Pilot Project to Enhance the Capacity of Ecuador to Use Genetic Identification Techniques to Assist in Implementation of CITES Shark Listings

Proyecto Piloto para la Capacidad de Ecuador para Utilizar Técnicas de Identificación Genética para Ayudar en la Aplicación de la CITES Listados de Tiburones

Projet Pilote pour Améliorer la Capacité Équateur à Utiliser des Techniques D'identification Génétique pour Aider è la Mise en œuvre D'annonces de Requins CITES

WESSLEY MERTEN[1], JIMMY MARTÍNEZ-ORTIZ[2], DIEGO CARDEÑOSA[3], SUSANNA CABALLERO[4], JENNY GILES[5], LAURA CIMO[1], and JOHN HYDE[6]

[1]*NMFS – Office of International Affairs and Seafood, 1315 East-West Highway, Silver Spring, Maryland 20910 USA. *wessleymerten@gmail.com.*

[2]*World Wildlife Fund- – Ecuador, Los Almendros, Mz K, villa 18., P.O. Box 13-01-8993, Guayaquil, Ecuador.*

[3]*School of Marine and Atmospheric Science, Stony Brook University, Stony Brook, New York 11794 USA.*

[4]*Laboratorio de Ecología Molecular de Vertebrados Acuáticos, Universidad de los Andes, Carrera 1 No. 18A-10, Bogotá, Columbia.*

[5]*National Academy of Sciences, NWFSC Forensic Laboratory, 2725 Montlake Blvd. E., East Bldg, Seattle, Washington 98112 USA.*

[6]*NMFS – Southwest Fisheries Science Center, 8901 La Jolla Shores Dr., La Jolla, California 92037 USA.*

ABSTRACT

A collaborative pilot project is underway between NOAA Fisheries, the World Wildlife Fund (WWF), and the Government of Ecuador to train Ecuadorian officials in genetic techniques to identify sharks incidentally landed and traded from Ecuador that are included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II. Collectively, 30 government officials from Ecuador's Vice-ministry of Aquaculture and Fisheries and Ministry of Environment have received training on species-specific genetic identification techniques over the course of two workshops. The hands-on training has been provided to Ecuador to help increase the country's capacity to monitor shark products in fisheries and trade and to ensure compliance with requirements under CITES. Furthermore, these trainings along with the provision of the necessary equipment have laid the foundation for Ecuador to improve their National Plan of Action for the Conservation and Management of Sharks by implementing new science and policy objectives to identify shark export subproducts to species level, insert an additional step in the shark product export process, and provide an important tool for continuous monitoring of shark landings. Improving the capacity of Ecuador to detect CITES Appendix II species bound for international markets can greatly aid in trade-monitoring and enforcement efforts to successfully implement CITES shark listings and serve as a model that can be replicated in the region to improve the conservation and management of global shark populations

KEY WORDS: CITES, Shark conservation, shark management, species-specific PCR, Ecuador

INTRODUCTION

Since 2003, the Convention on the International Trade of Endangered Species of Wild Flora and Fauna (CITES) has listed eight species of shark and manta rays (earliest to latest: *Cetorhinus maximus*; *Rhincodon typus*; *Carcharodon carcharias*; *Lamna nasus*; *Carcharinus longimanus*; *Sphyrna lewini*; *S. mokarran*; *S. zygaena*; *Manta alfredi*; *M. birostris*) to CITES Appendix II, a designation that imparts strict regulation in trade of these species by requiring import and export certificates by CITES parties including species-specific landings and origin of harvest information (Vincent et al. 2013). Accurate species-specific data of shark landings, discards, and traded products will allow better quantification of catch and trade trends and provide for more robust stock assessments of individual stocks which are essential for sustainable fisheries management (Stevens et al. 2000).

Both acts of species listing, and implementation of the listings, receive variable support from any given CITES party, largely due to the following issues: relations with other multilateral agreements, rules and procedures for issuance of permits and certificates, enforcement and compliance capacity (Vincent et al. 2013). This has resulted in very few shark and ray listings in Appendix II, and those that are may not reap the underlying conservation benefit if parties cannot properly implement science and policy initiatives to follow through on the capture and trade requirements. While morphological techniques (e.g., fin comparisons, morphometrics, distinguishable features) have been traditionally used to identify sharks and rays to species level when handled (e.g., dead or alive) (Strauss and Bond 1990, Hernández et al. 2010), these methods are often difficult to use when identifying sharks that have been landed fins detached, headless and gutted, or processed and traded (Hernández et al. 2010). As a result, genetic techniques (e.g., DNA barcoding, species-diagnostic PCR) are increasingly being used to identify sharks or rays to species level during any process of the harvest or trade supply chain (Wong et at. 2009, Caballero et al. 2012, Rocha et al. 2013). However, utilization of genetic techniques by CITES parties has been complicated due to lack of funding and resources, competence, and guidance. The objective of this paper is to provide an example of a capacity building project being conducted with the Government of Ecuador to implement the CITES Appendix II shark and ray listings using genetic identification techniques.

MATERIALS AND METHODS

A grant was provided from the National Oceanic Atmospheric Administration National Marine Fisheries Service (NOAA Fisheries) to World Wildlife Fund Ecuador who worked in conjunction with Ecuador's Ministry of Agriculture, Livestock, Aquaculture, and Fisheries (MAGAP), Vice-minister of Aquaculture and Fisheries (VMAP), and Ministry of Environment (MAE) to arrange for government officials and other personnel to be trained in genetic identification techniques. World Wildlife Fund Ecuador then worked in close consultation with genetic experts in NOAA Fisheries to purchase enough genetic equipment and supplies (Table 1) necessary to set up two functioning laboratories in the country. After the equipment was purchased, the United States Department of State worked with the U.S. Consulate in Ecuador to arrange for the equipment and supplies to be shipped down to Ecuador where World Wildlife Fund Ecuador could receive the equipment and deliver it to VMAP.

Following delivery of the equipment to VMAP, two workshops (April 27 - 29, 2015; July 7 - 9, 2015) were arranged where a group of genetic experts traveled to Ecuador to train government officials in the proper use and identification of CITES Appendix II shark and rays using genetic techniques. During the workshops, participants processed shark tissue samples collected from Playa de Tarqui (one of the main shark landings sites in Ecuador), Manta, Ecuador; the samples were collected by the workshop facilitation team the morning before each workshop began. In order to test the success of the participants in properly identifying the samples, the workshop facilitation team concealed the identity of the samples until participants acquired results (gels). To identify the samples collected, participants were trained to extract DNA from the tissue samples, amplify DNA using specific primers (Table 2) and the polymerase chain reaction, and identify samples to family and species level using gel electrophoresis and molecular barcoding (Figure 1). Currently, this project is ongoing and another workshop is scheduled to be held in January of 2016 to document the progress made since the last workshop held in July of 2015.

RESULTS

Over the course of two workshops held in Manta, Ecuador, a total of 30 participants from the Vice-ministry of Aquaculture and Fisheries, Ministry of Environment, and a local university were trained in the proper use of genetic equipment and techniques to identify shark and ray species landed in Ecuadorian large and small scale commercial fisheries destined for trade. Sharks identified using the techniques included *Alopias pelagicus*, *A. superciliosus*, *Sphyrna lewini*, *S. zygaena*, *Carcharhinus falciformis*, *C. longimanus*, *Isurus paucus*, *Prionace glauca*; of these, *S. lewini*, *S. zygaena*, and *C. longimanus* are listed on CITES Appendix II. Workshop participants were able to amplify and identify sharks to both family and species level (Figure 2).

Table 1. Equipment and materials checklist for genetic labs established in Ecuador.

Item Description	Supplier	Quantity
Digital camera workstation	Fotodyne	2
Spectrafuge Mini centrifuge	Fotodyne	4
FOTO/Force 300 Power Supply	Fotodyne	2
0.5-10 µL pipetor	BioExpress	4
10-100 µL pipetor	BioExpress	2
100-1000 µL pipetor	BioExpress	2
1-10 µL 8-channel pipetor	BioExpress	2
Genemate LE Quick Dissolve Agarose	BioExpress	1
Tris-Borate-EDTA Buffer Powder	BioExpress	4
Genemate strip tubes with attached caps	BioExpress	6
Extended Length 10 µL pipette tip racks case of 4800	BioExpress	2
1000 µL tips pack of 768	BioExpress	1
100 µL tips pack of 960	BioExpress	2
96-well PCR tube rack 5 pack	BioExpress	**5**
Unirack centrifuge rack 10 pack	BioExpress	1
BioExpress Comb 16 Well 1.5mm (E-1107-16-1.5)	BioExpress	4
Enduro Mini Gel Box, 7x10cm	BioExpress	2
Multigene Optimax Gradient Thermal Cyclers	BioExpress	2
Pyrex media bottle 10 pack	BioExpress	1
Nalgene 1L polyethylene bottle 6-pack	BioExpress	1
Ohaus 200g balance	Cole-Parmer	2
Tweezers 7" 12 pack	Cole-Parmer	1
Vortex mixer	Cole-Parmer	2
Magnetic stir plate	Argos Technologies	**4**
Magnetic stir bar	Argos Technologies	4
Ethidium bromide solution	BioExpress	1
100bp DNA ladder	BioExpress	1
HotstarTaq Plus Mastermix Kit (1000)	Qiagen	1
Chelex 100 resin 50g	BioRad	1

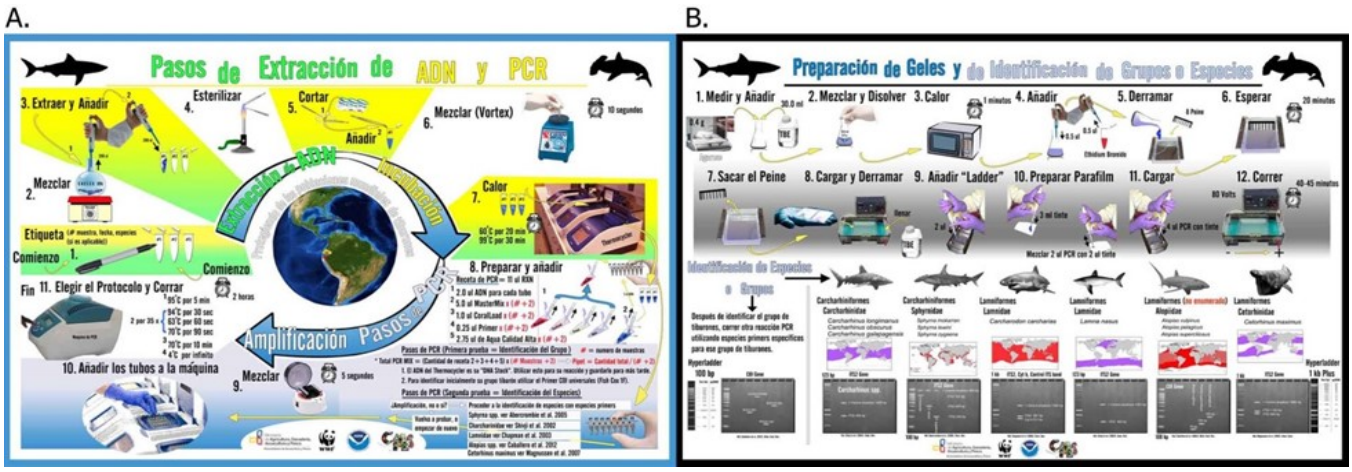

Figure 1. Workshop participants were trained in techniques used to (A.) extract and amplify DNA from tissues samples, then (B.) identify the samples using gel electrophoresis. These diagrams detail all the steps involved from sample collection to species identification and were provided to the participants as step-by-step guides in poster (width x height; 36" x 24") and document format (8.5" x 11") during the workshops.

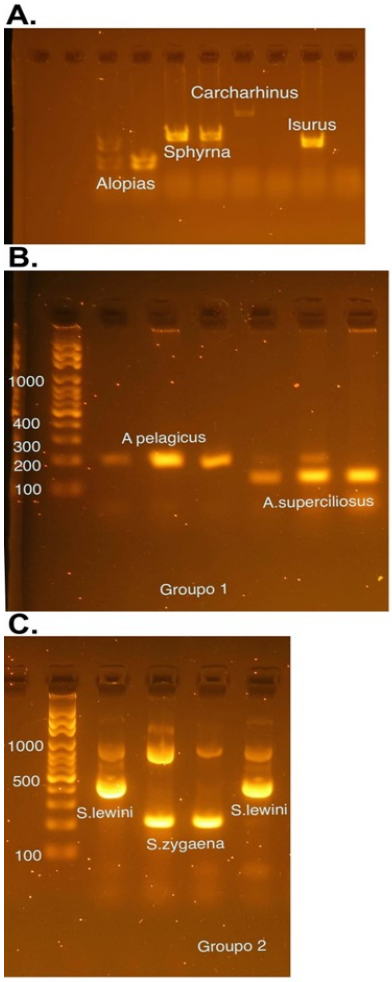

Figure 2. Workshop participants were trained on the steps involved in DNA extraction, amplification, and identification of (A.) families and (B.) species of sharks on agarose gels. These gels show successful amplifications from samples prepared solely by the participants trained during the workshop.

Table 2. Universal, family, and species-specific primers were provided to each of the laboratories established in Ecuador.

Sequence Name	Sequence
Fish Cox 1F	TCW ACC AAC CAC AAG AYA TYG GCA C
Thresher R (P)	TAG AAG TGA TCC TGG CTG TCC TAA
Thresher R (C)	TAG AAG TGA TCC GGG TTG TCC TAA
Thresher R(BE)	TAG GAG TGA TCC GGG CTG GCC TAA
Hammer R	GCT TCT ACY CCA GCR GAA GCT
Mako R (SF)	GCT ATG TCT GGT GCT CCG ATC
Mako R (LF)	CCA TAT CGG GTG CAC CGA TC
Carcharhinidae R	ACA TGA TAA AGG ATT GGA TCT CCT CCA
Universal thresher F	AGC TGG RGT TGA AGC YGG AG
Common thresher R	TCC AGC ATG TGC TAG ATT TCC C
Bigeye thresher R	TTG ATG AGA TAC CTG CTA AAT GAA GC
Pelagic thresher R	GTT TGA TAT TGG GAG ATT GCA GGG
Fish 5.8 S-F	TAG CGG TGG ATC ACT CGG CTC GT
Fish 28 S-R	TCC TCC GCT TAG TAA TAT GCT TAA ATT CAG C
GtHH123F	AGC AAA GAG CGT GGC TGG GGT TTC GA
ScHH401F	GGT AAA GGA TCC GCT TTG CTG GA
SmHH630F	TGA GTG CTG TGA GGG CAC GTG GCC T
Longfin mako	CCT CAA CGA CAC CCA ACG CGT TC
Shortfin mako	AGG TGC CTG TAG TGC TGG TAG ACA CA
Blue shark	AGA AGT GGA GCG ACT GTC TTC GCC
Silky shark	ACC GTG TGG GCC AGG GTC
Porbeagle	GTC GTC GGC GCC AGC CTT CTA AC
Dusky / Galapagos shark	GTG CCT TCC CAC CTT TTG GCG
GWSITS2F	GCT GGA GTT CAT TCT CCG TGC TG
GWScb-R	AGT CAG AAC TAG TAT GTT GGC TAC AAG AAT
LAM 499F	GCT TCT CAG TAG ACA ACG CCA CCC T

DISCUSSION

Ecuador has been at the forefront of efforts to effectively monitor and sustainably manage shark fisheries in the eastern tropical Pacific following the implementation of different management and regulatory policies. The Ministry of Agriculture, Livestock, Aquaculture and Fishing (MAGAP), through the Subsecretariat of Fishing Resources (SPR), issued a Ministerial Agreement managing incidental catches of scalloped and smooth hammerhead shark (MICIP 2006). They also adopted a system for monitoring and tracking shark products in trade from the point of harvest to the point of export (i.e., a chain of custody program). Therefore, as a supporter of the CITES shark listings that were adopted, and for the aforementioned policies, Ecuador was identified as a country where genetic capacity building assistance to implement the CITES Appendix II shark and ray listings could be effectively implemented. With the development of this capacity, Ecuador has proposed various science and policy based initiatives to both properly implement the CITES appendix II shark and ray listings but also explore other applications of the tools and skills obtained through this project (Table 3).

Species-diagnostic PCR based genetic techniques is a cost-effective ($5 - $10USD/sample) technique to gather species-specific identification information. Other genetic techniques that can be used to gather species-specific information include DNA sequencing (Rocha et al. 2013). However, in the context of capacity building, DNA sequencing are much more costly and sophisticated than species-diagnostic PCR techniques and therefore, more difficult to implement both financially and institutionally.

Personnel from Ecuador's Vice-Ministry of Aquaculture and Fisheries, Ministry and Environment, and local universities were trained during this project, however, individuals from customs were not. In the future, projects should focus on training Government officials from all relevant government authorities (Fisheries, Enforcement, and Customs).

The implementation of this project provides an additional tool to support enhanced collection of species-specific landings and trade data to support both the CITES Appendix II shark and ray listings but also improve shark and ray stock assessments and management within Ecuadorian waters. The successful implementation of this project within Ecuador provides a model framework that can be expanded to other CITES parties within Central and South America to improve the long-term management and conservation of commercially harvested shark and ray species.

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Table 3. Potential science and policy based outcomes as a result of project implementation. For each proposed outcome, a result and/or management implication is provided.

Proposed Science Outcome	Result/Management Implication
Genetic analysis of export subproducts (minor fins and parts)	Species-specific identification of shark sub-product trade/Improve species stock assessments and level of fisheries socio-economic impact
Genetic identification of shark product seizures	Species-specific identification of shark product seizures/Determine species harvested, number, and verify conservation status for enforcement
Genetic analysis of domestic consumption of sharks	Species-specific identification of shark product consumption/Reduce seafood fraud through species substitution
Population genetic studies	Determine population structure of shark and ray species/Determine extent of fishing pressure on shark and ray populations
Proposed Policy Outcome	Management Implications
Establish two genetic labs	Improve species-specific data collection; Improve stock assessments; Improve shark fishery socioeconomics; Improve identification of protected species landed and traded
Insert additional step in export process	Verify extent of species-specific trade to improve stock assessments; Understand species-specific trade trends; Improve conservation of sharks and rays
Provide recommendations to relevant fisheries authorities on use and implementation of genetic laboratories	Improve agency knowledge and communication; Guide funding and personnel support; improve agency impact on conservation and management of regional shark and ray resources