POPULATION STRUCTURE AND DISPERSAL OF BOTTLENOSE DOLPHINS
(TURSIOPS TRUNCATUS) OF THE INDIAN RIVER LAGOON ESTUARY,
FLORIDA, AND ADJACENT ATLANTIC WATERS

by

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POPULATION STRUCTURE AND DISPERUAL OF BOTTLENOSE DOLPHINS (TURSIOPS TRUNCATUS) OF THE INDIAN RIVER LAGOON ESTUARY, FLORIDA, AND ADJACENT ATLANTIC WATERS

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Greg O'Corry-Crowe, Department of Biological Sciences, and has been approved by the members of her supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

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Worldwide research of bottlenose dolphins (*Tursiops truncatus*) has led to varied definitions and terminology regarding ways to group dolphins for study and management. An understanding of the demographic history and population structure of bottlenose dolphins residing within the Indian River Lagoon Estuary System (IRLES), Florida, is needed to help define the IRLES dolphin population: ecotype, population, or community. Using mitochondrial DNA sequencing and microsatellite genotyping, this study detected: (1) genetic differentiation between estuarine and coastal individuals ($F_{st_{mtDNA}}=0.414$, $F_{st_{msat}}=0.057$; $p<0.05$; $K=2$), (2) genetic differentiation between the Indian River Lagoon (IRL) and Mosquito Lagoon (ML) ($F_{st_{mtDNA}}=0.0201$, $F_{st_{msat}}=0.0234$; $p<0.09$), and (3) minute undefined sub-structure within the IRLES ($F_{st_{mtDNA}}=-0.00$ -0.0379, $F_{st_{msat}}=0.00$ -
0.0445; $p \geq 0.1$). Additionally, within ML this study detected non-mixing cohabitation of two potential ecotypes, estuarine and coastal. These findings raise many questions regarding how dolphins are presently categorized and managed which are critical to population assessments including abundance, vital rates, and health.
DEDICATION

This manuscript is dedicated to my family, particularly to my understanding and patient husband, Derek, who has put up with these many years of schooling and research and has given me unconditional love throughout, and to my son, Jaxon, who was my motivation to finish. I wanted to show you to never quit something you set out to accomplish, no matter how impossible something may become; if you really want it, you will find a way to accomplish it. I also dedicate this work to my parents, James and Wendy, both of whom believed I was born a marine biologist and encouraged me throughout my life to pursue my dreams.
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INTRODUCTION

Bottlenose dolphins (*Tursiops truncatus* Montagu, 1821) are a common marine mammal found along the east coast of the United States as well as all temperate and tropical oceans of the world (Klinowska, 1991; Connor et al., 2000). Their habitat ranges from pelagic waters to tidal creeks and estuaries (Caldwell & Caldwell, 1972). Regardless of habitat preference, bottlenose dolphins live in fission-fusion societies, composed of small groups (10-12 individuals) of changing interacting individuals (Connor et al., 2000). Social relationships and patterns of sex-specific associations are similar those seen in common chimpanzees (*Pan troglodytes*) and spider monkeys (*Ateles paniscus*) (Connor et al., 1992, 1998; Smolker et al., 1992). Societies maintain consistent, discrete home ranges with fluid smaller internal groups (Wells et al., 1980). Adult females and associated calves are generally found together in groups and adult males are generally found in pairs or coalitions, or alliance formations (Wells et al., 1980; Connor et al., 1992; Reynolds et al., 2000; Gibson & Mann, 2008). Natal philopatry has been seen for males and females; further sub adult females are more readily found with adult females and sub adult males disperse to create bachelor groups (Wells et al., 1980). These relationships may vary within or between populations dependent on resource availability (itself dependent on prey distribution, conspecific territories, and hydrodynamics/topography/currents) and
Dolphin Morphotypes

The taxonomy of dolphins has been in flux since the species description in 1821. Over the last hundred years, *Tursiops sp.* have been divided into as many as 20 different genera (Hershkovitz, 1966). Historically, bottlenose dolphins are categorized into two morphotypes: offshore vs. inshore by several characters (habitat, morphometrics, hemoglobin content, diet, parasite burden, and genetics (Waring et al., 2011). Inshore dolphins include those living in near shore coastal waters and inside estuaries, bays, and rivers; offshore dolphins occupy further pelagic habitats (Mead & Potter, 1995; Hoelzel et al., 1998a; Rosel et al., 2009; Waring et al., 2011). Inshore dolphins are smaller in length, weight and skull size, lighter in color, and have larger pectoral fins, presumably for fine scale maneuvering in the shallows (Walker, 1981; Hersh & Duffield, 1990; Mead & Potter, 1990, 1995; van Waerebeek et al., 1990; Wang et al., 2000). Offshore dolphins have higher blood concentration of hemoglobin presumably for increased oxygen required for deeper foraging dives (Duffield et al., 1983; Hersh & Duffield, 1990). Diet, and parasite burden linked to diet, varies between the two morphotypes (Walker, 1981; Barros & Odell, 1990, 1994; Mead & Potter, 1990, 1995; van Waerebeek et al., 1990; Barros, 1993; Barros et al., 2000). Finally, some populations of the two morphotypes can be distinguished genetically; genetic diversity generally increases with distance from shore (Curry & Smith, 1997; Hoelzel et al., 1998a; Wang et al., 1999, Möller & Beheregaray, 2001;
Natoli et al., 2004; Quéréuil et al., 2007; Rosel et al., 2009; Tezanos-Pinto et al., 2009).

_Dolphin Ecotypes_

Turesson (1922) originally defined ‘ecotype’ as “an ecological unit to cover the product arising as a result of the genotypical response of an ecospecies to a particular habitat.” Interpretation of this definition has been varied over taxa and between species. A more current definition is by Cronin et al. 2009) has been used: a group of individuals in which adaptations to a specific habitat have altered morphometrics, demography, and behavior of the group.

In the past, the designations of offshore and inshore dolphin morphotypes were synonymous with offshore and coastal ecotypes (Yang, 1976; Walker, 1981; Leatherwood & Reeves, 1982; Duffield et al., 1983; Hersh & Duffield, 1990; Mead & Potter, 1990, 1995; Ross & Cockcroft, 1990; Torres et al., 2003). Recently, there have been suggested three ecotypes due to a divergence of the inshore morphotype into coastal and estuarine ecotypes via “unpublished” new genetic and observational evidence (Waring et al. 2011). Waring et al. (2011) suggested that three ecotypes may exist; ‘offshore’ migrating throughout pelagic waters, ‘coastal’ residential or migrating in near shore ocean waters, and ‘estuarine’ inhabiting semi-enclosed bays, estuaries and rivers. The three can also be distinguished by stomach content analysis and stable isotope ratios (Barros, 1993; Worthy et al., 2008); however, comparatively little research has been conducted to disseminate between inshore ecotypes.
**Dolphin Populations**

Within ecotypes, discrete populations can be recognized via geographic overlap of individuals that have the ability to interbreed (Hartl, 2007). *T. truncatus* populations are smaller geographic groupings of individuals with overlapping home ranges and, to complicate matters, these can be migratory or residential (Warring et al., 2011). In *T. truncatus* studies, the term ‘population’ is the most widely used term to group individuals, regardless of proper designation. Discriminating separate populations of bottlenose dolphins has been challenging, specifically within inshore ecotypes; approaches have used observation of ranging patterns and distribution of genetic diversity (Torres et al., 2003; Waring et al., 2011). Classic population studies incorporating both methods have been conducted in Sarasota Bay, Florida (Wells 1987, 1991; Sellas et al., 2005), Shark Bay, Australia (Smolker et al., 1992; Krutzen et al., 2004; Möller et al., 2007), and Moray Firth, Scotland (Wilson et al., 1985; Parsons et al., 2002).

**Dolphin Communities**

Within dolphin populations, distinct communities can be recognized. Community structure is defined as a natural division within a network in which the members of a community are more densely connected to each other than to the rest of the network (Girvan & Newman, 2002; Croft et al. 2008). This means different things to different people when considering *T. truncatus*, for example “resident dolphins that share large portions of their ranges, exhibit similar distinct genetic profiles, and interact with each other to a much greater extent than with
dolphins in adjacent waters” (Waring et al., 2011). More often than not, these definitions are structured around social affiliations derived from association patterns (Murdoch et al., in prep).

Communities have been identified by residency patterns, geographic boundaries, social affiliations, and genetics. Originally ‘community’ was a behavioral-based term adapted from Wells et al. (1987), however combination studies using genetics plus social or ranging data, are appearing more readily in the literature (Parsons et al., 2003; Fazioli et al., 2006; Möller et al., 2006, 2007; Wiszniewski et al., 2010a). Fine scale data pertaining to community population dynamics are necessary to determine differences in dispersal, behavior, and social associations that may exist.

Conservation and Management Issues of *Tursiops truncatus* in Florida waters

Bottlenose dolphins are a key element of a bionetwork functioning as apex predators (Wells et al., 2004). Additionally, bottlenose dolphins are considered a sentinel species capable of detecting slight changes in the environment that may alter their physiology or behavior; these changes ultimately serve as a warning sign to potential risks for the ecosystems in which they reside (Reddy et al., 2001; Wells et al., 2004; Bossart, 2006). As a long-lived species, dolphin’s bioaccumulate pollutants and contaminants that can cause individual stress and indicate presence of compounds likely to stress organisms at lower trophic levels (Wells et al., 2004). Thus, conservation of this top trophic species is expected to help to conserve the flora and fauna of the ecosystem.
Dolphin habitat within protected bays and estuaries decreases in both quantity and quality, as coastal human colonization increases along the east coast of Florida (Woodward-Clyde, 1994; Culliton, 1998; USCB, 2010; FLDEP, 2011). Pollution from agriculture production and diversion of freshwater with canals continue to alter the habitat in which estuarine dolphins reside (Woodward-Clyde, 1994; Miles & Pleuffer, 1997; Sigua et al., 2000). Canal and other diversions empty directly into Florida's estuaries and have resulted in the detectable levels of contaminants, such as polychlorinated biphenyl (PCBs), in the blubber of estuarine dolphins (Fair et al., 2007). Additionally, coastal and estuarine dolphins along Florida's east coast share many health risks with humans that can be acquired through ingestion (mercury contamination) environmental contact (lobomycosis), or respiration (brevetoxins) (Durden et al., 2007; Murdoch et al., 2008; Kirkpatrick et al., 2004; Glibert et al., 2005). Increasing boat traffic and construction further obstructs usable habitat and limits accessibility of the estuarine water ways which have caused direct and indirect bottlenose dolphin mortality (Bechdel et al., 2009). Florida dolphins are also killed by entanglement or ingestion of fishing gear and crab pots (Waring et al., 2009).

Residential dolphin populations of Florida’s estuaries and coastal regions may not be capable of adapting as disease prevalence and climate change apply additional stress (Bossart et al., 2005; Scavia et al., 2002). These compounded anthropogenic and environmental stressors may play a part in unusual mortality events (UME’s) in which several members of a population suddenly die over a short period of time (MMPA [16 U.S.C. 1362]). Mortality events can signal a
negative shift away from equilibrium between a population and its surrounding ecosystem (Lecky, 2006). In addition to resolving spatial structuring in more detail, defining the genetic relationships among dolphin communities may help localize potential human threats, (e.g. habitat destruction, physical injury such as boat strikes or fishing line entanglement, competition for prey, or harassment) which may cause mortality (Fair & Becker, 2000; Bechdel et al., 2009; Stolen et al., 2007; MMPA [16 U.S.C. 1362]). Defining margins of community structure could pinpoint disease hot-spots within populations and offer insight to the possibility of transmission between communities as well as among adjacent populations. Additionally, impacts of mortality may be more significant on smaller spatial scales (Waring et al., 2011). Therefore, identifying the boundaries of an affected ecotype/population/community and understanding the levels of demographic and genetic exchange involved, should help identify factor(s) causing a mass mortality event. In general, identifying dolphin structure and assessing dispersal and breeding patterns at multiple spatial, taxonomic, and organizational levels for dolphins is of conservation relevance. This is not only for the effective management of Florida east coast bottlenose dolphins, but is a way to monitor the health of our shared environment.

Stock structure and the genetics of management

The National Oceanic and Atmospheric Administration (NOAA) has been tasked with differentiating groups of dolphins in US waters into “management stocks” as defined by the Marine Mammal Protection Act (MMPA) (Waring et al.,
According to the MMPA, a management stock is “a group of marine mammals of the same species or smaller taxon in a common spatial arrangement, that interbreed when mature” (MMPA [16 U.S.C. 1362]). A stock is interpreted as a demographically distinct population having limited genetic exchange with other nearby populations and thus regulated by female patterns of dispersal (Wade & Angliss, 1997). Due to the increase of research on dolphin population structure, particularly within estuaries bays and rivers, NOAA has recently suggested a more “biologically community based” approach to management of dolphin stocks.

NOAA research has divided offshore and coastal dolphins of the US east coast into several separate stocks for management purposes using both observational and/or genetic means (Waring et al., 2011). Along Florida’s east coast, there are four additional estuarine stocks, Jacksonville Estuarine System (JES), Indian River Lagoon Estuarine System (IRLES), Biscayne Bay, and Florida Bay (Waring et al., 2011). Previous behavioral research in these areas clearly indicates that individual bottlenose dolphins can have restricted ranging patterns, and in many cases organize themselves into a number of discrete social communities with varying degrees of spatial overlap within populations (Caldwell, 2001; Litz, 2007; Mazzoil et al., 2008; Rosel et al., 2009; Murdoch et al., in prep). Further, genetic analyses in Jacksonville, Biscayne Bay and Florida Bay reveal significant sub structure below the population level (Caldwell, 2001; Litz, 2007; Rosel et al., 2009).
Management and conservation would be improved by parallel work examining population sub-structure within the IRLES. For example, two UME’s in the IRLES, in 2001 and 2008, resulted in mass strandings of dolphins in the northern portion of the Lagoon (Waring et al., 2009). However, it is unknown whether this area spans the range of one community or several. No genetic data has been published to date on the genetic structure of bottlenose dolphin in or adjacent to the IRLES and adjacent Atlantic coast.

Using a combination of both mtDNA (maternally inherited) and nDNA (bi-parentally inherited) to determine differences between groups such as ecotypes, stocks, populations, or communities is common in studies of *T. truncatus*. Within both the mitochondrial and nuclear genomes are regions of coding and non-coding DNA. The former tend to be highly conserved within taxa, and therefore harbor too little variation for intraspecific studies. Non-coding regions, however, are typically not under any selective constraints, making these regions hyper-variable (Kimura, 1968, 1987) and more appropriate for population studies aimed at disentangling evolutionary processes from those behavioral and demographic processes (i.e., gene flow, drift, and mutation) that may shape current patterns of population structure. The majority of molecular changes within individuals are suggested to arise from mutations within non-coding regions (Kimura, 1968, 1987); meaning mutations are more likely to be fixed (or lost) due to drift than removed due to selection. Depending on the rate at which these hyper-variable sites mutate and using multiple loci that change at differing rates, we can determine how genetically differentiated one group may be from another.
Mitochondrial DNA’s d-loop (further addressed as mtDNA throughout this thesis) is useful in phylogeography because this DNA is not a product of recombination during sexual reproduction, and thus is passed down maternally to the next generation (Avise et al., 1987). Evolutionary relationships among matrilines can then be studied with the addition of geography which can offer great insight to structure at the intraspecific level (Wilson et al., 1985; Avise, 1989). Further it has a relatively rapid pace of evolution and it can be used to investigate genetic ancestry and create evolutionary trees (Brown et al., 1979). However, mtDNA cannot be used to detect male patterns of dispersal, interactions on a more recent timescale, or differentiation between closely related individuals.

Another source of presumably neutral genetic variation are microsatellites, which are short (1-6bp) tandem repeating sequences in nuclear DNA (nDNA) (also called Simple Sequence Repeats or SSR’s) (Alberts et al., 1994; Turnpenny & Ellard, 2005). Microsatellites are a product of nDNA replication errors (slippage) during recombination (ex. DNA repair or chromosomal crossover during meiosis) (Blouin et al., 1996). Because nuclear microsatellites are bi-parentally inherited and are highly polymorphic, they are useful molecular markers for discerning genetic relatedness of individuals or groups, estimating inbreeding levels ($F_{IS}$), describing demographic history, and assessing the magnitude and directionality of gene flow between populations (Dizon et al., 1997). Microsatellites can be used to explain genetic histories of populations in large-scale geographic regions in relation to biogeography (phylogeography) as
well as explain fine-scale phylogenies of closely related species (Blouin et al., 1996).

Analyses of the mtDNA control region and microsatellites have been used to examine population structure in bottlenose dolphin populations world-wide. Genetic differences between ecotypes, stocks, populations or communities will arise from restricted dispersal and gene flow of each of these groups. The detection of this restricted dispersal and gene flow between different groups of dolphins could result from: habitat utilization or niche specialization (e.g. resource specialization due to ecotype evolution; Hoelzel et al., 1998a; Sellas et al., 2005; Segura et al., 2006), geographic or hydrologic parameters (e.g. distance, depth, barrier islands, separate water bodies; Parsons et al., 2002; Torres et al., 2003; Bilgmann et al., 2007; Tezanos-Pinto et al., 2009; Wiszniewski et al., 2010a), community structure (e.g. kin based groups; Parsons et al., 2003; Fazioli et al., 2006; Möller et al., 2006, 2007; Wiszniewski et al., 2010b), or behavioral isolating mechanisms (e.g. sex specific dispersal, non-random mating; Krutzen et al., 2004; Parsons et al., 2006; Ansmann et al., 2012). Studies like these, as well as the current one, are important for resolving the genetic structure of *T. truncatus*, especially in inshore areas where dolphins are more susceptible to anthropogenic and environmental disturbances, as mentioned above (Wiszniewski et al., 2010a; Waring et al., 2011). Thus, defining genetic structure of bottlenose dolphins has been and will continue to be needed for biological knowledge of *T. truncatus*, as well as for the proper management and conservation of this species.
Study Site & Adjacent Waters

The Indian River Lagoon Estuary System (IRLES) is one of the largest Florida estuarine systems in which bottlenose dolphins currently reside (Virnstein, 1990; Sigua et al., 2000). The IRL spans 256 km (1/3 of the east coast of Florida) and is connected to the Atlantic Ocean by five inlets and one lock (Figure 1). The IRL estuary includes the Mosquito Lagoon, Banana River, Indian River, and the St. Lucie River Estuary. The average depth is 1.5 m and the width ranges from 0.93-9.3 km. This estuary is surrounded by five counties from north to south: Volusia, Brevard, Indian River, St. Lucie, and Martin. The IRLES is a combination of two biogeographic habitats with a break point near Canaveral National Seashores for marine species (Gosner, 1971; Cerame-Viva & Gray, 1996) and a break point near St. Lucie Inlet for estuarine species (Engle & Summers, 1999). Influenced by subtropical climate to the south and warm temperate climate to the north, the IRLES houses a diverse array of species which gives it the distinction as one of the most biologically diverse estuaries in North America (species review in Drumm & Kreiser, 2012; Virnstein, 1990; Sigua et al., 2000). It was designated as an Estuary of National Significance in 1990 and a Comprehensive Conservation and Management Plan was created to protect this ecosystem (IRLCCMP, 1996).

As part of the conservation plan, several species of plants and animals have been identified as key resources within these waters, including the bottlenose dolphin. The IRL dolphin population has been studied since the 1970’s through the information obtained from stranded individuals, photo-
identified groups, and live capture/release events (Odell & Asper, 1990; Mazzoil et al., 2005, 2008; Bossart et al., 2006). These studies suggest that IRL dolphins are residents. Mazzoil (2008) defined three communities by a series of overlapping individual home ranges; a northern community connected to a southern community only by overlapping home ranges of a central community. A more recent study of dolphin association patterns in combination with kernel density analysis of dolphin utilization distributions using social network and time-lag analysis revealed a total of 6 dolphin communities in the entire estuarine system (Murdoch et al., in prep.) No genetic analysis of these communities has been completed, to date. Existence of community structure within the estuarine system, recurrent UME’s, and lack of current population abundance estimates causes concern: NOAA considers the IRL a “strategic” stock and a cause for concern, meaning minimal mortalities would cause a decline in population abundance and threaten sustainability (Lonergan, 2011).

Adjacent waters outside of the lagoon, along the Atlantic Ocean, are home to two known Florida coastal bottlenose dolphin populations determined through photo-identification and genetic research conducted by NOAA (Waring et al., 2009). There are a number of lines of evidence that suggest there may be mixing between these coastal stocks and the estuarine IRLES dolphin stock. First, current photo-identification studies have recorded minimal mixing of the coastal and estuarine populations (Mazzoil et al., 2011a). Monthly boat based surveys were conducted (2006-2008) outside of the IRL along the parallel Atlantic (ATL) coastal waters, identifying over 221 dolphins not previously recorded within the
Six dolphins were observed out of their known habitat range: four IRL dolphins were observed once within 1 km outside of an ocean inlet, and two ATL dolphins immigrated to the estuary (Mazzoil et al., 2011a). Second, a ‘point contact’ skin disease (*Lacazi lobo*) was recently identified in the coastal populations which was previously thought to be endemic only to the estuarine population (Murdoch et al., 2008, 2010), suggesting contact between the estuarine and coastal dolphins. Third, the five inlets allow for physical movement. To date, the degree of historic and contemporary mixing between these coastal and estuarine ecotypes is unknown.

The east coast of Florida is also home to other estuarine bottlenose dolphin populations. To the north of the IRL, the Jacksonville Estuarine System (JES) combines the St. Mary’s River, Amelia River, Nassau Sound, Fort George River and St. John’s River (Waring et al., 2009). This brackish habitat is similar to the IRL in that the Intracoastal Waterway shipping lane runs through estuarine tidal marshes and riverine systems averaging 2m in depth connected via few inlets to the Atlantic Ocean (Caldwell, 2001). Within the JES and adjacent Atlantic waters, Caldwell (2001) observed three communities (the term community was used interchangeably within her text with ‘populations’ and based on associations and genetic differentiation) through photo-identification analysis (Caldwell, 2001). Genetic differentiation among bottlenose dolphins at mitochondrial DNA and microsatellite loci supported the behavioral discrimination of the three communities: northern, southern and coastal (Caldwell, 2001; Rosel et al., 2009). The northern community was highly significantly genetically
differentiated from both the southern and coastal communities, however lower differentiation was observed between the southern and coastal communities. Genetic differentiation between JES and IRLES allow has not been assessed, thus NOAA considers JES to be a strategic stock (Warning et al., 2011).

To the south of the IRL, Biscayne Bay is a large, predominantly shallow embayment in Miami-Dade county, running 13 km wide and 56 km long (Waring et al., 2009). This bay is similar to the IRL in some general characteristics: 5m average depth with restricted water movement in some areas (specifically from island construction within the estuary) and unrestricted exchange with the Atlantic Ocean and Florida Bay through grass flats, tidal channels, and inlets (Waring et al., 2009). However, these bays are much wider and deeper in places and have more ocean access. Within the Biscayne Bay community (defined using definitions from Wells et al., 1987; Waring et al., 2009), two groups of dolphins, north and south, were designated based on photo-identification (Litz, 2007). Although the northern dolphins were significantly genetically differentiated from southern dolphins at nuclear markers, there was non-significant differentiation at mtDNA, perhaps due to differences in the power of analysis (Litz, 2007). Litz (2007) did find Biscayne Bay dolphins were significantly different in both mtDNA and nuclear DNA from Florida Bay supporting those as demographically distinct populations (Waring et al., 2009).

Dolphins within estuaries, sounds, and bays along the Gulf of Mexico (GOM) are also studied and managed using a community-based approach (Waring et al., 2009, 2011). Using both observational and genetic data, over 32
areas of enclosed or semi-enclosed bodies of water have been identified along the GOM shorelines as distinct bottlenose dolphin stock habitats (Waring et al., 2011).

Although the IRLES has similar estuarine qualities to other estuaries in Florida and the Gulf of Mexico, the IRLES has a unique linear shape to other areas, particularly long and narrow. The IRLES spans at least two geographically discrete water bodies: IRL proper and Mosquito Lagoon (ML), which were artificially connected in the 1850’s by the digging of Haulover Canal (Knetsch, 1994). Prior to Haulover and the creation of Canaveral lock system during the same time period, the northern portions of the IRL proper were isolated from Atlantic waters. Further, the first accounts of dolphins in this area were around the 1920’s from local fishermen and the first major documentation was the 1970’s when many dolphins were taken from the IRLES for the aquarium trade (Reeves & Leatherwood, 1984; Scott, 1990).

Study objectives

The objectives of this thesis were to discover and determine if population structure and dispersal are present between IRLES dolphins and adjacent Atlantic coastal dolphins and within the IRLES itself, and if possible, offer a definition for the grouping of bottlenose dolphins that reside within the IRLES as a separate ecotype, population, or community.

In this thesis, I used genetic methods in an attempt to understand patterns of differentiation within and between estuarine populations and adjacent coastal
bottlenose dolphins using four grouping strata: “Ecosystems” (estuarine, coastal), “Water bodies” (IRL, ATL, ML), hydrologic “Segments” (1A, 1B, 1C, 2, 3, 4), and social “Community” boundaries (C1, C2, C3, C4, C5, C6) (Figure 1). My four null hypotheses are:

\[ H_0^1 \text{ – Bottlenose dolphins sampled within the IRLES cannot be genetically differentiated from Atlantic Ocean coastal dolphins} \]

\[ H_0^2 \text{ – Bottlenose dolphins sampled within the Mosquito Lagoon cannot be genetically differentiated from bottlenose dolphin within IRL proper or coastal Atlantic Ocean} \]

\[ H_0^3 \text{ – Bottlenose dolphins sampled within the IRLES cannot be genetically differentiated by habitat boundaries (segments)} \]

\[ H_0^4 \text{ – Bottlenose dolphins sampled within the IRLES cannot be genetically differentiated by social boundaries (communities)} \]

My predictions were:

\[ H_0^1 \text{ – Yes, genetic differentiation between IRLES bottlenose dolphins and Atlantic Ocean coastal dolphins will be detected} \]

\[ H_0^2 \text{ – Yes, genetic differentiation between Mosquito Lagoon bottlenose dolphins from the IRL proper and Atlantic Ocean coastal dolphins will be detected} \]

\[ H_0^3 \text{ – Yes, genetic differentiation within IRLES bottlenose dolphins via segment boundaries will be detected} \]
$H_04$ – Yes, genetic differentiation within IRLES bottlenose dolphins via community boundaries will be detected

To test these hypotheses, 511 *T. truncatus* sampled from throughout the study area were genotyped at 11 markers (d-loop from the mtDNA control region and 10 nDNA microsatellites). Traditional F-statistics and Bayesian assignment methods were used to test each hypothesis. Inferences about the IRLES dolphins regarding their genetic structure within and in relation to the dolphins of the adjacent coastal waters were made, specific to the four hypotheses stated above.
MATERIALS AND METHODS

Sample collection and DNA extraction

Skin samples (n=511) collected from bottlenose dolphins stranded/beached during 1999-2009 and captured/released during health and ecological studies during 2003-2007 in the IRLES study area (NOAA permit no. 998-1678-00), were archived for future processing. Skin was excised immediately preserved in a mixture of saturated sodium chloride (NaCl) and 20% dimethly sulfoxide (DMSO), and stored in -20°C freezer (Seutin et al., 1991). Skin samples were removed from storage solution, rinsed with deionized water, and approximately 10-20 mg of skin was taken for DNA extraction. Cell lysis and protein digestion and was facilitated using FastPrep™ vortexing instrument (BIO 101, Carlsbad, California, USA). Using a standard NaCl method described by Miller et al. (1988), DNA was extracted from the homogenized tissue, precipitated in ethanol, and stored overnight at -20 °C. After resuspension in TE buffer, the concentration and quality of the purified DNA from all samples were estimated the following day by spectrophotometry (NanoDrop, Thermo Fisher Scientific, Wilmington, Delaware, USA).

Sites where live animals were captured or deceased dolphins were recovered were marked by GPS coordinates and used in analyses. Samples
from dead IRL dolphins were used in spatial analysis because they are known to
die and wash ashore within (90%) or close to (<10%, under 17 km) their known
home range (Mazzoil et al., 2011b). Location of each sample was plotted in
ArcGIS and samples were categorized in relation to the four hypotheses H01:
“Ecosystems” (estuarine, coastal), H02: “Water bodies” (IRL, ATL, ML), H03:
hydrologic “Segments” (1A, 1B, 1C, 2, 3, 4), and H04: social “Community”
boundaries (C1, C2, C3, C4, C5, C6) (Figure 2a-2d respectively). Hydrologic
segment boundaries were determined from analyses of geology and hydrology of
the IRL by the South Florida Water Management District (Woodward-Clyde,
1994) and represented a habitat based approach to clustering animals into
groups. Community boundaries, based on network analysis of social affiliations
among known dolphins across a 7 year study, were determined by kernel density
analysis of individuals with high association indices (Murdoch et al., in prep);
these boundaries represented a biological approach to grouping individuals.

*Mitochondrial DNA sequencing and analysis*

Mitochondrial DNA was sequenced using a polymerase chain reaction
(PCR), direct sequencing approach. Primers B (5’-TACCAATGTATGAAA-
CCTCAG-3’; H00034 in Rosel et al., 1995) and Threonine (5’-
TCAAAGCTTACACCAG-TCTTGTAAACC-3’; L15926 in Kocher et al., 1989)
were used to amplify the target 596 base pair segment of the mtDNA control
region. Amplification was performed using a 2720 ABI Thermo Cycler and
reactions contained approximately 1μL of 10ng genomic DNA, 0.25μL *Tag DNA*
polymerase (ABI/Life Technologies, Grand Island, New York, USA), 0.75\(\mu\)M of each primer, 0.375 mM of deoxyribonucleotide triphosphates (dNTP’s), 2.5\(\mu\)M of a 2.0mM MgCl₂ 10X buffer solution, and 19.375\(\mu\)L of H₂O. Thermocycling conditions included an initial 2.5 minute 90°C denature, followed by 30 cycles of 45 second denature at 94°C, 60 second annealing at 48°C, and 90 second extension at 72°C. The PCR was concluded with a final extension period of 5 minutes at 72°C.

Amplified DNA was cleaned with 3\(\mu\)L of ExoSap (Affymetrix, Santa Clara, California, USA) added to 7\(\mu\)L of PCR product and incubated for 15 minutes at 37°C followed by 15 minutes at 80°C. The sequencing reaction used the same forward and reverse primers listed above, and two separate reactions (one for each primer) contained 3.5\(\mu\)L of PCR product, 1\(\mu\)M primer, 1.5\(\mu\)L of Big Dye v.3.1 (ABI/Life Technologies, Grand Island, New York, USA), and 4\(\mu\)L of MQ H₂O. Thermocycling conditions included an initial 60 seconds 96°C denature, followed by 35 cycles of 10 second denature at 96°C, 5 second annealing at 50°C, and 4 minute extension at 60°C.

The sequencing preparation was modified from the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol. Ethanol concentration (95%, 40%) and volume (20\(\mu\)L, 42\(\mu\)L) varied for both centrifuge steps respectively, and time was decreased by 5 minutes for the second spin. The precipitate was then re-suspended in formamide (not injection buffer as protocol states), quickly spun to remove bubbles, and heat shocked at 90°C for 2 minutes. Plates were then
sequenced on a 3130 ABI Genetic Analyzer located in the Population Biology and Behavioral Ecology Lab at Harbor Branch Oceanographic Institute @ FAU. Analysis and alignment of sequences was conducted using SeqScape® and SeqAnalysis® programs to determine individual haplotype.

From 511 dolphin tissue samples analyzed, 308 yielded high quality data for 596bp of the mitochondrial DNA control region. However one third of the samples would not amplify after multiple extraction attempts, possibly due to DNA degradation or poor preservation. Additionally, several samples were not fully sequenced in both directions, and were eliminated from analysis. To reduce the possibility of mis-calls and contamination and to reinforce the dependability of the data, we conducted a secondary blind extraction of all distinct haplotypes, alignment of both 5’ and 3’ strands, and blind independent review and verification of haplotypic calls.

**Microsatellite genotyping and analysis**

From 511 dolphin tissue samples analyzed, 244 were genotyped at 10 independent polymorphic loci. Initial optimization of 20 previously published microsatellites resulted in 10 usable loci. Each microsatellite primer required different optimization protocols (Table 1). Adenylation, or the random addition of an A from Taq polymerase during the PCR process, was promoted via the addition of a G to the 5’ end of all reverse primers to ensure consistency in scoring.
Amplified DNA was prepared for genotyping by combining 13.75μL Hi-Di Formamide (ABI/Life Technologies, Grand Island, New York, USA), 0.25μL GeneScan 500-ROX (ABI/Life Technologies, Grand Island, New York, USA) and 1μL PCR product into each well of a 96well plate. Microsatellites were identified through gel electrophoresis using a 3130 ABI Genetic Analyzer located in the Behavioral Ecology and Population Biology Lab at Harbor Branch Oceanographic Institute @ FAU. Scoring of loci was conducted using GeneMapper (ABI/Life Technologies, Grand Island, New York, USA).

Loci were eliminated if allelic dropout (non-amplifying allele), null alleles (homozygote excess) and stutter bands/slippage were found by MICROCHECKER v.2.2.3 (Van Oosterhout et al., 2004), resulting in n=244 individuals genotyped over 10 loci. Mendelian inheritance was verified using known pedigrees (n=8 mom/calf pairs) and reproducibility was determined from n=8 individuals sampled twice or more over different years. Repeated verification/standardizations were conducted to ensure consistency throughout the study.

**mtDNA data analysis**

Variation within mtDNA was assessed by determining the number of unique haplotypes and number of variable sites found within the d-loop sequence using MEGA 5.1 (Tamura et al., 2011). ARLEQUIN 3.5 (Excoffier & Lischer, 2010) was used to calculate haplotype diversity, the probability that two randomly
selected individuals have different haplotypes \( (h; \text{Nei} \& \text{Tajima}, 1981) \), and nucleotide diversity, the average number of differences between homologous nucleotides in two sequences \( (\pi; \text{Nei}, 1978) \). Genetic differentiation using haplotype frequencies, \( F_{st_{mlDNA}} \) \( (\text{Wright}, 1965; \text{Weir} \& \text{Cockerham}, 1984) \) and haplotype frequencies combined with interhaplotypic evolutionary distances \( ST \) \( (\text{Tamura} \& \text{Nei}, 1993; \text{Weir} \& \text{Cockerham}, 1984) \) were estimated by ARLEQUIN 3.5 using an analysis of molecular variance framework (AMOVA) \( (\text{Excoffier} \& \text{Lischer}, 2010) \). Statistical homogeneity tests were conducted via 50,000 permutations of the original data. A median-joining (MJ) network of unique haplotypes was created using MEGA 5.1 and Adobe Photoshop 7.0 \( (\text{Tamura et al.}, 2011, \text{Adobe Systems Incorporated 1990-2002}) \).

**Microsatellite data analysis**

Hardy-Weinberg (HWE) and linkage equilibrium tests were computed with GENEPOP 4.0.10 \( (\text{Hardy 1908}; \text{Weinberg 1908}; \text{Raymond} \& \text{Rousset}, 1995; \text{Rousset 2008}) \). Sequential Bonferroni correction \( (\text{Rice}, 1989) \) was applied to adjust the significance levels for multiple tests. \( F_{st_{msat}} \) was calculated in both ARLEQUIN 3.5 and GENEPOP 4.0.10 for cross comparison, and \( R_{st} \), a measure that includes inter-allelic genetic distance, was generated in GENEPOP 4.0.10 \( (\text{Wright 1965}; \text{Slatkin 1995}; \text{Excoffier} \& \text{Lischer}, 2010; \text{Raymond} \& \text{Rousset}, 1995) \). Estimation of genetic diversity \( (H_e, H_o \text{ and no. of alleles}) \) for each locus was computed using GENEPOP 4.0.10 \( (\text{Raymond} \& \text{Rousset}, 1995) \).
We used STRUCTURE 2.3.3. (Pritchard et al., 2000), a Bayesian clustering analysis using Gibbs sampling MCMC methods (Geman & Geman, 1984), to investigate population and community structure by assigning individuals to populations both with and without a priori divisions for all hypotheses (Ecosystems, Water bodies, Segments, Communities). The current version of the program STRUCTURE altered the original model to incorporate location information as a prior distribution, and this modification was shown to detect lower levels of divergence with less data than the original version (Hubisz et al., 2009). Individuals were assigned into clusters such that Hardy Weinberg and Linkage Equilibriums were maximized (Pritchard et al., 2000). Log likelihood values \( \text{Ln} \ P(X|K) \) were created from running potential models \( H_01=7, H_02=4, H_03=7, H_04=6 \) of \( K \) (number of population clusters given the data) using a 50,000 burn-in over 1,000,000 iterations, and the average of 5 independent runs of all models were used to determine \( K \). Runs were performed both with and without admixture to discover mixture, if present, and to verify migrants.

Patterns of isolation by distance (IBD) were sought using \( Fst \) values verses distance \( (km) \) between each segment centroid point. IBD, reported as \( r \), was calculated using a Mantel test at 100,000 iterations via ARLEQUIN 3.5 (Excoffier & Lischer, 2010) and in Excel with PopTools 2.7 5 (Hood, 2006) for cross comparison. Assignment testing and stock mixture were conducted using BAYES (Pella & Masuda, 2001; Masuda, 2002), a Bayesian stock mixture analysis using FORTRAN routines (Lahey, 1997) and MCMC sampling to assign individuals of questionable origin to baseline stocks. Assignments were also
verified from Q-values reported in STRUCTURE 2.3.3 (Pritchard et al., 2000). Dispersal and migration rates, immigration ancestry, and identification of non-immigrants and their offspring were conducted using BAYESASS 1.3 (Wilson & Rannala, 2003), a Bayesian method using MCMC that allows deviations from HWE.

Additional H05 Data Analysis (to be identified in results below)

Approximately half of the samples with complete genetic profiles (n=116 of 244) have detailed home range data from sightings collected between 1996-2013 as part of an ongoing photo identification study within the IRLES and along adjacent portions of ATL coastline (details in Mazzoil et al., 2004; Mazzoil et al., 2005, 2008). Data from the photo-identification database (n=2261 sightings over 17 years) used when investigating migrants included: home range, latitude and longitude of sightings of individuals, year and month sighted, total number of sightings, birth year, and field estimated and total group size the individual was sighted with. Additionally, individual biological and morphometric data from strandings and live capture events (n=104) offered information on sex, length, and weight.
RESULTS

$H_{01}$-Ecosystems

A total of 308 samples yielded 18 mtDNA haplotypes (Table 2); 9 were observed in estuarine waters, 2 of which were private and 16 were observed in Atlantic coastal waters, 6 of which were private (Table 3). Six haplotypes were shared between the two ecosystems. One haplotype, Hap #1, was found in 94% of estuarine samples and 41% of coastal samples (Figure 3). The estuarine samples had lower haplotypic diversity ($h$=0.1017) and much lower nucleotide diversity ($\theta$=0.0003) than coastal samples ($h$=0.7913, $\pi$=0.0143) (Table 4a).

Similarly, the IRLES had lower heterozygosity ($H$) than coastal samples (0.575 vs. 0.703) for all 10 microsatellite loci combined (Table 3). Estimates of genetic diversity ($He$, $Ho$, and no. of alleles) for individual microsatellites were similar for both ecosystems (Figure 4). Four loci of 10 were found to be significantly out of Hardy-Weinberg Equilibrium (HWE) after Bonferroni correction (Rice, 1989). These departures from HWE were thought to be attributed to a biological factor or violation of HW assumptions (possibly non-random mating; Halliburton, 2004) or a sampling bias versus a mechanical problem with the loci. To verify this theory and validate dependability and use of the loci in further analyses: (1) all
loci were verified in MICROCHECKER showing no significant evidence for null alleles, allelic dropout, or scoring errors due to stuttering, (2) the data were redistributed and analyzed by Segment for $H_0$3 (discussed below) revealing four loci deviated from H-W randomly between loci and location, (3) analyses were repeated without these four loci; this did not significantly change the patterns of differentiation between the ecosystems as reported below. All but one combination of loci (TTr04 & KWM12) were in linkage equilibrium, but this combination conformed after Bonferroni correction.

Significant genetic differentiation was observed between ecosystems for both mtDNA and microsatellite markers, however the microsatellite value was substantially lower than mtDNA value ($F_{st_{mtDNA}}=0.414$, $F_{st_{msat}}=0.057$; $p<0.05$) (Table 4a). Similar differences when including genetic distance in the estimate of heterogeneity were also observed ($\Phi_{st}=0.329$, $p<0.05$; $R_{st}=0.077$) (Table 5a). Bayesian clustering analysis consistently chose two population clusters were most likely given the data ($K=2$), regardless of whether location priors were used or whether a model of admixture or non-admixture was applied (Table 6, Figure 5a). The 2 clusters were largely concordant with coastal (ATL) and estuarine (IRL proper) individuals (Figure 6a). However, half ($n=13$) the individuals in Mosquito Lagoon (ML) and a few throughout the rest of the estuarine system ($n=13$), were assigned to the coastal cluster with a high degree of confidence in all analyses; additionally, 7 of the 8 rarer estuarine mtDNA haplotypes were also found in these individuals. Migration and dispersal estimates (ATL into IRL=$0.015 \pm 0.03$; IRL into ATL=$0.155 \pm 0.24$) between the ecosystems estimated from
BAYESASS, however we caution the interpretation of these results as the data was found to not contain enough information to produce accurate results (Table 7).

\( H_0 \)2-Water bodies

By separating estuarine samples into two water bodies (ML and IRL proper), the mtDNA haplotype assemblage slightly shifted: 8 were observed in the IRL proper, 3 in the ML, and 16 in the ATL with 2 haplotypes shared among all three water bodies (Table 3). The IRL proper samples had the lowest haplotypic diversity \((h=0.0944)\) and lowest nucleotide diversity \((\pi=0.0003)\) versus both the ATL and ML samples \((h=0.7913, \pi=0.0143; h=0.2037, \pi=0.0005, \text{respectively})\) (Table 4b), with the ML intermediate in estimates of genetic diversity between the IRL and ATL. Similarly, the IRL proper had the lowest heterozygosity \((\hat{h}=0.575)\) when compared to ATL and ML samples (0.703, 0.595) for all 10 microsatellite loci combined (Table 3).

The ML was differentiated from the IRL proper for both mtDNA \((F_{st_{mtDNA}}=0.0201, p<0.09)\) and nDNA \((F_{st_{msat}}=0.0234, p<0.05)\) (Table 4b). The ML was also differentiated from the ATL \((F_{st_{mtDNA}}=0.1670, F_{st_{msat}}=0.0180, p<0.05; \text{Table 4b})\) but the level of differentiation was intermediate to that between ATL and IRL proper. Considerably larger genetic distance was observed between the ATL and IRL proper for both mtDNA \((\Phi_{st}=0.332, p<0.05)\) and microsatellites \((R_{st}=0.085)\) versus the ATL and ML \((\Phi_{st}=0.109, p<0.05; R_{ST}=0.027)\) (Table 5b). Bayesian clustering analysis marginally supported \(K=3\)
when using location prior and admixture; all other combinations supported $K=2$
as with the $H_01$-Ecosystem analysis (Table 6, Figure 5b). The three clusters did
not reveal further geo-spatial structure distinguishing the ML from the ATL or IRL,
rather exhibited minimal support for differentiation within the ATL (Figure 6;
bottom image).

$H_03$-Segments

MtDNA haplotypic diversity ($h$) ranged from 0.047-0.204 and nucleotide
diversity ($\pi$) ranged from 0.0001-0.0005, with the highest values for both in
segment 1A (ML) (Table 4c). Observed heterozygosity ($H$) across all nuclear loci
for segments, were similar and ranged from 0.546-0.604 (data not shown).

Low levels of genetic differentiation were detected among dolphins divided
into habitat segments and microsatellite estimates were considered significant at
$\alpha = 0.05$ (Table 4c). The largest $Fst$'s were observed between segment 1A (ML)
and all others, and the lowest $Fst$'s were observed between Segments 1B and
1C (Northern IRL proper). All pairwise estimates of differentiation were non-
significant ($p>0.1$) $\Phi st=0.019/RST=0.08$ with the marginal exception of segments
1A to 1B for $\Phi st=0.025$, $p \leq 0.06$ (Table 5c). The results of STRUCTURE analysis
were the same as $H_01$-Ecosystems and $H_02$-Water Bodies; with geo-structure for
both $K=2$ and $K=3$ generally concordant with ATL-IRL proper split with ML a mix
of the two predominant clusters (Table 6, Figure 5c). Using a Mantel test,
differentiation was only positively correlated with geographic distance among
habitat segments when data was analyzed including segment 1A (ML) in the
analysis \((r = 0.67, p \leq 0.05)\) but was negatively correlated when excluded \((r = -0.099, p \leq 0.05)\) (Table 8, Figure 7).

**H04-Communities**

MtDNA haplotypic diversity \((h)\) ranged from 0.000-0.165 and nucleotide diversity \((\pi)\) ranged from 0.0000-0.0004 in the six social communities identified to occur in the inshore waters of the IRLES, with the highest values for both markers in community C2 in ML (Table 4d). Observed heterozygosity \((H)\) across all loci for communities were similar (C1 excluded due to \(n=1\)), and ranged from 0.527-0.661 (data not shown).

Similar to segment analysis, minimal differentiation was found among social communities (Table 4d). The largest pairwise differentiations involved community C2, where the core area falls within the majority of the ML. Note that smaller sample sizes in the same strata (\(n=172\text{mtDNA}/156\text{msat}\)) compared to \(H03\)-Segments, likely influenced power to detect significant differences. All values found for genetic distance parameters were non-significant and/or negative (Table 5d). Bayesian clustering analysis marginally supporting \(K=3\) when using location prior and admixture; all other combinations supported \(K=2\) concurrent with \(H01\)-Ecosystem, \(H02\)-Water Bodies and \(H03\)-Segments analyses (Table 6, Figure 5d).
**H₀₅-Mosquito Lagoon Mixed Stock**

All cluster analyses consistently grouped ML individuals with either the ATL or IRL proper clusters with few \( n=4 \) exhibiting similar Q-values for both. Assuming therefore that the ML might be a mixture of IRL and ATL animals, we entertained a fifth hypothesis \( (H₀₅- “Mosquito Lagoon is not a combination of two genetically discrete mixed stocks”) \) and conducted a Bayesian stock mixture analysis using BAYES (Pella & Masuda, 2001). Individuals are either strongly assigned to IRL \( (n=11) \) or ATL \( (n=13) \) (with posterior probabilities \( >0.8 \)) (Table 9a). These results mirrored that of the Q-values STRUCTURE provided using location priors and admixture, however STRUCTURE reported four individuals within ML as “partial genotypes” defined as having similar genotypic ratios of each stock (Pritchard et al., 2000) (Table 9b).

Of the 13 ML samples with ATL genotypes, 11 are known residential individuals who have recurrent sighting histories within the IRLES. Using ATL Q-values generated in STRUCTURE and data derived from previous studies, we ran simple correlations to determine if any other data besides genotype could identify an estuarine individual as an ATL migrant (Pritchard et al., 2000). No significant correlations \( (r = -0.096 – 0.227) \) were found in estuarine individuals assigned ATL genotypes when using length, weight, sex, sighting history in months, birth year, and field estimate or total group size as independent variables (Table 10). Three additional variables were found to have significant \( (p<0.05) \) relationships with ATL Q-values: a weak positive relationship \( (r = 0.1628) \) using sighting history by year, a moderate positive relationship \( (r = \)
0.424) using longitude, and a moderate negative correlation ($r = -0.318$) using latitude (Table 10), the latter two consistent with the shape and geographic position of the IRLES and Atlantic Ocean.
DISCUSSION

This study has shown that the IRLES and ATL dolphins are genetically differentiated from one another, dolphins within ML are a mixed stock of estuarine and coastal dolphins, and minimal substructure was found within the IRLES. I discuss these findings with regard to the hypotheses originally proposed.

\( H_{0}1\)-Ecosystems

My data reject the null hypothesis of no differentiation between ATL and IRLES dolphins. Individuals within embayment’s have previously been found to be genetically differentiated from adjacent coastal populations in eastern Florida (inshore Jacksonville v. coastal north-eastern Florida; Caldwell, 2001), in the Gulf of Mexico (4 inshore populations - Sarasota Bay, FL, Tampa Bay, FL, Charlotte Harbor, FL and Matagorda Bay, TX v. one coastal Gulf of Mexico population; Sellas et al., 2005) and in south-eastern Australia (inshore v. coastal population; Möller et al., 2007).

Our study reports extremely low haplotypic and nucleotide diversities for mtDNA within the IRLES compared to these studies; most with smaller sample numbers in smaller study sites. Caldwell’s (2001) Southern seasonally resident community was the only comparable group for both haplotypic diversity (\(h=0.136\))
and nucleotide diversity ($\pi=0.0004$) in the literature to date. The Southern community’s limited mtDNA diversity was suggested to be a result of matrilineal social groups with female philopatry to foraging habitat or mating grounds. Many bottlenose dolphin studies report limited movement of females across all areas of the world (Krutzen et al., 2004; Möller & Beheregaray, 2004; Natoli et al., 2005; Sellas et al., 2005; Parsons et al., 2006; Möller et al., 2007). While female philopatry may be a common and acceptable explanation for the IRLES’s low mtDNA diversity, additional elements which may also limit genetic diversity include: (a) a recent founder event by one maternal group, (b) a genetic bottleneck event in which diversity is lost, or (c) geographic isolation leading to inbreeding and loss of allelic diversity. The evidence for and against these explanations are described below.

(a) recent founder event: Despite the IRL estuary population’s low haplotypic and nucleotide diversity, we report a high $\Phi_{st}$ value (when compared to the coastal samples) which suggests this low diversity may be long standing within the estuary. Founder events for inshore areas have been suggested for several bottlenose dolphin populations around the world where lower overall genetic diversity was found the farther inland one sampled (Hoelzel et al., 1998a; Natoli et al., 2004; Sellas et al., 2005; Möller et al., 2007; Tezanos-Pinto et al., 2009). When blasted on GenBank®, all our haplotypes were 97-99% matched with Quérouil et al.’s (2007) North East Atlantic haplotypes, which were found to be non-differentiated from the North West Atlantic Pelagic reported haplotypes
(i.e. the offshore ecotype of Florida). Past estimates of abundance for the IRLES in 2008 estimated a minimum of 615 distinctly identified individuals from vessel based surveys and recent estimates are suggested to range between 600 - 1,300 dependent on season via aerial surveys (Mazzoil et al., 2008; Durden et al., 2011). This large population size with limited matrilines could be possible via a historic founder event followed by a significant population expansion over many generations. The first records of dolphins in the IRLES date only to the last century; non-published accounts were around the 1920’s from local fishermen and between the late 1930’s-1950’s during aquarium trade captures for display, published accounts from wild observational studies have been ongoing since the 1970’s (Odell & Asper, 1990; Mazzoil et al., 2005, 2008; Waring et al., 2009). Further, life history data including fecundity and mortality rates for the IRLES dolphins suggest population growth between 0- 4.6% per year (Stolen & Barrow, 2003). Stolen & Barrow (2003) constructed a life table from age-at-death data, and inferred the IRLES population was approximately 250 individuals between 1978 and 1997. Thus using their stable estimated population growth at maximum 4.6%, the population should be around 1,200 presently and this estimation is near Durden et al.’s 2011 abundance maximum of 1,300 individuals. However, this simple calculation does not include increasing threats to mortality that IRLES dolphins have been exposed to over the past 35 years, and as mentioned in the introduction, play a significant part in dolphin survival. These findings form the support that (a), a recent founder event of one maternal group may be part of the explanation for low mtDNA diversity in the IRL.
(b) genetic bottleneck: Although we report low haplotypic diversity ($h$) within the IRL, many of the odd haplotypes within the estuary are singleton’s just a few steps diverged from one dominant haplotype. If a genetic bottleneck had occurred in the populations past, it would be expected that the most common haplotypes would be retained and the rarest haplotypes would be lost, often resulting in a sparse median joining network of a few distantly related haplotypes (Halliburton, 2004). Furthermore after such events occur, changes in patterns of diversity specifically resulting in sparse allelic distribution, can be seen during population expansion. An example of this can be seen in *T. truncatus* mtDNA haplotypes after mass mortality events (Rosel et al., 2009). A bimodal distribution of haplotype frequencies has been observed for recolonization of *T. truncatus*, as seen in coastal Virginia from the 1997-1998 epizootic morbillivirus die off along the western North Atlantic west coast and in the Gulf of Mexico from multiple HAB mortality events between 2000-2006 (Schulman et al., 1997; Gaydos, 2006; Rosel et al., 2009). Additionally, reduced diversity at the nuclear level would also have been expected. Within the IRLES in our study, the presence of rare haplotypes, the short relative distance between those haplotypes, and high levels of heterozygosity in nuclear markers suggest no such bottleneck has recently occurred; thus option (b), a genetic bottleneck, offers little help as explanation for the low mtDNA diversity in the IRLES.

(c) geographic isolation: Although there are several freely accessible inlets for both coastal and estuarine animals to disperse, this has not resulted in one
homogeneous inshore ecotype. Rather, higher pairwise $F_{st_{mtDNA}}$ versus
substantially lower $F_{st_{msat}}$ between the estuarine and coastal ecosystems is
consistent with male biased gene flow and philopatry of females to the IRLES.
This is a commonly reported for inshore *Tursiops sp.* along Australia's coasts
(Krutzen et al., 2004; Möller & Beheregaray, 2004; Möller et al., 2007), but other
studies report both sexes as philopatric (Natoli et al., 2005; Sellas et al., 2005;
Parsons et al., 2006; Rosel et al., 2009). Although several studies support similar
dispersal rates for both sexes in cetaceans, males are generally expected to
have higher dispersal ranges than females while still retaining their natal habitat
within their adult home range (Connor et al., 2000). It is suggested that
cooperative behaviors between related males to breed with females likely
increase fitness benefits for both males, thus supporting Connor’s theory
(Hamilton, 1964; Krutzen et al., 2003). This extended movement of males
between the IRLES and adjacent coastal animals was also detected during
clustering analysis in the form of ATL migrants, although both sexes were found
to be both immigrating and emigrating from the IRLES. Dispersal rates could not
be estimated from my data; however, dispersal was a full magnitude higher from
the estuary into the coast than the coast into the estuary. These findings above
do not sufficiently support (c) the idea that the IRLES is a genetically isolated
population from the male perspective, though we could argue that females are
genetically isolated. However, for isolation to cascade into loss of diversity (i.e.
genetic drift toward allele convergence or fixation within a population), the IRLES
population would have to be relatively small for a significant period of time to
reduce genetic variation within the population (Halliburton, 2004). This is further supported by the dramatic mtDNA differences between the estuarine and coastal populations, as drift causes divergence between populations (Halliburton, 2004).

Although the estuarine systems to the north in Jacksonville (Caldwell, 2001) and the embayment to the south in Biscayne Bay (Litz, 2007) are the closest inshore habitats occupied by bottlenose dolphins to our primary study area, it was surprising that our findings were not more consistent with these regions. A substantially higher microsatellite $Fst$ value was discovered when compared to many studies. The closest microsatellite $Fst$ value was reported between Florida dolphins from the Gulf of Mexico coastal waters versus dolphins from ATL coastal waters (Rosel et al., 2009). Further, Rosel et al. (2009) found a much higher $Fst$ value for mtDNA versus microsatellites for these two populations, but suggested social structure pressures and natal habitat familiarity were the reasons for her findings. Our levels of heterozygosity were similar to those reported from the west coast of Florida (Sellas et al., 2005) but also very similar to Scotland (Natoli et al., 2005) and New Castle, Australia (Möller et al., 2007). The similarities of our results to others vary between locations all across the world, and are most likely the result of how plastic this species appears to be: inhabiting all oceans of the world from pelagic waters to tidal creeks (Caldwell & Caldwell, 1972).

Genetic structure is influenced by many factors including social interactions, cultural learning/behavior, and habitat specialization (Avise, 2004; Rosel et al., 2009). Further, oceanographic and topographic features within each
habitat have been found to shape specific foraging strategies and possibly embed them genetically within social groups to be passed down generation to generation (Hoelzel et al., 1998b; Whitehead, 1998; Avise, 2004; Natoli et al., 2005). de Bruyn et al. (2013) stated that social patterns were the first clues toward the discovery of separate ecotypes in killer whales. Thus we suggest future studies to focus on the habitat and social interaction differences between these two ecosystems to determine if a third estuarine ecotype is diverging in the IRLES.

$H_{02}$-Water bodies

Analysis conducted between the three geographically discrete water bodies investigated in this study, ATL, IRL proper and the ML led us to perform additional analyses (details in $H_{05}$ discussion). At first glance, the ML composition has similarities to the STRUCTURE assignment results from the IRL proper: IRL individuals with scattered ATL genotypes. Such a pattern is typical of other estuarine systems with documented seasonal transients (Caldwell, 2001; Sellas et al., 2005; Litz, 2007; Rosel et al., 2009). One main difference between our study and the others listed above was, that one of our estuarine water bodies was less differentiated from the adjacent Atlantic coastal water body than to an interconnected estuarine water body. Specifically, microsatellite fixation indices were lower between the ML and ATL than the ML compared to the IRL proper, indicative of higher genetic exchange between ML and ATL. The closest large grouping of estuarine dolphins to the north of the ML, is within the JES.
(specifically the southern community of Jacksonville), and a mtDNA study and photo-identification matches suggested some individuals are shared between the areas (Bechdel et al., 2011; E. Howells, personal communication, November 1, 2011). The southern community of Jacksonville is seasonally resident and had partially overlapping ranges with the neighboring transient ATL community, and interbreeding between the two communities was suggested since they are not genetically differentiated (Caldwell, 2001). Further, the two coastal dolphin stocks, Atlantic Northern (outside JES) and Atlantic Central (outside IRLES), are suggested to have partial overlapping ranges near the latitude where Ponce Inlet, the Atlantic entrance to the ML, is located (Waring et al., 2009). Thus if either the southern or ATL JES communities have the opportunity to breed with dolphin of the ML, then this could explain why the ML might have higher shared allele frequencies to the ATL versus IRL proper.

The intermediate genetic composition of the ML dolphins could also suggest that this water body represents a region of interbreeding between inshore dolphins in the IRL proper and Atlantic coastal dolphins. However, the individuals sampled within the ML had strong assignments (Q>0.8, BAYES>0.8) to either the ATL or the IRL, with relatively few individuals with likely admixed genotypes as would be expected in a randomly mating population. The IRL proper assignments had more mixed genotypes. Additionally, when exploring the data with location prior (giving assignments to individuals to one of the three water bodies) and forcing the program to assign individuals to a population,
STRUCTURE still failed to identify the ML as a distinct separate population cluster.

Three different causes could lead to why the IRL proper and ML display differing patterns of genetic structure: (a) they may function as different habitats (host different prey and predator levels, varying water temperature or tides, nursery or breeding grounds), (b) house a different composition of individuals (i.e. territorial males, philopatric females, unrelated juveniles), or (c) historically had limited geographic access to one another or different levels of access to the ATL.

(a) habitat function: Many studies report lower diversity is found inland verses offshore (Rosel et al., 2009) and connected inshore populations are hypothesized to be genetically homogeneous (Duffield & Wells, 2002). However, proximity does not guarantee interbreeding (Sellas et al., 2005). Genetic differentiation within *Tursiops sp.* has been reported between neighboring water bodies in relatively close proximity to one another and without any obvious physical barriers (Caldwell, 2001; Parsons et al., 2006; Bilgmann et al., 2007; Litz, 2007; Möller et al., 2007). This could be a result of adjacent areas functioning as different habitats, such as “feeding grounds” or “nurseries” (Allen et al., 2001; Gibson et al., 2013). Further differences in predation pressures, prey availability, water temperatures and tidal fluxes all play a part to shape the environment and thus can eventually differentiate “forms” of a species, specialized to that habitat (Hoelzel, 1998; Hoelzel et al., 1998b; Natoli et al.,
This is further supported by the biogeographical break point near the southern end of the ML that may serve as a delineation line separating flora and fauna between the majority of the IRL proper and ML (Gosner, 1971; Virnstein, 1990; Cerame-Viva & Gray, 1996; Engle & Summers, 1999; Sigua et al., 2000; species review in Drumm & Kreiser, 2012). Many IRLES studies of bottlenose dolphins, both historic and current, have been conducted within portions of the estuary using inlets or county lines as study site boundaries or including the ML as part of the IRLES system. Few studies design research methods to test differences between the water bodies, specifically separating the ML from the IRL. We suggest, due to our findings here, that the ML be treated as a separate habitat, and suggest to others to take a cautious approach when studying adjacent water bodies as ‘all estuaries are not created equal’.

(b) individual composition: The composition of individuals (for example: territorial males, philopatric females, unrelated juveniles) and how these groups separately utilize the habitat can contribute to a populations genetic structure (Hoelzel, 1998; Sellas et al., 2005; Segura et al., 2006). If specific individuals (by sex, age, or source population) use certain estuaries and not others, the genetic signals between adjacent estuaries could be significantly different from one another. Mosquito Lagoon was considered part of the IRLES and has been studied, reported, and published on under this assumption for years. However,
my study shows this estuary contains dolphins genetically differentiated from the IRL proper, warranting further investigation.

*(c) limited historical access:* Historically, the ML was not naturally connected to the IRL proper. The Haulover canal, the channel connecting the two water bodies, was established between 1852-1854 to move supplies and men between these two water bodies during the Third Seminole War (Knetsch, 1994). Haulover Canal was not maintained as a consistent open passage until 1887, long enough ago for several generations of dolphins to move between the two estuaries and opportunities to interbreed (based on life tables in Stolen & Barlow, 2003). However, the earliest official documentation of IRL dolphins was during the capture and collection of wild dolphins in the 1970’s, and documentation of collections along the “east coast of Florida” was noted as early as 1938 (Leatherwood & Reeves, 1982). The two closest passages to the ATL in the ML, Ponce Inlet and Canaveral Locks, were not stabilized until 1951 and 1968, respectively (Purpura et al., 1974; Fields et al., 1989). Furthermore, the IRL proper had a minimum of three inlets to the ATL over the past 500 years (if not more during some time periods) which possibly allowed ATL immigrants earlier access to the IRL proper than to the ML. The inlets of the IRLES were documented to have opened and closed several times naturally and randomly over the years prior to permanent stabilization (Fineren, 1938; Mehta & Brooks, 1973; Walton, 1974). These geographic hindrances and the lack of observations of dolphins in the IRL until the middle of the 1900’s, offer a possible explanation
to the genetic differences discovered between the two estuaries. Perhaps the immigration of ATL dolphins into the ML is a more recent event which has not allowed sufficient time to pass to create panmixia within these two estuaries. More likely, it is a combination of the three explanations given above.

\textit{H}_{03}\text{-Segments} \& \textit{H}_{04}\text{-Communities}

The detection of fine scale structure within the IRL (\textit{H}_{03}-Segments and \textit{H}_{04}-Communities), also mirrored many estuarine and embayment studies (Krutzen et al., 2004; Sellas et al., 2005; Bilgmann et al., 2007; Möller et al., 2007; Rosel et al., 2009). Upon first inspection, the discovery of minor structure found within the IRLES for both \textit{H}_{03} and \textit{H}_{04} appear to support either hypothesis: habitat differences/geographic distance defined by segment or non-uniform association patterns defined by discrete social communities. However, further analysis revealed rejection of both hypotheses due to inconsistent results between analyses. Discussions of (a) why these two were rejected and (b) other possible hypotheses for the minimal genetic structure found using ‘segment’ and ‘community’ strata are discussed below.

\textit{(a) rejection support}: The \textit{a priori} stratification of samples into different spatial strata can yield misleading results if they do not represent underlying biological subdivisions. This may be the case with the hydro-geographic segments (Hypothesis 3). While they proved to show some statistically significant segregation yet minimal heterogeneity when using traditional fixation indices, the
clustering analysis ($K=2$, even with location priors) did not support sub-structure. Regrouping the samples using social affiliations (Hypothesis 4), eliminating the segment boundary issues, revealed some minimal structure via F-statistics but again was rejected using clustering analysis ($K=2$, with location priors). The differences between the two analyses in both hypotheses can possibly be explained; although the newest version of STRUCTURE claims to detect smaller internal structure than older versions, it still does not appear to have the resolution traditional F-statistics employ. Although our $F_{st}$ values suggest minimal structure, it is possible this structure may not be very meaningful or easy to interpret because the stratification used (segments and communities) were not ideal. To support this, issues with segment boarders became apparent after two segments (1C and 1B) showed no differentiation ($F_{st,mSAT} = -0.0017$) and segment 4 showed characteristics of the Wahlund effect (heterodeficit in many microsatellite loci, suggesting two populations were grouped as one). We concluded that while informative in detecting evidence of heterogeneity within water bodies and ecosystems (a) hydrologic and geographic boarders within the IRLES are not the driving factor in our internal genetic structure.

Deviations in HWE values of loci were variable from community to community and between differing loci per community suggesting structural issues with the boundaries each group had been conformed to and were also possibly the effects of smaller sample size. From these results, it was determined that (b) dolphin communities are not genetically discrete from one another within the IRLES, and that mate selection is most likely not driven by social affiliations. Due
to these inconclusive and varied results, further studies preferably using multiple methods are suggested to be needed to investigate sub and community structure within the IRLES estuarine dolphin population.

(b) other theories: Little evidence was found to support strong genetic subdivision at both the H03-Segments and H04-Community level, thus supplementary attempts were made to explain the minimal pattern of genetic variation in two other ways: (1) bias in location of sampled ATL individuals, or (2) isolation by distance. We surmised that the minimal structure observed among strata in the estuarine system could have been a result of a number of dolphins sampled in the IRLES that were adjudged to have genotypes more typical of the Atlantic. On the possibility that some were indeed Atlantic dolphins, we removed these samples and re-ran analyses. We obtained statistically similar $F_{st}$ results for both segment and communities respectively, and concluded that (1) ATL genotyped animals were not the source of IRL internal structure.

We then hypothesized that the structure could be a result of isolation by distance (IBD), as our study site is 256 km long and quite narrow. Krutzen et al. (2004) found patterns of IBD in Shark Bay, Australia, Ansmann et al. (2012) in Moreton Bay, Australia and Viaud-Martinez et al. (2008) in the Black Sea of the Mediterranean. Initially, our mantel test results appeared to support this theory. However, after taking into account the heavy weight of ATL genotypes in the ML (results from H02), we re-ran analysis without the ML, only the IRL proper, and
found no correlation to distance. We concluded that (2) isolation by distance was not a major cause for observed internal IRL genetic structure.

**H_{0.5}-Mosquito Lagoon Mixed Stock**

Mosquito lagoon appears to be a very dynamic area in terms of dolphin movements, dispersal and gene flow. Dolphins of estuarine ancestry co-occur with dolphins from the neighboring coastal waters. Furthermore, most of the animals we investigated in ML have clear estuarine or Atlantic genetic profiles indicating that many are immigrants, seasonal transients or descendants of immigrants that did not appear to interbreed with dolphins from the other source population. There are a few major explanations for these findings: (a) there are transient visitors in the estuary (b) transient dolphins immigrate to the ML, but occasional return to their source population, or (c) immigrants permanently move into the ML but selectively breed within their own source population.

**(a) seasonal transients:** One scenario explaining the ‘mixed’ ML stock is that the ATL genotypes found within the lagoon system are season transients from coastal waters into the inshore habitat. Seasonal or occasional movements of coastal dolphins into inshore waters have been observed elsewhere (Wells 1986, 1991; Weigle, 1990; Fazioli, 1999; Caldwell, 2001; Zolman, 2002; Litz, 2007; Laska et al., 2008; Rosel et al., 2009). Similarly, aerial studies of the IRLES found ML to exhibit the largest seasonal variability in abundance estimates in comparison to the IRL proper, with larger numbers in the winter
(Durden et al., 2011). Further Durden et al. (2011) suggest this variability is due to the influx of coastal ATL animals into the ML which would concur with our mixed stock results. She also notes unpublished accounts of groups of ML dolphins traveling out of Ponce Inlet into the coastal ATL waters during photo-identification surveys and distinctly marked IRL dolphins have been recovered outside the ML along the ATL coastline.

Our findings show that Mosquito Lagoon dolphins are clearly assigned to two separate genetic clusters, one of which characterizes the inshore estuarine system to the west and south (IRL proper), the other coastal Atlantic system to the east. Interestingly, ongoing photo-identification studies have documented ML dolphins from both genetic clusters co-habitated in this lagoon for over a decade (Mazzoil et al., 2008). Furthermore, among the 26 samples used in analysis, they lack genetic evidence of random mating. In addition, Mazzoil et al. (2008) found that dolphins within the Mosquito Lagoon had the highest re-sighting rate and the strongest site fidelity in comparison to all other segments studied within the IRL proper. Finally, using all known individuals in the photo-identification study that we were able to genotype, no correlation was found between month or year of sightings and whether the individual possess an estuarine or Atlantic/coastal genotype.

Most ATL immigrants were sampled within a home range length of an inlet so perhaps they only frequent in the lagoon when their coastal space is invaded by offshore ecotypes. Coastal and offshore ecotypes can be detected by the differences in diet; estuarine-single non-schooling soniferous fish, coastal-
exclusively small warm water fish, offshore-variety of large pelagic and schooling
cold water fish and cephalopods (Mead & Potter, 1990; Barros, 1993, Barros &
Stolen, 2001). The east central coast of Florida is known for its narrow
continental shelf and close proximity of the Gulf Stream and offshore dolphins
are suggested to come close to shore following their prey source during
upwelling events (Hersh & Duffield 1990; Barros & Stolen, 2001). If the offshore
ecotypes push into coastal territory, coastal dolphins could flood into estuarine
territory either from offshore animals in competition for space or by chasing their
food source as it is displaced by the cold offshore currents (Mendes et al., 2002).
It is possible some coastal animals would not return to the coast after such an
event would subside and become habituated to the lagoon. Future studies should
include tagging during upwelling events or possibly investigation for links
between ocean current changes (major fluctuations of the Gulf Stream perhaps)
with the start of sighting histories of known ATL genotyped estuarine individuals.

The mixed stock pattern could also be observed if the ML was dominated
by ATL genotyped animals as historically resident, and the newer residents are
dolphin from the IRL proper. The connection of ML to the north IRL proper at
Haulover Canal was no opened until 1854, and not stabilized until 1887
(Knetsch, 1994). Further, Ponce inlet was first described as a navigable inlet in
1605 by Alvaro Mexia during his exploration south of St. Augustine, and although
the inlet was known to have closed periodically due to natural environmental
fluctuations (Rouse, 1951), ML has been accessible to ATL dolphins far longer
than dolphins of the IRL proper.
(b) transient dolphins immigrate to the ML, but occasional return to their source population: The long-term photo-ID findings indicate that many of the Atlantic genotypes have long recorded histories of occurring in the Lagoon and associating with estuarine dolphins. In a recent study of dolphin social behavior and structure in the IRL and ML using photo-resight data, Murdoch et al. (in prep) found ML to have a discrete community of individuals (C2) who associate more with each other than with dolphins of other communities to the north above Ponce Inlet (C1), or to the south in IRL proper (C3-C6). Although 24 known individuals used in the Murdoch et al. (in prep) study were also genotyped in this study, only 3 individuals were assigned to ML or community C2. All three were assigned strongly to the estuarine genotype. Nevertheless, it is likely from the social network analysis and the present study that both genetic types, Atlantic/coastal and estuarine, are members of the same community.

Photo-identification surveys, however, are inevitably of limited duration and frequency, and a similar survey effort in the Atlantic Ocean has not been conducted and is prohibitive. Thus, while such studies can establish residency probabilities and associations for individual dolphins within a manageable inshore system such as an estuary, lagoon, or embayment, they cannot quantify shorter movements between such a system and an un-surveyed open coast. While it is plausible for dolphins to appear to live in the ML but occasionally leave for some reason, it would be expected this would correlate with feeding or breeding events which are suggested to be linked to environmental cues (prey shifting, temperature changes, migration). These events would be of longer duration than
the surveys intervals of the photo-ID study, thus we feel we would have seen some correlation with season but did not. Further, it might make more sense if a specific demographic of dolphin was immigrating, of a certain age or sex, but again no correlations were found using these parameters. We feel this option would need significantly more field work and extensive experimental design to properly address, as our conclusions are based upon data that was not collected for this study.

\[(c)\text{ assortative mating – immigrants permanently move into the ML but selectively breed within their own source population:}\] The genetic evidence indicates that dolphins from genetically discrete Atlantic population(s) co-occur with estuarine genotyped dolphins in the ML. We can’t, and shouldn’t exclude the possibility of interbreeding occurring as lower levels of admixture did indicate that some individuals likely have shared ancestry. However, interbreeding is not extensive, as if it were, we would not expect so many strong \((Q>0.8, \text{ BAYES}>0.8)\) assignments to one or the other source population. We would have expected to detect a single, cohesive genetic cluster for the ML.

It is possible dolphins only breeding within their source population may be by choice or some sort of “active avoidance” due to learned behavior from the lineage which they were derived. Learned behaviors are quite common for bottlenose dolphins (Krutzen et al., 2005) and have been suggested to be a cultural phenomenon passed down through maternal lines (Whitehead, 1998). This form of assortive mating may be beneficial for populations that have become
niche specialized, bet-hedging that mating within the source population will produce offspring with a higher probability of retaining any genetic specialization vs. risking new genes that would be less beneficial or detrimental to the niche (Wolf & Figueredo, 2011). Preferred and avoided companionships have been detected for IRL residential individuals within social communities (Hartel et al., 2013; Murdoch et al., in prep), but this phenomenon has not been investigated between IRL and ATL genotyped estuarine animals. It is also possible that morphologically ATL and IRL dolphins appear the same but they possibly behave (feed, breed, socialize) differently enough to be differentiated from each other. Future studies using the behavioral data collected via photo-identification in conjunction with genotypic data from this study, should be combined to investigate this possibility.

Staying with assortative mating is interesting to speculate on how dolphins might recognize like types. Kin recognition and mate selection has been linked to sense of smell and diversity in the major histocompatibility complex (MHC) gene family in other animals (Zelano & Edwards, 2002). The MHC is a multi-gene family that initiates the immune cascade, and is one of the most influential genetic systems for infectious disease resistance in vertebrates (Hill, 1996; Hedrick & Kim, 2000). While such a ‘mate-choice study’ has yet to be conducted on *T. truncatus*, Ferrer (2013) in a companion investigation to the current study, investigated MHC diversity in coastal and inshore populations of dolphins along Florida’s Atlantic coast in order to understand the factors influencing the adaptive immune response in this species. In this study she discovered two predominant
alleles in the MHC Class II locus DQA (loci linked to immunological responses) in IRL dolphins when compared to adjacent ATL populations (7 alleles total, 6 private). Perhaps the mtDNA haplotype 1 is specific to living in the estuary and linked to MHC or some other increased fitness/survival loci. Taking these findings in combination with those from the long-term photo-ID study that indicate long-term residency for most dolphins in the ML and little evidence as yet of extensive seasonal immigration, we concluded that of three options, option (c) ‘immigration with selective breeding’ is the most plausible explanation for the pattern of genetic variation observed in ML.

The previous three theories discuss non-breeding of estuarine and coastal dolphins on the basis that we did not find a strong genetic signal of interbreeding between the two source populations within ML. It is also possible that IRL and ATL genotyped animals do breed in the ML, just that we have misinterpreted our results. With the assumption that they do breed, the lack of discovery of an interbreeding genetic signal with ML could also be due to (d) dispersal of the progeny, (e) death of the progeny, or (f) recent colonization of the area.

(d) dispersal: Our markers can detect interbreeding (as they did within the IRL proper), but perhaps the resulting mixed young of the ML dispersed: north above Ponce Inlet, east to the adjacent ATL, or south into the North Indian River. The ML community (C2) found by Murdoch et al. in prep), is known to have a core area that extends through Haulover Canal and into the north parts of the IRL
proper. This happens to be the one area where the majority of my “mixed”
genotypes (i.e. potential offspring of ATL and IRL proper parents) were sampled. Because we do not know how dolphins within the ML utilize the habitat, it is possible that ML could be mating grounds for IRL proper and ATL dolphins, and because IRLES bottlenose dolphins are known to stay with their mothers for the first 2-3 years of life (Connor et al., 2000; Howells et al., 2008), this option would make sense as to why I did not find higher patterns of interbreeding within ML. Extensive genetic studies of mother calf pairs and relatedness of community individuals within the ML would help in the resolution of this theory.

(e) death: There also may be high neonate mortality, limiting the possibility of discovery of an interbreeding genetic signal. *Tursiops sp.* have been known to commit infanticide, or the killing of young, to decrease the fitness of others offspring and insure that of their own (Patterson et al., 1998; Dunn et al., 2002). Further, competition for limited habitat or receptive females may cause males to kill young offspring and this has been seen in *Tursiops sp.* (Patterson et al., 1998; Dunn et al., 2002; Kaplan et al., 2009) as well as other higher cognitive mammals such as primates (Hrdy, 1979). Another option for high neonate mortality could be the combination of ATL and IRL individuals possibly producing deleterious genotypes in their young, and natural selection removes these individuals from the population. Mortality rates are the highest in the Northern Indian River and two unusual mortality events, in 2001 and 2008, have been documented in this area (Stolen et al., 2007; Waring et al., 2009). Regardless of
hypothesis, data on calf survivorship and mortality for distinct water bodies need to be collected/analyzed before further interpretations can be made.

(f) recent colonization: As mentioned above, although most similar studies report coastal dolphins mixing within an established estuarine population, we have suggested and have some minimal support that it is also possible for the reverse to be true: coastal dolphins are the residents in ML and IRL proper dolphins are immigrants or the newest arrivals. Due to the time frame of which Haulover Canal was stabilized in 1887 or even accessible (est. in 1844) and Ponce Inlet offering access to the ATL some 300 years prior, it is plausible that the mixed stock we have discovered in the ML is a recent colonization event by IRL proper dolphins to established ATL habitat. It is possible that within the ML, the two mixed stocks have not had sufficient time to create a significant amount of genetically mixed offspring, which would result in the lack of detection of a breeding signal. This would explain why STRUCTURE failed to pull ML out as a third population. Further, $Fst$'s support ML as less differentiated to the ATL than the IRL in both mtDNA and nDNA markers regardless that an equal number of samples were assigned to each source population. This exciting possibility will need further genetic sampling within and around the ML: into the northern reaches of the IRL proper, coastally along ML, and north of Ponce Inlet past Halifax toward the southern community of JES.
Determining genetic structure is essential for understanding the behavioral ecology and evolution of the species, but it is also important knowledge that can be used for management and preservation of this environmentally dynamic cetacean. Our findings challenge conventional definitions of “ecotype” (Turesson, 1922; Hersh & Duffield, 1990; Walker et al., 1999; Torrez 2003; Segura et al., 2006), “stock” (Caldwell, 2001; Sellas et al., 2005; Litz, 2007; Waring et al., 2009), “population” (Mead & Potter, 1995; Hoelzel et al., 1998b; Natoli et al., 2004; Rosel et al., 2009; Mirimin et al., 2011), and “community” (Wells et al., 1987; Wells & Scott, 1999; Conner et al., 2000; Waring et al., 2009; Tyson et al., 2011; Kiszka et al., 2012) because these definitions are generally based on the concept that groups of individuals sharing the same areas have the opportunity to breed with adjacent groups but choose not to, thus they choose to mate with the group in which they are physically closest to. This would result in similar genetic profiles for individuals living in the same area.

Ecotypes are based on a species having the same genetic response to the environment (Turesson, 1922), which results in phenotypic changes as they specialize within their niche habitat. Various interpretations of Turesson’s initial definition have resulted in altered definitions and have caused debate among scientists (Cronin & Mech, 2009; de Bruyn et al., 2013). Current definitions term ‘ecotype’ as a group of individuals in which adaptations to a specific habitat include changes to morphometrics, demography and behavior of the group (Cronin et al., 2009). Killer whales (*Orcinus orca*) of the eastern North Pacific
have been separated into three distinct ecotypes (transient, resident, and offshore) based upon prey preference which altered social organization and reproductive strategies resulting in differing genetic profiles within the same local geographic area (Bigg et al., 1987; Hoelzel, 1998; Baird & Dill, 1995; Jones, 2006; Hoelzel et al., 2007; Dahlheim et al., 2008). Recent work has suggested 5 ecotypes (4 morphotypes) in Antarctic killer whales, but the authors caution that more data be obtained before making these designations due to the complex nature of this species (de Bruyn et al., 2013).

The differences between offshore and coastal/inshore *T. truncatus* ecotypes have been well documented. However, the majority of literature clumps estuarine and coastal *T. truncatus* together as the same ecotype due to lack of comparable studies between the two habitats. Barros (1993) found slight differences in prey type between coastal and estuarine IRL dolphins, suggesting IRL dolphins consume more soniferous (or sound producing) fish than coastal dolphins, which may reflect differing foraging strategies. Initial morphological comparisons of offshore dolphins versus inshore were differentiated by dramatically different skull shapes: further, inshore samples had extensive variance between the skulls (variance not seen in the offshore samples) thus inshore samples were suggested to be from more than one population (Hersh & Duffield, 1990; Mead & Potter 1995). The samples used in both studies referenced above sampled and grouped coastal and estuarine individuals together as “inshore”, thus perhaps this variation is actually differences between coastal and estuarine individuals. This could be one example of the first
phenotypic result from specialization of an estuarine ecotype. Waring et al., 2011), suggests there is much evidence that estuaries and costal animals are differentiated, with ‘unpublished references,’ perhaps this is one of them.

According to the definition of ecotype, all ML dolphins should have the same genetic response (shift in allele frequencies) to the environment if they are utilizing it in the same manner, but ML offers a dramatic mixed signal. Further the signal is strong enough to differentiate the IRL proper from ATL animals but is not so large as to cause changes where phenotype is dramatically altered between the two (or could be relatively recent as suggested in the previous section). Regardless of water body (ML or IRL proper), it is unknown if the ATL genotyped animal’s resident within the estuary are using the estuary in the same manner as the IRL genotyped individuals. If they are not, which is possible in the ML due to the evidence of selective mating, then it could be argued that there is a third ecotype of *T. truncatus* along the east coast of Florida, separating the coastal individuals from the estuarine individuals. This finding is quite imperative to the understanding of the IRL residential dolphins, as numerous studies have been and continue to be published with the assumption that the individuals within the lagoon are from one effectively closed estuarine population. Our study has already supported that approximately 10% of the supposed estuarine animals used in over 63+publications to date, many of which are related to the ‘health of the lagoon,’ have ATL genotypes in which one if not both parents were of ATL origin. A recent study on feeding ecology of IRL dolphins using fatty acid and stable isotope signatures (Worthy et al., 2008) in comparison to stomach content
analysis from 15 years past (Barros, 1993), proposes that IRL dolphins have prey shifted within the IRL, implying IRL dolphins will acclimate to their environment if need be. However, the blubber samples used in the contemporary portion of the analysis were derived from the same individuals used in our genetic work, and thus this prey shift phenomenon could simply be the result of using samples from estuarine and ATL genotyped individuals. It could be that the IRL dolphins cannot prey shift and could be negatively impacted by the changes in their prey source, the opposite conclusion that the authors suggest. In fact, several studies on dolphin habitat specialization suggest they do not adapt to prey changes, rather they seek out another habitat that their particular foraging specialty will apply to (Mann et al., 2008; Torres & Read, 2009). A future study between estuarine and coastal individuals needs to be addressed within the same study as this finding can support or disprove if these two groups of dolphins are using the habitat in the same manner. If not, this would reject these groups being considered one “inshore” ecotype, the theory which is currently in place.

When defining cetacean stocks, specifically coastal populations, oceanographic features and topography have been used (depth of water offshore, or water bodies such as a bay or river) to create stock boundaries (Rosel et al., 2009; Waring et al., 2011). Further, management stocks are identified on the basis of inferred demographic independence (Eagle et al., 2008). However, ML cannot be considered a separate stock because the potential ATL immigrants into the estuary are assigned fully to the coastal source population; so although the barrier island acts as partial geographic barrier, the
ML is not composed of a single interbreeding stock but rather two separate genetic clusters (likely stocks). Further, potential ATL immigrants are a mix of males and females, suggesting dispersal of both sexes from the coastal stock. However, the ML does not show increased haplotypic diversity usually associated with female dispersal and photo-identification data supports many of these ATL immigrants as residential in all seasons and years. These findings suggest that ML is a mixture of two separate stocks, a phenomenon with no current management guidelines.

It seems in the literature the term ‘populations’ is used interchangeably with ‘stocks’, but more often it is used as a term to describe smaller units of stocks or ecotypes. Additionally, the term ‘communities’ is very similar to ‘population’: only that it differs with the "association" component (defined in the introduction). Non-overlapping data between this study and the individuals used in Murdoch et al. in prep) association indices leave room for speculation in the ML. Although her study found one community within the ML, it is unknown if her samples contained ALT immigrants or not. Thus there is the possibility there are two communities present, and only one was sampled. If ATL immigrants were included in the analysis, then the two genotyped groups are associating together. However, association does not guarantee interbreeding as described above, therefore ML cannot conclusively be defined as a ‘community.' Waring et al. (2011) has suggested that stock designations take upon a more biological definition and incorporate a community based approach when attempting to create management units.
Clearly these two “groups” of resident ML dolphins (for lack of a better term) do not conform to any traditional definitions and raise many questions for future research. The mixed stock discovery in ML and the ATL immigrants within the IRL proper both highlight the need for genetic studies to be conducted prior to population assessments, and has been cautioned by other authors as well (Rosel et al., 2009). Using our findings as a baseline for how to construct new experimental designs, we suggest diurnal focal follows or satellite tagging of both IRL and ATL genotyped estuarine animals to solidify movement patterns, associations, habitat use of these individuals, and further genetic relatedness testing as this “key piece of evidence” is what separates our study from conforming to other *T. truncatus* estuarine studies. Our suggestions for immediate management concerns of the ML mixed-stock “population” would possibly be to split the PBR quotas for each of the IRL proper and ATL coastal stocks, and combine them for a new take value until further research can be conducted to fully investigate this interesting ecological situation.
PROBLEMS AND PITFALLS

Some problems that may arise when using mtDNA include its monomorphic nature, inability to determine relatedness between distantly related individuals/populations, and underestimation of gene flow if your study population has limited maternal gene flow. We experienced this problem with the marker early on, during my preliminary study when we were first seeing signs of one major dominate haplotype throughout the lagoon. However, coastal samples encouraged us with their variation that even with what appeared to be a marker with low diversity in our estuarine population, by using it as a comparison to the coastal samples it was highly informative regarding female dispersal and demography of the IRLES. Additionally, adding microsatellites to the mtDNA data tightened our results and added the male dispersal information we needed to support our mtDNA findings.

During this study, over half of the skin samples taken could not be successfully sequenced possibly due to DNA degradation from poor preservation methods. Additionally, several samples did not properly amplify both strands of the entire 596 bp (a QAQC standard within our lab) and were eliminated from the study analysis. Alternative extraction methods used with degraded DNA, such as using ancient DNA (aDNA) methods, were proposed to attempt to extract the “problem” samples (Hoess & Pääbo, 1993), however we felt after the first round
of 308 sequenced individuals and 244 genotyped individuals, the working sample sizes were of sufficient size to answer our current hypotheses. It’s not to say that future questions may require us to explore these methods specifically for relatedness studies where we have many additional samples from which we assume are mother calf pairs or full and half siblings from ongoing photo-identification studies. Thoughts to adjust the base pair length (less stringent/decrease the accepted length) to increase sample size was also proposed, however, variable sites towards the 3’ end of the forward sequences worried us that we would lose valuable haplotypic differences, and may cost us what little mtDNA variation differences we had.

Problems comparing distantly related groups (possibly estuary verses estuary) were a major concern as microsatellites can back mutate, leaving no consistent evolutionary signal. Pitfalls of microsatellites come by the dozens as each marker has its own quirks. Primarily time consuming optimization was one of the largest problems, and even when a primer does appear to work (bands on a gel, then successfully genotyped) one must still run a sufficient amount of samples with that primer before the resulting data can be run through QAQC programs to determine its stability. Allelic dropout (non-amplifying allele), null alleles (homozygote excess) and stutter bands/slippage are frequent problems with short tandem repeat (STR’s) analysis and were screened for several times during this study: initially using known pedigrees (n=8 mom/calf pairs, n=8 individuals sampled twice or more over different years), repeated verification/standardization during optimization as described above, and with the
final data set. Adenylation, or the random addition of an A from TAQ polymerase during the PCR process, was promoted via the addition of a G to the end of all microsatellite primers to further ensure consistency when scoring. Additionally our lab had both lengthy problems without the use of our in house sequencer (forcing us to initially send some samples out), and a major move of the machine; both of these requiring several re-running of older data to properly align old to new data ensuring consistent scoring of alleles throughout the study. These problems actually allowed me to verify my data so many times that I feel more confident in the oddities we found now verses just completing the standard QAQC.

Because many of the samples were from stranded individuals with potentially fragmented or damaged DNA, they were potentially unable to be analyzed as one long fragment. It was proposed that we would screen for single nucleotide polymorphisms (SNP’s), which can be used with degraded DNA, as they are often used to process problem samples. Although SNP’s are easier to use with fragmented DNA and more stable in terms of inheritance (higher mutation rate than STR’s), significantly more SNP’s (40-60) will have had to have been used in analysis to equal the informative power that few microsatellites will produce, thus was not applicable this our timeframe. Some additional problems with SNP’s include having to sequence both strands to verify the length of the SNP (costly), the difficulty for SNP’s to be amplified in GC heavy repetitive regions, and the inability to decipher mixtures (i.e. mutant/migrant, or
contamination). Future studies involving SNP’s are on the horizon for our lab as we move into processing genes under selection.
CONCLUSIONS

The IRLES dolphin population was previously considered a residential effectively closed population due to 40 years of observational studies that documented high levels of residency and suggested extremely limited movement between the inshore lagoon and Atlantic Ocean, via five open inlets (Odell & Asper, 1990; Mazzoil et al., 2005, 2008; Waring et al., 2011). Strong genetic subdivision was found between the inshore and coastal ecosystems. However, migrants from each primary cluster that characterize the two ecosystems were detected in the opposite non-source ecosystem. This detection suggests gene flow is occurring, but not at a high enough rate to eliminate the genetic signal separating the estuarine individuals from the coastal individuals.

Phylogeographic structure of the estuarine samples revealed that this population is highly philopatric with long standing differences. Substantially lower differentiation using microsatellites suggested males may be more of an influence on gene flow than the philopatric females. The ATL genotyped estuarine individuals or ATL immigrants or decedents of immigrants' had no correlation with length, weight, sex, sighting history in months, birth year, field estimate or total group size. Many coastal immigrants to the estuary have extended sighting histories with long lived residency, over all seasons and all years, however limited mixed genotypes were found within the estuary. Although
the estuarine and coastal ecotypes share the same habitat within the IRLES, these findings suggest minimal non-random mating occurs within the IRLES which may be linked to a learned behavior passed down through time.

To our surprise, coastal and estuarine genotypes were equally distributed within the Mosquito Lagoon with few combined genotypes, suggesting that each estuary contains different ratios of ecotypes. The ATL has more genetic influence on ML than it has on the IRL proper, and even with location information failed to separate ML as a separate population. Without the use of genetics, observational studies alone deemed ML “part of the IRL” closed population. Our findings not only reject this hypothesis but suggest that ML may be composed of residential ATL dolphins with recent IRL proper immigrants. The ML estuary may function more like an airport with a hotel, some individuals may come and go, some stay awhile, but random mixing/breeding was not detected. Future studies on social affiliations combined with genetics are already underway within this estuary.

Fine scale structure within the IRL was also detected; however neither habitat nor social boundaries were the cause of IRL internal genetic discreteness. Future studies will need to further investigate this phenomenon as both are suggested to be factors that can shape population structure in other similar studies.

By paring our genetic findings with the photo-identification data, we have not only shown that there are coastal individuals present within the lagoon, but they are residential with long sighting histories. Within the IRL proper, coastal and estuarine ecotypes have produced offspring with mixed genotypes. Coastal
immigrant home ranges were clustered near inlets and around Haulover Canal, but were absent from the north central part of the lagoon system. These findings compounded with the lack of haplotypic diversity and low number of combined genotypes found within the IRL proper, suggest the ATL genotypes found in the IRL proper could be a recent event in historical terms. Further, the instability of inlet accessibility to the Atlantic Ocean over the last hundred years supports this hypothesis.

Large differentiation between bottlenose dolphins within the IRL and adjacent coastal Atlantic waters was supported in our analyses, and evidence of some small intra-population subdivision within the IRL was also detected. However, this internal estuarine structure was not explained by habitat partitioning via hydrologic or geographic features, social community boundaries, or isolation by distance as many dolphin systems report (Krutzen et al., 2004; Natoli et al., 2004; Sellas et al., 2005; Parsons et al., 2006; Urian et al., 2009; Mendez et al., 2010; Wiszniewski et al., 2010a; Tyson et al., 2011; Kiszka et al., 2012) thus warrants further investigation. The mixed-stock discovery of animals from both Atlantic and Estuarine stocks co-occurring within Mosquito Lagoon (ML), a large estuarine water body with limited access to the Atlantic Ocean, compounds the current understanding of ecotypes, stocks, and populations in this adaptable species. Further, pairing our genetics findings with long-term photo-ID studies revealed that many of these ML individuals are long-term residents (Odell & Asper, 1990; Mazzoil et al., 2005) and that both Atlantic and estuarine genotypes may be members of the same distinct social community.
(Murdoch et al., in prep), which challenges us to expand our concept of community social structure in this species. And finally, these findings raise unique issues for the identification of management units where demographic independence of spatially discrete groups of individuals is the accepted guideline for the definition of population stocks under the U.S. Marine Mammal Protection Act.
Table 1. Microsatellite markers and associated information used to genotype *Tursiops truncatus* (*n* = 244) from within the IRLES and adjacent Atlantic coastal waters.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat Motif</th>
<th>No. Repeats</th>
<th>Seq 5'-3' (all reverse have GGTTCTT tails for plus A)</th>
<th>Source</th>
<th>Product Size</th>
<th>No. Alleles</th>
<th>MgCl conc.</th>
<th>Modified Protocols</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV37</td>
<td>AC&lt;sub&gt;24&lt;/sub&gt;</td>
<td>2</td>
<td>5'-AGCTTGATTTGGAAGTCATGA 5'-GGTTCTTTAGAGCCCTGTAAGGA</td>
<td>Valsecchi and Amos 1996</td>
<td>HEX</td>
<td>187-245</td>
<td>23</td>
<td>1.5mM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5'-GGTTCTTGTTTGTTTCCCAGGATTTAGTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 cycles(94° 20s, 54° 45s, 72° 60s), extend 72° 4m</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>denature 94° 3m,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>27 cycles(94° 40s, 48° 60s, 72° 100s), extend 72° 5m</td>
</tr>
<tr>
<td>KWM12a</td>
<td>AC&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>5'-CCATACACACACGACGTC 5'-GGTTCTCTACGAGAATGATGACC</td>
<td>Hoelzel et al. 1998</td>
<td>FLUOR</td>
<td>161-185</td>
<td>17</td>
<td>1.5mM</td>
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<td></td>
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<td>30 cycles(94° 20s, 62° 20s, 72° 40s), extend 72° 10m</td>
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<tr>
<td>Thr04</td>
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<td>2</td>
<td>5'-CTGACCAGGCACTTTCCAC 5'-GGTTCTTGGTTGATTTCCAGGATTTAGTC</td>
<td>Rosel et al. 2005</td>
<td>6-FAM</td>
<td>99-123</td>
<td>11</td>
<td>2.0mM</td>
</tr>
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<td></td>
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<td>denature 94° 3m,</td>
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<td>27 cycles(94° 40s, 48° 60s, 72° 100s), extend 72° 5m</td>
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<td>MK6</td>
<td>GT&lt;sub&gt;17&lt;/sub&gt;</td>
<td>2</td>
<td>5'-GTCTCTTTCCAGGTTAGGC 5'-GGTTCTGTGCCCCACTAAGGTGTTGACGC</td>
<td>Knutzen et al. 2001</td>
<td>FLUOR</td>
<td>147</td>
<td>12</td>
<td>1.5mM</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>30 cycles(92° 20s, 60° 45s, 72° 60s), extend 72° 4m</td>
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<tr>
<td>AAT40</td>
<td>AAT&lt;sub&gt;16&lt;/sub&gt;</td>
<td>3</td>
<td>5'-GCACCAGAAGAAAGTAG 5'-GGTTCTTCATGTGCTAGCAAGAA</td>
<td>Caldwell et al. 2002</td>
<td>TAMRA</td>
<td>181</td>
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</tr>
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<td>2</td>
<td>5'-TGGGTGGACCTCATAACAT 5'-GGTTCTGTGTTAAGGCTGTAAGAGG</td>
<td>Rosel et al. 2005</td>
<td>6-FAM</td>
<td>171-213</td>
<td>9</td>
<td>2.0mM</td>
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<td></td>
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<td>30 cycles(94° 20s, 60° 20s, 72° 40s), extend 72° 10m</td>
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<td>2</td>
<td>5'-TAAACATCAAGCAGACCC 5'-CGAGACGAGACAGAAGAGGA</td>
<td>Valsecchi and Amos 1996</td>
<td>HEX</td>
<td>123-159</td>
<td>19</td>
<td>1.5mM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 cycles(94° 20s, 54° 45s, 72° 60s), extend 72° 4m</td>
</tr>
<tr>
<td>TexVet5</td>
<td>CA&lt;sub&gt;24&lt;/sub&gt;</td>
<td>2</td>
<td>5'-GATTTGCCAAATGGAGAGCA 5'-GGTTCTTGTGTTAAGGCTGTAAGAGG</td>
<td>Rooney et al. 1999</td>
<td>TAMRA</td>
<td>236-260</td>
<td>12</td>
<td>2.0mM</td>
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<tr>
<td>TexVet7</td>
<td>CA&lt;sub&gt;12&lt;/sub&gt;</td>
<td>2</td>
<td>5'-TGCACTTGAGGTGTTGCTAG C 5'-GGTTCTTTCTCTTGGAGCGAGCTTGACC</td>
<td>Rooney et al. 1999</td>
<td>6-FAM</td>
<td>155-163</td>
<td>9</td>
<td>1.5mM</td>
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<tr>
<td>Utr+80</td>
<td>GATA&lt;sub&gt;10&lt;/sub&gt;</td>
<td>4</td>
<td>5'-AGCCATGTAGGGTGGTGAGAT 5'-GGTTCTTTGGGGCTTTGCGCTCTTGA</td>
<td>Nater et al. 2009</td>
<td>6-FAM</td>
<td>287-335</td>
<td>5</td>
<td>3.0mM</td>
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<td></td>
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<td>25 cycles(94° 30s, 60° 90s, 72° 90s), extend 72° 4m</td>
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Modified Protocols:
- denature 94° 3m,
- 32 cycles(94° 20s, 54° 45s, 72° 60s), extend 72° 4m
- denature 95° 3m,
- 27 cycles(94° 40s, 48° 60s, 72° 100s), extend 72° 5m
- denature 94° 3m,
- 30 cycles(92° 20s, 60° 45s, 72° 60s), extend 72° 4m
- denature 94° 3m,
- 27 cycles(94° 40s, 48° 60s, 72° 100s), extend 72° 5m
- denature 94° 3m,
- 30 cycles(94° 20s, 62° 20s, 72° 40s), extend 72° 10m
- denature 95° 5m,
- 27 cycles(94° 30s, 58° 30s, 72° 30s), extend 72° 10m
- denature 94° 3m,
- 30 cycles(92° 20s, 60° 45s, 72° 60s), extend 72° 4m
- denature 94° 3m,
- 27 cycles(94° 40s, 48° 60s, 72° 100s), extend 72° 5m
- denature 94° 3m,
- 30 cycles(94° 20s, 60° 20s, 72° 40s), extend 72° 10m
- denature 94° 3m,
- 32 cycles(94° 20s, 54° 45s, 72° 60s), extend 72° 4m
- denature 95° 5m,
- 35 cycles(94° 40s, 57° 60s, 72° 60s), extend 72° 4m
- denature 95° 5m,
- 35 cycles(94° 40s, 54° 60s, 72° 60s), extend 72° 10m
- denature 95° 5m,
- 35 cycles(94° 40s, 54° 60s, 72° 60s), extend 72° 10m
- denature 94° 3m,
Table 2. Haplotypes, variable sites, and associated GENBANK accession numbers determined from *Tursiops truncatus* (*n* = 308) within the Indian River Lagoon, Florida and adjacent coastal waters.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Variable Sites</th>
<th>Accession Number</th>
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<tbody>
<tr>
<td>Ttru_Hap1</td>
<td>C T - G C T A - G T A T A T T A T C A T A T C C C A C T T C T C T G G C C T C T C C C C G T C A G C</td>
<td>(Querouil et al. 2007, DQ073728.1 98%)</td>
</tr>
<tr>
<td>Ttru_Hap2</td>
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<td></td>
</tr>
<tr>
<td>Ttru_Hap3</td>
<td>T G - - - - - - C - - - - - - C - - - - - - T - - - - - - - A A - - - - - - - T - - - - - - - G</td>
<td>(Querouil et al. 2007, DQ073728.1 97%)</td>
</tr>
<tr>
<td>Ttru_Hap4</td>
<td>C G - - - - - - C G - - - - - - C - - - - - - T - - - - - - - T C C T - - - - - - - A A - - - - - - - T C T T - - - - - - - A C T - - - - - - - T</td>
<td>(Querouil et al. 2007, DQ073667.1 99%)</td>
</tr>
<tr>
<td>Ttru_Hap5</td>
<td>C G - - - - - - C G - - - - - - C - - - - - - T - - - - - - - T C C T - - - - - - - A A - - - - - - - T C T T - - - - - - - A - - - - - - - T</td>
<td>(Querouil et al. 2007, DQ073645.1 99%)</td>
</tr>
<tr>
<td>Ttru_Hap6</td>
<td>G - - - - - - G G - - - - - - C - - - - - - C - - - - - - A - - - - - - - T C T C - - - - - - - A - - - - - - - T C - - - - - - - C T - - - - - - - T</td>
<td>(Querouil et al. 2007, DQ073654.1 99%)</td>
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<tr>
<td>Ttru_Hap7</td>
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<td></td>
</tr>
<tr>
<td>Ttru_Hap8</td>
<td>A - - - - - - G - - - - - - A C - - - - - - C C - - - - - - T T G - - - - - - G - - - - - - - T C - - - - - - - C A A - - - - - - - C C T T - - - - - - - T A C T - - - - - - - T</td>
<td>(Querouil et al. 2007, DQ073728.1 97%)</td>
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<tr>
<td>Ttru_Hap9</td>
<td>G - - - - - -</td>
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<tr>
<td>Ttru_Hap10</td>
<td>- - - - - - - - - - - - C -</td>
<td></td>
</tr>
<tr>
<td>Ttru_Hap11</td>
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</tr>
<tr>
<td>Ttru_Hap12</td>
<td>G C - - - - - - C G - - - - - - T C - - - - - - C -</td>
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<tr>
<td>Ttru_Hap13</td>
<td>A - - - - - -</td>
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<tr>
<td>Ttru_Hap14</td>
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<td></td>
</tr>
<tr>
<td>Ttru_Hap15</td>
<td>G - - - - - - C G - - - - - - C - - - - - - T C - - - - - - C T T C - - - - - - A A - - - - - - - T C T T - - - - - - - A C - - - - - - - T</td>
<td></td>
</tr>
<tr>
<td>Ttru_Hap16</td>
<td>T G - - - - - - C - - - - - - C - - - - - - T - - - - - - - T - - - - - - - A A - - - - - - - T -</td>
<td></td>
</tr>
<tr>
<td>Ttru_Hap17</td>
<td>G - - - - - - G G - - - - - - C - - - - - - A - - - - - - - C T C T - - - - - - - A T T C - - - - - - - C T -</td>
<td></td>
</tr>
<tr>
<td>Ttru_Hap18</td>
<td>C G - - - - - - G - - - - - - C - - - - - - T - - - - - - - T - - - - - - - C C T T - - - - - - - A A - - - - - - - T C T T - - - - - - - A - - - - - - - T</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Standard and molecular diversity indices for 308 sequenced (top 6 rows) and for 244 genotyped (bottom 3 rows) *Tursiops truncatus* sampled within the IRLES and adjacent Atlantic coastal waters.

<table>
<thead>
<tr>
<th>Ecosystem</th>
<th>Water Body</th>
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<tbody>
<tr>
<td>IRLES</td>
<td>ATL</td>
</tr>
<tr>
<td>No. of haplotypes</td>
<td>9</td>
</tr>
<tr>
<td>No. of polymorphic sites</td>
<td>12</td>
</tr>
<tr>
<td>No. of transitions</td>
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<td>No. of substitutions</td>
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<td>No. of indels</td>
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<tr>
<td>Msat Heterozygosity</td>
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<tr>
<td><em>He</em> (all msat loci)</td>
<td>0.620</td>
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<tr>
<td><em>Ho</em> (all msat loci)</td>
<td>0.575</td>
</tr>
</tbody>
</table>
Table 4. Pairwise $F_{stmtDNA}$ values below diagonal, pairwise $F_{stmsat}$ above diagonal, and mtDNA haplotype ($h$) and nucleotide ($\pi$) diversity indices on the diagonal for each of the four Ho tested among *Tursiops truncatus* strata (a-d). Significance values $p<0.05$ are highlighted in dark grey, $0.1>p>0.05$ in light grey, and $p>0.1$ in white.

4a. Ho1-ECOSYSTEMS

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<tr>
<th>n</th>
<th>Coastal</th>
<th>Estuarine</th>
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<tbody>
<tr>
<td></td>
<td>$h = 0.7913$</td>
<td>$\pi = 0.0143$</td>
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<tr>
<td>Coastal</td>
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<td>$h = 0.1017$</td>
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<td>Estuarine</td>
<td>250/202</td>
<td>0.4143</td>
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4b. Ho2-WATER BODIES

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<th>n</th>
<th>ATL</th>
<th>ML</th>
<th>IRLprop</th>
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<td>$h = 0.7913$</td>
<td>$\pi = 0.0143$</td>
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<td>0.0180</td>
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<td>ML</td>
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<tr>
<td>IRLprop</td>
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4c. Ho3-SEGMENTS

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<th>1C</th>
<th>1B</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td></td>
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<td>0.0111</td>
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<tr>
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<td>71/49</td>
<td>0.0012</td>
<td>$h = 0.0526$</td>
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<td>0.0109</td>
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<tr>
<td>1B</td>
<td>38/28</td>
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<td>-0.0129</td>
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<tr>
<td>3</td>
<td>43/41</td>
<td>0.0339</td>
<td>0.0016</td>
<td>-0.0124</td>
<td>-0.01</td>
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<td>4</td>
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<td>-0.0075</td>
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4d. Ho4-COMMUNITIES

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<th>C4</th>
<th>C5</th>
<th>C6</th>
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<tr>
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<td>-0.0063</td>
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<td>$h = 0.0000$</td>
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<tr>
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<td>0.0000</td>
<td>0.0379</td>
<td>0.0086</td>
<td>0.0000</td>
<td>-0.0071</td>
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Table 5. Genetic distances reported as $F_{st}$ values below diagonal and $R_{st}$ above diagonal for each of the four $H_0$ tested among *Tursiops truncatus* strata (a-d). Significance values $p<0.05$ are highlighted in dark grey, $0.1>p>0.05$ in light grey, and $p>0.1$ in white.

5a. $H_01$-ECOSYSTEMS

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5b. $H_02$-WATER BODIES

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5c. $H_03$-SEGMENTS

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<th>4</th>
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5d. $H_04$-COMMUNITIES

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<th>C5</th>
<th>C6</th>
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<td>-0.0488</td>
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<td>0.0126</td>
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<tr>
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<td>-0.0014</td>
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<td>-0.0071</td>
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Table 6. Likelihood of the number of population clusters, $K$, and variance, $\text{Var}$ (reported for the 5th run only), when running STRUCTURE clustering analysis using admixture and location priors from *Tursiops truncatus* sampled within the IRLES and adjacent Atlantic coastal waters. Means and standard deviations, $SD$, are over all 5 runs.

| $K$   | 1         | 2         | 3         | 4         | 5         | $\text{Var} \ln P(D)$ | Mean $\ln P(X|K)$ | SD    |
|-------|-----------|-----------|-----------|-----------|-----------|------------------------|--------------------|-------|
| **Ecosystem**             |           |           |           |           |           |                       |                    |       |
| 1     | -6812.0   | -6812.0   | -6812.0   | -6812.0   | -6812.0   | 54.2                   | -6812.0            | 0.0   |
| 2     | -6552.0   | -6546.9   | -6546.0   | -6546.6   | -6550.1   | 329.1                  | -6548.3            | 2.6   |
| 3     | -6541.8   | -6536.5   | -6418.8   | -6478.0   | -6478.3   | 326.2                  | -6490.7            | 50.5  |
| 4     | -6591.8   | -6648.7   | -6603.9   | -6604.2   | -6812.2   | 1009.6                 | -6652.2            | 92.1  |
| **Water bodies**          |           |           |           |           |           |                       |                    |       |
| 1     | -6812.1   | -6812.2   | -6812.0   | -6812.0   | -6812.1   | 54.4                   | -6812.1            | 0.1   |
| 2     | -6554.0   | -6534.6   | -6522.4   | -6537.2   | -6536.2   | 306.9                  | -6536.9            | 11.3  |
| 3     | -6492.9   | -6479.5   | -6475.5   | -6484.3   | -6501.8   | 367.7                  | -6486.8            | 10.6  |
| 4     | -6678.1   | -6917.7   | -6490.0   | -6618.2   | -6594.2   | 684.2                  | -6659.6            | 159.5 |
| **Segments**              |           |           |           |           |           |                       |                    |       |
| 1     | -6812.0   | -6812.1   | -6812.0   | -6812.0   | -6812.0   | 54.3                   | -6812.0            | 0.1   |
| 2     | -6546.4   | -6518.1   | -6538.1   | -6545.8   | -6534.3   | 296.2                  | -6536.5            | 11.5  |
| 3     | -6476.4   | -6487.6   | -6476.7   | -6484.2   | -6496.0   | 349.7                  | -6484.2            | 8.2   |
| 4     | -6616.2   | -6672.0   | -6520.3   | -6787.5   | -6623.0   | 726.2                  | -6603.8            | 121.0 |
| 5     | -6691.0   | -6651.7   | -6811.2   | -6722.8   | -6651.3   | 818.3                  | -6698.4            | 74.9  |
| 6     | -6806.7   | -6826.2   | -6905.1   | -6683.7   | -6681.2   | 893.8                  | -6780.6            | 96.9  |
| 7     | -7035.7   | -7023.1   | -6927.1   | -6645.0   | -6771.6   | 1113.5                 | -6880.5            | 168.8 |
| **Communities**           |           |           |           |           |           |                       |                    |       |
| 1     | -3993.9   | -3993.8   | -3993.9   | -3993.9   | -3993.9   | 45.4                   | -3993.9            | 0.0   |
| 2     | -3939.8   | -3939.3   | -3943.6   | -3935.3   | -3932.3   | 162.9                  | -3938.1            | 4.4   |
| 3     | -3989.9   | -3999.2   | -4019.3   | -3994.2   | -3948.4   | 306.7                  | -3990.2            | 25.9  |
| 4     | -3707.6   | -4017.1   | -4034.6   | -3958.7   | -4073.0   | 545.2                  | -4019.7            | 52.3  |
| 5     | -4166.2   | -4053.1   | -4139.2   | -4088.5   | -4237.5   | 892.5                  | -4136.9            | 71.3  |
| 6     | -4175.1   | -4247.5   | -4056.3   | -4216.4   | -4093.2   | 484.1                  | -4145.7            | 96.0  |
Table 7. Genetic estimates of dispersal between Tursiops truncatus from the ATL and IRL proper conducted using BAYESASS (delta 0.05). Data may not contain enough information (not highly discriminatory) to produce accurate values.

<table>
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<th>ATL (n=46)</th>
<th>IRL proper (n=191)</th>
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<tbody>
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<td></td>
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<tr>
<td>ATL</td>
<td>0.8451 (CI 0.7616-0.9237)</td>
<td>0.1549 (CI 0.0762-0.2384)</td>
</tr>
<tr>
<td>IRL proper</td>
<td>0.0152 (CI 0.0054-0.0295)</td>
<td>0.9848 (CI 0.9705-0.9946)</td>
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</table>
Table 8. Mantel tests performed when investigating possible Isolation by Distance phenomenon for H03 -Segments using *Tursiops truncatus* $F_{stmsat}$ values verses path distance (km) from the central point within each segment. Correlation indices are reported as r using (a) segment and Atlantic values, (b) segments only, and (c) segment excluding Mosquito Lagoon.

### a.

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<th>SCIR</th>
<th>SLR</th>
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<tr>
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$r = 0.461$

### b.

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$r = 0.670$

### c.

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$r = -0.099$
Table 9a-9b. Assignment and proportion of ancestry for each *Tursiops truncatus* individual using (a.) BAYES Mixed Stock Analysis and (b.) STRUCTURE. BAYES chain lengths were set at 2000, with a burn-in of 1000, over 1000 iterations, for 3 independent chains. Structure used a burn in of 50,000 and 1,000,000 iterations. Orange highlighted individuals were considered "partial genotypes."

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Chains combined:

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<th>97.50%</th>
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<td>0.7103</td>
<td>3000</td>
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</tbody>
</table>
Table 10. Pearson's Simple Correlations, associated significant values ($p < 0.05$) for (a.) photo-indentification data variables and (b.) stranding/live capture data variables when correlated with ATL Q-Value's for genotyped *Tursiops truncatus* via STRUCTURE. Number of samples ($n$) used in each analysis varied.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Relation Coefficient = $r$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q vs. Easting</td>
<td>*-0.3181</td>
<td>of 2261 sightings from 104 individuals</td>
</tr>
<tr>
<td>Q vs. Month</td>
<td>-0.0141</td>
<td></td>
</tr>
<tr>
<td>Q vs. field est</td>
<td>0.0193</td>
<td></td>
</tr>
<tr>
<td>Q vs. group size</td>
<td>0.0333</td>
<td></td>
</tr>
<tr>
<td>Q vs. Northing</td>
<td>*0.4241</td>
<td></td>
</tr>
<tr>
<td>Q vs. Year</td>
<td>*0.1628</td>
<td>of 767 sightings from 104 individuals: 1 sighting/yr</td>
</tr>
<tr>
<td>Q vs. tot sight</td>
<td>0.0542</td>
<td>of 104</td>
</tr>
<tr>
<td>Q vs. birthyr</td>
<td>-0.0958</td>
<td></td>
</tr>
<tr>
<td>Q vs. sex</td>
<td>-0.0710</td>
<td>233</td>
</tr>
<tr>
<td>Q vs. length</td>
<td>0.0038</td>
<td>181</td>
</tr>
<tr>
<td>Q vs. weight</td>
<td>0.0769</td>
<td>76</td>
</tr>
<tr>
<td>Q vs. age</td>
<td>0.1331</td>
<td>78</td>
</tr>
<tr>
<td>Q vs. hap</td>
<td>*0.3646</td>
<td>244</td>
</tr>
</tbody>
</table>

*$p \leq 0.05$
Figure 1. Map of the study site, the Indian River Lagoon, FL and adjacent Atlantic waters.
Figure 2a-2d. The four hypotheses tested in a visual representation. Boxed terms are the compared strata for each hypothesis.
Figure 3. Minimum Spanning Network of haplotypes from H01- Ecosystems between *Tursiops truncatus* sampled within the Indian River Lagoon, FL and adjacent Atlantic waters.
Figure 4. Estimates of genetic diversity (He, Ho, and no. of alleles) between H01-Ecosystems for *Tursiops truncatus* sampled within the Indian River Lagoon, FL and adjacent Atlantic waters.
Figure 5a-5d. STRUCTURE estimated population clusters using log likelihood values for all four Hypotheses using admixture and location priors over 5 independent runs (identified in legend) for *Tursiops truncatus* sampled within the Indian River Lagoon, FL and adjacent Atlantic waters.
Figure 6a-6d. STRUCTURE results displayed per individual (organized by decreasing longitude) for all four Hypotheses.
Figure 7. Isolation by distance graphical representation for $H_04$ – Segment Hypothesis for *Tursiops truncatus* sampled within the Indian River Lagoon, FL.
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