Latin American Journal of Aquatic Research, 51(3): 388-403, 2023
DOI: 10.3856/vol51-issue3-fulltext-2984

*Research Article*

# Nuclear genetic structure of the white shark (*Carcharodon carcharias*) from the Northeastern Pacific

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**ABSTRACT.** The Northeastern Pacific (NEP) population of white sharks (*Carcharodon carcharias*) is genetically distinct from the rest of the world. This uniqueness results from adult fidelity to central California and Guadalupe Island aggregations sites. The strong mitochondrial genetic structure between the white sharks of central California and Guadalupe Island is also present, which indicates female philopatry. To date, few studies using nuclear DNA have found evidence of genetic patterns in the NEP white shark population, which could indicate that these sharks exhibit sex-biased dispersal. In this study, we evaluated the genetic structure, connectivity, and genetic diversity of NEP white sharks using samples from the southern California Bight (SCB), Baja California (including Sebastian Vizcaino Bay), the Gulf of California, and Guadalupe Island (GI) using nDNA (i.e. microsatellite loci). A total of five loci were successfully genotyped in 54 individuals. The patterns found in this study indicated low levels of genetic diversity among all localities (observed heterozygosity: *Ho* = 0.47), likely due to a founder effect. A slight genetic structure was present for NEP localities in this study ($F_{ST}$ = 0.045, *P* = 0.0001), mainly identified between the SCB and GI locations. A sibship assignment analysis indicated low and moderate probabilities of full- and half-siblings between white shark juveniles from coastal areas, suggesting a high degree of connectivity between nursery areas in the NEP. Our results suggest that juveniles can mask the genetic structure in coastal zones.

**Keywords:** *Carcharodon carcharias*; genetic connectivity; genetic structure; genetic diversity; microsatellite; sex-biased dispersal; white shark

## INTRODUCTION

Large marine vertebrates can migrate across entire ocean basins and do not recognize political boundaries (Costa et al. 2012). When multiple countries participate in developing conservation strategies for highly migratory species, the protection of these species depends on a complex network of political, cultural, social, and scientific interests (Kark et al. 2015). For these networks to act effectively, connectivity patterns and species-specific conservation challenges must be clearly understood (Martin et al. 2007). In fact, without proper species-specific information, it is challenging to design effective conservation strategies, which has been the case for some elasmobranch species. Sharks and rays constitute a group of particular interest for conservation due to their evolutionary history, broad geographical distributions, and sensitivity to factors that affect their survival, like fishing pressure (Ferretti et al. 2010).

The great white shark (*Carcharodon carcharias*) is an apex predator in almost all oceans in temperate and

Corresponding editor: José Gallardo

subtropical regions (Compagno et al. 2005) and is thus a species subject to both international and national laws. The white shark is categorized as Vulnerable to exploitation by the International Union for Conservation of Nature (IUCN, Fergusson et al. 2009) and is included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Inskipp & Gillett 2005). However, white sharks are exposed to various threats. For example, despite the protection of coastal nursery areas, young-of-the-year (YOY) and juvenile white sharks are vulnerable to fishing (Lowe et al. 2012, Santana-Morales et al. 2012, Lyons et al. 2013, Ramírez-Amaro et al. 2013, Curtis et al. 2014, Oñate-González et al. 2017, García-Rodríguez & Sosa-Nishizaki 2020). Moreover, total fishing-related mortality in international offshore waters remains unknown (Huvenners et al. 2018). In addition, long-term exposure to pollutants, disturbance due to maritime activities, and reductions in prey abundance indirectly threaten the species (Dewar et al. 2013, Mull et al. 2013, Huvenners et al. 2018).

The Northeastern Pacific (NEP) white shark population is one of the most studied worldwide. However, when compared to the Atlantic population, studies of population genetics in NEP white sharks that have evaluated patterns in a mitochondrial genetic structure are scarce (uniparental molecular markers; Jorgensen et al. 2010, Oñate-González et al. 2015, Díaz-Jaimes et al. 2016, Santana-Morales et al. 2020), and limited information on the patterns of nuclear genetic structure (biparental molecular markers) is available (Bernard et al. 2018). This is particularly important because differences between maternally inherited mitochondrial markers and biparental loci have been used to evaluate sex-biased dispersal and female philopatry in many shark species (Schrey & Heist 2003, Karl et al. 2011, Daly-Engel et al. 2012, Portnoy et al. 2015, Sandoval-Castillo & Beheregaray 2015, Momigliano et al. 2017, Day et al. 2019), including white sharks from the Atlantic, the western Pacific, and South Africa (Pardini et al. 2001, Blower et al. 2012, O'Leary et al. 2015). Further, the lack of genetic diversity information for populations based on multiple molecular markers is problematic given that international conservation policies for highly migratory species, such as the white shark, must include the protection of genetic diversity at the population level to safeguard the survival of these species (Domingues et al. 2018, Huveneers et al. 2018). Assessments of population genetics provide insights into multiple processes that reduce genetic diversity in wild populations and can negatively affect white shark survival, such as bottlenecks, inbreeding, or genetic drift (O'Leary et al. 2015).

Multiple studies have provided information on the population dynamics of NEP white sharks. The NEP white shark population spans two adult aggregation areas, Guadalupe Island (GI) and central California (CC), and two nursery grounds that are geographically distant from one another and located in Sebastian Vizcaino Bay (SVB) in Baja California (BC), Mexico, and the Southern California Bight (SCB) in the USA (Klimley 1985, Domeier 2012, Lowe et al. 2012, Santana-Morales et al. 2012, Oñate-González et al. 2017, Tamburin et al. 2019). Whereas newborn and YOY white sharks are seasonally present in nursery grounds, juvenile white sharks travel among coastal areas until they become sub-adults and recruit to adult populations (Dewar et al. 2004, Weng et al. 2007, 2012, Galván-Magaña et al. 2010, Domeier 2012, Hoyos-Padilla et al. 2016). By understanding the migratory and connectivity patterns among these areas and the genetic patterns of the population, a comprehensive picture of the ecological and evolutionary processes operating on and within the NEP white shark population may be generated.

Previous studies of white shark population genetics have shown that the NEP white shark population is demographically isolated from all other white shark populations (Jorgensen et al. 2010, Blower et al. 2012, O'Leary et al. 2015, Oñate-González et al. 2015, Andreotti et al. 2016b, Bernard et al. 2018, Leone et al. 2020). Moreover, the NEP population presents a marked mitochondrial genetic structure, which suggests the existence of female philopatry in each adult aggregation area (Oñate-González et al. 2015). A matrilineal origin analysis showed that young NEP white sharks were likelier to be born from GI females than CC females (Oñate-González et al. 2015). Also, juveniles from SVB and CC adults have not been found to show genetic differences (Díaz-Jaimes et al. 2016). On the other hand, the results of the only study with biparental molecular markers subject to selection (transcriptome-derived markers) did not indicate the presence of genetic structure between samples from GI and those from the coastal waters of California (Bernard et al. 2018). Therefore, we hypothesized no genetic structure would be apparent using bi-parental neutral molecular markers. This study aimed to evaluate the neutral nuclear variability of NEP white sharks to fill in gaps in their ecological and evolutionary history. This study will complement information on the migration patterns of adults and immature white sharks from the NEP by providing data

on population genetic structure with biparental molecular markers and new insights into their reproductive behavior.

## MATERIALS AND METHODS

### DNA extraction and microsatellite amplification

DNA samples (n = 121) were obtained from white shark (*Carcharodon carcharias*) muscle and skin tissue biopsies or necropsies that were collected from incidentally caught sharks in the coastal areas among the SCB, BC, SVB, Gulf of California (GC), and GI (Fig. 1), as reported by Oñate-González et al. (2015). The present study used 15 species-specific microsatellite markers designed by Pardini et al. (2000) and O'Leary et al. (2013) for *C. carcharias* (Supplemental Material; Table S1). Nevertheless, due to problems with DNA quality (28 and 39 samples were excluded due to low concentrations and degradation, respectively), only five loci (Ccar-1, Ccar-3.1, Ccar-9, Ccar-13, and Ccar-19) were successfully genotyped in 54 individuals.

Amplifications for each locus were performed individually in a total volume of 10 μL, which contained 0.2 μM each of dNTPs, 1X PCR buffer, 0.15 mM $MgCl_2$, 0.5 μM of each primer, 1/2 U Taq DNAPol (NEB, Ipswich, USA), and 10 ng of genomic DNA. The amplicons were fluorescently marked using the dye-labeled M13 universal primer method for genotyping proposed by De Arruda et al. (2010). The thermal cycling profile included two stages. The first stage included 5 min at 94°C, followed by 30 cycles of 60 s at 94°C, 60 s at the annealing temperature for each primer (Table S1), and 120 s at 72°C. After which, 0.5 μM of M13 universal primer was added to each reaction. The second stage consisted of 10 cycles of 60 s at 94°C, 60 s at 53°C, 120 s at 72°C, and a final extension of 10 min at 72°C. The PCR products were separated by capillary electrophoresis in an ABI 3100 automatic sequencer (Applied Biosystems Inc., Foster City, USA). The genotypes were scored with GeneMarker v.2.4.0 (Softgenetics LLC, State College, USA). Null alleles were assessed with Micro-checker (Van Oosterhout et al. 2004), and large allele dropout was assessed with MicroDrop v.1.01 (Wang & Rosenberg 2012). Allelic dropout is a common genotyping error for microsatellites due to either poor quality or low concentrations of DNA and results in missing genotypes (both alleles) and false homozygotes (one allele is missing in heterozygotes) (Hoffman & Amos 2005, Dewoody et al. 2006). MicroDrop assumes a positive correlation between the number of homozygotes and the amount of missing data at the individual and loci levels to identify allelic dropout in a data set.

### Data analyses

To evaluate the levels of genetic diversity, we estimated observed heterozygosity ($H_O$) and expected heterozygosity ($H_E$) using Genepop v.4.7 (Raymond & Rousset 1993, Rousset 2008). In addition, we calculated the number of alleles ($A$), allelic richness ($A_r$), and the inbreeding coefficient ($F_{IS}$) with FSTAT v.2.9.3.2 (Goudet 1995, Goudet et al. 2002). Departures from Hardy-Weinberg equilibrium (HWE) at each locus and locality were tested with Genepop v.4.7 (Raymond & Rousset 1993, Rousset 2008), and a sequential Bonferroni correction was applied (Rice 1989).

To identify if the loss of genetic diversity was due to a possible occurrence of a bottleneck, we used Bottleneck v. 1.2.02 (Piry et al. 1999). Bottleneck determines if a population shows a reduction in allele number ($k$) and $H_E$, which would indicate that the population experienced a recent reduction in its effective population size ($Ne$). Given the sample size and assumption of mutation-drift equilibrium, this reduction is evaluated by obtaining the distribution of $H_E$ from $k$ with three mutation models (i.e. infinite allele, IAM; two-phase, TPM; and single-step, SMM). The simulations are analyses that use three assessments: the sig, standardized differences (Cornuet & Luikart 1996), and Wilcoxon sign-rank (Luikart et al. 1998) tests. Also, the program uses a graphical method that illustrates the frequency of rare alleles in a population (i.e. an L-shaped graph; Luikart et al. 1998). In Bottleneck, 1000 iterations were used with a variance of 30 for a TPM and an SMM proportion of 70% in the TPM.

In order to evaluate the degree of nuclear genetic structure and contrast this with previous patterns of mitochondrial structure in the NEP to infer possible sex-biased dispersal, a hierarchical analysis of molecular variance (AMOVA) was implemented in Arlequin v.3.1 (Excoffier & Lischer 2010). AMOVA significance was estimated with a permutation test (10,100 permutations). To evaluate barriers to dispersal among localities, we estimated the levels of genetic differentiation using pairwise comparisons of genetic structure indices $F_{ST}$ (based in allele frequencies) and $R_{ST}$ (allele sizes and frequencies). We used a sequential Bonferroni correction to adjust the significance for multiple tests (Rice 1989). To identify which loci contributed to the pattern of genetic differentiation, we performed an AMOVA by locus.

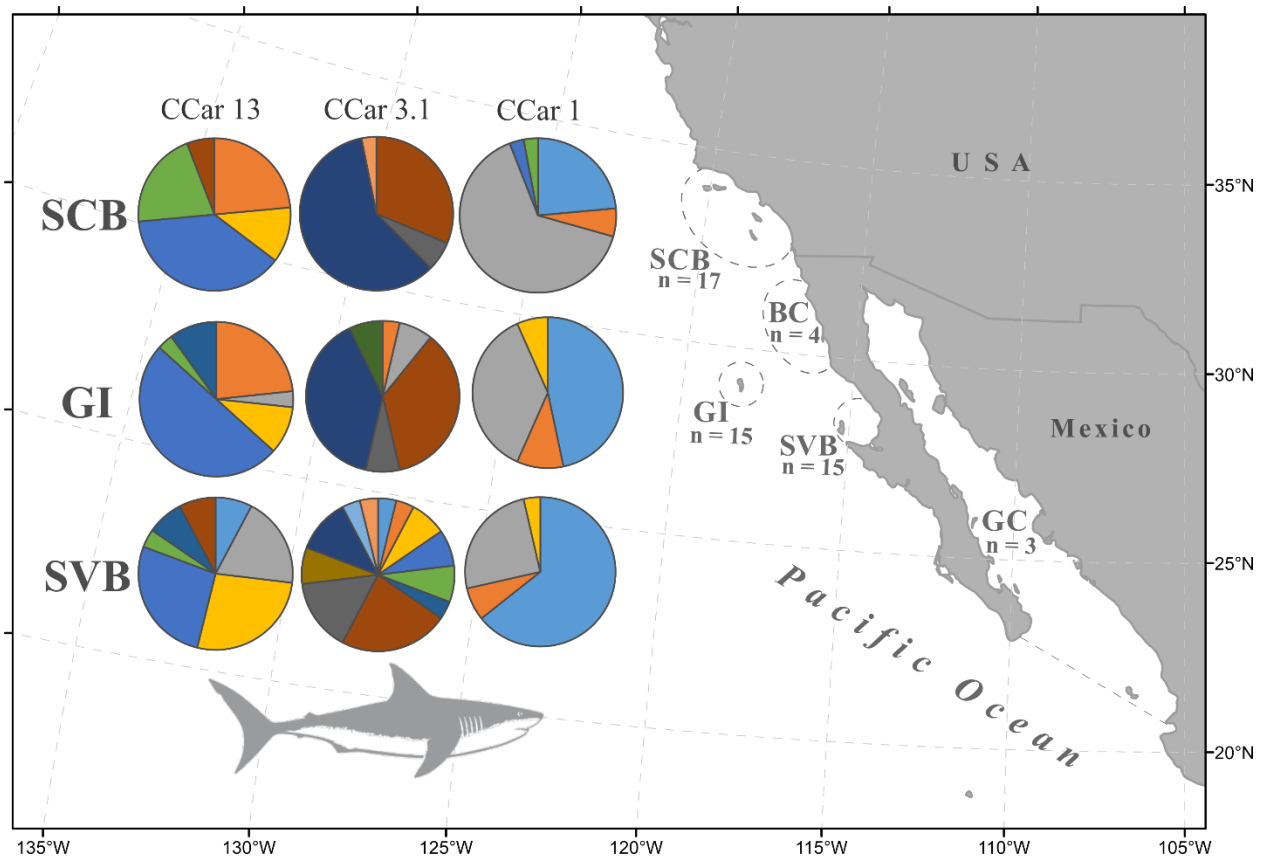


**Figure 1.** Map of sampled locations in the Northeastern Pacific (NEP): Southern California Bight (SCB), Baja California (BC), Guadalupe Island (GI), Sebastian Vizcaino Bay (SVB), and Gulf of California (GC). Pie charts represent the allele (colors) frequencies for each locus (Ccar-13, Ccar-3.1., and Ccar-1) in localities. Sample size (n).

To complete the statistical analyses of genetic structure, we estimated the number of subpopulations using a Bayesian analysis implemented in structure v.2.3.4 (Pritchard et al. 2000). The Structure software is based on the analysis of multilocus genotypes that have been grouped into clusters (*K*). The software adopts the assumptions of HWE and linkage equilibrium within each lineage. Parameters were adjusted according to O'Leary et al. (2015). They consisted of 10,000 iterations discarded as a burn-in, 100,000 Markov Chains Monte Carlo (MCMC) iterations, the ancestral mixing model (admixture), independence of allele frequencies, the *locprior* model, and ten runs with each assumed value of *K* (*K* = 2 to 5). The *K* number was estimated by Structure Harvester v.6.93 (Earl & Von Holdt 2012), which plots the log probability LnP(*K*) of the data over multiple runs and compares them via $\Delta K$ (Evanno et al. 2005). The results of the runs were merged with Clumpp (Jakobsson & Rosenberg 2007) and visualized with Distruct (Rosenberg 2004).

Kinship analyses were performed with Colony v.2.0.6.4 to assess the genealogical relationships between individuals to identify possible dispersal between nursery areas (Wang & Santure 2009, Jones & Wang 2010). The program uses a maximum likelihood algorithm to assign or infer parentage and kinship among individuals. The software was run several times using the population allele frequencies with different parameters to identify the best run (high probabilities of inclusion and exclusion). The best run had the following settings: "update allele frequency" unselected, "long-run" selected, allelic dropout set to 0, and an error rate of 0.01.

## RESULTS

Five loci were successfully genotyped in 54 individuals (i.e. Ccar-1, Ccar-3.1, Ccar-9, Ccar-13, and Ccar-19), all of which are from Pardini et al. (2000). There was no evidence of scoring error due to stuttering or large allele dropout in the five loci analyzed in this study.

Possible null alleles were detected in the samples (Table S2). At the loci level, these were mainly in Ccar-3.1 and Ccar-9. At the locality level, these were mainly in SVB ($P < 0.01$). The locus-specific dropout rates were low in Ccar-1, Ccar-19, and Ccar-1 (from 0 to 0.05%) and high in Ccar-3.1 and Ccar-9 (19.05 to 43.27%). However, there was no correlation between the number of homozygotes and the amount of missing data at the individual ($r = 0.015$; $P = 0.45$) and loci ($r = 0.071$; $P = 0.43$) levels.

### Genetic diversity

Low genetic diversity was recorded in almost all sampled localities in the NEP, and values of *Ho* oscillated between 0.3 and 0.7 (Table 1). The number of *A* averaged by locality ranged from 2.6 (BC) to 7 (GI). The average number of alleles per locus ranged between 3 (Ccar-19) and 16 (Ccar-3.1). Due to sample sizes among localities being highly variable, we calculated standardized $A_r$ with $n = 7$. The largest value of allelic richness by locality was found in GI ($A_r = 5.61$), while the lowest value was obtained for the SCB ($A_r = 4.14$). High $F_{IS}$ values were observed in SVB ($F_{IS} = 0.56$) and GI ($F_{IS} = 0.39$). Almost all HWE tests were significant, indicating disequilibrium due to a deficit of heterozygotes, especially in the SVB locality and loci Ccar-3.1 and Ccar-9 (Table 1).

The BC and GC localities were excluded from the remaining analyses because of their low sample sizes. Evidence of bottleneck events was absent except in SVB, which showed marginal significance with the IAM model ($P = 0.051$ and 0.031, Table 2). However, the allele frequency for all analyzed localities followed a normal L-shaped distribution (Table 2).

### Population genetic structure

A slight but significant genetic structure among localities in the NEP (AMOVA; $F_{ST} = 0.050$ and $R_{ST} = 0.055$, both $P < 0.001$). The Van Oosterhout tests indicated a probability of null alleles in three localities (i.e. SCB, SVB, and GI; Table S2), and an AMOVA re-analysis after correcting for the presence of null alleles resulted in minimal changes to the test values ($F_{ST} = 0.046$ and $R_{ST} = 0.054$, both $P < 0.001$). Nevertheless, pairwise comparisons were not significant after the Bonferroni correction. Due to the lack of significance in pairwise comparisons, we performed *a posteriori* AMOVA after eliminating the localities with low sample sizes (BC and GC). The slight genetic structure remained ($F_{ST} = 0.045$ and $R_{ST} = 0.039$, both $P < 0.001$), and after the Bonferroni correction, pairwise comparisons were significant only between SCB and IG for $F_{ST}$ (Table 3). Also, the AMOVA results by locus indicated that Ccar-1 and Ccar-3.1 showed the structure signal in both fixation indices ($P < 0.05$), while Ccar 13 showed marginal significance with $F_{ST}$ ($P = 0.052$; Table S3). The allelic frequency distribution of those loci showed latitudinal variation, which was mainly present between SCB and IG (Fig. 1). In the Bayesian analysis, the average LnP(*K*) value was maximal at 2 and to a lesser degree at 3 (Fig. S1a-b); however, the membership probabilities of the sampled individuals did not reflect a clear geographical pattern of genetic differentiation (Fig. S1c). The membership probability by population showed differences in the percentages of each cluster, mainly between SCB and GI (Fig. S1d).

### Genealogical analysis

The assigned parentage results showed over-split sibships when a low probability of exclusion (exclusive sibling group) was present despite a high probability of inclusion (group of full siblings). Over-split sibships result in an overestimation of assigned parentages. The sibling assignments showed five full sibling groups (Fig. S2), but only three showed probabilities of inclusion higher than 0.5 (Table S4), and the rest showed low probabilities of exclusion. Those full sibling groups included juveniles from SCB, BSV, and GC.

### Availability of data and material

The data that support the findings of this study are openly available in Mendeley data v.1 at http://doi.org/10.17632/96mzvs79kw.1

## DISCUSSION

### Nuclear genetic diversity

Some of the microsatellites used in this study were designed for sharks from the northwest Atlantic and South Africa. Both populations are geographically distant from the white sharks (*C. carcharias*) of the NEP (Jorgensen et al. 2010, Tanaka et al. 2011, Blower et al. 2012, Andreotti et al. 2016b, Leone et al. 2020). According to the null allele results, it is likely that mutations were present in the annealing sites of the primers, and thus the amplification success rates from these loci were low in sharks from the NEP. Also, some of the previously extracted DNA samples were degraded, and an allele dropout possibly occurred in at least one of five of the analyzed loci.

According to the genetic diversity patterns observed in the NEP white shark population, the low nuclear genetic diversity can be attributed to a founder effect

**Table 1.** Variation of five microsatellite loci in *Carcharodon carcharias* samples from the Northeastern Pacific (NEP). n: sample size, $H_o$: observed heterozygosity, $H_e$: expected heterozygosity, *A:* number of alleles, $A_{r(n)}$: standardized allelic richness, *pA*: private alleles, and $F_{IS}$: inbreeding coefficient. The superscripts indicate the significance of the HWE test (NS: not significant and *significant) of the *He* values after the Bonferroni correction. The values by locus included samples from Baja California and Gulf of California. SCB: Southern California Bight, SVB: Sebastian Vizcaino Bay, and GI: Guadalupe Island.

| Locations | | Values by locus | | | | | Values by location |
|---|---|---|---|---|---|---|---|
| | | Ccar-1 | Ccar-3.1 | Ccar-9 | Ccar-13 | Ccar-19 | |
| | $H_o$ | 0.412 | 0.313 | 0.538 | 0.588 | 0.438 | 0.458 |
| | $H_e$ | $0.537^{NS}$ | 0.563* | 0.874* | $0.761^{NS}$ | $0.433^{NS}$ | 0.633* |
| SCB | *A* | 5 | 4 | 9 | 5 | 3 | 5.2 |
| n = 17 | $A_{r\,(n=7)}$ | 3.48 | 3.13 | 7.15 | 4.54 | 2.43 | 4.14 |
| | *pA* | 2 | 0 | 1 | 0 | 0 | |
| | $F_{IS}$ | 0.24 | 0.45 | 0.39 | 0.23 | -0.01 | 0.29 |
| | $H_o$ | 0.267 | 0.286 | 0.286 | 0.400 | 0.267 | 0.301 |
| | $H_e$ | 0.655* | 0.728* | 0.901* | 0.697* | $0.331^{NS}$ | 0.662* |
| SVB | *A* | 4 | 6 | 7 | 6 | 2 | 5 |
| n = 15 | $A_{r\,(n=7)}$ | 3.59 | 4.78 | 7 | 4.65 | 1.99 | 4.40 |
| | *pA* | 0 | 2 | 1 | 0 | 0 | |
| | $F_{IS}$ | 0.60 | 0.62 | 0.7 | 0.43 | 0.20 | 0.56 |
| | $H_o$ | 0.286 | 0.385 | 0.455 | 0.615 | 0.533 | 0.455 |
| | $H_e$ | 0.537* | 0.914* | 0.853* | $0.831^{NS}$ | $0.517^{NS}$ | 0.730* |
| GI | *A* | 4 | 12 | 9 | 7 | 3 | 7 |
| n = 15 | $A_{r\,(n=7)}$ | 3.26 | 8.76 | 7.18 | 5.92 | 2.94 | 5.61 |
| | *pA* | 0 | 7 | 2 | 1 | 0 | |
| | $F_{IS}$ | 0.48 | 0.59 | 0.48 | 0.27 | -0.03 | 0.39 |
| | $H_o$ | 0.476 | 0.363 | 0.356 | 0.671 | 0.498 | 0.473 |
| Values by locus | $H_e$ | 0.601* | 0.661* | 0.759* | 0.774* | $0.453^{NS}$ | 0.680 |
| n = 54 | *A* | 6 | 16 | 14 | 8 | 3 | |
| | $A_{r\,(n=7)}$ | 3.40 | 5.66 | 7.50 | 5.68 | 2.61 | |

**Table 2.** Results of three probability tests of excess heterozygosity (i.e. ST: sig test, SDT: standardized differences test, WSR: two-tailed Wilcoxon sign-rank test), for the three mutation models (i.e. IAM: infinite allele, TPM: two-phase, SMM: single-step) and the allelic mode shift test. SCB: Southern California Bight, BC: Baja California, SVB: Sebastian Vizcaino Bay, GC: Gulf of California, and GI: Guadalupe Island.

| Locality | IAM | | | TPM | | | SMM | | | Mode-Shift |
|---|---|---|---|---|---|---|---|---|---|---|
| | ST | SDT | WSR | ST | SDT | WSR | ST | SDT | WSR | |
| SCB | 0.291 | 0.189 | 0.156 | 0.338 | 0.493 | 1.00 | 0.326 | 0.112 | 0.625 | Normal L-shaped |
| SVB | 0.051 | 0.083 | 0.031 | 0.617 | 0.251 | 0.219 | 0.640 | 0.410 | 0.594 | Normal L-shaped |
| GI | 0.322 | 0.161 | 0.062 | 0.662 | 0.431 | 1.00 | 0.335 | 0.190 | 0.922 | Normal L-shaped |

and not to a population bottleneck, as traditionally thought (Table 2). Likely, the NEP population was recently established at approximately 1.90 Mya (million years) by a small group of founders (Jorgensen et al. 2010) that were probably from the southwestern Pacific (Leone et al. 2020). Although the NEP population has been surveyed and is estimated to be comprised of >3000 sharks (Dewar et al. 2013, Burges et al. 2014), its size is not reflected in the levels of genetic diversity present (*Ho* = 0.47) or those from previous studies (*He* = 0.57, Bernard et al. 2018). Some authors have calculated that the minimum number of adult individuals required to maintain the genetic pool of a population and ensure its evolutionary potential falls between 500 and 1000 individuals (Franklin 1980, Franklin & Frankham 1998). Thus, it is likely that the observed discrepancy between the estimated population size and genetic diversity of NEP white sharks results from an insufficient number of generations following the founder effect needed to increase genetic diversity.

**Table 3.** Pairwise comparisons between locations, lower diagonal $F_{ST}$, and upper diagonal $R_{ST}$. SCB: Southern California Bight, SVB: Sebastian Vizcaino Bay, and GI: Guadalupe Island. Significant comparisons appear in bold after the Bonferroni correction.

| | SCB | SVB | GI |
|---|---|---|---|
| SCB | | 0.022 | -0.002 |
| SVB | 0.031 | | 0.145 |
| GI | **0.098** | 0.036 | |

Comparisons among genetic diversity patterns of white sharks in different ocean basins have found variations concerning the levels of genetic diversity. The southwestern Pacific population has been found to present the highest genetic diversity (*Ho* = 0.68, Blower et al. 2012; *He* = 0.60, Bernard et al. 2018), while populations of the northwest Atlantic and South Africa have been found to show lower values (*Ho* = 0.55 and 0.58, respectively; O'Leary et al. 2015, Andreotti et al. 2016b). Some interesting patterns emerge when comparing these genetic diversity patterns with population size (*N*) and *Ne*. The *N* of the southwestern Pacific population has been estimated to be between 2728-13,746 females (Malcolm et al. 2001), with both sexes showing *Ne* values of 1512 (Blower et al. 2012). The South African population has been estimated to be between 808-1008 individuals with a *Ne* of 333 (Towner et al. 2013, Andreotti et al. 2016a). The noticeable difference in *Ne* between the southwestern Pacific and South African populations is reflected in the levels of genetic diversity present. These results support the hypothesis that the NEP population is in the process of demographic expansion due to a founder effect in which moderate levels of genetic diversity still cannot be perceived.

**Nuclear genetic structure**

The genetic structure results from this study indicate that the NEP white shark population presents slight reproductive isolation among subpopulations ($F_{ST}$; Table 3). Isolated populations promote mating between consanguineous individuals; thus, these populations can present inbreeding (Wright 1922). This conclusion is supported by the degree of inbreeding in this study and the global *Ho* deficit (Table 1). The structure analyses showed that differences were mainly present concerning allele frequencies among populations ($F_{ST}$; Fig. 1); however, no differences were present at the molecular level ($R_{ST}$). This genetic pattern differs from the patterns derived from the results of the genome and transcriptome-derived markers in the same region ($F_{ST}$ = -0.007); however, the sample size in Bernard et al. (2018) was small (20 individuals) and lower than the sample size in this study, and thus this result is not conclusive.

The pattern of nuclear genetic structure, together with previous mitochondrial genetic structure results (Oñate-González et al. 2015) and those of tracking studies (Domeier & Nasby-Lucas 2008, 2012, Domeier 2012), suggest that females, as well as males, may present different degrees of philopatry. A genetic study of the Southwestern Pacific population recorded a similar pattern of genetic structure to that of the NEP white shark population (Blower et al. 2012). The population of the southwestern Pacific showed a greater degree of mitochondrial genetic structure than that of nDNA ($F_{STmtDNA}$ = 0.142, $P$ < 0.001; $F_{STnDNA}$ = 0.009, $P$ = 0.03). These differences may have been amplified by sex-biased dispersal, with females showing greater philopatry (i.e. less dispersal) than males. However, analyses by sex and developmental stage were not possible in this study due to the low sample size and many sampled individuals not being sexed (Table S5). In the NEP population, levels of genetic structure were more apparent for both molecular markers ($\Phi_{STmtDNA}$ = 0.351, $P$ < 0.001, Oñate-González et al. 2015; $F_{STnDNA}$ = 0.045, $P$ < 0.001, this study), which indicates that males generally tend to present less philopatry than females. However, this possible pattern of nuclear genetic structure in NEP white sharks must be cautiously viewed since we could not obtain adult samples from the California subpopulation to assess the degree of genetic differentiation between adults (Table S5).

The genetic structure results of both molecular markers showed contrasting patterns. The paired comparisons of $\Phi_{STmtDNA}$ showed genetic differences between SCB and SVB (Oñate-González et al. 2015); however, no differences were observed with the $F_{STnDNA}$ comparisons (this study). To reconcile these results, it is possible that females present parturition philopatry like other sharks and always choose the same coastal areas to give birth (DiBattista et al. 2007, Tillett et al. 2012, Feldheim et al. 2014, Félix-López et al. 2019), and thus there are differences at the level of mtDNA. However, we must consider that YOY and juveniles less than 200 cm in total length can migrate great distances in the coastal zones of the NEP (Weng et al. 2007, 2012, Benson et al. 2018, White et al. 2019, Santana-Morales et al. 2020). In the Atlantic Ocean, these ample migrations have been recorded over distances of 1500-2000 km in coastal zones (Curtis et al. 2018, Shaw et al. 2021). This ability to migrate could explain the lack of genetic differentiation concerning nDNA in this study, at least between SCB and BSV. As

such, no clear pattern of genetic structure is likely in the early ontogenetic stages. However, it is necessary to use other genetic markers to confirm this hypothesis and to generate robust genetic patterns that reflect the behavior of these sharks. Efforts are currently being made to address these issues.

### Connectivity patterns among juveniles from coastal zones

We attempted to evaluate the genealogical origin of immature sharks from SCB and SVB with those of the adults from GI; however, due to the low number of genotyped sharks, the results were not conclusive. Despite this, we detected moderate and low probabilities of full-siblings or half-siblings among juvenile sharks from coastal areas (i.e. SCB, SVB, BC, and GC), which indicates that individuals are migrating among nursery areas in the NEP. Coupled with this, pairwise comparisons of $F_{ST}$ did not show significant differences between the main juvenile aggregation areas (SCB and SVB), similar to what was found by Díaz-Jaimez et al. (2016). These results can be corroborated by tracking studies in which juveniles have been found to migrate from the SCB to SVB and even into the GC (Weng et al. 2007, 2012, White et al. 2019). This result may indicate that immature white sharks could seasonally occupy different coastal regions depending on their maturity or developmental stage, regardless of their place of origin.

The potential implications of the results of this and previous studies on white shark conservation in the coastal areas of the NEP are centered around immature individuals (i.e. newborns, YOYs, and juveniles). Two adult aggregation zones (in the USA and Mexico) are being conserved. As the abundance estimates reflect, local and international conservation strategies seem to positivile work in both zones (Dewar et al. 2013, Burges et al. 2014). Nevertheless, there are still many opportunities for improvement. In particular, conservation strategies must be modified to ensure that individuals are adequately protected throughout their ranges during each developmental stage. There appears to be mainly male-mediated genetic connectivity among aggregation zones, and the protection of immature organisms that have not yet been recruited to adult aggregations is important for the long-term survival of this top predator. Immature organisms migrate along the coastal areas of California, BC, and the GC and are thus at risk of being incidentally caught by local artisanal fisheries. For example, BSV has been identified as a nursery area for immature organisms (Oñate-González et al. 2017), yet these individuals are incidentally caught in gillnets within the bay. Given that the likelihood of post-release survival appears favorable, future regulatory actions in BSV should be focused on immature organisms, such as limiting the gillnet soak time (García-Rodríguez & Sosa-Nishizaki 2020). Similarly, local and international conservation strategies must protect immature and juvenile organisms as they migrate without recognizing political boundaries, and reductions in their numbers will affect both adult aggregations.

## CONCLUSIONS

We recognize the limitations of this study associated with the low number of molecular markers employed. The NEP population exhibits a slight nuclear genetic structure, which, together with previous results of mitochondrial structure, suggests sex-biased dispersal. Moreover, a high degree of genetic connectivity in immature individuals was observed between coastal aggregation zones in California and BC. As such, genetic data from other markers and samples from all regions of the NEP are needed to identify the genealogical relationships among aggregations of adult and juvenile white sharks. Also, the low genetic diversity results obtained in this study with nDNA suggest that the origin of the NEP white shark population results from a recent founder effect, as previously proposed (Jorgensen et al. 2010). This conclusion is supported by the results of a divergence time analysis in which the NEP population was found to have diverged from the Australian population during the late Pliocene and early Pleistocene (Leone et al. 2020). In future studies, alternative methodologies (e.g. SNPs) should be used to confirm the presence of sex-biased dispersal.

## ACKNOWLEDGMENTS

The study was funded by an internal project under the technical responsibility of Dr. Oscar Sosa Nishizaki of Centro de Investigación y Educación Superior de Ensenada (CICESE) [CICESE-625-320], the CONACyT Retos Nacionales project [PDCPN-CONACyT-2014 N°248076, under the technical responsibility of Dr. Nancy C. Saavedra-Sotelo (NCSS)], and the Universidad Autónoma de Sinaloa PROFAPI project [195, under the technical responsibility of NCSS]. We thank Dr. Oscar Sosa Nishizaki of the Laboratory of Fisheries Ecology of CICESE and Dr. Axayácatl Rocha Olivares of the Laboratory of Molecular and Evolutionary Ecology of CICESE for the use of their sample

collections and their support both in the field and laboratory. We thank John O'Sullivan of the Monterey Bay Aquarium, Dr. Christopher Lowe and Dr. Kady Lyons of California State University Long Beach (CSULB), and Dr. Michael Domeier and Dr. Nicole Nasby-Lucas of the Marine Conservation Science Institute for donating tissue samples. We would especially like to thank and recognize the fishers of the Baja California Peninsula, particularly those from Laguna Manuela and Las Casitas in Bahía Sebastián Vizcaíno and El Barril in the Gulf of California. We thank Juan Rendón for running Colony, Daniela Felix for help with figure editing, and Andrea Liévana Mactavish for English editing and comments on the manuscript.

## REFERENCES

Andreotti, S., Von-der-Heyden, S., Henriques, R., Rutzen, M., Meÿer, M., Osthuizen, H. & Matthee, C.A. 2016b. New insights into the evolutionary history of white sharks, *Carcharodon carcharias*. Journal of Biogeography, 43: 328-339.

Andreotti, S., Rutzen, M., Van-der-Walt, S., Von-der-Heyden, S., Henriques, R., Meyer, M., et al. 2016a. An integrated mark-recapture and genetic approach to estimate the population size of white sharks in South Africa. Marine Ecology Progress Series, 552: 241-253.

Benson, J.F., Jorgensen, S.J., O´Sullivan, J.B., Winkler, C., White, C.F., García-Rodríguez, E., et al. 2018. Juvenile survival, competing risks, and spatial variation in mortality risk of a marine apex predator. Journal of Applied Ecology, 2017: 1-10.

Bernard, A.M., Richards, V.P., Stanhope, M.J. & Shivji, M.S. 2018. Transcriptome-derived microsatellites demonstrate strong genetic differentiation in Pacific white sharks. Journal of Heredity, 109: 771-779.

Blower, D.C., Pandolfi, J.M., Bruce, B.D., Gomez-Cabrera, M. & Ovenden, J.R. 2012. Population genetics of Australian white shark reveals fine-scale spatial structure, transoceanic dispersal events and low effective population sizes. Marine Ecology Progress Series, 455: 229-244.

Burgess, G.H., Bruce, B.D., Cailliet, G.M., Goldman, K.J., Grubbs, R.D., Lowe, C.G., et al. 2014. A re-evaluation of the size of the white shark (*Carcharodon carcharias*) population of California, USA. Plos One, 9: e98078.

Compagno, L.J.V., Dando, M. & Fowler, S. 2005. Sharks of the world. Princeton University Press, New Jersey.

Cornuet, J.M. & Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, 144: 2001-2014.

Costa, D.P., Breed, G.A. & Robinson, P.W. 2012. New insights into pelagic migrations: implications for ecology and conservation. Annual Review of Ecology, Evolution, and Systematics, 43: 73-96.

Curtis, T.H., McCandless, C.T., Carlson, J.K., Skomal, G.B., Kohler, N.E., Natanson, L.J., et al. 2014. Seasonal distribution and historic trends in abundance of white sharks, *Carcharodon carcharias*, in the western North Atlantic Ocean. Plos One, 9: e99240.

Curtis, T.H., Metzger, G., Fischer, C., McBride, B., McCallister, M., Wiin, L.J., et al. 2018. First insights into the movements of young-of-the-year white sharks (*Carcharodon carcharias*) in the western north Atlantic Ocean. Scientific Reports, 8: 10794.

Daly-Engel, T.S., Seraphin, K.D., Holland, K.N., Coffey, J.P., Nance, H.A., Toonen, R.J. & Bowen, B.W. 2012. Global phylogeography with mixed-marker analysis reveals male-mediated dispersal in the endangered scalloped hammerhead shark (*Sphyrna lewini*). Plos One, 7: e29986.

Day, J., Clark, J.A., Williamson, J.E., Brown, C. & Gillings, M. 2019. Population genetic analyses reveal female reproductive philopatry in the oviparous Port Jackson shark. Marine and Freshwater Research, 70: 986-994.

De Arruda, M.P., Gonçalves, E.C., Schneider, M.P.C., Da Costa-Da Silva, A.L. & Morielle-Versute, E. 2010. An alternative genotyping method using a dye-labeled universal primer to reduce unspecific amplifications. Molecular Biology Reports, 37: 2031-2036.

Dewar, H., Domeier, M.L. & Nasby-Lucas, N. 2004. Insights into young of the year white shark, *Carcharodon carcharias*, behavior in the Southern California Bight. Environmental Biology of Fishes, 70: 133-143.

Dewar, H., Eguchi, T., Hyde, J., Kohin, S., Moore, J., Taylor, B.L. & Vetter, R. 2013. Status review of the Northeastern Pacific population of white sharks (*Carcharodon carcharias*) under the endangered species act (Research Report). National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center, La Jolla.

Dewoody, J., Nason, J.D. & Hipkins, V.D. 2006. Mitigating scoring errors in microsatellite data from wild populations. Molecular Ecology Notes, 6: 951-957.

Díaz-Jaimes, P., Reeb, C., Sosa-Nizhisaki, O. & O'Sullivan, J.B. 2016. Assessing genetic variation in juvenile white sharks using teeth collected from landfills in Baja California, México. Conservation Genetics Resource, 8: 431-434.

DiBattista, J.D., Feldheim, K.A., Gruber, S.H. & Hendry, A.P. 2007. When bigger is not better: selection against large size, high condition and fast growth in juvenile lemon sharks. Journal of Evolutionary Biology, 20: 201-212.

Domeier, M.L. 2012. A new life-history hypothesis for white sharks, *Carcharodon carcharias*, in the Northeastern Pacific. In: Domeier, M.L. (Ed.). Global perspectives on the biology and life history of the white shark. CRC Press, Boca Raton, pp. 199-223.

Domeier, M.L. & Nasby-Lucas, N. 2008. Migration patterns of white sharks *Carcharodon carcharias* tagged at Guadalupe Island, Mexico, and identification of an eastern Pacific shared offshore foraging area. Marine Ecology Progress Series, 370: 221-237.

Domeier, M.L. & Nasby-Lucas, N. 2012. Sex-specific migration patterns and sexual segregation of adult white sharks, *Carcharodon carcharias*, in the Northeastern Pacific. In: Domeier, M.L. (Ed.). Global perspectives on the biology and life history of the white shark. CRC Press, Boca Raton, pp. 133-146.

Domingues, R.R., Wagner, A., Hilsdorf, S., Bismarck, O. & Gadig, F. 2018. The importance of considering genetic diversity in shark and ray conservation policies. Conservation Genetics, 19: 501-525.

Earl, D.A. & Von Holdt, B.M. 2012. Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. Conservation Genetics Resource, 4: 359-361.

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. Molecular Ecology, 14: 2611-2620.

Excoffier, L. & Lischer, H.E.L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10: 564-567.

Feldheim, K.A., Gruber, S.M., DiBattista, J.D., Badcock, E.A., Kessel, S.T., Hendry, A.P., et al. 2014. Two decades of genetic profiling yields first evidence of natal philopatry and long-term fidelity to parturition sites in sharks. Molecular Ecology, 23: 110-117.

Félix-López, D.G., Bolaño-Martínez, N., Díaz-Jaimes, P., Oñate-González, E.C., Ramírez-Pérez, J.S., García-Rodríguez, E., et al. 2019. Possible female philopatry of the smooth hammerhead shark *Sphyrna zygaena* revealed by genetic structure patterns. Journal of Fish Biology, 94: 671-679.

Fergusson, I., Compagno, L.J.V. & Marks, M. 2009. *Carcharodon carcharias*, great white shark. The IUCN Red List of Threatened Species, 10: e.T3855A.

Ferretti, F., Worm, B., Britten, G.L., Heithaus, M.R. & Lotze, H.K. 2010. Patterns and ecosystem consequences of shark declines in the ocean. Ecology Letters, 13: 1055-1071.

Franklin, I.R. 1980. Evolutionary change in small populations. In: Soule, M.E. & Wilcox, B.A. (Eds.). Conservation biology - An evolutionary-ecological perspective. Sinauer Associates, Sunderland, pp. 135-150.

Franklin, I.R. & Frankham, R. 1998. How large must populations be to retain evolutionary potential? Animal Conservation, 1: 69-70.

Galván-Magaña, F., Hoyos-Padilla, E.M., Navarro-Serment, C.J. & Márquez-Farías, F. 2010. Records of white shark, *Carcharodon carcharias*, in the Gulf of California, Mexico. Marine Biodiversity Records, 3: e111.

García-Rodríguez, E. & Sosa-Nishizaki, O. 2020. Artisanal fishing activities and their documented interactions with juvenile white sharks inside a nursery area. Aquatic Conservation: Marine Freshwater Ecosystems, 30: 903-914.

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity, 86: 485-486.

Goudet, J., Perrin, N. & Waser, P. 2002. Tests for sex-biased dispersal using bi-parentally inherited genetic markers. Molecular Ecology, 11: 1103-1114.

Hoyos-Padilla, E.M., Klimley, A.P., Galván-Magaña, F. & Antoniou, A. 2016. Contrasts in the movements and habitat use of juvenile and adult white sharks (*Carcharodon carcharias*) at Guadalupe Island, Mexico. Animal Biotelemetry, 4: 1-14.

Hoffman, J.I. & Amos, W. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Molecular Ecology, 14: 599-612.

Huveneers, C., Apps, K., Becerril-García, E.E., Bruce, B., Butcher, P.A., Carlisle, A.B., et al. 2018. Future research directions on the "Elusive" white shark. Frontiers in Marine Science, 5: 1-15.

Inskipp, T. & Gillet, H.J. 2005. Checklist of CITES species and annotated CITES appendices and reservations. CITES Secretariat, Geneva & UNEP-WCMC, Cambridge.

Jakobsson, M. & Rosenberg, N.A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in the analysis of population structure. Bioinformatics, 23: 1801-1806.

Jones, O.R. & Wang, J. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources, 10: 551-555.

Jorgensen, S.J., Reeb, C.A., Chapple, T.K., Anderson, S., Perle, C., Van Sommeran, S.R., et al. 2010. Philopatry and migration of Pacific white sharks. Proceedings of the Royal Society B: Biological Sciences, 277: 679-688.

Kark, S., Tulloch, A., Gordon, A., Mazor, T., Bunnefeld, N. & Levin, N. 2015. Cross-boundary collaboration: key to the conservation puzzle. Current Opinion in Environmental Sustainability, 12: 12-24.

Karl, S.A., Castro, A.L.F., Lopez, J.A., Charvet, P. & Burgess, G.H. 2011. Phylogeography and conservation of the bull shark (*Carcharhinus leucas*) inferred from mitochondrial and microsatellite DNA. Conservation Genetics, 12: 371-378.

Klimley, A.P. 1985. The areal distribution and autoecology of the white shark, *Carcharodon carcharias*, off the west coast of North America. Memoirs of the Southern California Academy of Sciences, 9: 15-40.

Leone, A., Puncher, G.N., Ferretti, F., Sperone, E., Tripepi, S., Micarelli, P., et al. 2020. Pliocene colonization of the Mediterranean by great white shark inferred from fossil records, historical jaws, phylogeographic and divergence time analyses. Journal of Biogeography, 47: 1119-1129.

Lowe, C.G., Blasius, M.E., Jarvis, E.T., Mason, T.J., Goodmanlowe, G.D. & O'Sullivan, J.B. 2012. Historic fishery interactions with white sharks in the Southern California Bight. In: Domeier, M.L. (Ed.). Global perspectives on the biology and life history of the white shark. CRC Press, Boca Raton, pp. 169-186.

Luikart, G., Allendorf, F.W., Cournuet, J-M. & Sherwin, W.B. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity, 89: 238-247.

Lyons, K., Jarvis, E., Jorgensen, S.J., Weng, K., O'Sullivan, J., Winkler, C. & Lowe, C.G. 2013. The degree and result of gillnet fishery interactions with juvenile white sharks in southern California assessed by fishery-independent and-dependent methods. Fisheries Research, 147: 370-380.

Malcolm, H., Bruce, B.D. & Stevens, J.D. 2001. A review of the biology and status of white sharks in Australian waters. CSIRO Marine Research, Hobart.

Martin, T.G., Chadès, L., Arcese, P., Marra, P.P., Possingham, H.P. & Norris, D.R. 2007. Optimal migratory species. Plos One, 2: e751.

Momigliano, P., Harcourt, R., Robbins, W.D., Jaiteh, V., Mahardika, G.N., Sembiring, A. & Stow, A. 2017. Genetic structure and signatures of selection in grey reef sharks (*Carcharhinus amblyrhynchos*). Heredity, 119: 142-153.

Mull, C.G., Lyons, K., Blasius, M.E., Winkler, C., O'Sullivan, J.B. & Lowe, C.G. 2013. Evidence of maternal offloading of organic contaminants in white sharks (*Carcharodon carcharias*). Plos One, 8: e62886.

O'Leary, S.J., Feldheim, K.A. & Chapman, D.D. 2013. Novel microsatellite loci for white, *Carcharodon carcharias* and sand tiger sharks, *Carcharias taurus* (order Lamniformes). Conservation Genetics Resources, 4: 627-629.

O´Leary, S.J., Feldheim, K.A., Fields, A.T., Natanson, L.J., Wintner, S., Hussey, N., et al. 2015. Genetic diversity of white sharks, *Carcharodon carcharias*, in the Northwest Atlantic and Southern Africa. Journal of Heredity, 106: 258-265.

Oñate-González, E.C., Rocha-Olivares, A., Saavedra-Sotelo, N.C. & Sosa-Nishizaki, O. 2015. Mitochondrial genetic structure and matrilineal origin of white sharks, *Carcharodon carcharias*, in the northeastern Pacific: implications for their conservation. Journal of Heredity, 106: 347-354.

Oñate-González, E.C., Sosa-Nishizaki, O., Herzka, S.Z., Lowe, C.G., Lyons, K., Santana-Morales, O., et al. 2017. Importance of Bahia Sebastian Vizcaino as a nursery area for white sharks (*Carcharodon carcharias*) in the Northeastern Pacific: a fishery-dependent analysis. Fisheries Research, 188: 125-137.

Pardini, A.T., Jones, C.S., Scholl, M.C. & Noble, L.R. 2000. Isolation and characterization of dinucleotide microsatellite loci in the great white shark, *Carcharodon carcharias*. Molecular Ecology, 9: 1176-1179.

Pardini, A.T., Jones, C.S., Noble, L.R., Kreiser, B., Malcolm, H., Bruce, B.D., et al. 2001. Sex-biased dispersal of great white sharks. Nature, 412: 139-140.

Piry, S., Luikart, G. & Cornuet, J.M. 1999. Bottleneck: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity, 90: 502-503.

Portnoy, D.S., Puritz, J.B., Hollenbeck, C.M., Gelsleichter, J., Chapman, D. & Gold, J.R. 2015. Selection and sex-biased dispersal in a coastal shark: the influence of philopatry on adaptative variation. Molecular Ecology, 24: 5877-5885.

Pritchard, J.K., Stephens, M.M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959.

Ramírez-Amaro, S.R., Cartamil, D., Galván-Magaña, F., Gonzalez-Barba, G., Graham, J.B., Carrera-Fernández, M., et al. 2013. The artisanal elasmobranch fishery of the Pacific coast of Baja California Sur, Mexico, management implications. Scientia Marina, 77: 473-487.

Raymond, M. & Rousset, F. 1993. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86: 248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, 43: 223-225.

Rosenberg, N.A. 2004. DISTRUCT: a program for the graphical display of population structure. Molecular Ecology Notes, 4: 137-138.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Notes, 8: 103-106.

Sandoval-Castillo, J. & Beheregaray, L.B. 2015. Metapopulation structure informs conservation management in a heavily exploited coastal shark (*Mustelus henlei*). Marine Ecology Progress Series, 533: 191-203.

Santana-Morales, O., Abadía-Cardoso, A., Hoyos-Padilla, M., Naylor, J.P.G., Corrigan, S., Malpica-Cruz, L., et al. 2020. The smallest known free-living white shark *Carcharodon carcharias* (Lamniformes: Lamnidae): ecological and management implications. Copeia, 108: 39-46.

Santana-Morales, O., Sosa-Nishizaki, O., Escobedo-Olvera, M.A., Oñate-González, E.C., O'Sullivan, J.B. & Cartamil, D. 2012. Incidental catch and ecological observations of juvenile white sharks, *Carcharodon carcharias*, in Western Baja California, Mexico. Conservation implications. In: Domeier, M.L. (Ed.). Global perspectives on the biology and life history of the white shark. CRC Press, Boca Raton, pp. 187-198.

Shaw, R.L., Curtis, T.H., Metzger, G., McCallister, M.P., Newton, A., Fischer, G.G. & Ajemian, M.J. 2021. Three-dimensional movements and habitat selection of young white sharks (*Carcharodon carcharias*) across a temperate continental shelf ecosystem. Frontiers in Marine Science, 8: 643831.

Tamburin, E., Hoyos-Padilla, M., Sánchez-González, A., Hernández-Herrera, A., Elorriaga-Verplancken, F.R. & Galván-Magaña, F. 2019. New nursery area for white sharks (*Carcharodon carcharias*) in the Eastern Pacific Ocean. Turkysh Journal of Fisheries and Aquatic Sciences, 19: 325-329.

Tanaka, S., Kitamura, T., Mochizuki, T. & Kofuji, K. 2011. Age, growth and genetic status of the white shark (*Carcharodon carcharias*) from Kashima-nada, Japan. Marine and Freshwater Research, 62: 548-556.

Tillet, B.J., Meekan, M.G., Field, I.C., Thorburn, D.C. & Ovenden, J.R. 2012. Evidence for reproductive philopatry in the bull shark *Carcharhinus leucas*. Journal of Fish Biology, 80: 2140-2158.

Towner, A.V., Wcisel, M.A., Reisinger, R.R., Edwards, D. & Jewell, O.J.D. 2013. Gauging the threat: the first population estimate for white sharks in South Africa using photo identification and automated software. Plos One, 8: e66035.

Van Oosterhout, C., Hutchinson, C., Wills, D.P.M. & Shipley, P. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4: 535-538.

Wang, C. & Rosenberg, N.A. 2012. MicroDrop: a program for estimating and correcting for allelic dropout in nonreplicated microsatellite genotypes version 1.01. [http://rosenberglab.stanford.edu/software.html]. Reviewed: June 10, 2022.

Wang, J. & Santure, A.W. 2009. Parentage and sibship inference from multilocus genotype data under polygamy. Genetics, 181: 1579-1594.

Weng, K.C., O'Sullivan, J.B., Lowe, C.G., Winkler, C.E., Dewar, H. & Block, B.A. 2007. Movements, behavior and habitat preferences of juvenile white sharks *Carcharodon carcharias* in the eastern Pacific. Marine Ecology Progress Series, 338: 211-224.

Weng, K.C., O'Sullivan, J.B., Lowe, C.G., Winkler, C.E., Blasius, M.E., Locke-Smith, K.A., et al. 2012. Back to the wild: release of juvenile white sharks from the Monterey Bay Aquarium. In: Domeier, M.L. (Ed.). Global perspectives on the biology and life history of the white shark. CRC Press, Boca Raton, pp. 419-446.

White, C.F., Lyons, K., Jorgensen, S.J., O'Sullivan, J.B., Winkler, C., Weng, K.C. & Lowe, C.G. 2019. Quantifying habitat selection and variability in habitat suitability for juvenile white sharks. Plos One, 14: e0214642.

Wright, S. 1922. Coefficients of inbreeding and relationship. American Naturalist, 56: 330-338.

*Received: September 7, 2022; Accepted: March 5, 2023*

**Table S1.** List of microsatellite loci used in this study. Primers designed by Pardini et al. (2000). F: forward, R: reverse, T: annealing temperature.

| Locus | Fluorophore | Size (pb) | Sense | Sequence 5'- 3' | Motif | T (°C) |
|---|---|---|---|---|---|---|
| Ccar-9 | NED | 218 | F | AATGGGTTGTGATGGGAGTTT | $(TG)_{23}$ | 56 |
| | | | R | CAAGTGGAAGTCAAGCAGGTT | | |
| Ccar-19 | FAM | 205 | F | GCCAGACCGACACATCAGTAA | $(TG)_{16}CG(TG)_3$ | 55 |
| | | | R | GCAACGCCCACATCCCATAA | | |
| Ccar-1 | VIC | 169 | F | GCAGAGGTTGGGAAAGAGTT | $(AC)_{22}$ | 55 |
| | | | R | GCTATTCCAGTGACACTCTCC | | |
| Ccar-3.1 | PET | 149 | F | CTTGTGCTCGCTGCTCTAC | $(AC)_7$ | 56 |
| | | | R | GGTGGTTTGTGATTCTGTG | | |
| Ccar-13 | FAM | 288 | F | GCTGAGTGCTGGCTGACCT | $(TG)_4TT(TG)_9TT(TG)_3TTTT(TG)_{23}$ | 56 |
| | | | R | TATCCAGTTACCATCTCCAAAAA | | |

**Table S2.** Adjusted allele frequencies of amplified alleles based on the four methods of null allele estimation with Micro-Checker. SCB: Southern California Bight, SVB: Sebastian Vizcaino Bay, and GI: Guadalupe Island.

| Locus/Locality | Class | Obs. allele freq. | Oosterhout | Chakraborty | Brookfield 1 | Brookfield 2 |
|---|---|---|---|---|---|---|
| Ccar-1/SVB | 155 | 0.4667 | 0.3675 | 0.2765 | 0.3619 | 0.3619 |
| | 157 | 0.1 | 0.0691 | 0.0593 | 0.0776 | 0.0776 |
| | 163 | 0.3667 | 0.2254 | 0.2173 | 0.2844 | 0.2844 |
| | 165 | 0.0667 | 0.0691 | 0.0395 | 0.0517 | 0.0517 |
| Ccar-1/GI | 155 | 0.6429 | 0.4655 | 0.4571 | 0.5445 | 0.457 |
| | 157 | 0.0714 | 0.0742 | 0.0508 | 0.0605 | 0.0508 |
| | 163 | 0.25 | 0.1982 | 0.1778 | 0.2118 | 0.1777 |
| | 165 | 0.0357 | 0.0364 | 0.0254 | 0.0303 | 0.0254 |
| Ccar-3.1/SVB | 133 | 0.0357 | 0.0364 | 0.0207 | 0.027 | 0.0233 |
| | 139 | 0.0714 | 0.0364 | 0.0413 | 0.054 | 0.0465 |
| | 155 | 0.3571 | 0.2929 | 0.2067 | 0.2699 | 0.2326 |
| | 157 | 0.0714 | 0.0364 | 0.0413 | 0.054 | 0.0465 |
| | 163 | 0.3929 | 0.2441 | 0.2274 | 0.2969 | 0.2559 |
| | 165 | 0.0714 | 0.0742 | 0.0413 | 0.054 | 0.0465 |
| Ccar-3.1/GI | 123 | 0.0385 | 0.0392 | 0.0234 | 0.0283 | 0.0218 |
| | 133 | 0.0385 | 0.0392 | 0.0234 | 0.0283 | 0.0218 |
| | 141 | 0.0769 | 0.0392 | 0.0468 | 0.0567 | 0.0436 |
| | 143 | 0.0769 | 0.0392 | 0.0468 | 0.0567 | 0.0436 |
| | 147 | 0.0769 | 0.0392 | 0.0468 | 0.0567 | 0.0436 |
| | 153 | 0.0385 | 0.0392 | 0.0234 | 0.0283 | 0.0218 |
| | 155 | 0.2308 | 0.1679 | 0.1405 | 0.1701 | 0.1308 |
| | 157 | 0.1538 | 0.1229 | 0.0937 | 0.1134 | 0.0872 |
| | 159 | 0.0769 | 0.0392 | 0.0468 | 0.0567 | 0.0436 |
| | 163 | 0.1154 | 0.0801 | 0.0703 | 0.085 | 0.0654 |
| | 171 | 0.0385 | 0.0392 | 0.0234 | 0.0283 | 0.0218 |
| | 181 | 0.0385 | 0.0392 | 0.0234 | 0.0283 | 0.0218 |
| Ccar-9/SVB | 216 | 0.1429 | 0.0742 | 0.0727 | 0.1 | 0.0292 |
| | 220 | 0.2143 | 0.1548 | 0.1091 | 0.15 | 0.0438 |
| | 224 | 0.0714 | 0.0742 | 0.0364 | 0.05 | 0.0146 |
| | 226 | 0.2143 | 0.1548 | 0.1091 | 0.15 | 0.0438 |
| | 228 | 0.0714 | 0.0742 | 0.0364 | 0.05 | 0.0146 |
| | 232 | 0.1429 | 0.0742 | 0.0727 | 0.1 | 0.0292 |
| | 234 | 0.1429 | 0.0742 | 0.0727 | 0.1 | 0.0292 |

Continuation

| Locus/Locality | Class | Obs. allele freq. | Oosterhout | Chakraborty | Brookfield 1 | Brookfield 2 |
|---|---|---|---|---|---|---|
| | 212 | 0.0909 | 0.0465 | 0.0651 | 0.0729 | 0.043 |
| | 218 | 0.2273 | 0.2023 | 0.1629 | 0.1822 | 0.1076 |
| | 220 | 0.3182 | 0.2615 | 0.228 | 0.2551 | 0.1506 |
| | 224 | 0.0909 | 0.0465 | 0.0651 | 0.0729 | 0.043 |
| Ccar-9/GI | 226 | 0.0909 | 0.0465 | 0.0651 | 0.0729 | 0.043 |
| | 228 | 0.0455 | 0.0465 | 0.0326 | 0.0364 | 0.0215 |
| | 230 | 0.0455 | 0.0465 | 0.0326 | 0.0364 | 0.0215 |
| | 244 | 0.0455 | 0.0465 | 0.0326 | 0.0364 | 0.0215 |
| | 250 | 0.0455 | 0.0465 | 0.0326 | 0.0364 | 0.0215 |
| | 218 | 0.1154 | 0.0801 | 0.0901 | 0.0965 | 0.0612 |
| | 220 | 0.2692 | 0.1679 | 0.2103 | 0.2251 | 0.1428 |
| | 224 | 0.0769 | 0.0801 | 0.0601 | 0.0643 | 0.0408 |
| | 226 | 0.1923 | 0.1229 | 0.1502 | 0.1608 | 0.102 |
| Ccar-9/SCB | 230 | 0.0385 | 0.0392 | 0.03 | 0.0322 | 0.0204 |
| | 232 | 0.1154 | 0.1229 | 0.0901 | 0.0965 | 0.0612 |
| | 234 | 0.1154 | 0.1229 | 0.0901 | 0.0965 | 0.0612 |
| | 240 | 0.0385 | 0.0392 | 0.03 | 0.0322 | 0.0204 |
| | 250 | 0.0385 | 0.0392 | 0.03 | 0.0322 | 0.0204 |
| | 288 | 0.2333 | 0.1437 | 0.1739 | 0.1952 | 0.1952 |
| | 300 | 0.0333 | 0.0339 | 0.0248 | 0.0279 | 0.0279 |
| Ccar-13/SVB | 302 | 0.1 | 0.1056 | 0.0745 | 0.0837 | 0.0837 |
| | 304 | 0.5 | 0.3675 | 0.3727 | 0.4183 | 0.4183 |
| | 306 | 0.0333 | 0.0339 | 0.0248 | 0.0279 | 0.0279 |
| | 308 | 0.1 | 0.1056 | 0.0745 | 0.0837 | 0.0837 |

**Table S3.** AMOVA analyses by locus that pass the quality control.

| Locus | $F_{ST}$ | *P*-value | $R_{ST}$ | *P*-value |
|---|---|---|---|---|
| Ccar-1 | 0.107 | 0.031 | 0.158 | 0.012 |
| Ccar-3.1 | 0.067 | 0.065 | 0.129 | 0.024 |
| Ccar-9 | -0.002 | 0.877 | 0.009 | 0.557 |
| Ccar-13 | 0.048 | 0.052 | 0.006 | 0.593 |
| Ccar-19 | 0.000 | 0.404 | 0.031 | 0.161 |

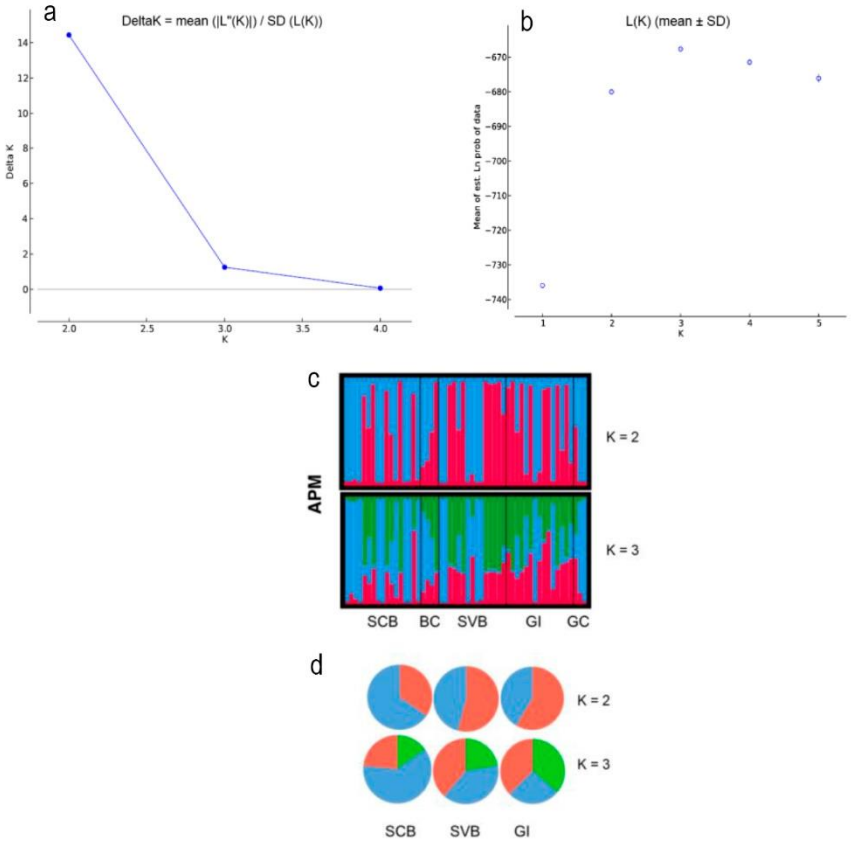


**Figure S1.** a) Plot of Evanno's $\Delta K$ values generated by Structure Harvester. $K$ = 2 was the best solution for *Carcharodon carcharias* of the Northeastern Pacific (NEP), b) plot of mean likelihood L($K$) and variance per $K$ value for a data set of 54 individuals genotyped for five microsatellite loci, c) plots of the average probability of membership (APM; y-axis; scale from 0 to 1) of individuals sampled in $K$ = 2 and 3 clusters as identified by structure, and d) plots of the APM of populations with larger sample sizes, each color represent percentage of each $K$. SCB: Southern California Bight, SVB: Sebastian Vizcaino Bay, and GI: Guadalupe Island.

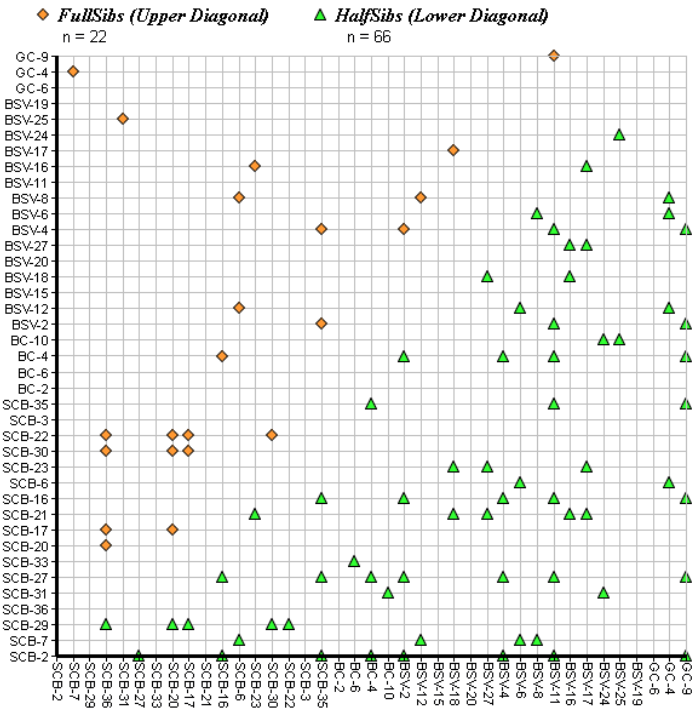


**Figure S2.** Plot of full-siblings and half-siblings among *Carcharodon carcharias* juveniles. SCB: Southern California Bight, BC: Baja California, SVB: Sebastian Vizcaino Bay, GC: Gulf of California, and GI: Guadalupe Island.

**Table S4.** Inclusion (IP) and exclusion (EP) probabilities of full siblings among *Carcharodon carcharias* juveniles. SCB: Southern California Bight, BC: Baja California, SVB: Sebastian Vizcaino Bay, GC: Gulf of California, and GI: Guadalupe Island.

| Group | IP | EP | Juveniles | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 0.049 | SCB-2 | | | | |
| 2 | 0.693 | 0.177 | SCB-7 | GC-4 | | | |
| 3 | 1 | 0.167 | SCB-29 | | | | |
| 4 | 0.285 | 0.280 | SCB-36 | SCB-20 | SCB-17 | SCB-30 | SCB-22 |
| 5 | 0.463 | 0.124 | SCB-31 | BSV-25 | | | |
| 6 | 1 | 0.148 | SCB-27 | | | | |
| 7 | 1 | 0.705 | SCB-33 | | | | |
| 8 | 1 | 0.191 | SCB-21 | | | | |
| 9 | 0.135 | 0.018 | SCB-16 | BC-4 | | | |
| 10 | 0.188 | 0.097 | SCB-6 | BSV-12 | BSV-8 | | |
| 11 | 0.665 | 0.145 | SCB-23 | BSV-16 | | | |
| 12 | 1 | 1 | SCB-3 | | | | |
| 13 | 0.423 | 0.103 | SCB-35 | BSV-2 | BSV-4 | | |
| 14 | 1 | 1 | BC-2 | | | | |
| 15 | 1 | 0.705 | BC-6 | | | | |
| 16 | 1 | 0.152 | BC-10 | | | | |
| 17 | 1 | 1 | BSV-15 | | | | |
| 18 | 0.769 | 0.231 | BSV-18 | BSV-17 | | | |
| 19 | 1 | 1 | BSV-20 | | | | |
| 20 | 1 | 0.199 | BSV-27 | | | | |
| 21 | 1 | 0.338 | BSV-6 | | | | |
| 22 | 0.154 | 0.019 | BSV-11 | GC-9 | | | |
| 23 | 1 | 0.182 | BSV-24 | | | | |
| 24 | 1 | 1 | BSV-19 | | | | |
| 25 | 1 | 1 | GC-6 | | | | |

**Table S5.** Sample size of each location and reproductive state (immature and mature).

| Location | Immature | Mature | Total |
|---|---|---|---|
| Southern California Bay | 17 | 0 | 17 |
| Baja California | 4 | 0 | 4 |
| Sebastián Vizcaíno Bay | 15 | 0 | 15 |
| Isla Guadalupe | | | |
| Females | 0 | 4 | 4 |
| Males | 0 | 7 | 7 |
| Indeterminate | 0 | 4 | 4 |
| Gulf of California | 2 | 1 | 3 |
| Total | 38 | 16 | 54 |