IOTC project summary

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

# Assessing the global genetic population structure and effective population size for the black marlin (*Istiompax indica*)

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## Executive summary

Genetic data are often used to identify the population structure of commercially exploited marine species. The identification of stocks is critical for fisheries management, particularly in highly migratory species that have few barriers to dispersal. This is true for the black marlin *Istiompax indica*, a valuable commercial and recreational species, whose population structure within the Indian and Pacific Oceans is unresolved. Without knowledge of how many stocks exist, the ability of fisheries managers to monitor and regulate exploitation of the species is restricted. This study will investigate the population structure for black marlin through its entire range by utilizing a suite of pre-developed microsatellite markers and next-generation DD-rad sequencing-based molecular methods. Compared to previous analyses of genetic population structure in marine fishes, the large number of molecular markers analyzed in this study will significantly increase the statistical power necessary to detect population structure. This research is in direct support of the Indian Ocean Tuna Commission (IOTC) priority research objective to identified the genetic analysis of istiophorid population structure.

## Background

The black marlin, *Istiompax indica* is one of the world's largest teleosts (females up to 700 kg) and individuals form seasonal aggregations to spawn and forage. Spawning aggregations of *I. indica* are documented to occur off north-eastern Australia from September to November (Domeier & Speare 2012) and south-western Taiwan in March and April (Nakamura 1975, Sun et al. 2014). Coinciding with these aggregations is increased commercial and recreational fishing pressure from longline, purse seine, harpoon and artisanal fishing. (Domeier & Speare 2012). The Indian Ocean Tuna Commission have recently noted that *I. Indica* are vulnerable to exploitation during spawning aggregations even by small-scale fisheries (IOTC 2015) and have expressed concern around the stock status of black marlin. As a result, a preliminary stock assessment

classified *I. indica* as currently experiencing overfishing and subsequently listed the collection of data on the biology, productivity and fisheries of *I. indica* as a high priority (IOTC 2014). This lack of information on the biology and ecology of *I. indica* is also reflected in their classification as 'Data Deficient' on the IUCN Red List (Collette *et al.* 2011a).

Population structure is an important ecological indice for informing stock assessments, as fisheries with separate populations require independent stock management (Shaklee and Bentzen 1998). A review of istiophorid billfish population structure recently noted *I. indica* as a species where uncertainty around the number of stocks remains (Graves and McDowell 2015). For other istiophorid billfishes the use of molecular markers to investigate stock identification has been able to identify succesfully population structure. In the blue marlin (*Makaira nigricans*) the presence of inter-oceanic structure (Indo-Pacific/Atlantic) has been revealed based on a combination of mitochondrial DNA as well as nuclear DNA markers (Buonaccorsi *et al.* 2001; Buonaccorsi *et al.* 1999). Similarly interoceanic structure between the Indo-pacific and Atlantic Ocean has also been detected for sailfish (*Istiophorus platypterus*) using nuclear markers, along with biogeographically discordant mitochondrial clade structure (McDowell 2002). However unlike blue marlin, sailfish have also demonstrated population subdivision within the Pacific Ocean (Graves and McDowell 1995; Graves and McDowell 2002). The striped marlin (*Kajikia audax*) appears to be the most structured of the Istiophorid billfishes representing four discrete breeding populations within the Pacific Ocean (Purcell 2009; Purcell and Edmands 2011).

Until recently, attempts to investigate the stock structure of black marlin using otolith microchemistry (Speare 1992), parasite assemblage (Speare 1999) as well as significant effort in pop-up satellite archival telemetry (PSAT) tags and standard tag-recapture (over 65,000 fish tagged and over 400 recaptured), have been lately unsuccessful in achieving this goal. However recent population genetics focusing on *I. indica* from the central Indo-Pacific research has identified three populations (Figure 1), consisting of; the southwestern Pacific Ocean, South China Sea and eastern Indian Ocean (Williams *et al.* 2015a). The south-western Pacific Ocean population was made up of fish along the Queensland and New South Wales coastlines, while the population in the eastern Indian Ocean was comprised of black marlin captured along the west Australian coast and Gulf of Carpentaria (Williams *et al.* 2015a). The southwestern Pacific Ocean and South China Sea populations are known to be centred on annual spawning aggregations that occur off north-eastern Australia from September to November (Domeier and Speare 2012; Leis *et al.* 1987) and south-western Taiwan in March and April (Sun *et al.* 2015), respectively. The identification of a separate population within the eastern Indian Ocean suggests that a putative spawning exists within the basin.

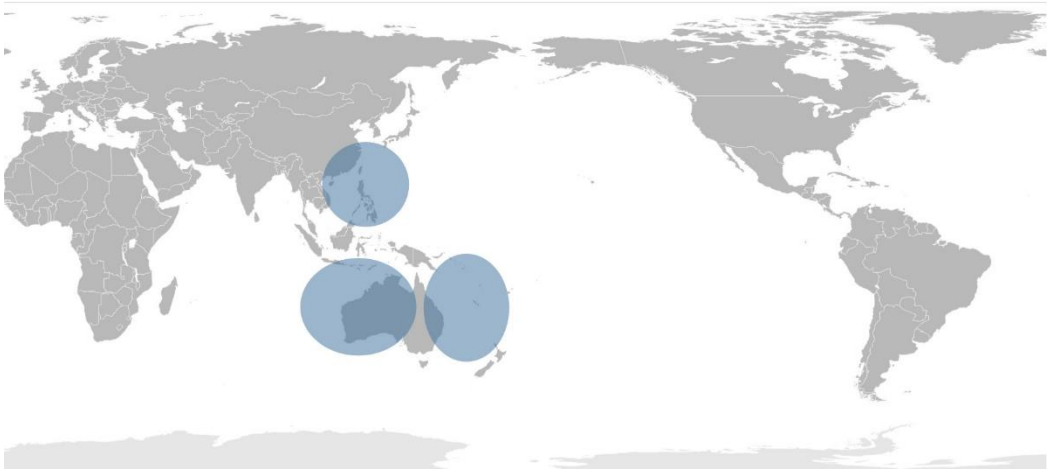

Figure 4. Figure 1: Separate populations of black marlin identified by recent genetics research

The aim of this study is to determine the genetic population structure of black marlin throughout its entire indo-pacific range. The study will test the null hypothesis that a single population exists in the Indian ocean and no more than two population exist in the Pacific Ocean. This investigation will also produce baseline estimates of effective population size among black marlin stocks using genetic data, to provide managers with novel insights into the spawning stock biomass.

**Sampling design**

This research will use state-of-the-art genetic techniques and collaboration with primary researchers throughout the world to identify and describe for the first time the population structure of black marlin (Table 1). It will collect genetic samples from fish markets in Taiwan, Vietnam, Ecuador, Mexico, Costa Rica, India, Sri Lanka, as well as expand on pre-collected samples in Australia, Kenya, South Africa and Mozambique through a citizen science research program.

In the Indian Ocean samples from commercially landed fish will be collected through collaborations with local researchers to samples the pectoral or dorsal fish once at localized artisanal ports. The collection of samples through citizen science sampling will be undertaken by members of the recreational fishing community using methods described by Williams et al (2015). This program will target anglers throughout Australia's no-take, tag-and-release billfish fishery and anglers on the east coast of Africa. Once collected, tissue samples were preserved in either 90% ethanol or 20% dimethyl sulphoxide in saturated NaCl solution, before being stored at -20 $^{o}$C until required for genetic analysis. Body mass will be used to estimate the age of individual fish using the growth key defined by Speare (2004).

Table 1. List of key personnel in the collection of tissue samples

| Country/region | Organization | Key personnel |
|---|---|---|
| Western Indian Ocean | The African Billfish Foundation | Roy Bealey and Tina Harris |
| Sri Lanka | National Aquatic Resources Research and Development Agency | Dr. Deishini Herath and Dr. Sisira Haputhantri |
| India | Annamalai University | Dr V. Ravi |
| Australia | The Game Fishing Association of Australia | Brett Cleary and Dr. Evan Jones |
| Vietnam | Fisheries Resources Department | Vu Viet Ha |
| Taiwan | Eastern Marine Biology Research Center, | Dr. Wei-Chuan Chiang |
| Central America | Depto de pesquerias | Dr. Sofia Garcia |
| Historic samples and global collaboration | Virginia Institute of Marine Science | Nadya Mamoozadeh and Dr. John Graves |
| Western Central Pacific | Western Central Pacific Fisheries Commission | Dr. Anthony Beeching |
| Eastern Pacific | National Oceanic and Atmospheric Administration<br>Inter-American Tropical Tuna Commission | Dr. Matthew Craig<br><br>Dr. Michael Hinton |

**Laboratory procedures**

Results from Williams *et al.,* (2015) suggest that the mitochondrial DNA provided a signal for historic clade structure however, was not sufficient for delineating contemporary population structure as defined by nuclear markers. Given this, the analysis of samples for the global population structure will be undertaken using the species specific microsatellite loci defined by Williams *et al.,* 2014 in combination with a genotype-by-sequencing SNP analysis.

In order to perform the SNP analysis Genomic DNA will be extracted from the tissue samples (consisting of muscle or fin tissue) using the salting out method. High quality tissue will then be sent off for RAD-Sequencing at DArT. SNP sequence data will then be analyzed using bioinformatics software.

The remaining samples will undergo Polymerase Chain Reactions (PCR's) by the University of Queensland's Animal Genetics Laboratory in order to genotype the DNA. The eighteen *I. indica*-specific microsatellite loci used in this study were described by Williams *et al.* (2014). The samples were amplified using a 12 µl PCR mixture containing approximately 10 ng of genomic DNA as per methods described by Williams et al 2015. Eighteen loci will be genotyped and assessed against the Single Nucleotide polymorphisms discovered using the DDRAD-sequencing method for their application to black marlin population genetics.

**Statistical analysis**

Resultant data from the molecular analysis will be used to evaluate population structure using estimates of genetic distance from each of the region. In analyzing the microsatellite loci, the allele frequencies will be pooled by locations to test for conformance to Hardy-Weinberg equilibrium (HWE). Once this is achieved, key indicators of nuclear genetic differentiation such as fixation index, alleles per locus and heterozygosity can be generated (GenAlex software). Standard population-pairwise F-statistics (Fst) will be produced to indicate genetic differentiation and fixation among the three regions sampled by this study (GenAlex or Arlequin software). Population structure results will also be compared across the nuclear marker types to check for correlation between findings. Identified populations will then have their allele frequencies simulated using *Ne Estimator* software in an attempt to determine their effective population size.

Microsatellite allele calling will be undertaken using Geneious version 7.0.5 (Biomatters Ltd., Auckland, NZ) against an internal size standard (GeneScan – 500 LIZ). The presence of null alleles and potential scoring errors is to be addressed using MICRO-CHECKER v. 2.2.3 (Van Oosterhout *et al.* 2004). Deviation from Hardy–Weinberg equilibrium (HWE), linkage disequilibrium (LD) and single locus $F_{IS}$ (inbreeding coefficient, ratio of expected versus observed heterozygosity) will be evaluated for each locus and sampling location combination using GENEPOP 4.0 (Rousset 2008). The exact test for linkage disequilibrium (LD), as implemented in GENEPOP, will be used to detect non-random association of alleles among multiple loci after a straight Bonferroni adjustment. Loci can be deemed unsuitable for estimating population structure and will discarded if they exhibited significant departures from HWE or LD in one or more locus-by-population comparison.

The numbers of alleles ($N_A$) and their frequencies, the effective number of alleles ($N_E$) as well as expected ($H_E$) and observed heterozygosities ($H_O$) will be estimated using GenAlEx ver. 6.5 (Peakall & Smouse 2012). To measure the extent of divergence among populations of the same species relative to the net genetic diversity within the species pairwise $F_{ST}$ values will be undertaken using Arlequin v3.11 (Excoffier *et al.* 2005) with *p*-values calculated by permutation of allele frequencies between populations.

SNP data will be analyzed by separating the data set into panels of neutral and adaptive loci. To identify putative loci under selection Lositan (Antao et al 2008) will be used by computing the pairwise population outlier test. Pairwise Fsts will be calculated in Arlequin v3.5 (Excoffier et al 2010) on both outlier and neutral SNP loci panels for the same purpose as the microsatellite loci in order to evaluate subdivision between populations. Similarly, structure analysis will be used as a Bayesian approach to investigate the number of genetically distinct populations. A Discriminant Principle Component Analysis (DAPC) will also be run in R Studio using the Adegenet package (Jombart and Ahmed 2011) for neutral and outlier loci to investigate the relationship between SNPs, individuals and their clustered population.

**Preliminary Results**

Substantial effort has been made to collect samples from all regions mentioned. The samples have been collected through both landed commercial fish and recreational fisher's catching them as part of tag and release practices. At current 812 samples have been acquired (Table 2). These samples

span up to multiple decades in some regions and with previously identified stocks in the South China Sea and western Pacific Ocean containing up to four decades of variation in sample collections. This availability or historic and contemporary samples will allow for temporal shifts in allele frequencies to be evaluated. In addition, the large sample sizes for the aforementioned regions will permit attempts to determine the effective population size for black marlin, which will represent the first time such a technique has been applied to a billfish stock.

A total of 94 tissue samples have been extracted representing fish from the western Pacific Ocean (20), South China Sea (20), eastern Indian Ocean (20), eastern Pacific Ocean (19) and western Indian Ocean (15). These samples have been sent off to Diversity Array Technology's for preliminary SNP discovery and genotype by sequencing. Additional samples from Sri Lanka, the Eastern Pacific and Western Indian Ocean will be sent off for a second and final round of SNP analysis in October this year. Microsatellite genotyping will be undertaken on all samples collected by January 2017, with a final report to be produced by July 2017.

Table 1. Overview of black marlin tissue samples collected for genetic analysis

| Sampling Location | Year | No. of Samples | Storage Solution | Region |
|---|---|---|---|---|
| South Africa | 1997 | 12 | DMSO | west Indian Ocean |
| South Africa | 1998-1999 | 36 | DMSO | west Indian Ocean |
| South Africa | 2015 | 33 | DMSO | west Indian Ocean |
| Kenya | 2015 | 32 | RNAlater | west Indian Ocean |
| Mozambique | 2015 | 22 | DMSO | west Indian Ocean |
| Seychelles | 1997 | 7 | DMSO | west Indian Ocean |
| E. Australia | 1996-1997 | 45 | DMSO | west Pacific Ocean |
| E. Australia | 1985 | 45 | DMSO | west Pacific Ocean |
| E. Australia | 1984-1989 | 45 | DMSO | west Pacific Ocean |
| E. Australia | 2006-2013 | 50 | EtOH | west Pacific Ocean |
| E. Australia | 2012-2014 | 77 | EtOH | west Pacific Ocean |
| W. Australia | 2012-2015 | 110 | EtOH | east Indian Ocean |
| Gulf of Carpentaria | 2012-2015 | 30 | EtOH | east Indian Ocean |
| Galapagos | 2004-2006 | 14 | EtOH | east Pacific Ocean |
| Central America | 2004 | 26 | EtOH | east Pacific Ocean |
| South America | 2007 | 5 | EtOH | east Pacific Ocean |
| Mexico | 2006-2016 | 11 | EtOH | east Pacific Ocean |
| Ecuador | 1995-97 | 17 | DMSO | east Pacific Ocean |
| Panama | 2004 | 3 | DMSO | east Pacific Ocean |
| Panama | 2001 | 6 | DMSO | east Pacific Ocean |
| Taiwan | 2004 | 39 | DMSO | South China Sea |
| Taiwan | 1998-1999 | 34 | DMSO | South China Sea |
| Taiwan | 2014 | 41 | DMSO | South China Sea |
| Vietnam | 2015 | 27 | EtOH | South China Sea |

| | | | | |
|---|---|---|---|---|
| Eastern Pacific | 2004-2015 | 19 | EtOH | east Pacific Ocean |
| Central pacific | 2014-2016 | 11 | EtOH | central Pacific Ocean |
| India | 2016 | ? | DMSO | central Indian Ocean |
| Sri Lanka | 2016 | 15 | DMSO | central Indian Ocean |
| Total | | 812 | | |

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