Ministerio de Ambiente, Vivienda
Desarrollo Territorial
República de Colombia

Libertad y Orden

Universidad de los Andes
Colombia

Prosperidad para todos

Genetic characterization of the main tuna species (Yellow Fin Tuna, Bigeye Tuna and Skipjack Tuna) and Tuna Bycatch species (Sharks, Mahi-Mahi, Wahoo, Sea Turtles, Marine Mammals and Billfishes) regulated by the Interamerican Tropical Tuna Commission - IATTC - in the Eastern Pacific Ocean

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May, 2011

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On behalf of the Government of Colombia:

Libertad y Orden

Ministerio de Ambiente, Vivienda
y Desarrollo Territorial

Universidad de los Andes
Colombia

In collaboration with:

Inter-American Tropical Tuna Commission - IATTC
National Oceanographic and Atmospheric Administration – NOAA
University of Stony Brook
University of Stanford
Centro de Educación Científica y de Educación Superior de Ensenada - CICESE
Instituto Colombiano de Desarrollo Rural –INCODER
MarViva Colombia
Fundación Malpelo y Otros Ecosistemas Marinos
Centro de Investigación para el Manejo Ambiental y Desarrollo –CIMAD
Conservation International Colombia
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ABSTRACT

This research project aims to contribute to the management of the main tuna species and bycatch species in the eastern Pacific Ocean, managed by the Inter-American Tropical Tuna Commission (IATTC), in terms of the genetic characterization of these populations, in order to have more reliable management tools which, together with stock assessments, will give the IATTC staff better scientific information to support conservation measures for these species. The project aims to work with strategic stakeholders of the scientific community in Colombia and other countries of the region, in order to obtain tissue samples of yellowfin tuna (*Thunnus albacares*), bigeye tuna (*T. obesus*), skipjack tuna (*Katsuwonus pelamis*), mahi-mahi (*Coryphaena hippurus*), some shark species such as the thresher shark (*Alopias pelagicus*), hammerhead shark (*Sphyrna lewini*) and silky shark (*Charcharhinus falciformis*), among others. Other species include wahoo (*Acanthocybium solandri),* striped marlin (*Tetrapturus audax*) and blue marlin (*Makaira nigricans*), sea turtles (*Chelonia mydas*, *Caretta caretta*) and marine mammals such as several dolphin species or whales that may be captured as bycatch in the tuna fisheries. The research work will include the scientific staff of the IATTC, universities, research institutions, NGOs and government staff involved with this kind of work

RESUMEN

Este proyecto de investigación pretende contribuir a la ordenación de las principales especies de atunes y especies de captura incidental en el Océano Pacífico Oriental, gestionados por la Comisión Interamericana del Atún Tropical – CIAT – , en términos de la caracterización genética de estas poblaciones, a fin de contar con herramientas de ordenación más fiables que, junto con las evaluaciones de las poblaciones, brindará al personal de la CIAT una mejor información científica para apoyar las medidas de conservación de estas especies. El proyecto pretende trabajar con los actores estratégicos de la comunidad científica en Colombia y otros países de la región, a fin de obtener muestras de tejido de atún aleta amarilla (*Thunnus albacares*), atún patudo (*T. obesus*), atún barrilete (*Katsuwonus pelamis*), dorado (*Coryphaena hippurus*), ciertas especies de tiburones tales como el tiburón zorro (*Alopias pelagicus*), cornuda (*Sphyrna lewini*) y tiburón sedoso (*Charcharhinus falciformis*), entre otros. Otras especies incluyen el peto o sierra wahoo (*Acanthocybium solandri),* marlín rayado (*Tetrapturus audax*) y marlín azul (*Makaira nigricans*), tortugas marinas (*Chelonia mydas*, *Caretta caretta*) y mamíferos marinos tales como varias especies de delfines o ballenas que podrían ser capturadas incidentalmente en las pesquerías atuneras. El trabajo de investigación incluirá el personal científico de la CIAT, universidades, instituciones de investigación, ONG, y personal del gobierno relacionado con este tipo de trabajo.

1. INTRODUCTION

Tuna fish are pelagic species that are commercially very important, due to their tropical and subtropical distribution and their big size and abundance in all oceans (Griffiths 2010; Wu *et al.* 2009; Wu *et al.* 2010). It has been suggested that yellow-fin tuna (*Thunnus albacares*) seems to be the most important species fished in the Eastern Tropical Pacific (Díaz-Jaimes & Uribe-Alcocer 2006), followed by big-eye tuna (*Thunnus obesus*) and skipjack tuna (*Euthynnus pelamis*) (Aires-da-Silva & Maunder 2010;

Maunder 2010; Maunder & Aires-da-SIlva 2010). These species are the focus of commercial, artisanal and recreational fisheries (Griffiths 2010). For this reason, it is important to understand their biology, life cycles and stock structure, in order to be able to manage these resources in a long term, sustainable way. For some areas, including the Eastern Tropical Pacific, it has been suggested that restricted longitudinal migrations and restricted gene flow among northern and southern populations of yellow-fin tuna exist (Díaz-Jaimes & Uribe-Alcocer 2006). In this species, it is know that spawning occurs in particular warm water, tropical, locations and may be influenced by upwelling events closer to coastal areas (Wexler *et al.* 2003). Generally speaking, it is believed that overfishing is not a problem confronted by these tuna species. This has been suggested based on studies on the population genetic structure and genetic diversity, that show big population sizes (Ely *et al.* 2005; Wu *et al.* 2009), accompanied by relatively high genetic diversity (Griffiths 2010). To date, only the Southern blue- fin tuna (*Thunnus maccoyii*) has been classified as in critical risk of extinction (IUCN, 2009).

Tuna is an important resource for commercial and artisanal fisheries and local communities. That is why it is important to study them and to be able to predict possible effects of anthropogenic activities on their population. This will allow for implementation of management and conservation programs (IUCN, 2009) and also to determine if one section of the population or one sex is at higher risk due to particular fishing practices or timing (Robertson & Gemmell 2006). Tuna plays an important role in maintaining ecosystem health, as they are top predators and, therefore, this characteristic needs to be taken into consideration when determining for fishing quotes (Chiang *et al.* 2008; Wu *et al.* 2009).

Although tuna species have some morphological characteristics that allow for their identification at sea (Chiang *et al.* 2008; Griffiths 2010; Wu *et al.* 2009; Zagaglia *et al.* 2004), some species are morphologically quite similar, and may be easily confused at sea. For this reason using molecular techniques to accurate identify species based on

their differences at the genetic level (DNA Barcoding) (Hajibabaei *et al.* 2007). Is recommended in order to have consistent species capture data (Teletchea 2009). "DNA barcoding" is done by amplifying and sequencing a region of the mitochondrial Citochrome Oxidase subunit 1 gene (COI). This technique has been widely applied to a series of marine organisms, including sharks (Abercrombie *et al.* 2005). In previous studies on tune developed by our research group, with support of Mariva Foundation, we have been able to identify not only one but to species of *Euthynus* found in artisanal catches in Northern Colombia (i.e. *Eutynnus affinis* and *Euthynus lineatus*). *Euthynus affinis* is considered a species common to the Indopacific, but we found that it is also frequently found in the Pacific Coast of Colombia (Mariño, 2010).

In Colombia, there are very few studies regarding the population structure and population biology of tuna species. For the Eastern Tropical Pacific, Díaz-Jaimes and Uribe-Acolocer (2009) researched the population structure of yellow-fin tuna. These researchers used a series of molecular markers, including seven microsatellite loci. Samples included in this study were obtained mainly from the U. S. A, Peru and México, but they did not include samples from the Pacific Coast of Colombia. This study showed differentiation among tuna stocks north and south of the Ecuador, but, to date, there is no published information assigning tuna caught in Colombia to either of these populations. A preliminary study by Mariño (2010), analyzing microsatellite markers on yellow-fin tuna captured in artisanal fisheries in the Northern Pacific coast, showed that this population seems to have high levels of genetic diversity.

One problem that affects commercial tuna fisheries is bycatch of a series of large pelagic organisms, including sharks, turtles, billfishes, wahoo, mahi-mahi and small marine mammals (Hall 1996). Bycatch destiny is, in the best case, freed and returned alive to sea (turtles and marine mammals), but in many cases, it is used, especially when bycatch are species that may have high commercial value, including billfishes, mahi-mahi, wahoo and some shark species (Graves 1998). Some of the species

affected by bycatch in tuna fisheries are considered as vulnerable, species, specially sea turtles and sharks, whose population have shown recent declines (Lewison *et al.* 2004a; Lewison *et al.* 2004b). In other cases, it has been suggested that bycatch may also affect whole marine communities and their structure and that their effect may not only be lethal but may be sublethal (reduced reproduction and fitness) (Lewison *et al.* 2004a). It is difficult to quantify the effect of bycatch on populations due to data recording and spatial scale (Lewison *et al.* 2004a). One way to better understand possible effects of bycatch on particular marine pelagic species and problems with data reporting, may be to use samples obtained from animals captured as bycatch to determine species identity and provide important data to population genetic studies. This may be a good starting point to understanding what species are more affected and how to improve their management and the policies and techniques to try to reduce bycatch to a sustainable level (Lewison *et al.* 2004a).

The project pretends to find key information of the genetic structures and biodiversity of target and bycatch species of the tuna fishery in the Eastern Pacific Ocean (EPO) including Colombian waters. For this purpose, the project has been divided into 8 sub-projects that deal with the main species captured by the tuna fleet in the EPO.

2. OBJECTIVES

2.1 Main objective

- To genetically characterize tuna and bycatch species, as a tool to get more scientific supported criteria to improve the tuna fisheries management, including an ecosystem approach in the Eastern Pacific Ocean.

2.2 Specific Objectives

- To understand the population structure and genetic diversity of yellow-fin tuna, Bigeye Tuna and Skipjack Tuna captured in the Eastern Pacific Ocean, including Colombian waters, and to understand where these population belong in terms of genetic stock assignment.
- To identify species of marine pelagic species commonly captured as bycatch in tuna fisheries in the Eastern Pacific Ocean, including Colombian waters, using molecular markers, such as sequencing of the mitochondrial COI, including sea turtles, marine mammals (if available), wahoo, mahi-mahi, billfishes, and sharks.
- To identify the population structure, migration and genetic diversity of bycatch species (sea turtles, marine mammals, wahoo, mahi-mahi, billfishes, and sharks) distributed along the Eastern Pacific Ocean, including Colombian waters.

3. GENERAL METHOD

Observers an board from the IATTC and IDCP observers Program, National Observers Programs and Colombian Pilot Observer Program will collect samples an board during their trips during one (1) year. Samples will be collected for target and bycatch species and number of samples will depend on fishing grounds, time of the current month of the sample, number of species to collect samples, and special circumstances on board. Sampling won't damage captured fish or any bycatch species that will be released alive. Observers will follow all safety procedures to obtain samples and sampling will depend on available time to fulfill their duties under the International Dolphin Conservation Program –IDCP-, IATTC or under any other National Pilot Programs.

Skin and/or muscle samples will be collected in 90% ethanol or 20% DMSO solution saturated with NaCl. The samples size will be between 0.5-1 cm^3. Samples will be collected in 2 ml (total volume) plastic tubes and will be stored frozen or at room

temperature if no refrigeration is available. Once samples are shipped to the lab, total DNA will be extracted following traditional phenol- chlorophorm protocols of by digestion with chelex resin solution (Sambrook *et al.* 1989). Depending on the project or research question, particular gene sections (mitochondrial or nuclear DNA) will be amplified by the polymerase chain reaction (PCR) (Palumbi 1996).

Samples taken by the IDCP and IATTC program will be gathered by IATTC staff. Samples taken by National Observers Programs will be gathered by IATTC staff or fisheries authorities, and samples gathered by Pilot observers Programs will be gathered by the Fisheries authorities. All samples will be sent to the Molecular Ecology Laboratory of the Andes University (Bogotá, Colombia) to be processed. Collected samples will be divided depending on species and eight (8) sub-projects will be developed to analyze each group of species or a specific species if required. Results to be applied in a regional level will be covered by confidential clauses under the IATTC rules, or by agreements to be arranged with the main stakeholders involved, so that those results are warranted with impartial analysis and correspond strictly to scientific findings. The sub-projects developed are as follows.

3.1 Population structure of Yellow fin Tuna (*Thunnus albacores*), Bigeye Tuna (*Thunnus obesus*) and Skipjack Tuna (*Katsowonus pelamis*) in the Eastern Pacific Ocean

Tuna is an important resource for commercial and artisanal fisheries and local communities. That is why it is important to know how their populations are doing, if they are genetically healthy to assure long term fisheries sustainability. There are a number of studies that have tried to understand the genetic diversity and stock structure of a variety of tuna species in the Pacif Ocean (Díaz-Jaimes & Uribe-Alcocer 2006). However, to date, very little is known about the stock structure of tuna species in the Eastern Tropical Pacific. We would like to understand the levels of genetic diversity, the

phylogeographic and population genetic structure patterns in three tuna species, yellow fin Tuna (*Thunnus albacores*), bigeye Tuna (*Thunnus obesus*) and skipjack Tuna (*Katsowonus pelamis*) in the Pacific Coast of Colombia, using microsatellite markers. This will also allow us for direct comparisons and assignment test to understand how tuna in Pacific Colombian fit into the Pacific stock structure found previously by other researchers.

We also want to compare information from animals fished in oceanic waters with animals obtained from artisanal fisheries in the Pacific Coast of Colombia, to understand possible populations dynamics such as migration and current gene flow between pelagic and coastal populations.

3.2 Population structure of mahi-mahi (*Coryphaena hippurus*) in the Eastern Tropical Pacific: different stocks or one panmictic population?

Mahi-mahi is a cosmopolitan fish species, usually found in tropical and subtropical waters (Palko *et al.* 1982). They are considered abundant in the Eastern Tropical Pacific and are part of directed fisheries and also captured as tuna bycatch. Data from 1994 y 1996 show captures of about para Colombia 1322.230 tons (Zapata 1993). Some seasonality has been observed for this fisheries in the Eastern Tropical Pacific (Lasso& Zapata 1999). This seasonality can be related to seasonal migrations from reproductions areas or be related to changes in water temperatures (Diaz-Jaimes *et al.* 2006). This apparent spatial and temporal seasonality may have the effect of driving populations or stocks through genetic differentiation processes. Results from previous studies on mahi-mahi population structure undertaken in Mexico, show some heterogeneity among populations but slight differentiation, suggesting a big panmictic population (Diaz-Jaimes *et al.* 2010; Tripp-Valdez *et al.* 2010)

To date, no information is available regarding population structure and genetic diversity of mahi-mahi in the Pacific Coast of Colombia. We plan to analyze five microsatellite

loci previously described by (Tripp-Valdez *et al.* 2010) and sequencing of two mitochondrial gene regions (NADH1 and cytochrome *b*)(Rocha-Olivares& Chávez-González 2008). Our analyses will be divided *a priori* by catch month, so that it may be possible to detect if genetic heterogeneity corresponds to differential migration of particular stocks along the Pacific of Colombia, in order to compare these data with information gathered by other researchers studying this species in other points of the Eastern Tropical Pacific.

3.3 Species identification of sharks captured as bycatch in tuna fisheries

Molecular methods have been successfully used tools to identify shark species and body parts in markets and ports (Clarke *et al.* 2006; Shivji *et al.* 2002). In a recent interregional shark management workshop, organized in Manta, Ecuador, in July 2010, we started a very productive collaboration with Dr. John Hyde (NOAA), who developed new molecular methods for identification of sharks from the Eastern Tropical Pacific based on multiplexing and sequencing of the mitochondrial gene Cytochrome Oxidase subunit II. As a result from this collaboration, we are about to submit a first manuscript, presenting the methodology and first results of molecular identification of sharks species from the Pacific Coast of Colombia (Caballero et al., in prep). Our idea is to continue using these methods in the identification of species affected by bycatch in the Eastern Tropical Pacific.

3.4 Phylogeography, genetic diversity and population structure of scalloped hammerhead sharks (*Sphyrna lewini*) and silky sharks (*Carcharhinus falciformis*).

Very little is known about the population structure, phylogeography and genetic diversity of these two sharks species in the Eastern Tropical Pacific. The scalloped hammerhead shark is distributed in tropical waters and there is some evidence of possible female phylopatry to particular nursery areas (Compagno 1984; Duncan *et al.* 2006). The taxonomy of scalloped hammerhead sharks is still unclear, with possible cryptic species

found in the Eastern Tropical Pacific (Quattro *et al.* 2006). The first phylogeographic study of scalloped hammerhead sharks revealed connectivity between close coastal areas, but low levels of female dispersal in open ocean, and therefore, the possibility of female phylopatry to particular coastal areas. This study also revealed distinct populations between ocean basins (Duncan *et al.* 2006). Only eight samples from the Eastern Tropical Pacific were included in this first study, so information regarding population structure and phylopatry for this population is still needed. To our knowledge, there is currently no published information regarding the population diversity and genetic diversity of silky sharks, although is one of the species strongly affected by tuna fisheries bycatch (Watson *et al.* 2009).

These two species are considered high priority in the Colombian shark Action Plan (PAN-Tiburones Colombia) (Caldas-Aristizábal *et al.* 2010) and, therefore, strong interest exists in trying to understand their reproduction, biology, population structure and genetic diversity. For both species we intend to determine the genetic diversity, population structure and possible female phylopatry of this species in the Eastern Tropical Pacific by means of analyses of mitochondrial DNA (Duncan *et al.* 2006; Keeney & Heist 2006) and microsatellite genotyping (Nance *et al.* 2009; Ovenden *et al.* 2009).

3.5 Migratory patterns and population structure of the pelagic thresher shark (*Alopias pelagicus*) in the Tropical Eastern Pacific: key information for sustainable management plans and long term conservation.

Thresher sharks seem to be abundant in the Eastern Tropical Pacific and the pelagic thresher shark appears to be an important species for local fisheries in the Pacific Coast of Colombia (Caballero *et al*. In prep). It is also one of the prioritized species in the Colombian Action Plan (PAN-Tiburones Colombia)(Caldas-Aristizábal *et al.* 2010). This study will allow us to look into the migratory patterns and population structure of the pelagic thresher shark a top oceanic predator in the TEP in order to (i) assess seasonal

migrations, (ii) determine the locations of aggregation sites of different populations detected by genetic analyses, (iii) describe for the first time the migratory behavior and habitat use of the pelagic thresher shark, (iv) to use mitochondrial and nuclear molecular markers to study the population structure of *Alopias pelagicus* in the TEP, levels of gene flow mediated by females and males and (v) determine genetic diversity of *Alopias pelagicus* in the TEP.

This is an international project, involving scientist and students from Colombia, USA and Mexico, including academic institutions (Universidad de los Andes, Stanford University, Stony Brok University, CICESE), governmental institutions (Ministry of Environment-Colombia, NOAA-SWFSC) and non-governmental organizations (Fundación Malpelo, Colombia). As part of this international collaboration, one colombian student will have the opportunity to get trained on satellite tracking techniques and genetics and will possibly develop his PhD project working in collaboration with Dr. Salvador Jorgensen (Stanford University).

This project will benefit immensely from samples from this species collected by observers along the Eastern Tropical Pacific, as these will give us a much more complete picture of population structure and migratory patterns of *Alopias pelagicus* in this location.

3.6 Phylogeography, genetic diversity and population structure of billfishes, including wahoo (*Acanthocybium solandri*), striped marlins (*Tetrapturus audax*) and blue marlins (*Makaira nigricans*).

Previous studies have detected little genetic structure and high levels of genetic diversity among stocks of wahoo (Garber *et al.* 2005; Theisen *et al.* 2008). However, to date, no or little data from samples from the Eastern Tropical Pacific has been included in these analyses. We would like to then analyze wahoo samples obtained as bycatch, in order to understand where the Eastern Tropical Pacific groups fit into this worldwide phylogeographic analyses. For the striped marlins and blue marlins, we would like to

confirm species identification using molecular techniques and contribute data to previous phylogeographic analyses for this species, that show some genetic differentiation among ocean basins (Graves & McDowell 1995, 2003).

3.7 Population structure, differential sex-mediated gene flow and paternity analysis in green turtles (*Chelonia mydas*) and loggerhead turtles (*Caretta caretta*)

Sea turtles are an important component of bycatch in tuna fisheries (Lewison *et al.* 2004b). Some of the species that are greatly affected by bycatch are the green and loggerhead turtles. To date, some information on the biology of these species exists for the Pacific Coast of Colombia, including information about their diet and digestion (Amorocho & Reina 2008) and some initial information regarding their population structure (Amorocho 2009). Most of this information has been obtained from turtles found in Gorgona Island in the Colombian Pacific. However, although sea turtles are also found on beaches along the Pacific Coast very little information exists about these populations. No information exists regarding their connectivity, if any, to populations studied in Gorgona or to other nesting beaches in the Eastern Tropical Pacific. No information exists either about female phylopatry and male mediated gene flow that may be occurring among these groups in the mainland and in Gorgona Island. For this reason, we would like to investigate the population structure, connectivity and male and female mediated gene flow (Moore & Jr 2002) among sea turtle populations in the Pacific Coast of Colombia and with other populations in the eastern Tropical Pacific by means of analyses of mitochondrial DNA (Bjorndal *et al.* 2005; Bowen *et al.* 1995; Bowen *et al.* 2005) and microsatellite analyses (Dutton& Frey 2009; Shamblin *et al.* 2009) We would also like to be able to determine, via population assignment methods, to which population turtles caught belong to.

This section of the project will be worked in collaboration with Dr. D. Amorocho (CIMAD), who leads sea turtle research in the Pacific Coast of Colombia.

3.8 Species identification of Marine Mammals (cetaceans)

We would like to obtain samples from any marine mammal caught has bycatch in tuna fisheries. Initially, we would just confirm identification of the sample by means of molecular identification. If eventually we obtain sufficient numbers of samples, we may consider starting a project on population structure for one particular marine mammal species from the Eastern Pacific Ocean, including Colombian waters. This part of the research will be developed in collaboration with Dr. Phil Morin (NOAA-SWFSC).

After the sampling period (1 year), an additional year will be dedicated to analyze each species or group of species for each sub-project, and to prepare official final reports of each sub-project. A complete final report, considering results from all subprojects will also be prepared.

4. PARTICIPATING INSTITUTIONS AND THEIR ROLE

4.1 Universidad de Los Andes, LEMVA (Colombia):

The Aquatic Vertebrates Molecular Ecology Laboratory -LEMVA, is part of the Biological Sciences Department at Universidad de los Andes and has developed projects working on the genetic structure and conservation genetics of various aquatic vertebrate species, including sharks, dolphins, whales, river otters, tunas and ornamental fish species. In this particular project, scientist from LEMVA, including Susana Caballero and a number of MSc and PhD students will lead genetic analyses for all samples collected as bycatch. Data analyses will also be part of the analyses undertaken by scientist from this institution. Financial resources will be managed with the support of the financial office of the Science Faculty at Universidad de los Andes.

4.2 Ministry of Environment, Housing and Territorial Development of Colombia

The Ministry will work together with the Universidad de los Andes to process genetic access permits required according to current international conventions the country is

part of. A fisheries expert from the Ministry will be part of the analysis and results implementation. According to results, the Ministry will work together with strategic stakeholders to propose management and conservation measures for all species included in these analyses in the Eastern Pacific Ocean and jurisdictional waters, together with the National Fisheries Authority.

4.3 Interamerican Tropical Tuna Commission –IATTC- and the Observers program of IDCP.

IATTC and IDCP will participate in sample collection on vessels included in its observer programs. Scientific collaboration with scientist from IATTC will be also part of this active collaboration and commitment. National Observer Programs are also encouraged to participate in the sample collection during the trips they are in charge of.

4.4 Fisheries and Aquaculture National Authority –INCODER- Colombia

The Colombian Fisheries authority is already collaborating with Universidad de los Andes with sample collection done by their national observers in small to medium tuna fishing vessels of foreign countries legally allowed to fish in Colombian jurisdictional waters. Samples are of few species and it is pretended to open the range of other species included in the main project.

4.5 National Oceanographic and Atmospheric Administration NOAA- Southwest Fisheries Science Center

We are already actively collaborating with researchers at NOAA-SWFSC, including Dr. John Hyde, with whom we have been working on the shark species molecular identification project and we are looking for funding so that we can also start collaborating in the *Alopias pelagicus* project.

We are also currently collaborating on other projects, including cetacean phylogenetics and phylogeography with Dr. Phil Morin and Dr. Barbara Taylor.

4.6 Non Government Organizations

Universidad de los Andes, Ministry of Environment and the National Fisheries Authority –INCODER – are currently working with Colombian and International NGOs interested in research, conservation and fisheries management of marine resources. NGOs already working or willing to work in this kind of projects are:

- *Fundación Marviva Colombia*: It is supporting a project to do molecular species identification and genetic diversity of tuna species captured in artisanal fisheries in the Northern Colombian Pacific (Chocó). Interests are focused in comparing population structure of stocks from industrial tuna fisheries and artisanal tuna fisheries in order to understand if they are part of a common stock or different pelagic vs. coastal stocks.

- *Fundación Malpelo*: It is supporting a research work on genetic identification of sharks species landed at the Buenaventura port, as well as sharks confiscated from illegal fishing around Malpelo Island National Marine Sanctuary. Research projects on phylogeography and female phylppatry of the scalloped hammerhead shark are currently being developed, and results might be helpful for this regional project.

- *CIMAD (Centro de Investigaciones para el Manejo Ambiental y el Desarrollo):* Interests is focused on genetic diversity, phylogeography and populations structure of sea turtles in the Eastern Pacific Ocean, with enphasis of those visiting the Colombian Pacific coast.

- *Conservation International Colombia*: Interests are focused on migratory species management, and it has been supporting research work with sea turtles, sharks and fisheries management in general.

Another NGOs are welcome to be part of this initiative, as soon as it is clarified their role in the Project or each sub-projects.

4.7 Academia

We are already collaborating for particular sub-projects, with researchers from a number of Universities, particularly in the United States (Stony Brook, Stanford) and Mexico (CICESE), but many opportunities will exist to contact other Universities in Central and South America, or even in eastern countries.

4.8 Fisheries Authorities

We will invite a number of fisheries institutes and fisheries authorities from a number of countries in the Eastern Pacific Ocean, to be part of this initiative. Some of these include: Instituto del Mar de Perú– IMARPE- (PERU) , Instituto Nacional de Pesca– INP- (ECUADOR), Subsecretaria de Pesca SRP. (Ecuador), Autoridad de Recursos Acuáticos de Panamá– ARAP, Instituto Costaricense de Pesca y Acuicultura– INCOPESCA-, OSPESCA, and national observers programs in the United States, Colombia, Panamá and Venezuela, the European Union fisheries authorities, eastern countries fisheries authorities or research institutions and fishing entities.

4.9. Other Entities

Fisheries Project of the Eastern Tropical Pacific Marine Corridor – CMAR-:

The CMAR fisheries project in Colombia is working together with the national fisheries authority in Pilot observer programs, bycatch, Mahi- Mahi and Sharks projects. The

support of these projects may help to gather tissue samples for the species involved in this project.

5. EXPECTED RESULTS

We expect to obtain important information regarding the species, population structure, phylogeographic and migratory pattern of Tuna species currently fished in the Eastern Tropical Pacific, as well as crucial genetic information for a series of important bycatch species in tuna fisheries. These results will be helpful for the IATTC counterparts as another scientific supported tool to take decisions on conservation measures and stock assessment analyses of tuna species. Results on bycatch species will significantly help to understand the impact of tuna fisheries on these populations, taking these species into account in the conservation measures, in order to apply an ecosystem approach. Results will also help countries or fisheries authorities involved to understand better migratory species behavior during their journey in their jurisdictional waters, giving tools to make agreements among them regarding the management of these species in their waters, which will have to be in accordance to the management of the same species in international waters.

Results are expected to be presented in a number of international scientific meetings and published in per-review indexed international scientific journals. Information gathered in these projects may be presented in the meetings of the IATTC scientific advisory committee, and fisheries authorities of different Governments. We hope that the information obtained in this project can also be shared with other scientist already working in some of these subjects at the national level.

6. ACTIVITIES SCHEDULE

Table 1 shows the general activities and the time schedule to do each one. The table may be modified, according to more detailed activities, if required.

Table1. Activities schedule for the Project

Genetic characterization of the main tuna species (Yellow Fin Tuna, Bigeye Tuna and Skipjack Tuna) and Tuna Bycatch species (sharks, mahi-mahi, wahoo, sea turtles, marine mammals and billfishes) regulated by de Interamerican Tropical Tuna Commission – IATTC - in the Eastern Pacific Ocean																								
Item	Activities	Months																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	Sample collection																								
2	Sample shipping to Bogotá, Colombia (Universidad de los Andes)																								
3	DNA extraction																								
4	DNA amplification, sequencing and microsatellite genotyping																								
5	Statistical analyses and phylogeographic comparisons																								
6	Partial reports (each sub-project)																								
7	Final reporting (full project)																								
8	Preparation for publication																								

	Reports
	Activities to perform

7. BUDGET

General budget are detailed in table 2. The budget provides information based on the general activities provided in table 1 and explain in detail the staff involved in the project, sample shipping to the Andes University, laboratory equipment, materials and other items that will cover the project. For some items, detailed budget is not described, since this depend on facilities of each agency depending on their role in the project. Anyway, we decided to estimated an approximate cost (with an over cost) that may change. Therefore the overall cost of the project may change as well with a trend to reduce costs.

Table 2: General budget

Activities	Items	Quantity	Provided by:						
			Univ. Andes	Min. Env.	INCODER	IATTC	NGO	Others	TOTAL
	1. Human Resources								
	University experts	1							
	Agency experts	5							
	Laboratory staff	2							
	Other staff	8							
	Graduate students (draduate assistanceship)	8							
	Undergraduate students	2							
	SubTotal								**110615***
	2. Sample shipping								
	Packaging								
	shipping								
	Subtotal:								**18000***
	3. Laboratory Equipment								
	Biosafety cabinet class I Type A2	2							
	Vapor extraction hood	1							
	Micropipettes (P-10, P-100, P-1000)	10							
	Fridge	2							
	Orbital Shaker	1							
1. Sample collection	Fourth decimal place analytical balance	1							
	Vacuum pump	1							
	Magnetic stirrer with heating plate	1							
2. Sample shipping to Bogotá, Colombia (Universidad de los Andes)	Vortex	2							
	Horizontal electrophoresis chamber	5							
	Vertical electrophoreis chamber	1							
	DNA fluorometer	1							
	Microcentrifuge	2							
	Thermocycler (PCR)	2							
	Sequencer ABI 3100	1							
3. DNA extraction	REVCO freezer -80oC	1							
	Computer	2							
	Subtotal:		22130						**22130**
4. DNA amplification, sequencing and microsatellite genotyping									
	4. Laboratory materials								
	500 sampling tubes (2 ml) per subproject	4800 tubes							748
	Absolute ethanol (1 Lt) per subproject	8 Lt							528
	DMSO solution (1 Lt) pero subproject	8 Lt							528
	Chelex DNA extraction for 300 samples per subproject	2400							1725
5. Statistical analyses and phylogeographic comparisons	Pipette tips 100-1000ul Axygen, price for five bags per subproject	48 bags							1276
	Pipette tips 1-10 ul Axygen, price for five bags per subproject	48 bags							968
	Pipette tips 10-200 ul Axygen, price for five bags per subproject	48 bags							942
	Proteinase k 10 mg (3 vials per subproject)	24 vials							1769
6. Partial reports (each sub-project)	Latex gloves (3 boxes per subprojects)	24 boxes							198
	Eppendorf 1.5 ml tubes, 500 tubes per bag,five bags per subproject	48 bags							1276
7. Final reporting (full project)	Biolase DNA polymerase, 3 tubes per subproject	24 tubes							2640
	dNTP mix 100 ml, 1 tube per subproject	8 tubes							528
	Flat cap thin wall tubes 0.2 ml, 2000 per subproject	tubes							1056
8. Preparation for publication	Primers, 10 in average, unlabelled, per subproject	80 primers							1936
	Primers, labelled, 10 average per subproject	80 primers							11440
	Agarose, 100 gr, per subproject	800 gr							1584
	Blue juice, loading buffer 10X x 3 tubes 1 ul each, per subproject	24 tubes							880
	Boric Acid x 500 gr, per subproject	4800 gr							396
	EDTA x 500 gr, per subproject	4800 gr							1628
	Tris Base x 500 gr	4800 gr							484
	Ethidium Bromide x 10 ml, 10mg/ml	80 ml							528
	Hyperladder IV Bioline, range 100-1000 bp	8 tubes							1188
	Hi-Di formamide x 25 ml, 2 per subproject	16 vials							880
	subproject	16 tubes							11440
	PCR purification via polyetylenglyco-ethanol, 3,3 USD per sample	samples							7920
	Sequencing at Macrogen, Korea, 5,5 USD pr sample	2400 samples							13200
	Subtotal:								**67686**
	5. Air tickets								**2000**
	6. Printing and publications								**3000**
	7. Events participation								**2000**
	TOTAL PROJECT COST								**225431***

	item provided by one or more agencies
*	aproximate cost

8. REFERENCES

Abercrombie DL, Clarke SC, Shivji MS (2005) Global-scale genetic identification of hammerhead sharks: application to the assessment of the international fin trade and law enforcement. *Conservation genetics* **6**, 775-788.

Aires-da-Silva A, Maunder MN (2010) Condición del atún patudo en el Océano Pacífico Oriental en 2008 y perspectivas para el futuro (ed. IATTC), La Jolla, CA.

Amorocho DF (2009) *Foraging ecology and population structure of the green sea turtle (*Chelonia mydas*) in the Eastrn Pacific Coast of Colombia*, Monash University.

Amorocho DF, Reina RD (2008) Intake passage time, digesta composition and digestibility in East Pacific green turtles (*Chelonia mydas agassizii)* at Gorgona National Park, Colombian Pacific. *Journal of Experimental Marine Biology and Ecology* **360**, 117-124.

Bjorndal KA, Bolten AB, Troeng S (2005) Population structure and genetic diversity in green turtles nesting at Tortuguero, Costa Rica, based on mitocondrial control region sequences. *Marine Biology* **147**, 1449-1457.

Bowen BW, Abreu-Grobois FA, Balazs GH*, et al.* (1995) Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers. *Proceedings of the National Academy of Sciences of the USA* **92**, 3731-3734.

Bowen BW, Bass AL, Soares L, Toonen RJ (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). *Molecular Ecology* **14**, 2389-2402.

Caballero S, Cardeñosa D, Soler G, Hyde, J. In prep. Application of multiplex PCR approaches for shark molecular identification: feasibility and applications for fisheries management and conservation in the Eastern Tropical Pacific.

Caldas-Aristizábal JP, Castro-González E, Puentes V*, et al.* (2010) Plan de acción nacional para la conservación y manejo de tiburones, rayas y quimeras de Colombia (PAN-Tiburones Colombia), p. 70. Instituto Colombiano Agropecuario, Secretaria Agricultura y Pesca de San Andrés, Ministerio de Ambiente, Vivienda y Desarrollo Territorial, Insituto de Investigaciones Marinas y Costeras, Instituto Alexander von Humboldt, Universidad del Magdalena, Universidad jorge Tadeo Lozano, Pontificia Universidad Javeriana, Fundación Squalus, Fundación Malpelo y otros Ecosistemas Marinos, Conservación Internacional, WWF Colombia.

Chiang H-C, Hsu C-C, Wu GC-C, Chang S-K, Yang H-Y (2008) Population structure of bigeye tuna (*Thunnus obesus*) in the Indian Ocean inferred from mitochondrial DNA. *Fisheries Research* **90**, 305-312.

Clarke SC, Magnussen JE, Abercrombie DL, Mcallister MK, Shivji MS (2006) Identification of shark species composition and proportion in the Hong Kong shark fin market based on molecular genetics and trade records. *Conservation Biology* **20**, 201-211.

Compagno LJV (1984) *Sharks of the world*.

Díaz-Jaimes P, Uribe-Alcocer M (2006) Spacial differentiation in the eastern Pacific yellowfin tuna revealed by microsatellites variation. *Fisheries Science* **72**, 590-596.

Diaz-Jaimes P, Uribe-Alcocer M, Ortega-García S, Durand JD (2006) Spatial and temporal mitochondrial DNA genetic homogenetiy in dolphinfish populations (*Coryphaena hippurus*) in the eastern central Pacific. *Fisheries Research* **80**, 333-338.

Diaz-Jaimes P, Uribe-Alcocer M, Richa-Olivares A, *et al.* (2010) Global phylogeograahy of the dolphinfish (*Coryphaena hippurus*): the influence of large effective population size nd recent dispersal on the divergence of a marine pelagic cosmopolitan species. *Molecular Phylogenetics and Evolution* **57**, 1209-1218.

Duncan KM, Martin AP, Bowen BW, DeCouet HG (2006) Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini)*. *Molecular Ecology* **15**, 2239-2251.

Dutton PH, Frey A (2009) Characterization of polymorphic microsatellite markers for the green turtle (*Chelonia mydas*). *Molecular Ecology Resources* **9**, 354-356.

Ely B, Viñas J, Alvarado-Bremer JR, *et al.* (2005) Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonus pelamis*). *BMC Evolutionary Biology*.

Garber AF, Tringali MD, Franks JS (2005) Population genetic and phylogeogrpahic structure of wahoo, *Acanthocybium solandri,* from the western central Atlantic and central Pacific Oceans. *Marine Biology* **147**, 205-214.

Graves JE (1998) Molecular Insights Into the Population Structures of Cosmopolitan Marine Fishes. *Journal of Heredity* **89**, 427-437.

Graves JE, McDowell JR (1995) Inter-ocean genetic divergence of istiophorid billfishes. *Marine Biology* **122**, 193-203.

Graves JE, McDowell JR (2003) Stock structure of the world´s istiophorid billfishes: a genetic perspective. *Marine and freshwater research* **54**, 287-298.

Griffiths SP (2010) Stock assessment and efficacy of size limits on longtail tuna (*Thunnus tonggol*) caught in Australian waters. *Fisheries Research* **102**, 248-257.

Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA (2007) DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* **23**, 167-172.

Hall MA (1996) On bycatches. *Reviews in Fish Biology and Fisheries* **6**, 319-352.

Hinton MG, Maunder MN (2002) Status of striped marlin in the Eastern Pacific Ocean in 2002 and outlook for 2003-2004.

Keeney DB, Heist EJ (2006) Worldwide phylogeography of the blacktip shark (*Carcharinus limbatus*) inferred from mitochondrial DNA reveals isolation of western Atlantic populations coupled with recent Pacific dispersal. *Molecular Ecology*.

Lasso J, Zapata L (1999) Fisheries and biology of *Coryphaena hippurus* (Pisces: Coryphaenidae) in the Pacific coast of Colombia and Panama. *Scientia Marina* **63**, 387-399.

Lewison RL, Crowder LB, Read AJ, freeman SA (2004a) Understanding impacts of fisheries bycatch on marine megafauna. *Trends in Ecology and Evolution* **19**, 598-604.

Lewison RL, Freeman SA, Crowder LB (2004b) Quantifying the effects of fisheries on threatened species: the impact of pelagic longlines on loggerhead and leatherback turtles. *Ecology Letters* **7**, 221-231.

Mariño, C. 2010. Identificaión de especies y genética poblacional del atún (tribu Thunnini:Scombridae) en las costas del Pacífico Norte Colombiano. BSc thesis, Universidad de los Andes, Bogotña, Colombia.

Maunder MN (2010) Indicadores actualizados de la condición de la población de atún barrilete en el Océano Pacífico Oriental (ed. IATCC), La Jolla, CA.

Maunder MN, Aires-da-SIlva A (2010) Condición del atún aleta amarilla en el Océano Pacífico Oriental en 2008 y perspectivas para el futuro (ed. IATTC).

Moore MK, Jr RMB (2002) Multiple paternity in loggerhead turtle (*Caretta caretta*) nests on Melbourne Beach, Florida: a microsatellite analysis. *Molecular Ecology* **11**, 281-288.

Nance HA, Daly-Engel TS, Marko PB (2009) New microsatellite loci for the endangered scalloped hammerhead shark, *Sphyrna lewini*. *Molecular Ecology Resources* doi: 10.1111/j.1755-0998.2008.02510.x

Ovenden JR, Street R, Broderick D (2009) New microsatellite loci for Carcharhinid sharks (*Carcharhinus tilstoni* and *C. sorrah*) and their cross-amplification in other shark species. *Molecular Ecology Notes* **6**, 415-418.

Palko JB, Beardsley GL, Richards WJ (1982) Synopsis of the biological data on dolphin-fishes, *Coryphaena hippurus* and *Coryphaena equiselis*. In: *FAo Fisheries Synopsis*. NOAA Technical Report.

Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In: *Molecular Systematics* (eds. Hillis D, Moritz C, Mable BK). Sinauer Associates, Suderland, MA.

Quattro JM, Stoner DS, Driggers WB, *et al.* (2006) Genetic evidence of cryptic speciation within hammerhead sharks (genus *Sphyrna*). *Marine Biology* **148**, 1143-1155.

Robertson BC, Gemmell NJ (2006) PCR-based sexing in conservation biology: Wrong answers from an accurate methodology? *Conservation Genetics* **7**, 267-271.

Rocha-Olivares A, Chávez-González JP (2008) Molecular identification of dolphinfish species (genus *Coryphaena*)using multiplex haplotype-specific PCR of mitochondrial DNA. *Ichthyological Research* **55**, 389-393.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York, U.S.A.

Shamblin BM, Faircloth BC, Dodd MG, *et al.* (2009) Tetranucleotide markers from the loggerhead sea turtle (*Caretta caretta*) and their cross-amplification in other marine turtle species. *Conservation genetics* **10**, 577-580.

Shivji M, Clarke S, Pank M, *et al.* (2002) Genetic identification of pelagic shark body parts for conservation and trade monitoring. *Conservation Biology* **16**, 1036-1047.

Teletchea F (2009) Molecular identification methods of fish species: reassessment and possible applications. *Reviews in Fish Biology and Fisheries* **19**, 265-293.

Theisen TC, Bowen BW, Lanier W, Baldwin JD (2008) High connectivity on a global scale in the pelagic wahoo, *Acanthocybium solandri* (tuna family Scombridae). *Molecular Ecology* **17**, 4233-4247.

Tripp-Valdez MA, GarcíadeLeón FJ, Ortega-García S, *et al.* (2010) Population genetic structure of dolphinfish (*Coryphaena hippurus*) in the Gulf of California, using microsatellite loci. *Fisheries Research* **105**, 172-177.

Watson JT, Essington TE, Lennert-Cody CE, Hall MA (2009) Trade-offs in the design of fishery closures: management of silky shark bycatch in the Eastern Pacific Ocean tuna fishery. *Conservation Biology* **23**, 626-635.

Wexler JB, Scholey VP, Olson RJ, *et al.* (2003) Tank culture of yellowfin tuna, *Thunnus albacares*: developing a spawning population for research purposes. *Aquaculture* **220**, 327-353.

Wu GC-C, Chiang H-C, Chen K-S, Hsu C-C, Yang H-Y (2009) Population structure of albacore (*Thunnus alalunga*) in the Northwestern Pacific Ocean inferred from mitochondrial DNA. *Fisheries Research* **95**, 125-131.

Wu GC-C, Chiang H-C, Chou Y-W, *et al.* (2010) Phylogeography of yellowfin tuna (*Thunnus albacares*) in the Western Pacific and the Western Indian Oceans inferred from mitochondrial DNA. *Fisheries Research* **In Press, Corrected Proof**.

Zagaglia CuR, Lorenzzetti JoA, Stech JL (2004) Remote sensing data and longline catches of yellowfin tuna (*Thunnus albacares*) in the equatorial Atlantic. *Remote Sensing of Environment* **93**, 267-281.

Zapata LA (1993) Evaluación preliminar de la pesca blanca en el Pacífico Colombiano. In: *Informe Técnico INPA/Colciencias*, p. 6, Buenaventura.

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