

**NESTING ECOLOGY AND POPULATION GENOMICS OF AMERICAN  
BLACK DUCKS IN NORTH CAROLINA**

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment  
of the requirements for the degree of Master of Science in Wildlife Ecology

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## TABLE OF CONTENTS

LIST OF TABLES .....	vi
LIST OF FIGURES .....	viii
ABSTRACT .....	xi
Chapter	
1 NESTING ECOLOGY OF AMERICAN BLACK DUCKS IN COASTAL NORTH CAROLINA.....	1
Introduction .....	1
Study area .....	4
Methods .....	7
Results .....	13
Discussion.....	23
Management Implications .....	28
2 NESTING HABITAT SELECTION OF AMERICAN BLACK DUCKS IN COASTAL NORTH CAROLINA, WITH IMPLICATIONS IN FUTURE SEA LEVEL RISE .....	30
Introduction .....	30
Study area .....	32
Methods .....	34
Results .....	42
Discussion.....	51
Management Implications .....	55
3 MALLARD–BLACK DUCK HYBRIDIZATION AND POPULATION STRUCTURE IN COASTAL NORTH CAROLINA.....	56
Introduction .....	56
Study area .....	60
Methods .....	62
Results .....	67
Discussion.....	74
Management Implications .....	78
REFERENCES .....	79

Appendix

SLAMM GMSLR SIMULATIONS OF VARYING INTENSITY IN  
COASTAL NORTH CAROLINA, USA, 2019–2100 ..... 96

## LIST OF TABLES

Table 1	MCestimate® predictor of nest failure: abbreviation, definition, and data type of variables predicting American black duck nest success in coastal North Carolina, USA, 2017–2018. ....	12
Table 2	Mean nest initiation date (Julian Day), day of first nest initiation, last nest initiation, and 25th, 50th, and 75th quartiles of black ducks in coastal North Carolina, USA, 2017–2018. ....	15
Table 3	Mean initiation dates (Julian Day) and analysis of variance (ANOVA) of nesting black ducks in mainland marsh, natural island, and spoil-island habitats in coastal North Carolina, USA, 2017–2018. ....	16
Table 4	Contributions to American black duck nest fates (i.e. successful, abandoned, nonviable eggs, unknown, destroyed) from 2017–2018 with percent contributions for each category and sub-category (i.e. natural, investigator, avian, flooding, mammal, unknown) within Hyde and Dare counties of coastal North Carolina, USA. ....	18
Table 5	MCestimate 12-variable nest failure model rank, name, K, AICc, delta AICc, and weight of black ducks in coastal North Carolina, USA, 2017–2018. ....	20
Table 6	Marsh (natural-island and mainland marsh) nesting versus spoil-island nesting black duck incubation recess chronologies and sample sizes, recess duration, recess frequencies, recess duration time per day, and incubation constancy estimates for each.in Hyde and Dare counties of coastal North Carolina, USA, 2017–2018. ....	23
Table 7	SLAMM input parameters for the simulation of GMSLR for mainland Hyde and Dare Counties, North Carolina, USA, 2019–2100. Values for parameters with no specific local information were kept at the model default value. ....	40
Table 8	Macro-habitat description of searched area (ha), black duck nests found per habitat and nest density per habitat, coastal North Carolina, USA, 2017–2018. ....	44

Table 9	Spoil-island, natural island, and mainland marsh micro-habitat characteristics of nest sites (use). Group-wise ANOVA results are recorded for distance to open water (DOW), elevation, visual obstruction (VOR), vegetation height (Veg height), and ground cover relative percent composition. 2017–2018 Hyde and Dare Counties, North Carolina, USA. ....	45
Table 10	Natural island and mainland marsh micro-habitat characteristics of nest sites (use) and randomly sampled (non-use) points. T-test results are recorded for elevation, visual obstruction, vegetation height, and ground cover relative percent composition. 2017–2018 Hyde and Dare Counties, North Carolina, USA. ....	47
Table 11	Spoil-island micro-habitat characteristics of nest sites (use) and randomly sampled (non-use) points. T-test results are recorded for elevation, visual obstruction, vegetation height, and ground cover relative percent composition. 2017–2018 Hyde and Dare Counties, North Carolina, USA. ....	48
Table 12	Predicted change of regularly and irregularly-flooded marsh area and predicted number of black duck nests (assuming 1 nest per 122 ha in regularly-flooded marsh and 137 ha in irregularly flooded marsh) by 2100 given simulated scenarios of eustatic SLR in mainland Hyde County, mainland Dare County, and Outer Banks (made up of both Dare and Hyde Counties), North Carolina, USA. ....	50
Table 13	Simulation-based indices for “pure” black ducks, “pure” mallards, F1 hybrids, F2-black duck backcrosses, as well as F3-black duck backcrosses. Per index, assignment probabilities are based on the proportion of intra- and inter-specific assignment. Purity assignments based on percentage assigned to black duck populations. Location is coastal North Carolina with samples provided (n = 40). ....	71

## LIST OF FIGURES

Figure 1	American black duck nesting ecology study area encompassing Hyde and Dare Counties, N.C., USA, 2017–2018.....	5
Figure 2	American black duck nest locations in Hyde and Dare counties of coastal North Carolina, USA, 2017–2018.....	15
Figure 3	Bimodal distribution of American black duck nesting (Nest Counts by Julian Day) in Hyde and Dare counties of coastal North Carolina, USA, 2017.....	16
Figure 4	Unimodal distribution of American black duck nesting (Nest Counts by Julian Day) in Hyde and Dare counties of coastal North Carolina, USA, 2018.....	16
Figure 5	Peak nesting distribution of American black ducks (Nest Counts by Julian Day) in Hyde and Dare counties of coastal North Carolina, USA, 2017–2018.....	17
Figure 6	Daily nest survival (DSR) probabilities in relation to vegetation visual obstruction (m). MCestimate model of nest failure for nesting black ducks in coastal North Carolina, USA, 2017–2018.....	21
Figure 7	Daily nest survival (DSR) probabilities in relation to maximum vegetation height (m). MCestimate model of nest failure for nesting black ducks in coastal North Carolina, USA, 2017–2018.....	21
Figure 8	Daily nest survival (DSR) probabilities in relation to digital elevation reading (m). MCestimate model of nest failure for nesting black ducks in coastal North Carolina, USA, 2017–2018.....	22
Figure 9	Daily nest survival (DSR) probabilities in relation to nesting initiation date (Julian day). MCestimate model of nest failure for nesting black ducks in coastal North Carolina, USA, 2017–2018.....	22
Figure 10	ADMIXTURE assignment probabilities from $K$ of 5 analyses across reference and North Carolina American black ducks, mallard, game farm mallards, and hybrid samples. Location is coastal North Carolina with samples provided ( $n = 40$ ).....	69
Figure 11	Co-ancestry plot of North Carolina black duck samples ( $n = 40$ ). Sample relation pairings are estimated in Program COLONY, to determine full and half sibship.....	70

Figure 12	Autosomal DNA pairwise $\Phi_{ST}$ estimates for genetically vetted mallards, American black ducks, known game-farm mallards, and North Carolina ADMIXTURE assigned samples. 2017–2018. ....	72
Figure 13	Nucleotide Diversity ( $\pi$ ) among reference mallard, black duck, and game-farm mallard samples along with admixture assigned groups within coastal North Carolina.....	73
Figure 14	Haplotype Network of reference and North Carolina American black ducks, mallard, game farm mallards, and hybrids where graduated circles represent sample size and network lines represent genetic distance between and within the OW A and NW B mitochondrial haplogroups. ....	74
Figure 15	SLAMM habitat change predictions: Initial condition (2019) for mainland Hyde County, North Carolina, USA.....	97
Figure 16	SLAMM habitat change predictions: A1B Mean (2100) for mainland Hyde County, North Carolina, USA.....	98
Figure 17	SLAMM habitat change predictions: RCP 2.6 (2100) for mainland Hyde County, North Carolina, USA.....	99
Figure 18	SLAMM habitat change predictions: RCP 4.5 (2100) for mainland Hyde County, North Carolina, USA.....	100
Figure 19	SLAMM habitat change predictions: RCP 8.5 (2100) for mainland Hyde County, North Carolina, USA.....	101
Figure 20	SLAMM habitat change predictions: 1-m fixed (2100) for mainland Hyde County, North Carolina, USA.....	102
Figure 21	SLAMM habitat change predictions: Initial condition (2019) for mainland Dare County, North Carolina, USA.....	103
Figure 22	SLAMM habitat change predictions: A1B Mean (2100) for mainland Dare County, North Carolina, USA. ....	104
Figure 23	SLAMM habitat change predictions: RCP 2.6 (2100) for mainland Dare County, North Carolina, USA. ....	105
Figure 24	SLAMM habitat change predictions: RCP 4.5 (2100) for mainland Dare County, North Carolina, USA. ....	106

Figure 25	SLAMM habitat change predictions: RCP 8.5 (2100) for mainland Dare County, North Carolina, USA. ....	107
Figure 26	SLAMM habitat change predictions: 1-m fixed (2100) for mainland Dare County, North Carolina, USA. ....	108
Figure 27	SLAMM habitat change predictions: Initial condition (2019) for the Outer Banks, North Carolina, USA. ....	109
Figure 28	SLAMM habitat change predictions: A1B Mean (2100) for the Outer Banks, North Carolina, USA. ....	110
Figure 29	SLAMM habitat change predictions: RCP 2.6 (2100) for the Outer Banks, North Carolina, USA. ....	111
Figure 30	SLAMM habitat change predictions: RCP 4.5 (2100) for the Outer Banks, North Carolina, USA. ....	112
Figure 31	SLAMM habitat change predictions: RCP 8.5 (2100) for the Outer Banks, North Carolina, USA. ....	113
Figure 32	SLAMM habitat change predictions: 1-m fixed (2100) for the Outer Banks, North Carolina, USA. ....	114

## ABSTRACT

North Carolina represents the southernmost extent of the American black duck's (*Anas rubripes*) breeding range; however, little is known about their breeding ecology in this region. I located and monitored 140 nesting black ducks over two years (2017–18) to assess nesting productivity and quantify nesting habitat within coastal North Carolina. Specifically, I quantified nest initiation, peak nesting dates, nest success rates, and causes of failure. I further assessed genetic integrity and population structure using high-throughput DNA sequencing methods and identified *Anas platyrhynchos* × *A. rubripes* hybrids using ADMIXTURE hybrid filial generation thresholds. Nests were found in brackish marshes (n = 105, 75%) within the Pamlico Sound, and on man-made dredge spoil-islands (n = 35, 25%) along the Outer Banks. The average nest initiation date over the study was April 16 with a peak nesting date of May 7. Apparent nest success rates varied from 31% (2017) to 63% (2018). Predation rates were 42% in 2017 and 28% in 2018. Nest predators included crows (*Corvidae*), raccoons (*Procyon lotor*), American mink (*Neovison vison*), and bald eagles (*Haliaeetus leucocephalus*). Using MCEstimate, I found that the majority (73%) of variability in nest success was modeled best by nest location, vegetation density, maximum vegetation height, and elevation. Nesting black ducks selected high-marsh (NWI code E2EM1P) habitats where nests were located an average of 21.81 m from open water at mean elevation of 1.36 m. In these habitats visual obstruction readings were 0.5 m with a maximum mean vegetation height of 0.81 m mainly consisting of grass (84.61%). Nesting density was 1 nest per 22 ha. Under the threat of sea level rise, identified selected nesting habitat will increase under the most liberal simulations, allowing for 42 more black duck nests with the study area. Results from my genetic

analysis reveal that introgressive hybridization is occurring from the non-western game-farm mallard genome into this population of breeding black ducks. Breeding black duck management efforts in coastal North Carolina should focus on promoting selected nesting habitat, reducing nest predators, conserving lands for future marsh travel, and reducing game-farm mallard contact with black ducks.

## Chapter 1

# NESTING ECOLOGY OF AMERICAN BLACK DUCKS IN COASTAL NORTH CAROLINA

### Introduction

The American black duck (*Anas rubripes*, hereafter ‘black duck’) is a large species of dabbling duck endemic to eastern North America found within the Mississippi and Atlantic flyways. Black ducks breed along the Atlantic coast from eastern Canada to North Carolina and winter throughout the eastern United States and southern reaches of the Maritime Provinces (Baldassarre 2014). Historically, the black duck was the most numerous duck in the Atlantic flyway but is now a species of greatest conservation concern in 14 of the 17 states in the Atlantic flyway (Devers and Collins 2011) after dramatic declines (>50%) from the 1950’s through the 1990’s. There are several potential explanations for this decline, including overharvest (Nichols et al. 1987, Francis et al. 1998, Longcore et al. 2000), loss in the quantity and quality of breeding and non-breeding habitats, and interactions (competition, hybridization) with mallards (*Anas platyrhynchos*) (Anderson et al. 1987, Conroy et al. 2002). Since 1990, the black duck population has stabilized in the Atlantic Flyway (U.S. Fish and Wildlife Service 2019a). However, continued losses in breeding and non-breeding habitat, competition with mallards, and rising sea levels (Tiner 1987, Dahl 1990, Dahl 2000, Dahl 2006, Stedman and Dahl 2008, Dahl and Stedman 2013) now threaten the ability of the Atlantic Flyway to support populations at North American Waterfowl Management Plan (NAWMP) goals (Morton et al. 1989, North

American Waterfowl Management Plan Committee 2014). To enable targeted conservation goals, there is a need to quantify regional differences and factors influencing productivity (U.S. Fish and Wildlife Service 2008).

Black ducks breed in greatest concentrations in the North Atlantic Region (northern Atlantic U.S. states and eastern Canadian provinces) (Rusch et al. 1989) and apparent nest success (the proportion of successful nests calculated by simple division of successful nests/total nests) is highly variable (22–76 Reed 1975, Longcore et al. 1990, B elanger et al. 1997, Petrie et al. 2000). A smaller population of black ducks nest in the mid-Atlantic (Greene et al. 2004) and three studies conducted on islands in the Chesapeake Bay found apparent nest success ranged from 31–54% (Stotts and Davis 1960, Krementz et al. 1992, Haramis et al. 1996). Haramis et al. (1996) attributed nest failures to high spring tides while Krementz et al. (1992) attributed the majority of nest failures to depredations by avian predators, snakes, and humans. Stotts and Davis (1960) attributed black duck nest failures to avian predation and nest abandonment.

North Carolina is the southernmost extent of the mid-Atlantic (Greene et al. 2004) and the black duck’s breeding range (Stewart 1958, Parnell and Quay 1962, Bellrose 1980). However, black duck breeding ecology in this state is poorly understood. While breeding populations in Virginia and Maryland (Costanzo and Hindman 2007) have declined, there is no research to show if this trend is occurring in North Carolina. In 2016, the North Carolina Wildlife Resources Commission (NCWRC) began an operational breeding season black duck population survey to quantify annual abundance, distribution, and density of black ducks they identified in suitable nesting habitats within coastal marshes of North Carolina (NCWRC

unpublished data). Breeding season surveys conducted in 2016–2017 in North Carolina coastal marshes estimated 2,270–2,404 total black ducks with 694–1048 nesting pairs. There have been a few past attempts to quantify the nest selection and nest success of black ducks in North Carolina. Parnell and Quay (1962) reported black duck productivity on Pea Island, North Carolina, however they were only able to record one black duck nest in a thin stand of saltmeadow cordgrass (*Spartina patens*) just behind the ocean dunes. Fleming et al. (1996) located 12 nests within brackish needlerush (*Juncus spp.*) marsh on Piney Island, North Carolina and observed an apparent nest success of 67%. Wave action during a storm destroyed one nest, two were predated, and a predator killed one hen near her nest.

Although there have been ongoing efforts to understand the population limiting factors of mid-Atlantic (Greene et al. 2004) black ducks during the nonbreeding season (Cramer et al. 2012, Livolsi 2015, Ringelman et al. 2015), there is a need to better understand the breeding season limiting factors as well, especially in light of analyses that suggest long-term declines are occurring in recruitment (Brook 2009). Recognizing this, the Black Duck Joint Venture (a conservation partnership established in 1989 to stabilize and restore the population of the American black duck) has determined that there is a need to quantify regional differences and factors influencing black duck productivity. Because there is little knowledge about black duck nest success in coastal North Carolina, breeding population and habitat management decisions are limited. Further, in North Carolina, the U.S. Fish and Wildlife Service, North Carolina Forest Service and NCWRC regularly conduct prescribed burns within coastal marshes in early spring to reduce fuel loads, or to alter vegetative structure and composition. However, it is unclear if the timing of these

burns coincides with black duck nest initiation and peak nesting periods, therefore these management practices are uninformed and could potentially lead to direct losses of black ducks, their nests, and broods. Therefore, the goals of this research include quantification of 1) nest initiation and peak nesting dates to provide marsh burning timing guidelines, 2) nest success and causes of failure, including identification of nest predators, and 3) nesting constancy (the percentage of time the hen spends incubating nest) within the North Carolina breeding black duck population.

### **Study area**

I conducted research in Hyde and Dare counties within the Albemarle-Pamlico Peninsula and the Outer Banks region of North Carolina from late March to the end of June in 2017 and 2018 ([Figure 1](#)). Specifically, my research focal area ranged from the Pamlico and Pungo River convergence northeast along the Pamlico Sound to the intersection of US 64 and the Croatan Sound and throughout the Outer Banks of Hyde and Dare Counties. Study sites included Swanquarter National Wildlife Refuge (35°21'59.99" N -76°19'18.00" W), Alligator River National Wildlife Refuge (35°46'59.99" N -75°50'59.99" W), Pea Island National Wildlife Refuge (35°41'17.99" N -75°32'26.99" W), and private and state-owned lands along the Pamlico Sound. Study sites also included a series of man-made dredge spoil-islands (hereafter spoil-islands) located in a north-south direction from Wanchese to Oregon Inlet. These spoil-islands were created because of dredging operations that keep navigation channels open and the commercial fishing industry in Wanchese connected to the Atlantic Ocean through Oregon Inlet.

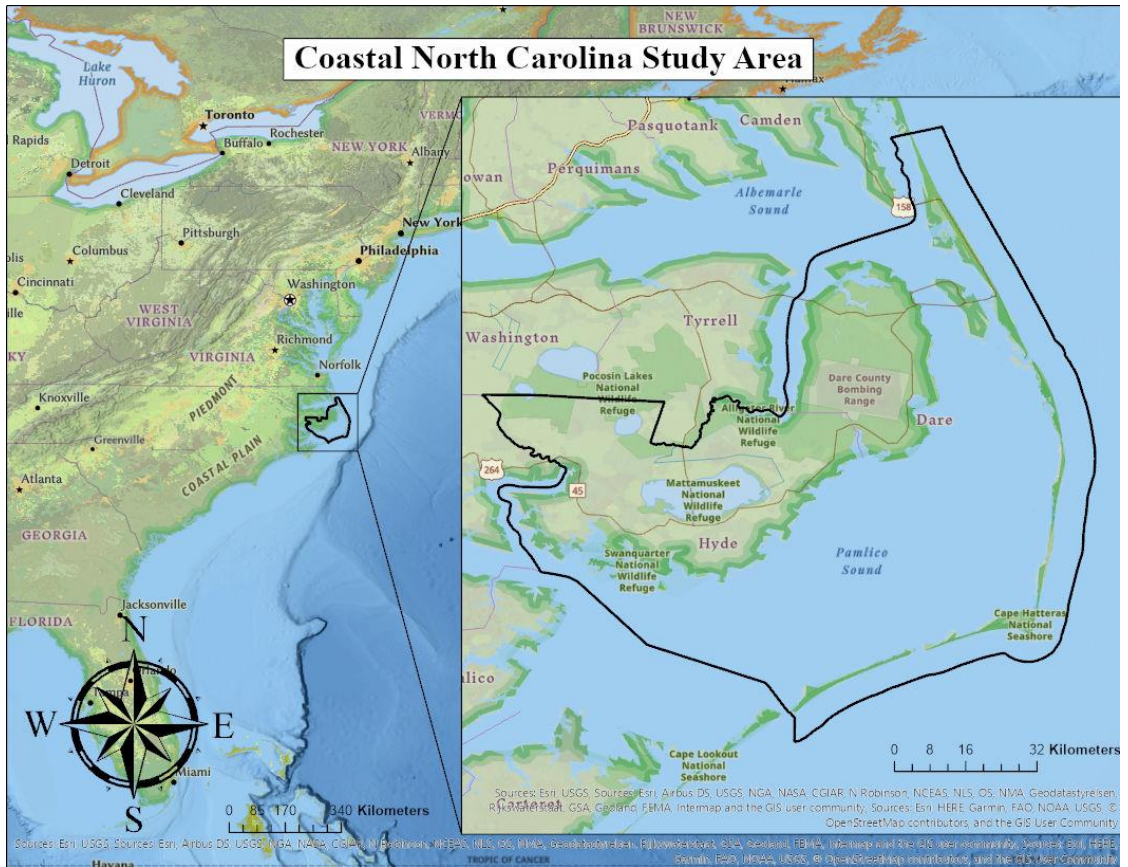


Figure 1 American black duck nesting ecology study area encompassing Hyde and Dare Counties, N.C., USA, 2017–2018

The Albemarle-Pamlico Peninsula was historically dense in wetland habitats but most of the wetlands were drained and converted to agricultural cropland (Devers and Collins 2011). Extant wetland habitats include large natural lake basins (including Lake Mattamuskeet which is the largest in North Carolina), river drainage systems of the Alligator, Pungo, and Long Shoal rivers, large contiguous blocks of semi-permanently flooded swamp, pocosins, and freshwater and brackish marshes. Freshwater lacustrine and riverine habitats in this region are dominated by panic grasses (*Panicum spp.*), cattail (*Typha spp.*), sawgrass (*Cladium spp.*), sedges (family

*Cyperaceae*), rushes (family *Juncaceae*), and duck weeds (*Lemna spp.*) and edges transition into mixed hardwood swamps. The dominant overstory is composed of red maple (*Acer rubrum*), black gum (*Nyssa sylvatica*), bald cypress (*Taxodium distichum*), and swamp tupelo (*Nyssa biflora*), or hardwood and mixed-pine habitats. Pocosins cover the freshwater-brackish water transition zone and consist primarily of pond pine (*Pinus serotina*), loblolly bay (*Gordonia lasianthus*), red bay (*Persea borbonia*), and sweet bay (*Laurus nobilis*), with an understory of titi (*Cyrilla racemiflora*), honeycup (*Zenobia pulverulenta*), fetterbush (*Lyonia lucida*), and gallberry (*Ilex coriacea*). Brackish marshes adjacent to the mainland and natural islands within the Pamlico Sound are low-lying and are primarily composed of black needlerush (*Juncus roemarianus*) with patches of cordgrasses (i.e. *Spartina patens* and *S. alterniflora*). The Outer Banks region within the study area is a barrier island complex bordered by Pamlico, Croatan, and Core sounds to the west and the Atlantic Ocean to the east (Devers and Collins 2011). Within this area, Pea Island National Wildlife Refuge is a 20-km long, narrow island with a 91–244 m wide line of beach dunes on the island's eastern border, several large submerged aquatic vegetation (SAV) impoundments at its core, and extensive brackish marsh dominated by black needlerush, saltmeadow cordgrass, and sawgrass, on its western border. The remaining portion of this region, including the spoil-islands, contains beach dunes (composed of sea oats [*Uniola paniculata*], panic grasses, and seaside bluestem [*Schizachyrium littorale*]); maritime shrub and dry grassland (e.g. wax myrtle [*Morella cerifera*], red cedar [*Juniperus virginiana*], yaupon holly [*Ilex vomitoria*], greenbrier [*Smilax spp.*], blackberry [*Rubus spp.*], poison ivy [*Toxicodendron radicans*], and saltmeadow

cordgrass), and various early successional habitats. Typically, older spoil-islands are at a more advanced successional stage.

### **Methods**

Technicians and I searched for black duck nests from March 24–June 23, 2017–2018. In 2017, I began my search efforts in what NCWRC biologists identified as suitable coastal marsh nesting habitats, as determined via breeding black duck surveys (NCWRC unpublished data). In 2017, initial searches were conducted systematically then became more focused in 2018. To increase nest sample size and more accurately address research objectives, I high-graded searches after developing a search image of nesting habitat cues. I conducted nest searches in the mornings as hens are more likely to attend nests during morning hours (Gloutney et al. 1993). To limit hen disturbance and possible nest abandonment, I did not nest search in inclement weather. To search for nests, I hand dragged a 30.5 m rope across potential nest locations. I attached aluminum cans with rocks inside at ~2 m intervals along the entire length of the rope to serve as noisemakers (modified from all-terrain vehicle chain drags used for upland nesting waterfowl, Klett et al. 1986). A third technician acted as a spotter to identify flushing hens and the exact location and distance that the hen flushed from the nearest investigator. In areas where I could not drag the rope due to vegetation height and rigidity, technicians distanced themselves 5–25 m apart and walked transects of the selected area.

Once I identified an active nest (i.e. nests between laying and hatching) location, one technician approached the nest and initiated data collection while the other remained >5 m away and recorded data. To mark nest locations, I attached flagging ribbon to a nearby (10–15 m) piece of rigid vegetation and recorded GPS

(Garmin GPSmap 62s) coordinates of the nest. I monitored nests at 7–10 day intervals either when the incubating adult was located away from the suspected nest site or by flushing the hen from the nest (Weller 1956). I estimated incubation date based on candling (Weller 1956) and floating of eggs (Westerskov 1950). When nests were inspected, I recorded hen status and condition of nest (i.e. hen absent–nest successful, hen absent–eggs warm/covered, hen absent–eggs warm/uncovered, hen absent–eggs cold/covered, hen absent–eggs cold/uncovered, hen absent–all eggs missing/destroyed, hen present–alive, and hen present–dead), nest status (i.e. normal, investigator damage, partly/totally destroyed by predator, or eggs missing), number of eggs in nest, and incubation stage. I recorded hen status based on several criteria: hen presence at the nest upon approach, hen vitality (i.e. alive vs. dead) if present, presence or absence of down lining covering eggs, and relative temperature of eggs (i.e. warm vs. cold). I determined nest status based on visual evidence of predation (e.g. small fragmented or large parts of eggshell without chorioallantoic membranes present), missing eggs, and normality (i.e. eggs present, unharmed, and continuing in incubation). At the conclusion of every visit, I covered the nest bowl with down and other present nest materials to mimic a normal nest departure (Götmark 1992). I monitored selected nests (i.e. all nests in active incubation with sufficient cover) with trail cameras (Reconyx® Hyperfire HC600) set to take photos on one-minute intervals to aid in determining nest fate (including identification of predating species), assessing nesting behavior, and measuring incubation constancy.

I returned to nests every 7–10 days until the nest was terminated (i.e. successful, abandoned, destroyed, nonviable, or unknown). Nests where  $\geq 1$  egg hatched, denoted by presence of detached shell membranes and yellowish feather

sheaths or small egg fragments without membranes in nest material, or presence of ducklings in the nest bowl, were defined as successful (Klett et al. 1986). At the conclusion of monitoring visits, I covered the nest bowl with down and other present nest materials and made a distinct “X” on top with two pieces of contrasting vegetation to determine if the hen returned to the nest between subsequent visits. At the previous nest visit where the hen was noted as present or the eggs were warm, persistence of the “X” at the subsequent visit confirmed nest abandonment was investigator-induced. Likewise, I used the progression of embryonic development and the “condition of the nest” to determine if the hen was still actively incubating the nest or if a natural abandonment (e.g. abandoned due to partial depredation, hen mortality, or other unknown factors) had occurred. Nests were fated as “naturally” abandoned if the incubation stage did not progress according to the span between nest visits and if the eggs were cold/uncovered or cold/covered without persistence of the “X”. Nest predators were determined via trail camera when present and according to predation characteristics of waterfowl nest predators according to Rearden (1951) when trail cameras were not present or did not capture the nest predator. Other causes of destroyed nests (i.e. investigator, machinery, flooding, or other) were determined by trail cameras when present and/or by evidence at the nest (e.g. ATV tracks or standing water) when trail cameras were not present or did not capture the cause of destruction. I fated nests as nonviable when eggs addled or did not progress past day zero under active hen incubation.

I quantified 4<sup>th</sup> order habitat characteristics (Johnson 1980) at all terminated nest sites to assess their impact on nest success. Technicians and I measured ground cover (i.e. relative percentages of bare ground, grass, forbs, water, woody vegetation,

and litter that summed to 100%) within a 50 x 50 cm sampling Daubenmire frame (modified from Daubenmire 1959) centered on the nest bowl. I also measured the maximum height of vegetation directly over the nest and distance (m) from the nest to open water. I placed a visual obstruction pole (Robel et al. 1970) in the nest bowl to determine the lowermost 0.1 m stratum that was  $\geq 50\%$  visible through existing vegetation. Visual obstruction readings (VORs) were collected 3.5 m from the pole in each cardinal direction, and the mean of the four measurements was defined as a single VOR for each nest (Varner et al. 2013). Further, I quantified presence of down in the nest bowl (i.e. 0 = absent, 1 = present), National Wetland Inventory (NWI) habitat classification, elevation at the nest (i.e. m above NAVD88 vertical datum), nest location (i.e. mainland marsh, natural island, or spoil-island), distance to anthropogenic disturbance (i.e. distance to primary and secondary roads), year of study, age of nest when found (i.e. day after incubation initiation), and nest initiation date (i.e. the day the first egg was laid) to explain nest success variability in program MCestimate (Etterson 2014).

First, I compiled nest initiation and peak nesting chronologies over the 2017 and 2018 nesting seasons. I estimated laying dates for all nests by backdating (Klett et al. 1986), and quantified the 25<sup>th</sup>, 50<sup>th</sup>, and 75<sup>th</sup> quantiles along with peak initiation date and date of earliest and latest initiation. Similarly, I determined peak nesting dates by calculating gaussian peak (SPSS®, Version. 26.0. IBM Corp. Armonk, NY. 2018). I compared mean initiation dates across years via pooled t-test ( $P < 0.05$ ; SPSS®, Version. 26.0. IBM Corp. Armonk, NY. 2018). Additionally, I ran an analysis of variance (ANOVA; SPSS®, Version. 26.0. IBM Corp. Armonk, NY. 2018) to

determine if mean initiation dates were significantly different between mainland marsh, natural islands, and spoil-islands.

Second, I estimated apparent and Mayfield (1961, 1975) nest success to compare to previous black duck nesting studies in other states and provinces in the Atlantic Flyway. Following Beauchamp et al. (1996a), I defined apparent nest success as the proportion of successful nests and is calculated by simple division of successful nests/total nests. The Mayfield nest success estimator calculates nest success by using the number of days that a nest is monitored (i.e., exposure period) to account for and to reduce bias associated with differential detection probabilities. Additionally, I derived binomial success estimates in program MCestimate (Etterson 2014), where daily probabilities of nest failure are calculated using the Markov chain algorithms described by Etterson et al. (2007). I calculated daily survival rates (DSR) in MCestimate as a function of the null model. Daily survival rates were based on multinomial logit scale estimates and subsequent fitted probabilities corresponding to the values of covariates in the model (Etterson 2014). I derived overall nest success estimates by extrapolating DSR's over the length of the nest cycle. To determine nest cycle length, I averaged full clutch sizes and incubation lengths of observed black ducks. Finally, I reported standard error of the fitted probability (calculated via the delta method, Seber 1982) and lower and upper confidence limits of the parameter (Sambamoorthi et al. 1994) for both survival estimates. Sample sizes varied among analyses because of different criteria used in selecting samples (Klett et al. 1986). I excluded nests from nest success analyses when the eggs were destroyed or were abandoned due to clear observer disturbance (Gloutney et al. 1993).

Third, using program MCEstimate (Etterson 2014) and Akaike’s Information Criteria (AICc) model selection theory (Burnham and Anderson 2002), I developed and tested a series of 25 a priori competing models of black duck nest failure as a function of 12 variables including: visual obstruction readings (m), maximum vegetation height (m), and relative grass composition (%) taken at the nest, distance to open water from the nest (m), presence of down in nest bowl (i.e. 0 = absent, 1 = present), National Wetland Inventory classification, elevation at the nest (i.e. m above NAVD88 vertical datum), nest location (i.e. mainland marsh, natural islands, or spoil-islands), distance to anthropogenic disturbance (i.e. distance to primary and secondary roads), year of study, age of nest when found (i.e. day after incubation initiation), and nest initiation date ( i.e. the day the first egg was laid) ([Table 1](#)).

Table 1 MCEstimate® predictor of nest failure: abbreviation, definition, and data type of variables predicting American black duck nest success in coastal North Carolina, USA, 2017–2018.

Abbreviation	Covariate Definition	Type
.	Null	
VEGD	Visual Obstruction Reading at Nest	Continuous
VEGH	Maximum Vegetation Height at Nest	Continuous
VEGC	Relative Ground Cover Composition at Nest	Continuous
DOW	Distance to Open Water from Nest	Continuous
DWN	Presence of Down in Nest Bowl (No=0, Yes=1)	Categorical
ATEN	Proportion of observed time spent incubating	Continuous
HAB	National Wetland Inventory	Categorical
DEM	Digital Elevation Model Value	Continuous
LOC	Location: Mainland Marsh, Natural Island, and Spoil-island	Categorical
ANT	Distance to Anthropogenic Disturbance	Continuous
YEAR	Year of Study (2017=0 and 2018=1)	Categorical
AGE	Age of Nest When Found	Continuous
INIT	Nest Initiation Date (Julian Date)	Continuous

I used parametric bootstrap algorithms with 100 iterations to assess goodness-of-fit of top-ranking models of nest failure ( $\Delta AICc \leq 2$ ). Further, if multiple models were plausible, I calculated a weighted average of parameter estimates using the zero method (Burnham and Anderson 2002) and interpreted the direction (positive or negative) and magnitudes (effect sizes) in relation to one another (Grueber et al. 2011).

Finally, I estimated incubation constancy according to Skutch (1962) and defined it as the percentage of time the hen spends incubating the nest. I derived nest attentiveness estimates on all black duck nests that had  $\geq 7$  days of trail camera records logged at one-minute intervals within incubation time parameters (i.e. nest attendance between conclusion of egg-laying and nest fate). I assessed the effect of nesting constancy on nest success separately from the previous models because including the nest attentiveness estimate reduced the sample size of qualifying nests for modeling. Additionally, I compared incubation constancy estimates of marsh (i.e. nests on mainland marsh and natural islands) and spoil-island nesting black ducks via pooled t-test ( $P \leq 0.05$ ; SPSS®, Version. 26.0. IBM Corp. Armonk, NY. 2018).

## Results

During the 2017 and 2018 breeding seasons, 140 black duck nests were found on spoil-islands ( $n = 35$ , 25%), natural islands ( $n = 54$ , ~39%), and in mainland marshes of the Pamlico Sound ( $n = 51$ , ~36%) ([Figure 2](#)). I monitored nests from 8 April until 23 June in 2017 and from 24 March until 14 June in 2018. The earliest nest initiation dates varied by 6 days across the years surveyed ([Table 2](#)). The earliest mean nest initiation date for both years was 28 February (Julian day (JD) = 59). I observed a bimodal pattern of nest initiation in 2017 ([Figure 3](#)), but a more unimodal pattern in

2018 (Figure 4). Mean nest initiation was 22 April in 2017 (JD = 111.68, 95% CI = 104.96–118.40) and 12 April in 2018 (JD = 101.71, 95% CI = 97.69–105.73) and were significantly different ( $t_{80} = -2.48, P < 0.01$ ). Mean nest initiation date was also significantly different ( $F_{136} = 22.49, P < 0.01$ ) across mainland marsh, natural island, and spoil-island habitats with a mean initiation date of 28 March (JD = 87.26) on spoil-islands and mean initiation dates of 23 April (JD = 113.04) and 18 April (JD = 108.63) for mainland marsh and natural islands, respectively (Table 3). In 2017, nest initiation peaked in the first week of April, followed by a 41% decline in initiation effort which spanned 9 days (Julian day 105–114), followed by another initiation peak in mid-May (Julian day 130–140). Spring floods in the mainland marshes and natural islands caused by heavy rain and high wind-tides coincided with the observed 41% decline in 2017. The latest nest initiation dates varied by 1 day across years; 25 May (JD = 145) was the mean initiation date in the last nests. Backdating revealed that 25% of all nests had initiated by 2 April (JD = 92), 50% by 13 April (JD = 103), and 75% by 4 May (JD = 124, Table 2). Mean nesting season length was 110 days with a peak on 26 April (JD = 116, 95% CI = 115–117, Figure 5). Mean clutch size was 9.86 eggs (95% CI = 9.76–9.96) with an average incubation period of 26.38 days (95% CI = 26.31–26.45). Therefore, nest success calculations were based on a 36-day survival period (10 egg-days + 26 incubation days).

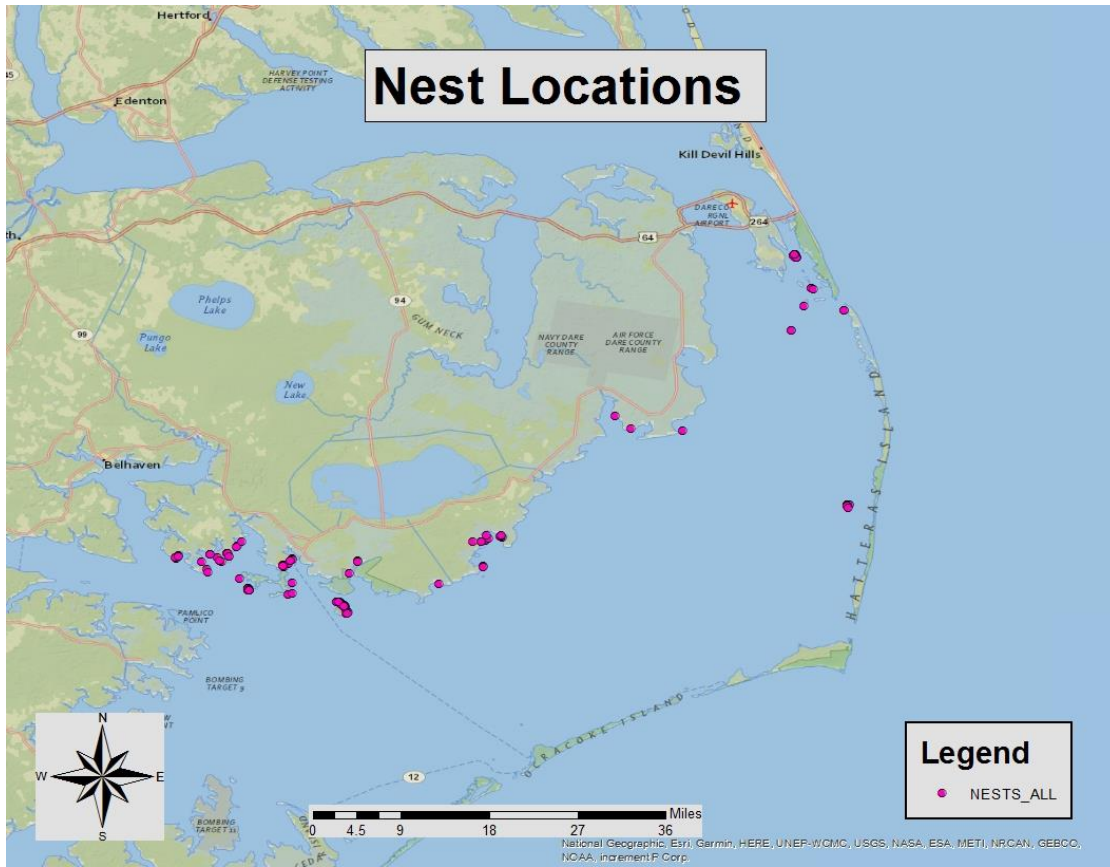


Figure 2 American black duck nest locations in Hyde and Dare counties of coastal North Carolina, USA, 2017–2018.

Table 2 Mean nest initiation date (Julian Day), day of first nest initiation, last nest initiation, and 25th, 50th, and 75th quartiles of black ducks in coastal North Carolina, USA, 2017–2018.

Year	Peak	Initiation of first nest	Initiation date quartiles			Initiation of last nest
			25 %	50 %	75 %	
2017	111.68	62	93	117	131	144
2018	101.71	56	91	101	117	145
Mean	105.00	59	92	109	124	145

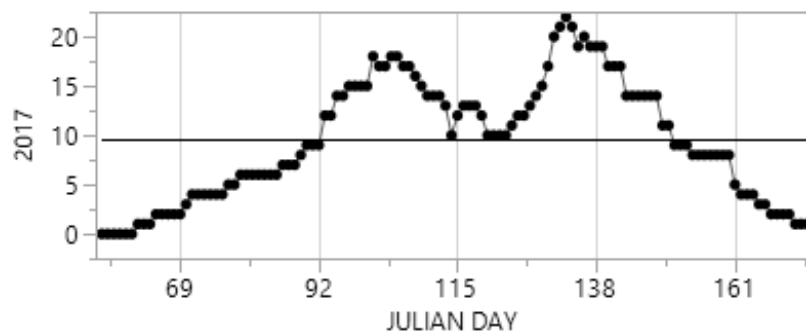


Figure 3 Bimodal distribution of American black duck nesting (Nest Counts by Julian Day) in Hyde and Dare counties of coastal North Carolina, USA, 2017.

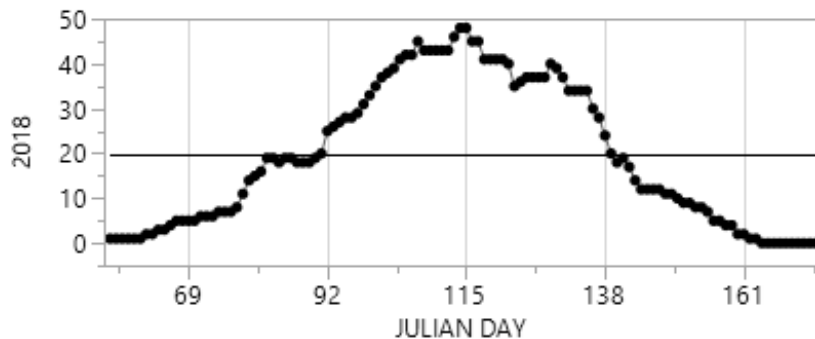


Figure 4 Unimodal distribution of American black duck nesting (Nest Counts by Julian Day) in Hyde and Dare counties of coastal North Carolina, USA, 2018.

Table 3 Mean initiation dates (Julian Day) and analysis of variance (ANOVA) of nesting black ducks in mainland marsh, natural island, and spoil-island habitats in coastal North Carolina, USA, 2017–2018.

Mean Initiation Day + SE			<i>F</i> -test
Mainland Marsh ( <i>n</i> = 50)	Natural Island ( <i>n</i> = 54)	Spoil-island ( <i>n</i> = 35)	
113.04 ± 2.84	108.63 ± 2.20	87.26 ± 3.12	$F_{136} = 22.49, P < 0.01$

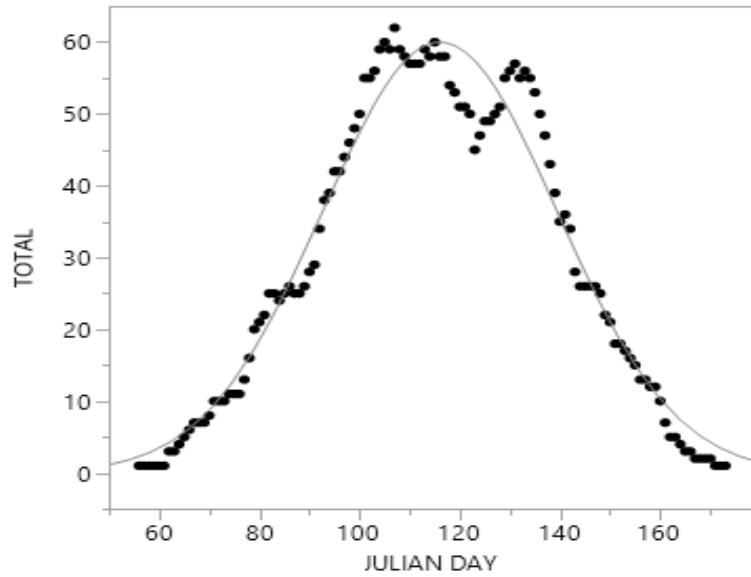


Figure 5 Peak nesting distribution of American black ducks (Nest Counts by Julian Day) in Hyde and Dare counties of coastal North Carolina, USA, 2017–2018.

Predation was the leading cause of natural nest failure for both years (Table 4). Nest predators included raccoon (*Procyon lotor*), mink (*Neovison vison*), American crow (*Corvus brachyrhynchos*), fish crow (*Corvus ossifragus*), bald eagle (*Haliaeetus leucocephalus*), red imported fire ant (*Solenopsis invicta*), eastern rat snake (*Pantherophis alleghaniensis*), and corn snake (*Pantherophis guttatus*). However, I also report a high occurrence of investigator-induced abandonments ( $n = 38$ ,  $\bar{x} = 27.14\%$ ), which occurred during the egg-laying period and early stages of incubation. These nests were not included in any estimates of nest success. The number of clutches with nonviable eggs and the number of nests of unknown fate were minimal ( $n = 4$ ,  $\bar{x} = 2.86\%$ ). However, I did observe two separate occasions where nests contained at least one runt egg (i.e. an abnormally small egg). All runt eggs observed

were nonviable. Nests that failed due to flooding occurred in 2017 over the course of two days when flood water inundated the brackish marshes of Pamlico Sound.

Table 4 Contributions to American black duck nest fates (i.e. successful, abandoned, nonviable eggs, unknown, destroyed) from 2017–2018 with percent contributions for each category and sub-category (i.e. natural, investigator, avian, flooding, mammal, unknown) within Hyde and Dare counties of coastal North Carolina, USA.

Fate	Year		Total	Percent Contribution
	2017	2018		
Successful	9	39	48	34.29
Abandoned	18	34	52	37.14
Natural	8	6	14	(10.00)
Investigator	10	28	38	(27.14)
Nonviable eggs	3	1	4	2.86
Unknown	2	0	2	1.43
Destroyed	15	19	34	24.28
Avian	5	2	7	(5.00)
Flooding	4	0	4	(2.86)
Mammal	4	15	19	(13.57)
Unknown	2	2	4	(2.86)
Total found	47	93	140	100.00

Apparent nest success varied with year (2017 = 29.16%, 2018 = 77.55%,  $\bar{x}$  = 61.64%) and nest location (mainland marsh = 20%, natural- and spoil-islands = 77.36%). Mayfield and MCestimate nest success estimates were based on 1534 exposure days (218 observations,  $\bar{x}$  duration =  $7.04 \pm 1.05$  days) between 73 nests. Daily survival rates (DSR) were 0.9780 and 0.9806 (SE = 0.0036, 95% CI = 0.9720-0.9866) for Mayfield and MCestimate methods, respectively. Overall nest success estimates ( $\hat{S} \pm SE$ ), based on a 36-day survival period, were three times lower in 2017 ( $22.85 \pm 8.24\%$ ) than in 2018 ( $67.60 \pm 7.99\%$ ). Further, mainland nests ( $15.35 \pm$

7.24%) were less successful than natural- and spoil-island nests ( $67.94 \pm 7.59\%$ ) across years.

Nest failure ( $n = 71$ , exposure days = 1,360) was predicted by three plausible models (Table 5, summed model weight = 0.73) with six variables including: LOC (Location: mainland marsh, natural island, and spoil-island), VEGD (Visual obstruction reading), VEGH (Maximum vegetation height), DEM (Digital elevation model value), YEAR (Year of study, 2017=1 and 2018=2), and INIT (Nest initiation date) represented by the model averaged equation:

$$\text{fail} = -2.1365 (\text{LOC: natural island, SE} = 1.0087) - 0.8032 (\text{LOC: spoil-island, SE} = 1.2505) - 1.0198 (\text{VEGD, SE} = 0.8073) + 0.8809 (\text{VEGH, SE} = 0.4808) - 0.0549 (\text{DEM, SE} = 0.0700) - 0.1988 (\text{YEAR: 2, SE} = 0.1172) - 0.0004 (\text{INIT, SE} = 0.0030) - 2.8587 (\text{SE} = 0.8164)$$

Failure was negatively correlated with nest location, where spoil-island had the strongest and mainland marsh had the weakest negative correlation. Additionally, visual obstruction had a high negative correlation ([Figure 6](#)), maximum vegetation height had a moderate positive correlation ([Figure 7](#)), and elevation ([Figure 8](#)), year 2 (2018), and nest initiation date ([Figure 9](#)) had a weak negative correlation with nest failure.

Table 5 MCEstimate 12-variable nest failure model rank, name, K, AICc, delta AICc, and weight of black ducks in coastal North Carolina, USA, 2017–2018.

Rank	Model Name <sup>a</sup>	K	AICc	$\Delta$ AICc	Weight
1	fail(LOC+VEGD+VEGH+DEM)	6	138.61	0	0.37
2	fail(LOC)	3	139.94	1.33	0.19
3	fail(LOC+YEAR+INIT)	5	140.2	1.59	0.17
4	fail(VEGC+LOC+VEGH)	5	141.81	3.2	0.07
5	fail(Global)	18	142.57	3.96	0.05
6	fail(DWN+LOC+YEAR+INIT+DEM+VEGD)	8	142.59	3.98	0.05
7	fail(LOC+YEAR+DWN+HAB)	10	142.87	4.26	0.04
8	fail(LOC+YEAR+INIT+VEGD+VEGC)	7	143.69	5.08	0.03
9	fail(DWN+LOC+INIT)	5	143.72	5.11	0.03
10	fail(VEGD)	2	151.16	12.54	0
11	fail(DWN)	2	151.3	12.69	0
12	fail(YEAR)	2	151.71	13.09	0
13	fail(VEGD+VEGC)	3	152.19	13.58	0
14	fail(VEGD+VEGH+HAB)	8	153	14.38	0
15	fail(DEM)	2	155.25	16.64	0
16	fail(DEM+INIT)	3	156.44	17.83	0
17	fail(INIT)	2	156.49	17.88	0
18	fail(.)	1	157.48	18.87	0
19	fail(HAB)	6	157.86	19.25	0
20	fail(DOW+HAB)	7	158.8	20.19	0
21	fail(ANT)	2	158.83	20.22	0
22	fail(VEGC)	2	159.09	20.48	0
23	fail(DOW)	2	159.14	20.53	0
24	fail(VEGH)	2	159.35	20.74	0
25	fail(AGE)	2	159.49	20.87	0

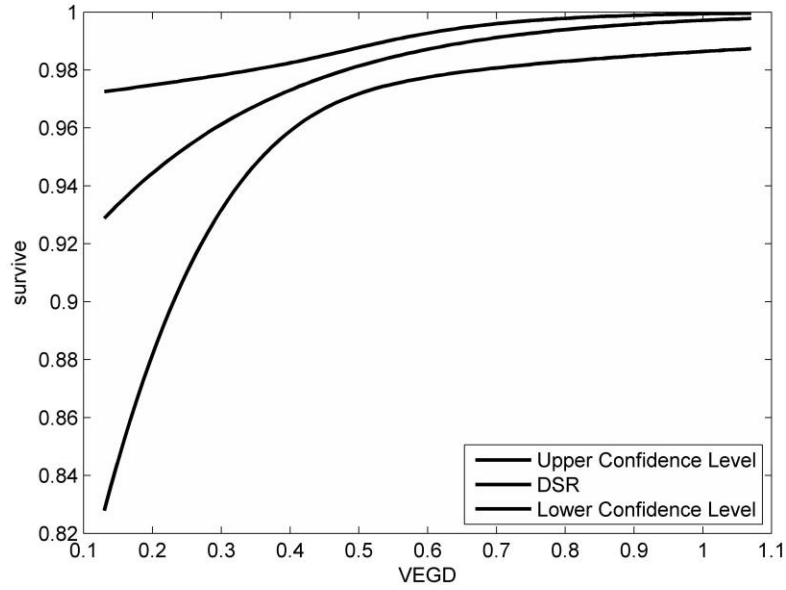


Figure 6 Daily nest survival (DSR) probabilities in relation to vegetation visual obstruction (m). MCEstimate model of nest failure for nesting black ducks in coastal North Carolina, USA, 2017–2018.

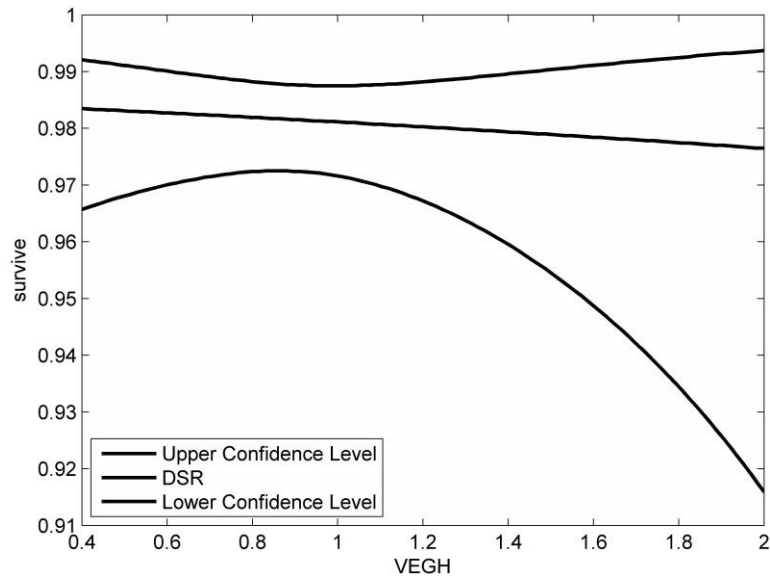


Figure 7 Daily nest survival (DSR) probabilities in relation to maximum vegetation height (m). MCEstimate model of nest failure for nesting black ducks in coastal North Carolina, USA, 2017–2018.

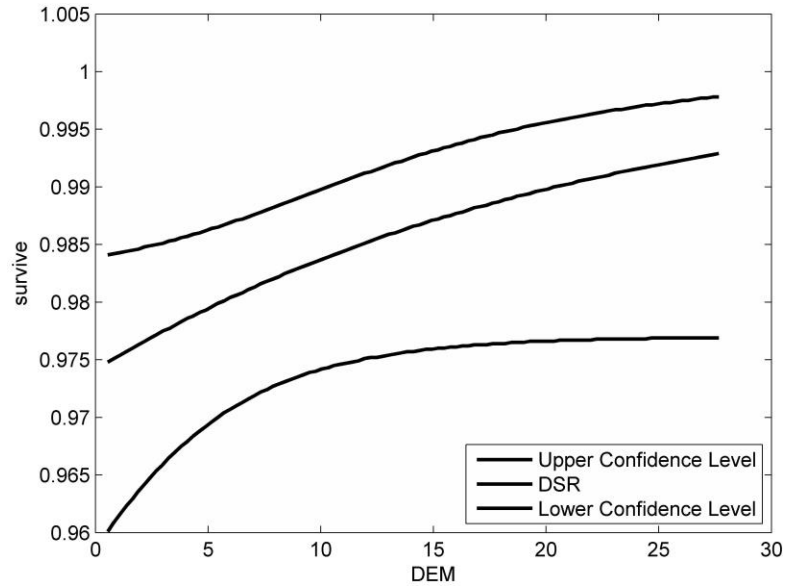


Figure 8 Daily nest survival (DSR) probabilities in relation to digital elevation reading (m). MCEstimate model of nest failure for nesting black ducks in coastal North Carolina, USA, 2017–2018.

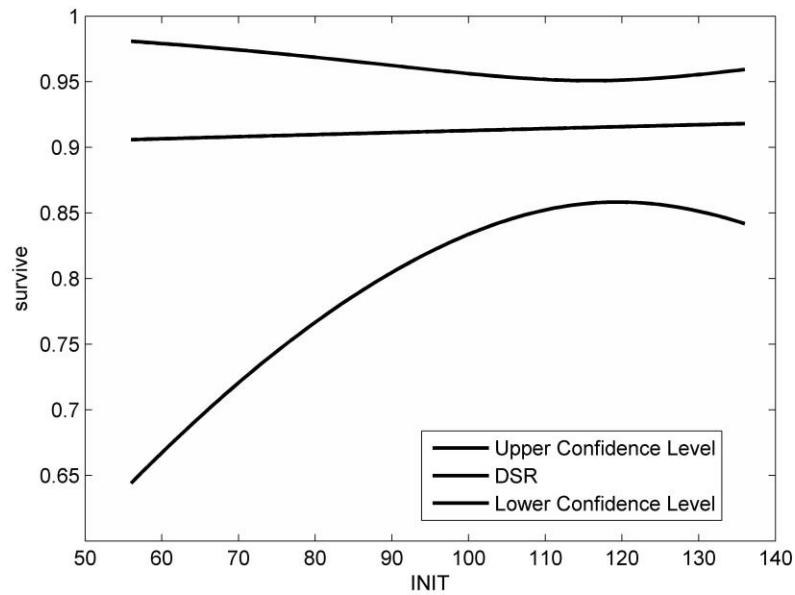


Figure 9 Daily nest survival (DSR) probabilities in relation to nesting initiation date (Julian day). MCEstimate model of nest failure for nesting black ducks in coastal North Carolina, USA, 2017–2018.

To determine incubation rhythms for black ducks in North Carolina, I monitored 39 nests via trail cameras (Reconyx<sup>®</sup> Hyperfire HC600; 1-minute recording intervals) for a combined total of 552 exposure days. I estimated mean incubation constancy (Skutch 1962) at 83.47% (95% CI = 81.81–85.11%). Hens averaged 1.71 recesses/day with a mean recess duration of 143.41 min (95% CI = 137.53–149.28 min). Natural island- and mainland marsh-nesting hens took more frequent and longer recesses than did spoil-island-nesting hens resulting in significantly different nesting constancy estimates ( $t_{37} = -6.85, P < 0.01$ , [Table 6](#)). Additionally, I remodeled nest failure as a function of the preceding 12 variables but included nesting constancy (ATEN); however, sample size was greatly reduced. Inclusion of this variable did not change the top ranked models of nest failure.

Table 6 Marsh (natural-island and mainland marsh) nesting versus spoil-island nesting black duck incubation recess chronologies and sample sizes, recess duration, recess frequencies, recess duration time per day, and incubation constancy estimates for each in Hyde and Dare counties of coastal North Carolina, USA, 2017–2018.

N	Nest location	Days monitored	Recess $\pm$ SE		Duration time/day (min)	Incubation constancy %
			Duration (min)	Frequency (N/day)		
23	Marsh	314	144.23 $\pm$ 5.58	1.89 $\pm$ 0.08	272.07	79.97
16	Spoil-island	237	123.41 $\pm$ 6.44	1.47 $\pm$ 0.08	181.42	87.74

## Discussion

Unlike black duck nesting studies in the Chesapeake Bay where mean nest initiation was April 26 (range 23 March - 10 June; Stotts and Davis 1960; Krementz et al. 1991, 1992), black ducks in North Carolina initiated nests 11 days earlier, from 28

February until 25 May, (range = 86 days) with a mean initiation date of April 15 (JD = 105, SE = 1.8). Additionally, comparing my findings to nesting black ducks in eastern Canada, where the mean initiation date was May 1 (JD = 121, SE = 6.7; Bélanger et al. 1997), it appears that black ducks initiate nests following a latitudinal photoperiod gradient (Murton and Kear 1973) in conjunction with location-specific subsidiary factors (Farner and Lewis 1971, Wingfield and Farner 1980).

Krementz et al. (1992) observed a bimodal pattern of egg laying in black ducks nesting in the Chesapeake Bay. They attributed this pattern to renesting effort or later nesting initiation by first year females. I observed a bimodal pattern of nest initiation in 2017 ([Figure 3](#)) that coincided with flooding due to heavy rain and high wind-tides. Stotts and Davis (1960) found that flooding of a nest, even if not destroyed, usually caused the hen to abandon the nest. The flooding observed in 2017 destroyed all nests located in the mainland marshes monitored during this period and additionally damaged available nesting cover. Therefore, marsh flooding could set back initiation and result in a bimodal distribution resembling a natural renesting or a delayed first-year hen nesting effort. Confirming this, there were no significant spring floods in 2018 nor a subsequent bimodal pattern in nest initiation.

I found significant differentiation in mean nest initiation date across mainland marsh, natural islands, and spoil-islands ([Table 3](#)). Mean initiation on spoil-islands occurred  $\geq 21$  days earlier than mean initiation in natural island and mainland marsh habitats. This finding could be consistent with a bimodal pattern of nest initiation, where older hens initiate nests earlier than first-year nesting hens (Krementz et al. 1992). Specifically, older hens may be selecting to nest on the few spoil-islands, which are immune to overwash and high spring tides, at the beginning of the breeding

season, thereby forcing the less-experienced, young hens to nest on natural islands and in mainland marsh habitats. Alternatively, early selection of spoil-island habitats could be an indication of optimal vegetation height and density. Frequent winter flooding events could alter residual perennial vegetation in natural island and mainland marsh habitats making them unsuitable for nesting until spring growth commences and an optimal vegetation structure is reached.

I estimated apparent mean nest success at ~62%, which is comparable to the findings of Fleming et al. ( $\bar{x} = 67\%$ ; 1996) for black ducks in coastal North Carolina. Chesapeake Bay studies estimated apparent mean nest success at 31–55% ( $\bar{x} = 38.68$ ; Stotts and Davis 1960, Krementz et al. 1992, Haramis et al. 1996), and in eastern Canada, apparent mean nest success ranged from 22–42% ( $\bar{x} = 39.05$ ; Reed 1975, Bélanger et al. 1997, Petrie et al. 2000). Considering nest success as the only measure of reproductive productivity, black duck nest success in coastal North Carolina is comparable, if not greater, than the rest of its Atlantic Flyway breeding range. However, nest success alone is insufficient in determining local recruitment (Cowardin and Blohm 1992). Thus, future research in this area should be aimed at estimating nesting fecundity and propensity, and brood and hen survival.

The results of nest failure modeling indicated that variability in nest failure is best represented by nest location (i.e. mainland marsh, natural island, and spoil-island), vegetation visual obstruction, maximum vegetation height, elevation, year, and nest initiation date. Nest location was the strongest driver as it had the highest beta values and was in all top models. This is consistent with the observed disparity in apparent nest success estimates of mainland marshes ( $\bar{x} = 15.35\%$ ) and natural- and spoil-islands ( $\bar{x} = 67.94\%$ ). Unlike natural- and spoil-islands, mainland marshes have a

high land-based predation rate. This is best explained by the apparent accessibility of the mainland marshes to predators like the raccoon. However, location alone does not fully explain nest failure variability. Represented by the model, as the visual obstruction of nesting vegetation increases, so does nest success (Figure 6). Increased vegetation visual obstruction certainly contributes to concealment of the nest from both aerial and ground-based predators. Elevation of the nest also increases nest success. Spring marsh floods are prominent in coastal North Carolina, thus, nest success likely increases when nest elevation exceeds regular overwash elevation.

Flooding accounted for ~7% of observed nest failures during my study, compared to Chesapeake Bay studies which estimated ~4% (Stotts and Davis 1960), ~4% (Krementz et al. 1991), and ~38% (Haramis et al. 1996). Consistent with other observations of marsh-nesting black ducks, I noted that nests located in flood-prone spots, brackish marsh especially, were slightly elevated (15–30 cm) structures that consisted of needlerush or *Spartina spp.* leaves and resembled overwater-nesting diving duck nests. Additionally, trail camera monitoring allowed us to document two hens elevating nests prior-to and during marsh floods in 2017.

Unlike black duck nesting studies in the Chesapeake Bay (Stotts and Davis 1960; Krementz 1991, 1992; Haramis 1996), I attributed more total predations to raccoons (60%) than to crows (~17%). However, crows were more prominent predators on islands (60%) than were raccoons, which is more consistent with findings in the Chesapeake Bay (60%, Stotts and Davis 1960). Therefore, the occurrence of raccoon predations appears to be limited by accessibility. For example, Pea Island National Wildlife Refuge, located on the eastern edge of my study site, was once reported to be a highly productive black duck nesting area (Parnell and Quay 1962).

Prior to 1984, raccoons were a rare occurrence on the refuge, however they were reported as common nest predators on neighboring Bodie Island (Parnell and Quay 1962, U.S. Fish and Wildlife Service 1984). During the first year of this study, I intensively searched the entirety of the refuge for black duck nests but was unsuccessful. I did however note an overabundance of raccoons on the refuge, as evidenced by tracks, scat, and very common diurnal sightings. Human habitation on the Outer Banks has likely allowed raccoons access to Pea Island, thereby possibly deterring use by nesting black ducks (Garrott et al. 1993). In the mainland marshes where raccoon predation was highest, black ducks nested in remote high marsh directly adjacent ( $\bar{x} = 23.38 \pm 0.99$  m) to open water of back bays and points jutting into the sound. Contrary to nesting habitat selection in the Chesapeake Bay, described by Stotts and Davis (1960), I did not locate any nests in the marsh-upland transition zone or on spoil deposits (i.e. dikes or levees) adjacent to ditches and canals in the mainland marshes. Although these areas are less prone to spring floods and have enough nesting cover, they also serve as potential travel corridors for ground-based predators. In contrast, the spoil-islands of the Pamlico Sound provided a quasi-upland nesting habitat refuge where predation was extremely low (<10%). These islands proved to be very diverse in topography, and vegetation composition and structure.

Previous black duck nesting studies suggest 10–30 % of total discovered nests abandon due to investigator activity (Stotts and Davis 1960, Krementz et al. 1992, Haramis et al. 1996). Our estimates (~27%) were consistent with these findings. The majority (~90%) of investigator-induced abandonments occurred the day of initial nest discovery when nests were in laying or early incubation stages and it was rare for a hen to abandon late in nest incubation.

Ringelman et al. (1982) monitored black duck nests in Maine to estimate incubation rhythms from 1977–1980. They found estimated mean incubation constancy (89.4%), recess time/day (153 min), recess frequency/day (1.5), and recess duration/day (102 min.) for six black duck hens. Calculating the same values for 39 nests for a total of 552 exposure days, I found that North Carolina black ducks exhibited lower nesting constancy and took longer and more frequent recesses/day. Ringelman et al. (1982) additionally noted that wetland nesting black ducks averaged more recesses/day ( $\bar{x} = 2.3$ ) with shorter average recess duration ( $\bar{x} = 82$  min.) than did upland nesters (recesses/day = 1.1, recess duration = 183 min). I discovered a similar trend between spoil-island and marsh nesting black ducks in North Carolina, where marsh nesting hens took more frequent and longer recesses than did spoil-island nesting hens.

### **Management Implications**

Conservation and management efforts of black ducks in coastal North Carolina would benefit from the creation and maintenance of natural islands and spoil-islands, where predation pressure is the lowest and nests are less prone to spring overwash. If nest depredation on these islands increases, ground-based predators (i.e. raccoons) should be trapped annually or semi-annually rather than one single, large effort (Engeman et al. 2002, Stocking et al. 2017). Ground-based predator trapping efforts have been effective at increasing nesting productivity in upland nesting waterfowl (Duebbert and Kantrud 1974, Beauchamp et al. 1996*b*, Pieron and Rohwer 2010). Thus, semi-annual raccoon trapping regimes aimed at reducing the raccoon population on Pea Island National Wildlife Refuge by >50% will likely be effective in reestablishing nesting black ducks to the refuge. However, careful monitoring of both

black duck nesting and raccoon population responses are required to determine the optimal target raccoon population goal for the refuge and to determine if trapping has been effective. Additionally, maintenance of impoundment dikes on the refuge and spoil-islands of the Pamlico Sound aimed at promoting observed selected relative ground cover compositions (largely composed of grasses; see Chapter 2) at visual obstruction levels  $>0.40$  m will ensure that black ducks have sufficient nesting cover from raccoons. Managers can achieve this through prescribed burning and/or woody vegetation herbicide treatments implemented at 3–5 year intervals. Maintaining early successional habitats also benefit colonial-nesting birds, which also have high utilization of spoil islands. Timing of prescribed burns on spoil islands and Pea Island National Wildlife Refuge should occur in late February to early March to set back vegetative succession, maintain a vegetation visual obstruction  $>0.40$  m, and avoid black duck peak nest initiation. Although, regular inundation and overwash events in the mainland brackish marshes are likely sufficient in maintaining optimal vegetation composition (see Chapter 2), properly timed marsh burns can increase plant production, reduce wildfire fuel loads, and in tidal fresh marshes reduce woody vegetation encroachment (Nyman and Chabreck 1995). Prescribed burning events employed by United States Fish and Wildlife Service, the North Carolina Wildlife Resources Commission, and North Carolina Forest Service intended to reduce fuel loads and/ or reduce woody encroachment should be conducted in the winter or in the early growing season not to exceed the 25<sup>th</sup> quantile date of black duck nest initiation (2 April). Future research would benefit from brood survival and brood habitat selection studies where brood survival is estimated, to gain a more holistic understanding of breeding productivity and recruitment in coastal North Carolina.

## Chapter 2

# NESTING HABITAT SELECTION OF AMERICAN BLACK DUCKS IN COASTAL NORTH CAROLINA, WITH IMPLICATIONS IN FUTURE SEA LEVEL RISE

### Introduction

The American black duck (*Anas rubripes*; hereafter black duck) was once the most numerous duck in the Atlantic flyway but after population declines (>50%) from 1950–1990, it is now a species of greatest conservation concern in 14 of the 17 states in the Atlantic flyway (Devers and Collins 2011). There are several potential explanations for this decline, including overharvest (Nichols et al. 1987, Francis et al. 1998, Longcore et al. 2000), loss in the quantity and quality of breeding and non-breeding habitats (Dennis et al. 1985, Diefenbach and Owen 1989), and interactions (competition, hybridization) with mallards (*Anas platyrhynchos*) (Anderson et al. 1987, Conroy et al. 2002). Additionally, recent losses in Atlantic coastal breeding and wintering habitat from Global Mean Sea Level Rise (GMSLR) (Krementz et al. 1991, Costanzo and Hindman 2007, Dahl and Stedman 2013, Livolsi 2015) now potentially threaten the ability of the Atlantic Flyway to support populations at North American Waterfowl Management Plan (NAWMP) goals (Morton et al. 1989, North American Waterfowl Management Plan Committee 2014).

Coastal North Carolina represents the southern extent of the species' breeding range (Stewart 1958, Parnell and Quay 1962, Bellrose 1980) and there is little knowledge about black duck nesting habitat selection. In the mid-Atlantic region, black ducks in New York prefer upland and wetland forested areas (Cowardin et al. 1967, Dwyer and Baldassarre 1993) while coastal mid-Atlantic nesting habitat selection shifts to islands and brackish marshes (Stewart 1958, Bellrose 1980). In

North Carolina, limited field observations have shown black ducks nesting in low-elevation brackish marsh containing cordgrasses (i.e. *Spartina patens* and *Spartina alterniflora*), and black needlerush (*Juncus roemarianus*) as well as higher elevation man-made dredge spoil islands (hereafter spoil-islands) with early successional and shrub species (Parnell and Quay 1962, Parnell et al. 1986, Fleming et al. 1996). In 2016, the North Carolina Wildlife Resources Commission (NCWRC) began an operational breeding season black duck population survey to quantify the annual abundance, distribution, and density of breeding black ducks in coastal North Carolina (NCWRC unpublished data). This annual survey provides additional evidence that black ducks are primarily nesting in low-elevation brackish marshes of the Pamlico Sound and on higher elevation spoil islands.

Because this preliminary evidence (Parnell and Quay 1962, Parnell et al. 1986, Fleming et al. 1996, NCWRC unpublished data) suggests coastal North Carolina black ducks actively utilize low-elevation brackish marsh, there is concern that GMSLR over the next century could influence the availability of black duck nesting habitats. However, projections of sea level rise vary due to future greenhouse gas concentration and emission scenarios under human-induced environmental change. The “A1B scenario” predicts 39 cm GMSLR above historic levels by 2100 based on a future world of rapid economic growth, global population that peaks in mid-century and declines thereafter, and the rapid introduction of new and more efficient technologies (Clough et al. 2016a). The “Representative Concentration Pathway (RCP) 2.6” scenario predicts 44 cm GMSLR above historic levels by 2100 based on mitigation scenarios aiming to limit the increase of global mean temperature to 2°C (Van Vuuren et al. 2011). The “RCP 4.5” scenario predicts 53 cm GMSLR above historic levels by

2100 based on a scenario of long-term, global greenhouse gas emissions and land-use-land-cover which stabilizes radiative forcing at 4.5 W/m<sup>2</sup> in the year 2100 (Thomson et al. 2011). The “RCP 8.5” scenario predicts 74 cm GMSLR above historic levels by 2100 based on a ‘baseline’ scenario with high greenhouse gas emissions, no specific climate mitigation target, and a radiative forcing of 8.5 W/m<sup>2</sup> at the end of the century (Riahi et al. 2011).

Because there has not been a large scale and comprehensive evaluation of preferred black duck nesting habitat in North Carolina, and thus no knowledge on how the GMSLR scenarios may impact these preferred habitats, it is critical to quantify both components to inform long-term management plans for black duck breeding habitat conservation. Therefore, the goal of this research is to first quantify black duck nest-site selection including micro- and macro-habitat preferences. The second goal is to determine the availability of these preferred nesting habitats under five GMSLR sea level rise scenarios projected by the Intergovernmental Panel on Climate Change (IPCC) Fifth Annual Report (AR5) (Church et al. 2013) including 1) A1B mean scenario, 39 cm rise by 2100, 2) RCP 2.6, 44 cm rise by 2100, 3) RCP 4.5, 53 cm rise by 2100, 4) RCP 8.5, 74 cm rise by 2100, and 5) a 1 m fixed rise by 2100.

### **Study area**

I conducted research in Hyde and Dare counties in the Albemarle-Pamlico Peninsula and the Outer Banks regions of North Carolina from late March to the end of June in 2017 and 2018 ([Figure 1](#)). Specifically, my research focal area ranged from the Pamlico and Pungo River convergence northeast along the Pamlico Sound to the intersection of US 64 and the Croatan Sound and throughout the Outer Banks of Hyde and Dare Counties. Study sites included Swanquarter National Wildlife Refuge

(35°21'59.99" N -76°19'18.00" W), Alligator River National Wildlife Refuge (35°46'59.99" N -75°50'59.99" W), Pea Island National Wildlife Refuge (35°41'17.99" N -75°32'26.99" W), and private and state-owned lands along the Pamlico Sound. Study sites also included a series of man-made dredge spoil islands (hereafter spoil-islands) located in a north-south direction from Wanchese to Oregon Inlet. These spoil islands were created as a result of dredging operations that keep navigation channels open and the commercial fishing industry in Wanchese connected to the Atlantic Ocean through Oregon Inlet.

The Albemarle-Pamlico Peninsula was historically dense in wetland habitats but most of the wetlands were drained and converted to agricultural cropland. Extant wetland habitats include large natural lakes (including Lake Mattamuskeet which is the largest in North Carolina), river drainage systems of the Alligator, Pungo, and Long Shoal rivers, large contiguous blocks of semi-permanently flooded swamp, pocosins, and freshwater and brackish marsh. Freshwater lacustrine and riverine habitats in this region are dominated by panic grasses (*Panicum* spp.), cattail (*Typha* spp.), sawgrass (*Cladium* spp.), sedges (family *Cyperaceae*), rushes (family *Juncaceae*), and duck weeds (*Lemna* spp.) and edges transition into mixed hardwood swamps, where the dominant overstory is composed of red maple (*Acer rubrum*), black gum (*Nyssa sylvatica*), bald cypress (*Taxodium distichum*), and swamp tupelo (*Nyssa biflora*), or hardwood and mixed pine habitats. Pocosins cover the freshwater–brackish water transition zone and consist primarily of pond pine (*Pinus serotina*), loblolly bay (*Gordonia lasianthus*), red bay (*Persea borbonia*), and sweet bay (*Laurus nobilis*), with an understory of titi (*Cyrilla racemiflora*), honeycup (*Zenobia pulverulenta*), fetterbush (*Lyonia lucida*), and gallberry (*Ilex coriacea*). Vegetation in

the brackish marshes adjacent to the mainland and natural islands within the Pamlico Sound are primarily composed of black needlerush with patches of cordgrasses. The Outer Banks region in my study area is a barrier island complex bordered by Pamlico, Croatan, and Core sounds to the west and the Atlantic Ocean to the east (Devers and Collins 2011). Within this area, Pea Island National Wildlife Refuge is a 12-mile long, narrow island with 91–244 m wide line of beach dunes on the island’s eastern border, several large submerged aquatic vegetation (SAV) impoundments at its core, and extensive brackish marshes dominated by black needlerush, saltmeadow cordgrass (*Spartina patens*), and sawgrass, on its western border. The remaining portion of this region, including the spoil-islands, contains beach dunes (composed of sea oats [*Uniola paniculata*], panic grasses, and seaside bluestem [*Schizachyrium littorale*]); maritime shrub and dry grassland (dominant shrubs include: wax myrtle [*Morella cerifera*], red cedar [*Juniperus virginiana*], yaupon holly [*Ilex vomitoria*], greenbrier [*Smilax* spp.], blackberry [*Rubus* spp.], and poison ivy [*Toxicodendron radicans*]); and various early successional habitats.

## **Methods**

I searched for black duck nests from March 19–June 30, 2017–2018. Nest searching was conducted in the mornings as nests are more likely to be attended during morning hours and females serve as indicators of nest location when present (Gloutney et al. 1993). To limit hen disturbance and possible nest abandonment, I did not nest search in inclement weather. To search for nests, two technicians hand-dragged a 30.5 m rope with aluminum cans with rocks inside attached at ~2 m intervals along the entire length of the rope to serve as noisemakers (modified from all-terrain vehicle chain drags used for upland nesting waterfowl, Klett et al. 1986). A

third technician served as a spotter used to identify flushing hens and the exact location and distance that the hen flushed from the rope or nearest investigator. In areas where I could not drag the rope due to vegetation height and rigidity, technicians distanced themselves 5–25 m apart and walked transects of the selected area. Once the nest location was identified, one technician approached the nest and began data collection while the other remained >5 m away and recorded data. I recorded all active nests found, between lay and hatch using GPS waypoints. Nests were monitored on 7 to 10-day intervals until termination.

I quantified micro-habitat characteristics at all terminated nest sites. I measured ground cover (relative percentages of bare ground, grass, forbs, woody vegetation, water, and litter summed to 100%) within a 50 x 50 cm sampling Daubenmire frame (modified from Daubenmire 1959) centered on the nest bowl. I also measured the maximum height of vegetation directly over nest bowls. Finally, I placed a visual obstruction pole (Robel et al. 1970) in the nest bowl to determine the lowermost 0.1 m stratum that was  $\geq 50\%$  visible through existing vegetation. Visual obstruction readings (VORs) were taken from vantage points 3.5 m from the pole in each cardinal direction, and the mean of the four measurements were defined as a single VOR for each nest (Varner et al. 2013). I then repeated micro-habitat measurements at two randomly selected points, as determined through random number generation of azimuth (0–360°) and distance (10–50 m) from the nest, to compare use (nest) vs. non-use (randomly-selected) sites. The nest metrics collected from the two random points were averaged to provide a single value for non-use nesting habitat.

I used ANOVA (SPSS®, Version. 26.0. IBM Corp. Armonk, NY. 2018) for visual obstruction reading (VOR), vegetation height, and percent grass, forb, woody

vegetation, litter, bare soil, and water composition measurements to determine if there were significant ( $P \leq 0.05$ ) differences in nesting micro-habitat preference across mainland marsh, natural island and spoil-island habitats. To assess micro-habitat preference of nesting black ducks per habitat, I compared visual obstruction readings (VOR), vegetation height measurements, and percent grass, forb, wood vegetation, litter, bare soil, and water composition at the nest site to average values measured at the random points using a paired t-test ( $P \leq 0.05$ ; SPSS®, Version. 26.0. IBM Corp. Armonk, NY. 2018). Further, DEM elevation (value procurement explained below) was quantified via paired t-test, in the same manner, at use and non-use sites. Distributions of all measurements at nest and random sites were then qualitatively assessed for biological significance.

I imported all known nest locations into ArcGIS pro (v. 10.7, ESRI 2018) as point shapefiles and overlaid them with the United States Fish and Wildlife Service National Wetland Inventory (NWI) geographic information system shapefile (U.S. Fish and Wildlife Service 2019b) to project and identify different classifications of wetland habitat within the study area. In conjunction with the NWI layer, I also utilized 1 m<sup>2</sup> resolution Light Detection and Ranging (LiDAR) digital elevation models (DEM), developed by the United States Fish and Wildlife Service (U.S. Fish and Wildlife Service 2018) to procure elevation values at use and non-use sites. I broke NWI wetland and deepwater habitat classifications down to modifiers within the Estuarine, Marine, Riverine, Lacustrine, and Palustrine classes to explain habitat selection at a fine scale. To estimate the amount of habitat searched over the study area, as well as estimate macro-habitat nest selection, I created buffers around GPS tracks made throughout the field seasons. Buffer width was determined by averaging

black duck flush distances from closest search party observer as observed in the field. I then converted the buffer polygon layer into a raster with a resolution equal to 1 m<sup>2</sup> using the “polygon to raster” tool in ArcGIS pro. I then utilized the “Extract Values to Table” tool within ArcGIS pro to determine the total amount of ha of each NWI habitat category that was searched. To estimate the effect of single categorical response variable (NWI habitat types in the searched buffered area) on resource selection (nest present/absent) I used an initial Chi-square test ( $\alpha \leq 0.05$ ) for overall difference in use versus availability (Neu et al. 1974), followed by 2 proportions z test ( $\alpha \leq 0.05$ ) to determine macro-habitat preference/avoidance (McDonald et al. 2012).

To quantify the impacts of GMSLR on nest site availability, I forecasted changes in available nesting habitat under several GMSLR model predictions projected by climate change scenarios in the Intergovernmental Panel on Climate Change (IPCC) Fifth Annual Report (AR5) (Church et al. 2013). I created GIS spatial models for Hyde and Dare counties, North Carolina using the Sea Level Affecting Marshes Model (SLAMM v. 6.7, Warren Pinnacle Consulting Inc., Waitsfield, VT). This program predicts land cover changes in wetland habitats based on specified climate change scenarios, while accounting for processes such as marsh erosion and accretion. This model uses a complex decision tree incorporating geometric and qualitative relationships based upon a succession of equilibrium states with sea level to simulate the dominant processes affecting coastal marshes affected by long-term sea level rise. Outputs from the model include mapped wetland distribution, tabular, and graphical data at specified time steps. Each study site is divided into homogenous cells (5×5 m for these simulations) in which the SLAMM model computes local sea level rise and conversion from one wetland class to another after considering the new cell

elevation at a given time step with respect to the class in that cell and its inundation frequency. Inundation, erosion, overwash, saturation, and accretion are the five primary processes that determine wetland fate in this model (Clough et al. 2015).

High vertical-resolution elevation data may be the most important SLAMM data requirement (Clough et al. 2016a). Elevation data delineate the inland penetration of predicted sea level rise, frequency of wetland inundation, and lower elevation ranges for wetlands, which serves to predict wetland conversion in the model. I downloaded 1 m<sup>2</sup> resolution digital elevation data (U.S. Fish and Wildlife Service 2018) at an extent encompassing Hyde and Dare Counties. I derived elevation rasters from the “Digital Surface Model in Eastern 59 Counties of North Carolina from 2014–2015 LiDAR” project and USGS NED 1/9 arc-second 15 × 15 min topobathymetric imagery (U.S. Fish and Wildlife Service 2018, U.S. Geological Survey 2013a, and U.S. Geological Survey 2013b). Using ArcGIS pro (v. 10.7, ESRI 2018), I resampled elevation imagery to a 5 m<sup>2</sup> resolution, merged all images into a raster mosaic, and clipped to mainland Hyde County, mainland Dare County, and the Outer Banks regions of both counties for a total of 3 study areas. Using ArcGIS pro raster surface tools, I created slope rasters from each clipped elevation raster with a matching resolution (5 m<sup>2</sup>) and spatial extent (GRS 1980 Lambert Conformal Conic [m]). The elevation and slope rasters were then exported as ASCII files for SLAMM model input. Next, I reprojected the NWI polygon layer under the GRS 1980 Lambert Conformal Conic (m) projected coordinate system and then clipped the layer to mainland Hyde County, mainland Dare County, and the Outer Banks regions of both counties for a total of 3 study areas. Translation of NWI wetland categories to SLAMM wetland categories followed the crosswalk information provided in the

SLAMM 6.7 technical documentation (Clough et al. 2016a). Next, I reformatted the converted NWI layer to a 5 m<sup>2</sup> GRID raster using the “resample” tool in ArcGIS pro. National Land Cover Database (NLCD) Imperviousness (CONUS) 2016, 30 m<sup>2</sup> resolution data (Yang et al. 2018) was resampled to the NWI and elevation raster resolution, spatial extent, and projection. Further, I reclassified percent imperviousness into two categories: Imperviousness = 0–25% and  $\geq 26\%$ . These categories represented code 2 (Undeveloped Dry Land) and 1 (Developed Dry Land) of the SLAMM categories, respectively. Finally, I created a mosaic raster dataset, where the NWI raster was the operator classifying wetland categories and the NLCD raster classifying upland categories (i.e. SLAMM codes 1 and 2). The resulting mosaic raster was then exported as an ASCII file for SLAMM model input.

The historic trend of sea level rise for all study sites was estimated at 4.69 mm/year (Table 7) using the approximate average of long-term trends measured at Oregon Inlet Marina, North Carolina (NOAA station 8652587). Great diurnal tide range (mean higher high water – mean lower low water) for mainland Dare County and the Outer Banks was estimated at 0.360 m using present-day (1995–2011) measurements at Oregon Inlet Marina, North Carolina (NOAA station 8652587) (Table 2.3). Great diurnal tide range for mainland Hyde County was estimated at 0.127 m using present-day (1995–2011) measurements at Cedar Island, North Carolina (NOAA station 8652587). For mainland Dare county and the Outer Banks, the LiDAR digital elevation model of NAVD88 was related to mean tide level (MTL-NAVD88) using the NOAA station at Oregon Inlet Marina, North Carolina (NOAA station 8652587). For mainland Hyde County, MTL-NAVD88 estimates were derived from Clough (2012).

Table 7 SLAMM input parameters for the simulation of GMSLR for mainland Hyde and Dare Counties, North Carolina, USA, 2019–2100. Values for parameters with no specific local information were kept at the model default value.

Parameter	Main. Dare	Main. Hyde	Outer Banks
NWI photo date (YYYY)	2019	2019	2019
DEM date (YYYY)	2015	2015	2015
Direction offshore [N, S, E, W]	East	South	East
Historic trend (mm/yr)	4.69	4.69	4.69
MTL-NAVD88 (m)	-0.037	-0.030	-0.037
GT great diurnal tide range (m)	0.36	0.127	0.36
Salt elevation (m above MTL)	0.128	0.19	0.127
Marsh erosion (horizontal m/yr)	1.8	1.8	1.8
Marsh erosion fetch (km)	9	9	9
Swamp erosion (horizontal m/yr)	1	1	1
T.Flat erosion (horizontal m/yr)	0.5	0.5	1
Regularly-flooded marsh accretion (mm/yr)	3.85	3.7	3.85
Irregularly-flooded marsh accretion (mm/yr)	4.7	4.1	4.7
Tidal-freshwater marsh accretion (mm/yr)	5.9	5.9	5.9
Tidal swamp accretion (mm/yr)	1.1	1.1	1.1
Swamp accretion (mm/yr)	0.3	0.3	0.3
Beach sedimentation rate (mm/yr)	0.5	0.5	0.5
Irregular-flood collapse (m)	0.07	0.07	0.07
Regular-flood collapse (m)	0.19	0.19	0.19
Use wave erosion model [true,false]	TRUE	TRUE	TRUE
Wave erosion alpha (unitless)	0.00085	0.00085	0.00085
Regular flood use model [true,false]	TRUE	TRUE	TRUE
Regular flood maximum accretion (mm/year)	3.85	3.7	3.85
Regular flood minimum accretion (mm/year)	0	0	0
Regular flood elevation a (mm/(year HTU <sup>3</sup> ))	-0.2272	-0.21834888	-0.22720086
Regular flood elevation b (mm/(year HTU <sup>2</sup> ))	-3.00002	-2.8831368	-3.0000208
Regular flood elevation c (mm/(year*HTU))	0.758432	0.72888261	0.7584319
Regular flood elevation d (mm/year)	3.802515	3.6543648	3.8025147
Irregular flood use model [true,false]	TRUE	TRUE	TRUE
Irregular flood maximum accretion (mm/year)	4.7	4.1	4.7
Irregular flood minimum accretion (mm/year)	0	0	0
Irregular flood elevation a (mm/(year HTU <sup>3</sup> ))	-0.23935	-0.45009826	-0.5159663
Irregular flood elevation b (mm/(year HTU <sup>2</sup> ))	-3.1604	-5.9432175	-6.8129566
Irregular flood elevation c (mm/(year*HTU))	0.798978	1.5024982	1.722376
Irregular flood elevation d (mm/year)	5.12053	4.8908176	5.606547

Referencing previous studies, I set salt elevation at 0.128 m above MTL for mainland Dare County (Clough 2013), 0.19 m above MTL for mainland Hyde County (Clough 2012), and 0.127 m above MTL for the Outer Banks (Clough 2008) (Table 2.3). For mainland Dare County and the Outer Banks, I set regularly-flooded and irregularly-flooded marsh accretion rates at 3.85 and 4.7 mm/year, respectively, based on a study of vertical marsh accretion in Cedar Island, NC (Cahoon et al. 1995). In mainland Hyde County, I set regularly-flooded and irregularly-flooded marsh accretion rates at 3.7 mm/year (Cahoon et al. 1999) and 4.1 mm/year (Reed et al. 2008), respectively. Tidal flat erosion rates were based on SLAMM defaults for Hyde and Dare counties and input estimates from Clough (2008) for the Outer Banks. Tidal-fresh marsh accretion values were set to 5.9 mm/year based upon an average of fresh marsh accretion rates within the region (Clough 2012). Estimates of marsh erosion, swamp erosion, tidal swamp accretion, swamp accretion, and beach sedimentation were set following SLAMM parameter default settings. Marsh erosion fetch (minimum distance required for erosion causing waves to form) was set at 9 km following Leonardi et al. (2016) for all sites. Irreg-Flood Collapse (vertical loss of elevation when irregularly-flooded marsh falls below its lower elevation boundary and is converted to regularly-flooded marsh) and Reg-Flood Collapse (vertical loss of elevation when regularly-flooded marsh falls below its lower elevation boundary and is converted to tidal flats) rates were set to 70 mm and 190 mm, respectively, based on estimates measured in Connecticut marshes (Clough et al. 2016b) (Table 7).

Wind rose data were derived from wind speed (m/s) and direction measurements (16 cardinal directions) taken at Manteo, North Carolina Airport (WBAN 13766) and Cape Hatteras, North Carolina Airport (WBAN 93729) spanning

12/12/17–12/12/19. These data were used in conjunction with marsh erosion fetch and a wave erosion alpha of 0.00085 (Marani et al. 2011) to simulate wave erosion in the SLAMM model. Minimum and maximum accretion rates and site-specific elevation statistics were used to calculate accretion curves and SLAMM rescaled elevation coefficients a-d for irregularly- and regularly-flooded marsh.

SLAMM models were set to run five sea level rise scenarios that represented low (A1B Mean and RCP 2.6 Scenarios), intermediate (RCP 4.5 Scenario), and high (RCP 8.5 scenario and 1 m fixed rise by 2100) levels. Model forecast data is output for the year 2100 with the initial condition date set to 2019, the date of the most recent wetland data available. GIS raster outputs and resulting wetland areas (ha) were reported for the first and last year of the simulation ([Appendix](#)).

## Results

During the 2017 and 2018 breeding seasons, I searched 461.12 ha of 12 wetland habitat types including 109.69 ha of regularly-flooded low marsh and 294.68 ha of irregularly-flooded high marsh ([Table 8](#)). Black duck nests were found on spoil-islands (n = 35, 25%), natural islands (n = 54, ~39%), and in mainland marshes of the Pamlico Sound (n = 51, ~36%) ([Figure 2](#)). Spoil-islands were typically elevated and contained a diversity of dune grasslands, maritime shrublands, upper and lower beach, and maritime vine tangles. Typically, older islands were at a higher successional stage. Naturally formed islands were low-lying and were broadly classified as brackish marsh. Black ducks favored nesting in the high marsh perimeter directly adjacent to the open water of back bays and points jutting into the sound. Of the 140 nests found, 116 (82.86%) were in irregularly-flooded, estuarine “high marsh” habitats consisting of *Spartina alterniflora*, *Spartina patens*, *Juncus roemerianus*, and *Distichlis spicata*

(NWI wetland classes E2EM1P [82.86%]). An additional 15 nests were found in irregularly-flooded estuarine marshes (NWI wetland classes E2EM5Pd [0.71%], E2SS(1,3,4)P [8.57%], and E2USP [1.42%]), with differing prominent vegetation types, where E2EM5Pd consists mainly of *Phragmites australis*, E2SS(1,3,4)P is scrub-shrub, and E2USP has an unconsolidated bottom with only early-successional salt-tolerant plants (commonly used to classify new spoil-islands). Last, I found 9 nests in estuarine “low marsh” that was regularly-flooded consisting of vegetation *Juncus roemerianus* and short-form *Spartina alterniflora* (NWI wetland class E2EMIN [6.43%]). I found no nests in the remaining 8 macro-habitat types of “E2EM1/SS(1,3,4)P”, “M2US2P”, “PEM1”, “PF”, “PFO(1,3,4)(A,B,C)”, “PFO(3,4)(R,S)”, “PSS(1,3,4)(A,B,C,F)”, and “PSS(1,3,4)(R,T)” (Table 8). Of the 5 used habitat types, I can reject the null hypothesis that nesting black ducks use all habitat types in proportion to their availability ( $\chi_4^2 = 33.85, P < 0.01$ ). Within that, high marsh (NWI code E2EM1P, 116 nests found in 256.78 ha) was a preferred habitat for nesting (used 82.9 % versus 64.2 % available,  $z = -13.03, P < 0.01$ ), whereas the other habitat types were selected for in proportion to their availability (low marsh [ $z = 1.41, P = 0.16$ ], *Phragmites* [ $z = 0.09, P = 0.93$ ], shrub-scrub wetland [ $z = -0.47, P = 0.64$ ] and unconsolidated sandy habitat [ $z = -0.07, P = 0.94$ ], Table 8).

Table 8 Macro-habitat description of searched area (ha), black duck nests found per habitat and nest density per habitat, coastal North Carolina, USA, 2017–2018.

Macro-habitat type	NWI Code	Searched (ha)	Proportion available of used habitats	Nests present	Proportion selected of used habitats	ha/nest
Estuarine, emergent/ scrub-shrub, irregularly-flooded,	E2EMI/SS(1,3,4)P	8.03	-	0	-	1
Estuarine, emergent, regularly-flooded, tidal low marsh	E2EM1N	109.69	27.4%	9	6.4%	122
Estuarine, emergent, irregularly-flooded, tidal high marsh	E2EM1P	256.78	64.2%	116	82.9%	22
Estuarine, emergent, irregularly-flooded, tidal,	E2EM5Pd	7.71	1.9%	1	0.7%	77
Estuarine, scrub-shrub, irregularly-flooded, tidal marsh	E2SS(1,3,4)P	22.16	5.5%	12	8.6%	18
Estuarine, unconsolidated bottom, tidal	E2US	3.77	0.9%	2	1.4%	19
Marine, unconsolidated bottom, irregularly-flooded, tidal	M2US2P	0.00	-	0	-	0
Palustrine, emergent, persistent marsh	PEM1	5.07	-	0	-	0
Managed impoundment	Pf	2.82	-	0	-	0
Freshwater, non-tidal, forested wetland	PFO(1,3,4)(A,B,C)	8.10	-	0	-	0
Freshwater, tidal, forested wetland	PFO(3,4)(R,S)	22.78	-	0	-	0
Freshwater, non-tidal, shrub-scrub wetland	PSS(1,3,4)(A,B,C,F)	10.41	-	0	-	0
Freshwater, tidal, shrub-scrub wetland	PSS(1,3,4)(R,T)	2.76	-	0	-	0
Total		461.12	-	140	-	N/A

Table 9 Spoil-island, natural island, and mainland marsh micro-habitat characteristics of nest sites (use). Group-wise ANOVA results are recorded for distance to open water (DOW), elevation, visual obstruction (VOR), vegetation height (Veg height), and ground cover relative percent composition. 2017–2018 Hyde and Dare Counties, North Carolina, USA.

Micro-habitat	Mean + SE			<i>F</i> -test
	Mainland Marsh	Natural Island	Spoil-island	
DOW (m)	21.32 ± 1.62	22.26 ± 1.42	29.80 ± 1.05	$F_{136} = 5.87, P < 0.05$
Elevation (m)	1.31 ± 0.06	1.41 ± 0.06	19.88 ± 1.38	$F_{134} = 283.54, P < 0.01$
VOR(m)	0.46 ± 0.02	0.54 ± 0.02	0.59 ± 0.04	$F_{136} = 6.31, P < 0.01$
Veg height (m)	0.84 ± 0.04	0.79 ± 0.03	1.02 ± 0.07	$F_{136} = 6.10, P < 0.01$
% grass	82.38 ± 3.16	86.67 ± 2.50	62.49 ± 4.99	$F_{136} = 12.55, P < 0.01$
% forbs	0.00 ± 0.00	1.20 ± 0.93	8.86 ± 3.05	$F_{136} = 9.10, P < 0.01$
% woody	1.40 ± 1.30	3.70 ± 1.37	17.03 ± 4.18	$F_{136} = 12.54, P < 0.01$
% litter	12.90 ± 2.63	7.41 ± 1.45	8.85 ± 1.72	$F_{136} = 2.11, P = 0.13$
% bare soil	0.92 ± 0.49	0.37 ± 0.22	2.49 ± 0.68	$F_{136} = 5.20, P < 0.01$
% water	2.40 ± 0.77	0.65 ± 0.35	0.29 ± 0.29	$F_{136} = 4.19, P < 0.05$

Habitats that were not significantly different in the majority ( $\geq 75\%$ ) of micro-habitat measurements were combined into a broader habitat category. Mainland marsh and natural island nests were not significantly different in any micro-habitat measurement except for water composition (Mean difference = 1.75%,  $P = 0.049$ ). Thus, for this study natural island and mainland marsh were combined as “marsh” (Table 9). Mainland marsh nest micro-habitat significantly differed ( $P \leq 0.05$ ) from spoil-island nest micro-habitat in all measurements but litter composition ( $\bar{x}$  difference = 4.04%,  $P = 0.390$ ) and bare soil composition ( $\bar{x}$  difference = -1.57%,  $P = 0.056$ ). Finally, natural island nest micro-habitat significantly differed ( $P \leq 0.05$ ) from spoil-island nest micro-habitat in all measurements but litter composition ( $\bar{x}$  difference = -1.45%,  $P = 0.882$ ), water composition ( $\bar{x}$  difference = 0.36%,  $P = 0.896$ ), and vegetation visual obstruction readings ( $\bar{x}$  difference = -0.06 m,  $P = 0.249$ ).

Nests in the marshes of the mainland and natural islands (hereafter marsh) were located an average of 21.81 m (SE = 1.10 m) away from open water (Table 10). Nests located in flood prone spots, were slightly elevated (15–30 cm) structures that consisted of *Juncus* spp. or *Spartina* spp. leaves. Nests that were not elevated, were tucked deep into surrounding cover (*Spartina patens*, *Distichlis spicata*, and *Juncus roemerianus*) and the bowl was low in profile and mainly constructed of *Spartina patens*. Micro-habitat elevation difference between nest and random sites was not significant ( $t_{103} = -0.07$ ,  $P = 0.95$ ), mean elevation at nest sites was  $1.36 \pm \text{SE } 0.04$  m above sea-level according to the NAVD88 vertical datum (Table 10), Marsh nest sites compared to random marsh sites within 50 m of the nest had significantly more grass ( $84.61 \pm \text{SE } 2.00\%$  vs  $78.85 \pm \text{SE } 2.15\%$ ,  $t_{103} = 2.51$ ,  $P < 0.05$ ), less bare soil ( $0.63 \pm \text{SE } 0.26\%$  vs  $4.95 \pm \text{SE } 1.16\%$ ,  $t_{103} = -3.84$ ,  $P < 0.01$ ), and less water ( $1.49 \pm \text{SE } 0.42\%$  vs  $7.19 \pm \text{SE } 1.20\%$ ,  $t_{103} = -5.28$ ,  $P < 0.01$ , Table 10). Nest site versus random sites were also surrounded by higher vegetation ( $0.81 \pm \text{SE } 0.03$  m vs  $0.73 \pm 0.02$  m,  $t_{103} = 2.76$ ,  $P < 0.01$ ) but did not have significant differences in vegetation visual obstruction ( $0.50 \pm 0.01$  m vs  $0.47 \pm 0.01$  m,  $t_{103} = 1.76$ ,  $P = 0.08$ , Table 10). Other micro-habitat variables (% forbs, woody vegetation, and litter) were not significantly different between selected and random points ( $t_{139} < 1.47$ ,  $P > 0.15$ , Table 10).

Table 10 Natural island and mainland marsh micro-habitat characteristics of nest sites (use) and randomly sampled (non-use) points. T-test results are recorded for elevation, visual obstruction, vegetation height, and ground cover relative percent composition. 2017–2018 Hyde and Dare Counties, North Carolina, USA.

Micro-habitat	Mean + SE		t-test
	Nest site	Random site	
Distance to open water (m)	21.81 ± 1.10	-	-
Elevation (m)	1.36 ± 0.04	1.37 ± 0.04	$t_{103} = -0.07, P = 0.95$
Visual obstruction (m)	0.50 ± 0.01	0.47 ± 0.01	$t_{103} = 1.76, P = 0.08$
Vegetation height (m)	0.81 ± 0.03	0.73 ± 0.02	$t_{103} = 2.76, P < 0.01$
% grass	84.61 ± 2.00	78.85 ± 2.15	$t_{103} = 2.51, P < 0.05$
% forbs	0.63 ± 0.49	0.63 ± 0.37	$t_{103} = 0.00, P = 1$
% woody vegetation	2.60 ± 0.95	1.06 ± 0.51	$t_{103} = 1.47, P = 0.15$
% litter	10.05 ± 1.49	7.34 ± 1.28	$t_{103} = 1.42, P = 0.16$
% bare soil	0.63 ± 0.26	4.95 ± 1.16	$t_{103} = -3.84, P < 0.01$
% water	1.49 ± 0.42	7.19 ± 1.20	$t_{103} = -5.28, P < 0.01$

Nests on spoil-islands were located an average of 29.80 m (SE = 1.05 m) away from open water (Table 11) within dense vegetation in combinations of warm season grasses, *Rubus* spp., and forbs. Micro-habitat elevation difference between nest and random sites was significant ( $t_{33} = 2.14, P = 0.040$ ), with measurements of 19.88 m and 16.06 m above sea-level according to the NAVD88 vertical datum, respectively (Table 11). On spoil-islands, nest sites compared to random sites within 50 m of the nest had significantly more grass ( $62.49 \pm \text{SE } 5.00\%$  vs  $46.27 \pm \text{SE } 4.68\%$ ,  $t_{35} = 4.66, P < 0.01$ ) and less bare soil ( $2.49 \pm \text{SE } 0.68\%$  vs  $12.94 \pm \text{SE } 3.10$ ,  $t_{35} = -3.44, P < 0.01$ , Table 11). Nest site versus random sites were also surrounded by higher vegetation ( $1.03 \pm \text{SE } 0.07$  m vs  $0.70 \pm \text{SE } 0.06$  m,  $t_{35} = 4.66, P < 0.01$ ) and had a greater vegetation visual obstruction ( $0.60 \pm \text{SE } 0.04$  m vs  $0.42 \pm \text{SE } 0.04$  m,  $t_{35} = 4.61, P < 0.01$ , Table 11). Other micro-habitat variables (% forbs, woody vegetation, water, and litter) were not significantly different between selected and random points ( $t_{139} < 1.76, P > 0.09$ , Table 11).

Table 11 Spoil-island micro-habitat characteristics of nest sites (use) and randomly sampled (non-use) points. T-test results are recorded for elevation, visual obstruction, vegetation height, and ground cover relative percent composition. 2017–2018 Hyde and Dare Counties, North Carolina, USA.

Micro-habitat	Mean + SE		t-test
	Nest site	Random site	
Distance to open water (m)	29.80 + 1.05	-	-
Elevation (m)	19.88 ± 1.38	16.06 ± 1.49	$t_{33} = 2.14, P < 0.05$
Visual obstruction (m)	0.60 ± 0.04	0.42 ± 0.04	$t_{35} = 4.61, P < 0.01$
Vegetation height (m)	1.03 ± 0.07	0.70 ± 0.06	$t_{35} = 4.66, P < 0.01$
% grass	62.49 ± 5.00	46.27 ± 4.68	$t_{35} = 2.76, P < 0.01$
% forbs	8.86 ± 3.05	13.03 ± 2.65	$t_{35} = -1.76, P = 0.09$
% woody vegetation	17.03 ± 4.18	13.27 ± 3.31	$t_{35} = 0.85, P = 0.40$
% litter	8.86 ± 1.73	14.49 ± 2.92	$t_{35} = -1.74, P = 0.09$
% bare soil	2.49 ± 0.68	12.94 ± 3.10	$t_{35} = -3.44, P < 0.01$
% water	0.29 ± 0.29	0.00 ± 0.00	$t_{35} = 1.00, P = 0.32$

For SLAMM modeling, E2EM1N fell into the “Regularly-flooded Marsh” and E2EM1P, E2EM1Pd, E2EM5Pd, E2SS(1,3,4)P, and E2USP were combined into the “Irregularly-flooded Marsh” category. Thus, the average density of observed nests was 1 nest per 122 ha in regularly flooded marsh and 1 nest per 137 ha in irregularly flooded marsh (Table 8). The initial wetland land cover area (ha), percentage losses by 2100, and projected black duck nest counts for Mainland Hyde County, Mainland Dare County, and the Outer Banks under different SLR scenario are presented in Table 12 with graphical representations of NWI land-cover losses in the Appendix. In 2019, mainland Hyde County had an initial coverage of 7,561 ha of regularly-flooded marsh and 7,037 ha of irregularly-flooded marsh. Under the conservative GMSLR pathways, A1B Mean and RCP 2.6, there is a gain of 37.81% and 38.23% of regularly-flooded marsh for each scenario, respectively. Under these scenarios, I projected a 2.12% and 1.94% loss of irregularly-flooded marsh under A1B Mean and RCP 2.6, respectively. Under the intermediate projection, RCP 4.5, I projected a

41.06% gain in regularly-flooded marsh and a 3.27% loss in irregularly-flooded marsh. The more liberal projections, RCP 8.5 and 1 m fixed rise by 2100, simulate 47.15% and 64.91% increases in regularly-flooded marsh and 6.54% and 12.68% loss in irregularly-flooded marsh by 2100. In 2019, mainland Dare County had an initial coverage of 2,603 ha of regularly-flooded marsh and 3,320 ha of irregularly-flooded marsh. Under the conservative GMSLR pathways, A1B Mean and RCP 2.6, there is a gain of 32.17% and 34.94% of regularly-flooded marsh for each scenario, respectively. Under these scenarios, I projected a 0.35% and 0.32% loss of irregularly-flooded marsh under A1B Mean and RCP 2.6, respectively. Under the intermediate projection, RCP4.5, I projected a 33.12% gain in regularly-flooded marsh and a 0.57% loss in irregularly-flooded marsh. The more liberal projections, RCP 8.5 and 1 m fixed rise by 2100, simulate 32.10% and 54.43% increases in regularly-flooded marsh and 1.26% and 0.65% loss in irregularly-flooded marsh by 2100. In 2019, the Outer Banks had an initial coverage of 2,165 ha of regularly-flooded marsh and 4,753 ha of irregularly-flooded marsh. Under the conservative GMSLR pathways, A1B Mean and RCP 2.6, I projected a 5.91% and 6.49% loss of regularly-flooded marsh for each scenario, respectively. Under these scenarios, there was a 0.22% and 0.12% loss of irregularly-flooded marsh under A1B Mean and RCP 2.6, respectively. Under the intermediate projection, RCP 4.5, there was a 7.30% loss in regularly-flooded marsh and a 0.64% loss in irregularly-flooded marsh. The more liberal projections, RCP 8.5 and 1 m fixed rise by 2100, simulated 8.69% and 7.17% loss in regularly-flooded marsh and 2.47% and 8.79% loss in irregularly-flooded marsh by 2100. Across all study areas, under the most conservative GMSLR pathway, there was an overall gain of 3,660 ha (22.23%) of regularly-flooded marsh and an overall loss of 153 ha (1.01%)

of irregularly-flooded marsh by the year 2100. Under the most liberal GMSLR pathway, there was an overall gain of 6,170 ha (50.04%) of regularly-flooded marsh and an overall loss of 1,332 ha (8.81%) of irregularly-flooded marsh by the year 2100.

Table 12 Predicted change of regularly and irregularly-flooded marsh area and predicted number of black duck nests (assuming 1 nest per 122 ha in regularly-flooded marsh and 137 ha in irregularly flooded marsh) by 2100 given simulated scenarios of eustatic SLR in mainland Hyde County, mainland Dare County, and Outer Banks (made up of both Dare and Hyde Counties), North Carolina, USA.

Land cover category	County	Initial cover (ha)	A1B	RCP	RCP	RCP	1 m
			Mean	2.6	4.5	8.5	fixed
			% Land cover change by 2100				
Regularly-flooded marsh	Mainland Hyde	7561	37.81	38.23	41.06	47.15	64.91
	Mainland Dare	2603	32.17	34.94	33.12	32.10	54.43
	Outer Banks	2165	-5.91	-6.49	-7.30	-8.69	-7.17
	Total	12329	28.94	29.68	30.90	34.17	50.04
Irregularly-flooded marsh	Mainland Hyde	7037	-2.12	-1.94	-3.27	-6.54	-12.68
	Mainland Dare	3320	-0.35	-0.32	-0.57	-1.26	-0.65
	Outer Banks	4753	-0.22	-0.12	-0.64	-2.47	-8.79
	Total	15110	-1.13	-1.01	-1.85	-4.10	-8.81
		Predicted initial number of nests	Predicted future number of nests				
Regularly-flooded marsh	Mainland Hyde	62	85	86	87	91	102
	Mainland Dare	21	28	29	28	28	33
	Outer Banks	18	17	17	16	16	16
	Total	101	130	131	132	136	152
Irregularly-flooded marsh	Mainland Hyde	51	50	50	50	48	45
	Mainland Dare	24	24	24	24	24	24
	Outer Banks	35	35	35	34	34	32
	Total	110	109	109	108	106	101

Under the most liberal sea-level rise model (1 m fixed rise by 2100), regularly-flooded marsh within the study area increased, translating in a 50.5% relative increase in the projected number of black duck nests within Hyde and Dare Counties (Table 12).

Even under the most conservative model (A1B Mean Scenario), increased regularly-flooded marsh results in a 28.7% relative increase in the projected number of black duck nests. Conversely, decreasing irregularly-flooded marsh observed under the A1B Mean Scenario, resulted in a 0.9% relative decrease in projected black duck nests.

Finally, 1 m of GMSLR by 2100 translated to an 8.2% relative decrease in black duck nests (Table 12).

## **Discussion**

In the Atlantic flyway, black ducks nest in a variety of habitats. In the northern reaches of their breeding range (e.g. New Brunswick, Nova Scotia, Maine, Vermont, and New York), nesting black ducks have a high affinity for thick grasslands, shrublands, and wooded habitats of islands when available (Reed 1975, Bélanger et al. 1997). Mainland nest sites in this northern region have been documented in live conifers, downed trees, herbaceous or shrub vegetation and recent wood cuts (Gross 1945, Coulter and Miller 1968, Dwyer and Baldassarre 1993), forested wetlands (Wright 1954, Cowardin et al. 1967, Seymour 1984, Erskine 1987, Petrie et al. 2000) agricultural areas (Petrie et al. 2000, Lieske et al. 2012), early-successional habitat edges (e.g. abandoned farmland, hedgerows, and riparian areas; Gross 1945, Wright 1954, Coulter and Miller 1968, Seymour 1984), peatlands (Bélanger et al. 1997, Maisonneuve et al. 2000), and other various marsh habitats (Reed 1975). Further south in the mid-Atlantic, while some nesting has been noted in forested habitat (Van

Huizen 1932, Stotts and Davis 1960, Krementz et al. 1992), habitat selection begins to shift to coastal islands and marshes (Van Huizen 1932, Stewart 1958, Stotts and Davis 1960, Bellrose 1980, Krementz et al. 1992). For example, in coastal New Jersey, Gavutis (1966) and Kirby (1988) noted nests in clumps of *Spartina patens* and *Spartina alterniflora* within brackish marshes. In Maryland on Smith Island, Haramis (1996) monitored black duck nests located in needlerush (*Juncus* spp.) marsh where he described nests as “large elevated structures built of fragments of needlerush leaves to a height of 20–33 cm above the marsh floor with distinct ramps similar in structure to the overwater nests of North American diving ducks”.

North Carolina represents the southern extent of the species’ breeding range in the Atlantic flyway (Stewart 1958, Parnell and Quay 1962, Bellrose 1980). There have been a few past attempts to quantify the nesting habitat selection of American black ducks in North Carolina. Parnell and Quay (1962) reported black duck productivity on Pea Island, North Carolina; however, were only able to record one black duck nest in a thin stand of saltmeadow cordgrass (*Spartina patens*) just behind the ocean dunes. Parnell et al. (1986) conducted a study of breeding bird use of dredge islands within the Pamlico Sound and found several black duck nests in smooth cordgrass (*Spartina alterniflora*). Fleming et al. (1996) located 12 nests within brackish needlerush (*Juncus roemarianus*) marsh on Piney Island, North Carolina. Black ducks in this current study selected high marsh for nesting habitat. Contrary to Fleming et al.’s (1996) study in Piney Island, NC, I found relatively few nests (<7%) in needlerush-dominated low marsh. Nests that were located in these flood prone areas were elevated as described by Haramis (1996). Low-marsh habitat was used in proportion to its availability, likely due to its regular inundation coupled with availability of less flood-

prone high marsh habitats in Hyde and Dare counties. Indeed, I noted that in the brackish marsh, black ducks almost exclusively nested in *Spartina patens* patches on the open-water perimeter of the high marsh. Given the mosaic vegetation structure of the high marsh perimeter, I found habitat use was predicted to more often occur in higher cover (0.81 m) with a high grass component (~85%). Black ducks that chose spoil-islands as nest sites gravitated toward a mean vegetation height of 1.03 m with 0.60 m of visual obstruction. Vegetation composition at the nest was as follows:~62% grass, 17% woody vegetation, ~9% forbs, ~9% litter, ~2% bare soil, and negligible water. This differs from marsh habitat, having a lower grass composition and resultant higher woody vegetation and forb composition. Additionally, maximum vegetation height and vegetation visual obstruction were significantly higher at nests on spoil islands.

Unlike the findings of Van Huizen (1932), Stotts and Davis (1960), and Krementz et al. (1992), my searches in flooded oak-pine forests, early successional upland marsh perimeters, and spoil deposits on ditches and canals yielded no nests. Further, nest sites in this study system greatly differed from the mainland agricultural, early-successional, and peatland habitats selected by North Atlantic breeders, although similar habitats are spatially available in North Carolina. These habitats are likely selected against due to impeding nest predator pressure created by human habitation and upland refugia (Garrott et al. 1993). For example, Pea Island National Wildlife Refuge, located on the eastern edge of my study site, was once a highly productive black duck nesting area (Parnell and Quay 1962). Prior to 1984, raccoons were a rare occurrence on the refuge, however they were reported as common nest predators on neighboring Bodie Island (Parnell and Quay 1962, U.S. Fish and Wildlife Service

1984). During the first year of this study, I intensively searched the entirety of the refuge for black duck nests but was unsuccessful. I did however note an overabundance of raccoons on the refuge, as evidenced by tracks, scat, and very common diurnal sightings. I hypothesize that the increased human habitation on the Outer Banks and upland refugia has allowed raccoons to thrive on Pea Island, thereby possibly deterring use by nesting black ducks. In contrast, as referenced in chapter I, the spoil-islands of the Pamlico Sound, provided a quasi-upland nesting habitat refuge where predation was extremely low (>10%), likely attributable to inaccessibility to ground-based predators.

Over the next century GMSLR is projected to drastically alter the landcover of Hyde and Dare Counties, North Carolina. However, SLAMM simulations indicated that regularly-flooded and irregularly flooded marsh habitats important to black duck breeding, may be resilient. Contrary to the extreme local wetland losses projected by Clough (2008, 2012, and 2013), my SLAMM simulations reflected a dynamic, traveling marsh edge creeping into the mainland with slight losses of irregularly-flooded marsh and increases of regularly-flooded marsh even under the most liberal GMSLR pathway. Consistent with the high-resolution elevation data and technological advances published in the latest version of SLAMM (v.6.7), wave erosion calculated with wind rose data and marsh fetch distances allowed me to the likely routes of vertical marsh accretion. The consequence of this marsh resilience will result in an increased potential to support nesting black duck nests across these different SLR scenarios by 2100.

## **Management Implications**

In the face of climate change, nesting black ducks in this study system will benefit the most from the creation and maintenance of spoil-islands where predation pressure and sea-level rise affects are the lowest. Habitat management efforts on these islands should aim to maintain succession via prescribed fire and/or woody vegetation herbicide treatments implemented every 3 to 5 years to match a composition as follows: ~62% grass, 17% woody vegetation, ~9% forbs, ~9% litter, and ~2% bare soil.. Selected high marsh habitats should also be carefully managed to insure their fruition. However, it is unknown how frequent prescribed burning in the brackish marsh affects vertical accretion (Nyman and Chabreck 1995). If vertical accretion in North Carolina brackish marsh is driven by litter/peat build-up then prescribed burning would reduce vertical accretion rates. Conversely, if vertical accretion is maintained through root growth then returning nutrients to the soil through prescribed burning would promote growth and thereby increase accretion rates (Nyman and Chabreck 1995). Future management of black duck nesting habitat in the brackish marshes of North Carolina would benefit from research determining how fire frequency affects vertical accretion rates. Additionally, managers need to take consideration of likely marsh travel corridors as simulated in SLAMM models. Sea level rise is imminent and will change existing North Carolina marshes indefinitely, thus land acquisition and mitigation projects should target lands in SLAMM simulated marsh travel corridors to ensure the persistence of identified black duck nesting habitats. Future research in North Carolina, along with the rest of the coastal mid-Atlantic, would benefit from black duck habitat selection studies during the brood-rearing and wintering period to identify the possible limitations and of black duck productivity with implications of future sea level rise.

## Chapter 3

# **MALLARD–BLACK DUCK HYBRIDIZATION AND POPULATION STRUCTURE IN COASTAL NORTH CAROLINA**

### **Introduction**

A growing body of literature suggests that while gene flow is naturally a widespread evolutionary phenomenon across taxonomic lineages (Mallet 2007, Arnold and Kunte 2017), the increasing incidence of anthropogenic hybridization between taxa poses great conservation concern with important implications that vary temporally, spatially, and across biological systems (Arnold 1992, Barton 2001, Seehausen et al. 2008). Specifically, anthropogenic hybridization as a result of habitat change(s) and/or the direct movement of individuals are resulting in higher than natural rates of interspecific interaction among closely related species (Vallejo-Marin and Hiscock 2016). Consequences of such anthropogenic introgressive hybridization (i.e. when hybrids backcross to parental populations, Anderson 1953) range from the breakup of co-adapted gene complexes (i.e., outbreeding depression) to the possibility of adaptive introgression (Arnold and Martin 2009). In extreme cases, prolonged or frequent events of introgression can result in a “hybrid swarm” in which individual gene pools are eliminated, causing extinction in one or both parental taxa (Simberloff 1996, Rhymer and Simberloff 1996, Rhymer 2006, Seehausen 2006, Allendorf 2017). Effects of hybridization are further amplified when dealing with small populations, which are innately more susceptible to becoming genetically swamped (Lavretsky et al. 2019c). Thus, understanding the frequency and extent of introgressive hybridization among interacting species is increasingly critical when attempting to conserve or manage populations (Allendorf et al. 2001, Randi 2008, McFarlane and Pemberton 2019).

Ducks and geese (Order Anseriformes) exhibit some of the highest levels of hybridization in class Aves (Johnsgard 1960, Scherer and Hilsberg 1982, Grant and Grant 1992). Notably in Anatine ducks, the mallard (*Anas platyrhynchos*) readily hybridizes with many other mallard-like species including the American black duck (*Anas rubripes*; hereafter, black duck). Black ducks belong to a paraphyletic group with an estimated divergence time from mallards ~600,000 years before present (Lavretsky et al. 2019a). Prior to the 1950's, black ducks and mallards were largely allopatric, however, increasing conversion of boreal forests to open habitats in eastern Canada allowed mallard ranges to extend eastward (Livezey 1991, Green 1996, Johnson and Sorenson 1999, Mank et al. 2004). Additionally, state and private entities have facilitated large-scale game-farm mallard (hereon, GFM) releases, with ~500,000 GFM annually released through the first half of the 20th century (Smith 1999), and nearly 210,000 GFM/year still being released today (Guay and Tracey 2009, Champagnon et al. 2013, U.S. Fish and Wildlife Service 2013). In general, these events resulted in a ~600% increase in mallard 381,179 abundance east of the Mississippi River by the mid-1960s (Heusmann 1974, Soutiere 1986, Hepp et al. 1988), which translated to unnaturally high rates of interspecific interactions with black ducks in eastern North America (Anderson et al. 1987, Ankney et al. 1987, Avise et al. 1990, Conroy et al. 2002, Mank et al. 2004). Moreover, recent molecular work identified that the release of GFM in eastern North America has resulted in an established feral (i.e., individuals of domestic origins but found in wild settings) and feral × wild mallard hybrid swarm (Lavretsky et al. 2019b). Thus, black ducks can potentially interact with wild and/or feral origin mallards.

Historically, the black duck was the most abundant duck species in the Atlantic flyway, but due to dramatic population declines (>50%) between the 1950's and 1990's, black ducks are now a species of greatest conservation concern in 14 of 17 states (Devers and Collins 2011). Potential explanations for this decline include overharvest (Nichols et al. 1987, Francis et al. 1998, Longcore et al. 2000), loss of breeding and non-breeding habitat quantity and quality (Conroy et al. 2002, Zimpfer 2004), and interactions (competition, hybridization) with mallards (Anderson et al. 1987, Conroy et al. 2002, Mank et al. 2004). In fact, recent landscape-level sampling coupled with high-throughput DNA sequencing methods confirmed a substantially high rate of hybridization of 25% between mallards and black ducks, which is highest of all comparisons between mallards and other North American monochromatic mallard-like ducks (Lavretsky et al. 2015c, Peters et al. 2016, Ford et al. 2017). However, introgression of mallard genes into black ducks remained relatively low (Lavretsky et al. 2015a, Lavretsky et al. 2019c), suggesting that backcrossing of F1 mallard x black duck hybrids into the black duck gene pool was somehow limited (e.g., assortative mating, hybrid-breakdown), and largely can be associated with lost reproductive potential. More concerning, however, was that many of the F1 hybrids and the few black duck-backcrossed samples recovered by Lavretsky et al. (2019c) showed nuclear genetic assignment to GFM. Additionally, mallards in North America carry two mitochondrial haplogroups; Old World (OW) A and New World (NW) B (Awise et al. 1990, Johnson and Sorenson 1999, Kulikova et al. 2004; Kulikova et al. 2005, Lavretsky et al. 2014a). Although several hypotheses have been formulated to explain the presence of these haplogroups in North America (Awise et al. 1990, Johnson and Sorenson 1999, Kulikova et al. 2004, Kulikova et al. 2005, Lavretsky et

al. 2014a), Lavretsky et al. (2019b) provided conclusive evidence supporting that the majority of OW A mtDNA haplotypes in North America are due to gene flow from GFM. Thus, the possession of an OW A mtDNA haplotype would be strong evidence of GFM ancestry. Importantly, these results suggest that much of the interspecific interaction that has been discussed between mallards and black ducks is likely largely the result of the recently established eastern North American feral GFM population.

The objective of this study is to assess the genetic integrity and population structure of a black duck population at the southernmost extent of their breeding range. In general, the black duck population in the Albemarle-Pamlico Peninsula and Outer Banks regions of coastal North Carolina (Parnell and Quay 1962, Bellrose 1980, Baldassarre 2014) is thought to be small, and largely comprised of locally breeding individuals that rarely conduct long-distance movements (NCWRC *pers. comm*). In addition to the fact that genetic drift disproportionately impacts populations of small size (Wang et al. 1999), inbreeding may also be a particular issue for ducks that exhibit seasonal monogamy and female nest-site fidelity (Coulter and Miller 1968). Thus, it is important to understand whether any observable population structure is due to sibling relationship, inbreeding, or both.

Here, I utilized high-throughput DNA sequencing methods to assay thousands of nuclear loci, as well as the mitochondrial control region across samples, and compare them to published reference black duck, mallard, and GFM samples (Lavretsky et al 2019c). First, I aimed to attain individual ancestry assignments to determine hybridization rates, including the mallard source (wild or feral) that may be interacting with the local black duck population. Given that several GFM release preserves are in the vicinity of the study site ([Figure 1](#)), I expected that most hybrids

will be of GFM × black duck ancestry. Furthermore, I expected to see multiple filial generations of hybrids if hybrids readily backcross. Alternatively, if hybrid survival is low or selected against during pair-bonding, then I expected to find little evidence of backcrossed hybrids. Next, I measured the relationship among samples (i.e. sibship, parentage) to determine the extent to which relatedness is present in the North Carolina black duck population. Moreover, I compared molecular diversity estimates for the local North Carolina to reference black duck populations to determine whether higher rates of relatedness translate to loss of diversity and increased potential for inbreeding depression (Charlesworth and Charlesworth 1987). Specifically, if the North Carolina breeding black duck population is in immediate risk of inbreeding depression, I expected to find low genetic structure, high levels of interrelatedness, as well as low haplotype and nucleotide diversity within genetically “pure” North Carolina black ducks as compared to reference continental black duck populations.

### **Study area**

I conducted research in Hyde and Dare counties in the Albemarle-Pamlico Peninsula and the Outer Banks regions of North Carolina from late March to the end of June in 2017 and 2018 ([Figure 1](#)). Specifically, my research focal area ranged from the Pamlico and Pungo River convergence northeast along the Pamlico Sound to the intersection of US 64 and the Croatan Sound and throughout the Outer Banks of Hyde and Dare Counties. Study sites included Swanquarter National Wildlife Refuge (35°21'59.99" N -76°19'18.00" W), Alligator River National Wildlife Refuge (35°46'59.99" N -75°50'59.99" W), Pea Island National Wildlife Refuge (35°41'17.99" N -75°32'26.99" W), and private and state-owned lands along the Pamlico Sound.

The Albemarle-Pamlico Peninsula was historically dense in wetland habitats but most of the wetlands were drained and converted to agricultural cropland. Extant wetland habitats include large natural lakes (including Lake Mattamuskeet which is the largest in North Carolina), river drainage systems of the Alligator, Pungo, and Long Shoal rivers, large contiguous blocks of semi-permanently flooded swamp, pocosins, and brackish and freshwater marsh. Freshwater Lacustrine and Riverine habitats in this region are dominated by panic grasses (*Panicum spp.*), cattail (*Typha spp.*), sawgrass (*Cladium spp.*), sedges (family *Cyperaceae*), rushes (family *Juncaceae*), and duck weeds (*Lemna spp.*) and edges transition into mixed hardwood swamps, where the dominant overstory is composed of red maple (*Acer rubrum*), black gum (*Nyssa sylvatica*), bald cypress (*Taxodium distichum*), and swamp tupelo (*Nyssa biflora*), or hardwood and mixed pine habitats. Pocosins cover the fresh-saltwater transition zone and consist primarily of pond pine (*Pinus serotina*), loblolly bay (*Gordonia lasianthus*), red bay (*Persea borbonia*), and sweet bay (*Laurus nobilis*), with an understory of titi (*Cyrilla racemiflora*), honeycup (*Zenobia pulverulenta*), fetterbush (*Lyonia lucida*), and gallberry (*Ilex coriacea*). Vegetation in the brackish marsh is primarily composed of black needlerush (*Juncus roemarianus*) with patches of cordgrasses (i.e. *Spartina patens* and *S. alterniflora*). The Outer Banks region is a barrier island complex bordered by Pamlico, Croatan, and Core sounds to the west and the Atlantic Ocean to the east (Devers and Collins 2011). Within this area, Pea Island National Wildlife Refuge is a long and narrow island with 91–244 m wide line of beach dunes on the island's eastern border, several large submerged aquatic vegetation (SAV) impoundments at its core, and extensive brackish and saltmarsh dominated by black needlerush, saltmeadow cordgrass (*Spartina patens*), and sawgrass, on its

western border. The remaining portion of this region contains beach dunes (composed of sea oats [*Uniola paniculata*], panic grasses, and seaside bluestem [*Schizachyrium littorale*]); maritime shrub and dry grassland (dominant shrubs include: wax myrtle [*Morella cerifera*], red cedar [*Juniperus virginiana*], yaupon holly [*Ilex vomitoria*], greenbrier [*Smilax spp.*], blackberry [*Rubus spp.*], and poison ivy [*Toxicodendron radicans*]); and various early successional habitats.

### **Methods**

Over the 2017 and 2018 nesting seasons, I collected chorioallantoic membranes and/or maternal contour feathers from 140 black duck nests. DNA extraction and isolation were attempted across all available chorioallantoic membranes (n = 85) and maternal contour feathers (n = 40) using a Qiagen DNAeasy blood and tissue kit (Qiagen, Valencia, CA) and following manufacturer's protocol. In general, the contour feather calami (Pearce et al. 1997, Williams et al. 2005) and vascularized chorioallantoic membrane tissue represented the maternal and offspring genotypes, respectively. Note that mtDNA sequences were used to determine the maternal lineage in cases where the mother failed to successfully sequence. Extractions were quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific Inc.) to ensure a minimum concentration of 0.02 µg/µl.

Library preparation for multiplexing followed double digest restriction-site associated DNA (ddRAD-seq) protocol described by Lavretsky et al. (2015b). In brief, 10 U of SbfI and EcoRI restriction enzymes were used to digest 1 µg of gDNA and then the ligated samples were run on a 2% low-melt agarose gel. I excised DNA ranging from 300 to 450 bp and purified it using a MinElute Gel Extraction Kit (Qiagen, Valencia, CA). Selected DNA was then amplified using standard PCR with

Phusion high-fidelity DNA polymerase (Thermo Scientific, Pittsburgh, PA). Products were purified using magnetic AMPure XP beads (Beckman Coulter, Inc., Indianapolis, IN) and quantified using real-time PCR. All samples were then pooled in equimolar concentrations, and 150 base pair, single-end sequencing was completed on an Illumina HiSeq 4000 at the University of Oregon, Genomics & Cell Characterization Core Facility (GC3F).

After sequencing, I followed methods described by Lavretsky et al. (2015a) to demultiplex and process sequences from 40 putative NC breeding black ducks using the computational pipeline described by DaCosta and Sorenson (2014; <http://github.com/BU-RAD-seq/ddRAD-seq-Pipeline>). Once again, I compared NC mtDNA sequences to the same reference sample set of genetically-vetted black duck, wild mallard, GFM, and F1 mallard × black duck hybrids (sequences attained from Lavretsky et al. 2019c). For each sample, identical reads were collapsed and filtered based on a Phred score < 20 in UCLUST (USEARCH v. 5; Edgar 2010). Following Lavretsky et al. (2014b), I then clustered and concatenated reads with an `-id` setting of 0.85 in UCLUST. The reads within each cluster were aligned using MUSCLE v. 3 (Edgar, 2004), and samples within each aligned cluster were genotyped using the Python script RADGenotypes.py. Alignments with end gaps due to indels and/or a polymorphism in one of the restriction sites were either automatically trimmed or flagged for manual editing during genotyping. Alignments with  $\geq 2$  polymorphisms in the first or last five base-pairs were also flagged for manual inspection. Polymorphisms were scored using read depths for major and minor alleles and a population-aware algorithm. Individual genotypes fall into four general categories: “missing” (no data), “good” (unambiguously genotyped), “low depth” (recovered data,

but could not reliably score as 91 homozygous or heterozygous because of low depth), and “flagged” (recovered heterozygous genotype, but with counts of major and other alleles below acceptable thresholds). Loci with <10% missing genotypes and  $\leq 6$  flagged genotypes were retained for downstream analyses. Unlike other protocols, the developed pipeline retains loci containing indels and high variability by flagging them for manual editing. By including these flagged loci, I increased the total number of retained markers by ~15%, while reducing any bias resulting from discarding loci with indels or high variability.

Because of its maternal inheritance, lack of meiotic recombination, and rapid rate of evolution relative to the nuclear genome, mitochondrial DNA (mtDNA) has been extensively used to determine gene flow and population structure within the mallard complex (Awise et al. 1990, Johnson and Sorenson 1999, Kulikova et al. 2004, Lavretsky et al. 2014*b*). Primers L78 and H774 were used to PCR (polymerase chain reaction) amplify and sequence 653 bp of the mtDNA control region (Sorenson and Fleischer 1996, Sorenson et al. 1999) across samples. In short, the mtDNA control region was amplified with the following PCR mixture: 1.5  $\mu$ L of template DNA (10 ng/  $\mu$ L), 2x GoTaq Green Master Mix (Promega), and 1.0 nM of each primer, in a total volume of 15  $\mu$ L (Lavretsky et al. 2014*b*). PCR was conducted following Lavretsky et al. (2014*b*) using an Eppendorf Mastercycler (epgradient) under the following conditions: DNA denaturation at 94°C for 7 min, followed by 45 cycles of denaturation at 94°C for 20 s, primer annealing at 58°C (at 52°C for mtDNA) for 20 s, and extension at 72°C for 1 min, and a final DNA extension at 72°C for 7 min. Amplification was verified using gel electrophoresis with a 1.5% agarose gel, and PCR products were cleaned with AMPure XP beads following the Agencourt protocol

(Beckman Coulter Co.). Sanger sequencing of PCR products were done on an ABI 3730 at the University of Texas - El Paso. Finally, all sequences were aligned and edited using Sequencher v. 4.8 (Gene Codes, Inc.).

For mtDNA, 108 North Carolina samples and an additional 199 genetically-vetted wild mallard, GFM, black ducks, and vetted F1 black duck × mallard hybrids were included in analyses (sequences attained from Lavretsky et al. 2019c).

Population structure was visualized via a haplotype network reconstructed in the program Network v 5 (Bandelt et al. 1999; fluxus-engineering.com), which were also used to determine samples carrying OW A versus NW B mtDNA haplotypes.

Next, I visualized population structure across ddRAD-seq autosomal loci only using bi-allelic SNPS with singletons removed and then formatted as required for the program ADMIXTURE v.1.3 (Alexander et al. 2009, Alexander and Lange 2011). Analyzing autosomal markers, admixture analysis was run with a 10-fold cross-validation, and with a quasi-Newton algorithm employed to accelerate convergence (Zhou et al. 2011). To limit any possible stochastic effects from single analyses, I ran 100 iterations at each population of  $K$  (from  $K$  of 1–10). Each analysis used a block relaxation algorithm for point estimation and terminated once the change (i.e., delta) in the log-likelihood of the point estimations increased by  $<0.0001$ . The optimum  $K$  was based on the average of CV errors across the 100 analyses per  $K$ ; however, additional  $K$ s were analyzed for further population structure resolution. I then used the program CLUMPP v.1.1 (Jakobsson and Rosenberg 2007) to determine the robustness of the assignments of individuals to populations at each  $K$ . First, the R program PopHelper (Francis 2017) was used to efficiently convert ADMIXTURE outputs into CLUMPP input files at each  $K$ . In CLUMPP, I employed the Large Greedy algorithm and 1,000

random permutations. Final admixture proportions for each  $K$  and per sample assignment probabilities (Q estimates; the log likelihood of group assignment) were based on CLUMPP analyses of all 100 replicates per  $K$ . Interpreting mixed ancestry based on maximum likelihood assignments in admixture alone can lead to potential pitfalls (Lawson et al. 2018). Therefore, I additionally evaluated recent patterns of coancestry using fineRADstructure (Malinsky et al. 2018), which includes RADpainter v 0.1 and finestructure (Lawson et al. 2012). Briefly, fineRADstructure derives a matrix of coancestry coefficients following the distribution of identical or nearest-neighbor haplotypes within samples, where each individual's coancestry per locus is equally divided among all other individuals with identical haplotypes, or in the case of a unique allele, all other individuals with the “nearest-neighbor” haplotype. Thus, rare haplotypes defined by rare SNPs contribute the most to the coancestry index, providing a measure emphasizing recent coancestry. This analysis was completed without a priori information on population or species identity. A burn-in of 100,000 iterations, followed by 100,000 Markov chain Monte Carlo iterations were completed, followed by tree building using default parameters. To visualize the results, I used the Program R scripts `fineradstructureplot.r` and `finestructurelibrary.r` (available at <http://cichlid.gurdon.cam.ac.uk/fineRADstructure.html>). I then analyzed optimum  $K$  and per sample assignment probabilities to assign “pure” black ducks, wild mallards, GFM, and mallard  $\times$  black duck hybrids (1<sup>st</sup>–3<sup>rd</sup> filial generations and direction of backcross) following purity thresholds defined by Lavretsky et al. (2019c). Finally, I independently calculated composite pair-wise estimates of relative divergence ( $\Phi_{ST}$ ) and nucleotide diversity ( $\pi$ ) in the R package PopGenome (Pfeifer et al. 2014) across ddRAD-seq loci and the mtDNA control region.

Relatedness was quantified across samples using the program COLONY v. 2.0.6.5 (Jones and Wang 2010). COLONY implements full-pedigree likelihood methods to simultaneously infer sibship and parentage among individuals using multilocus genotype data. COLONY analyses were based on ddRAD-seq autosomal loci with <5% missingness and a minimum allele frequency (MAF) of 0.5 across samples. To reduce the risks of type I error, I only report parental, full-sibling, and half-sibling dyads with pairwise relatedness estimates that were greater than 0.2 (Lebigre et al. 2010). If there are few migrants into the North Carolina population, I expect to find substantial relatedness given the hypothesis that the local breeding population is small and likely disjunct from the main black duck population breeding in northern Canadian Boreal forests (NCWRC *pers. comm.*).

## Results

I achieved sufficient DNA quality and quantity required to construct ddRAD libraries for 32 chorioallantoic membranes (38% success) and 8 maternal contour feather (20% success) samples. A total of 1,330 ddRAD-seq autosomal loci (195,984 base pairs; 22,155 polymorphisms) met my coverage and missing data criteria for these 40 samples. Final datasets comprised loci with an average median sequencing depth of 220 reads per locus per individual (median range = 10–2,344 reads/ locus/ individual), and on average, 94% of alleles per individual per locus were scored.

Individual assignment probabilities based on 8,737 bi-allelic single nucleotide polymorphisms (SNPs) and excluding singletons, were attained in the program ADMIXTURE to genetically assign samples prior to further analysis. Given inherent bias favoring lower  $K$  values common in analyses of closely related taxa, and the linear increase in cross-validation (CV) values indicating that higher values of  $K$  might

reveal biologically meaningful information (Janes et al. 2017), additional values of  $K$  were explored for further resolution of population structure. At a  $K$  of 4, previously described structure across known GFM, wild mallards, and parental black duck samples was achieved (Lavretsky et al. 2019c), as well as recovery of a unique genetic structure within North Carolina samples (Figure 10). Comparing those samples assigned to the unique ADMIXTURE genetic cluster with coancestry assignments in fineRADstructure, I determined that these samples indeed showed high levels of relatedness. Moreover, using 262 ddRAD-seq autosomal SNPs that met my coverage and minimum allele frequency requirements to quantify relatedness among samples in the program COLONY revealed 32 parentage assignments, 5 full-sib pairings, and 38 half-sib pairings with pairwise relatedness estimates that were greater than 0.2 (Figure 11). Thus, the unique genetic cluster of black ducks in North Carolina is best explained by high inter-relatedness. Together, for the 40 NC samples, I assigned 12 as related NC black ducks, 5 as second cousin black ducks, 8 as unrelated wild black ducks, 2 as putative GFM  $\times$  black duck hybrids, and 9 as putative mallard  $\times$  black duck hybrids. I used purity thresholds as defined by Lavretsky et al. (2019c) to assign samples across pure parental and several hybrid classes (i.e., F1–F3, Table 13). Of putative North Carolina black ducks, 45% were classified as mallard  $\times$  black duck hybrids representing F1 (25%), F2 (15%), and F3 (5%) hybrid generations. A significant portion of hybrids (~ 55%) had contributions from GFM, including all samples identified as putative F3 hybrids.

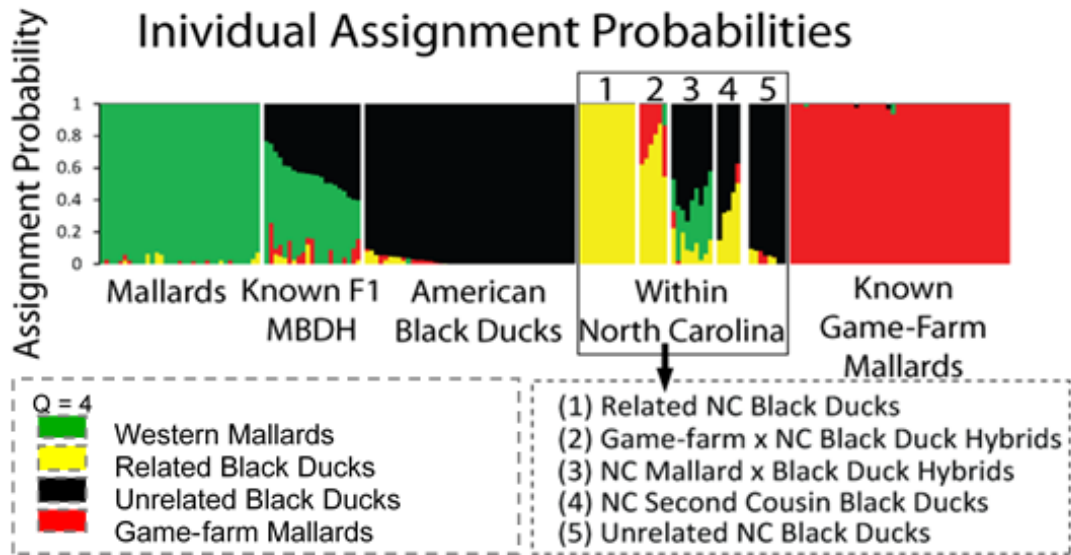


Figure 10 ADMIXTURE assignment probabilities from  $K$  of 5 analyses across reference and North Carolina American black ducks, mallard, game farm mallards, and hybrid samples. Location is coastal North Carolina with samples provided ( $n = 40$ ).

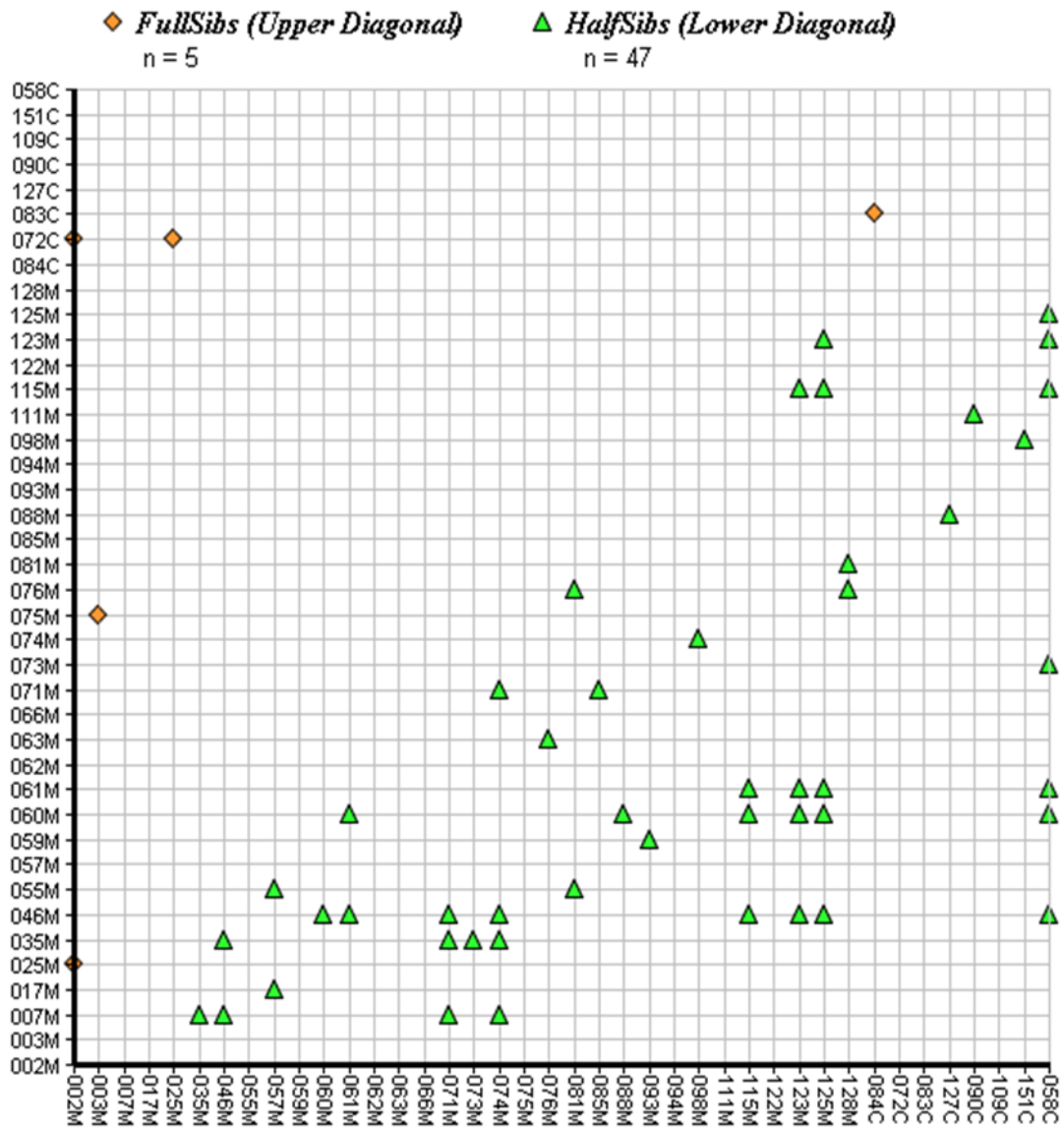


Figure 11 Co-ancestry plot of North Carolina black duck samples (n = 40). Sample relation pairings are estimated in Program COLONY, to determine full and half sibship.

Table 13 Simulation-based indices for “pure” black ducks, “pure” mallards, F1 hybrids, F2-black duck backcrosses, as well as F3-black duck backcrosses. Per index, assignment probabilities are based on the proportion of intra- and inter-specific assignment. Purity assignments based on percentage assigned to black duck populations. Location is coastal North Carolina with samples provided (n = 40).

<b>Group</b>	<b>Index</b>	<b>NC (n = 40)</b>
American Black Duck	Pure $\geq 95\%$	22 (0.55)
	Prop. Assigned to Secondary Mallard Group	0
	Prop. A mtDNA haplogroup	0
Hybrid (MBDX F1)	$27\% < F1 \leq 72\%$	10 (0.25)
	Prop. Assigned to Secondary Mallard Group	4 (0.40)
	Prop. A mtDNA haplogroup	0
F2 Toward ABDU (ABDX F2)	$10\% < F2 \leq 27\%$	6 (0.15)
	Prop. Assigned to Secondary Mallard Group	4 (0.64)
	Prop. A mtDNA haplogroup	1 (0.03)
F3 Toward ABDU (ABDX F3)	$5\% < F3 \leq 10\%$	2 (0.05)
	Prop. Assigned to Secondary Mallard Group	2 (1.00)
	Prop. A mtDNA haplogroup	0

For autosomal loci within North Carolina samples, related black ducks exhibited significant amounts of differentiation ( $\Phi_{ST} = 0.06$ ) from unrelated black ducks (Figure 12). Comparisons of North Carolina and reference samples of mallards yielded differentiation estimates of 0.05 and 0.025, for related black ducks and unrelated black ducks, respectively. Similarly, estimated differentiation from reference black ducks was 0.05 and 0.01 for related black ducks and unrelated black ducks, respectively. Significant differentiation was apparent for related black ducks and unrelated black ducks, ( $\Phi_{ST} = 0.12$  and 0.11, respectively) when compared against known game-farm mallards. Collectively, sample differentiation ( $\Phi_{ST}$ ) was  $\sim 0.019$  from reference mallards and black ducks, and  $\sim 0.09$  from known game-farm mallards (Figure 12).

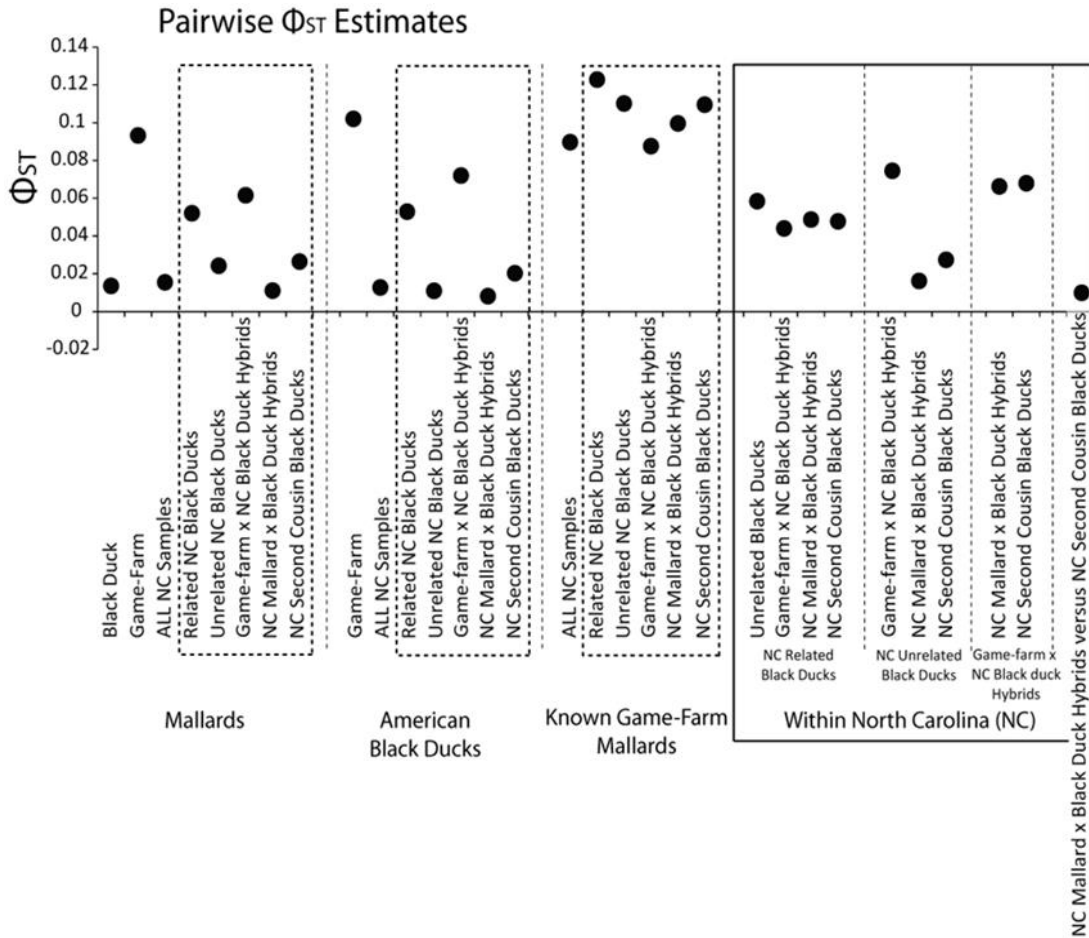


Figure 12 Autosomal DNA pairwise  $\Phi_{ST}$  estimates for genetically vetted mallards, American black ducks, known game-farm mallards, and North Carolina ADMIXTURE assigned samples. 2017–2018.

Nucleotide diversity ( $\pi$ ) in related and unrelated North Carolina black ducks was 0.0070 and 0.0072, respectively, and was comparable to reference black ducks and mallards ( $\pi \sim 0.0071$ , [Figure 13](#)). Game-farm mallard nucleotide diversity was the least at 0.0058. Collectively, nucleotide diversity across North Carolina samples was 0.0075 ([Figure 13](#)).

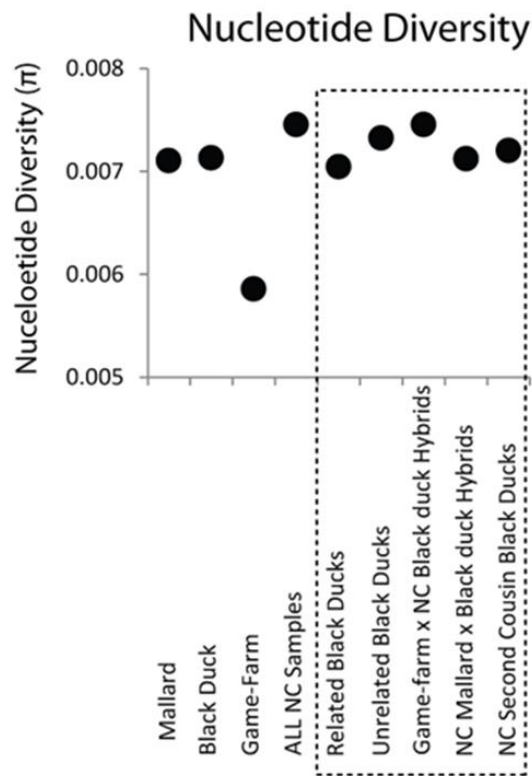


Figure 13 Nucleotide Diversity ( $\pi$ ) among reference mallard, black duck, and game-farm mallard samples along with admixture assigned groups within coastal North Carolina.

A total of 624 base-pairs of the mtDNA control region was successfully amplified and sequenced across 108 samples, 93 of which were phenotypic black ducks. Previously described OW A and NW B mtDNA haplogroups (Avisé et al. 1990, Johnson and Sorenson 1999, Kulikova et al. 2005, Lavretsky et al. 2019a) were recovered in the mtDNA haplotype network (Figure 14). Of 108 phenotypically-assigned black ducks and mallards, 9 and 3 possessed OW A haplotypes, respectively. Finally, of the 40 samples for which ddRAD autosomal loci were sequenced for, only

a single wild mallard x black duck F2 hybrid possessed an OW A haplotype, with all remaining samples possessing NW B haplotypes ([Figure 14](#)).

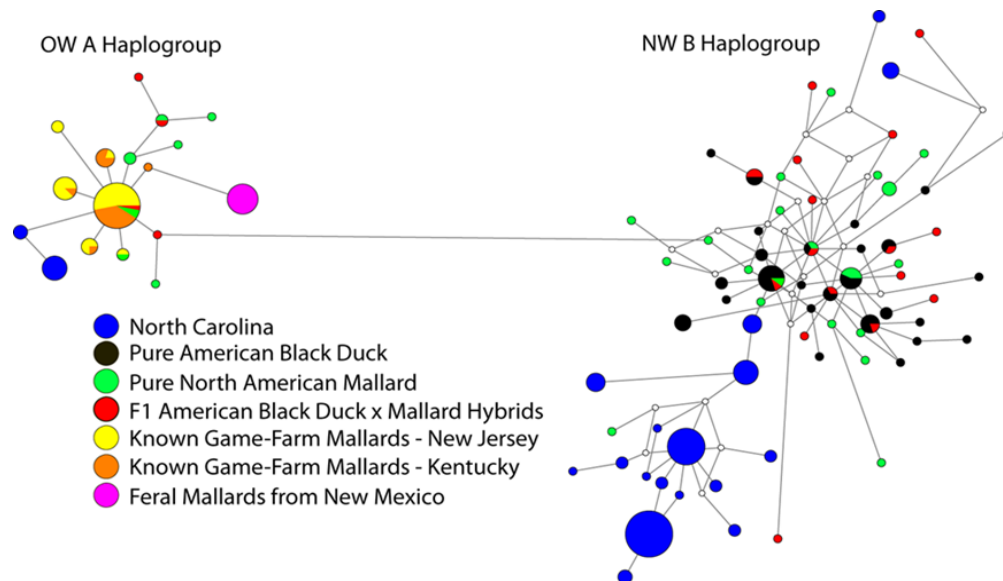


Figure 14 Haplotype Network of reference and North Carolina American black ducks, mallard, game farm mallards, and hybrids where graduated circles represent sample size and network lines represent genetic distance between and within the OW A and NW B mitochondrial haplogroups.

## Discussion

Molecular assessment of a local breeding black duck population in North Carolina reveals unique genetic structure largely explained by high relatedness among sampled individuals. Specifically, I found unique population structure when analyzing mtDNA ([Figure 14](#)) and nuclear ([Figure 12](#)) variation, which I conclude is due to high co-ancestry among related samples (i.e., siblings and parent-offspring; [Figure 11](#)). Comparing North Carolina's related black ducks or unrelated black ducks to the reference black duck population, I found that related black ducks are significantly

differentiated, whereas unrelated black ducks are genetically identical to the continental black duck population ( $\Phi_{ST} \sim 0.055$ , [Figure 12](#)). Moreover, related North Carolina black ducks largely possess a unique NW B mitochondrial haplogroup, further suggesting localized population structure, specifically among breeding females. Unlike most families in the class Aves, Anatine waterfowl exhibit female-biased natal and breeding philopatry (Coulter and Miller 1968, Greenwood and Harvey 1982, Clarke et al. 1997, Zhuravlev and Kulikova 2014), which often results in strongly structured mitochondrial lineages. Among black ducks, ~36 % of adult hens are philopatric to natal breeding sites and in extreme cases, have been found to repeatedly nest within the same wetland (i.e., < 0.25 km away annually; Reed 1975, Ringelman et al. 1982, Seymour 1991). In North Carolina, I recovered 2 re-nesting hens, 3 returning hens, and 2 breeding sisters that nested with <0.05 km in distance from previous nests of one another. Thus, the possession of a unique mitochondrial haplogroup among North Carolina black ducks suggests that same lineage of females have been breeding in the area for some time and is consistent with the philopatric nature of these birds.

Despite strong population structure due to elevated levels of interrelatedness, nucleotide diversity remains high ( $\pi \geq 0.007$ ) and similar to reference populations ([Figure 13](#)). Among my samples, I recovered 8 unrelated black ducks that were genetically most similar to the reference black ducks in my dataset, supporting that non-residents do come and breed in North Carolina. Thus, despite the potential for bottlenecking due to founder events and high interrelatedness, I conclude that the influx of genetic material from non-resident birds likely maintains high molecular diversity in North Carolina. I note that the maintenance of a unique North Carolina

mtDNA haplogroup (Figure 14) suggests that nuclear variation is maintained by breeding with migrating males. Together, I conclude that further bottlenecking, including inbreeding is not currently in immediate risk to the North Carolina black duck population.

Based on my sample set, 55% of samples were characterized as “pure” black ducks. Of the 18 samples (~45%) that I found to be hybrids, 10 were recovered with game-farm mallard (GFM) ancestry and included three filial generations of hybrids (Table 13). Additionally, of 108 mtDNA samples, I recovered 12 with OW A haplotypes, which is a direct result of gene flow from GFM in North America (Lavretsky et al. 2019b). Though, there are relatively few GFM releases within coastal North Carolina (~15,000 annually, NCWRC *pers. com.*), my results suggest that these birds perhaps pose a threat to breeding wild birds in North Carolina through anthropogenic hybridization. Alternatively, the recently described wild × GFM hybrid swarm breeding in the northeastern United States (Lavretsky et al. 2019b) may be moving into the area, breeding, and subsequently moving GFM genetic variation into North Carolina’s gene pool. The effects of hybridization are further amplified when dealing with small populations, which are innately more susceptible to becoming genetically swamped (Lavretsky et al. 2019c). In addition to decreasing molecular diversity, the movement of maladaptive domestic traits into wild populations can result in outbreeding and decreased adaptive potential of those wild populations. Importantly, the negative effects from interbreeding between domestic and wild types have been shown to often take several generations (i.e., hybrid breakdown) to be observed (Ellison and Burton 2008, Johansen-Morris and Latta 2007, Arcella et al. 2014, Stelkens et al. 2015, Bolstad et al. 2017). Thus, understanding the frequency and

extent of anthropogenic hybridization among interacting species is increasingly critical when attempting to conserve or manage populations (Allendorf et al. 2001).

Hybridization levels reported here are similar to those reported for the continental population of black ducks (~42%, Lavretsky et al. 2019c), and which remains significantly higher than for other North American monochromatic mallard-like taxa (i.e., ~5%, Lavretsky et al. 2015a, Peters et al. 2016, Ford et al. 2017). However, despite being a relatively small and isolated population that is surrounded by hunting preserves that release GFMs, I found no evidence in support of a hybrid swarm (Simberloff 1996, Rhymer and Simberloff 1996, Rhymer 2006, Lavretsky et al. 2019b). The latter results suggest that while hybridization does occur, introgression or gene flow is somehow limited. In addition to the potential that mallard (wild or GFM) × black duck hybrids may be less adaptive on the landscape (i.e. Haldane's Rule, Kirby et al. 2004; and/or hybrid breakdown, Dobzhansky and Dobzhansky 1971, Armbruster et al. 1997, Galloway and Fenster 1999, Hall and Willis 2005), assortative mating may also be resulting in low levels of incorrect mate pair selection, including mating with hybrids by black ducks.

Future research will benefit from investigating the dynamics of GFM × black duck extra-pair copulations and hybridization. This can be accomplished by genetically-sampling ducklings (as described in this chapter, i.e. ddRAD-seq) in future black duck brood studies and employing the programs, ADMIXTURE and COLONY to detect and detail extra-pair copulations. I predict that results would identify male GFM as the primary contributor in black duck extra-pair interspecies copulation events. If this were the case, then GFM gene flow into the black duck genome could

be reduced by only allowing hen GFM as candidates for captive-reared releases on shooting preserves.

### **Management Implications**

Based on these genetic results, I contend that conservation and management efforts of American black ducks in North Carolina, as well as the Atlantic flyway, would benefit from heavier restrictions on GFM stocking practices on shooting preserves. While Federal Code (§ 21.13 of title 50) provides logistics for GFM birds, there is no limitation on the number of GFM's released nor spatial constraints thus there is unlimited potential for spatial mixing and genetic swamping of wild birds. To limit the occurrence of GFM gene flow into wild relatives, state policies may want to consider reducing the number of GFM released or allow licensed shooting preserves only be allowed to practice tower shoots, where GFM are released from a designated station and are shot on the preserve the same day of the release. Further, shooting preserves could be required to submit GFM release and retrieval data to their respective state governing agency and potentially be fined per bird when released birds are not retrieved. Additionally, GFM recovered by hunters outside of their released shooting area should not count toward that hunter's daily bag limit thus helping to remove excess GFMs from the landscape. This would require amendments be made to the Migratory Bird Treaty Act (16 U.S.C. 703–712), where definitions of migratory waterfowl will not include captive-reared GFM.

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Appendix

**SLAMM GMSLR SIMULATIONS OF VARYING INTENSITY IN COASTAL  
NORTH CAROLINA, USA, 2019–2100**

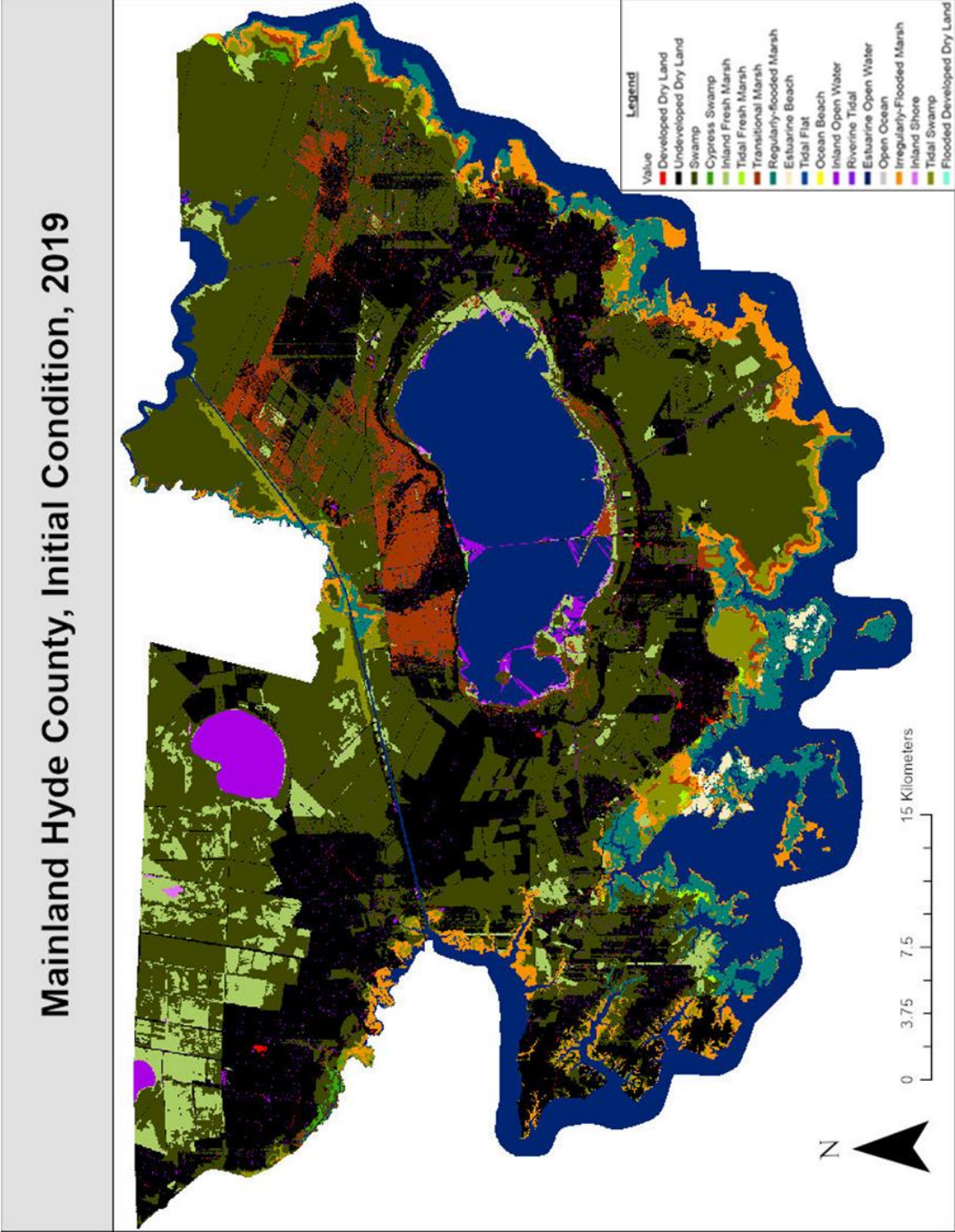


Figure 15 SLAMM habitat change predictions: Initial condition (2019) for mainland Hyde County, North Carolina, USA.

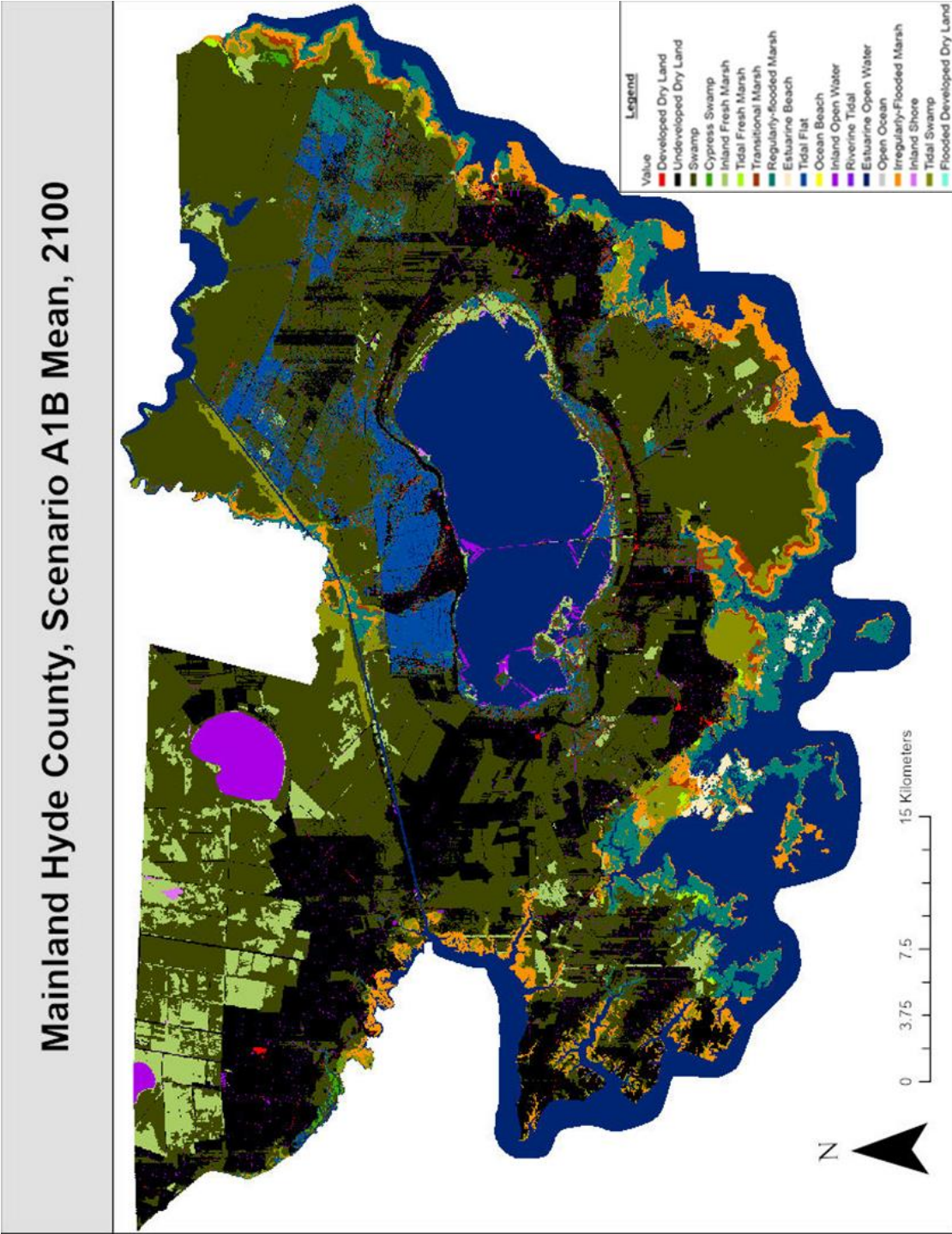


Figure 16 SLAMM habitat change predictions: A1B Mean (2100) for mainland Hyde County, North Carolina, USA.

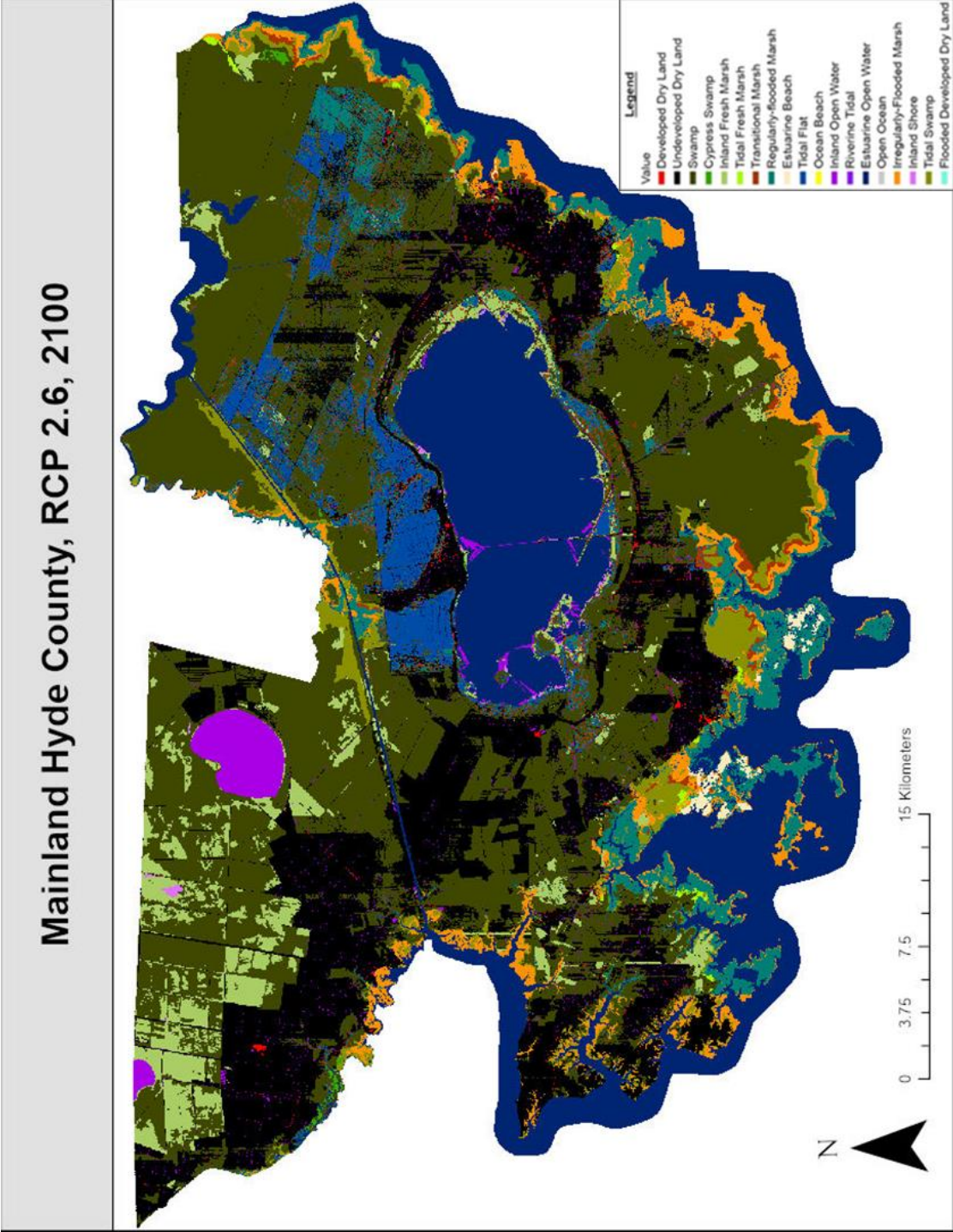


Figure 17 SLAMM habitat change predictions: RCP 2.6 (2100) for mainland Hyde County, North Carolina, USA.

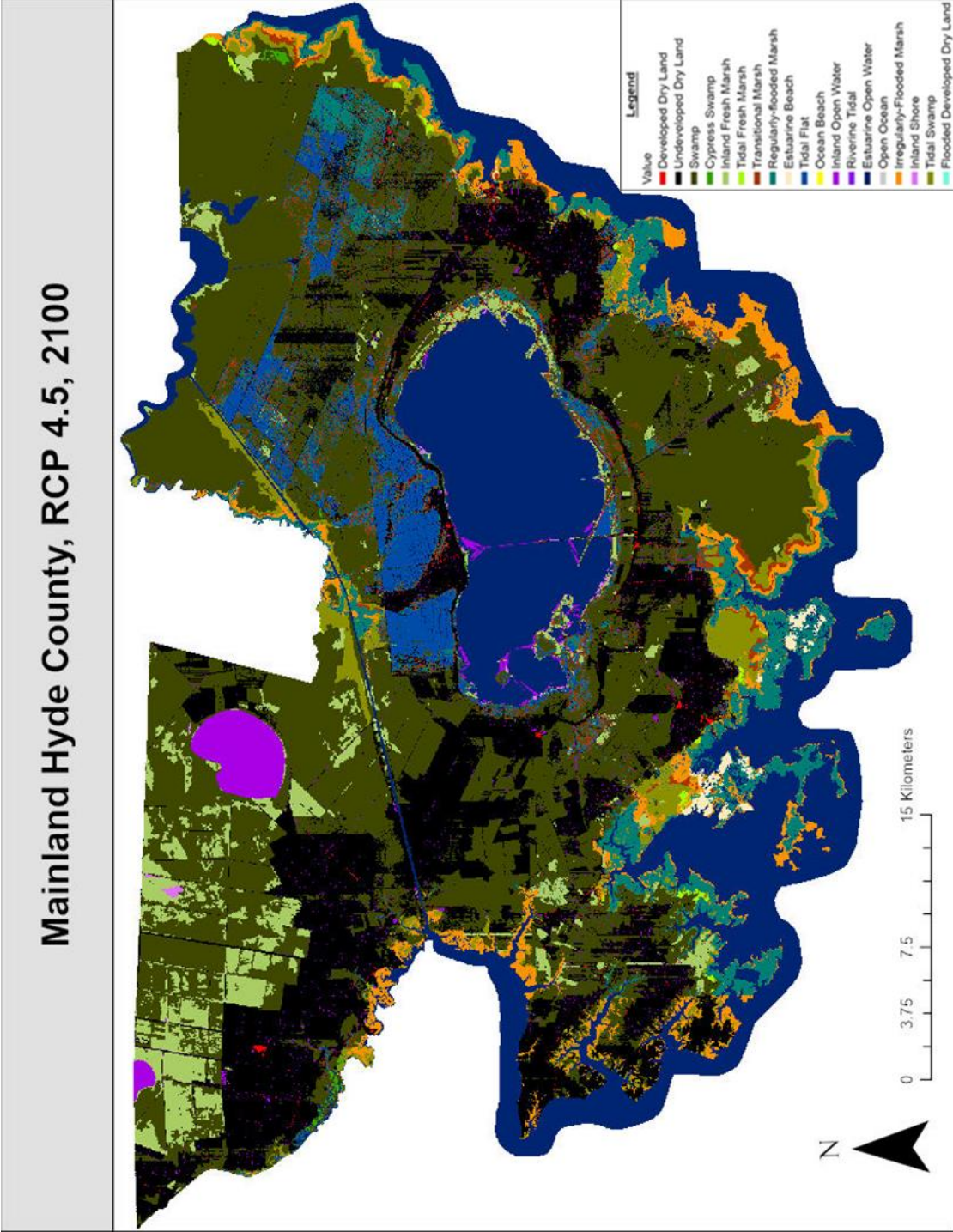


Figure 18 SLAMM habitat change predictions: RCP 4.5 (2100) for mainland Hyde County, North Carolina, USA.

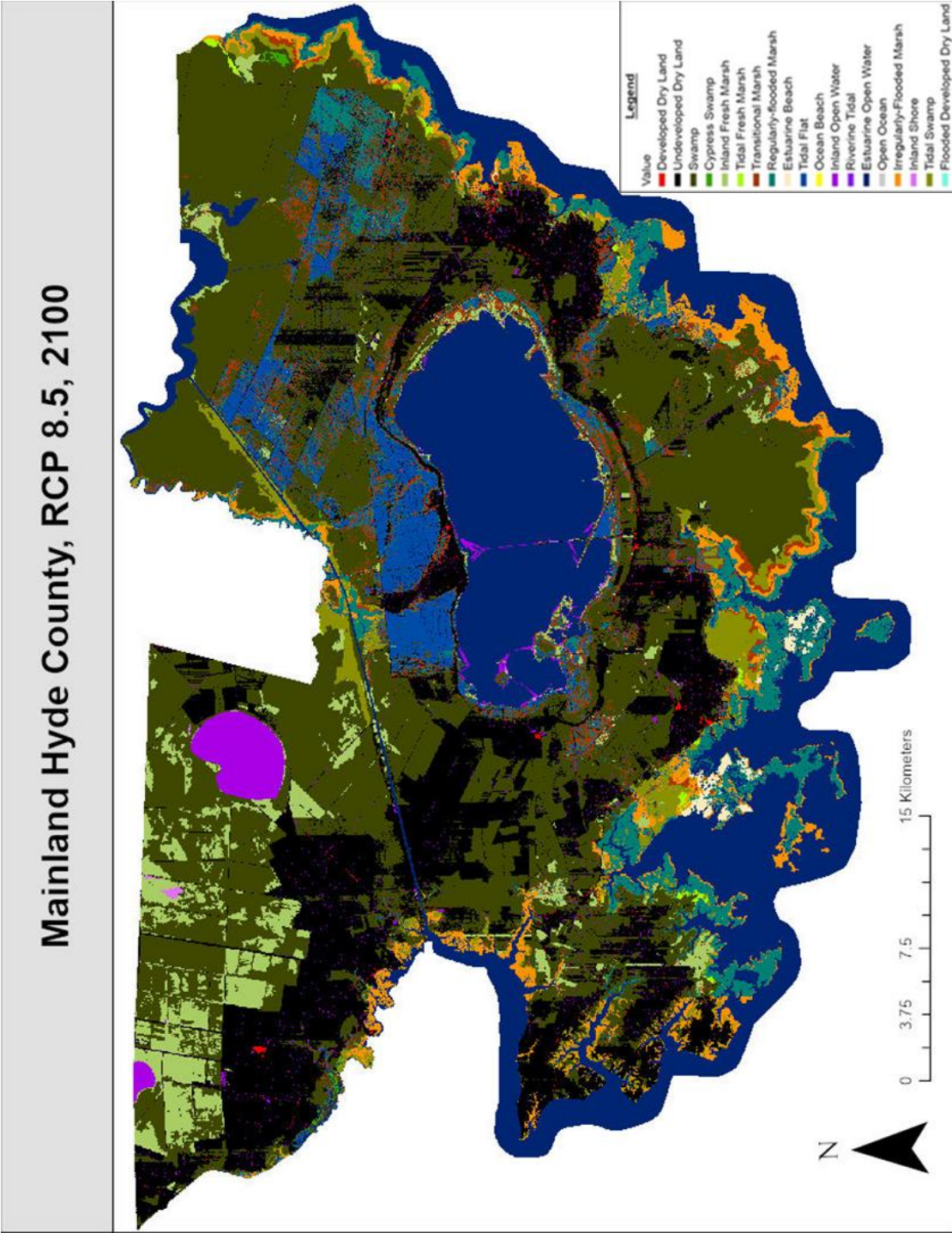


Figure 19 SLAMM habitat change predictions: RCP 8.5 (2100) for mainland Hyde County, North Carolina, USA.

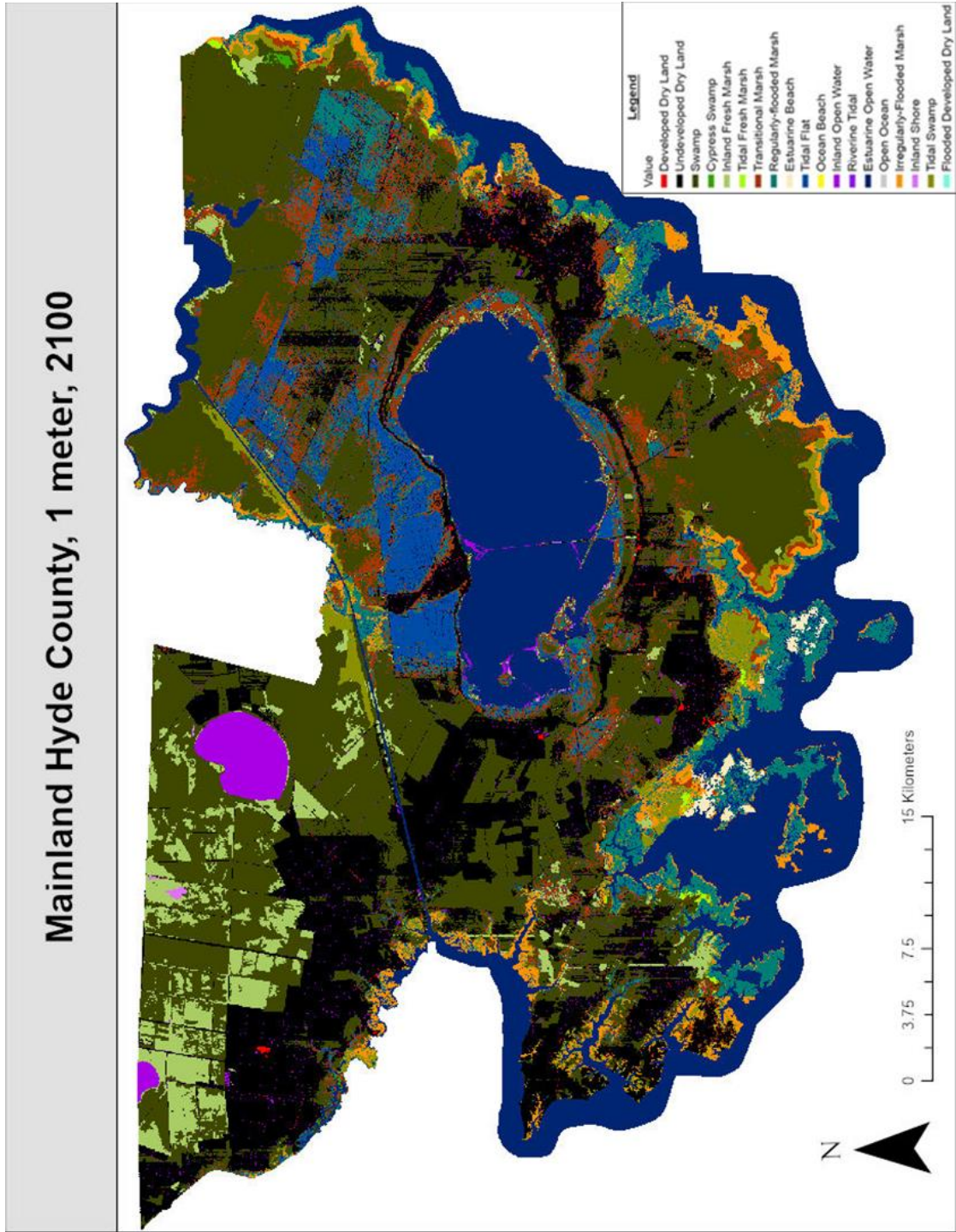


Figure 20 SLAMM habitat change predictions: 1-m fixed (2100) for mainland Hyde County, North Carolina, USA.

# Mainland Dare County, Initial Condition, 2019

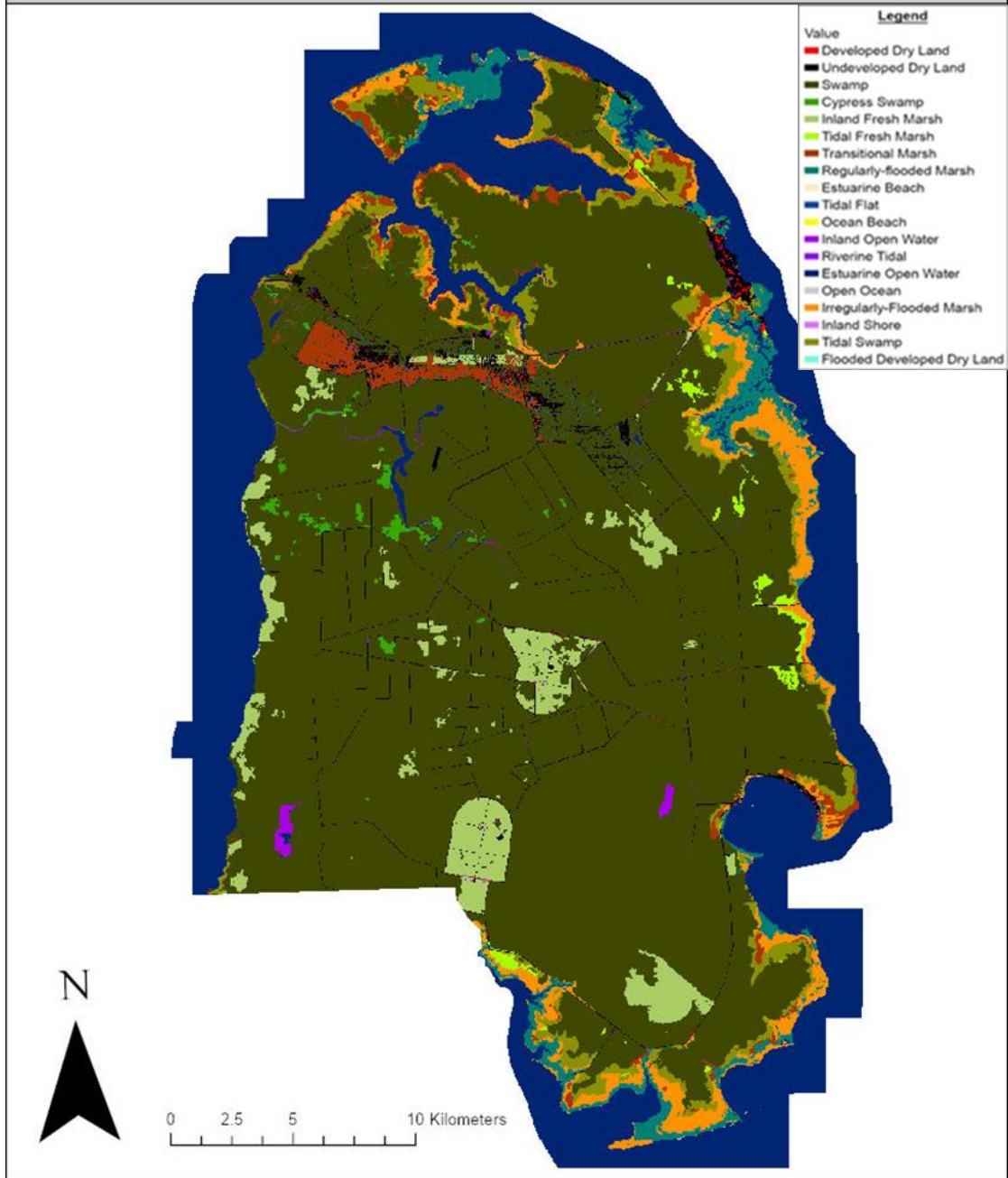


Figure 21 SLAMM habitat change predictions: Initial condition (2019) for mainland Dare County, North Carolina, USA.

## Mainland Dare County, Scenario A1B Mean, 2100

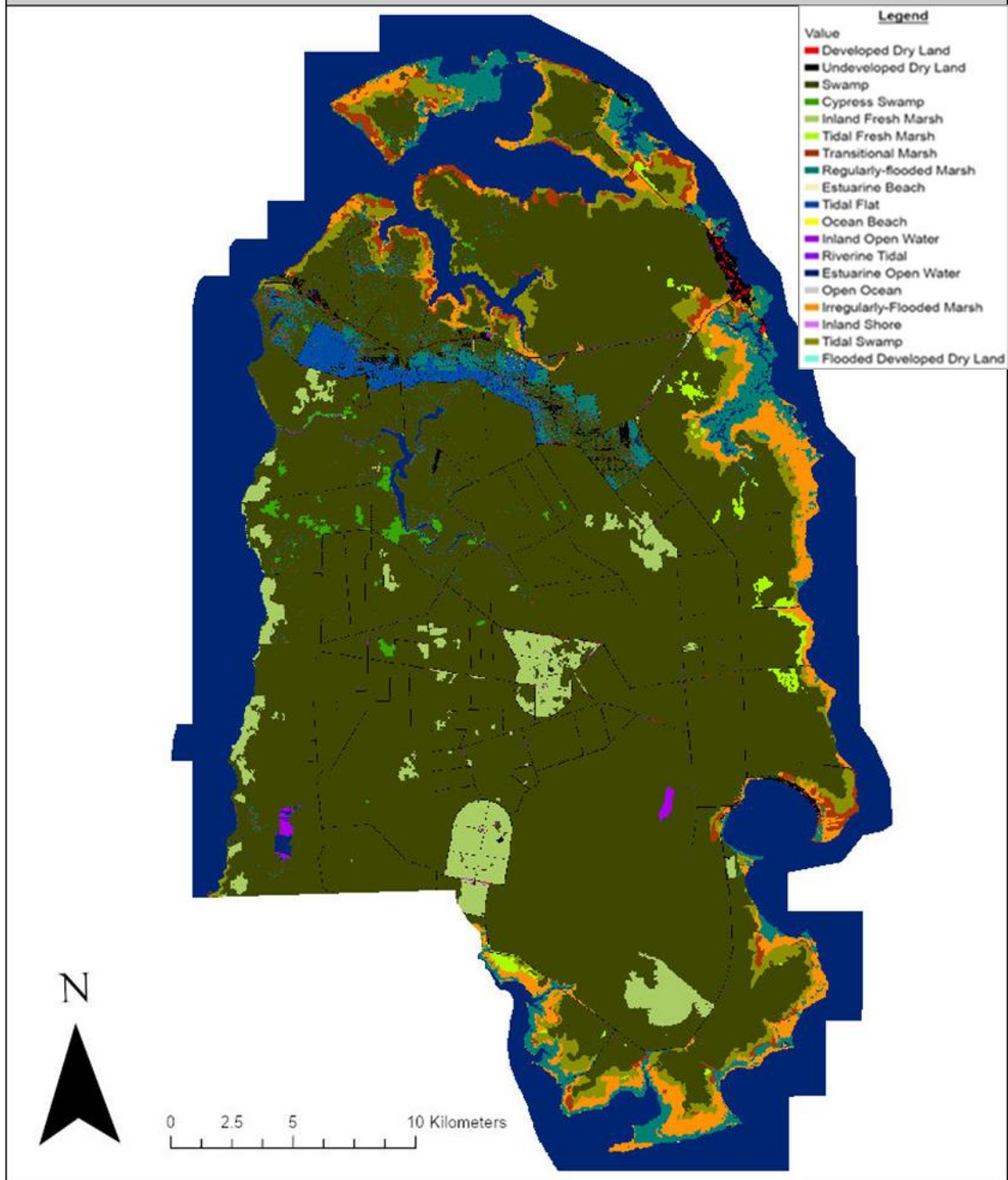


Figure 22 SLAMM habitat change predictions: A1B Mean (2100) for mainland Dare County, North Carolina, USA.

# Mainland Dare County, RCP 2.6, 2100

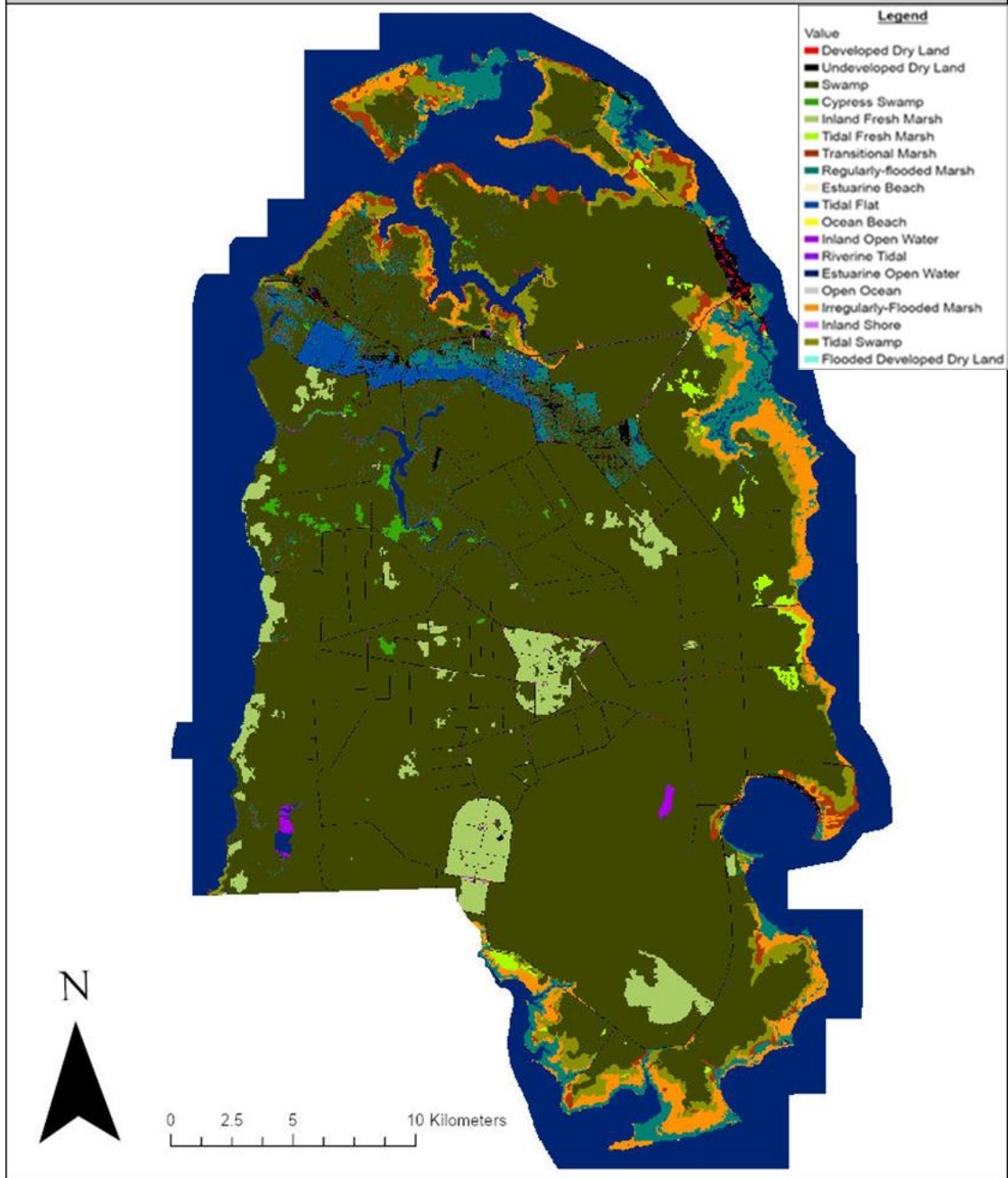


Figure 23 SLAMM habitat change predictions: RCP 2.6 (2100) for mainland Dare County, North Carolina, USA.

# Mainland Dare County, RCP 4.5, 2100

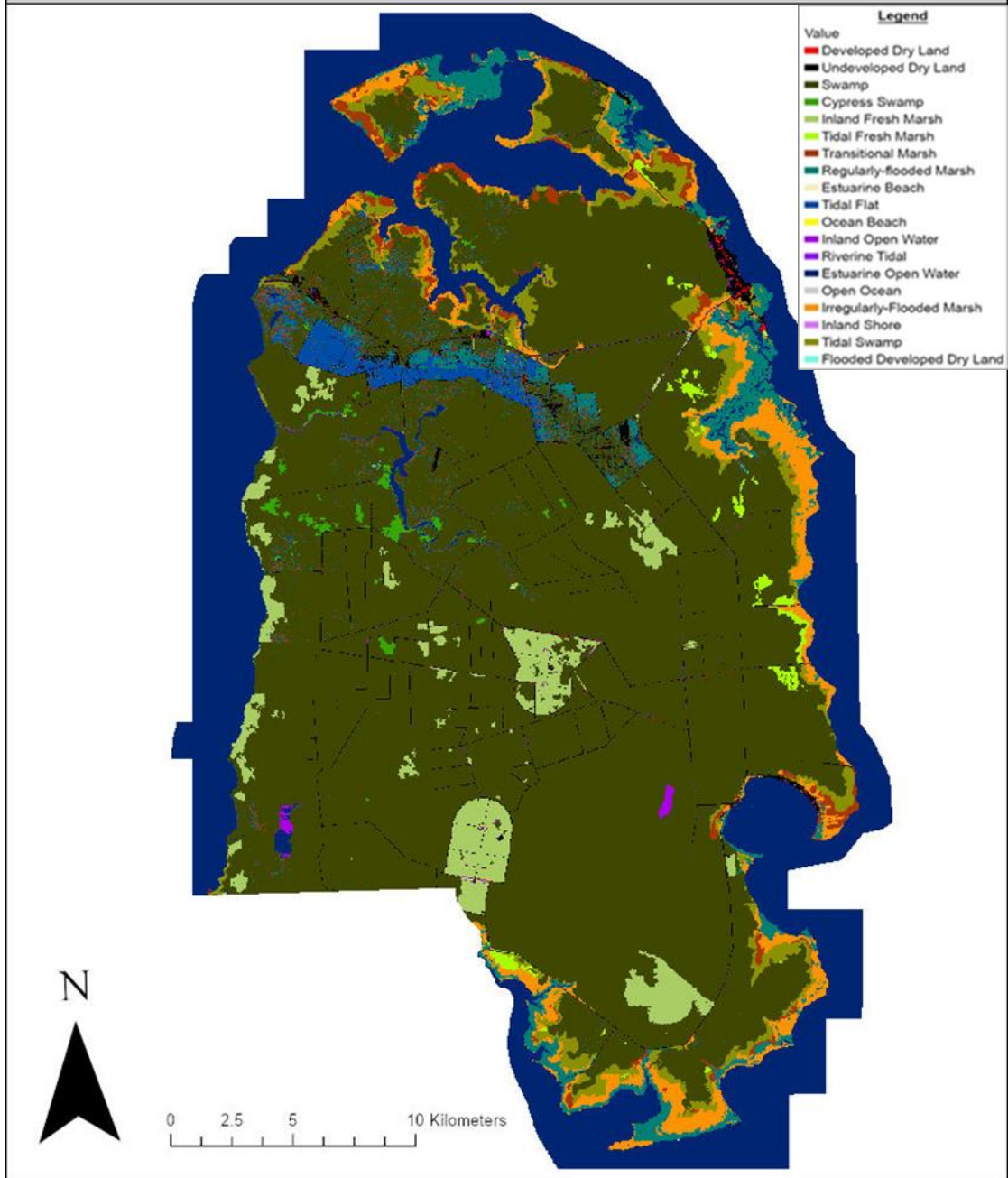


Figure 24 SLAMM habitat change predictions: RCP 4.5 (2100) for mainland Dare County, North Carolina, USA.

# Mainland Dare County, RCP 8.5, 2100

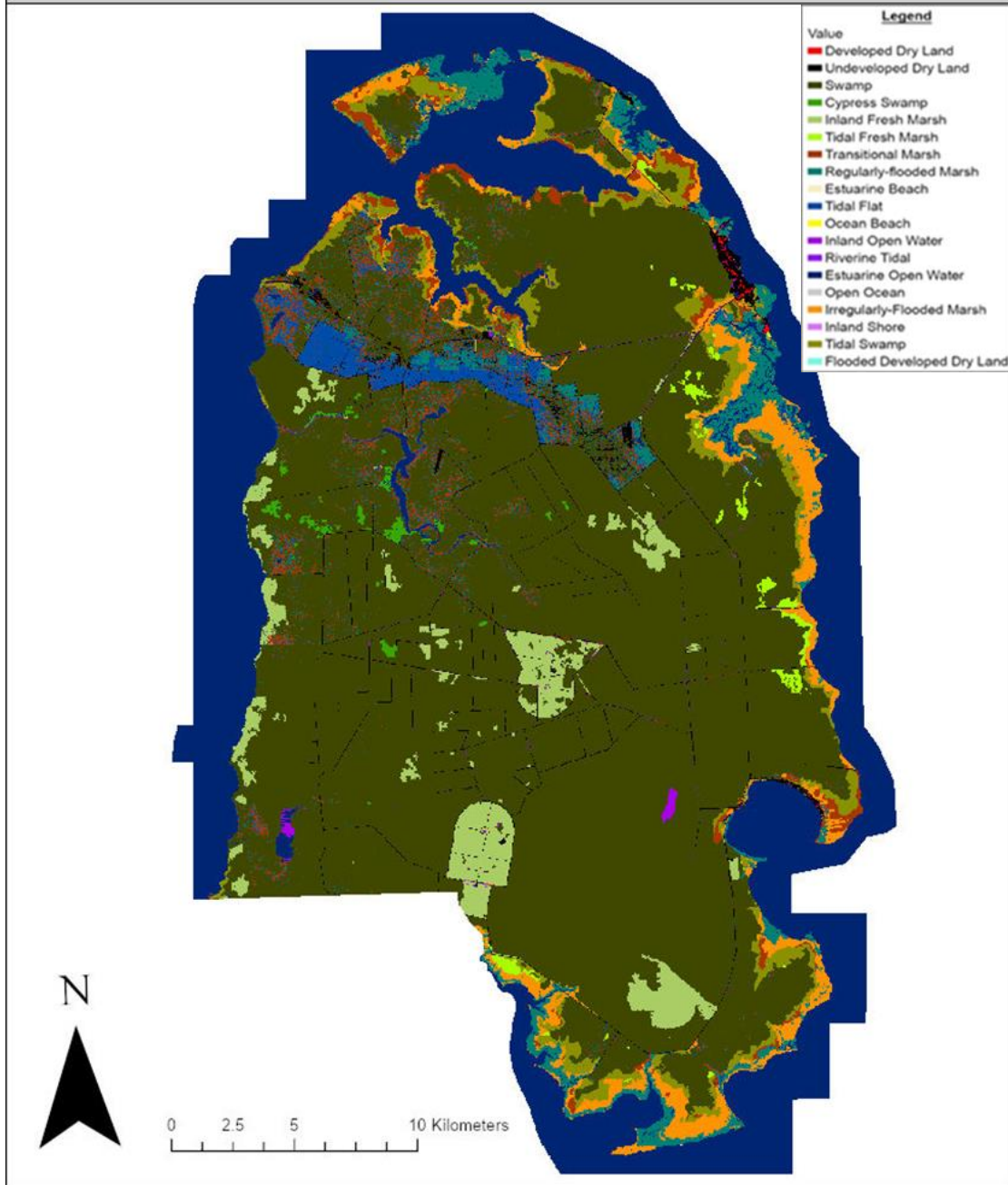


Figure 25 SLAMM habitat change predictions: RCP 8.5 (2100) for mainland Dare County, North Carolina, USA.

# Mainland Dare County, 1 meter, 2100

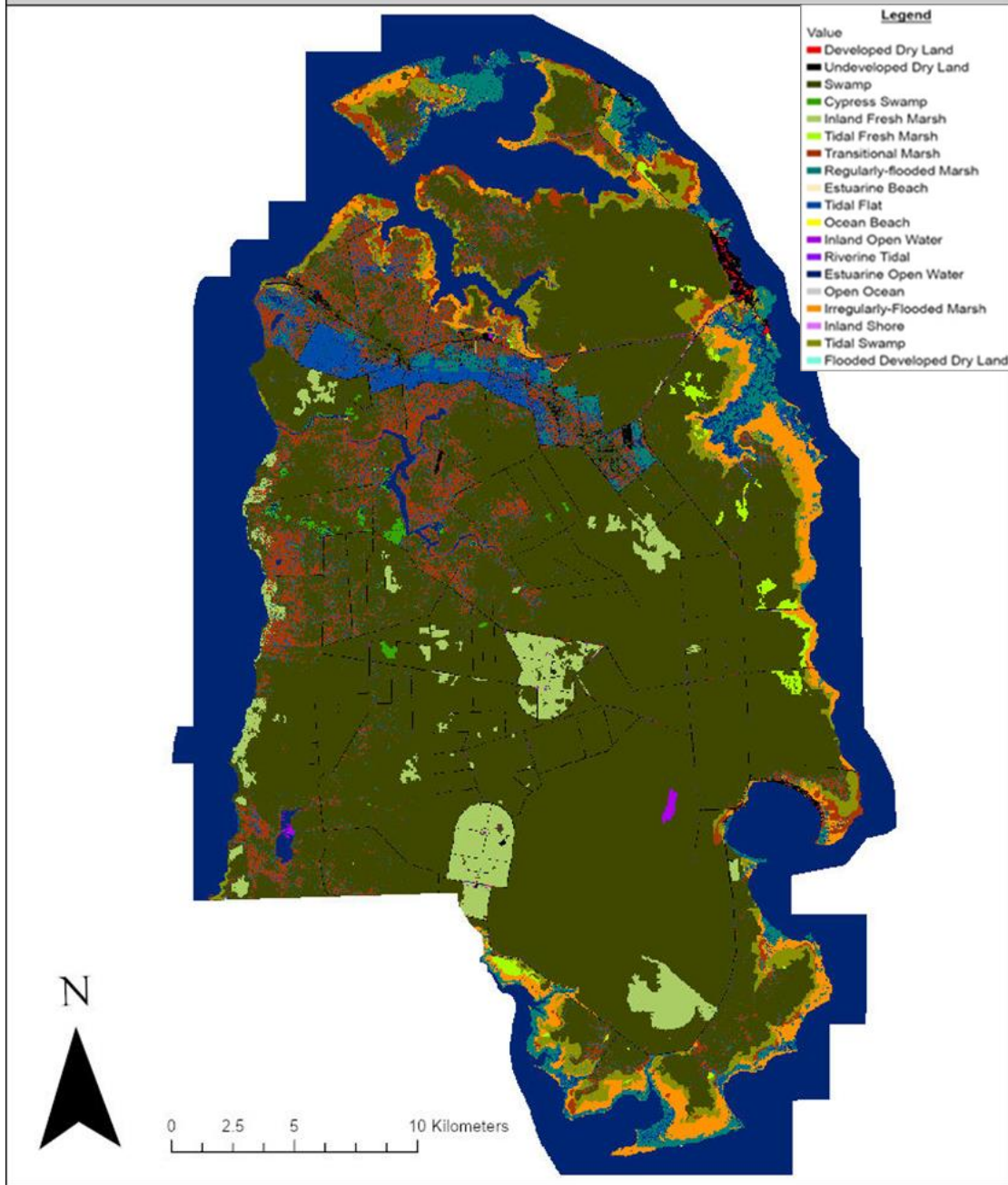


Figure 26 SLAMM habitat change predictions: 1-m fixed (2100) for mainland Dare County, North Carolina, USA.

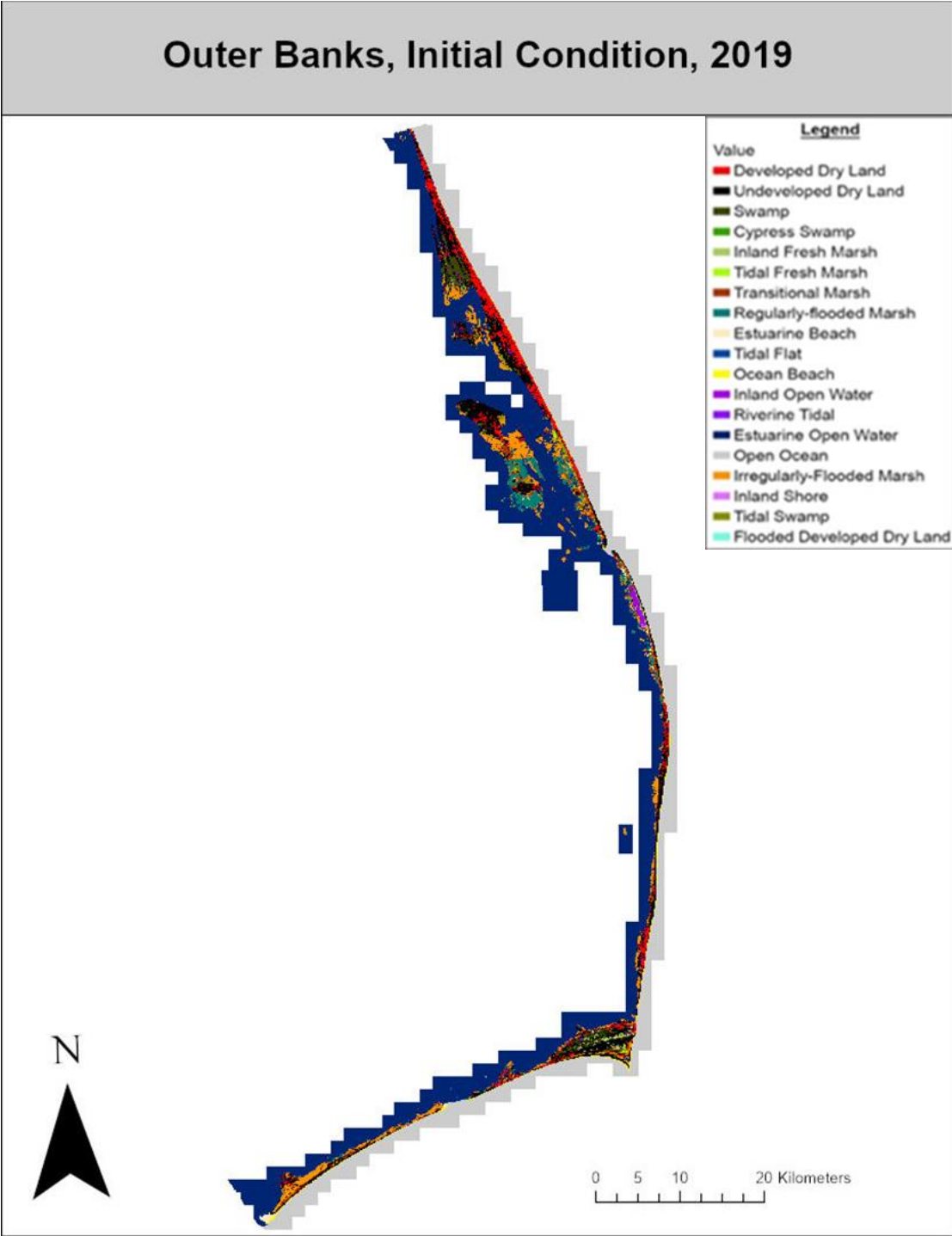


Figure 27 SLAMM habitat change predictions: Initial condition (2019) for the Outer Banks, North Carolina, USA.

## Outer Banks, Scenario A1B Mean, 2100

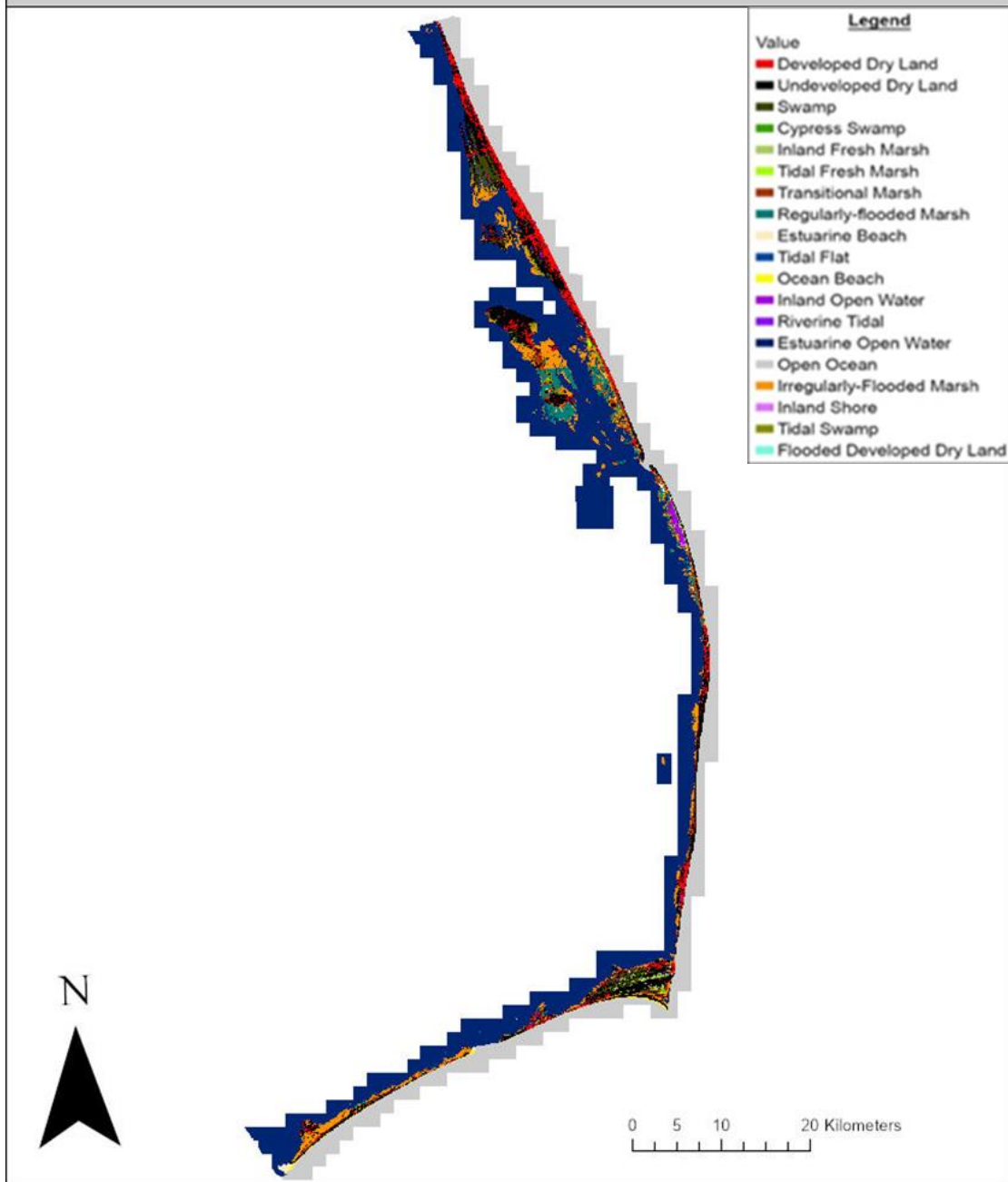


Figure 28 SLAMM habitat change predictions: A1B Mean (2100) for the Outer Banks, North Carolina, USA.

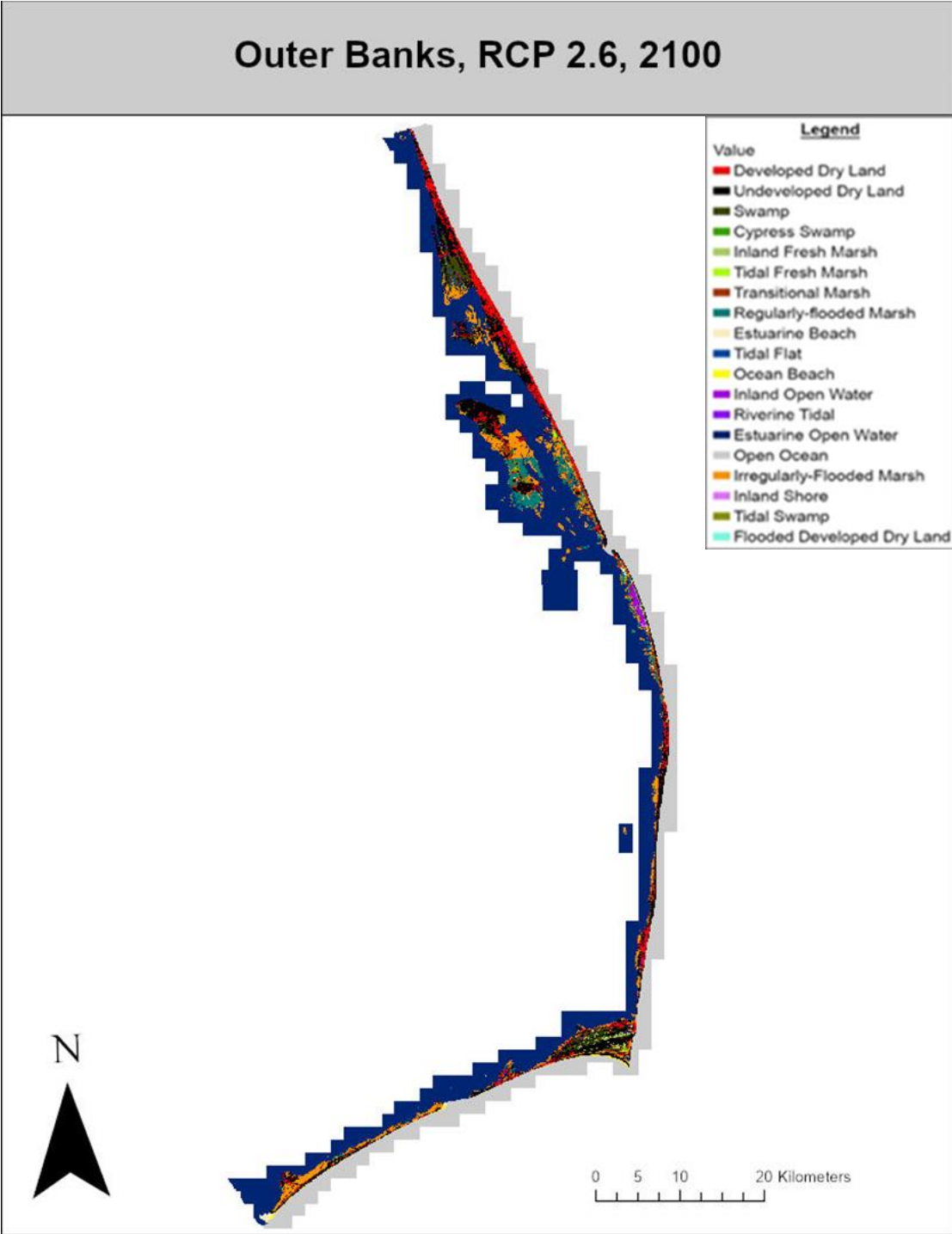


Figure 29 SLAMM habitat change predictions: RCP 2.6 (2100) for the Outer Banks, North Carolina, USA.

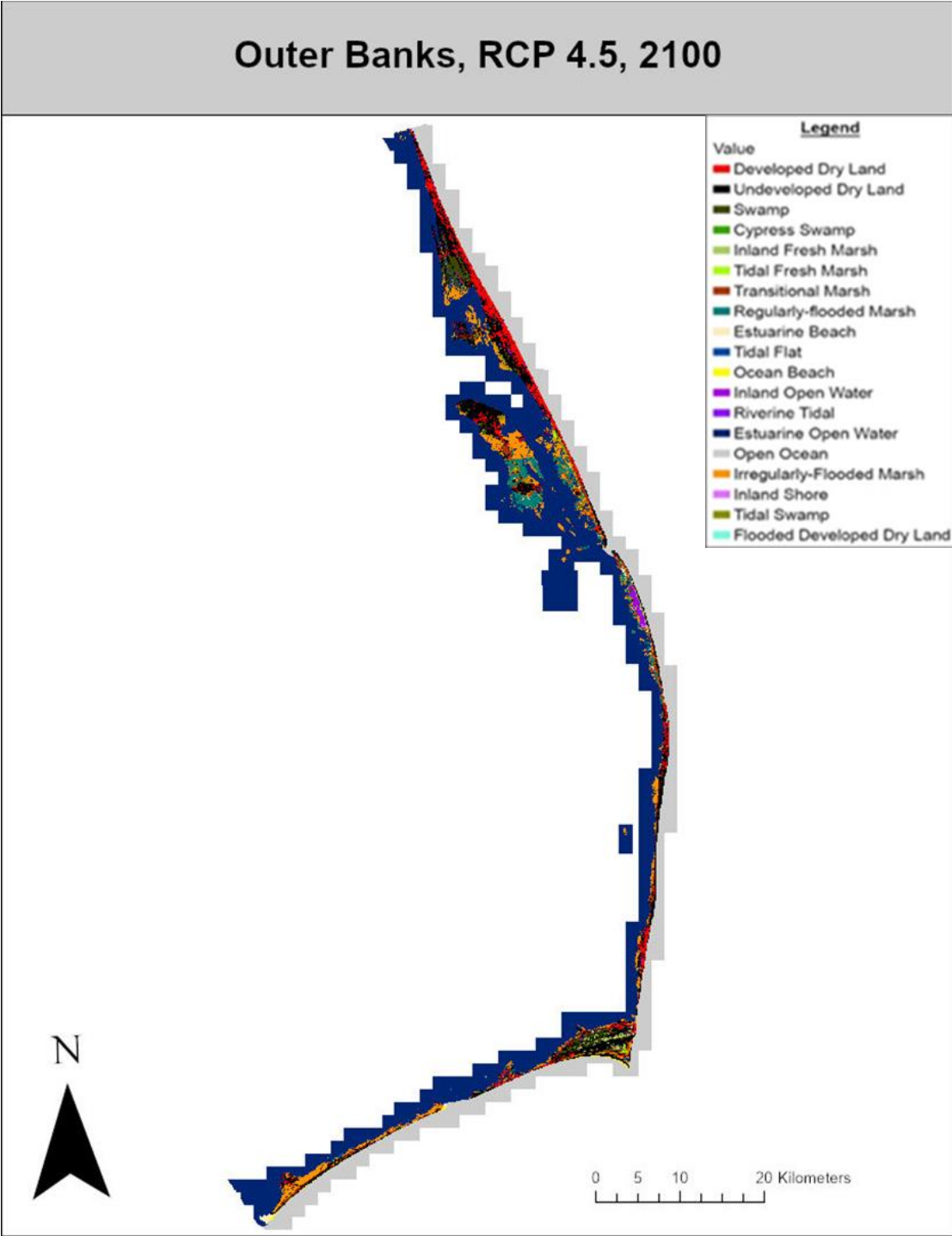


Figure 30 SLAMM habitat change predictions: RCP 4.5 (2100) for the Outer Banks, North Carolina, USA.

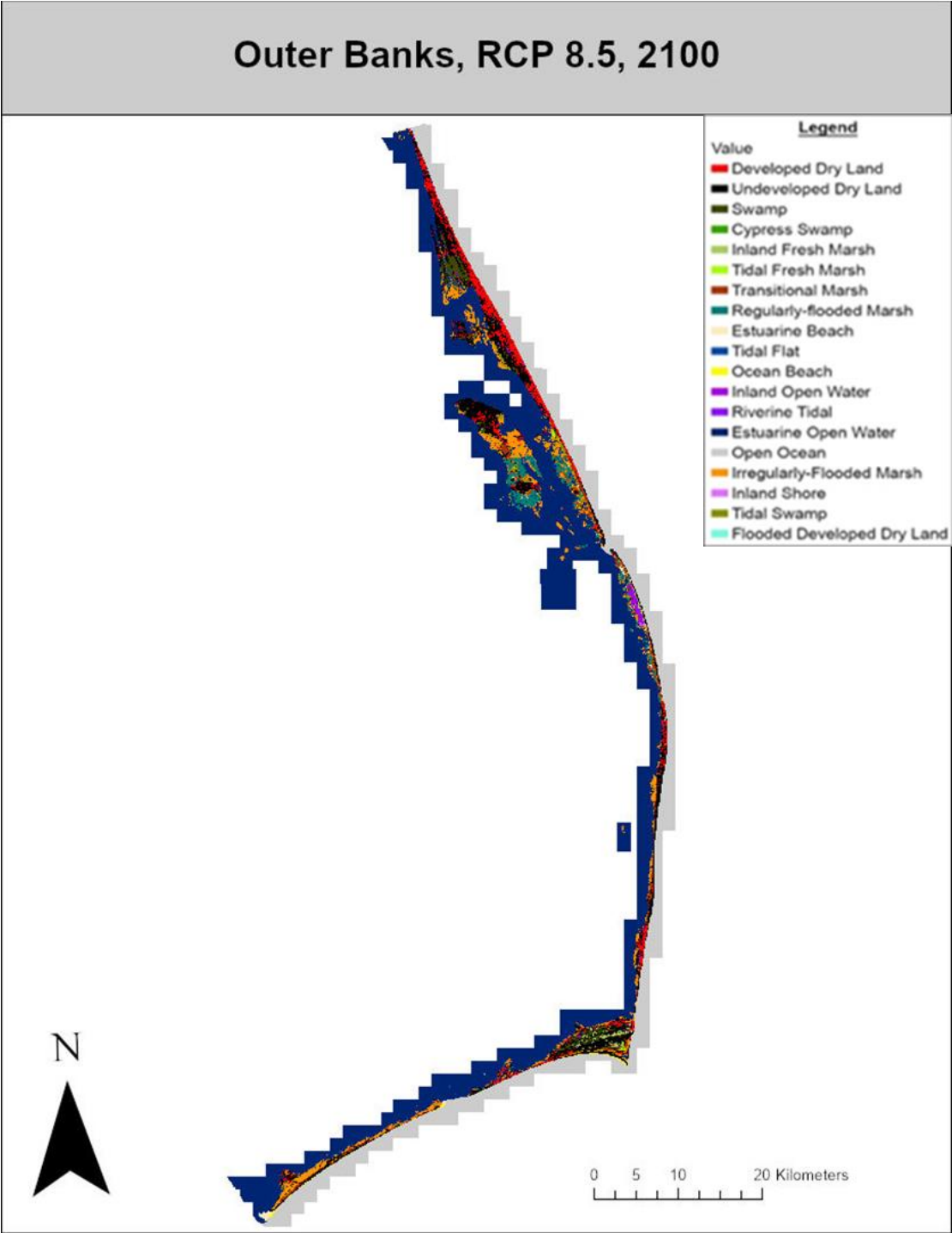


Figure 31 SLAMM habitat change predictions: RCP 8.5 (2100) for the Outer Banks, North Carolina, USA.

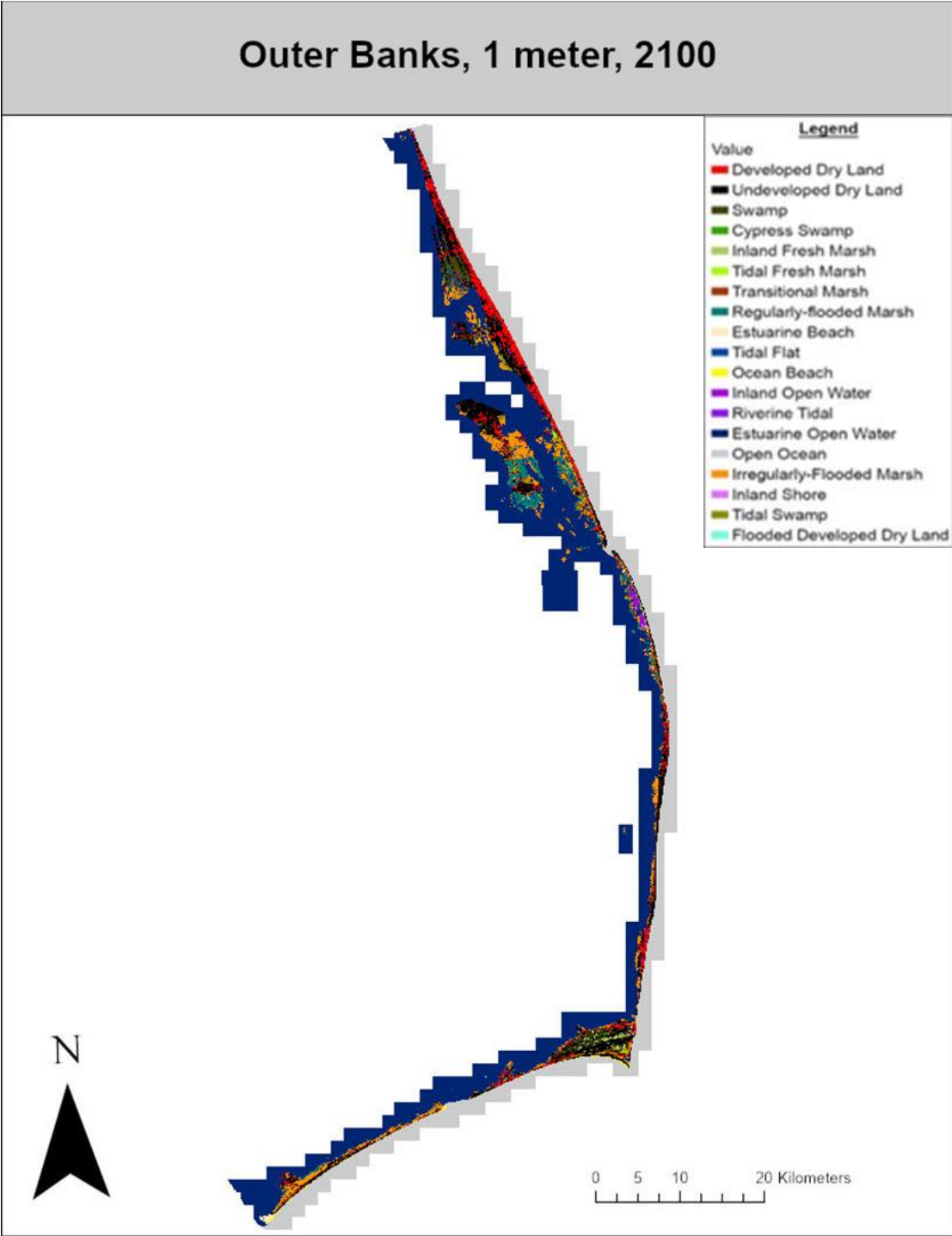


Figure 32 SLAMM habitat change predictions: 1-m fixed (2100) for the Outer Banks, North Carolina, USA.