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Genetic structure of leopard shark (*Triakis semifasciata*) populations along the Pacific coast of North America

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ABSTRACT

Poorly understood genetic structure throughout a species' range can impede the implementation of effective management strategies. Leopard sharks (*Triakis semifasciata*) are known to form seasonal aggregations along the California coastline, but little is known about broad-scale movement patterns once aggregations disperse. If aggregations represent isolated populations, increased protective measures of known aggregation sites may be required to reduce the risk of local extirpation. Five highly polymorphic microsatellite markers were used to analyze the genetic population structure of *T. semifasciata*. A total of 382 individuals were genotyped from six locations in California and one location in Mexico, spanning approximately 1800 km of coastline. In addition, the mitochondrial DNA control region was sequenced for five individuals in Mexico to compare to a previous analysis of *T. semifasciata* haplotypes found in California. Analyses of the genetic data show that *T. semifasciata* does not form one panmictic population and significant population structure is present. Pairwise F_{ST} tests and Bayesian clustering analyses show significant differentiation between northern and southern California populations (north and south of Point Conception, CA). Based on limited sampling, analyses of both microsatellites and mitochondrial DNA indicate that the Mexican population is genetically isolated from the California populations, making it more susceptible to local decline.

1. Introduction

The combination of overfishing and a K-selected life history makes sharks and rays particularly vulnerable to population decline (Musick et al., 2000), and recent estimates indicate that a quarter of all elasmobranch species are threatened with extinction (Dulvy et al., 2014). 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).

Many shark populations are subject to directed fisheries, non-target bycatch, or both. 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 may need to be managed as discrete units to lower the risk of extirpation (Begg and Waldman, 1999). Moreover, many species display philopatric behavior, in which female sharks demonstrate fidelity to specific geographic locations (Feldheim et al., 2014; Hueter et al., 2004; Mourier and Planes, 2013; Nosal et al., 2014; Pardini et al., 2001; Portnoy et al., 2010). Consequently, sexual segregation can lead to differential fishing pressure between sexes, and female stocks can rapidly crash if exploited (Mucientes et al., 2009). As ecologically and commercially important resources, it is essential to understand shark species' population structure if effective management strategies are to be implemented.

Leopard sharks (*Triakis semifasciata*) are benthic elasmobranchs found in shallow coastal waters from Samish Bay, Washington, USA to Mazatlan, Sinaloa, Mexico, and are particularly common in California (Castro, 2011; Farrer, 2009; Love, 2011). *T. semifasciata* are typically found in water ≤4 m, although a maximum depth of 156 m has been documented (Love, 2011). *T. semifasciata* form seasonal aggregations, and local movement patterns during times of aggregations have been well studied; diel and tidal patterns, water temperature, and access to prey items drive fine-scale movements (Ackerman et al., 2000; Carlisle and Starr, 2010; Hight and Lowe, 2007; Nosal et al., 2013a). Temperature and photoperiod have been suggested as the primary cues for dissolution of aggregations in the fall (Nosal et al., 2014). Once aggregations disperse, the movement patterns of *T. semifasciata* are poorly understood, making population connectivity difficult to ascertain by studying behavior alone. During a three-year acoustic monitoring period, tagged *T. semifasciata* from aggregation sites in La Jolla and Del Mar, CA were never detected or reported north of Palos Verdes,

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CA (approximately 130 km north) or south of Puerto Nuevo, Baja California, Mexico (approximately 100 km south), suggesting that dispersal from these sites is limited in southern California (Nosal et al., 2014). Notably, *T. semifasciata* in San Francisco Bay are believed to be a resident population, with most individuals remaining in the bay year-round (Smith, 2001). Furthermore, because aggregations are often comprised mostly of adult females (Ebert and Ebert, 2005; Hight and Lowe, 2007; Hopkins and Cech, 2003; Nosal et al., 2013a), the potential role of males in modulating gene flow is unknown.

Currently *T.* semifasciata is listed as "least concern" under the IUCN Red List. However, strong seasonal philopatry and aggregation behavior make *T. semifasciata* vulnerable to local extirpation (Nosal et al., 2014). A recreational fishery accounts for most annual catch of *T. semifasciata*, and although they are subject to incidental catch and a small commercial fishery, landings declined after the ban of nearshore gillnetting in California (Smith, 2001). Little is known about the status of *T. semifasciata* in Mexico.

Previous work on *T. semifasciata* has examined population structure using mitochondrial DNA (mtDNA) and inter simple sequence repeats (ISSR) (Lewallen et al., 2007). Sequences of mtDNA control region revealed only low levels of genetic diversity, a result that is not atypical due to slow mutation rates in shark mtDNA (Martin et al., 1992). However, despite finding only five mtDNA haplotypes throughout California, Lewallen et al. (2007) found evidence for significant differentiation between northern and southern California 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.

A second finding of Lewallen et al. (2007) came from the analysis of ISSR data. A Bayesian assignment test using these data identified seven putative genetic clusters in California, although these clusters did not correspond to distinct geographic locations. Further, the data support genetic differentiation between Santa Catalina Island and mainland California *T. semifasciata* populations. Given that *T. semifasciata* is a shallow-water benthic shark, the deep-water channel between Santa Catalina Island and mainland California (with a maximum depth of 1100 m) may act as a barrier to dispersal. However acoustic telemetry has provided evidence that *T. semifasciata* do travel across the channel, at least occasionally (Hight and Lowe, 2007; Nosal et al., 2014), so further studies of connectivity between these populations are warranted.

In this study, we use nuclear microsatellite markers to analyze the genetic population structure of *T. semifasciata*. In contrast to dominantly-inherited ISSRs, microsatellites are co-dominantly inherited, meaning that assignments of allelism can be more readily achieved. Furthermore, microsatellite loci show high mutation rates and are highly polymorphic, making them ideal molecular markers for elucidating temporally sensitive patterns of recent population structure.

Microsatellite markers can identify divergence at relatively fine spatial scales and reflect recent patterns of gene flow (Selkoe and Toonen, 2006). As a result, microsatellite data combined with additional population sampling may provide further insight into *T. semifasciata* population structure. Here, we use samples obtained from the previous study, combined with additional samples from Santa Catalina Island and La Jolla, to gain a better understanding of population connectivity between island and mainland populations. In addition, samples from one location in Mexico allow us to analyze a previously unsampled portion of this species' range, and compare their mtDNA haplotypes to those found in California populations by Lewallen et al. (2007).

2. Materials and methods

2.1. Sample collection and DNA extraction

Fin clips were taken from individuals at three locations in northern California (Humboldt Bay n = 21, San Francisco Bay n = 32, Elkhorn Slough n = 62), three locations in southern California (Santa Barbara n = 18, Santa Catalina Island n = 96, La Jolla n = 148) and one location in Mexico (Bahía Tortugas n = 5; Fig. 1). Five sharks from neighboring Ventura, CA were pooled with Santa Barbara for analysis due to their geographic proximity (<50 km) and lack of genetic divergence (F_{ST} = 0.007, p = 0.607). Fin clips from Humboldt Bay, San Francisco Bay, La Jolla, and Bahía Tortugas were collected for the present study. Fin clips from Elkhorn Slough, Santa Barbara, and Santa Catalina Island include a combination of new samples and samples from Lewallen et al. (2007). To simplify discussion, northern California (NCA) refers to Humboldt Bay, San Francisco Bay, and Elkhorn Slough, while southern California (SCA) refers to Santa Barbara, Santa Catalina Island, and La Jolla (Table 1). Tissue samples were stored in 95% ethanol at −80 °C and DNA was extracted using DNeasy Blood and Tissue Kit (QIAGEN).

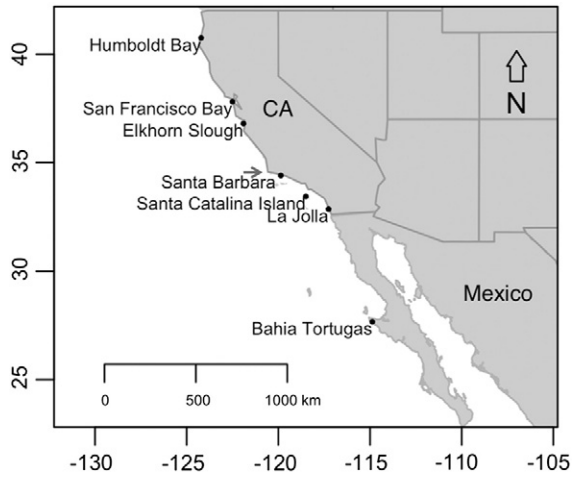

Fig. 1. Map of California, USA and Baja California, Mexico showing the location of sample sites. Gray arrow indicates location of Point Conception, CA.

2.2. Microsatellite methods

Sampled individuals were genotyped at five microsatellite loci developed specifically for *T. semifasciata*; four of these were previously identified and optimized (*Tse*01, *Tse*02, *Tse*03, and *Tse*04; (Nosal et al., 2013b). Using the same methods, a fifth locus (*Tse*05) was developed for the present study. Forward primers were fluorescently labeled on the 5′ end with one of three dyes (6-FAM, HEX, TET). PCR amplifications were performed under the following conditions: 95 °C for 3 min (hot start), followed by 28–35 cycles of 95 °C for 30 s, 55–56 °C for 30 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 45 min (Table 2). Genotypes and a marker ladder (MegaBACE ET550-R; GE Healthcare Life Sciences, Piscataway, NJ) for sizing were resolved on large format (33 × 39 cm), 0.4 mm thick 5% polyacrylamide denaturing gels (Gruenthal and Burton, 2008) and visualized by fluorescent scanning with a Typhoon 9410 Variable Mode Imager (Molecular Dynamics). Genotypes were scored manually with the aid of ImageQuant software (Molecular Dynamics). Individuals were included for analysis

Table 1
Summary of sample locations and sample sizes. Sample sites are separated into three regions: northern California (NCA), southern California (SCA), and Mexico (MEX).

Region	Sample location	N
NCA	Humboldt Bay	21
	San Francisco Bay	32
	Elkhorn Slough	62
SCA	Santa Barbara	18
	Santa Catalina Island	96
	La Jolla	148
MEX	Bahía Tortugas	5

Table 2
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
Trse01	F: 5′-TGTGCTTTTGTATTCCTAATCC-3′ R: 5′-CGGGAGTATGGTGGTATTC-3′	HEX	56	33	9	0.759	0.767	0.867
Trse02	F: 5′-CACCAGCAATCTGTCACTTG-3′ R: 5′-CTGTCTTAGCAATGGGTCTGT-3′	FAM	56	28	27	0.79	0.815	0.079
Trse03	F: 5′-CAGTATICTGGGATGGACTCTA-3′ R: 5′-MGCAGTGTCAGTGGTAGTAGG-3′	TET	56	32	18	0.853	0.865	0.596
Trse04	F: 5′-CCTGCCTGGTTATTGACC-3′ R: 5′-CCTGACTGAGGTGTGTAAGATT-3′	HEX	56	35	19	0.845	0.876	0.981
Trse05	F: 5′-TGGCATTTAGCGATGGAC-3′ R: 5′-TCAGGCGGGTAAGTTGTG-3′	TET	55	33	10	0.802	0.767	0.596

if they were successfully genotyped for at least four of the five microsatellite loci.

2.3. MtDNA methods

MtDNA sequencing of the five samples from Bahía Tortugas, Mexico, followed the methods of Lewallen et al. (2007). The control region was amplified using forward (CR1 5′-CCTGCCC TTGGCTCCCAAAGCCAAGAT TC-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 a 3730 xl DNA Analyzer (Applied Biosystems). Sequences were aligned, trimmed, and edited using CLC Genomics Workbench (QIAGEN), and compared to known haplotypes available in GenBank (Lewallen et al., 2007).

2.4. Analyses

Conformance to Hardy–Weinberg equilibrium was calculated for each population and microsatellite locus using Genepop (Rousset, 2008), and expected and observed heterozygosity were calculated using GenoDive (Meirmans and Van Tienderen, 2004). Allelic richness was calculated using the R package diveRsity (Keenan et al., 2013), and linkage disequilibrium was tested using MultiLocus (Agapow and Burt, 2001).

F_{ST} and corresponding significance values were calculated for each population pair, and region, using GenoDive. P-values were adjusted for multiple comparisons using the FDR method implemented in R (Benjamini and Hochberg, 1995). To assess microsatellite variation within and among populations, analysis of molecular variance (AMOVA) was conducted using GenAlEx (Peakall and Smouse, 2012). An AMOVA was first conducted on each population separately, and then on groups of populations that were pooled by region.

Genepop was used to test for isolation by distance (IBD) in microsatellite data by plotting $F_{ST} / (1 - F_{ST})$ against the geographic distance between sampling sites. Geographic distance was defined as the straight-line path between two sampling sites, and F_{ST} values previously calculated in GenoDive were used.

The Bayesian clustering software Structure (Falush et al., 2003; Pritchard et al., 2000) was also used to assess population structure. Structure assigns individuals into a predetermined number of populations (K) based on HWE assumptions. Simulations were run from K = 1 to K = 8, setting the theoretical maximum number of populations to one more than the number of sites sampled. Sampling locations were integrated into simulations, which 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 100 thousand steps following a 50 thousand step burn-in period. For each value of K, 20 iterations were conducted. K was chosen using the Evanno method (Evanno et al., 2005) as implemented in Clumpak (Kopelman et al., 2015). Structure plots were created with Clumpak, which compares all runs at each value of K to identify optimal clustering scenarios and uses Distruct (Rosenberg, 2004) to create resulting figures.

MtDNA analysis was performed on haplotypes obtained from Lewallen et al. and Bahía Tortugas samples sequenced for the present study. For all mtDNA analyses, individuals were grouped by region: NCA, SCA, and MEX. A median-joining mtDNA haplotype network was drawn in Network (fluxus-engineering.com) (Bandelt et al., 1999), and manually re-drawn for publication using Inkscape (https://inkscape.org/en/). Arelequin was used to calculate Φ_{ST} and corresponding significance values (Excoffier and Lischer, 2010).

Table 3
Summary of sampling location 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	0.8	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	0.76	0.8	0.668	3.77

3. Results

No significant deviations from Hardy–Weinberg equilibrium were detected at any microsatellite locus or in any population (Tables 2 and 3). Additionally, evidence of microsatellite linkage disequilibrium between loci was not observed. Allelic richness was similar across all sampling locations (Table 3).

Pairwise differentiation tests of microsatellite data showed a pattern of divergence between northern and southern California (Table 4). Within northern California, pairwise tests suggested connectivity between San Francisco Bay and the neighboring Humboldt Bay to the north ($F_{ST} = 0.000$, $p = 0.507$) and Elkhorn Slough to the south ($F_{ST} = 0.000$, $p = 0.499$), however differentiation was supported between Humboldt Bay and Elkhorn Slough ($F_{ST} = 0.010$, $p = 0.026$). Within southern California, pairwise tests suggested connectivity between all sampling locations, with the exception of Santa Catalina Island and La Jolla ($F_{ST} = 0.003$, $p = 0.026$). Pairwise tests for differentiation between Bahía Tortugas and all other sampling locations were significant (Table 4). When pairwise tests for differentiation were performed with sampling locations pooled into regions (NCA, SCA, MEX), all comparisons were significant (Table 5). Plotting pairwise $F_{ST} / (1 - F_{ST})$ values against geographic distance yielded a significant pattern of isolation by distance ($r^2 = 0.665$, $p = 0.004$; Fig. 2a). When the southernmost sampling location (Bahía Tortugas) was excluded from analysis, the pattern of IBD remained significant, although the relationship was not as strong ($r^2 = 0.477$, $p = 0.039$, Fig. 2b).

Application of the Evanno et al. (2005) method to results from Structure suggests that *T.* semifasciata form two population clusters (Fig. 3). When two populations are inferred, northern California forms the first cluster (Humboldt Bay, San Francisco Bay, and Elkhorn Slough), while the remaining sampling locations in southern California and Mexico form the second (Santa Barbara, Santa Catalina Island, La Jolla, and Bahía Tortugas).

When all sampling locations were analyzed separately, an AMOVA showed that the variation among locations accounted for 8.1% of the

Table 4
Pairwise F_{ST} values between sampling locations below the line with corresponding p-values above the line.

Sampling location	Humboldt Bay	San Francisco Bay	Elkhorn Slough	Santa Barbara	Santa Catalina Island	La Jolla	Bahía Tortugas
Humboldt Bay	–	0.507	0.0256*	0.002*	0.002*	0.002*	0.002*
San Francisco Bay	0	–	0.499	0.015*	0.002*	0.002*	0.003*
Elkhorn Slough	0.01	0	–	0.002*	0.002*	0.002*	0.002*
Santa Barbara	0.037	0.024	0.027	–	0.499	0.247	0.020*
Santa Catalina Island	0.028	0.018	0.024	0	–	0.026*	0.002*
La Jolla	0.021	0.017	0.018	0.003	0.003	–	0.002*
Bahía Tortugas	0.079	0.07	0.066	0.047	0.061	0.061	–

* Asterisk indicates a significant value.

overall variation ($F_{ST} = 0.081$, $p = 0.001$; Table 6). Sampling locations were then pooled according to the clusters identified by Structure, 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 variation among regions accounted for 3.1% of the total variation ($F_{ST} = 0.031$, $p = 0.001$). When sampling locations were pooled into three groups, with northern California representing the first group, southern California representing the second group, and Bahía Tortugas representing the third, the variation among regions accounted for 3.7% of the total variation ($F_{ST} = 0.037$, $p = 0.001$).

Of the five individuals sampled in Bahía Tortugas, we found two unique mtDNA haplotypes that were not observed by Lewallen et al. (2007) (Fig. 4; GenBank accession nos. KM358169, KM358170). For both new haplotypes, sequences differed by one base pair from the common haplotype. These two new haplotypes were confirmed by sequencing a second time. The other Bahía Tortugas individuals had the haplotype that was found in approximately 92% of all California individuals sequenced by Lewallen et al. (2007). Comparisons of Φ_{ST} between regions suggest differentiation between northern California and southern California ($\Phi_{ST} = 0.046$, $p = 0.00$; Table 7). Differentiation between southern California and Mexico was also significant ($\Phi_{ST} = 0.175$, $p = 0.045$).

4. Discussion

The genetic data provide strong evidence for genetic structure among *T. semifasciata* populations along the Pacific coast of North America. There is a clear pattern of IBD with significant differentiation between northern and southern California populations. The small sample size from Mexican waters results in a lack of statistical power to definitively assess connectivity between southern California and Mexico. However, we argue below that our data supports divergence between Bahía Tortugas and California populations, resulting in at least three leopard shark population clusters throughout the sampled range.

We did not find significant structuring between the mainland and offshore island populations as expected from a deep-water barrier to this generally nearshore benthic shark (Ebert, 2003). All analyses indicated little divergence between Santa Catalina Island and Santa Barbara, and despite the fact that the F_{ST} value between La Jolla and Santa Catalina Island is statistically significant ($F_{ST} = 0.003$, $p = 0.038$), its low value suggests that it may not be biologically meaningful (Waples, 1998). The results of the Structure clustering analysis support connectivity between La Jolla and Santa Catalina Island.

Table 5
Pairwise F_{ST} values between northern California (NCA), southern California (SCA), and Mexico (MEX) below the line with corresponding p-values above the line.

Region	NCA	SCA	MEX
NCA	–	0.001*	0.002*
SCA	0.02	–	0.001*
MEX	0.066	0.059	–

* Asterisk indicates a significant value.

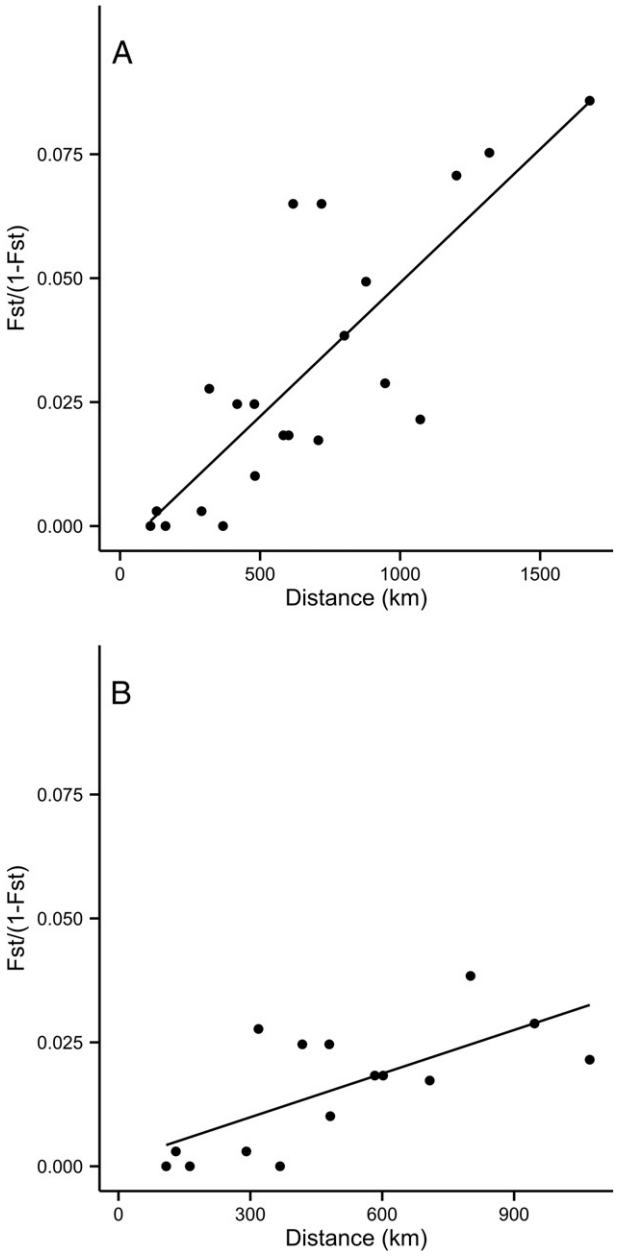

Fig. 2. Isolation by distance (IBD) plots including all sample sites (A) and excluding the sample site in Mexico (B).

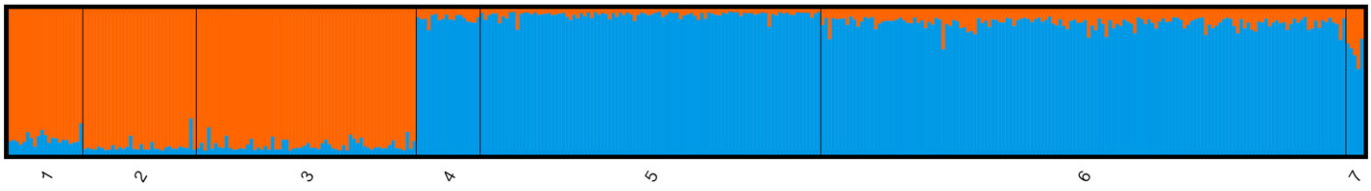

Fig. 3. Structure clustering analysis. Sampling sites are arranged north to south: Humboldt Bay (1), San Francisco Bay (2), Elkhorn Slough (3), Santa Barbara (4), Santa Catalina Island (5), La Jolla (6), and Bahía Tortugas (7).

Evidence of migration between Santa Catalina Island and the mainland is also supported by previous acoustic monitoring studies (Hight and Lowe, 2007; Nosal et al., 2014), where onshore–offshore movements have been reported for at least four tagged sharks. In one instance, Hight and Lowe (2007) reported that one shark detected at Santa Catalina Island moved approximately 100 km and was relocated near the mainland at Carlsbad, California only seven days later; remarkably, the same shark was detected back at Santa Catalina Island a month later, suggesting that crossing may be routine for some individuals. Although leopard sharks are considered a benthic species, it is unlikely that they continue to swim close to the seafloor with bottom depths in excess of 1000 m. *T. semifasciata* that were transported and released offshore generally swam within 30 m of the surface as they traveled back to shore (A. Nosal, unpublished), suggesting the deep-water channels separating the mainland and Channel Islands may not be a strong barrier to gene flow in this species.

Within northern California, pairwise comparisons revealed differentiation among populations that was not corroborated by Structure analysis. 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 and San Francisco Bay may represent a transition region within northern California. Although San Francisco Bay has been considered a resident population with few sharks leaving the bay (Smith, 2001), evidence of divergence due to isolation was not detected for this population in the present study. Similarly, although previous work found Humboldt Bay to be the most divergent leopard shark population (Lewallen et al., 2007), our results did not reveal pronounced divergence of this population. This difference in results could potentially be due to the differences in the types of molecular markers used in each study (ISSRs vs. microsatellites); or reflect limitations of sampling few individuals near the extreme end of the species' range; this limitation may apply to our limited sampling in the southern end of the species range as well.

Although results from Structure analysis suggest that *T. semifasciata* form two genetic clusters, we argue that three clusters are likely when results from all analyses are considered together. F_{ST} values indicate that Bahía Tortugas is distinct from all other populations. Indeed, despite the small sample size from this population, Φ_{ST} between southern California and Bahía Tortugas was significant, and 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 determine if these haplotypes are common in Bahía Tortugas and possibly additional unsampled populations in coastal Mexico.

Our results show some pronounced differences from those of the only previous genetic analysis of *T. semifasciata* population structure (Lewallen et al., 2007). Previously, a high level of connectivity between Elkhorn Slough and three populations in southern California was found. This is a stark contrast to our data, which indicates 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. Possible reasons for these differences include: (1) stochastic differences in sampling or increased analytical power contributed by larger sample sizes, (2) temporal changes in the population structure of leopard sharks over time, (3) different patterns revealed by different molecular markers (ISSR vs microsatellites). In contrast with ISSRs, microsatellites are co-dominant and highly polymorphic, and allow researchers to better elucidate fine-scale patterns of population differentiation (Selkoe and Toonen, 2006).

Our work has identified two regions where additional sampling and genetic analysis will be particularly useful. First, our analysis of the population structure and status of *T. semifasciata* in Mexico is based on a single small sample, yet observed differentiation from California samples suggests that Mexico can harbor leopard sharks that are genetically distinct. The continued use of nearshore gill nets in Mexico may pose a substantial threat to these populations (Ramirez-Amaro et al., 2013),

Table 6
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 (northern California) or south of Point Conception (southern California and Mexico). For three-group analysis, northern California, southern California, and Mexico each formed a 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: NCA/SCA + MEX	3%	97%	0.031	0.001
3: NCA/SCA/MEX	4%	96%	0.037	0.001

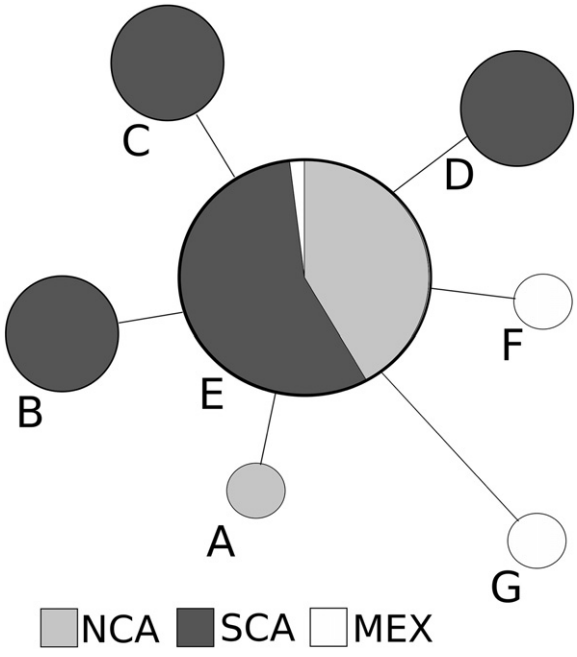

Fig. 4. Haplotype network with pie charts showing frequency in northern California (NCA), southern California (SCA), and Mexico (MEX). Nodes are roughly representative of total frequency (not to scale). Total frequency is as follows: A = 1, B = 3, C = 4, D = 6, E = 158, F = 1, and G = 1.

Table 7
Pairwise Φ_{ST} values between northern California (NCA), southern California (SCA), and Mexico (MEX) below the line with corresponding p-values above the line.

Region	NCA	SCA	MEX
NCA	–	0.000*	0.009*
SCA	0.046	–	0.045*
MEX	0.611	0.175	–

* Asterisk indicates a significant value.

especially if they lack connectivity with more protected California populations. Second, although our results indicate an abrupt genetic divergence between northern and southern California populations, distances between sampled locations are large. Given the general pattern of isolation by distance, it remains possible that gene flow between northern and southern California populations gradually decreases through a transitioning region. The transition region may occur around Point Conception, although genetic breaks that coincide with this biogeographic barrier are uncommon (Burton, 1998; Dawson, 2001). Additional samples from locations between Elkhorn Slough and Santa Barbara would be necessary to identify where the transition between northern and southern populations occurs.

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