Dynamics of the bottlenose dolphin, *Tursiops truncatus*, off the west and south coast of Puerto Rico: population, distribution, threats, and genetic structure

By

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ABSTRACT

To understand the dynamics of a species, information on the status, abundance, and relationship of contiguous populations is essential, along with information on its geographic range, habitat analysis, and needs. Although the bottlenose dolphin (Tursiops *truncatus*) is commonly found around Puerto Rico, no assessments have been done to determine the population's size, extent, and distribution, and this presents management challenges as defined under the Marine Mammal Protection Act of 1972. To address these needs, a multidisciplinary approach was utilized to develop a comprehensive picture of the bottlenose dolphin population in Puerto Rico. This approach included a boat-based survey for distribution and abundance estimates and a skin biopsy sampling of free ranging individuals to determine ecotype composition. The study areas were the south and west coasts of Puerto Rico between the years 2002 and 2015. The distribution was modeled to determine the spatio-temporal distribution patterns of sighting location, group size, composition and behavior in terms of key features such as distance from shore, depth and habitat type. It was found that dolphins had a more prevalent nearshore distribution within 5 km of the coast, but they are also uniquely associated with edge and slope habitats of the platform and bank/islands off the west coast. Dolphin's sightings were rare on the central portion of the insular shelf and on deep waters outside the shelf edge. From 2013 to 2015, mark-recapture methods of photographic surveys yielded a current abundance estimate of 127 dolphins within the study area. Using a catalog of 2,270 photographs, scarring patterns were used to determine factors affecting the population. Among all cataloged dolphins, 15% showed scar patterns attributed to natural causes, 28% had scars attributed to

anthropogenic causes, and 9% had scars of unidentified origin. Over time, there were significant increases in the proportion of scars caused by anthropogenic interactions and the proportion of individuals having two or three such scars. The mitochondrial control region was sequenced from 27 live dolphins in the study area, plus from 11 stranded dolphins from around Puerto Rico and five stranded dolphins from Guadeloupe; the and results were combined with sequences available from the Atlantic and the Pacific Ocean. Although only one ecotype was observed morphologically, the genetic data showed the presence of two distinct genetic lineages in Puerto Rico. Given the largely coastal distribution of dolphins and the demonstration of increasing anthropogenic impacts, management should prioritize further studies on (1) population health, (2) mapping potential hotspots of human dolphin interaction, and (3) the movements of individuals that may affect population size and interactions between the two genetic lineages.

RESUMEN

Para determinar la dinámica de una especie, es esencial contar con información del estatus, la abundancia y la relación que pueda tener con otras poblaciones, esto sumado a información de su distribución geográfica, análisis de hábitat y necesidades. A pesar que el delfín hocico de botella (*Tursiops truncatus*) se encuentra comúnmente alrededor de Puerto Rico, no se ha realizado un estudio para determinar el tamaño poblacional, su extensión y distribución y esto representa un reto para el manejo de la especie según definido por el Acta de Protección de Mamíferos Marinos del 1972. Para abordar estas necesidades, se utilizó un enfoque multidisciplinario para desarrollar una imagen completa de la población de delfines hocico de botella en Puerto Rico Este enfoque incluyó una

encuesta basada en embarcaciones para estimaciones de distribución y abundancia y un muestreo de biopsia de piel de individuos de rango libre para determinar la composición del ecotipo. Las áreas de estudio fueron las costas sur y oeste de Puerto Rico durante el período 2002-2015. La distribución se modeló para determinar los patrones de distribución espaciotemporal de la ubicación del avistamiento, el tamaño del grupo, la composición y el comportamiento en términos de características clave como la distancia desde la orilla, la profundidad y el tipo de hábitat. Se descubrió que los delfines tenían una distribución nearshore más prevalente dentro de los 5 km de la costa, pero también están asociados de forma única con los hábitats de borde y pendiente de la plataforma y bancos e islas de la zona oeste. Los delfines raramente de observaban en la parte central de la plataforma insular y en aguas profundas fuera del borde de la plataforma. De 2013 a 2015, los métodos de marca-recaptura fotográficas produjeron un estimado de abundancia actual de 127 delfines dentro del área de estudio. Utilizando un catálogo de 2,270 fotografías, se determinaron patrones de cicatrización para establecer los factores que afectaban a la población. Entre todos los delfines catalogados, el 15% mostraba patrones de cicatrices atribuidos a causas naturales, el 28% tenía cicatrices atribuidas a causas antropogénicas, y el 9% tenía cicatrices de origen no identificado. Con el tiempo hubo aumentos significativos en la proporción de cicatrices causadas por interacciones antropogénicas y la proporción de individuos que tienen dos o tres cicatrices. Para estudiar la estructura genética de la población, se secuenció la región de control mitocondria en muestras tomadas mediante el método de biopsia remota a 27 delfines vivos en el área de estudio,

además se secuenciaron muestras de 11 delfines varados de todo Puerto Rico y cinco delfines varados de Guadalupe. Los resultados se combinaron con secuencias disponibles del océano Atlántico y Pacífico. Aunque sólo se observó un ecotipo morfológicamente, los datos genéticos mostraron la presencia de los distintos linajes genéticos en Puerto Rico. Dada la gran mayoría de la distribución costera de los delfines y la demostración de un aumento de los impactos antropogénicos, la administración debe priorizar estudios adicionales sobre (1) la salud de la población, (2) cartografíar los puntos críticos potenciales de la interacción de delfines humanos, y (3) el movimientos de individuos que pueden afectar el tamaño de la población y la estructura genética.

Dedicated to my sister Yamitza; thanks for all the help encouragement, and support.

In memory of Dr. John E. Reynolds III

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GLOSSARY OF TERMS

ArcGISArc Geographical Information SystemsNOAANational Oceanic and Atmospheric Administration

CHAPTER ONE – INTRODUCTION

In the Caribbean region, the bottlenose dolphins, *Tursiops truncatus* (Montagu 1821) are considered the most common cetacean (Ward et al. 2001). A few long-term studies have noted site fidelity and small group size over the region (Belize; Grigg and Markowitz 1997, Kerr et al. 2005, Bahamas; Fearnbach et al. 2012). Other short-term studies have also noted site fidelity and residency patterns (Whaley et al. 2005 in Dominican Republic and Rodriguez-Ferrer 2001 in Puerto Rico). Throughout the region *Tursiops* spp. have been identified on several broad-scale surveys such as seismic surveys, aerial and ship surveys, and opportunistic sightings (Puerto Rico; Miguncci-Giannoni 1998 and Rodriguez-Ferrer 2001, Monserrat; Weir et al. 2011, Dutch Islands; Debrot et al. 1998, Colombia; Fraija et al. 2009, and Suriname Manocci et al. 2013, Do Boer 2015).

Two ecotypes of *Tursiops truncatus* have been described in the Atlantic and Pacific Oceans. The distinction is based on DNA, hemoglobin, parasite loads, prey preferences, morphology, and distribution (Hersh and Duffield 1990; Mead and Potter 1995; Hoelzel et al. 1998; Segura et al. 2006). The coastal ecotype is found mainly in rivers, channels, waterways, estuaries, and on continental/insular shelves and shelf breaks (Mead and Potter 1995), while the offshore or pelagic ecotype is found in zones close to oceanic islands or offshore (Hersh and Duffield 1990). Despite the evidence supporting the existence of these two ecotypes, distinguishing them in the field is not straightforward, and this has hindered assessment efforts. The most recent NOAA Marine Mammal stock assessment for the Atlantic Ocean and Gulf of Mexico recognized that additional morphological, genetic and/or behavioral data are needed to provide further information on stock delineation (Waring et al. 2011). At present time, US Caribbean dolphins are considered to be a single and separate stock for management purposes,

despite the fact that "there is currently no information to differentiate this stock from the Atlantic Ocean and Gulf of Mexico stocks" (Waring et al. 2011). Recent analysis of mitochondrial DNA from stranded bottlenose dolphins from Puerto Rico revealed the presence of the inshore and "a worldwide-distributed form" comparable to the offshore or pelagic ecotype (Caballero et al. 2011). True stock structure is unknown both at large and small geographic scales. Yet, management actions based on such data are required. Bottlenose dolphins are the second most stranded marine mammal in Puerto Rico (Mignucci-Giannoni et al. 1999, 2009), with human interactions directly implicated in some of these standings. The lack of knowledge on dolphin population structure and biology has detrimental implications for the species, and correct management decisions are hindered because of insufficient data.

Bottlenose dolphins are considered to be trans-boundary, so it is important to delineate each stock in order to manage them according to their respective threats. Dolphins face different threats according to their habitat. It is more common for coastal dolphins to interact with nearshore artisanal and recreational fisheries (Wells et al. 1998; Waring et al. 2011) whereas offshore dolphins interact more with pelagic long-line fisheries (Cupka and Murphy 2007). Small cetaceans, such as the bottlenose dolphin, are exposed to perturbations from natural environmental causes (e.g., storms, hurricanes, red tides) and human activities (e.g., marine transportation, fishing, dredging, sewage outfalls, etc.). They are vulnerable to the effects of these activities, which can include habitat loss (Wells and Scott 1999), pollution (Moore 2008), incidental capture in fishing gear (Fruet et al. 2012, Diaz-Lopez 2012), harassment (Witt and Read 2006, Vail, 2016), and collisions with ships (Wells and Scott 1997, Van Waerebeek et al. 2013, Luxenburg 2014).

The bathymetry of Puerto Rico, with deep-water basins, estuaries, and steep-drop offs as well as reefs, provides a diverse set of conditions that suit several species of cetaceans including the common bottlenose dolphin. In 2001, Rodriguez-Ferrer reported a population size of 314 individuals for the southwest coast of Puerto Rico with a more coastal distribution. The author concluded that a low sighting rate could be indicative of a broader distribution. Since this research took place no other project has assessed the population of bottlenose dolphins.

The southwest coast of Puerto Rico is a very important zone for fisheries due to its extended insular shelf (Tonioli and Agar, 2011). Matos Caraballo, 2001, reported the municipality of Cabo Rojo on the southwest coast of Puerto Rico as the most productive municipality for commercial fisheries; however it has shown a steady decrease the total percentage of landings. The author described the fishery resource as overfished. Another issue is coastal development and recreational activities that have increased all over the Island, especially on the West Coast (Díaz and Hevia, 2011). There are no data relating the effects of these activities on marine mammals. This decrease in resource availability and increase in human interactions could be affecting coastal species such as the bottlenose dolphin, therefore baseline data on population dynamics is important to understand the distribution of the species.

In this dissertation the bottlenose dolphin population off the south and west coasts of Puerto Rico were assessed by means of a photographic survey. The objectives were to: 1) model current distribution for the species, 2) determine abundance and trends of population dynamics, and 3) ascertain possible factors affecting the bottlenose dolphins. Additionally, genetic studies of live dolphins and stranded dolphins from Puerto Rico were conducted to determine the presence, extent and possible interactions of the reported ecotypes in the region.

This study is divided into four independent chapters:

- Abundance of the common bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821), off the south and west coasts of Puerto Rico.
- Modeling distribution of the common bottlenose dolphin, *Tursiops truncatus* off the southwest coast of Puerto Rico.
- Scar patterns in common bottlenose dolphin (*Tursiops truncatus*) off southwest Puerto Rico: potential causes and changes over time.
- The presence of two distinct mitochondrial lineages in the bottlenose dolphin (*Tursiops truncatus*) in Puerto Rico and their affinities with previously reported lineages.

Chapter 2 provides an abundance estimate of the bottlenose dolphin population in the west and southwest coasts of Puerto Rico using photo-based mark and recapture data collected from 2013-2015. Mark-recapture techniques also provide information on movements that can be used to determine if the bottlenose dolphins in this area are a subset of a larger, mobile population, or if there is local spatial structure and a smaller local population. This distinction has important management implications.

In Chapter 3, sighting data collected from boat-based surveys from 2002 to 2015 were modeled against benthic habitat maps to determine distribution patterns of free ranging dolphins. This information was then analyzed with spatial analysis to determine habitat use patterns and distribution. In studying aspects of dolphin distribution, the first priority is to map where they occur, while the second is to relate observed differences in abundance/behavior to various spatiotemporal factors that may serve (1) as a guide to predict where dolphins can found in other areas and (2) to elucidate the underlying causes of observed distribution patterns. Many studies commonly used simple factors, such as distance from shore, depth (plus derived characters) and relation to the shelf to describe dolphin distributions, as these features are easily derived from nautical charts. In this study, several spatio-temporal analyses were combined with benthic map data to give a more detailed description of the distribution and habitat used of bottlenose dolphins.

In Chapter 4, the photo identification data were again used to explore the nature and frequency of scarring patterns and their change over time. Using scars to identify fisheries interactions has proven to be effective and constitutes a proactive step in targeting management towards the conservation of marine mammals (Read and Murray 2000, Kiszka et al 2008, Robins 2010). The classification system of Luksenburg (2014) was used to determine the possible source of such scars, plus four new scar categories were added and traced to trap fisheries interactions.

Chapter 5 presents the results obtained from sequencing the control region of live dolphins from the south, southwest and west coast, and stranded dolphins from all of Puerto Rico, combined with sequences available from the Atlantic and the Pacific Ocean. These data were used to infer the presence of the two described ecotypes (inshore vs offshore) and the genetic relation between Puerto Rico and the Caribbean-wide population of bottlenose dolphins.

Chapter 6 presents a brief summary of the findings of the dissertation to give a appropriate assessment of the bottlenose dolphins in the south and west coast of Puerto Rico, and this is followed by several recommendations for future research and management stemming from this work.

5

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CHAPTER TWO-ABUNDANCE OF THE COMMON BOTTLENOSE DOLPHIN, TURSIOPS TRUNCATUS (MONTAGU, 1821), OFF THE SOUTH AND WEST COASTS OF PUERTO RICO

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2.1 Abstract

The abundance of the common bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821), was investigated by mark-recapture methods using photographic surveys on the south and west coasts of Puerto Rico from 2013 to 2015. The number of individuals having distinctive marks was 62, representing 46.5% of dolphins observed. Additionally, two dorsal fins were matched with photos taken during a study in 2000, making this the first report of long-term resightings in Puerto Rico. The abundance estimate for the south and west coast of Puerto Rico was of 127 dolphins. This represents a statistically significant, 60% decrease from the reported estimate back in 2001. It is unclear whether this decline is due to emigration, mortality, or a combination of the two. Management should prioritize further studies on the health of this population and the potential factors contributing to the decline, such as overfishing or excessive boat traffic.

Key Words: common bottlenose dolphin, Puerto Rico, abundance

2.2 Introduction

The common bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821), is one of the most familiar and studied marine mammal species in the world (Figure 2.1; Jefferson et al. 2008). This species is found worldwide in tropical and temperate zones. The International Union for Conservation of Nature (IUCN) estimated a global population of 600,000 individuals (Hammond et al. 2012). In the Caribbean, bottlenose dolphins are considered the most common cetacean species (Ward et al. 2001). In Puerto Rico, bottlenose dolphin is the most abundant cetacean and the second most common marine mammal to strand on the Island (Mignucci-Giannoni et al. 1989, Mignucci-Giannoni et al. 1999). For management purposes, dolphins in the US Caribbean are currently considered one stock separate from the Atlantic Ocean and Gulf of Mexico stocks, despite the lack of studies and data for such differentiation (Waring et al. 2011). True stock structure and population size of the bottlenose dolphin population of Puerto Rico are unknown both at large and small geographic scales. The current definition of stock structure is based on management need and not necessarily on available information.



Figure 2.1. The common bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821), one of the most familiar and studied marine mammal species in the world. Photo taken by Grisel Rodríguez-Ferrer on July 17, 2014 approximately two miles off Puerto Angelino, Cabo Rojo, Puerto Rico.

Two factors complicate our understanding of the local dolphin population. One is that there are two ecotypes of *Tursiops truncatus*. The distinction is based on morphology, but this is strongly supported by differences in DNA, hemoglobin, parasite loads, prey preferences, morphology, and distribution (Hersh and Duffield 1990, Mead and Potter 1995, Hoelzel et al. 1998, Segura et al. 2006). The coastal ecotype is found mainly in rivers, channels, waterways, estuaries, and on continental/insular shelfs and shelf breaks (Mead and Potter 1995), while the offshore or pelagic ecotype is found in zones close to oceanic islands, or offshore (Hersh and Duffield 1990). Despite the evidence supporting the existence of these two ecotypes, distinguishing them in the field is not straightforward, and this has hindered assessment efforts. In Puerto Rico, recent analysis of mitochondrial DNA from stranded bottlenose dolphins revealed the presence of both the inshore ecotype and a worldwide-distributed form comparable to the offshore or pelagic ecotype (Caballero et al. 2011). Yet, survey data to date indicate that within the platform-shelf edge environment only the inshore variety is present (Rodríguez-Ferrer 2001). The second factor is our understanding of the residency patterns of dolphins, as this directly affects the spatial scale of the population and thus, population size. A few long-term studies have noted site fidelity and small group size over the region (Grigg and Markowitz 1997 and Kerr et al. 2005) - Belize, Fearnbach et al. 2012 – Bahamas). Other short-term studies have also noted site fidelity and residency patterns (Whaley et al. 2006 - Dominican Republic; Rodríguez-Ferrer 2001 - Puerto Rico). Combined, these studies suggest that dolphins within Puerto Rico consist solely of the coastal ecotype and that they are limited in their movements, at least over the short-term relative to life span, such that populations are structured at a local scale relative to important ecological and population processes.

The purpose of this study was to assess the bottlenose dolphin population in the west and southwest coasts of Puerto Rico by using photo identification of dorsal fins as a tool for mark and recapture. Mark-recapture techniques allow not only for an estimate of population abundance, but also provide information on movements that can be used to determine if the bottlenose dolphins in this area are a subset of a larger, mobile population, or if there is local spatial structure and a smaller local population.

This distinction has important management implications. Understanding population size is one of the most crucial factors for assessing population health and vulnerability. A low population size can make a species vulnerable to threats affecting individuals directly or indirectly through impacts on its preferred environment (Bejder et al. 2006, Tezanos-Pinto et al. 2009). Anthropogenic effects such as pollution, fisheries, increased coastal development, habitat depletion, and human interactions could be detrimental on survival and population persistence. For this reason, one of the focal points of the US Marine Mammal Protection Act of 1972, which covers the US Caribbean, is to "prevent marine mammal species and population stocks from declining beyond the point where they ceased to be significant functioning elements of the ecosystems of which they are a part". Bottlenose dolphins are the second most frequently stranded marine mammal in Puerto Rico (Mignucci-Giannoni 1989, Mignucci-Giannoni et al. 1999), with human interactions directly implicated in some of these strandings. Additionally, the southwest area of Puerto Rico is the most important fishery zone due to its extended insular shelf (Tonioli and Agar 2011), yet landings have steadily decreased from overfishing (Matos-Caraballo 2002). Such a decline in the fish supply could impact the bottlenose dolphin population and potentially increase dolphin-gear interactions. Other potential stressors of marine nearshore communities are coastal development and recreational activities, which have increased throughout Puerto Rico, especially on the west coast (Díaz and Hevia 2011). While there are no direct data on the effects of these activities (e.g., increased sedimentation, turbidity caused by terrestrial run-off, increased recreational boating) on the marine mammal species of Puerto Rico, the resulting decrease in resource availability could be having negative impacts.

In the only study to date in Puerto Rico, Rodríguez-Ferrer, 2001 reported a population size of 314 individuals for the southwest insular shelf. That estimate was based on a line transect survey of the area, but included dorsal fin photographs for identification purposes. The resulting resighting rate in that study led Rodríguez-Ferrer (2001) to conclude that the dolphins could be moving over a broader distribution, such that true population size was larger. Using this previous study as a baseline, the present survey seeks to update the estimate of population size while revisiting the assumptions regarding movement and spatial distribution.

2.3 Methods

2.3.1 Study Area

The study area covered the waters off the southwest coast of Puerto Rico from Aguada in the north to Punta Ballenas, Guánica in the south (Figure 2). The area was composed of broad and shallow insular shelf on the west coast that extends seawards up to 26 km and between 10 to 20 m deep (Schlee et al. 1999, Ballantine et al. 2008). The coast was characterized by low-wave energy conditions, and two major rivers, the Añasco and Guanajibo (Schlee et al. 1999), discharge in the area. Also included is Bajo de Sico, an isolated seamount off the shelf about 25 km west of Puerto Rico (Armstrong and Singh 2012).



Figure 2.2. Survey areas off the west and south coasts of Puerto Rico. Black lines are the survey tracks for bottlenose dolphins sampled in 2013-2015. Blue (paler) line represents the 100-m isobath. Dotted lines represent the survey areas divided by municipality. Along the south coast the area is characterized by a narrower insular shelf that tapers eastwardly. Off La Parguera, in the municipality of Lajas, the shelf extends from six to ten km in width with an average depth of 18 to 20 m, but off Guánica it narrows to only three to four km width and an average depth of 12 m (Morelock et al. 1994). This zone is characterized by a series of small mangrove cays that extend over the south coast, interrupted by Guánica Bay.

Survey

Boat-based dolphin surveys were conducted in the study area from January 2013 to October 2015 in an open seven meter boat, offering a 360° field of view. Survey speed while searching was 10 knots (20 km/hr) (Rosel et al. 2011). The survey team included the boat captain, a photographer, data recorder, and an additional observer or dolphin spotter. The area covered was recorded using the tracking mode of a handheld Global Positioning System (GPS). All surveys were conducted at a Beaufort Sea State scale of three or less, the equivalent of a wave height 0.9 m or less, to ensure that encounter rate was unaffected by poor visibility. On-going surveys were terminated if the sea state increased above Beaufort 3.

The following information was recorded for each dolphin sighting: 1) time of first sighting and position of the animal were recorded using a GPS unit, and 2) weather conditions (i.e., cloud cover, sea state, approximate wind speed and direction) were recorded and depth determined with the boat's depth finder.

Group characteristics were also recorded. Dolphin were classified *all adults* = a group composed of individuals greater than about 250 cm in length, *all juveniles* = all individuals were less than 250 cm, *mixed group* = a group composed of several age classes including calves and neonates, *mom and calf pairs* = female and calf (Figure 2.3), *mixed species* = two distinct species clearly interacting (swimming together, social behavior) during the sighting. The number of individuals and group the direction of travel was also recorded when the group was first sighted.



Figure 2.3. A *Tursiops truncatus* mom and calf pair swimming by *Sargassum* sp. brown algae. The calf has a *Remora remora* (Linnaeus, 1758) on the right side anterior to dorsal fin. Photo taken by Grisel Rodríguez-Ferrer on June 19, 2015, Mayagüez Bay, Puerto Rico.

A group was defined as all dolphins sighted within a 100-m radius of its estimated center and that were engaged in similar activities (= school, Wells et al. 1987, Quintana-Risso and Wells 2001, Zoolman 2002). An "offshore" Tursiops was defined as an animal with dark gray coloration, adults approximately 290 cm, small flippers, and small beak, whereas a "nearshore/coastal" Tursiops was defined as an animal with light gray coloration, adults no larger than 260 cm, long beak and large flippers (Mead and Potter 1995, Wells and Scott 2002). Dolphin age categories were defined following guidelines established in Shane (1990) and Wells et al. (1987). A juvenile was defined as any individual approximately less than two meters long that swam independent of the adult; calves were defined as individuals two-thirds or less the length of an adult and swimming alongside or slightly behind the adult, while a neonate was characterized by the presence of fetal folds (stripes on the sides of a new born dolphin that last a couple of weeks after birth) and a charcoal coloring with an uncoordinated surfacing pattern. Group behavior was recorded when first sighted, during the sighting and at the end of the sighting. Behaviors were classified according to Melancon et al.'s (2011) behavioral categories for bottlenose dolphins. The behavior categories used were

- *travel* = directional movement
- *social behavior* = when animals have bodily contact including sexual interactions, chasing one another and fighting
- probable feeding = some indications of feeding behavior (repeated dives, variable movement)
- *feeding* = fish in mouth is observed
- *other* = any activity not described, such as interactions with the research boat, e.g., bow riding, boat avoidance, interactions with other boats, etc.

In addition, photographs of the dolphin dorsal fins were taken using a Nikon D 7100 with a 300-mm lens. During the sightings, effort was focused on photographing all the individuals within the group. Four conditions were used to end a sighting: a) all the dolphins were photographed by a single, experienced dolphin photographer (GRF), b) the group was lost, c) sighting time surpassed 30 mins or d) dolphins were clearly avoiding the boat (Melancon et al. 2011). All research activities described in this manuscript are under the auspices of U.S. National Marine Fisheries Service Permit No. 14450-04, and Puerto Rico Department of Natural and Environmental Resources Permit number DRN-2015I-C32.

Photoidentification of dolphins

The photographs were used to identify dolphins based on the location, size and patterns of notches on the dorsal fin (Wells and Scott 1990, Würsig and Jefferson 1990). Photographs of both sides of the fins, peduncle and tail, when possible, were taken to identify scars, notches and any other unique natural markings that helped identify individuals. Notches and or permanent scars were used as the primary distinctive elements for photoidentification (Figure 4, Gnone et al. 2011). These photographs were catalogued using the software application FinBase (Adams et al. 2006). Images were sorted and processed for photographic quality. This was based on the degree to which the fin was in focus (excellent, moderate or poor), contrast (ideal or excessive/minimal), angle of the dorsal fin relative to the camera (perpendicular, slight angle or oblique angle), if the whole fin was visible and the estimated distance of the camera to the dorsal fin (Melancon et al 2011). Each of the factors affecting the quality of the image was ranked from one (good) to three (poor) and a composite score was calculated by adding across all five factors. To be entered into the catalog, a dolphin had to have a photograph with a score of 12 or less. For each dolphin, the photograph with the lowest score was used.



Figure 2.4. Dorsal fin of two *T. truncatus* showing diagnostic shape, color, and notches that enable researchers to identify individual dolphins. A. Specimen 7010, unknown sex. B. Specimen 1001, unknown sex. Images by Grisel Rodríguez-Ferrer on July 17, 2015 (7010), May 20, 2014 (1001).

The proportion of distinct individuals to all individuals was calculated separately for each sighting. These proportions were based on the catalogued photographs. The overall proportion for the entire population was taken as the mean across all sightings. This was then used as the proportion of marked dolphins in the population. Following the recommendations of Urian et al. (2015), only individuals with distinctive fin notches and or permanent scarring were considered for the estimation of population size. Dorsal fin photographs were also compared with those from
the southwest coast taken in 1999-2001, available in the Puerto Rico Bottlenose Dolphin Catalog (Rodríguez-Ferrer 2001).

Population Estimation

The population size of dolphins in southwest Puerto Rico was estimated by the mark and recapture method (Würsig and Jefferson 1990, Gormely et al. 2005, Speakman 2010). In this project, a marked individual was defined as a dolphin photographed with an identifiable dorsal fin. Sampling was broken into periods to be able to meet Jolly-Seber assumptions. The sampling was characterized by short sampling periods (1-56 days) relative to longer periods between sampling periods (68-139 days) (Table 1).

The CloseTest program was used to test if the data were coming from a closed or open population. A closed population assumes no individuals were added to or lost from the population over the mark-recapture period (Stanley and Richards 2004). This program uses two closure tests; the Stanley and Burnham (1999), which allows time specific variations in capture probabilities, and the Otis et al. (1978), which allows for heterogeneity in capture probabilities.

The program MARK (White and Burnham, 1999) was used to estimate population abundance, employing the POPAN module of the Jolly-Seber formulation (Schwarz and Arnason 2006) for open-populations. POPAN estimates the parameter N (super population), which is the total number of animals available for capture at any time in a study, i.e., the total number of animals ever in the sample area between the first and last sampling occasion (Nichols et al. 2000). Other parameters estimated are net births (B) = number of animals that enter the population between two sampling periods and survive to the next occasion, apparent survival rate (φ) between sampling periods, where permanent emigration is treated as mortality, probability of capture (P) within each sampling period, and the probability (b) that an animal from the super population enters the subpopulation, where subpopulation refers to the animals in the study area. Separate models were constructed making these parameters either vary with time (t) (time dependence) or leaving the parameters constant. This estimate by POPAN gives a gross population size. The total population size (N) was obtained by dividing the gross population estimate obtained using POPAN (Ň) by the proportion of identifiable dolphins (Θ), that is N= Ň / Θ (Williams et. al. 1993).

Date	Survey Area	Num. Sightings	Num. Dolphins	Num. Calves	Num. Neonates	Dolphins Marked	Dolphins Unmarked	Resights
1/23/2013	Guánica	0	0	0	0	0	0	0
2/7/2013	Guánica	1	2	1	0	1	1	0
2/8/2013	Cabo Rojo	1	2		0	0	0	0
2/15/2013	Guánica	0	0	0	0	0	0	0
2/28/2013	Cabo Rojo	1	2	0	0	0	0	0
3/1/2013	Lajas	1	0	0	0	0	0	0
3/14/2013	Lajas	1	0	0	0	0	0	0
11/21/2013	Guanica	1	8	2	2	2	1	0
1/3/2014	Bajo Sico	1	15	2	0	L	8	0
1/24/2014	Bajo Sico	1	3	0	0	0	0	0
5/22/2014	Cabo Rojo	1	8	0	0	5	3	0
5/23/2014	Mayagüez	1	2	0	0	0	2	0
6/13/2014	Guánica	3	33	4	0	18	4	0
6/18/2014	Cabo Rojo	0	0	0	0	0	0	0
6/19/2014	Cabo Rojo	0	0	0	0	0	0	0
7/8/2014	Guánica	c	0	C	c	c	c	C

Table 1. Summary of surveys, sightings, marks and recaptures and sampling periods for surveys of bottlenose dolphin, *Tursiops truncatus*, in Puerto Rico. A marked dolphin is a photographed dolphin that has a distinctive dorsal fin, and ummarked dolphin is a photographed dolphin that has a compare. Group size field estimates including presence of calves and or neonates are presented.

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7/17/2014	Lajas	1	13	0	0	12	6	6
7/23/2014	Lajas	0	0	0	0	0	0	0
8/1/2014	Guanica	0	0	0	0	0	0	0
9/10/2014	Lajas	0	0	0	0	0	0	0
9/11/2014	Lajas	0	0	0	0	0	0	0
10/2/2014	Guánica	0	0	0	0	0	0	0
10/3/2014	Lajas	0	0	0	0	0	0	0
10/8/2014	Cabo Rojo	0	0	0	0	0	0	0
10/10/2014	Lajas	1	ষ	0	0	0	2	0
10/16/2014	Lajas	1	2	0	1	1	1	0
10/30/2014	Lajas	0	0	0	0	0	0	0
10/31/2014	Guayanilla	1	3	1	0	1	2	0
1/23/2015	Cabo Rojo	1	2	1	0	0	0	0
2/27/2015	Lajas	0	0	0	0	0	0	0
3/12/2015	Cabo Rojo	1	20	1	0	13	4	2
3/13/2015	Cabo Rojo	1	9	1	0	5	ŝ	1
5/20/2015	Cabo Rojo	1	15	1	0	12	4	7
5/21/2015	Cabo Rojo	1	1	0	0	0	1	0

5/27/2015	Lajas	0	0	0	0	0	0	0
5/28/2015	Lajas	0	0	0	0	0	0	0
5/29/2015	Cabo Rojo	1	7	1	0	7	4	7
6/2/2015	Guánica	0	0	0	0	0	0	0
6/3/2015	Cabo Rojo	1	3	1	0	1	0	0
6/19/2015	Rincón	1	3	1	0	0	0	0
6/24/2015	Guánica	1	4	0	0	0	3	0
7/10/2015	Mayagüez	0	0	0	0	0	0	0
7/23/2015	Mayagüez	0	0	0	0	0	0	0
8/6/2015	Mayagüez	0	0	0	0	0	0	0
9/16/2015	Rincón	0	0	0	0	0	0	0
9/17/2015	Mayagüez	2	30	æ	0	٢	5	2
9/18/2015	Guanica	0	0	0	0	0	0	0
9/25/2015	Cabo Rojo	0	0	0	0	0	0	0
9/24/2015	Cabo Rojo	0	0	0	0	0	0	0
10/2/2015	Lajas	0	0	0	0	0	0	0
0/14/2015	Aguada	1	10	1	0	4	9	0
Totals		34	198	ដ	6	93	63	30

The program MARK uses Maximum Likelihood models to estimate population parameters (Cooch and White 2006). The models were ranked using the Akaike Information Criterion (AIC) (Burnham and Anderson, 2002) as implemented within MARK (White and Burnham, 1999). The Goodness of Fit test (Test 2 and Test 3) run in the program U-Care (Choquet et al. 2005) to

evaluate potential violations to the Jolly-Seber assumptions. TEST 2 evaluates heterogeneity in the data, with Test 2CT evaluating if animals were photo happy or photo shy, and Test 2CL examining if this potential effect lasts more than one interval. TEST 3 evaluates the probability of survival, with Test 3SR assessing if there is an effect of capture on resighting (transience), and Test 3SM examining if there is an effect of capture on survival.

2.4 Results

Field Effort

Fifty photographic surveys were completed during January 2013 to October 2015 for a total effort of 217 hours (time searching for dolphins). Consistency of mid-morning/afternoon trade winds made surveys possible only early in the morning. Surveys covered the whole of the shelf totaling a distance traveled of 4,417 km across all surveys. The average distance per survey day was 88.34 km. (Figure 1). The distribution by area is given in Table 1. Survey areas were assigned to adjacent municipalities (Figure 1). The Cabo Rojo area, which contained most of the southwest insular platform, was the zone most visited, with 15 trips; these surveys included an inshore or coastal portion and an offshore portion. Sightings were obtained on 20 (39%) of the 51 surveys. During these 20 surveys, a total of 26 sightings were recorded (mean = 0.12 sightings/hr). Sightings per day ranged from 1-3 (1.13 ± 0.68). A total of 186 dolphins were observed during the 26 sightings.

Abundance Capture/Recapture

Sixty-two dolphins with distinct dorsal fins were observed and catalogued during the survey period between 2013 and 2015. The estimated number of dolphins with non-distinct dorsal fins (i.e., unidentified) was 65. Thus, the proportion of marked individuals was 0.465 (Standard Deviation= 0.315). Of those 62 dolphins with distinctive fins, the overall recapture rate was 50%, including multiple recaptures of individuals within and across time periods. For the Jolly-Seber analysis, the respective recaptures were as follows: 66.1 % (n = 41) were only sighted once (i.e., the initial capture event), while 22.6% (n = 14) were resigned once, 6.5% resigned twice (n = 4), and 4.8% three times (n = 3). Additionally, there were two dolphin fins that matched with the 1999-2000 Bottlenose Dolphin Photo Identification Catalog (Rodríguez-Ferrer 2001). Including these, the number of days between first and last signting ranged from five to 5,935 days (mean = 900.3 ±1,248.7). Table 2 shows the marked dolphins and the years they were signted.

2003-201(4								5	4	4		•	
Ē	Number	1000	0000	1006	000	2003	2004	2005	2006	2007	2006	2000	0100	2013	1014	2015
9	sightings	CCCT	0007	1007	1001	C007		2007	0007	1007	0007	2007	0107	CINT		CT07
1000***								2							1	
1001	ε	1						1		1						
1002	2	1							1							
2002	1								1							
2003	1												1			
2004	1														1	
2005	4														ŝ	1
2006	9														ŝ	ъ
2007	4														ŝ	1
2009***	2	1														1
3002	1												1			
3003	4													1	1	2
3005	3														1	2
3006	4															4
5000	2						1								1	
5003	1	1														
6000	2						1	-								
6001	1							1								
6002	1							-								
6005	1												1			

Table 2. Marked and recapture history of individual bottlenose dolphins off of southwest Puerto Rico. Data prior to 2013 are from the Puerto Rico Dolphin Survey 1999-2000 Photo Identification Catalog and from opportunistic surveys from 2003-2010.

Table 2. Marked and recapture history of individual bottlenose dolphins off of southwest Puerto Rico (continuation).

6006	1				
6008	6			ŝ	e
6009	1			1	
7003	ŝ	1	1	1	
7004	1	1			
7005	2	1 1			
7008	1			1	
7009	1			1	
7010*	8			4	83
7011	2			1	
7012	1			1	
7013*	8			4	
7014	ŝ			ŝ	
7015	1			1	
7016	6			m	
7017**	10			9	
7018	ŝ			2	
7019	2			1	
7021	1			1	
7022	1			-	
7023	1			1	
7024	1			1	

Dolphin No. 2009, sighted July 30, 1999 and resighted after 16.3 years on October 14, 2015, represents the longest period between sightings. The second longest period between sightings was 8.9 years (dolphin 1000, sighted March 3, 2005; resighted January 3, 2014.

The CloseTest analysis determined that the population was opened (z-value = -0.99817, p-value = 0.059, p <0.05, α = 0.05). The POPAN model of JollySeber for open populations successfully fit the data, with survival (phi) and population size (N) varying through time and with probabilities of capture and entrance constant [phi (t), p (.), pent (.), N (t)] being selected using the AIC evaluation (Table 3).

For analysis purposes, sampling periods were divided into approximate three-month intervals, except for 2013 (Table 1, Appendixes 1-2). This was the year with the least field work, and all samplings for that year then were combined in a single four-month period. A sampling day is considered a day wherein dolphins were sighted and positively marked (new or resighting) by photographs. JollySeber analysis showed that the influx of new individuals with distinctive, identifiable fins was essentially zero. Thus, the study effectively monitored a fixed initial population of marked individuals over the study period. That no new individuals entered the identifiable dolphin population, despite evidence of juveniles and neonates (Table 1), raises the question as to whether the ratio of identifiable to non-identifiable dolphins within the population changed during the study. Consequently, the population size reported here is restricted to that of the initial population estimate. The estimate of N (super population) is 58.8 (SE = 6.42, 95% confidence interval, or CI = 51.4-79.0 dolphins). The corresponding estimate for the distinctive individuals was 59 (SE = 6.42, 95% CI = 47.5-72.8). Taking the 46.5% photographed individuals

with distinctive fins into account, the best estimate of population size (N) for the bottlenose dolphin in the study area is 127 individuals.

The Global test result for the Goodness of Fit statistic (Program U-Care) indicated that there is no significant overdispersion in the data. The test for transience (Test 3 SR) was not significant [N (0, 1) z = 0.76, *p*-value (two-sided) = 0.445], indicating that there is no difference in the probability of the animals being reencounter. Test 2 CT indicates that the animals are neither camera shy nor camera happy [N (0, 1) z = 0.85, *p*-value (two-sided) = 0.393]. The test 2CL was also not significant ($X^2 = 2.3427^{-30}$, df = 1 *p*-value = 1), an expected result given there was no initial photo effect detected.

bundance for marked bottlenose dolphins, Tursiops truncates, on the	per population) = the total number of animals available for capture at	er of animals ever in the sample area between the first and last sampling	phi = apparent survival rate, $p = probability$ of capture, $(.) = constant$. AIC = Akaine Information Criterion.
ole 3. Open population models for abundance for marked bot	hwest coast of Puerto Rico: N (super population) = the tota	ime in a study, or as the total number of animals ever in the sa	sion, pent= probability of entrance, phi = apparent survival 1	neter, (t) = time varying parameter. AIC = Akaine Informat

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Phi(t) p(t) pent(.) N(.)	170.3957	170.3957	0	0	10	-62.5847
{Phi(.) p(.) pent(.) N(.)	227.5423	227.5423	0	0	4	9.3831
{Phi(t) p(.) pent(.) N(t)	46855.76	46855.76	0	0	7	46630.517
{Phi(t) p(.) pent(t) N(t)	46859.489	46859.489	0	0	10	46626.508
{Phi(t) p(t) pent(t) N(.)	46863.396	46863.396	0	0	13	46621.929
{Phi(.) p(.) pent(t) N(.)	46866.695	46866.695	0	0	7	46641.452

2.5 Discussion

We took several steps to validate Jolly-Seber assumptions. The Jolly-Seber assumption of equal capture probabilities (complete mixing) was addressed by having a high sampling effort spread out over the whole study area. In addition, complete mixing was supported by movements documented by the mark-recapture histories of individual dolphins (e.g., Table 1, Figure 2); during the study dolphins were found to move over broad areas multiple times (e.g., Figure 2, dolphin 7011 is an example moving from Rincon into one of the more heavily surveyed areas in the south). To validate mark recognition, only superior quality images were used, and only of animals with long lasting marks; any fins not distinctive enough were counted as unmarked. Sampling was done on a regular basis over the three-year period to be able to detect any dramatic changes on fin shape or new scarring. Two experienced persons catalogued and validate fins, thus controlling for observer error and providing consistency to the analysis. Photo identification is a non-invasive method that reduces the probability of an adverse behavioral response to sampling; therefore, there is no behavioral response to "marking" that would violate the assumptions of equal behavior and probability of capture, and this was supported by the results of Tests 2CT, 2CL and 3SR. The resulting capture probability was relatively high for mark-recapture studies of open populations. A high probability of capture suggests that the population is resident or semi-resident on the scale of the area surveyed.

Having surveys and sightings in all months of the year over the study period helps confirm that bottlenose dolphins are resident in Puerto Rico. Furthermore, the observed sighting rate (39% of the surveys with sightings) is similar to that reported by Rodríguez-Ferrer (2001): 39.8% of

surveys with sightings (41 of 103 surveys). Of the marked individuals, the majority (61%) were sighted only once, with 22% of the animals resighted within only one sampling period and 11% on two or more sampling periods. This supports Rodríguez-Ferrer's (2001) conclusion that the dolphins of south and west coasts of Puerto Rico constitute a semi-resident population, where most of the individuals surveyed are staying within the study area most of the time. Nevertheless, dolphins are capable of long distance movement, potentially covering a larger area over multiyear time periods. If so, residency might more properly be viewed in the context of animals using the areas on the west and south coasts as important habitats over multiple years. Further study is clearly needed on dolphin home range boundaries.

One noticeable difference in this study was the fact that dolphins on the south coast were not sighted in offshore waters during the surveys, but on the west coast they were sighted over deep waters. Figure 2.5 illustrates the observed movements for two such dolphins. One potential explanation is that dolphins would have no incentive or navigational cues that would direct them into the deep waters off the south coast, while off the west coast there could be multiple geomorphic and acoustical cues that would lead them to navigate across the Mona Channel to connect to the island of Hispaniola. Dolphins are capable of long movements, and it has been noted that they have different movement patters depending on habitat (Würsig et al. 1991) and/or fish abundance (Würsig and Würsig 1979).



Figure 2.5. Example of movement patterns of bottlenose dolphins (7011 and 8009) in the waters off the southwest platform of Puerto Rico. Sighting days ranged from November 21, 2013 to September 17, 2015. Numbers for dolphin 8009 indicate the sighting sequence. Arrows connect the observation locations in sequence but do not reflect actual paths of movement. Outer two depth isobaths are for 30 m and 50 m depth, representing the top and slope of the insular platform, respectively.

The distance between Puerto Rico and Hispaniola is about 120 km (64 Nmiles); however, the extended insular platforms of the two islands reduce this distance to 95 km. Furthermore, there is a ridge of several sea mounts and banks connecting the two, where depths can be less than 360 m (e.g., see dolphin 7011 in Figure 3), while the islands of Mona and Monito in the middle of the

channel are additional features that could facilitate inter-island movement. Dolphins of the coastal ecotype have been routinely observed around these islands. The depths within the Mona Passage, especially along the submerged ridge running off the northwest portion of the western platform, are within known dolphin diving limits. Klasky et al. (2007) reported dives that were from 50 to 450m deep for satellite tracked individuals off Bermuda.

The above distances are small relative to known movement patterns elsewhere. For example, long-distance movements of satellite tracked offshore Tursiops truncatus have been reported of up to 4,200 km in depths that ranged from 10-5,000 m (Wells et al. 1999), while Würsig (1978) reported a round trip of 600 km for a group or bottlenose dolphins in Argentina, thus making interisland movements plausible. However, most studies of dolphin movements do not sufficiently report information (ecotype, distance, depth and driving mechanism, e.g., following migratory fish stocks) necessary to assess the likelihood of interisland movement between Puerto Rico and Hispaniola. One exception is Tobeña et al. (2014), who report that coastal dolphins inhabiting the Canary Islands exhibit regular inter-island movements, which would involve distances of 6080km over waters of 2000+m depth. If there is any connectivity between dolphins off the west coast of Puerto Rico and eastern Hispaniola, this implies either a migratory stock or a larger population boundary. This would have a potentially profound impact on population size estimates, genetic structure, ecological resilience and therefore conservation and management, and as such would warrant confirmation studies based on genetic analysis and on individual movements.

The abundance estimated for the studied population was 127 dolphins. Given the 95% confidence limits of this estimate, this is a statistically significant 60% decrease from the 2001

estimate of 314 individuals (Rodríguez-Ferrer 2001). The 2001 study targeted the west coast insular platform and used a line transect survey analysis. The present study covered not only the area studied previously, but also added the south coast from Guánica Bay. The difference between survey methods is that mark and recapture provides an estimate of the abundance of all dolphins (present or not present) using the area during the study (Daura-Jorge and Lopez 2016), while line transect only estimates the abundances of the animals present now of the survey in each area (Calambokidis and Barlow 2004). For coastal dolphin populations found in small groups, mark and recapture methods have proven to be more precise in abundance estimates than line transect methods (Lukoschek and Chilvers 2008, Daura-Jorge and Lopez 2016). Thus, based on both area surveyed and methodology, if the dolphin population was stable, a higher population estimate would have been expected for the recent survey, and this was not the case. As a check on the current estimate, a separate calculation based on the proportion of the shelf surveyed was made using the following parameters: Transect width = 1km, Mean distance/trip = 67.8km. Surveys during first sampling period = 9, Shelf area to 50m contour = $980km^2$. Using these parameters, the total area surveyed was 611km², or 62.3% of the shelf. The 69 dolphins observed during the first sampling period would then represent the same percentage, for a total population estimate of 110 individuals. Given that the area surveyed went outside the 50-m contour demarcating the insular platform, this calculation would slightly underestimate population size, but the result is consistent with that obtained using the mark-recapture approach.

A 60% decline in the dolphin population off southwest Puerto Rico in 15 years is substantial but represents only a 4.06% annual loss. Daura-Jorge and Simões-Lopes (2016) used power analysis in their mark-recapture study of a bottlenose dolphin population in Brazil to estimate that it would take 11 years of continued sampling to determine a 5% decrease per year with a 95% level of confidence. They further point out that, given their small population size, the resulting 40% decline would already have devastating effects long before such significance was achieved. This is on a scale with that observed in Puerto Rico. Given the magnitude of the change already observed, and the difficulty in detecting slight changes, more frequent and intense sampling is recommended.

The observed population decline represents a high degree of either mortality or emigration, or some combination of the two. Given that dolphin home ranges and movements are not well known, it could be that the population has shifted its location to other areas. One possible factor related to either mortality or emigration could be the decrease on prey abundance. Puerto Rico fish stocks were declared by Matos-Caraballo in 2001 as overfished, which was supported by a more quantitative analysis by Ault et al. 2008; it has not shown signs of recuperation since, although there has been a shift in fishing pressure toward recreational and coastal migrating species (Appeldoorn et al. 2015). Declines in habitat quality due to land-based anthropogenic inputs could also contribute to a decline in food resources (Appeldoorn et al. 2009, Hernandez-Delgado et al. 2010). A significant decline in food availability could either force dolphins to seek new feeding areas or lead to increased mortality through starvation or increased stress. The Puerto Rico Marine Mammal Rescue Program reported an average of 1-3 bottlenose dolphin strandings per year in the last 10 years (unpublished), a rate that has not changed over that period. Therefore, there has not been an increase in reported deaths or a massive die-off that could explain such a decline. Yet, some of these strandings show unambiguous evidence of human, particularly fisheries interactions indicating potential competition for scarce resources.

Another factor that could contribute to permanent emigration and increased stress is the high boat traffic associated with recreational activities and fishing that characterizes the southwest coast of Puerto Rico. High boat traffic has proven to negatively impact dolphin behavior (Nowacek et al. 2001, Hastie et al. 2003). It can affect habitat selection for foraging by changing preferred areas, either directly to avoid boat traffic or indirectly because of the prey response to high boat traffic (Allen and Read 2000), which can result in changed residency patterns (Lusseau 2005). The noise created by high boat traffic has been categorized as a source of acoustic harassment for the species (Haviland-Howell et al. 2007). Small dolphin populations, such as the one presented here, have been shown to be negatively affected by dolphin watching operations. In several areas where dolphin-based tourism is practiced the population has declined (Constantine 2002, Bejder et al. 2006, Lusseau et al. 2006, Currey et al. 2009).

While there are no data on reproductive success or related life history parameters for the bottlenose dolphin population in Puerto Rico, long-term studies elsewhere have reported an estimated age at first birth from 5-12 years (Wells and Scott 1987, Mann et al. 2000,) and a calving interval of 3-6 years for Australia (Connor et al. 1996, Mann et al. 2000), 2-10 years for Florida (Scott et al. 1996), and 3 years for North Carolina (Thayer 2008). This lengthy time to reach age of reproduction, coupled with low fecundity and long time between births limits the ability of dolphins to respond to either a sudden population decrease, by mass die offs or slow sustained declines resulting from anthropogenic impacts. Yet, the majority (38%) of the dolphin sightings during this study were composed of mixed groups of adults, juveniles and calves, with most of the sightings having one calf and 1-2 juveniles per group (Table 4); but only 3 neonates were

observed. This suggests that there is at least some active level of reproduction occurring, but there is insufficient information to interpret if this is adequate to sustain the population.

Table 2.4. Description of the group compositions for bottlenose sightings, *Tursiops truncatus*, encountered in Puerto Rico on surveys between 2013 and 2015.

Solitary	Mom and calf pairs	Adults only	Mixed (adults ar calves in group	Pair of adults
2 (12%)	4 (15%)	7 (27%)	10 (38%)	3 (12%)

A minimum effective breeding population size is estimated to be 500 individuals (Franklin 1980, Lande and Barrowclough 1987), and small populations generally show greater variability in population size. Even if the change reported here represents such natural variation, given the low reproductive potential for dolphins it would represent a serious threat to local population persistence. This significant decline in population abundance means that the conservation and management of bottlenose dolphins in Puerto Rico needs to be more aggressive. However, further efforts should focus on determining the causes of this decline so that management actions can be targeted to reduce overall societal impact, for example, if overall reductions in fishing or boating activities are warranted. There is a need for research on the effect of anthropogenic factors on bottlenose dolphins in Puerto Rico. Factors such as overfishing, coastal development, boat traffic, human interactions, and pollution should be studied in relation to the species' distribution, behavior, and population dynamics, and further mark-recapture on studies are needed to assess if there is in fact a decreasing abundance trend in this population.

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pendix 1. Summary of the field information by day for the survey on bottlenose dolphin, <i>Tursiops truncatus</i> , the south and west coast of Puerto Rico. Photographs by day, number of individuals present as well as mber of animals marked is presented.
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Date	SubArea	Number of sightings	Number of pictures	Number of photographed dolphins	Cataloged	Marked dolphins	Unmarked
07-Feb-13	Parguera	1	43	2	6	1	1
28-Feb-13	Combate	1	5	2	0	0	0
21-Nov-13	Parguera Puerto	7	87	2	0	2	0
03-Jan-14	Real	1	140	15	15	7	8
24-Jan-14	Joyuda	1	0	0	0	0	0
22-May-14	Combate	1	72	8	8	5	3
23-May-14	Bouy 8	1	23	7	2	0	7
13-Jun-14	Combate	3	436	36	21	18	3
17-Jul-14	Combate	1	285	22	21	12	6

II the south a	nd west coast o	I Puerto K	cico (conunua	lion).			
10-Oct-14	Parguera	1	38	3	2	0	2
16-Oct-14	Parguera	1	66	2	2	1	1
31-Oct-14	Guanica	1	138	4	3	1	2
23-Jan-15	Combate	1	0	0	0	0	0
12-Mar-15	Combate	1	0	0	0	0	0
13-Mar-15	Bouy 4	1	147	9	5	2	ŝ
20-May-15	Combate	1	114	15	15	11	4
21-May-15	Bouy 8	1	82	1	1	0	1
29-May-15	Combate	1	510	10	10	6	4
03-Jun-15	Boquerón	Ē	63	2	T	1	0
19-Jun-15	INIAYABUCZ Bay	1	125	5	5	1	4
24-Jun-15	Guánica	1	64	5	3	0	ŝ
17-Sep-15	Boquerón	1	0	0	0	0	0
17-Sep-15	Combate	2	193	20	12	7	5
14-Oct-15	Aguada	1	49	10	10	4	9

Appendix 1. Summary of the field information by day for the survey on bottlenose dolphin, *Tursiops truncatus*, off the south and west coast of Puerto Rico (continuation)

days were col dolphin is a I dolphin that th of calves and	mpiled into si photographed ie fin is not di or neonates ar	x sampling p dolphin that stinctive and e presented.	eriods starti has a distin will be hard	ing 11/21/2 active dorsa I to recogni	013 as indic ll fin, and u ze. Group siz	ated by horiz ımarked dol ce field estim	zontal lines. phin is a pho ates including	A marked tographed g presence
Date	Survey Area	Number of Sightings	Number of Dolphins	Number of Calves	Number of Neonates	Dolphins Marked	Dolphins Unmarked	Resights
11/21/2013	Guánica	1	80	0	6	ы	1	0
1/3/2014	Bajo Sico	1	15	2	0	7	8	0
1/24/2014	Bajo Sico	1	3	0	0	1	5	0
5/22/2014	Cabo Rojo	I	8	0	0	5	ю	0
5/23/2014	Mayagüez	1	2	0	0	0	7	0
6/13/2014	Guánica	3	33	4	0	18	3	0

Appendix 2. Summary of the data used for the mark recapture analysis. For mark-recapture analysis, survey do do of

Appendix 2.	Summary of the	data used f	for the marl	k recapture	analysis (co	ntinuation).		
7/17/2014	Lajas	1	13	0	0	12	6	9
10/10/2014	Lajas	1	4	0	0	0	2	0
10/16/2014	Lajas	1	7	0	1	1	1	0
10/31/2014	Guayanilla	1	3	1	0	ч	2	0
1/23/2015	Cabo Rojo	1	2	1	0	0	0	0
3/12/2015	Cabo Rojo	н	20	1	0	13	4	7
3/13/2015	Cabo Rojo	1	9	1	0	0	3	1
5/20/2015	Cabo Rojo	7	15	1	0	12	4	7
5/21/2015	Cabo Rojo	1	1	0	0	0	1	0
5/29/2015	Cabo Rojo	1	7	1	0	7	4	7
6/3/2015	Cabo Rojo	1	3	1	0	1	0	0
6/19/2015	Rincón	1	3	1	0	0	0	0
6/24/2015	Guánica	1	4	0	0	0	3	0
9/17/2015	Mayagüez	2	30	3	0	7	5	2

CHAPTER THREE MODELING DISTRIBUTION OF THE COMMON BOTTLENOSE DOLPHIN, *TURSIOPS TRUNCATUS* OFF THE SOUTHWEST COAST OF PUERTO RICO

Submitted to Journal of Marine Systems

3.1 Abstract

Standardized sightings of the common bottlenose dolphin (*Tursiops truncatus*) from the south and west coasts of Puerto Rico were quantitatively analyzed to determine the spatio-temporal distribution patterns of sighting location, group size, composition and behavior in relation to key features such as distance from shore, depth and habitat type. Dolphins inhabit primarily the nearshore zone within five kilometers of the coast, but they are also uniquely associated with edge and slope habitats of the platform and bank/islands off the west coast. Dolphins were rare both over the central portion of the western platform, characterized as broad area of sand, as in deep water outside the shelf edge zone. Although dolphins were sighted in various habitat types, the most predominant were sea grass beds (44%), sand (25%), and reef (22%). Only 17 % of the sightings were in deeper waters, such as the shelf edge and mid platform, which were dominated by hard bottom. Multiple, multivariate correlations based on distances (DistLM), showed that the only statistical significant predictor for dolphin abundances was Habitat Evenness (AIC = 250.89; $R^2 = 0.12$; p = 0.003). On the other hand, for behavior data, DistLM showed that the only significant predictor was average rugosity (AICc = 551.21, R²= 0.09, p=0.02). Given the predominately inshore distribution of bottlenose dolphins, the area off the west coast of Puerto Rico should be designated a Critical Habitat, as the species is resident (Rodriguez et al. 2017), in small numbers and could be affected by a number of anthropogenic stressors.

3.2 Introduction

One of the most familiar and studied marine mammal species is the common bottlenose dolphin, Tursiops truncatus (Montagu, 1821). It is found worldwide in tropical and temperate zones, and in the Caribbean it is considered the most common cetacean (Ward et al. 2001). The bottlenose dolphin is divided into two ecotypes based on morphology, DNA, hemoglobin, parasite loads and prey preferences (Duffield et al. 1983; Perrin et al. 2011; Félix et al. 2017; Fruet et al. 2017). The offshore or pelagic ecotype is less studied as their populations are generally less accessible to researchers (Silva et al. 2008). In contrast, the coastal ecotype is widely distributed and can be found in rivers, channels, waterways, estuaries, and on continental/insular shelves and shelf breaks (Mead and Potter, 1995); and associated with a wide range of habitat types, such as seagrass beds, sandy substrates, reefs and even pelagic waters (Ballance 1992; Grigg and Markowitz, 1997; Cribb et al. 2018). While dolphins can be characterized as opportunistic in habitat use, some studies have shown that there is a differential habitat use, leading to the formation of distinct groups, even within close proximity (Rossbach and Herzing, 1999; Ansmann et al. 2012). For example, in Hawaii there are two subgroups of coastal dolphins, one primarily located < 200 m depth and the other in waters between 200 - 1,000 m (Baird et al. 2009). Similarly, in the Bahamas, Rossbach and Herzing (1999) found two subgroups along the leeward margin of Little Bahama Bank, a southern group that extends from Grand Bahama Island up to 19 km from the coast and a northern group that extends further north up to 27 km from the coast. Such partitioning of distribution patterns alone has important ramifications for conservation and management, especially when threats tend to be habitat specific (Wells et al. 1998; Whitt and Read, 2006; Moore, 2008; Diaz-Lopez, 2012; Vail, 2016). This is even more so when such partitions are related to important aspects of behavior, such as feeding strategy, calving or nursery functions. In Florida, for example, distinct depth distributions were related to dolphins that fed exclusively using deep diving versus those that employed mud ring feeding in shallower locations (Torres and Read, 2009).

In studying aspects of dolphin distribution, the first priority is to map where they occur, while the second is to relate observed differences in abundance/behavior to various spatiotemporal factors that may serve (1) as a guide to predict where dolphins can be found in other areas and (2) to elucidate the underlying causes of observed distribution patterns (Palacios et al. 2013). Many studies describing dolphin distributions, commonly used simple factors such as distance from shore, depth (plus derived characters) and relation to the shelf, as these features are easily derived from nautical charts. In a more detailed analysis, Blasi and Boitani (2012) modeled the distribution of dolphins using encounter rate and behavior within 1x1-km cells in relation to 18 physiographic variables to identify key feeding and resting/calving areas. In another approach, Grigg and Martkowitz (1997) analyzed dolphin distribution on Glovers Reef, Belize, related to large scale seascape features (e.g., central lagoon, proximity to reef cuts or mangrove channels) and found that encounters were more common near reef cuts and less common in the central lagoon. These studies suggest that dolphin distributions seem to respond to large scale features of the seascape, which might be expected for a highly mobile species. However, it is only recently,
with the advent of synoptic mapping of habitat types and depth via airborne or satellite sensors, that fine-scale aspects of habitat use can be addressed.

In Puerto Rico, bottlenose dolphin is the most abundant and frequently encountered marine mammal, and the second most frequently stranded marine mammal (Mignucci-Giannoni et al. 1999; Mignucci-Giannoni et al. 2009). Until recently, data on habitat characterization was not available for Puerto Rico, and habitat descriptions for bottlenose dolphins were based on distance from shore and bathymetry only (Mignucci-Giannoni 1989; Mignucci-Giannoni 1998; Rodriguez-Ferrer 2001). For example, in studying dolphin abundance off the west coast, Rodriguez-Ferrer, (2001) only gave a crude description of their distribution, stating they had an average depth of 10.4m and a mean distance from land of 4.0km (SD= ± 0.74). Using a new set of distribution data, we propose to take this analysis further by modeling the spatial distribution of bottlenose dolphins, along with group size, group composition, seasonality and behavioral activity and determine the species spatio-temporal patterns related to habitat type and bathymetric parameters. Using models to describe a species' distribution enhances ecological understanding (Zanardo et al. 2017) and facilitates the design of appropriate conservation measures (Passadore et al. 2018). An in-depth look at habitat preferences should help delineate the bottlenose dolphin population in this poorly known area.

3.3 Materials and Methods

3.3.1 Study Area

The study area covered the waters off the southwest coast of Puerto Rico from Aguada in the north to Punta Ballenas, Guánica in the south (Figure 3.1a). The area is composed of a broad

and shallow (10-20 m) insular shelf on the west coast that extends seawards up to 26 km (Schlee et al. 1999, Ballantine et al. 2008). It consists of nine major reefs plus Banco de Esponja and Bajo de Sico Banks, isolated seamounts about 25 and 36 km west of Puerto Rico, respectively (Armstrong and Singh 2012), and Desecheo Island 20.92 km from the coast (Figuerola-Hernández et al. 2017). The coast is characterized by low-wave energy conditions and two major rivers, the Añasco and Guanajibo, discharge in the area (Schlee et al. 1999).

The south coast of Puerto Rico is characterized by a narrow insular shelf that tapers eastwardly. Off La Parguera, Lajas the shelf extends from six to 10 km in width with an average depth of 18 to 20 m, but off Guánica it narrows to only three to four km width and an average depth of 12 m (Morelock et al. 1999). This zone is characterized by a series of small mangrove cays that extend over the south coast, interrupted by Guanica Bay. This Bay is approximately three km long and 2.5 km wide, with an entrance to the open ocean (Whitall et al. 2013).

3.3.2 Survey

Boat-based surveys were conducted in the study area from April 2002 to October 2015 in an open 7-m boat, offering a 360° field of view (see Rodriguez-Ferrer et al. 2017 for survey details). This study represented a combination of opportunistic surveys that were more limited and variable year-to-year and dedicated surveys, especially in the latter years. All research activities described in this manuscript were under the auspices of National Marine Fisheries Service Permit No. 14450-04, and Department of Natural and Environmental Resources Permit number DRN--2015-I-C32.

Survey tracks (Figure 3.1b) were recorded using the tracking mode of a GPS (Global Positioning System). Surveys were conducted at Beaufort Sea States of 3 or less (wave height of

0.91 m or less). When dolphins were sighted, their location was recorded using a GPS unit, as well as the direction of travel and depth (boat's depth finder). Weather conditions (i.e., cloud cover, sea state, approximate wind speed and direction) were also recorded.

In this study, a group was defined as all dolphins sighted within a 100-m radius of the estimated center that were engaged in similar activities (= school, Wells et al. 1987, Quintana-Risso and Wells 2001, Zoolman 2002). To describe group composition, dolphin categories were defined using Shane's (1990) and Wells et al.'s (1987) dolphin categories. The categories were

- all adults = a group composed of individuals greater than about 250 cm in length
- all juveniles = a group of dolphins where the length of the individuals was less than
 250 cm
- mixed group = a group composed of individuals of several age classes including calves and neonates
- mom and calf pairs = female and calf/neonate (calves were defined as individuals two-thirds or less the length of an adult and swimming besides or slightly behind the adult, while a neonate was characterized by the presence of fetal folds and a charcoal coloring with an uncoordinated surfacing pattern)
- mixed species = two different species clearly interacting (swimming together, social behavior) during the sighting.

Behaviors were classified according to Melancon et al. (2011). The behavior categories used were:

• travel = directional movement

- social behavior = when animals have bodily contact including sexual interactions, chasing one another and fighting
- probable feeding = some indications of a feeding behavior (e.g. repeated dives, variable movement)
- feeding = fish in mouth is observed,
- other = any activity not described such as interactions with the research boat (e.g. bow riding, boat avoidance, interactions with other boats etc.)

3.3.3 Distribution

Sightings of dolphins were plotted in Arc GIS 10.6 and associated data: location, group size and composition and behavior) were initially analyzed relative to season and to variables based on geomorphology: distance from coast, depth, and zone. Four zones were established because there was not a direct correspondence between distance from shore, depth and position on the shelf due to the large variations in the width of the shelf across the study area (Figure 3.1a). A nearshore zone comprised all areas within a distance of five km from the coast. A shelf-edge zone was delimited as 2.5 km either side of the shelf-edge drop (defined as the 50 m isobath) (Fig 3.1a). This latter contains the shelf edge reef environments on the platform and the upper insular slope, as well as similar areas associated to offshore islands and banks. Where the shelf is narrow and the nearshore and self-edge zones overlap, distribution was assigned to the nearshore zone. The third or mid zone was the central portion of the western platform that fell in between the first two zones. The fourth or deep zone constituted the deep waters off the shelf and outer island/banks. Seasons were defined as fall: October-December, winter: January-March, spring: April-June and summer July to September.

3.3.4 Habitat characterization

Habitat characterization was conducted by extracting information from existing spatial data sets. Habitat parameters were developed for a 200-m radius buffer from the sighting point given that: 1) the 100-m radius used to define a dolphin group, 2) the fact that dolphins were frequently moving, and 3) as such, they may respond to larger scale seascape distributions of habitat (Grigg and Markowitz, 1997). Primary sources of habitat-type information were the NOAA benthic habitat maps of Puerto Rico and the U.S. Virgin Islands (Kendall et al. 2002, side-scan sonar habitat maps along the coastal margin (J.A. Rivera, personal communicaton), US Geological Survey benthic maps (Buczkowski et al. 2006), high resolution (5-m lidar, (Battista, 2016); 3-m multibeam, (Battista , 2015) bathymetry and backscatter data, and a habitat map of Abrir la Sierra on the northwest shelf margin (Costa et al. 2009). Depth and rugosity information were derived primarily from the lidar data, supplemented where needed with the multibeam data.

The following parameters were extracted from the spatial data for each 200-m radius circle: mean depth, mean slope, mean rugosity, habitat richness, habitat evenness, percentage of hard bottom, percentage of unconsolidated sediment, and percentage of vegetated bottom. Depth, slope, and rugosity were derived from lidar bathymetry using Focal Statistics in Spatial Analysis (ARC GIS 10.6). Rugosity was calculated as the slope of the slope (Pittman et al. 2007; Pittman et al. 2009). The NOAA benthic habitat map, which has a minimum mapping unit of 4047 m² (one acre), was the primary source of habitat information. Where gaps existed, habitat information from the other sources were used, scaled as near as possible to the same minimum mapping unit. Simpson's habitat evenness (Prada et al. 2008), based on the area of habitat patches within the 200-m radius circle, was calculated as Evenness = D/D_{max}, where $D = 1/\Sigma pi^2$ with p_i being the proportional area of each habitat type (i) and D_{max} being the total number of habitats present. Evenness = 1 when all habitat types in the circle have equal area. To examine the relationship to percent habitat type, habitats were reduced to the three basic types (hard bottom, unconsolidated sediment, vegetated) to minimize habitat definitions across data sets, but also to reduce the number of predictor variables given the number of dolphin sightings.

3.3.5 Analysis

A detailed analysis was performed on the sightings occurring on top of the insular platform (< 30 m deep), for which the above eight detailed habitat and bathymetry variables were available. This information was used to create a more rigorous model to correlate sightings with habitat characterizations. For the model, a total of 90 non-dolphin points were also characterized. Using ArcGIS, non-dolphin points were generated randomly along track lines from survey days when no dolphins were sighted.

For analyses, there were two sets of response variables: 1) total counts of dolphins per sampling station, and 2) recorded behavior per sampling station, which was recorded as presence/absence such that they were mutually exclusive across the three behavioral categories: travel, feeding and social. A resemblance matrix that related all pairs of observations was constructed for each data set of variables. To do this, Euclidean distances were used for dolphin counts, whereas Jaccard coefficients were used for behavioral data. The Euclidean distance is the recommended resemblance measure for univariate data, whereas Jaccard is recommended whenever double zeros are present (Legendre and Legendre, 2012).

Based on these two resemblance matrices, Distance Based Linear Models (DISTLM) were used to identify the suit of predictor variables that were most related to patterns of spatial

distribution of the response variables (i.e., dolphin counts and behavioral data). DISTLM is a distance-based regression approach that can be used on univariate (counts) or multivariate data (behavior) in response to continuous (or categorical) predictor variables (Anderson et al. 2008). The predictor variables included the eight detailed habitat variables plus latitude and longitude. To construct the models, the *Best* procedure was used to select the best fitting model, as this approach examines all possible models and combinations of predictor variables and selects the optimal solution by comparing all selection criteria. In this particular case, the modified AIC criterion (AICc) was used as selection criteria because it tends to provide more conservative (less spurious results/correlations) than the traditional AIC (Anderson et al. 2008).

In the case of the multivariate analysis (behavior data), Distance-based Redundancy Analyses (dbRDA) were done to ordinate and illustrate the fitted model detected with DISTLM. dbRDA is a constrained ordination of the fitted values (i.e., fitting response resemblance matrix on to the predictor matrix) from a multivariate regression model. The fitting process uses only those variables that were statistically significant in the previous procedure (i.e. DISTLM).

3.4 Results

3.4.1 Field Effort

During the period from April 2002 to October 2015 a total of 109 photographic surveys were completed for a total of 625 effort hours (time searching for dolphins). Mean daily effort was 5.73 hours. Consistency of mid-morning/afternoon trade winds made surveys possible only early in the morning (Table 3-1). We covered the whole platform extensively for a total area covered of 8,729 km² (Figure 3.1). From 2002-2010, surveys were based from the P.R. Fisheries Research Laboratory in Joyuda, Cabo Rojo, and from 2013-2015 surveys were based from the

Magueyes Island Marine Laboratory of the University of Puerto Rico-Mayagüez, in La Parguera, Lajas. Seasonal effort was almost constant, with an average of 27 trips per season (Table 3-2).

Sightings were obtained on 61 (56%) of the 109 surveys. During these 61 surveys, a total of 72 sightings were recorded (mean= 0.115 sightings/effort hour; 1-3 sightings per day (mean =1.13, SD±0.68)) (Table 3-2). The estimated total number of dolphins observed was 476, all of the inshore ecotype. Spring was the season with the most sightings (23), followed by fall (22, Table 3-2), but the number of sightings increased with field effort (Pearson's correlation r=0.349, n=50, p=0.0130). Accounting for differences in effort, sightings/field effort hour was similar for all seasons (Kruskal-Wallis test: Chi Square=8, df= 6, p=0.238).

Dolphin groups ranged from one to 40 individuals (Figure 3.2). Over all sightings, 88% were of groups of less than 15 individuals (Figure 3.2). About 9% of the time groups larger than 20 individuals were sighted. These largest groups were composed of individuals of different dolphin groups interacting together. There was no significant difference in group size within the nearshore and shelf-edge zones (Kruskal Wallis; H=0.739, *d*,*f*.=1, *p*=0.390). Also, There was no significant difference on mean group size by survey year (Figure 3a) Kruskal Wallis H=2.819, *d*,*f*.=9 *p*=0.971) or by season (Figure 3b; Kruskal Wallis H=1.172 *d*,*f*.=3, *p*=0.760,). As there were no significant temporal differences (season or year), comparison across depths were done for all year and seasons together. In this sense, there was a significant difference among water depth ranges (Figure 3.4; Kruskal-Wallis H=134.69, d.f.=3, *p*=<0.001,); larger groups being sighted in waters between 51 and 75m, depths that would be associated with the insular slope.

Mixed groups (adults and calves within the same group) were the group type most frequently (35%) encountered, followed by groups of adults only (27%). Solitary adults composed

12 % of sightings, followed by mom and calf pairs (8%), and adult pairs (18%). In the fall and winter months mixed groups dominated the sightings, whereas single animals or only adults were mostly sighted in warmer months (Table 3-3). Number of calves per group followed the same pattern. No differences were found among the presence of calves by year (Kruskal Wallis H=10.021, df=9 p= 0.349, 95 % confidence level) or by season (H= 2.363 df=3 p=0.501, 95 % confidence level). There were no statistical difference in seasons but a significant difference among depths and the presence of calves. The presence of calves generally (in mixed groups or in mom and calf pairs) varied significantly with depth (Krusal Wallis H=8.892 df=3 p= 0.031, 95 % confidence level), being more prevalent in shallow waters.

3.4.2 Overall Distribution

Bottlenose dolphin sightings were present over the study area, but their distribution was not uniform. The majority of sightings occurred near shore (70.83%) while another cluster was found associated with the shelf edge and drop-offs (16.6% occurred in the shelf-edge zone). Only 8.33% occurred in the mid zone, and one of the most conspicuous patterns was the near absence of sightings from the middle portion of the shelf (Figure 5). The deep zone contained only 4.16% of the sightings. Of the five sightings not seen in either the nearshore or shelf-edge zones, three of them were just outside, leaving only two sightings in deep water, both located in the Mona Channel (Figure 5). No dolphins within the study area were sighted in deeper waters off the south coast. Most of the shelf edge sightings were either within or very close to the 100 m contour, indicating that they are tightly associated with the shelf. Seventy-four percent of sightings were in the 0-25 m depth range, 6.94 % at 26-75 m, 1.38% 76-100, and 16.66 % in the >100m depth range (Figure

5). There was no significant difference among seasons and sighting depths (Kruskal Wallis H=4.78, d.f.=3, p=0.188)

3.4.2.1 Insular platform distribution and habitat

Most sightings on the platform (93%) were in nearshore waters, whereas 3.63% were at the shelf edge, and 5.45% of sightings where in the mid zone, but closer to the shelf edge (Figure 3.1a). Depth range for sightings in the platform was between 2-70.4 m with an average depth of 13.1 m SE \pm 1.5m. Although dolphins were sighted in various habitat types, the most predominant were sea grass beds (44%), sand (25%), and reef (22%) (Figure 3.6). The nearshore waters had the greatest variety of habitats available (Figure 3.6), and of the sightings in these waters 49% were over sea grass beds (Figure 3.6). Deeper waters such as the shelf edge and mid platform zone were dominated by hard bottom, and just 17% of the sightings were in this area. In the nearshore environment, dolphins were sighted feeding on four occasions (one each over seagrass, algae, coral reef, silty clay and sand), socializing 14 occasions (over seagrass, sand, algae, and coral reef,) and traveling on 32 occasions (over seagrass, coral reef, silty clay, hard ground and sand). For sightings in the deep water zones the behavior that dominated sightings was travelling.

3.4.3 Modelling dolphin distribution in the insular platform

Multiple, multivariate correlations based on distances (DistLM), showed that the only statistical significant predictor for dolphin abundances was Habitat Evenness (AIC = 250.89; $R^2 = 0.12$; p = 0.003). However, this statistically significant relationship only explained 12% of the total number of dolphins found in a particular place, which is why the shape of the relationship is not clear (Figure 3.7).

For behavior data, DistLM showed that the only significant predictor was average rugosity $(AICc = 551.21, R^2 = 0.09, p=0.02)$. As per the previous case, this significant relationship only explains 9% of the total variance in the type of behavior. As a consequence, constrained ordinations (RDA) did not showed clear patterns of distribution in relation to behavior. Nevertheless, it would appear that social behavior is more frequent in highly rugose areas, whereas traveling is more frequent in areas of lower rugosity (Figure 3.8).

3.5 Discussion

The factors determining dolphin distribution are variable, occur on different spatial scales, and may only be loosely correlated with measurable parameters, and this presents challenges to general characterization and modeling. Thus, understanding the factors affecting dolphin distribution patterns may not be generally ascertained from a single study, but rather may require a comparative approach, especially among areas with significantly different geomorphologies. The study area was unique in that it consisted of a broad (26-km wide) insular shelf on the western margin that became quite narrow to the north and southeast, and this resulted in a non-monotonic relationship between depth and distance from shore. This contrasts with previous studies around islands (e.g., Blasi and Boitani, 2012; Dinis et al. 2016) in two ways. First, there is significant shallow, inshore habitat. Second, with the shelf edge not parallel to the coast line, predictive variables such as depth and distance from shore or a specific depth contour are not confounded. While this also introduces a problem of nonlinearity with respect to these variables, the contrast

does allow for partitioning out effects of larger geomorphology, distance from shore, and potentially relationship to food supply (Hastie et al. 2004) and behavior (Pereira, 2012).

The most dominant pattern observed in this study was that dolphins were distributed preferentially with respect to two features. Most were found nearshore, but there was a secondary association of dolphins with the shelf-edge environment, extending 26 km offshore, even including the offshore banks and islands. In contrast, few dolphins were observed over the central portion of the western platform. This pattern may be driven by prey abundance (Wilson et al. 1997; Blanco et al. 2001; Hastie et al. 2004; Cribb et al. 2008; Blasi and Boitani, 2012), as the distribution of prey species is associated to habitat and geomorphological features (Davis et al. 2002). Inshore waters have a greater variety of habitats, and more developed reefs and sheltered waters, where fish abundances and feeding opportunities are going to be higher. Inshore areas are characterized by higher habitat diversity, and consequently higher fish abundance and diversity due to spatial connectivity (Appeldoorn et al. 2003; Kendall et al. 2003; Pittman, et al. 2007). Habitat heterogeneity has been observed to be one of the important factors for development of variable feeding strategies within dolphins (Sergeant et al. 2007), and for a generalist species that means focusing on several different prey types to more efficiently utilize an area's available resources.

The shelf edge is also characterized by coral reef development and includes the insular slope, both of which are generally correlated with higher fish abundance and richness (Pittman et al. 2007; Pitman et al. 2009; García-Sais et al. 2012; Berjano et al. 2014; Tzadik et al. 2017), including the presence of important fish spawning aggregation sites (Ojeda-Serrano et al. 2007; Rowell et al. 2012; Schärer et al. 2012). Shelf edge sites are also proximate to the potential prey species that constitute the deep scattering layer located in the Mona Channel. The association of

dolphins with deeper, high slope environments has also been reported in other areas (e.g., Rossbach and Herzing, 1999; Ingram and Rogan, 2002; Blasi and Boitani, 2012)

The central portion of the western platform is largely devoid of dolphins, despite substantial sampling effort in the area. The composition of this habitat is silty-clay in the central region and sand along the majority of the outer portion. The lack of habitat diversity and distance to reef/seagrass, where fish abundance is higher, may then make this habitat less suitable for dolphin's prey. This broad sand area is characterized as queen conch (*Lobatus gigas*) habitat (Mateo et al. 1998; Baker et al. 2016), which is not a dolphin prey item (dos Santos et al. 2009). From this perspective, it is not surprising that dolphins would not spend time in this habitat if there is no benefit for the acquisition of food. A similar situation may have been responsible for the dolphin distributions reported by Rossbach and Herzing (1999) on Little Bahama Bank. Although not specifically mentioned in their study, the dolphins they studied were limited to the leeward margin of the bank, the only area where there is any benthic structure on the bank, which is otherwise uniformly covered by sand.

In other studies, distinct distributional subgroups have been observed (Kiszka et al. 2012). In Hawaii, Baird at al. (2009) found coastal dolphins to be separated into two conspicuous groups discriminated by depth (< or > 200m), and in the Bahamas Rossbach and Herzing (1999) found two groups of coastal dolphins separated by distance from shore (< or > 19km). Furthermore, bottlenose dolphins have shown marked prey preferences and foraging tactics depending on the habitat and prey availability (Sergeant et al. 2007; Millman et al. 2016), and in some areas stomach content analyses have revealed different diets of nearby groups (Hernandez-Milian et al. 2015; Millman et al. 2016). It is proposed that the nearshore and shelf-edge dolphins off the west coast

of Puerto Rico may similarly constitute different groups, with different foraging habits and prey preferences. However, Rodriguez-Ferrer et al. (2017) showed movement patterns for two marked individuals using the shelf-edge environment. One of these moved further offshore to a deep bank, while the other was found primarily in nearshore waters but occasionally traveled to the outer shelf-edge, or beyond, before returning the same inshore area. While these events are not conclusive, they suggest that the shelf edge and nearshore dolphins are part of the same (or some larger) population. One possibility is that the study area is just part of the population range, and the deep water sightings are indicative of a larger distribution, possibly including waters of the Mona Channel and maybe the Dominican Republic. The particular situation of the West coast of Puerto Rico, with a large insular shelf, yet with inshore and shelf edge areas in close connective proximity makes it feasible for the dolphin population to have developed variable feeding strategies (Sergeant et al. 2007) allowing it to exploit both habitats, having then a wider range than expected for populations associated with islands (Baird, et al., 2009).

While prey abundance may be a major driver of dolphin distribution (Hastie et al. 2004), risk of predation may also play a significant role (Wirsing et al. 2008). Tiger sharks (*Galeocerdo cuvier*) are known to affect dolphin (*Tursiops aduncus*) feeding behavior and distribution (Heithaus and Hill, 2002; 2006), and in Puerto Rico they are typically distributed along the shelf edge, especially during the day (Randall 1967; Rivera-Lopez, 1970). This would suggest that dolphins may reduce time foraging along the shelf edge despite an abundance of prey, preferring to remain in inshore waters. While this cannot be address this directly, it was found a difference between group size and water depth, with larger groups being sighted in waters between 51 and

75m, depths that would be associated with the insular slope. It is plausible that in these waters several dolphin groups come together for protection (Campbell et al. 2002).

Dolphin distribution was quantitatively modeled against habitat and other parameters across the insular shelf. On this more restricted spatial scale, habitat evenness accounted for 12% of the variability of abundance. Although it is a small percentage, it does agree with the nearshore distribution, where habitat evenness is higher. While the distribution of dolphins has been shown to be influenced by environmental heterogeneity (Ingram and Rogan, 2002), the results suggest that this is more likely driven by habitat evenness rather than by heterogeneity per se. Inshore waters characterized by an abundance of different habitat types, including sea grass, highly develop reef systems and mangroves as found off the west coast of Puerto Rico, support a higher diversity and abundance of fishes (Pittman et al. 2007), and therefore present higher feeding opportunities.

Rugosity explained 9% of the distribution based on behavior. Although a small percentage, this is of great interest as it suggests social behavior is more prevalent in rugose areas. This could be explained by differential habitat use across group structures relative to sex and age. For example, groups of mothers and calves or lactating females tend to use inshore waters, where well-developed reefs provide sheltered waters and also high and diverse prey availability (Hiethaus and Dill, 2002; Martínez-Serrano et al. 2011). Reef as well as sand-dwelling fishes have been reported in the stomachs of calves (Cock-Croft and Ross, 1990), meaning that these high rugose areas could be used as zones of high socialization, especially for calves. This socialization could also include teaching behavioral tactics for prey hunting.

Modeling species distributions has become an important tool (Robinson et al. 2011) for understanding species ecology, including marine mammals (Gregr et al. 2013), and aiding in their management and conservation. The quantitative approach to describe dolphin distribution was limited by the geographic range for which detailed habitat data were available, and this may represent only part of the population's distributional range (Rodriguez-Ferrer et al. 2017). However, one explicit advantage to restricting the modeling to the area on top of the platform was the elimination of what would otherwise have been a non-monotonic distribution in relation to key parameters (e.g., depth, slope), thereby allowing linear models to be employed. And while the results explained only a small proportion of the variation, they were significant in further suggesting potential reasons why dolphins are preferentially distributed in inshore waters.

Nevertheless, while the correlative approach used in this analysis can predict what factors are important, it lacks a way to discover the mechanisms underlying such correlations (Dormann et al. 2012) and ought to be considered as only the first method in understanding the key processes that could influence dolphin distribution (Palacios et al. 2013), especially where little additional population information is available. Given that the main objective of such models is to describe how much data variability can be explained with independent variables, thus allowing predictions (Palacios et al. 2013), these models often lack direct measures of important drivers that determine animal distribution. Indeed, habitat and geomorphic data may only be indirectly related to important determining factors, although Torres et al. (2008) found that for inshore areas use of habitat characteristics yielded better results than using direct measures of prey abundance. Bottlenose dolphins have a very complex social structure, so their distributions could be influenced not only by prey availability (Hartel 2010; McCluskey et al. 2016; Zanardo et al. 2017) but also

by group dynamics (Lois et al. 2018; Sprogis et al. 2018), predator avoidance (Hiethaus and Dill, 2002; Hiethaus, 2006), competition (Lois et al. 2018)), foraging specialization (Sprogis et al. 2017), and even human disturbance (Bossley et al. 2016); and these variables should be considered in greater detail in future analysis of the population. In this context, the observations on group size versus possible predation threat and of social behavior related to inshore distribution represent only a first approach into these additional drivers.

The results also question the scale at which such modeling efforts can be applied. The most revealing distribution patterns were fortunately obvious as the detailed habitat information was absent to cover the full distributional range of the observations, nor would linear models have been practical if such data were available. It is unlikely that detailed habitat data, or even sufficiently detailed geomorphic data would ever be forthcoming for deeper areas off the platform. Thus, the geographic range over which such data can be used in modeling will be small, especially in island environments where platforms can be quite narrow (e.g., Blasi and Butani, 2012; Luksenburg, 2014), and this may represent just too small a proportion of the distributional range for such an analysis to yield useful results, especially for such a mobile species. Indeed, it is not even clear at what scale habitat information should be assessed. The use of a 200-m radius around the center of a maximum 100-m radius sighting is a first approximation given dolphin movement patterns, and the analysis was based on the assumption that there exists a consistent correlation between distribution and bottom characteristics. Yet, since most (81%) sightings were classified as traveling, there may be no association between position and underlying bottom characteristics, or that the characteristics of movement corridors may differ from those associated with feeding and social activities.

Understanding habitat use and the different aspects of a species distribution over an area are important to help delineate zones that need protection for the sake of the species. This study shows that the bottlenose dolphin off the west coast of Puerto Rico has a predominant nearshore distribution, which make the species subject to a wide array of anthropogenic stressors, both directly and indirectly. Not only is the west coast of Puerto Rico an important zone for both commercial (Matos-Caraballo and Agar, 2011) and recreational (Lilyestrom and Hoffmaster, 2002) fishing, but it is also frequently visit by for recreational boaters and beach goers (García-Sais et al. 2008). Fishing/recreational boating interactions with dolphins have been recorded via scars and marks of anthropogenic origin, and these have increased in number in recent years, with propeller scar wounds being the more prevalent (Rodriguez-Ferrer and Appeldoorn submitted Marine Biology Journal). The data now suggest that there may be a spatial component to these interactions.

For example, recreational boating is a year-round activity, especially off the west coast due to its calmer seas (Garcia Sais et al. 2008), with the number of boats increasing yearly (Garcia Sais et al. 2008). With recreational boating activities occurring primarily in nearshore waters, this may impact dolphins in several ways. First, recreational boating may contribute substantially to the increasing proportion of propeller scars. For a highly vocal species, the increase on underwater noise may also affect communication. In areas with high boat traffic dolphins decrease and "simplify" their vocal repertoire, which can affect communication between mom/calf pairs or group cohesion (Fouda et al. 2018). Also, an increase in ambient noise decreases the effectiveness to search for prey (Pirotta et al. 2015). Further, an increase in boat traffic has been related to changes in group behavior (Nowacek, Wells and Solow, 2001; Hastie et al. 2003; Lemon et al.

2006), and dolphins avoid areas where there is high boat traffic (Lusseau, 2005). Educational activities directed at recreational boat users or spatial restriction of boating activities may help alleviate such problems.

Additionally, dolphins are known to interact with fishing gear such as traps and nets, which are used substantially in both inshore and near the shelf edge and slope regions (Marshak et al. 2008), the two areas where bottlenose dolphins are mostly found. Trap flipping by dolphins to obtain prey (Aiken and Pal, 2008), dolphins entangled on fish traps have been documented for this population on the inshore waters (Rodriguez-Ferrer and Appeldoorn, submitted Marine Biology Journal). Given that fish abundance drives the location of fishing activities and likely dolphin distribution, it is not surprising that these interactions occur. This suggests that management should prioritize studying the extent and impact of these interactions off the west coast, potentially leading to gear or spatial restrictions to reduce losses.

Lastly, the nearshore distribution of dolphins increases the probability of detrimental human-dolphin interactions through habituation to humans (Lockyer, 1990; Cunningham-Smith et al. 2006). These include harassment and potential poisoning by chasing, swimming with, or feeding dolphins (Samuels and Bejder, 2004; Christiansen et al. 2016) leading to undesirable behavioral changes (Foroughirad and Mann, 2013; Hazelkorn et al. 2016). Again, the extent to which this is occurring in Puerto Rico has not been studied.

Indirect stressors such as coastal development, river runoff, sewage disposal and chemical pollution would also likely affect the majority of the population inhabiting nearshore waters. The degradation and loss of inshore habitats could also affect the distribution of prey species, particularly those dependent upon inshore nursery areas (Garcia-Sais et al. 2008; Wilson et al.

2013; Eirman and Connor, 2014). Sewage is of particular concern, as the Mayagüez regional treatment plant discharges primary-treated effluent via a submarine outfall (Garcia-Sais et al. 2008) in the same area dolphins were observed, and more worrisome is the significant nonpoint-source seepage of sewage across the western platform, presumably from the high density and poor maintenance of septic tanks (Bonkosky et al. 2008). Additionally, concentrations of fecal coliforms and fecal streptococcus above regulatory standards have been reported for many rivers in Puerto Rico (Larsen and Webb, 2009). Bacteria associated with human illnesses, such as those belonging to genus *Vibrio* spp., *Shawanella* spp., *Pseudomonas* spp. and *Staphylococcus* spp., have been identified in free ranging dolphins of the southeastern United Stated (Stewart et al. 2014), and there is a direct correlation between infections in bottlenose dolphins with bacteria of human fecal origin, such as *E. coli*, and sewage discharges and increase precipitation (Schafer et al. 2011). Thus, the potential for human sewage to directly affect the health of dolphins (Esperón et al. 2008, Schaefer et al. 2011), particularly the majority found in nearshore waters, is a clear management concern.

The nearshore discharge of contaminants such as heavy metals and PCBs can also have direct effects. While detailed studies are lacking on the west coast, unusually elevated concentrations of pollutants, such as PCBs, nickel, chromium, DDT, PAHs, As, Cu, Hg, and Zn, were found in sediments of Guanica Bay (Whitall et al. 2014), one of the areas frequented by dolphins. Immune response in dolphins decreases with high concentrations of PCB and DDT (Lahvis et al. 1995). Dolphins tend to bioaccumulate toxins in the blubber layers, with detrimental effects (Bossart, 2011), and Rodriguez-Sierra and Jiménez (2002), reported the presence of trace metals in diseased stripped mojarra (*Eugerres plumieri*), an inshore prey item that has been

collected in bottlenose dolphins stranded in Puerto Rico (Mignucci-Giannoni et al. 2009). The concentration and effects of these toxins in the bottlenose dolphins off of western Puerto Rico are unknown, but given that the population appears to be small (Rodriguez et al. 2017) and predominately inshore, it is of obvious management concern.

Given the predominately inshore distribution of bottlenose dolphins, the area off the west coast of Puerto Rico should be designated a Critical Habitat, as the species is resident (Rodriguez et al. 2017), in small numbers and could be affected by a number of anthropogenic stressors. The distribution results further suggest the dolphin prey should be more thoroughly characterized to determine their role as a driver of space and habitat use. This could be approached using nonlethal techniques such as stable isotopes (Knoff et al. 2008; Barros et al. 2010) and perhaps eDNA of dolphin feces (Foote et al. 2012). The overlap of nearshore and shelf-edge environments at the northern and southern extents of the western platform suggests the potential use of these areas as movement corridors. If so, these may represent distributional bottlenecks. Thus, a study documenting the movement patterns and ecology of dolphins would be a priority. Such a study would also help determine resident times, the spatial bounds of the population, and exchange rates with other areas; together these make a strong argument for satellite tagging resident dolphins (Balmer et al. 2013). Such a combination of applied and basic biological studies will ultimately be needed to not only help quantify population dynamics but also to proactively assess threats that could jeopardize the west coast dolphin population.

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Figure 3.1. Study area southwest Puerto Rico, the nearshore zone is represented by light gray and shelf edge zone is represented by dark gray (a) Black lines show the tracks of surveys for bottlenose dolphin (*Tursiops truncatus*) off of southwest Puerto from 2002 to 2015 (b). Blue line is the 100-m depth contour.

 Table 3-1. Survey effort and encounter rate for bottlenose dolphins (*Tursiops truncatus*) off of southwest

 Puerto Rico. Surveys are divided on opportunistic sightings (sightings done from other research platforms)

 and dolphin survey (research focused on bottlenose dolphin).

Year	# of surveys	Survey effort (km)	Total number of sightings	Total number of dolphins	Encounter rate (sightings/km)	Dolphins per km
2002	2	194	1	1	0.005	0.01
2004	11	995	10	83	0.010	0.08
2005	28	1,637	15	84	0.009	0.05
2006	9	702	6	41	0.009	0.06
2007	7	662	5	11	0.008	0.02
2008	3	305	3	10	0.010	0.03
2009	1	47	0	0	0.000	0.00
2010	2	201	2	11	0.010	0.06
2013	8	704	4	11	0.006	0.02
2014	18	1,578	14	122	0.009	0.08
2015	20	1,704	12	102	0.007	0.06
Total	109	8729	72	476	0.008	0.05

Table 3-2. Sampling effort by season and number of dolphins sighted by season and for the opportunistic as well as the systematic surveys for bottlenose dolphins (*Tursiops truncatus*) off southwest Puerto Rico. N=number of surveys per season. *Effort hour=time spent searching for dolphins. The time frame used to photograph animals and document behavior was counted as off-effort and it is not part of this analysis.

Season	Ν	Mean hours	Total effort hours*	# of sightings	Sighting per effort hour
Spring	26	5.73	148.9	23	0.154
Summer	24	5.05	121.29	14	0.115
Fall	29	5.96	172.93	22	0.127
Winter	30	6.06	181.86	16	0.088
Total	109	5.73	624.98	72	0.115



Figure 3.2. Total group size range distribution of bottlenose dolphins (*Tursiops truncatus*) sighted during dolphin surveys of southwest Puerto Rico 2002-2015. Total group size includes calves and neonates.



Figure 3.3. Mean group size per year (a) and mean group size per season (b) for bottlenose dolphins (*Tursiops truncatus*) sighted between 2002 and 2015 off southwest Puerto Rico.



Figure 3.4. Mean group size per depth range for bottlenose dolphin (*Tursiops truncatus*) sighted between 2002 and 2015 off southwest Puerto Rico.

Mom and calf pair	Fall 4.2	Winter 5.3	Spring 5.3	Summer 14.3
Solitary adults	12.5	10.5	10.5	14.3
Adults only	20.8	31.6	36.8	21.4
Pair of adults	16.7	15.8	15.8	28.6
Mixed	45.8	36.8	31.6	21.4

 Table 3-3. Percent of sightings encountered with the different group composition per seasons for bottlenose

 dolphin sightings (*Tursiops truncatus*) encountered on between 2002 and 2015 off southwest Puerto Rico.



Figure 3.5. Distribution of bottlenose dolphin (*Tursiops truncatus*) sightings off southwest Puerto Rico between 2002 and 2015. Light orange shaded area is within 5 km of the coast and marks the nearshore zone; dark shaded area is within 2.5 km of the 100-m depth contour and marks the shelfedge zone. The midshelf zone lies between the shaded areas, while the offshore zone is the area outside of the shelfedge zone. The thin black line is the 100-m depth contour.



Figure 3.6. Habitat distribution by zone for the south west coast of Puerto Rico. Data source from Kendall et al. 2001, Prada & Rivera 2008, Costa Pittman et al. 2009. Habitat composition of the nearshore zone bottlenose dolphin (*Tursiops truncatus*) sightings between 2000-2015 in the south and west coast of Puerto Rico.



Figure 3.7. Relationship between Habitat Evenness and Total number of dolphin per sampling site.



Figure 3.8. Constrained multivariate ordination (dbRDA) showing relationship between three types of dolphin behavior and average rugosity. 1 = travel, 2 = feeding, 3 = social.

CHAPTER FOUR- SCAR PATTERNS IN COMMON BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) OFF SOUTHWEST PUERTO RICO: POTENTIAL CAUSES AND CHANGES OVER TIME

Submitted to Marine Biology Journal

4.1 Abstract

Scars from a total of 2,270 photographs of bottlenose dolphins (*Tursiops truncatus*) taken during three survey periods from 1999 to 2015 off the south and west coasts of Puerto Rico were analyzed using the classification scheme by Lukserburg [1]. Among all cataloged dolphins, 15% showed scar patterns attributed to natural causes (tooth rakes, bite marks), 28% had scars attributed to anthropogenic causes (propeller impacts, interactions with fishing gear), and 9% had scars whose potential origin could not be identified. Over time, there were significant increases in the proportion of scars caused by anthropogenic interactions, as well as the proportion of individuals having two or three such scars. Importantly, the proportion of obtuse, short and cut-like indentations, consider to be propeller scars, have increased over the years. Scars indicative of interactions with fishing traps were significant (12%) and consistent over time, despite an overall reduction in trap use. These results suggest that targeted measures mapping potential hotspots and educating boaters and trap fishers may help reduce impacts to the local dolphin population.

4.2 Introduction

The study of cetaceans is often difficult because of the expense of at-sea sampling, widelyranging populations and the limited sampling and experimental opportunities available due to their protected status [2]. Thus, to understand the interactions between cetaceans and their habitat it is necessary to take into consideration all the available information, even data that can only infer process or status when direct observations are not possible or extremely rare [3]. The realization in the 1970's that patterns in scars and/or natural markings are useful for identifying individuals [4] gave cetacean biologists an important tool to understand species distribution and population dynamics [5]. This tool has also been used to identify interactions between cetaceans and their immediate environment; for example, scars have been used in the past to describe antagonistic behavior with conspecifics [6-8], interspecific aggressions [9], predator-prey interactions [3], and to describe anthropogenic interactions [10-12].

Social interactions among pod members can include aggressiveness to determine reproductive dominance [6, 13] and coercion [14]. Displays of aggression (i.e., bites, hitting, chasing) can leave permanent scars that can then be used to characterize the occurrence of this behavior. Predator-prey interactions can also leave characteristic scars. Bottlenose dolphins (*Tursiops truncatus*) can be preyed upon by several shark species [15]. Scars (or teeth) from non-fatal interactions have even helped identify the predator shark species [3, 16, 17]. Known predatory shark species on dolphins, such as the tiger (*Galaeocerdo cuvier*), bull (*Carcharhinus leucas*), and shortfin mako (*Isurus oxyrinchus*) [15], are present in the Puerto Rico [18]. So, the presence of shark inflicted wounds is expected in local populations. Typical shark wounds are easy to

recognize by the half-moon shaped bite scars, typically located toward the tail and peduncle area [15]. The south and west coasts of Puerto Rico where this analysis is based, is characterized by a large insular platform and reef rich zones that are habitat for several shark [19] and cetacean species [20].

Dolphins are also impacted by anthropogenic activities. With the rapid development of the coastal zone and the human dependency on the sea and its products, the opportunity for dolphin-human interactions has increased [21], and these interactions can leave distinctive scarring patterns [22-23]. One of the most common scarring patterns is a propeller scar. Although these are typically more related to manatees [24-25], they are also common in other marine mammals, even in fast swimming cetaceans [26-28], especially given the increase in boating activity within dolphin habitat [21].

Another important anthropogenic activity affecting dolphins is fishing. Reports of dolphinfishery interactions within the Caribbean are limited. A global review of threats to cetaceans listed nine cetacean species, including the common bottlenose dolphin, that are known to occur as fisheries by-catch [29]. Cetaceans and sirenians are affected mainly by gillnets and traps. However, no annual incidental mortality assessments have ever been estimated in the Caribbean region to evaluate the impact of fisheries on the marine mammal population. For other regions, such as Venezuela, they report a low prevalence of injuries in Atlantic spotted dolphins (*Stenella frontalis*) [30]. In contrast, almost half of the external injuries of common bottlenose dolphin, Atlantic spotted dolphin and false killer whale (*Pseudorca crassidens*) in Aruba have been attributed to human interactions [1] In neither of these cases are the temporal dynamics known. Fisheries in Puerto Rico, as in much of the Caribbean, are dominated by the artisanal commercial fishing sector and a large recreational sector. While there is broad overlap between these sectors, there are also substantial differences. The commercial sector consists of over 800 registered fishers, who use a variety of gears: scuba (mainly spearing), traps, lobster pots, nets and lines, with traps comprising 43.8% of all gears. However, this proportion varies by coast, with traps being more prevalent (51% of all gear) along the south and east coasts, 45% on the west coast, and only 13% along the north coast [31]. The recreational sector consists of over 124,000 anglers (U.S. National Marine Fisheries Service, Fisheries Statistics Division, Personal communication, September 9, 2018) and has a shift in focus toward pelagic species, using mainly rod and real and to lesser extent spear fishing [32].

The common bottlenose dolphin is not considered a common bycatch species in Puerto Rico [33]. However, not all such interactions may be reported, and non-fatal interactions are known to occur elsewhere. In Jamaica, dolphins are known to interact with fishing gears, which vary from rubbing against floating trap lines to flipping traps to get the fish inside [34]. Injuries from fisheries interactions have been proven to affect dolphins, potentially including reproduction and ultimately causing mortality [35]. Evidence of fisheries interactions has been documented and characterized through identification of scarring patterns on live and stranded marine mammals [36-37, 11, 38]. This tool has proven to be effective in assessing the frequency and potential impacts of fishery interactions [10, 1, and 39].

We explore the evidence for natural and anthropogenic interactions within the common bottlenose dolphin population in Puerto Rico by analyzing scar patterns in photographs from the Puerto Rico Bottlenose Dolphin Photo Identification Catalog. Our first goal is to determine the type and frequency of scarring patterns relative to their known or likely cause. Our second goal is then to explore changes in these patterns over three time periods. Using scars to identify potential interactions has proven to be effective and has the potential to inform management measures aimed at conservation of marine mammals [40, 10, 41].

4.3 Materials and Methods

4.3.1 Study Area

This study focused on the bottlenose dolphins occurring along the southwest coast of Puerto Rico (Figure 1). This zone is composed of a broad and shallow insular shelf on the west coast that extends seawards up to 26 km at depths of 10-20 m [42-43]. Low-wave energy conditions are prevalent, and two major rivers, the Añasco and Guanajibo [42], discharge in the area. Also included is Bajo de Sico, an isolated seamount off the shelf about 2 km west of Puerto Rico [44]. Along the south coast of Puerto Rico, the insular shelf is narrow and extends from 3-10 km in width; average depth on the outer platform is 20 m [45]. Closer to the coast, it is characterized by a series of small mangrove cays that extend along the south coast, interrupted by Guanica Bay.

4.3.2 Photographic analysis

Photographs analyzed were part of the Puerto Rico Bottlenose Dolphin Catalog [46-47]. This catalog has been progressively constructed with pictures taken by the same photographer using the same protocol over the past 18 years. Photographs were taken with a Nikon D 7100 with a 300mm lens. Photographs of both sides of the fins, peduncle and tail, when possible, were taken to identify scars, notches and any other unique natural markings that helped identify individuals.

For analysis of temporal trends, these were divided into three periods. Photographs from

1999-2001 were part of the Puerto Rico Bottlenose Survey [46]. This study was a standard line transects boat-based survey focusing on species abundance. The period from 2002-2010 contains photographs taken opportunistically during a study on wahoo (*Acanthocybium solandari*) reproduction [48]. No surveys were conducted during 2011-2012. Lastly, the period from 2013-2015 is composed of pictures taken during a bottlenose dolphin mark/recapture and biopsy sampling project [47]

For all sampling periods, information recorded for each dolphin sighting included group size, behavior and presence of fishing or fishing gear (fishing gear could only be detected by the presence of buoys, if there were submersed we depended on the water visibility to be able to identified it). Each of these was counted only if they were within a 100-m radius of the estimated center of the dolphin pod. An attempt was made to photograph the dorsal fin of all animals present in each sighting, although this was not possible if the group was too large or if individuals actively avoided the boat.

Scars, notches and distinctive patterns in the dolphin's body are typically used as the standard for individual identification [4, 5]. Each photograph was analyzed for quality, and only those that were in focus and clear were chosen. Analysis focused on photographs showing dorsal fins, the whole body, head peduncle and/or tail. We followed Luksenburg's [1] scar and injury categories for our characterization (Table 1) and added four new scar categories using the nomenclature of Moore and Barco [49]. The new categories are **Leading edge abrasion** = leading edge of dorsal fin is missing epidermis. An abrasion occurs when the skin is scraped or rubbed away by a rough surface [50]. This is characteristic of animals that were entangled in heavy line, or where gears/debris rub parts of the body [49]. **Side cut** = a deep scar on any side of the body.

Dorsal fin penetrating incised wound = an incised penetrating wound that cleanly cuts into the skin and is longer than deep [50]. "Sharp-edged objects such as knives or propellers" [49] could cause these wounds.

Each scar category was then classified as scars from anthropogenic, natural or unknown origin. The former included scars suspected to have resulted from either interactions with fishing gear (probable fishing gear) or from propellers. Propeller scars could also result from fishing activities, from either the commercial or recreational sectors, but also from non-fishing related boating activities. Scars considered as being of natural origin included tooth rakes (indicative of conspecific interactions) and shark bites as determined by the typical half-moon impression. The unidentified category included scars of unknown origin that could not be related to any of the other categories (Table 2).

4.4 Results

Over the three time periods, 360 dolphins were cataloged, with 222 of these having distinctly identifying features. Of these, 187 were identifiable due to scar patterns, representing 52% of cataloged individuals. Among the scarred dolphins, 53 (15% of all dolphins) had natural marking scars (12% tooth rakes and 3% shark bites), while 34 (9%) had scars whose cause (natural or anthropogenic) could not be determined (Table 2). Scars of anthropogenic origin dominated (H=65.909, df=1, P=<0.001, Kruskall Wallis). A total of 101 (28%) dolphins had scars of anthropogenic origin: 43 likely due to fishing gear interactions and 59 likely due to propellers (Table 2).

The percentage of distinct individuals among catalogued dolphins (including calves and juveniles) increased over time (43%, 56%, and 68% for the three time periods, respectively (X^2

=142.50, df=2, P \leq 0.001), and this was driven entirely by the increase in the percentage of scarred individuals. The percentage of distinct individuals without scars actually dropped (60%, 12% and 5%) for the three time periods, respectively (X²=32.11, df=2, P \leq , 0.001).

Individual dolphins can exhibit one or more types of scars, and there appears to be an increasing trend over time (Table 3, H=125.84, df=2, P<0.001). Ten different scar or lesion categories were present in the analyzed photographs (Table 1, Figure 2). For the 1999-2000 and 2002-2010 periods only, 2 (25%) and 1 (4%), dolphins, respectively, showed more than one anthropogenic-related scar (Table 4). However, in the 2013-2015 period there was a notable increase, with 15 (23%) individuals with two scars and 6 (6%) with three or more scars (Table 4).

Scars from natural origin were dominated by tooth rakes, possibly from conspecifics followed by round cuts (Tables 2 and 3). Non-linear severed dorsal fins were observed only during the first period. Both round cuts and non-linear severed dorsal fins are interpreted as being caused by shark bites. Tooth rakes were significantly more prevalent than shark bites (H=48.547, df=1, P=<0.001) (Table 2). Furthermore, there was a significant increase in the last sampling period in the presence of tooth rakes (H=43.33 df=2, P=<0.001,) relative to the total number of scarred dolphins, but no significant change in shark bites (H=5.74, P=0.057, df=2). The proportion of shark bites to scarred dolphins appears to have declined across the three time periods (H=5.74, df=2, P=0.057).

Within the anthropogenic category, there was a significant difference in the prevalence of scars attributed to fishing gear interactions and those scars possibly caused by propellers (H=18.353, df=1, P=<0.001), with the latter being more frequent (Table 2). The obtuse, short, cut like indentation, attributed to propellers, was by far the most commonly observed scar across all

periods and across both the propeller and fishery interaction categories (Tables 5 and 6). Among scars attributed to fishery interactions, only single occurrences of side cuts and leading-edge abrasions were observed in 1999-2000. During the period 2002-2010, scars like side cut, leading edge abrasion and again straight deep cut were identified. During 2013-2015 the dominant scar was the dorsal fin penetrating wound (38% of all fishery-related scars) followed by the leading-edge abrasion (26%) (Table 6).

Temporal patterns were tested for the five scar categories showing the greatest percent change across time periods. Significant increases were noted in the two most common scars attributed to propellers. Relative to the number of scarred dolphins, the obtuse, short, cut like indentation increased in occurrence between the first period (15%) and the last two periods (23%, 37%, respectively) (F=37.91, df=2, p=0.00001, One-Way ANOVA), while the dorsal fin incised penetrating wound increased, from 0% and 1.6% in the first two periods, respectively, to 11.7% in the last (F=10.88, df=2, p=0.00034, α =0.05, One-Way ANOVA), although this scar type may also result from fisheries interactions. No significant changes were found among other scar types related to fisheries interactions or natural causes, specifically the percentage occurrence of leading-edge abrasions (F=1.73, df=2 p=0.185, One-Way ANOVA) and non-linear severed dorsal fins (F=0.5, df=2, p=0.610, One-Way ANOVA).

4.4.1 Dolphin behavior with fishing gear

Fishing gear was present at 11.18% of all sightings. Fish traps (58%) and trammel nets (42%) were the active gears present during these sightings (Table 7). The average group size for dolphins close to the nets was 5, and only in the 2013-2015 period were calves present in the area. Dolphins were only seen once, in 2015, interacting in the immediate area where a net was set,

which was used to catch the baitfish ballyhoo (*Hemiramphus brasiliensis*). The animals were seen surfacing less than 1 m from net and circling the boat. No negative reactions from the fishermen were observed.

Dolphins behaved differently in the vicinity of fish traps than when associated with nets, often being observed swimming either close to or between traps buoys. No fishermen were ever observed during these trap-associated sightings. Average group size was 2.3 (SE=1.52). During the course of these surveys, we witnessed on three occasions fish trap interference (*sensu* [34]). This behavior consists of a dolphin shaking and moving the trap to obtain the fish inside. We observed the animal grabbing the side of the fish trap with its teeth and flipping it until the trap was positioned vertically, i.e., on its side. Once vertical, the fish escaped, and the dolphin grabbed the fish. The trap was left in the vertical position. This behavior was seen two times with single adults and once with a mother-calf pair, but never with larger groups.

4.5 Discussion

The presence of natural markings such as tooth rakes in the population can be considered normal for a social species such as the bottlenose dolphin [13]. Aggression is an important part of the interactions for establishing dominance in the dolphin community, e.g., for sexual cohesion or male/male competition [6, 14]. Tooth rakes were present in 11% of all pictures analyzed (1999-2013), although the proportion changed across time periods. In our survey, we did encounter dolphins during aggressive interactions, but these were very scattered across the years, thus supporting the generally low percentage of tooth-rake scarred individuals. While Marley [13] showed that males are likely to be more scarred than females, and juvenile males are more likely

to show scars than adult males, we were not able to assess these patterns as our sample size was small and sex could not always be determined. Interspecific scarring such as round cut scars from a failed shark attack were present in the population but to a lesser degree, occurring in 3% of the analyzed photographs. We suspect that these attacks are either rare or, when they happen, are mostly lethal, thus limiting the number of remaining scarred individuals.

The noticeable increase in scars observed over the three time periods indicates that the frequency of possible anthropogenic interactions has increased over the years. This was particularly evident for penetrating wounds (49.7% of all scars, Table 5 and 6), which are indicative of interactions with sharp objects, such as propellers, or the materials used in the construction of fish traps. Other gears types, such as nets, tend to leave impressions or lacerations [49].

There has been an increase in the number of documented cetacean/vessel collisions worldwide [51]. Fast swimming small cetaceans can also be impacted by watercraft [27], but because it occurs to a lesser degree compared to slow moving species such as manatees [22] and large whales [52], the impact to the populations has not been assessed fully. In Florida, the risk of a dolphin being injured increased during holiday weekends when there is more boating activity [22]. With increasing boating activity in Puerto Rico, up to an estimated 24,458 registered vessels in 2018 [53], coupled with increased scarring, we consider the risk of injury by propeller impact in bottlenose dolphins to be high. Additionally, dolphins do react, perhaps negatively, to high boat traffic, stopping activities such as feeding and socializing when a fast-moving boat approaches [54].

Commercial fishing in Puerto Rico is considered artisanal. There are currently 580 registered commercial boats [53], with the majority of boats less than 9 m. This contrasts with the large recreational sector, which is composed of both recreational anglers and boating enthusiasts that transit the waters year-round. In particular, the southwest coast of Puerto Rico, where this study was conducted, is the second most important area for local tourism, especially for the recreational boating sector. The average number of boats greater that 4 m for the years of the study is 12,678 vessels. Even if they were used only one day a month, which could potentially be 152,140 boat-days/year. This would greatly exceed yearly boat-days of commercial fishermen. With an average of 421 registered commercial vessels over the study period used 5 days/week, the annual boat days would be 25,296. Thus, recreational vessels could be the dominant source of these negative interactions.

We believe that a second possible interaction causing penetrating wounds could be dolphins interacting with fish or lobster traps. The sighting locations of individuals with scar types indicative of trap fishery interactions correlates with trap-fishing grounds reported by Koeneke [55]. The similarity in scar types, the observed trap-flipping behavior, and the overlap of dolphin and fish-trap distributions are clear indications that these interactions are occurring in the dolphin population of southwest Puerto Rico and that the rate has been at least consistent over time. These observations relating scars patterns to trap entanglement are also supported by studies elsewhere. For example, McFee et al. [56] showed how the shaking and movement of fish traps not only could cause scars directly but also lead to dolphins accidentally entangling on buoy lines, leaving scar patterns similar to those observed in our study.

One possible factor driving sustained trap-dolphin interactions is a potential decrease in prey abundance due to overfishing [57]. This may drive individuals toward the opportunity of "getting an easy meal" from trap-caught fish, thus making this gear a frequent target for dolphins. Species reported to be caught in traps, such as snappers, grunts and groupers [58-59], have also been reported as prey items for bottlenose dolphins in other areas [60-62].

It is further interesting that consistent trap-dolphin interactions have been occurring during a period when trap use has significantly declined. Matos-Caraballo et al. [31] reported a decline in the use of traps and nets since the 1990's, and there has been a shift in fishery target species, with trap fishing now more focused on spiny lobster [31]. This suggests that, if prey competition is an important driver of these interactions, this effect must be strong. Nevertheless, although the use of traps has declined, they are still abundant, and the risk of entanglement still exists. Furthermore, trap-dolphin interactions are not limited to traps currently in use. Traps that have been moved by currents or bad weather, or that otherwise have been abandoned (i.e., ghost traps) are still functional and could therefore continue to be a source of entanglements and dolphin-gear interactions. There is no estimate of the number of ghost traps for the south and west coasts of Puerto Rico. However, in the neighboring U.S. Virgin Islands, Clark et al. [63] reported that 8% of commercial fish traps were lost over a three-year period, and that in a survey of 8.6km², total density of ghost traps was 4.9 traps/km². An associated experimental ghost trap study [64] showed that the average number of fish/trap over 19 weeks varied from 1.28 to 7.44, with surgeon fishes and snappers dominating the catch; hence these traps would still be attractive to dolphin. A big step in understanding the effect of these abandoned traps would be a similar island-wide survey of abandoned gears.

Other observations support the significance of trap-dolphin interactions. For example, in 2008, fishermen cut off a dolphin carcass that had died entangled in a pot rope [65], and in 2014, the Marine Mammal Rescue Program of the Puerto Rico Department of Natural and Environmental Resources reported a juvenile bottlenose dolphin entangled in the rope along a train of two fish traps (Case DRN0119-Monday September 15, 2014, 17.92337° N, 066.22878° W, Boca del Infierno, Municipality of Salinas). The dolphin was found alive but subsequently died of dry drowning. The scars present on this individual are similar to those found on photographed animals and in dolphins that had been observed flipping traps (i.e., dorsal fin penetrating wound and side cut) (Figure 3).

Stranded animals around Puerto Rico do show scarring indicative of trap-dolphin interactions (Rodriguez-Ferrer personal observation), which would perhaps suggest that lethal interactions are more common than observed. However, these data are not useful for any quantitative analysis, given that the large proportion of stranded individuals consist of highly decomposed carcasses. This prevents determination of the potential cause of death and also indicates there was substantial drift before stranding, making it unlikely the dolphin was from the local population. Supporting this latter point, no stranded animals have ever been matched to an identified individual in the population.

4.6 Conclusions

The bottlenose dolphin population of southwest Puerto Rico has been described as a small, coastal resident group [47], and any negative interactions, natural or anthropogenic, could be

detrimental for the population. The study zone is an important boating area and the increase in scars that could be attributable to propeller impacts could be indicative of an increase in the interactions between dolphins and boaters. The prevalence of propeller-induced scarring suggests this as an area of management concern. Although such impacts may result in non-lethal injuries [66], the proportion of lethal impacts is not known and even non-lethal impacts can have severe negative impacts. There are examples of the negative impacts of boating on cetaceans worldwide, especially bottlenose dolphins [54]. While these include death from extreme boating impacts, documented non-lethal impacts include animals leaving prey rich habitats to avoid human interactions, food begging and consequent aggressiveness towards humans, and reproductive impairment.

The overall level of anthropogenic scarring shown in this study and the fact that it is increasing over time represent evidence that the bottlenose dolphin population in Puerto Rico is being impacted. Although the exact causes of the scars are unknown, we suggest that the increase in boating activities around Puerto Rico is a potentially significant threat to the population. Mark-recapture studies using photo identification have shown that many dolphins off the southwest coast are resident, with the longest period between first and last capture being 16 yr. [47]. A long residence time would contribute to the increased probability of anthropogenic scarring, both in overall incidence and in number of scars per animal.

There are a number of activities that management could pursue that would help decrease the effect of boating activities on the dolphin population. One would be to map boating activities around Puerto Rico and compare this against the known dolphin distribution. This would identify potential hot spots for interactions. This could be implemented through a survey among boat drivers to determine the location and frequency of incidents and/or just dolphin encounters. Another activity would be to develop an outreach campaign targeting boat drivers and focused on ways to diminish such incidents. This could be implemented al multiple points, such as part of training to obtain a license to operate a motored vessel, issuing of educational material when boat registrations are renewed, or through ongoing training sessions targeting yacht clubs, fishing tournaments, or other organizations.

There is clear evidence that bottlenose dolphins in Puerto Rico are interacting with the trap gear present along the south and west coasts of Puerto Rico. Specific management needs include more detailed mapping of fishing activities by gear, and continued mapping of dolphin distributions to identify fishery-dolphin interaction hot spots. A second step might be to assess the severity of these scars to dolphin health by continuously monitoring injured individuals to determine survival rate. To evaluate if prey limitation is driving these interactions, abundance levels of suspected prey species and their change over time would need to be assessed relative to dolphin distributions (e.g. [67-68]). Relating these factors to fishery practices can benefit both fishermen and animals, as subsequent management efforts to reduce interactions, if warranted, can be more targeted to achieve greater benefits with minimal impacts to the gear.

4.7 Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

4.8 Funding Statement

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Figure 4.1. Map showing the area surveyed from 1999-2015 as part of photographic surveys of the bottlenose dolphin

(Tursiops truncatus).

Scar category	Description	Fisheries cause	Non-fisheries cause
Linear severed dorsal fin	Cleanly severed part of the dorsal	propeller hit, gear	propeller hit
	fin	interaction	
Tooth rakes	parallel linear skin wounds or		Intra or intraspecific
	scars		interactions
Non-linear severed dorsal	Non-cleanly severed part of		inter or intraspecific
fin	dorsal fin with irregular borders		interactions
Straight deep cut	v-shape wound that is more deep	lines/nets cutting	
	than high	into the tissue	
Opposing cuts	Cuts or cut-like indentations on	fishing line wrapped	
	opposing sides of the dorsal fin	around the dorsal fin	
	(leading and trailing edge)	11 1 1	11 1 4
Parallel cuts	Multiple (straight, curved or semi	propeller hit	propeller hit
	curved) incisions, cuts or stasnes,		
	speed of variable length		
Collapse dorsal fin	Dorsal fin that is completely or	entanglement with	poor health or stress
Conapse dorsar mi	partially bent over	fishing gear	poor health of sitess
Obtuse short cut-like	Wound characterized by a blunt	nroneller hit gear	propeller hit
indentation	cut-like indentation	interaction	propender int
Indentation	Indentation or laceration in the	entanglement in	
	epidermis	lines and/or nets	
Round cut	half round or oval shaped cut		inter- or intraspecific
	•		interactions
Leading-edge abrasion	Leading edge of dorsal fin is	entanglement in	
	missing epidermis	lines; rubbing	
		against gear	
Side cut	A deep scar on any side of the	Sharp object	
	body	interaction such as	
		wire from traps,	
		knife, metal	
Dorsal fin penetrating	Incised penetrating wound that	sharp-edged objects	propeller hit
incised wound	cleanly cuts into the skin and is	such as knives or	
	longer than deep	propellers	

 Table 4-1. List of scar categories used to characterize bottlenose dolphins (*Tursiops truncatus*) photographed off the south and west coasts of Puerto Rico from 1999 to 2015. The first nine categories are as defined by Luksenburg (2014), the last three use the nomenclature of Toughey-Moore and Barco (2013).

Table 4-2. Number of catalogued and scarred (with	probable origin) bottlenose dolphins (Tursiops truncatus) per sampling
period off the south and west coast of Puerto Rico.	
	Tatal

Year	Catalogued Dolphins	Not distinct*	Distinct No Scars	dolphins with scars	Anthropoge	Anthropogenic scars		thropogenic scars Natural causes Unider		Unidentified
					Probable fishing gear	Propeller	Tooth rakes	Shark bite (Round cut, or nonlinear severed dorsal fin)		
1999-2000	89	52	24	13	2	3	2	2	4	
2002-2010	100	32	5	63	9	15	22	6	11	
2013-2015	171	54	6	111	32	41	18	3	17	
Total % total	360	138	35	187	43	59	42	11	32	
dolphins with scars					11.94%	16.38%	11.66%	3.05%	8.88%	
					Anthropogenic		Natural Causes		Unidentified	
					28.33%		14.72%		8.88%	

*Not distinct = dolphin with no recognizable feature that would permit identification across sightings.

Table 4-3.	Total number	of scars from na	atural causes pe	r type obser	ved in bottleno	ose dolphins ((Tursiops truncatus) per
survey per	iod off the sout	h and west coast	s of Puerto Rico	A dolphin	can have scars	from more th	an one category

Natural causes	1999-2000	2002-2010	2013-2015
Tooth rakes	5	8	14
Nonlinear severed dorsal fin	2	0	0
Round cut	0	5	6
Total number of scars	7	13	20
Number of dolphins with scars	13	63	111

 Table 4-4. Number of anthropogenic related scars per dolphin per survey period for the bottlenose dolphin photographed from 1999-2015 off the south and west coast of Puerto Rico.

Year	One scar	Two Scars	Three or more scars
1999-2000	6	1	1
2002-2010	27	1	1
2013-2015	72	15	6

Table 4-5. Total number of propeller related scars per type shown by bottlenose dolphins (*Tursiops truncatus*) per survey period off the south and west coasts of Puerto Rico. A dolphin can have scars from more than one category

Possible propeller related scar type	1999-2000	2002- 2010	2013- 2015
Linear severed dorsal fin	1	1	0
Parallel cuts	0	0	2
Obtuse, short, cut like indentation	2	15	42
Total number of scars	3	16	44
Number of dolphins with scars (all types)	13	63	111

Table 4-6. Total number of fishery gear related scars per type shown by bottlenose dolphins (*Tursiops truncatus*) per survey period off the south and west coasts of Puerto Rico. A dolphin can have scars from more than one category.

Possible Fishery related scar type	1999-2000	2002- 2010	2013- 2015
Straight deep cut	0	2010	5
Opposing cut	0	0	1
Side cut	1	2	3
Leading-edge abrasion	1	2	9
Dorsal fin incised penetrating wound	0	1	13
Healed puncture wound	0	0	2
Protrusion	0	0	1
Total number of fishery related scars	2	7	34
Number of dolphins with scars (all types)	13	63	111

Table 4-7. Summary of presence of fishing boats and gear in the water and behavior of bottlenose dolphins in the surveys from 1999-2015 in the south and west coast of Puerto Rico.

				Fisł	ı traps			Fishi	ng Nets	
Period	Total sightings	Sightings with fishing boats	Number of sightings	Average number of dolphins	Presence of calves	Occasions dolphins flipping trap	Number of sightings	Average number of dolphins	Presence of calves	Occasions dolphins interacting with nets
1999-	50	7	3	4.3	No	2	6	3	No	0
2000										
2002-	48	1	2	2.1	No	0	0	-	-	-
2010										
2013-	54	3	5	1.3	Yes	1	1	7	Yes	1
2015										
Total/	152/	11/	10/	7/		3/	7/	10/		1
Mean	50.6	3.66	3.33	2.3		1.5	3.5	5.0		



Figure 4.2. Examples of fisheries related scars in bottlenose dolphin (*Tursiops truncatus*) in Puerto Rico (a) Linear severed dorsal fin, (b) non-linear severed dorsal fin, (c) straight deep cut, (d) parallel cuts, (e) obtuse, short, cut like indentation, (f) round cut, (g) side cut, (h) dorsal fin penetrating incise wound, i) healed puncture wound, (j) dorsal fin leading edge abrasion.



Figure 4.3. Examples of scars present in dolphins 7010 (top left) and 3007 (bottom left) comparable with the scars inflicted by the trap entanglement in case DRN0119 (right).

CHAPTER FIVE-THE PRESENCE OF TWO DISTINCT MITOCHONDRIAL LINEAGES IN THE BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) IN PUERTO RICO AND THEIR AFFINITIES WITH PREVIOUSLY REPORTED LINEAGES

5.1 Abstract

Sound management of coastal resources is based on science-based decisions. Bottlenose dolphins are found around Puerto Rico, yet very limited information exists on the ecology, behavior, distribution patterns, habitat uses, and population structure. We sequenced the mitochondrial control region of 27 live dolphins from the south, southwest and west coast, 11 stranded dolphins from Puerto Rico and five stranded dolphins from Guadeloupe, we then combined our data set with sequences available from the Atlantic Ocean. Throughout the known range of bottlenose dolphins, two distinct ecotypes are usually found, however, we did not detect distinct morphologies in the live and or stranded specimens. Despite the absence of distinct morphologies, our genetic data from the new samples indicates the presence of the distinct genetic lineages in Puerto Rico, the first time this amount of population subdivision is observed in this Caribbean Island. The unclear composition (e.g., numbers, distribution) and relationship between the two ecotypes present challenges the management of this species as defined in the Marine Mammal Protection Act of 1972.

Key words: cetacean ecotypes, haplotype network, mitochondrial DNA, Caribbean

5.2 Introduction

The common bottlenose dolphin (*Tursiops truncatus*) is considered the most common nearshore cetacean in the Caribbean (Ward et al. 2001). Geographical variations in size, coloration, habitats and cranial characteristics of bottlenose dolphins across the world's oceans have led researchers to differentiate two ecotypes (or morphotypes) (Hersh & Duffield, 1990; Mead & Potter, 1995): an offshore or worldwide distributed form and an inshore (nearshore or coastal) form. This distinction is based on mtDNA, hemoglobin, parasite loads, prey preferences and distribution (Hersh & Duffield, 1990; Mead & Potter, 1995; Hoezel, et al., 1998). Typically, the offshore ecotype is characterized by a falcated dorsal fin, short rostrum, bulky body, dark cape pattern, a white saddle patch in the peduncle area behind dorsal fin (Herzing & Elliser, 2016; Van Waerebeek, et al., 2017), and is found in deep zones close to oceanic islands, or in the open sea (Hersh & Duffield, 1990).

On the other hand, the inshore ecotype has light coloration, larger flippers, and is smaller in size (Mead & Potter, 1995; Ramos, et al., 2016). However, the features of the two types are not consistent worldwide (Curry & Smith, 1997). For example, in the Pacific, offshore *Tursiops* tend to be smaller than their nearshore counterparts (Curry & Smith, 1997; Bearzi, et al., 2009). The inshore ecotype is found mainly in rivers, channels, waterways, estuaries, and on continental/insular shelf and shelf breaks (Mead & Potter, 1995; Ramos, et al., 2016).

Analysis of mitochondrial DNA from bottlenose dolphins from the Caribbean revealed the presence of the inshore and a worldwide-distributed form comparable to the offshore or pelagic ecotype (Tezanos-Pinto, et al., 2009; Caballero, et al., 2011), but no corresponding morphological assessment was made. In the Caballero et al. (2011) study, 26 of the analyzed samples were from

dolphins that stranded in Puerto Rico, and based on the genetic analysis of these, both ecotypes were identified (24 offshore and 2 inshore dolphins). As these samples came from stranded individuals, no data were available on the geographic origin of the dolphins. Ocean currents can move cetacean carcasses far from residence sites (Peltier et al. 2012). Determining population structure based only on carcasses can fail to detect or infer erroneous patterns of population differentiation; those patterns are crucial for understanding population structure and dynamics and imperative for management decision-making (Bilgmann, et al., 2011). Absence of data from living specimens from Puerto Rico that could lead to a better understanding of the population dynamics of dolphins was one of the motivations for undertaking this study.

In the Caribbean Sea and adjacent waters there are few studies of genetic structure of known populations, but results suggest there is significant population differentiation (Caballero et al. 2011). In the northern Bahamas, a fine-scale population structure was found between three *Tursiops* populations, suggesting different units for conservation and management (Parsons et al. 2006). In Bocas del Toro, Panama, low genetic diversity was found within a well-monitored population (Barragán-Barrera, et al. 2013; Barragán-Barrera, et al. 2017). Similar results have been reported elsewhere (i.e., Australia; Allen et al. 2016, South Pacific; Sanino et al. 2005, Black Sea; Viaud-Martinez et al. 2008) showing genetic differentiation among regional populations and in some cases low diversity (Fruet et al. 2014) in this highly mobile species. However, there are reports of populations that do not show differentiation, as in the case of the bottlenose dolphins off the mid-Atlantic that exhibited shared haplotypes with both inshore and offshore types (Castilho et al. 2015).

Although the presence of both ecotypes has been reported in Puerto Rico (Tezanos-Pinto et al. 2009; Caballero et al. 2011; Waring et al. 2011), no assessment has been done to determine their extent, distribution and if there are any interactions between the two ecotypes. The unclear composition (e.g., numbers, distribution) and relationship between the two ecotypes presents challenges in the management of this species as defined in the Marine Mammal Protection Act of 1972 and the mandatory stock assessments for marine mammals in the U.S. Caribbean.

Rodriguez-Ferrer (2001) reported on the abundance and distribution of bottlenose dolphins off the southwest coast of Puerto Rico, which showed a more coastal distribution. However, no information was collected relative to the population structure and the presence/absence of the two ecotypes for that region. Thus, the objectives of this work were: 1) to characterize the genetic variability and structure, sex ratio, and group composition of bottlenose dolphins throughout Puerto Rico by analyzing mitochondrial DNA from live, biopsied individuals from the south and west coast as well as opportunistic, island-wide strandings and, 2) determine the genetic relationships between dolphins from Puerto Rico and the Caribbean and also worldwide.

5.3 Materials and Methods

5.3.1 Study area – Puerto Rico

Sampling of free raging dolphins was focused on the waters off the south and west coasts of Puerto Rico (18° 12'N, 66° 39'W) (Figure 1). Puerto Rico is an archipelago of approximately 140 structures that include islands, islets and keys of various sizes, surrounded by deep waters (Méndez-Méndez & Fernández, 2015). Surrounding the island is an insular shelf that is narrow on the northern coast (Scheneidermann et al. 1976). The western insular shelf is wide and extends

from six to 26 km in width with an average depth of 18 to 20 m (Schlee et al. 1999; Ballantine et al. 2008). In the south coast, the shelf extends east and narrows again (Morelock et al. 1994).

Biopsy sampling surveys were conducted from Aguada in the northwest to Caja de Muertos Island in the south (Figure 1) during two periods (August 18-31, 2014 and October 19-30, 2015) when dolphin sightings were reported to peak (Rodriguez-Ferrer, 2001). The surveys were conducted in an open 7-m boat, offering a 360° field of view. Sampling was attempted only under favorable weather conditions (Beaufort scale up to 3; equivalent of a wave height 0.91 m or less). The survey effort focused on known areas of dolphin distribution (Rodriguez-Ferreret al. 2017). Once a group of dolphins was sighted, data were first recorded on behavior prior to sampling, group size, and composition. In addition, visible diagnostic offshore/inshore ecotype characteristics were recorded to distinguish among ecotypes. For the offshore ecotype, the characters used were large size and bulky body, falcated fin, dark coloration, short rostrum in proportion to body size, and/or a white saddle patch (Herzing & Elliser, 2016; Van Waerebeeket al. 2017). For the inshore ecotype, key characteristics were light coloration, no saddle patch, and rostrum in proportion to body size (Mead & Potter, 1995).

After recording initial sighting data the boat was positioned parallel to the swimming group. Skin samples of free ranging dolphins were collected using a standard biopsy protocol (Sinclair et al. 2015). Darts and tips especially designed for small cetaceans (F. Larsen, Ceta-Dart, ACC darts, with floats and vanes for crossbow and sampling heads M8/40 mm) were deployed with a crossbow from a trained, federally-licensed marksperson. Adult dolphins were biopsied along their flank below the dorsal fin (Gorgone, et al., 2008). At the moment of sampling the individual was also photographed for identification and cataloguing purposes based on dorsal fin

morphology and/or any scarring present. Pictures were then compared and included in an existing dorsal fin catalog (http://seamap.env.duke.edu/species/180426). Tissue samples were preserved in liquid nitrogen and then stored in a -80°C freezer. We conducted fieldwork under permits from the National Marine Fisheries Service, Southeast Fisheries Science Center, Marine Mammal Protection Act (Scientific Permit Number 14450-01) and the Puerto Rico Department of Natural and Environmental Resources (Permit 2015-IC-047).

5.3.2 The Total Data Set of this Study

Skin samples collected from stranded dolphins around Puerto Rico were included in the data set. Necropsy reports, if available were reviewed for pictures and/or description of the specimen to infer gender and ecotype. The samples included eight stranded dolphins covering the years between 2006-2018 from the Puerto Rico Department of Natural and Environmental Resources tissue bank and three samples from Puerto Rico from the Center for Manatee Conservation (2001-2016) (Figure 2). Also, for comparison purposes, five samples from the Guadeloupe Stranding Network (2013-2015) were included in the set. Finally, a total of 308 control region sequences were extracted from GenBank to augment our dataset (Table 1), to bring the total to 357 sequences. The new samples from Guadeloupe, as well as the GenBank records were included for comparison purposes since the second objective of this research project was to place the Puerto Rico dolphins in the context of the wider distribution of the species in the Caribbean, the Atlantic and the Pacific.

5.3.3 DNA Extraction, PCR and Sexing

DNA was extracted from skin samples using the DNeasy kit (Qiagen, Valencia, CA, USA). A 550-bp region of DNA was amplified using the primers tPro-whale (5'-TCACCC AAAGCTGRARTTCTA-3') and Dlp-5 (5'CCATCGWGATGTCTTATTTAAGRGGAA-3') (Baker et al. 1998) following the same amplification conditions as in Caballero et al. (2011). PCR products were cleaned from excess primers and dNTPs with the ExoSAP-ITTM PCR Product Cleanup Reagent kit (Fisher Scientific, Pittsburgh, PA) and sequenced with Sanger sequencing. Sex of live animals was determined by a molecular assay where a PCR reaction was performed with the primers TtSRYR (5'-ACCGGCTTCCATTCGTGAACG-3'), PMSRYF (5'-CATTGTGTGGTGGTCTCGTGATC-3') (Richard, al., 1994), ZFX0582F (5'et ATAGGTCTGCAGA CTCTTCTA-3') (Bérubé & Palsboll, 1996), ZFX0923R (5'-AGAATATGGC GACTTAAGAACG-3') (Bérubé & Palsboll, 1996). We followed the PCR conditions as outlined in Rosel (2003).

5.3.4 Data Analysis

All successful PCR amplicons were purified from excess primers and unincorporated dNTPs using four μ L of ExoSAP-IT per five μ L of PCR product. Samples were plated on 96-well sequencing plates and were processed for Sanger sequencing in both directions using the Big Dye 3.1 Terminator Cycle Sequencing Kit. The ethanol-precipitated products were loaded into an ABI 3130xl 16-capillary Genetic Analyzer at the Sequencing and Genomics Facility of the University of Puerto Rico, Rio Piedras. All DNA sequences have been submitted to GenBank (control region: Accession Numbers XXXXX).

The DNA traces produced were visually inspected for quality and accuracy in nucleotide base assignment in Codon Code Aligner v. 8.0.2 (Codon Code Corp.). Sequences were trimmed in Codon Code Aligner then aligned by the MAFFT Algorithm v. 7 (Bandelt, et al., 1999; Katoh & Standley, 2013) for further analyses. DnaSP v.6 (Rozas, et al., 2017) was used to calculate number of variable sites (S), number of haplotypes (Hap), and assigned sequences to groups. The program Arlequin v 3.5.2.2 (Excoffier & Lischer, 2010) was used then to estimate nucleotide diversity indices (π and θ), neutrality test statistics (Tajima D and Fu's Fs) and analysis of molecular variance (AMOVA) (Excoffier et al. 1992). *F*-statistics were used to estimate the proportion of genetic variability found among populations (F_{ST}), among populations within groups (F_{SC}) and among groups (F_{CT}). Pairwise F_{ST} population comparisons were run between all sample locations (Weir & Cockerham, 1984) as implemented in <u>Arlequin</u> and statistical significance was <u>assessed</u> against the null hypothesis of panmixia <u>by 10000 permutations</u>. The female effective population size (N_ef) for Puerto Rico populations was estimated using the formula N_ef = $\theta/2\mu g$, where μ = bp substitution rate per generation and θ = genetic diversity. We used generation time (g = 10 years) as estimated for bottlenose dolphins (Cassens et al., 2005) with a mutation rate of 1.5^{E-7} (Hoelzel et al., 1991).

Haplotype networks were illustrated with a median-joining network algorithm (Bandelt, et al., 1999) using the software PopART v. 1.7.2 (Leigh & Bryant, 2015) to depict visually the geographic distribution of haplotypes as well as their relatedness. Sequence divergences between sequences and inferred populations were estimated in PAUP* (Swofford, 2001) using the appropriate model of nucleotide substitution as estimated by the BIC criterion in jModelTest2 (Darriba, et al., 2012). Phylogenetic relationships among dolphin sequences were inferred in RaxML-ng (Kozlov et al., 2018 maximum likehood) using 200 bootstrap replicates to assess branch support. Trees were visualized in iToL (Letunic & Bork, 2016) and improved with Adobe Illustrator.

5.4 Results

Weather conditions restricted the survey time in the offshore waters and all sightings were recorded within nearshore waters (Figure 1), therefore the sampling is biased towards nearshore environment. None of the sighted individuals had notable offshore ecotype characteristics (Herzing & Elliser, 2016; Van Waerebeek, et al., 2017). A total of 25 biopsy samples were collected from the free ranging population during two sampling periods; nine samples off the south, thirteen samples from the southwest and three samples from the west coast of Puerto Rico (Figure 2). Two more samples from live, biopsied dolphins provided by AAMG from the 2001 Marine Mammal Survey NOAA Gordon Gunter trip (NMFS Permit No. 779-1339-02, DNER Permit No. 99-EPE-18) for a total of 27 live animal biopsy samples (Figure 2).

Stranded samples included 11 from recent strandings (years 2001 to 2018) and the set of 23 samples from dolphins stranded in Puerto Rico (1994 to 2003) reported in Caballero et al. (2011). The distribution of stranding sites extends to the north and east coast (Figure 2). Sex was determined for all samples. For biopsied dolphins this was by a molecular assay (Rosel, 2003) using the amplification patterns of ZXY and ZXX genes. For stranded dolphins, the sex was determined visually during necropsy. For two stranded dolphins that sex could not be determined on-site, it was inferred by the molecular essay.

Only live adult individuals were biopsied. Twelve of the 27 biopsied dolphins were animals that have been identified on previous surveys (re-sighting interval ranges from months to a year) and 15 dolphins were sighted for the first time the day of the biopsy. The sex ratio for biopsied dolphins was 10 females and 17 males and for stranded dolphins 21 females and 13 males including the 22 Puerto Rico samples reported in Caballero et al. (2011). There were four males and one

female from Guadeloupe. Of the re-sighted dolphins only one individual was a female, the rest were males.

Nineteen of the biopsied dolphins were of the inshore ecotype, and eight biopsied dolphins were of the offshore ecotype. Two of the eight dolphins exhibiting the offshore ecotype have been sighted before in previous surveys, these dolphins were identified as males by the molecular essay. The sex ratio for the offshore ecotype was six males and two females. For the inshore ecotype, eight dolphins were re-sighted. Sex ratio of the re-sighted inshore dolphins was seven males and one female. Samples that came from recent strandings included nine offshore and two inshore dolphins. The sex ratio for stranded offshore dolphins was six males and three females and for the inshore ecotype two females and no males. None of the stranded dolphins could be matched with fins from the fin catalog (http://seamap.env.duke.edu/species/180426). The sex ratio reported in the Caballero et al. (2011) study was 7 males and 13 females (offshore) and 1 male and 1 female (inshore) dolphins.

Size range for stranded dolphins in Puerto Rico ranged from 111 to 259 cm total length (DNER/NMFS/SEFCS-Stranding Agreement). The average length for a stranded offshore dolphin was 232 cm, while the average for an inshore dolphin was 226 cm. There is no significant difference between the total length of stranded offshore versus the stranded inshore dolphins (One-Way-ANOVA, f=0.18218, p= 0.675573). Offshore dolphins are the dominant ecotype in strandings for both sexes and year classes. The north coast of Puerto Rico was the coast with the most offshore strandings followed by the south coast (Figure 2). Strandings of the inshore ecotype were present on all coasts but to a lesser degree (1-3 animals per coast).

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DNA sequences were imported in DNAsp v6 and were allocated to four geographical groups (Caribbean, Eastern North Atlantic, Western North Atlantic, and Pacific (Table 1). Identical sequences were collapsed to haplotypes in DNAsp with sites with gaps and missing data considered and non-considered. When all gaps/missing data were included, 204 haplotypes were extracted, and 130 haplotypes were extracted when they were excluded. We used the more conservative approach of excluding the sites with missing data. Arlequin files were then generated in DNAsp for downstream analysis. Table 2 summarizes the DNA statistics of the four geographical groups.

The best-fit-model of nucleotide substitution (-lnL = 2001.3542, HKY+I+G) for our data set was selected in jModelTest2, where I = 0.5600 and gamma shape (alpha) = 0.3590. The model HKY+I+G was applied in PAUP to estimate sequence divergence among haplotypes from Puerto Rico (Table 6).

To reconstruct the regional haplotype network, the 46 newly generated control region sequences were combined with 313 sequences from GenBank (Figure 3, Table 1). Haplotype analysis based on the Median-Joining network (Figure 3) shows a complex haplotypic structure characterized by the high abundance of singleton sequences (n=96). The geographic subdivision of haplotypes is mostly visually detected in the Pacific and the eastern Atlantic groups. The most common haplotype of our data set (Hap 124; n = 29) consisted mostly of Caribbean dolphins, and the second most common, (Hap 93; n = 26) was exclusively present in the Pacific. Hap 46 (n = 21) was mostly present in eastern Atlantic, however several dolphins from the northern Atlantic and Caribbean shared this haplotype.

The majority of the sequences generated from live dolphins of Puerto Rico in the current study belonged to Hap 124 (n=20), followed by Hap 72 (n=5) and Hap 46 (n=2) (Figure 3 and 4). Hap 124 was shared with previously sampled dolphins from Mexico and Puerto Rico (Caballero, et al., 2011), and with the Bahamas (Parsons, et al., 2006) (Figure 3). Haps 46, 76, 72 were shared with dolphins from Costa Rica (Barragán-Barrera, et al., 2017), and the Puerto Rico Caballero's data set (Caballero, et al., 2011). Hap 124 (n=5) and Hap 72 (n=4) were also present in the stranded dolphins (Figure 5a, b). Three additional haplotypes were detected from stranded dolphins (Hap 76 (n=5), Hap 78 (n=1), and Hap 12 (n=1)). The dolphin represented by Hap 78 was from Guadeloupe, and Hap 12 represents a female who stranded on the north coast of Puerto Rico (Figure 3). Interestingly, Hap 12 is predominantly present (n=10) in the Azores (Querouil, 2007).

The offshore haplotypes of Puerto Rico differed from 4.34% to 6.58% from the inshore haplotypes (Table 6, Figure 5). The smallest sequence divergence was observed between the offshore Haps 12 and 14 (0.27%) and the largest between the offshore Hap 12 and the inshore Hap 108 (6.5%). The range of sequence divergence within inshore and offshore dolphin haplotypes was 2.17% and 0.27-3.86%, respectively (Table 6). The effective female population size of the inshore dolphins ranged from 867 to 2400, (Ne= 0.0026/(2*10*0.00000015)) and (Ne= 0.0072/(2*10*0.00000015)), respectively. The effective female population size of the offshore dolphins ranged from 1400 to 3333, (Ne=0.0042/(2*10*0.00000015)) and (Ne= 0.01/(2*10*0.00000015)), respectively.

Where possible dolphins from previous studies were assigned to either inshore or offshore ecotype as indicated by the authors (Table 1). None of these ecotype classifications was based on morphometrics, rather they were based on DNA sequence clustering. We followed the clustering scheme of the previous studies and classified the live and stranded dolphins by ecotype. 29 out of 313 dolphins included in this study were not classified by the authors. The inshore and offshore ecotypes formed two visually distinct groups (Figure 3), however not as distinct as expected. The inshore dolphins are present in the Pacific, W. North Atlantic, and Caribbean including Central America. The offshore dolphins are more numerous in the data set and are present in all sampled areas, including eastern North Atlantic (Figure 3). A few reported inshore and offshore haplotypes (e.g., 32-35, 40-44, and 99-102) are genetically similar, oftentimes different by 1 base substitution. For Puerto Rico, we identified two haplotypes (108 and 124) as inshore haplotypes and five haplotypes (9, 12, 46, 72 and 76) as offshore haplotypes (Figures 4, 5). Most live dolphins were inshore ecotypes (e.g., Hap 124, n = 20 live, n = 2 stranded from Caballero et al., (2011), n =1 stranded, current study). Haplotype 76 (n=5) was recovered only from stranded animals and Hap 72 was equally represented in live and stranded dolphins.

5.4.1 Arlequin Results

The data set was divided into four groups (Caribbean, W. North Atlantic, E. North Atlantic, and Pacific) and within each group, the dolphins were labeled as inshore or offshore. This was not possible in all cases; for example the dolphins from New Zealand (Tezanos-Pinto et al. 2009) had no ecotype information and were excluded from the Pacific group in all statistical tests performed in Arlequin (Tables 2, 3, 4, 5). The highest number of haplotypes were found in the western North Atlantic inshore dolphins (n=39, h=0.9947), a population flagged as very distinct by Caballero et al. (2011). The second highest number of haplotypes were recorded in eastern North Atlantic offshore (n=31, h=0.9431) but also this group has the highest number of individuals. Both groups exhibited very high haplotypic diversity with 92 and 40 haplotypes, respectively. The Caribbean

population exhibited a lower haplotypic diversity than those in the Atlantic. The highest pi and Theta were recorded in W. North Atlantic Inshore, as expected from the high haplotypic diversity. The lowest values of pi and theta are recorded in Puerto Rico, which is a subgroup with the Caribbean population. In all populations, the offshore ecotype harbors the highest genetic diversity; the glaring exception is the western North Atlantic. Unfortunately, there were only three dolphins in this inshore group so it is not possible to infer any meaningful trends from such a small sample size. Tajima's D statistic was only significant in the inshore dolphins of Puerto Rico, indicating the lack of diversity in the group or the presence of negative selection. The Fu's Fs test statistic was significantly different than expected under neutrality in W. North Atlantic and Pacific Offshore. Highly negative values of Fu's Fs statistic are driven by the excess of singletons, suggesting a possible past population expansion event.

Genetic differentiation was examined using mean F_{ST} values (Table 3), where all population comparisons except those involved the small sample size (n=3) of W. North Atlantic deviated significantly from zero (Table 3). The two highest pairwise F_{STs} values were reported between Caribbean inshore vs. offshore and between Pacific Inshore and Caribbean Offshore. Similarly, for Puerto Rico, which is a sub-sample of Caribbean dolphins, the inshore and offshore ecotypes are highly differentiated (F_{ST} =0.87206). One of the lowest pairwise F_{STs} values were reported between Eastern North Atlantic Inshore versus Offshore.

The results of the AMOVA (Table 4) indicated the presence of significant population subdivision (F_{ST} = 0.45802) based on the control region data, with most of the variation distributed among populations within groups. The presence of highly differentiated ecotypes within each geographical group drives the results of the AMOVA test. The AMOVA of the two ecotypes

(within each ecotype all four regions were included) (Table 5) showed that 51.9% of the genetic variation was attributed to differences within populations and 39.5% of the variation to differences among geographical populations within each ecotype. The fixation index among groups (F_{CT}) was not significant in either of the AMOVA designs. These results suggest that *Tursiops* populations are structured not just within regions (e.g., Caribbean Inshore vs. Offshore) but also within each ecotype (e.g., Caribbean Offshore vs. Eastern and Western Atlantic Offshore. This is especially evident in Table 3, where most pairwise F_{ST} comparisons were significant at the P<0.001 level.

5.4.2 RAxML Results

The phylogenetic analysis based on maximum likelihood (Figure 5) yielded rather similar groups as the haplotype network in Figure 3. The inshore dolphin group, with representatives in the Pacific, W. North Atlantic, Caribbean and Central America, was also detected with the ML analysis and supported by a bootstrap value >50. The eight haplotypes from Puerto Rico were divided in three visible groups as in Figure 4. The group of genetically similar inshore and offshore haplotypes (e.g., 32-35, 40-44, 99-102) were clustered near the inshore clade, which consisted of the haplotypes 107-120 and 124-130.

5.5 Discussion

This study confirms the presence of both ecotypes in Puerto Rico, not only in stranded dolphins but also in the free ranging population of the south and west coasts. Furthermore, we found their distributions to differ. The local population, which is mostly distributed in nearshore waters, was dominated by the inshore ecotype, while the offshore ecotype dominated strandings.

In this study, we assume that stranded dolphins had some level of association with Puerto Rican waters, but in reality stranded carcasses can be carried far from their area of origin. The unknown origin of stranded dolphins prevented Caballero et al. (2011) from confirming the occurrence of both ecotypes within the Puerto Rico population even though they found both types among stranded individuals.

Even though all 25 dolphins biopsied in this study were sampled in nearshore waters, we genetically identified six dolphins as belonging to the offshore ecotype. At the time of sampling we assumed that the dolphins were of the inshore ecotype because of their smaller size and lack of other cranial or fin diagnostic characters of the offshore ecotype. Interestingly, two of six offshore dolphins were males, and both were sighted interacting within a group of inshore ecotype dolphins. Such cross-ecotype interactions increase the likelihood of breeding between ecotypes, as opposed to just an overlap in distribution (Segura et al., 2006). Overall, the sex ratio for biopsied individuals (17 males: 10 females) is not considered to be representative but rather due mainly to dolphin behavior as males tend to interact more with sampling boats (Quérouil, et al., 2009).

The inconsistency between morphological ecotype classification (all dolphins were identified as inshore) and that determined by genetic analysis is interesting. This trend was consistent among live animals (eight of 27 individuals were of the offshore ecotype) and those from strandings, which also consisted of both ecotypes. Further analysis of this is hindered because most other genetic studies did not differentiate ecotype base on morphology. Studies have shown morphological differences using morphometrics, coloration, and differences in fin shape and or sizes (Mead and Potter 1995, Wang 2000, Felix et al. 2017). Most of the studies were morphometrics have accompanied mtDNA data have been done on carcasses from temperate and

in areas of large continental shelf waters, where they show marked differences between morphotypes (Mead & Potter, 1995; Perrin et al. 2011). Our live animal data set included eight offshore *Tursiops* that when sighted on the field were identified as inshore, due to the size and coloration. The non-significant difference in total length among the ecotypes for stranded dolphins, could indicate that *Tursiops* in the Caribbean have adapted to warmer conditions, therefore size of offshore would be similar to inshore to the naked eye. Contrary to continental regions, the islands of the Caribbean have on average narrow insular shelves (Hubbard et al. 1981; Smithet al. 1997; Claro & Lindeman, 2003; Betancourtet al. 2012); therefore, the inshore ecotype has adapted to the morphological differences are well marked, and the morphological distinction between ecotypes is clear. When we compare these areas with the Caribbean, these zones have large shelves, enclosed bays or estuaries with calmer and shallow waters (Mead & Potter, 1995; Segura et al., 2006; Fruet et al., 2017).

All haplotypes present in the live samples from Puerto Rico have been reported elsewhere in the Caribbean, a similar observation that was made previously using only stranded animals (Caballero, et al., 2011). Haplotype 124 is the most common one in the Caribbean, indicating the fact that what is common in Puerto Rico is common in the Caribbean. On the other hand, the presence of Hap 12 from a stranded animal from Puerto Rico is curious since this haplotype primarily occurs within the Azores. Alternative hypotheses have been suggested to explain the presence of shared haplotypes among distant regions. These include an evolutionary interconnection between bottlenose dolphins worldwide (Caballero et al. 2011), possible founder events by the offshore ecotype (Natoli, et al., 2004; Tezanos-Pinto et al., 2009; Caballero et al. 2011), or drift of a dead dolphin from far or nearby waters. The Puerto Rico sample comes from a stranding, and it could be possible that a migratory population passed by the north coast of Puerto Rico at the time of death. Carcass condition was in moderate stage of decomposition. Although carcass drift hinders determination of source population, this may indicate that a migratory population could be pass close to the coastal waters of Puerto Rico. If this stranding was of Puerto Rico origin, this could indicate a potential for long-range gene flow between the two sides of the Atlantic (Querouil, 2007). Sampling of free ranging individuals off the north coast of Puerto Rico or that the population shared mtDNA with individuals from the North Atlantic, suggesting then recent gene flow among regions (Silva, et al., 2008; Castilho et al., 2015).

Rodriguez-Ferrer (2001) and Rodriguez et al. (2017) estimated the population size (Nc) of bottlenose dolphins off southwest Puerto Rico as several hundred individuals, including juveniles. Thus the female breeding population would be substantiall smaller. However, these may have underestimated true population size due to both methodological issues and because sampling was limited to the southwest coast, when the full geographic range of the population is unknown. The female effective population size of the inshore dolphins (867-2400) is substantially higher, supporting the idea that the Puerto Rico population covers a much larger area than previously studied. However, a significant increase in available resources (e.g., boats, trained observers and photographers, cameras) would be required to improve the estimate of population size and range using survey mark-recapture techniques. Thus, genetic data offers an alternative, cost effective approach to estimate population statistics. The phylogenetic analysis generated similar groups as the haplotype network, which supports our classification of the inshore and offshore ecotypes. This is more evident in the Caribbean and subsequently in Puerto Rico where, genetically there are two ecotypes. These ecotypes are considered parapatric populations, they have the potential to overlap in distribution and they do as offshore dolphins were observed to interact with inshore dolphins in one occasion in the current study. Yet, they are not interbreeding as far as our samples indicate. Of the regions analyzed, the Eastern North Atlantic is characterized by high gene diversity, the majority of the Eastern North Atlantic samples were identified as offshore (Natoli, et al., 2005;Querouil, 2007). Offshore dolphins seem to harbor higher genetic diversity even across considerable spatial scales (Quérouil et al. 2007; Tenzanos-Pinto et al. 2009); the exception being the western North Atlantic inshore population.

Haplotype diversity for Puerto Rico is comparable with other studies of the Caribbean region. Generally, inshore populations are characterized by low haplotype diversity. In the Caribbean low haplotype diversity has been reported for the Bahamas and Panama (Parsons et al. 2006; Barragan-Barrea et al. 2013). An exception is the inshore Caribbean-wide population reported by Caballero et al. (2011) (h= 0.578), where haplotype diversity is much higher than reported for the inshore population of Puerto Rico is low (h=0.0800). However, estimates based on large geographic scales, drawing from multiple populations may overestimate the haplotype diversity within specific, localized populations, such as Puerto Rico. Puerto Rico has only ¼ (8 out of 32) of the haplotypes reported for the Caribbean as a whole. This lower haplotype diversity has obvious management implications. Rodriguez-Ferrer et al. (submitted) reported a prevalent nearshore distribution of the population off southwest Puerto Rico, where risk of anthropogenic

impacts is significantly higher. Anthropogenic impacts therefore could be detrimental for a small population with low genetic diversity. In contrast, as expected haplotype diversity was high for the Puerto Rico offshore ecotype, similar to what Caballero et al. (2011) reported for the region (Puerto Rico h=0.7246 vs. Caribbean h=0.71). The Puerto Rico population showed a high degree of genetic sequence divergence among the two ecotypes, but when compared to the rest of the region there is no genetic differentiation. Since all haplotypes of Puerto Rico are shared with those of the Caribbean, this indicates that long swimming distances for a strong swimming mammal and lack of natural barriers do not hinder gene flow among Caribbean populations.

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5.7 Acknowledgements

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Table 5.1. Location, ecotype, live or stranded, GenBank accession numbers, and publication source. Ecotype naming is presented on the source paper where sequences were extracted. For the purpose of the study coastal, alongshore = inshore, while pelagic, worldwide distributed form=offshore.

Location	Ecotype	Haplotype	Assigned Haplotype for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Sex	Genbank accession number	Publication source
Azores	Pelagic	TT001	Hap 5	Offshore	L	М	DQ073641	Quérouil et al. (2007)
Azores	Pelagic	TT002	Hap 12	Offshore	L	М	DQ073642	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT003	Hap 46	Offshore	L	М	DQ073643	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT004	Hap 46	Offshore	L	М	DQ073644	Quérouil et al. (2007)
Azores	Pelagic	TT005	Hap 1	Offshore	L	F	DQ073645	Quérouil et al. (2007)
Azores	Pelagic	TT006	Hap 12	Offshore	L	М	DQ073646	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT007	Hap 12	Offshore	L	М	DQ073647	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT008	Hap 49	Offshore	L	F	DQ073648	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT009	Hap 46	Offshore	L	F	DQ073649	Quérouil et al. (2007)
Azores	Pelagic	TT010	Hap 73	Offshore	L	М	DQ073650	Quérouil et al. (2007)
Azores	Pelagic	TT012	Hap 50	Offshore	L	М	DQ073652	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT013	Hap 53	Offshore	L	М	DQ073653	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT014	Hap 46	Offshore	L	М	DQ073654	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT015	Hap 13	Offshore	L	М	DQ073655	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT017	Hap 65	Offshore	L	F	DQ073657	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT018	Hap 12	Offshore	L	F	DQ073658	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT019	Hap 45	Offshore	L	М	DQ073659	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT020	Hap 8	Offshore	L	F	DQ073660	Quérouil et al. (2007)

Table	5.1.	Cont.
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Location	Ecotype	Haplotyp e	Assigned Haplotyp e for this analysis	Assigne d Ecotype for this analysis	Live or Strande d	Sex	Genbank accession number	Publication source
Azores	Pelagic	TT021	Hap 45	Offshore	L	F	DQ073661	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT022	Hap 12	Offshore	L	М	DQ073662	Quérouil et al. (2007)
Azores	Pelagic	TT023	Hap 6	Offshore	L	F	DQ073663	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT024	Hap 65	Offshore	L	М	DQ073664	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT025	Hap 65	Offshore	L	F	DQ073665	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT026	Hap 65	Offshore	L	М	DQ073666	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT027	Hap 12	Offshore	L	М	DQ073667	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT029	Hap 64	Offshore	L	М	DQ073669	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT030	Hap 45	Offshore	L	М	DQ073670	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT031	Hap 57	Offshore	L	М	DQ073671	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT032	Hap 46	Offshore	L	М	DQ073672	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT033	Hap 30	Offshore	L	М	DQ073673	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT035	Hap 52	Offshore	L	F	DQ073675	Quérouil et al. (2007)
Azores	Central Group/Pelagi c	TT036	Hap 1	Offshore	L	М	DQ073676	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT037	Hap 12	Offshore	L	М	DQ073677	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT038	Hap 30	Offshore	L	М	DQ073678	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT039	Hap 8	Offshore	L	F	DQ073679	Quérouil et al. (2007)
Azores	Pelagic	TT041	Hap 3	Offshore	L	М	DQ073681	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT042	Hap 5	Offshore	L	F	DQ073682	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT043	Hap 70	Offshore	L	М	DQ073683	Quérouil et al. (2007)

Table 5.1. Cont.

Location	Ecotype	Haplot ype	Assigned Haplotype for this analysis	Assigne d Ecotype for this analysis	Live or Strande d	Sex	Genbank accession number	Publication source
Azores	Central Group/Pelagic	TT044	Hap 45	Offshore	L	М	DQ073684	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT046	Hap 70	Offshore	L	М	DQ073686	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT047	Hap 45	Offshore	L	F	DQ073687	Quérouil et al. (2007)
Azores	Pelagic	TT048	Hap 4	Offshore	L	М	DQ073688	Quérouil et al. (2007)
Azores	Western Group/Pelagic	TT051	Hap 45	Offshore	L	М	DQ073691	Quérouil et al. (2007)
Azores	Western Group/Pelagic	TT052	Hap 51	Offshore	L	М	DQ073692	Quérouil et al. (2007)
Azores	Pelagic	TT053	Hap 56	Offshore	L	М	DQ073693	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT054	Hap 1	Offshor e	L	М	DQ073694	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT055	Hap 50	Offshore	L	М	DQ073695	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT056	Hap 6	Offshore	L	М	DQ073696	Quérouil et al. (2007)
Azores	Pelagic	TT058	Hap 77	Offshore	L	М	DQ073698	Quérouil et al. (2007)
Azores	Pelagic	TT059	Hap 60	Offshore	L	М	DQ073699	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT060	Hap 77	Offshore	L	М	DQ073700	Quérouil et al. (2007)
Azores	Pelagic	TT061	Hap 11	Offshore	L	М	DQ073701	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT063	Hap 50	Offshore	L	М	DQ073702	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT065	Hap 5	Offshore	L	F	DQ073704	Quérouil et al. (2007)
Azores	Pelagic	TT066	Hap 46	Offshore	L	М	DQ073705.	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT067	Hap 8	Offshore	L	F	DQ073706	Quérouil et al. (2007)
Azores	Pelagic	TT069	Hap 52	Offshore	L	F	DQ073707	Quérouil et al. (2007)

Table 5.1. Cont.

Location	Ecotype	Haplotype	Assigned Haplotype for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Sex	Genbank accession number	Publication source
Azores	Central Group/Pelagic	TT070	Hap 1	Offshore	L	М	DQ073708	Quérouil et al. (2007)
Azores	Western Group/Pelagic	TT072	Hap 49	Offshore	L	М	DQ073709	Quérouil et al. (2007)
Azores	Western Group/Pelagic	TT073			L	F	DQ073710	Quérouil et al. (2007)
Azores	Western Group/Pelagic	TT075	Hap 46	Offshore	L	М	DQ073712	Quérouil et al. (2007)
Azores	Western Group/Pelagic	TT076	Hap 65	Offshore	L	М	DQ073713	Quérouil et al. (2007)
Azores	Eastern Group/Pelagic	TT016	Hap 9	Offshore	L	F	DQ073656	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT071	Hap 2	Offshore	L	F	DQ525357	Quérouil et al. (2007)
Azores	Seamount/Pelagic	TT083	Hap 9	Offshore	L	М	DQ525358	Quérouil et al. (2007)
Azores	Seamount/Pelagic	TT084	Hap 64	Offshore	L	М	DQ525359	Quérouil et al. (2007)
Azores	Seamount/Pelagic	TT085	Hap 30	Offshore	L	М	DQ525360	Quérouil et al. (2007)
Azores	Seamount/Pelagic	TT086	Hap 46	Offshore	L	М	DQ525361	Quérouil et al. (2007)
Azores	Seamount/Pelagic	TT087	Hap 1	Offshore	L	М	DQ525362	Quérouil et al. (2007)
Azores	Western Group/Pelagic	TT077	Hap 12	Offshore	L	М	DQ073714	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT079	Hap 52	Offshore	L	М	DQ073716	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT080	Hap 12	Offshore	L	F	DQ073717	Quérouil et al. (2007)
Azores	Pelagic	TT011	Hap 12	Offshore	S	U	DQ073651	Quérouil et al. (2007)
Azores	Pelagic	TT049	Hap 1	Offshore	L	М	DQ073689	Quérouil et al. (2007)
Azores	Pelagic	TT057	Hap 1	Offshore	L	М	DQ073697	Quérouil et al. (2007)
Azores	Pelagic	TT064	Hap 1	Offshore	L	М	DQ073703	Quérouil et al. (2007)

Table 5.1. Cont.

Location	Ecotype	Haplotype	Assigned Haplotype for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Sex	Genbank accession number	Publication source
Azores	Pelagic	TT028	Hap 7	Offshore	L	М	DQ073668	Quérouil et al. (2007)
Azores	Azores,Central Group	TT050	Hap 9	Offshore	S	М	DQ073690	Quérouil et al. (2007)
Azores	Azores,Central Group	TT034	Hap 9	Offshore	L	М	DQ073674	Quérouil et al. (2007)
Azores	Central Group/Pelagic	TT0045	Hap 12	Offshore	S	М	DQ073685	Quérouil et al. (2007)
Azores	Pelagic	TT074	Hap 46	Offshore	L	М	DQ073711	Quérouil et al. (2007)
Azores	Pelagic	TT078	Hap 53	Offshore	S	F	DQ073715	Quérouil et al. (2007)
Bahamas	Coastal	U	Hap 110	Inshore	L	U	AF155160	Parsons et al. (1999)
Bahamas	Coastal	U	Hap 115	Inshore	L	U	AF155161	Parsons et al. (1999)
Bahamas	Coastal	U	Hap 109	Inshore	L	U	AF155162	Parsons et al. (1999)
Bahamas	Coastal	HapB	Hap 109	Inshore	L	U	AF378176	Parsons et al. (2006)
Bahamas	Coastal	HapD	Hap 124	Inshore	L	U	AF378177	Parsons et al. (2006)
Bahamas	Coastal	HapE	Hap 111	Inshore	L	U	AF378178	Parsons et al. (2006)
Bahamas	Coastal	HapF	Hap 109	Inshore	L	U	DQ118180	Parsons et al. (2006)
Bahamas	Coastal	HapG	Hap 116	Inshore	L	U	DQ118181	Parsons et al. (2006)
Bahamas	Worldwide Distributed form	НарК	Hap 72	Offshore	L	U	DQ118182	Parsons et al. (2006)
Bahamas	Coastal	HapL	Hap 114	Inshore	L	U	DQ118183	Parsons et al. (2006)
California	Offshore	GC1	Hap 43	Unknown	U	U	KF570389	Moura et al (2013)
Caribbean	Inshore	TrtuCARA	Hap 109	Inshore	U	U	JN596281	Caballero et al. (2011)
Caribbean	Inshore	TtruCARD	Hap 109	Inshore	U	U	JN596284	Caballero et al. (2011)
Caribbean	Inshore	TtruCARE	Hap 109	Inshore	U	U	JN596285	Caballero et al. (2011)
Caribbean	Inshore	TtruCARF	Hap 112	Inshore	U	U	JN596286	Caballero et al. (2011)
Caribbean	Worldwide Distributed form	TtruCARG	Hap 82	Offshore	U	U	JN596287	Caballero et al. (2011)
Caribbean	Inshore	TtruCARK	Hap 109	Inshore	U	U	JN596291	Caballero et al. (2011)
Caribbean	Inshore	TtruCARM	Hap 109	Inshore	U	U	JN596293.	Caballero et al. (2011)
Caribbean	Inshore	TtruCARN	Hap 113	Inshore	U	U	JN596294	Caballero et al. (2011)
Caribbean	Worldwide Distributed form	TtruCAR- O	Hap 84	Offshore	U	U	JN596295	Caballero et al. (2011)
Caribbean	Worldwide Distributed form	TtruCARP	Hap 72	Offshore	U	U	JN596296	Caballero et al. (2011)
Caribbean	Worldwide Distributed form	TtruCARR	Hap 84	Offshore	U	U	JN596298	Caballero et al. (2011)

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Location	Ecotype	Haplotype	Assigned Haplotype for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Sex	Genbank accession number	Publication source
Caribbean	Inshore	TtruCARS	Hap 113	Inshore	U	U	JN596299	Caballero et al. (2011)
Caribbean	Worldwide Distributed form	TtruCART	Hap 47	Offshore	U	U	JN596300	Caballero et al. (2011)
Caribbean	Inshore	TtruCARU	Hap 125	Inshore	U	U	JN596301	Caballero et al. (2011)
Caribbean	Inshore	TtruCARV	Hap 129	Inshore	U	U	JN596302	Caballero et al. (2011)
Caribbean	Worldwide Distributed form	TtruCARW	Hap 81	Offshore	U	U	JN596303	Caballero et al. (2011)
Caribbean	Inshore	TtruCARX	Hap 109	Inshore	U	U	JN596304	Caballero et al. (2011)
Caribbean	Inshore	TRUCARZ	Hap 124	Inshore	U	U	JN596306	Caballero et al. (2011)
Caribbean	Inshore	TtruCAR-BB	Hap 129	Inshore	U	U	JN596308	Caballero et al. (2011)
Caribbean	Inshore	TtruCARFF	Hap 126	Inshore	U	U	JN596312	Caballero et al. (2011)
Caribbean	Worldwide Distributed form	TturCARKK	Hap 76	Offshore	U	U	JN596317	Caballero et al. (2011)
Caribbean	Worldwide Distributed form	TtruCARMM	Hap 74	Offshore	U	U	JN596319	Caballero et al. (2011)
Costa Rica	Worldwide Distributed Form	TTruCAR- CR1 tRNA- Pro	Hap 72	Offshore	L	U	KY817220	Barragan et al. (2017)
Costa Rica	Worldwide Distributed Form	TtruCAR- CR2 tRNA- Pro	Hap 72	Offshore	L	U	KY817221	Barragan et al. (2017)
Cuba	Worldwide Distributed form	TtruCARJ	Hap 72	Offshore	U	U	JN596290	Caballero et al. (2011)
Cuba	Inshore	TtruCARL	Hap 109	Inshore	L	U	JN596292	Caballero et al. (2011)
Cuba	Inshore	TtruCARQ	Hap 117	Inshore	U	U	JN596297	Caballero et al. (2011)
Eastern North Atlantic	Coastal	TTR8	Hap 71	Offshore	L or S	U	AY963617	Natoli et al. (2005)
Eastern North Atlantic	Coastal	AAT49	Hap 46	Offshore	L or S	U	AY963618	Natoli et al. (2005)
Eastern North Atlantic	Coastal	AAT54	Hap 77	Offshore	L or S	U	AY963620	Natoli et al. (2005)

Table 5.1. Cont.

Location	Ecotype	Haplotype	Assigned Haplotype for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Sex	Genbank accession number	Publication source
Eastern North Atlantic	Coastal	TtAL5	Hap 79	Offshore	L or S	U	AY963621	Natoli et al. (2005)
Eastern North Atlantic	Coastal	TTR1	Hap 46	Offshore	L or S	U	AY963626	Natoli et al. (2005)
Ecuador	Inshore	Ttr_SA2	Hap 92	Inshore	L	М	KU992130	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_SA3	Hap 44	Unknown	L	U	KU992131	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_SA4	Hap 40	Unknown	L	F	KU992132	Bayas-Rea et al. (2018)
Ecuador	Offshore	Ttr_SA5	Hap 26	Offshore	L	F	KU992133	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_SA6	Hap 41	Unknown	L	U	KU992134	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_SA7	Hap 92	Inshore	L	F	KU992135	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG37	Hap 93	Inshore	S	U	KU992136	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG39	Hap 94	Inshore	S	U	KU992137	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG44	Hap 93	Inshore	S	U	KU992138	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_SA12	Hap 67	Offshore	S	U	KU992139	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_SA13	Hap 40	Unknown	S	U	KU992140	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG1	Hap 93	Inshore	L	М	KU992141	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG2	Hap 93	Inshore	L	М	KU992142	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG3	Hap 93	Inshore	L	М	KU992143	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG4	Hap 93	Inshore	L	F	KU992144	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG5	Hap 95	Inshore	L	М	KU992145	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG6	Hap 93	Inshore	L	F	KU992146	Bayas-Rea et al. (2018)

Table 5.1. Cont.

Location	Ecotyp e	Haplotyp e	Assigned Haplotyp e for this analysis	Assigned Ecotype for this analysis	Live or Strande d	Sex	Genbank accession number	Publication source
Ecuador	Inshore	Ttr_GG7	Hap 93	Inshore	L	М	KU99214 7	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG8	Hap 93	Inshore	L	F	KU99214 8	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG9	Hap 94	Inshore	L	М	KU99214 9	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG10	Hap 93	Inshore	L	F	KU99215 0	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG11	Hap 93	Inshore	L	F	KU99215 1	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG12	Hap 93	Inshore	L	М	KU99215 2	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GI1	Hap 95	Inshore	L	F	KU99215 3	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG14	Hap 95	Inshore	L	М	KU99215 4	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG15	Hap 93	Inshore	L	F	KU99215 5	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG16	Hap 93	Inshore	L	М	KU99215 6	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG17	Hap 93	Inshore	L	М	KU99215 7	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG18	Hap 94	Inshore	L	М	KU99215 8	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG19	Hap 93	Inshore	L	F	KU99215 9	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG20	Hap 93	Inshore	L	F	KU99216 0	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG21	Hap 93	Inshore	L	М	KU99216 1	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG22	Hap 93	Inshore	L	М	KU99216 2	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG23	Hap 93	Inshore	L	М	KU99216 3	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG24	Hap 93	Inshore	L	М	KU99216 4	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG25	Hap 93	Inshore	L	М	KU99216 5	Bayas-Rea et al. (2018)

Table 5.1. Cont.

Location	Ecotype	Haplotyp e	Assigned Haplotyp e for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Sex	Genbank accession number	Publication source
Ecuador	Inshore	Ttr_GG26	Hap 93	Inshore	L	М	KU992166	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG27	Hap 93	Inshore	L	М	KU992167	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG28	Hap 93	Inshore	L	М	KU992168	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG29	Hap 94	Inshore	L	М	KU992169	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG30	Hap 94	Inshore	L	F	KU992170	Bayas-Rea et al. (2018)
Ecuador	Inshore	Ttr_GG31	Hap 93	Inshore	L	М	KU992171	Bayas-Rea et al. (2018)
Ecuador	Estuarine	Ttr_PE1	Hap 96	Inshore	L	U	KU992172	Bayas-Rea et al. (2018)
Ecuador	U	Ttr_GI1	Hap 26	Offshore	L	U	KU99212 8	Bayas-Rea et al. (2018)
Ecuador	Estuarine	Ttr SA1	Hap 38	Unknow n	S	U	KU99212 9	Bayas-Rea et al. (2018)
Florida	Alongshor	h4 D-loop	Hap 116	Inshore	L	U	KF366720.	Richards et al. (2013)
Florida	Alongshor e	h5 D-loop	Hap 118	Inshore	L	U	KF366721	Richards et al. (2013)
Gulf of Mexico, USA	Coastal	GTtr19 tRNA- Pro	Hap 123	Inshore	L	U	AY99730 7	Sellas et al. (2005)
Gulf of Mexico, USA	Coastal	GTtr20 tRNA- Pro	Hap 123	Inshore	L	U	AY99731 0	Sellas et al. (2005)
Gulf of Mexico, USA	Offshore	OTtr49	Hap 46	Offshore	U	U	HQ38368 5	Litz et al. (2012)
Gulf of Mexico, USA	Coastal	Ttr41	Hap 109	Inshore	U	U	HQ38386	Litz et al (2012)
Gulf of Mexico,US A	Coastal	Ttr16 tRNA- Pro	Hap 129	Inshore	L	U	AY99730 9	Sellas et al. (2005)
Gulf of Mexico,US A	Coastal	GTtr30 tRNA- Pro	Hap 130	Inshore	L	U	AY99731 1	Sellas et al. (2005)
Madeira	Pelagic	TTM02	Hap 7	Offshore	L	F	DQ525386	Quérouil et al. (2007)
Madeira	Pelagic	TTM029	Hap 1	Offshore	L	М	DQ52538 7	Quérouil et al. (2007)

Table 5.1. Cont.

Location	Ecotype	Haplotype	Assigned Haplotype for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Sex	Genbank accession number	Publication source
Madeira	U	TTM001	Hap 52	Offshore	U	F	DQ525364	Quérouil et al. (2007)
Madeira	U	TTM002	Hap 45	Offshore	U	М	DQ525365	Quérouil et al. (2007)
Madeira	U	TTM003	Hap 8	Offshore	U	М	DQ525366	Quérouil et al. (2007)
Madeira	Pelagic	TTM004	Hap 53	Offshore	L	F	DQ525367	Quérouil et al. (2007)
Madeira	Pelagic	TTM005	Hap 46	Offshore	L	М	DQ525368	Quérouil et al. (2007)
Madeira	Pelagic	TTM006	Hap 57	Offshore	L	F	DQ525369	Quérouil et al. (2007)
Madeira	Pelagic	TTM007	Hap 70	Offshore	L	F	DQ525370	Quérouil et al. (2007)
Madeira	Pelagic	TTM008	Hap 60	Offshore	L	F	DQ525371	Quérouil et al. (2007)
Madeira	Pelagic	TTM009	Hap 7	Offshore	L	М	DQ525372	Quérouil et al. (2007)
Madeira	Pelagic	TTM010	Hap 46	Offshore	L	М	DQ525373	Quérouil et al. (2007)
Madeira	Pelagic	TTM012	Hap 45	Offshore	L	М	DQ525374	Quérouil et al. (2007)
Madeira	Pelagic	TTM013	Hap 46	Offshore	L	М	DQ525375	Quérouil et al. (2007)
Madeira	Pelagic	TTM014	Hap 53	Offshore	L	F	DQ525376	Quérouil et al. (2007)
Madeira	Pelagic	TTM015	Hap 75	Offshore	L	М	DQ525377	Quérouil et al. (2007)
Madeira	Pelagic	TTM016	Hap 68	Offshore	L	М	DQ525378	Quérouil et al. (2007)
Madeira	Pelagic	TTM019	Hap 46	Offshore	L	F	DQ525379	Quérouil et al. (2007)
Madeira	Pelagic	TTM020	Hap 53	Offshore	L	F	DQ525380	Quérouil et al. (2007)
Madeira	Pelagic	TTM022	Hap 65	Offshore	L	М	DQ525381	Quérouil et al. (2007)
Madeira	Pelagic	TTM023	Hap 46	Offshore	L	М	DQ525382	Quérouil et al. (2007)

Tsble 5.1. Cont.

Location	Ecotype	Haplotype	Assigned Haplotype for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Sex	Genbank accession number	Publication source
Madeira	Pelagic	TTM025	Hap 46	Offshore	L	М	DQ525383	Quérouil et al. (2007)
Madeira	Pelagic	TTM026	Hap 46	Offshore	L	М	DQ525384	Quérouil et al. (2007)
Madeira	Pelagic	TTM027	Hap 46	Offshore	L	F	DQ525385	Quérouil et al. (2007)
Madeira	Pelagic	TTM030	Hap 65	Offshore	L	F	DQ525388	Quérouil et al. (2007)
Mexico	Worldwide Distributed form	TtruCARY	Hap 85	Offshore	U	U	JN596305	Caballero et al. (2011)
Mexico	Inshore	TtruCARAA	Hap 112	Inshore	L	U	JN596307	Caballero et al. (2011)
Mexico	Inshore	TtruCARCC	Hap 127	Inshore	L	U	JN596309	Caballero et al. (2011)
Mexico	Inshore	TtruCARDD	Hap 128	Inshore	L	U	JN596310	Caballero et al. (2011)
Mexico	Inshore	TtruCAREE	Hap 126	Inshore	L	U	JN596311	Caballero et al. (2011)
Mexico	Inshore	TtruCar1Ta02	Hap 113	Inshore	L	U	JN596320	Caballero et al. (2011)
Mexico	Worldwide Distributed form	TtruCARQR1	Hap 88	Offshore	L	U	JN596321	Caballero et al. (2011)
New Zealand	U	NZ-N01	Hap 66	Offshore	L,S	U	EU276389	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N18	Hap 15	Offshore	L,S	U	EU276390	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N05	Hap 10	Offshore	L,S, MP	U	EU276391	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N06	Hap 32	Unknown	L,S	U	EU276392	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N09	Hap 17	Offshore	L,S	U	EU276393	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N12	Hap 34	Unknown	L,S	U	EU276394	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N14	Hap 22	Offshore	L,S	U	EU276395	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N15	Hap 23	Offshore	L,S	U	EU276396	Tezanos-Pinto et al. (2009)

Table 5.1. Cont.

Location	Ecotype	Haplotype	Assigned Haplotype for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Sex	Genbank accession number	Publication source
New Zealand	U	NZ-N26	Hap 89	Offshore	L,S	U	EU276397	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N35	Hap 37	Unknown	L	U	EU276398	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N37	Hap 69	Offshore	L	U	EU276399	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N38	Hap 10	Offshore	L	U	EU276400	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N04	Hap 22	Offshore	L	U	EU276401	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N49	Hap 89	Offshore	L	U	EU276402	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-N132	Hap 36	Unknown	L	U	EU276403	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-F01	Hap 35	Unknown	L,S	U	EU276404	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-F02	Hap 33	Unknown	L,S	U	EU276405	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-MS01	Hap 24	Offshore	L	U	EU276406	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-MS10	Hap 18	Offshore	L	U	EU276407	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-MS08	Hap 25	Offshore	L	U	EU276408	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-MS25	Hap 19	Offshore	L	U	EU276409	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-MS34	Hap 24	Offshore	L	U	EU276410	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-F10	Hap 33	Unknown	L	U	EU276411	Tezanos-Pinto et al. (2009)
New Zealand	U	NZ-FJB2	Hap 15	Offshore	L	U	EU276412	Tezanos-Pinto et al. (2009)
Panama	Inshore	TTruCAR- BOC D	Hap 107	Inshore	L	U	KX833116	Barragan et al. (2017)
Peru	Offshore	AGG601	Hap 106	Offshore	U	U	AF323893	Barreto et al. (2000)
Peru	Coastal Isolate	AGG741	Hap 97	Inshore	U	U	AF323894	Barreto et al. (2000)

Table 5.1. Cont.

Locatio n	Ecotype	Haplotype	Assigned Haplotype for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Se x	Genbank accession number	Publication source
Peru	Offshore	JAS12	Hap 100	Unknow n	U	U	AF323895	Barreto et al. (2000) *
Peru	Coastal Isolate	JAS47	Hap 97	Inshore	U	U	AF323896	Barreto et al. (2000) *
Peru	Offshore	KVW239	Hap 99	Unknow n	U	U	AF323897	Barreto et al. (2000) *
Peru	Offshore	KVW241	Hap 105	Offshore	U	U	AF323898	Barreto et al. (2000) *
Peru	Offshore	KVW243	Hap 102	Unknow n	U	U	AF323899	Barreto et al. (2000) *
Peru	Offshore	KVW244	Hap 101	Unknow n	U	U	AF323900	Barreto et al. (2000) *
Peru	Offshore	MFB441	Hap 98	Unknow n	U	U	AF323901	Barreto et al. (2000) *
Peru	Offshore	MFB701	Hap 103	Offshore	U	U	AF323902	Barreto et al. (2000) *
Peru	Offshore	MFB702	Hap 104	Offshore	U	U	AF323903	Barreto et al. (2000) *
Portugal	Mainlan d	Hap 64	TT/03/199 7	Offshore	S	F	DQ07371 9	Quérouil et al. (2007)
Portugal	Mainland Portugal	LUA	Hap 64	Offshore	S	М	DQ073726	Quérouil et al. (2007)
Portugal	Mainland Portugal	TT/43/2004	Hap 45	Offshore	S	F	DQ073727	Quérouil et al. (2007)
Portugal	Mainland Portugal	TT/141/2004	Hap 46	Offshore	S	М	DQ073728	Quérouil et al. (2007)
Portugal	Mainland Portugal	TT/152/2004	Hap 30	Offshore	S	F	DQ073729	Quérouil et al. (2007)
Portugal	Mainland Portugal	TT/54/2005	Hap 51	Offshore	S	М	DQ525363	Quérouil et al. (2007)
Portugal	Mainland Portugal	TT/31/1996	Hap 46	Offshore	S	М	DQ073718	Quérouil et al. (2007)
Portugal	Mainland Portugal	TT/71/1997	Hap 45	Offshore	S	F	DQ073720	Quérouil et al. (2007)
Portugal	Mainland Portugal	TT/102/1997	Hap 8	Offshore	S	М	DQ073721	Quérouil et al. (2007)
Portugal	Mainland Portugal	TT/01/1998	Hap 45	Offshore	S	М	DQ073722	Quérouil et al. (2007)

Table 5.1. Cont.

Location	Ecotype	Haplotype	Assigned Haplotype for this analysis	Assigned Ecotype for this analysis	Live or Stranded	Sex	Genbank accession number	Publication source
Portugal	Mainland Portugal	TT/15/1998	Hap 31	Offshore	S	F	DQ073723	Quérouil et al. (2007)
Portugal	Mainland Portugal	TT/31/2000	Hap 77	Offshore	S	М	DQ073724	Quérouil et al. (2007)
Portugal	Mainland Portugal	TT/61/2002	Hap 64	Offshore	S	М	DQ073725	Quérouil et al. (2007)
Portugal	U	TT040	Hap 8	Offshore	S	М	DQ073680	Quérouil et al. (2007)
South Carolina	Alongshore	h1 D-loop	Hap 121	Inshore	L	U	KF366717	Richards et al. (2013)
South Carolina	Alongshore	h2 D-loop	Hap 119	Inshore	L	U	KF366718	Richards et al. (2013)
South Carolina	Alongshore	h3D-loop	Hap 120	Inshore	L	U	KF366719	Richards et al. (2013)
Southwestern Atlantic	Inshore	Hap_003	Hap 39	Unknown	L	U	MF405801	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_004	Hap 54	Offshore	L	U	MF405802	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_005	Hap 38	Unknown	L	U	MF405803	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_006	Hap 55	Offshore	L	U	MF405804	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_014	Hap 58	Offshore	L	U	MF405805	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_008	Hap 54	Offshore	L	U	MF405806	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_007	Hap 61	Offshore	L	U	MF405807	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_021	Hap 62	Offshore	L	U	MF405808	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_020	Hap 3	Offshore	L	U	MF405809	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_024	Hap 59	Offshore	L	U	MF405810	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_025	Hap 63	Offshore	L	U	MF405811	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_012	Hjap 46	Offshore	L	U	MF405812	Fruet et al. (2017)

Table 5.1. Cont.

Location	Ecotype	Haplotyp e	Assigned Haplotyp e for this analysis	Assigne d Ecotype for this analysis	Live or Strande d	Se x	Genbank accession number	Publication source
Southwestern Atlantic	Offshore	Hap_026	Hap 28	Offshore	L	U	MF405813	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_027	Hap 29	Offshore	L	U	MF405814	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_009	Hap 86	Offshore	L	U	MF405815	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_013	Hap 21	Offshore	L	U	MF405816	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_023	Hap 46	Offshore	L	U	MF405817	Fruet et al. (2017)
Southwester n Atlantic	Offshor e	Hap_016	Hap 1	Offshore	L	U	MF40581 8	Fruet et al. (2017)
Southwestern Atlantic	Inshore	TLD012	Hap 83	Offshore	L	U	MF405819	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_028	Hap 16	Offshore	L	U	MF405820	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_030	Hap 52	Offshore	L	U	MF405821	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_019	Hap 48	Offshore	L	U	MF405822	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_031	Hap 91	Offshore	L	U	MF405823	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_032	Hap 60	Offshore	L	U	MF405824	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_033	Hap 42	Unknown	L	U	MF405825	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_034	Hap 90	Offshore	L	U	MF405826	Fruet et al. (2017)
Southwestern Atlantic	Inshore	Hap_035	Hap 41	Unknown	L	U	MF405827	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_036	Hap 46	Offshore	L	U	MF405828	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_037	Hap 27	Offshore	L	U	MF405829	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_038	Hap 20	Offshore	L	U	MF405830	Fruet et al. (2017)
Southwestern Atlantic	Offshore	Hap_039	Hap 21	Offshore	L	U	MF405831	Fruet et al. (2017)

Table 5.1. Cont.

Location	Ecotype	Haplotyp e	Assigned Haplotyp e for this analysis	Assigned Ecotype for this analysis	Live or Stran ded	Sex	Genban k accessio n number	Publicatio n source
Cataño, Puerto Rico	Worldwi de Distribute d form	TtruCARG G	Hap 72	Offshore	S	U	JN59631 3	Caballero et al. (2011)
Lajas,Puerto Rico	Inshore	TtruCARB	Hap 124	Inshore	S	F	JN59628 2	Caballero et al. (2011)
Manati, Puerto Rico	Worldwi de Distribute d form	TtruCARC	Hap 72	Offshore	U	U	JN59628 3	Caballero et al. (2011)
Ponce, Puerto Rico	Worldwi de Distribute d form	TtruCARI	Hap 46	Offshore	U	U	JN59628 9.	Caballero et al. (2011)
San Juan, Puerto Rico	Worldwi de Distribute d form	TtruCARH H	Hap 9	Offshore	U	U	JN59631 4	Caballero et al. (2011)
San Juan, Puerto Rico	Worldwi de Distribute d form	TtruCARL LCommon	Hap 14	Offshore	U	U	JN59631 8	Caballero et al. (2011)
San Juan, Puerto Rico	Inshore	TtruCARJ J	Hap 108	Inshore	U	U	JN59631 6	Caballero et al. (2011)
Vega Baja, Puerto Rico	Worldwi de Distribute d form	TtruCARII	Hap 76	Offshore	U	U	JN59631 5	Caballero et al. (2011)
Vieques, Puerto Rico	Worldwi de Distribute d form	TtruCARH	Hap 76	Offshore	U	U	JN59628 8.	Caballero et al. (2011)
Aguadilla,Puer to Rico			Hap72	Offshore	S	F		this study
Cabo Rojo , Puerto Rico			Hap 124	Inshore	L	F		this study
Cabo Rojo, Puerto Rico			Hap 124	Inshore	L	F		this study
Cabo Rojo, Puerto Rico			Hap 124	Inshore	L	М		this study
Cabo Rojo , Puerto Rico			Hap 124	Inshore	L	М		this study
Cabo Rojo , Puerto Rico			Hap 124	Inshore	L	М		this study
Cabo Rojo , Puerto Rico			Hap 124	Inshore	L	F		this study

Table 5.1. Cont.

Location	Ecotyp e	Haplotype	Assigned Haplotyp e for this analysis	Assigne d Ecotype for this analysis	Live or Strande d	Se x	Genbank accessio n number	Publicatio n source
Cabo Rojo , Puerto Rico			Hap 124	Inshore	L	М		this study
Cabo Rojo , Puerto Rico			Hap 124	Inshore	L	М		this study
Cabo Rojo , Puerto Rico			Hap 124	Inshore	L	М		this study
Cabo Rojo , Puerto Rico			Hap 124	Inshore	L	М		this study
Cabo Rojo , Puerto Rico			Hap 124	Inshore	L	М		this study
Cabo Rojo , Puerto Rico			Hap76	Offshore	L	М		this study
Guayanilla,Puerto Rico			Hap 72	Offshore	S	М		this study
Lajas, Puerto Rico			Hap 124	Inshore	L	М		this study
Lajas, Puerto Rico			Hap 124	Inshore	L	М		this study
Lajas,Puerto Rico			Hap 124	Inshore	L	F		this study
Mayaguez,Puerto Rico			Hap 124	Inshore	L	F		this study
Mayaguez, Puerto Rico			Hap 124	Inshore	L	F		this study
Salinas, Puerto Rico			Hap 124	Inshore	S	М		this study

Table 5.2. Summary of DNA statistics of *Tursiops truncatus* inshore and offshore populations based on the control region dataset. Puerto Rico sequences include the new sequences and those of Caballero et al. (2011). In parentheses the values of one standard deviation of the mean are indicated. (*) Asterisks denote significant values (P <0.05).

Location	N	No. of Hapl otype	Haplotype diversity (h)	Theta (θ)	Pi (π)	Tajima' s D	Fu's Fs
W. North Atlantic -Inshore	4 4	39	0.9947(0.0061)	0.0301(0.0097)	0.0310(0.0161)	-0.1622	- 24.75 83*
W. North Atlantic -Offshore	3	3	1.0000(0.2722)	0.0253(0.0161)	0.0271(0.0214)	0	1.139 4
E. North Atlantic - Inshore	9	6	0.9167(0.0725)	0.0116(0.0057)	0.0135(0.0082)	1.1506	0.045 8
E. North Atlantic - Offhore	1 2 0	31	0.9431(0.0086)	0.0236(0.0064)	0.0205(0.0107)	-0.4531	- 4.012 8
Caribbean-Inshore	6 1	18	0.7344(0.0558)	0.0168(0.0053)	0.0125(0.0069)	-1.1083	- 2.757 0
Caribbean- Offshore	4 3	14	0.8151(0.0431)	0.0109(0.0039)	0.0097(0.0055)	-0.6025	- 2.621 4
Pacific-Inshore	4 6	13	0.6725(0.0759)	0.0192(0.0063)	0.0128(0.0071)	-1.1244	- 0.414 9
Pacific-Offshore	1 1	11	1.0000(0.0388)	0.0273(0.0119)	0.0280(0.0159)	1.1175	- 4.963 8*
Puerto Rico- Inshore	2 5	2	0.0800(0.0722)	0.0049(0.0023)	0.0017(0.0015)	- 2.1581*	2.040 1
Puerto Rico- Offshore	2 4	6	0.7246(0.0606)	0.0076(0.0034)	0.0094(0.0056)	0.2257	1.253 8

Table 5.3. F_{ST} population comparisons (Weir and Cochran, 1984) of *Tursiops truncatus* based on the control region sequences. All comparisons are based on pairwise differences and are all statistically significant except those comparisons indicated with an NS (Not significant).

Caribbean_inshore Caribbean	Offshore East	ern_NA_Offshor	re Eastern_NA_	inshore Wester	n_NA_inshore V	Vestern_NA_off	shore Pacific_in	shore Pacific_offshore
Caribbean_inshore	340							
Caribbean_Offshore	0.70176	1.72						
Eastern_NA_Offshore	0.55107	0.27079	-					
Eastern_NA_inshore	0.64316	0.26098	0.17983	-				
Western_NA_inshore	0.41292	0.27693	0.07756	0.12371	-			
Western_NA_offshore	0.59400	0.27700	-0.08359,NS	0.19429	-0.02687, NS	-		
Pacific_inshore	0.67582	0.70744	0.55268	0.63698	0.46967	0.59196	Ξ.	
Pacific_offshore	0.53047	0.44070	0.09789	0.25882	0.02397	0.01125, NS	0.49482, NS	-

Table 5.4. Analysis of molecular variance (AMOVA) results for *Tursiops truncatus* inshore and offshore based on the mitochondrial control region among four groups: Caribbean, W. North Atlantic, E. North Atlantic, and Pacific sequences. F_{ST} = 0.45802*, F_{SC} =0.51626*, F_{CT} =-0.12041. *denotes statistical significance.

Source of	d.f.	Sum of	Variance	%
variation		squares	components	of variation
Among four groups	3	414.772	-0.67707	-12.04
Among populations within groups	4	306.730	3.25250	57.84
Within populations	329	1002.652	3.04757	54.20

Table 5.5. Analysis of molecular variance (AMOVA) results for *Tursiops truncatus* based on the mitochondrial control region. All inshore and offshore populations from the four regions were grouped together. The four regions are Caribbean, W. North Atlantic, E. North Atlantic, and Pacific. F_{ST} = 0.48112*, F_{SC} =0.43239*, F_{CT} =0.08586. *denotes statistical significance.

Source of variation	d.f.	Sum of squares	Variance components	% of variation
Among Inshore vs. Offshore	1	249.649	0.50428	8.59
Among populations within Inshore vs. Offshore	6	471.853	2.32152	39.53
Within populations	329	1002.652	3.04757	51.89

Table 5.6. A Maximum-likelihood distance matrix of the eight *Tursiops truncatus* haplotypes of Puerto Rico based on the control region dataset. Genetic distances were estimated in PAUP* and were corrected with the HKY85 variant model of nucleotide substitution. Haplotype numbers refer to haplotypes of Figure 5.

1 2 3 4 5 6 7 8 1 Hap 9 _ 2 Hap 12 0.00546 -3 Hap 14 0.00838 0.00268 4 Hap 46 0.03392 0.02986 0.02596 5 Hap 72 0.03014 0.02620 0.02243 0.00849 6 Hap 76 0.03424 0.03857 0.03429 0.01156 0.00842 7 Hap 108 0.05546 0.06578 0.06062 0.04765 0.04811 0.04336 8 Hap 124 0.05046 0.06037 0.05541 0.05304 0.05354 0.04854 0.02175



Figure 5.1. Study area and survey effort (thick black line) for the bottlenose dolphin *Tursiops truncatus*. Live animal biopsy sampling took place during August 18-31, 2014 and October 19-30, 2015. Sampling of free ranging dolphins was focused on the waters off the south and west coasts of Puerto Rico (18° 12'N, 66° 39' W).



Figure 5.2. Distribution of samples of *Tursiops truncatus* from the current study and those of Caballero et al. (2011). Triangles (▲) represent live animals sampled in this study, circles (●) represent stranded dolphins (years 2001 to 2018), squares (■) dolphins stranded in Puerto Rico (1994 to 2003) reported in Caballero et al. (2011).



Figure 5.3. Haplotype network based on the mtDNA control region of *Tursiops truncatus* from the Atlantic and Pacific Ocean. The median-joining network algorithm (epsilon = 0) was used as implemented in PopArt.



Figure 5.4. Haplotype sequences based on the mtDNA control region of *Tursiops truncatus* from Puerto Rico. Live and stranded animals have been included from the current study and those reported in Caballero et al. (2011). The median-joining network algorithm (epsilon = 0) was used as implemented in PopArt.



Figure 5.5. Maximum likelihood tree depicting the phylogenetic relationships of the 130 haplotypes based on the mtDNA control region of *Tursiops truncatus* from the Atlantic and Pacific Ocean. Blue circles on the branches indicate bootstrap values above 50%. The eight haplotypes found in Puerto Rico are indicated in red, bold letters. Hap2 was represented by a long branch that has been truncated for better viewing of the tree.

CHAPTER SIX - CONCLUSION

The bottlenose dolphin population that inhabits the south and west coasts of Puerto Rico exhibits complex dynamics and distribution patterns. The abundance estimate of the free ranging individuals, show a small population, semi-resident with periods of no presence. The extension of the insular shelf on the West coast and the lack of natural barriers make this environment suitable for the species to explore both inshore and offshore waters. Genetic data show the presence of an inshore and offshore ecotype, with a prevalence of the offshore ecotype in stranded dolphins, while the inshore ecotype dominates the live dolphins sampled in this study. The presence of only eight mitochondrial haplotypes in Puerto Rico should be of concern, since it indicates low levels of genetic variability. The estimated population size based on mark-recapture modeling is small but there is a large discrepancy with the estimated population size based on genetic data and needs to be explored further by more observations and higher resolution genetic markers. Puerto Rico is a small island where boating activity is high and year around. The nearshore distribution of the species put them in direct contact with humans. The dolphins already exhibit the effects from anthropogenic interactions in the form of increased number of scars. Dolphins interact with fishing gear and boat activities, therefore steps should be taken to minimize these negative interactions. These results could be used to establish conservation measures to help protect the bottlenose dolphins in Puerto Rico.

This project should be the first step towards understanding the dolphins in Puerto Rico and then relate them to the Caribbean population. There is a need for sampling the free ranging population island wide to help determine population structure. Especially using next generation techniques such as SNPs to help understand the true structure. The interactions of both ecotypes as well as their distribution should be study using telemetry and focusing on the deep water zone. Stable isotopes, should be incorporated learn about the species feeding habits, prey preferences and how this relate to fisheries.