Journal of Heredity 2013:104(6):765–778
doi:10.1093/jhered/est070

Patterns of Population Structure for Inshore Bottlenose Dolphins along the Eastern United States

Vincent P. Richards, Thomas W. Greig, Patricia A. Fair, Stephen D. McCulloch, Christine Politz, Ada Natoli, Carlos A. Driscoll, A. Rus Hoelzel, Victor David, Gregory D. Bossart, and Jose V. Lopez

From the Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 (Richards); the Marine Mammal Research and Conservation Program, Harbor Branch Oceanographic Institute at Florida Atlantic University, Ft. Pierce, FL (Greig and Fair); the National Oceanic and Atmospheric Administration, Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC (McCulloch, Politz, Driscoll, Bossart, and Lopez); the School of Biological and Biomedical Sciences, University of Durham, South Road Durham, UK (Natoli and Hoelzel); the Basic Research Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD (David); the Georgia Aquarium, NW Atlanta, GA (Bossart); and the Nova Southeastern University Oceanographic Center, Dania Beach, FL (Lopez). Ada Natoli is now at the Biology Department, United Arab Emirates University, Al-Ain, Abu Dhabi, United Arab Emirates.

Address correspondence to Vincent P. Richards at the address above, or e-mail: vpr3@cornell.edu.

Data deposited at Dryad: http://dx.doi.org/10.5061/dryad.v2m91

Abstract

Globally distributed, the bottlenose dolphin (*Tursiops truncatus*) is found in a range of offshore and coastal habitats. Using 15 microsatellite loci and mtDNA control region sequences, we investigated patterns of genetic differentiation among putative populations along the eastern US shoreline (the Indian River Lagoon, Florida, and Charleston Harbor, South Carolina) (microsatellite analyses: $n = 125$, mtDNA analyses: $n = 132$). We further utilized the mtDNA to compare these populations with those from the Northwest Atlantic, Gulf of Mexico, and Caribbean. Results showed strong differentiation among inshore, alongshore, and offshore habitats ($\Phi_{ST} = 0.744$). In addition, Bayesian clustering analyses revealed the presence of 2 genetic clusters (populations) within the 250 km Indian River Lagoon. Habitat heterogeneity is likely an important force diversifying bottlenose dolphin populations through its influence on social behavior and foraging strategy. We propose that the spatial pattern of genetic variation within the lagoon reflects both its steep longitudinal transition of climate and also its historical discontinuity and recent connection as part of Intracoastal Waterway development. These findings have important management implications as they emphasize the role of habitat and the consequence of its modification in shaping bottlenose dolphin population structure and highlight the possibility of multiple management units existing in discrete inshore habitats along the entire eastern US shoreline.

Key words: *Indian River Lagoon, microsatellite, mtDNA,* Tursiops truncatus

Determining population structure in highly mobile organisms is important for facilitating effective conservation management and advances our understanding of the mechanisms that drive the evolution of population genetic structure (Hoelzel 1998). Here, we focus on a marine species with a global geographic distribution, the common bottlenose dolphin (*Tursiops truncatus*). Despite this species' ability for long-range dispersal (Wells et al. 1990; Wursig and Harris 1990; Defran et al. 1999; Wells et al. 1999), populations often show measurable morphological and genetic differentiation, possibly due to complex social behavior and/or habitat specialization. Dolphin habitat is often described as either "coastal" or "offshore," and numerous studies have detected morphological and genetic differentiation between coastal and offshore habitats for *T. truncatus* (Hoelzel, Potter, et al. 1998; Natoli et al. 2005; Sellas et al. 2005; Segura et al. 2006). Furthermore, genetic differentiation has been detected among multiple coastal populations occupying discrete habitat zones for bottlenose dolphins in

general (Natoli et al. 2005; Möller et al. 2007; Rosel et al. 2009; Mirimin et al. 2011). Coastal habitat can be further subdivided into inshore and alongshore. Here, we define inshore habitat as bays, lagoons, sounds, tidal marshes, or estuarine waters (Leatherwood and Reeves 1983; Sellas et al. 2005) and refer to the remaining coastal habitat as alongshore. Despite the ubiquity of inshore habitat, little work has been done to investigate genetic population structure among *T. truncatus* populations occupying this type of habitat. A rare study of this kind was that of Sellas et al. (2005) who found these populations to be highly differentiated. Inshore habitat also has great potential for environmental heterogeneity within the bay or estuary, which increases the potential for fine-scale population subdivision, and studies of Indo-Pacific bottlenose dolphin populations (*Tursiops aduncus*) within Australian embayments have confirmed these expectations (Krützen, Sherwin, et al. 2004; Wiszniewski et al. 2010; Ansmann, Parra, Lanyon, et al. 2012).

The purpose of our study was to test hypotheses regarding patterns of genetic population structure for this species in the context of habitat variation over a fine geographic scale. To this end, we investigated 2 inshore habitats along the eastern US coastline and compared these 2 regions with proximate alongshore and offshore populations in the Northwest Atlantic, Gulf of Mexico, and Caribbean. The 2 inshore dolphin sample sets studied were from the Indian River Lagoon, Florida (IRL), and Charleston Harbor, South Carolina and its adjacent estuarine area (CHS) (Figures 1 and 2). The US National Marine Fisheries Service now lists dolphins in these regions as distinct management stocks: the Indian River Lagoon Estuarine System Stock and the Charleston Estuarine System Stock (Waring et al. 2010). The management of cetaceans is a difficult problem as populations often show complex patterns of sympatry, parapatry, and mixed assembly (Hoelzel 1998). Consequently, genetic

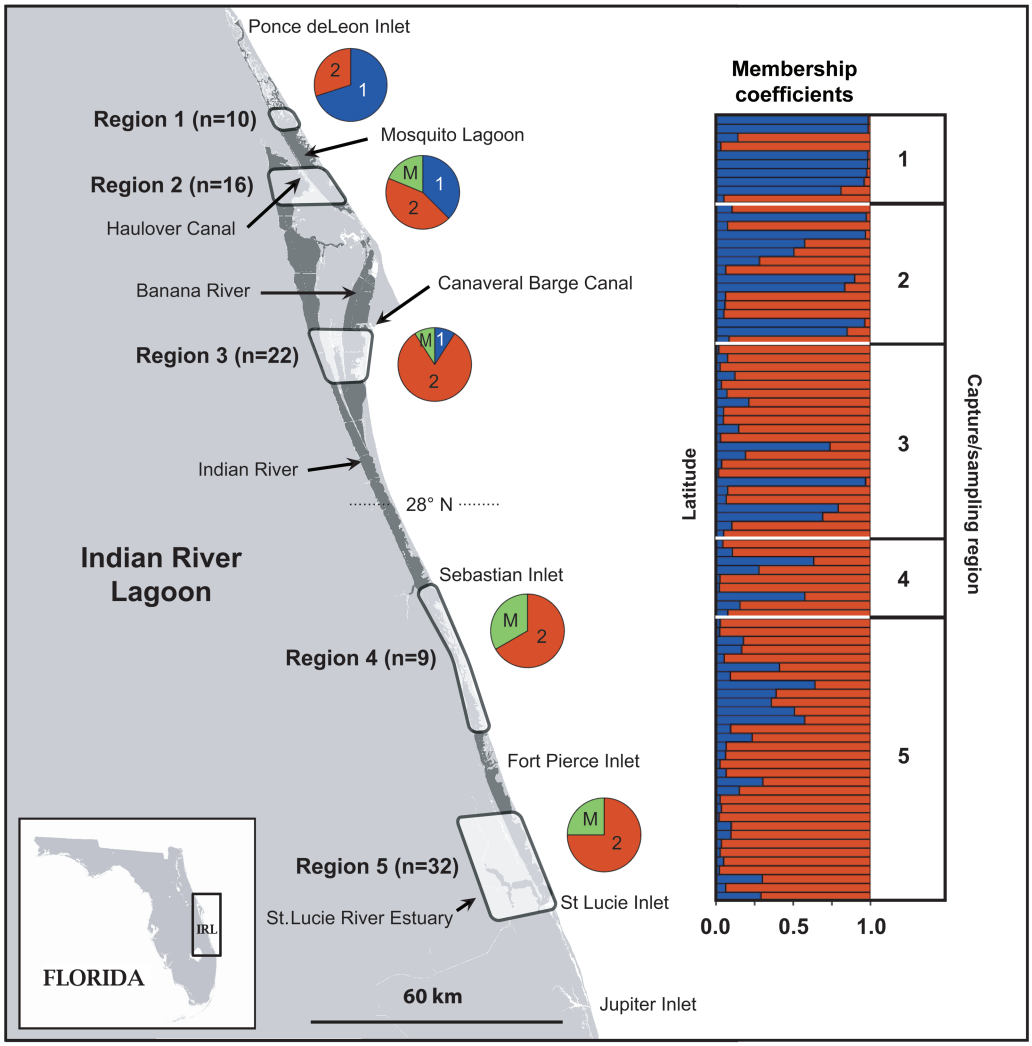

Figure 1 Map of the Indian River Lagoon showing capture/sampling regions (1–5). Membership coefficients (Q) generated by STRUCTURE for each individual are shown in the bar chart. Each individual is positioned in the chart according to its capture/sampling latitude (region is shown in column to the right). The distribution of individuals assigned to clusters 1 and 2 for each region is shown in pie charts (1 = cluster 1, 2 = cluster 2, and M = individuals with mixed ancestry [$Q < 0.75$]).

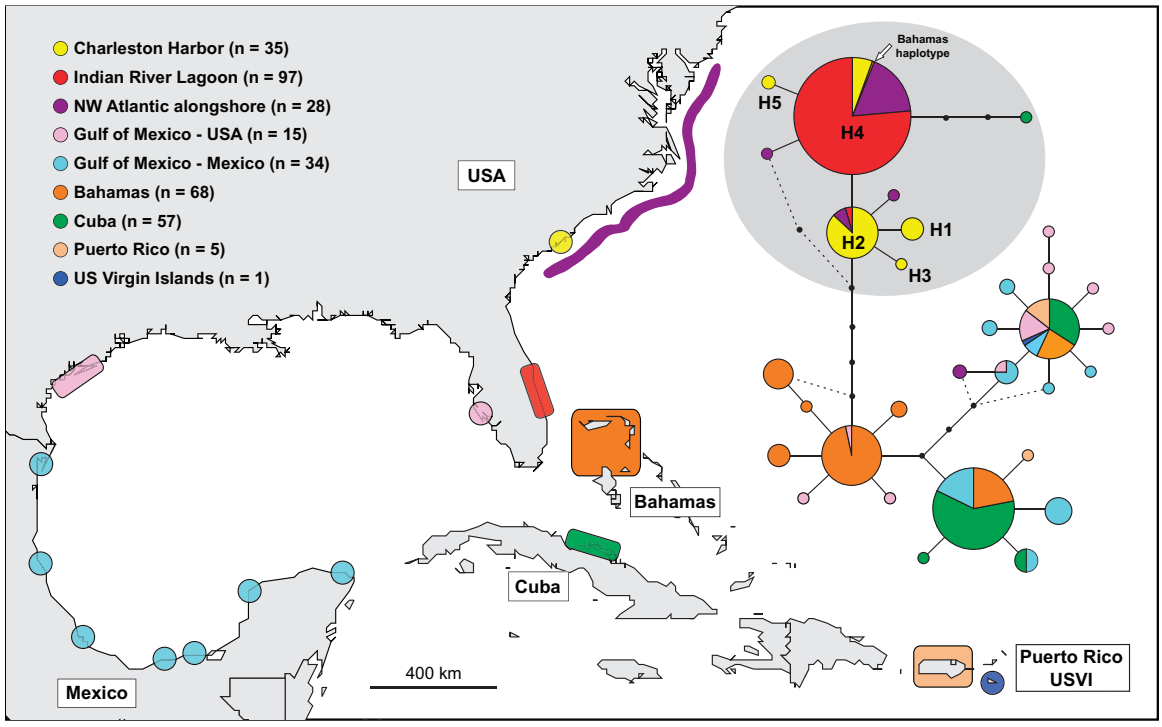

Figure 2 Map showing geographic location of mitochondrial control region sequences used in the statistical parsimony analysis and the resulting network. Northwest Atlantic offshore haplotypes and haplotypes described as "worldwide form" (Caballero et al. 2012) were not included in the analysis. Sample sizes are shown top left (also see Table 1). In the network, circles represent different haplotypes, color indicates geographic sampling location, and size represents frequency of occurrence. Lines connecting haplotypes represent mutational steps and small black circles represent hypothetical haplotypes that were not sampled. Dashed lines show haplotype connections considered less likely following criteria based on coalescent theory (Crandall and Templeton 1993). The 5 haplotypes discovered as part of this study are indicated as follows: H1–H5. A single individual sampled in the Bahamas possessing haplotype H4 is indicated.

population data are valuable for reliable stock identification and effective management of bottlenose dolphins (Hoelzel 1998; Waring et al. 2002), and the data provided here will be directly applicable to US National Marine Fisheries Service management of the Indian River Lagoon Estuarine System Stock and Charleston Estuarine System Stock (Hoelzel 1998; Waring et al. 2002). The habitats studied have contrasting biogeographic features. For example, the Indian River Lagoon on Florida's east coast experiences a dynamic range of hydrologic and climatic conditions that has created a highly heterogeneous environment possessing resident fauna adapted to both temperate and tropical conditions (White 1970; Steward and VanArman 1987; Gilmore 1995; McRae et al. 1998). The lagoon is a shallow estuary encompassing approximately 3600 km^2 that stretches 250 km from the Jupiter Inlet in the south to the Ponce de Leon Inlet in the north (Steward et al. 1994) and is considered one of the most biodiverse estuaries in North America (Gilmore 1995). Furthermore, in addition to the Indian River, which runs the length of the estuary, the Indian River Lagoon system also includes smaller, less accessible areas, such as the Mosquito Lagoon, Banana River, St. Lucie River, and several tributaries (Figure 1). The lagoon has only 6 inlets from the Atlantic Ocean, which could serve as potential factors shaping population substructure. In contrast, Charleston Harbor and its adjacent estuarine area (including the Ashley, Cooper, and Wando Rivers, and Stono River Estuary), which is about 426 km from the most northern Indian River Lagoon inlet, is considerably smaller, possesses temperate estuaries, and is more accessible to open ocean. Here, we test the hypothesis that these differences in habitat will be reflected in differences in the structure of local populations and that the inshore populations will be differentiated from each other and from alongshore and offshore populations (as seen in earlier, similar studies in the Gulf of Mexico and Australia).

Materials and Methods

Sample Collection and DNA Extraction

Dolphin skin samples were collected using capture–release over a 5-year period (1999–2004) under the appropriate National Marine Fisheries Service (NMFS)/National Oceanic and Atmospheric Administration (NOAA) permits for the Dolphin Health and Risk Assessment (HERA) Project (see Acknowledgments). IRL samples were collected from 5

subregions of the lagoon system that included the Banana River, Mosquito Lagoon, Indian River, and St. Lucie River (Figure 1). Charleston Harbor samples included individuals from the Wando River, and the adjacent Stono River Estuary. Tissue samples were stored in NaCl-saturated 20% dimethyl sulfoxide (DMSO) (Amos and Hoelzel 1990) at 4 °C. For the microsatellite analyses, 125 samples were collected (CHS = 36 and IRL = 89) (see Figure 1 for the distribution of samples within the IRL). The CHS samples included 3 from the Wando River and 10 from the Stono River Estuary. Genomic DNA was isolated from skin using Qiagen DNeasy Tissue Kits (Qiagen, Inc., Valencia, CA) following manufacturer's instructions for animal tissue. Extracted DNA quality was evaluated by electrophoresis on 0.7% agarose gels and quantified by spectrophotometer, ND1000 (ThermoScientific, Waltham, MA).

For the mitochondrial analyses, 82 samples were collected by capture–release (CHS = 35 and IRL = 47). To these samples, we added 11 acquired via biopsy dart and 39 opportunistic samples from dolphin strandings either from within the Indian River Lagoon proper or from the Atlantic Ocean side of the lagoon (n = 3). IRL samples were collected from the same 5 subregions described above. Charleston Harbor samples again included individuals from the Wando River (n = 7), and the adjacent Stono River Estuary (n = 15). The additional tissue samples were stored in NaCl-saturated 20% DMSO (Amos and Hoelzel 1990) at 4 °C. Total genomic DNA was extracted using a standard proteinase K digestion for 4 h at 45 °C, followed by 3 phenol/chloroform extractions (Sambrook and Russell 2001). The DNA was then resuspended in Tris–ethylenediaminetetraacetic acid buffer and stored at −20 °C. Overlap between the 2 data sets was 49 individuals for the microsatellite data and 10 individuals for the mitochondrial data. Sex of individuals was determined in the field for capture–release individuals and strandings. Dart biopsy samples were sexed using the polymerase chain reaction (PCR) test of Rosel (2003).

Microsatellite DNA Genotyping and Analysis

Using 18 microsatellite loci, 125 individuals (CHS = 36 and IRL = 89) were genotyped. We employed 14 *Tursiops*-specific loci: MK5, MK6, MK8, MK9 (Krützen et al. 2001), Ttr04, Ttr11, Ttr19, Ttr34, Ttr48, Ttr58, Ttr63, TtrFF6 (Rosel et al. 2005), TexVet5, and TexVet7 (Rooney et al. 1999), and 4 additional loci from other cetacean species: EV14, EV94 (Valsecchi and Amos 1996), KWM12a (Hoelzel, Dahlheim, et al. 1998), and PPHO130 (Rosel et al. 1999). Reaction conditions and methods followed that of Rosel et al. (2009) and consisted of 25–50 µg of sample DNA in 25 µL reaction volumes. All reactions included both positive and negative controls. Samples were genotyped on an Applied Biosystems 3130 xl Genetic Analyzer using the internal size standard GS-Liz 500 and GENEMAPPER v3.7 (Applied Biosystems, Foster City, CA).

GENEPOP v4.1 (Rousset 2008) was used to calculate Weir and Cockerham F-statistics (Weir and Cockerham 1984) and perform exact tests for significant population differentiation, Hardy–Weinberg equilibrium, and linkage disequilibrium between all locus pairs. The false discovery rate (FDR) procedure of Benjamini and Hochberg (1995) was used to correct for multiple hypothesis testing (FDR = 0.05). Observed and expected frequencies of heterozygotes were calculated using GENETIX v4.03 (Belkhir et al. 1996–2004). Each locus was checked for null alleles, scoring errors, and large allele dropout using MICRO-CHECKER v2.2.3 (Van Oosterhout et al. 2004). The discriminatory power of the microsatellite data was assessed by calculating the probability of identity (PI) for the combined set of loci using GENALEX v6.5 (Peakall and Smouse 2012). PI is an estimate of the average probability that 2 unrelated individuals drawn from the same randomly mating population will have the same multilocus genotype by chance. To exclude the possibility that population structure analyses were influenced by the presence of related individuals (i.e., the family affect), we determined whether individuals were more closely related than expected in a randomly mating population as follows: 1) average pairwise relatedness among CHS and IRL individuals was calculated using the Queller and Goodnight (1989) relatedness index (r) and 2) the null hypothesis of no relatedness was tested by comparing this average to that of a null distribution created using IDENTIX v1.1 (Belkhir et al. 2002) (1000 permutations).

The number of genetic clusters (K) was estimated by performing a Bayesian evaluation of genetic partitioning, as implemented in the program STRUCTURE v2.2.3 (Pritchard et al. 2000) and then calculating the ad hoc statistic ΔK (Evanno et al. 2005). ΔK was calculated for K = 1–5 by averaging 20 Markov chains for each value of K. Length of each chain was 50 000 steps following an initial burn in of 50 000 steps. The admixture ancestry and correlated allele frequency models were used. ΔK is a measure of the second-order rate of change of the probability of the data $\Pr(X|K)$ for each value of K. The highest value for ΔK identifies the optimum value of K, and the method has been shown to perform well when differentiation among populations is modest ($F_{ST} \geq 0.03$) (Latch et al. 2006). However, when hierarchal levels of population structure exist, this method usually detects the uppermost level of partitioning (Evanno et al. 2005). To estimate the number of clusters at the next level of structure (i.e., population substructuring), we repeated the analysis on each of the separate genetic clusters identified in the previous run, as suggested by Pritchard (2007). Final individual membership coefficients (Q) were obtained using a run of 5 000 000 steps with a 20% burn in at the optimum overall value of K. Individuals with $Q \geq 0.75$ were considered a member of a cluster.

Estimates of migration rates within the IRL were calculated using the program MIGRATE version 3.51 (Beerli 2006). Using the Bayesian framework, we estimated parameters Θ and m/μ using a chain of 6 000 000 steps with a burn in of 2 000 000 steps. Boundaries for uniform prior distributions were established empirically via shorter preliminary runs. The microsatellite stepwise mutation model was employed.

Spatial genetic structure (SGS) within the IRL was assessed by comparing genetic similarity to spatial distance using GENALEX. Spatial distance was calculated using individual sampling coordinates. Multilocus genetic distances as

described by Smouse and Peakall (1999) were plotted against geographic distances between pairs of individuals using the spatial autocorrelation method of Smouse and Peakall (1999). SGS was calculated at 10 km distance intervals (distance classes) up to 200 km. Significance of the spatial autocorrelation coefficient (r) was tested by constructing a 2-tailed 95% confidence interval (95% CI) around the null hypothesis of no SGS (i.e., $r = 0$) by performing 999 random permutations of genotypes among geographic locations. The FDR procedure of Benjamini and Hochberg (1995) was used to correct for multiple hypothesis testing (FDR = 0.05).

We tested for sex-biased dispersal within the IRL, CHS, and both regions combined using the assignment method of Favre et al. (1997) and Mossman and Waser (1999) as implemented in GENALEX. The method calculates the log-likelihood of an individual's assignment to its population (in this case, male or female). Log-likelihoods are corrected using an assignment index correction (AIc) as follows: individual log-likelihood minus mean log-likelihood of the population. Negative values of AIc indicate individuals with a high probability of being immigrants. Sex-biased dispersal is inferred if there is significant difference in the frequency distribution of AIc values for males versus females.

Mitochondrial DNA Sequencing and Analysis

Using established PCR primers (Rosel et al. 1995), we obtained 296 bp of DNA sequence data from the 5' end of the mtDNA control region for 132 individuals (CHS = 35 and IRL = 97). PCR amplification conditions were as follows: 10–100 ng of genomic DNA, 2 mM deoxyribonucleotide triphosphates, 10× PCR buffer (1.5 mM $MgCl_2$), 25 pmol of each primer, and 0.5 U Taq DNA polymerase. The amplification reactions followed the profile: 1 cycle at 94 °C for 1 min, followed by 30 cycles of 94 °C for 40 s, 53 °C for 1 min, 72 °C for 1 min, and ending with 1 cycle at 72 °C for 30 min. PCR products were purified with Qiaquick PCR purification columns (Qiagen) for DNA sequencing. Both forward and reverse strands were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Products were separated and bases called by the Interdisciplinary Center for Biotechnology Research at the University of Florida. Haplotype sequences are available from GenBank (accession numbers: KF366717–KF366721).

Mitochondrial control region sequences ($n = 279$) from 11 additional geographic regions were obtained from GenBank and/or previous publications (Table 1). Including the Charleston Harbor area and the Indian River Lagoon, these sampling sites represented a range of temperate and tropical offshore, alongshore, and inshore habitats.

Individual sequences were cropped to the same length (258 bp) and aligned using MAFFT v6.814b (Katoh et al. 2002) as implemented in GENEIOUS PRO v5.5.3 (Drummond et al. 2010). Genetic population structure among the following 8 geographic regions was examined by an analysis of molecular variance and exact tests of haplotype frequency differentiation as implemented in ARLEQUIN v3.11 (Excoffier et al. 2005): CHS, IRL, Northwest Atlantic offshore (NWAo), Northwest Atlantic alongshore (NWAa), and coastal animals from the Gulf of Mexico (United States of America), the Gulf of Mexico (Mexico), Bahamas, and Cuba (see Table 1 for sample sizes). Significance of Φ statistics was determined by permuting haplotypes among populations 10 000 times (Excoffier et al. 2005). Molecular diversity indices were calculated using ARLEQUIN. Evolutionary relationships among inshore/alongshore haplotypes ($n = 31$) (see Table 1 for individuals included) were inferred by constructing an unrooted statistical parsimony network using the method of Templeton et al. (1992) as implemented in the software package TCS v1.13 (Clement et al. 2000). Alignment gaps (indels) were treated as a fifth state. Evolutionary relationships among inshore/alongshore ($n = 31$) and offshore/worldwide form haplotypes ($n = 25$) were inferred by maximum likelihood phylogenetic analysis (see Supplementary Table S1 for accession numbers). PhyML v3.0 (Guindon et al. 2010) was used to generate 500 bootstrap replicates using the HKY+I+G substitution model, which was determined as the best fit for the data using MODELTEST v3.7 (Posada and Crandall 1998). A bootstrap consensus tree was constructed using the Triple Construction Method as implemented in the program TRIPLEC (Ewing et al. 2008). This procedure is based on the observation that the most probable 3-taxon gene tree consistently matches the species tree (Degnan and Rosenberg 2006). The method searches all input trees for the most frequent of the 3 possible rooted triples for each set of 3 taxa. Once found, the set of rooted triples are joined to form the consensus tree using the quartet puzzling heuristic (Strimmer and von Haeseler 1996). The procedure has been shown to be a statistically consistent estimator of the species tree topology and to out perform majority rule and greedy consensus methods (Degnan et al. 2009).

In fulfillment of data archiving guidelines (Baker 2013), we have deposited the primary data underlying these analyses with Dryad (http://datadryad.org).

Results

Microsatellite Characterization

None of the 18 microsatellite loci showed significant deviation from Hardy–Weinberg equilibrium (Table 2). However, 8 loci showed significant linkage disequilibrium (FF6, MK8, EV14, TTR11, MK6, PPHO130, TXVT7, and TTR58) (10 pairwise comparisons). Multiple population events could be responsible for this disequilibrium, for example, admixture between populations with different allele frequencies (Smith et al. 2001), inbreeding (Chakraborty and Weiss 1988), the founding of populations by a small number of individuals followed by rapid population growth (Sheffield et al. 1998; Shifman and Darvasi 2001), and a relatively small and stable population size (Hill and Robertson 1968). None of the loci showed evidence for large allele drop out. However, 3 loci showed evidence for the presence of null alleles (EV94, MK9, and TTR11). We excluded these 3 loci from all subsequent analyses. Rosel et al. (2009) also detected null alleles for EV94. Average pairwise relatedness among CHS and IRL individuals using the remaining 15 loci was −0.0301

Table 1 Description of *Tursiops truncatus* mtDNA samples included in various analyses and population diversity indices

Region	*N*	*n*-pop	*H*	*h*	π	θ_S	θ_π	TCS	Source
Charleston Harbor (inshore)	35	35	5	0.63	0.0032	0.97	0.81	35 (yellow)	1
Indian River Lagoon (inshore)	97	97	2	0.02	0.0001	0.19	0.02	97 (red)	1
Northwest Atlantic offshore (NWAo)	25	25	11	0.88	0.0224	4.24	5.74		2
Northwest Atlantic alongshore (NWAa)	28	28	5	0.38	0.0065	2.31	1.68	28 (purple)	2
Gulf of Mexico (USA): Texas alongshore	10							10 (pink)	3
Gulf of Mexico (USA): Texas and central/west coast of Florida, mixed alongshore/inshore	5	15	9	0.88	0.0113	3.07	2.91	5 (pink)	4
Gulf of Mexico (Mexico): inshore ecotype = 34, worldwide form = 6	40	34	9	0.82	0.0114	1.96	2.95	34 (light blue)	5
Caribbean, Bahamas: Grand Bahama and Abaco Islands: alongshore = 53, offshore = 2	55							53 (orange)	6
Caribbean, Bahamas: Grand Bahama, Abaco Islands, and New Providence, inshore ecotype	13	66	6	0.72	0.0088	2.71	2.26	13 (orange)	5
Caribbean, Cuba: inshore ecotype = 57, worldwide form = 8	65	57	5	0.44	0.0079	2.6	2.04	57 (green)	5
Caribbean, Puerto Rico: inshore ecotype = 5, worldwide form = 21	26							5 (brown)	5
Caribbean, US Virgin Islands: inshore ecotype	1							1 (dark blue)	5
Caribbean, Colombia: worldwide form	4								5
Caribbean, Jamaica: worldwide form	1								5
Caribbean, Honduras: worldwide form	6								5

N = total number of samples for each geographic region; *n*-pop = number of samples included in the analysis of population structure (all alongshore/inshore samples from the Gulf of Mexico [United States] were pooled, all alongshore [53] and inshore ecotype samples [13] from the Bahamas were pooled); *H* = number of haplotypes; *h* = haplotype diversity; π = nucleotide diversity. θ_S (Tajima 1989), and θ_π (Tajima 1983). The Northwest Atlantic alongshore samples (NWAa) ranged from Georgia to New Jersey and the offshore samples (NWAo) were collected 160–480 km offshore this same stretch of coastline (Hoelzel, Potter, et al. 1998). TCS = samples included in statistical parsimony analysis (colors corresponding to those used to indicate geographic sampling region in Figure 2 are shown in parentheses). 1 = this study, 2 = Hoelzel, Potter, et al. (1998), 3 = Natoli et al. (2004), 4 = Sellas et al. (2005), 5 = Caballero et al. (2012), and 6 = Parsons et al. (2006). See Caballero et al. (2012) for description of inshore ecotype and worldwide form. *Note*: according to our delineation of environments, the sampling location for individuals described as "inshore ecotype" by Caballero et al. (2012) is regarded here as coastal as it is unclear if any of these animals were located in an inshore environment as we define it.

and −0.0105, respectively, and neither population contained individuals more related than expected in a randomly mating population (CHS: $P = 0.059$; IRL: $P = 0.258$). PI was 2.8E-12, indicating a very low probability that 2 unrelated individuals would share the same multilocus genotype by chance.

Microsatellite Population Structure and Migration Rates

The Charleston Harbor and Indian River Lagoon populations were highly differentiated ($F_{ST} = 0.131$, $P < 0.0001$), and the STRUCTURE analysis reflected this differentiation with a ΔK of 2 (Supplementary Figure S1a). Analysis of substructuring for IRL produced a ΔK of 2 (Supplementary Figure S1b). The same analysis for CHS was not conclusive. ΔK could not be used due to a drastic drop in the mean log probability score [$\Pr(X|K)$] for $K = 2$ relative to $K = 1$ (Supplementary Figure S1c). The score for $K = 3$ was also lower than the score for $K = 1$. The score for $K = 4$ was slightly higher than for $K = 1$ and the score for $K = 5$ slightly higher still. We performed additional STRUCTURE runs to determine if the scores continued to rise for $K = 6$–8. Results showed the scores to decline steadily, with all scores lower than that for $K = 1$. Consequently, the highest score was for $K = 5$ followed by $K = 4$ and then $K = 1$. Without additional samples to further explore these results, we suggest $K = 1$ as the most biologically realistic result.

Table 2 Number of alleles, expected and observed heterozygote frequencies, and F_{IS} values for each microsatellite locus within Charleston Harbor and the Indian River Lagoon for *Tursiops truncatus*

Locus	CHS	IRL	Locus	CHS	IRL
EV14			Ttr04		
N_a	5	6	N_a	8	5
H_E	0.683	0.649	H_E	0.767	0.602
H_O	0.611	0.602	H_O	0.750	0.618
F_{IS}	0.120	0.078	F_{IS}	0.036	−0.021
EV94			Ttr11		
N_a	3	2	N_a	4	6
H_E	0.574	0.487	H_E	0.559	0.728
H_O	0.417	0.546	H_O	0.472	0.607
F_{IS}	0.287	−0.114	F_{IS}	0.169	0.172
TtrFF6			Ttr19		
N_a	8	7	N_a	5	5
H_E	0.702	0.649	H_E	0.510	0.546
H_O	0.694	0.685	H_O	0.472	0.598
F_{IS}	0.025	−0.050	F_{IS}	0.087	−0.090
KWM12a			Ttr34		
N_a	5	6	N_a	2	5
H_E	0.547	0.467	H_E	0.105	0.638
H_O	0.686	0.409	H_O	0.056	0.682
F_{IS}	−0.240	0.130	F_{IS}	0.482	−0.062
MK5			Ttr48		
N_a	5	4	N_a	4	4
H_E	0.699	0.604	H_E	0.496	0.277
H_O	0.667	0.674	H_O	0.457	0.291
F_{IS}	0.060	−0.110	F_{IS}	0.093	−0.045
MK6			Ttr58		
N_a	7	7	N_a	4	5
H_E	0.738	0.615	H_E	0.641	0.594
H_O	0.722	0.553	H_O	0.611	0.562
F_{IS}	0.036	0.107	F_{IS}	0.060	0.060
MK8			Ttr63		
N_a	9	5	N_a	12	11
H_E	0.801	0.632	H_E	0.874	0.767
H_O	0.750	0.640	H_O	0.806	0.809
F_{IS}	0.078	−0.008	F_{IS}	0.093	−0.049
MK9			TexVet5		
N_a	4	3	N_a	4	4
H_E	0.436	0.437	H_E	0.705	0.400
H_O	0.472	0.356	H_O	0.657	0.432
F_{IS}	−0.070	0.190	F_{IS}	0.082	-0.073
PPHO130			TexVet7		
N_a	6	5	N_a	4	4
H_E	0.619	0.638	H_E	0.681	0.530
H_O	0.543	0.562	H_O	0.639	0.466
F_{IS}	0.138	0.126	F_{IS}	0.075	0.127

N_a = number of observed alleles; H_E = expected heterozygosity; H_O = observed heterozygosity. No F_{IS} values were significant after FDR correction ($\alpha = 0.05$).

For the IRL, the proportion of individuals assigned to the 2 clusters and those with mixed ancestry ($Q < 0.75$) was not evenly distributed in each of the 5 sampling regions (Figure 1). Individuals assigned to cluster 1 were restricted to the 3 northern regions (1–3), whereas individuals assigned to cluster 2 occurred in all sampling regions. Individuals with mixed ancestry occurred in all regions except the most northern (region 1). Based on these findings, we investigated differentiation between northern and southern regions. We explored 2 groupings: (I) regions 1 and 2 versus regions 3–5 and (II) regions 1–3 versus regions 4 and 5. There was significant differentiation between both groupings; however, the first grouping showed the highest level of differentiation (I: $F_{ST} = 0.0241$, $P < 0.0001$; II: $F_{ST} = 0.0120$, $P < 0.0001$). Using the grouping that showed the highest differentiation (I), we additionally explored differentiation between IRL regions and CHS as follows: (i) regions 1 and 2 versus CHS and (ii) regions 3–5 versus CHS. The second comparison showed the highest differentiation (i: $F_{ST} = 0.3687$, $P < 0.0001$; ii: $F_{ST} = 0.4102$, $P < 0.0001$). The migration rates between regions 1 and 2 versus regions 3–5 showed a strong bias. The number of migrants per generation from regions 1

and 2 into regions 3–5 was 7.8 (95% CI = 2.1–18.4), whereas the number of migrants per generation from regions 3–5 into regions 1 and 2 was 27.9 (95% CI = 14.6–47.0).

SGS and Sex-Biased Dispersal

The SGS analysis detected significant genetic structure at the extremes of the distance classes (0–10, 20–30, and 190–200 km) (Figure 3). We detected no signal of sex-biased dispersal within the IRL, CHS, or both regions combined. IRL: mean male AIc = -0.190 ± 0.392 (standard error, SE), mean female AIc = 0.338 ± 0.281 (SE), and no significant difference in the male and female AIc distributions ($Z = 0.166$, $P = 0.868$). CHS: mean male AIc = 0.029 ± 0.466 (SE), mean female AIc = -0.033 ± 0.362 (SE), and AIc distributions ($Z = -0.283$, $P = 0.777$). Both regions combined: mean male AIc = 0.051 ± 0381 (SE), mean female AIc = -0.079 ± 0492 (SE), and AIc distributions ($Z = -0.172$, $P = 0.863$).

Mitochondrial Population Differentiation and Diversity

For the samples collected in CHS and the IRL, we detected 5 haplotypes (H1–H5) (Figure 2), with only 2 occurring within the IRL (H2 and H4). H4 was considerably more frequent within the IRL than H2 (H4 = 96 and H2 = 1). For CHS, haplotype frequencies were as follows: H1 = 1, H2 = 20, H3 = 5, H4 = 7, and H5 = 2. Due to the low number of haplotypes within the IRL, it was not possible to examine population structure among the 5 regions within the lagoon. However, examination of population structure over a wider geographic scale (eastern USA, Gulf of Mexico, and Caribbean) revealed a high level of overall genetic partitioning among the 8 regions ($\Phi_{ST} = 0.744$, $P < 0.0001$), with all pairwise comparisons significant after FDR correction (Φ_{ST} and exact tests). With 3 exceptions, all pairwise comparisons showed high differentiation (Table 3). The 3 exceptions were the comparisons of CHS and IRL to the Northwest Atlantic alongshore population (CHS vs. NWAa: $\Phi_{ST} = 0.231$, IRL vs. NWAa: $\Phi_{ST} = 0.160$), and in particular, Gulf of Mexico (Mexico) to Cuba ($\Phi_{ST} = 0.052$). Genetic diversity indices showed that nucleotide diversity and theta were highest for the Northwest Atlantic offshore population, followed by the 5 alongshore/coastal populations, and finally, the inshore populations (CHS and IRL) showed the lowest values (Table 1).

Evolutionary Relationships

The statistical parsimony analysis clustered CHS, IRL, and Northwest Atlantic alongshore haplotypes into a distinct group separate from haplotypes from the remaining 6 geographic regions (5 steps separated them) (Figure 2). A single haplotype sampled in Cuba was connected to the CHS/IRL/NWAa grouping by 3 steps, and a single individual sampled in the Bahamas shared the most frequent haplotype (H4) within the CHS/IRL/NWAa grouping. The first group of haplotypes connected to the CHS/IRL/NWAa grouping was dominated by Bahamas haplotypes. The maximum likelihood phylogeny was concordant with the statistical parsimony analysis as it clustered CHS, IRL, and NWAa haplotypes into a single strongly supported group that was separate from Caribbean and Gulf of Mexico inshore haplotypes (Figure 4). These latter haplotypes were also clustered into a single strongly supported group. All offshore/worldwide form haplotype groupings were basal to the inshore/alongshore group (also strongly supported). The CHS/IRL/NWAa grouping again contained the single haplotype from Cuba.

Discussion

Patterns of Population Structure within Inshore Habitat

Both the mitochondrial and microsatellite data showed the 2 inshore populations of CHS and IRL to be highly

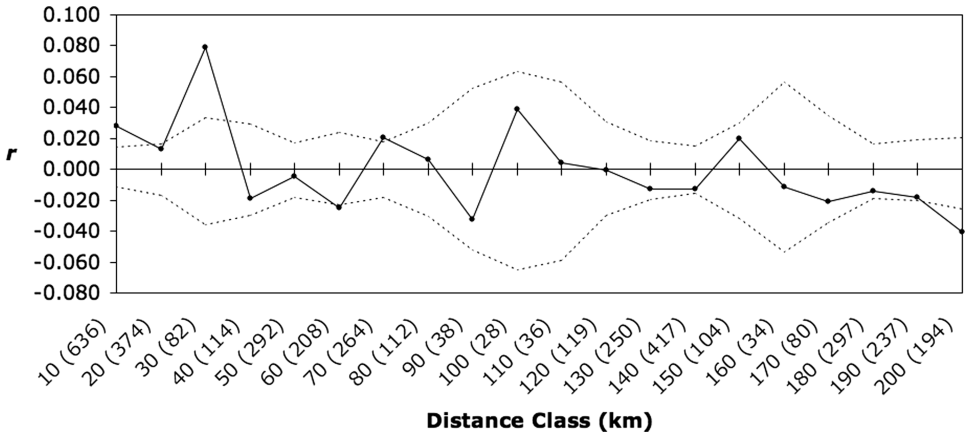

Figure 3 Correlogram showing the spatial correlation *r* as a function of distance (20 distance classes, 10 km each). Sample size (the number of pairs compared) for each distance class is shown in parentheses. *r* values are shown at the end point of each distance class. Dashed lines show the 95% CI around the null hypothesis of a random distribution of dolphins for each distance class. *r* shows significant deviation from a random distribution for 3 distance classes (0–10, 20–30, and 190–200 km).

Table 3 mtDNA pairwise Φ_{ST} values among 8 geographic regions (inshore, alongshore, and offshore individuals) for *Tursiops truncatus*

	CHS	IRL	NWAa	GOM-USA	GOM-Mexico	Bahamas	Cuba
IRL	0.710	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NWAa	0.231	0.160	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
GOM-USA	0.852	0.960	0.784	<0.0001	<0.0001	<0.0001	<0.0001
GOM-Mexico	0.789	0.911	0.733	0.315	<0.0001	<0.0001	0.0411
Bahamas	0.750	0.872	0.706	0.496	0.286	<0.0001	<0.0001
Cuba	0.808	0.908	0.765	0.470	0.052	0.289	<0.0001
NWAo	0.762	0.886	0.696	0.644	0.674	0.688	0.732

CHS = Charleston Harbor; IRL = Indian River Lagoon; NWAa = Northwest Atlantic alongshore; NWAo = Northwest Atlantic offshore; GOM = Gulf of Mexico. Φ_{ST} values are shown below diagonal, with associated *P* values shown above diagonal (all significant after FDR correction; $\alpha = 0.05$).

differentiated, and the microsatellite data revealed the presence of 2 distinct populations (genetic clusters) within the IRL. Individuals assigned to cluster 1 were restricted to the northern most sampling regions (1–3), occurring predominately in regions 1 and 2 (87%), whereas individuals assigned to cluster 2 were seen throughout the IRL. Maximum differentiation was attained when regions 1 and 2 were grouped separately from the rest of the IRL, confirming the distinctiveness of the Mosquito Lagoon. These findings are concordant with the photoidentification study of Mazzoil et al. (2008), suggesting a distinct community within the Mosquito Lagoon. However, although their study also suggested the presence of 2 additional communities in the remainder of the IRL, we only find evidence for 1 additional population (cluster 2).

The Mosquito Lagoon and the remainder of the IRL (the Indian and Banana rivers) are connected by the Haulover canal, which traverses a narrow strip of land approximately 0.75 km wide that separates the 2 bodies of water. The canal was constructed circa 1887 (Florida Department of State: Division of Historical Recourses). For approximately 35 years prior to this, a smaller canal had existed a mile to the south. Consequently, the Mosquito Lagoon and Indian/Banana Rivers have only been connected for approximately 160 years. Prior to this, and the construction of the Canaveral Barge Canal (1965) and Sebastian Inlet (1924), the only connection between the lagoon and rivers would have been via 4 natural inlets and the ocean. Specifically, the Ponce deLeon Inlet in the north of the Mosquito Lagoon (northern most extremity of the IRL) and the Fort Pierce, St. Lucie, and Jupiter inlets in the south of the IRL (Figure 1). Therefore, it is possible that the Mosquito Lagoon and Indian/Banana Rivers were colonized independently prior to construction of the canal, with cluster 1 representing the Mosquito Lagoon population and cluster 2 the Indian/Banana River population. The SGS analysis provided good support for this hypothesis, as the Haulover canal is approximately 187 km from the southern most sampling location and the analysis showed the IRL to be genetically structured at the distance class of 190–200 km.

Numerous studies have shown high population differentiation between alongshore and offshore habitat (Hoelzel, Potter, et al. 1998; Natoli et al. 2005; Sellas et al. 2005; Segura et al. 2006), and environmental factors such as temperature, salinity, and productivity may differentiate bottlenose dolphin populations (Natoli et al. 2005). Consequently, the environmental transition between inshore and alongshore habitat may be contributing to differentiation among inshore populations. A likely example of this process was reported for 3 inshore populations along the west coast of Florida that were strongly differentiated and separated by approximately 130 km (a distance approximately half the length of the IRL) (Sellas et al. 2005). These findings and other recent studies showing fine-scale differentiation for bottlenose dolphins (Wiszniewski et al. 2010; Mirimin et al. 2011; Ansmann, Parra, Lanyon, et al. 2012) suggest that once the Mosquito Lagoon and Indian/Banana Rivers were colonized, there would have been limited genetic exchange between the 2 regions via the ocean. Subsequent to construction of the Haulover canal, resident populations in the Mosquito Lagoon and Indian/Banana Rivers would have had direct access to each other and the presence of individuals with mixed ancestry within the IRL possibly reflects subsequent genetic exchange. Alternative hypotheses, where either the Mosquito Lagoon or Indian/Banana Rivers lacked resident dolphin populations prior to the construction of the canal, are perhaps less likely given the availability of such suitable habitat.

For bottlenose dolphins, habitat heterogeneity has been shown to be an important factor affecting the development of distinct habitat utilization skills (foraging strategies) (Chilvers and Corkeron 2001; Sargeant et al. 2007; Wiszniewski et al. 2009; Olin et al. 2012). The IRL crosses a zone of climatic transition along the eastern Florida coast, with the boundary typically described as occurring at Cape Canaveral (Briggs 1974; Briggs and Bowen 2012). This location is also identified as a phylogeographic boundary for numerous coastal species (Bowen and Avise 1990; Avise 1992). However, the work of Gilmore (1995) showed that the biogeographic boundary within the IRL existed slightly further south approximately midway along its length at 28°00'N (Figure 1). This climatic transition has created distinct environments in the north and south of the lagoon. For example, tropical conditions in the south allow permanent residence of many tropical fish species and mangroves to dominate the wetlands, whereas wetlands in the north are dominated by extensive meadows of temperate marsh grasses (Gilmore 1995). Also, moving north through the lagoon, there is a general trend of decreasing diversity for many seagrass associated species (Virnstein 1995). Therefore, a possible explanation for the restricted distribution of cluster 1 individuals and not cluster 2 could

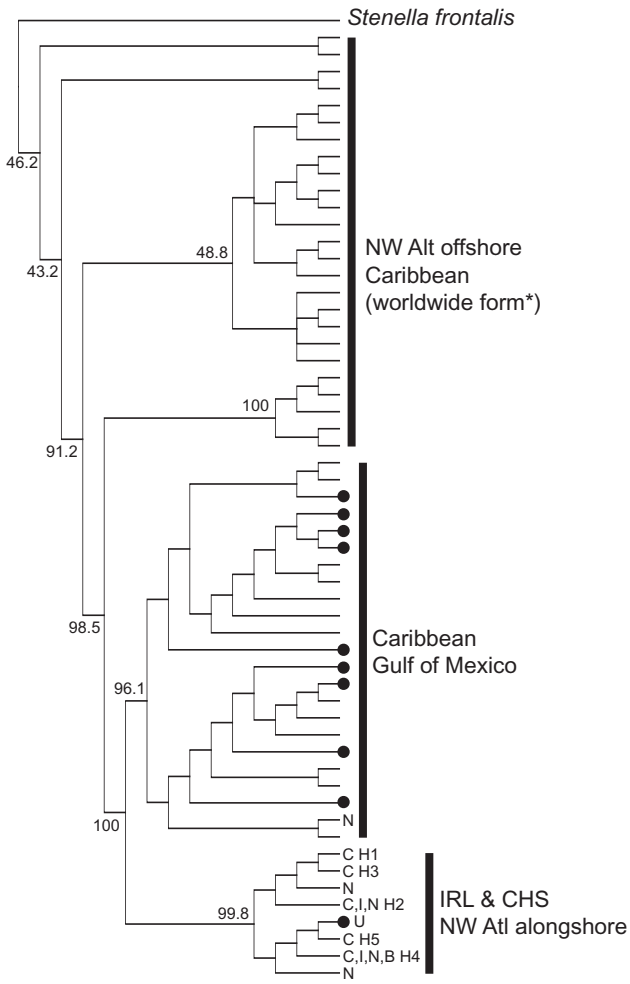

Figure 4 Maximum likelihood phylogeny showing relationship among 56 *Tursiops truncatus* mitochondrial control region haplotypes sampled from the Northwest Atlantic, Gulf of Mexico, and Caribbean (see Materials and Methods). Asterisk indicates haplotypes described as "worldwide form" (Caballero et al. 2012). Solid black circles show haplotypes described as "inshore ecotype" (Caballero et al. 2012). Haplotypes generated as part of this study are shown (H1–H5). Haplotype sampling regions for the IRL and CHS, NW Atlantic alongshore grouping are indicated as follows: C = CHS, I = IRL, N = NW Atlantic alongshore, B = Bahamas, and U = Cuba. Numbers over branches show bootstrap support.

be due to reduced or more specialized habitat utilization skills of cluster 1 individuals when compared with cluster 2 individuals. Specifically, the cluster 2 population has likely developed habitat utilization skills suited to both northern and southern environments, whereas the cluster 1 population only has skills suited to the northern environment (as a consequence of being restricted to the very north of the IRL until relatively recently). The finding of considerably more migration from regions 3–5 into regions 1 and 2 than in the opposite direction, coupled with the strong site fidelity for Mosquito Lagoon individuals detected by Mazzoil et al. (2008) despite the availability of habitat in the south lends support to this hypothesis, and studies of inshore bottlenose dolphins within embayments in Australia have provided evidence for fine-scale population structure resulting from utilization of different habitats (Wiszniewski et al. 2010; Ansmann, Parra, Lanyon, et al. 2012).

Communities with different habitat utilization skills may become reproductively isolated if each group continues to invest in learned strategies and associate with social groups using the same strategies, as suggested for killer whale populations (Hoelzel et al. 2007). However, the presence of mixed ancestry individuals within the IRL indicates some level of genetic exchange between cluster 1 and 2 populations, and the distribution of these individuals throughout most of the IRL suggests that they are generalists. One interpretation of this observation is that the descendants of cluster 1 population and cluster 2 population genetic exchange are more likely to be retained in generalist communities. Alternatively, it might reflect the gradual erosion of partitioning between these populations subsequent to their recent connection. A similar pattern has been observed for bottlenose dolphins in Moreton Bay, Australia. Twenty years ago, 2 communities appeared partitioned due to different foraging strategies (one of the communities fed on commercial trawling vessel bycatch). However, trawling activity since then has been dramatically reduced and this community structure now appears to be breaking down (Ansmann, Parra, Chilvers, et al. 2012).

An interesting finding from the SGS analysis was the presence of genetic structure over very short geographic distances (distance classes of 0–10 and 20–30 km). This finding might be explained by any one or combination of the following factors: 1) close associations among related females (Möller et al. 2004), 2) male alliances among related individuals (Krützen et al. 2003; Parsons et al. 2003), 3) community subdivision into temporarily dynamic social groups (Wiszniewski et al. 2009), and 4) geographically restricted and/or unique foraging bases (Olin et al. 2012).

Sex-Biased Dispersal

Previous studies of sex-biased dispersal for bottlenose dolphins have shown differing results. For example, several studies of inshore populations have shown male-biased dispersal (Krützen, Barré, et al. 2004; Bilgmann et al. 2007; Wiszniewski et al. 2010), whereas studies within more homogeneous environments have found no evidence for sex-biased dispersal (Natoli et al. 2005; Parsons et al. 2006; Bilgmann et al. 2007; Rosel et al. 2009). However, concordant with our findings, Ansmann, Parra, Lanyon, et al. (2012) found no evidence for sex-biased dispersal within an inshore environment. One hypothesis for increased female philopatry is that it may be advantageous for rearing offspring and defense from predators (sharks) (Möller and Beheregaray 2004). Due to low salinities, the only year-round resident shark species within the IRL is the euryhaline bull shark (*Carcharhinus leucas*) and size distribution of individuals suggests that adults spend most of their time outside the lagoon (Snelson and Williams

1981). Although these observations suggest that there may be a reduced threat from sharks within the IRL, a study comparing the prevalence of shark bites/individual between IRL and CHS dolphins (Fair PA, unpublished data) showed no significant difference (IRL = 28.9%, CHS = 31.0%), with these frequencies similar to those of other studies (Corkeron et al. 1987; Urian et al. 1998), suggesting a similar risk of shark predation. Lastly, despite our findings here of no sex-biased dispersal, additional samples may be required before it can be confidently rejected.

Evolutionary Perspective

Concordant with previous studies, the mtDNA showed an overall pattern of strong genetic differentiation (all comparisons were significant). Nevertheless, the statistical parsimony network showed the Northwest Atlantic alongshore, CHS, and IRL haplotypes to be closely related, and the levels of mitochondrial differentiation among these regions were relatively low, suggesting elevated levels of genetic exchange. For CHS, long-term sighting studies indicate migration of individuals in and out of the area and therefore the potential for genetic exchange. For example, the photoidentification study of Zolman (2002) suggested that a small number of individuals might seasonally migrate in and out of the area, and the more recent mark–recapture study of Speakman et al. (2010) identified 3 distinct groups: year-round residents, seasonal residents, and transients. In addition, the photoidentification study of Laska et al. (2011) documented frequent mixing of individuals between an estuarine and coastal community. The only comparison to show less differentiation than these 3 regions was the comparison between Cuba and the Gulf of Mexico (Mexico), suggesting elevated levels of genetic exchange across the Yucatán Channel. The highest level of differentiation, on the other hand, was between the IRL and all regions in the Gulf of Mexico and Caribbean, this despite the relatively short geographic distance from the IRL to most of these regions. Similar to the previous work of Rosel et al. (2009), the overall pattern of differentiation we detected combined with the statistical parsimony and phylogenetic analyses showed dolphins along the eastern US coastline to be strongly partitioned from the Gulf of Mexico and Caribbean. Differentiation between populations in the Gulf of Mexico and the NW Atlantic has been observed for numerous taxa (Avise 2000; Soltis et al. 2006), with the phylogeographic break typically located along the east coast of Florida. Our results suggest that the break for *T. truncatus* extends at least as far south as the IRL. Additional samples from the southern tip of Florida including the Florida Keys would be informative in determining the precise location of the break.

Overall, eastern US bottlenose dolphins show a hierarchal pattern of population structure, with the strongest signal grouping dolphins along the eastern US shoreline separately from those in the Gulf of Mexico and the Caribbean. Within these regions, dolphins are further subdivided into distinct along and inshore populations. Lastly, as shown in this study and previously for Australian embayments, dolphins within inshore habitat can be subdivided still further. Despite this general pattern, long-range dispersal does appear possible, as we detected 2 haplotypes, one sampled in the Bahamas and the other in Cuba, that were more closely related to eastern US coast individuals than to the Bahamas or Cuban, respectively. However, our data suggest that this type of migration is rare.

The phylogenetic analysis showed the offshore/worldwide form haplotypes to be ancestral to the alongshore/inshore (coastal) haplotypes, and a younger age for the coastal populations was supported by the measures of mitochondrial molecular diversity, which showed these populations to have lower diversity than the Northwest Atlantic offshore population. Furthermore, the levels of diversity showed a general decrease when moving from offshore to alongshore and then inshore. Rosel et al. (2009) found a similar pattern of lower diversity for 2 estuarine (inshore) populations (Charleston Harbor, South Carolina and Jacksonville, Florida) along the eastern US coastline when compared with alongshore populations. A possible explanation for these observations is that the inshore populations were recently founded, possibly from an alongshore population. Detecting a similar pattern of diversity for bottlenose dolphins along the west coast of Florida, Sellas et al. (2005) also argued that this pattern was the signature of a founder event. Similarly, Natoli et al. (2004) and Hoelzel, Potter, et al. (1998) suggested that the less diverse alongshore populations of the Western Atlantic were founded by the more diverse offshore population. An additional consideration is that low molecular diversity can also reflect low population size, which could be due to limiting factors based on the physical size of the inshore habitat.

Conclusion

Our study reports fine-scale genetic structure over very short geographic distances within heterogeneous inshore habitat for a highly mobile marine mammal, with habitat and its utilization likely important mechanisms shaping population structure. Community structure and close association among related individuals may also be important factors shaping structure within inshore habitat. Importantly, we show for the first time that the Indian River Lagoon Estuarine System Stock (IRLES) is comprised of 2 distinct genetic populations that are partially sympatric and strongly differentiated from other eastern US coastal populations and stocks. Furthermore, the high level of population differentiation between the inshore habitats highlights the possibility of multiple discrete management units existing within this type of habitat along the entire length of the eastern US shoreline. Of concern is the possible amalgamation of 2 discrete units of genetic diversity (populations) within the IRL as a consequence of habitat restructuring. Consideration of these inshore population characteristics and the potential detrimental effects of anthropogenic disturbance should contribute to the long-term success of bottlenose dolphin management strategies.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

Funding

State of Florida's Protect Wild Dolphins Specialty License Plate Fund; NOAA/Center for Coastal Environmental Health and Biomolecular Research (CCEHBR); NOAA/NMFS Marine Mammal Health and Stranding Response Program; Intramural Research Program of the National Institutes of Health, National Cancer Institute.

Acknowledgments

This research was conducted under NMFS permit 998-1678-00 issued to G.D.B. The authors are grateful to Harbor Branch Oceanographic Institute at Florida Atlantic University, the CCEHBR, and all associated collaborators who participated and aided in this research. The authors acknowledge L. Hansen, M. Mazzoil, L. Fulford, F. Townsend, E. Zolman, T. Speakman, J. Adams, W. McFee, M. de Sieyes, K. Kroell, E. Murdoch-Titcolmb, E. Howells, S. Bechdel-Rodgers, J. Goldstein, and all of the many dedicated staff and volunteers who assisted with IRL and CHS HERA collections. A special thanks to P. Rosel and the NMFS MMMGL for technical assistance with microsatellites. Additional recognition is also given to the many dedicated members of the SEUS Marine Mammal Health and Stranding Network who work tirelessly to respond, recover, and bring aid to sick and injured marine mammals. The content of this publication does not necessarily reflect the views of the Department of Health and Human Services nor does the mention of trade names, commercial products, or organizations imply endorsement by the US Government.

References

Amos B, Hoelzel AR. 1990. DNA fingerprinting cetacean biopsy samples for individual identification. Report of the International Whaling Commission Special Issue (No. 12). p. 79–85.

Ansmann IC, Parra GJ, Chilvers BL, Lanyon JM. 2012. Dolphins restructure social system after reduction of commercial fisheries. Anim Behav. 84:575–581.

Ansmann IC, Parra GJ, Lanyon JM, Seddon JM. 2012. Fine-scale genetic population structure in a mobile marine mammal: inshore bottlenose dolphins in Moreton Bay, Australia. Mol Ecol. 21:4472–4485.

Avise J. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos. 63:62–76.

Avise J. 2000. Phylogeography: the history and formation of species. Cambridge (MA): Harvard University Press.

Baker CS. 2013. Journal of heredity adopts joint data archiving policy. J Hered. 104:1.

Beerli P. 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics. 22:341–345.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 1996–2004. GENETIX 4.03, logiciel sous Windows TM pour la génétique des populations. Montpellier (France): Université de Montpellier II.

Belkhir K, Castric V, Bonhomme F. 2002. IDENTIX, a software to test for relatedness in a population using permutation methods. Mol Ecol Notes. 2:611–614.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B. 57:289–300.

Bilgmann K, Moeller LM, Harcourt RG, Gibbs SE, Beheregaray LB. 2007. Genetic differentiation in bottlenose dolphins from South Australia: association with local oceanography and coastal geography. Mar Ecol Prog Ser. 341:265–276.

Bowen B, Avise J. 1990. The genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: the influence of zoogeographic factors and life history patterns. Mar Biol. 107:371–381.

Briggs J. 1974. Marine zoogeography. New York: McGraw-Hill.

Briggs J, Bowen B. 2012. A realignment of marine biogeographic provinces with particular reference to fish distributions. J Biogeogr. 39:12–30.

Caballero S, Islas-Villanueva V, Tezanos-Pinto G, Duchene S, Delgado-Estrella A, Sanchez-Okrucky R, Mignucci-Giannoni A. 2012. Phylogeography, genetic diversity and population structure of common bottlenose dolphins in the Wider Caribbean inferred from analyses of mitochondrial DNA control region sequences and microsatellite loci: conservation and management implications. Anim Conserv. 15:95–112.

Chakraborty R, Weiss K. 1988. Admixture as a tool for finding linked genes and detecting that difference from allelic association between loci. Proc Natl Acad Sci USA. 85:9119–9123.

Chilvers BL, Corkeron PJ. 2001. Trawling and bottlenose dolphins' social structure. Proc Biol Sci. 268:1901–1905.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. Mol Ecol. 9:1657–1659.

Corkeron P, Morris R, Bryden M. 1987. Interactions between bottlenose dolphins and sharks in Moreton Bay, Queensland. Aquat Mamm. 13:109–113.

Crandall KA, Templeton AR. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. Genetics. 134:959–969.

Defran RH, Weller DW, Kelly DL, Espinosa MA. 1999. Range characteristics of Pacific coast bottlenose dolphins (*Tursiops truncatus*) in the Southern California Bight. Mar Mamm Sci. 15:381–393.

Degnan JH, DeGiorgio M, Bryant D, Rosenberg NA. 2009. Properties of consensus methods for inferring species trees from gene trees. Syst Biol. 58:35–54.

Degnan JH, Rosenberg NA. 2006. Discordance of species trees with their most likely gene trees. PLoS Genet. 2:e68.

Drummond A, Ashton B, Buxton S, Cheung M, Cooper A, Heled J, Kearse M, Moir R, Stones-Havas S. 2010. Geneious v5.1. Available from: http://www.geneious.com.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 14:2611–2620.

Ewing GB, Ebersberger I, Schmidt HA, von Haeseler A. 2008. Rooted triple consensus and anomalous gene trees. BMC Evol Biol. 8:118.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online. 1:47–50.

Favre L, Balloux F, Goudet J, Perrin N. 1997. Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. Proc Biol Sci. 264:127–132.

Gilmore R. 1995. Environmental and biogeographic factors influencing ichthyofaunal diversity: Indian River Lagoon. Bull Mar Sci. 57:153–170.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 59:307–321.

Hill W, Robertson A. 1968. Linkage disequilibrium in finite populations. Theor Appl Genet. 38:226–231.

Hoelzel AR. 1998. Genetic structure of cetacean populations in sympatry, parapatry, and mixed assemblages: implications for conservation policy. J Hered. 89:451–458.

Hoelzel AR, Dahlheim M, Stern SJ. 1998. Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and genetic differentiation between foraging specialists. J Hered. 89:121–128.

Hoelzel AR, Hey J, Dahlheim ME, Nicholson C, Burkanov V, Black N. 2007. Evolution of population structure in a highly social top predator, the killer whale. Mol Biol Evol. 24:1407–1415.

Hoelzel AR, Potter CW, Best PB. 1998. Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. Proc Biol Sci. 265:1177–1183.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30:3059–3066.

Krützen M, Barré LM, Connor RC, Mann J, Sherwin WB. 2004. 'O father: where art thou?'—Paternity assessment in an open fission-fusion society of wild bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. Mol Ecol. 13:1975–1990.

Krützen M, Sherwin WB, Berggren P, Gales N. 2004. Population structure in an inshore cetacean revealed by microsatellite and mtDNA analysis: bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. Mar Mamm Sci. 20:28–47.

Krützen M, Sherwin WB, Connor RC, Barré LM, Van de Casteele T, Mann J, Brooks R. 2003. Contrasting relatedness patterns in bottlenose dolphins (*Tursiops* sp.) with different alliance strategies. Proc Biol Sci. 270:497–502.

Krützen M, Valsecchi E, Connor R, Sherwin W. 2001. Characterization of microsatellite loci in *Tursiops aduncus*. Mol Ecol Notes. 1:170–172.

Laska D, Speakman T, Fair PA. 2011. Community overlap of bottlenose dolphins (*Tursiops truncatus*) found in coastal waters near Charleston, South Carolina. J Mar Anim Ecol. 4:10–18.

Latch E, Dharmarajan G, Glaubitz J, Rhodes O Jr. 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. Conserv Genet. 7:295–302.

Leatherwood S, Reeves RR. 1983. The Sierra Club handbook of whales and dolphins. San Francisco (CA): Sierra Club Books. p. i–xviii, 1–302.

Mazzoil M, Reif JS, Youngbluth M, Murdoch ME, Bechdel SE, Howells E, McCulloch SD, Hansen LJ, Bossart GD. 2008. Home ranges of bottlenose dolphins (*Tursiops truncatus*) in the Indian River Lagoon, Florida: environmental correlates and implications for management strategies. EcoHealth. 5:278–288.

McRae G, Camp D, Lyons W, Dix T. 1998. Relating benthic infaunal community structure to environmental variables in estuaries using nonmetric multidimensional scaling and similarity analysis. Environ Monit Assess. 51:233–246.

Mirimin L, Miller R, Dillane E, Berrow SD, Ingram S, Cross TF, Rogan E. 2011. Fine-scale population genetic structuring of bottlenose dolphins in Irish coastal waters. Anim Conserv. 14:342–353.

Möller L, Beheregaray L, Allen S, Harcourt R. 2004. Association patterns and kinship in female Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) of southeastern Australia. Behav Ecol Sociobiol. 61:109–117.

Möller L, Wiszniewski J, Allen S. 2007. Habitat type promotes rapid and extremely localised genetic differentiation in dolphins. Mar Freshw Res. 58:640–648.

Möller LM, Beheregaray LB. 2004. Genetic evidence for sex-biased dispersal in resident bottlenose dolphins (*Tursiops aduncus*). Mol Ecol. 13:1607–1612.

Mossman CA, Waser PM. 1999. Genetic detection of sex-biased dispersal. Mol Ecol. 8:1063–1067.

Natoli A, Birkun A, Aguilar A, Lopez A, Hoelzel AR. 2005. Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). Proc Biol Sci. 272:1217–1226.

Natoli A, Peddemors VM, Hoelzel AR. 2004. Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. J Evol Biol. 17:363–375.

Olin JA, Fair PA, Recks MA, Zolman E, Adams J, Fisk AT. 2012. Unique seasonal forage bases within a local population of bottlenose dolphin (*Tursiops truncatus*). Mar Mamm Sci. 28:E28–E40.

Parsons KM, Durban JW, Claridge DE, Balcomb KC, Noble LR, Thompson PM. 2003. Kinship as a basis for alliance formation between male bottlenose dolphins, *Tursiops truncatus*, in the Bahamas. Anim Behav. 66:185–194.

Parsons KM, Durban JW, Claridge DE, Herzing DL, Balcomb KC, Noble LR. 2006. Population genetic structure of coastal bottlenose dolphins (*Tursiops truncatus*) in the northern Bahamas. Mar Mamm Sci. 22:276–298.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics. 28:2537–2539.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics. 14:817–818.

Pritchard J. 2007. Documentation for STRUCTURE software: version 2.2. Available from: http://pritch.bsd.uchicago.edu/structure.html.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics. 155:945–959.

Queller DC, Goodnight KF. 1989. Estimating relatedness using genetic markers. Evolution. 43:258–275.

Rooney AP, Merritt DB, Derr JN. 1999. Microsatellite diversity in captive bottlenose dolphins (*Tursiops truncatus*). J Hered. 90:228–231.

Rosel P. 2003. PCR-based sex determination in *Odontocete* cetaceans. Conserv Genet. 4:647–649.

Rosel PE, Forgetta V, Dewar K. 2005. Isolation and characterization of twelve polymorphic microsatellite markers in bottlenose dolphins (*Tursiops truncatus*). Mol Ecol Notes. 5:830–833.

Rosel PE, France SC, Wang JY, Kocher TD. 1999. Genetic structure of harbour porpoise *Phocoena phocoena* populations in the northwest Atlantic based on mitochondrial and nuclear markers. Mol Ecol. 8:S41–S54.

Rosel PE, Hansen L, Hohn AA. 2009. Restricted dispersal in a continuously distributed marine species: common bottlenose dolphins *Tursiops truncatus* in coastal waters of the western North Atlantic. Mol Ecol. 18:5030–5045.

Rosel PE, Haygood MG, Perrin WF. 1995. Phylogenetic relationships among the true porpoises (*Cetacea:Phocoenidae*). Mol Phylogenet Evol. 4:463–474.

Rousset F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour. 8:103–106.

Sambrook J, Russell D. 2001. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press.

Sargeant BL, Wirsing AJ, Heithaus MR, Mann J. 2007. Can environmental heterogeneity explain individual foraging variation in wild bottlenose dolphins (*Tursiops* sp.)? Behav Ecol Sociobiol. 61:679–688.

Segura I, Rocha-Olivares A, Flores-Ramirez S, Rojas-Bracho L. 2006. Conservation implications of the genetic and ecological distinction of *Tursiops truncatus* ecotypes in the Gulf of California. Biol Conserv. 133:336–346.

Sellas AB, Wells RS, Rosel PE. 2005. Mitochondrial and nuclear DNA analyses reveal fine scale geographic structure in bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico. Conserv Genet. 6:715–728.

Sheffield VC, Stone EM, Carmi R. 1998. Use of isolated inbred human populations for identification of disease genes. Trends Genet. 14:391–396.

Shifman S, Darvasi A. 2001. The value of isolated populations. Nat Genet. 28:309–310.

Smith MW, Lautenberger JA, Shin HD, Chretien JP, Shrestha S, Gilbert DA, O'Brien SJ. 2001. Markers for mapping by admixture linkage disequilibrium in African American and Hispanic populations. Am J Hum Genet. 69:1080–1094.

Smouse PE, Peakall R. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity (Edinb). 82(Pt 5):561–573.

Snelson FF Jr, Williams SE. 1981. Notes on the occurrence, distribution, and biology of elasmobranch fishes in the Indian River Lagoon system, Florida. Estuaries. 4:110–120.

Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS. 2006. Comparative phylogeography of unglaciated eastern North America. Mol Ecol. 15:4261–4293.

Speakman TR, Lane SM, Schwacke LH, Fair PA, Zolman ES. 2010. Mark-recapture estimated of seasonal abundance and survivorship for bottlenose dolphins (*Tursiops truncatus*) near Charleston, South Carolina, USA. J Cetacean Res Manage. 11:153–162.

Steward J, VanArman J. 1987. Indian River Lagoon Joint Reconnaissance Report for CM-137 and CM-138. Contract.

Steward J, Virnstein R, Haunert D, Lund F. 1994. Indian River Lagoon surface water improvement and management (SWIM) plan. St. Johns River Water Management District, Palatka FL and South Florida Water Management District, West Palm Beach, FL, USA.

Strimmer K, von Haeseler A. 1996. Quartet puzzling: a quartet maximum-likelihood method for reconstructing tree topologies. Mol Biol Evol. 13:964–969.

Tajima F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics. 105: 437–460.

Tajima F. 1989. The effect of change in population size on DNA polymorphism. Genetics. 123: 597–601.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics. 132:619–633.

Urian K, Wells R, Scott M, Irvine A, Read A, Hohn A. 1998. When the shark bites: an analysis of shark bite scars on wild bottlenose dolphins (*Tursiops truncatus*) from Sarasota, Florida. The World Marine Mammal Conference, Monaco. p. 20–24.

Valsecchi E, Amos W. 1996. Microsatellite markers for the study of cetacean populations. Mol Ecol. 5:151–156.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 4:535–538.

Virnstein RW. 1995. Anomalous diversity of some seagrass-associated fauna in the Indian River Lagoon, Florida. Bull Mar Sci. 57:75–78.

Waring G, Josephson E, Maze-Foley K, Rosel PE. 2010. U.S. Atlantic and Gulf of Mexico Marine Mammal Stock Assessments. Contract.

Waring G, Quintal J, Fairfield C. 2002. US Atlantic and Gulf of Mexico stock assessments. Contract.

Weir BS, Cockerham CC. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution. 38:1358–1370.

Wells RS, Boness D, Rathbun G. 1999. Behavior. In: Reynolds J, Rommel S, editors. Biology of marine mammals. Washington (DC): Smithsonian Institution Press. p. 324–422.

Wells RS, Hansen LJ, Baldridge A, Dohl TP, Kelly DL, Defran RH. 1990. Distribution, movements and abundance. In: Leatherwood S, Reeves RR, editors. The bottlenose dolphin. San Diego (CA): Academic Press, Inc. p. 369–434.

White W. 1970. The geomorphology of the Florida Peninsula. Contract.

Wiszniewski J, Allen SJ, Moeller LM. 2009. Social cohesion in a hierarchically structured embayment population of Indo-Pacific bottlenose dolphins. Anim Behav. 77:1449–1457.

Wiszniewski J, Beheregaray LB, Allen SJ, Moeller LM. 2010. Environmental and social influences on the genetic structure of bottlenose dolphins (*Tursiops aduncus*) in Southeastern Australia. Conserv Genet. 11:1405–1419.

Wursig B, Harris G. 1990. Behavior and ecology. In: Leatherwood S, Reeves RR, editors. The bottlenose dolphin. San Diego (CA): Academic Press, Inc. p. 199–368.

Zolman ES. 2002. Residence patterns of bottlenose dolphins (*Tursiops truncatus*) in the Stono River estuary, Charleston County, South Carolina, U.S.A. Mar Mamm Sci. 18:879–892.

Received February 12, 2013; First decision May 2, 2013; Accepted September 6, 2013

Corresponding Editor: Brian W. Bowen