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Genetic Diversity, Gene Flow, and Polyandry of Northeastern Pacific Triakid Sharks

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Genetic Diversity, Gene Flow, and Polyandry of Northeastern Pacific Triakid Sharks

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Biology

by

Chris Lance Chabot

2013

ABSTRACT OF THE DISSERTATION

Genetic Diversity, Gene Flow, and Polyandry

of Northeastern Pacific Triakid Sharks

by

Chris Lance Chabot

Doctor of Philosophy in Biology

University of California, Los Angeles, 2013

Professor Donald G. Buth, Chair

Exploitation of sharks by both commercial and recreational fisheries has caused decreasing numbers resulting in population collapses. Sharks are extremely vulnerable to the effects of exploitation due to life-history characteristics including long lifespans, low fecundities, and late maturities. One family of shark, the Triakidae, has been the subject of global exploitation for greater than 80 years, especially within the eastern Pacific. The Triakidae is a primary component of the eastern Pacific elasmobranch assemblage considered both commercially and recreationally important with several endemic species found within the region including *Mustelus albipinnis, M. californicus, M. dorsalis, M. henlei*, *M. lunulatus,* and *Triakis semifasciata.* In regards to conservation efforts, there is a general paucity of information regarding the status of triakid sharks. As a result of this paucity of information, this study used mitochondrial and nuclear genetic markers to investigate: 1) gene flow among globally distributed populations of the tope shark, *Galeorhinus galeus*; 2) determine the

existence of multiple paternity in *M. henlei* from within the northern portion of the species' range; and, 3) explore the population connectivity of *M. henlei* among localities distributed throughout the range of the species from along the northeastern Pacific coast.

A lack of gene flow among globally distributed populations of the tope shark, *G. galeus* from North America, South America, Western Europe, South Africa, and Australia has been observed based on data from thirteen species-specific microsatellite loci. These data support previous patterns of gene flow based on mitochondrial control region sequence data and indicate the isolated nature of globally distributed populations of *G. galeus*. As a result, these populations should be managed separately and the currently recognized species status of *G. galeus* as monotypic should be reconsidered.

The existence of polyandry and multiple paternity within a population of *M. henlei* at Santa Catalina Island, CA over a period of eight years has been confirmed based on data from 18 litters and four species-specific microsatellites. The observed range of multiple paternity, 0-40%, is significantly lower than that observed in another population of *M. henlei* from Baja California (93% of 14 litters were observed to be sired by multiple males within a single year). As population sizes differ between the two localities (Baja California > Santa Catalina Island), the rate at which females encounter mates may be responsible for the difference in the frequencies of multiple paternity observed between Santa Catalina Island and Baja California.

Data from six species-specific microsatellites and the mitochondrial control region have detected three distinct populations of *M. henlei* in the northeastern Pacific (Northern (San

Francisco), Central (Santa Barbara, CA, Santa Catalina Island, CA, Punta Lobos, Baja California, Mexico, and San Felipe, Baja California, Mexico), and Southern (Costa Rica)). Reduced mitochondrial genetic diversity was observed in San Francisco and Santa Catalina relative to all other localities. Possible reasons for the observed reductions in genetic diversity may be the recent establishment of isolated populations after Pleistocene glaciations, female philopatry to nursery areas, or a combination of the two. Long-term gene flow was asymmetric among localities with the predominating pattern being from the north to the south. Gene flow within the past two to three generations appears to be virtually nonexistent among localities with the exception of Punta Lobos that demonstrated significant gene flow to all other localities within the central population. This pattern may be the result of changing climate and may reflect the dispersal of tropical *M. henlei* into subtropical and temperate regions.

The dissertation of Chris Lance Chabot is approved.

Paul H. Barber

David Jacobs

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Larry G. Allen

Edward J. Heist

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2013

Dedication

This work is dedicated to the memory of my mother, Karen Lyn Ruhl. She was always there for me and supported me in every way possible. From getting me on to the "turtle bus" and picking me up at the bus stop every day after kindergarten, through the numerous adversities that life has thrown my way, and during all of the greatest moments of my life including the birth of my daughter Bailey. The selflessness and courage that she displayed when facing the end of her life just before the completion of this work has inspired me and I can only hope to live up to her memory. Without her, I would have been nothing. With her, I have been able to achieve everything.

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Acknowledgments

This work would not have been possible without the support and collaboration of numerous individuals. The planning, implementation, and critique of the studies contained within are a direct result of the guidance provided by my outstanding dissertation committee consisting of Don Buth, Paul Barber, Dave Jacobs, Reggie Edgerton, Larry Allen, and Ed Heist (and John Novembre who unfortunately left UCLA prior to its completion). Don took a chance on this "older" grad student (although not his oldest) and provided the opportunity for me to obtain my PhD at UCLA and maintained the highest standards of quality throughout my work. I am truly indebted to him for the rest of my life. Paul provided pointed critiques of my work in an effort to improve their overall impact as well as no-nonsense feedback on the relevance and future direction of my research that has broadened the scope of my work. Although I'm sure that Reggie would say that he didn't contribute much to this work, nothing could be further from the truth. Reggie has always asked some of the best questions that have forced me to think of the "big picture" and how to present my research. I'll never forget him asking at my orals, "what is the coolest thing that you expect to find?" A question that made me think quickly on my feet and one that I now ask of everyone that presents their data to me. Although Dave was a late addition to my committee, he provided excellent feedback on my chapters as well as clear explanations of the patterns that were observed. One of my favorite insights from Dave pertained to the migration patterns of *Mustelus henlei* in the Gulf where he simply stated that "maybe they just moved". Believe it or not, these words were profound and cleared my mind and helped me to think simply. Larry has always been one of my staunchest supporters and has been an inspiration for my success as a researcher. He has always been there for me and is almost like a second father. Ed is a true expert in the field of elasmobranch genetics and I am so

very lucky to have had him serve on my committee. The feedback provided by Ed has been some of the most influential of my career and I can only hope to obtain the level of impact and respect that he truly deserves within the field. Finally, although John had to leave prior to the completion of my dissertation, his impact throughout this work was definitely noticed. There's nothing quite like speaking with a highly respected theoretical population geneticist about such trivial things like gene flow when he is busy scanning the entire human genome and revolutionizing his field. All in all, this is the best committee that I could have ever hoped for and they are the reason that I have been successful in obtaining my PhD.

As sampling is one of the most difficult things to accomplish with relatively large, extremely vagile vertebrates like sharks, numerous individuals deserve my deepest appreciation for all of their efforts to provide samples for this work. These individuals have all been properly acknowledged within the following chapters; however, one individual deserves to be given additional recognition. Brent Haggin has been a long-time collaborator and was instrumental in obtaining samples of *Galeorhinus galeus, Mustelus Californicus,* and *M. henlei* (including virtually all of the litters used for the multiple paternity chapter and the specimens of *M. californicus* and *M. henlei* used for a forthcoming allozyme paper). Without his assistance, much of this work would not have been possible. Developing the tools necessary to generate the majority of data for this work would have been impossible without John Pollinger, Sergio Nigenda, and Bob Wayne. John was instrumental in the development of the microsatellite libraries for *G. galeus* and *M. henlei* and was always willing to offer assistance with the analyses of data and the use of software. Sergio taught me the techniques necessary to screen microsatellite libraries for suitable loci that are presented in Chapter I. Chapter I is a version of

Chabot, C.L., and S. Nigenda. 2011. Characterization of 13 microsatellite loci for the tope shark, *Galeorhinus galeus*, discovered with next-generation sequencing and their utility for eastern Pacific smooth-hound sharks (*Mustelus*). Conservation Genetics Resources. 3:553-555. Bob provided lab space and equipment for the generation of data. Without these vital components, this work would have been far more difficult.

My parents have always supported me in all of my endeavors and are largely responsible for my successes. Unfortunately, my mother couldn't witness the conclusion of this work as she passed away just before its completion. However, she was well aware of its progress and knew that it was just a matter of time before it would be finished. She was very influential and always provided encouragement and support when things seemed their darkest. My father was also extremely encouraging as he would ask, "when are you going to be done?" This question kept me on task and made me always aware of the passing of time as my dissertation evolved.

Finally, the love and support of my daughter Bailey and the love of my life, Sarah, made all of this possible. I can only hope that this work serves as inspiration for Bailey as she makes her way through life and faces adversity and challenge. Anything is possible as long as you know what you want and have the tenacity to obtain it. Sarah has been there throughout the completion of my master's and PhD. She has listened to me speak incessantly of sharks, gene flow, evolutionary patterns, and the trials and tribulations associated with performing research and surviving as a graduate student. This PhD is as much hers as it is mine.

Vita

Degrees

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Teaching Experience and Employment

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Honors and Awards

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2011 American Institute of Fisheries Research Biologists: Student Presentation Award

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Chapter I

Characterization of 13 microsatellite loci for the tope shark, *Galeorhinus galeus,* discovered with next-generation sequencing and their utility for eastern Pacific smoothhound sharks (*Mustelus*)

Abstract

The tope shark, *Galeorhinus galeus*, is a commercially important member of the Triakidae that has been exploited globally for the past 80 years. Here 13 microsatellite loci for *G. galeus* discovered by next-generation sequencing (Roche 454 pyrosequencing) and their utility for eastern Pacific smoothhound sharks (*Mustelus*) are described. These loci were polymorphic (3-12 alleles) with observed heterozygosity between 0.11 and 0.86 and expected heterozygosity between 0.24 and 0.87. Several loci (7 of 13) amplified consistently for *Mustelus californicus* and *M. henlei*. These loci are the first to be characterized explicitly for *G. galeus* and should be useful in the investigation of population structure of this vulnerable elasmobranch.

The tope shark, *Galeorhinus galeus* (Triakidae), has been commercially exploited for greater than 80 years with populations demonstrating historic collapses (Ebert, 2001; Walker et al., 2006). Continued exploitation of the species has resulted in a classification of vulnerable by the IUCN (Walker et al. 2006) and a need to determine the connectivity of globally distributed individuals in order to generate conservation strategies. Nuclear microsatellites have been used to reveal patterns of population connectivity in numerous taxa. Therefore, this study sought to generate a library of microsatellite markers for *G. galeus* using next-generation sequencing technology (Roche 454 pyrosequencing) in order to elucidate the patterns of population structure and gene flow in *G. galeus*.

DNA used for the generation of the microsatellite library was extracted from the fin clip of an Australian sample using the DNeasy blood and tissue extraction kit (Qiagen, Valencia, USA) following the manufacturer's protocols. 500 ng of DNA was prepared for whole genome shotgun sequencing on the Roche Genome Sequencer FLX instrument utilizing the GS FLX Titanium Rapid Library Preparation Kit (Roche Applied Sciences, Indianapolis, USA) following the manufacturer's protocol. The library was quantified for DNA fragment size distribution and concentration (Agilent 2100 Bioanalyzer) and then processed with the GS FLX emulsion polymerase chain reaction (PCR) and sequencing kits. Sequencing was performed using $1/16^{th}$ of a picotiterplate and yielded 40,156 sequences.

The sequences were screened for potential microsatellite loci by MSATCOMMANDER (Faircloth, 2008) under the default settings. Of the 40,156 sequences, 1,344 contained putative microsatellite loci. Similar to a previous study of the Australian gummy shark (Boomer and

Stow, 2010), the majority of microsatellite motifs identified were dinucleotide in nature (~80%). Primers for dinucleotide (minimum repeat number (mrn) = 8), tetranucleotide (mrn = 4), and pentanucleotide (mrn = 4) loci were designed by the PRIMER3 software (Rozen and Skaletsky 2000) embedded in MSATCOMMANDER using the default settings. In total, 32 primer pairs were used for amplification trials consisting of 18 dinucleotide, 11 tetranucleotide, and 2 pentanucleotide loci. For all loci, the forward primer was synthesized with an M13F(-20) sequence (GTAAAACGACGGCCAG) added to the 5' end to incorporate a 5′ fluorescent label per the technique of (Boutin-Ganache et al., 2001). Initially, eight samples from four subpopulations (2 samples per population: North America (California), South Africa, Australia (Australian Bight and Tasmania), and the U.K. (Irish Sea)) were used to test amplification of loci and evaluate polymorphic content. The PCR protocol was as follows: A 10 μL touchdown PCR was performed using an Eppendorf Mastercycler *epgradient* S thermal cycler and the following reaction conditions: 10-100 ng template DNA, 0.2 μM reverse primer, 0.01 μM forward primer, 0.01 μM dye labeled M13 primer, 0.4 mg/mL BSA, and 5.0 μL of Qiagen Multiplex Mastermix (Qiagen, Valencia, USA). Initial denaturation was at 95°C for 15 minutes followed by 25 cycles of denaturation (94°C for 30 seconds), annealing (59°C for 90 seconds), extension (72°C for 60 seconds) and another 20 cycles of denaturation (94°C for 30 seconds), annealing (53°C for 90 seconds), extension (72°C for 60 seconds), and terminating with a final extension (60°C for 30 minutes). All PCR products were then electrophoresed on an Applied Biosystems (ABI) 3730*xl* DNA Analyzer. Allele sizes were determined by using an internal lane standard LIZ 500 (ABI) and GeneMapper® 3.7 (ABI). Out of the 32 primer pairs tested, 13 were successfully amplified by PCR and further characterized using additional samples from the Australian Bight and Tasmania (n = 28). In order to validate the dataset, 30% of our samples were reanalyzed at all

loci producing identical genotypes between reads.

MICROCHECKER (Van Oosterhout et al., 2003) was used to investigate the existence of null alleles, large allele dropout, and stuttering. With the exception of Gg2, Gg17, Gg18, and Gg22, all loci demonstrated a lack of null alleles. GENEPOP 4.0 (Raymond and Rousset, 1995; Rousset, 2008) was used to estimate allele frequencies, observed heterozygosity (H_O) and expected heterozygosity (H_E), and determine departures from Hardy-Weinberg equilibrium (HWE). All 13 loci of *G. galeus* were polymorphic (3-12 alleles). H_O and H_E were 0.11-0.86 and 0.24-0.87 respectively (Table 1.1) and after Bonferroni correction all loci were in HWE with the exception of Gg4 and Gg17. FSTAT 2.9.4 (Goudet, 2003) was used to test for linkage disequilibrium and estimate F_{IS}. All loci were in linkage equilibrium and F_{IS} ranged between -0.132-0.721 (Table 1.1).

To determine the utility of these markers for genotyping species of eastern Pacific smoothhound sharks (*Mustelus*), the 13 loci were tested on *Mustelus californicus* and *M. henlei* using the PCR reactions and analyses described above. Seven of the loci successfully amplified for both species and two loci produced stutter products (Table 1.2). The development of these 13 microsatellite loci from *G. galeus* using next-generation sequencing technology, along with those of Boomer and Stow (2010), should aid in the elucidation of gene flow within species of the Triakidae and provide valuable tools for the conservation of threatened and data deficient shark species.

Acknowledgments

I would like to thank John Pollinger for the preparation of the 454 library, Sergio Nigenda for his assistance and guidance with the characterization of the microsatellite loci, and Robert K. Wayne for laboratory equipment and reagents. Funding was provided by the Southern California Academy of Sciences and the U.S. Department of Education (GAANN Fellowship).

Table 1.1 Characteristics of microsatellite loci for *Galeorhinus galeus.*
N number of Australian samples, *Size* based on all samples, *A* number of alleles, H_O observed heterozygosity for Australian samples, H_E expected heterozygosity for Australian samples.

Locus	Forward Primer 5' - 3'	Reverse Primer 5' - 3'	Motif	N	Size (bp)	A	H_O	H_E	F_{IS}
Gg2	TGGCTCAGTCCAGAAACCC	CCCTATTCGAGAGGCCCAG	(TG)n	29	249-259	6	0.30	0.55	0.336
Gg3	CCGTGACTGAAAGCAGCC	CCCTCAACCATGGCAAGTG	(GATT)n	28	257-265	4	0.43	0.46	0.128
Gg4	CTGGAATACATGCCGAGCAC	CCCGAAAGGTCTTAGTTCGC	(GA)n	29	179-213	3	0	0	NA
Gg7	CTGTGGAACCAAACTCCAGC	AGCTGGTCGAGGTGAATGC	(AG)n	29	296-312	5	0.48	0.51	0.060
Gg11	AAGTTGCACGTTTCCCAGC	TACTGCAGGACCGGTTTCC	(TCCC)n	28	329-363	8	0.68	0.60	-0.132
Gg12	TGTCAAACACCATCGCAGG	TGCTCTGAAGTCTACAAGAATGG	(TA)n	25	276-296	11	0.70	0.72	0.024
Gg15	GGCTGAATGGTTTCCCAGC	GCCTCCAACTTAGCATAGCC	(GA)n	27	147-169	12	0.85	0.87	0.027
Gg16	AGTGTGGTCTCACCAATGC	TGGAAGGGTAAGGAAATTGGC	(GA)n	27	174-182	3	0.41	0.43	0.047
Gg17	CCTGCTTGTGACAGTTACCC	ACAGGCATCACCTCTGTGC	(AC)n	27	159-181	5	0.15	0.52	0.721
Gg18	TCCACTTCAGGAAGGCCAG	CAAAGCCAGGTGGTTCTCC	(GA)n	28	179-187	4	0.11	0.31	0.661
Gg20	GACCAAGGGTCATCCAGAC	TCAGCTTGGGCAATTCCAG	(TC)n	29	194-202	3	0.21	0.24	0.147
Gg22	TCCTGGGATGGCAAGTTCG	AGGCCACCCAACTATCCTG	(GT)n	30	237-247	7	0.63	0.82	0.229
Gg23	ACAGACCACAGGGCATGG	TGCAGAGCAGGCTAGATGG	(AC)n	28	258-278	9	0.86	0.83	-0.029

Table 1.2 PCR results of the 13 loci for *Mustelus californicus* and *M. henlei.* Successful PCRs indicated by +, stutter products by S, and failed reactions by 0.

	Gg2	Gg3	Gg4	Gg7	Gg11	Gg12	Gg15	Gg16	Gg17	Gg18	Gg20	Gg22	Gg23
Mustelus californicus	S	0	+	+	+	0	0	+	+	+	+	S	0
Mustelus henlei	S	+	+	+	+	0	0	+	+	+	+	+	0

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Chapter II

Microsatellite loci confirm a lack of gene flow among globally distributed populations of the tope shark, *Galeorhinus galeus* (Triakidae)

Abstract

The tope shark, *Galeorhinus galeus,* is a commercially important member of the Triakidae that has been globally exploited for greater than 80 years resulting in historic declines in population numbers and its placement into the IUCN threat category of Vulnerable. In order to effectively manage and conserve populations of *G. galeus*, it is important to determine the levels of connectivity among globally distributed populations and assess the taxonomic status of the species. This study used 13 polymorphic nuclear microsatellite loci to determine the population connectivity of five geographically isolated populations of *G. galeus* (Africa, Australia, North America, South America, and Western Europe). Genetic analyses revealed significant structure among all populations indicating a lack of gene flow. These findings indicate that globally distributed populations of *G. galeus* are isolated and should be managed as distinct, independent stocks.

Introduction

Within the marine environment it is commonly assumed that physical barriers are few and that the virtually continuous connectivity of bodies of water should enhance population connectivity (Rocha et al., 2005) resulting in shallow population structure (Waples, 1998). Based on this assumption, highly vagile marine species, such as pelagic wahoo (Theisen et al., 2008), whale sharks (Castro et al., 2007), and juvenile sea turtles (Bowen and Karl, 2007), or those species with extended periods of pelagic larval dispersal within currents, as observed in moray eels (Reece et al., 2010), are expected to and have indeed demonstrated weak to non-existent genetic population structure due to high levels of gene flow. Population connectivity is important for maintaining genetic diversity in the face of exploitation and the over-exploitation of natural resources is considered to be a significant threat to marine biodiversity. Fisheries targeting multiple taxa such as fishes, reptiles, and cetaceans have significantly reduced the abundances of numerous species resulting in their protection by various agencies (Bowen and Karl, 2007; Roman and Palumbi, 2003; Bowen et al., 2005). As a result of this over-exploitation, it has been estimated that commercial fisheries targeting large predatory fishes have reduced numbers by approximately 90% resulting in decreased landings and smaller fish (Myers and Worm, 2003; Myers et al., 2007).

Numerous populations of elasmobranchs are in widespread decline due to fisheries over-exploitation (Ferretti et al., 2010). These declines are a result of life-history characteristics including long life-spans, slow maturation rates, long gestation periods, and low fecundity (Hoenig and Gruber, 1990; Bonfil, 1994; Smith et al., 1998; Musick et al., 2000; Frisk et al., 2001) that impair their ability to rebound from such intense pressure. One such species, the tope

shark, *Galeorhinus galeus*, has had a long history of global exploitation predating the second World War in which fisheries were established to harvest their vitamin-A rich livers (Ebert, 2001). As a consequence of this intense fishery pressure, populations of *G. galeus* collapsed along the California coast, and nursery areas, such as Tomales Bay, California, have yet to recover (Ebert, 2001). It has been observed recently that populations of *G. galeus* are increasing off of the coast of southern California (Pondella and Allen, 2008). However, *G. galeus* is still being intensely harvested with ~50 million kilograms landed between 2000 and 2010 globally (FAO: http://www.fao.org/fishery/species/2828/en). This continuing pressure, along with its history of exploitation and population collapse, has led to its placement into a threat category (Vulnerable) by The World Conservation Union (Walker et al., 2006).

Galeorhinus galeus is a cosmopolitan, benthopelagic member of the Triakidae distributed anti-tropically in temperate waters (Compagno, 1984; Riede, 2004). Considered a relatively large member of the Triakidae with a total length of 200 cm and a maximum weight of 41 kg (Ripley, 1946; Capape and Mellinger, 1988), *G. galeus* has been observed traveling distances greater than 4,000 km between New Zealand and southern Australia in less than a 10 year period (Hurst et al., 1999). Vertical migration has also been observed in *G. galeus* with individuals occupying coastal shelf depths of 600 m during the day followed by migrations to shallower depths for hours at night (West and Stevens, 2001). Demographic patterns have been observed in *G. galeus* off of the California coast in which sexes are stratified both vertically (males regularly occupying depths between 38 and 220 m and females in shallower waters < 38 m) and latitudinally with males dominating the waters of northern California and females dominating the waters of southern California (Ripley, 1946). Similar to many elasmobranchs, life-history characteristics

of *G. galeus* include an estimated life-span of 40 years (Smith et al., 1998) with a generation time of 17.7 years (Cortes, 2002), age of sexual maturity between 8-10 years for males and females, respectively (Olsen, 1954), gestation approximately 12 months (Ripley, 1946; Lucifora et al., 2004), and litter sizes range between 6-54 pups with an average of 35 (Ripley, 1946).

Effective fisheries management requires confidence in the taxonomy of the managed species as well as information as to the number of fishery stocks. However, there has been some uncertainty as to the species' status and distribution of *G. galeus*. Prior to 1984, several species of *Galeorhinus* were recognized worldwide including *G. galeus* (Linnaeus, 1758) in Europe, *G. zyopterus* Jordan and Gilbert, 1883 in the northeastern Pacific, *G. australis* Macleay, 1881 in Australia, and *G. chilensis* Perez Canto, 1886 in the southeastern Pacific. Compagno (1984) placed all species into synonomy under *G. galeus.* However, Compagno (1984) did note that *G. galeus* in the northeastern Pacific has more vertebrae and reaches maturity later than its globally distributed conspecifics. Based on this observation, he left open the possibility of subdividing globally distributed populations of *G. galeus* into subspecies. With the potential uncertainty of the species' status, designating management units for *G. galeus* is difficult, especially if globally distributed populations are connected via migration. One possibility is to manage the species as a single panmictic stock capable of replenishing exploited populations from neighboring populations (i.e., a source-sink dynamic). A second management strategy would be to treat each globally distributed population as isolated and only capable of self-recruitment. However, data are currently insufficient to support either course of action.

To determine the degree of global population connectivity in *G. galeus*, Chabot and Allen (2009) used DNA sequence data obtained from the mitochondrial control region to investigate gene flow among five geographically distributed populations: Australia (Great Australian Bight and Tasmania), South Africa, North America (California), South America (Argentina and Peru), and Western Europe (the Irish Sea of the United Kingdom). Chabot and Allen (2009) discovered a lack of shared haplotypes (with the exception of the populations off South Africa and Australia that shared 1 haplotype) and a significant global Φ_{ST} value (0.84) among populations indicating significant genetic structuring in *G. galeus* globally. However, due to the matrilineal transmission of the mitochondrial control region (mtCR), patterns of gene flow are generally ascribed to female mediated gene flow (Avise, 2004). Based on this, nuclear microsatellites have been used to determine gene flow in numerous shark species because of their codominance, high mutation rates, and selective neutrality (Pardini et al., 2001; Keeney et al., 2005; Schultz et al., 2008; Karl et al., 2010; Veríssimo et al., 2010; Daly-Engel et al., 2012). This study used polymorphic nuclear microsatellites to test the findings of Chabot and Allen (2009) by exploring two questions: 1) do globally distributed populations of *G. galeus* belong to a single panmictic population, and, 2) what are the barriers, if any, to gene flow among populations of the species?

Materials and Methods

Sampling

Tissue samples were collected as described in Chabot and Allen (2009) from both male and female adult *G. galeus* from throughout the range of the species with the exception of two neonates from the northeastern Pacific (Figure 2.1) between 1997 and 2006. In total, 114 samples were genotyped from six geographically distributed populations: Argentina (n =1),

Australia (AUS) (Great Australian Bight n = 24 and Tasmania n = 25), North America (NA) (California n = 25), Peru (n = 11), South Africa (AF) (n = 16), and Western Europe (UK) (Irish Sea n = 12). Because only a single individual was obtained from the southwestern Atlantic (Argentina) and the haplotype for this individual was identical to those from Peru (Chabot and Allen, 2009), a single population from South America (SA = Peru and Argentina) was used for analyses.

DNA Extraction and Amplification

DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. As sample sizes were relatively small in three of the five populations, maximizing the power to detect genetic variation within and among populations was a necessity. Although increasing sample sizes is expected to increase the power to detect patterns of genetic variation marginally, increasing the number of loci used in genetic analyses has been demonstrated to have a greater effect on the power to detect genetic variation when compared to that of increasing sample size (Cornuet and Luikart, 1996). Therefore, thirteen polymorphic microsatellite loci from Chabot and Nigenda (2011) were used for this study and all polymerase chain reactions (PCR) were performed as described in Chabot and Nigenda (2011).

Statistical Analyses

Genetic Diversity

MICRO-CHECKER 2.2.3 (van Oosterhout et al., 2004) was used to detect the presence of null alleles, large allele dropout, and stuttering. Departures from Hardy-Weinberg Equilibrium and Observed heterozygosity (H_O) and expected heterozygosity (H_E) were estimated in GENEPOP

4.0 (Raymond and Rousset, 1995; Rousset, 2008). FSTAT 2.9.3.2 (Goudet, 2003) was used to test linkage disequilibrium (LD), provide the total number of alleles, and estimate allelic richness (A_R).

Genetic Divergence and Population Structure

STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003; Falush et al., 2007) was used to test for the presence of multiple, independent spawning stocks of *G. galeus*. Number of subpopulations (K) was estimated with five independent runs of K = 1 - 10. Each run was performed with 900,000 MCMC repetitions and a burn-in of 100,000 steps under the admixture model with correlated allele frequencies. The optimal number of subpopulations was estimated using ΔK of Evanno et al. (2005) as implemented in STRUCTURE HARVESTER (Earl and vonHoldt, 2012).

Population structure was estimated using analysis of molecular variance (AMOVA) and pairwise population F_{ST} significance tests as implemented in ARLEQUIN 3.5.1.2 (Excoffier and Lischer, 2010). Overall F_{ST} for *G. galeus* was estimated by assuming a lack of *a priori* structure in an AMOVA analysis and the mtCR population structure observed in Chabot and Allen (2009) was tested by pairwise F_{ST}. To test for population connectivity across ocean basins and equatorial waters, hierarchical AMOVA analyses were employed in which the genetic variance among groups (F_{CT}), among populations within group*s* (F_{SC}), and within populations (F_{ST}) was estimated. To determine the effect of ocean basins, three groups were analyzed including one group consisting of North American and South American samples, a second group consisting of Western European and African samples, and a final group of Australian samples. In order to

determine the effect of warm equatorial waters on gene flow, samples were grouped by hemisphere (e.g., Northern = North America and Western Europe and Southern = South America, Africa, and Australia). Estimates were tested nonparametrically (5,000 bootstraped replicates) and significance was adjusted for simultaneous pairwise tests using the sequential Bonferroni correction of Rice (1989). To determine the statistical power of the microsatellite loci used in the present study to detect genetic divergence and to reject the null hypothesis of panmixia among localities of *G. galeus*, power simulations were conducted in POWSIM 4.1 (Ryman and Palm, 2006). Simulation settings were a minimum F_{ST} of 0.05, a value indicated by Balloux and Lugon-Moulin (2002) to be the upper threshold of weak divergence for microsatellite loci, 500 replicates, and sample sizes from populations after the simulated drift process equal to those of the present study (n = 49, 25, 16, 12, and 11). F_{ST} is commonly used to assess population subdivision. However, due to the high mutation rate of microsatellites, F_{ST} may underestimate population subdivision (Rousset, 1996). Therefore, Hedrick's G'_{ST} (Hedrick, 2005) and Jost's D (Jost, 2008) were estimated in SMOGD (Crawford, 2010) and averaged across all loci. Both estimators produce values between 0 and 1 with 0 indicating panmixia and 1 being indicative of a lack of migration.

Demographic Analyses

When populations undergo recent declines in effective population sizes (N_e) due to bottlenecks, observed heterozygosity at neutral loci is generally much greater than that expected by the number of alleles (i.e., an excess in heterozygosity is observed). To detect recent declines in N_e within populations of *G. galeus*, BOTTLENECK 1.2.02 (Piry et al., 1999) was implemented under the two phase model (TPM) with 20,000 replications, 5% of multistep mutations, and

variance among multiple steps of 12 as recommended for microsatellites (Piry et al., 1999). The significance of any observed heterozygote excess was assessed by a one-tailed Wilcoxon's signed rank test; a test considered to be the most informative and robust for microsatellites (Piry et al., 1999). To further investigate the possibility of bottlenecked populations of *G. galeus,* the modified *M* ratio test of Garza and Williamson (2001) was also calculated for each population in ARLEQUIN 3.5.1.2. This test calculates the ratio of the number of alleles at a given locus and the range of allele sizes with the expectation that the number of alleles in a population that has experienced a bottleneck will be reduced faster than the range of allele sizes (Garza and Williamson, 2001).

Results

Genetic Diversity

MICRO-CHECKER detected an excess of homozygotes and the possibility of null alleles for GG4, GG17, GG18, GG22, and GG23. However, these results were generally population specific (GG4 and GG22 were detected in North America, GG23 in Africa, and GG17 and GG18 were found in all populations with the exception of Africa (0 null alleles for either) and Western Europe (only GG17 was detected)). All loci were in Hardy-Weinberg equilibrium after corrections for multiple tests with the exception of GG17 and GG18. However, as above, this finding was observed in only two populations, Australia and North America. All other populations were in Hardy-Weinberg equilibrium at these loci after corrections for multiple tests. To determine the effect of these potentially problematic loci on estimates of gene flow and population connectivity, statistical analyses were performed with and without the problematic loci mentioned above. Removal of the loci did not change the general outcome of the analyses (Appendix 2 and Appendix 3) and the following results are based on all 13 loci. Observed heterozygosities (H_O) ranged between 0.31 - 0.45 and expected heterozygosities (H_E) were between 0.39 - 0.56 (Table 2.1). No evidence of LD was detected for any of the loci. The observed number of alleles was between 36 - 71 and allelic richness ranged between 2.50 - 3.52 (Table 2.1). Private alleles were detected in each population with the greatest number (n =14) observed in Australia and the least (n = 1) observed in South America (Table 2.1).

Genetic Divergence and Population Structure

Results of the STRUCTURE analysis indicated the greatest likelihood of $K = 5$ populations comprised of distinct clusters of individuals from the African, Australian, and Western European

populations. A single cluster of individuals from the South American and North American populations was observed and the Australian sample was subdivided into two clusters (Figure 2.2). To test these findings, STRUCTURE analyses were performed on both the Australian population and the hypothesized North American + South American population using the parameters described above (with the exception of K being tested between 1 - 3). In both cases, $K = 1$ had the greatest posterior probability (data not shown).

The AMOVA analysis without *a priori* population groupings revealed significant structure in *G. galeus* with an overall F_{ST} of 0.245 ($p < 0.0001$). Results of pairwise F_{ST} analyses revealed significant structuring among all populations with Africa and Western Europe demonstrating the greatest structure and North America and South America the least (Table 2.2). Hierarchical AMOVA analyses demonstrated non-significant F_{CT} values for groups of populations across both ocean basins and equatorial waters (Table 2.3). However, significant divergences of populations within groups across both ocean basins and equatorial waters were observed with significant F_{SC} values ranging between 0.217 - 0.233 ($p < 0.0001$) (Table 2.3). Power simulations from POWSIM demonstrated the power of the microsatellite loci and sample sizes used in the present study to detect divergence among localities of *G. galeus* with a power of 100% and a minimum F_{ST} of at least 0.05. G'_{ST} and Jost's D also demonstrated significant divergence among populations and the structure obtained by pairwise F_{ST} analyses was also recovered (Table 2.4).

Evidence of Bottlenecks

BOTTLENECK did not reveal any significant heterozygote excess in any of the populations of *G. galeus* (Table 2.1). However, modified Garza-Williamson M ratio values were low (0.15 - 0.29)

(Table 2.1) for all populations suggesting historic reductions in population sizes. Garza and Williamson (2001) have indicated that *M* ratios > 0.8 represent stable populations and that ratios < 0.69 represent reduced or island populations.

Discussion

Despite having the potential to migrate over thousands of kilometers (Hurst et al., 1999) at both mesopelagic and bathypelagic depths (Riede, 2004), a life-history characteristic that would be expected to promote high levels of connectivity and gene flow among global populations of *G. galeus*, results from 13 polymorphic microsatellite loci indicate profound barriers to genetic exchange among these populations. Significant structure was observed among populations of *G. galeus* across ocean basins (pairwise F_{ST} values between 0.23 - 0.37 and $F_{SC} = 0.26$; Tables 2.2 and 2.3) and across the Equator (pairwise F_{ST} values between 0.1 - 0.32 and $F_{SC} = 0.23$; Tables 2.2 and 2.3). These findings support those of Chabot and Allen (2009) in which the global phylogeography of *G. galeus,* determined by mitochondrial control region sequence data, also demonstrated significant structure across ocean basins and the Equator.

Unlike many marine taxa with limited dispersal potential, due primarily to planktonic larval dispersal in which the dispersal of individuals is dependent on the speed and direction of currents (Palumbi, 1994; Rodriguez-Lanetty and Hoegh-Guldberg, 2002; Ackiss et al., 2013), sharks typically give birth to live young that are capable of both swimming and feeding shortly after parturition (Lowe, 2002; Duncan et al., 2006). This combination of life-history traits in neonate sharks would be expected to increase dispersal potential and increase population connectivity among geographically distributed populations. Despite the high dispersal potential of sharks, barriers and behaviors capable of affecting population connectivity in globally distributed species of sharks are being observed more commonly as the number of studies increases (Schrey and Heist, 2003; Keeney et al., 2005; Duncan et al., 2006; Keeney and Heist, 2006; Schultz et al.,

2008; Chabot and Allen, 2009; Veríssimo et al., 2010; Blower et al., 2012; Daly-Engel et al., 2012).

Distance across ocean basins has been indicated as a barrier to population connectivity in numerous marine taxa with coastal distributions (Palumbi, 1994; Natoli et al., 2006; Oremus et al., 2009; Luiz et al., 2012) including sharks (Duncan et al., 2006; Keeney and Heist, 2006). However, this pattern is not consistent among all shark species as contemporary gene flow across ocean basins has been observed in the scalloped hammerhead shark (*Sphryna lewini*) (Daly-Engel et al., 2012) and lemon sharks (*Negaprion*) (Schultz et al., 2008). Furthermore, Schultz et al. (2008) determined that ocean basin depth provided greater explanatory power than distance across ocean basins alone for the lack of observed gene flow across ocean basins in certain species of lemon sharks. Although ocean basins may present an obstacle to population connectivity in many coastal shark species, genetic data and tagging studies from pelagic species have demonstrated that this barrier is not insurmountable (Bonfil et al., 2005; Castro et al., 2007; Rus Hoelzel et al., 2006; Gore et al., 2008; da Silva et al., 2010; Veríssimo et al., 2010). It has been suggested that *G. galeus* be considered a pelagic species of shark (Duncan et al., 2006). Based on this, it would be expected that patterns of population connectivity in *G. galeus* would be similar to that of a pelagic species. However, patterns demonstrated in Chabot and Allen (2009) and from the present study are more consistent with those demonstrated by coastal species in which ocean basins play a significant role in divergence. Although populations of *G. galeus* possess region-specific haplotypes that are separated by basins (Chabot and Allen, 2009), a single shared haplotype was recovered between Africa and Australia. It is generally accepted that one effective migrant per generation is capable of reducing divergence due to drift (Kimura

and Ohta, 1971; Spieth, 1974; Mills and Allendorf, 1996). Based on the sharing of a haplotype between the two populations, it can be assumed that migration may occasionally occur between Africa and Australia and that divergence would be expected to take a greater amount of time. However, Chabot and Allen (2009) concluded that the shared haplotype observed between the two populations could be ancestral (based on the observation of significant Φ_{ST} values and a low migration rate between the two populations) and not indicative of current migration. The lack of observed gene flow between Africa and Australia demonstrated by this study (significant F_{ST} values between Africa and Australia and STRUCTURE analyses demonstrating a lack of admixture; Table 2.2 and Figure 2.2) would seem to support the hypothesis of Chabot and Allen (2009).

In addition to distance across ocean basins, temperature may also affect the population connectivity of marine taxa (Bowen and Grant, 1997; Scoles et al., 1998; Tranah and Allen, 1999) including temperate sharks. For example, Veríssimo et al. (2010) observed a lack of gene flow and differences in life-history traits between North and South Pacific populations of spiny dogfish and concluded that long-term isolation across the Pacific equator was responsible for their observations. Similarly, Ahonen et al. (2009) detected a lack of gene flow across the western Atlantic equator in the sand-tiger shark, *Carcharias taurus*. In contrast to these findings, both Schrey and Heist (2003) and Skomal et al. (2009) have observed shark species capable of crossing warm equatorial waters. Using satellite pop-up tags, Skomal et al. (2009) observed basking sharks tagged in the northwestern Atlantic crossing equatorial waters to South America and into the Southern Hemisphere. While crossing equatorial waters, these sharks descended to mesopelagic depths (between 200 - 1000 m) and remained there for periods of

weeks to months (Skomal et al., 2009). Similar to the spiny dogfish (Veríssimo et al., 2010) and the sand tiger shark (Ahonen et al., 2009), populations of *G. galeus* separated by warm equatorial waters appear to be isolated from one another. Significant population structure was observed between North America and South America as well as between Western Europe and Africa (Table 2.3) in regions with virtually continuous coastlines. This is somewhat unexpected because *G. galeus* is commonly found at depths between the lower mesopelagic and upper bathypelagic (Riede, 2004) where temperatures are quite low. Crossing beneath warm equatorial waters, as observed in the basking shark (Skomal et al., 2009), would appear to be a prospect available to *G. galeus.* However, unlike the basking shark, which is capable of spending weeks to months in this zone (Skomal et al., 2009), *G. galeus* is known to undergo vertical migrations and commonly travels to shallower depths at night (West and Stevens, 2001). If *G. galeus* were to cross equatorial waters, this daily vertical migration would be expected to expose *G. galeus* to temperatures warmer than those generally preferred by the species. This general absence in warmer waters has been observed in fossil distributions of the genus *Galeorhinus*. Prior to the closure of the Isthmus of Panama in the Pliocene and the subsequent warming of the northwestern Atlantic, *Galeorhinus* was distributed in the Gulf of Mexico and along the east coast of North America (Musick et al., 2004). It has been suggested that the change in temperature within the region that followed the closure of the Isthmus is most likely responsible for the extirpation of the genus in the region (Musick et al., 2004).

Although temperature has been indicated as a barrier to dispersal in temperate species of shark, incongruence between molecular markers may be indicative of the effect of behavior on dispersal. For example, using microsatellites, Schrey and Heist (2003) did not detect population

structure in Atlantic and Pacific Ocean populations of the mako shark, *Isurus oxyrinchus*, across by the equator. However, a previous study by Heist et al. (1996) detected significant population structure among mako shark populations using mitochondrial RFLP data leading Schrey and Heist (2003) to hypothesize that sex biased dispersal (i.e., female philopatry to natal sites) was most likely responsible for their observations. Philopatry, the return of a migrating animal to a specific location for the purpose of feeding or breeding, can produce significant levels of genetic divergence between populations of marine species considered to have high dispersal potentials (Baker et al., 1990; Meylan et al., 1990; Palumbi, 1994; Palumbi and Baker, 1994; Keeney et al., 2005; Bowen and Karl, 2007). The incongruence of genetic structure demonstrated by the matrilineally transmitted mitochondrial DNA and biparentally inherited nuclear microsatellites of *I. oxyrhinchus* described above (Heist et al., 1996; Schrey and Heist, 2003) is consistent with the expectation of female philopatry and male mediated gene flow. Several globally distributed shark species have demonstrated this pattern including the blacktip shark, *Carcharhinus limbatus* (Keeney et al., 2005), *C. carcharias* (Pardini et al., 2001), the sandbar shark, *Carcharhinus plumbeus* (Portnoy et al., 2010), *R. typus* (Castro et al., 2007; Schmidt et al., 2009) an *S. lewini* (Duncan et al., 2006; Daly-Engel et al., 2012). Although many species of shark appear to exhibit female philopatry and male mediated gene flow (see above), patterns of gene flow in *G. galeus* do not appear to support male mediated gene flow among the sampled locations. Both mitochondrial sequence data (Chabot and Allen 2009) and microsatellite data from the present study demonstrate a significant lack of gene flow among all globally distributed populations. Therefore, based on the genetic criteria for female philopatry presented above, female philopatry does not appear to play a role in the structuring of populations of *G. galeus* on a global scale.

However, female philopatric behavior has not been evaluated within regions and may occur at a smaller scale.

Demographic History, Species Status, and Conservation Implications

Galeorhinus galeus has been exploited globally for greater than 80 years earning the species a listing of Vulnerable by the IUCN (Walker et al., 2006). Similar to the spiny dogfish, a species of shark with the lowest intrinsic rebound potential of any shark species (Smith et al., 1998), *G. galeus* has an extremely low rebound potential due to a long life-span and late maturity (Smith et al., 1998). As a consequence of these life-history traits, populations have crashed historically in the northeastern Pacific (Ebert, 2001) and have been extirpated from the coast of Uruguay (Walker et al., 2006). Although BOTTLENECK failed to detect an excess of heterozygosity in any of the populations of *G. galeus*, low Garza-Williamson modified *M* ratios were observed in these populations (Table 2.1) indicating a reduction in the number of alleles and a possibly greater sensitivity of the index to detect past reductions in population size. These results allude to historic reductions in population sizes in *G. galeus,* however, the scale at which genetic estimates of population decline are most credible is evolutionary and not ecological (i.e., contemporary) and should therefore be considered with caution.

Data from Chabot and Allen (2009) and the present study demonstrate a lack of gene flow among globally distributed populations of *G. galeus* consistent with reproductive isolation. Similar observations to *G. galeus* have been made within the North Pacific. Veríssimo et al. (2010) performed a global phylogeographic study of the spiny dogfish and demonstrated a complete lack of gene flow between North Pacific populations and all other populations

(Veríssimo et al., 2010). Furthermore, these authors also noted that North Pacific populations of spiny dogfish mature later, have greater maximum sizes, and live longer than individuals from all other populations (Veríssimo et al., 2010). Based on the observed lack of gene flow, differences in life-history traits and maximum size, these authors argued for the taxonomic separation of spiny dogfish in the North Pacific and a re-evaluation of the species based on morphological and molecular analyses (Veríssimo et al., 2010). Based on the lack of population connectivity observed in Chabot and Allen (2009) and the present study along with the observations of Compagno (1984) that *G. galeus* in the northeastern Pacific possess a greater number of vertebrae on average and mature at a later age when compared to other globally distributed populations of the species, the currently accepted placement of *G. australis*, *G. chilensis*, and *G. zyopterus* into synonomy with *G. galeus* should be re-examined and the taxonomic status of the northeastern Pacific *G. galeus* should be re-considered as suggested for *S. acanthias*. At the very least, each population should be considered a unique evolutionary significant unit (ESU) (Moritz, 1994) and managed as such.

From a conservation perspective, the removal of genetic diversity from any of the populations studied will result in the loss of unique genetic variation within regions. The continuing pressure of global fisheries on *G. galeus* is reducing population numbers and has the potential to regionally extirpate species of the genus. Historically, collapses in populations have been observed in *G. galeus* in the northeastern Pacific and the southwestern Atlantic (see above). These collapses, coupled with a low rebound potential (Smith et al., 1998), have earned *G. galeus* the conservation status of Vulnerable by the IUCN. Based on data from Chabot and Allen (2009) and the present study, the current conservation status of globally distributed

populations of *G. galeus* should be reassessed with a recommendation that the species be listed as endangered.

Conclusion

Microsatellite data from the present study support the findings of Chabot and Allen (2009) based on mitochondrial control region sequence data. As a result, all globally distributed populations of *G. galeus* should be considered distinct and isolated from one another and not members of a single panmictic population. Ocean basins and temperature appear to have the greatest affect on gene flow among populations of *G. galeus* and female philopatry and male mediated gene flow are not supported by genetic data. The currently accepted placement of *G. australis*, *G. chilensis*, and *G. zyopterus* into synonomy with *G. galeus* should be re-examined based on the genetic isolation of globally distributed populations and the observed variation in morphology and life-histories. Regardless of taxonomic status, each population of *G. galeus* represents an evolutionarily significant unit and should be managed accordingly. Furthermore, the conservation status of populations of *Galeorhinus* should be re-considered in light of their significant isolation and history of over-exploitation. Although this study attempted to survey the complete global distribution of *G. galeus*, sample numbers were low in certain regions and all potential sample localities were not surveyed (e.g., the Mediterranean Sea and New Zealand). Therefore, increasing sample sizes from South America, Africa, and the U.K., as well as obtaining samples from the Mediterranean Sea and New Zealand should further elucidate gene flow among populations of this commercially important elasmobranch.

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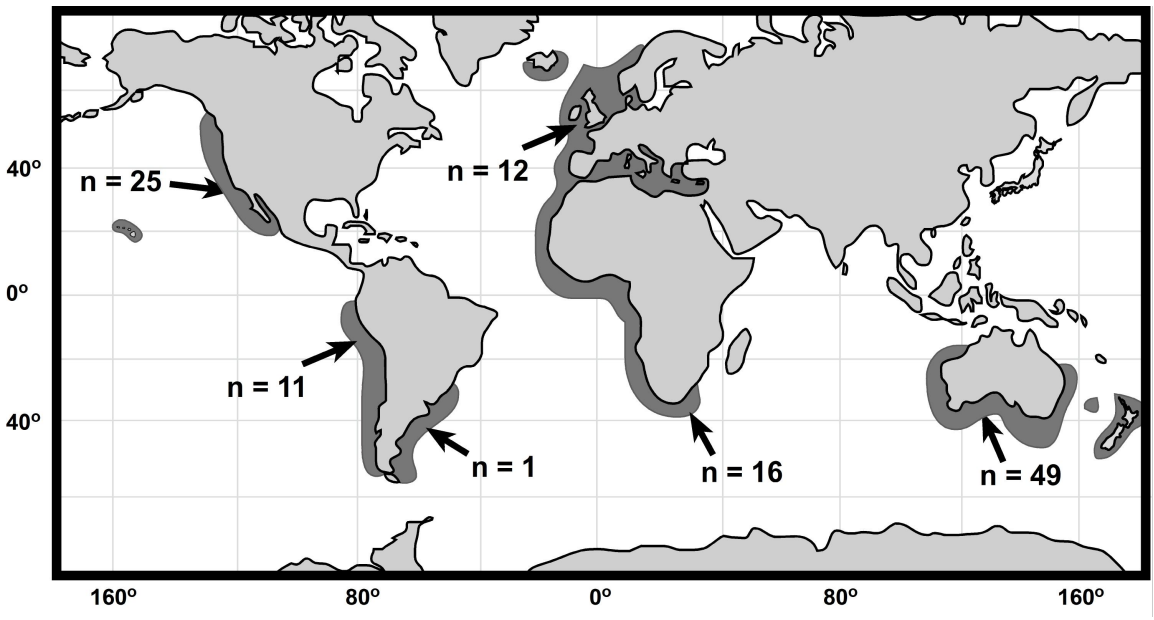


Figure 2.1 Global distribution of *Galeorhinus galeus* (shaded areas) and sample localities with sample numbers indicated by *n.*

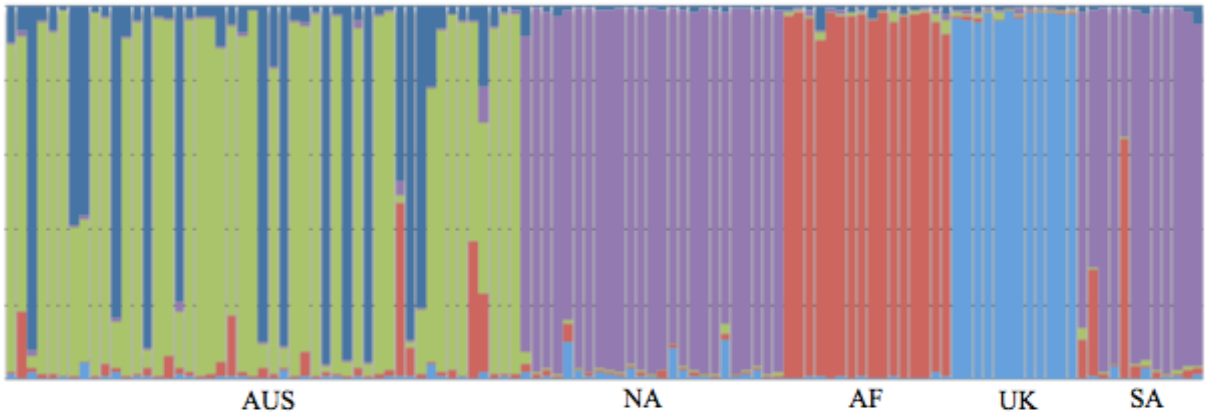


Figure 2.2 STRUCTURE analysis for *Galeorhinus galeus*.

Table 2.1 Summary statistics for *Galeorhinus galeus.*
N, Number of individuals; H_O, average observed heterozygosity; H_E, average expected heterozygosity; *A*, number of alleles; A_R, average allelic richness after rarefaction; *PA*, private alleles; *B*, Wilcoxon's test probability of heterozygote excess under the two phase model (TPM); *GW,* average modified Garza-Williamson *M* ratio.

Locality	N	H_O	H_E	A	A_R	PA	B	GW
Overall	114	0.393	0.45	98	3.84	—	p = 0.892	0.20 +/- 0.05
Australia	49	0.45	0.56	71	3.52	14	p = 0.339	0.29 +/- 0.14
N. America	25	0.33	0.47	47	2.78	7	p = 0.139	0.20 +/- 0.16
Africa	16	0.38	0.44	47	2.85	5	p = 0.924	0.19 +/- 0.10
W. Europe	12	0.35	0.42	40	2.67	6	p = 0.585	0.19 +/- 0.11
S. America	12	0.31	0.39	36	2.50	1	p = 0.515	0.15 +/- 0.07

Table 2.2 Pairwise F_{ST} values for *Galeorhinus galeus.*
F_{ST} values below diagonal *p* values above (* Indicates *P* values < 0.000001).

	Australia	N. America	Africa	W. Europe	S. America
Australia	—	*	*	*	*
N. America	0.24	—	*	*	*
Africa	0.23	0.24	—	*	*
W. Europe	0.26	0.25	0.37	—	*
S. America	0.23	0.10	0.23	0.32	—

Table 2.3 Hierarchical AMOVA for populations of *Galeorhinus galeus.*

Northern vs. Southern Hemisphere	
F_{CT}	0.017
F_{SC}	0.233***
F_{ST}	0.250***
Across ocean basins	
F_{CT}	0.027
F_{SC}	0.217***
F_{ST}	0.247***

P values < 0.0001 indicated by ***
Northern vs. Southern Hemisphere = NA/WEU vs AF/AUS/SA
Across ocean basins = NA/SA vs WEU/AF vs AUS

Table 2.4 Average G'_{ST} and Jost's D values for populations of *Galeorhinus galeus* including Australia, North America (N. America), Africa, Western Europe (W. Europe), and South America (S. America). G'_{ST} below diagonal and Jost's D above.

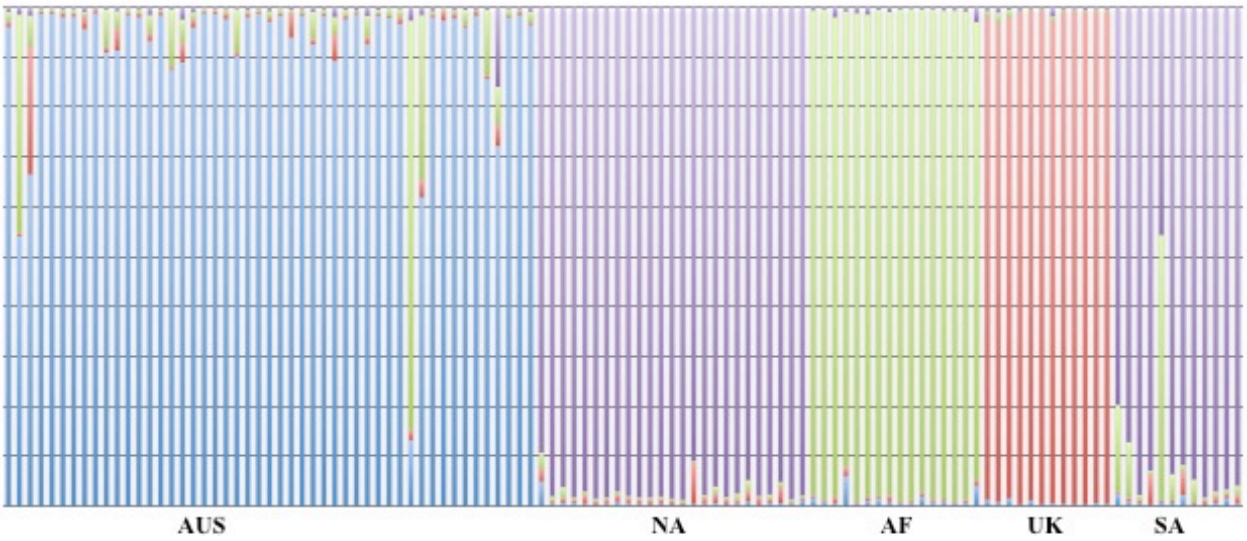
	Australia	**N. America**	**Africa**	**W. Europe**	**S. America**
Australia	—	0.34	0.33	0.40	0.37
N. America	0.41	—	0.29	0.28	0.15
Africa	0.40	0.35	—	0.47	0.24
W. Europe	0.50	0.37	0.53	—	0.41
S. America	0.43	0.19	0.29	0.47	—

Appendix 2.1 Summary statistics for 13 loci from within 5 populations of *Galeorhinus galeus*: Africa (AF), Australia (AUS), North America (NA), South America (SA), and the Western Europe (UK). *N*, number of samples; *A*, number of alleles; A_R, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; $F_{IS,}$ correlation of alleles within individuals compared to their subpopulation; *p*, F_{IS} *p* values (significant F_{IS} *P* values < 0.00077 indicated by *).

		AF	AUS	NA	SA	UK
GG2	N	16	49	24	12	12
	A	3	4	4	3	6
	A_R	2.9	2.7	2.6	2.5	4.4
	H_O	0.38	0.35	0.42	0.33	0.67
	H_E	0.55	0.4	0.41	0.55	0.73
	F_{IS}	0.32	0.12	-0.02	0.41	0.009
	p	0.07	0.15	0.68	0.1	0.4
GG3	N	16	45	25	12	12
	A	2	5	1	2	2
	A_R	1.8	2.4	1	1.8	1.8
	H_O	0.18	0.51	-	0.17	0.17
	H_E	0.18	0.52	-	0.16	0.16
	F_{IS}	-0.07	0.01	-	-0.05	-0.05
	p	1	0.54	-	1	1
GG4	N	16	49	25	12	12
	A	3	1	2	2	1
	A_R	2	1	2	1.8	1
	H_O	0.19	-	0.24	0	-
	H_E	0.18	-	0.47	0.16	-
	F_{IS}	-0.05	-	0.5	1	-
	p	1	-	0.02	0.04	-
GG7	N	16	49	25	10	12
	A	4	5	2	2	3
	A_R	2.7	3.4	2	2	2
	H_O	0.5	0.49	0.48	0.6	0.17
	H_E	0.41	0.57	0.47	0.44	0.16
	F_{IS}	-0.22	0.15	-0.02	-0.39	-0.02
	p	1	0.1	0.7	1	1
GG11	N	16	47	25	12	12
	A	4	8	1	3	1
	A_R	3.9	3.5	1	2	1
	H_O	0.75	0.64	-	0.17	-
	H_E	0.75	0.55	-	0.16	-
	F_{IS}	0	-0.16	-	-0.2	-
	p	0.6	0.98	-	1	-
GG12	N	15	46	24	11	12
	A	6	9	2	2	4
	A_R	4.8	5.3	1.4	2	3.7
	H_O	0.8	0.72	0.08	0.09	0.67
	H_E	0.69	0.77	0.08	0.25	0.68
	F_{IS}	-0.16	0.07	-0.02	0.64	0.02
	p	1	0.22	1	0.16	0.58

Appendix 2.1 continued

		AF	AUS	NA	SA	UK
GG15						
	A	6	11	3	5	2
	A_R	4.1	6.8	3	4.7	2
	H_O	0.6	0.8	0.54	0.82	0.67
	H_E	0.67	0.89	0.64	0.8	0.46
	F_{IS}	0.11	0.09	0.16	-0.02	-0.5
	p	0.36	0.07	0.19	0.69	1
GG16	N	15	46	24	12	12
	A	2	3	3	1	3
	A_R	1.8	2.2	2.2	1	2.7
	H_O	0.2	0.43	0.29	-	0.33
	H_E	0.19	0.45	0.26	-	0.42
	F_{IS}	-0.08	0.05	-0.11	-	0.21
	p	1	0.42	1	-	0.31
GG17	N	16	49	23	8	11
	A	4	6	6	3	4
	A_R	2.8	4.1	4.1	2.5	3.1
	H_O	0.25	0.14	0.04	0.13	0.27
	H_E	0.34	0.65	0.65	0.24	0.61
	F_{IS}	0.26	0.78	0.94	0.5	0.57
	p	0.17	0.0008	0.0008	0.07	0.021
GG18	N	16	45	22	12	12
	A	1	3	4	2	3
	A_R	1	2.5	3.4	2	2.9
	H_O	-	0.11	0.46	0	0.58
	H_E	-	0.53	0.68	0.39	0.48
	F_{IS}	-	0.79	0.34	1	-0.24
	p	-	0.0008	0.02	0.0008	1
GG20	N	16	49	25	11	10
	A	4	2	2	3	2
	A_R	2.7	1.9	2	2.8	2
	H_O	0.56	0.25	0.28	0.36	0.1
	H_E	0.45	0.28	0.51	0.54	0.27
	F_{IS}	-0.27	0.11	0.45	0.33	0.64
	p	1	0.38	0.031	0.15	0.15
GG22	N	15	49	23	6	12
	A	5	6	5	3	4
	A_R	3.8	4.2	4	3	3.7
	H_O	0.6	0.65	0.52	0.67	0.42
	H_E	0.7	0.74	0.74	0.68	0.68
	F_{IS}	0.15	0.12	0.3	0.02	0.4
	p	0.26	0.1	0.02	0.59	0.32
GG23	N	15	45	22	9	11
	A	3	8	12	5	5
	A_R	2.9	5.9	7.3	4.5	4.6
	H_O	0	0.78	0.91	0.67	0.55
	H_E	0.61	0.84	0.9	0.72	0.75
	F_{IS}	1	0.07	-0.01	0.08	0.28
	p	0.0008	-0.16	0.7	0.47	0.1



Appendix 2.2 STRUCTURE analysis for *Galeorhinus galeus* without loci GG17 and GG18.

Appendix 2.3 Pairwise F_{ST} values for *Galeorhinus galeus*.
Values above diagonal without loci GG17 and GG18 and values below with GG17 and GG18.

	Australia	N. America	Africa	W. Europe	S. America
Australia	—	0.27*	0.21*	0.26*	0.27*
N. America	0.24*	—	0.21*	0.27*	0.11*
Africa	0.23*	0.24*	—	0.32*	0.17*
W. Europe	0.26*	0.25*	0.37*	—	0.3*
S. America	0.23*	0.1*	0.23*	0.32*	—

Significant F_{ST} values ($P < 0.000001$) indicated by *

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Chapter III

Characterization of 11 microsatellite loci for the brown smoothhound shark, *Mustelus henlei* (Triakidae), discovered with next-generation sequencing

Abstract

The brown smoothhound shark, *Mustelus henlei* (Triakidae), is an endemic member of the eastern Pacific shark assemblage considered both commercially and recreationally important. Here, eleven microsatellite loci for *M. henlei* discovered by next-generation sequencing (Roche 454 pyrosequencing) are described. All loci were polymorphic (3-10 alleles) with observed heterozygosities between 0.24-0.89 and expected heterozygosities between 0.23-0.86. These loci are the first to be characterized explicitly for *M. henlei* and should be useful for the investigation of population structure and gene flow in this species and for other members of the Triakidae.

The houndshark family, Triakidae, is a primary component of the eastern Pacific elasmobranch assemblage considered both commercially and recreationally important (Compagno et al., 2005; Compagno, 1984). Several species are considered endemic to the eastern Pacific including *Mustelus henlei*, *Mustelus californicus*, *Triakis semifasciata*, *Mustelus hacat*, and *Mustelus lunulatus* (Compagno, 1984; Compagno et al., 2005; Pérez Jiménez et al., 2005; Ebert, 2003). Although *M. henlei* is a common component of the nearshore shark assemblage in the northeastern Pacific, there is a lack of information pertaining to the population structure and gene flow of the species. Therefore, the goal of this project was to generate a library of microsatellite markers for *M. henlei* using next-generation sequencing technology (Roche 454 pyrosequencing) in order to measure population connectivity along the eastern Pacific and provide vital information for the management and conservation of this species.

DNA used for the generation of the microsatellite library was extracted from the fin clip of an individual from Santa Barbara, California using the DNeasy blood and tissue extraction kit (Qiagen, Valencia, USA) following the manufacturer's protocols. 500 ng of DNA was prepared for whole genome shotgun sequencing on the Roche Genome Sequencer FLX instrument utilizing the GS FLX Titanium Rapid Library Preparation Kit (Roche Applied Sciences, Indianapolis, USA) following the manufacturer's protocol. The library was quantified for DNA fragment size distribution and concentration (Agilent 2100 Bioanalyzer) and then processed through the GS FLX emulsion polymerase chain reaction (PCR). Sequencing was performed in a GS FLX Titanium picotiterplate and yielded 22,309 sequences.

The sequences were screened for potential microsatellite loci by MSATCOMMANDER

(Faircloth, 2008) under the default settings. Of the 22,309 sequences, 1,124 contained putative microsatellite loci. Similar to the studies of the Australian gummy shark (Boomer and Stow, 2010) and the tope shark (Chabot and Nigenda, 2011), the majority of microsatellite motifs identified were dinucleotide in nature (68%). Primers for dinucleotide (minimum repeat number (mrn) = 8) and tetranucleotide (mrn = 4) loci were designed by PRIMER3 (Rozen and Skaletsky, 2000) embedded in MSATCOMMANDER using the default settings. In total, 28 primer pairs were used for amplification trials consisting of 22 dinucleotide and six tetranucleotide loci. For all loci, the forward primer was synthesized with an M13F(-20) sequence (GTAAAACGACGGCCAG) added to the 5' end to incorporate a 5′ fluorescent label per the technique of (Boutin-Ganache et al., 2001). Chabot and Nigenda (2011) identified nine microsatellite loci from *Galeorhinus galeus* (Gg3, Gg4, Gg7, Gg11, Gg16, Gg17, Gg18, Gg20, and Gg22) that cross-amplified in *M. henlei*. These loci were screened alongside those obtained from *M. henlei.* Initially, eight samples from Santa Barbara, California were used to test the amplification of loci and evaluate polymorphic content. The PCR protocol was as follows: A 10 μL touchdown PCR was performed using an Eppendorf Mastercycler *epgradient* S thermal cycler and the following reaction conditions: 10-100 ng template DNA, 0.2 μM reverse primer, 0.01 μM forward primer, 0.01 μM dye labeled M13 primer, 0.4 mg/mL BSA, and 5.0 μL of Qiagen Multiplex Mastermix (Qiagen, Valencia, USA). Initial denaturation was at 95°C for 15 minutes followed by 25 cycles of denaturation (94°C for 30 seconds), annealing (59°C for 90 seconds), extension (72°C for 60 seconds) and another 20 cycles of denaturation (94°C for 30 seconds), annealing (53°C for 90 seconds), extension (72°C for 60 seconds), and terminating with a final extension (60°C for 30 minutes). All PCR products were then electrophoresed on an Applied Biosystems (ABI) 3730*xl* DNA Analyzer. Allele sizes were determined by using an

internal lane standard LIZ 500 (ABI) and GeneMapper® 3.7 (ABI). Out of the 37 primer pairs tested, 11 loci (10 from *M. henlei* and one from *G. galeus*) were successfully amplified by PCR and further characterized using additional samples from Santa Barbara, California (n = 24).

MICROCHECKER (Van Oosterhout et al., 2003) was used to investigate the existence of null alleles, large allele dropout, and stuttering. With the exception of Mh5, Mh29, and Gg4, all loci demonstrated a lack of null alleles. GENEPOP 4.0 (Raymond and Rousset, 1995; Rousset, 2008) was used to estimate allele frequencies, observed heterozygosity (H_O) and expected heterozygosity (H_E), and determine departures from Hardy-Weinberg equilibrium (HWE). All 11 loci were polymorphic (3-10 alleles). H_O and H_E were 0.24-0.89 and 0.23-0.86 respectively (Table 3.1) and after Bonferroni correction all loci were in HWE with the exception of Mh5, Mh29, and Gg4. FSTAT 2.9.4 (Goudet, 2003) was used to test for linkage disequilibrium and estimate F_{IS}. All loci were in linkage equilibrium and F_{IS} ranged between -0.155-0.625 (Table 3.1).

The development of these 10 microsatellite loci from *M.henlei* using next-generation sequencing technology, along with those of Boomer and Stow (2010) and Chabot and Nigenda (2011), should aid in the elucidation of gene flow within species of the Triakidae and provide valuable tools for the conservation of potentially threatened or exploited shark species.

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Table 3.1 Characterization of 11 microsatellite loci for *Mustelus henlei*.
N number of samples used for characterization, *Size* size of alleles, *A* number of alleles, H_O observed heterozygosity, H_E expected heterozygosity.

Locus	Forward Primer 5' - 3'	Reverse Primer 5' - 3'	Motif	N	Size (bp)	A	H_O	H_E	F_{IS}
Mh1	GGAGGAGGGAAGCCTATGG	TCTCTGGCTCCATTCAGGG	(AG)n	27	178-222	10	0.89	0.83	-0.075
Mh5	CCATTCAAAGGTCTGGTGGC	GCAGCTTCTGGCACTTGAG	(AT)n	29	302-366	10	0.55	0.86	0.361
Mh6	CATGTCCACTTCCCATCGC	GGAGAGATTAGAACAGGTGGC	(CT)n	31	191-203	6	0.44	0.58	0.242
Mh13	ACTCGATAGGCCAAAGGGC	ATGACTGGGCACCTCCAAG	(ATCT)n	32	194-212	6	0.34	0.34	-0.021
Mh15	TGCCCTTCGAGGTGGTAAG	TAGGCTGGAAAGTTGGGAG	(ATTT)n	25	416-426	4	0.24	0.23	-0.071
Mh16	GTTGATGCGGACTCACTGG	TGTCATCTGCTCCTCACCG	(GGGT)n	30	224-226	2	0.37	0.38	0.039
Mh25	TGCAATAACCGTTCTGCGTC	TCACACCCGCAGTTAGATCC	(CT)n	32	156-170	6	0.63	0.64	0.027
Mh29	ATCAGCCCAGATTGTCCGC	AGACATTCCGCCTTCCAGC	(CT)n	28	196-204	5	0.25	0.66	0.625
Mh34	CCCTTTCTAGGCTTGGCAC	CCCTCTCTCTGGAGTTGGAAG	(AG)n	30	221-225	3	0.3	0.34	0.111
Mh36	ACGATGGAGTTGACATGTATGC	ATGAGCAGCCTGGGAATGG	(AT)n	26	245-251	4	0.42	0.37	-0.155
Gg4	CTGGAATACATGCCGAGCAC	CCCGAAAGGTCTTAGTTCGC	(GA)n	27	210-214	3	0.37	0.63	0.415

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Chapter IV

Frequency of multiple paternity varies between two populations of brown smoothhound shark, *Mustelus henlei*

Abstract

Multiple paternity was recently observed in a population of the brown smoothhound shark, *Mustelus henlei*, from Las Barrancas, Baja California Sur, Mexico with litters demonstrating the greatest percentage of multiple paternity for any shark species (93% of litters and an average number of sires = 2.3). To determine if this frequency is consistent elsewhere in the species' range, 4 polymorphic microsatellite loci were used to determine the frequency of multiple paternity in 18 litters of *M. henlei* from Santa Catalina Island, CA sampled in 2004, 2008, and 2012. Multiple paternity varied among sampling years with 2004 demonstrating multiple sires for 40% of sampled litters (n = 10) with an average of 1.4 sires per litter and 2008/2012 demonstrating a total lack of multiply sired litters (n = 8). Although multiple paternity was detected in this study, the range of frequencies observed are lower than that observed in the Mexican population. Based on these findings, investigators should take location into consideration when assessing the existence of multiple paternity in future studies of elasmobranch species.

Introduction

Polyandry has been demonstrated in several vertebrate taxa including mammals (marmots and meerkats), birds (tits, warblers, and red-winged blackbirds), reptiles (tuatara, box turtles, pythons, and *Anolis*), and amphibians (salamanders and frogs) (Kempenaers et al., 1992; Stutchbury et al., 1994; Kempenaers et al., 1997; Krokene et al., 1998; Pearse et al., 2002; Lodé and Lesbarrères, 2004; Myers and Zamudio, 2004; Madsen et al., 2005; Westneat and Mays, 2005; Cohas et al., 2006; Calsbeek et al., 2007; Young et al., 2007; Moore et al., 2008). Numerous theories have been proposed to explain this phenomenon with the majority assuming that females increase their fitness due to multiple mating. Of these theories, reducing the damage inflicted by aggressive males (Chapman et al., 1995), trading up in order to mate with males of higher quality (Kempenaers et al., 1992), increasing the genetic variation of offspring to compensate for environmental instability (Yasui, 1998; 2001), the sexy sperm hypothesis in which sons have competitive dominance in regards to sperm function (Keller and Reeve, 1995), and bet hedging in which females reduce the probability of mating with genetically incompatible males (Zeh and Zeh, 1996; Zeh, 1997) are cited most commonly within the primary literature.

Globally, both numbers and sizes of large marine predators are decreasing at an alarming rate due to the over-exploitation of fisheries (Myers and Worm, 2003; Coleman et al., 2004). Especially vulnerable to exploitation are the numerous elasmobranch species known to possess life-history characteristics including long lifespans, late maturity, and low numbers of offspring that make their recovery from intense over-exploitation slow to impossible (Hoenig and Gruber, 1990; Bonfil, 1994; Smith et al., 1998; Musick et al., 2000; Frisk et al., 2001). Among elasmobranchs, polyandrous behavior has been demonstrated in populations of thornback rays

(*Raja clavata*), nurse sharks (*Ginglymostoma cirratum*), sandbar sharks (*Carcharhinus plumbeus*), lemon sharks (*Negaprion brevirostris*), bonnethead sharks (*Sphyrna tiburo*), spiny dogfish (*Squalus acanthias*), rig (*Mustelus lenticulatus*), gummy shark (*Mustelus antarcticus*), sand tiger shark (*Carcharias taurus*), and leopard shark (*Triakis semifasciata*) (Saville et al., 2002; Chapman et al., 2004; Feldheim et al., 2004; Chevolot et al., 2007; Daly-Engel et al., 2007; Lage et al., 2008; Heist et al., 2011; Veríssimo et al., 2011; Boomer et al., 2013; Chapman et al., 2013; Nosal et al., 2013). Several hypotheses have been put forward to explain the observation of polyandry within elasmobranchs. Mating in sharks is a violent process in which males bite females in order to secure copulations. When genetic benefits are not indicated by polyandrous behavior, the reduction of physical damage inflicted by coercive males has been attributed to the evolution of polyandrous behavior (Keeney et al., 2005; Portnoy et al., 2007). Aside from coercion, assuming that each female mates with every male she encounters during a breeding season, it has been suggested that the occurrence of polyandry between conspecific males and females may simply be a function of encounter rate (Daly-Engel et al., 2007). Finally, it has also been suggested that polyandry may have evolved as a possible consequence of exploitation and significant population declines (Chevolot et al., 2007).

Houndsharks (Triakidae) are a primary component of the northeastern Pacific elasmobranch assemblage considered both commercially and recreationally important (Compagno, 1984; Compagno et al., 2005). Several members of the group are endemic to the temperate eastern Pacific including *Mustelus henlei*, *Mustelus californicus*, *Triakis semifasciata*, *Mustelus albipinnis*, and *Mustelus lunulatus* (Compagno, 1984; Ebert, 2003; Castro-Aguirre et al., 2005; Compagno et al., 2005; Pérez-Jiménez et al., 2005) with *M. californicus*, *M. albipinnis*, and *T.*

semifasciata occurring north of the equator in cooler subtropical to temperate waters (Miller and Lea, 1972; Compagno, 1984; Ebert, 2003; Compagno et al., 2005). *Mustelus henlei* is distributed within temperate coastal waters at depths ranging from the shallow intertidal to 200 m (Ebert, 2003) with a geographic range spanning Coos Bay, Oregon (North America) to Peru and Ecuador (South America) (Compagno, 1984; Eschmeyer et al., 1999). Estimates of maximum total length and age are reported as 100 cm and 13 years (Yudin and Cailliet, 1990; Smith et al., 1998) with estimates of age at first maturity occurring at 2-3 years (Yudin and Cailliet, 1990). Reproduction is through placental viviparity with periods of gestation lasting one year and each female capable of producing between 1-10 pups (Yudin, 1987). Recently, Byrne and Avise (2012) observed the occurrence of polyandry in *M. henlei* from Las Barrancas, Baja California Sur, Mexico. Based on four microsatellite markers, Byrne and Avise (2012) observed the highest frequency of multiply sired litters of any elasmobranch to date with 13 of 14 litters (93%) demonstrating multiple paternity and a minimum number of sires ranging from one to three.

Variation in the frequency of multiple paternity has been observed geographically within elasmobranchs (Daly-Engel et al., 2007; Portnoy et al., 2007; Lage et al., 2008; Veríssimo et al., 2011). As the majority of studies on elasmobranch polyandry have focused on single populations, demographic processes that can potentially influence the frequency of this reproductive mode, such as varying mate encounter rates among populations, may be overlooked and the characterization of a species' reproductive biology will be biased towards a single observation. To explore this possibility, this study sought to determine the level of polyandry within a geographically distant population of *M. henlei* from that of Byrne and Avise (2012) across several time periods and to compare the frequencies of both regions in order to determine

the effect of varying demographic processes between geographically distant populations on the reproductive strategies of the brown smoothhound shark in the northeastern Pacific.

Materials and Methods

Sampling

Mustelus henlei was collected at Santa Catalina Island, CA by gill netting (OREHP (Ocean Resources Enhancement Hatchery Program) white seabass monitoring program) in June and October of 2004 and June of 2008, and by hook and line in June of 2012. Prior to the initiation of this study, 10 pregnant sharks collected in 2004 (n = 10) were brought to the California State University, Northridge where fetuses were removed and placed into 70% ethanol. Unfortunately, Tissue samples from the mothers of these litters were not secured prior to their disposal. Pregnant females from 2008 (n = 5) and 2012 (n = 3) were placed on dry ice in the field and brought back to the University of California, Los Angeles (UCLA) and stored at -20^{o} C until litters could be removed. An adult male collected with the females from 2008 was deposited at the Scripps Institution of Oceanography as a voucher specimen (SIO 13-25).

Extraction and Amplification

Due to the improper storage of fetuses from 2004 (70% ethanol for eight years) two methods were used to extract DNA from litters of *M. henlei.* A standard phenol chloroform-iso-amyl alcohol extraction (Sambrook et al., 1989) was used to extract DNA from the 2004 fetuses and the DNeasy Blood and Tissue Kit (Qiagen) was used to extract DNA from mothers and their respective embryos obtained in 2008 and 2012. Four microsatellite loci (Mh1, Mh6, Mh13, and Mh25) ranging in size from 156-251 bp from Chabot (2012) were used to genotype available mothers and their litters. Polymerase chain reactions (PCR) were carried out as described in Chabot (2012). A subset of samples (~20%) was genotyped a second time and reanalyzed to validate the dataset.

Genetic Analyses

Litter size, the polymorphism of microsatellite loci, and the number of putative fathers and their reproductive success can affect the probability of detecting and quantifying multiply paternity within a litter. To determine the power of the microsatellite markers used in this study to detect multiple paternity in litters of *M. henlei*, simulations were run in PrDM (Neff and Pitcher, 2002). Several simulations were run varying parameters including number of sires, reproductive skew of putative fathers, litter size, and inclusion or exclusion of maternal genotypes. Studies of polyandry in sharks have detected a range of 1-7 sires per brood (Saville et al., 2002; Chapman et al., 2004; Feldheim et al., 2004; Daly-Engel et al., 2007; Portnoy et al., 2007; DiBattista et al., 2008; Heist et al., 2011; Byrne and Avise, 2012) so this study assumed a conservative range of 2-5 sires based on the findings of Byrne and Avise (2012). Each simulation was run with litter sizes ranging between 3-10 that corresponded to the minimum and maximum size of observed litters. GERUD2.0 (Jones, 2005) was used to detect the minimum number of sires per litter under an exhaustive search. Allele frequencies for GERUD2.0 were based on the reference population of 31 *M. henlei* from Chabot (2012). Loci in the reference population were in Hardy-Weinberg equilibrium, lacked null alleles, were in linkage equilibrium, and had between 4-10 alleles (Chabot, 2012). Alleles observed in mothers and/or pups but not in the reference population were set to a frequency of 0.01 and all other frequencies were adjusted accordingly. Where a maternal genotype was not available, GERUD2.0 was used to reconstruct all putative maternal genotypes prior to the exhaustive search. Statistical differences in observed frequencies of multiple paternity among sampled years and between the study site and Las Barrancas were assessed by chi-square tests performed in Microsoft® Excel for Mac 2011. To determine significance, a single proportion for the species was estimated (i.e., the average of the

frequencies of multiply paternity observed in both Las Barrancas and Santa Catalina Island or the average of the frequencies observed at Santa Catalina Island among sampling periods) and used for all tests.

Results

Simulations indicated that the power to detect multiple paternity increased with both clutch size and the number of fathers (Table 4.1). Power to detect multiple paternity was moderate for litter sizes of three to five (0.49 to 0.74) but quickly improved with litters between six to ten (0.82 to 0.98). As the average number of offspring per sampled time period was seven or more (Table 4.2), these loci should be reliable for identifying the presence of polyandry in *M. henlei*. Exclusion probabilities were higher ($p = 0.89$) in 2008 and 2012 when maternal genotype was available than in 2004 ($p = 0.7$) when it was not.

The observed frequency of multiple paternity varied among sampling periods with 40% (4 of 10) of the litters from 2004 being fathered by multiple sires with an average of 1.4 sires per litter and 100% (8 of 8) of the litters from 2008 and 2012 being sired by a single father (Table 4.2). Chi-square tests revealed significant differences in the frequency of multiple paternity between Las Barrancas and Santa Catalina Island ($X^2 = 8.73$, $p = 0.013$, $df = 2$), however, there was no significant difference observed among sampling years at Santa Catalina Island.

Discussion

Similar to Byrne and Avise (2012), the use of four polymorphic microsatellite loci from Chabot (2012) has allowed for the detection of multiple paternity in litters of *M. henlei*. However, unlike the observed frequency of 93% from Byrne and Avise (2012), the maximum frequency of 40% observed in this study is more in line with frequencies observed in other triakid sharks including *M. antarcticus* (31%) (Boomer et al., 2013), *M. lenticulatus* (41%) (Boomer et al., 2013), and *T. semifasciata* (Nosal et al., 2013). Varying frequencies of multiple paternity between sample localities have been observed for other species of shark. For example, the tropical population of the sandbar shark, *Carcharhinus plumbeus*, demonstrated a frequency of multiple paternity less than half that of its counterpart in the temperate northwestern Atlantic (0.40 versus 0.85, respectively) (Daly-Engel et al., 2007; Portnoy et al., 2007) and northwestern Atlantic populations of the spiny dogfish, *Squalus acanthias*, demonstrated an almost two-fold difference in observed frequencies of multiple paternity (0.17 versus 0.30) (Lage et al., 2008; Veríssimo et al., 2011). Due to the observation that brown smoothhounds generally occur in abundance where sampled (Compagno, 1984), Byrne and Avise (2012) hypothesized that large aggregations of brown smoothhounds may potentially increase the opportunity of females to multiply mate.

Data from Chapter V may support this hypothesis and explain the divergence in the frequency of multiple paternity observed between the two localities. Estimates of θ (a proxy for effective population size (N_e) assuming equal mutation rates among populations) were greater in Baja California, Sur, Mexico than in Santa Catalina Island. Estimates of θ obtained from MIGRATE (Beerli and Felsenstein, 1999; Beerli and Felsenstein, 2001; Beerli, 2006) using mitochondrial

control region sequence data (mtCR θ) and nuclear microsatellite data (MS θ) were at least two fold greater at Baja California, Sur than Santa Catalina Island (Table 4.3). Although no overlap in confidence intervals for microsatellite derived values of θ were observed, an overlap in θ derived from mitochondrial data was observed (Table 4.3). This difference in effective population size is not surprising as catch per unit effort between the two populations is strikingly different. Observed incidental landings of only 40 *M. henlei* at Santa Catalina Island on a single day during OREHP surveys in June 2004 (n = 35 females and 5 males) and 27 *M. henlei* in June 2008 (n = 22 females and 5 males) (Haggin, pers. obs.) were far less than those observed in populations around La Paz in which many small fishing boats returned to port daily with at least 200 *M. henlei* on board (Castro, 2011).

As the estimated number of brown smoothhounds at Santa Catalina Island is less than that of Punta Lobos, Santa Catalina Island females may be under less reproductive pressure due to a reduced number of males and may also be able to employ reproductive strategies that reduce multiple mating opportunities such as "refuging" behavior in which females avoid mating by making use of different habitats from those of males or by posturing the body in a manner that is not conducive to mating (Pratt and Carrier, 2001). Many species of triakid sharks demonstrate sex-specific segregation in which females occupy different depths or are distributed at different frequencies latitudinally when compared to males (Ripley, 1946; Ebert, 2003; Nosal et al., 2013). This pattern of sexual segregation has been observed in *M. henlei* (Love, 1996; Ebert, 2003; Love, 2011) and may be effective as a strategy to reduce mate encounter rates. However, as mating has not been observed in *M. henlei*, the utilization of "refuging" and body posturing in

the species are speculative and further investigations into these possibilities for *M. henlei* would seem to be warranted in both localities.

Polyandry in elasmobranchs has been described in several species (see Byrne and Avise, 2012; and Fitzpatrick et al., 2012 for recent reviews). Although the reason for the evolution of polyandrous behavior in sharks is not yet clear and may be species-specific, the overall effect on effective population size and genetic diversity within a population may be detrimental. Theoretically, an increase in polyandry may reduce the effective population size and genetic diversity of a population due to the increased variance in male reproductive success (Karl, 2008). However, it is possible for polyandry to boost the effective population size under certain scenarios. One such scenario is a population in which polyandry and sperm storage co-occur and the population undergoes a severe population bottleneck. Due to the retention of paternal alleles from multiple males in individual females, this population would be expected to have a greater effective population size following the population bottleneck than a population lacking polyandrous behavior and sperm storage (Karl, 2008).

Within *Mustelus,* sperm storage has been detected in the terminal zone of the oviducal gland of *M. antarcticus*, *M. asterias*, and *M. canis* (Conrath and Musick, 2002; Hamlett et al., 2002; Storrie et al., 2008; Farrell et al., 2010). Mating patterns and the period of sperm storage varies among species of *Mustelus.* Due to the presence of sperm throughout the reproductive tract of *M. antarcticus* and the biennial reproductive cycle of the species, Storrie et al. (2008) concluded that *M. antarcticus* mates throughout the year and that sperm have the potential to be stored for at least 10 months and upwards of two years. Similarly, Farrell et al. (2010) observed sperm

storage in *M. asterias* throughout the species' gestation period of 12 months indicating that sperm are stored for at least that amount of time. Sperm storage has also been detected in immature females of *Mustelus* indicating that mating occurs prior to the first ovulation (Hamlett et al., 2002; Storrie et al., 2008; Farrell et al., 2010). With the observation of sperm storage in *Mustelus*, it is possible that *M. henlei* also employs this reproductive strategy and that the existence of multiply sired litters may be the result of sperm storage and should not only be attributed to multiple matings occurring during the course of a single reproductive cycle. Additional investigation into the existence of sperm storage in *M. henlei* is needed to elucidate the reproductive strategy of the species and is warranted based on the results of this study.

Finally, the effect of polyandry on the effective population size of elasmobranch species may be overestimated as the potential for males to secure multiple matings during a season, as well as over their lifetime, has not been addressed. As sharks are long-lived, utilize internal fertilization, do not form stable pair-bonds, and are iteroparous (Cortés, 2000; Chapman et al., 2004), the contribution of males to the effective population size of a species over the course of their lifetime would be expected to offset the male reproductive variance generated by multiple paternity within a single season. As monogamy or polygyny are considered to be the dominant reproductive strategies in vertebrates with internal fertilization (Portnoy, 2010), sharks with mating systems composed of both polyandry and polygyny would best be described as polygynandrous. Based on the observation of polyandry in Byrne and Avise (2012) and the present study, the description of *M. henlei* as a polygynandrous species would seem to be warranted and brings to light the need to consider the reproductive potential of both males and females when investigating the mating systems of a species and the impact of these systems on

effective population size. Further investigation through pedigree analysis on populations of *M. henlei* and the success of sires in producing offspring within resulting cohorts would be expected to elucidate the frequency of polygyny in the species and provide a greater understanding of the reproductive dynamics of the species.

Conclusion

Based on the findings of this study, the existence of multiple paternity in *M. henlei* has been confirmed at Santa Catalina Island, CA, albeit, at a reduced frequency compared to that of Las Barrancas, Baja California Sur, Mexico. These findings, along with those multi-litter investigations of other shark species, would suggest that polyandry is a common life-history strategy for elasmobranchs and that researchers should take location and density into consideration when assessing the existence of multiple paternity in future studies. Furthermore, it is most likely that *M. henlei* is polygynandrous and that the observed occurrence of multiple paternity in the species hardly affects its evolutionary potential.

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Table 4.1 PRDM simulation results for observed litter sizes of *Mustelus henlei* without maternal genotypes (2004) and with maternal genotypes (2008/12).

# Sires (paternal skew)	Litter Size															
	3		4		5		6		7		8		9		10	
	2004	2008/12	2004	2008/12	2004	2008/12	2004	2008/12	2004	2008/12	2004	2008/12	2004	2008/12	2004	2008/12
2(0.5:0.5)	0.28	0.30	0.50	0.56	0.64	0.67	0.73	0.76	0.79	0.81	0.83	0.86	0.85	0.85	0.88	0.88
2(0.667:0.333)	0.25	0.25	0.45	0.50	0.58	0.55	0.68	0.63	0.74	0.70	0.78	0.81	0.82	0.77	0.84	0.80
2(0.75:0.25)	0.21	0.25	0.38	0.42	0.51	0.55	0.60	0.63	0.66	0.70	0.71	0.74	0.75	0.77	0.78	0.80
3(0.333:0.333:0.333)	0.40	0.46	0.65	0.71	0.79	0.84	0.87	0.90	0.91	0.94	0.94	0.96	0.96	0.97	0.97	0.98
3(0.57:0.285:0.145)	0.34	0.39	0.56	0.62	0.72	0.75	0.80	0.83	0.85	0.88	0.89	0.91	0.91	0.93	0.93	0.95
4(0.25:0.25:0.25:0.25)	0.46	0.52	0.72	0.78	0.85	0.89	0.92	0.94	0.95	0.97	0.97	0.98	0.98	0.99	0.99	0.99
4(0.52:0.27:0.14:0.07)	0.38	0.43	0.62	0.68	0.76	0.80	0.84	0.87	0.89	0.91	0.92	0.94	0.94	0.96	0.96	0.97
5(0.20:0.20:0.20:0.20:0.20)	0.50	0.56	0.76	0.81	0.88	0.92	0.94	0.96	0.97	0.98	0.98	0.99	0.99	0.99	0.99	1.00
5(0.50:0.26:0.13:0.07:0.04)	0.40	0.45	0.64	0.70	0.78	0.82	0.86	0.88	0.91	0.92	0.93	0.95	0.95	0.96	0.96	0.97

Table 4.2 Summary of analyzed litters of *Mustelus henlei* including litter size, putative number of maternal genotypes (PMG), estimated number of sires, and male reproductive skew.

	Mother	Litter Size	PMG	Sires	Skew
2004	SC0455	7	4	2	(0.71:0.29)
	SC0459	5	18	1	-
	SC0479	7	6	1	-
	SC0480	3	3	1	-
	SC0482	5	6	2	(0.6:0.4)
	SC0483	7	6	1	-
	SC0484	5	2	1	-
	SC04101	6	2	2	(0.83:0.17)
	SC04121	10	8	2	(0.5:0.5)
	SC04123	4	8	1	-
2008	SC081	10	1	1	-
	SC0811	10	1	1	-
	SC0812	10	1	1	-
	SC0813	9	1	1	-
	SC0816	9	1	1	-
2012	SC129	7	1	1	-
	SC1210	3	1	1	-
	SC1211	8	1	1	-

Table 4.3 Mitochondrial control region (mtCR) and microsatellite (MS) theta values for two populations of *Mustelus henlei*.

	mtCR θ	95% CI	MS θ	95% CI
Santa Catalina Island	0.0007	0-0.0027	0.1835	0.1613-0.2042
Baja California, Sur	0.0094	0.0018-0.0581	0.4193	0.3664-0.4816

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Chapter V

Population structure and gene flow in the brown smoothhound shark, *Mustelus henlei*, in the northeastern Pacific

Abstract

To determine the effects of the prominent biogeographic (Point Conception and the Peninsula of Baja California) and phylogeographic barriers (Los Angeles Region) of the northeastern Pacific on the population connectivity of the brown smoothhound shark, *Mustelus henlei* (Triakidae), data from the mitochondrial control region (mtCR) and six nuclear microsatellite markers were used to measure gene flow among sample localities from throughout the range of the species (San Francisco Bay, CA, Santa Barbara, CA, Santa Catalina Island, CA, Punta Lobos, Baja California Sur, San Felipe, Baja California, Mexico, and Costa Rica). Mitochondrial and microsatellite data demonstrated significant population structure resulting in the designation of three populations: northern (San Francisco), central (Santa Barbara, Santa Catalina, Punta Lobos, and San Felipe), and southern (Costa Rica). Patterns of long-term and contemporary migration were incongruent with long-term migration being asymmetric and occurring in a north to south direction and a lack of significant contemporary migration observed between localities with the exception of Punta Lobos that contributed migrants to all localities within the central population. Based on these findings, Point Conception may be affecting gene flow between the northern and central populations, barriers to gene flow within the central population would seem to be ineffective, and a contemporary expansion of tropical *M. henlei* into subtropical and temperate waters in response to climate change may have been observed.

Introduction

Global climate change is expected to have an effect on the distribution of temperate marine taxa due to increases in sea surface temperature, alteration of current patterns, decreased salinity, and shifts in primary productivity (Worm and Lotze, 2009). Based on recent modeling, it is expected that subtropical marine taxa will expand their distributions into temperate latitudes and displace a proportion of the current taxa by the year 2050 (Kaschner et al., 2011). As a result, at the cost of pre-existing temperate diversity, overall species richness will increase in temperate waters as subtropical species expand their distributions. Possibly exacerbating this phenomenon would be the effect of biogeographic and phylogeographic barriers on the connectivity of populations being potentially displaced by subtropical species.

Biogeographic and phylogeographic barriers are known to disrupt population connectivity and are produced by currents, changes in sea surface temperature (SST), physical barriers, upwelling, and resource availability (Palumbi, 1994; Dawson, 2001; Dawson et al., 2001; Jacobs et al., 2004). The eastern Pacific is an area composed of numerous biogeographic and phylogeographic barriers. Of these barriers, the most prominent are located at Cape Mendocino, Point Conception, the Los Angeles Region (LAR), Punta Eugenia, the Peninsula of Baja California, the Sonoran Gap, the Central American Gap, the Isthmus of Panama, and the Equator (Rawson et al., 1999; Stepien et al., 2000; Dawson et al., 2001; Dawson, 2001; Jacobs et al., 2004; Dawson et al., 2006; Robertson and Cramer, 2009). The impact of these barriers can vary in magnitude depending on age and degree of obstruction. For example, the Isthmus of Panama, which is a relatively recent biogeographic barrier (~3.5-3.1 mya), has been demonstrated to alter current patterns, increase tidal amplitudes, affect upwelling, and sever gene flow between eastern Pacific,

western Atlantic, and Caribbean taxa (Coates and Obando, 1996; Duncan et al., 2006; Keeney and Heist, 2006; Dick et al., 2003; Coates et al., 2004). Barriers can also produce population structure along latitudinal gradients. Chabot and Allen (2009) and Chabot (Chapter I) have demonstrated significant population structure among populations of the soupfin shark, *Galeorhinus galeus*, straddling the Equator and have hypothesized that it is the species affinity for cool temperate waters that inhibits its ability to cross warm equatorial waters.

Fluctuating temperatures may have had an integral role in shaping eastern Pacific distributions of numerous marine organisms including those of the houndsharks (Triakidae). It has been suggested that increases in upwelling during the Miocene may have been responsible for the origins of numerous eastern Pacific lineages (Jacobs et al., 2004). Furthermore, cooler equatorial waters and South American extirpations during the Pleistocene may have allowed northeastern Pacific lineages to invade South American waters (Jacobs et al., 2004). Glacial periods have also had a significant effect on the distribution of lineages along the northeastern Pacific coast through the displacement of populations during periods of declining sea surface temperature (SST) and the retention of populations within glacial refugia (Hickerson and Ross, 2001; Dawson et al., 2006). During glacial periods, the ranges of eastern Pacific triakids may have undergone repeated contractions and expansions in response to cyclic periods of increasing and decreasing glaciations. If these hypotheses do indeed reflect the history of the eastern Pacific Triakidae, then members of this group would be expected to possess the genetic signatures of these paleogeographic events.

Houndsharks are a primary component of the northeastern Pacific elasmobranch assemblage considered both commercially and recreationally important (Compagno, 1984; Compagno et al., 2005). Several members of the group are considered endemic to the temperate eastern Pacific including *Mustelus henlei*, *Mustelus californicus*, *Triakis semifasciata*, *Mustelus albipinnis*, and *Mustelus lunulatus* (Compagno, 1984; Ebert, 2003; Compagno et al., 2005; Pérez-Jiménez et al., 2005) with *M. californicus*, *M. albipinnis*, and *T. semifasciata* occurring north of the equator in cooler subtropical to temperate waters (Miller and Lea, 1972; Compagno, 1984; Ebert, 2003; Compagno et al., 2005). Although *M. henlei* is considered to occur primarily in the northeastern Pacific, observations of this species off the coasts of Peru and Ecuador (Compagno, 1984) may indicate the ability of this species to cross warm equatorial waters unlike other members of the eastern Pacific triakid assemblage.

Mustelus henlei is distributed within temperate coastal waters at depths ranging from the shallow intertidal to 200 m (Ebert, 2003) with a geographic range spanning Coos Bay, Oregon (North America) to Peru and Ecuador (South America) (Compagno, 1984; Eschmeyer et al., 1999) and a recent discovery of the species off the coast of Washington (North America) (Dave Ebert, pers. comm.). Estimates of maximum total length and age are reported as 100 cm and 13 years (Yudin and Cailliet, 1990; Smith et al., 1998) with estimates of age at first maturity occurring between 2-3 years (Yudin and Cailliet, 1990). Reproduction is through placental viviparity with periods of gestation lasting ~1 year and each female capable of producing between 1-20 pups depending on geographic locality (n = 1-10 in Central California vs. 1-21 in the northern Gulf of California, respectively) (Yudin, 1987; Pérez-Jiménez and Sosa-Nishizaki, 2008). During the winter, *M. henlei* from San Francisco Bay are known to migrate out of the Bay when salinity decreases due

to increased rainfall and have been observed offshore from November to April (Compagno, 1984; Yudin and Cailliet, 1990). However, it is currently unknown as to where these sharks migrate to or the extent of their migration.

In order to determine the impact of eastern Pacific biogeographic and phylogeographic barriers on gene flow within the Triakidae, both nuclear (microsatellite) and mitochondrial (control region) genetic markers were used to reconstruct the phylogeographic history of *M. henlei* as well as elucidate contemporary gene flow within the distribution of the species. The matrilinealy transmitted, non-coding, mitochondrial control region (mtCR) is considered a selectively neutral marker that lacks recombination and mutates at a relatively high rate making it effective for detecting historic or evolutionary structure among populations (Avise, 2004; Wang, 2010). Due to their relatively high mutation rates and bi-parental mode of transmission, microsatellites are also ideal for testing gene flow among populations and provide a more contemporary glimpse into the gene flow of species (Avise, 2004; Wang, 2010). The purpose of this study was to determine the patterns of population connectivity among sample localities of *M. henlei* from throughout its northeastern Pacific range and to identify the biogeographic and phylogeographic barriers, if any, that may be responsible for shaping these patterns.

Materials and Methods

Sample Acquisition and DNA Extraction

Specimens of both male and female adult *M. henlei* were collected from throughout the range of the species by state agency and commercial trawling (California Department of Fish and Game's San Francisco Bay Study Project and Mike McCorkle Enterprises), beach seine, and gill netting (artisanal fisheries in Punta Lobos and the Ocean Resources Enhancement Hatchery Program white seabass monitoring program in 2000, 2008, 2010, and 2012. An adult *M. henlei* from 2012 was deposited at the Scripps Institute of Oceanography as a voucher specimen (SIO 13-25). In total 157 tissue samples were collected from six geographic locations: 31 from San Francisco Bay, CA (37.7750° N, 122.4183° W), 34 from Santa Barbara, CA (34.4208° N, 119.6972° W), 30 from Santa Catalina Island, CA (33.3749° N, 118.4199° W), 30 from Punta Lobos, Baja California Sur, Mexico (26.9841667° N, 113.9994° W), 12 from the northern Gulf of California (G.O.C.) at San Felipe, Baja California, Mexico (31.0275° N, 114.8353° W) and 32 from Costa Rica (9.9500^{O} N, 84.0000^{O} W). Fin clips (1 cm^2) were taken from the first dorsal fin, placed in 95% ethanol, and stored at -20 ^{O}C at UCLA for long-term storage until DNA could be extracted. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, USA) following the manufacturer's protocols.

Mitochondrial Sequencing

The mitochondrial control region was amplified as described in Chabot and Allen (2009) using universal shark mitochondrial control region primers. BigDye 3.1 (Applied Biosystems) dye-termination sequencing was carried out using sequencing primers designed for *M. henlei*: MhenFSeqPrimer 5′- TGC TAC GAC GCG CAA AAG CC and MhenRSeqPrimer 5′-CGT CGG

CCC TCG TTT TAG GGG. Sequencing reactions were performed in an Applied Biosystems GeneAmp 9700 thermocycler for 35 cycles of 90 ^{O}C for 10 s, 50 ^{O}C for 10 s, and 60 ^{O}C for 4 min, followed by direct sequencing in an Applied Biosystems 3130X Genetic Analyzer. Sequencing products validated by eye in GENEIOUS PRO 5.1.4 (Biomatters Ltd.) and aligned using CLUSTALW (Thompson et al., 1994).

Genetic Diversity and Gene Flow

ARLEQUIN 3.5.1.2 (Excoffier and Lischer, 2010) was used to estimate haplotype diversity (h), mean nucleotide diversity (π), mean pairwise difference, and the population mutation parameter θ_s (based on the number of segregating sites, sample size, and θ for a sample of non-recombining DNA). An analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was performed in ARLEQUIN to calculate the divergence estimator Φ_{ST} for all sample localities and JMODELTEST (Posada, 2008) was used to determine the best model of sequence evolution for AMOVA analyses. Based on the Akaike Information Criterion, the best model of sequence evolution provided by JMODELTEST was the HKY+I. However, ARLEQUIN does not include this model, therefore, the next best model, TN+I, was used for AMOVA analyses. Estimates were tested non-parametrically (10,000 bootstrapped replicates) and significance was adjusted for simultaneous pairwise tests using the sequential Bonferroni correction of Rice (1989). Pairwise estimates of Φ_{ST} were generated for all pairs of sample localities in ARLEQUIN and significance was tested via permutation and significance was adjusted for simultaneous pairwise tests using the sequential Bonferroni correction of Rice (1989). Spatial autocorrelation was tested using an isolation by distance model in GENALEX (Peakall and Smouse, 2006) and statistical significance was determined by permutation (9,999 replicates). Effective female

population size, N_{ef}, was estimated for each sample locality and based on the equation $\theta_s = 2N_{ef}u$ ($u = 2\mu k$, where μ is the mutation rate and k is the number of nucleotides). An average mutation rate, μ, of 0.0074 sequence divergence per million years based on the scalloped hammerhead shark, *Sphyrna lewini,* (0.008/my) and lemon sharks, *Negaprion*, (0.0067/my) (Duncan et al., 2006; Schultz et al., 2008) was used in all calculations of N_{ef}. Number of migrants per generation, M, ($M = N_f m$ for haploid data, with N_f = the effective female population size and m = the migration rate) was estimated in ARLEQUIN. Coalescent based estimates of long-term migration among the six sample localities were generated in MIGRATE-N (Beerli and Felsenstein, 2001) and based on M (where $M = m/\mu$ and m is the migration rate per generation and μ is the mutation rate). For the MIGRATE-N analysis, an initial analysis under the default parameters was used to estimate θ for all populations as well as migration rates for all population pairwise comparisons. Following this, two subsequent runs with different starting seeds using these estimates as priors were performed and averaged to generate θ and M. A median joining network of haplotypes was constructed in NETWORK 4.6.1 (www.fluxus-engineering.com; Bandelt et al., 1999) to visualize haplotype clustering and diversity. Following the generation of an initial median joining network, the MP option (Polzin and Daneshmand, 2003) was used to calculate and screen the network to delete superfluous median vectors and links that are not contained in the shortest trees.

Population Expansion

Tajima's D (Tajima, 1989; Tajima, 1993; Tajima, 1996), and Fu's F_S (Fu, 1997) were calculated in ARLEQUIN and used to detect historic demographic expansions. Tajima's D compares two estimators of the population parameter θ (θ_S based on the number of segregating sites and θ_π

based on the mean number of pairwise differences between haplotypes) (Tajima, 1989; Tajima, 1996). Under the infinite sites model, both estimates should be equal indicating a population at equilibrium and a D of ~0. In contrast, significantly negative values of D ($P \leq 0.05$) are indicative of populations not in equilibrium due possibly to a recent range expansion or recovery from a population bottleneck (Tajima, 1989; Tajima, 1993; Tajima, 1996; Aris-Brosou and Excoffier, 1996). Similar to Tajima's D, Fu's F_S statistic is also sensitive to population expansion under the infinite sites model with significantly negative F_S values ($P \leq 0.02$) indicating an excess of novel haplotypes and a departure from equilibrium (Fu, 1997). Population expansion times were estimated from Tau ($\boldsymbol{\tau}$) values, the amount of mutational time in which all lineages within a sample coalesce, derived from mismatch distributions (Rogers and Harpending, 1992) calculated in ARLEQUIN. Generations since divergence, t, was estimated by the equation $\boldsymbol{\tau} = 2\mu t$ (with t = the number of generations and μ = the mutation rate) (Rogers and Harpending, 1992). Expansion time, T, was estimated by multiplying t by 4.7, the average generation time of *M. henlei* (Cortes, 2002).

Microsatellite Genotyping and Analyses

Six nuclear microsatellite loci from Chabot (2012) (Mh1, Mh6, Mh13, Mh25, Mh34, and Mh36) were used to genotype individuals of *M. henlei* from all sample localities following the procedures of Chabot (2012). MICRO-CHECKER 2.2.3 (van Oosterhout et al., 2004) was used to detect the presence of null alleles, large allele dropout, and stuttering. Departures from Hardy-Weinberg Equilibrium (HWE), observed heterozygosity (H_O), and expected heterozygosity (H_E) were estimated in GENEPOP 4.0 (Raymond and Rousset, 1995; Rousset, 2008). FSTAT 2.9.3.2

(Goudet, 2003) was used to test linkage disequilibrium (LD), provide the total number of alleles, and estimate allelic richness (A_R).

Genetic Divergence and Population Structure

STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003; Falush et al., 2007) was used to estimate population subdivision in *M. henlei*. Number of subpopulations (*K*) was estimated with five independent runs of K = 1-10. Each run was performed with 1,000,000 MCMC repetitions and a burn-in of 100,000 steps under the admixture model with correlated allele frequencies. The optimal number of subpopulations was estimated using ΔK of Evanno et al. (2005) as implemented in STRUCTURE HARVESTER (Earl and vonHoldt, 2012). Overall population structure was estimated by AMOVA as implemented in ARLEQUIN. Estimates were tested nonparametrically (10,000 bootstrapped replicates) and significance was adjusted for simultaneous pairwise tests using the sequential Bonferroni correction of Rice (1989). Pairwise population estimates of F_{ST} were generated for all pairs of sample localities in ARLEQUIN and significance was tested via permutation and adjusted for simultaneous pairwise tests using the sequential Bonferroni correction of Rice (1989). Spatial autocorrelation was tested using an isolation by distance model in GENALEX (Peakall and Smouse, 2006) and statistical significance was determined by permutation (9,999 replicates). To determine the statistical power of the microsatellite loci used in the present study to detect genetic divergence and to reject the null hypothesis of panmixia among localities of *M. henlei*, power simulations were conducted in POWSIM 4.1 (Ryman and Palm, 2006). Simulation settings were a minimum F_{ST} of 0.05, a value indicated by Balloux and Lugon-Moulin (2002) to be the upper threshold of weak divergence for microsatellite loci, 500 replicates, and sample sizes from populations after

the simulated drift process equal to those of the present study. F_{ST} is commonly used to assess population subdivision, however, due to the high mutation rate of microsatellites, F_{ST} may underestimate population subdivision (Rousset, 1996). Therefore, Hedrick's G'_{ST} (Hedrick, 2005) and Jost's D (2008) were estimated in GENALEX and statistical significance was evaluated by permutation (9,999 replicates). Both estimators produce values between 0 and 1 with 0 indicating complete panmixia and 1 being indicative of a lack of migration.

Migration

Historic and contemporary estimates of migration were generated in MIGRATE-N and BAYESASS 3.0.3 (Wilson and Rannala, 2003). For the MIGRATE-N analysis, an initial analysis under the default parameters was used to estimate θ for all populations as well as migration rates for all population pairwise comparisons. Following this, five subsequent runs with different starting seeds using these estimates as priors were performed and averaged to generate θ and M. For BAYESASS, five analyses were performed with different starting seeds and were averaged to produce a single migration estimate with 95% credible sets for each pairwise comparison. Each analysis consisted of twenty million iterations with the first two million iterations discarded as burn-in and Δ_A, Δ_F, and Δ_M set to 0.3, 0.6, and 0.1, respectively. To determine the convergence of runs, mean log-probabilities were compared among runs and total log-likelihood was plotted versus iteration in TRACER 1.5 (Rambaut and Drummond, 2007) to determine if runs consisted of regular oscillations (i.e., no persistent peaks and valleys).

Population Bottleneck

When populations undergo recent declines in effective population sizes (N_e) due to bottlenecks, observed heterozygosity at neutral loci is generally greater than that expected by the number of alleles. To detect recent declines in N_e within populations of *M. henlei*, BOTTLENECK 1.2.02 (Piry et al., 1999) was used under the two phase model (TPM) with 20,000 replications, 5% of multistep mutations, and variance among multiple steps of 12 as recommended for microsatellites (Piry et al., 1999). The significance of any observed heterozygote excess was assessed by a one-tailed Wilcoxon's signed rank test; a test considered to be the most informative and robust for microsatellites (Piry et al., 1999). To further investigate the possibility of bottlenecked populations of *M. henlei,* the M ratio test of Garza and Williamson (2001) was calculated for each population in M_P_val (Garza and Williamson, 2001) and a critical value of M_C was estimated by CRITICAL_M (Garza and Williamson, 2001). The M ratio test calculates the ratio of the number of alleles at a given locus and the range of allele sizes with the expectation that the number of alleles in a population that has experienced a bottleneck will be reduced faster than the range of allele sizes (Garza and Williamson, 2001). Parameters for the M ratio test θ, p_s (proportion of single-step mutations), and Δ_g (average size of non-single-step mutations) were obtained from MIGRATE-N (θ) and Garza and Williamson (2001) (p_s = 0.88 and Δ_g = 2.8). Pre-bottleneck values of θ for CRITICAL_M were estimated from equilibrium heterozygosities (H_e) (Table 1B) and the equation $1\text{-}H_e = 1/1+ \theta$. Conservative values recommended by Garza and Williamson (2001) for p_s = 0.9 and Δ_g = 3.5 were used for CRITICAL_M.

Results

Mitochondrial Data

Nucleotide and Haplotype Diversity

Out of the 169 tissue samples of *M. henlei* used in the present study, 126 control region sequences of approximately 700 bp were obtained. Nucleotide composition was similar to that of *G. galeus* (Chabot and Allen, 2009) with cytosine, thymine, adenine, and guanine comprising 19.00%, 35.84%, 30.14%, and 15.01% respectively of control region sequences. Overall, 28 polymorphic sites were observed in control region sequences of *M. henlei* consisting of 16 transitions, 11 transversions, and a single insertion/deletion (Appendix 5.1) defining 27 haplotypes (GenBank accession numbers KC208467- KC208482). Of the 27 haplotypes, 21 were unique to their sample locality (San Francisco = SF1; Santa Barbara = SB4, SB9, SB17, SB28; Punta Lobos = PL1, PL10, PL27, PL30, PL39; San Felipe = GOC11; Costa Rica = CR1, CR2, CR6, CR11, CR16, CR20, CR26, CR38, CR40, CR43). Of the remaining six haplotypes, SB15 was observed in all localities (excluding Costa Rica) with frequencies ranging from 0.39-0.95, SB11, SB14, and PL18 were observed at Santa Barbara, Punta Lobos, and San Felipe, and SB19 was observed at Santa Barbara and Punta Lobos. Although the Costa Rica sample was composed almost entirely of unique haplotypes, haplotype SB14 was observed at this locality as well. Of the sampled localities, only Santa Catalina lacked any unique haplotypes and was dominated by the haplotypes SB15 (0.95) and SB23 (0.05).

Divergences, Gene Flow, and Migration

Observed sequence divergences of *M. henlei* were low in this study and ranged between 0.1% and 0.6% (Table 5.1A) and average pairwise nucleotide differences ranged between 0-2.75

among localities (Table 5.5). Effective female population sizes varied among sample localities with San Francisco demonstrating the lowest female effective population size (12,586) and Punta Lobos the greatest (179,849) (Table 5.1A). Significant genetic structure was observed in *M. henlei* with 34.61% ($P < 0.00001$) of the variation being observed among sample localities (Table 5.2A). Pairwise Φ_{ST} values ranged between -0.02 to 0.66 with San Francisco and Costa Rica demonstrating significant pairwise divergences ($P \leq 0.0033$) between all other localities and Santa Catalina Island demonstrating significant pairwise divergences ($P \leq 0.0033$) between all other localities with the exception of Santa Barbara (Table 5.3). Based on analyses from ARLEQUIN, estimated number of migrants per generation varied with sample locality and ranged between 0.28 and infinity (Table 5.4). Overall, migration rates determined by MIGRATE-N were asymmetric with gene flow occurring predominantly in a north to south direction (Table 5.5A). On regional scales, migration between Santa Catalina and Santa Barbara demonstrated asymmetric rates with Santa Barbara receiving immigrants from Santa Catalina and the three southern localities (Punta Lobos, San Felipe, and Costa Rica) demonstrating a complete lack of emigration into neighboring localities (Table 5.5A). Significant isolation by distance was observed ($P < 0.00001$) when all sample localities were analyzed together. As the Costa Rican sample locality has the greatest geographic distance from all sample localities, it is possible that this locality may have led to the significance of the spatial autocorrelation. To determine if this was the case, an analysis was performed without this locality resulting in a non-significant spatial autocorrelation ($P = 0.451$). The median joining network demonstrated a general lack of divergence among localities with many of the haplotypes diverging from the most frequently observed haplotype, SB15, by a single mutation (Figure 5.1).

Population expansion

Of the localities, Santa Barbara, Santa Catalina, Punta Lobos, and Costa Rica demonstrated negative Tajima's D values with significance ($P \leq 0.05$) being observed in all but Costa Rica (Table 5.1A). Negative F_S values were observed in Santa Barbara, Punta Lobos, and Costa Rica with only Santa Barbara and Costa Rica being significant ($P \leq 0.02$) (Table 5.1A). Results of mismatch distributions were not significant for all sample localities and therefore the null hypothesis of recent demographic expansion could not be rejected (Table 5.6). Based on the results of the mismatch distributions, tau values were used to estimate expansion times for all localities. Expansion estimates ranged from 317,569-3,130,308 years before present (ybp) with San Francisco demonstrating the most recent expansion and Punta Lobos the greatest (Table 5.6).

Microsatellite Data

Genetic Diversity

MICRO-CHECKER detected an excess of homozygotes and the possibility of null alleles due to stuttering for Mh6, Mh13, and Mh36. However, these results were generally locality specific (Mh6 was only observed in San Francisco, Mh13 was only observed in Santa Barbara and Punta Lobos, and Mh36 was observed only in San Francisco and Santa Barbara). As putative null alleles were not consistently observed across-sample, and given that null alleles have demonstrated only minor effects on estimates of F_{ST} and assignment testing (Carlsson, 2008), all loci were used for subsequent analyses. Observed heterozygosities (H_O) ranged between 0.4-0.54 and expected heterozygosities (H_E) were between 0.4-0.56 (Table 5.1B). All loci were in HWE after corrections for multiple tests and there was no evidence of LD detected for any of the loci. Observed number of alleles ranged between 22-35 and allelic richness ranged between

2.77-4.64 (Table 5.1B). Private alleles were detected in San Francisco, Santa Barbara, and Costa Rica with Santa Barbara possessing the greatest number of alleles (Table 5.1B).

Genetic Divergence, Population Structure, and *Migration*

Results of ΔK from STRUCTURE HARVESTER based on the STRUCTURE analysis (Figure 5,2) indicated the greatest posterior probability of $K = 3$ (Figure 5.3) for all sample localities of *M. henlei*. Overall, AMOVA demonstrated significant structure among sample localities ($F_{ST} = 0.12$; $P < 0.00001$) after Bonferroni correction (Table 5.2B). Significant genetic structure based on pairwise F_{ST} values was detected between San Francisco, Santa Catalina, and Costa Rica and all other localities after Bonferroni corrections with the exceptions of San Felipe and Santa Barbara that were not significantly different from San Francisco and Santa Catalina respectively (Table 5.3). Pairwise G'_{ST} values were significant for all comparisons with Costa Rica demonstrating the greatest divergence (Table 5.7). Significant estimates of Jost's D recovered the same pattern of genetic divergence as those of pairwise F_{ST} presented above (Table 5.7). Long-term estimates of migration among population pairs were asymmetric with the predominant direction of gene flow being in a north-south direction (Table 5.1B). Interestingly, the opposite pattern is observed in Costa Rica as the direction of gene flow is from the south to the north (Table 5.1B). Overall, estimates of contemporary gene flow demonstrated a lack of significant gene flow among sample localities (Table 5.8). However, Punta Lobos did contribute significantly to Santa Barbara, Santa Catalina, and San Felipe but not to San Francisco and Costa Rica (Table 5.8). Of note, algorithm bounds in BAYESASS limits the proportion of non-migrants and migrants to 0.67 and 0.33 respectively and values in these ranges may not be indicative of actual migration proportions. As the estimates of migration (0.138-0.186; Table

5.8) and 95% credible sets (0.007-0.318; Table 5.8) from Punta Lobos to Santa Barbara, Santa Catalina, and San Felipe are less than the lower model bound, these estimates are not representative of the limitations imposed by the model.

Population Bottleneck

BOTTLENECK did not reveal any significant heterozygote excess in any of the populations of *M. henlei* (Table 5.1B). Garza-Williamson M ratio values were all between 0.74-0.83 and above critical values of M_C with the exception of San Francisco (Table 5.1B). Garza and Williamson (2001) have indicated that M ratios > 0.8 represent stable populations and that ratios < 0.69 represent reduced or island populations.

Discussion

Biogeographic and Phylogeographic Barriers

This is the first range-wide genetic investigation of gene flow of a member of *Mustelus* within the northeastern Pacific. Significant structure was observed among sample localities of *M. henlei* based on mitochondrial control region sequence data and microsatellite data (Table 5.2, Table 5.3, and Figure 5.2). Based on these data, three distinct populations of *M. henlei* can be described: a northern population made up entirely of individuals from San Francisco, a central population composed of individuals from Santa Barbara, Santa Catalina, Punta Lobos, and San Felipe, and a southern population composed entirely of individuals from Costa Rica. These populations correspond to the biogeographic provinces of the Oregonian, a blending of the San Diegan and Cortez Provinces, and the Panamanian Provinces (Horn et al., 2006; Stephens Jr. et al., 2006; Robertson and Cramer, 2009). Throughout these provinces there are several biogeographic and phylogeographic barriers that have been described for various taxa with Point Conception, the Los Angeles Region (LAR), and the Peninsula of Baja California exerting the most influence on the population connectivity of northeastern Pacific temperate taxa (Rawson et al., 1999; Stepien et al., 2000; Dawson et al., 2001; Dawson, 2001; Bernardi et al., 2003; Jacobs et al., 2004; Hyde and Vetter, 2009).

The classification of Point Conception as a biogeographic barrier (Briggs, 1974) and the LAR as a phylogeographic barrier (Dawson, 2001; Dawson et al., 2001) is generally based on the patterns of observed disjunctions between populations of relatively small-sized taxa with limited adult dispersal that rely on currents to distribute larvae. However, as further studies of taxa with distributions spanning this area have demonstrated varying degrees of disjunction across the

barrier, Point Conception has been re-classified as a gradual transition zone between northern and southern lineages (Horn et al., 2006). Species with large, actively dispersing adults such as the leopard shark, *Triakis semifasciata* (Triakidae), and the California halibut, *Paralicthys californicus*, from the northeastern Pacific with distributions spanning the LAR and Point Conception have revealed similar patterns of gene flow across Point Conception but contrasting patterns across the LAR. Craig et al. (2011) used mitochondrial data to reveal an overall lack of structure among populations of *P. californicus* sampled from throughout the northeastern Pacific and a lack of structure specifically across the LAR and Point Conception and concluded that the species is comprised of a single panmictic population, at least over evolutionary time scales. Lewallen et al. (2007) observed significant structure among sample localities of *T. semifasciata* based on mitochondrial data and reduced nuclear genetic variation among sample localities between Elkhorn Slough (Monterey Bay, CA) and the LAR. Based on these observations, Lewallen et al. (2007) concluded that the LAR provided a diffuse barrier to gene flow and acted as a transition zone between populations North and South of the LAR and that Point Conception had no effect on population connectivity. Along the California coast, *P. californicus*, *T. semifasciata*, and *M. henlei* are commonly encountered together. Commercial halibut trawlers commonly encounter *M. henlei* during trawls (Mike McCorkle, pers. comm.) and leopard sharks have been observed among aggregations of *M. henlei* (Ebert, 2003) and share various life-history characteristics including preferences in habitat (Love, 1996) and food (Haeseker and Cech Jr, 1993; Webber and Cech Jr, 1998). As the northern population of San Francisco is significantly separated from the central population, Point Conception may be acting as a barrier to dispersal between the northern and central populations. However, based on the co-occurrences and shared life-history characteristics of *P. californicus*, *T. semifasciata*, and *M. henlei*, it would be expected

that *M. henlei* maintain gene flow across Point Conception and that the barrier to dispersal is most likely further north of this transition zone.

Horn and Allen (1978) described a discontinuity between northern and southern taxa at approximately Monterey Bay, CA where mean maximum temperature declines and numerous taxa encounter critically low temperatures. Similarly, a cline in genetic diversity has been observed within the range of the acorn barnacle, *Balanus glandula*, in the vicinity of central California (Sotka et al., 2004) and Lewallen et al. (2007) also observed a peak in divergence between San Francisco and Monterey Bay populations of *T. semifasciata* as well as a shift in the composition of northern and southern populations south of this area. As samples of *M. henlei* were not available from Morro Bay, CA and Monterey Bay for the present study (areas below San Francisco but above Point Conception), the existence of this barrier is hypothetical and should be tested in future studies. In regards to the LAR, patterns of population connectivity throughout this area among localities of *M. henlei* (Table 5.3 and Figure 5.2) are similar to those of Craig et al. (2011) and Lewallen et al. (2007) and indicate that this phylogeographic barrier is not effective at reducing gene flow among localities within the central population of *M. henlei*. Based on the combined observations of the studies above with those of the present study, the biogeographic and phylogeographic barriers at Point Conception and the LAR would seem to be largely ineffective in inhibiting contemporary gene flow in relatively large, vagile species in contrast to smaller, less vagile species that are dependent on shallow coastal habitats.

The Peninsula of Baja California is a tectonically active region that has undergone plate spreading, uplifting, and subduction as the Gulf of California has expanded in size over the past

3.5-12 million years (Holt et al., 2000). As a result, it has been hypothesized that ephemeral seaways have existed between the Pacific and the Gulf of California over this time period with the most recent occurring in the La Paz region during the Pleistocene (Walker, 1960). This seaway would have allowed for the migration of marine species across the Peninsula while maintaining the barrier produced by the warm surface waters of the Cape (Bernardi et al., 2003). Population disjunction has been observed in numerous species with distributions spanning both sides of the Cape including spotted sandbass, *Paralabrax maculatofasciatus*, (Tranah and Allen, 1999; Stepien et al., 2000), opaleye, *Girella nigricans*, (Bernardi et al., 2003), grunion, *Leuresthis tenuis/sardina* (Bernardi et al., 2003), sargo, *Anisotremus davidsoni* (Bernardi et al., 2003), longjaw mudsucker, *Gillichthys mirabilis* (Bernardi et al., 2003), shovelnose guitarfish, *Rhinobatus productus* (Sandoval-Castillo et al., 2004), golden cownose ray, *Rhinoptera steindachnari* (Sandoval-Castillo and Rocha-Olivares, 2011), and banded guitarfish, *Zapteryx exasperate* (Castillo-Páez et al., 2013). As demonstrated by the variety of species listed above, this barrier is effective at inhibiting gene flow among species with varying size, vagility, and reproductive strategies including but not limited to broadcast spawners or internal fertilizers followed by either bipartite life-history stages that utilize planktonic larval dispersal or highly vagile neonates as observed in sharks, skates, and rays. The generally accepted mechanism associated with the lack of gene flow observed between Pacific and Baja California populations at the Peninsula is the convergence of Pacific and Gulf Currents at the Cape where cold-temperate species are unable to round the Cape due to elevated sea-surface temperatures (Bernardi et al., 2003). Although this mechanism may be effective at impeding gene flow in the majority of observed species, this barrier may not exert as much of an influence on taxa capable of swimming beneath warmer surface waters like the California sheephead, *Semicossyphus*

pulcher, and the round stingray, *Urobatis halleri*, species that have been collected at depths greater than 50 m within this region (Bernardi et al., 2003) and have demonstrated gene flow between the Pacific and the Gulf of California (Bernardi et al., 2003; Plank et al., 2010). Similar to *S. pulcher* and *U. halleri*, *M. henlei* is commonly collected at depths between 60 m and 200 m throughout its distribution (Ebert, 2003), a range of depths that may allow the species to swim beneath the warm surface waters of the Cape and to disperse around the Cape of the Baja California Peninsula resulting in the population connectivity observed in the present study between sample localities on both sides of the Peninsula (Table 5.3 and Figure 5.2).

The observation of a distinct southern population of *M. henlei* at Costa Rica in the present study corresponding to the Panamanian Province may be the result of various biogeographic barriers distributed along the coast of Central America that have acted in concert to isolate populations (Robertson and Cramer, 2009). Of these barriers, the Sonoran and the Central American Gaps that are composed of long stretches of sandy substrate appear to restrict the population connectivity of numerous taxa throughout the Province (Walker, 1960; Hastings, 2000; Robertson and Cramer, 2009). Of the two gaps, the Sonoran Gap has been associated with the southern boundary of the Cortez Province for soft-bottomed species (Robertson and Cramer, 2009). Based on the affinity of *M. henlei* for soft-bottomed habitat (Compagno, 1984; Castro, 2011), this region may indicate a possible location as to where the disjunction between the central and southern populations occurs. However, these barriers do not act ubiquitously on all species (Craig et al., 2006) and their effect on the distribution of regional fauna has been questioned (Robertson and Cramer, 2009). Furthermore, these barriers are based primarily on reef-associated fishes that are restricted to rocky substrate (Hastings, 2000) that would be

expected to demonstrate a lack of connectivity across long stretches of unsuitable soft-bottomed habitat and may not reflect the dispersal potential of highly vagile species of shark, such as *M. henlei*, that are commonly encountered over soft-bottomed habitats (Compagno, 1984; Castro, 2011).

Aside from the barriers described above, distance may also be responsible for the lack of connectivity between the central and southern populations of *M. henlei*. Analyses of spatial autocorrelation from the present study supported a model of isolation by distance between Costa Rica and all other sample localities. As the Costa Rican population is the greatest distance from all other populations (> 5,000 km to the nearest localities of San Felipe and Punta Lobos), this finding is not surprising based on a tagging study of *M. henlei* in which an individual traveled a maximum distance of ~150 km over a three month period (Ebert, 2003). Regardless of isolating mechanism, the observation of virtually all of the haplotypes from Costa Rica being unique to the population, the existence of private microsatellite alleles, and a shift in the preferred diet of the species when compared to northern and central populations (fish and cephalopods versus crustaceans respectively) (Espinoza et al., 2012), the southern population of *M. henlei* has most likely been isolated from northern populations for some time and may be undergoing incipient speciation.

Demographic Expansions and Bottlenecks

Historic changes in the intensity of upwelling, varying sea surface temperatures, and repeated glaciations have resulted in a climatically dynamic coastline throughout the northeastern Pacific that has affected the distributions of numerous marine taxa (Hickerson and Ross, 2001; Dawson

et al., 2006). One expected effect of oscillating climate cycles along the coastline is the expansion or contraction (i.e., population bottleneck) of marine populations. Expansion times for *M. henlei* based on mean Tau values support an expansion from the central population of *M. henlei* to the north and to the south (Table 5.6). Specifically, Punta Lobos and Santa Barbara have expansion times well into the Pliocene followed by San Felipe in the Plio-Pleistocene, Santa Catalina and Costa Rica in the early to mid Pleistocene, and San Francisco within the mid to late Pleistocene (Table 5.6). Demographic expansions or population bottlenecks can leave a mark on the genetic diversity of populations by either generating novel genetic variants as a result of rapidly expanding effective population sizes (Rogers and Harpending, 1992) or significantly reduce genetic diversity due to severe reductions in population sizes (Cornuet and Luikart, 1996; Piry et al., 1999; Garza and Williamson, 2001). BOTTLENECK and M ratio tests did not detect any significant excesses in heterozygosity or reductions in the number of alleles relative to the range of allele sizes that would be indicative of bottlenecks for sample localities of *M. henlei* with the exception of San Francisco (Table 5.1B). The value of M for San Francisco was well below the 0.69 threshold established for populations that have undergone a historic bottleneck as described by Garza and Williamson (2001). However, BOTTLENECK did not detect the signature of a bottleneck for this locality. It has been noted by Williamson-Natesan (2005) that the two methods are generally better for detecting bottlenecks over different time scales as well as under different mutation rates and pre-bottleneck population sizes. Generally, detecting a bottleneck using heterozygote excess was more effective when the bottleneck occurred recently, mutation rates were low, and the pre-bottleneck population sizes were small (Williamson-Natesan, 2005). In contrast, using the range in allele size conditioned on the number of alleles worked best when bottlenecks occurred over several generations, mutation

rates were high, and pre-bottleneck population sizes were large (Williamson-Natesan, 2005). Therefore, the observed incongruence between the two methods at San Francisco would seem to indicate a historic population bottleneck that may be due to a founder's event associated with the expansion of the species' range during periods of climate change.

Of the sample localities, San Francisco and Santa Catalina demonstrated the lowest mtDNA diversity (Table 5.1A) as both were composed of only two haplotypes with the majority being the most frequent haplotype observed in the present study. In San Francisco, this apparent reduction in diversity may be due to a historic range expansion northward into the area that would later become San Francisco Bay followed by the subsequent colonization of the bay after its formation ~10,000 ybp (Cohen, 2000) at the end of the last glacial maximum. However, mean coalescent times for *M. henlei* in San Francisco are well in excess of the formation of San Francisco Bay < 10,000 ybp (Table 5.6). This apparent lack of concordance could be due to the use of a mutation rate in the present study that is not specific to the species or genus. Therefore, caution must be used when interpreting expansion times as they may be under/overestimates and are only used here to describe the magnitude of divergence among expansion estimates. Similar to the present study, a cline of genetic variation in the northeastern Pacific has also been observed in the acorn barnacle, *Balanus glandula*, by Sotka et al. (2004). Sotka et al. (2004) suggested that one explanation for the genetic pattern that they observed was that populations on both sides of the observed cline might have been isolated historically during glacial periods followed by range expansions and secondary contact once glacial ice retreated.

In regards to Santa Catalina, it is possible that during the early Pleistocene gene flow may have been relatively uninterrupted between this locality and coastal localities (e.g., Santa Barbara) due to lowered sea levels and shallower coastal basins. However, as sea level began to rise and coastal basins became deeper, population connectivity may have been reduced resulting in the more recent expansion estimate for Santa Catalina in the early to mid Pleistocene and the observation of relatively low mitochondrial diversity (e.g., only two haplotypes). The impact of deep-water basins on population connectivity has been observed among the northern Channel Islands within the Southern California Bight where populations of the black surfperch, *Embiotica jacksoni*, from the northern channel islands shared numerous mitochondrial haplotypes among islands but were separated from southern Channel Island populations by deep basins (Bernardi, 2000). Similarly, taxa with distributions on both Santa Catalina and along the southern California coastline demonstrate a general lack of gene flow across the San Pedro Basin (Gaida, 1997; Bernardi, 2000; Plank et al., 2010) and would seem to support the role of basins as barriers to population connectivity. However, the effect of basins on population connectivity is likely species-specific based on life-history characteristics and may not be generalizable to all marine taxa with both island and mainland distributions. For example, leopard sharks, *T. semifasciata*, tagged at Santa Catalina have been observed some time later along the mainland of southern California (Hight and Lowe, 2007) indicating the species' ability to cross the San Pedro Basin. As *M. henlei* is commonly encountered with aggregations of *T. semifasciata* (Ebert, 2003) and shares various life-history characteristics including preferences in habitat (Love, 1996) and food (Haeseker and Cech Jr, 1993; Webber and Cech Jr, 1998), it is plausible that *M. henlei* is also capable of crossing the San Pedro Basin in a similar fashion to that of *T. semifasciata*. As estimates of genetic divergence were non-significant between Santa Catalina and Santa Barbara

(Table 3) and long-term migration estimates for both mitochondrial and nuclear markers (Table 5.5) demonstrate a sufficient number of migrants per generation to reduce the effect of drift and homogenize gene pools (Mills and Allendorf, 1996), population connectivity between the two localities across the San Pedro Basin would seem to be ongoing.

Aside from post-glacial expansions and the effect of deep-water basins, the reduced mitochondrial diversity observed in *M. henlei* at San Francisco and Santa Catalina could also be due to the existence of discrete nursery areas promoted by female philopatry in which females return to natal sites to breed and undergo parturition. Philopatry, the return of a migrating animal to a specific location for the purpose of feeding or breeding, can produce significant levels of genetic divergence between populations of marine species considered to have high dispersal potentials as related females return consistently to natal nursery areas over a period of several generations (Baker et al., 1990; Meylan et al., 1990; Palumbi, 1994; Keeney et al., 2003; Keeney et al., 2005). Several nurseries may exist for *M. henlei* along the Pacific coast of California and Baja California. In northern California, *M. henlei* is reported to have nursery areas in Humboldt Bay, Tomales Bay, and San Francisco Bay (Bane and Bane, 1971; DeWit, 1975; Yudin, 1987). Similarly, Santa Catalina may also serve as a putative nursery area in southern California as a recent study of multiple paternity in *M. henlei* sampled pregnant individuals at various stages of development consistently in this region (Chapter IV). Further supporting this hypothesis are the levels of microsatellite diversity observed at San Francisco and Santa Catalina. Both localities are as genetically diverse as all of the other localities sampled in the present study with similar values of observed and expected heterozygosities, number of alleles, and allelic richness (Table

5.1B) indicating that the lack of mitochondrial diversity may be driven by asymmetric migration between males and females due to female philopatry to natal nursery sites.

Historic and Contemporary Gene Flow and the Effect of Climate Change

The North Pacific has been described as an "evolutionary engine that produces flora and fauna that have been able to transgress biogeographic boundaries and become established elsewhere" (Briggs and Bowen, 2013) with interhemispheric dispersals generally occurring from the north to the south (Briggs and Bowen, 2013). In regards to the invasion of South American waters by northern taxa, cooler equatorial waters and South American extirpations during the Pleistocene may have allowed northeastern Pacific lineages to invade South American waters and establish populations within the region (Jacobs et al., 2004). Results of the MIGRATE analyses in the present study support a general pattern of long-term north to south dispersal (Table 5.5) that may have led to the establishment of *M. henlei* in South America. As populations of *M. henlei* are known from Peru and Ecuador (Compagno, 1984; Eschmeyer et al., 1999) and this region comprises the southern limit of the species' distribution, it would be expected that this region would have reduced genetic diversity and a more recent expansion time when compared to the populations in the North. Further investigation of this region is needed to confirm this hypothesis and elucidate the patterns of gene flow within the terminus of the southern distribution of *M. henlei*.

Contemporary migration estimates from BAYESASS would seem to paint a different picture from those based on long-term migration. Overall, a lack of significant migration was observed to other localities from San Francisco, Santa Barbara, Santa Catalina, San Felipe, and Costa Rica

(Table 5.8). However, significant migration was observed within the central population with Punta Lobos contributing migrants to all localities (Table 5.8). This south to north pattern, with the exception of the northern Gulf of California, may be indicative of the expansion of tropical *M. henlei* into subtropical and temperate waters. Although the northern Gulf of California is separated from the temperate waters of the San Diegan Province, the northern Gulf of California is considered to have a temperate climate as well as temperate taxa associated with California (Robertson and Cramer, 2009). Based on the temperate nature of the northern Gulf of California and its temperate taxa, the assertion that tropical *M. henlei* is dispersing into the temperate waters of the northern Gulf of California would seem to be supported. As BAYESASS estimates gene flow over the course of two to three generations, *M. henlei* possessing an estimated generation time of 4.7 years (Cortes, 2002), and samples from the present study all being obtained within the past ten years, the observed expansion of the Punta Lobos population into adjacent waters of higher latitude may be a result of climate change as water temperatures increase. This pattern has been observed in the distributional shift of subtropical taxa into cooler, temperate waters along the northeastern Pacific coastline of California (Horn et al., 2006).

Based on recent climate change modeling, it is expected that subtropical marine taxa will expand their distributions into temperate latitudes and displace a proportion of the current taxa by the year 2050 (Kaschner et al., 2011). As the range of *M. henlei* spans tropical to temperate waters along the northeastern Pacific coastlines of North and Central America and the species is considered to be the only member of *Mustelus* to be cold-tolerant (Compagno, 1984), the loss of habitat would seem to be marginal for the central population based on the significant gene flow observed in this study (Table 5.3 and Figure 5.2). However, the isolated nature of the northern

population makes it vulnerable to invasion from the South and the swamping out of its novel genetic variants via intraspecific hybridization with migrants from the central population. However, it is possible that this process may proceed in the opposite direction with the genome of the invading population/species being introgressed by the local population assuming that hybrid fitness is not too low (Excoffier et al. 2009).

Further compounding the situation are the congeneric grey smoothhound, *Mustelus californicus*, and the sicklefin smoothhound, *Mustelus lunulatus*, that might expand their ranges northward under a scenario of climate change. Like *M. henlei, M. californicus* is also distributed within temperate coastal waters at depths ranging from the shallows to 60 m (Sandell, 1973) with a geographic range spanning Cape Mendocino, California to Mazatlan, Mexico (Eschmeyer et al., 1999). Both species demonstrate patterns of seasonal migration with *M. californicus* migrating north during the summer and occurring primarily offshore during this period (Compagno, 1984; Yudin and Cailliet, 1990). Unlike *M. californicus,* the warm-temperate to tropical *M. lunulatus* is known to only occur in Californian waters during warm-water summers (Compagno, 1984) and is generally absent from the region. An increase in sea surface temperatures at greater latitudes might facilitate the range expansion of these species and increase the competition for resources, at least at shallower depths (< 60 m) where the species would be expected to interact the most frequently. As the three species regularly co-occur within the Gulf of California (Compagno, 1984; Ebert, 2003; Pérez-Jiménez et al., 2012), modeling the future interaction of increasing subtropical and tropical *Mustelus* along greater Pacific latitudes should focus on this region first in order to generate a baseline for predictions.

Conservation

As a family, the Triakidae has been the subject of global exploitation for greater than 80 years with members being exploited by commercial fisheries for their large vitamin-A-rich livers (e.g., *Galeorhinus galeus*), by artisanal and recreational fisheries for consumption, and by the aquarium trade (e.g., *Mustelus henlei, Mustelus californicus, and Triakis semifasciata*) (Yudin and Cailliet, 1990; Ebert, 2003; Compagno et al., 2005; Lewallen et al., 2007). Fishery pressure on *M. henlei* is regionally variable with interest north of Mexican waters being minimal and the majority of landings of *M. henlei* being attributed to by-catch or by recreational anglers as a commercial fishery no longer exists in these waters (Pérez-Jimenéz and Carlisle, 2009). However, in the northern waters of the Gulf of California, *M. henlei* is still of considerable commercial and artisanal importance and has been the most abundant species of shark caught during fall and winter months as landings have been in excess of 150 kg per hour (Pérez-Jimenéz and Carlisle, 2009). Currently, the IUCN Red List has listed *M. henlei* with the threat category Least Concern due to the observed lack of over-fishing of the species, its fast growth-rate coupled with a relatively low longevity, an early age at first maturity with a relatively high fecundity, and a lack of evidence of catch declines (Pérez-Jimenéz and Carlisle, 2009). Estimated female effective population sizes (N_{ef}) for *M. henlei* observed in this study were all relatively high ranging from ~13,000 to 180,000 (Table 5.1A). As N_{ef} is generally considered to be ~10% of the female census size of a population (Frankham et al., 2010), estimated census sizes of sample localities from this study would be expected to be rather large and would support the threat category assigned by the IUCN. However, the generalization of Frankham et al. (2010) is based on an average obtained from many studies with a huge range in both effective population and census sizes and may not be generalizable to sharks. Recent observations in the

sandbar shark, *C. plumbeus*, have demonstrated that the effective number of breeders within a population can approximate the census size (Portnoy et al., 2009). Therefore, based on the observation of Portnoy et al. (2009), it is possible that the estimates of effective female population size obtained in the present study may approximate the census size of female populations of *M. henlei.* Aside from estimates of effective population size, the significant gene flow observed among localities, at least within the central population, detected in this study along with the observed genetic variation observed in Santa Barbara (greatest observed genetic diversity with several locality specific haplotypes and private alleles) and Punta Lobos (several locality specific haplotypes) would be expected to genetically buffer *M. henlei* from short-term population declines of small effect.

Conclusion

As this study is the first range-wide investigation into the gene flow and population connectivity of a member of the northeastern Pacific *Mustelus*, three patterns have been observed. First, throughout the range of *M. henlei* there exists three distinct populations with the northern and southern populations demonstrating significant divergence from the central population. Second, traditional biogeographic and phylogeographic barriers within the central population appear to have little effect on the population connectivity of *M. henlei.* And third, contemporary expansion of *M. henlei* from the tropics to subtropical and temperate regions in response to changing climate may have been revealed. As always, further sampling from different localities would be expected to bolster the results of any population genetic study of a species in the wild. With this in mind, the further sampling of *M. henlei* from Washington, Humboldt Bay, Tomales Bay, Morro Bay, Peru, and Ecuador would seem appropriate in order to elucidate patterns of

gene flow among localities within the northern and southern limits of the species' range. Finally, when these findings are integrated with those of other nearshore, northeastern Pacific taxa, the effect of global climate change on temperate biodiversity in the region will be made far clearer and our ability to predict and manage marine resources will be greatly improved.

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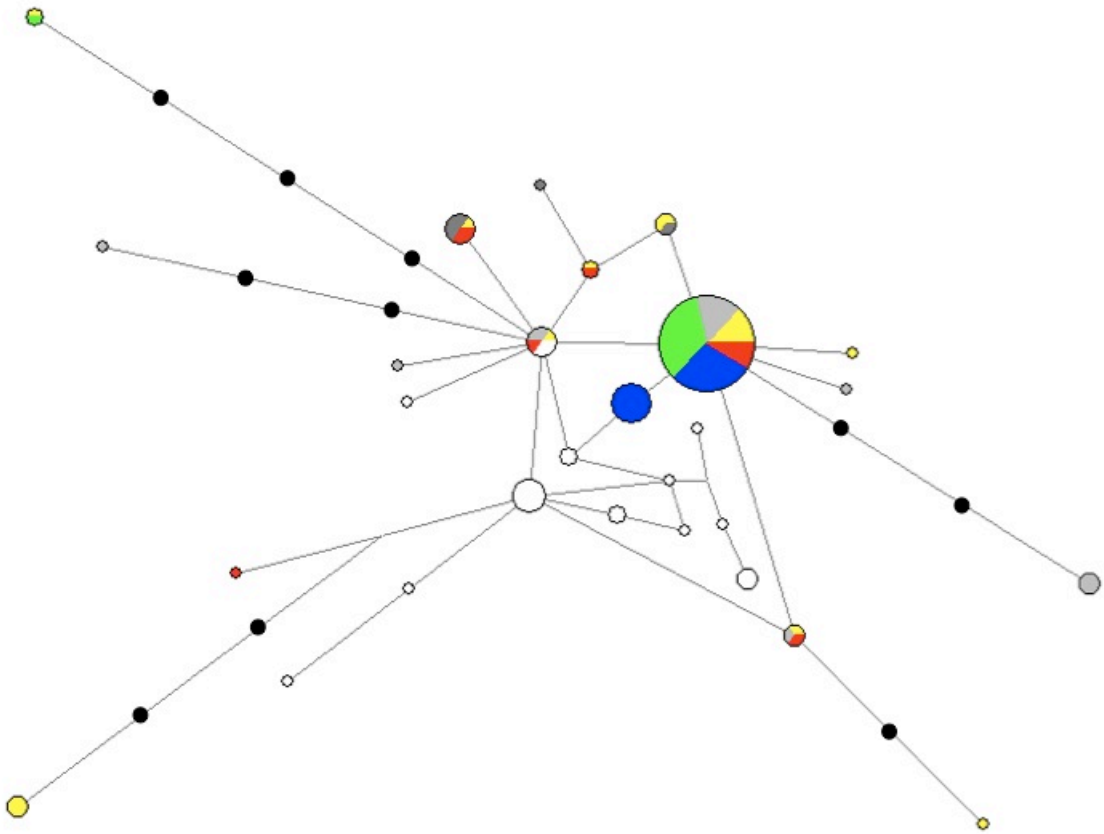

Figure 5.1 Median joining network of mtCR haplotypes for *Mustelus henlei.*
Circles represent individual haplotypes with size proportional to frequency, branches indicate mutations, and black circles are hypothetical ancestors. Localities are as follows: San Francisco Bay (Blue), Santa Barbara (Yellow), Santa Catalina Island (Green), Punta Lobos (Gray), San Felipe (Red), and Costa Rica (White).

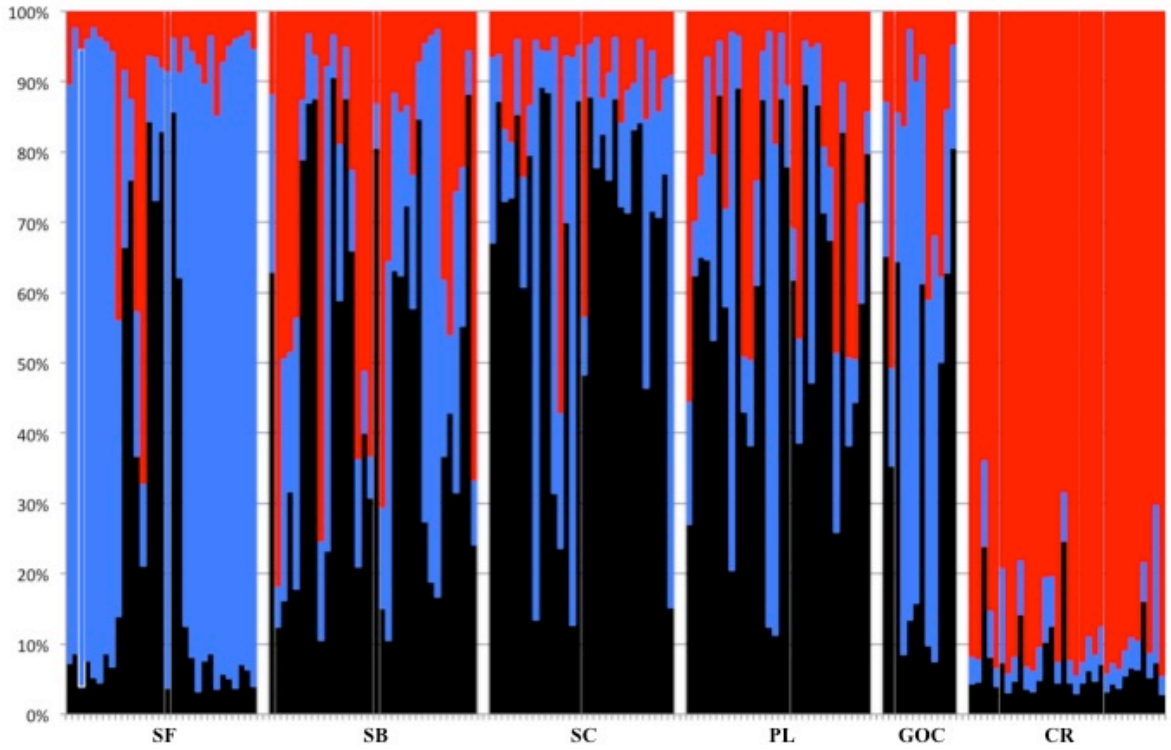

Figure 5.2 Population assignment of *Mustelus henlei* as estimated by STRUCTURE. Each individual is represented as a single histogram with percentage of ancestry on the y-axis and populations on the x-axis (SF = San Francisco, SB = Santa Barbara, SC = Santa Catalina, PL = Punta Lobos, GOC = San Felipe, and CR = Costa Rica). Spaces have been inserted between sample localities.

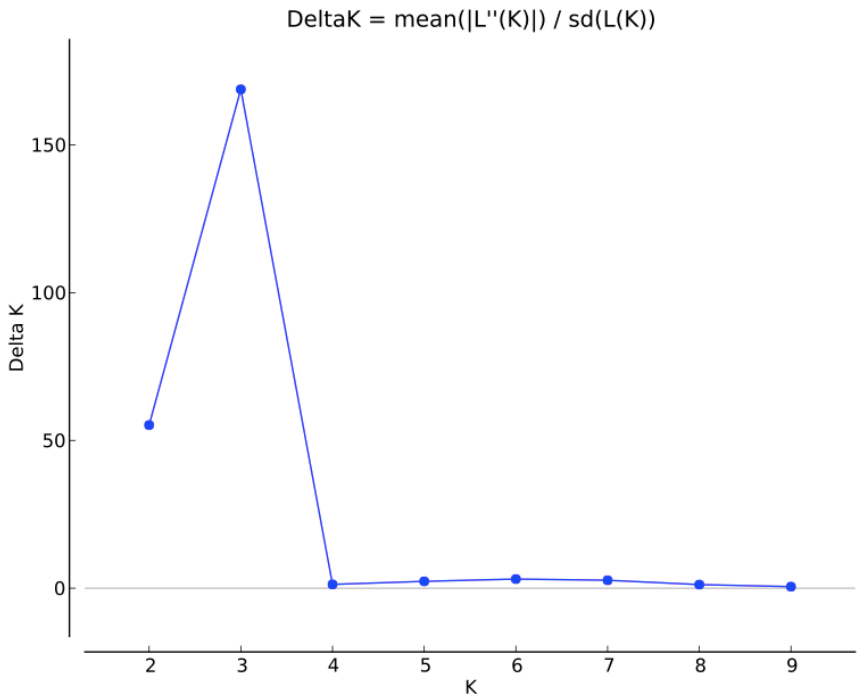

Figure 5.3 ΔK indicating most likely value of K for *Mustelus henlei*.

Table 5.1 Genetic diversity of *Mustelus henlei.*

A) Summary of mtDNA statistics for *Mustelus henlei.*
N, haplotype number; haplotype diversity (h); mean nucleotide diversity (π); mean pairwise difference (*MPD*); coancestry coefficient (θ_S); effective female population size (N_{ef}); * indicates significant p values for Tajima's D and Fu's F < 0.05 and 0.02 respectively.

Locality	N	h	π	MPD	θ_S	N_{ef}	Tajima's D	Fu's F
Overall	126	0.77 ± 0.04	0.004 ± 0.003	2.94 ± 1.55	4.81	245,408	-1.53*	-10.34*
San Francisco	27	0.48 ± 0.05	0.001 ± 0.001	0.484 ± 0.43	0.26	12,586	1.4	1.51
Santa Barbara	20	0.83 ± 0.08	0.005 ± 0.003	3.41 ± 1.8	3.38	172,449	-0.6*	-2.23*
Santa Catalina	21	0.1 ± 0.8	0.001 ± 0.001	0.86 ± 0.6	1.11	56,633	-1.39*	2.59
Punta Lobos	23	0.83 ± 0.07	0.006 ± 0.004	4.27 ± 2.2	3.52	179,849	-0.79*	-0.93
San Felipe	11	0.8 ± 0.11	0.004 ± 0.003	2.69 ± 1.55	1.71	87,370	0.01	-0.8
Costa Rica	24	0.87 ± 0.05	0.004 ± 0.003	2.26 ± 1.29	2.41	150,474	-0.21	-3.31*

B) Summary microsatellite statistics for *Mustelus henlei.*
N, number of individuals, H_O avg. observed heterozygosity; H_E avg. expected heterozygosity; *B*, Wilconxon's test probability of heterozygote excess under the Two-Phase Model (*TPM*); *GW*, avg. modified Garza-Williamson M ratio; *A*, number of alleles; *AR*, avg. allelic richness; *PA*, private alleles.

Locality	N	H_O	H_E	A	A_R	PA	B	GW
Overall	169	0.45	0.56	44	4.11	-	NS	0.37
San Francisco	31	0.45	0.49	28	2.77	4	NS	0.37
Santa Barbara	34	0.45	0.56	35	4.64	5	NS	0.32
Santa Catalina	30	0.47	0.54	26	3.73	0	NS	0.24
Punta Lobos	30	0.45	0.52	28	4.04	0	NS	0.26
San Felipe	12	0.54	0.52	22	3.44	0	NS	0.2
Costa Rica	32	0.4	0.4	22	2.82	3	NS	0.5

Table 5.2 Fixation indices for *Mustelus henlei.*

A) Φ_{ST} values for ***Mustelus henlei.***

Φ-Statistics

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	5	53.493	0.47231 Va	34.61
Within populations	120	107.096	0.89246 Vb	65.39
Total	125	160.589	1.36478	
Fixation index (Φ_{ST})	0.35			

$P < 0.00001$

b) F_{ST} values for ***Mustelus henlei.***

F-Statistics

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	5	63.26	0.19951 Va	12.18
Within populations	163	255.177	0.12742 Vb	7.78
Within Individuals	169	221.500	1.31065 Vc	80.04
Total	337	539.932	1.63758	
Fixation index (F_{ST})	0.12			

$P < 0.00001$

Table 5.3 Pairwise F_{ST} and Φ_{ST} values for *Mustelus henlei*.
F_{ST} values are presented below the diagonal and Φ_{ST} values above.

	San Francisco	Santa Barbara	Santa Catalina	Punta Lobos	San Felipe	Costa Rica
San Francisco	—	0.20*	0.19*	0.14*	0.33*	0.64*
Santa Barbara	0.08*	—	0.09	0.04	-0.02	0.41*
Santa Catalina	0.12*	0.02	—	0.06	0.2*	0.66*
Punta Lobos	0.13*	0.03	0.03*	—	0.01	0.45*
San Felipe	0.04	0.02	0.05*	0.03	—	0.42*
Costa Rica	0.27*	0.15*	0.23*	0.23*	0.21*	—

* indicates P values ≤ 0.0033 after sequential Bonferroni correction

Table 5.4 Estimated number of migrants per generation (*M*) and average pairwise nucleotide differences between sample localities of *Mustelus henlei* based on the mtCR. *M* values are presented above the diagonal and average pairwise nucleotide differences below.

	San Francisco	Santa Barbara	Santa Catalina	Punta Lobos	San Felipe	Costa Rica
San Francisco	—	2	2.12	3.18	1	0.28
Santa Barbara	0.4	—	4.97	10.98	Inf	0.73
Santa Catalina	0.12	0.18	—	7.95	1.97	0.26
Punta Lobos	0.23	0.13	0.11	—	71.65	0.62
San Felipe	0.41	0	0.21	0.04	—	0.7
Costa Rica	2.44	1.85	2.75	2	1.57	—

Table 5.5 Average long-term migration rates between sample localities of *Mustelus henlei* as estimated by MIGRATE-N.

A Estimates of migration based on mtCR haplotype data.
Bold values with a credible set not including 0 are provided below each pairwise population estimate and theta values with credible sets in italics on the diagonal.

	Donor					
Recipient	San Francisco	Santa Barbara	Santa Catalina	Punta Lobos	San Felipe	Costa Rica
San Francisco	*0.0004* *(0-0.0021)*	0.0001 (0-1.5216)	0.0001 (0-1.8587)	0.0001 (0-1.4944)	0.0042 (0-1.4798)	0.0003 (0-1.1961)
Santa Barbara	**3.7742 (0.0867-38.6626)**	*0.0073* *(0.0012-0.0389)*	**4.6539 (0.0875-38.4464)**	0.8561 (0-34.0621)	0.0024 (0-34.5204)	0.0024 (0-18.4277)
Santa Catalina	0.0601 (0-2.4515)	0.0577 (0-2.3015)	*0.0007* *(0-0.0027)*	0.0079 (0-2.0958)	0.0002 (0-2.088)	0.0002 (0-1.5835)
Punta Lobos	**5.3353 (0.1426-57.9913)**	3.0656 (0-52.3072)	**5.3457 (0.1422-57.6811)**	*0.0094* *(0.0018-0.0581)*	0.9026 (0-51.7129)	0.0031 (0-27.3486)
San Felipe	**3.3044 (0.0465-80.7758)**	**1.5974 (0-73.8086)**	**3.2685 (0.0442-80.9026)**	0.7197 (0-72.085)	*0.0062* *(0.0007-0.0817)*	0.0016 (0-49.6591)
Costa Rica	0.0016 (0-4.8325)	0.0016 (0-5.8874)	0.0016 (0-4.9925)	0.0175 (0-6.099)	0.0484 (0-6.2508)	*0.0048* *(0.001-0.0122)*

B Estimates of migration based on microsatellite data.
Bold values indicate greatest estimate of migration between population pairs and theta values with credible sets in italics on the diagonal.

	Donor					
Recipient	San Francisco	Santa Barbara	Santa Catalina	Punta Lobos	San Felipe	Costa Rica
San Francisco	*0.0147* *(0.0134-0.0162)*	0.9010 (0.641-1.2561)	0.6002 (0.3975-0.8595)	0.9217 (0.6526-1.2447)	0.2178 (0.0418-0.3402)	**0.0793 (0.0418-0.1696)**
Santa Barbara	**0.9760 (0.6867-1.5035)**	*3.4214* *(3.1822-3.6937)*	3.9544 (1.3778-2.4163)	1.2477 (0.8743-1.7356)	0.8837 (0.61-1.2528)	**1.2404 (0.8913-1.7587)**
Santa Catalina	**3.4835 (2.5181-4.9922)**	**6.7848 (1.3579-8.797)**	*0.1835* *(0.1613-0.2042)*	2.1257 (1.3579-3.1228)	1.0410 (0.6064-1.8421)	**0.9849 (0.5898-1.5941)**
Punta Lobos	**6.2031 (4.2669-8.7339)**	**40.6253 (12.8731-21.8942)**	**6.0108 (4.2395-8.7223)**	*0.4193* *(0.3664-0.4816)*	4.3938 (2.8646-6.3627)	**12.4103 (9.1537-16.7075)**
San Felipe	**7.9350 (3.3371-20.066)**	**33.9624 (18.9356-99.9823)**	**9.0114 (4.2577-21.8866)**	**19.0584 (9.5847-39.7582)**	*0.4123* *(0.27644-0.6976)*	**22.4760 (11.778-47.2809)**
Costa Rica	0.0618 (0.0424-0.1247)	0.4735 (0.2408-0.6541)	0.3345 (0.2408-0.4677)	0.6337 (0.4765-0.8287)	0.0089 (0.0177-0.0272)	*0.0083* *(0.00766-0.0092)*

Table 5.6 Estimates of Tau, θ_0, θ_1, and expansion times (T) for sample localities of *Mustelus henlei*. SSD is the sum of squared differences from mismatch distributions with significant values ($P \leq 0.05$) indicated by *.

Locality	Lower Bound	Mean	Upper Bound
San Francisco			
SSD = 0.019			
Tau	0	0.7	1.38086
θ_0	0	0.00176	0.49395
θ_1	9.37109	99999	99999
T	0	317,569	626,454
Santa Barbara			
SSD = 0.028			
Tau	0.28125	6.70000	12.35352
θ_0	0	0	1.83164
θ_1	1.41582	4.34082	99999
T	127,597	3,039,575	5,604,398
Santa Catalina			
SSD = 0.013			
Tau	0.62891	3.0	3.0
θ_0	0	0	0
θ_1	0	0.05391	99999
T	285,318	1,361,003	1,361,003
Punta Lobos			
SSD = 0.032			
Tau	1.10156	6.90	11.64844
θ_0	0	0	3.00059
θ_1	4.66797	8.88672	99999
T	499,742	3,130,308	5,279,825
San Felipe			
SSD = 0.024			
Tau	0.12109000	5.40	30.38086
θ_0	0	0.00176	3.32578
θ_1	0.96084	4.26392	99999
T	54,934	2,449,809	13,782,821
Costa Rica			
SSD = 0.003			
Tau	0.31445	2.0	5.80664
θ_0	0	0.2127	1.06875
θ_1	2.68677	5.92773	99999
T	174,577	1,110,375	685,910

Table 5.7 Average G'_{ST} and Jost's D for *Mustelus henlei.*
G'_{ST} values are presented below the diagonal and Jost's D values above.

	San Francisco	Santa Barbara	Santa Catalina	Punta Lobos	San Felipe	Costa Rica
San Francisco	—	0.1*	0.12*	0.12*	0.04	0.31*
Santa Barbara	0.13*	—	0.02	0.03	0.03	0.15*
Santa Catalina	0.2*	0.03*	—	0.03*	0.06*	0.26*
Punta Lobos	0.2*	0.04*	0.05*	—	0.03	0.24*
San Felipe	0.06*	0.04*	0.08*	0.05*	—	0.21*
Costa Rica	0.41*	0.22*	0.35*	0.34*	0.3*	—

* indicates *P* values ≤ 0.0033 after sequential Bonferroni correction

Table 5.8 Average **c**ontemporary migration rates (N_em) between sample localities of *Mustelus henlei* and self-recruitment (e.g., from donor locality San Francisco into recipient locality San Francisco) as estimated by BAYESASS. Bold values with a credible set (CS) not including 0 are provided below each pairwise population estimate and diagonal is in italics.

	Donor					
Recipient	San Francisco	Santa Barbara	Santa Catalina	Punta Lobos	San Felipe	Costa Rica
San Francisco	***0.907***	0.017	0.016	0.038	0.009	0.012
	(0.840 - 0.975)	(-0.017 - 0.051)	(-0.014 - 0.046)	(-0.019 - 0.096)	(-0.009 - 0.028)	(-0.01 - 0.034)
Santa Barbara	0.027	***0.733***	0.021	**0.186**	0.010	0.024
	(-0.0193 - 0.072)	***(0.634 - 0.832)***	(-0.029 - 0.071)	**(0.053 - 0.318)**	(-0.009 - 0.028)	(-0.017 - 0.065)
Santa Catalina	0.020	0.029	*0.772*	**0.156**	0.010	0.013
	(-0.014 - 0.053)	(-0.045 - 0.103)	*(-0.023 - 0.053)*	**(0.027 - 0.285)**	(-0.09 - 0.029)	(-0.011 - 0.037)
Punta Lobos	0.019	0.046	0.015	***0.895***	0.010	0.015
	(-0.016 - 0.053)	(-0.062 - 0.154)	(-0.004 - 0.035)	***(0.770 - 1.02)***	(-0.009 - 0.028)	(-0.012 - 0.042)
San Felipe	0.097	0.030	0.031	**0.138**	***0.687***	0.017
	(-0.014 - 0.208)	(-0.042 - 0.102)	(-0.02 - 0.082)	**(0.007 - 0.270)**	***(0.651 - 0.724)***	(-0.015 - 0.048)
Costa Rica	0.010	0.011	0.010	0.011	0.009	***0.951***
	(-0.009 - 0.028)	(-0.01 - 0.031)	(-0.009 - 0.028)	(-0.01 - 0.031)	(-0.008 - 0.026)	***(0.911 - 0.99)***

95% CSs are constructed as ± 1.96*SDs

Appendix 5.1 Nucleotide changes representative of the haplotypes of *Mustelus henlei.*

Haplotypes	N	Sites																											
		5	**29**	**51**	**52**	**57**	**70**	**98**	**116**	**138**	**140**	**153**	**174**	**210**	**220**	**231**	**271**	**294**	**394**	**429**	**435**	**443**	**466**	**600**	**623**	**645**	**647**	**671**	**685**
SF1	10	C	C	T	T	C	C	T	A	A	T	G	T	C	A	T	T	G	C	C	G	C	G	G	T	A	-	T	C
SB4	3	.	.	C	.	.	.	C	.	.	.	A	C	.	.	.	C	A	.	.	.	.	.	.	.	.	.	.	.
SB9	2	.	.	C	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
SB11	3	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
SB14	6	A	.	C	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SB15	59	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SB17	1	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	T	A	.	.	.	.	.	.	.	.
SB19	3	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
SB23	2	.	.	C	.	.	.	C	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	A	T	A	.	.
SB28	1	.	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PL1	1	.	A	C	.	.	.	.	.	.	.	A	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PL10	1	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
PL18	6	.	.	C	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PL27	1	.	.	C	.	.	A	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
PL30	3	.	.	C	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T
PL39	1	.	.	C	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
GOC11	1	.	.	C	.	.	.	.	.	.	.	A	.	.	.	.	C	A	T	.	.	.	.	.	.	.	.	.	.
CR1	4	.	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.
CR2	2	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CR6	1	.	.	C	.	A	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CR11	1	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	A	T	.	.	.	A	.	.	.	.	.	.
CR16	1	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	A		.	.	.	.	.	.	.	.	.	.
CR20	8	.	.	C	.	.	.	.	.	.	.	A	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
CR26	1	.	.	.	.	.	.	.	.	C	.	A	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
CR38	1	.	.	C	A	.	.	.	.	.	C	A	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
CR40	2	.	.	C	.	.	.	.	.	C	.	A	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
CR43	1	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.

Appendix 5.2 Summary statistics for 6 loci from within 5 localities of *Mustelus henlei*: San Francisco (SF), Santa Barbara (SB), Santa Catalina (SC), Punta Lobos (PL), San Felipe (GOC), and Costa Rica *(CR)*. *N*, number of samples; *A*, number of alleles; A_R, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; $F_{IS,}$ correlation of alleles within individuals compared to their subpopulation; *p*, F_{IS} *p* values (significant F_{IS} *p* values ≤ 0.00167 indicated by *).

		SF	SB	SC	PL	GOC	CR
Mh1	N	30	32	30	29	12	32
	A	6	9	6	6	6	5
	A_R	4.09	6.636	5.339	5.829	5.833	4.042
	H_O	0.6	0.843	0.733	0.793	1	0.66
	H_E	0.61	0.819	0.779	0.79	0.79	0.66
	F_{IS}	0.0076	-0.031	0.06	-0.005	-0.282	0.0023
	p	0.5472	0.725	0.33	0.585	1	0.3914
Mh6	N	31	32	29	30	12	32
	A	7	6	6	6	3	2
	A_R	4.239	4.515	5.357	3.703	2.917	2
	H_O	0.36	0.406	0.621	0.533	0.333	0.47
	H_E	0.54	0.55	0.628	0.547	0.54	0.47
	F_{IS}	0.351	0.265	0.012	0.025	0.393	-0.0022
	p	0.002	0.0217	0.53	0.535	0.11	0.6493
Mh13	N	31	33	30	30	12	32
	A	5	5	3	4	4	3
	A_R	4.178	4.263	2.367	3.196	3.913	2.068
	H_O	0.77	0.353	0.167	0.233	0.75	0.13
	H_E	0.68	0.646	0.438	0.37	0.591	0.12
	F_{IS}	-0.1348	0.458	0.622	0.373	-0.286	-0.0377
	p	0.8884	0.0017	0.0017	0.0133	0.965	1

Appendix 5.2 Continued

		SF	SB	SC	PL	GOC	CR
Mh25	N	31	32	29	29	11	30
	A	3	6	4	5	5	7
	A_R	2.736	4.506	3.352	4.813	5	4.551
	H_O	0.32	0.656	0.724	0.724	0.455	0.6
	H_E	0.39	0.668	0.624	0.694	0.532	0.64
	F_{IS}	0.1792	0.017	-0.164	-0.044	0.153	0.0662
	p	0.1932	0.52	0.9067	0.7367	0.3233	0.0095
Mh34	N	31	33	24	30	11	31
	A	4	3	2	3	1	3
	A_R	2.88	2.51	1.991	2.356	1	2.253
	H_O	0.29	0.242	0.292	0.1	-	0.38
	H_E	0.26	0.246	0.254	0.159	-	0.36
	F_{IS}	-0.1066	0.013	-0.15	0.374	-	-0.0746
	p	1	0.48	1	0.0617	-	1
Mh36	N	31	33	30	30	12	29
	A	3	4	3	3	2	2
	A_R	2.588	3.141	2.89	2.948	2	1.999
	H_O	0.07	0.212	0.267	0.3	0.167	0.19
	H_E	0.49	0.418	0.346	0.413	0.464	0.18
	F_{IS}	0.8693	0.497	0.232	0.277	0.651	-0.0732
	p	0.0105	0.0017	0.105	0.0517	0.0667	0.8258

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