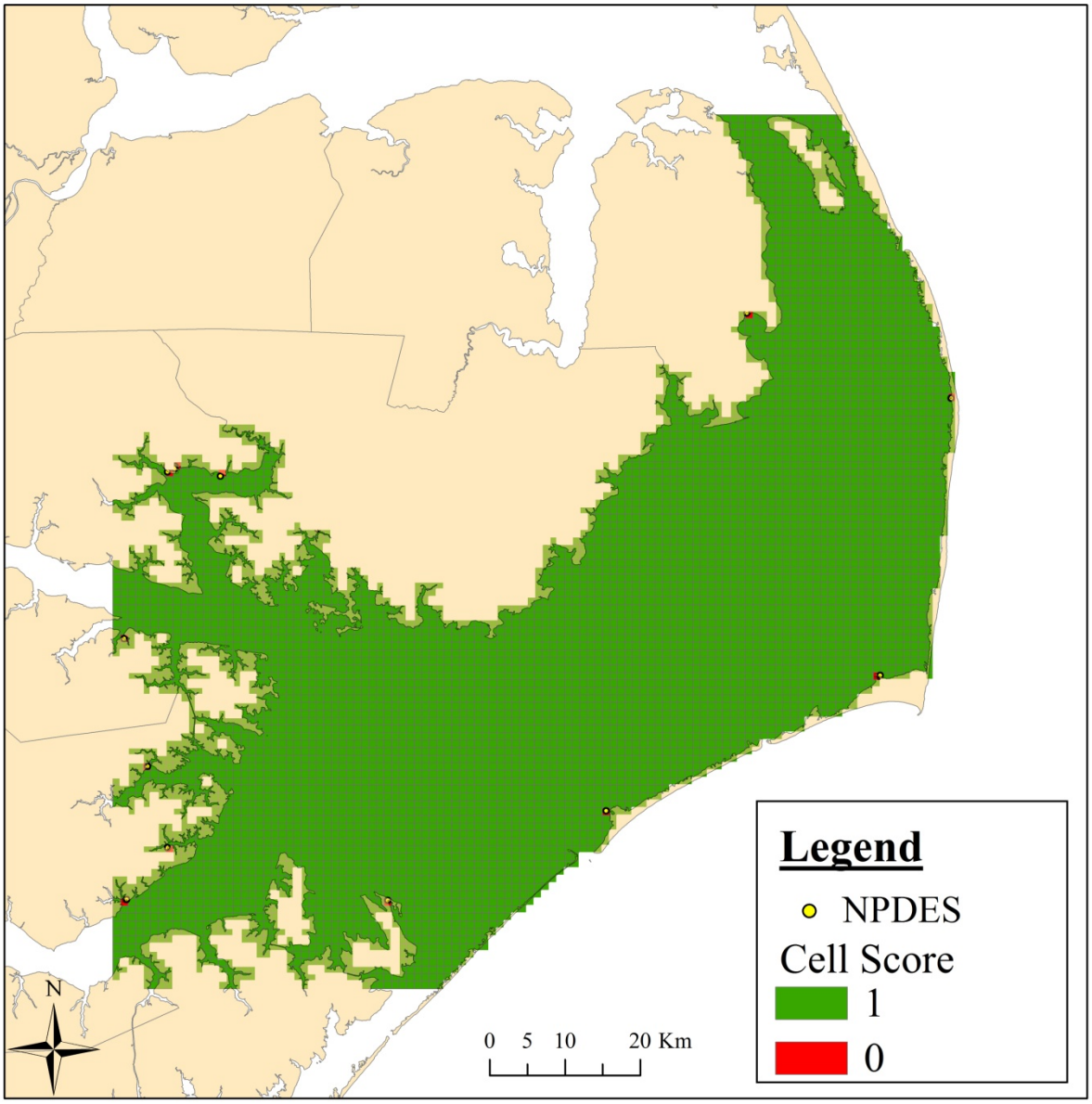

Figure 18. Pamlico Sound National Pollutant Discharge Elimination System (NPDES) threshold layer for oyster sanctuaries and artificial reefs. Threshold scores were 1 for cells without NPDES (green) and 0 for cells with NPDES (red). For layer weighting, see Table 1.

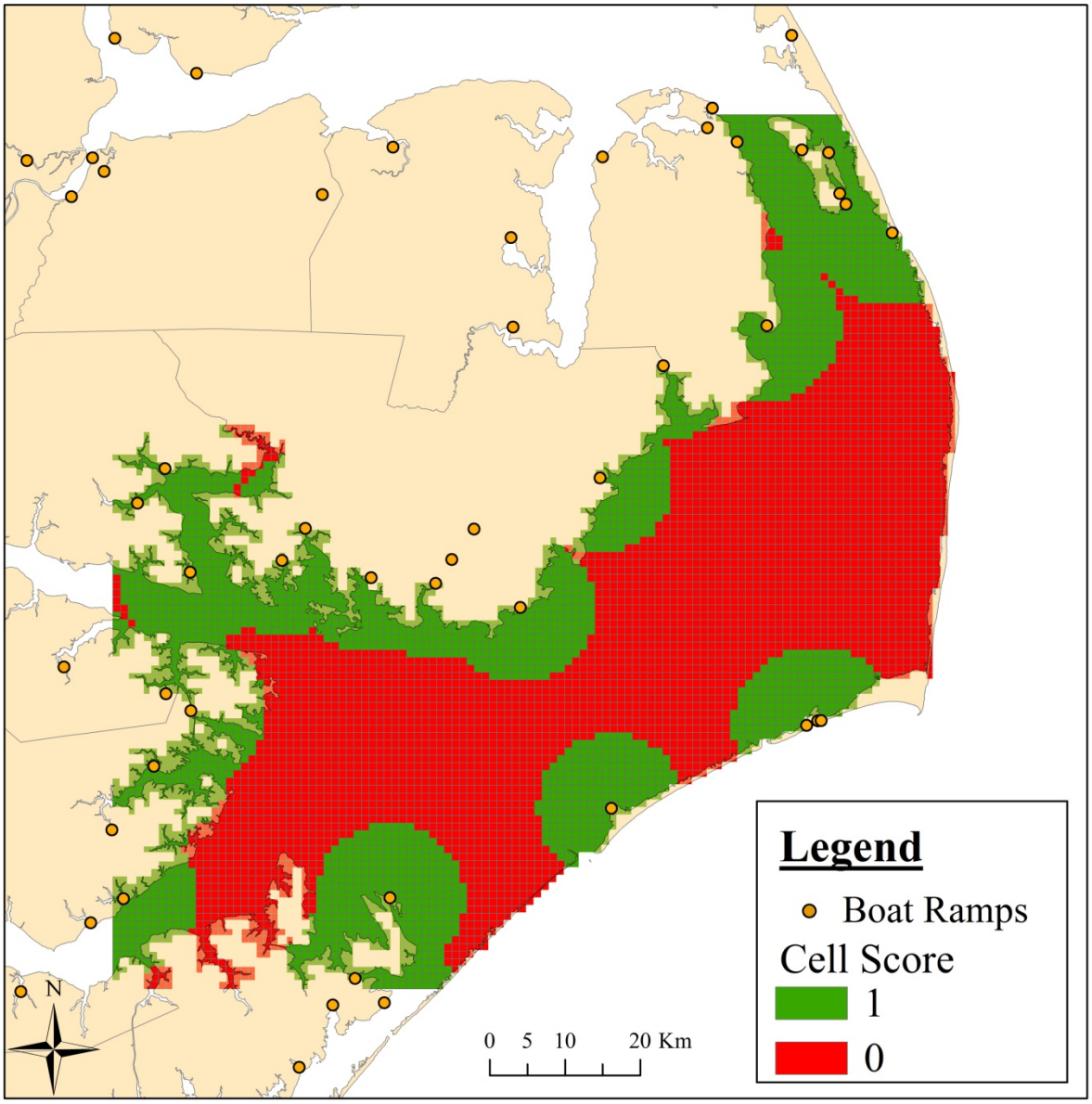

Figure 19. Pamlico Sound boat ramp threshold layer for oyster sanctuaries and artificial reefs. Threshold scores were 1 for cells within a 10 nautical mile buffer distance of boat ramps (green) and 0 for cells outside of a 10 nautical mile buffer distance of boat ramps (red). Buffer distance thresholds were based on Ramos et al. 2006. For layer weighting, see Table 1.

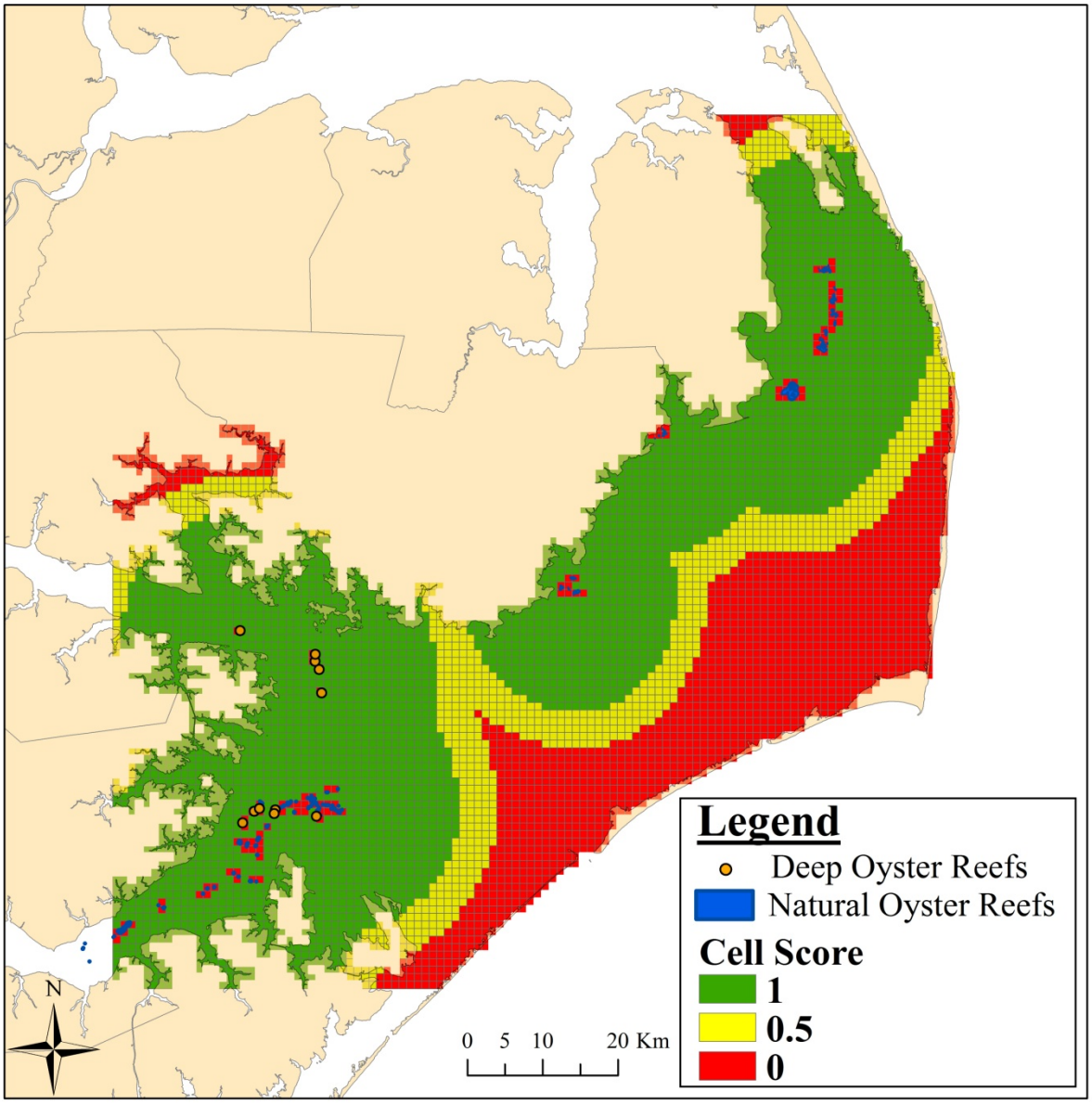

Figure 20. Pamlico Sound natural oyster reef threshold layer for oyster sanctuaries. Threshold scores were 1 for cells within a 15 km buffer distance of natural oyster reefs (green), 0.5 for cells between a 15-20 km buffer distance of natural oyster reefs (yellow), and 0 for cells beyond a 20 km buffer distance of natural oyster reefs (red). Buffer distance thresholds were based on average dispersal distances of oyster larvae such that new oyster sanctuaries provide and receive biological contributions to natural oyster reefs. For layer weighting, see Table 1.

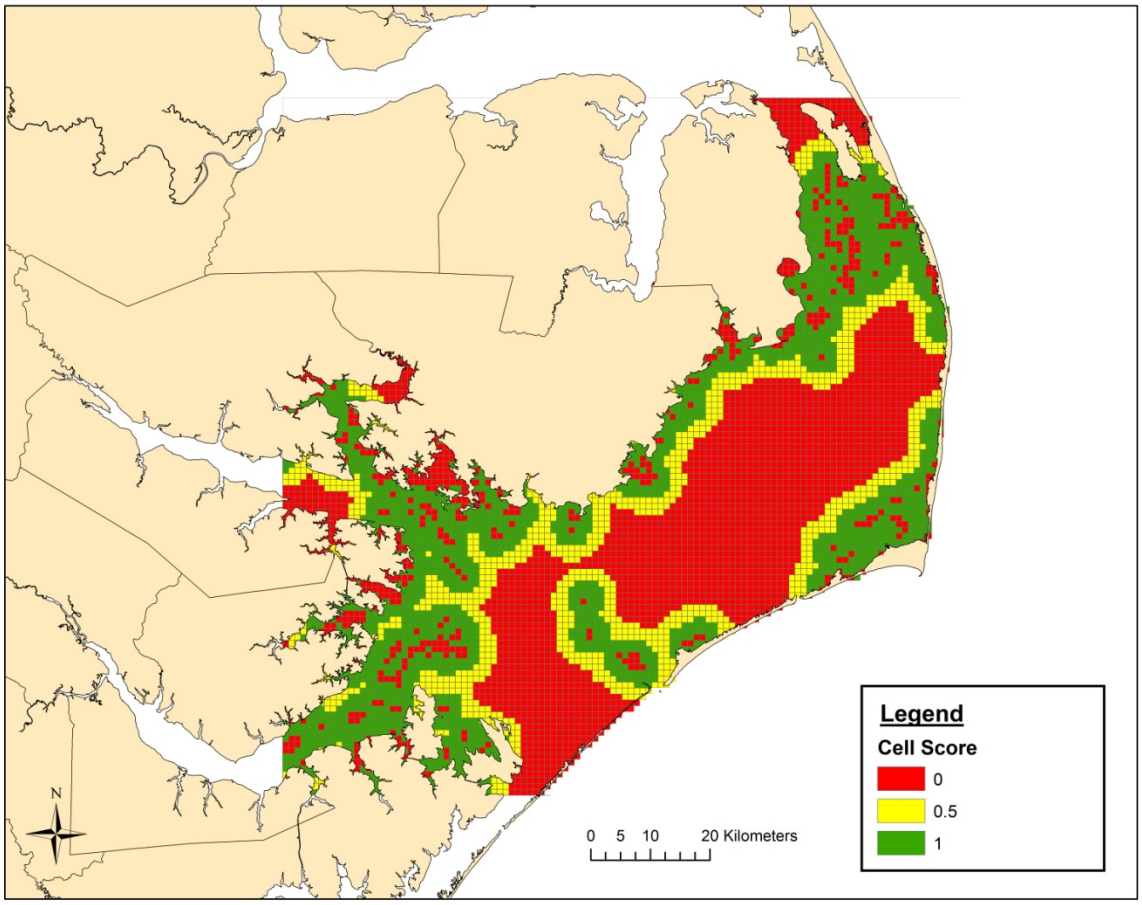

Figure 21. Pamlico Sound natural oyster reef threshold layer for artificial reefs. Threshold scores were 1 for cells within a 2.5 km buffer distance of natural oyster reefs (green), 0.5 for cells between a 2.5-5 km buffer distance of natural oyster reefs (yellow), and 0 for cells beyond a 5 km buffer distance of natural oyster reefs (red). Buffer distance thresholds were based on average dispersal distances of ingressing gag postlarvae such that new artificial reefs provide and receive biological contributions to natural oyster reefs. For layer weighting, see Table 1.

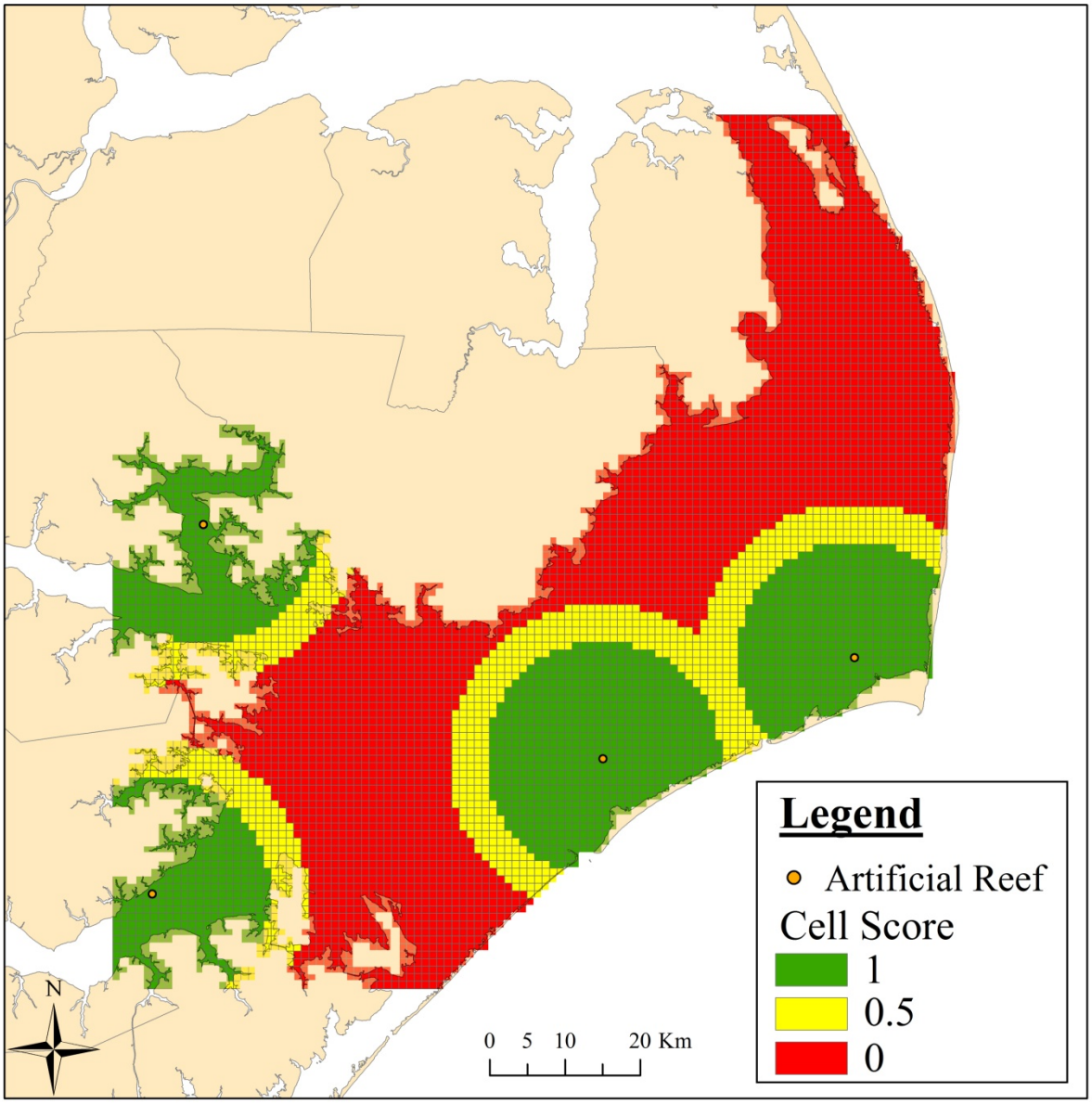

Figure 22. Pamlico Sound artificial reef threshold layer for oyster sanctuaries. Threshold scores were 1 for cells within a 15 km buffer distance of artificial reefs (green), 0.5 for cells between a 15-20 km buffer distance of artificial reefs (yellow), and 0 for cells beyond a 20 km buffer distance of artificial reefs (red). Buffer distance thresholds were based on average dispersal distances of oyster larvae such that new oyster sanctuaries provide and receive biological contributions to existing artificial reefs. For layer weighting, see Table 1.

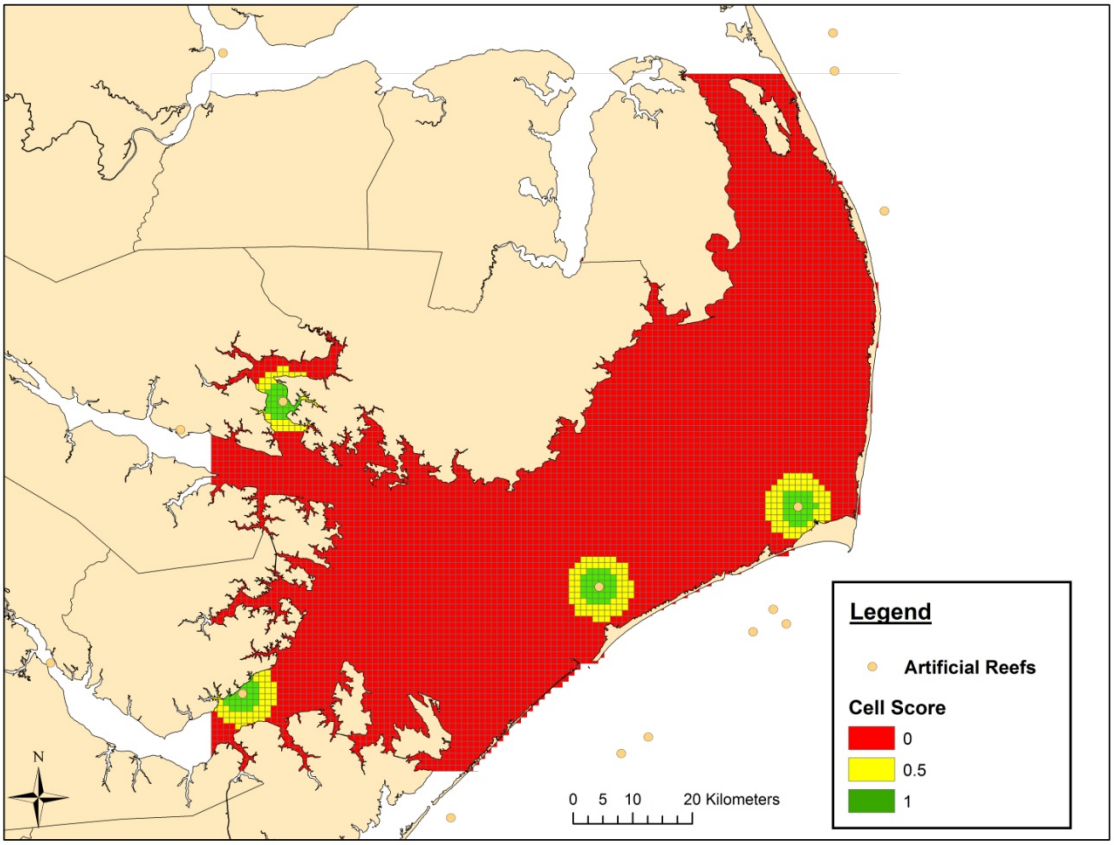

Figure 23. Pamlico Sound artificial reef threshold layer for artificial reefs. Threshold scores were 1 for cells within a 2.5 km buffer distance of artificial reefs (green), 0.5 for cells between a 2.5-5 km buffer distance of artificial reefs (yellow), and 0 for cells beyond a 5 km buffer distance of artificial reefs (red). Buffer distance thresholds were based on average dispersal distances of ingressing gag postlarvae such that new artificial reefs provide and receive biological contributions to existing artificial reefs. For layer weighting, see Table 1.

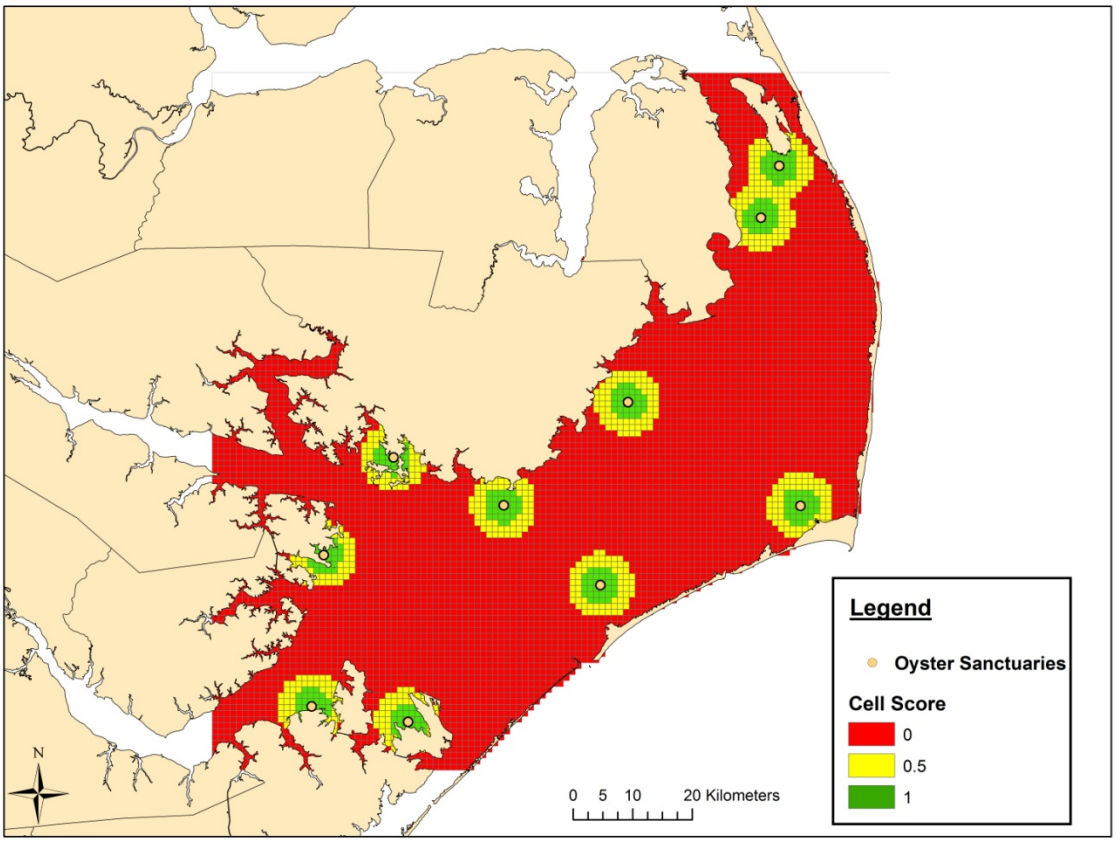

Figure 24. Pamlico Sound oyster sanctuary threshold layer for artificial reefs. Threshold scores were 1 for cells within a 2.5 km buffer distance of oyster sanctuaries (green), 0.5 for cells between a 2.5-5 km buffer distance of oyster sanctuaries (yellow), and 0 for cells beyond a 5 km buffer distance of oyster sanctuaries (red). Buffer distance thresholds were based on average dispersal distances of ingressing gag postlarvae such that new artificial reefs provide and receive biological contributions to existing oyster sanctuaries. For layer weighting, see Table 1.

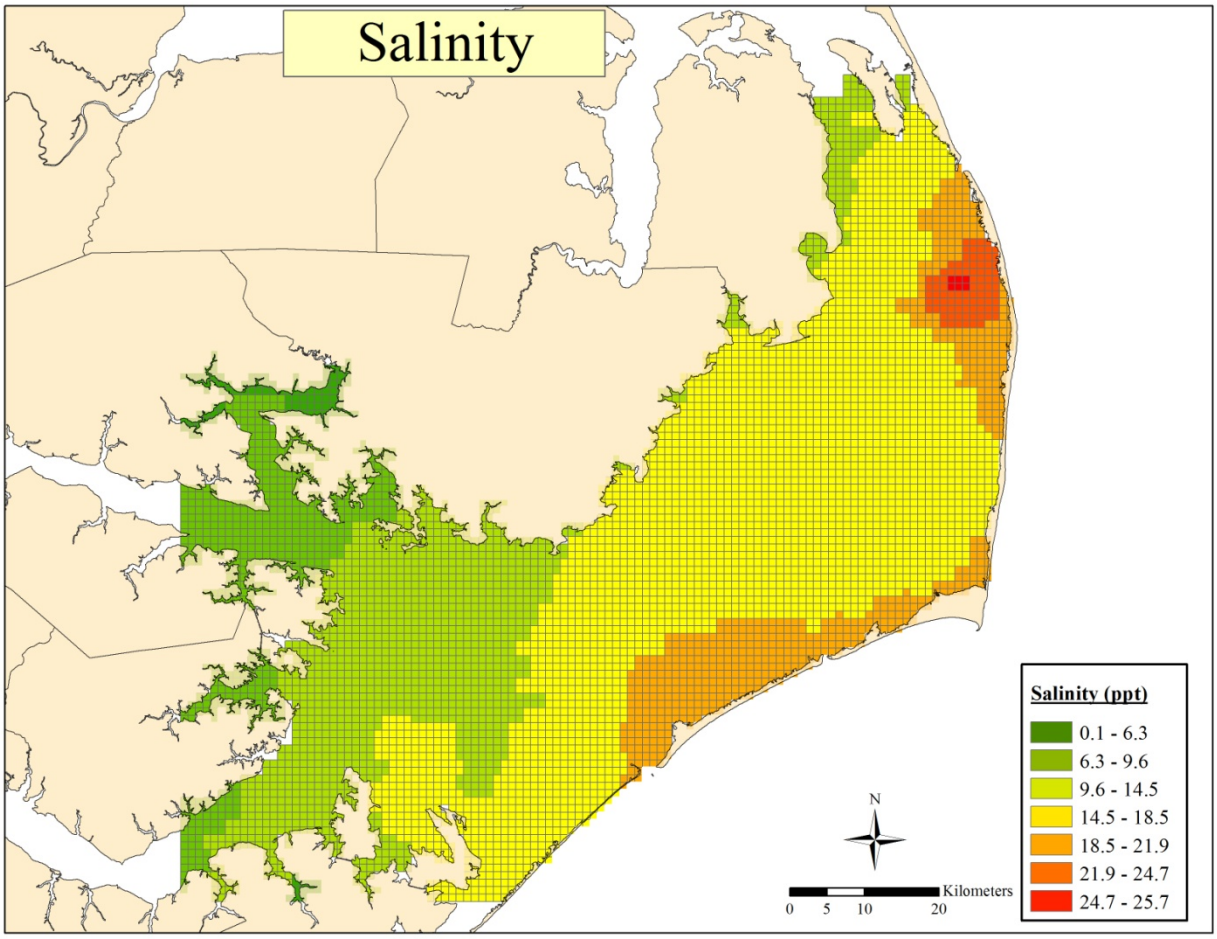

Figure 25. Pamlico Sound salinity map during long-term average conditions of freshwater influx. Salinity map was generated from statistical modeling and interpolation of NC DMF Program 195 data.

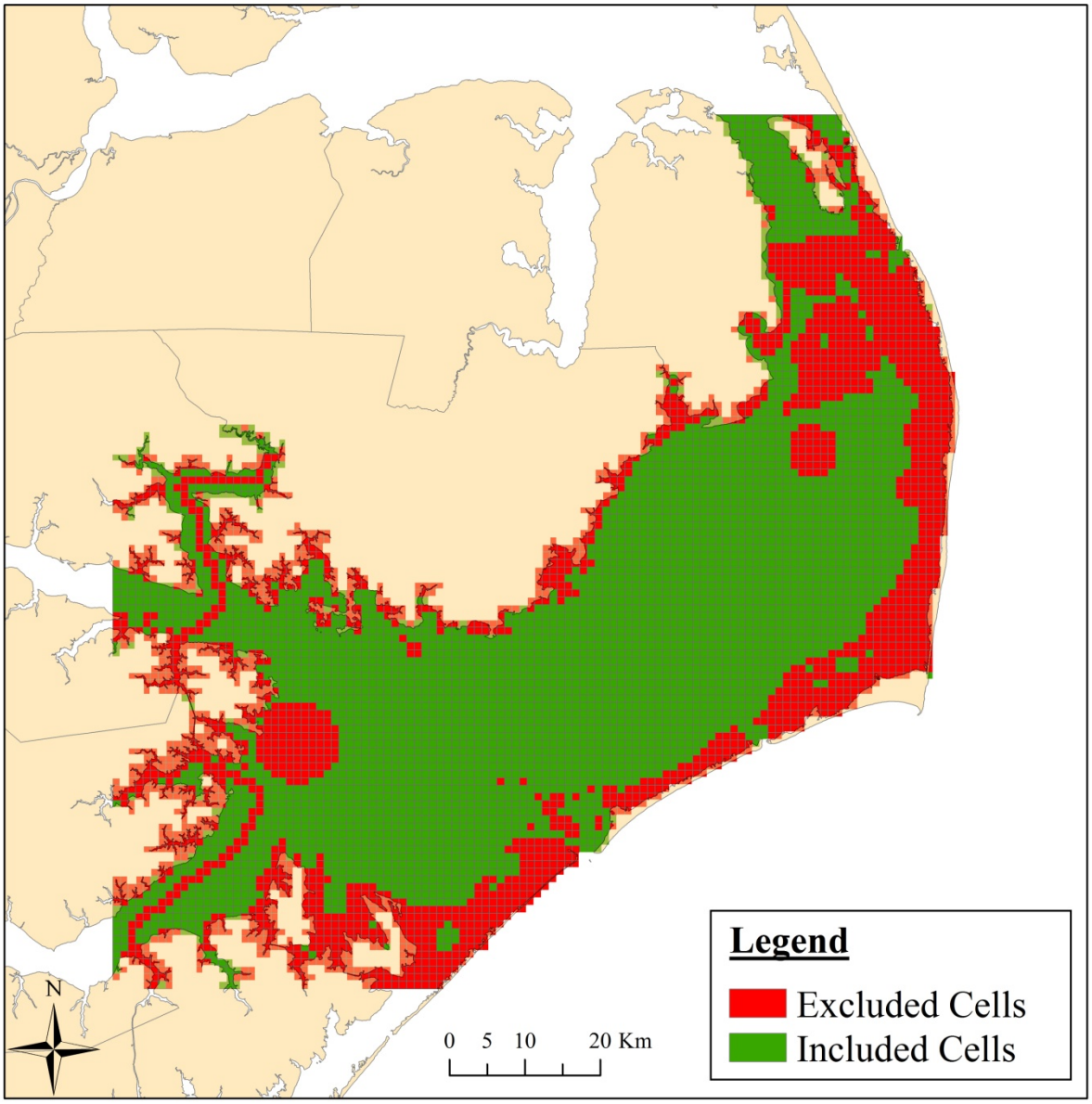

Figure 26. Pamlico Sound exclusion map based on 7 exclusion layers (Figures 2-8). Cells excluded from consideration for habitat enhancement (red) covered 39% of Pamlico Sound.

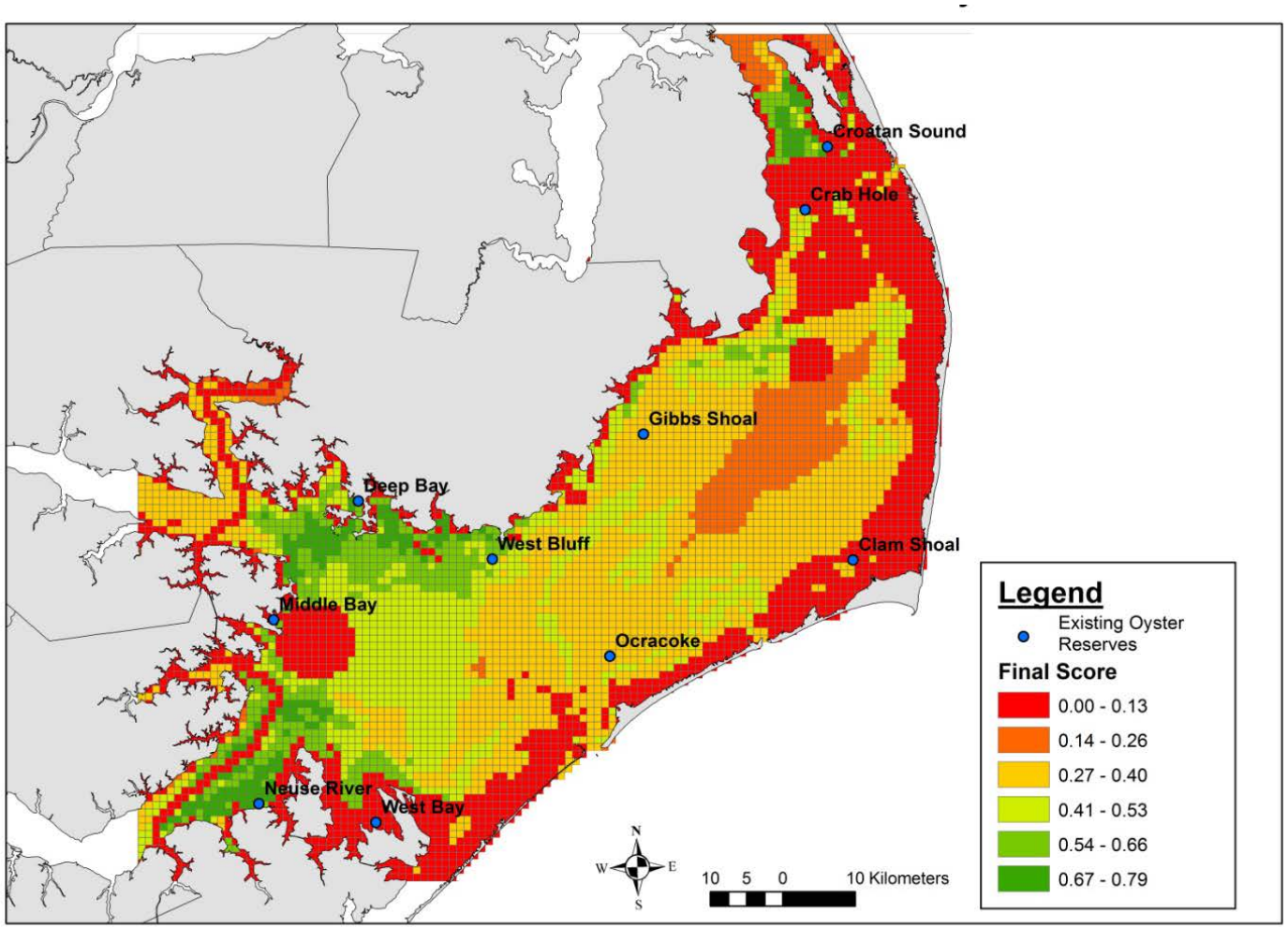

Figure 27. Pamlico Sound suitability map for oyster sanctuaries. Suitability map was based on 7 exclusion layers (Figures 2-8) and 10 threshold layers (Figures 9, 11-12, 14-15, 17-20, 22). Cell suitability increases from red (unsuitable or least suitable) to green (optimal).

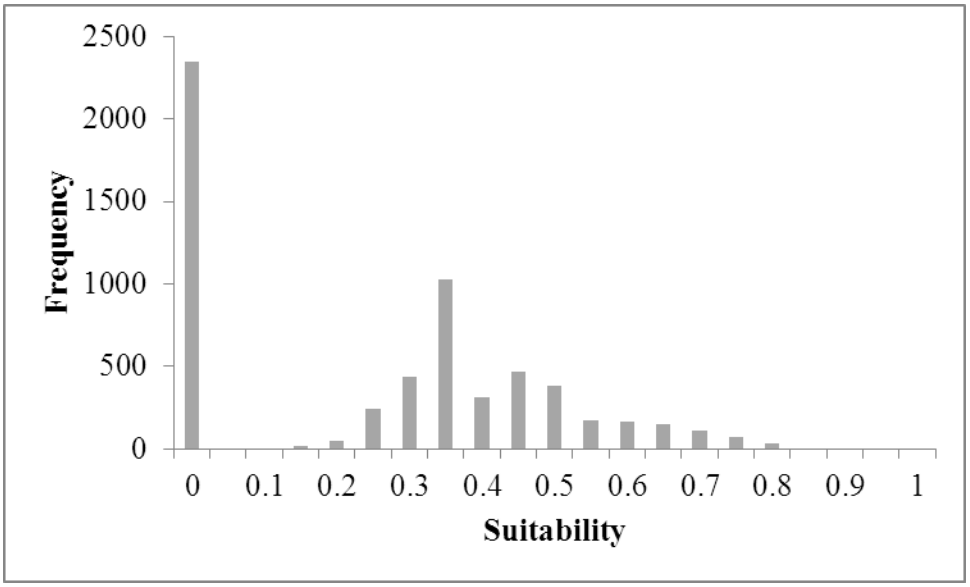

Figure 28. Cell suitability frequency plot for oyster sanctuaries in Pamlico Sound. Suitability scores of 0 were based on exclusion layers (Figure 2-8). Suitability scores >0 were based on threshold layers (Figures 9, 11-12, 14-15, 17-20, 22).

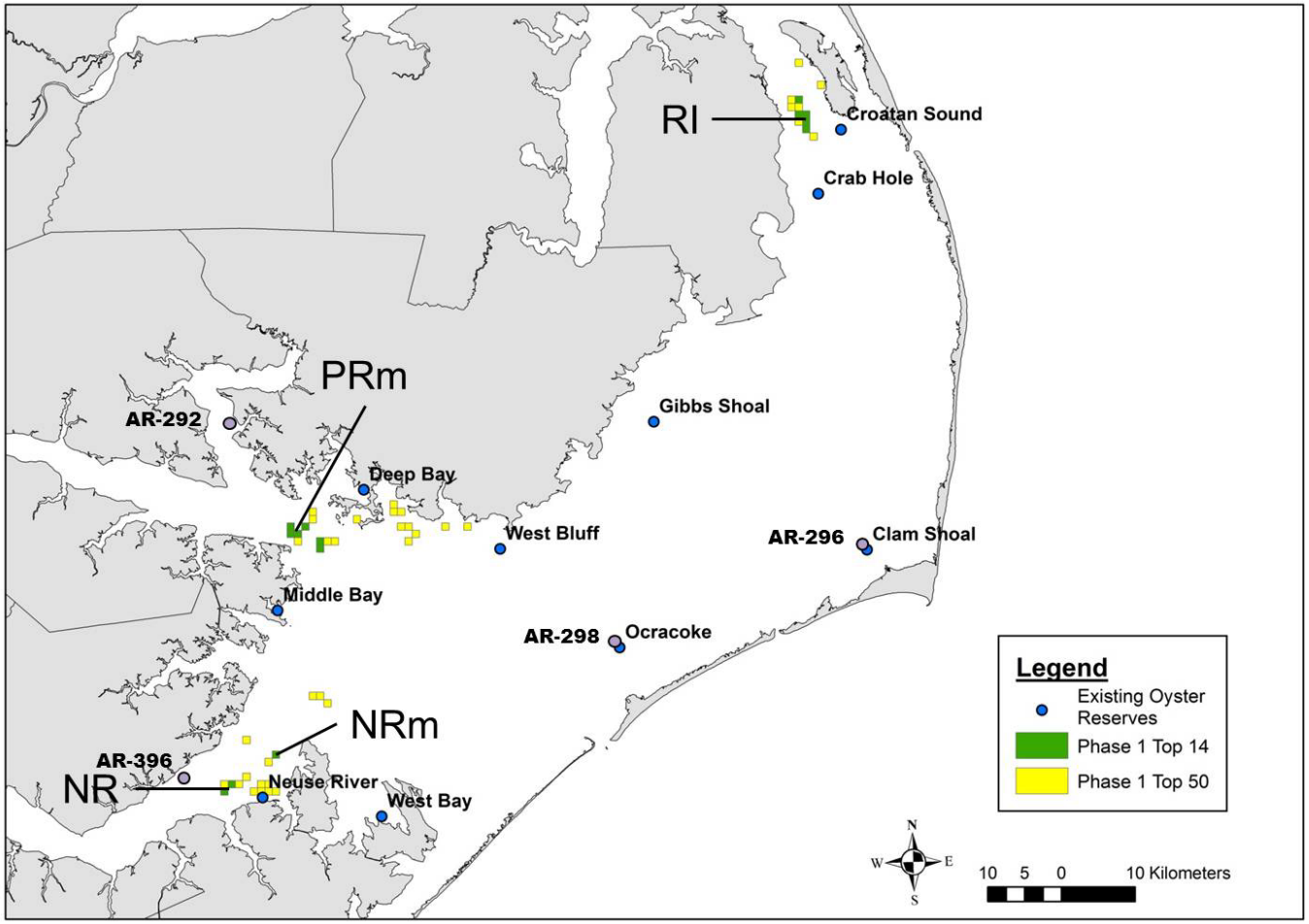

Figure 29. Location of 50 most suitable (yellow) and 14 most suitable (green) 1 km^2 cells for oyster sanctuaries in Pamlico Sound. Existing oyster sanctuaries and artificial reefs are denoted by blue and purple circles, respectively. Location of data sonde deployment for field verification of water quality near Roanoke Island (RI), Pamlico River mouth (PRm), Neuse River (NR), and Neuse River mouth (NRm) are also shown on the map.

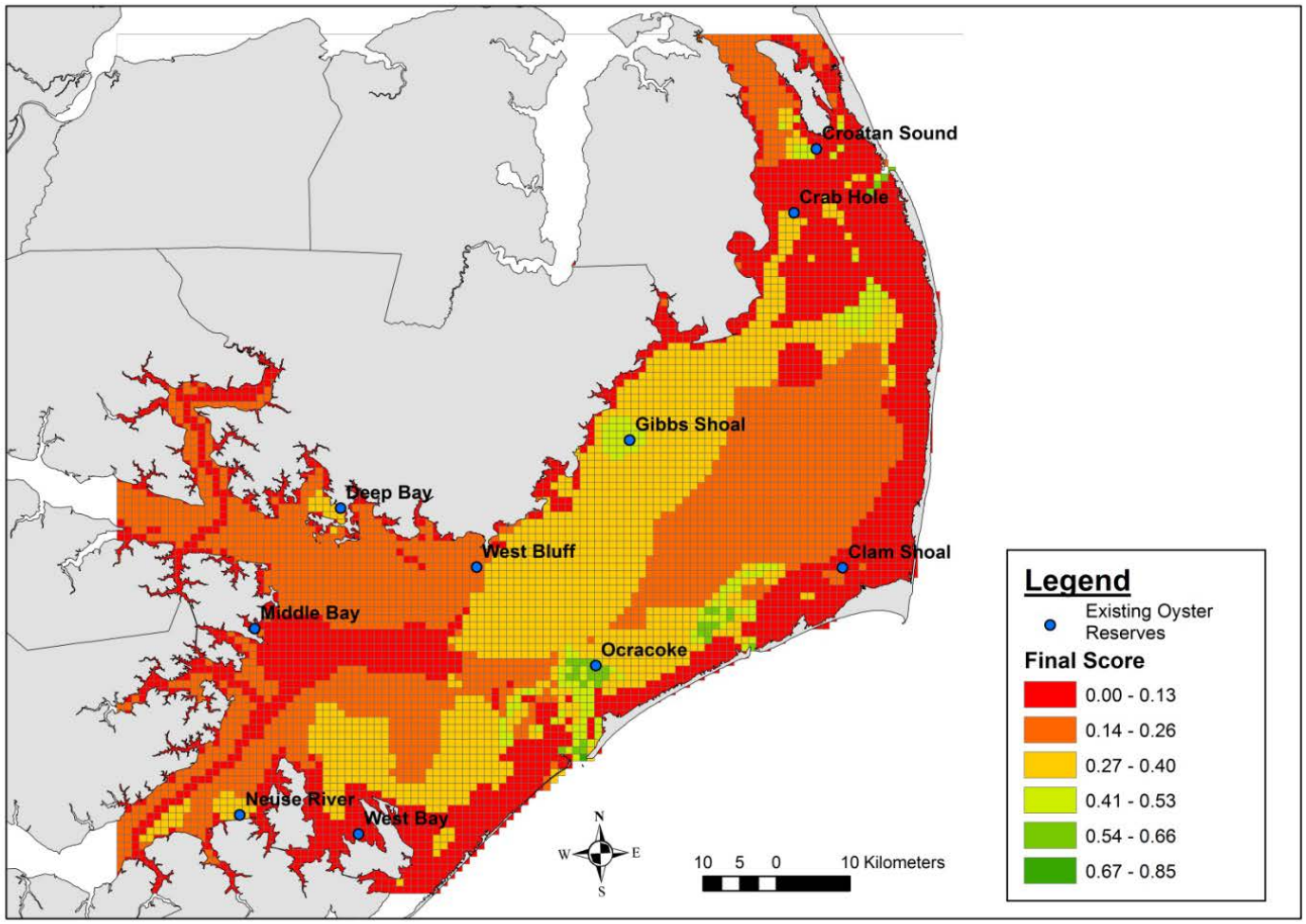

Figure 30. Pamlico Sound suitability map for artificial reefs. Suitability map was based on 7 exclusion layers (Figures 2-8) and 9 threshold layers (Figures 10, 13, 16-19, 21, 23-24). Cell suitability increases from red (unsuitable or least suitable) to green (optimal).

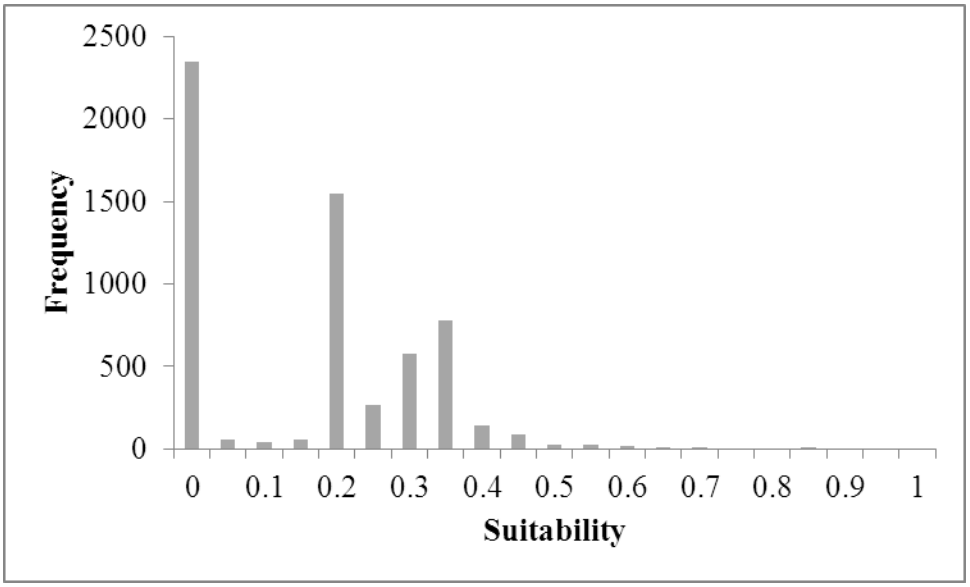

Figure 31. Cell suitability frequency plot for artificial reefs in Pamlico Sound. Suitability scores of 0 were based on exclusion layers (Figure 2-8). Suitability scores >0 were based on threshold layers (Figures 10, 13, 16-19, 21, 23-24).

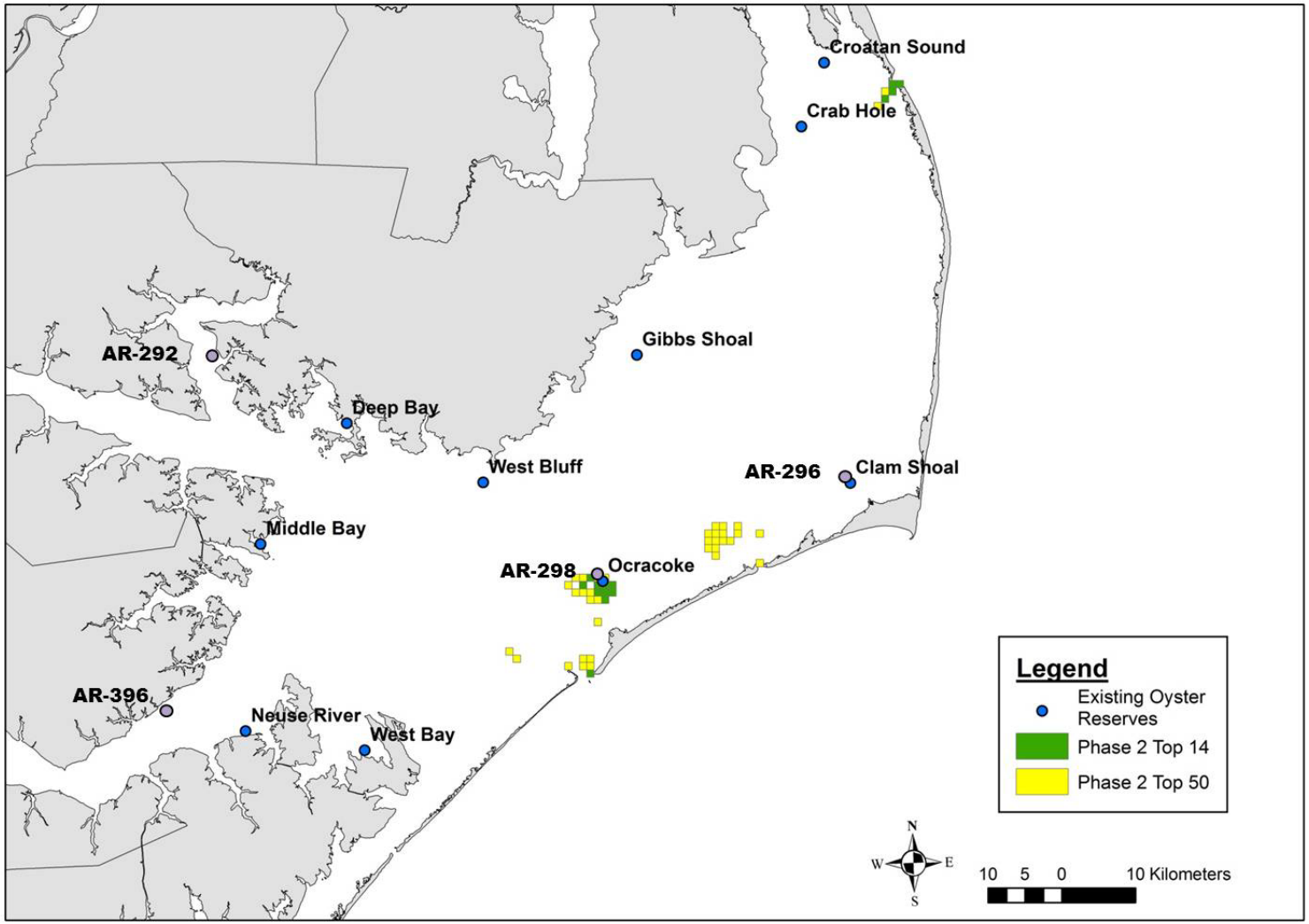

Figure 32. Location of 50 most suitable (yellow) and 14 most suitable (green) 1 km^2 cells for artificial reefs in Pamlico Sound. Existing oyster sanctuaries and artificial reefs are denoted by blue and purple circles, respectively.

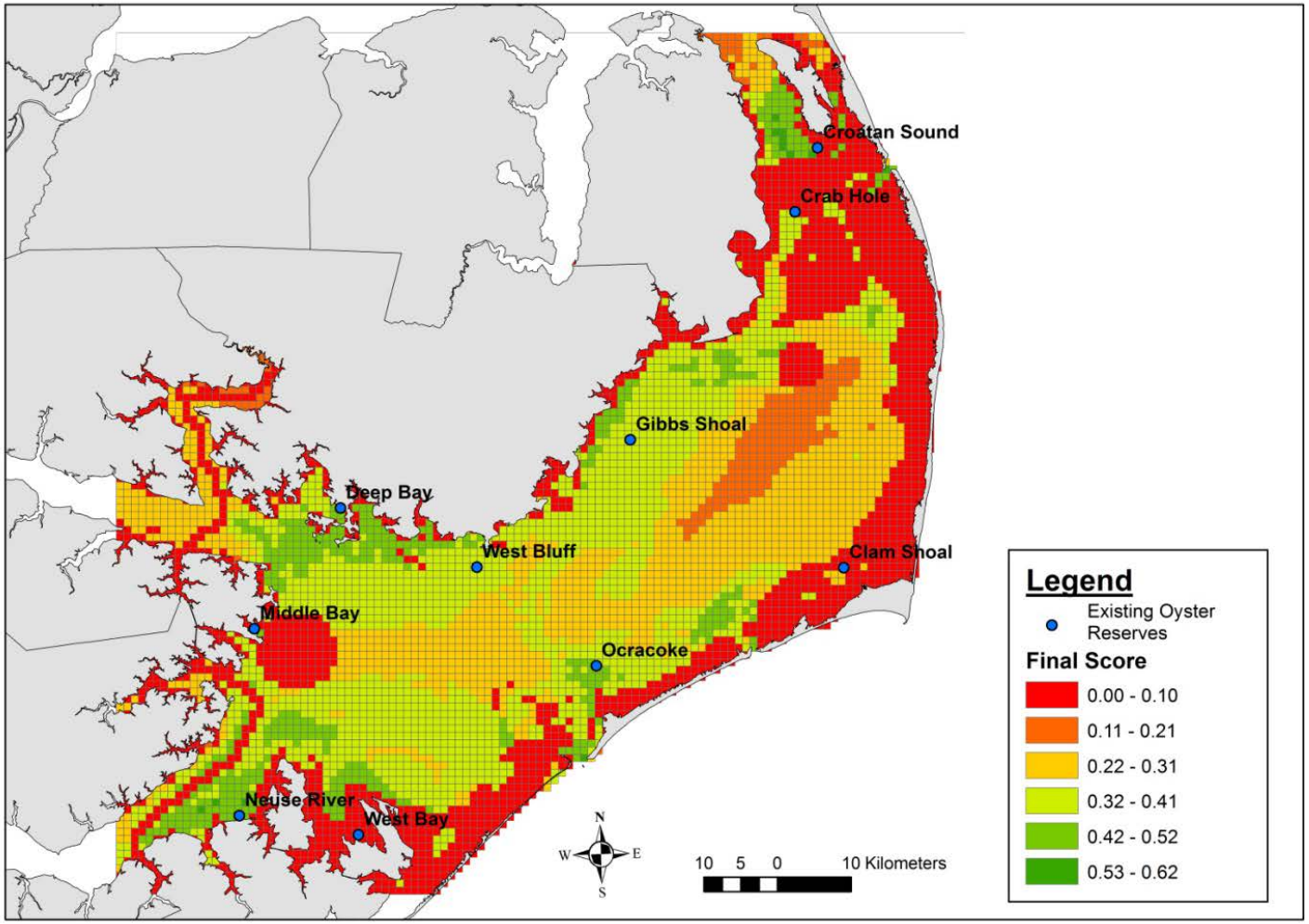

Figure 33. Pamlico Sound suitability map for general habitat enhancement based on average cell suitability for both oyster sanctuaries and artificial reefs. Suitability map was based on 7 exclusion layers (Figures 2-8) and 12 threshold layers (Figures 9-24). Cell suitability increases from red (unsuitable or least suitable) to green (optimal).

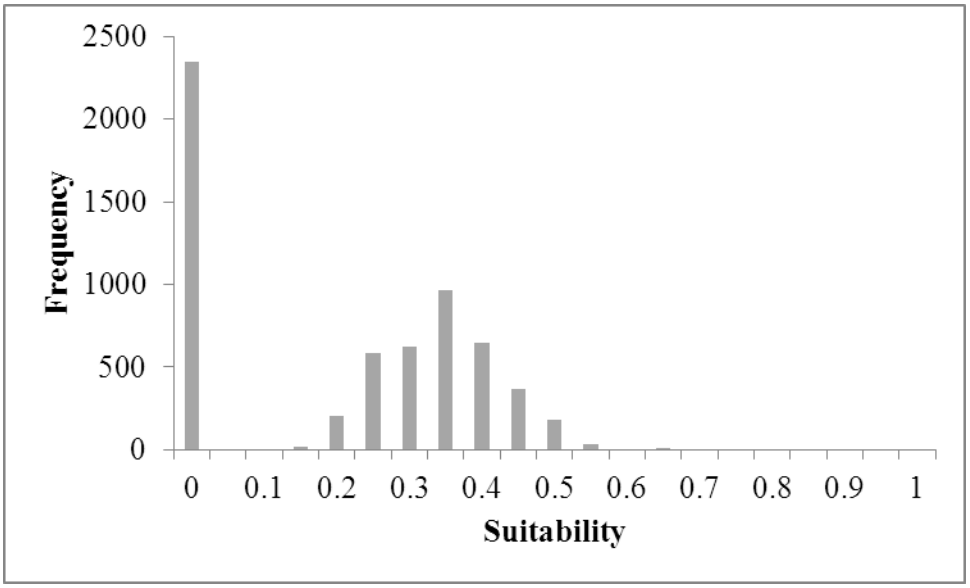

Figure 34. Cell suitability frequency plot for general habitat enhancement based on average cell suitability for both oyster sanctuaries and artificial reefs (Figures 30 and 33).

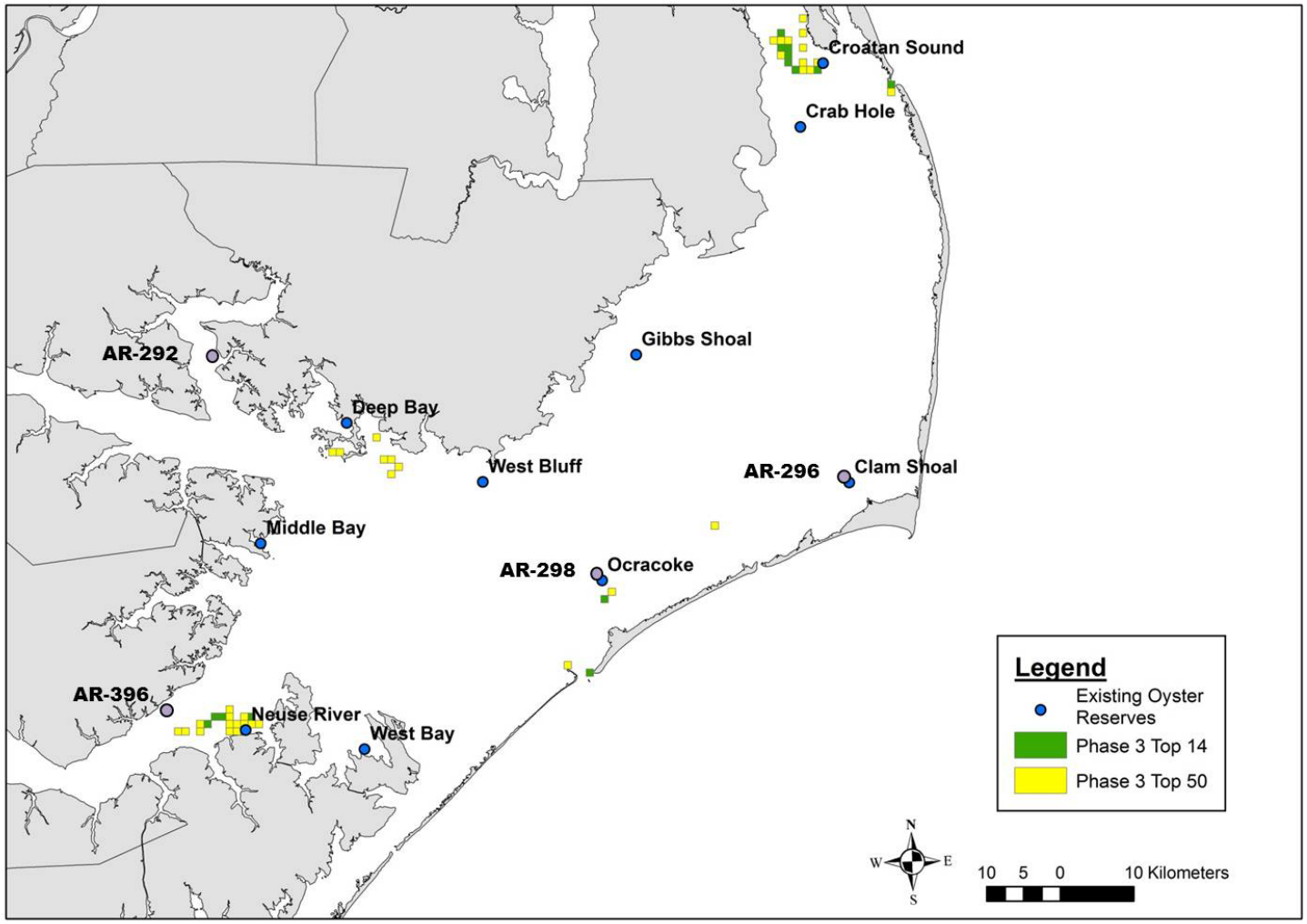

Figure 35. Location of 50 most suitable (yellow) and 14 most suitable (green) 1 km^2 cells for general habitat enhancement in Pamlico Sound based on mean cell suitability scores for oyster sanctuaries and artificial reefs.

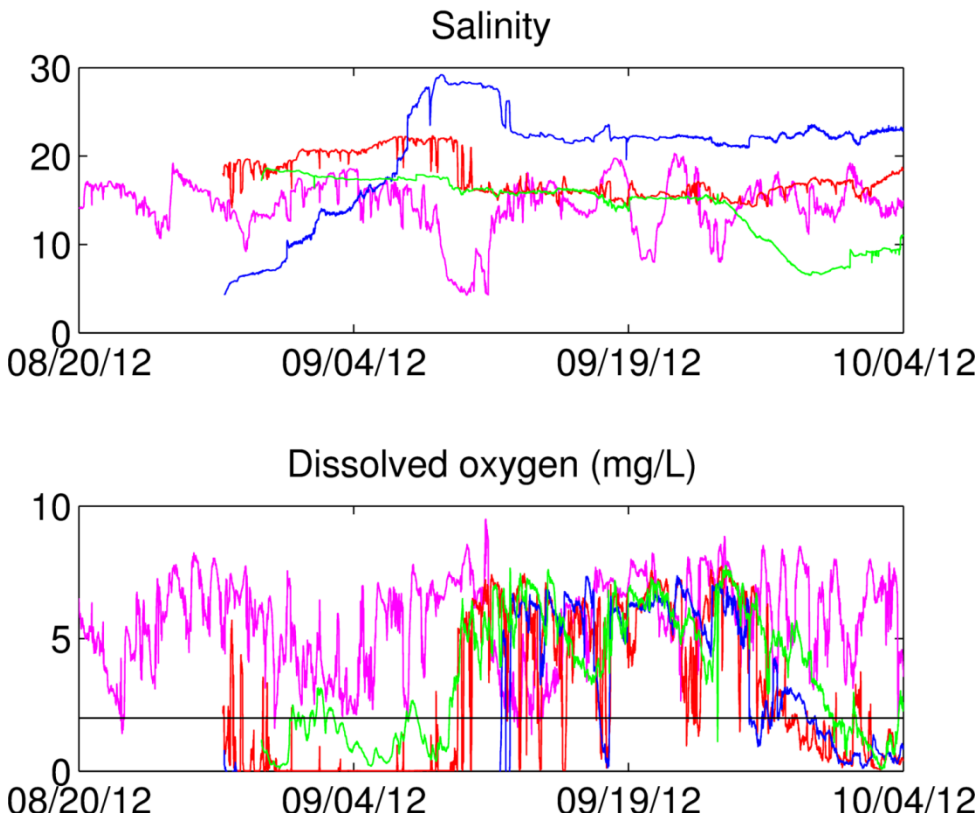

Figure 36. Field observations of salinity and dissolved oxygen at 4 sites proximal to optimal sites for the location of oyster sanctuaries based on GIS-modeling. Site abbreviations are as follows: Roanoke Island (RI), Neuse River (NR), Neuse River mouth (NRm), Pamlico River mouth (PRm). For site locations, see Figure 29.

Appendix 1. Rank order of latitude and longitude coordinates for the center of the 50 most suitable 1 km^2 cells (in descending order) for siting oyster sanctuaries in Pamlico Sound.

Latitude	Longitude	Oyster sanctuary suitability
35.01578	-76.5902	0.79
35.84256	-75.7017	0.79
35.81524	-75.6918	0.79
35.82424	-75.6914	0.79
35.32895	-76.4709	0.79
35.82454	-75.7024	0.79
35.80623	-75.6921	0.79
35.33773	-76.4596	0.78
35.05928	-76.5121	0.78
35.02457	-76.579	0.78
35.31925	-76.4382	0.78
35.33819	-76.4816	0.78
35.32918	-76.4819	0.77
35.31024	-76.4384	0.77
35.83355	-75.7021	0.77
35.05049	-76.5234	0.77
35.02435	-76.568	0.77
35.12076	-76.4334	0.76
35.02346	-76.5242	0.76
35.83385	-75.7131	0.76
35.79692	-75.6814	0.76
35.81554	-75.7028	0.76
35.36193	-76.3267	0.76
35.33465	-76.3166	0.76
35.13	-76.4441	0.76
35.0782	-76.5554	0.75
35.31994	-76.4711	0.75
35.02368	-76.5351	0.75
35.31638	-76.3062	0.75
35.33441	-76.3056	0.75
35.01445	-76.5244	0.75
35.31902	-76.4272	0.75
35.01422	-76.5135	0.75
35.03314	-76.5568	0.75
35.01467	-76.5354	0.75
35.0149	-76.5464	0.74
35.32515	-76.2949	0.74
35.8876	-75.6999	0.74
35.33317	-76.2506	0.74

35.35553	-76.448	0.74
35.85967	-75.6678	0.74
35.13023	-76.4551	0.74
35.33242	-76.2177	0.74
35.84286	-75.7128	0.74
35.31878	-76.4162	0.74
35.34651	-76.4483	0.74
35.34511	-76.3823	0.74
35.35292	-76.327	0.74
35.35267	-76.316	0.74
35.02479	-76.5899	0.73

Appendix 2. Rank order of latitude and longitude coordinates for the center of the 50 most suitable 1 km^2 cells (in descending order) for siting artificial reefs in Pamlico Sound.

Latitude	Longitude	Artificial reef suitability
35.7749	-75.5385	0.84
35.06613	-76.0183	0.68
35.17372	-75.9924	0.65
35.76589	-75.5389	0.65
35.15569	-75.9931	0.63
35.17344	-75.9814	0.61
35.17399	-76.0034	0.61
35.1647	-75.9927	0.60
35.18327	-76.014	0.60
35.7572	-75.5503	0.60
35.77459	-75.5274	0.60
35.17453	-76.0253	0.59
35.16443	-75.9818	0.59
35.16498	-76.0037	0.59
35.18354	-76.025	0.58
35.16552	-76.0257	0.58
35.16579	-76.0366	0.57
35.22357	-75.8257	0.57
35.22328	-75.8147	0.57
35.18381	-76.036	0.57
35.07541	-76.029	0.56
35.21484	-75.837	0.56
35.183	-76.0031	0.56
35.24072	-75.7921	0.55
35.07514	-76.018	0.55
35.20555	-75.8264	0.55
35.7485	-75.5617	0.55
35.07595	-76.0509	0.55
35.24159	-75.825	0.54
35.22386	-75.8367	0.54
35.08415	-76.0177	0.54
35.2413	-75.814	0.54
35.19479	-75.7609	0.53
35.15624	-76.015	0.53
35.23171	-75.7924	0.53
35.21456	-75.8261	0.53
35.76621	-75.5499	0.53
35.23083	-75.7595	0.53
35.16525	-76.0147	0.53

35.15597	-76.0041	0.52
35.09608	-76.1379	0.52
35.23258	-75.8254	0.52
35.08681	-76.1273	0.52
35.08442	-76.0286	0.52
35.23286	-75.8363	0.51
35.12893	-76.005	0.51
35.18272	-75.9921	0.51
35.17507	-76.0473	0.51
35.23229	-75.8144	0.51
35.22299	-75.8037	0.51

Appendix 3. Rank order of latitude and longitude coordinates within the center of the 50 most suitable 1 km^2 cells (in descending order) for siting habitat enhancement (oyster sanctuaries + artificial reefs) in Pamlico Sound based on mean cell suitability scores.

			Suitability	
Latitude	**Longitude**	**Habitat enhancement**	**Oyster sanctuary**	**Artificial reef**
35.7749	-75.5385	0.62	0.40	0.84
35.79601	-75.6483	0.55	0.68	0.41
35.06613	-76.0183	0.54	0.39	0.68
35.01578	-76.5902	0.53	0.79	0.26
35.81524	-75.6918	0.53	0.79	0.26
35.82424	-75.6914	0.53	0.79	0.26
35.80623	-75.6921	0.52	0.79	0.26
35.82454	-75.7024	0.52	0.79	0.26
35.02435	-76.568	0.52	0.77	0.28
35.02346	-76.5242	0.52	0.76	0.28
35.79692	-75.6814	0.52	0.76	0.28
35.84256	-75.7017	0.52	0.79	0.25
35.15569	-75.9931	0.52	0.41	0.63
35.02457	-76.579	0.52	0.78	0.25
35.83355	-75.7021	0.51	0.77	0.26
35.02368	-76.5351	0.51	0.75	0.28
35.79662	-75.6704	0.51	0.61	0.41
35.01445	-76.5244	0.51	0.75	0.28
35.76589	-75.5389	0.51	0.38	0.65
35.81554	-75.7028	0.51	0.76	0.26
35.01422	-76.5135	0.51	0.75	0.28
35.01467	-76.5354	0.51	0.75	0.28
35.36193	-76.3267	0.51	0.76	0.26
35.03314	-76.5568	0.51	0.75	0.27
35.84166	-75.6685	0.51	0.62	0.40
35.0149	-76.5464	0.51	0.74	0.27
35.16443	-75.9818	0.51	0.42	0.59
35.79631	-75.6593	0.51	0.60	0.41
35.07595	-76.0509	0.50	0.46	0.55
35.00588	-76.5466	0.50	0.73	0.28
35.83385	-75.7131	0.50	0.76	0.25
35.33465	-76.3166	0.50	0.76	0.25
35.00763	-76.6343	0.50	0.73	0.27
35.00742	-76.6233	0.50	0.73	0.28
35.82364	-75.6693	0.50	0.73	0.28
35.24159	-75.825	0.50	0.46	0.54
35.31638	-76.3062	0.50	0.75	0.25

35.0061	-76.5576	0.50	0.73	0.28
35.33441	-76.3056	0.50	0.75	0.25
35.34511	-76.3823	0.50	0.74	0.26
35.01512	-76.5573	0.50	0.72	0.28
35.02413	-76.557	0.50	0.73	0.27
35.016	-76.6011	0.50	0.73	0.26
35.34534	-76.3933	0.50	0.73	0.26
35.83325	-75.691	0.50	0.59	0.40
35.32515	-76.2949	0.50	0.74	0.25
35.80562	-75.67	0.50	0.58	0.41
35.85967	-75.6678	0.50	0.74	0.25
35.80502	-75.6479	0.50	0.58	0.41
35.00698	-76.6014	0.49	0.73	0.26