MEMORANDUM December 1, 2017

TO: Robert F. Kennedy Library

FROM: Diona L. Drake

SUBJECT: THESIS

****

Transmitted herewith is an original copy of the above subject from:

Student's Name:	Diona L. Drake
Title of Thesis:	THE POPULATION STRUCTURE OF THE WHITETIP REEF SHARK (*Triaenodon obesus*) IN THE MARIANA ARCHIPELAGO
Number of Pages:	47 (including abstract and approval pages)

****

I hereby grant permission to the University of Guam to furnish upon request copies in whole of the Thesis described above, in written or electronic format, as determined by the library.

I am aware that a graduate student of the University of Guam owns the copyright of his or her thesis or special project. However, as a condition of being awarded the degree, I grant the University the non-exclusive right to retain, copy, use and distribute the thesis or special project, together with the right to require its publication for archival use.

I am further aware that a graduate student may delay or restrict release of his or her thesis or special project or a portion thereof for up to two years in cases where part or all of the thesis or special project is being published elsewhere or the work is subject to patent disclosure requirements.

SIGNATURE

12/1/17

DATE

AN ABSTRACT OF THE THESIS of Diona L. Drake for the Master of Science in Biology presented, November 27, 2017

Title: The population structure of the whitetip reef shark (*Triaenodon obesus*) in the Mariana Archipelago

Approved: _____

Terry J. Donaldson, Ph.D, Chairman, Thesis Committee

Microsatellite markers were used to determine the population structure of whitetip reef sharks (*Triaenodon obesus*) in the Mariana Archipelago. Sharks in the Marianas are understudied and more information on their population structure is vital to their management and protection. Whitetip reef sharks are not known to travel long distances between islands in many locations which presents an interesting question of their genetic structure across a chain of islands. Whitetip reef sharks were caught in baited traps from six islands in the archipelago and tissue samples were taken from each individual. DNA was extracted from all tissue samples and successfully analyzed employing four microsatellite markers developed for the blacknose shark (*Carcharhinus acronotus*). It was found that the Mariana Archipelago contains one interbreeding population of whitetip reef sharks that have the potential to travel between islands. Given the existence of a single interbreeding population, management which takes this into account is in order.

TO THE OFFICE OF GRADUATE STUDIES

The members of the committee approve the thesis of Diona L. Drake presented November 27, 2017.

Dr. Terry J. Donaldson, Ph. D
Chairman

Dr. Daniel P. Lindstrom, Ph. D
Member

Dr. Sarah Lemer, Ph. D
Member

Dr. Christopher Lowe, Ph. D
Member

ACCEPTED:

Lee Yudin, Ph.D.
Dean, College of Natural and Applied Sciences

Date December 5, 2017

THE POPULATION STRUCTURE OF THE WHITETIP REEF SHARK (*Triaenodon obesus*) IN THE MARIANA ARCHIPELAGO

BY

Diona L. Drake

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

IN

BIOLOGY

UNIVERSITY OF GUAM
NOVEMBER 27, 2017

ACKNOWLEDGEMENTS

I would first like to thank my amazing fiancé Victor Snyder for helping to support me through these last three years and his endless encouragement and humor. I could not have done this without him. I would also like to thank my committee, Dr. Terry Donaldson, Dr. Daniel Lindstrom, Dr. Sarah Lemer, and Dr. Christopher Lowe, for their tireless help and hard work through the completion of this thesis. I want to thank my family for their endless love and support through the years, and all the sacrifices they made to help me get to where I am today pursuing my passions. I would like to thank NOAA and PIFSC for allowing me to be a part of their research cruise on R/V Oscar Elton Sette, without which this project would not have been possible. I would like to thank all of the faculty, staff, and students at the UOG Marine Laboratory, my Poksai paddling team, and Chamorri Familia for supporting me and giving me a family while enjoying life on Guam.

This material is based upon work supported by the National Science Foundation under Grant Number OIA-1457769. *Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Sharks are important in the maintenance of healthy coral reef ecosystems due to the stabilizing effect they have on food webs. Many sharks are trophic generalists and feed upon a wide range of reef species. This strategy prevents any one species from dominating the reef and thus promotes a diverse assemblage of larger, more ecologically valuable herbivores (Paine 1966, Sandin et al. 2008). The overall effect of the removal of sharks from a reef system may not be limited solely to direct predation effects, but also risk effects (changes in prey species behavior due to predation risk) and indirect species interactions (Heithaus et al. 2008, Madin et al. 2010). For example, a study on Pacific sleeper sharks (*Somniosus pacificus*) in Alaska found that they exhibit a strong effect on harbor seals, effecting where the seals hunted for food, and even caused the seals to underutilize a food source due to predation risk (Frid et al. 2007). Removing sleeper sharks from the ecosystem would change how the seals effect their environment. Sharks may change their behavior based on environmental conditions and reef impacts, such as reef degradation. An example of an environmental condition changing the behavior of a shark includes feeding behaviors associated with diel changes in body temperature. Papastamatiou et al. (2015) showed that the blacktip reef sharks (*Carcharhinus melanopterus*) would enter warmer, shallow water during low tide to use the heated water to aid in digestion and while hunting at night to maximize their rates of ingestion. Densities of sharks have been shown to increase with high coral cover, larger reef size, and more reef biomass (Espinoza et al. 2014).

Extensive exploitation of sharks in various fisheries, and as incidental bycatch, threatens wild shark populations and depleted populations of many species may not

recover. Most fisheries do not specifically target shark species in their operations, but they are an incidental casualty. The array of threats or stressors inhibiting the recovery of depleted populations can result in a permanent loss of genetic diversity because of life history characteristics of most shark species (i.e., low fecundity, slow growth, late sexual maturity, etc.) that ultimately render them vulnerable to overexploitation/extinction (Dulvy et al. 2004, Rodrigues-Filho et al. 2012). Despite these factors, it has been reported that some shark species still carry a high level of genetic diversity within and between populations. Examples include blacktip reef sharks (*Carcharhinus limbatus*) and scalloped hammerhead sharks (*Sphyrna lewini*, Sphraenidae) (Keeney et al. 2005, Duncan et al. 2006).

Protection of shark populations in Micronesia has progressed during the last six years with the enactment of several new laws to protect resident species. The island nation of Palau made its waters a shark sanctuary in 2009 and Guam enacted a "no shark finning" law in 2011. Guam (including Cocos Island) is the largest and southern-most island of the Mariana Archipelago. A key management issue of Guam's sharks, as well their management globally, is the lack of knowledge regarding their population structure, reproductive/demographic dynamics, and life histories. Such information is vital for the management and conservation of these "charismatic" megafauna under exploitation (Crandall et al. 2000, Robbins et al. 2006). Studies conducted on the Great Barrier Reef (GBR) suggest that differences in behavior between sexes and life stages can influence the level of protection from management strategies like Marine Protected Areas (MPAs). Protection in semi-isolated reefs may be lower compared to more isolated reefs and island-wide movements by sharks makes management harder if MPAs are small

(Espinoza et al. 2014, Bradley et al. 2017b). In general, the status of reef-associated sharks is unclear due to limited quantitative data and analyses because reef sharks are often not a high research priority and are usually not targeted directly by commercial fishing (Nadon et al. 2012).

Research on Guam's shark populations has been relatively limited. The University of Guam Marine Laboratory (UoGML) produced a technical report describing the inshore sharks of Guam and methods of catching sharks using small boats (Bryan 1972). The technical report described catching relatively few sharks using their methods, but was able to document the presence of three previously undocumented species in Guam's inshore waters. The Guam Department of Agriculture's Division of Aquatic and Wildlife Resources (DAWR) conducted aerial surveys that documented fishing activity, and the inshore presence of elasmobranchs, sea turtles, and cetaceans, over a five-decade period (Martin et al. 2016). The aerial surveys lacked sufficient visual resolution to identify sharks to species, but patterns of occurrence in inshore habitats could be discerned. The surveys conducted by DAWR showed a negative trend in sightings of reef sharks over time. Shark sightings decreased five-fold between 1963-2012. Most sharks were sighted in areas with high reef cover and low human density along the eastern (windward) side of the island (Martin et al. 2016). Much of the decline in shark sightings may be attributed, as elsewhere, to reef degradation, overfishing, and other human impacts (Baum et al. 2003, Ferretti et al. 2008, Ward-Paige et al. 2010), but some declines may be overestimated due to the difficulty establishing a proper baseline (Bradley et al. 2017a, 2017b). There is also incidental and largely unpublished data collected by UOGML researchers and others conducting visual surveys of fishes for the

last 45 years (i.e., UOGML technical reports by various authors; T.J. Donaldson, unpublished data, etc.) that have documented the presence of sharks in inshore waters around Guam. Clearly, more data are needed for managers to develop and implement plans that would aid in the proper management of Guam's shark populations.

One species, the whitetip reef shark, *Triaenodon obesus*, (Family Carcharhinidae), is known to inhabit many different areas in Guam's waters. The whitetip reef shark is one of the most common and broadly distributed species of coral reef shark in the Indo-West Pacific region (Randall 1977). This species is known to frequent shallower reef areas and is often seen resting on the bottom or in caves. Whitetip reef sharks are known to inhabit the same resting site for long periods of time, maintaining a small, well-defined daily home range, and returning to the same resting site afterwards (Randall 1977, Nelson and Johnson 1980). One study in Hawaii has shown that whitetip reef sharks may have a maximum dispersal distance of 9-24 km over a timespan of several years (Whitney et al. 2012a).

Whitetip reef sharks are known to feed upon fishes and mollusks, with some records indicating they also on occasion consume crustaceans (Randall 1977, Whitney et al. 2012a). This species feeds primarily at night, but some cases of daytime feeding have been observed (Randall 1977). Due to the slender shape of the whitetip's body, this species can swim through narrow crevices and holes within the reef. The ability to maneuver through or into these spaces is beneficial for hunting and acquiring food (Randall 1977). Whitetip sharks can capture prey that most other shark species, including grey reef sharks (*Carcharhinus amblyrhynchos*) and blacktip reef sharks (*C. melanopterus*), are unable to reach.

Whitetip reef sharks are viviparous and thus lack a pelagic dispersal stage that would aid in greater geographic colonization potential (Randall 1977, Tricas and Le Feuvre 1985, Whitney et al. 2012b). Female whitetips do not reach sexual maturity for eight years and exhibit a K-selected reproductive strategy, only producing an average of two pups per litter with females producing an estimated 12 pups over their lifetime (Robbins 2006, Robbins et al. 2006). Due to the slow rate of sexual maturity and the small number of offspring produced, whitetip reef sharks are particularly vulnerable to the detrimental effects of overfishing leading to a potential collapse of the population.

Previous research has examined the geographic distribution and population connectivity of adult sharks that make long distance migrations across ocean basins as well as shorter ones along coastlines. Many species of pelagic, ocean-traversing sharks, including the white shark (*Carcharadon carcharias*, Lamnidae), whale shark (*Rhincodon typus*, Rhincodontidae), basking shark (*Cetorhinus maximus*, Cetorhinidae), and shortfin mako (*Isurus oxyrhincus*, Lamnidae), demonstrate very little genetic structure across their ranges (Schrey and Heist 2003, Hoelzel et al. 2006, Castro et al. 2007, Jorgensen et al. 2010). Whitney et al. (2012b) investigated the widespread dispersal and connectivity of whitetip reef sharks across the Indo-Pacific Ocean. Biogeographical barriers that coincide with past glacial cycles appear to constrain the dispersal of *T. obesus*, aiding in genetic differences between the Pacific and Indian oceans. Whitetip reef sharks in the continuous reefs of the GBR exhibited genetic structure between neighboring reefs, thus demonstrating low connectivity. Surprisingly, the opposite however was found in the Hawaiian Archipelago, which exhibited high connectivity between islands and no genetic structure.

The mechanism that has allowed these sharks to become so widespread is still not fully understood since they are not generally known to travel long distances. Higher occurrence of sharks moving between reefs, and potentially between islands, would create more connectivity and less genetic structure. This data is important for the development of management strategies that correctly take into account the specific needs of this Archipelago based on the determined population structure. If population structure does exist between islands and is ignored or goes undetected the removal of too many individuals from these subpopulations would be detrimental to the system as a whole (Keeney et al. 2005).

The aim of this study is to determine if the genetic structure of whitetip reef shark populations in the Mariana Archipelago follows the pattern of the GBR (limited gene flow) or of the Hawaiian Archipelago (genetic connectivity). Because the whitetip reef shark is so widespread in the Indo-West Pacific it is suspected that there may be more genetic connectivity between neighboring islands than thought previously (Whitney et al. 2012b). Specifically, genetic sequencing was conducted to determine if this shark species is one or more distinct populations based upon significant differences in allele frequencies in populations between islands. If sharks migrate between neighboring islands, or along coastlines to more distant parts of an island, this would allow for a measurement of genetic diversity that could show genetic connectivity between the islands in an archipelago. Population connectivity of the whitetip reef sharks in the Marianas was assessed by using genetic and geographic distance comparisons, as well as analysis of molecular variance. If there is a depletion of whitetip reef sharks in the Mariana Archipelago due to overfishing, on one hand genetic structure among

islands/populations would be high due to isolation of populations. On the other hand, genetic diversity would be small in each island/population due to lower effective population size and genetic drift.

I hypothesized that populations of *T. obesus* in the Mariana Archipelago show varying levels of genetic structure, diversity and connectivity within and between islands. Genetic connectivity should depend upon the varying distances and depths between each island in the archipelago. I predicted that populations of whitetip reef sharks distributed within the Marianas would exhibit varying degrees of genetic diversity based upon the location of the island where they were sampled.

METHODS

Study Sites

The Mariana Islands (Figure 1) are located in the western Pacific Ocean between 13.4443°N, 144.7937°E and 20.5421°N, 144.8924°E.

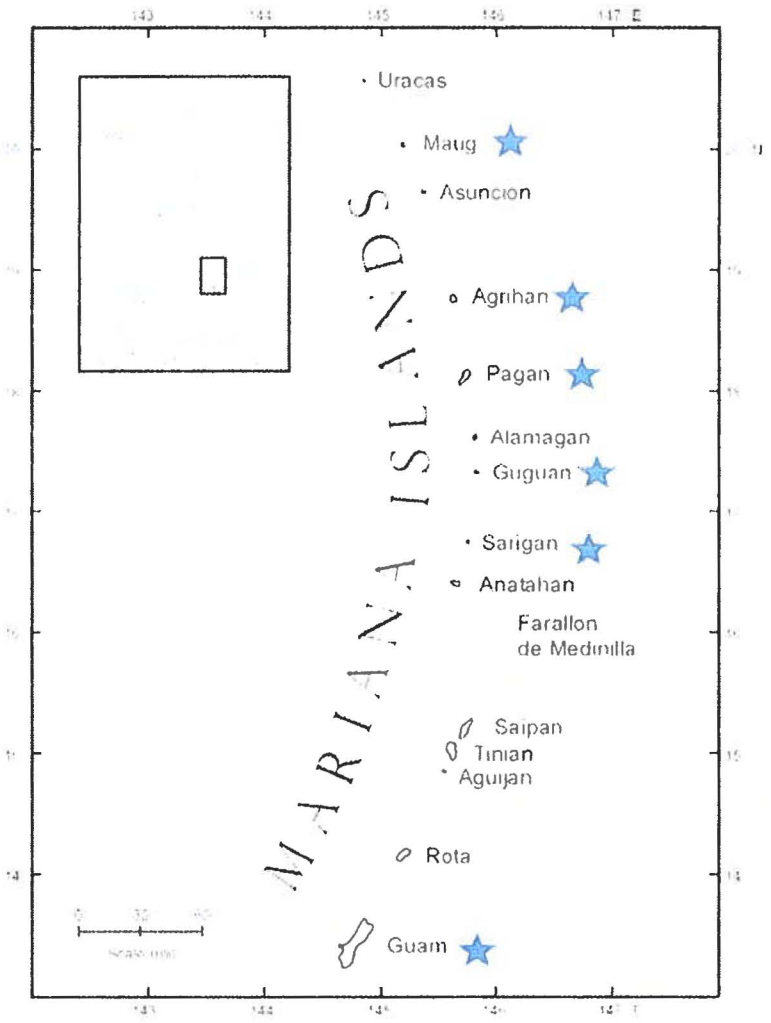


Figure 1. The Mariana Archipelago. Stars indicate islands where specimens were obtained.

The 10 northern islands (Uracas – Farallon de Medinilla) are geologically young (<1.5 million years), volcanic in origin, and mostly uninhabited. Four of the six southern islands, Saipan, Tinian, Rota, and Guam, are much older and comprised of a mix of volcanic and coral limestone or karst and support virtually all human populations found in the archipelago. Guam, the southernmost and largest island in the archipelago, has the largest human population, ca.160,000 (US Census Bureau, 2010). Guam is an

unincorporated territory of the United States while the remaining islands in the archipelago form the U.S. Commonwealth of the Northern Mariana Islands (CNMI).

Tissue Sample Acquisition and Processing

Thirty-seven whitetip reef sharks were caught opportunistically in conjunction with a NOAA research cruise (SE15-03) aboard the NOAA Ship Oscar Elton Sette to the Northern Mariana Islands (June 11-27, 2015). Sharks were caught in waters around the islands of Maug, Agrihan, Pagan, Guguan, and Sarigan (Fig. 1). Specific locations of where sharks were caught on each island are shown in Figure 2.

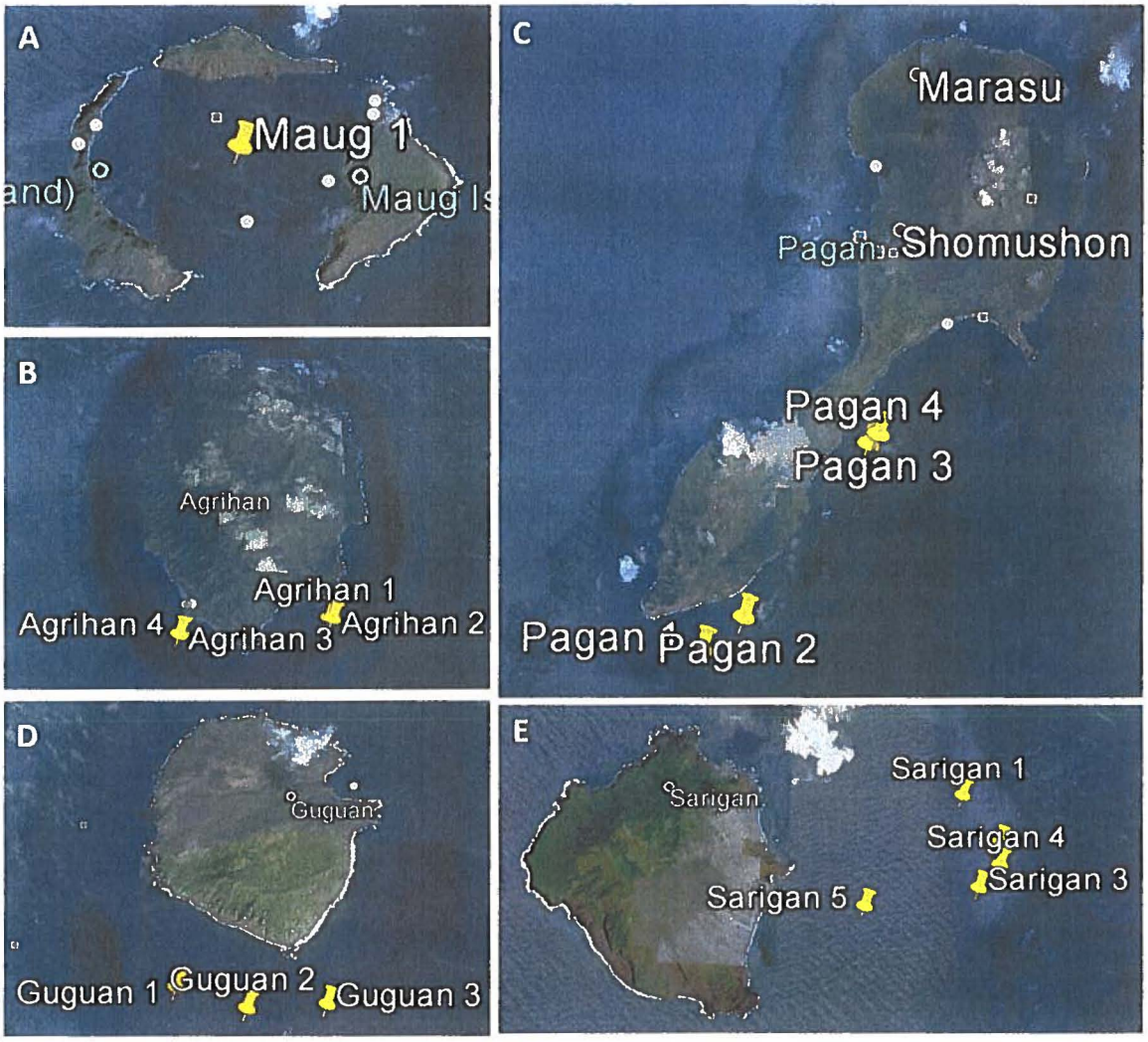


Figure 2. Sampling locations around the islands. (A) Maug, (B) Agrihan, (C) Pagan, (D) Guguan, and (E) Sarigan.

Sharks were caught either in baited lobster pots or a large rebar fish trap deployed from the NOAA Research Vessel Oscar Elton Sette during daytime and soaked overnight. For each trap deployment, a string of six lobster pots alternating with minnow traps (attached to gangions every 40 m of line with 100 m of float line from the last gangion) was deployed. Water depth and GPS coordinates were recorded with every deployment and the depth of the traps ranged from 39-136 m overall. Once sharks were brought on board, metadata were collected. These data included sex determination

(presence or absence of claspers) and body length (total length, TL and fork length, FL). Body size ranges of sharks collected with either method depended upon the size of the trap's entry hole. Tissues were sampled from each shark by making two non-lethal, minimally-invasive dorsal fin clips (Hussey et al. 2011, Matich et al. 2011) (Fig. 3). The fin-clips were preserved in vials containing 95% ethanol (EtOH). Each sample was then stored at -20°C while awaiting processing and analysis. After sampling, sharks were returned to the water alive and swam off under their own power.

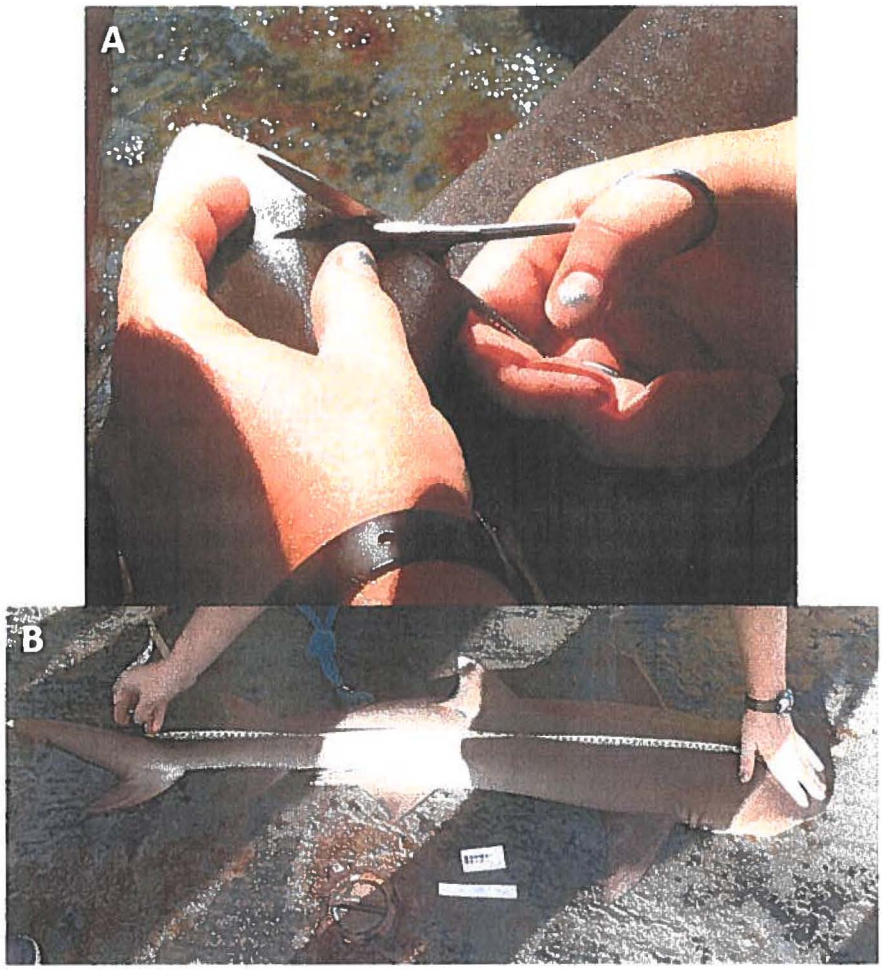


Figure 3. Shark specimen processing. (A) Two minimally-invasive fin clips were taken from the dorsal fin of each shark. (B) Each shark was measured and photographed.

Four additional whitetip reef sharks were caught off Guam aboard the NOAA R/V Oscar Elton Sette during a cruise between 25 July – 8 August, 2014 (Cruise Number SE14-06). All GPS and sampling data is located in Appendix A and Table 1. All these sharks were collected with lobster pots that soaked overnight. One dorsal fin clip and a photograph were obtained from each individual by NOAA scientists before the sharks were returned to the water. Each fin clip was stored in 95% ethanol and then stored at -20°C. The sex and GPS data of the four sharks captured from this cruise were recorded but went missing before being turned over for this study.

DNA Extraction and Polymerase Chain Reactions (PCR)

The population genetics of whitetip reef sharks was investigated using DNA obtained from dorsal fin clips. The method focused on microsatellites which are small (1-6 base pair) repeats (di-, tri-, or tetranucleotide) of non-coding DNA. Microsatellite sequences are usually flanked by unique non-repetitive DNA sequences, making it possible to reliably utilize PCR to target and amplify homologous microsatellites across samples (Tautz 1989, Bruford and Wayne 1993, Abdul-Muneer 2014). Microsatellites have many advantages since they are diploid, co-dominant and capable of being analysed from very small amounts of template DNA (Jarne and Lagoda 1996). Microsatellites are also used extensively to evaluate, paternity and relatedness, population structure, conservation management strategies, and determine genetic variability between individuals and population This results from the fact that there is little to no selective pressure on these introns allowing for higher mutation rates even within populations, making them ideal for the discernment of population structure over evolutionarily short

time frames (Bruford and Wayne 1993). Microsatellites are have been used elsewhere to evaluate genetic structure and gene flow in wild populations of many different species of elasmobranchs (Hernández et al. 2014, Vignaud et al. 2014, Spaet et al. 2015). Prior to the present study, microsatellite markers specifically developed for the whitetip reef shark did not exist, so microsatellite markers for a closely-related species (*Carcharhinus acronotus*) were tested and used. These microsatellite markers were obtained from a previous study into whitetip parthenogenesis (Portnoy et al. 2014). Eight microsatellite primer pairs were first tested to identify which ones worked with whitetip DNA. From the loci tested, four amplified well and showed a good range of polymorphisms, were employed in this study and lead to the same conclusions.

DNA was extracted using a DNeasy Blood and Tissue sampling kit (Qiagen, Inc.) following the kit protocol. All extracted DNA template concentrations were tested using a Qubit™ fluorometer to confirm appropriate yields before proceeding to PCR amplification. All samples were genotyped using four microsatellite loci developed for a close relative of *T. obesus*, the blacknose shark (*Carcharhinus acronotus*) (Giresi et al. 2012). The loci targeted in this study were *Cac40* (S9), *Cac54* (S11), *Cac57* (S13), and *Cac67* (S15). The PCRs were completed in a 10 µl reaction volume that contained a 1µl DNA template, 5µl of AmpliTaq™ Gold 360 Master Mix, 3µl nuclease free H_20; and 0.5 µl of each the forward and reverse primer at 1 pmol concentrations.

Thermocycling conditions varied for each locus. For loci S9, the PCR consisted of a denature step of 95°C for 10 min followed by 25 cycles of 95°C for 1 min, 52°C for 30 sec, and 72°C for 30 sec and a final extension step of 72°C for 4 min. The PCR for S11 and S15 consisted of a denature step of 95°C for 10 min, followed by 27 cycles of 95°C

for 1 min, 52°C for 30 sec, and 72°C for 30 sec and a final extension step of 72°C for 4 min. For loci S13, the PCR consisted of a denature step of 95°C for 10 min, followed by 36 cycles of 95°C for 1 min, 50°C for 30 sec, and 72°C for 30 sec and a final extension step of 72°C for 4 min. After the final extension step in all PCR runs, samples were then brought down to a 4°C soak until the PCR product was stored in a freezer at -20°C. All PCR amplification products were verified by electrophoresis of a 2µl aliquot through a 1% agarose gel stained with EtBr prior to the acrylamide gel analysis below.

Acrylamide Gel Electrophoresis

Gels were made with 20 ml 6.5% KB^{Plus} acrylamide matrix, with the addition of 150µl 10% ammonium persulfate and 15 µl Temed solutions. The gel matrix solution was then injected between the plate assembly with a syringe. A 0.25 mm sharks tooth comb was inserted to create the loading wells/lanes. The gel matrix was allowed to polymerize for 1.5 hours before cleaning and reassembly and placement of the rig into the DNA Analyzer 4300 for electrophoresis (LI-COR, Inc., USA). PCR products and size standard ladders were loaded in individual lanes in 1µl amounts after a pre-run/warm up of the gel and electrophoresed for analysis.

Data Analysis

Genotyping of all gel outputs was conducted on a DNA Analyzer 4300 sequencer with $SAGA^{GT}$ (LI-COR, Inc., USA) software. The $SAGA^{GT}$ software creates a data management system, automates lane finding and loci detection and provides automated genotyping. All genotypes were then accessed and checked manually to account and

adjust for any discrepancies prior to analysis. The genotypes were then exported to analysis software to estimate molecular variance. GenAlEx v. 6.503 (Peakall and Smouse, 2006) was used to evaluate the number of alleles, number of effective alleles, and expected and observed heterozygosities, as well as the fixation index. GenAlEx was also used to determine allele frequencies, private alleles, and allelic patterns. The allelic richness was calculated using FSTAT v. 2.9.3.2 (Goudet 1995).

Genetic structure within populations and among populations was first assessed by completing an analysis of molecular variance (AMOVA) in Arlequin v 3.5.2.2 (Excoffier et al. 2005). Population pairwise genetic structure was assessed using F_{ST} values and Nei's Genetic distance in GenAlEx. Results were then visualized by conducting a principal coordinate analysis (PCA) on the matrices created from both genetic distance tests (PCA plots do not show any relevant patterning-See Appendix B). In order to test for an isolation by distance (IBD) pattern of dispersal, two Mantel tests were conducted using both the pairwise F_{ST} values and Nei's genetic distances indices and the geographic distances between islands. The distance between each island was measured in kilometers (km) using Google Earth. Each distance was determined by drawing a straight line from the middle of one island to the middle of the next island. Because only one individual was caught at Maug, data for this shark were excluded from all statistical analyses.

RESULTS

Forty-one whitetip reef sharks were captured and biopsied from six different islands and subjected to microsatellite analysis at four different loci. The average TL of the 37 sharks caught in the northern Mariana Archipelago was 127.5 cm, with the shortest measuring 112 cm and the longest measuring 138 cm (see Table 1 for the size

distribution of the 37 whitetip reef sharks caught in the Northern Mariana Islands). Of the 37 whitetips caught on the Northern Mariana cruise, only 3 were female.

Table 1. The size distribution of the 37 *T. obesus* from the Northern Mariana Islands.

	111-115 cm	*116-120 cm*	*121-125 cm*	*126-130 cm*	*131-135 cm*	*136-140 cm*
Male	1	5	5	11	9	3
Female	0	0	0	2	1	0

Genetic diversity estimates were generally low for populations within each island. The mean number of alleles per locus ranged from 3.75 to 5.75, with standard error ranging from 1.109 to 2.213. The mean number of private alleles ranged from 0.50 to 1.00 with standard error ranging from 0.289 to 0.707 (Table 2). Characteristics of all four microsatellite loci are given in Table 3. The observed heterozygosities in the majority of loci were higher than the expected, except in the case of S13 and S15 from Agrihan, S11 from Pagan, and Guguan. Observed and expected heterozygosity indices per locus were nearly the same indicating that the microsatellite markers showed no bias. The fixation index for all islands was low indicating a lack of inbreeding.

Table 2. Summary statistics describing genetic diversity patterns over all microsatellite loci. Observations include the number of sample per island (N), mean number of alleles (N_A), standard error of the number of alleles (N_A SE) mean number of private alleles (N_{PA}), standard error of private alleles (N_{PA} SE) and mean allelic richness (AR), or average number of alleles per locus.

	N	N_A	N_A *SE*	N_{PA}	N_{PA} *SE*	*AR*
Agrihan	**6**	**4.00**	**1.225**	**0.50**	**0.289**	**3.353**
Pagan	**7**	**4.00**	**1.225**	**0.50**	**0.289**	**3.279**
Guguan	**11**	**4.25**	**1.315**	**0.75**	**0.479**	**2.994**
Sarigan	**12**	**5.75**	**2.213**	**1.00**	**0.707**	**3.515**
Guam	**4**	**3.75**	**1.109**	**0.50**	**0.289**	**3.750**

Table 3. Characteristics of four microsatellite loci from each island. The number of alleles (A), Shannon's information index (I), observed heterozygosity (H_O), expected heterozygosity (H_E), unbiased expected heterozygosity (uH_E), and the inbreeding coefficient (F_{IS}) are given.

Island		*S9*	*S11*	*S13*	*S15*	*Overall*
Agrihan	**Na**	3	1	6	6	4
	Ho	0.667	0.000	0.667	0.667	0.500
	He	0.486	0.000	0.792	0.750	0.507
	F_{IS}	-0.371	N/A	0.158	0.111	-0.034
Pagan	**Na**	2	2	5	7	4
	Ho	0.571	0.000	0.667	0.857	0.524
	He	0.408	0.245	0.667	0.816	0.534
	F_{IS}	-0.400	1.000	0.000	-0.050	0.138
Guguan	**Na**	3	2	4	8	4.250
	Ho	0.286	0.000	0.600	0.909	0.449
	He	0.255	0.165	0.480	0.773	0.418
	F_{IS}	-0.120	1.000	-0.250	-0.176	0.113
Sarigan	**Na**	3	1	9	10	5.750
	Ho	0.455	0.000	0.900	0.917	0.568
	He	0.368	0.000	0.815	0.847	0.507
	F_{IS}	-0.236	N/A	-0.104	-0.082	-0.141
Guam	**Na**	3	1	5	6	3.750
	Ho	0.500	0.000	0.750	1.000	0.563
	He	0.406	0.000	0.688	0.813	0.477
	F_{IS}	-0.231	N/A	-0.091	-0.231	-0.184

The AMOVA analysis (Table 4) shows that the majority of the variation within the population is within individuals. Genetic variation is essentially 0 which means there is only one population. The pairwise F_{ST} values (Table 5) showed little genetic differentiation between islands due to their values being closer to 0 than 1; this suggests absence genetic structure in the Marianas population. The same lack of genetic differentiation was found in Nei's genetic distance (Table 7). Both tests of isolation-by-distance (Tables 5, 6 and 7; Figure 4 and Figure 5) showed no correlation between genetic and geographic distances (linear regression; $R^2 = 0.0029$ and $R^2 = 0.043$, respectively).

Table 4. Summary of the analysis of molecular variance (AMOVA) to examine the structure of *T. obesus*.

Source of Variation	*Sum of Squares*	*Variance Components*	*Percentage Variation*	*P-Value*
Among Populations	3.925	-0.00636	-0.59792	1.000
Among Individuals within Populations	33.443	0.01859	1.74800	0.36364
Within Individuals	37.500	1.05132	98.84992	0.36364
Total	*74.869*	*1.06356*		

Table 5. Matrix of pairwise F_{ST} values between sampled islands.

Agrihan	*Pagan*	*Guguan*	*Sarigan*	*Guam*	
0.000					***Agrihan***
0.036	0.000				***Pagan***
0.062	0.042	0.000			***Guguan***
0.017	0.033	0.047	0.000		***Sarigan***
0.051	0.041	0.041	0.029	0.000	***Guam***

Table 6. Matrix of geographic distances (km) between all the Mariana Islands sampled for *T. obesus*.

Agrihan	*Pagan*	*Guguan*	*Sarigan*	*Guam*	
0					***Agrihan***
73.01	0				***Pagan***
162.78	89.31	0			***Guguan***
227.5	155.01	67.24	0		***Sarigan***
592.83	523.97	439.7	376.13	0	***Guam***

Table 7. Matrix of pairwise Nei's genetic distances for each island.

Agrihan	*Pagan*	*Guguan*	*Sarigan*	*Guam*	
0.000					***Agrihan***
0.064	0.000				***Pagan***
0.129	0.073	0.000			***Guguan***
0.036	0.054	0.097	0.000		***Sarigan***
0.120	0.073	0.079	0.070	0.000	***Guam***

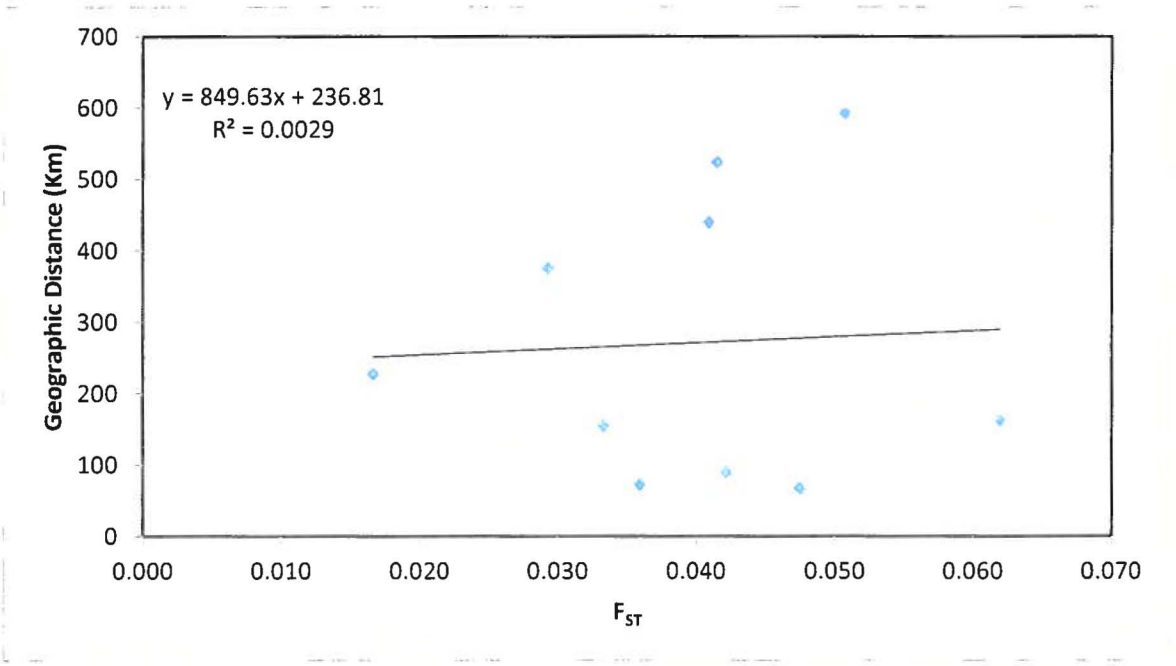


Figure 4. Relationship between pairwise F_{ST} indices and geographic distance (km) between islands sampled for *T. obesus*.

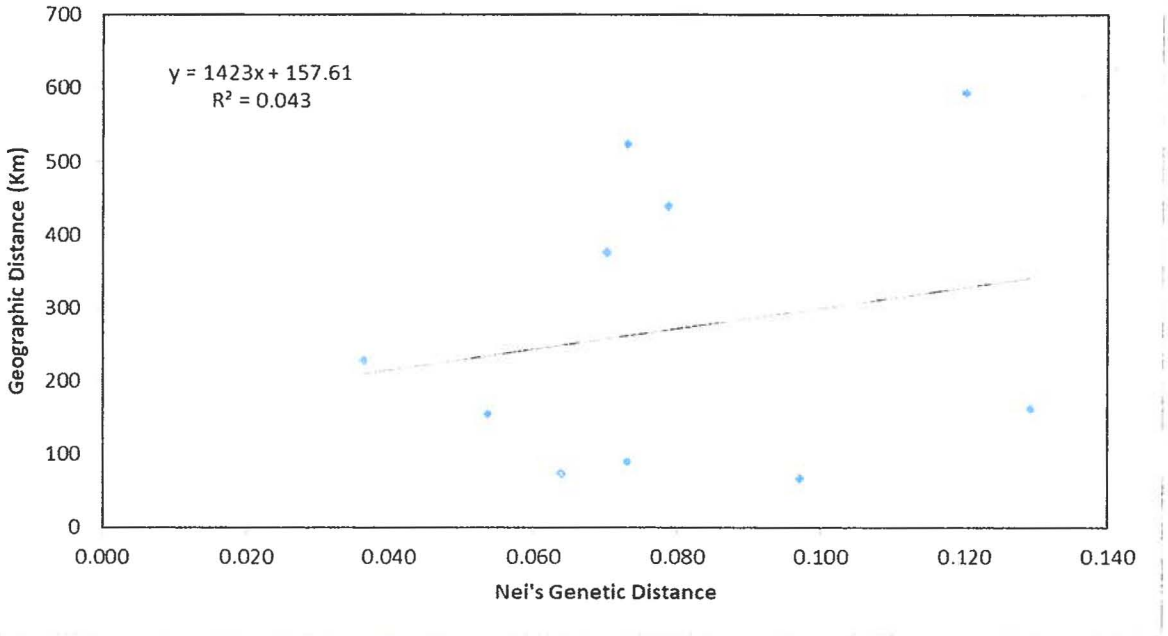


Figure 5. Relationship between Nei's genetic distance and distance (km) between islands sampled for *T. obesus*.

DISCUSSION

The population structure of whitetip reef sharks in the Mariana Archipelago was investigated through microsatellite analysis. Each of the Mariana islands sampled do not possess a genetically distinct population of whitetip sharks. Evidence of a lack of genetic differentiation suggests that the Mariana Archipelago has a single reproductively-mobile population. Each of the localities sampled share nearly identical allele parameters for the four microsatellite loci analyzed. This indicates that no islands have sharks that possess unique genetic patterns. Allelic patterns show that islands with a greater sample sizes have a greater number of alleles. This suggests there is more genetic diversity present but that my low sample sizes prevented complete quantification of this diversity. The low number of markers prevented some analyses from having significant p-values but each analysis indicated the same general conclusion, thus adding to its collective strength.

The observed heterozygosity (0.449-0.568) of whitetip sharks from these islands is low, but is within the same range as the expected heterozygosity (0.418-0.534); this indicates that these are ranges expected with the four genetic markers used in this study. Other microsatellite and population genomic studies on sharks have observed heterozygosity and expected heterozygosity within or near this same range (Heist et al. 2003, Keeney et al. 2005, Veríssimo et al. 2017). This indicates that there is not an issue with number or variability of the markers used in this study. Overall, there is no lack of heterozygosity between the islands suggesting that the populations are not genetically isolated and enough migration/genetic mixing between islands occurs to maintain homogeneity and prevent inbreeding through time. This supports the existence of a single interbreeding population within the Mariana Archipelago whose members migrate

between islands to mate at levels sufficient to negate any effect of geographic isolation. Because this connectivity pattern is observed in the whitetip reef sharks of the Mariana Archipelago, genetic structure is suppressed between islands. Connectivity of whitetip reef sharks in the Mariana Archipelago resemble the pattern of whitetip reef sharks in the Hawaiian Archipelago, but not the GBR, as reported by Whitney et al (2012b). The whitetip reef sharks in the GBR must have other factors resulting in population genetic structure besides deep-water barriers. These could include behavioral, oceanographic or ecologically derived barriers.

Previously, population genetic connectivity in the Mariana Archipelago has been demonstrated in studies of larval fishes and corals, that have a transient planktonic larval stage that promotes dispersal (Priest et al. 2012, Kendall and Poti 2014). Among terrestrial species of the archipelago, the Mariana crow (*Corvus kubaryi*) and the Mariana common moorhen (*Gallinula chloropus guami*) have been shown to exhibit inter-island connectivity due to the dispersal ability afforded by flight (Tarr and Fleischer 1999, Miller et al. 2015)

The lack of a significant relationship in the linear regressions (Nei's and Pairwise F_{ST}) between genetic and geographic distance supports the finding that there is no isolation by distance between islands. If isolation by distance did exist, the IBD plot would be linear. Degrees of isolation are important in creating population structure, which was found in whitetip reef sharks in the GBR (Whitney et al. 2012b), as well as another reef-associated shark, *Carcharhinus amblyrhynchos* (Espinoza et al. 2014). Since the IBD plots show a cluster of points this also suggests that sharks migrate between islands and further suggests that some sharks may be capable of traveling beyond the next

closest island. The population shows no signs of inbreeding, therefore is panmictic, meaning that every shark has an equal chance of combining with any other in the population regardless of genotype, and that no selective mating is indicated. This finding suggests also that depth and distance between islands may not be a limit whitetip dispersal.

No study of this kind has been reported previously for sharks in the Mariana Islands. Connectivity studies on other reef sharks in the Pacific have been undertaken and the results also support the finding that reef sharks are capable of migrating between islands separated by deep channels. Mourier and Planes (2013) demonstrated that female blacktip reef sharks (*C. melanopterus*) in French Polynesia, which also exhibit high reef fidelity and home ranges, can travel great distances over deep open ocean (up to 2000 m depth) to reach birthing sites and nurseries. They will also travel up to 50 km to reach neighboring atolls and islands. This is not surprising based on the wide distribution patterns of reef sharks in the Pacific. Microsatellite analysis of blacktip reef sharks in French Polynesia indicated that all individuals consisted of a single gene pool. Other species of reef sharks can also travel considerable distances over deep water. For example, grey reef sharks (*C. amblyrhynchos*) in Australia were found to traverse open ocean in order to reach an island 134 km away (Heupel et al. 2010). Similarly, a male Caribbean reef shark (*C. perezi*) traveled at least 50 km between two atolls with 30 km of that over open water (Chapman et al. 2005).

The sharks that were caught in the Northern Marianas were mostly male caught at greater depths far from most reefs, with only three out of the 37 being females. This is an interesting result. All individuals were shown in the analysis to be part of the same

interbreeding population. This suggests that the sampled individuals could have been migrating between islands, or at minimum they regularly make forays into deeper waters away from shallow near shore reef habitats. That males may navigate over longer distances while females do not suggests that the latter may be restricted to suitable parturition sites in order to breed successfully, and it is the movement of males that results in genetic connectivity across large distances between islands in the Marianas archipelago (Chapman et al. 2015). The decline in reef shark populations, which may be the result of overfishing and reef degradation, may motivate sharks to move between islands to find greater mating or feeding opportunities.

CONCLUSIONS

Evidence from the four microsatellite markers used in this study suggest that whitetip reef sharks in the Mariana Archipelago comprise a single population. Therefore, it is likely that individuals are migrating between islands within the archipelago, and that distance and depth may not be limiting factors. Potentially, more tissue samples from sharks along the entire archipelago with a more targeted sampling regime that includes shallow reefs and greater coverage and the development of additional genetic markers may yield more complete and informative results. There are many ways to address questions raised by this study. If sampling were increased to target shallow reef habitats, and not just deeper sandy slope areas, microsatellites could be used to determine parental relationships. This could determine if offspring were living near their parents, or if offspring were residing on reefs on different islands. This could further support the finding that the Mariana Archipelago consists of one population of whitetip

reef sharks. Studies of differences in migratory behavior between males and females, such as employing modern acoustic tagging/tracking technology, are suggested in order to determine if there is mating site fidelity, or if there are one or more patterns of migration associated with a breeding cycle. A logical continuation of this project would be to focus on mitochondrial genomics (maternally derived) or sex-linked information to discern if female whitetips show site fidelity. This could be determined by obtaining a greater sample size. The results of such studies will better inform fisheries and conservation managers.

REFERENCES CITED

Abdul-Muneer, P. M. 2014. Application of microsatellite markers in conservation genetics and fisheries management : Recent advances in population structure analysis. Genetics Research International 2014:1–11.

Baum, J. K., R. A. Myers, D. G. Kehler, B. Worm, S. J. Harley, and P. A. Doherty. 2003. Collapse and conservation of shark populations in the Northwest Atlantic. Science 299:389–92.

Bradley, D., E. Conklin, Y. P. Papastamatiou, D. J. McCauley, K. Pollock, B. E. Kendall, S. D. Gaines, and J. E. Caselle. 2017a. Growth and life history variability of the grey reef shark (*Carcharhinus amblyrhynchos*) across its range. PLoS ONE 12:1–20.

Bradley, D., E. Conklin, Y. P. Papastamatiou, D. J. Mccauley, K. Pollock, A. Pollock, B. E. Kendall, S. D. Gaines, and J. E. Caselle. 2017b. Resetting predator baselines in coral reef ecosystems. Nature Publishing Group:1–9.

Bruford, M. W., and R. K. Wayne. 1993. Microsatellites and their application to population genetic studies. Current Opinion in Genetics and Development 3:939–943.

Bryan, P. G. 1972. The inshore sharks of Guam: methods of small-boat shark fishing. University of Guam Marine Laboratory Technical Report 4:1–26.

Castro, A. L. F., B. S. Stewart, S. G. Wilson, R. E. Hueter, M. G. Meekan, P. J. Motta, B. W. Bowen, and S. A. Karl. 2007. Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). Molecular Ecology 16:5183–5192.

Chapman, D. D., K. A. Feldheim, Y. P. Papastamatiou, and R. E. Hueter. 2015. There and back again: A review of residency and return migrations in sharks, with implications for population structure and management. Annual Review of Marine Science 7:547–570.

Chapman, D. D., E. K. Pikitch, E. Babcock, and M. S. Shivji. 2005. Marine reserve design and evaluation using automated acoustic telemetry: a case-study involving coral reef-associated sharks in the mesoamerican caribbean. Marine Technology Society Journal 39:42–55.

Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace, and R. K. Wayne. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution 15:290–295.

Dulvy, N. K., J. R. Ellis, N. B. Goodwin, A. Grant, J. D. Reynolds, and S. Jennings. 2004. Methods of assessing extinction risk in marine fishes. Fish and Fisheries 5:255–276.

Duncan, K. M., A. P. Martin, B. W. Bowen, and H. G. De Couet. 2006. Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). Molecular Ecology 15:2239–2251.

Espinoza, M., M. R. Heupel, A. J. Tobin, and C. A. Simpfendorfer. 2014. Residency patterns and movements of grey reef sharks (*Carcharhinus amblyrhynchos*) in semi-isolated coral reef habitats. Marine Biology 162:343–358.

Excoffier L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): an intergrated software package of population genetics data analysis. Evol Bioinfrom Online 1:47-50.

Ferretti, F., R. A. Myers, F. Serena, and H. K. Lotze. 2008. Loss of large predatory sharks from the Mediterranean Sea. Conservation Biology 22:952–964.

Frid, A., L. M. Dill, R. E. Thorne, and G. M. Blundell. 2007. Inferring prey perception of relative danger in large-scale marine systems. Evolutionary Ecology Research 9:635–649.

Giresi, M., M. A. Renshaw, S. Portnoy, and J. R. Gold. 2012. Isolation and characterization of microsatellite markers for the blacknose shark, Carcharhinus acronotus. Conservation Genetics Resources.

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

Heist, E. J., J. L. Jenkot, D. B. Keeney, R. L. Lane, G. R. Moyer, B. J. Reading, and N. L. Smith. 2003. Isolation and characterization of polymorphic microsatellite loci in nurse shark (*Ginglymostoma cirratum*). Molecular Ecology Notes 3:59–61.

Heithaus, M. R., A. Frid, A. J. Wirsing, and B. Worm. 2008. Predicting ecological consequences of marine top predator declines. Trends in Ecology and Evolution 23:202–210.

Hernández, S., C. Duffy, M. P. Francis, and P. a. Ritchie. 2014. Evidence for multiple paternity in the school shark *Galeorhinus galeus* found in New Zealand waters. Journal of Fish Biology 85:1739–1745.

Heupel, M. R., C. A. Simpfendorfer, and R. Fitzpatrick. 2010. Large-scale movement and reef fidelity of grey reef sharks. PLoS ONE 5:1–5.

Hoelzel, A. R., M. S. Shivji, J. Magnussen, and M. P. Francis. 2006. Low worldwide genetic diversity in the basking shark (*Cetorhinus maximus*). Biol Lett. 2:639–642.

Hussey, N. E., D. D. Chapman, E. Donnelly, D. L. Abercrombie, and A. T. Fisk. 2011. Fin-icky samples : an assessment of shark fin as a source material for stable isotope analysis. Limnology and Oceanography: Methods 9:524–532.

Jarne, P., and P. J. L. Lagoda. 1996. Microsatellites, from molecules to populations and back. Trends in Ecology and Evolution 11:424–429.

Jorgensen, S. J., C. A. Reeb, T. K. Chapple, S. Anderson, C. Perle, S. R. Van Sommeran, C. Fritz-Cope, A. C. Brown, A. P. Klimley, and B. A. Block. 2010. Philopatry and migration of Pacific white sharks. Proceedings of the Royal Society B 277:679–688.

Keeney, D. B., M. R. Heupel, R. E. Hueter, and E. J. Heist. 2005. Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. Molecular Ecology 14:1911–1923.

Kendall, M. S., and M. Poti. 2014. Potential larval sources, destinations, and self-seeding in the Mariana Archipelago documented using ocean drifters. Journal of Oceanography 70:549–557.

Madin, E. M. P., S. D. Gaines, and R. R. Warner. 2010. Field evidence for pervasive indirect effects of fishing on prey foraging behavior. Ecology 91:3563–3571.

Martin, S. L., K. S. Van Houtan, T. T. Jones, C. F. Aguon, J. T. Gutierrez, R. B. Tibbatts, S. B. Wusstig, and J. D. Bass. 2016. Five decades of marine megafauna surveys from Micronesia. Frontiers in Marine Science 2:1–13.

Matich, P., M. R. Heithaus, and C. A. Layman. 2011. Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. Journal of Animal Ecology 80:294–305.

Miller, M. P., T. D. Mullins, S. M. Haig, L. Takano, and K. Garcia. 2015. Genetic structure, diversity, and interisland dispersal in the endangered Mariana Common Moorhen (*Gallinula chloropus guami*). The Condor 117:660–669.

Mourier, J., and S. Planes. 2013. Direct genetic evidence for reproductive philopatry and associated fine-scale migrations in female blacktip reef sharks (*Carcharhinus melanopterus*) in French Polynesia. Molecular Ecology 22:201–214.

Nadon, M. O., J. K. Baum, I. D. Williams, J. M. Mcpherson, B. J. Zgliczynski, B. L. Richards, R. E. Schroeder, and R. E. Brainard. 2012. Re-creating missing population baselines for Pacific reef sharks. Conservation Biology 26:493–503.

Nelson, D. R., and R. H. Johnson. 1980. Behavior of reef sharks of Rangiroa, French Polynesia. Natl Geogr Soc Res Rep 12:479-499.

Paine, R. T. 1966. Food web complexity and species diversity.The American Naturalist 100:65-75.

Papastamatiou, Y. P., Y. Y. Watanabe, D. Bradley, L. E. Dee, K. Weng, C. G. Lowe, and J. E. Caselle. 2015. Drivers of daily routines in an ectothermic marine predator: hunt warm, rest warmer? PLoS ONE 10:1–16.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288-295.

Portnoy, D. S., C. M. Hollenbeck, J. S. Johnston, H. M. Casman, and J. R. Gold. 2014. Parthenogenesis in a whitetip reef shark *Triaenodon obesus* involves a reduction in ploidy. Journal of Fish Biology 85:502–508.

Priest, M. A., A. R. Halford, and J. L. McIlwain. 2012. Evidence of stable genetic structure across a remote island archipelago through self-recruitment in a widely dispersed coral reef fish. Ecology and Evolution 2:3195–3213.

Randall, J. E. 1977. Contribution to the biology of the whitetip reef shark (*Triaenodon obesus*). Pacific Science 31:143–164.

Robbins, W. D. 2006. Abundance, demography and population structure of the grey reef shark (*Carcharhinus amblyrhynchos*) and the white tip reef shark (*Triaenodon obesus*) (Fam. Charcharhinidae). PhD Thesis, James Cook University:1–215.

Robbins, W. D., M. Hisano, S. R. Connolly, and J. H. Choat. 2006. Ongoing collapse of coral-reef shark populations. Current Biology 16:2314–2319.

Rodrigues-Filho, L. F., D. Pinhal, D. Sodré, and M. Vallinoto. 2012. Shark DNA forensics : applications and impacts on genetic diversity. Analysis of Genetic Variation in Animals:269–286.

Sandin, S. A., J. E. Smith, E. E. DeMartini, E. A. Dinsdale, S. D. Donner, A. M. Friedlander, T. Konotchick, M. Malay, J. E. Maragos, D. Obura, O. Pantos, G. Paulay, M. Richie, F. Rohwer, R. E. Schroeder, S. Walsh, J. B. C. Jackson, N. Knowlton, and E. Sala. 2008. Baselines and degradation of coral reefs in the Northern Line Islands. PLoS ONE 3:e1548. doi:10.1371/journal.pone.0001548.

Schrey, A. W., and E. J. Heist. 2003. Microsatellite analysis of population structure in the shortfin mako (*Isurus oxyrinchus*). Canadian Journal of Fisheries and Aquatic Sciences 60:670–675.

Spaet, J. L. Y., R. W. Jabado, A. C. Henderson, A. B. M. Moore, and M. L. Berumen. 2015. Population genetics of four heavily exploited shark species around the Arabian Peninsula. Ecology and Evolution 5:2317–2332.

Tarr, C. L., and R. C. Fleischer. 1999. Population boundaries and genetic diversity in the endangered Mariana crow (*Corvus kubaryi*). Molecular Ecology 8:941–949.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17:6463–6471.

Tricas, T. C., and E. M. Le Feuvre. 1985. Mating in the reef white-tip shark *Triaenodon obesus*. Marine Biology 84:233-237.

Veríssimo, A., Í. Sampaio, J. R. McDowell, P. Alexandrino, G. Mucientes, N. Queiroz, C. da Silva, C. S. Jones, and L. R. Noble. 2017. World without borders—genetic population structure of a highly migratory marine predator, the blue shark (*Prionace glauca*). Ecology and Evolution 7:4768–4781.

Vignaud, T. M., J. Mourier, J. A. Maynard, R. Leblois, J. Spaet, E. Clua, V. Neglia, and S. Planes. 2014. Blacktip reef sharks, *Carcharhinus melanopterus*, have high genetic structure and varying demographic histories in their Indo-Pacific range. Molecular Ecology 23:5193–5207.

Ward-Paige, C. A., C. Mora, H. K. Lotze, C. Pattengill-Semmens, L. McClenachan, E. Arias-Castro, and R. A. Myers. 2010. Large-scale absence of sharks on reefs in the greater-Caribbean: A footprint of human pressures. PLoS ONE 5:e11968. doi:10.1371/journal.pone.0011968.

Whitney, N. M., R. L. Pyle, K. N. Holland, and J. T. Barcz. 2012a. Movements, reproductive seasonality, and fisheries interactions in the whitetip reef shark (*Triaenodon obesus*) from community-contributed photographs. Environmental Biology of Fishes 93:121–136.

Whitney, N. M., W. D. Robbins, J. K. Schultz, B. W. Bowen, and K. N. Holland. 2012b. Oceanic dispersal in a sedentary reef shark (*Triaenodon obesus*): Genetic evidence for extensive connectivity without a pelagic larval stage. Journal of Biogeography 39:1144–1156.

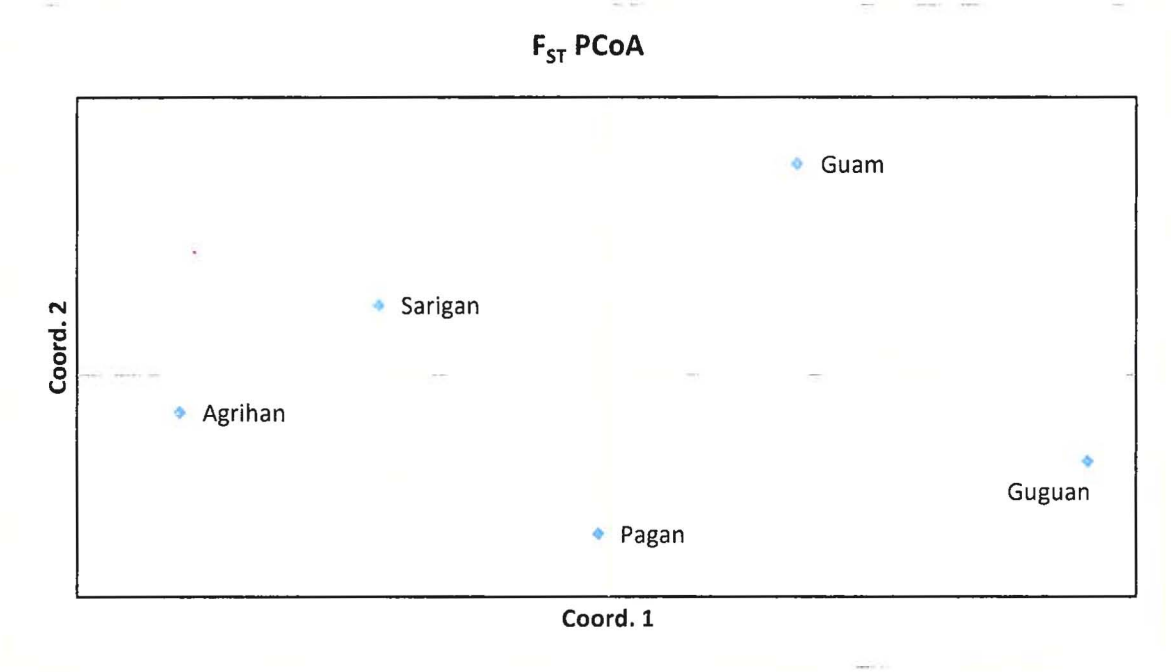
APPENDIX A

Summary of *Triaenodon obesus* captured by island. Multiple trap deployments were possible on some islands, so all latitude and longitude measurements are included. The sex and GPS data of the Guam samples are missing.

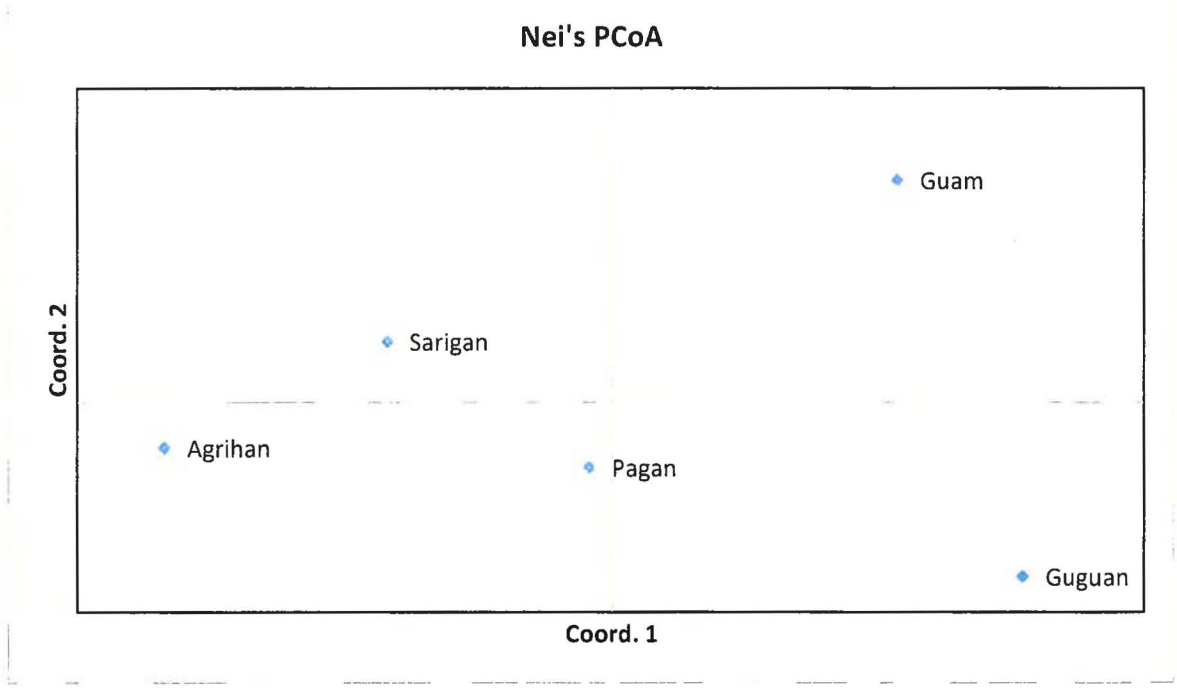
Island	Latitude	Longitude	N	Males	Females
Maug	**20.022127**	**145.2213**	**1**	**1**	**0**
Agrihan	**18.730488**	**145.687605**	**1**	**1**	**0**
	18.7303832	**145.688450**	**2**	**2**	**0**
	18.726528	**145.649460**	**1**	**1**	**0**
	18.726808	**145.649910**	**2**	**2**	**0**
Pagan	**18.033840**	**145.719829**	**2**	**2**	**0**
	18.040713	**145.729447**	**2**	**2**	**0**
	18.079940	**145.760547**	**1**	**1**	**0**
	18.077880	**145.758213**	**2**	**1**	**1**
Guguan	**17.292153**	**145.834790**	**1**	**1**	**0**
	17.290385	**145.8404507**	**1**	**1**	**0**
	17.290698	**145.846772**	**9**	**8**	**1**
Sarigan	**16.709980**	**145.805650**	**3**	**3**	**0**
	16.705424	**145.808738**	**1**	**1**	**0**
	16.701208	**145.806620**	**3**	**3**	**0**
	16.703330	**145.808632**	**1**	**1**	**0**
	16.699577	**145.796460**	**4**	**3**	**1**
Guam	**13.4443**	**144.7937**	**4**	**-**	**-**

APPENDIX B

The PCoA plots associated with the Fst and Nei's Genetic Distance matrices.



The F_{ST} PCoA plot accounts for 92.51% of variation over the first 3 axes.



The Nei's PCoA plot accounts for 91.80% of variation over the first 3 axes.