Original Article

Genetic Diversity of White Sharks, *Carcharodon carcharias*, in the Northwest Atlantic and Southern Africa

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Abstract

The white shark, *Carcharodon carcharias*, is both one of the largest apex predators in the world and among the most heavily protected marine fish. Population genetic diversity is in part shaped by recent demographic history and can thus provide information complementary to more traditional population assessments, which are difficult to obtain for white sharks and have at times been controversial. Here, we use the mitochondrial control region and 14 nuclear-encoded microsatellite loci to assess white shark genetic diversity in 2 regions: the Northwest Atlantic (NWA, *N* = 35) and southern Africa (SA, *N* = 131). We find that these 2 regions harbor genetically distinct white shark populations (*Φ*<sub>ST</sub> = 0.10, *P* < 0.00001; microsatellite *F*<sub>ST</sub> = 0.1057, *P* < 0.021). M-ratios were low and indicative of a genetic bottleneck in the NWA (M-ratio = 0.71, *P* < 0.004) but not SA (M-ratio = 0.85, *P* = 0.39). This is consistent with other evidence showing a steep population decline occurring in the mid to late 20th century in the NWA, whereas the SA population appears to have been relatively stable. Estimates of effective population size ranged from 22.6 to 66.3 (NWA) and 188 to 1998.3 (SA) and evidence of inbreeding was found (primarily in NWA). Overall, our findings indicate that white population dynamics within NWA and SA are determined more by intrinsic reproduction than immigration and there is genetic evidence of a population decline in the NWA, further justifying the strong domestic protective measures that have been taken for this species in this region. Our study also highlights how assessment of genetic diversity can complement other sources of information to better understand the status of threatened marine fish populations.

Subject areas: Conservation genetics and biodiversity; Population structure and phylogeography

Key words: bottleneck, effective population size *N*<sub>e</sub>, inbreeding

258
Understanding the contemporary size and trajectory of marine fish populations remains a significant challenge that has serious economic and environmental implications (Reynolds et al. 2005; Hare et al. 2011; Burgess et al. 2014). Important insights can be gleaned from the assessment of population genetic diversity because processes such as genetic bottlenecks, genetic drift, and inbreeding reduce genetic diversity of small populations and, in some cases, leave detectable signatures in the population genetic architecture (Ward et al. 1994; DeWoody and Avise 2000). For these reasons, genetic diversity is frequently assessed and used to inform the conservation of terrestrial endangered species (Frankham 1996). However, a variety of obstacles have slowed the integration of these types of analyses into the conservation of wild marine fish populations. For example, marine fish have relatively large populations compared with most terrestrial vertebrates, which is thought to make them more resilient to processes influencing their genetic diversity (Waples and Do 2009). Genetic studies are now starting to be used to inform fisheries management and conservation for the elasmobranchs (sharks and batoids), a group of primarily marine fish that are subject to heavy fishing pressure and a high proportion of which are considered threatened species (Worm et al. 2013; Dulvy et al. 2014).

White sharks (Carcharodon carcharias) are large apex predators that primarily occur in the temperate and subtropical zones of 7 regions: the Northwest Atlantic (NWA), Northwest Pacific, Northeast Pacific, Australasia, southern Africa (SA), South America, and the Mediterranean (Compagno et al. 1997). Domestic or state legislation prohibiting targeted fishing and retention of incidentally captured white sharks has been established in several of these regions based on concerns about the inherent vulnerability of this species and/or evidence of regional population declines (Compagno 1991; Blower et al. 2012; Burgess et al. 2014; Curtis et al. 2014 and references therein). Despite these recently implemented management measures, very little is known about the recent trajectories of white shark populations or their current population size and status. In SA, Cliff et al. (1996) used tag-recapture methods to estimate that the population consisted of 1279 individuals between the Cape of Good Hope and Richards Bay (South Africa), the geographic extent of the shark beach-meshing program where tagged sharks were potentially recaptured. Another tag-recapture study using photographic identification methods conducted in Gansbaai, South Africa, from 2007–2011 estimated that the population size was 808–1008 individuals (Towner et al. 2013). These authors suggested that they sampled the same population as Cliff et al. (1996) and speculated that the SA population was therefore stable over this period (Towner et al. 2013). Indeed several authors have presented evidence of population stability in SA white sharks (Compagno 1991; Dudley and Simpfendorfer 2006; FAO 2004). In contrast, commercial long-line fishery logbooks analyzed from the NWA indicated that the white shark population declined 59–89% between 1986 and 2000 (Baum et al. 2003). This has proven controversial as Burgess et al. (2005) highlighted some shortcomings in these analyses, for example due to fisherman logbook errors concerning species identity (Baum et al. 2005 countered some of these arguments). Curtis et al. (2014) subsequently estimated that this population declined by 27–86% from 1961 to the late 1980s using several additional data sources. They also reported evidence that the population was in the beginning stages of recovery since the species was protected in the region in the late 1990s (Curtis et al. 2014). There has also been recent controversy about the status, size and trajectory of white shark populations in other regions (e.g., the Northeast Pacific; Chapple et al. 2011; Burgess et al. 2014).

Population genetic data could contribute to our understanding of white shark status, recent trajectory, and aid in management decisions. First, delineating population structure is a fundamental step for establishing management units and interpreting population dynamics. Correspondingly, regional white shark population structure has been delineated for the Pacific and Indian ocean white sharks (e.g., Pardini et al. 2001; Gubile et al. 2010; Jorgensen et al. 2010; Tanaka et al. 2011; Blower et al. 2012), but the Atlantic ocean has been neglected in this regard. In addition, little attention has been paid to levels of intrapopulation genetic diversity of this species and what this information suggests about regional population sizes and trajectories. A population decline in the NWA of the magnitude suggested by Baum et al. (2003) and Curtis et al. (2014), for example, could have caused a genetic bottleneck. Quantitative detection of this bottleneck could help validate these studies that were based on time-series white shark catch or sightings analysis. In addition, Blower et al. (2012) used microsatellite DNA profiling to estimate the effective population size (Ne) of white sharks in Australia at Ne = 1512; though so far genetic data have not been used to determine Ne for white shark populations in other parts of the world. The effective population size is the size of an idealized population that loses genetic diversity to genetic drift at the same rate as the study population (Wright 1984). Although it is difficult to reconcile the exact relationship between effective and total population size, this parameter is by itself useful because genetic diversity loss through drift is proportional to Ne (Waples and Do 2009; Hare et al. 2011; Palstra and Fraser 2012). It is important to note that while neutral genetic diversity as determined with microsatellite data can be correlated to Ne, it is not always correlated to adaptive potential (Reed and Frankham 2003). Nevertheless, estimating Ne, testing for genetic bottlenecks and further delineating population structure using genetic approaches may provide complementary information useful for understanding the status of white shark populations.

Here, we report patterns of genetic diversity among white sharks sampled in 2 population centers for this species: SA and the NWA, the latter of which may have recently experienced a severe size reduction (Baum et al. 2003; Curtis et al. 2014). Our objectives were to assess whether these populations are genetically isolated from one another and other regional populations (Australia, New Zealand, California, the Mediterranean, and Japan), and to test for evidence of a genetic bottleneck associated with population decline. We also tested for evidence of inbreeding within each population and estimated Ne.

Materials and Methods

In fulfillment of data archiving guidelines (Baker 2013), primary data have been deposited with Dryad. Individual white sharks were sampled from 2001 to 2008 in the NWA (N = 35) and 2003 to 2010 in SA (N = 131). NWA specimens consisted of primarily of individuals captured by fishers and sampled by the US National Marine Fisheries Service from Massachusetts to the northern Gulf coast of Florida, including individuals sampled in New York, Rhode Island, Virginia (see Supplementary Material online). Most of the NWA individuals in our sample were not measured accurately because only part of the individual was available for sampling, but all were estimated to be less than 3.0 m total length and therefore immature (Compagno et al. 1997). The mean precaudal length of individuals in the SA sample was 2.14 m (standard deviation = 0.55 m), and all were judged to be immature based on dissection results. The sex ratio was 1:2 females to males. More details about the size, sex, and geographic origin of individuals are available upon request to the
corresponding author. We suggest our sampling is representative of regional genetic diversity in NWA and SA because it was widespread along the coast and juvenile white sharks are known to make long-distance coastal movements in both regions (see www.ocearch.org). Genomic DNA was extracted from 0.015 to 0.035 g of tissue using the Qiagen Blood and Tissue Extraction Kit (Qiagen, Valencia, CA). The mitochondrial control region (mtCR) was amplified in all NWA specimens and an equivalent number of randomly selected SA specimens using species-specific primers and a modified PCR protocol as described in Blower et al. 2012 and run on a 3730 DNA Analyzer (Life Technologies). Forward and reverse mtCR sequences were individually checked for quality and aligned using ClustalX (Thompson et al. 2002) along with haplotypes from previous studies (GenBank accession numbers: HQ414073–HQ414086 [Blower et al. 2012], AY026196–AY026224 [Pardini et al. 2001], GU002302–GU002322 [Jorgensen et al. 2010], HQ540294–HQ540298 [Gubli et al. 2010]), then trimmed to 826 matching base pairs. The alignments were exported into Arlequin v3.5 (Excoffier and Lischer 2010) to characterize haplotypes using the Tamura and Nei model and calculate pairwise $\Phi_\text{ST}$. A minimum spanning tree was created and visualized using HapStar (Teacher and Griffiths 2011).

All individuals were genotyped at 14 polymorphic microsatellite loci (Table 1) as described in Shrey and Heist (2002), Gubli et al. (2010), and O’Leary et al. (2013a). Even though this led to an imbalance in sample size between NWA and SA, several of the analyses that we subsequently performed on each sample benefit from larger sample sizes so we used the maximum number of individuals available. Amplicons were run on a 3730 DNA Analyzer (Life Technologies) using an internal size standard (500 LIZ™; Life Technologies) and scored using Peakscan v1.0 (Applied Biosystems). Eight randomly selected homozygotes per locus were re-amplified at 3°C below the melting temperature to expose possible null alleles. Approximately, 10% of all genotypes were re-amplified and 40% re-scored by a second analyst to avoid genotyping error. Quality control was performed using Microsatellite Toolkit for Excel (Park 2001; scoring errors, duplicate genotypes) and Microchecker (van Oosterhout et al. 2004; large allelic dropouts, null alleles, large allelic gaps). We used exact tests implemented in Genepop (Raymond and Rousset 1995; Raymond et al. 1997) to determine deviations from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD). We calculated $\Phi_\text{ST}$ as well as the standardized measures $G_{ST}$ (Hedrick 2005) and $F_{ST}$ (Jost 2008). The Bayesian clustering program Structure (Pritchard et al. 2000) was run for a burn-in period of 50,000 MCMC steps followed by 100,000 MCMC steps using the admixture model (with a priori location) for K = 1–5 for 10 independent runs each to determine convergence. To infer the correct number of clusters K, Pritchard et al. (2000) suggest determining the convergence of the mean estimate of the ln probability of K. Further, we used the $\Delta K$ metric suggested by Evanno et al. (2005) to determine the statistically most supported number of clusters as implemented in StructureHarvester (Earl and von Holdt 2012).

The genetic diversity of each sample was assessed by calculating the haplotype and nucleotide diversities of the mitochondrial haplotypes using Arlequin v3.5. Expected and observed heterozygosity and allelic richness of the microsatellite loci was assessed using Fstat (Goudet 1995). ADZE (Szpiech et al. 2008) was used to determine allelic richness for NWA and SA using rarefaction, as the sample size for SA was substantially larger than NWA. We tested for bottlenecks using the mode-shift test, heterozygosity excess (HE) test (Cornuet and Luikart 1996; Piry et al. 1999), and $M$-ratios (Garza and Williamson 2001). When a locus is in mutation-drift-equilibrium, most alleles occur at low frequencies, whereas a bottleneck causes a disproportionate loss of these low-frequency alleles that can cause a mode shift in the allele frequency distribution (Luikart et al. 1998). When a bottleneck occurs, the allelic diversity is also reduced faster than heterozygosity (Cornuet and Luikart 1996). The heterozygosity excess method tests whether the observed heterozygosity is higher than it would be under mutation-drift equilibrium (Cornuet and Luikart 1996; Piry et al. 1999). We performed the heterozygosity excess test using the program Bottleneck (Piry et al. 1999) under the infinite allele model (IAM), step-wise mutation model (SMM), and 2-phase model (TPM; proportion of step-wise mutations in TPM 60%, 70%, 80%, 90%) including all microsatellite loci and excluding any loci

### Table 1. Microsatellite marker diversity of SA and NWA populations described by observed ($H_o$) and expected heterozygosity ($H_e$), allelic richness ($A$), and inbreeding coefficient ($F_{IS}$)

| Locus | NWA | SA |
|-------|-----|----|
| Cca9  | 0.733 | 0.887 |
| Cca1466 | 0.565 | 0.338 |
| H' | 0.636 | 0.803 |
| $P$ value | 0.0706 | 0.6485 |
| A | 10 | 19 |
| $F_{IS}$ | 0.134 | 0.095 |
| Cca1077 | 0.134 | 0.095 |
| Cca1419 | 0.849 | 0.808 |
| Cca1536 | 0.765 | 0.648 |
| $P$ value | 0.1362 | 0.1001 |
| A | 12 | 9 |
| $F_{IS}$ | 0.101 | 0.198 |
| Cca6.27 | 0.859 | 0.598 |
| Cca1226 | 0.853 | 0.527 |
| $P$ value | 0.1254 | 0.4001 |
| A | 10 | 7 |
| $F_{IS}$ | 0.007 | 0.119 |
| Cca1536 | 0.689 | 0.419 |
| Cca1536 | 0.676 | 0.719 |
| $P$ value | 0.0566 | 0.0001 |
| A | 10 | 11 |
| $F_{IS}$ | 0.089 | 0.138 |
| Cca85 | 0.844 | 0.827 |
| Cca1276 | 0.636 | 0.699 |
| $P$ value | 0.2533 | 0.3107 |
| A | 10 | 12 |
| $F_{IS}$ | 0.249 | 0.155 |
| Cca1276 | 0.791 | 0.875 |
| Cca1276 | 0.647 | 0.740 |
| $P$ value | 0.0207 | 0.0688 |
| A | 12 | 16 |
| $F_{IS}$ | 0.184 | 0.154 |

*Significant deviations from HWE ($P$ values).

Unpublished locus developed from a shortfin mako shark (Isurus oxyrinchus) library.
that potentially exhibited null alleles. The M-ratio calculates the ratio of total number of extant alleles in a population (k) to the total number of alleles expected given the range of allele sizes (r). The loss of rare alleles during population bottlenecks tends to affect k more than r, thus reducing the ratio (Garza and Williamson 2001). The M-ratio was calculated per locus and population (Table 2); we used the program M_P_Val (available at https://swfsc.noaa.gov) to determine if M-ratios were lower than expected for a population at equilibrium. We determined theta using Arlequin v3.5 assuming that the percentage of multistep mutations was 22% (following the recommendation of Peery et al. [2012] to minimize the probability of type I error) and mean size of mutations larger than single-step mutations was 2.8 (following Garza and Williamson 2001).

Effective population size for each population was estimated using the LD method (Waples and Do 2008) as implemented in NeEstimator2.0 (Do et al. 2014). LD is the association of alleles at an unlinked loci resulting from random genetic drift during reproduction and thus reflects Ne (Hill 1981). The LD method is the most widely applied single-sample Ne estimator and is especially useful for species with overlapping generations (Waples and Do 2009). When sample sizes are not representative of the entire generation, the Ne estimates are equivalent to the effective number of breeders that produced offspring during that time period (Palstra and Fraser 2012). The LD method has proven to be very robust for small sample sizes and for small populations and thus relevant to our data set.

Levels of inbreeding were estimated in NWA and SA using 2 measures: the inbreeding coefficient F<subIEEE</sub>, calculated using Fstat, which measures intrapopulation heterozygote deficiency and the internal relatedness (IR) using Storm (Fraser 2008), which measures the relatedness of an individual’s parents (Amos et al. 2001). For outbred individuals, IR-values should be below or close to 0. For parents who share many alleles (i.e., are closely related), IR should be positive, with one being the maximum value. We also used Storm to determine pairwise relatedness to determine if sampled juveniles were closely related.

Results
Fifteen mtCR haplotypes were identified in the 34 individuals sampled in SA, whereas 12 haplotypes were identified in the NWA (N = 44), with a haplotype diversity of 0.7237 and 0.7495, for SA and United States, respectively, and nucleotide diversity of 0.0059 and 0.003. Although SA and NWA shared the most common haplotype, there was significant partitioning of genetic diversity (Φ<sub>ST</sub> = 0.10, P < 0.00001; Figure 1), NWA and SA did not share previously published haplotypes with Mediterranean or Pacific samples published elsewhere (Pardini et al. 2001; Gubili et al. 2010; Jorgensen et al. 2010; Blower et al. 2012; Figure 1A). Similarly, all 3 measures of differentiation calculated using microsatellites showed NWA (N = 35) and SA (N = 131) to be distinct (F<sub>ST</sub> = 0.1057, P = 0.021; G<sub>ST</sub> = 0.278, P = 0.002; D = 0.237, P = 0.001). Further, Bayesian clustering revealed 2 distinct clusters corresponding to individuals from NWA and SA (Figure 1B, C). A small number of individuals sampled in the NWA, however, had a higher membership coefficient to the second cluster and vice versa (Figure 1B). White sharks from NWA and SA were thus treated as separate, isolated populations for all subsequent analyses.

Microsatellite loci had from 3 to 19 alleles per sample and observed heterozygosities ranging from 0.029–0.853 for NWA and 0.252–0.803 for SA. The mean observed heterozygosity (H<sub>o</sub>) was 0.578 for SA and 0.548 for the NWA sample. The allelic richness (A) was 9.07 for the SA and 7.86 for the NWA sample. After using rarefaction to compensate for differences in sample size (Figure 2), this difference was not significant (t-test, P < 0.161). Average pairwise relatedness for NWA individuals was −0.14 and for SA individuals was 0.05. Four loci in NWA and 2 in SA were out of HWE, all but one of which was a deficit of heterozygotes (Table 1). Microchecker did not find evidence of large allelic dropouts or large allelic gaps, but 3 loci could have null alleles: CCA1273 (NWA), CCA1226 (NWA), and CCA418 (SA, NWA). Although we cannot entirely rule out null alleles at these loci, no new alleles were exposed after reducing the annealing temperature. Null alleles have little effect on population structure analysis or testing for bottlenecks using M-ratio or mode shifts but can influence results of the heterozygote excess test, estimates of effective population size and estimates of inbreeding. Population structure, mode shift, and M-ratio analyses are therefore based on the complete set of 14 loci. We report results with and without those loci that potentially have null alleles for the other analyses.

Only 1 of the bottleneck detection methods detected clear evidence of a genetic bottleneck. No mode shift was detected for NWA or SA as allele frequencies exhibited a normal L-shape distribution. The heterozygosity excess test was inconclusive. The P values for both populations under all 3 models using 2 different tests of significance are reported in Table 2. Peery et al. (2012) demonstrate that the Wilcoxon test is the more reliable test for significance, but we have also included the results testing for significance using the sign test. For the IAM and TPM (60%, 70%, 80%) none of the HE tests are significant. For TPM with 90% step-wise mutations, the HE is significant for NWA and SA when the 3 loci suspected of null alleles are excluded. Assuming the SMM, the HE test is significant for both populations with and without loci with null alleles. The M-ratio test, however, indicated evidence of a recent bottleneck for NWA (M = 0.71, P < 0.004), whereas the M-ratio for SA (M = 0.85) was not significantly different from one at mutation-drift equilibrium (P = 0.39; Table 3).

Table 2. P values for heterozygosity excess test under the IAM, SMM, and TPM for NWA and SA populations

|          | NWA (14 loci) | NWA (11 loci) | SA (14 loci) | SA (11 loci) |
|----------|---------------|---------------|--------------|--------------|
| SMM      | 0.00074 (0.00061) | 0.0071 (0.0068) | 0.00009 (0.00031) | 0.00095 (0.0024) |
| TPM      |                |               |              |              |
| 90%      | 0.207 (0.029) | 0.119 (0.123) | 0.004 (0.029) | 0.037 (0.206) |
| 80%      | 0.164 (0.172) | 0.256 (0.278) | 0.172 (0.153) | 0.523 (0.764) |
| 70%      | 0.163 (0.295) | 0.269 (0.413) | 0.177 (0.357) | 0.512 (0.898) |
| 60%      | 0.318 (0.584) | 0.484 (0.700) | 0.339 (0.714) | 0.506 (0.464) |
| IAM      | 0.266 (0.041) | 0.529 (0.067) | 0.234 (0.090) | 0.025 (0.00098) |

Test was performed using all 14 loci and excluding 3 loci with potentially exhibiting null alleles. Results reported for sign test and Wilcoxon test in parentheses.
Figure 1. Population structure of white sharks assessed using nuclear and mitochondrial markers. (A) Haplotype network of the mtCR. Haplotypes from SA and the NWA are scaled based on frequency of occurrence in the sample set. Small black dots indicate hypothetical haplotypes. (B) Bayesian inference of genetic structure. Sample locations are indicated on the x axis. Each individual is represented by a single vertical column with the y axis indicating the membership coefficient for 1 of 2 clusters. (C) $\Delta K$ values determined using Evanno et al. (2005) method indicating most likely number of clusters K.

Figure 2. Determination of allelic richness of NWA (dashed line) and SA (solid line) populations based on rarefaction using ADZE (Szpiech et al. 2008). Actual sample sizes: $N_{\text{NWA}} = 35$, $N_{\text{SA}} = 131$.

Table 3. Comparison of M-ratios per locus and population

| Locus   | NWA | SA  |
|---------|-----|-----|
| CCa9    | 0.77| 0.72|
| CCa1027 | 0.86| 0.69|
| CCa6.27 | 1.00| 0.88|
| CCa1226 | 0.86| 0.83|
| CCa1536 | 0.53| 0.92|
| CCa1466 | 0.67| 1.00|
| CCa85   | 0.77| 0.92|
| CCa711  | 0.53| 0.90|
| CCa1276 | 0.56| 0.89|
| CCa1273 | 0.63| 0.82|
| CCa1419 | 0.70| 1.00|
| m59     | 0.67| 1.00|
| CCa418  | 0.80| 0.78|
| IoX10   | 0.80| 0.83|
For $p_{null} = 0.02$ (i.e., lowest included allele frequency), the $Ne$ estimate for SA was $346.6$ (95% confidence interval [CI]: 220.2–728.1) and 32.2 (95% CI: 25.2–42.6) for the NWA. After removing the 3 loci with potential null alleles, the results were not substantially different with $Ne = 364.6$ (95% CI: 188.0–1998.3) and 43.5 (95% CI: 31.3–66.3) for the NWA. The multilocus inbreeding coefficient $F_{IS}$ was significantly different from 0 for SA ($P = 0.003$) and NWA ($P = 0.001$) and $F_{ST}$ was significantly higher ($t$-test, $P = 0.0018$) for NWA ($F_{ST} = 0.222$) than for SA ($F_{ST} = 0.102$). Removing the 3 suspect loci did lower $F_{ST}$ in both locations though the difference was not significant ($p_{null} = 0.073, F_{ST} = 0.337$). The mean IR for NWA white sharks (IR = 0.257) was significantly higher ($t$-test, $P = 0.006$) than for SA white sharks (IR = 0.1134). After removing the 3 problematic loci, IR was 0.236 for NWA and 0.134 for SA.

**Discussion**

White shark populations in the NWA and SA are genetically differentiated from one another and, at least at mitochondrial loci, from Pacific and Mediterranean populations. Differentiation was observed at nuclear-encoded and mitochondrial markers, indicating restricted gene flow by both sexes. Overall genetic diversity as measured by heterozygosity, allelic richness, haplotype, and nucleotide diversity were generally similar between these populations. Although white sharks are wide ranging, they are not known to make trans-equatorial migrations (Jorgensen et al. 2010), which would be necessary to maintain gene flow between NWA and SA. Although clearly differentiated, there was evidence of potential migrants in the cluster analyses, which indicates that occasional migration (possibly ephemeral) or reproductive mixing between these populations occurs. There is evidence of similar exchange between South Africa and Australia even though these populations are genetically subdivided (Pardini et al. 2001; Bonfil et al. 2005; Blower et al. 2012). Similar to some other sharks (e.g., Feldheim et al. 2014), white sharks are thought to be philopatric to their natal region or nursery area for purposes of reproduction, (i.e., they preferentially return to the broad area where they were born to give birth or mate). Movement between NWA and SA could therefore occur without gene flow if individuals subsequently return to their natal region to breed (Jorgensen et al. 2010; Blower et al. 2012). The presence of potential migrants in these samples might also be a result of recent secondary contact after a long period of isolation.

There was evidence of a genetic bottleneck in the NWA based on the M-ratio test but not the mode shift or heterozygosity excess approaches, though the results of the heterozygosity excess test varied depending on the model of microsatellite evolution and whether or not all loci were included in the analysis. The mode shift is generally considered a very weak bottleneck test and in a review comparing the statistical power of the M-ratio and heterozygosity excess tests, Peery et al. (2012) determined that the power of the heterozygosity excess test is much lower than the M-ratio. This is especially true when sample size is similar to what we had for the NWA ($N = 35$). We therefore consider the M-ratio test result to better reflect reality than either of the other tests. It is also important to consider the possibility of a type I error in the M-ratio test. Although we only sampled 35 NWA individuals and may therefore have missed some alleles and artificially lowered the M-ratio, the NWA rarefaction curve is approaching an asymptote at this sampling level (Figure 2). In general, samples size of approximately 30 individuals is considered sufficient to determine allelic richness (Hoehn et al. 2012). The M-ratio test is also sensitive to the assumption about the proportion of multistep mutations and can produce false-positive bottleneck detections when this class of mutation is common (Peery et al. 2012). We assumed that the proportion of multistep mutations was 22%, which is what Peery et al. (2012) recommend as a conservative assumption based on empirical data from vertebrates. We found that even at proportions of up to 30%, the probability that the population was in mutation-drift equilibrium was below 5%. We therefore conclude that a genetic bottleneck has occurred in NWA and could be associated with the recent demographic bottleneck documented by Baum et al. (2003) and Curtis et al. (2014). Although there has been some controversy about the magnitude of the NWA white shark population decline (see Baum et al. 2003, 2005; Burgess et al. 2005), our analysis provides independent genetic evidence of a recent reduction in population size severe enough to impact the population’s genetic architecture. Without surveying genetic diversity before and after the decline we cannot, however, prove that it is associated with this particular period as opposed to one occurring at an earlier time. The SA white shark population is thought to have been comparatively stable in recent times (Compagno 1991; Clift et al. 1996; Dudley and Simpfendorfer 2006; FAO 2004; Towner et al. 2013) and, correspondingly, we found no evidence for a genetic bottleneck even despite having a large sample of this population ($N = 131$ individuals).

The estimated effective population size of the NWA is substantially smaller than SA. However, it is important to consider some of the potential sampling biases that may affect this estimate. Estimating $Ne$ hinges upon randomly sampling the generation (Palstra and Fraser 2012 and references therein). Our sampling of both NWA and SA occurred over 7–8 years, which is smaller than the generation time of white sharks (Compagno 1997; Hamady et al. 2014) and therefore precludes random sampling. It is, therefore, possible that our estimates are actually closer to $Ne$ for these cohorts alone rather than of the whole generation. We also caution that it is generally difficult to reconcile $Ne$ with the actual numbers of adults alive at the time (Palstra and Fraser 2012). As a result, our estimates of $Ne$ are probably best used to illustrate that the NWA population is or was recently smaller than the SA population as opposed to making inferences about absolute population sizes in these regions.

Small, isolated populations are vulnerable to inbreeding as reproducing adults have an elevated probability of mating with relatives (Wright 1922; Wright 1984). Nonrandom mating of this nature generates deviations in allele frequencies from theoretical expectations in the form of deficiencies in heterozygotes. We observed heterozygote deficiencies in both populations that for several reasons are unlikely to result entirely from technical or sampling artifacts. Some of our loci probably have null alleles, which can artificially inflate estimates of inbreeding by generating false homozygotes. Despite this, our estimates of both $IR$ and $F_{ST}$ were robust to the removal of the 3 loci most likely to have null alleles. Although we acknowledge that we cannot entirely rule out the possibility that null alleles at other loci inflated IR and $F_{ST}$, our results suggest that white shark mating pairs in SA and even more so in NWA occasionally exhibit coancestry.

Genetic diversity assessments and analyses are commonly integrated into the conservation of threatened terrestrial species and can be similarly useful for the management of threatened marine fish. We found that NWA and SA white shark populations are genetically distinct and are likely to be demographically independent. This means that resource managers charged with conserving this species in these regions should recognize that intrinsic reproduction is a far more important process for sustaining or recovering each of these populations than immigration, despite white sharks being highly mobile and oceanic. We also find that the isolated NWA white shark population exhibits evidence of a genetic bottleneck, which is concordant with other studies that found a severe population decline from the 1960s to the 1990s. As is quite common with marine species, there has been some uncertainty and debate about this decline and genetic data in this case provide independent...
corroborative evidence supporting the hypothesis that a severe decline took place. Although bottleneck detection is not without its own caveats, this study highlights the value of including it together with other types of evidence (e.g., catch series analysis, demographic analysis) when assessing the status of marine fish populations. We also found evidence of low effective population size and inbreeding in NWA white sharks but highlight some methodological and sampling issues that should be taken into consideration before accepting these findings and integrating them into management decisions. Nonetheless, genetic assessments of other species of sharks and rays have found evidence of inbreeding, or modest Ne (Hoelzel et al. 2006; Chapman et al. 2011; Mourier and Planes 2013), highlighting that analysis of intrapopulation genetic diversity can potentially yield important information useful for the conservation of these threatened marine fish.

**Supplementary Material**

Supplementary material can be found at http://www.jhered.oxford-journals.org/

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