FINAL REPORT: Characterizing shark depredation in the fisheries of Guam and Saipan

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Prepared for:

Western Pacific Regional Fishery Management Council
Sponsor Representative: Dr. Mark Fitchett

Prepared by:

Hawaii Institute of Marine Biology,
University of Hawaii at Manoa,
46-007 Lilipuna Rd.,
Kaneohe, Hi. 96744.
Principal Investigator: Dr. Carl Meyer

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Executive Summary

Shark depredation in the bottomfishing and troll fisheries is a long-running concern in Guam and Saipan. To date there has been uncertainty and speculation about which shark species are responsible for depredating catches because most incidents are obscured from view below the surface and, even when sighted, sharks can be difficult to visually identify. We collaborated with Guam and Saipan fishers to identify depredating shark species by analyzing transfer DNA collected from swab samples taken from shark-bitten fishes (when sharks bite hooked fish they leave traces of their DNA in the bite impression). Fishers were trained at workshops held in Guam and Saipan in early 2020 and equipped with swab kits to take on fishing trips. Fishing was disrupted by the Covid-19 pandemic but 4 fishers provided swab samples from 29 bottomfishing depredation events in waters off Guam and Saipan. Using DNA barcoding, we successfully identified the shark species responsible for depredating catches in 26 (90%) out of 29 cases. A total of 5 shark species were identified from depredation swabs: silvertip shark (*Carcharhinus albimarginatus*), silky shark (*Carcharhinus falciformis*), grey reef shark (*Carcharhinus amblyrhynchos*), whitetip reef shark (*Triaenodon obesus*) and tiger shark (*Galeocerdo cuvier*). Three of five species identified (silvertip shark, grey reef shark and whitetip reef shark) are strongly reef-associated and the remaining two (tiger shark, silky shark) are strongly associated with shelf habitats and drop-offs. No threatened or endangered shark species (e.g. oceanic whitetip sharks, *Carcharhinus longimanus*) were detected interacting with the Marianas bottomfish fishery. The transfer DNA swab method is effective for identifying depredating sharks from damaged catches and was successfully implemented by fisher citizen scientists. Future research should focus on additional depredation swab sampling to further characterise the depredating species and tracking studies of the known depredating species to determine their fidelity to fishing sites. Incorporating University of Guam graduate students into the research team could facilitate engagement with the local fishing community and build local technical capacity for this type of applied research. Chartering local fishing vessels would provide financial incentives to participate in shark research while leveraging fisher skill and expertise in identifying fishing sites for shark tagging and receiver placement.

Introduction

Fishers in the CNMI and Guam troll and bottomfish fisheries report frequent interactions with sharks resulting in (1) economic losses due to catch depredation and gear loss, and (2) 'hidden' indirect fisheries mortality in target species (fishers can lose multiple fish to depredation for each one successfully landed). Shark depredation in these fisheries has been identified as a major problem since at least 2004 (WPRMC 2004) and in 2015, 338 (55%) of 617 fishers interviewed reported shark interactions (WPRMC 2016). Fishers report losing up to 60% of catches to shark depredation (WPRMC 2006), and describe significant increases in fish loss to shark depredation over time, accompanied by changing patterns of behaviour with sharks becoming more aggressive around fishing activities (WPRMC 2013).

Troll and bottomfishing are the two most popular methods of small boat fishing around Guam and Saipan (Myers 1993, Dalzell et al. 1996, Ayers 2018). These fishing methods are important to the local communities, providing a source of fresh food and basic income and are an integral part of the islands' traditional and modern cultures (Amesbury et al., 1986, Ayers 2018, Chan and Pan 2019). In 2017, the small boat fisheries provided approximately 8 pounds of fresh fish per capita in the CNMI and 5 pounds per capita in Guam (Chan and Pan 2019). The major species taken in the troll fishery are mahi-mahi (*Coryphaena hippurus*), wahoo (*Acanthocybium solandri*), skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacares*) tunas, and blue marlin (*Makaira sp.*) (Amesbury et al. 1986). Catches of large pelagic fishes around Guam and the CNMI are strongly seasonal (Amesbury & Babin 1990). Bottomfish fishing targets a variety of reef-associated species including high value snappers. In Guam, the average potential sales value of fish caught by trolling and bottomfishing ranged from $117 to $176 per trip between 2015 and 2017, with average trip costs of around $100. In the CNMI, the average potential ex-vessel sales value ranged between $200–300 from 2012 to 2017, with trip costs under $90 (Chan and Pan 2019). With an average per trip profit margin of $17 to $200 even a small number of depredation losses are economically significant to fishers.

Based on anecdotal observations and reported shark landings at Western Pacific Regional Fishery Management Council meetings (WPRMC, 2013), the species most frequently involved in troll fishery interactions are thought to be silky sharks (*Carcharhinus falciformis*), Galapagos sharks (*Carcharhinus galapagensis*) and oceanic whitetip sharks (*Carcharhinus longimanus*), whereas bottomfish fishers also report interactions with tiger sharks (*Galeocerdo cuvier*), blacktip sharks (presumably *Carcharhinus limbatus*) and whitetip sharks (presumably *Triaenodon obesus*). However, depredating species require further characterization as most interactions are not directly observed because they occur at depth making it impossible to visually identify the species involved. Even when sharks are clearly observed or captured, many species can only be distinguished by subtle differences in morphological characteristics (e.g. Galapagos shark versus silky shark) making identification challenging even for trained

observers. We need a clearer understanding of shark interactions in order to develop effective strategies to reduce shark bycatch and depredation in the Marianas fisheries.

To help create a clearer picture of depredation incidents, we developed easy-to-use forensic DNA kits to identify depredating shark species via simple swabbing of shark-damaged fishes. We conducted volunteer training and recruitment workshops in Guam and Saipan, and distributed these kits to local volunteer fishers participating in the study. Despite major disruption from the Covid-19 pandemic, Guam and Saipan fishers collected shark depredation swab samples and we used these to successfully identify the depredating shark species in bottomfishing depredation incidents.

Goals and objectives

Our overarching goal was to characterize shark interactions with the CNMI and Guam troll and bottomfish fisheries. Specific objectives included: (1) Engaging the fishing community in this research from the outset of the project, (2) Implementing a new, easy-to-use DNA swab test to identify depredating species, (3) Collecting swab samples from depredated catches in the troll and bottomfish fisheries, (4) Deploying underwater cameras to document depredation events in the troll and bottomfish fisheries, (5) Identifying species involved and quantifying shark interactions and bycatch, and (6) Disseminating project information and results through education and outreach efforts.

Methods

Overview

We utilized DNA barcoding to identify shark species from mucus swabs taken from depredated fishes. In broad terms, the process involved (1) local fishers in Guam and CNMI thoroughly swabbing shark bite wounds on depredated fishes, with three replicates for each fish; (2) storing the swabs in an appropriate medium to avoid DNA degradation while shipping samples to Hawai‘i for genetic analysis; (3) DNA extraction and targeted gene amplification; and (4) Sequencing of amplified DNA and comparison to known genetic references to identify species. Barcodes targeted the cytochrome oxidase subunit 1 (COI) region of mitochondrial DNA (mtDNA), a gene region often used in studies to identify species across various taxa. We compared our barcoding data to the open source genetic reference databases NCBI’s GenBank and the Barcode of Life Database (BOLD), in order to identify depredating shark species.

Initial validation of transfer DNA swabbing technique using captive sharks

Prior to applying our methods to real-world depredation incidents, we validated the technique with experimental depredation events by captive sharks under controlled conditions (Figure 1).

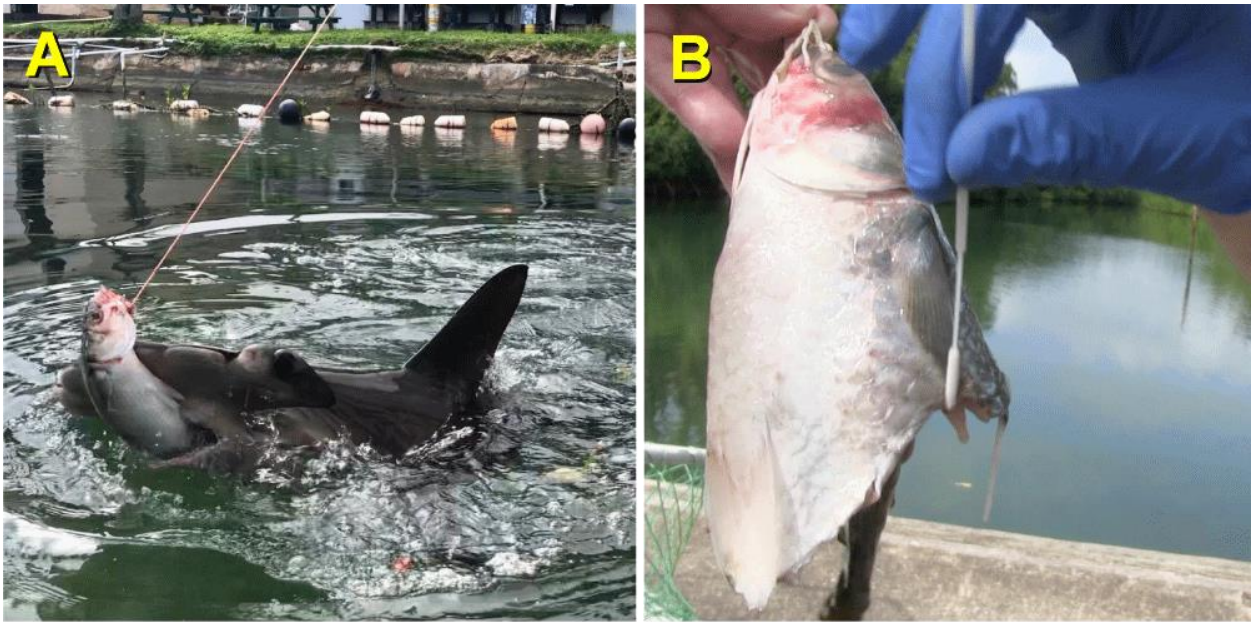

Figure 1. Experimental depredation experiments with captive adult scalloped hammerhead sharks (*Sphyrna lewini*) and mullet (*Mugil cephalus*). A. Scalloped hammerhead shark biting mullet. B. Swab sampling of bitten fish.

To obtain experimentally depredated fish for transfer DNA trials, dead striped mullet (*Mugil cephalus*) were tied to a handline and presented to adult sandbar (*Carcharhinus plumbeus*) and scalloped hammerhead (*Sphyrna lewini*) sharks held in captivity at the Hawaii Institute of Marine Biology (Figure 1a). The bitten fish were recovered and the bite margins were thoroughly swabbed with sterile swabs to collect shark transfer DNA (3 replicates per fish) (Fotedar et al. 2019)(Figure 1b). The tips of the swabs were carefully cut off with scissors and stored in vials containing DNA extraction buffer (Buffer ATL from Qiagen DNeasy Blood & Tissue Kit) and stored frozen prior to analysis. The experimental procedures were recorded to create training materials for Guam and Saipan fishers:

(https://www.youtube.com/watch?v=UhTXW-JQA_s).

<u>Real-world depredation sampling in Guam and Saipan fisheries</u>

Twenty-five fisher volunteers were recruited and trained to use DNA swab kits at project workshops held in Guam and Saipan in January 2020. Each fisher received a DNA swab kit containing sterile swabs, nitrile gloves, storage vials containing DNA extraction buffer, scissors, written instructions, and a data sheet for recording depredation metadata. Between February and August 2020, captured fishes with depredation damage were swabbed by fisher volunteers using protocols described above. Swab samples were placed on ice for the remainder of the fishing trip and then stored frozen before being collected by project collaborators and shipped to Hawai‘i for analysis in December 2020. Fishers recorded metadata for each depredation sample including

date and time, fishing location and method, and fish species damaged. Data sheets were shipped along with swabs.

DNA Extraction and Sequencing

DNA extraction and amplification was carried out at the Hawai'i Institute of Marine Biology (University of Hawai'i at Mānoa). DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Mississauga, ON, Canada) with some minor changes to the manufacturer protocol for tissue specimens; one third of the swab along with 360 µl of the associated extraction buffer (Buffer ATL) and 40 µl of Proteinase K were used in an initial digestion of 2 hours (rather than overnight). Double volumes were used until all of the digested samples were transferred through the filter column. Lastly, two elutions were performed with 100 µl HPLC water each. Polymerase chain reactions (PCR) were used to amplify the extracted DNA using shark-specific primers to target the cytochrome c oxidase subunit 1 (COI) region of the mitochondrial genome. The shark-specific primers described in Fotedar et al. (2019) were able to amplify shark DNA from a wide variety of species without co-amplifying contaminate DNA:

CO1shark25F -5' AGCAGGTATAGTTGGAACAGCCC 3'

CO1shark 315R -5' GCTCCAGCTTCTACTCCAGC 3'

PCR reactions included 8.6 µl of ultrapure water, 10 µl of GoTaq Green Master Mix (Promega Corporation, USA), 0.2 µl of each primer (10 mM), and 1 µl of template DNA for a total reaction volume of 20 µl. Amplification was performed using a T100 thermal cycler (Bio-Rad Laboratories, Inc.) with an initial denaturation at 95 ºC for 3 minutes followed by 35 cycles of 95 ºC for 30 seconds, annealing at 62 ºC for 45 seconds, and one minute at 72 ºC, with a final extension at 72 ºC for 5 minutes. Products were examined using 1% agarose gel stained with GelRed, and samples that failed to amplify were re-tested. Samples that appeared in gel images were purified using ExoFap (Exonuclease I and FastAP - Life Technologies, Carlsbad, CA). If gels showed multiple bands, samples were cleaned using gel excision by loading remaining pcr product (~17 ul) into a 2% agarose gel using 1 x modified TAE (no EDTA) and run at 50 mV for 90 minutes. Bands were then excised from the gel using a sterile scalpel and loaded into a filter column and centrifuged for 10 min at 5000 rpm. The filtered volume was then used in sequencing reactions. 5 µl ExoFap or Gel Excision products were mixed with 1 µl of either forward or reverse primer (3.2 mM), and shipped to Genewiz Inc (South Plainfield, NJ) for sequencing using Applied Biosystems BigDye version 3.1 and Applied Biosystems 3730xl DNA Analyzer.

Sequence data were processed in Geneious 10.0.9 (Kearse et al. 2012). Sequences were trimmed at an error probability limit of 0.05, and forward and reverse sequences were assembled and edited visually. Assembled sequences were exported as a fasta file and placed in a BLAST

search function from GenBank (Altschul et al. 1990). Species identification of bite incidents were considered valid if at least one of three replicate swabs were >98% homologous with multiple reference barcodes of the same species. Sequencing results were also aligned by bite (results from more than one swab taken from the same bite impression) to gain confidence that genetic material from all successful replicates matched. A third layer of confirmation was achieved by inputting aligned bite sequences to the BLASTn function to check for >98% identity between combined bite data and reference barcodes.

Camera deployments to record images of depredating sharks

The Covid-19 pandemic prevented us from fully implementing the camera component of the study but preliminary camera deployments were conducted with local fishers during the January-February 2020 visit to Guam. GoFish Cams were deployed on trolling lines by project personnel accompanying local fishers during fishing activities around Guam.

Social media outreach

To reach a broad audience, we created Facebook and Instagram pages to advertise the project and provide regular updates on project activities and progress.

https://www.facebook.com/sharkbiteforensics

https://www.instagram.com/sharkbiteforensics/

Permitting

DNA swab sampling activities were conducted under the University of Hawai‘i Institutional Animal Care and Use (IACUC) protocol # 19-3168 and. CNMI Scientific Research License: SRC21-05-RE.

Results

Initial validation of transfer DNA swabbing technique using captive sharks

Swab samples were collected from 2 shark-bitten striped mullet during proof-of-concept experiments with one fish bitten by a captive scalloped hammerhead and the other by a sandbar shark. The depredating species for each event were clearly observed (e.g. Figure 1b). In both cases, DNA barcoding of swab samples clearly identified the species observed biting the striped mullet.

Real-world depredation sampling in Guam and Saipan fisheries

Swab samples were collected from a total of 29 real-world shark depredation events in waters off Guam and Saipan with metadata supplied for 22 of these events (Table 1). All samples for which metadata were provided were collected during bottomfishing trips, with 17 samples

collected by 2 Guam-based fishers and the remaining 5 samples collected by 1 Saipan-based fisher. Depredated fishes included several species of snapper, dogtooth tuna and trevally (Table 1, Figure 2).

Figure 2. Guam fisher James Borja swabs a shark-bitten Onaga head.

Barcoding analyses successfully identified the culprit shark species in 26 (90%) of 29 depredation events (Table 1). A total of 5 shark species were identified from depredation swabs: silvertip shark (*Carcharhinus albimarginatus*), silky shark (*Carcharhinus falciformis*), grey reef shark (*Carcharhinus amblyrhynchos*), whitetip reef shark (*Triaenodon obesus*) and tiger shark (*Galeocerdo cuvier*)(Table 1).

Camera results

We deployed cameras (GoFish Pro) on trolling lines on two fishing trips during our initial visit to Guam (bad weather prevented additional trips). No depredation occurred but a grey reef shark was recorded shadowing a mahimahi (*Coryphaena hippurus*) approaching a lure (Figure 3).

Figure 3. A trolled camera captures a grey reef shark stalking a mahimahi approaching a trolling lure.

Table 1. Summary of shark depredation events and depredating shark species identified through DNA barcoding. Superscript numbers after depredating species names indicate the number of swabs yielding useable transfer DNA.

Depredation Event #	Date	Time	Home Port	Fishing Location	Fishing Activity	Damaged Fish Species	Depredating Shark Species
1	4/19/20	13:30	Guam	South Rosa Banks	Bottom	Red Gill Emperor	Inconclusive
2	4/19/20	13:40	Guam	South Rosa Banks	Bottom	Red Gill Emperor	Silvertip Shark[3]
3	4/19/20	14:15	Guam	South Rosa Banks	Bottom	Red Gill Emperor	Grey Reef Shark[3]
4	8/15/20	15:20	Guam	Rota Banks	Bottom	Dog Tooth Tuna	Silvertip Shark[3]
5	8/15/20	16:00	Guam	Rota Banks	Bottom	Dog Tooth Tuna	Silvertip Shark[3]
6	8/15/20	17:15	Guam	Rota banks	Bottom	Amberjack	Grey Reef Shark[1]
7	4/13/20	12:00	Saipan	1 miles east of marine beach	Bottom	Emperor snapper	Silvertip Shark[1]
8	5/28/20	21:30	Saipan	2 miles SW of Saipan	Bottom	Blue stripe snapper	Whitetip reef shark[3]
9	6/18/20	11:00	Saipan	1/2 mile south obyan	Bottom	Emperor snapper	Whitetip reef shark[3]
10	4/8/20	17:00	Saipan	NW Pt. of Tinian	Bottom	Emperor snapper	Inconclusive
11	3/9/20	11:50	Saipan	1 Miles east of tank beach	Bottom	Trevally	Silvertip Shark[1]
12	6/6/20	15:20	Guam	Off North	Deep Bottom	Onaga	Silvertip Shark[2]
13	6/6/20	15:40	Guam	Off North	Deep Bottom	Onaga	Silvertip Shark[1]
14	5/23/20	15:10	Guam	Off North	Deep Bottom	Onaga	Silvertip Shark[1]
15	5/9/20	15:15	Guam	Off North	Deep Bottom	Onaga	Silvertip Shark[3]
16	5/16/20	12:30	Guam	Off North	Deep Bottom	Onaga	Silvertip Shark[3]
17	2/8/20	12:50	Guam	Off North 2 miles	Deep Bottom	Onaga	Silvertip Shark[2]
18	4/11/20	14:10	Guam	Off North	Deep Bottom	Ehu	Silvertip Shark[1]
19	7/21/20	9:10	Guam	3 miles north of Ritidian	Deep Bottom	Pale snapper	Silvertip Shark[1]
20	7/25/20	16:35	Guam	North	Deep Bottom	Chum Bag	Tiger Shark[1]
21	8/15/20	13:55	Guam	North	Deep Bottom	Dog tooth tuna	Silky Shark[2]
22	8/29/20	14:50	Guam	North	Deep Bottom	Onaga	Silky Shark[2]
23	N/A	N/A	N/A	N/A	N/A	N/A	Inconclusive
24	N/A	N/A	N/A	N/A	N/A	N/A	Tiger shark[1]
25	N/A	N/A	N/A	N/A	N/A	N/A	Tiger shark[1]
26	N/A	N/A	N/A	N/A	N/A	N/A	Tiger shark[1]
27	N/A	N/A	N/A	N/A	N/A	N/A	Silky Shark[1]
28	N/A	N/A	N/A	N/A	N/A	N/A	Silky Shark[1]
29	N/A	N/A	N/A	N/A	N/A	N/A	Silky Shark[1]

Discussion

Our results confirm that swab sampling of fish damaged by shark depredation is a viable method for identifying the depredating shark species via mtDNA barcoding analysis. Previous proof-of-concept studies relied on researchers to swab depredated target species (Fotedar et al. 2019, Drymon et al. 2019) whereas we demonstrated that citizen scientist fishers can collect viable transfer DNA samples from shark-bitten fishes with minimal training. Our results suggest that this approach could be easily transposed to other fisheries where shark depredation occurs (e.g. high-seas longline fisheries) to definitively identify the depredating species. Depredation swab kits and online training could be provided to fishers and fisheries observers. This method is directly applicable to the NMFS management priority of improving understanding of indirect and unaccounted mortality resulting from predation associated with commercial and recreational fisheries.

Only 4 of 25 fishers who originally volunteered for the study followed through on sample collection. The global Covid-19 pandemic was probably the major factor contributing to this low uptake rate. The pandemic disrupted fishing activities and prevented project personnel from returning to Guam and Saipan in order to work directly with fishers and encourage continued participation in the project. Although direct correspondence and social media outreach was conducted throughout 2020, these remote efforts were not enough to encourage a higher level of ongoing participation by the original volunteers. Clearly these were unprecedented circumstances but future efforts to characterize shark depredation in Guam and Saipan should prioritize field visits by project personnel to keep local fishers engaged. Incorporating University of Guam graduate students into the research team could facilitate engagement with the local fishing community and build local technical capacity for this type of applied research.

Despite a limited sample size (n=29 depredation events) we provided the first clear insights into shark species depredating bottomfish catches in Guam and Saipan. The depredating shark species identified in our study were representative of the coastal shark assemblage in the Marianas Archipelago (Compagno 1984, Compagno and Niem 1998). Three of five species identified (silvertip shark, grey reef shark and whitetip reef shark) are strongly reef-associated (Last and Stevens 1994) and the remaining two (tiger shark, silky shark) are strongly associated with shelf habitats and drop-offs (Compagno 1984, Compagno and Niem 1998). No threatened or endangered shark species (e.g. oceanic whitetip sharks, *Carcharhinus longimanus*) were detected interacting with the Marianas bottomfish fishery. Our limited camera results revealed a clearly identifiable grey reef shark stalking a mahimahi following a trolled lure confirming that in-line cameras attached to fishing gear are also a viable method for identifying depredating shark species.

Future research should focus on (1) additional depredation swab sampling to further characterise the depredating species (2) additional trolling camera work to characterise

depredation in the troll fisheries, and (3) telemetry studies of the known depredating species to understand their movements and site fidelity. Passive acoustic monitoring (implanting sharks with acoustic transmitters and tracking them with underwater receivers) could be used to monitor shark visits to bottomfishing sites and identify any predictable periods of low-shark presence when depredation will be naturally lowest, or periods where fishing should be avoided due to high shark abundance. This information could be developed into simple shark avoidance guidelines based on locations, times, moon phases, and seasons. An acoustic telemetry study could have direct industry involvement with chartered bottomfishing vessels used as platforms for shark tagging and receiver deployment and recovery. Chartering bottomfishing vessels would provide financial incentives to participate in the project while leveraging fisher skill and expertise in identifying bottomfishing sites for shark tagging and receiver placement.

Shark depredation is a long-running and ongoing problem in the CNMI and Guam troll and bottomfish fisheries. It is a fundamentally difficult problem to mitigate but gaining a clearer understanding of the problem through the development and use of new techniques can contribute to potential solutions.

References

Altschul SF, Gish W, Miller W, Meyers EW, Lipman DJ. (1990) Basic local alignment search tool. J Mol Biol 215:403-410.

Amesbury SA, Cushing FA, Sakamoto RK (1986) Guide to the coastal resources of Guam: Vol. 3, Fishing on Guam. Univ. Guam Press, 110 p.

Amesbury SA, Babin M (1990) Ocean temperature structure and the seasonality of pelagic fish species near Guam, Mariana Islands. Micronesica 23: 131-138.

Ayers AL (2018) The Commonwealth of the Northern Mariana Islands Fishing Community Profile: 2017 Update. NOAA Tech. Memo. NMFS-PIFSC-66 57 p.

Compagno LJV (1984). FAO species catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 2. Carcharhiniformes. FAO Fisheries Synopsis 125:251-655.

Compagno LJV, Niem VH (1998). Carcharhinidae. Requiem sharks. p. 1312-1360. In K.E. Carpenter and V.H. Niem (eds.) FAO Identification Guide for Fishery Purposes. The Living Marine Resources of the Western Central Pacific. FAO, Rome.

Chan HL, Pan M (2019) Tracking economic performance indicators for small boat fisheries in American Samoa, Guam, and the Commonwealth of the Northern Mariana Islands. NOAA Tech. Memo. NMFS-PIFSC -79, 76 p.

Dalzell P, Adams TJH, Polunin NVC (1996) Coastal fisheries in the Pacific Islands. Oceanography and Marine Biology: an Annual Review 34:395-531.

Drymon JM, Cooper PT, Powers SP, Miller MM, Magnuson S, Krell E, Bird C (2019) Genetic identification of depredator species in commercial and recreational fisheries. North American Journal of Fisheries Management. https://doi.org/10.1002/nafm.10292

Fotedar S, Lukehurst S, Jackson G, Snow M (2019) Molecular tools for identification of shark species involved in depredation incidents in Western Australian fisheries. PLoS ONE 14(1): e0210500. https://doi.org/10.1371/journal. pone.0210500

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S et al. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics;28:1647-1649.

Last PR, Stevens JD (1994) Sharks and rays of Australia. CSIRO, Australia. 513 p.

Myers RF (1993) Guam's Small-Boat-based Fisheries. Marine Fisheries Review 55:117-128

Western Pacific Regional Fishery Management Council (2004) – Minutes of the 123rd meeting.

Western Pacific Regional Fishery Management Council (2006) - Minutes of the 135th meeting.

Western Pacific Regional Fishery Management Council (2013) - Minutes of the 158th meeting.

Western Pacific Regional Fishery Management Council (2016) – Minutes of the 166th meeting.