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Low genetic diversity and minimal population substructure in the endangered Florida manatee: implications for conservation

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Species of management concern that have been affected by human activities typically are characterized by low genetic diversity, which can adversely affect their ability to adapt to environmental changes. We used 18 microsatellite markers to genotype 362 Florida manatees (*Trichechus manatus latirostris*), and investigated genetic diversity, population structure, and estimated genetically effective population size (N_e). The observed and expected heterozygosity and average number of alleles were 0.455 ± 0.04, 0.479 ± 0.04, and 4.77 ± 0.51, respectively. All measures of Florida manatee genetic diversity were less than averages reported for placental mammals, including fragmented or nonideal populations. Overall estimates of differentiation were low, though significantly greater than zero, and analysis of molecular variance revealed that over 95% of the total variance was among individuals within predefined management units or among individuals along the coastal subpopulations, with only minor portions of variance explained by between group variance. Although genetic issues, as inferred by neutral genetic markers, appear not to be critical at present, the Florida manatee continues to face demographic challenges due to anthropogenic activities and stochastic factors such as red tides, oil spills, and disease outbreaks; these can further reduce genetic diversity of the manatee population.

Key words: AMOVA, conservation genetics, effective population size (N_e), genetic diversity, microsatellites, population differentiation, *Trichechus manatus latirostris*

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Many species of conservation concern exist in environments that are continuously changing either naturally or due to anthropogenic influences; genetic diversity is a prerequisite for species to be able to adapt to the ever-changing environment (Frankham et al. 2002). However, genetic diversity may be reduced or lost in small, isolated, or fragmented populations (Bouzat et al. 1998; Dixon et al. 2007; Frankham et al. 2002). The loss of genetic variation and ensuing inbreeding depression has been shown to reduce survival, reproduction, and ultimately fitness and population persistence of many wildlife species (Bouzat et al. 1998; Hostetler et al. 2010; Johnson et al. 2010; Mills, in press). In addition to genetic diversity, the effective population size (N_e) is a critical parameter in conservation as it determines the rate of loss of heterozygosity (Allendorf and Luikart 2007; Wright 1931, 1938). Conservation of small populations thus necessitates quantification and monitoring of genetic diversity such that appropriate management actions may be taken for genetic or demographic restorations when necessary and appropriate (e.g., Johnson et al. 2010; Westemeier et al. 1998).

The Florida manatee (*Trichechus manatus latirostris*), a subspecies of the West Indian manatee (*T. manatus*), is a long-

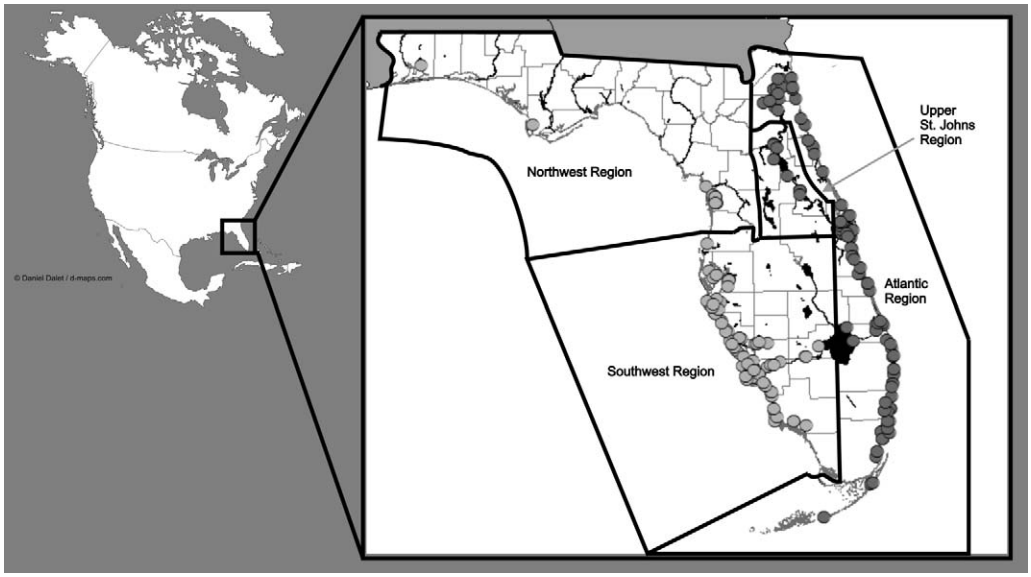

Fig. 1.—The Florida manatee management units as established by the United States Fish and Wildlife Service (USFWS 2001). The four regions are the Upper St. Johns River, Atlantic Coast, Southwest, and Northwest. The circles indicate location of sample collection. The map of North America was obtained from http://d-maps.com, and the map of the management unit delineations was obtained from the Florida Fish and Wildlife Conservation Commission (Haubold et al. 2006).

lived mammal (~60 years) with a lengthy generation time (16–23 years) and a low reproductive rate (Haubold et al. 2006; Marmontel et al. 1996; Rathbun et al. 1995). Geographic distribution of the subspecies includes coastal regions of the southeastern United States from Georgia through Alabama in the summer. The Florida manatee is susceptible to cold-related illnesses, and depends on warm-water effluents and natural springs in Florida for thermoregulation during winter (Ackerman 1995; FWRI 2010b; Lefebvre et al. 1989). Florida manatees were listed as federally endangered by the United States Endangered Species Preservation Act in 1967 and 1973, and received additional protection from the Marine Mammal Protection Act of 1972. Internationally, the Florida manatee is classified as endangered by the International Union for the Conservation of Nature (IUCN; Deutsch 2008) and listed in Appendix I in the Convention on International Trade in Endangered Species (CITES) of Wild Flora and Fauna (CITES 2008). The long-term persistence of the Florida manatee is uncertain due to threats such as small population size, collision with watercraft (the largest known cause of anthropogenic mortality in Florida), loss and degradation of habitat, uncertain future of warm water refuges, entrapment, and entanglement (Beck and Barros 1991; Beck et al. 1982; Buckingham et al. 1999; FWC 2006; Laist and Reynolds 2005a; Lightsey et al. 2006; Sorice et al. 2006).

For conservation purposes, the Florida manatee population has been divided into 4 management units (MUs): the Atlantic Coast (ATL) and Upper St. Johns River (SJR) on the East Coast (EC), and the Northwest (NW) and Southwest (SW) MUs on the Gulf Coast (GC) of Florida (Fig. 1; Haubold et al. 2006; USFWS 2001). These MUs were designated on the basis of telemetry, photoidentification, and mortality studies, as well as the threats faced by manatees in each region. However, it is unknown if the currently delineated MUs represent genetic management units (gMUs—Moritz 1994; Palsbll et al. 2007); population genetic studies would help determine whether, and to what extent, the currently delineated MUs reflect any existing genetic substructure (Runge et al. 2007a).

Earlier studies indicated that Florida manatees were characterized by low genetic diversity (e.g., Garcia-Rodriguez et al. 1998, 2000; McClenaghan and O'Shea 1988); however, these studies were limited in sample size or lacking highly variable molecular markers. There remains a need to determine whether genetic structure exists along the Florida peninsula. We developed a panel of 18 microsatellite markers, and used these markers to genotype 362 Florida manatees sampled from coastal waters in Florida. Our specific objectives were to quantify neutral genetic diversity and to investigate population substructure across the distribution and determine the degree to which variation is partitioned among Florida manatee MUs. Furthermore, existing population models have used total adult population size as a surrogate for genetically effective population size (N_e; Runge et al. 2007a); however, the use of N_e when available is generally preferable. Thus, we also estimated N_e for the Florida manatee population.

Materials and Methods

We obtained tissue samples from 362 manatees from the United States Geological Survey (USGS) Sirenia Project, Gainesville, Florida. All samples were collected from coastal waters in Florida. Skin samples were collected from the tail region of manatee carcasses examined at necropsy or from live manatees using a cattle ear-notcher. Sampling protocols followed recommendations of the American Society of Mammalogists (Sikes et al. 2011). All samples were stored in

a high-salt buffer (0.24 M ethylenediaminetetra-acetic acid, pH 7.5, 2.99 M NaCl, 20% dimethyl sulfoxide—Amos and Hoelzel 1991; Proebstel et al. 1993). Deoxyribonucleic acid (DNA) was extracted from carcass specimens using standard phenol/chloroform methods (Hillis et al. 1996) and from live manatees using the QIAGEN DNeasy kit (Valencia, California).

We genotyped each individual manatee for 18 microsatellite loci: Tma-E1, Tma-E4, Tma-E7, Tma-E14, Tma-H13, Tma-J02, Tma-K01, Tma-SC5, Tma-SC13, Tma-KB60 (Pause et al. 2007), Tma-A02, Tma-E02, Tma-E08, Tma-E11, Tma-E26, Tma-F14, Tma-M79 (Garcia-Rodriguez et al. 2000), and Tma-H23 (Hunter et al. 2009). Polymerase chain reaction (PCR) was performed as described in Pause et al. (2007). Reactions were carried out in a total volume of 12.5 μl containing 10 ng of template DNA, 0.8 mM deoxynucleotide triphosphates, 1× Sigma PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.001% gelatin), 0.04 units Sigma JumpStart Taq polymerase, 3 mM $MgCl_2$, and 0.24 mM each primer. Bovine serum albumin was added as described in Pause et al. (2007), with 0.2 mg ml^{-1} added to reactions for locus TmaM79. Annealing temperatures were reoptimized for the Garcia-Rodriguez et al. (2000) primers as follows: TmaA02 = 56°C, TmaE26 = 58°C, TmaF14 = 60°C, TmaM79 = 54°C. All PCR products were sequenced on an ABI3730xl (Applied Biosystems, Foster City, California) with the GeneScan500-LIZ size standard at the Genetic Analysis Facility at the Hospital for Sick Children (Toronto, Canada). Fragment data were scored using GeneMarker, version 1.4 (Soft Genetics, LLC, State College, Pennsylvania), and stored in the Manatee Individual Genetic-Identification System (MIGS), an Access (Microsoft, Seattle, Washington) database developed by the University of Florida and currently maintained by the USGS, Sirenia Project. Genotypes were checked for errors by both repeating the PCR amplification and re-extracting DNA and conducting a second amplification.

We applied the exact test for deviation from Hardy–Weinberg proportions and tested for linkage equilibrium using the Markov chain procedure implemented in Genepop 4.0.10 (Rousset 2008). We examined deviation from Hardy–Weinberg equilibrium (HWE) at 2 spatial scales; 1st, across samples grouped by MU, and 2nd, among samples collected from the EC and GC. Runs included 1,000 batches of 5,000 iterations each. Summary statistics (number and effective number of alleles, and heterozygosity) were calculated using GenAlEx 6.3 (Peakall and Smouse 2006). Micro-Checker (Van Oosterhout et al. 2004) was used to identify loci with evidence of null alleles. Genecap was used to minimize the effect of genotyping error by ensuring that no multilocus genotypes were identical for any individuals (Wilberg and Dreher 2004).

Global genetic differentiation among populations was estimated in GenoDive ver. 2 by computing the fixation index, G_{ST} (Nei 1987), and the standardized fixation index, G'_{ST} (Hedrick 2005), which control for downward bias of G_{ST} in highly variable markers like microsatellites, and Jost's (2008) differentiation (D), which is independent of the amount of within-population diversity. We also estimated pair-wise differentiation (F_{ST}—Slatkin 1995; Weir and Cockerham 1984) between the EC and GC, and testing for deviation from panmixia with 9,999 permutations. We used analysis of molecular variance (AMOVA) to evaluate the partitioning of genetic variation within and among management units, and coasts (GC versus AC). AMOVA was conducted using GenAlEx 6.41 (Peakall and Smouse 2006). As AMOVA are sensitive to missing data, we pruned the data set of genotypes missing more than 2 loci, then applied the "interpolate missing data" option for the remainder.

To estimate the actual number of genetic clusters that exists in the data set, and to evaluate the degree of population structuring along MUs, we applied a Bayesian clustering method implemented in Structure 2.3.1 (Pritchard et al. 2000). We examined the data in 2 ways. First we conducted a naïve analysis where no prior knowledge of sample location was included. Second, we used an identifier of MU as a prior (Locprior) that can help inform the search for the "true" number of genetic groups (K) when data are weakly informative (Hubisz et al. 2009). We explored the effect of including all markers and a subset on the basis of their fit to Hardy–Weinberg expectation. Each analysis was implemented using the admixture model and we ran 500,000 Markov chain Monte Carlo iterations following a burn-in period of 100,000 generations. We explored values of K ranging from 1 to 4. For the Locprior analyses we identified samples from the 4 MUs (Fig. 1) and samples collected from the East and West coasts. Each analysis was replicated 10 times to evaluate convergence.

We used LDN_e (Waples and Do 2008) to estimate N_e from the microsatellite allele frequencies using the bias-corrected method of Waples (2006). This approach, like other single point estimates, uses random deviations from linkage equilibrium that occurs stochastically in small populations. This method does not require an assumption of random mating, and allows evaluation of the impact of varying levels of rare alleles (which can bias estimates), and for random or monogamous mating systems. Furthermore, the method removes the downward bias associated with the true N_e being greater than the sample size used to estimate it (Waples 2006). The N_e was estimated for the entire Florida population, and for the EC and GC separately. Using the full 18-locus data set, we pruned our data set of genotypes from immature individuals to minimize the impact of multiple generations biasing results. In addition, genotypes missing more than 2 loci were omitted (final sample size for $N_e = 255$).

We used the software package Bottleneck to test for heterozygosity excess and to evaluate the potential effect of recent population bottleneck (Cornuet and Luikart 1996). The allele frequency distribution was also examined using the mode-shift indicator. We analyzed data using both the stepwise mutation model (SMM) and the two-phased model of mutation (TPM) of Di Rienzo et al. (1994). The TPM was used with variance = 12.0 and 90% of the mutations following a 2-phase mutation pattern, rather than a strict SMM pattern.

Results

Tests of HWE revealed deviation from HWE at 1 to 13 loci, depending on how samples were grouped for analysis (Appendix I). However, significant departures were consider-

Table 1.—Measures of Florida manatee genetic diversity for each management unit (SJR, St. Johns River; ATL, Atlantic; SW, Southwest Gulf Coast; and NW, Northwest Gulf Coast), for the east coast (EC) and Gulf Coast (GC), and for the entire Florida (FL) population. Values represent averages across 18 loci. Symbols used are: N, average number of genotypes sampled; A, average number of alleles per locus; N_e average effective number of alleles; F_{IS}, inbreeding coefficient; H_O and H_E, average observed and expected heterozygosity, respectively.

Grouping	N	A	N_e	F_{IS}	H_O	H_E
Management unit						
SJR	51.1	4	2.015	0.052	0.444	0.467
ATL	121.8	4	2.057	0.051	0.461	0.482
SW	90.7	4.3	2.212	0.049	0.436	0.471
NW	77.4	4	2.092	0.031	0.455	0.465
Coastal subpopulations						
EC	172.9	4.2	2.043	0.027	0.465	0.478
GC	168.2	4.5	2.120	0.046	0.444	0.471
Florida-wide						
FL	341.1	4.8	2.082	0.045	0.455	0.478

ably lower when samples were divided by coasts (EC = 5 versus GC = 7), and lower still when examined by MU. We evaluated the effect of reducing the data set to 11 of the 18 loci on our results by removing those loci that had deviated from HWE across multiple data partitions (Tma-E02, Tma-E11, Tma-SC5, Tma-K01, Tma-J02, Tma-KB60, and Tma-E08). Micro-Checker detected evidence for null alleles in the EC at Tma-E01, Tma-E7, and Tma-KB60. Null alleles were inferred among GC samples at Tma-E7, Tma-KB60, Tma-E02, and Tma-E14. All pairs of loci were estimated to be in linkage equilibrium after a sequential Bonferroni correction.

The overall (i.e., for the entire population) average (± 1 *SE*) number of alleles per locus (A), observed heterozygosity (H_O), and expected heterozygosity (H_E) were 4.77 ± 0.51, 0.455 ± 0.04, and 0.479 ± 0.04, respectively, indicating relatively low genetic diversity in Florida manatees. Measures of genetic diversity were generally similar among MUs or between the EC and GC populations (Table 1).

Overall differentiation among samples collected within the 4 MUs was low across 11 loci ($G_{ST} = 0.018 \pm 0.007$), even after correction for biases associated with highly variable markers ($G'_{ST} = 0.024 \pm 0.010$; $D = 0.017 \pm 0.008$). Though differentiation was low overall, the permutated data sets yielded values of test statistics that were all smaller than the observed values (all $P < 0.001$), suggestive of low though statistically significant differentiation overall. Similar results were observed for the full data set ($G_{ST} = 0.012 \pm 0.004$; $G'_{ST} = 0.016 \pm 0.006$; $D = 0.015 \pm 0.005$). The pair-wise F_{ST} estimated between the EC and GC was 0.02 ($P < 0.001$) when calculated across 11 loci. Results from AMOVA revealed that ~95% of the measured variation was at the within-MU level, with values ranging from 1% to 3% of the variation apportioned among MUs or coastal subpopulations (Table 2). The overall mean inbreeding coefficient was 0.039 ($P = 0.032$). We found no evidence for population bottleneck using the Wilcoxon sign-rank test. However, the heterozygote excess tests identified a possible bottleneck for the GC (SMM, $P = 0.05$; TPM, $P = 0.06$), but no bottleneck for the EC. The "mode-shift" indicator suggested a normal L-shaped distribution with no distortion of allele frequencies for either coast.

Table 2.—Analysis of molecular variance (AMOVA) results from the reduced (11 loci) and full (18 loci) data sets.

Source	*d.f.*	Sum of squares	Mean square	Estimated variance	% Variance
11-locus data set					
Among coasts	1	34.403	34.403	0.101	2%
Among MUs	2	31.292	15.646	0.148	3%
Within MUs	322	1,505.041	4.674	4.674	95%
Total	325	1,570.736		4.924	100%
18-locus data set					
Among coasts	1	44.617	44.617	0.114	1%
Among MUs	2	46.255	23.128	0.193	2%
Within MUs	322	2,855.962	8.869	8.869	97%
Total	325	2,946.834		9.176	100%

Naïve Structure analyses revealed different patterns between the 11-locus and 18-locus data sets. For the 11-locus data set $K = 1$ had the highest likelihood and increasing K-values were ambiguous due to poor information content in the genotypes (i.e., all individual genomes apportioned [q] equally among K). When all 18 loci were included, support increased from $K = 1$ to $K = 3$; however, at $K = 2$ and $K = 3$ there was no discernible pattern of genetic structure (see Appendix II). The incorporation of the Locprior option using MUs or EC versus GC produced genome-assignment patterns that appeared to split all individuals in an a priori group into similar introgressed genomes (Appendices II and III).

The N_e for the EC and GC were estimated to range from 197.2 (EC) and 1,106.0 (GC) depending on allele frequencies included in the estimate. Estimates of N_e increased as more rare alleles were included (Table 3). Both the GC and total Florida population estimates had upper confidence intervals (*CI*s) that were undefined.

Discussion

Biodiversity conservation policies have traditionally focused on species-level diversity and on the maintenance of viable populations of species under threat. However, genetic diversity has more recently received prominence in conservation biology literature because of the recognition of the fact that genetic diversity provides raw material for adaptation to climate change and other anthropogenic or naturally occurring perturbations to the environment (Frankel and Soulé 1981; Frankham et al. 2002; Lacy 1987). Furthermore, many wildlife species of conservation concern occur in small numbers and also are generally characterized by low genetic diversity; the latter has been shown to reduce survival, reproduction, and population growth rate, and may increase the probability of extinction (Frankham et al. 2002; Johnson et al. 2010; Mills, in press). Consideration of genetic diversity is particularly important for small, isolated populations or those occupying

Table 3.—Estimates of effective population size (N_e) for Florida manatees using the unbiased method implemented in LDN_e using the random mating model (Waples and Do 2008). The lowest frequency of rare alleles included in the analysis, the number of independent comparisons for linkage disequilibrium between all pair-wise comparisons of loci and alleles, and resulting estimates of N_e (95% *CI*) are given for the total Florida population and for the East and Gulf coasts separately. Estimates of N_e are presented after excluding all alleles with frequencies of less than 0.05, 0.02, and 0.01.

Sample	Lowest frequency of rare alleles included	No. of independent comparisons	N_e (95% *CI*)
Total population	0.05	843	1,260.0 (297.8–∞)
	0.02	1,158	1,327.7 (362.9–∞)
	0.01	1,437	1,404.3 (386.2–∞)
East Coast	0.05	809	197.2 (92.7–571.4)
	0.02	928	200.6 (106.7–580.1)
	0.01	1,098	310.1 (120.3–1,013.3)
Gulf Coast	0.05	776	429.4 (153.4–∞)
	0.02	1,102	647.9 (198.3–∞)
	0.01	1,323	1,106.0 (253.2–∞)

fragmented habitats, and it may be critical for anthropogenically affected large mammals that tend to have reduced genetic diversity (DiBattista 2008; Dixon et al. 2007; Wooten and Smith 1985). The Florida manatee is such a species—a large mammal that has been severely threatened by human activities including habitat fragmentation and degradation, marine debris (Beck and Barros 1991), boat strikes (Beck et al. 1982; Lightsey et al. 2006), and human recreational activities (Buckingham et al. 1999; Sorice et al. 2006).

The expected heterozygosity for the overall Florida manatee population was lower than the average expected heterozygosity reported for all placental mammals including demographically challenged placental mammals (Garner et al. 2005). Similarly, H_E and allelic richness in Florida manatees was lower than the average reported for hunted or fragmented populations of mammals (DiBattista 2008). Although expected heterozygosity for the Antillean manatees sampled in Belize and Mexico was slightly higher than what we found for the Florida manatee (Hunter et al. 2010, Nourisson et al. 2011), they were still lower than averages reported for mammals (DiBattista 2008; Garner et al. 2005). Expected heterozygosity for Antillean manatees in Puerto Rico was slightly lower even than that reported here for Florida manatees (Hunter et. al., in press). Null alleles are not expected to play a major role in this pattern of excess homozygosity, since they were detected at only a few loci. These results suggest that manatee populations in general, and Florida manatees in particular, are characterized by low levels of genetic diversity; measures of genetic diversity for the Florida manatee were lower than those reported for other disturbed and fragmented populations (DiBattista 2008; Garner et al. 2005). Furthermore, the estimate of F_{IS} indicated moderate inbreeding in our study population, and there was evidence for a population bottleneck in the GC population. In addition to the reduction in population size and population fragmentation due to anthropogenic influences, a founder effect or major population bottleneck likely contributed to the low level of genetic variation observed in our study population (Garcia-Rodriguez et al. 1998; McClenaghan and O'Shea 1988; Vianna et al. 2006).

For both management and research purposes and to ensure that manatees thrive in all parts of Florida, the Florida manatee population has been divided into 4 MUs (Fig. 1; FWC 2007; USFWS 2001). We sought to determine if these MUs represented gMUs. We found no evidence to support that the 4 MUs represented genetic groups. Similarly, although applying Locprior option in Structure tended to delineate the groups qualitatively, the patterns observed were not those expected from informative data, meaning the apportionment of q values under the Locprior runs tends to divide each individual's q value similarly among clusters (Appendix II). Only when all 18 loci were included, without applying Locprior option, did results appear to accurately evaluate individual q values. Although $K = 2$ or 3 had higher likelihoods than did $K = 1$, the proportion of each group (e.g., MU or coast) assigned to each K was roughly symmetric, a pattern that is indicative of no structure (Pritchard et al. 2010). These results are reflected in the AMOVA results where very little (at most 3%) of the total variance could be explained by intercoast or inter-MU variation (Table 3). Though evidence for population substructure is limited, some of our results may reflect weak differentiation between coasts. For example, the high level of deviation from HWE across most loci when calculated at the total population level compared with coastal or even MU levels may partially reflect admixture of allele pools. Pair-wise F_{ST} indicated very weak differentiation between the EC and GC. Structure is also expected to perform poorly when there is limited differentiation and when samples do not represent geographically clustered samples but are more evenly distributed across a landscape, as is the case here. The clustering pattern produced under some models did reflect differences between coastal subpopulations. Although our results indicate that the current MUs do not represent gMUs, they have proven useful for devising and implementing site-specific management actions to address specific threats faced by manatees within each MU.

Runge et al. (2007a, 2007b) used adult population size as a surrogate for genetically effective population size (N_e). They estimated a current adult population size of less than 2,500. The published aerial surveys conducted by the Florida Fish and Wildlife Conservation Commission (FFWCC) place the minimum population counts at 1,372 in 1991, and increasing to ~5,000 individuals in 2010 (http://myfwc.com/research/manatee/projects/population-monitoring/synoptic-surveys/; accessed 24 February 2012). Using the most conservative estimate (allele frequencies ≥5%), our estimate of N_e for the whole population was 1,260. It is important to note that the linkage disequilibrium estimate of N_e may be biased by the fact that manatees have nondiscrete generations. By removing genotypes from calves and known second-generation individuals we hoped to minimize this issue. Perhaps more important here is the decreased precision and increased variance of N_e

when true N_e is large (e.g., >100). Under these circumstances, sampling error can result in either negative estimates of N_e or *CI*s that are infinite. Waples and Do (2010) suggested that undefined upper *CI* could be interpreted as the lack of evidence that the population is not very large. However, the well-defined lower *CI* within a reasonably close range of the estimate of N_e (Table 3) does suggest that our estimates of N_e are plausible, even in the cases where the upper *CI* are not well defined (Waples and Do 2010). Directly comparing our estimates of N_e with demographic estimates of adult population size would be ideal as the ratio of N_e/N provides valuable insight to the potential risks of demographic contraction due to a lack of genetic variation even if the number of individuals in the population is still large (Saarinen et al. 2010). However, the time frame that our estimate of N_e provides is for the effective number of breeders in the previous generation, not the current generation's N_e. The lack of a strong signal for a bottleneck hints against a large change in demographics over the past 2–3 generations. Thus, if adult population size has been relatively stable, an approximate estimate of N_e/N would be ~0.5. This is at the higher end of typical reported ratios (0.1–0.5—e.g., Frankham 1995; Nunney and Elam 1994; Palstra and Ruzzante 2008). The annual FFWCC surveys do not provide information on the population's age structure, so the adult census size would be lower than the number used here. Regardless of the imprecision on the census size and N_e, these values, together with our estimates of inbreeding, would suggest that N_e is not critically low in Florida manatees, and that demographic concerns related to low genetic diversity (e.g., inbreeding depression) are not severe at this time. However, managers should be cognizant of the fact that further reduction in population size or disruption to gene flow within and between the coasts could alter this situation drastically.

A recent quantitative threat analysis estimated that the probability of the Florida manatee population falling below 500 individuals within 100 years was 49.32% (Runge et al. 2007a). Our results suggest that this outcome would likely be due to stochastic demographic factors like increased mortality rather than factors associated with inbreeding. This is in contrast to another prominent Florida mammal of conservation concern, the Florida panther (*Puma concolor coryi*), whose population was at the brink of extinction due to small population size, with biomedical abnormalities and poor demographic performance attributed to inbreeding depression (Hostetler et al. 2010; Johnson et al. 2010). In the decades to come, it is important that threats to manatees are well understood, and that prudent management actions are taken to minimize such threats. A reduction in the number of available warm-water sites (Laist and Reynolds 2005b), coupled with the colder-than-average winters as predicted by some climate change models, could result in a significant reduction in the Florida manatee population size, and can potentially intensify genetic drift and inbreeding on the population (FWRI 2010a). Over 60% of manatees utilize warm water from power-plant effluent (Laist and Reynolds 2005a), and the closing and repowering of power plants will affect manatee winter distribution patterns (FWC 2007; Laist and Reynolds 2005a). Additionally, the long-term effects of the 2010 Deepwater Horizon Gulf of Mexico oil spill on the Florida manatee population remain unknown, and the possibility of similar disastrous human accidents cannot be ruled out, resulting in unknown effects on Florida manatees and their habitat. Also, red tides caused by the dinoflagellate *Karenia brevis* cannot be prevented and also affect the manatee population. Furthermore, the human population of coastal Florida continues to grow, causing increased boat traffic and alteration of natural habitats, both of which can have profound negative effects on the manatees. A daunting challenge for the wildlife managers in Florida, therefore, is to identify or anticipate threats faced by the Florida manatee, and to take appropriate management actions to ensure long-term persistence of a species that has not only been a symbol of Florida wilderness but also a major tourist attraction.

Acknowledgments

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Supporting Information

Table S1. Measures of genetic diversity in Florida manatees. Available online at: http://dx.doi.org/10.1644/12-MAMM-A-048.1S1.

Appendix I. Structure results from 11 microsatellite loci. Available online at: http://dx.doi.org/10.1644/12-MAMM-A-048.1S2.

Appendix II. Structure results from 18 microsatellite loci. Available online at: http://dx.doi.org/10.1644/12-MAMM-A-048.1S3.

Appendix III. Mean (SD) of the log probablility of each model examined using Structure. Available online at: http://dx.doi.org/10.1644/12-MAMM-A-048.1S4.

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