Genetic Diversity Despite Population Collapse in a Critically Endangered Marine Fish: The Smalltooth Sawfish (*Pristis pectinata*)

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**Abstract**

Sawfish (family Pristidae) are among the most critically endangered marine fish in the world, yet very little is known about how genetic bottlenecks, genetic drift, and inbreeding depression may be affecting these elasmobranchs. In the US Atlantic, the smalltooth sawfish (*Pristis pectinata*) has declined to 1–5% of its abundance in the 1900s, and its core distribution has contracted to southwest Florida. We used 8 polymorphic microsatellite markers to show that this remnant population still exhibits high genetic diversity in terms of average allelic richness (18.23), average alleles per locus (18.75, standard deviation [SD] 6.6) and observed heterozygosity (0.43–0.98). Inbreeding is rare (mean individual internal relatedness \( R_I = 0.02, \) SD 0.14; \( F_{IS} = -0.011, \) 95% confidence interval [CI] = -0.039 to 0.011), even though the estimated effective population size \( (N_e) \) is modest (250–350, 95% CI = 142–955). Simulations suggest that the remnant smalltooth sawfish population will probably retain >90% of its current genetic diversity over the next century even at the lower estimate of \( N_e \). There is no evidence of a genetic bottleneck accompanying last century’s demographic bottleneck, and we discuss hypotheses that could explain this. We also discuss features of elasmobranch life history and population biology that could make them less vulnerable than other large marine vertebrates to genetic change associated with reduced population size.

**Key words:** elasmobranch, genetic bottleneck, genetic drift, inbreeding, K-selected species, microsatellites

Inbreeding depression and the loss of genetic diversity due to population decline and drift are all serious concerns for the management of endangered species in terrestrial and freshwater ecosystems (Frankham 2010). Marine species often have such large populations and wide geographic ranges that it is difficult to imagine that they are also vulnerable to these processes (Hauser et al. 2002; Hutchinson et al. 2003; Hoarau et al. 2005). Nevertheless, these types of genetic change have been observed in a variety of marine vertebrates, ranging from r-selected bony fish (Hauser et al. 2002; Hutchinson et al. 2003; Hoarau et al. 2005) to K-selected marine mammals (Acevedo-Whitehouse et al. 2003; Frère et al. 2010). But what about elasmobranchs (e.g., sharks, rays, sawfish, and skates), primarily marine fish that exhibit a K-selected life history? Very little is known about genetic changes occurring in elasmobranchs, despite evidence of heavy exploitation and declining populations (Simpfendorfer 2000, 2005; Clarke et al. 2006; Dulvy et al. 2008; Hayes et al. 2009).

Sawfish (family Pristidae) are among the most endangered marine fish in the world (Wueringer et al. 2009), prompting reasonable concerns that they exhibit especially
low effective population sizes, inbreeding depression, and depleted genetic diversity (e.g., Simpfendorfer 2000; National Marine Fisheries Service [NMFS] 2009). These hypotheses are all plausible for the critically endangered smalltooth sawfish Pristis pectinata of the Western Atlantic. Growing to lengths of 500–600 cm, this species is one of the largest marine animals occurring in the coastal waters of the United States and is the only fully marine fish listed under the U.S. Endangered Species Act (Simpfendorfer 2005). Smalltooth sawfish were extremely common in the littoral zone of the US Atlantic Ocean and Gulf of Mexico at the turn of last century. They were present in southern Florida year round in very large numbers and ranged from Texas to as far north as New York in the summer months (Bigelow and Schroeder 1953; NMFS 2009). Sawfish are vulnerable to entanglement in fishing nets, and as nearshore net fisheries developed in the early 1900s, they experienced high fishing mortality (Simpfendorfer 2000, 2005; Wiley and Simpfendorfer 2007, 2010). Though not the target of these fisheries, sawfish were generally killed because they were a nuisance or so that their rostrum could be sold as a trophy or novelty item (Simpfendorfer 2000, 2005; Wiley and Simpfendorfer 2007, 2010). Many of the wetland areas smalltooth sawfish relied on for reproduction were also developed or degraded over this period (Seitz and Poulakis 2002; Poulakis and Seitz 2004; Simpfendorfer 2005; Seitz and Poulakis 2006; Wiley and Simpfendorfer 2010). As a consequence of fishing mortality and habitat loss, smalltooth sawfish declined by 95–99% last century (Simpfendorfer 2000; NMFS 2009). The only time series of sawfish abundance (from Louisiana) suggests that the species reached extremely low levels, thereby 1950–1960 (Simpfendorfer 2000, 2005), which coincides with their disappearance from other many areas where they were formerly common (e.g., Texas, Louisiana, Florida’s east coast, the Indian River Lagoon system, and Florida; Snelson 1981; NMFS 2009; Wiley and Simpfendorfer 2010). By 1985, smalltooth sawfish were virtually absent from anywhere other than a small area of southwest Florida (hereafter referred to as SWFL), centered around the Everglades National Park (ENP; Seitz and Poulakis 2002, 2006; Poulakis and Seitz 2004; Wiley and Simpfendorfer 2007, 2010). At present, there is no evidence of any substantial breeding occurring elsewhere in the continental United States or neighboring regions (e.g., Mexico, Caribbean islands, and Bahamas).

Suffice to say they have gone from being extremely abundant to being extremely rare, it is not known how large the remnant SWFL smalltooth sawfish population is or how small it became last century. The species generation time is not known with certainty but has been estimated to be 27 years (NMFS 2009). If sawfish abundance troughed in SWFL around 1950–1960, this suggests that they have had ~2–3 generations to recover. Carlson et al. (2007) analyzed recreational catch data in the ENP (1972–2005) and suggested that the species may have recently stabilized at a very low level. Anglers did not report capturing any smalltooth sawfish from the onset of the survey in 1972–1988, with the small numbers of the species being reported each year from 1989 onward (Carlson et al. 2007). One explanation for the absence of smalltooth sawfish in these surveys prior to 1989 is that the species may have been close to extinction even in SWFL. If this is true, we predict that the population experienced a genetic bottleneck that should be detectable in its contemporary genetic architecture. If the current population is extremely small, we predict that inbreeding may also be prevalent.

Given the degree of decline and range contraction, this species has experienced over the last few generations, we hypothesize that 1) the remnant smalltooth sawfish population in SWFL has experienced a genetic bottleneck, 2) inbreeding is occurring among surviving individuals, and 3) effective population size is low. Simpfendorfer (2000) suggest that population fragmentation may also be a problem in this species, so we also test the hypothesis that there is only one smalltooth sawfish population SWFL. As one of the first microsatellite-based studies of the conservation genetics of a critically endangered elasmobranch fish, this study also aimed to provide insight into how vulnerable these increasingly overexploited species may be to genetic bottlenecks, drift, and inbreeding depression relative to other marine vertebrates.

**Materials and Methods**

Between 2002 and 2008, live smalltooth sawfish (N = 137) were collected in SWFL following ESA permitting guidelines (see Simpfendorfer et al. 2008 for detailed field methods). Seven sites were regularly sampled, with several additional sawfish being opportunistically sampled outside of these locations. Captured sawfish were measured, tagged, tissue sampled via a small (~1 x 1 cm) fin clip taken in the field, and released alive after data and sample collection. Tissue samples were placed in ~4 ml of 95% reagent grade ethanol and stored at room temperature until processing. Total genomic DNA was extracted from 25 mg of fin tissue using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) following the manufacturer’s protocol. Isolated DNA was checked for quality and approximate concentration following electrophoresis on a 1.2% agarose gel. We amplified 8 polymorphic microsatellite loci using previously published primer sets and PCR protocols (Feldheim et al. 2010). Amplification products were run with an internal size standard (LIZ-500; Applied Biosystems) on an ABI 3730 Genetic Analyzer and scored by 2 experienced analysts (D.D.C., K.A.F.) using ABI PRISM GeneMapper Software v3.7 (Applied Biosystems). Genotypes were checked for scoring errors, large allele drop out, large allelic gaps, identical genotypes, and null alleles using Microsatellite Toolkit for Excel and Microchecker (Oostesthaut et al. 2004).

Some subtropical elasmobranchs exhibit natal site fidelity for several years and therefore live in close proximity to littermates (e.g., Chapman et al. 2009). This could occur in smalltooth sawfish because juveniles are restricted to shallow nearshore habitats that Simpfendorfer et al. (2010) hypothesized were within or very close to their natal area. If this hypothesis is correct, then concentrated sampling of
juvenile sawfish from a small area would result in a disproportionate occurrence of full or half siblings in the analysis. Therefore, to minimize bias from oversampling littermates, we used the pairwise hypothesis testing option in the program ML-RELATE (Kalinowski et al. 2006) to identify potential siblings among juvenile sawfish captured in the same location (i.e., within 0–1000 m of each other). ML-RELATE calculates a likelihood ratio of relatedness between pairs of individuals and then assesses the probability of obtaining this ratio given the hypothesized relationship using a simulated null distribution based on observed population allele frequencies. We simulated likelihood ratios of 10 000 random dyads and accepted the hypothesis that pairs were full siblings as opposed to unrelated individuals when the probability of their likelihood ratio was < 0.01. If $P > 0.01$ but < 0.05, we tested the hypothesis that pairs were half siblings as opposed to being unrelated, allowing for the fact that littermates could also be half siblings in sawfish because polyandry and multiple maternity are known to occur in other batoids (Chapman et al. 2003; Chevrot et al. 2007). In cases where we found probable siblings, we excluded all but one randomly selected member of the group from downstream analysis.

The program FSTAT 2.9.3.2. (Goudet 1995) was used to describe the genetic diversity of the sample, and Genepop 4.0 (Rousset 2008) was used to test for linkage disequilibrium and deviations from Hardy–Weinberg expectations. A Bonferroni adjustment of $\alpha$ was employed to correct for multiple tests. We tested for population differentiation in 2 ways. First, individual sawfish were divided a priori into 2 geographic groups: Everglades-Florida Bay (E-FB) and Caloosahatchee River (CR). We split out these putative “populations” on the grounds that they occupy areas designated by the United States as 2 separate units of “Critical Habitat” that could be discrete breeding areas (Figure 1). We did not include the samples from the Florida Keys in this analysis given that we had relatively few samples from this area. We calculated $F_{ST}$ between the E-FB and CR sampling areas and tested for population differentiation using an exact test in Genepop 4.0 (Rousset 2008). Tests for genetic differentiation within our data set were also run without establishing putative populations a priori by using the program STRUCTURE 2.3 to estimate the total number of populations ($K$) and the geographic origins of individuals within any populations defined by the analysis (Pritchard et al. 2000). STRUCTURE 2.3 was run using the admixture model with correlated allele frequencies, simulating $K = 1–3$ with 350 000 Markov Chain Monte Carlo steps proceeding after a burn-in period of 15 000 steps. No a priori information about sampling location was used in the simulation, and 8 independent runs for each value of $K$ were conducted to check for convergence.

The current effective population size of SWFL smalltooth sawfish was estimated using the linkage disequilibrium approach for a single temporal sample (Waples 2006) using LDNe ver 1.31 (Waples and Do 2008). This approach uses the slight linkage of alleles that arises from sampling error in the union of gametes in a small population to estimate the current generational population size from a single temporal sample. It assumes that the population sample comprises individuals from a single generation and that related individuals occur in the sample at the same frequency as they do in the population. We pared our total sample set to meet the first assumption by removing all animals that could have reached sexual maturity within our most active sampling period (2005–2008) to ensure that we sampled individuals from a single generation. We therefore only included sawfish that were collected from 2005 to 2008 and were less than 250 cm stretched total length (STL) at the time of sampling (i.e., these individuals were all many years from reaching sexual maturity, Simpfendorfer et al. 2008; $N = 72$). We met the second assumption by keeping probable littermates captured in the same location out of the final data set, as previously described. We suggest that the frequency of littermates in our complete sample of 137 sawfish (see Results) is an artifact of relatively large litters, natal site fidelity, and repeated sampling of several nursery locations and is therefore not representative of the true frequency of first-order relatives in the SWFL population. We made 3 estimates of effective population size, excluding alleles with frequencies less than 0.01, 0.02, and 0.05, respectively. The 95% confidence interval for each was estimated using a jack-knifing approach.

We evaluated contemporary levels of inbreeding in 2 ways. We first calculated $F_{IS}$, as a measure of intrapopulation heterozygote deficit due to inbreeding, together with 95% CIs by bootstrapping over all loci, in FSTAT 2.9.3.2. (Goudet 1995). If inbreeding is prevalent in the contemporary smalltooth sawfish population, then mating pairs will frequently share alleles by descent as opposed to rarely sharing them by chance. We therefore calculated internal relatedness (IR) of every individual in our study as a measure of how related their parents were to assess the prevalence of inbreeding, the first step toward assessing how threatened the current population is by inbreeding depression (Amos et al. 2001). IR expresses the proportion of typed loci that are homozygous in an individual scaled by population allele frequencies, so that homozygotes with rare alleles are given more weight than homozygotes with common alleles. IR therefore provides a measure of allele sharing between parents due to recent common ancestry. Inbred individuals will exhibit high IR, whereas outbred individuals will exhibit IR close to or below zero. IR was calculated for all smalltooth sawfish in the program STORM (Frasier 2008). We also determined whether there was a significant negative correlation between IR and body size (STL), which we would expect to observe if individuals with high IR tend to die at an early age.

Three types of test were used to detect a genetic bottleneck signature in SWFL smalltooth sawfish: the mode shift test, heterozygote excess tests, and the $M$-ratio test. Each of these tests require a single temporal sample and is capable of detecting bottlenecks given the sample size and number of loci used in this study. We first examined the allele frequency distribution for all loci combined looking for a “mode shift” in the distribution (Luikart et al. 1998). This qualitative test is based on the premise that a severe
bottleneck causes a reduction in the relative abundance of rare alleles (which are collectively the most common category of alleles), resulting in a mode shift in allele frequency distribution that persists for a few generations after the bottleneck. Second, we also implemented the Sign and Wilcoxon tests in the program Bottleneck 1.2.02 (Piry et al. 1999) to test for an excess of heterozygosity brought about by the loss of rare alleles following a population bottleneck. We assumed a two-phase mutation (TPM) model consisting of 70% stepwise and 30% non-stepwise mutations and run 5000 iterations. To test how sensitive these data were to variations in the assumed mutation model, the Sign and Wilcoxon tests were also run assuming a strict stepwise mutation model (SMM), an infinite allele mutation model, and TPM with different percentage of stepwise mutations (i.e., 80%, 60%, and 50%). The third method we used to test for a recent genetic bottleneck was the $M$-ratio test (Garza and Williamson 2001). The $M$-ratio test is based on the premise that most allelic states (i.e., repeat units) of any microsatellite locus should be occupied, assuming a SMM and a robust population size. Because rare alleles are lost during a bottleneck, there tends to be an increase in the number

Figure 1. Map of smalltooth sawfish (*Pristis pectinata*) sampling locations in SWFL. The 2 stippled areas denote federally designated “Critical Habitat” for this species. The Caloosahatchee River area is indicated by “CR,” the Everglades National Park/Florida Bay area is designated as “E-FB,” and the Florida Keys are designated as “FK.” Sample sizes, after removal of all but one member of probable sibling groups, are shown for each collection area.
of unoccupied allelic states. The degree to which allelic states are unoccupied can be quantified by expressing the ratio of the number of alleles observed in a single population sample (\(k\)) to the number of potential allelic states (\(K\)) given the size range of alleles observed in the sample (see Garza and Williamson 2001). Population samples from species known to have experienced a recent bottleneck have been shown to exhibit a low \(M\)-ratio (Garza and Williamson 2001). We calculated mean \(M\) across all 8 loci and estimated the 95% CI using a t-distribution.

In order to assess how likely this endangered population is to lose genetic diversity over time, we used BottleSim2.6 (Kuo and Janzen 2003) to simulate the change in genetic diversity (observed number of alleles) across our 8 loci in SWFL smalltooth sawfish after a severe reduction in \(N_e\). For 3 simulations, we assumed that a 95% decline in \(N_e\) occurred, resulting in a stable \(N_e = 150\) individuals (i.e., at the lower end of our 95% CI) for the next 200 years. We were especially interested in how longevity and age at first maturity affected post-bottleneck genetic diversity. Because these 2 parameters are not currently known with certainty (Simpfendorfer 2000; NMFS 2009), we ran simulations (1000 iterations) with 2 published estimates of longevity (30 and 60 years) and up to 3 published estimates of age at first maturity (10, 20, and 33 years; Simpfendorfer 2000; NMFS 2009). We assumed a randomly mating population with overlapping generations and a 1:1 sex ratio that initially exhibited the same allele frequencies as our study population. We also simulated population declines to \(N_e = 20\) and 50 for comparison.

**Results**

A total of 137 smalltooth sawfish (62–426 cm STL, mean 143 cm STL) were collected and sampled in SWFL (Figure 1). Most individuals were smaller than the estimated age at first maturity and were probably less than 10 years old (Simpfendorfer 2000; NMFS 2009). After testing for relatedness between individuals captured in the same location, we opted to remove 33 individuals on suspicions that we had sampled littermates at some sampling sites (i.e., ML-RELATE indicated that 2 or more individuals have >1% probability of being unrelated vs. being full or half siblings). Whether or not these individuals are littermates will be further explored in future publications, but we subsequently analyzed the genetic diversity of 104 individuals that we were confident were not littermates (Figure 1).

All 8 loci exhibited high allelic diversity and heterozygosity. Average allelic richness was 18.23 with the number of alleles per locus (\(k\)) ranging from 10 to 30 (mean 18.75, standard deviation [SD] 6.6) and observed heterozygosities ranging from 0.43 to 0.98 (Table 1). Allele frequency distributions, divided into E-FB and CR sampling areas, are shown for the 6 most polymorphic loci in Figure 2. All 8 loci conformed to Hardy–Weinberg expectations (Table 1), and no pair exhibited linkage disequilibrium. Standard \(F_{st}\) analysis failed to differentiate the E-FB and CR sampling areas (\(F_{st} = 0.0014\), Exact test \(P > 0.06\)). Bayesian cluster analysis run in STRUCTURE 2.3 also failed to detect population structure. The hypothesis that \(K = 1\) consistently exhibited a higher probability than \(K = 2\) or 3, and each individual sawfish was assigned to each of the 2 or 3 populations (where \(K\) was set to 2 or 3, respectively) in approximately equal proportions (i.e., apparently at random). Individuals from all sampling regions were therefore pooled for subsequent analysis.

After isolating a subsample (\(n = 72\) of smalltooth sawfish that were 1) captured from 2005 to 2008 and 2) were <250 cm STL at the time of capture, we estimated the effective population size of smalltooth sawfish in SWFL. Estimates were 250.4 (95% CI = 142.4–796.1), 342.5 (95% CI = 202.8–954.8), and 295.5 (95% CI = 170.6–901.4) excluding alleles at a frequency of less than 0.05, 0.02, and 0.01, respectively. IR was calculated for all 137 sawfish we sampled and was close to zero in most individuals (mean \(-0.02\), SD 0.14, Figure 3). There was no significant correlation between sawfish body size (ca. age) and IR (\(r^2 = 0.0004\)). There was also no evidence of intrapopulation heterozygote deficit due to contemporary inbreeding (\(F_{is} = -0.011\), 95% CI = –0.039 to 0.011).

None of the qualitative or quantitative indicators employed provided evidence of a population bottleneck. There was no evidence of a mode shift in the allele frequency distribution as ~88% of alleles occurred at a frequency <0.1 (Figure 4). The expected number of loci with heterozygosity excess assuming a TPM model with 70% stepwise mutations was 4.74, and the smalltooth sawfish population conformed to this expectation (3 loci with heterozygote deficiency and 5 loci with heterozygote excess, Sign test: \(P < 0.58\)). The Wilcoxon test also failed to detect an excess of heterozygotes assuming the same mutation model (\(P < 0.68\)). Changing the mutation model assumed in the test had no impact on the test results. \(M\)-ratios were high for each locus (0.66–0.90, mean 0.81, 95% CI = 0.76–0.86; Table 1), reflecting that most allelic states were occupied at all 8 loci.

Our simulations showed that when \(N_e = 150\), near the lower 95% CI of our \(N_e\) estimate for SWFL, the number of alleles remained >90% of virgin levels (i.e., year 0) 100 years after the population decline. After 100–200 years, diversity only dropped below 90% only when lifespan was assumed to be 30 years (Figure 5). When we simulated \(N_e = 20\) and \(N_e = 50\), there was a much sharper decline in diversity, and >10% of alleles were consistently lost within 100 years (Figure 5). Increased lifespan (30 vs. 60 years) had a substantial positive impact on the retention of genetic diversity (Figure 5).

**Discussion**

We were unable to detect statistically significant population structure between the 2 sampling areas CR and E-FB. These findings do not preclude the possibility that female smalltooth sawfish exhibit philopatry to these breeding areas because the genetic signature of this pattern would be...
obscured at nuclear microsatellite loci if males mated with females at random with respect to which nursery area they use for parturition (Keeney et al. 2003; Feldheim et al. 2004; Portnoy et al. 2010). Mitochondrial control region sequences of 3 sawfish species (P. microdon, P. zijsron, and P. clavata) are geographically partitioned across Northern Australia (~4000 km), which is likely attributable to female philopatry (Phillips et al. 2011). We suggest future mitochondrial studies of smalltooth sawfish to further resolve this issue.

The negative $F_{IS}$ and the near zero IR values we observed for most of the specimens we sampled indicate that mating between close relatives is not common in SWFL. Although it is possible that inbred individuals with high IR experience early mortality and are therefore underrepresented in our database, >75% of the smalltooth sawfish we sampled were from young-of-the-year to ~2 years old. Moreover, there was no significant negative correlation between sawfish body size (ca. age) and IR ($r^2 = 0.0004$), which is contrary to expectations if individuals with high IR were dying earlier than outbred conspecifics. Unless mortality of inbred individuals occurs in utero or within the first few months of life, our data are consistent with consanguineous mating being unusual in this population despite its small size. Nevertheless, small populations are vulnerable to random fixation of deleterious alleles that can cause reduced fitness even in the absence of true inbreeding (Madsen et al. 1994).

If any elasmobranch was to exhibit low genetic diversity due to population decline, we would have expected to observe it in the remnant smalltooth sawfish population of SWFL. Yet despite this, the population exhibits genetic diversity that compares favorably with those of other, less depleted elasmobranch populations (e.g., Feldheim et al. 2001; Keeney et al. 2003; Feldheim et al. 2004; Portnoy et al. 2010).

### Table 1

<table>
<thead>
<tr>
<th>Locus</th>
<th>$k$</th>
<th>All. Rich.</th>
<th>$H_e$</th>
<th>$H_o$</th>
<th>$P &lt;$</th>
<th>$R$</th>
<th>$M$</th>
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<td>18.23</td>
<td>0.83589</td>
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</table>

$k = \text{number of alleles, All. Rich. = Allelic Richness, } H_e = \text{expected heterozygosity assuming Hardy–Weinberg equilibrium (HWE), } H_o = \text{observed heterozygosity, } P < = \text{P value for hypothesis that } H_o \text{ conforms to HWE, } R = \text{size range of alleles, and } M = M\text{-ratio (Garza and Williamson 2001). Mean values for } k, H_o, \text{ and } M \text{ are shown in the bottom row.}

Figure 2. Allele frequency histograms for the 6 most polymorphic microsatellite loci used in this study (the 2 remaining loci show the same pattern of few or no gaps in the allele frequency distribution and are not shown). Solid bars denote allele frequencies for the CR sampling area, and open bars denote allele frequencies for the E-FB sampling area. All but one member of every full sibling cluster has been removed from the data set prior to generating these distributions.
The estimated effective population size, however, is small in the low to high hundreds. Assuming that longevity is from 30 to 60 years, our simulations suggest that the SWFL population will likely maintain >90% of its current genetic diversity in the next century if it stays at this size or grows. Immediate aggressive efforts to facilitate population recovery in the wild are therefore warranted in order to naturally raise effective population size and preserve the remaining genetic diversity.

One caveat about our estimate of effective population size is that we removed 33 individuals that are probably littermates with ones that we kept in the analysis. The inclusion of these samples leads to an estimated effective population size of 106–201 individuals, which aligns with the low end of our estimates made without them. All 33 of the excluded individuals fit the criteria of 1) being very young, 2) being captured in a nursery area, and 3) being captured in close proximity to their putative siblings (within from 0 to 1000 m). In many cases, 2 or more putative littermates were captured on the same mudflat at the same time. We therefore defend our decision to remove these individuals on the grounds that their inclusion artificially lowers estimated effective population size due to an unrepresentative high proportion of relatives. Even so, the estimated effective population size with these samples included is still sufficiently large for the species to retain allelic diversity in the coming decades (see Figure 5).

There is no evidence of a loss of genetic diversity during the collapse of the smalltooth sawfish population last century. None of the tests detected a genetic bottleneck occurring in SWFL, despite an adequate number of loci, high polymorphism, and a large sample size. Bottlenecks of a variety of strengths and lengths should be detectable for many generations (tens to hundreds, depending on the length and severity of the bottleneck) using these tests (Luikart et al. 1998; Piry et al. 1999; Garza and Williamson 2001; Williamson-Natesan 2005). Moreover, our simulations of a decline to effective population sizes of 20 or 50 were all accompanied by a rapid decline in allelic diversity, which suggests that if a severe genetic bottleneck had taken place in the 1950s–1960s then there should be many missing alleles in the extant population. These data do not, however, preclude the possibility that the US population as a whole lost genetic diversity as it collapsed. For example, any unique genetic variation associated with populations that were extirpated may have been lost. Moreover, we have only surveyed neutral microsatellite loci in the present study and caution that future surveys should examine genetic diversity in coding regions. For example, it would be prudent to assess major histocompatibility complex diversity in SWFL given that variation at these loci, low diversity in these genes can increase susceptibility to pathogens and lead to sudden dramatic decline in endangered species (Siddle et al. 2007).

Why is there no evidence of a genetic bottleneck in this severely depleted species? Depleted populations avoid genetic bottlenecks when 1) the population rapidly recovers (i.e., the duration of the bottleneck is short), 2) gene flow
counteracts genetic drift, 3) the species is long lived, and 4) the demographic bottleneck is not severe enough to produce a genetic bottleneck over the period of observation. Given the low intrinsic rate of population increase (Simpfendorfer 2000) and its extreme rarity for at least the last 2–3 generations (NMFS 2009), the hypothesis that the smalltooth sawfish has recovered from a severe genetic bottleneck is biologically unrealistic. Indeed, the best available evidence suggests that stabilization of this species only appears to have occurred in the late 1980s or 1990s (Carlson et al. 2007). Recovery from or avoidance of a genetic bottleneck through gene flow is also unlikely given the lack of potential source populations for immigrants. Sawfish are large conspicuous animals that live in shallow habitats that are frequented by a wide variety of ocean users, yet the capture or sighting of one outside of SWFL has been newsworthy for decades given their rarity (Simpfendorfer 2000; 2005; Seitz and Poulakis 2006; Wiley and Simpfendorfer 2007, 2010). There is also very little evidence of substantial breeding occurring outside of the United States (e.g., Mexico, Caribbean islands, and Bahamas; NMFS 2009). Recent captures of large juvenile sawfish at Andros Island (Bahamas), however, suggest that this area could harbor a breeding population (Grubbs D, personal communication). Future genetic survey and telemetry studies are needed to more fully evaluate whether or not gene flow contributes to the high genetic diversity observed in this species.

We suggest that the 2 leading hypotheses for the absence of a genetic bottleneck are 1) the longevity of smalltooth sawfish has slowed genetic drift and/or 2) the SWFL population never reached the severely low level (i.e., an effective population size in the tens rather than the hundreds) needed to produce one in the time elapsed. The longevity of smalltooth sawfish is not known, with Simpfendorfer (2000) and NMFS (2009) suggesting that their lifespan could be from 30 to 60 years. Longevity slows genetic drift when using a fixed time period as point of reference and explains the absence of genetic bottlenecks in other depleted long-lived vertebrates (Haier et al. 2006; Lippé et al. 2006; Marsack and Swanson 2009). Our simulations show that if these estimates of longevity for sawfish are accurate, then the species is likely to retain significant genetic diversity in the next 100–200 years even at effective population sizes at the low end of what we estimate for the remnant SWFL population. Resolving the lifespan of this species should be a research priority for refining our understanding of how the genetic architecture of the species will respond to changes in effective population size. The hypothesis that smalltooth sawfish never dropped to an effective population size that was low enough for them to lose significant genetic diversity is also plausible. We know that in other parts of the United States, smalltooth sawfish clearly subsided below this level for a period of time before being extirpated, but there are no estimates of their population size in SWFL at any time in the last century. However, we suggest that even an extremely severe proportional population decline (e.g., 95–99%), as estimated for the species throughout the United States; Simpfendorfer 2000) might have failed to produce a genetic bottleneck in SWFL if the initial population was so large that there were enough survivors to have maintained an effective population size in the low hundreds. Historical photographs, naturalist accounts, and catch records all indicate that the species was extremely abundant at the turn of last century (Bigelow and Schroeder 1953), which could explain the absence of a genetic bottleneck even despite a large proportional population decline as the 20th century progressed.

We also suggest a more general hypothesis that elasmobranchs may be less vulnerable than other marine vertebrates to anthropogenic-induced genetic change. Marine vertebrates share several general characteristics that were once thought to buffer them from exploitation-driven genetic bottlenecks, including large virgin population sizes, wide geographic ranges, and high gene flow. We now know that genetic bottlenecks have recently occurred in some marine vertebrates (Hauser et al. 2002; Hutchinson et al. 2003; Hoarau et al. 2005), whereas others are suffering the effects of inbreeding depression (Acevedo-Whitehouse et al. 2003; Frère et al. 2010). Marine bony fish appear to be quite vulnerable to bottlenecks because their effective population sizes tend to be several orders of magnitude lower than census population sizes, due to high variance in reproductive success associated with the larval life stage in these r-selected species (Hauser et al. 2002; Hutchinson et al. 2003; Hoarau et al. 2005). On the other hand, elasmobranchs exhibit direct development and a K-selected life history that promotes a more comparable ratio of effective to census population size (Portnoy et al. 2009). It is therefore not until a population reaches extremely low levels that genetic diversity is likely to be lost. Of course, once K-selected species like elasmobranchs reach critical levels, it potentially takes a long time for them to recover, thus increasing the risk of genetic change over many generations.

Depleted K-selected elasmobranchs may be vulnerable to inbreeding depression, as has been documented in some marine mammals (Acevedo-Whitehouse et al. 2003; Frère et al. 2010). We did not find evidence of inbreeding in critically endangered smalltooth sawfish, and we are not aware of any studies that have documented inbreeding or inbreeding depression in any elasmobranch. Inbreeding in marine mammals has been documented in species that exhibit philopatry to reproductive sites by both sexes and have mating systems characterized by extreme polygyny (Acevedo-Whitehouse et al. 2003; Frère et al. 2010), both of which can facilitate consanguineous mating. Although they exhibit a broadly similar life-history strategy, there are some fundamental differences in the population biology of elasmobranchs and marine mammals that we propose reduces the likelihood of inbreeding in the
former. First, there is no evidence that certain males dominate paternity in elasmobranchs, reducing the proportion of paternal half siblings in each breeding area relative to highly polygynous marine mammals (Feldheim et al. 2004). Moreover, male-biased dispersal from the natal area appears to be common in elasmobranchs, which would further reduce the likelihood of females encountering and mating with related males (Feldheim et al. 2004; Portnoy et al. 2010). We therefore hypothesize that the combination of comparable effective and census population sizes, male-biased dispersal, and lack of extreme polygyny could reduce the susceptibility of elasmobranchs to anthropogenic-induced genetic bottlenecks, genetic drift, and inbreeding relative to bony fish or marine mammals. More genetic studies of critically endangered elasmobranch species are needed to evaluate this hypothesis.

In conclusion, the present study indicates that this critically endangered marine vertebrate still retains significant genetic diversity. We suggest 3 not necessarily mutually exclusive hypotheses to explain this: 1) the species is long lived, which would slow genetic drift; 2) at its lowest, the remnant SWFL population was still large enough to maintain genetic diversity, perhaps due to its large virgin size and the refuge from human activities provided by the ENP; and 3) general features of elasmobranch life history and reproductive behavior reduce their susceptibility to genetic bottlenecks and inbreeding. We show that although the smalltooth sawfish currently has a small effective population size, especially for a marine fish, immediate and aggressive management action to promote recovery will probably preserve its remaining genetic diversity.

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