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The Genetic Structure of Leopard Shark (Triakis semifasciata) Populations Along the Pacific Coast of North America

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UNIVERSITY OF CALIFORNIA, SAN DIEGO

The Genetic Structure of Leopard Shark (*Triakis semifasciata*) Populations Along
the Pacific Coast of North America

A Thesis submitted in partial satisfaction of the requirements for the degree
Master of Science

in

Biology

by

Amanda Marie Barker

Committee in charge:

      Professor Ronald S. Burton, Chair
      Professor Carolyn Kurle, Co-Chair
      Professor Kaustuv Roy

2014

The Thesis of Amanda Marie Barker is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

_____
Chair

University of California, San Diego

2014

EPIGRAPH

How inappropriate to call this planet Earth,
when clearly it is Ocean.

Arthur C. Clarke

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Finally, this thesis would not have been possible without the love and support of my family, friends, and partner.

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Discussion, in part is currently being prepared for submission for publication of the material. Barker, Amanda M.; Nosal, Andrew P.; Lewallen, Eric A.; Burton, Ronald S. The thesis author was the primary investigator and author of this material.

ABSTRACT OF THE THESIS

The Genetic Structure of Leopard Shark (*Triakis Semifasciata*) Populations Along the Pacific Coast of North America

by

Amanda Marie Barker

Master of Science in Biology

University of California, San Diego, 2014

Professor Ronald S. Burton, Chair

Professor Carolyn Kurle, Co-Chair

The leopard shark (*Triakis semifasciata*) is a common nearshore benthic elasmobranch endemic to the Pacific coast of North America, from Samish Bay, Washington, USA to Mazatlan, Mexico. Leopard sharks aggregate at specific coastal locations in the spring and summer, but little is known about leopard

shark movement patterns once aggregations disperse. As a result, the extent of potential gene flow remains to be fully elucidated. Five microsatellite markers were used to analyze the genetic population structure of *T. semifasciata* throughout much of its range. Fin clips were collected from six locations in California and one location in Mexico (total n= 382). Analyses of the genetic data show a significant pattern of isolation by distance and structuring among several locations. Overall, pairwise differentiation tests showed a general pattern of northern California populations being significantly different than southern California populations. We conclude that *T. semifasciata* does not form one panmictic population and significant population structure is present. While *T. semifasciata* is not currently a threatened species, understanding gene flow throughout the species' range may provide insight into the population structure of similar species.

Introduction

      As high trophic level predators, sharks play a key role in maintaining the

health of ecosystems by providing top-down control of mesopredators. A trophic

cascade caused by the removal of apex predators can have devastating effects on

community functioning and biodiversity, and result in the loss of economically

important fisheries (Myers et al., 2007). The combination of overfishing and a k-

selected life history strategy make sharks and rays particularly vulnerable to

population decline (Musick et al., 2000). In fact, it has recently been estimated

that a quarter of all elasmobranch species are threatened with extinction (Dulvy

et al., 2014). Because fishing pressure varies across the range of many species,

appropriate management strategies require an understanding of population

structure. When a population is highly structured, regional stocks must be

managed as discrete units to lower the risk of extirpation. Sharks are important

both ecologically and commercially, and it is essential to understand a species'

population structure if effective management strategies are to be implemented.

      Sharks are targeted both recreationally and commercially, with additional

losses from incidental catch. It has been estimated that the global chondrichthyan

catch is only about half of the true global catch due to unreported bycatch (Myers

et al., 2007; Stevens, 2000). Commercial markets exist for shark meat, cartilage,

and liver, though the shark finning industry remains the most lucrative. The

process of shark finning is both cruel and incredibly wasteful. Fins are removed

and kept, while the body is discarded back into the ocean, oftentimes when the shark is still alive. Fins are used to make shark fin soup, an expensive dish that is considered a status symbol in many Asian cultures. A 2006 report estimated that 26-73 million fins are traded annually (Clarke et al., 2006; Musick et al., 2000). Conservation efforts and a surge in public awareness have pushed for shark fin bans in recent years. In 2010 Hawaii became the first state to ban the sale and possession of shark fins (*S.B. 2169*, 2010), and other states have begun to follow suit .

Shark population structure is influenced by several factors. First, although species differ in mode of reproduction, they all lack pelagic larvae. In most species, female sharks give birth to live, fully developed pups with strong swimming capabilities. In oviparous shark species, the morphology of egg cases typically includes features that prevent dispersal, such as tendrils or adhesive fibers that secure the egg case in place (Dulvy et al., 2014; Klimley and Oerding, 2013). In an interesting display of parental care, female horn sharks (*Heterodontus francisci*) use their mouths to pick up their egg cases and push them into crevices, relocating them weekly (Ebert, 2003; Klimley and Oerding, 2013). The lack of a dispersive larval stage in the life histories of sharks likely plays an important role in the way populations are structured.

Two additional features of shark biology also impact population structure. First, many species show evidence of natal philopatry, a behavior in which an organism returns to its birthplace to reproduce; over time, fidelity to different geographically distinct breeding sites can lead to genetically divergent populations. Although philopatric behavior is best known in species of salmon and sea turtles, there is increasing evidence that such behaviors exists in some species of sharks, such as lemon sharks (*Negaprion brevirostris*) and blacktip reef sharks (*Carcharhinus melanopterus*) (Feldheim et al., 2013). Second, sex-biased dispersal in many pelagic species must be taken into consideration when investigating questions regarding population structure and stock management. Because mitochondrial DNA (mtDNA) and nuclear DNA have different modes of inheritance, a variety of markers should be used to gain a clear picture of population structure. When female sharks are philopatric and males disperse, there may be discordance between mtDNA and nuclear marker analyses (Portnoy et al., 2010). In species with male-mediated gene flow, mtDNA markers may suggest highly structured populations, while nuclear markers may indicate that populations are well mixed. Sex-biased dispersal and sexual segregation can lead to differential fishing pressure between sexes (Mucientes et al., 2009). If philopatric females do not typically move between populations, female stocks can rapidly crash if exploited.

Leopard sharks (*Triakis semifascia*ta) are common benthic elasmobranchs found in coastal waters from Samish Bay, Washington state, USA to Mazatlan, Mexico. In the northern part of their range they are typically found in bays and estuaries, while in the southern portion they are generally found along sandy flats and kelp beds. *T. semifasciata* most commonly occupy shallow water, although the maximum depth of 156 meters has been documented (Love, 2011). Leopard sharks are opportunistic feeders whose diet typically includes benthic invertebrates, fish eggs, and small fish. Leopard sharks are aplacental viviparous, giving live birth to one to thirty-seven pups on what is believed to be an annual reproduction cycle (Castro, 2011).

Leopard sharks are known to aggregate at specific coastal locations beginning in spring. Local movement patterns during times of aggregations have been well studied at several aggregation sites. For example, it has been shown that the *T. semifasciata* aggregation in La Jolla, CA is centered in an area of warm shallow water with low wave energy during the day, with individuals dispersing to deeper and colder waters at night, presumably to feed on market squid (*Doryteuthis opalescens*) in nearby canyons (Nosal et al., 2012). The sharks that form this aggregation are almost exclusively pregnant females, and it is has been suggested that they use these warm waters to accelerate gestation. At other aggregation sites, movements are dictated by tidal patterns, access to prey items, and water temperature (Ackerman et al., 2000; Carlisle and Starr, 2010).

Heithaus (2007) has proposed that bays and estuaries serve as nursery areas for many shark species, however very little is known about the habitat, behavior, and dispersal of juvenile leopard sharks.

Once aggregations disperse in the fall, knowledge is limited with respect to broad scale movement patterns. When sharks in La Jolla disperse, tagging data has shown that some individuals consistently travel north, while others consistently travel south year to year (Nosal, unpublished), however the reason for this remains unclear. Notably, the leopard sharks found in San Francisco Bay are unique in that they are considered to be a resident population, with most sharks remaining in the bay year-round (Smith, 2001). If few sharks migrate in and out of the bay each year, this population may become distinct over time. Again, it is unknown why this behavior occurs. As a result of this gap in knowledge, the extent of potential gene flow between populations remains to be fully elucidated.

Although it is difficult to envision barriers to leopard shark dispersal along coastal habitats, apparently isolated populations occur offshore, on island Santa Catalina Island. In order for leopard sharks to travel to this island, they must swim across a deep-water channel that reaches a maximum depth of 1100 meters. Although leopard sharks are benthic, it is unlikely that they continue to swim along the sea floor at such great depth. It is more likely that they behave as

a pelagic shark while making this journey. Due to the lack of leopard shark prey items in the pelagic zone in addition to an increased exposure to predators, such as larger sharks, we hypothesize that leopard sharks do not cross from the mainland to the island and vice-versa with any frequency, resulting in differentiation between island and mainland populations. The nurse shark (*Ginglymastoma cirratum*) is another common shallow water benthic elasmobranch with both mainland and offshore island populations. Analysis of nurse shark population structure has revealed pronounced divergence between mainland and island populations, suggesting that deep water is indeed acting as a barrier to dispersal (Karl et al., 2011). This supports our hypothesis that leopard sharks will have significant structuring between mainland and island populations.

Previous work on *T. semifasciata* has examined population structure using mitochondrial DNA and inter simple sequence repeats (ISSR) (Lewallen et al., 2007). A Bayesian assignment test using ISSR data identified seven putative genetic clusters in California, however these clusters did not correspond to distinct geographic locations. Sequences of mtDNA control region revealed low levels of genetic diversity, with only five haplotypes occurring throughout California. This result is not atypical due to slow mutation rates in shark mtDNA (Martin et al., 1992). Despite low mtDNA diversity, significant structuring was found between northern and southern California populations; three haplotypes

were observed multiple times in southern populations, but not in northern populations. Given that mtDNA variation reflects historic gene flow, evidence of population differentiation revealed through mtDNA may indicate an especially pronounced level of divergence in leopard sharks.

In this study, we use nuclear microsatellite markers to analyze the genetic population structure of leopard sharks. In contrast to ISSRs, microsatellites are highly polymorphic, co-dominantly inherited, and mutate rapidly, making them an ideal molecular marker for elucidating population structure. The use of microsatellite markers provides high resolution, and may be able to identify divergence at a finer scale, reflecting more recent patterns of gene flow (Selkoe and Toonen, 2006). As a result, we will gain a better understanding of leopard shark population structure. While the leopard shark is not currently a threatened species, understanding gene flow throughout the species' range may provide insight into the population structure of similar species.

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Methods and Materials

Sample Collection

   Fin clips were collected from a total of 382 individuals from six locations in California (Humboldt Bay n=21, San Francisco Bay n=32, Elkhorn Slough n=62, Santa Barbara n=18, Santa Catalina Island n=96, La Jolla n=148) and one location in Mexico (Bahía Tortugas n=5) (Figure 1). Five samples collected in Ventura, CA were tested for genetic difference between eighteen samples from Santa Barbara. Due to a lack of genetic differences and close proximity (<50 km), Ventura and Santa Barbara samples were pooled for analyses; all references to the Santa Barbara population include the pooled Ventura samples. California populations north of Point Conception (Humboldt Bay, San Francisco Bay, Elkhorn Slough) will be collectively referred to as the northern California populations. California populations south of Point Conception (Santa Barbara, Santa Catalina Island, La Jolla) will be referred to as southern California populations. With the exception of all La Jolla and 72 Santa Catalina Island samples, fin clips were donated by either Lewallen et al. or recreational fishermen. Sharks were captured by hook and line, and small fin clips were removed from the trailing tip of the first dorsal fin. Upon collection, each individual was tagged with an ID number to prevent resampling and immediately released. Tissue samples were stored in 95% ethanol at -80C. DNA was extracted using the QIAGEN DNeasy kit.

Microsatellite Methods

A total of five microsatellite markers were selected for population analysis. Four microsatellite loci (A1, A103, D2, D12) were developed and optimized by Nosal et al. (2013). A fifth locus (D122), also developed by Nosal et al., was optimized for the present study. Forward primers were fluorescently labeled on the 5' end by one of three dyes (FAM, HEX, TET). PCR amplifications were performed under the following conditions: hot start at 95°C for 3 min, followed by 32-35 cycles of 95°C for 30s, 55-56°C for 30s, 72°C for 1 min, and ended with a final extension of 72°C for 45 min. Genotypes and a marker ladder for sizing were resolved on 0.4 mm thick 5% polyacrylamide denaturing gels and visualized by fluorescent scanning with a Molecular Dynamics Typhoon 9410 Variable Mode Imager. Genotypes were scored manually, and ambiguous genotypes were confirmed by running a second time adjacent to alleles of known size. If genotypes could not be confirmed, they were discarded. Individuals were included for analysis if they were successfully genotyped for at least four of the five loci.

MtDNA Methods

Five samples from Bahia Tortugas, Mexico were selected for mitochondrial DNA analysis for comparison to mtDNA haplotypes found in California by Lewallen et al. (2007). Five samples from Humboldt Bay were also selected for mitochondrial DNA analysis to see if any contained a unique

haplotype previously observed in one individual from Humboldt Bay. The control region was amplified using forward (CR1 5'-CCTGCCC TTGGCTCCCAAAGCCAAGATTC-3') and reverse (CR2 5'-TTACAATTAARAC TAAGGCRAGGACCAAA-3') primers. PCR amplifications were performed as follows: 94°C for 2 min, followed by 34 cycles of 94°C for 30 s, 57°C for 1 min, 72°C for 1 min, and ended with a final extension of 72°C for 7 min. Successful amplifications were purified using Sephadex G-50 Fine filtration medium and sequenced by Retrogen Corp (San Diego) using Applied Biosystems 3730 xl DNA Analyzer. Sequences were aligned, trimmed, and edited using CLC Genomics Workbench. Sequences were compared to known haplotypes available in GenBank (Lewallen et al., 2007).

Analysis

Conformance to Hardy-Weinberg equilibrium was calculated using Genepop (Rousset, 2008), and expected and observed heterozygosity were calculated using GenoDive (Meirmans and van Tiendered, 2004). Effective population size (N_e) was estimated using the linkage disequilibrium method with random mating (Waples and Do, 2008) implemented in NeEstimator v2 (Do et al., 2014). Allelic richness was calculated using the R package diveRsity (Keenan et al., 2013).

F_{ST} and corresponding significance values were calculated for each population pair using GenoDive. Linkage disequilibrium was tested using MultiLocus (Agapow and Burt, 2001). To evaluate pairwise population differentiation, allele frequencies of each population were compared using a Fisher's exact test for genic differentiation in Genepop. P-values were adjusted for multiple comparisons using the FDR method implemented in R (Benjamini and Hochberg, 1995).

An analysis of molecular variance (AMOVA) was used to assess variation within and among populations using GelAlEx (Peakall and Smouse, 2012). An AMOVA was conducted on all populations separately, and then populations were pooled into geographical configurations and analyzed again. First, populations north of Point Conception were pooled as one group, and populations south of Point Conception were pooled as the second. Next, populations north of Point Conception were pooled as one group, populations south of Point Conception in California were pooled as the second, and the single Mexican population constituted the third group.

Genepop was used to test for isolation by distance (IBD) by plotting $F_{ST}/(1- F_{ST})$ against the geographic distance between sampling sites. Geographic distance was defined as coastal kilometers between two sampling sites to reflect

the typical behavior of *T. semifasciata*, and F_{ST} values previously calculated in GenoDive were used.

The clustering software Structure (Falush et al., 2003; Pritchard et al., 2000) was also used to assess population structure. Simulations were run from K=1 to K=7, setting the maximum populations to the number of sites sampled. We chose the model that integrates sampling site information into simulations. This assists in population detection when the genetic signal may be low, but does not increase the risk of finding structure when there is none (Hubisz et al., 2009). Structure was run for 1 million steps following a 500 thousand burn-in period. For each value of K, 20 iterations were conducted. K was estimated by plotting the likelihood probability (lnP(D)) of each simulation as well as the average lnP(D) against the corresponding value of k. K values with high likelihood probability and low variability between runs were excluded from further analysis. Figures for Structure output were created using Distruct (Rosenberg, 2003).

Methods and Materials, in part is currently being prepared for submission for publication of the material. Barker, Amanda M.; Nosal, Andrew P.; Lewallen, Eric A.; Burton, Ronald S. The thesis author was the primary investigator and author of this material

Results

We found no significant deviations from Hardy-Weinberg equilibrium in all loci and population (Tables 1 and 2). We did not find evidence of linkage disequilibrium between loci. Plotting pairwise F_{ST} values against geographic distance yielded a significant pattern of isolation by distance (p=0.004, R^2=0.6647) (Figure 2). This indicates that populations that are further apart geographically are more genetically distinct. The points representing the relationship between Bahía Tortugas and the two closest populations of San Diego and Santa Catalina Island strayed far above the regression line, indicating that the differences between these populations is greater than what is expected due to geographic distance alone. Interestingly, comparisons between Santa Catalina Island and the closest mainland populations of Santa Barbara and La Jolla did not fall above the line, suggesting that the deep water does not prevent sharks from crossing back and forth any more than the distance.

Overall, pairwise differentiation tests showed a general pattern of northern California populations being significantly different than southern California populations (Tables 3 and 4). Results varied greatly when each locus was analyzed separately, and no clear pattern was present. Most notably, most pairwise comparisons disagreed with the overall pattern when D122 was analyzed separately; D122 indicated that significant structuring only occurs

between San Francisco Bay and La Jolla. This may be due to the relatively low polymorphism at this locus. Allelic richness was similar in all populations. Within northern California, both Fisher's exact test and F_{ST} indicated that Humboldt Bay is divergent from Elkhorn Slough (p=0.0057; F_{ST}=0.010, p=0.0383) The Fisher's exact test did not suggest divergence between Santa Catalina Island and the closest mainland populations of Santa Barbara and La Jolla (p=0.535, p=0.153).

In contrast to Fisher's exact test, F_{ST} values did indicate a significant difference between La Jolla and Santa Catalina Island (F_{ST} =0.003, p=0.038). Furthermore, the Fisher's exact test indicated that Bahía Tortugas is divergent from all other populations, with the exception of Santa Barbara. F_{ST} values did not indicate the same exception, and suggested that Bahía Tortugas is divergent from all other populations. Apart from these two incongruences, F_{ST} results agreed with the results of the Fisher's exact test.

Results from Structure analysis (using K=1 to K=7) suggest that leopard sharks form two or three population clusters (Figures 3 and 4). Both of these scenarios had high likelihood probabilities that varied little between runs (Figure 5). When two populations are inferred, northern California (Humboldt Bay, San Francisco Bay, Elkhorn Slough) forms the first cluster, while the remaining populations form the second. When three populations are inferred, northern California forms the first cluster, southern California forms the second cluster,

and Bahía Tortugas separates into a third cluster. In all runs, Catalina was not distinct from other southern California populations.

Of the five individuals sampled in Bahía Tortugas, we found two unique haplotypes that have not been observed in California populations. For both new haplotypes, sequences differed by one base pair from the common haplotype. These two individuals were sequenced a second time to ensure this result was not due to a sequencing error. The other three individuals had the haplotype that was found to be most common in California, as did the five newly sequenced individuals from Humboldt Bay.

When all sampling locations were analyzed separately, an AMOVA showed that the variation among populations accounted for 8% of the overall variation (F_{ST}=0.081, p=0.001). The populations were then pooled into two groups, with Humboldt Bay, San Francisco Bay, and Elkhorn Slough representing the first group, and Santa Barbara, Santa Catalina Island, La Jolla, and Bahía Tortugas forming the second group. In this case, an AMOVA showed that the variation among regions accounted for 3% of the total variation (F_{ST}=0.031, p=0.001). Populations were then pooled into three groups, with northern California representing the first group, southern California representing the second group, and Bahía Tortugas representing the third. With this grouping, the variation

among regions accounted for 4% of the total variation (F_{ST}=0.037, p=0.001) (Table 5).

Estimates of effective population size failed to converge; although three population estimates were obtained (San Francisco Bay, N_e=374.5, Elkhorn Slough, N_e= 389.3, and La Jolla, N_e=767.7), the 95% confidence interval for all populations included infinity (Table 6).

Results, in part is currently being prepared for submission for publication of the material. Barker, Amanda M.; Nosal, Andrew P.; Lewallen, Eric A.; Burton, Ronald S. The thesis author was the primary investigator and author of this material.

Discussion

Our results suggest an overall trend of IBD with significant structuring between northern and southern California populations. Although we lack the statistical power to determine whether there are two or three population clusters present, we argue that our data support the hypothesis that Bahía Tortugas is divergent from California populations, resulting in at least three leopard sharks population clusters throughout the sampled range. Estimates of effective population size (N_e) failed to converge for most populations. An infinite effective population size suggests that the amount of allelic variation can be explained by sampling error, rather than genetic drift due to a finite number of breeders in a population.

Our results did not always agree with those of Lewallen et al. (2007). They found an unusually high level of connectivity between Elkhorn Slough and three populations in southern California. This is a stark contrast to our data, which indicated a strong divergence between Elkhorn Slough and southern California populations in all analyses. Additionally, Lewallen et al. found some evidence of genetic discontinuity between Santa Catalina Island and southern California mainland populations, while our results suggest connectivity between these populations. Our data agreed with their conclusion that there is a high level of genetic connectivity between mainland southern California populations.

Interestingly, we did not find significant structuring between the mainland and offshore island populations as expected. Although the extent of gene flow between Santa Catalina Island and La Jolla remains unclear, all tests indicated that there is no divergence between Santa Catalina Island and Santa Barbara. Despite a low F_{ST} value between La Jolla and Santa Catalina Island, this was found to be significant (F_{ST} =0.003, p=0.038). Future work that corrects potential sampling bias could provide insight into whether this result is biologically significant, such as sampling over several years and in different areas of a particular site (Knutsen et al., 2010).

Evidence of gene flow between Santa Catalina Island and the mainland is supported by previous work that monitored the movements of leopard sharks at Santa Catalina Island (Hight and Lowe, 2007). Although this study focused on localized movements around Santa Catalina Island, the authors noted that two female leopard sharks tagged at Santa Catalina Island were detected along the mainland. The first shark was detected approximately 100 kilometers away near the mainland at Carlsbad only seven days after it's last detection at Santa Catalina Island. The shark was detected back at Santa Catalina Island a month later, which suggests that crossing may be routine for some individuals. The second shark was detected in Anaheim Bay a year after the study ended (Hight and Lowe, 2007). The purpose of these mainland-island crossings is unclear, however the authors also noted that both detections along the mainland were near known

breeding sites. The occurrence of gene flow between mainland and island leopard shark populations provides an interesting contrast to their typically benthic lifestyle.

Within northern California, pairwise population differentiation analyses revealed minor structuring between these populations. Gene flow appears to occur between San Francisco Bay and the other populations north of Point Conception, Humboldt Bay and Elkhorn Slough. However, there is evidence that Humboldt Bay and Elkhorn Slough are divergent from each other, suggesting that San Francisco Bay may represent a transitioning region within northern California. Previous work that found Humboldt Bay to be the most divergent leopard shark population suggested that this site may provide a model for future study on local adaptation in elasmobranchs (Lewallen et al., 2007), however our results did not reveal a particularly pronounced level of divergence unique to this population. While San Francisco Bay is considered a resident population with few sharks leaving the bay (Smith, 2001), we did not find evidence of divergence in this population. It may be that the San Francisco Bay population has not been isolated for a sufficiently long time for divergence to occur.

Results from Structure analysis indicate that leopard sharks form two or three genetic clusters that correspond to geographic populations. When K=2, the northern California populations form one cluster, while the southern California

populations and Bahía Tortugas form the second. In the case of K=3, the clustering of northern California populations remains the same, however a separation between the southern California populations and Bahía Tortugas becomes apparent. Small samples sizes at some locations in addition to a low number of markers limit our ability to make a distinction between these two scenarios, however K=3 appears to be most likely when results from all analyses are considered together. F_{ST} values indicate that Bahía Tortugas is significantly divergent from all other populations. Fisher's exact test revealed a similar pattern, with an exception of Santa Barbara, although the lack of a significant difference between Santa Barbara and Bahía Tortugas after correction for multiple comparisons is likely due to small sample sizes at these locations. The presence of two unique mtDNA haplotypes found in Bahía Tortugas highlights an area of potential future research. Because these haplotypes were only found in one individual each, additional samples are required to assess whether these haplotypes are common in Bahía Tortugas and represent true population divergence.

The current status of leopard sharks in Mexico remains unclear. Further study with additional sampling locations in Mexico, along the Pacific coast as well as inside the Gulf of California, is required to accurately assess population structure throughout the species' range. Our results suggest that there is a very distinct break between northern and southern California populations. It is

unlikely that there is a distinct line that northern and southern sharks do not cross, but rather a transitioning region with gradually decreasing gene flow between northern and southern populations. Additional samples from locations between Elkhorn Slough and Santa Barbara would be necessary to identify where the transition between these populations occurs.

Discussion, in part is currently being prepared for submission for publication of the material. Barker, Amanda M.; Nosal, Andrew P.; Lewallen, Eric A.; Burton, Ronald S. The thesis author was the primary investigator and author of this material.
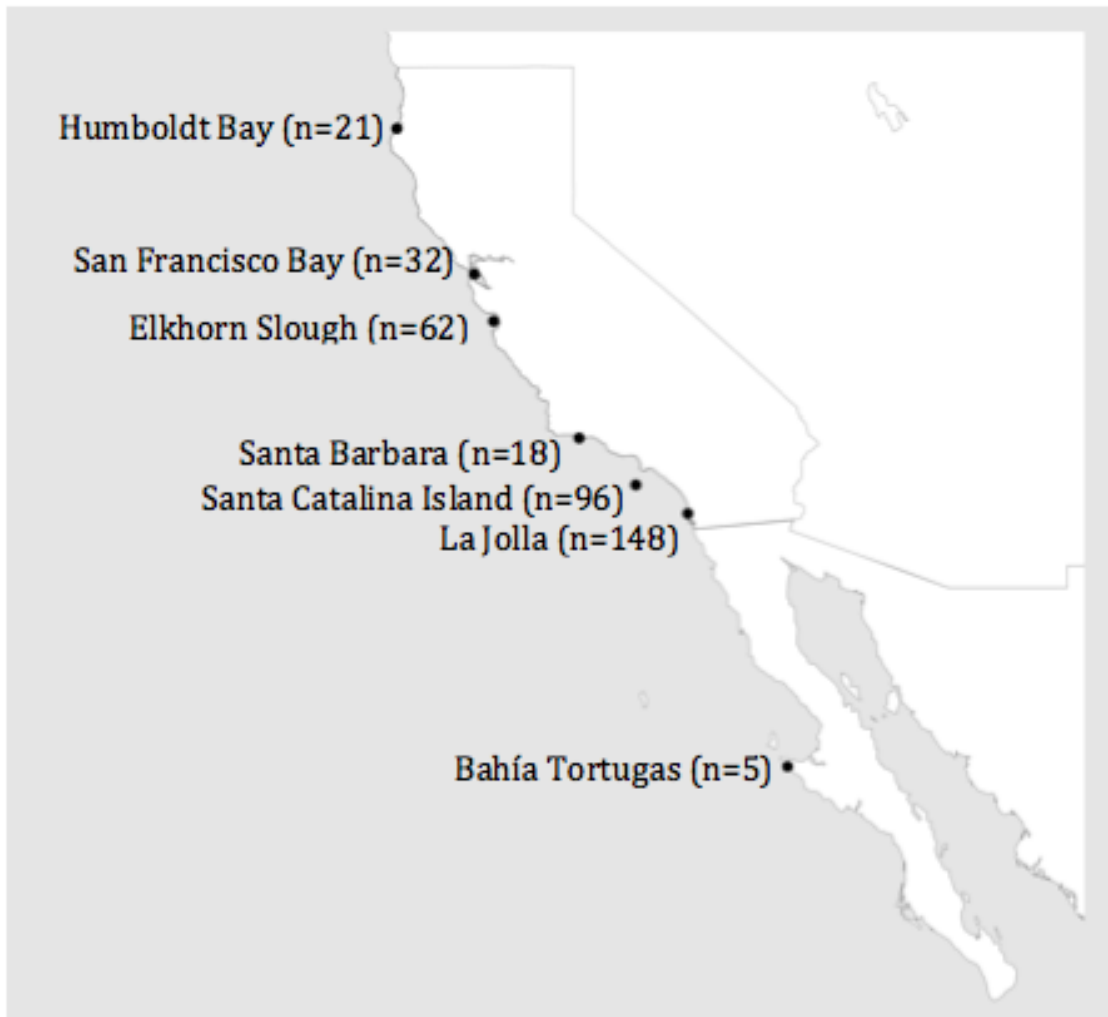
Figure 1. Map of California, USA and Baja California, Mexico showing the location of sample sites and the corresponding sample size. Total n=382.
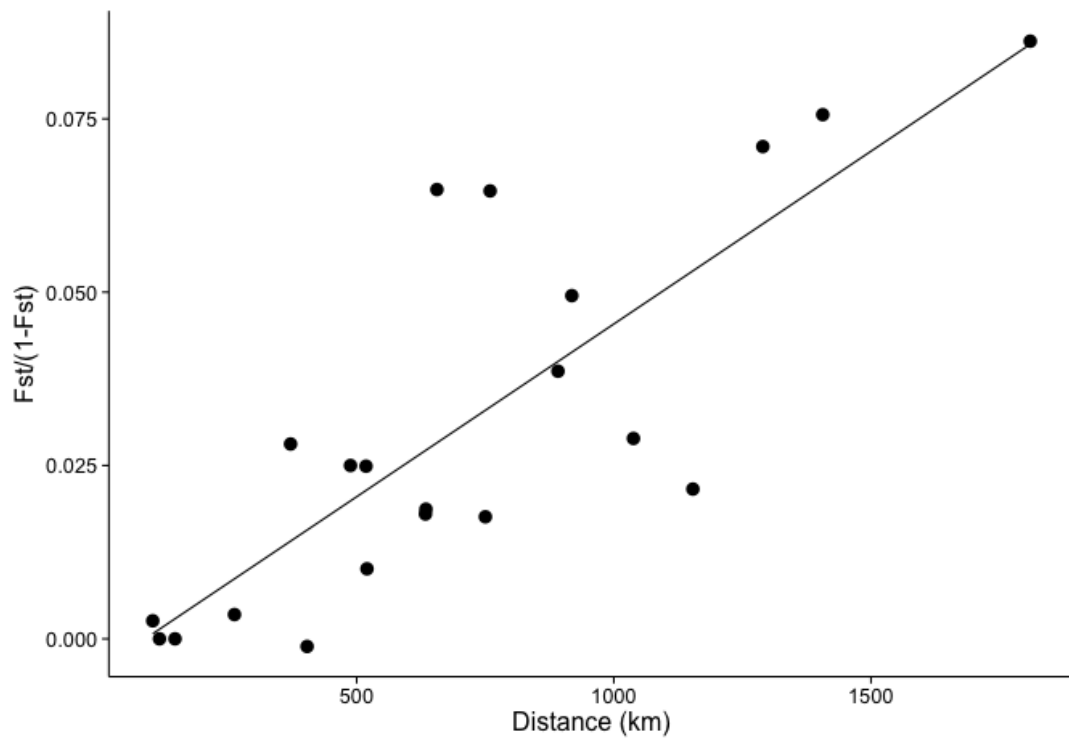
Figure 2. Isolation by Distance (IBD) plot. R^2=0.6647, p=0.004.

HB **SFB** **ES** **SB** **SCI** **LJ** **BT**

Figure 3. Structure clustering analysis when K=2. Sampling sites are arranged north to south: Humboldt Bay (HB), San Francisco Bay (SFB), Elkhorn Slough (ES), Santa Barbara (SB), Santa Catalina Island (SCI), La Jolla (LJ), Bahía Tortugas (BT).
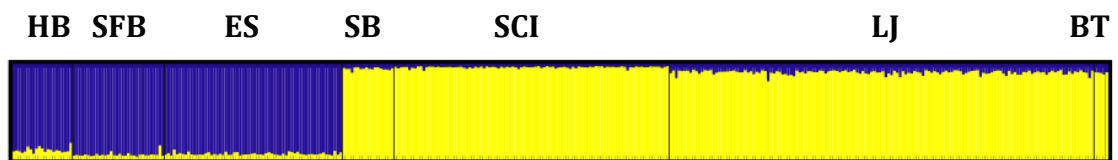
Figure 4. Structure clustering analysis when K=3. Sampling sites are arranged north to south: Humboldt Bay (HB), San Francisco Bay (SFB), Elkhorn Slough (ES), Santa Barbara (SB), Santa Catalina Island (SCI), La Jolla (LJ), Bahía Tortugas (BT).
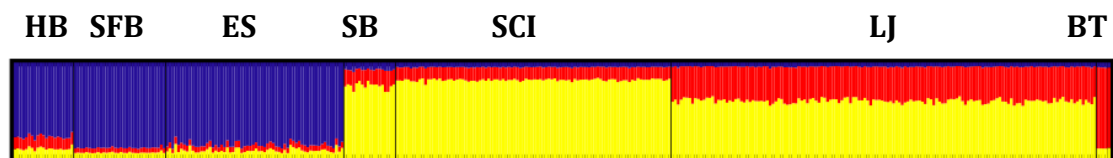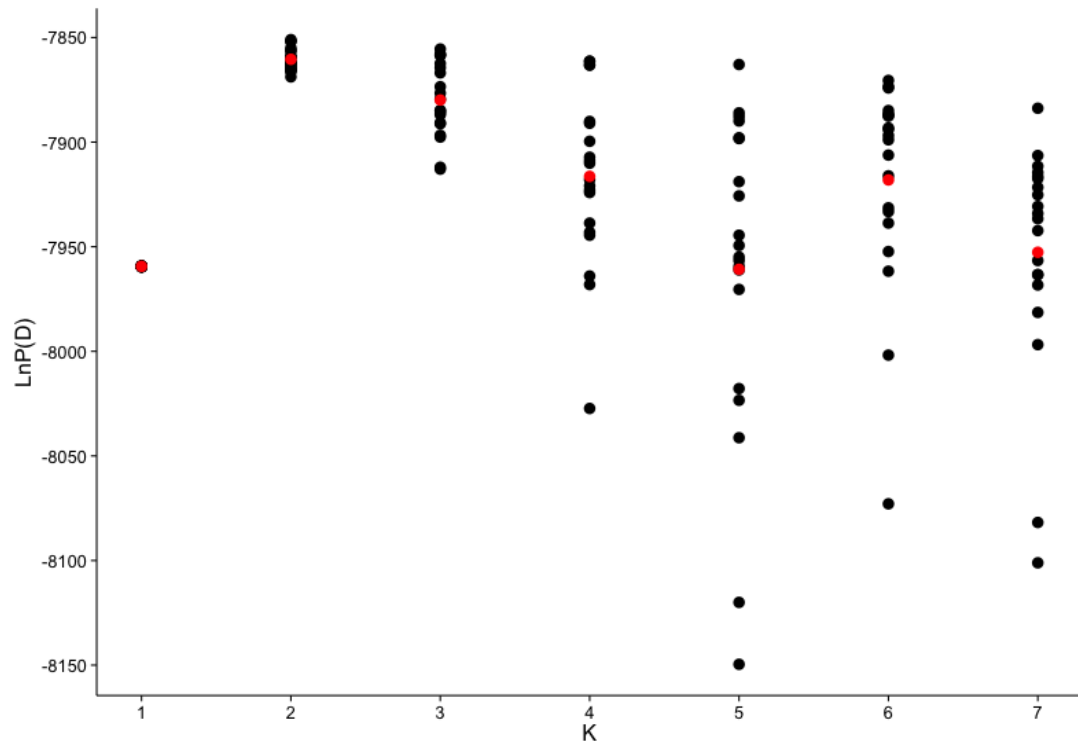
Figure 5. Plot of Structure likelihood probabilities (LnP(D)) for all 20 iterations at each value ok K. The mean lnP(D) for each value of K is indicated by a red point.

Table 1. Summary of microsatellite characteristics. Primer sequence; fluorescent label; annealing temperature (T_a) in °C; number of PCR cycles (# Cycles); allelic diversity (N_a); expected heterozygosity (H_e); observed heterozygosity (H_o); P-values from Hardy-Weinberg exact test for heterozygote deficit (P).

Locus	Primer Sequence	5' Label	T_a (°C)	# Cycles	N_a	H_e	H_o	P
A1	F: 5'-TGTGCTTTTGTATTCCTAATCC-3' R: 5'-CGGGAGTATGGTGGTATTC-3'	HEX	56	33	9	0.759	0.767	0.867
A103	F: 5'-CACCAGCAATCTGTCACTTG-3' R: 5'-CTGTCTTAGCAATGGGTCTGT-3'	FAM	56	28	27	0.79	0.815	0.079
D2	F: 5'-CAGTATICTGGGATGGACTCTA-3' R: 5'-MGCAGTGTCAGTGGTAGTAGG-3'	TET	56	32	18	0.853	0.865	0.596
D12	F: 5'-CCTGCCTGGTTATTGACC-3' R: 5'-CCTGACTGAGGTGTGTAAGATT-3'	HEX	56	35	19	0.845	0.876	0.981
D122	F: 5'-TGGCATTTAGCGATGGAC-3' R: 5'-TCAGGCGGGTAAGTTGTG-3'	TET	55	33	10	0.802	0.767	0.596

Table 2. Summary of population characteristics. Expected heterozygosity (H_E); observed heterozygosity (H_O); p-values from Hardy-Weinberg exact test for heterozygote deficit by population (P); allelic richness (A_R).

Population	H_E	H_O	P	A_R
Humboldt Bay	0.838	0.819	0.563	4.97
San Francisco Bay	0.739	0.828	0.563	4.13
Elkhorn Slough	0.841	0.832	0.668	5.57
Santa Barbara	0.800	0.767	0.563	4.94
Santa Catalina Island	0.809	0.811	0.668	5.28
La Jolla	0.835	0.837	0.563	5.53
Bahía Tortugas	0.760	0.800	0.668	3.77

Table 3. P-values for Fisher's exact test for pairwise population differentiation. Asterisk (*) indicates a significant value.

	Humboldt Bay	San Francisco Bay	Elkhorn Slough	Santa Barbara	Santa Catalina Island	La Jolla
San Francisco Bay	0.7325					
Elkhorn Slough	0.0057*	0.2338				
Santa Barbara	0.0002*	0.0064*	<0.0001*			
Santa Catalina Island	<0.0001*	<0.0001*	<0.0001*	0.5354		
La Jolla	<0.0001*	<0.0001*	<0.0001*	0.6346	0.1532	
Bahía Tortugas	<0.0001*	0.0059*	0.0023*	0.0539	0.0015*	0.0030*

Table 4. Pairwise F_{ST} values below the line with corresponding p-values above the line. Asterisk (*) indicates a significant value.

	Humboldt Bay	San Francisco Bay	Elkhorn Slough	Santa Barbara	Santa Catalina Island	La Jolla	Bahía Tortugas
Humboldt Bay	-----	0.524	0.0383*	0.0019*	0.0019*	0.0019*	0.0019*
San Francisco Bay	-0.001	-----	0.4998	0.0165*	0.0019*	0.0019*	0.0032*
Elkhorn Slough	0.01	0.000	-----	0.0019*	0.0019*	0.0019*	0.0032*
Santa Barbara	0.037	0.024	0.027	-----	0.4998	0.2007	0.0336*
Santa Catalina Island	0.028	0.018	0.024	0.000	-----	0.0383*	0.0019*
La Jolla	0.021	0.017	0.018	0.003	0.003	-----	0.0019*
Bahía Tortugas	0.079	0.07	0.066	0.047	0.061	0.061	-----

Table 5. Results from AMOVA showing the percentage of molecular variance among and within populations. No grouping indicates that all populations were analyzed separately. For two-group analysis, populations were pooled as north of Point Conception (Humboldt Bay, San Francisco Bay, Elkhorn Slough) or south of Point Conception (Santa Barbara, Santa Catalina Island, La Jolla, Bahía Tortugas). For three-group analysis, Bahía Tortugas was pulled from the south of Point Conception group to form its own group. F_{ST} values and significance (P) for each grouping are shown.

Groups	Percentage of molecular variance		F_{ST}	P
	Among pop	Within pop		
None	8%	92%	0.081	0.001
2: North Pt. Conception/South Pt. Conception	3%	97%	0.031	0.001
3: North Pt. Conception/South Pt. conception/ Mexico	4%	96%	0.037	0.001

Table 6. Estimation of effective population size (N_e) with 95% confidence intervals for each sampling site.

Sampling Site	N_e	95% Confidence Intervals	
		(Parametric)	(Jackknife)
Humboldt Bay	Infinite	41.9-infinite	26.7- infinite
San Francisco Bay	374.5	31.1-infinite	41.3-infinite
Elkhorn Slough	389.3	83.8-infinite	57.1-infinite
Santa Barbara	Infinite	42-infinite	20.1-infinite
Santa Catalina Island	Infinite	182.9-infinite	220.6-infinite
La Jolla	767.7	204.5-infinite	184.8-infinite
Bahía Tortugas	Infinite	1.7-infinite	14.6-infinite

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