# Population Genomics of Bottlenose Dolphins (*Tursiops truncatus*) in the Northwest Atlantic

By

Nikki Shintaku

Dr. Tom Schultz, Advisor

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## Executive Summary

Bottlenose dolphins (*Tursiops truncatus)* are widely accepted as belonging to one of two ecotypes: offshore or inshore. These ecotypes exhibit remarkable differences in ecology, morphology, and genetic diversity, and the distinction between the two ecotypes has been described in a number of geographic locations. However, regional patterns of genetic differentiation and stock delineation remain poorly defined for both ecotypes. The objectives of this project are to: (1) exploit next-generation sequencing techniques to identify genome-wide genetic variation, (2) provide unprecedented genetic resolution of population structure, and (3) aid to inform management of specific stocks along the U.S. east coast.

To improve our understanding of the population structures among these groups we investigated genome-wide genetic variation from 96 biopsy samples collected from bottlenose dolphins in inshore and offshore waters of the northwest Atlantic from North Carolina to Florida using restriction site associated DNA sequencing to infer population structure. Analysis of 14,783 single nucleotide polymorphisms revealed at least three genetically differentiated populations through both Bayesian clustering analysis and Discriminate Analysis of Principal Components (DAPC). Our results suggest an inshore population along North Carolina's Outer Banks (n=32), an offshore population off the continental shelf break from North Carolina to Jacksonville, Florida (n= 38), and a shelf population off Jacksonville, Florida (n=26).

Bayesian clustering showed significant admixture between the North Carolina and Jacksonville populations, providing potential evidence of historical or current gene flow. 30 out of the 32 inshore North Carolina population are confirmed to belong in the Western North Atlantic Southern Migratory Coastal Stock (SM), which is thought to make seasonal migrations as far south as northern Florida. The spatial overlap of the SM stock with the various coastal stocks along the Atlantic coast may explain the reason for this admixture. Most of the offshore samples were collected off Cape Hatteras, but this population also includes four individuals sampled beyond the continental shelf break off Jacksonville, FL, in close spatial proximity to shelf animals. This suggests a sharp distinction between shelf and offshore individuals structured by the shelf break itself. Such habitat heterogeneity is likely a driver in diversifying populations through influences on social behavior and foraging strategies.

As genomic techniques become more widely adopted in conservation, it can have a positive influence on management and policy decisions. Genetic population data is valuable for reliable stock identification and effective management since anthropogenic pressure can be different in coastal and pelagic environments. Additionally, genetically linking individuals to specific stocks through photo ID can help understand migratory patterns along the coast or habitat range. By knowing seasonal migratory patterns, management solutions could be to set seasonal and spatial regulations. Genomic data can also be used to tell if inbreeding is occurring and to find genes and alleles that are adaptive under pressure, such as disease.

The three distinct clusters found in this study have management implications of local stocks and highlights the importance of utilizing genetics to reconstruct populations that may differ in resource use. Genomics is a power tool that provides unprecedented genetic resolution to help further aid past findings in an attempt to better define the populations along the Western North Atlantic coast as well as answer evolutionary questions.

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## Introduction

Common bottlenose dolphins (*Tursiops truncatus)* are one of the well-known and most studied out of the cetaceans. They are observed in temperate and tropical marine waters around the world with an estimated global population of 600,000 (Wells and Scott 2018). Bottlenose dolphins are protected under the Marine Mammal Protection Act (MMPA), which established a moratorium on taking or importing marine mammals. A "take" is defined as to harass, hunt, capture, or kill any marine mammal or attempt of any of these activities (Fisheries 2021a). Bottlenose dolphin stocks are managed by the National Marine Fisheries Services (NFMS) under the MMPA. The MMPA defines a "stock" as a species or subspecies in a common spatial arrangement that interbreed when mature (Fisheries 2019). Under Section 117 of the MMPA, stocks of marine mammals are considered either strategic or non-strategic depending on their conservation status. A stock is considered strategic if it's declining, likely to be listed as a threatened species under the Endangered Species Act, designated as depleted, or the level of bycatch exceeds the potential biological removal (Fisheries 2019). The MMPA requires stock assessment reports for all marine mammals in U.S. waters, and those that are "strategic" are reviewed annually (Fisheries 2021b).

The worldwide distribution of the bottlenose dolphin (*Tursiops truncatus*) is found in a range of offshore (pelagic) and coastal habitats. Moura et al. 2013 used mitochondrial sequences and climatological records to propose that *Tursiops* originated in Australasian coastal habitats based on patterns of lineage formation. With this, it was then inferred that radiation into pelagic environments was relatively recent and likely followed by multiple returns to coastal habitats in regions like the Western North Atlantic (Moura et al. 2013). Today, bottlenose dolphins are widely accepted as belonging to one of two ecotypes: offshore or inshore (also referred to as coastal). These ecotypes can be differentiated at multiple levels including ecology, morphology, and genetics, and the distinction between the two ecotypes has been described for this species in a number of geographic locations (Tezanos-Pinto et al. 2009; Segura et al. 2006; Hoelzel, Potter, and Best 1998; Fruet et al. 2017).

Differences between inshore and offshore ecotypes are remarkably distinct in several morphological and biological characteristics. Regions, such as the northwest Atlantic, show unique characteristics where inshore dolphins consist of stocky body size, no speckling on the abdomen, and pectoral fins that tuck to the sides, which are effective in shallow waters to maneuver quickly (Moser 2012). Offshore dolphins characteristics include, thin body size, longer in length, darker in color, speckling on the abdomen, wider nasal bones, and pectoral fins that do not tuck to the sides. These dolphins live in deeper waters of the open ocean and are capable of swimming at high speeds (Moser 2012).

In terms of population structuring, inshore ecotypes tend to form small, fragmented populations, and offshore ecotypes form larger populations up to thousands of individuals connected across vast geographical scales (Wells and Scott 2018). Due to different habitats, parasite load, food habits, and diving capabilities differ greatly between the two ecotypes. Mead and Potter 1995 found differences in the amount and types of parasites between the ecotypes. Strandings of offshore dolphins reveal stomach contents to have pelagic squid and deep water fish of the Myctophidae family. In contrast, inshore dolphin stomachs consisted mainly of four near-shore sciaenid fishes: trout, croaker, spot, and white perch (Mead and Potter 1995). Inshore bottlenose dolphins make brief dives surfacing on average twice every minute, whereas dives for offshore dolphins have been documented to last longer than 13 minutes at depths of more than 1,000 meters . The deeper dives exhibited by offshore dolphins correspond to the reported nightly vertical migrations of mesopelagic prey (Klatsky, Wells, and Sweeney 2007). Furthermore, these two ecotypes also express differences in the number of hemoglobin profiles. Inshore dolphins exhibit only 1 hemoglobin type, and the offshore expresses 2 types along with a higher percentage of hematocrit values suggesting an adaptation of the deep-diving capabilities of the offshore ecotype (Duffield, Ridgway, and Cornell 1983; Hersh and Duffield 1990).

Easy access has resulted in more research on inshore dolphin populations compared to offshore populations. Little is known about the genetic differences between inshore and offshore

bottlenose dolphins, and the difference in morphometrics, diving behavior, and habitat range between the two make this an interesting topic to study. This study focuses on bottlenose dolphins (*Tursiops truncatus)* in the Western North Atlantic ranging from Florida to North Carolina. This study area overlaps with many estuarine, coastal, and offshore stocks. Stock delineation is difficult due to complex patterns of distribution, seasonal variation, and overlap, which can lead to stocks being poorly understood and managed (National Marine Fisheries Services 2018b). Management of these bottlenose dolphins are conducted on a stock-by-stock basis. Therefore, successful management relies on the use of photo ID, aerial surveillance, satellite tag-telemetry, and genetics to help properly estimate abundance and stock assignment.

Determining population structure of bottlenose dolphins along the western north Atlantic can help facilitate effective conservation management as well as advance the understanding of processes that may drive the evolution of population genetic structure. As next-generation genomic technologies are advancing, the ability to analyze large genetic datasets are increasingly accessible. High-throughput techniques, such as Restriction site-Associated DNA sequencing (RADseq), are now available as a low-cost option to genotype thousands of genetic markers for any species (Andrews et al. 2016). With no prior genomic information required, RADseq is particularly advantageous for studies of non-model organisms and has provided scientists with a method that is revolutionizing ecological, evolutionary, and conservation genetics (Andrews et al. 2016).

This study uses RAD sequencing to investigate genome-wide genetic variation of 96 bottlenose dolphins in the Western North Atlantic. The objectives are to: (1) exploit next-generation sequencing techniques to identify genome-wide genetic variation, (2) provide unprecedented genetic resolution of population structure, and (3) aid to inform management of specific stocks along the U.S. east coast.

# Materials and Methods

## *Samples and DNA Extraction*

Bottlenose Dolphin skin tissue samples were previously collected through biopsies under the appropriate permit (NMFS Scientific Permit #14241, #779-1633, #14450, #14809, & #22156) and stored at -80°C until DNA extraction. Biopsy sampling dates ranged from 2008-2020 with a group sampled along the outer banks North Carolina (inshore), a group off the continental shelf (offshore) and a group off the coast of Jacksonville, FL (shelf). Biopsy samples were obtained using a crossbow and custom-made stainless steel biopsy sampling tip designed to extract a small piece of skin tissue from the animal (Torres et al. 2003; Read et al. 2013). In depth sample information can be found in Appendix Table 1.

RAD sequencing was conducted on bottlenose dolphin DNA samples defined by geographic sampling location: inshore or offshore. DNA was extracted from a total of 123 biopsies. From those, 96 were chosen for RAD library preparation based on DNA concentration – 33 were classified as inshore and 63 were classified as offshore. DNA was extracted from tissue samples using the Wizard SV Genomic DNA Purification Kit (Promega). Roughly 100-200mg of the tissue sample was dissected using a sterile scalpel. Quantity of the genomic DNA was determined by Qubit 2.0 dsDNA Broad Range assay. Samples were then stored at 20°C until RAD library preparation.

## *RAD Library Preparation*

Double digest RADseq libraries were prepared as previously described with minor modifications (Peterson et al. 2012). 500 ng of DNA from each sample was double digested with enzymes SbfI and MspI at 37°C for two hours followed by heat inactivation of the restriction enzymes at 65°C for 10 minutes. Digested DNA was then purified using SPRI magnetic beads. Barcoded (6-bp) P1 adapters were ligated to the SbfI cut sites, and P2 adapters to the MspI cut sites. The samples were then pooled, 32 samples per library, and adapter-ligated products purified by magnetic bead purification again. The libraries were then PCR amplified with limited cycles (18x) using Phusion PCR Mix (New England Biolabs), purified on SPRI magnetic beads, and sent for

sequencing (50bp paired-end) on a Illumina NovaSeq 6000 at the Sequencing and Genomic Technologies facility at Duke University (Osterberg et al. 2018; Cammen et al. 2015).

### *SNP Identification & Genotyping*

The resulting RAD sequences were analyzed using Stacks v2.55 pipeline and Freebayes v1.3.2 following the schematic in Figure 1 (CATCHEN et al. 2013; Garrison and Marth 2012). Reads were demultiplexed and filtered for quality using process_radtags. Fast QC was run on the fastq files to check quality of the data. Quality-filtered reads were then mapped onto the bottlenose dolphin genome assembly (GenBank assembly accession: GCA_011762595.1) using BWA v0.7.17 under the bwa mem algorithm.

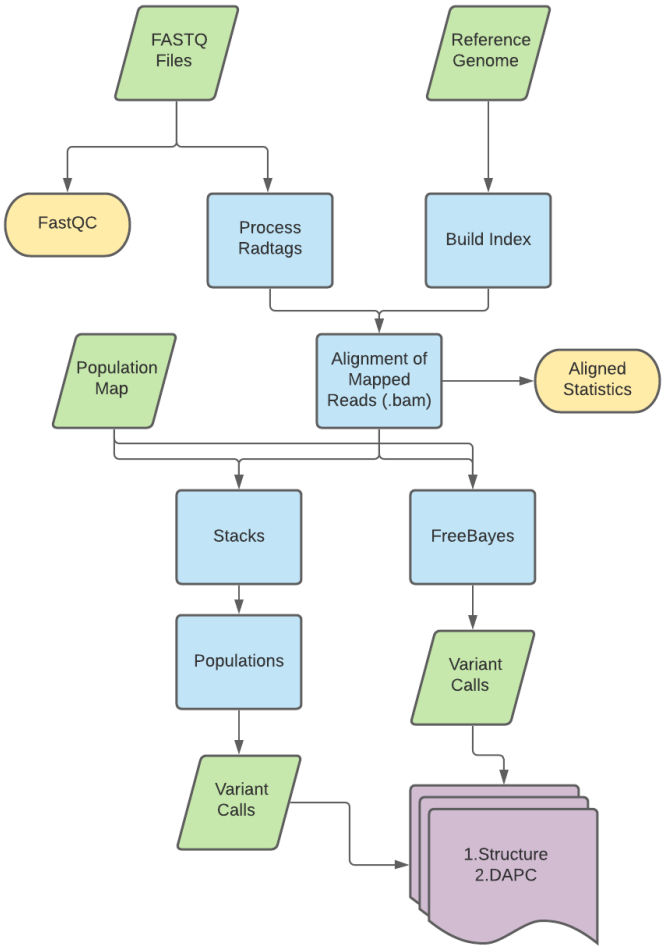


*Figure 1: In depth step-by-step flow chart of RAD Seq data process. The green shapes indicate a data input file. The yellow shapes indicate a terminal output. The blue shapes indicate a process flow, and purple indicates multiple outputs. The filtered vcf output from Freebayes was used as the final dataset in this study.*

I compared two different pipelines to identify variants and call genotypes across all 96 individuals: Stacks and Freebayes. Gstacks and Populations programs in Stacks were run using Refmap perl wrapper. Populations was run with a minimum of 1 population and a minimum of 50% individuals in a population required to process a locus.

The FreeBayes program was used to call variants. The following parameters were set:

- Minimum map quality was set to 30, which excludes alignments from analysis if there is a mapping quality less than the value.
- Minimum base quality was set to 20, which excludes alleles from analysis if their supporting base quality is less than the value.
- Min-coverage was set to 1500 and requires this coverage to process a site.
- Skip processing of alignments overlapping positions with coverage greater than 30000.

The output file of Stacks Populations and FreeBayes were in variant call format (vcf). The final vcfs were filtered based on these parameters:

- Exclude missing data less than 80%
- Only include bi-allelic SNPs
- Include sites with minor allele count greater than or equal to 3
- Remove indels
- Minor allele frequency greater than or equal to 0.05

### *Genetic Variation and Population Structure*

The filtered vcf output from Freebayes was used as the dataset for analyzing genetic variation and population structure. Structure 2.3.4 (Pritchard, Stephens, and Donnelly 2000) was used to run a Bayesian model-based clustering to infer population structure. This model calculates the log-likelihood value of the data to determine the most likely number of clusters (K). Simulations were performed using 10,000 burn-in and 25,000 repetitions, assuming values of K varying between 2 and 5. Five independent runs were performed to limit the influence of stochasticity (Fruet et al. 2017). The method of Evanno et al. (2005), which determines the second-order rate of change of the likelihood function on K, was used to determine the most likely value of K over multiple runs, as implemented in Structure Harvester (Evanno G., S. Regnaut, J. Goudet 2005). In addition, discriminant analysis of principal components (DAPC) implemented in ADEGENET 2.1.3. in R 3.6.2 was performed to further corroborate genetic variation and population

structure findings. The optimal number of clusters for DAPC was determined using Bayesian information criterion (BIC) (Attard et al. 2018; Jombart, Devillard, and Balloux 2010).

# Results

## *Samples*

A total of 96 bottlenose dolphin individuals were used in this study. There is 64 males, 28 females, and 4 were undetermined. Samples were collected between May 2008 and October 2020. Sampling effort over time is shown in Figure 2. Over half of the individuals were sampled during the summer of 2012 (N=49). Inshore sampling occurred only in 2011, 2012, and 2020 whereas offshore and shelf sampling occurred across multiple years and various months. Based off geographical sampling location, 33 individuals categorized as inshore and 63 categorized as offshore. Out of the 63 offshore, 26 were part of the continental shelf dolphin population off the coast of Jacksonville, Florida. Additionally, 30 inshore individuals are confirmed to be a part of the Western North Atlantic Southern Migratory Coastal Stock as delineated by the National Marine Fisheries Service.

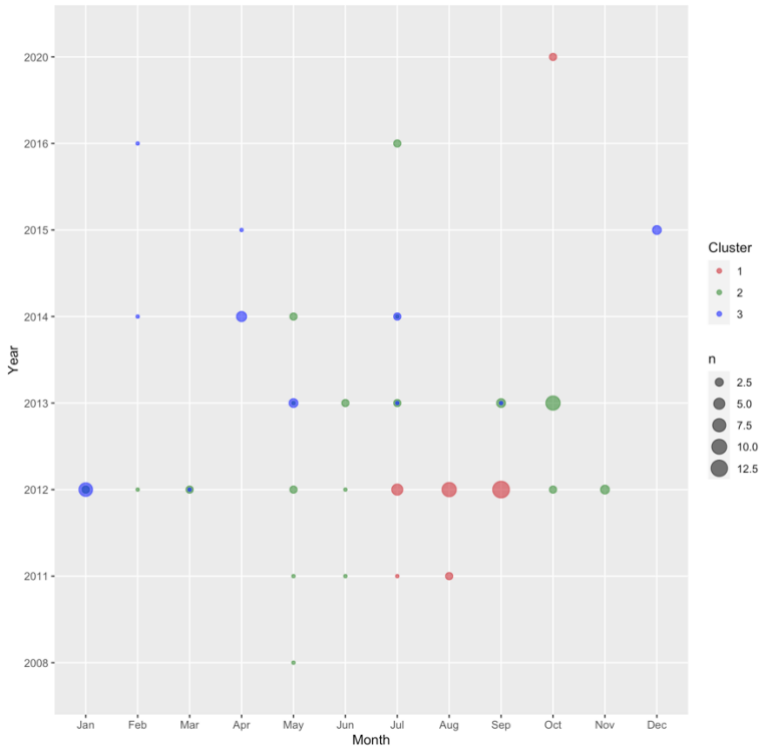


*Figure 2: Plot of sampling effort over time. n refers to the number of samples and is reflected by circle size. Cluster 1 (inshore), Cluster 2 (offshore), and Cluster 3 (shelf).*

## *SNP Identification & Genotyping*

A total of 803,511,134 reads were generated on one lane from the Illumina platform. After demultiplexing and quality-filtering, 549,449,265 reads were retained. Organized by cluster, Cluster 1 (OBX) had 181,598,858 retained reads, Cluster 2 (OFF) had 194,933,589 retained reads, and Cluster 3 (Shelf) had 172,916,818 retained reads (Figure 3). The number of retained reads were relatively similar across all three clusters.


*Figure 3: Distribution of retained sequence reads by population cluster assignment. Cluster 1 (inshore), Cluster 2 (offshore), and Cluster 3 (shelf)*

Gstacks genotyped 925,512 loci with an effective per-sample coverage mean of 23.6x. The gstacks program examines a RAD data set one locus at a time across all individuals. It identifies SNPs within the meta population for each locus and then genotypes each individual at each identified SNP. On the 925,512 genotyped loci, Stacks Populations then removed 830,606 loci that did not have a minimum of 50% individuals in a population leaving a total of 94,906 loci with a mean Fst = 0.03 ($p < 0.05$) for population pair divergence.

Stacks Populations and Freebayes were both used to detect SNPs as a comparison of methods used to process RAD seq data. The filtering of the Stacks Populations vcf file lead to 29,327 SNPS out of a possible 148,819 sites. In comparison, the filtering of the FreeBayes vcf file led to a final dataset of 14,783 SNPs out of a possible 188,791 sites. Pairwise Fst values from Freebayes are shown below in Table 1. Populations calculates genetic statistics at every variant site and provides strong filtering options. Freebayes is haplotype-based and uses a Bayesian approach to detect genetic variants along with avoiding identical sequences that may have

multiple possible alignments. The results presented in the rest of this manuscript are based off the Freebayes analysis generating of 14,783 SNPs.

| Cluster | 1 | 3 | 2 |
|---|---|---|---|
| **1** | | 0.052 | 0.16 |
| **3** | | | 0.12 |

*Table 1: Pairwise Fst values by cluster ($p < 0.05$)*

## *Population Structure*

In order to infer population structure, we determined the optimal number of clusters observed within the 14,783 SNPs without prior knowledge of sampling information using Discriminant Analysis of Principal Components (DAPC) (ADEGENET 2.1.3.) and STRUCTURE (Pritchard, Wen, and Falush 2009). Both analyses resulted in three distinct groups with the optimal clustering solution determined as three clusters (Figure 5 & 6), and both programs yielded identical clustering results down to the individual (Figure 4).

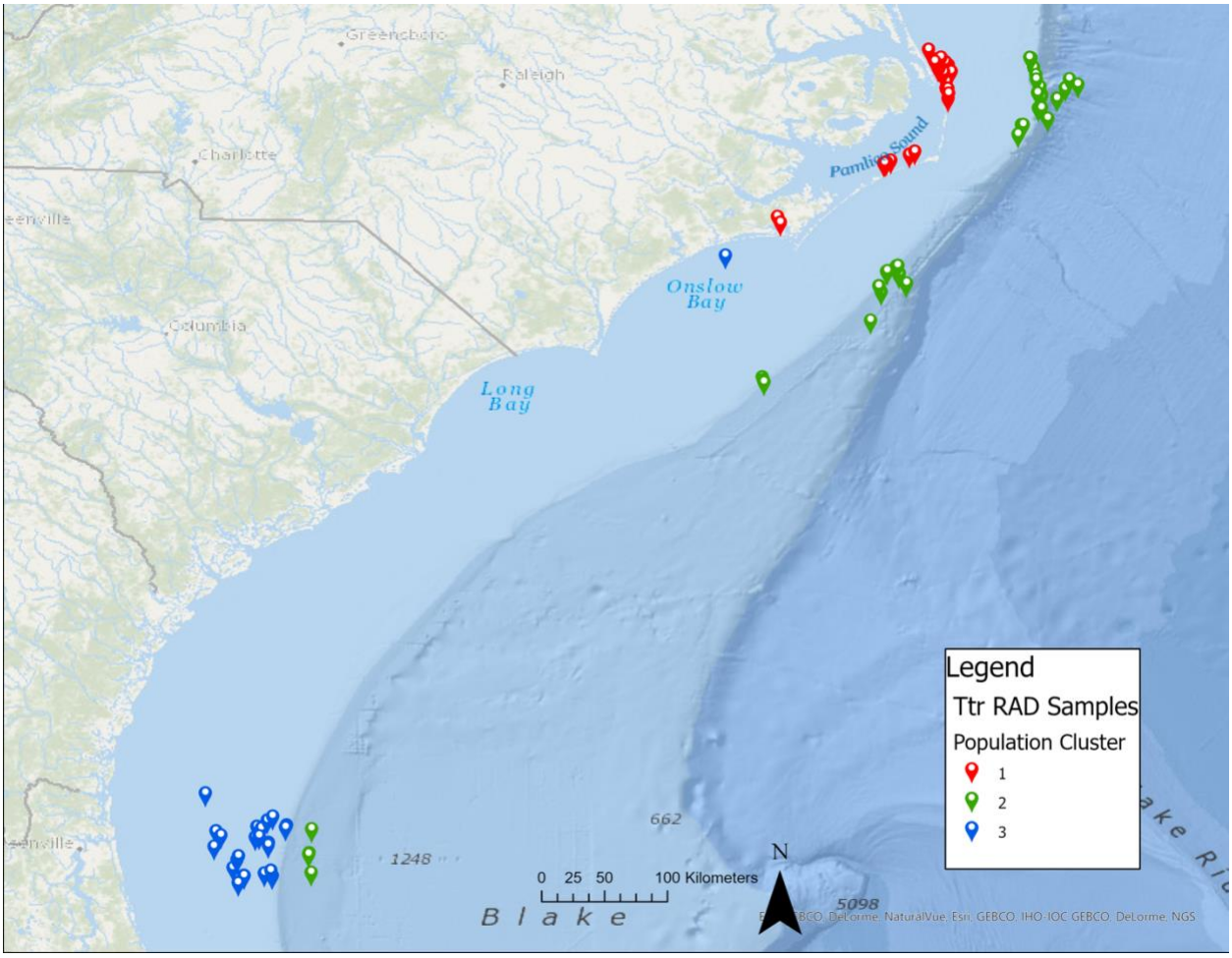


*Figure 4: Map of Tursiops Truncatus samples displaying population cluster assignment from DAPC and Structure results. DAPC and Structure results were identical.*

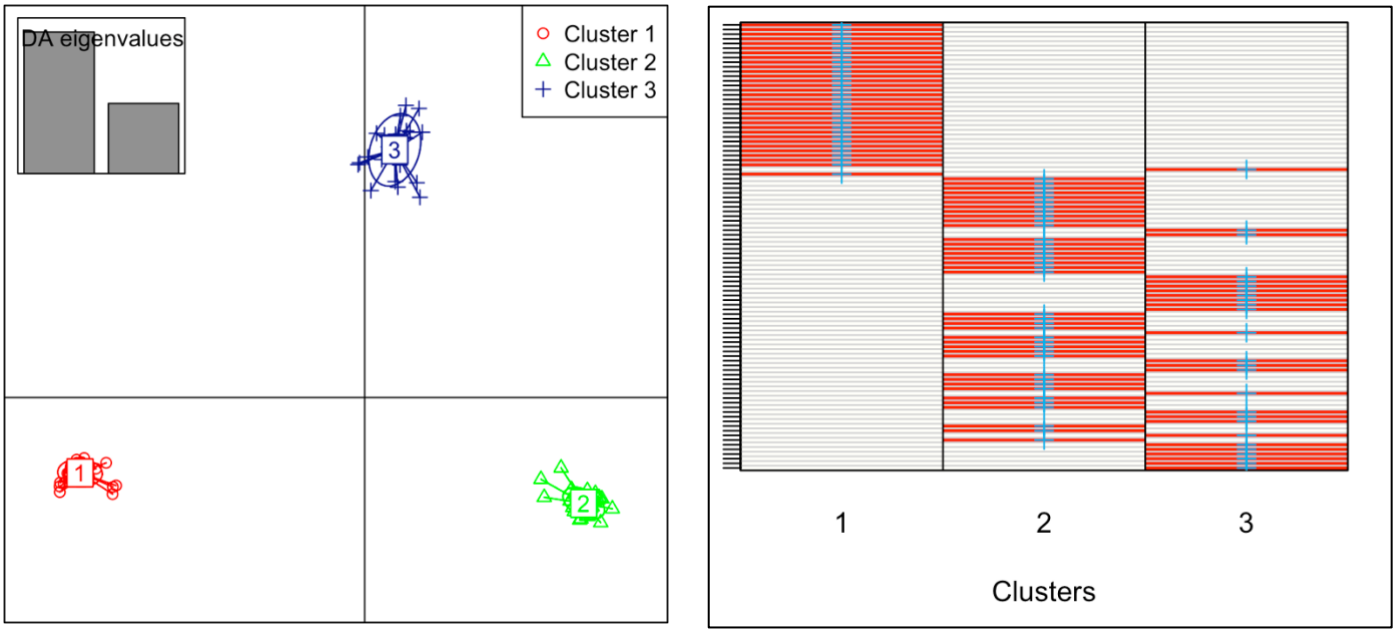


*Figure 5: (Left) DAPC results color coded by cluster. Eigenvalues shown in the top-left correspond to the ratio of the variance between groups over the variance within groups for each discriminant function. (Right) Cluster assignment with each bar representing a different individual. The dark red color indicates strong assignment.*

For DAPC, data is first transformed using a principal components analysis (PCA) and subsequently clusters are identified using discriminant analysis (DA) (Jombart, Devillard, and Balloux 2010). The discriminant analysis functions as a way to show difference between groups as best as possible while minimizing variation within clusters (Jombart and Collins 2015). Using k-means, the optimal clustering solution corresponding to the lowest Bayesian information criterion was K = 3. Figure 5 shows DAPC cluster results; the scatterplot on the left is color coded by cluster with the points representing individuals, and the visual on the right is cluster assignment with each bar representing an individual. In the scatterplot, Cluster 1 (red) is geographically linked to the OBX cluster; Cluster 2 (green) is geographically linked to the OFF cluster, and Cluster 3 (blue) is geographically linked to the Shelf cluster. Based off Figure 5 results, each of the three clusters are very well-resolved as there is no overlap between clusters or individuals.

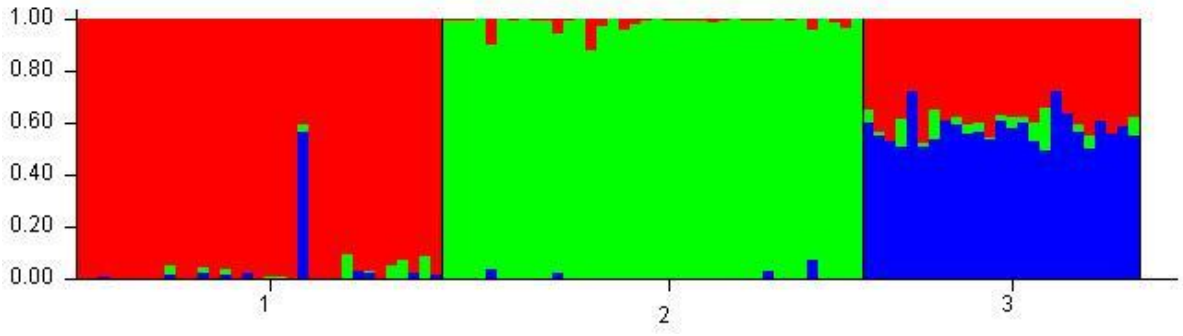


*Figure 6: Structure results ordered by geographic sampling location. Cluster 1 represents the OBX cluster. Cluster 2 represents the OFF cluster, and Cluster 3 represents the Shelf cluster.*

Structure implements a Bayesian model-based clustering method for inferring population structure using genotype data consisting of unlinked markers (Pritchard, Wen, and Falush 2009). Individuals are assigned to a population or to two or more populations if their genotypes are admixed. When applying the Evanno method (Evanno G., S. Regnaut, J. Goudet 2005) in Structure Harvester, the best estimate was K = 3 for the 96 dolphins. Individuals sampled from the outer banks of North Carolina all matched genetically (N = 32; shown as red in Figure 6). However, one individual that was geographically sampled from coastal North Carolina genetically matched to the Shelf cluster shown as half red and half blue under Cluster 1 in Figure 6. Furthermore, the individuals sampled geographically from the Shelf cluster show a strong signal of admixture with the OBX cluster as seen with half red and half blue (N = 26). The individuals sampled along the continental shelf, including four individuals sampled at the shelf off Jacksonville, FL, all matched genetically as shown in green with slight admixture (N= 38; Figure 6).

The results of population structure and genetic diversity indicated in both DAPC and Structure consider offshore (OFF) and inshore (OBX) dolphins as different populations along with a third population (Shelf) that shares half of its genetic information with that of the inshore dolphins. Figure 4 summarizes DAPC and Structure results with individuals mapped and color-coded by the cluster they were assigned based on genetic similarity.

# Discussion

## *Population Structure in the Western North Atlantic*

There are strong levels of structuring and genetic diversity between bottlenose dolphin ecotypes along the northwest Atlantic coastline. Results from DAPC and Structure were in agreement. Fine scale population structure can be surprising given the absence of geographical barriers to gene flow and the potential for significant habitat zone overlap between the populations. However, admixture is explicitly demonstrated by the Shelf cluster with the OBX cluster suggesting potential evidence of historical or current gene flow.

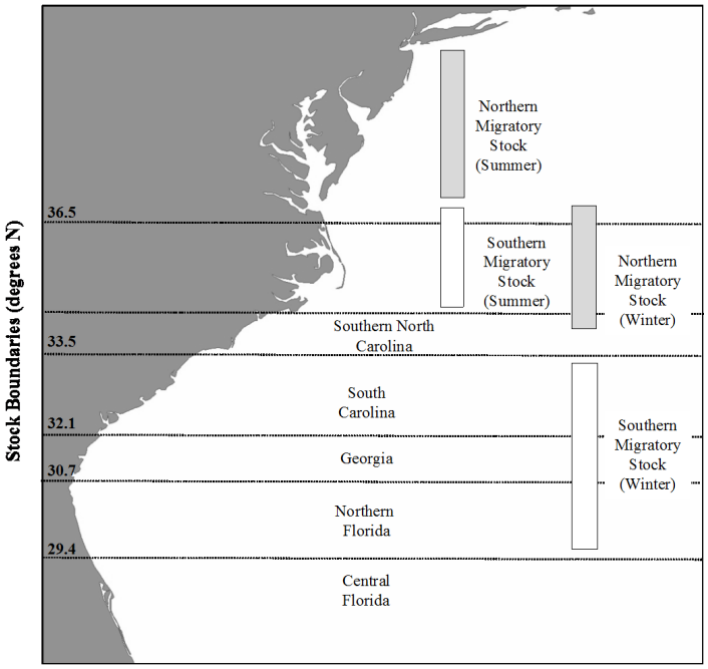


*Figure 7: Stock distribution along the western north Atlantic. Source: National Marine Fisheries Service*

Using photo ID and genetic analysis from a past study, 30 out of the 32 OBX cluster are confirmed to belong in the Western North Atlantic Southern Migratory Coastal Stock (SM) as described by the National Marine Fisheries Services (NMFS) for bottlenose dolphins along the Atlantic coast (Read et al. 2013; National Marine Fisheries Services 2018b). This stock is known to occur in coastal waters and overlap in ocean waters along the Outer Banks (Figure 7). The Southern Migratory stock is also thought to make broad-scale, seasonal migrations along the

coast between North Carolina and northern Florida making it very likely to come in contact with the Shelf cluster (National Marine Fisheries Services 2018b). In the months of January-March, the Southern Migratory stock is observed as far south at northern Florida where it would overlap spatially with the South Carolina/Georgia and Northern Florida Coastal stocks (National Marine Fisheries Services 2018b). The spatial overlap of the SM stock with the various coastal stocks along the Atlantic coast may explain the reason for the Shelf cluster to have half of the same genetic material as the OBX cluster and the other genetic half completely different. The spatial distribution and migratory movements of the SM stock still remains poorly understood, and this stock is "strategic" due to its designation as depleted under the MMPA (National Marine Fisheries Services 2018b). Thus, these confirmed identifications can help estimate abundance and habitat range.

The sampling dates of the Shelf cluster occur across various months and multiple years suggesting that this cluster may be residents of that area year round. On the other hand, the sampling dates of the OBX cluster occurred in the summer months from June – September in 2011 and 2012 respectively (except 2 samples that were from 2020 located in Beaufort Inlet Channel) where the SM stock is known to occupy these coastal waters north of Cape Lookout, North Carolina (National Marine Fisheries Services 2018b). The summer sampling dates combined with geographic coordinates add valuable knowledge to the lack of known seasonal migration patterns of the SM stock and help to confirm where the stock is located in the summer months. However, the sampling timeframe and seasonal distribution of the OBX cluster may play a bias in the resulting three distinct geographic and genetic clusters. There is also a known Northern Migratory Coastal Stock (NM) that frequents the waters north of Cape Lookout, North Carolina in the winter months that was not sampled in this study – Figure 7 (National Marine Fisheries Services 2018a). Additional sampling of the NM stock would aid in genetic resolution of population structure, seasonal migratory patterns, and spatial distributions of these two migratory coastal stocks.

### *Evolutionary Adaptations Driving Population Structure*

Since 1972, the coastal populations of bottlenose dolphins around Cape Hatteras, North Carolina have been studied mainly through examining stranded specimens (Mead and Potter 1990). Several studies since have differentiated coastal and offshore ecotypes in the Western North Atlantic through ecology, morphology, and genetics (Torres et al. 2003; Hoelzel, Potter, and Best 1998; Richards et al. 2013; Rosel, Hansen, and Hohn 2009; Duffield, Ridgway, and Cornell 1983; Mead and Potter 1995). Mead and Potter (1995) were one of the first to differentiate two ecotypes of *Tursiops* in this area by finding differences in distribution, overall size, skull morphology, food habitat, and parasite burden. Further studies led to the conclusion of distinct populations of inshore and offshore ecotypes in the western North Atlantic using mitochondrial and nuclear genetic markers (Hoelzel, Potter, and Best 1998).

Measurable morphological and genetic differentiation between the ecotypes is hypothesized to stem from complex social behavior and/or habitat specialization (Richards et al. 2013). Additionally, environmental factors such as temperature, salinity, and productivity could structure the populations (Natoli et al. 2005). Thus, the environmental transition between coastal and offshore habitats along the western Atlantic coastline may contribute to this differentiation. Torres et al. (2003) used mitochondrial DNA to differentiate coastal and offshore ecotypes in the Northwest Atlantic and found a statistically significant break in ecotype at 34 km from shore. Offshore ecotypes were found in waters beyond 34 km from shore and deeper than 34m whereas coastal ecotypes were found within 7.5 km of shore. Most of the offshore samples were collected off Cape Hatteras, but the offshore population also includes four individuals sampled beyond the continental shelf break off Jacksonville, FL, in close spatial proximity to shelf animals. This suggests a sharp distinction between shelf and offshore individuals structured by the shelf break itself. Habitat heterogeneity could be a likely force in diversifying bottlenose dolphin populations through its pressure on social behavior and foraging specialization (Richards et al. 2013; Rosel et al. 2009). Foraging specialization are learned behaviors usually passed down from the matrilineal lines. Dolphins specializing in a specific foraging type don't switch their foraging when found in a different habitat type, but

instead will seek habitats that allow them to exercise their specialization (Rosel et al. 2009). Because of these learned behaviors, bottlenose dolphins often exhibit site-fidelity and remain in their respective habitat. As stated previously, offshore dolphins forage in deep, open ocean waters on squid and mesopelagic fish, whereas inshore dolphins forage in shallow waters on sciaenid fishes (Mead and Potter 1995). Therefore, feeding specialization is a likely factor in the evolutionary divergence of the two ecotypes. There is also differences in hemoglobin profiles most likely resulting from selective pressures for deep-diving foraging in the offshore ecotype (Hersh and Duffield 1990).

Phylogenetic analysis on population structure patterns for inshore bottlenose dolphins in the Western North Atlantic revealed that offshore form haplotypes to be ancestral to the inshore haplotypes. Mitochondrial molecular diversity supported a younger age of the coastal populations along with lower diversity than that of the Northwest Atlantic offshore population (Richards et al. 2013). This coastline exhibits a general decrease in levels of diversity when moving from offshore to inshore (Rosel et al. 2009; Richards et al. 2013; Natoli et al. 2004). These observations may indicate that the inshore populations were recently founded from a more diverse offshore population (Hoelzel, Potter, and Best 1998; Natoli, Peddemors, and Hoelzel 2004; Richards et al. 2013). Sellas et al. (2005) discovered a similar pattern of diversity for bottlenose dolphins along the west coast of Florida suggesting this to be a typical founder event pattern. This pattern is experienced elsewhere in the world among bottlenose dolphin populations (Louis et al. 2014; Fruet et al. 2014; Lowther-Thieleking et al. 2015; Fruet et al. 2017). High resolution genomic data allow researchers to ask questions related to ancestry and evolutionary history that can then reconstruct phylogeny.

### *Management Implications*

As genomic techniques become more widely adopted in conservation, it can have a positive influence on management and policy decisions. Conservation policies and laws rely on defined and distinct units of conservation to support enforcement and resources allocation. Genetic population data is valuable for reliable stock identification and effective management since

anthropogenic pressure can be different in coastal and pelagic environments. As population structure may be driven by ecological adaptation to specific habitats, anthropogenic pressure, such as habitat degradation from organic contaminants or noise pollution from boat traffic and construction, could strongly affect locally adapted coastal populations (Louis et al. 2014). Chen et al. (2017) discovered there are at least two populations of bottlenose dolphins distributed in the coastal waters around Taiwan and Japan that are affected by different threats. They argue that the population in the waters on the east coasts of the countries are specifically targeted by a small-scale dolphin drive fishery. On the other hand, the population in the west coast waters suffer from habitat loss and degradation, pollution, acoustic disturbances, and other fisheries interactions. It would be justifiable to manage these populations separately as they are both genetically different while also facing extremely different anthropogenic pressure.

The same evaluation of anthropogenic threats is applicable to the three clusters found here. Bycatch in fishing gear is a leading cause of bottlenose dolphin deaths and injuries (Fisheries 2021c). Inshore and coastal fishing gear and pressure could vary significantly between North Carolina and Florida leading to different threats to the OBX and Shelf cluster. Using genetics to define populations in coastal waters is especially pertinent because each state has jurisdiction over coastal waters extending to 3 miles offshore. Therefore, any regulations to reduce interactions between bottlenose dolphins and the inshore fishery comes from the state. Regulations may not be consistent across states' coastlines posing a major problem for the highly mobile species. Consequently, the offshore population also faces bycatch pressure from offshore fisheries, which are managed federally, bringing in another involved agency. Additionally, genetically linking individuals to specific stocks through photo ID can help understand migratory patterns along the coast or habitat range. By knowing seasonal migratory patterns, possible management solutions could set seasonal restrictions on certain fisheries, fishing gear, or locations to reduce fishery interactions.

Inshore dolphin populations face further differences in threats than offshore populations, such as habitat destruction and degradation by contaminants and oil spills (Fisheries 2021c). For

example, dolphins living along the coastal waters of Georgia in the presence of high levels of PCBs where found to have impacts to their immune systems (Schwacke et al. 2012). Another concern is shoreline development and boat traffic noise pollution, which can disrupt foraging and communication (Fisheries 2021c). All of these threats are restricted nearshore activities. The offshore population along the Northwest Atlantic coast also faces noise pollution, but through sources like seismic surveys and active naval sonars. Also, there is now unknown threats that offshore wind development may pose to this offshore stock with underwater noise, vessel collision, benthic habitat loss, dredging, and contamination from construction (National Marine Fisheries Services 2020). Conservation and management plans need to consider how varied habitat range will produce different sources of threats to bottlenose dolphin populations, and the use of genetics can help piece together locations of population clusters, link individuals to certain stocks, and assist in abundance estimates.

Genomic data can also be used to tell if a population is inbreeding. That information can be used to determine whether the level of inbreeding is predicted to have negative consequences on the fitness of the population so the proper actions can be taken to conserve that population (Supple and Shapiro 2018). Additionally, genomic data can be used assess overall genetic variation and find genes and alleles that are adaptive, such as to resist certain diseases (Supple and Shapiro 2018). It's important to ensure that populations sustain enough genetic variation throughout the process of adaptation. Knowledge of candidate loci can be applied to help other populations of the same species or even to a closely related species as well as help prioritize populations for conservation. As genomic technologies strengthen, its potential to impact conservation and management decisions also increases and will help answer questions we weren't able to answer before.

Fine-scale population differentiation in these globally distributed and mobile species creates difficult management scenarios especially with overlapping stocks. Managers need to take into account respective threats, genetic and ecological processes, and geographical distribution spatially and temporally because a poorly managed stock leading to possible eradication can

leave a long-term gap in a species' range and decrease its overall genetic diversity. The study would further benefit by collecting more biopsies from additional known stocks and increasing the geographic biopsy range to collect biopsies along the coast of southern North Carolina, South Carolina, and Georgia as there was a spatial gap in this study. Doing this would provide more fine-scale population differentiation along the southeast U.S. and could help to explain past evolutionary trajectories. The three distinct clusters found in this study have management implications of local stocks and highlights the importance of utilizing genetics to reconstruct populations that may differ in resource use. Genomics is a power tool that provides unprecedented genetic resolution to help further aid past findings in an attempt to better define the populations along the Western North Atlantic coast as well as answer evolutionary questions.

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# Appendix

*Table 1.* List of dolphin samples (N=96) used in this study with corresponding sample information.

| Biopsy Sample ID | Date | Latitude | Longitude | Barcode-P1 | Barcode-P2 | White Peduncle | Sex | Pop Map Assignment | DAPC Cluster | Structure Cluster | Inshore Stock Assignment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **AJR-08-024** | 26-May-08 | 35.66584 | -74.79782 | P1-3 | P2-2 | | M | Offshore | 2 | 2-Green | |
| **AJR-12-001** | 3-May-12 | 35.51199 | -74.80915 | P1-2 | P2-2 | Yes | F | Offshore | 2 | 2-Green | |
| **AJR-13-003** | 6-Jul-13 | 35.65599 | -74.61683 | P1-17 | P2-2 | | M | Offshore | 2 | 2-Green | |
| **AJR-13-004** | 4-Oct-13 | 34.34721 | -75.89349 | P1-9 | P2-3 | Yes | M | Offshore | 2 | 2-Green | |
| **AJR-13-005** | 4-Oct-13 | 34.35155 | -75.88708 | P1-3 | P2-3 | Yes | | Offshore | 2 | 2-Green | |
| **AJR-13-006** | 4-Oct-13 | 34.3241 | -75.80606 | P1-30 | P2-2 | Yes | M | Offshore | 2 | 2-Green | |
| **AJR-13-007** | 4-Oct-13 | 34.33288 | -75.80244 | P1-27 | P2-2 | Yes | F | Offshore | 2 | 2-Green | |
| **AJR-13-008** | 4-Oct-13 | 34.38503 | -75.82321 | P1-22 | P2-2 | Yes | F | Offshore | 2 | 2-Green | |
| **AJR-13-009** | 4-Oct-13 | 34.38914 | -75.8125 | P1-7 | P2-2 | Yes | F | Offshore | 2 | 2-Green | |
| **ASF-11-003** | 28-May-11 | 35.80134 | -74.84249 | P1-4 | P2-2 | Yes | F | Offshore | 2 | 2-Green | |
| **ASF-11-007** | 4-Jun-11 | 35.7499 | -74.82753 | P1-5 | P2-2 | Yes | F | Offshore | 2 | 2-Green | |
| **DMW-12-001** | 25-Jan-12 | 30.27371 | -80.30949 | P1-12 | P2-2 | | M | Offshore | 3 | 3-Red/Blue | |
| **DMW-12-002** | 25-Jan-12 | 30.25899 | -80.31058 | P1-6 | P2-3 | | M | Offshore | 3 | 3-Red/Blue | |
| **DMW-12-005** | 12-Jul-12 | 35.18205 | -75.7309 | P1-1 | P2-2 | | M | Inshore | 1 | 1-Red | SM |
| **DMW-12-006** | 14-Jul-12 | 35.11899 | -75.91439 | P1-29 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **DMW-12-007** | 6-Aug-12 | 35.58128 | -75.4544 | P1-11 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **DMW-12-009** | 17-Aug-12 | 35.79872 | -75.52661 | P1-9 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **DMW-12-010** | 17-Aug-12 | 35.79836 | -75.51955 | P1-27 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **DMW-12-013** | 26-Sep-12 | 35.81932 | -75.45215 | P1-13 | P2-1 | | F | Inshore | 1 | 1-Red | SM |
| **DMW-12-014** | 26-Sep-12 | 35.79067 | -75.44691 | P1-22 | P2-1 | | M | Inshore | 1 | 1-Red | SM |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **DMW-12-015** | 26-Sep-12 | 35.77412 | -75.43166 | P1-4 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **DMW-12-017** | 30-Sep-12 | 35.70135 | -75.46675 | P1-14 | P2-1 | | F | Inshore | 1 | 1-Red | SM |
| **DMW-13-005** | 5-Sep-13 | 35.71997 | -74.58727 | P1-10 | P2-3 | | M | Offshore | 2 | 2-Green | |
| **DMW-16-001** | 21-Jul-16 | 30.20054 | -80.01912 | P1-13 | P2-3 | Yes | F | Offshore | 2 | 2-Green | |
| **DWJ-12-006** | 4-May-12 | 35.7177 | -74.82387 | P1-24 | P2-2 | | F | Offshore | 2 | 2-Green | |
| **HJF-12-005** | 24-Jan-12 | 30.0607 | -80.00417 | P1-20 | P2-2 | Yes | M | Offshore | 2 | 2-Green | |
| **HJF-12-014** | 17-Aug-12 | 35.93074 | -75.59363 | P1-25 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **HJF-12-015** | 26-Sep-12 | 35.86261 | -75.49637 | P1-24 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **HJF-12-016** | 26-Sep-12 | 35.85824 | -75.49498 | P1-20 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **HJF-12-017** | 26-Sep-12 | 35.84271 | -75.47404 | P1-28 | P2-1 | | F | Inshore | 1 | 1-Red | SM |
| **HJF-12-019** | 10-Nov-12 | 33.59426 | -76.78155 | P1-29 | P2-3 | | M | Offshore | 2 | 2-Green | |
| **HJF-13-005** | 4-Oct-13 | 35.39319 | -74.93014 | P1-1 | P2-3 | | | Offshore | 2 | 2-Green | |
| **HJF-13-006** | 4-Oct-13 | 35.394 | -74.91683 | P1-32 | P2-2 | | M | Offshore | 2 | 2-Green | |
| **HJF-16-001** | 21-Jul-16 | 30.19157 | -80.02223 | P1-18 | P2-3 | Yes | | Offshore | 2 | 2-Green | |
| **JHW-20-001** | 7-Oct-20 | 34.738049 | -76.672673 | P1-19 | P2-1 | No | M | Inshore | 1 | 1-Red | |
| **RJM-14-002** | 12-Apr-14 | 30.39841 | -80.18007 | P1-31 | P2-2 | Yes | M | Offshore | 3 | 3-Red/Blue | |
| **RJM-14-003** | 12-Apr-14 | 30.38292 | -80.18761 | P1-24 | P2-3 | Yes | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-11-004** | 31-Jul-11 | 35.20372 | -75.69218 | P1-31 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-11-006** | 2-Aug-11 | 35.13985 | -75.86349 | P1-32 | P2-1 | | F | Inshore | 1 | 1-Red | SM |
| **ZTS-11-014** | 19-Aug-11 | 35.80327 | -75.52497 | P1-26 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-12-002** | 8-Jan-12 | 30.09573 | -80.56193 | P1-15 | P2-2 | | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-12-004** | 8-Jan-12 | 30.03923 | -80.4843 | P1-5 | P2-3 | | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-12-005** | 8-Jan-12 | 30.03811 | -80.48276 | P1-23 | P2-2 | | F | Offshore | 3 | 3-Red/Blue | |
| **ZTS-12-010** | 24-Jan-12 | 30.03811 | -80.28133 | P1-11 | P2-3 | | M | Offshore | 3 | 3-Red/Blue | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **ZTS-12-011** | 24-Jan-12 | 30.04162 | -80.28565 | P1-13 | P2-2 | | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-12-012** | 25-Jan-12 | 30.38851 | -80.39202 | P1-8 | P2-2 | | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-12-015** | 31-Jan-12 | 33.99776 | -76.00808 | P1-26 | P2-3 | Yes | M | Offshore | 2 | 2-Green | |
| **ZTS-12-017** | 27-Feb-12 | 35.32565 | -74.95434 | P1-29 | P2-2 | | F | Offshore | 2 | 2-Green | |
| **ZTS-12-018** | 15-Mar-12 | 35.60738 | -74.7857 | P1-2 | P2-3 | | F | Offshore | 2 | 2-Green | |
| **ZTS-12-019** | 15-Mar-12 | 35.59564 | -74.78589 | P1-8 | P2-3 | | M | Offshore | 2 | 2-Green | |
| **ZTS-12-020** | 30-Mar-12 | 29.98758 | -80.52506 | P1-16 | P2-2 | No | F | Offshore | 3 | 3-Red/Blue | |
| **ZTS-12-026** | 1-Jun-12 | 34.20215 | -75.93194 | P1-25 | P2-3 | | F | Offshore | 2 | 2-Green | |
| **ZTS-12-032** | 14-Jul-12 | 35.12216 | -75.90426 | P1-1 | P2-1 | | F | Inshore | 1 | 1-Red | SM |
| **ZTS-12-033** | 14-Jul-12 | 35.12776 | -75.89448 | P1-6 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-12-034** | 14-Jul-12 | 35.12256 | -75.90559 | P1-12 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-12-035** | 3-Aug-12 | 35.65164 | -75.469 | P1-2 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-12-036** | 3-Aug-12 | 35.65471 | -75.46254 | P1-5 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-12-038** | 3-Aug-12 | 35.65112 | -75.45577 | P1-8 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-12-039** | 4-Aug-12 | 35.78293 | -75.49182 | P1-18 | P2-1 | | F | Inshore | 1 | 1-Red | SM |
| **ZTS-12-040** | 4-Aug-12 | 35.76964 | -75.48522 | P1-30 | P2-1 | | F | Inshore | 1 | 1-Red | SM |
| **ZTS-12-045** | 26-Sep-12 | 35.79889 | -75.51747 | P1-7 | P2-1 | | F | Inshore | 1 | 1-Red | SM |
| **ZTS-12-046** | 26-Sep-12 | 35.82956 | -75.53396 | P1-10 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-12-047** | 26-Sep-12 | 35.86025 | -75.54328 | P1-3 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-12-048** | 26-Sep-12 | 35.87405 | -75.50475 | P1-23 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-12-051** | 27-Sep-12 | 35.61801 | -75.44995 | P1-17 | P2-1 | | M | Inshore | 1 | 1-Red | SM |
| **ZTS-12-052** | 28-Sep-12 | 35.84497 | -75.54865 | P1-15 | P2-1 | | F | Inshore | 1 | 1-Red | SM |
| **ZTS-12-058** | 24-Oct-12 | 34.26005 | -75.75545 | P1-27 | P2-3 | | M | Offshore | 2 | 2-Green | |
| **ZTS-12-059** | 24-Oct-12 | 34.26382 | -75.7512 | P1-28 | P2-3 | | M | Offshore | 2 | 2-Green | |
| **ZTS-12-062** | 10-Nov-12 | 33.57456 | -76.77519 | P1-30 | P2-3 | | M | Offshore | 2 | 2-Green | |
| **ZTS-12-063** | 10-Nov-12 | 33.55908 | -76.76921 | P1-31 | P2-3 | | F | Offshore | 2 | 2-Green | |

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| **ZTS-13-001** | 10-May-13 | 30.05559 | -80.33879 | P1-4 | P2-3 | | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-13-002** | 10-May-13 | 30.07555 | -80.29203 | P1-7 | P2-3 | | F | Offshore | 3 | 3-Red/Blue | |
| **ZTS-13-003** | 10-May-13 | 30.07555 | -80.29203 | P1-28 | P2-2 | | F | Offshore | 3 | 3-Red/Blue | |
| **ZTS-13-013** | 29-May-13 | 35.68084 | -74.52648 | P1-18 | P2-2 | | M | Offshore | 2 | 2-Green | |
| **ZTS-13-014** | 23-Jun-13 | 35.86878 | -74.8613 | P1-19 | P2-2 | | F | Offshore | 2 | 2-Green | |
| **ZTS-13-015** | 23-Jun-13 | 35.87283 | -74.87057 | P1-11 | P2-2 | | M | Offshore | 2 | 2-Green | |
| **ZTS-13-023** | 16-Jul-13 | 34.24412 | -75.94815 | P1-32 | P2-3 | | M | Offshore | 2 | 2-Green | |
| **ZTS-13-024** | 18-Jul-13 | 30.15254 | -80.53394 | P1-26 | P2-2 | | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-13-025** | 5-Sep-13 | 35.51531 | -74.78606 | P1-12 | P2-3 | | F | Offshore | 2 | 2-Green | |
| **ZTS-13-026** | 5-Sep-13 | 35.58155 | -74.6761 | P1-14 | P2-2 | | | Offshore | 2 | 2-Green | |
| **ZTS-13-029** | 10-Sep-13 | 34.46246 | -77.04485 | P1-21 | P2-1 | | M | Inshore | 3 | 3-Red/Blue | |
| **ZTS-13-030** | 4-Oct-13 | 35.43879 | -74.74184 | P1-21 | P2-2 | | F | Offshore | 2 | 2-Green | |
| **ZTS-14-007** | 17-Feb-14 | 30.62691 | -80.76006 | P1-6 | P2-2 | Yes | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-14-009** | 11-Apr-14 | 30.37738 | -80.3591 | P1-10 | P2-2 | Yes | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-14-012** | 12-Apr-14 | 30.35679 | -80.68403 | P1-14 | P2-3 | No | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-14-014** | 18-May-14 | 35.62204 | -74.81515 | P1-9 | P2-2 | Yes | M | Offshore | 2 | 2-Green | |
| **ZTS-14-015** | 18-May-14 | 35.61964 | -74.81217 | P1-25 | P2-2 | Yes | M | Offshore | 2 | 2-Green | |
| **ZTS-14-020** | 22-Jul-14 | 30.2461 | -80.69749 | P1-15 | P2-3 | No | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-14-023** | 23-Jul-14 | 30.37076 | -80.00085 | P1-17 | P2-3 | Yes | M | Offshore | 2 | 2-Green | |
| **ZTS-14-025** | 23-Jul-14 | 30.43387 | -80.31759 | P1-21 | P2-3 | | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-15-002** | 23-Apr-15 | 30.17852 | -80.52339 | P1-16 | P2-3 | No | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-15-029** | 16-Dec-15 | 30.32629 | -80.64908 | P1-22 | P2-3 | | M | Offshore | 3 | 3-Red/Blue | |

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| **ZTS-15-030** | 16-Dec-15 | 30.31304 | -80.40348 | P1-20 | P2-3 | | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-15-031** | 16-Dec-15 | 30.3206 | -80.37577 | P1-19 | P2-3 | | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-16-001** | 28-Feb-16 | 30.46124 | -80.27882 | P1-23 | P2-3 | | M | Offshore | 3 | 3-Red/Blue | |
| **ZTS-20-002** | 7-Oct-20 | 34.6971083 | -76.652392 | P1-16 | P2-1 | No | M | Inshore | 1 | 1-Red | |